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NEURAL CONTROL OF TRACHEAL SMOOTH MUSCLE FROM CONTROL AND IMMUNOLOGICALLY SENSITIZED DOGS

BY

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ABSTRACT

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The precise role of airway smooth muscle in pulmonary physiology is not known; however its regulation of airway calibre and therefore resistance to airflow is appreciated. The regulation of its contractile state is dependent largely on neural and pharmacological mechanisms which are poorly understood. Our ignorance of the interactions of regulation mechanisms is particularly acute as it relates to disease states such as asthma in which the airway smooth muscle may hyperreact to certain stimuli.

The present studies were designed to assess the functional contributions of endogenous neural elements, their prejunctional control mechanisms and the relevant post-junctional receptors in canine tracheal smooth muscle (TSM). The specific objectives were to: 1) Examine adrenoceptor mediated responses in isolated TSM, and the relationship of these responses to active tone, 2) compare adrenoceptor mediated responses of TSM taken from dogs immunologically sensitized to ovallbumin (OA) with a control population, 3) examine the effect of histamine and 5-hydroxytryptamine (5-HT) on isometric tension development by muscles from control and OA-sensitized dogs, and 4) determine the effect of a variety of stimulatory conditions on overflow of acetylcholine and norepinephrine from non electrically stimulated endogenous cholinergic and adrenergic nerves respectively. The effect of propranolol on acetylcholine overflow was also studied. Isometric tension measurements demonstrated physiological importance of neuromodulation.

The effect of tone on responses of canine tracheal smooth muscle (TSM) to norepinephrine (NE) was studied to elucidate the role of sympathetic innervation and adrenoceptors in the control of the airways. Electrical field stimulation produced contraction of TSM *in vitro* which was augmented by eserine, depressed by phentolamine and almost eliminated by TTX or hyoscyamine. Resting TSM did not contract in response to NE (10^{-8} - 10^{-4}M)) normally or in the presence of propranolol (10^{-5}M). The addition of NE (10^{-8} - 10^{-6}M) at the plateaux of contractions produced by K^+ (22.8 mM), histamine (10^{-6}M) or acetylcholine (ACh, 5 x 10^{-8}M) produced a further phentolamine-sensitive contraction which was potentiated by beta-adrenoceptor blockade with propranolol (10^{-5}M). The addition of tyramine (10^{-5} or 10^{-4}M) at the plateau of contraction produced by K^+ (22.8 mM) produced a further contraction which was potentiated by propranolol (10^{-5}M) and reduced by phentolamine (10^{-5}M). Following cold storage (4°C for 3 days) the response to tyramine was inhibited while responses to NE remained. While NE in the presence of active tone caused a further increase in tension at low concentration (10^{-8} to 10^{-6}M), a propranolol-sensitive relaxant response was elicited at higher concentrations (10^{-5} and 10^{-4}M). Maximum contractile responses to NE in the absence or presence of beta-blockade were dependent on the tone of the muscle. These findings suggest a functional adrenergic innervation of canine TSM and the presence of alpha- and betaadrenoceptors which mediate contractile and relaxant responses respectively. The functional dominance of either of the latter is related to the active tone in the muscle and the concentration of NE.

The biphasic responses, involving alpha-adrenoceptor mediated contraction and beta-adrenoceptor mediated relaxation in OA sensitized and control TSM were both qualitatively and quantitatively similar. Unlike control TSM, sensitized tissue at basal tone contracted when exposed to tyramine 10^{-4}M or the combination of propranolol (10^{-5}M) and norepinephrine (10^{-5}M). Beta-blockade with propranolol (10^{-5}M) enhanced the tyramine-stimulated contraction while phentolamine (10^{-5}M) abolished it. These studies attribute the increased alpha-adrenoceptor-mediated responsiveness of sensitized TSM to an increased resting basal tone although this was not measured directly.

The observation that the histamine (10^{-5}M)-stimulated contraction of TSM is about 50% atropine-sensitive suggests a possible action of histamine in promoting acetylcholine release. The addition of eserine (10^{-6}M, an anticholinesterase) prior to histamine enhanced the contraction to 703% of the initial histamine-induced contraction. The further addition of hyoscyamine reduced the contraction to 41% of the initial histamine contraction. A similar pattern although not as pronounced was noted in the atropine-sensitive (10%) component of the 5-HT 10^{-6}M contraction and enhancement (245% of initial) by eserine 10^{-6}M. Eserine (10^{-6}M) was also observed to enhance K^+ (23mM) and electrically stimulated isometric tension development an effect blocked by hyoscyamine. The addition of eserine to unstimulated muscles was without effect. The addition of phentolamine (10^{-5}M) reduced isometric tension of both histamine (10^{-5}M) and 5-HT (10^{-6}M)-induced contractions suggesting a role for these substances in the neuromodulation of norepinephrine

release from adrenergic nerves in addition to their direct contractile effect on TSM.

A similar protocol for histamine and 5-HT in OA-sensitized TSM demonstrated a qualitative and quantitative similarity to control tissues.

Acetylcholine (10^{-8}M)-contracted TSM was partially inhibited (57% of initial) by phentolamine (10^{-5}M) and further addition of atropine (10^{-7}) abolished the contraction. Eserine 10^{-6}M enhanced isometric tension developed following acetylcholine or K^+ (23 mM) stimulation.

These studies suggest a role of 5-HT, histamine and acetylcholine in prejunctional neuromodulation on both cholinergic and adrenergic nerves in TSM. Further studies utilize TSM preincubated in either ^{3}H-norepinephrine or ^{14}C-choline to demonstrate overflow in the unstimulated preparation. ^{3}H-norepinephrine overflow was increased by electrical field stimulation (EFS, 15V, 60 H_z, AC), phentolamine (10^{-5}M), 23mM K^+ nonepinephrine (10^{-6}M) and acetylcholine 10^{-6}M in cocaine (10^{-6}M)-pretreated muscles. When cocaine was absent only electrical and K^+ (23mM) stimulation produced measureable increases in ^{3}H-NE overflow Tyramine (10^{-5}M) was without effect on ^{3}H-NE overflow in the presence or absence of cocaine. ^{14}C-choline overflow was increased by electrical field stimulation and K^+ (23 mM). When eserine (10^{-6}M) was present throughout the experiment histamine (10^{-5}M), 5-HT (10^{-6}M), and norepinephrine (10^{-7}M) also enhanced ^{14}C-choline overflow. The further addition of tetrodotoxin (TTX, 10^{-6}M) abolished the effect of histamine and 5-HT. Norepinephrine (10^{-4}M) and propranolol (10^{-5}M)

had a significant inhibitory action on ^{14}C-choline overflow applied in the presence of eserine $10^{-6}M$ and tetrodotoxin ($10^{-6}M$).

These studies suggest the presence of prejunctional alpha and beta-adrenoceptors on both adrenergic and cholinergic nerves, prejunctional muscarinic receptors on adrenergic nerves and 5-HT and histamine receptors at the level of the ganglia on cholinergic nerves.

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REVIEW OF LITERATURE

FUNCTION OF AIRWAY SMOOTH MUSCLE

The physiological significance of smooth muscle in the tracheobronchial tree is not well understood. Several theories have been put forward to explain the possible roles for this muscle in normal "healthy" states. Firstly, airway smooth muscle, by shortening and therefore producing various degrees of bronchoconstriction may divert ventilation to areas of the lung which are well perfused so as to maintain homogeneity of ventilation/perfusion (Williams, 1981). This does occur in patients with pulmonary embolism; quantitatively, however, this is not adequate to be of physiologic benefit as measured by lung scanning (Williams, 1981). The bronchoconstriction is also short lived and is unlikely to provide adequate adjustment to a chronic clinical condition.

A second theory put forward by Widdicombe and Nadel (1963) suggested that airway smooth muscle tone may balance the amount of anatomical dead space and airway resistance. Macklem et. al. (1969) support this theory for larger airways (>2mm inside diameter) in dogs. It should be noted however, that humans prefer to breathe through the nasal passage (high resistance) as opposed to the lower resistance oral passage (Macklem and Engel, 1975). An additional consideration is that the volume of anatomical dead space is small compared to tidal volume and reduction in dead space occurs at the expense of great increases in airway resistance; it is therefore difficult to appreciate the physiologic benefit of such an alteration.

A third theory relates to the effect of smooth muscle on airway rigidity. Olsen et. al., (1967) have demonstrated that increasing bronchomotor tone provides cartilage-containing airways with increased

rigidity and therefore less compressibility. This increased stability may prevent airway closure during forced expiratory flow with high intrathoracic pressures. This benefit is limited however, in that smaller, non-cartilage containing airways may narrow the airway sufficiently to reduce maximum expiratory flow rates (Macklem and Engel, 1975). Williams (1981) has suggested a dual action for muscle in the airways: one to stabilize larger airways during forced expiration and another to regulate maximum expiratory flow. When bronchodilating agents are administered airway resistance continues to drop past the point which permits maximum expiratory flow and the further relaxation of the airway smooth muscle allows some mechanical instability of the larger airways which then become the flow-limiting segments.

Another hypothesis regarding the physiological significance of airway smooth muscle suggests that constriction of airways may function to pull open connecting alveoli and therefore maintain isotropic airways and parenchyma with identical specific compliances (Macklem and Engel, 1975). The benefit of this effect is likely to occur at low lung volumes where collapse of alveoli is more likely.

That bronchoconstriction and increased airway resistance occur in disease states such as chronic bronchitis, hay fever and asthma is well known (Hogg, 1981; Boushey, 1981; Orehek, et. al., 1977).

Recently, McFadden (1981) has demonstrated that excessive heat loss from airways may induce bronchoconstriction in asthmatics and normals. It has been suggested that the bronchoconstriction measured may be a mechanism of thermoregulation especially in cold climates (Williams, 1981).

Finally and perhaps most importantly airway smooth muscle may function to protect the alveoli from irritant inhaled particulate or gaseous matter. Airway constriction may in itself mechanically stimulate irritant receptors in the mucosa and produce cough. Narrower airways (depending on degree of resistance) will result in a higher linear velocity of airflow and therefore a more efficient cough (Macklem and Mead, 1968). Bronchoconstriction further results in the formation of intraluminal folds and with increased velocity the turbulence produced will likely impact particulate matter in the larger airways where turbulence is greatest (Williams, 1981). Smaldone and Bergofsky (1976) have demonstrated that bronchoconstriction in small airways would move the flow limiting segment towards the trachea. This should increase the velocity of flow in the periphery and aid in the clearance of particulate matter from small airways.

The mechanisms by which overactivity in airway smooth muscle occurs are seen from the anatomic immunologic, physiologic, pharmacologic, genetic, biochemical and pathologic perspectives. However, since it is unlikely that the function of airway smooth muscle is to produce disease states, a more complete understanding of normal physiological mechanisms of this muscle are required in order to evaluate the homeostatic significance of changes observed in disease states.

TRACHEAL SMOOTH MUSCLE

Previous studies have demonstrated differences in airway smooth muscle between and within species in terms of neural control (Richardson, 1979; Richardson and Ferguson, 1980) receptor status (Fleisch, 1980) and

site of response along the tracheobronchial tree (Fleisch and Calkins, 1976). Other factors such as age dependence (Pandya, 1977) multi unit and single unit behavior (Kroeger and Stephens, 1975) membrane potential (Stephens and Kroeger, 1980) resting tone (Bergen and Kroeger, 1980; Ohno et. al., 1981) and mechanical responsiveness (Stephens, et. al., 1975; Stephens and Kroeger, 1970) all contribute to the interpretation of airway smooth muscle function. Therefore in making comparisons between species and between smooth muscles (e.g. vascular and bronchial) one must keep in mind the various factors mentioned above. The focus of this thesis will be primarily concerned with the neural and pharmacological regulation of canine tracheal smooth muscle (Stephens et. al., 1979).

Since it is also possible to sensitize dogs immunologically to allergen such that they respond to specific antigen challenge with bronchoconstriction as measured by airway resistance in vivo (Kepron et. al., Hirshman and Downes, 1981; Kessler et. al., 1973; Krell et. al., 1976) and smooth muscle contraction in vitro (Antonissen, et. al., 1979; Bergen and Kroeger, 1979) this provides a model analogous to allergic asthma in humans (Yu et. al., 1972). The observation that the intact human (Permutt, 1973; Nadel, 1973) and dog (Stephens and Kroeger, 1980) show similar responses of both large and small resistance airways to various agonists and nerve stimulation suggests that canine tracheal smooth muscle is a useful model in studying normal and disease states such as asthma. This model provides a unique experimental tool in gaining an understanding of alterations in smooth muscle receptor status and innervation of the large airways with allergic disease and an appreciation of the actions of the wide variety of agents administered in the treatment of asthma.

RELATION OF STRUCTURE TO ELECTROPHYSIOLOGY OF TRACHEAL SMOOTH MUSCLE

The structure and ultrastructure of canine tracheal smooth muscle has been reviewed by Stephens, et. al. (1979), Stephens and Kroeger (1980), Daniel et. al. (1979) and Richardson and Ferguson (1980). A systematic description of this subject is beyond the scope of the present treatment and only those aspects which relate to phenomena of interest will be considered. Canine TSM shows multiunit properties *in vitro* in that no spontaneous electrical or mechanical activity is observed and a myogenic response to stretch is absent (Stephens and Kroeger, 1975). Gap junctions and electrotonic current spread (as measured by the space constant) suggest good cell-to-cell coupling as might be expected in single unit smooth muscle. Single unit smooth muscle generally demonstrates spontaneous electrical activity and myogenic responses (Bozler, 1948; Prosser, et. al., 1960). *In vivo* studies (Akasaka, et. al., 1975) have shown the presence of spontaneous action potentials in canine and human bronchial smooth muscle, the frequency of which was increased in human asthmatics during bronchospasm. Kroeger and Stephens (1975) and Bose and Bose (1977) were able to convert airway smooth muscle multiunit behaviour *in vitro* to single unit responses (spontaneous phasic activity) by the use of a potassium channel blocker (tetraethylammonium) or glucose depletion respectively. Kirkpatrick (1975) demonstrated spontaneous phasic mechanical activity with bovine tracheal smooth muscle *in vitro* in response to histamine. These results suggest that while tracheal smooth muscle appears to resemble multiunit type histologically by its sparse innervation (Daniel et. al., 1979) and *in vitro* responses (absence of myogenic activity; Stephens and Kroeger, 1975) appropriate conditions may

alter the response to that of single unit behaviour. Whether there is a change to spontaneous mechanical and electrical activity or perhaps an increase of this activity in disease states such as asthma in intact humans is not known. Antonissen et. al. (1979) have demonstrated that in allergen sensitized dogs, basal resting tone is increased, spontaneous mechanical activity and myogenic response are present in vitro, but not in littermate controls.

The resting membrane potential of canine TSM is -50 $\pm$ 2 mV (SE) (Stephens, et al., 1979) and demonstrates no phasic electrical fluctuations. The contractile state of canine TSM is largely dependent on the degree of depolarization. Suzuki, et. al., (1976) demonstrated that a 4 mV depolarization from rest produced measurable contraction (i.e. mechanical threshold) in canine trachealis while Cameron and Kirkpatrick (1977) using bovine trachealis showed that graded depolarization produced graded mehanical responses. Stephens et. al. (1979) used increasing concentrations of K^+ in the bathing solution to obtain both graded mechanical responses and graded depolarization. These graded responses to K^+ and TEA are abolished in Ca^{++}- free solutions demonstrating that the mechanical responses to phasic and tonic depolarization are dependant on extracellular Ca^{++}. (Stephens, et. al., 1975; Stephens, 1976).

The unmasking of a potential for spontaneity with TEA is of special interest regarding the single unit characteristics of the airways in disease (Akasaka et al., 1975). In addition to blocking potassium conductance (Kroeger and Stephens, 1975), TEA increases the number of gap junctions (Kannan and Daniel, 1978), and increases the space constant

(Stephens, et. al., 1979). The action potentials recorded in canine TSM following treatment with TEA (Suzuki, et. al., 1976; Kirkpatrick, 1975; Kroeger and Stephens, 1975) were blocked when D-600 (a calcium channel blocker) was added. These findings suggest an important role for a Ca-current in the development of action potentials.

Graded stimulation using a 60 Hz AC electrical source applied with platinum plate electrodes, produced maximal isometric tetanus at 13V in canine trachealis in vitro (Stephens and Kroeger, 1980). These contractions, which are atropine-sensitive, are not sustained with continuous electrical field stimulation as observed by a 75% reduction from peak tension after approximately 20 seconds of applying the stimulus. This is thought to be the result of acetylcholine depletion in cholinergic nerves. In contrast exogenous carbamylcholine or acetylcholine produces a sustained isometric contraction which gradually decreases to approximately 85% of the initial response after four hours. Also of interest is the finding that the time to peak tension development is the same, regardless of voltage applied which implies a voltage independent rate of contraction. This finding suggests a functional similarity among the contractile units of canine TSM and that as more voltage is applied, cells with lower excitabilities, or cells farther from the electrode are recruited in the response. Therefore unlike the all-or-none response observed for cardiac muscle in which the depolarization spreads throughout the functional syncytium the trachealis appears to be able to maintain various degrees of tonic activity dependant on the stimulus input. The results with carbamylcholine also suggest that airway smooth muscle is capable of maintaining a prolonged tonic contraction as observed in

asthma where there is prolonged bronchoconstriction.

Since this thesis focusses on the neural and pharmacologic control of tracheal smooth muscle the reader is referred to other reviews for further information on the biochemistry, biophysics and ultrastructure (Richardson and Ferguson, 1980; Daniel, et. al., 1979; Stephens and Kroeger, 1980) of smooth muscle in airways.

NEURAL CONTROL OF AIRWAY SMOOTH MUSCLE

Parasympathetic System

The parasympathetic system is the major neural mechanism for bronchocontriction in humans (Richardson and Beland, 1976) dogs (Brown, et. al., 1982; Russell, 1978) cats (Olsen et al., 1965) guinea pigs (Coburn and Tomita, 1973) baboons (Middendorf and Russell, 1980) and cows (Kirkpatrick, 1975). Stimulation of the vagus nerve results in widespread airway narrowing (Woolcock, et. al., 1969) thus increasing resistance to airflow. The latter can be enhanced by cholinesterase inhibitors or blocked at the postsynaptic muscarinic receptor by atropine (Colebatch and Halmagyi, 1963). Stimulation of vagal fibers is also blocked by tetrodotoxin (TTX) and hexamethonium. The blockade by hexamethonium (which blocks nicotinic receptors) suggests the involvement of ganglia in the parasympathetic pathway. Histologically these ganglia have been located in airway muscle or adjacent to it (Richardson and Ferguson, 1980).

Cholinergic nerve terminals or varicosities can be identified with electron microscopy by demonstrating small agranular vesicles (SAV) of

50-70 nm diameter (Burnstock, 1970), or by histochemical techniques demonstrating the presence of acetylcholinesterase (Mann, 1971). The ganglia receive preganglionic cholinergic and adrenergic fibers (Richardson and Ferguson, 1980) and some may have purinergic fibers (non-adrenergic fibers) depending on the species. In the human the cholinergic and nonadrenergic fibers (containing large agranular vesicles, 80-120 nm in diameter (Burnstock, 1970; Burnstock, 1972)) predominate, while in the dog both cholinergic and adrenergic nerves (as demonstrated by histochemical staining for norepinephrine (Fillenz, 1970), or small dense cored granular vesicles of 40-70 nm diameter (Burnstock, 1972)) are present. Present evidence suggests a paucity of adrenergic fibers in the human (Richardson, 1979) and no purinergic fibres in the dog tracheal smooth muscle. Postganglionic vagal fibers leave the ganglia, which are situated just outside the smooth muscle and cartilage of the airways to travel through the smooth muscle giving off branches with multiple neurotransmitter-containing varicosities (Richardson and Ferguson, 1980). Direct innervation of airway smooth muscle or associated blood vessels by adrenergic fibers has not been clearly shown and it has been suggested that adrenergic fibers may not control airway smooth muscle. Several studies, however, using electrical stimulation of nerves have suggested a functional role of adrenergic nerves in airway smooth muscle tone (Suzuki et. al., 1976, Cabezas et. al., 1971) Cholinergic, adrenergic and non-adrenergic fibers may be influenced by preganglionic vagal fiber stimuli.

Interactions involving reflexes (Coleridge and Coleridge, 1977; Russell and Lai-Fook, 1979; Widdecombe, 1975) and modulating influences of circulating adrenaline, serotonin, locally released prostaglandins,

histamine and slow reacting substance of anaphylaxis (SRS-A) at the level of the ganglia are not presently understood. The importance of controls at the level of the ganglia in the regulation of the effector tissue is relevant for the understanding of neural mechanisms regulating airway smooth muscle.

As was mentioned above, vagal stimulation releases acetycholine (ACh) from small agranular or electron-transparent vesicles. The ACh is rapidly hydrolysed by acetylcholinesterase forming choline and acetate; choline is taken up actively by the nerve varicosity to synthesize ACh and thereby recycles and conserves choline (Burnstock, 1979).

Acetylcholine is released from cholinergic nerves following electrical stimulation (Colebatch and Halmagyi, 1963). The contractile response acompanying ACh release is blocked by atropine but not hexamethonium thus demonstrating that the contraction is mediated by post ganglionic cholinergic nerve stimulation. In vitro studies in the dog have also demonstrated similar responses (Russell, 1978; Stephens and Kroeger, 1980). In vitro studies of guinea pig trachea (Carlyle, 1963) show that at rest, cholinergic nerves release ACh at a constant rate. It is likely that in vivo the airways "at rest" are also under the influence of some continuous ACh release which may serve to provide a resting tone in airway smooth muscle. Addition of atropine or ipratropium bromide results in bronchodilation in human airways from healthy individuals (Vincent et. al., 1970) and asthmatics (Gold, 1975; Gandevia, 1975) as well as dogs (Severinghaus and Stupfel, 1955). This basal or resting tone was also abolished by vagotomy or cooling the vagus, thus demonstrating that a low level of cholinergic activity maintains airway smooth muscle tone (Severinghaus and Stupfel, 1955).

EFFECTS OF ACH ON THE POSTJUNCTIONAL MEMBRANE

ACh released from nerve varicosities in tracheal smooth muscle binds to postjunctional muscarinic receptors with a resultant depolarization of the muscle membrane. Coburn (1979) has demonstrated that while ACh produces depolarization it is not required for the production of contractions. In fact hyperpolarization of the cell produced by anodal current pulses did not inhibit ACh-induced contractions and using the Ca^{++} channel blockers verapamil, D-600 or lanthanum had minimal effect on these contractions. This study demonstrates a pharmacomechanical coupling independent of membrane potential or external Ca^{++}. In contrast high-K and 5-hydroxytryptamine (5-HT) stimulated contractions demonstrated electromechanical coupling and a dependence on external Ca^{++} (Coburn, 1979).

The action of acetylcholine also appears to stimulate increases in cellular levels of cyclic guanosine monophosphate (c-GMP) (Schultz et. al., 1972). This response, however, did not correlate well temporaley with the increase in intracellular Ca^{++} which preceeded the rise in c-GMP (Lundholm et. al., 1976; Andersson et. al., 1975). These data illustrate the variety of mechanisms producing contraction in airway smooth muscle. For complete reviews on these mechanisms in smooth muscle the reader is referred to articles by Bolton (1979) Coburn and Yamaguchi (1977) and Simonsson and Svedmyr (1977).

SYMPATHETIC SYSTEM

Neural control of tracheal smooth muscle (TSM) occurs largely through the postganglionic parasympathetic neural release of acetylcholine (ACh), which stimulates postjunctional muscarinic receptors

(Widdicombe, 1963; Russell, 1978; Stephens, 1979) to produce contraction. This appears to be true of most species studied as mentioned above. The presence of an adrenergic system innervating airway smooth muscle is less clear. Histological evidence indicates the presence of adrenergic nerves in airways of cats (Silvia and Ross, 1974), guinea pigs (Richardson and Ferguson, 1979) and dogs (Suzuki, 1976). Evidence for a direct innervation of TSM by adrenergic nerves in humans is lacking, however adrenergic fibers are observed in the ganglia (Richardson and Beland, 1976). The role of these fibers is not known but it is possible that norepinephrine released from adrenergic nerves in the ganglia could act on cholinergic fibers. Adrenergic fibers in ganglia of the canine lung have been demonstrated (Jacobowitz et. al., 1973). Since many adrenergic fibers accompany blood vessels in the lung, (Fillenz, 1970, Kadowitz et. al., 1976), it is difficult to demonstrate histologically a direct control of airway smooth muscle.

A functional role of sympathetic neurons in canine airway smooth muscle has been suggested by Cabezas et. al., (1971) who have shown that in vivo electrical stimulation of these nerves produces a beta-adrenoceptor mediated bronchodilation which is blocked by propranolol. Russell (1980) has shown similar results for canine TSM in vitro. Further studies have shown that sectioning sympathetic fibers to the airways in dogs resulted in a mild bronchoconstriction and suggested that the bronchodilator activity serves to counterbalance the bronchoconstricting effect of parasympathetic activity (Green and Widdicombe, 1966). These results suggest a basal rate of sympathetic and parasympathelic activity in airways, and direct measurements bear this out (Widdicombe, 1966). It is

concluded that although adrenergic innervation to airways is sparse (Mann, 1971) a functional role does exist. Controversy as to that role is abundant. Electrical stimulation of thoracic sympathetic nerves has been shown to produce bronchodilation (Castro de la Mata, et. al., 1962; Cabezas et. al., 1971) and this response is dependent on bronchomotor tone which is abolished by vagotomy (Cabezas et. al., 1971). Interestingly, these studies found that when bronchoconstrictor tone was high the bronchodilator effect of sympathetic nerve stimulation was greatest (Cabezas et. al., 1971). Similar results were found for cat airways, in which the administration of eserine (which increased cholinergic tone) also enhanced the effects of sympathetic nerve stimulation (Daly et. al., 1951). These results illustrate a relationship between active tone and the bronchodilating action of sympathetic nerve stimulation.

A bronchoconstrictor action of the sympathetic system in airways has also been suggested (Adolphson, et. al., 1971). $Beta_2$-adrenoceptors which mediate relaxation have been identified in the airways (Fleish, 1980) and thus forms the basis of many bronchodilating therapies (Jack, 1973; Wilson and McPhillips, 1978). The existence of alpha-adrenoceptors mediating contraction of TSM is however, controversial. Evidence for the existence of alpha-adrenoceptors in the tracheobronchial tree was obtained for guinea pigs (Everitt and Cairncross, 1969) dogs (Beinfield and Seifter, 1980; Castro de al Mata et. al., 1962; Kneussl and Richardson, 1978; Suzuki et. al., 1976), rabbits, cats, rats and guinea pigs (Fleish et. al., 1970) and humans (Kneussl and Richardson 1978; Simonsson et. al., 1972; Mathe, et. al., 1971). Foster (1966), Stone, et. al. (1973) and Cabezas et. al. (1971) were unable to find evidence for

alpha-adrenoceptor mediated responses in guinea pigs, humans and dogs respectively. Species differences in the amount of contraction produced by alpha-adrenoceptor stimulation, have been noted but could be demonstrated only after beta-adrenoceptor blockade (Fleish et. al., 1970; Mathe et. al., 1971). While beta-blockade was used to demonstrate an alpha-adrenoceptor mediated contraction in canine trachealis (Suzuki, et. al., 1976) with electrical field stimulation in vitro, others (Beinfield and Seifter, 1980) were able to demonstrate phentolamine-sensitive contractions following low doses of norepinephrine (i.v.) in canine TSM in the absence of propranolol.

Variability of the adrenergically mediated responses noted above might be attributed to age-related effects. Pandya (1977), on the basis of observations that the TSM of younger dogs responded more strongly to norepinephrine than that of older dogs, concluded that the number and/or activity of alpha-adrenoceptors decreased with increasing age. Another possibility is that the pre-existent tone of the muscles qualitatively influences the response to alpha-adrenergic stimulation (Ohno et. al., 1981). Thus Richardson and Ferguson (1979) demonstrated greater alpha-adrenoceptor mediated (phentolamine sensitive) contractions in tracheal and bronchial smooth muscle strips taken from humans with pneumonia or chronic obstructive airway disease. We (Antonissen et. al., 1979) have also noted that in conditions in which the normally passive basal tension is increased, as with immunological sensitization, responses to adrenergic agents are altered and have suggested (Bergen and Kroeger, 1979) the possibility of increased alpha-adrenoceptor mediated responses with sensitization. Whether this reflected a primary effect of sensitization or

a consequence of increased basal tone was not clear and preliminary studies with a variety of stimulatory agonists supported the notion of a controlling influence of pre-existent tone in these responses.

POTASSIUM-INDUCED RESPONSES

Potassium-rich saline (high-K) contracts tracheal smooth muscle of rabbits, guinea pigs, rats (Akcasu, 1959), dogs (Kneussl and Richardson, 1978; Coburn, 1979) and humans (Kneussl and Richardson). High-K treatment results in a depolarization dependent Ca^{++}influx (Somylo and Somlyo, 1970) producing subsequent smooth muscle contraction. Cholinergic receptor antagonists in intestinal smooth muscle and alpha-adrenoceptor antagonists in vascular smooth muscle reduce the contractile response to high-K indicating that neural depolarization and transmitter release constitute a component of the response (Lundholm et. al., 1976).

PREJUNCTIONAL NEUROMODULATION

The frequency of action potentials arriving at the axon terminal or varicosities was thought to be the only determinant of neurotransmitter release until recently when it was shown that for the adrenergic system hormones, neurotransmitters and local microenvironmental conditions could influence this quantal release (Westfall, 1977). The adrenergic system has come to be the most widely studied with respect to neuromodulation, and four general mechanisms have been described (Westfall, 1977).

1) Locally, norepinephrine released may bind to presynaptic or prejunctional receptors and either increase (positive feedback) or decrease (negative feedback) further norepinephrine released by action potentials. This provides a means of autoregulation.

2) Transynaptic regulation, where postsynaptic stimulation by neurotransmitter binding to postjunctional receptors results in an increased production of a substance or substances (e.g. prostaglandin or adenosine containing compounds) which provide feedback and neuromodulate further transmitter release.

3) Contralateral neuronal controls whereby neurotransmitters from different nerves (cholinergic, dopaminergic or serotonergic) adjacent to adrenergic fibers may neuromodulate norepinephrine release and vice versa.

4) Neuromodulation of norepinephrine release by substances in the blood such as angiotension, epinephrine or 5-hydroxy-tryptamine.

These mechanisms of neuromodulation for the adrenergic system, especially the vascular component, illustrate an important regulatory function. These controls, in other neural networks (e.g. cholinergic) are presently not well known. For purposes of this review several of the factors important in prejunctional neuromodulation will be considered, although these factors have not been studied in airway smooth muscle.

ADRENERGIC TRANSMITTER RELEASE

Norepinephrine is synthesized from tyrosine in adrenergic nerves and stored in granules until an increase of intracellular calcium promotes release of the granules by exocytosis (Katz and Miledi, 1970; Kirpekar, Prat and Wakade, 1975). Thus action potentials, increased extracellular potassium concentration or calcium ionophore A23187 increase intracellular Ca^{++} and all release norepinephrine (Mellow, 1979; Tauc, 1982). Released norepinephrine may be recycled by an active neuronal uptake

(Uptake 1). This rapid uptake is inhibited by cocaine, imipramine and amitriptyline antidepressant drugs (Iverson, 1974; Langer, 1977; Nigro and Enero, 1981). A small amount of the NE in the nerve is metabolized via mitochondrial monamine oxidase system (MAO) to 3, 4, dehydroyphenylglycol (DOPEG) and other metabolites. Unstimulated adrenergic nerves passively release DOPEG continuously and only small quantities of NE are released. Recently, it has been shown that NE is released at a basal rate which may come not from the vesicles of the neuron but the cytoplasm. This release produces an estimated 10^{-8}M concentration of NE in the immediate extracellular area of the nerve varicosity and corresponds to the quantal release estimated to occur when the action potential frequency is 2 hertz (Tauc, 1982). Stimulation of the nerve greatly increases dopamine beta hydroxylase and NE release while DOPEG release remains low (Vanhoutte, 1978) Therefore stimuli which increase ^{3}H-norepinephrine overflow do not increase NE metabolite overflow substantially. Released NE may bind to effector or target cells at specific receptors (alpha or beta) or be taken up extraneurally by a membrane transport system (Uptake 2). Uptake 2 has a much lower affinity for NE and therefore the majority of NE released is removed from the junctional cleft by neural uptake (Uptake 1) (Langer, 1977). This difference in transport systems is vital to the loading of adrenergic nerves with radiolabelled norepinephrine (Iversen, 1974; Westfall, 1977). The loading of adrenergic nerves with ^{3}H-norepinephrine has been used in many tissues for the purpose of examining the prejunctional influence of many substances (Duckles and Rapoport, 1979; Nigro and Enero, 1981; Dixon et. al., 1979; Weiner, 1979; Lorenz, Vanhoutte and Shepherd, 1979; Wemer et.

al., 1979; Russell and Bartlett, 1981). Foster (1975) demonstrated that [^{3}H]-noradrenaline neural uptake in guinea pig trachea was almost eliminated when nerves were destroyed with 6-hydroxydopamine. Subsequent transmural electrical stimulation resulted in a marked reduction of relaxant responses and ^{3}[H]-NE efflux.

Use of ^{3}H-norepinephrine has been applied to the study of nerves in airways. After loading adrenergic nerves with ^{3}H-norepinephrine Vermiere and Vanhoutte (1979) were able to demonstrate a release of ^{3}H-NE with electrical stimulation of canine tracheal smooth muscle strips in vitro. They also demonstrated that exogenous NE reduced contractile responses following parasympathetic stimulation more so than contraction to eogenous acetylcholine in canine airways, an effect which was abolished with propranolol. It was suggested that NE released from adrenergic nerves may inhibit ACh release via prejunctional mechanisms (Vermiere and Vanhoutte, 1979). In contrast, a stimulatory role has been described for serotonin on the cholinergic system in canine airways (Sheller et. al., 1982). Recently, Russell and Bartlett (1981) measured ^{3}H NE and norepinephrine metabolites in the superfusate of canine TSM strips following electrical or tyramine stimulation. While both stimuli increased overflow of intact norepinephrine and NE metabolites the percentage increase was greater for norepinephrine. Using ^{3}H-NE as a qualitative indicator of norepinephrine release these authors also demonstrated an atropine-sensitive inhibitory effect of exogenous acetylcholine on electrically stimulated ^{3}H-NE overflow and that TTX blocked all overflow produced by electrical stimulation but had no effect on the action of tyramine. Other preparations, most notably those of the vasculature or vessel-rich organs such as the spleen have provided much

information about the nature of prejunctional receptors. Prejunctional alpha-receptors (termed $alpha_2$) have been widely studied. $Alpha_2$ receptors as opposed to postjunctional $alpha_1$ receptors have higher affinities for the agonist clonidine (Doxey et. al., 1977) and the antagonist yohimbine (Wood et. al., 1979). Postsynaptic $alpha_1$ receptors bind the agonist phenylephrine (Starke, et. al., 1975) and antagonist prazosin (Cavero, et. al., 1977; Cambridge, et. al., 1977) with greater affinity. Utilizing these tools to separate pre- from postjunctional receptors has demonstrated that stimulation of $alpha_2$ receptors decreases Ca^{++} movement into the nerve and thereby reduces NE released by nerve stimulation. By blocking presynaptic $alpha_2$ receptors with yohimbine, phentolamine, or phenoxybenzamine, (Langer, 1977) electrically stimulated NE-overflow is increased. On the basis of these studies it has been suggested that when nerves are firing at a low frequency the amount of NE released is small and binds to the higher affinity prejunctional $beta_2$ receptor to promote NE release (i.e. positive feedback). This has been demonstrated by Dixon, et. al. (1979) using isoproterenol (10^{-7}-10^{-6}M) to enhance ^{3}H-NE overflow, an effect which was propranolol-sensitive in isolated cat spleen. As action potential frequency increases more NE is released (Langer, 1977) and upon reaching a threshold concentration stimulates prejuntional $alpha_2$ receptors which reduce the amount of transmitter release (negative feedback). The physiologic importance of alpha-mediated prejunctional neuromodulation was demonstrated by Starke (1972) who showed that phenoxybenzamine enhanced electrically stimulated NE overflow and this was accompanied by increased heart rate in the rabbit. While the role of

the presynaptic $beta_2$ receptor is probably less important than the presynaptic $alpha_2$ receptor Dixon et. al. (1979) have suggested that circulating catecholamines whose levels increase under stress may activate presynaptic $beta_2$ adrenoceptors thus enhancing NE release from adrenergic nerves.

Acetylcholine (ACh) appears to act in a neuromodulating capacity on adrenergic NE release. Low concentrations of ACh inhibit release of NE in response to electrical stimulation (Fozard and Muscholl, 1972) and this effect can be eliminated by elevating extracellular calcium (Dubey, et. al., 1975). While atropine antagonises the inhibitory action of ACh on NE release (Russell and Bartlett, 1981) increasing the ACh concentration in the presence of atropine results in augmented NE overflow upon electrical stimulation (Lindmar, et. al., 1968). The physiologic importance of muscarinic inhibition and nicotinic augmentation of NE release is not known. Of interest is the finding that in the gastrointestinal tract (Manber and Gershon, 1979) and airway smooth muscle (Richardson and Ferguson, 1980; Jacobowitz, et. al., 1973) adrenergic and cholinergic axons are often seen side by side and even within the same Schwann cell sheath. The role of this close association seen on electron microscopy is not known, but presynaptic mechanisms may be important in such areas.

Other presynaptic receptors have been postulated and they are listed here with a brief account of their action on NE release from adrenergic nerves when stimulated electrically.

1) Dopamine receptors which inhibit NE release (Enero and Langer, 1975; Long, et. al., 1975; Hope, et. al., 1978).

2) Opiate receptors which, when activated by morphine (Hughes, et.

al., 1975) or pentapeptides met and leu-enkephalin (Langer, 1977) decrease NE release.

3) Inhibitory prostaglandin receptors stimulated by prostaglandins E_1 and E_2 (Stjarne, 1973; Hedqvist, 1976).

4) Adenosine receptors which decrease NE release (Hedqvist and Fredholm, 1976; Verhaeghe, Vanhoutte and Shepherd, 1977).

5) Angiotensin II receptors which increase or facilitate NE release (Zimmerman, 1978).

6) Histamine (H_2) receptors which inhibit NE release (McGrath and Shepherd, 1976; Foldes and Hall, 1979; Powell, 1979).

7) 5-hydroxytryptamine (5-HT) receptors.

Presynaptic sites for 5-HT are suggested by indirect evidence from rabbit heart (Fozard and Mivaluko, 1976) and isolated cat spleen (Pluchino, 1972). Westfall (1977) suggested that 5-HT may increase NE release from nerves during low frequency firing whereas Fenuik, et. al., (1979) have demonstrated a reduction of NE release produced by potassium depolarization or electrical stimulation. Whether 5-HT does release NE from nerves at rest has not been determined by direct measurement of ^{3}H-NE overflow.

This review of presynaptic receptors illustrates the presynaptic regulation for the adrenergic system in several tissue types and species. Physiologic and pharmacologic importance are ascribed to these presynaptic receptors. The work described has mainly centered on adrenergic neuromodulation in tissues where adrenergic innervation is dominant. In the gastrointestinal tract and tracheobronchial tree the parasympathetic (cholinergic) system is dominant. Only a few studies examining presynap-

tic regulation of the cholinergic system have been undertaken, those in the gut (Powell and Tapper, 1979; Vizi and Knoll, 1971; Drew, 1977) and canine airways (Vermiere and Vanhoutte, 1979; Sheller, et. al., 1982).

Generally, depolarization of nervous tissue is accompanied by increases of intraneuronal cyclic AMP (Kakiuchi, et. al., 1969) and cyclic GMP (Ferrendelli, et. al., 1973). These nucleotides have been associated with calcium movements in a variety of smooth muscles (Marshall and Kroeger, 1973; Bolton, 1979; Diamond, 1978) and in nervous tissue with a release of neurotransmitter that is dependent on Ca^{++} movements (Weiner, 1979). Since many of the factors known to have a role in prejunctional neuromodulation also act postsynaptically to affect cyclic-AMP and cyclic-GMP levels in effector tissues, it is conceivable that they may produce similiar effects in nerves. This has been studied for several substances (Westfall, 1977), however little attention has been given to the effects of presynaptic receptor activation in nerves at rest which have a basal rate of firing and hence transmitter efflux.

Presynaptic modulation at low frequencies may be quite different from that at higher stimulus frequency (Sax and Westfall, 1981). A biphasic regulation is found for norepinephrine (Langer, 1977; Langer, et. al., 1975) and perhaps 5-HT (Westfall, 1977) as mentioned previously. The relative importance of these possible regulatory mechanisms physiologically, pharmacologically, therapeutically in diseases such as asthma are presently unknown. The widespread use of substances such as propranolol and clonidine for hypertension have shown the important application gained by understanding presynaptic neuromodulation.

Several excellent reviews on presynaptic regulation serve to provide an overall evaluation of these mechanisms (Langer, 1977; Westfall, 1977; Weiner, 1979; Starke, 1977).

AIRWAY SMOOTH MUSCLE HYPERREACTIVITY

That patients with asthma show increased bronchoconstrictor responses to a variety of stimuli has been known for some time (Curry, 1946; Weiss et. al., 1932). Further studies have demonstrated increased bronchoconstrictor responses of asthmatics to serotonin (Panzani, 1962), acetylcholine, methacholine and carbachol (Curry, 1947; Parker, et. al., 1965; Orehek, et. al., 1977), prostaglandin F_2 alpha (Mathe, et. al., 1973), bradykinin (Varonier and Panzani, 1968), following exercise (McNeill, et. al., 1966), cold air inhalation (Simonsson, et. al., 1967), dust (Dubois and Dautrebande, 1958) and chemical irritants such as sulfur dioxide (Nadel, et. al., 1965) as well as drugs (Rebon and Parikh, 1980). Certain patients develop specific allergies and when exposed to the relevant allergen develop narrowing of the airways (Killian, et. al., 1976). Although bronchial hyperreactivity has been used to describe the smooth muscle constriction producing airway narrowing in asthmatics other factors such as mucosal edema, excessive mucous production, pooling and alterations in mucous viscosity all serve to increase resistance to airflow in the tracheobronchial tree. The focus of this review will be on mechanisms involved in asthma leading to a bronchoconstrictor response mediated by airway smooth muscle.

Cross sectional area of the airways at different levels reveals that the resistance to airflow decreases progressively from the nasal passages

and large airways, to the smaller bronchi (Hogg, 1981). Bronchoconstrictor stimuli may constrict airways at various levels including the trachea where large increases in resistance to airflow can occur. In humans and in dogs the major airway narrowing appears to occur at the level of smaller (1-5mm) bronchi. The site of increased resistance is also quite variable as observed in humans (Ruffin, et. al., 1978) and dogs (Woolcock, et. al., 1969). The mechanisms producing this resistance to airflow have been studied from a variety of perspectives and have led to several theories concerning bronchial hyperreactivity. Bronchial hyperreactivity is not exclusive to asthma since it occurs in patients with chronic bronchitis (Simonsson et. al., 1970), viral infections of the respiratory tract (Parker et. al., 1965) and hay fever (Townley, et. al., 1965). It is likely that the mechanisms involved in the different disorders which have some increased resistance to airflow are different or overlap each other. Therefore it is simplistic to view asthma as a disease involving only one mechanism; it should probably be viewed as a syndrome of defined symptoms (e.g. increased resistance to airflow, increased reserve volume of the lung) which may be produced by single or multiple mechanisms. Studies on asthmatic patients treated with several therapies do suggest multiple causes since various individuals respond to cholinergic antagonists (Snow, et. al., 1979; Lightbody, et. al., 1978), beta-adrenoceptor agonists (Wilson and McPhillips, 1978; Laarson and Svedemyr, 1977), adrenoceptor antagonists (Patel and Kerr, 1975), steroids such as dexamethasone (Leitch, 1982), the mast cell stabilizer, disodium cromoglycate (Bernstein, 1981), antihistamines (Karlin, 1972; Popa, 1977) and prostaglandins (Mathe and Hedqvist, 1975).

Hyperreactivity of airway smooth muscle, as occurs in asthma may be conveniently divided into two groups regarding mechanisms: abnormal stimulatory mediators and abnormal smooth muscle responses to normal stimuli. Within this framework further subdivisions can be made. While a complete review of all the mechanisms is not the purpose of this thesis, it is hoped that an outline may give the reader a perspective on the multiple approaches to this subject.

I ABNORMAL MEDIATOR STIMULI

The first major group refers to alterations in the mediator inputs to produce either relaxation or contraction of smooth muscle. It may be further subdivided into extrinsic factors and intrinsic factors.

A) Extrinsic Factors

1) Allergy - This approach to the study of asthma involves immune mechanisms leading to airway narrowing. Allergic reactions may be of the immediate variety (Type I) or involve a delayed reaction (Type IV). Immediate hypersensitivity reactions may be further subdivided into atopic and non-atopic types, the former involving quantitatively and qualitatively abnormal reactivity to concentrations of endogenous substances or exogenously administered pharmacologic mediators which otherwise produce no reaction. Non-atopic disease involves a normal antibody response to unnatural exposure of antigen. Atopic disease involves overproduction of antibody to a wide variety of antigens such as are found in house dust or tree pollens whereas non-atopic disease occurs only on exposure to the specific antigen to which the individual is sensitized.

Both mechanisms involve mast cells located in the epithelium of airways (Salato, 1976) which degranulate following crossbridging of immunoglobulin E (IgE) attached via their F_c portions to mast cell membranes (Gold et. al., 1977, Ishizaka and Ishizaka, 1975). Degranulation leads to release of histamine, eosinophil-chemotactic factor (ECF), prostaglandins, SRS-A (slow reacting substance of anaphylaxis now thought to be a mixture of leukotrienes), various kinins and other substances (Wilson and Galant, 1974). Histamine (Yanta, et. al., 1981; Loring et. al., 1978) SRS-A (Krell and Chakrin, 1978) and several prostaglandins, excepting the E series (Snapper, et. al., 1979; Malo, et. al., 1982) can all produce tracheal smooth muscle contraction and airway narrowing. The response to allergen inhalation can be partially blocked by H_1 antagonists (Karlin, 1972), atropine (Yu, et. al., 1972; Gold et. al., 1972) and disodium cromoglycate (Berstein, 1981). Inhibition of mast cell release of mediators can be affected by histamine (H_2 receptors), beta-adrenoceptor activation, prostaglandin E_1 and glucocorticoids acting on mast cell membranes to inhibit degranulation (Lichtenstein and Gillespie, 1973; Norn and Skov, 1979). The process of degranulation is dependent on Ca^{++} movement into mast cells or basophils (Kazimierczak, 1978) and a decrease in cellular cyclic-AMP (Norn et. al., 1977). Substances such as alpha-adrenoceptor agonists, acetylcholine and prostaglandin F_2 alpha are associated with decreasing cellular cyclic-AMP levels and thereby enhance mast cell degranulation (Krell and Chakrin, 1976; Tung and Lichtenstein, 1981). The histamine released may therefore act on several sites: H_1 receptors on airway smooth muscle producing contraction (Chand et. al., 1979; Himori and Taisa, 1978) H_2 receptors on

mast cells to increase cycle AMP intracellularly and inhibit further histamine release (Lichtenstein and Gillespie, 1973; Norn, et. al., 1977); receptors on adjacent supporting cells and smooth muscle cells to stimulate prostaglandin and leukotriene synthesis (Lewis and Austin, 1981; Mitchell, 1981; Orange, 1977), or on irritant receptors located in airway epithelium (Vidruk, et. al., 1976) to produce bronchoconstriction by a vagally mediated reflex (Drazen and Austen, 1975; Gold et. al., 1977; Wasserman, 1975).

SRS-A, presently thought to be leukotrienes C_4, D_4 and E_4 (Leitch, 1982) produces prolonged bronchoconstriction. The role of prostaglandins and other arachidonic acid metabolites is currently of great interest as to their role in many of the bronchoconstrictor responses, however the subject is too broad for review here. The reader is referred to the following references (Nakanishi, et. al., 1978; Anderson, et. al., 1980; Wasserman, 1976; Gardiner and Collier, 1980; Adcock and Garland, 1980; Dahlen, et. al., 1980, Brink et. al., 1980).

Late asthmatic allergic reactions may appear gradually over several hours following exposure to a specific allergen. The reaction is thought to be of the type III, immune complex type, and likely involves bronchoconstrictor prostaglandins, histamine and bradykinin (Pepys and Hutchcroft, 1975).

Models of allergic asthma have been developed (Hirshman et. al., 1980; Wanner et. al., 1979) and serve to provide a means by which *in vivo* and *in vitro* studies can investigate muscle responses to antigen challenge. The animals used are sensitized to a specific antigen such as ovalbumin (Antonissen et. al., 1979; Kepron, et. al., 1977), ragweed

pollen (Patterson, 1969) or ascaris antigen (Hirshman, et. al., 1980; Krell and Chakrin, 1976). Antonissen et. al., (1979) have demonstrated that canine tracheal smooth muscle strips isolated from ovalbumin-sensitized dogs contract when exposed to the specific antigen ovalabumin (OA). Mepyramine a specific H_1 antagonist blocks the response to OA challenge (Antonissen, et. al., 1980). In vivo studies on the dogs sensitized to OA (Kepron et. al., 1977) demonstrated a marked increase in airflow resistance following aerosol challenge with antigen. Antonissen et al. (1979) also demonstrated tracheal smooth muscle of dogs sensitized to OA had increased rates and amounts of shortening as compared to non-sensitized littermate control tissue in vitro. These results illustrate the complexity of allergic reactions in allergic disease which may involve not only differences in mediator release from mast cells but also changes in the smooth muscle itself.

2) Irritant Receptor Reflex - The irritant or fast adapting receptor has been described by Widdicombe (1954) as being involved in mediating cough responses to upper airway stimuli (Nadel, 1980; Mortola, et. al., 1975) and bronchoconstriction with lower airway stimulation. The irritant receptor is thought to be located within the airway epithelium (Mortola, et. al., 1975) and is stimulated by histamine (Sampson and Vidruk, 1975; Vidruk, et. al., 1976; Gold et. al., 1972) ozone (Goldsmith and Nadel, 1969) dust (Kessler, et. al., 1973) ammonia (Widdicombe, 1975) sulfur dioxide (Nadel et. al., 1965) mechanical stimuli (Boushey, et. al., 1972) and other substances (Nadel, 1980). The reflex bronchoconstriction produced is mediated by the vagus nerve as demonstrated by its sensitivity to atropine (Gold et. al., 1972) hexamethonium (Simonsson et.

al., 1967) or vagotomy (Gold et. al., 1972; De Kock et. al., 1966). Since histamine appears to stimulate this reflex strongly in humans (Simonsson, et. al., 1967) and also to some extent in dogs (Yanta et. al., 1981; Kessler, et. al., 1973) the relationship to allergy and mast cell degranulation is apparent. Other factors such as viral infections which damage airway epithelium (Dixon, et. al., 1979) may increase exposure or sensitivity of these irritant receptors to the stimuli mentioned. In fact many individuals do show an atropine-sensitive increase in airflow resistance following a viral respiratory tract infection (Empey, et. al., 1976; Dixon, et. al., 1979). The reflex seems to be involved predominantly with extrinsic factors such as allergens or air contaminants. Other considerations such as alterations in mucosal permeability (Hogg et. al., 1979) or damage to airway epithelium (Lee, et. al., 1977) may explain why only certain persons develop the bronchoconstrictor response following chronic exposure to ozone or a viral infection and suggest intrinsic changes, perhaps in the neural activity of the airways. Histamine may interact with vagal efferents to alter activity either at the level of the ganglia or central nervous system (Loring et. al., 1978; Douglas, et. al., 1973). Others (Hahn, et. al., 1978; Sheller et. al., 1982) have demonstrated a facilitation by 5-HT of bronchoconstriction produced by vagal stimulation but not exogenous ACh. These results suggest a possible influence of histamine and 5-HT on vagal efferent activity, acetylcholine release or postjunctional changes in the smooth muscle itself.

The reflex involving the vagus nerve has also been implicated in exercise induced asthma (Simonsson, et. al., 1967). Cold dry air

(Strauss, et. al., 1977) and rapid respiratory rates (Haynes, et. al., 1976) may precipitate bronchoconstriction. Since exercise in a humid warm environment, such as swimming is associated with milder bronchoconstriction in exercise-induced asthmatics, it has been suggested that cold, drying and mechanical stimulation of irritant receptors may be the major factors involved (Strauss, et. al., 1977; 1978) although atropine is not always effective in blocking the response (Deal, et. al., 1976). It has been shown (Gross, et. al., 1974) that some exercise-induced asthmatics respond to treatment with phentolamine, thus showing that other neural or receptor mechanisms are also likely involved.

B) Intrinsic Factors

1) Cholinergic Overactivity - The cholinergic system which is active in dogs (Severinghaus and Stupfel, 1975) and humans (Vincent et. al., 1970) to provide a basal tone to airway smooth muscle, may be overactive due to reflexes, psychogenic stimuli or abnormalities in regulation at the ganglia (Boushey, et. al., 1980). Since the resting baseline caliber of the airways can be altered by cholinergic stimulation (Colebatch and Halmagyi, 1963) it seems probable that a wide range of resting values exist. Boushey et. al. (1980) have suggested that since resistance to airflow is inversely proportional to the fourth power of the radius, equal absolute changes in radii will produce greater increases in airflow resistance in the smaller airways.

Experiments on asthmatics and normal individuals have indicated that large differences in bronchial reactivity occur following carbachol challenge even when baseline values of airway resistance are similar (Rubinfeld and Pain, 1977). De Kock et. al. (1966) demonstrated that

changes in baseline airflow resistance by stimulation of sectioned cervical vagus nerves in dogs did not alter the bronchoconstrictor response to histamine aerosol. These studies indicate that resting airway caliber does not influence the further contraction of airway smooth muscle in response to histamine or carbachol. Whether this is true of other bronchoconstricting agonists is not known.

Asthmatics (Cropp, 1975) and patients with chronic bronchitis (Klock, et. al., 1975) may have increased airway smooth muscle tone produced by increased vagal activity. This conclusion is suggested by the finding that atropine reduces airway resistance to airflow and that airways constrict markedly in the presence of a beta antagonist, (which is blocked by atropine, McNeill and Ingram, 1966). The cause of this cholinergic overactivity in asthmatics is not known but, as mentioned above, several factors (reflexes, CNS, ganglionic regulation) must be considered.

2) Alterations in Mediator Production and/or Release - Since asthmatics have marked airway hyperreactivity (Orehek, et. al., 1977; Curry, 1947) to aerosolized histamine or carbachol, much study has sought to explain these phenomena without considering the possibility of alteration in the quantities of mediator released from nerves, mast cells, platelets, lymphocytes, basophils and eosinophils. Chronic asthmatics do have an increased number of eosinophils infiltrating airway muscle and muscosa especially around blood vessels (Kay et. al., 1971). Their role in inflamatory responses is partially understood while information concerning mediators from eosinophils which alter muscle contractility are not known.

In some asthmatics, changes in the number of mast cells may occur thus allowing for more mediator release following degranulation and therefore greater bronchoconstriction. Following antigen challenge circulating levels of histamine do increase in asthmatics (Gold, et. al., 1977) but these levels do not correlate well with the severity of bronchoconstriction.

Studies measuring catecholamines, cortisol and histamine (Barnes et. al., 1980) in asthmatics at various times have suggested that when circulating adrenaline is low, as occurs at night and early morning, the beta-adrenoceptor activity mediating a negative feedback on mast cell histamine release and smooth muscle relaxation is reduced and the greater amount of circulating histamine could produce an asthmatic attack or at least increase its severity.

The role of 5-HT in producing bronchoconstriction in vivo is not clear although it is a potent pulmonary vasoconstrictive agent (Sterling, et. al., 1972). In rats serotonin appears to play a major role in anaphylactic bronchoconstriction since the latter is largely (70%) blocked by methysergide (Church, et. al., 1972). Exogenous serotonin administered to dogs (Sheller, et. al., 1982) or humans (Hajos, 1962; Rodbard and Kira, 1972) does produce contraction of tracheal smooth muscle. The action of serotonin appears to be mediated via a D receptor located on the muscle membrane (which is blocked by lysergic acid diethylamide, LSD; Born, 1970) and a M receptor on nerve cells which is blocked by atropine or morphine and is therefore thought to be located on cholinergic neurons (Simonsson and Svedmyr, 1977). Evidence for an interaction between serotonin and the cholinergic system has been adduced for

the dog (Hahn, et. al., 1978; Sheller, et. al., 1982) but the importance or role of this interaction in disease states is unknown. In vivo studies of humans suggest that normal subjects do not produce bronchoconstriction following inhalation of aerosolized serotonin (Panzani, 1962) whereas the majority of those with asthma do respond as measured by a 20% decrease in FEV_1 (Hajos, 1962).

3) Sympathetic System - Some controversy as to the presence and functional role of adrenergic fibers in airway smooth muscle was mentioned above. The role of the sympathetics in human airway muscle is not clear (Richardson and Beland, 1976) while in dogs (Cabezas, et. al., 1971) they are thought to be predominantly involved in effecting bronchodilation or counteracting the vagal constrictor influence. When sympathetic nerves to canine airways were sectioned (Woolcock, et. al., 1969) a small increase in airflow resistance resulted. In humans, pretreatment with the beta-adrenoceptor blocker propranolol potentiates bronchoconstrictor responses to acetylcholine (Orehek, et. al., 1975) cigarette smoke (Zuskin, et. al., 1974) and histamine (Ploy-Song-Sang, 1978). These studies suggest a functionally significant inhibitory role for sympathetics in humans. It is conceivable that alterations in sympathetic nervous activity are involved in disease states with airway hyperreactivity. The level at which the possible defect in the sympathetic system occurs may be at the receptor, nerve or ganglion. Defects or changes at the receptor level are postjunctional and are discussed as abnormal smooth muscle response mechanisms.

Sympathetic nerve changes, as in rate of activity or in the varying density of innervation may occur. Damage to sympathetic nerves selective-

ly is unlikely, however genetic factors may contribute since it is known that asthma has some familial tendancy (Bias, 1973). Hirschsprung's disease, for example, in which a section of the colon is devoid of non-adrenergic nerves thus leading to defects in smooth muscle control is genetic in origin, (Frigo, et. al., 1973; Hukuhara, et. al., 1961). Whether an analagous situation involving sympathetics occurs in airways of asthmatics is unknown. The density of adrenergic innervation to airways is low and therefore changes in density are difficult to determine (Mann, 1971).

The presence of adrenergic nerve endings in ganglia of airways of several species (Mann, 1971; Jacobowitz, et. al., 1973) has led to the discovery that noradrenaline release is able to influence cholinergic nerves and reduce ACh release (Vermiere and Vanhoutte, 1979). A loss of such regulation could conceivably lead to unopposed parasympathetic bronchoconstrictor effects and may play a role in the etiology of asthma but evidence is lacking.

4) Nonadrenergic System - As mentioned previously, a functional role of this system in promoting relaxation in humans (Richardson, 1977) and guinea pigs (Coburn and Tomita, 1973; Coleman and Levy, 1974) is likely, whereas in canine airways it is absent. Since the role of the nonadrenergic system is greater than that of the adrenergic system in human airway bronchodilation (Richardson, 1977) it is more likely to be faulty in disease states. It has been suggested that, analagous to Hirschsprungs disease of the colon, a similar disorder occurring in the lungs may lead to a loss of the inhibitory action of the nonadrenergic system and therefore allow for the increased airway smooth muscle responses of

bronchial hyperreactivity (Richardson and Bouchard, 1975). Evidence for changes in the nonadrenergic system in asthma and normals are lacking due in part to the paucity of information concerning the neurotransmitter involved and lack of specific antagonists.

II ABNORMAL RESPONSES OF SMOOTH MUSCLE

Abnormal responses of smooth muscle include those which involve the postjunctional membrane. While a nerve in a disease state may release more or less neurotransmitter than a normal nerve in response to the same stimulus, the postjunctional or muscle response itself may be different even in response to the same quantities of agonist or antagonist. Abnormal responses of smooth muscle may be subdivided into those which occur due to changes at the level of the cell membrane (e.g. receptors or ion channels) and those which occur intracellularly (e.g. enzyme activities or molecular configurations). The focus of this section will be primarily on those changes in contractile activity which are mediated by receptors on airway smooth muscle cell membranes.

1) Changes in Muscle Mass - Patients with asthma (Takizawa and Thurlbeck, 1971) and some with chronic bronchitis (Hossain, 1970) develop hypertrophy and hyperplasia of airway smooth muscle. This increased muscle mass capable of developing more tension, could probably contribute to excessive airway narrowing. By analogy, wall thickness of arterioles may also contribute significantly to the increased blood flow resistance observed in hypertensive rats (Folkow, 1971). While airway smooth muscle mass may contribute to the chronic asthmatic it has been suggested that hypertrophy and hyperplasia of smooth muscle are unlikely to occur in a

short period of time as observed in airway hyperreactivity following viral infections (Empey et. al., 1976) or air pollutants such as ozone (Holtzman, et. al., 1979). While muscle mass is an important feature contributing to the severity of the disease, it is likely not the cause of the hyperreactivity of smooth muscle. Animal models of asthma suggest that greater contractile responses (normalized in g/cm^2) are observed in response to the agonist histamine in vitro (Antonissen et. al., 1980) and therefore implicate factors other than muscle mass in the increased reactivity of airway smooth muscle in asthma.

2) Changes at receptor level - Szentivanyi (1968) proposed that patients with asthma had reduced numbers of beta-adrenoceptors. Direct binding studies of beta-receptors in airway smooth muscle were not carried out, but much support for this concept came from studies showing reduced beta-adrenoceptor stimulated relaxation in some asthmatics (Cookson and Reed, 1967), increased atropine-sensitive bronchoconstrictor responses to a number of stimuli (Orehek et. al., 1975) changes in beta-receptors on leukocytes (Parker and Smith, 1973) and mast cells (Austen and Orange, 1975) and increased alpha-adrenoceptor mediated constriction (Adolphson et. al., 1971). Beta-adrenoceptor blockade produced increased bronchoconstriction in asthmatics (McNeill and Ingram, 1966; Grieco and Pierson, 1971) but not in normals (Tattersfield et. al., 1973; Townley et. al., 1976). Beinfeld and Seifter (1980) have shown increased alpha-adrenoceptor-mediated bronchoconstriction following propranolol administration in dogs, while Patel and Kerr (1975) demonstrated beneficial treatment of thymoxamine in asthmatics. Whether this indicates reduced numbers of beta-receptors is not known without direct binding data.

Others have measured levels of cyclic-AMP in TSM and suggest that for dogs (Rinard, et. al., 1979), asthma may involve lower cyclic-AMP levels and perhaps fewer associated $beta_2$ receptors. Reduced cyclic-AMP at the level of smooth muscle or mast cell (Orange, 1971; Conolly, 1970) would potentiate bronchoconstriction, but Gold (1975) questions the cyclic-AMP evidence on the basis of poor temporal correlation of bronchoconstriction with rises of histamine in serum following antigen challenge. Further studies have shown that long term treatment with beta agonists may themselves lead to reduced beta-adrenoceptor responses (Busse et. al., 1979; Chuang and Costa, 1979) while others have shown that the long term use of terbutaline did not alter beta-adrenoceptor responsiveness (Tashkin et. al., 1982). Recently Szentivanyi (1979), using [^{3}H]- dihydroalprenolol and [^{3}H]- dihydroergocryptine to bind to beta- and alpha adrenoceptors respectively, has shown that lung tissue of patients with reversible airway obstruction (asthma) had increased alpha-receptor and reduced beta-receptor complements compared to persons without airway obstruction. Receptor binding studies in the guinea pig model of chronic asthma support the concept of a shift to an increased number of alphaadrenoceptors and less beta-andrenoceptors in obstructive airway disease (Barnes, et. al., 1980). Szentivanyi (1979) also showed that the adrenoceptor shift occurred in lymphocytes of patients with asthma but not in normals and that hydrocortisone could restore the normal balance <u>in vitro</u> (Szentvanyi, et. al., 1979).

Kneussl and Richardson (1979) demonstrated increased alpha-adrenoceptor responses in tissues obtained from patients with airway diseases. Several studies on humans (Kneussl and Richardson, 1979) and dogs (Bergen

and Kroeger, 1980; Ohno et. al., 1981) demonstrated a relationship of muscle tone to the expression of alpha-adrenoceptor mediated contraction. Therefore it appears that numbers of adrenoceptors (both alpha and beta) and the tone of the preparation influence the amount of isometric tension developed.

Asthmatics demonstrate a hyperreactivity in terms of bronchoconstriction to acetylcholine or methacholine challenge. Whether this is due to increased numbers of ACh receptors, decreased acetylcholinesterase or changes in intracellular events leading to actomyosin interaction is not known. Sensitized canine TSM does not show differences in electrical stimulus-response and carbachol dose-response relationships compared to littermate controls in vitro, suggesting that if hyperreactivity to acetylcholine were present it occurs via prejunctional mechanisms (Antonissen et. al., 1979). Ozone which acts as an irritant may produce airway hyperreactivity (Golden et. al., 1978) and increased responses to acetylcholine. Although ozone may have several effects, it is of interest that ozone decreases acetylcholinesterase concentrations in red blood cells (Goldstein, et. al., 1968).

Histamine may act via a direct pathway on smooth muscle cells (Antonissen et. al., 1980; Nathan et. al., 1979) or indirectly on a vagal reflex (Yanta et. al., 1981). Direct actions are mediated by bronchoconstrictor H_1 (mepyramine-(pyrilamine maleate) sensitive) receptors or H_2 (metiamide sensitive) bronchodilator receptors (Antonissen et. al., 1980; Nathan, et. al., 1979). Variability of the response to histamine is dependant on species differences (Eyre, 1977; Fleisch, 1980), distribution of histamine receptors in the tracheobronchial tree (Chand et.

al., 1980; Eyre, 1969; Eyre, 1973), responses of humans (Habib et. al., 1979) or dogs (Snapper et. al., 1979) to aerosolized histamine. H_2 receptors are also important in providing a negative feedback to mast cells modulating histamine release. It is conceivable that changes in histamine receptor status (number, affinity) may be altered in asthmatics since airway hyperreactivity to histamine aerosol challenge is observed (Mathe et. al., 1973; Townley et. al., 1965). The author is unaware of any studies measuring histamine binding or its alteration in airway disease. Our studies on ovalbumin-sensitized dogs demonstrate increased isometric tension responses to histamine compared with littermate control tracheal smooth muscle in vitro (Antonissen, et. al., 1980). We interpret these findings in terms of a homogeneous complement of H_1 receptors in control and sensitized muscles with respect to their affinity for histamine but that the greater response of sensitized tissue results from a higher efficacy of the agonist or an increased number of H_1 receptors with the same affinity and intrinisic activity as controls, such that the increased response is due to an additive cellular action.

3) Changes at the Intracellular Level - Hyperreactivity may occur to many agents (vide supra). Alterations which may occur at the intracellular level of the muscle are little known. Increases in velocity of shortening observed for TSM from allergic dogs suggest changes occurring at the level of actomyosin interaction (Antonissen, et. al., 1979). A complete review of possible intrinisic changes is beyond the scope of the present treatment, so only a list of possibilities is provided:

1) abnormal membrane permeability to Ca^{++} and other ions or changes in membrane leakiness (Weiss and Viswanath, 1979).

2) altered membrane potential in disease.

3) changes in Ca^{++} regulatory proteins such as protein kinases and calmodulin.

4) changes in enzyme activities regulating Ca^{++} movements phosphorylation reactions, activation of actin or the myosin head, the troponin system, cross bridge formation and cycling.

STATEMENT OF THE PROBLEM

While a great deal is known concerning neural and pharmacological control in airway smooth muscle particularly the canine tracheal smooth muscle (TSM), several areas are poorly understood. One of these concerns is the role of adrenoceptors in mediating contractile responses, and the relationship of the receptors to tonus of TSM and possible changes which may occur in airway hyperreactivity observed in diseases such as asthma. Factors which may modulate neurotransmitter release from neurons innervating the airways is also poorly understood. Present knowledge has been gleaned primarily from blood vessels and the central nervous system. Although previous studies on airways have uncovered interesting interactions of various isolated agonists and neural stimulation of TSM in vivo understanding of these interactions is poor.

The purpose of the present studies was to examine adrenoceptor-mediated responses in isolated strips of canine TSM, and the relationship of these responses to active tone. Electrical field stimulation which excites neural elements was considered to be a useful adjunct to the pharmacological approach. A comparison of adrenoceptor-mediated responses between a mongrel control population and dogs specifically sensitized

to ovalbumin will be made.

Several substances which may act as neuromodulators of cholinergic and or adrenergic nerves in canine TSM will be studied with nonelectrically stimulated nerves. Changes in neurotransmitter overflow will be measured by means of appropriate radiolabelled transmitters. It is hoped that the physiological importance of the factors studied can be demonstrated by mechanical measurements of isometric tension.

The results of this study may provide information pertinent to the understanding of neural control in airways smooth muscle.

METHODS

SENSITIZED CANINE TSM

Dogs were sensitized to ovalbumin (OA) by a technique developed by Pinckard et. al. (1972) to increase OA-specific IgE antibodies, thus resulting in anaphylactic sensitivity to OA. Dogs immunized at birth and receiving booster injections of 10 ug of dinitrophenol conjugated to ovalbumin (DNP_2-OA) mixed with 30 mg of A $(OH)_3$ developed high titers of IgE antibodies specific for OA and DNP (Kepron et. al., 1977). Animals with serum IgE titers greater than 255, as determined by passive cutaneous anaphylaxis (PCA) in non-sensitized dogs, were used for the present studies. Dogs with titers in excess of 64 developed large increases in specific airflow resistance when challenged by OA given via a nebulizer (Kepron, et. al., 1977).

In vitro experiments with tracheal smooth muscle strips obtained from OA-sensitized dogs (6 mo. to 1 year of age) were treated in similar manner as control tissue from the mongrel dog population. At the end of each experiment TSM from OA sensitized dogs was exposed to OA (0.3 mg/ml). The OA challenge resulted in contraction in TSM from sensitized dogs only. The response to OA is specific as was shown (Antonissen, et. al., 1979) by lack of cross reactivity to bovine serum albumin.

DISSECTION

Tracheal smooth muscle (TSM) was obtained from the cervical trachea of adult mongrel dogs (8 to 20 kg.). The animals were anesthetized with 30 mg/kg sodium pentobarbital (Nembutal, Abbott) injected intravenously, following which the trachea was excised and placed in cold oxygenated Krebs-Henseleit solution. The anesthetized dog was then killed by intra-

cardial injection of saturated KCl solution (10 ml.). After removal of adhering connective tissue, the tracheal rings (in groups of 3 rings) were separated and bisected through the anterior cartilage. Eversion of the bisected rings exposed the mucosa over the smooth muscle on the luminal aspect. Removal of the tunica fibrosa left a clean strip of smooth muscle with parallel fibers joining the dorsal ends of the bisected cartilage. Incisions, parallel to the muscle fibers produced 5 to 6 strips of TSM which were of approximately equal size (averaging 0.8 - 1.4 cm. in length, 0.1 - 0.2 cm in width, less than 0.1 cm in thickness and 10 - 25 mg. in weight). These strips were dissected and mounted in organ baths for isometric tension recording and radiolabelled neurotransmitter efflux studies.

ISOMETRIC TENSION MEASUREMENTS

The canine TSM strips obtained are ideally suited to studies of isometric tension development due to the large smooth muscle component (>75%) and the parallel nature of the fibers (Stephens et. al., 1969). The lower ends of the muscle strips were fastened to a rigid aerating tube by means of 000 braided surgical silk. The upper end was attached by a length of the surgical silk to a Statham UC-3 force transducer through a lever assembly for isometric tension recording on a Gould-Brush 2400 recorder. The muscle strips were immersed in a modified Krebs-Henseleit solution of the following composition (mM): NaCl, 115; $N_a HCO_3$, 25; NaH_2PO_4, 1.38; KCl, 2.51; $MgSO_4 .7H_2O$, 2.46; $CaCl_2$, 1.91; and dextrose, 5.56. Aeration of the organ bath (15 ml) was provided by a 95% O_2 - 5% CO_2 mixture which maintained pH at 7.40 while

the bath temperature was held constant at 37°C. Strips of TSM were maintained at a resting tension of 1g (near optimal length; Stephens, et. al., 1969), and allowed to equilibrate for 90 minutes prior to stimulation. A high KCl solution (127 mM) was prepared by substituting KCl isotonically for NaCl and added to the bath quantitatively so as to provide the desired K^+ concentration while maintaining a final bath volume of 15 ml.

Electrical field stimulation (EFS) was applied by rectangular platinum plate electrodes placed parallel to the smooth muscle strips in the bath and connected to a variable-voltage 60-Hz AC source. All electrical stimuli were applied at an experimentally determined optimum voltage (15 V set at source, see also Brown et. al., 1980) and of duration defined in the experiments. Electrical stimuli were given at intervals greater than 5 minutes to allow for recovery of the muscle and nerve.

The isometric tension results are expressed in g/cm^2 of muscle cross-sectional area above resting tension. Due to variability of the responses with cumulative additions of drugs it was convenient to express the initial response as 100% and subsequent responses as a percentage of the initial value. This maneuver decreased variability substantially and statistical testing was performed using these percentages.

Drugs used in the experiments are listed in table A. All drug concentrations expressed in the results are final bath concentrations and stock solutions were made at a concentration which was 10^3 greater than desired bath concentrations so as to minimize the volume of fluid involved in drug additions. After addition of a drug to the bath sufficient time was allowed for responses to stabilize prior to the addition of any subsequent drug. When a washout period occurred a time interval of

TABLE A Drugs Used in Experiments

Drug	Abbreviation	Action	Source
acetylcholine	ACh	muscarinic and nicotinic receptor agonist	Sigma
atropine	ATR	muscarinic receptor antagonist	Sigma
[^{14}C]-choline		acetylcholine precursor	New England Nuclear
cocaine		inhibits neural uptake of NE	Health Protection Branch
eserine		antiacetylcholine-sterase	Sigma
histamine	HIST	H_1 and H_2 receptor agonist	Sigma
5-hydroxytryptamine	5-HT	M and D receptor agonist	Sigma
hyoscyamine	HYO	muscarinic receptor antagonist	Sigma
norepinephrine	NE	Alpha and Beta adrenoceptor agonist	Sigma
[^{3}H]- norepinephrine	^{3}H-NE	tritiated nore-pinephrine	New England Nuclear
phentolamine	PHENT	Alpha adrenoceptor antagonist	Ciba
propranolol	PROP	Beta adrenoceptor antagonist	Sigma
tetrodotoxin	TTX	selective neural sodium channel blocker	Sigma
tyramine	TYR	displaces NE from adrenergic nerves	Sigma

20 minutes was usually allowed with at least 3 rinses of fresh KrebsHenseleit solution. When muscles were pretreated with an antagonist such as atropine, phentolamine or propranolol a period of 20 minutes was allowed before addition of further drugs, so as to permit antagonistreceptor equilibration.

Statistical means and standard errors are included for all data. The results for the actions of norepinephine on TSM after development of tone (results section B) are graphically presented as schematic mechanograms. Although standard errors for these studies are not shown, they can be obtained from the tables in the appendix. The other results of isometric tension development are graphed as a line graph joining the mean values with standard error bars indicated.

STATISTICS USED

Three statistical tests used are as follows: 1) the paired T-test for experiments excluding sections B, F and G in the results, 2) the non-parametric sign test for section B, 3) the Students' T-test for sections F and G. Significant effects were determined at $p < 0.05$. An appendix is provided for individual muscle responses for results sections A, B, C and D.

RADIOLABELLED TRANSMITTER EFFLUX EXPERIMENTS

Tracheal smooth muscle strips of size and weight range similar to those used in studies of isometric tension were used. Strips were mounted in an organ bath with radiolabelled transmitter (see below) for a period of 1 hour, during which a supramaximal electrical field stimulus

(15 V, 60 cycle, 15 sec duration) was applied by platinum plate electrodes at 10 minute intervals as for isometric tension studies in order to increase transmitter turnover and therefore promote neural uptake of the label. After the incubation period a collection period began which involved collecting the entire organ bath volume (10 ml) for each 3 minute sample interval. The organ baths were filled with fresh Krebs-Henseleit solution immediately after each sample was collected. Appropriate drugs were added for each specific protocol outlined below. All specific stimuli to be tested were given during the collection interval from 24 to 27 minutes from the start of the sampling period. The first 3 samples, representing primarily extracellular label, were discarded such that the first of the ten consecutive samples analyzed began at the 12 minute mark from the start of the collection. It was found that a reasonably stable basal rate of transmitter efflux (as measured by radioactivity) occurred by the sixth sample (15-18 min.) from the start of the collection period. After the sampling period all samples were allowed to dry over low heat (100°F) in a fume hood for 3 days after which 7 ml of scintillation fluid (Beckman, HP scintillation cocktail) was added to each sample vial and radioactivity was measured on a Beckman LS-350 scintillation counter. Counting efficiency was 55% for ^{3}H and 90% for ^{14}C. After the last sample the muscles were removed, weighed and solubilized in scintillation vials to which 250 ul NCS (Amersham/Searle) were added. The NCS, a surfaceactive organic base dissolved the tissues over a period of 6 days which freed radiolabelled transmitter in nerve endings. Scintillation fluid was added to the muscles following this solubilization and radio-activity in counts

per minute (cpm) determined as for previous samples. A wide range of radioactivity remaining in muscle was found, from approximately 2,000 cpm to 100,000 cpm. The reasons for this are not known but may be an indication for the variability of nerve density in canine TSM. In order to express the release of radioactivity for each sample in a normalized fashion the rate coefficient (Shanes and Bianchi, 1959) was used. The rate coefficient was obtained as follows:

$$\frac{\text{number of cpm released per minute per sample}}{\text{number of cpm remaining in the muscle at the start of the sample used in the numerator}} \times 100$$

This rate coefficient results are expressed in units of % tissue radioactivity released per minute. It is a good method of normalizing the wide variability obtained for cpm released and those cpm remaining in the muscle.

Rate coefficients were used to plot efflux in order to graphically illustrate the effect of the treatments on radiolabelled transmitter overflow. An increase in rate coefficient therefore indicates an increase in radioactivity efflux. Efflux curves were obtained by calculating means for the first 5 samples (min. 12-24 in the sample period) for all the muscles in the same experiment such that all 49 muscles for example, in the [^{3}H]- norepinephrine efflux experiment were used to calculate these means. The function which best described these 5 means was found to be a power curve. For all curves calculated the correlation coefficient was $r > .96$. From the power curve, the number of samples which were used to obtain the means and a theoretical t it was possible to calculate the 95% confidence limits about the efflux curve. From the

power curve function expected values for samples from 27 to 39 minutes were calculated and the confidence limits extended around these points. This allowed a comparison of treatment effects for the interval (24-27 minutes) to be compared with the expected value by use of a t-test. Only the periods in which the treatments were applied are compared with expected values.

Control curves, to which no stimulus was given, were compared with the expected calculated control curves in order to support the validity of calculated values. Figure 28 and 36 illustrate these findings for [^{3}H]- norepinephrine and [^{14}C]- acetylcholine efflux respectively.

Four different radiolabelled transmitter efflux studies were carried out. Specifics of each are described below, while the general methods described previously apply to all of these experiments.

[^{3}H] NOREPINEPHRINE EFFLUX

The incubation period was carried out in the presence of [^{3}H]-norepinephrine, specific activity 13.4 Ci/mmol at a concentration of 10^{-6} M. All radiolabelled drugs [^{3}H]- norepinephrine or [^{14}C] choline were obtained from New England Nuclear, Boston, Mass. After incubation with [^{3}H]-norepinephrine and electrical stimulation as previously described, cocaine was added to all muscles to a final bath concentration of 10^{-7} M and maintained throughout the sampling period (Nigro and Enero, 1981). Isometric tension was recorded thorughout the experiment.

[^{14}C]-ACETYLCHOLINE EFFLUX

TSM strips were incubated with [^{14}C] choline (10^{-6} M and specific activity 2-5 mCi/mol) for 1 hour and electrical stimulation as described previously. Choline rather than acetylcholine was used since only choline is taken up by nerve endings which then synthesize acetylcholine. By electrical stimulation the turnover of acetylcholine (ACh) is increased and radiolabelled choline becomes part of the vesicular pool of acetylcholine. [^{14}C]-acetylcholine overflow experiments were undertaken in 1) control muscles (done in the absence of eserine or tetrodotoxin) 2) in eserine (10^{-6} M)-treated muscles (eserine added at the end of the incubation period and throughout the experiment and 3) in muscles treated with eserine (10^{-6} M) and tetrodotoxin (TTX, 10^{-6} M) throughout the sampling period. Efflux curves and tests of significance were determined as for [^{3}H]-norepinephrine efflux studies. Simultaneous isometric tension measurements were made only for [^{14}C]-acetylcholine efflux in the absence of eserine and TTX.

NOREPINEPHRINE OVERFLOW

The demonstration of norepinephrine release from adrenergic nerves is dependent on showing overflow of intact norepinephrine from a stimulated (i.e. electrically) preparation. The present studies with those of others fulfil these criteria for electrically stimulated canine TSM in vitro. Foster and O'Donnell (1975) demonstrated that adrenergic nerves in guinea pig trachea take up [^{3}H] noradrenaline and that ^{3}H-NE is depleted by electrical stimulation. When 6-hydroxydopamine was used to

destroy adrenergic nerves both [^{3}H]-noradrenaline uptake and efflux following electrical stimulation were reduced. While not all the radiolabelled -NE enters nerves the great majority is processed via uptake 1 and subsequent stimulation results in an efflux of the labelled transmitter (Russell and Bartlett, 1981). Studies using canine TSM have also shown (Russell and Bartlett, 1981) that the majority of the efflux is intact norepinephrine with a lesser quantity of norepinephrine metabolites. Vermiere and Vanhoutte (1979) have applied this information to the study of adrenergic nerves in dog trachealis, and shown an increase of [^{3}H] norepinephrine released following an electrical stimulus.

In the present studies the release of intact norepinephrine from adrenergic nerves in canine TSM was demonstrated using electrical field stimulation (EFS, 15 V, 60 HZ, AC, 3 minute duration) and high performance liquid chromatography. The total bath volume (20 ml) for each sample period was subjected to catecholamine extraction following the procedure of Hallman et. al. 1978. The catecholamine extracts were purified on activated alumina, followed by electrochemical detection using a Spectra-Physics SP8700 solvent delivery system linked to a LC-4A amperometric detector (Bioanalytical Systems, Inc.) on an oxidation mode. The column used was a RPI8SPHERI-5 reverse phase (Brownlee Laboratory) and results recorded on a Hewlett-Packard Integrator 3390A.

Table B demonstrates a large increase of norepinephrine overflow during EFS during sample period 2, while no increase of epinephrine overflow is noted. After the EFS, norepinephrine release continues to rise in sample interval 3 accompanied by a small increase of epinephrine. The reason for the continued release of NE following the stimulus interval is

not known but is likely related to a diffusion delay. Similar results are noted for the ^{3}H-norepinephrine overflow studied (Fig. 29). The present study confirms that the use of ^{3}H-norepinephrine tracer can be used to determine qualitatively norepinephrine release from adrenergic nerves of canine TSM (Russell and Bartlett, 1981; Vermiere and Vahoutte, 1979).

TABLE B Effect of electrical field stimulation (EFS; 15 V, 60 HZ, AC, 3 minute duration) on norepinephrine overflow in tracheal smooth muscle. Values are expressed in pg. NE/mg tissue. Prior to sampling, the muscles (150 mg total; 14 strips) were incubated in a 10^{-4} M norepinephrine-containing Krebs-Henseleit solution. Following incubation cocaine (10^{-7} M) was present throughout the experiment. Each sample interval was 3 minutes.

Amount of Norepinephrine Released (pg/mg)

Sample Interval	1	2 (EFS)	3
Norepinephrine	7.4	167	514
Epinephrine	13.7	14	19.7

RESULTS

A) Electrical Field Stimulation of canine TSM, in vitro

Eletrical field stimulation (15V, 60 Hz, AC, 15 sec. duration) applied to tracheal smooth muscle strips showed stable reproducible contractile responses to repeated stimulation. Addition of physostigmine (eserine) 10^{-7}M augmented the response to EFS to 136% of the initial value and the further addition of hyoscyamine (10^{-5}M), still in the presence of eserine reduced the contraction to 4% of the initial (Figure 1). Responses to electrical field stimulation (EFS) also showed a 24% decline in isometric tension development in the presence of phentolamine (10^{-5}M). Addition of tetrodotoxin reduced the response to 2% of the initial electrically stimulated contraction (Figure 2). Figure 3 indicates a spontaneous increase of $6^{+}4$% over the first 5 isometric tension responses to EFS which was not significant and due mainly to increased responsiveness of two of the nine trachealis strips in the study (Table 3 appendix). Addition of propranolol (10^{-5}M) non significantly reduced the response to EFS to $97^{+}4$% whereas the further addition of phentolamine (10^{-5}M), still in the presence of propranolol, significantly ($p<0.05$) reduced the contraction to $64^{+}9$% of the initial response (100%, Figure 3).

Addition of norepinephrine (NE 10^{-6}M) was without effect on responses to EFS, but the further addition of NE (10^{-4}M) reduced the response of four trachealis strips and was without effect on the other one, resulting in a mean value of $95^{+}7$% (Figure 4). This reduction was significant ($p<.05$) with the use of a non parametric sign test (used to evaluate a trend in response). Addition of propranolol (10^{-5}M) and NE (10^{-4}M) further reduced the response to $85^{+}6$% of the initial value.

FIG. 1 Effect of eserine and hyoscyamine on isometric tension developed by TSM following electrical field stimulation (E). Means of isometric contractions (n=7), are expressed as a % of the initial contraction (100%), following E. Actual responses are 20 seconds in duration occurring at 10 minute intervals with electrical stimulus alone and 20 minute intervals following addition of drug to allow for equilibration. Initial tension produced was 1347 + 172 g/cm^2. Significant differences ($p < 0.05$) in response to E following exposure to eserine (136 + 6%) and hyoscyamine (4 + 1%) are indicated *. See table 1 appendix.

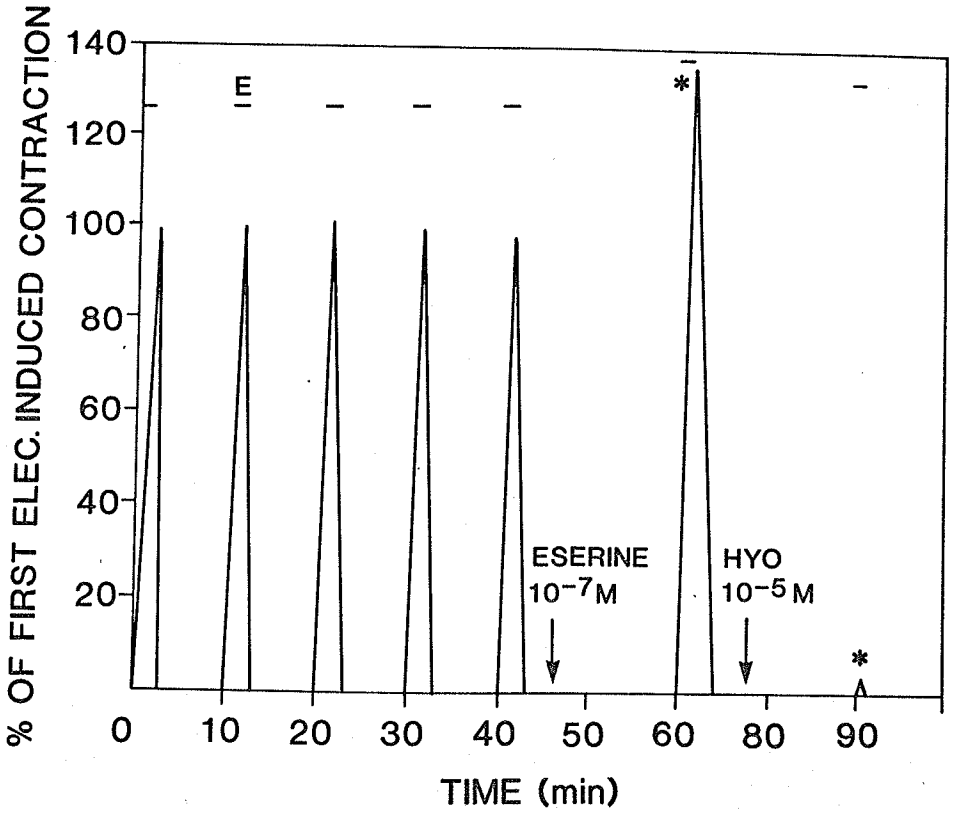


FIG. 2 Effect of phentolamine and tetrodotoxin on isometric tension developed following electrical field stimulation (E). Means of isometric contractions (n=8) are expressed as a % of the initial contraction (100%), following E. Actual responses are 20 seconds in duration occurring at 10 minute intervals with electrical stimulus alone and 20 minute intervals following addition of drugs to allow for equilibration. Initial tension produced was 1218 + 102 g/cm^2. Significant differences ($p < 0.05$) in response to E following exposure to phentolamine (PHENT 10^{-5}M) (79 + 12%) and tetrodotoxin (TTX 10^{-6}M) (2 + 1%) are indicated *. See table 2 appendix.

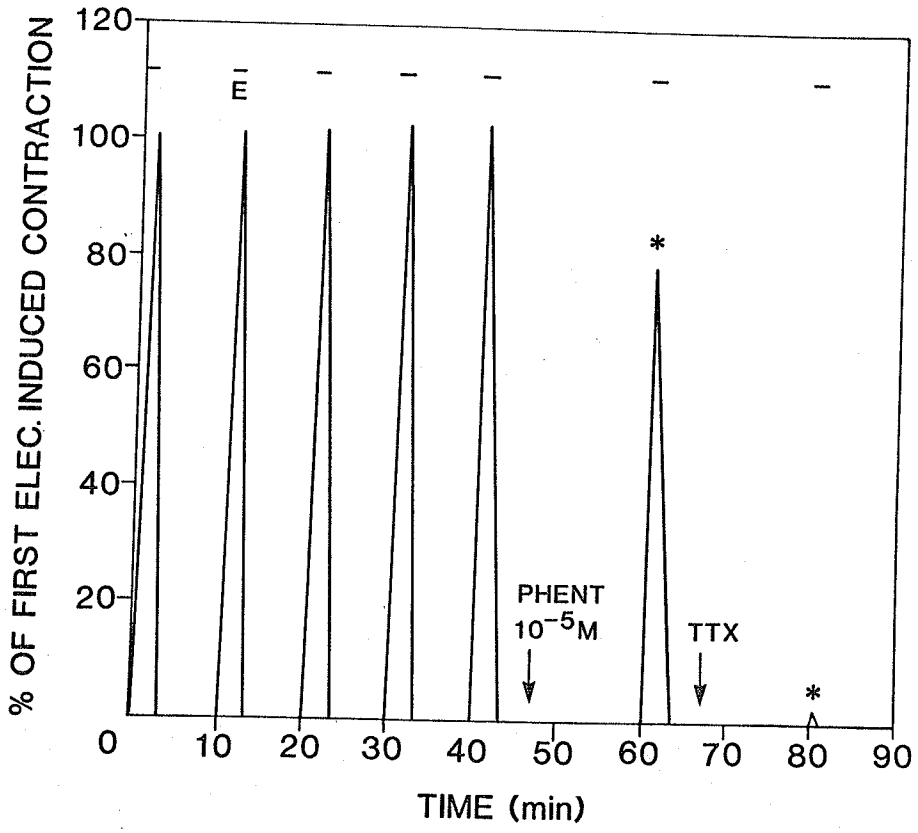


FIG. 3 Effect of propranolol and phentolamine on isometric tension developed by TSM following electrical field stimulation (E). Means of isometric contractions (n=9) are expressed as a % of the initial contraction (100%), following E. Actual responses are 20 seconds in duration occurring at 10 minute intervals with electrical stimulus alone and 20 minute intervals following addition of drugs to allow for equilibration. Initial tension produced was 1,082 $\pm$ 90 g/cm^2. Significant difference ($p < 0.05$) in response to E following exposure to phentolamine (PHENT 10^{-5} M) (64 $\pm$ 9%) is indicated. Propranolol (PROP 10^{-5}M) (97 $\pm$ 4%) was without significant effect. See table 3 appendix.

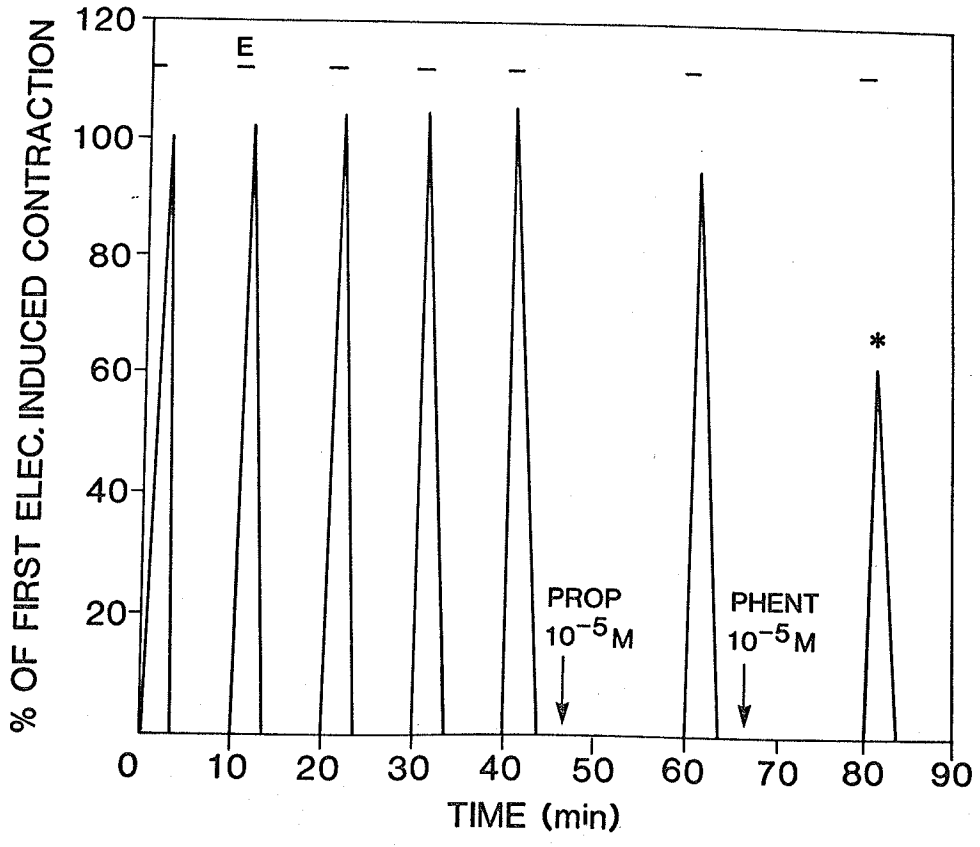
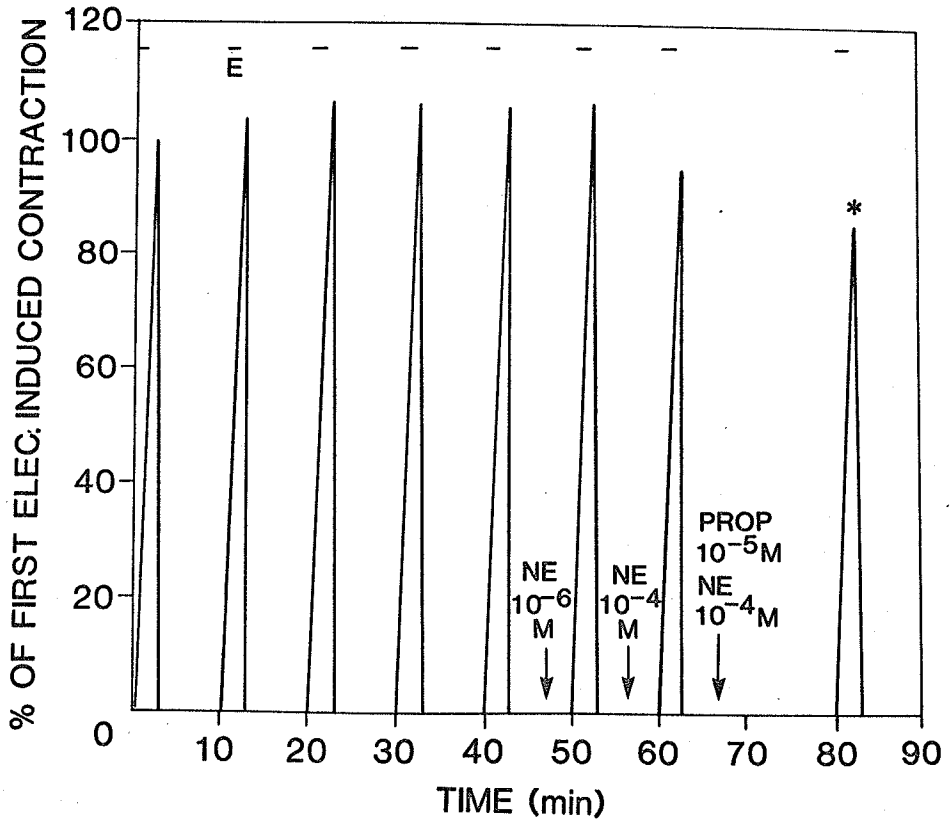


FIG. 4 Effect of norepinephrine and propranolol on isometric tension developed by TSM following electrical field stimulation (E). Means of isometric contractions (n=5), expressed as a % of the initial contraction (100%), following E. Actual responses are 20 seconds in duration occurring at 10 minute intervals, and a 20 minute interval after addition of propranolol (PROP 10^{-5}M) to allow for equilibration of the drug. Initial tension produced was 1304 $\pm$ 152 g/cm^2. Significant difference ($p < 0.05$) in response to E following exposure to PROP (10^{-5}M) and norepinephrine (NE 10^{-4}M) (85 $\pm$ 6%) is indicated *. A significant ($p < 0.05$) trend to a lower response following NE (10^{-4}M) is found using a non parametric sign test. See table 4 appendix.



value. The trend of increasing tension following repeated EFS was mainly because of exaggerated responses produced by two of the five muscle strips used, and was not significant (Figure 4).

B) Effect of Tone on Adrenoceptor Mediated Responses in Canine TSM

Unstimulated tracheal smooth muscle strips showed no contractile or relaxant response to norepinephrine (10^{-8}M to 10^{-4}M) or tyramine (10^{-5}M and 10^{-4}M) even following beta-adrenoceptor blockade with propranolol (10^{-5}M). However when isometric tension was developed to high-K (22.8 mM) exposure, the subsequent addition of norepinephrine (NE 10^{-8} - 10^{-6}M) always produced a further contractile response with a mean maximum rise to 17% greater than the response to K alone (Fig. 5). A further increase in NE concentration to 10^{-5}M resulted in partial relaxation which reached a maximum at 61% of the response to K. Phentolamine (10^{-5}M) further enhanced this relaxation to 29% (at 10 min) of the response to K. When phentolamine was present throughout the exposure to NE (Fig. 5), the results show a relaxant response to NE with a threshold at 10^{-8}M and maximum at 10^{-5}M. The response to 10^{-4}M NE consisted of a small non significant increase in mean tension which could be abolished by the addition of more phentolamine (2×10^{-5}M).

In order to examine the possibility of a cholinergic contribution to the increased tension developed in response to low doses of NE (in the presence of 22.8 mM K), atropine (10^{-6}M) was introduced at the plateau of the K-induced contraction (prior to the administration of graded increases of NE). Figure 6 illustrates the cholinergic component of the K contraction in that the tension declined by 20% after treatment with

FIG. 5 Response of TSM to norepinephrine (NE) in the presence of 22.8 mM K^+. The schematic mechanogram is representative of 7 experiments in which the dose-response relation of the muscle to NE was conducted at the plateau of a K-induced contracture in the absence (solid line) and presence (broken line) of phentolamine (PHENT. 10^{-5}M). In separate experiments the K-induced contracture was stable for the duration of the experiment. Numbers below drugs indicate final bath concentrations (M). See tables 5 and 6 appendix.

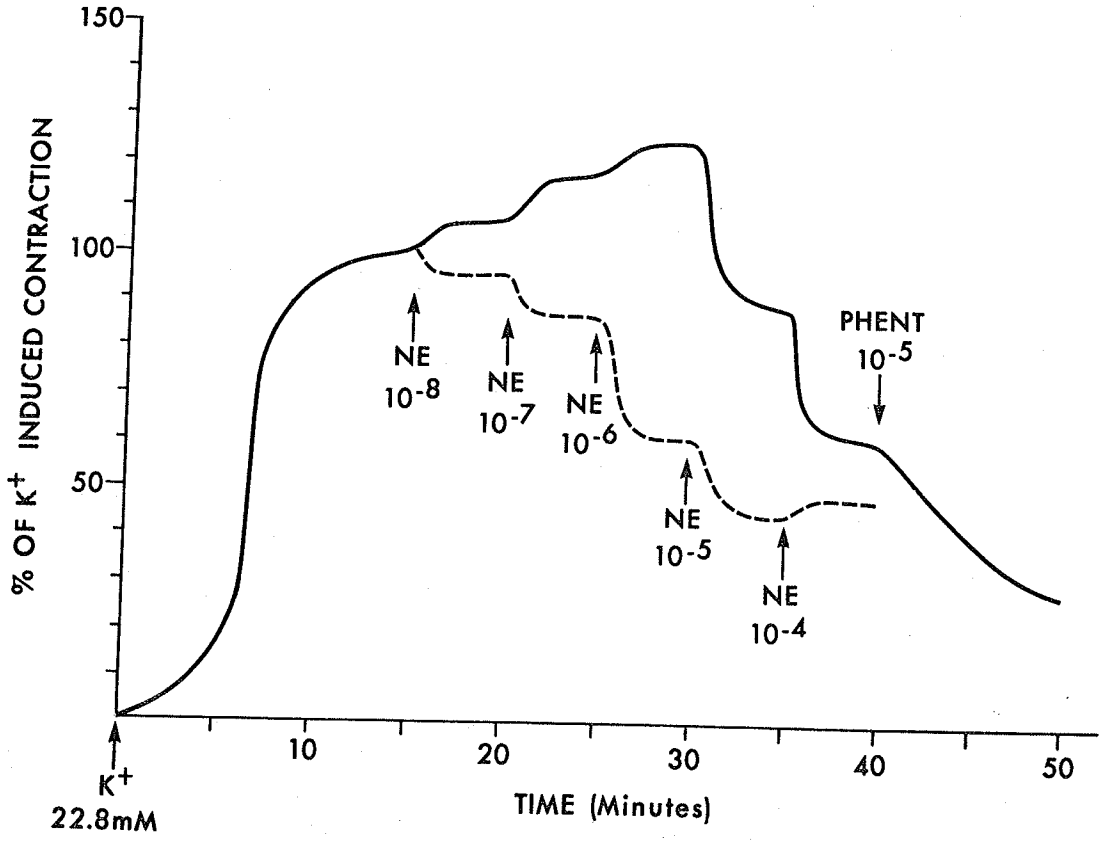
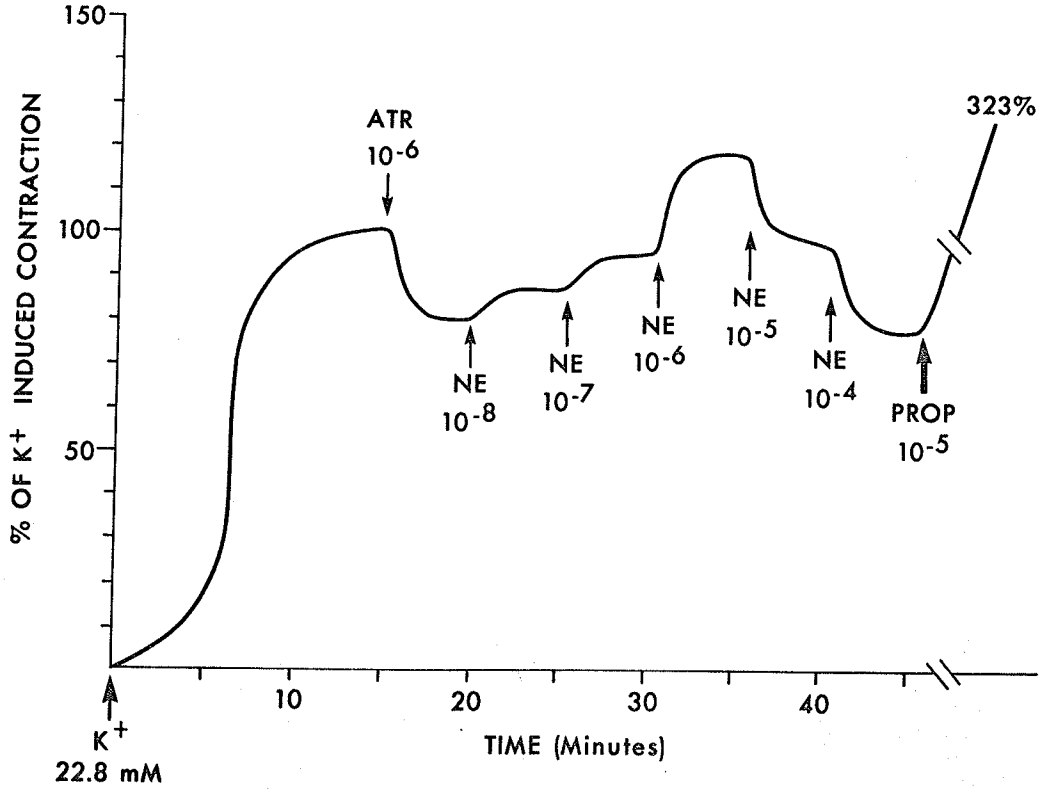


FIG. 6 Effect of atropine on the response of TSM to norepinephrine. Pre-existent tone was established with K^+ (22.8 mM) as in Fig. 1. The response to propranolol (PROP.) plateaued after about 10 min. The mechanogram shown is representative of 7 experiments. See table 7, appendix.



atropine. The subsequent contractile response to NE (10^{-8} to 10^{-6}M) however was not altered following atropine treatment, reaching a mean maximum of 18% greater than the K-induced contraction. Relaxation produced by NE (10^{-4}M) was reversed by beta-adrenoceptor blockade with propranolol (10^{-5}M), resulting in a mean isometric tension at 323% of the K-induced contraction. Due to large differences in tension development (normalized in g/cm^2) between muscles in both group (responses to NE in the presence and absence of atropine) qualitative measures of the responses were compared by use of the non-parametric sign test. Therefore, although the schematic mechanograms are based on the means of the various muscle responses, it is the qualitative aspect (i.e. relaxation or contraction) which is the major focus of this study.

When the same muscles (Fig. 6) were exposed to atropine (10^{-5}M) and propranolol (10^{-5}M) 20 minutes prior to K-stimulation (Fig. 7) the response to NE (10^{-8} to 10^{-4}M) was not biphasic but increased progressively to a maximum of 257% greater than the K-induced contraction. The contractile response to NE was reversed to relaxation at all doses of NE (10^{-8} - 10^{-4}M) by alpha-adrenoceptor blockade with phentolamine (10^{-5}M). The threshold dose of NE (10^{-8}M) was not altered by alpha - or beta-blockers.

Tyramine (10^{-5}M and 10^{-4}M), which acts to displace NE from adrenergic nerve terminals was shown to produce an increase in isometric tension in addition to that produced by K (22.8 mM). This contractile response was not blocked by atropine but was enhanced by propranolol and blocked by phentolamine (10^{-5}M, Fig. 8). To test the possible direct effects of tyramine on post-junctional receptors the effectiveness of

FIG. 7 Effect of propranolol pretreatment on the response of K-contracted TSM to norepinephrine. Propranolol (10^{-5}M) was introduced 20 minutes prior to elevation of the K^+-concentration and was present throughout the experiment. Phentolamine (PHENT.) was introduced at the plateau of the response to 10^{-4}M NE. The mechanogram shown is representative of 7 experiments. See table 8 appendix.

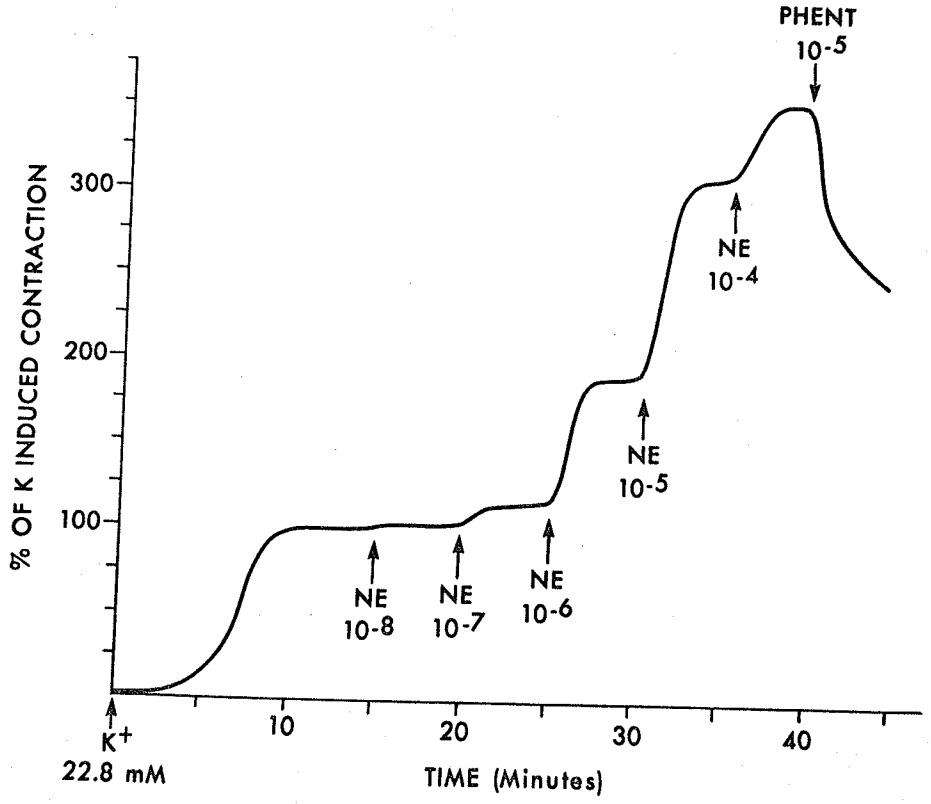
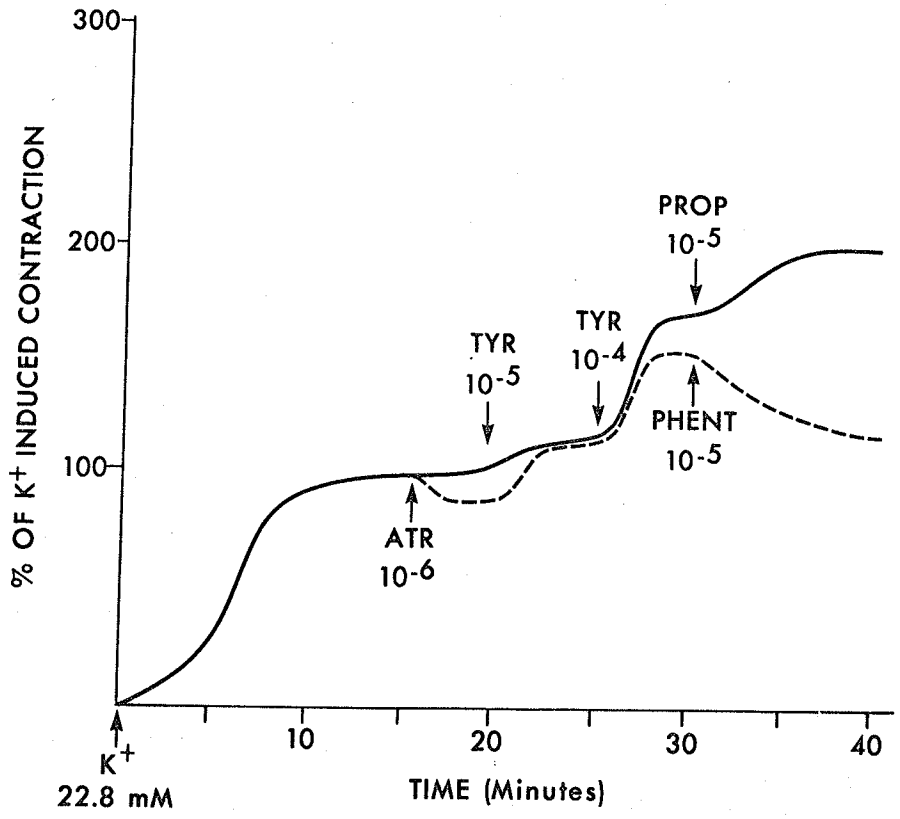


FIG. 8 Effect of atropine (ATR) and tyramine (TYR) on the K-contracture of TSM. Propranolol (PROP, 10^{-5}M) and phentolamine (PHENT. 10^{-5}M), resp. were added at the arrows. The mechanogram shown is representative of 7 experiments. See table 9 and 10 appendix.



this agent was examined in cold stored muscles. After 3 days of cold storage (4°C) TSM showed diminished response to tyramine (10^{-5} or 10^{-4}M), while responses to exogenous NE were not markedly affected (data not shown).

In order to examine the relation between the level of pre-existent tone and the magnitude of alpha-adrenoceptor mediated responses, atropinized (10^{-6}M) muscles were exposed to various $[K^+]_o$ prior to the addition of NE. At 48.2 mM K^+ the contractile response to NE (10^{-8} - 10^{6}M) was greatly reduced in spite of the fact that the cholinergic component could still be seen (Fig. 9). The relaxation response to NE (10^{-5}M) was markedly reduced (Fig. 9). This relaxation was propranolol-sensitive as was observed for lower $[K^+]_o$ (Fig. 1 and 2). The contraction produced by NE (10^{-8} to 10^{-6}M) at the plateau of the K(48.2mM) contraction was enhanced after pretreatment with propranolol (10^{-5}M, Fig. 7), but was much less than that observed at lower $[K^+]_o$ (22.8mM, Fig. 7). Similar experiments were conducted over the range of $[K^+]_o$ from 17.4 to 64.2 mM and responses to NE (10^{-8} to 10^{-4}M, Fig. 10) are expressed as the absolute tension above or below the K-induced contracture at the respective $[K]_o$. It can be seen that the optimal $[K]_o$ for demonstrating the alpha-adrenoceptor mediated contraction, both in the presence and absence of propranolol was 22.8 mM. Increases in K systematically potentiated the relaxant component and inhibited the alpha-adrenoceptor-mediated contractions; thus, while 10^{-6} M NE produced contraction at the lower $[K]_o$, it produced relaxation at the higher $[K]_o$.

In order to test whether the biphasic response to NE was specific to

FIG. 9 Representative mechanogram showing the effect of antagonists on the response of TSM to 48.2 mM K^+. The broken line documents the response of muscle pretreated (20 min) with propranolol (PROP. 10^{-5}M) and atropine (ATR, 10^{-6}M). The solid line represents the responses of non-pretreated muscles to the agents as indicated (n=7). See table 13 appendix.

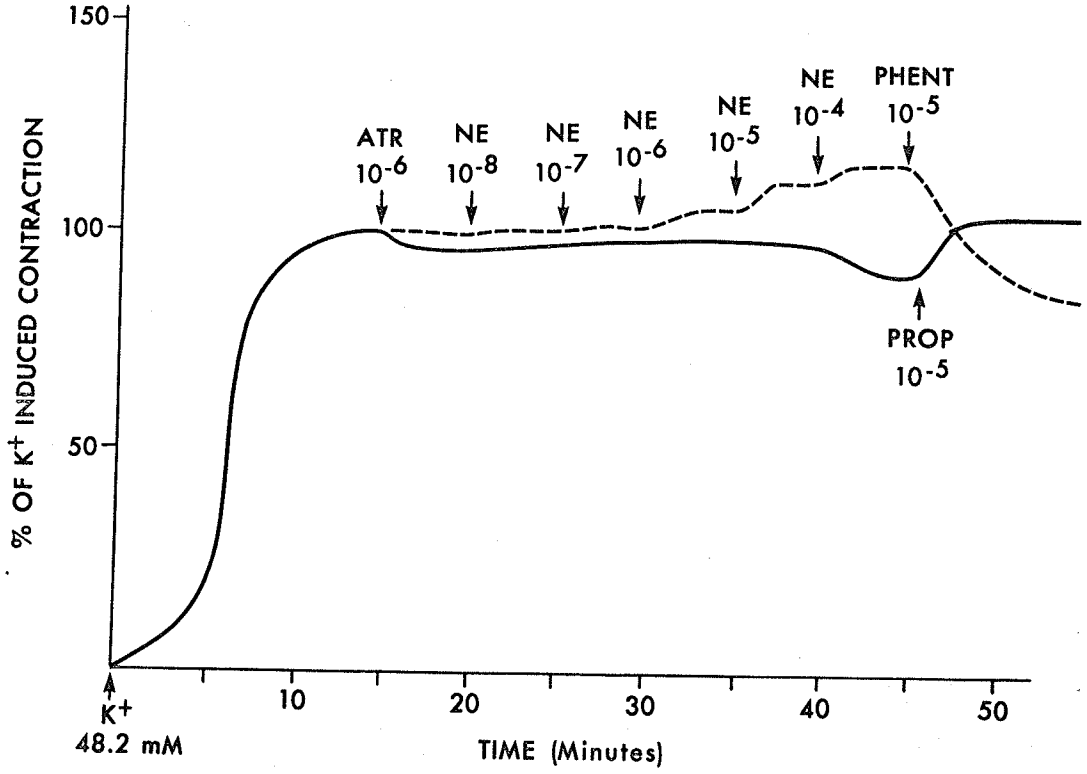


FIG. 10 Responses of atropinized TSM to NE at various $[K]_o$. The alterations in tension produced by NE are plotted for each NE concentration in M; a, 10^{-8}; b, 10^{-7}; c, 10^{-6}; d, 10^{-5}; e, 10^{-4} and f, 10^{-4} plus propranolol 10^{-5}M. The zero basline indicates the respective plateau tension responses to various $[K]_o$ in g/cm^2: 17.4 mM = 187; 22.8 mM = 508; 35.2 mM = 1080; 48.2 mM = 1230; 64.2 mM = 1503. Inset: Dose-response relation of atropinized (10^{-6}M) TSM to K^+. Means ± SEM of 7 experiments are presented.

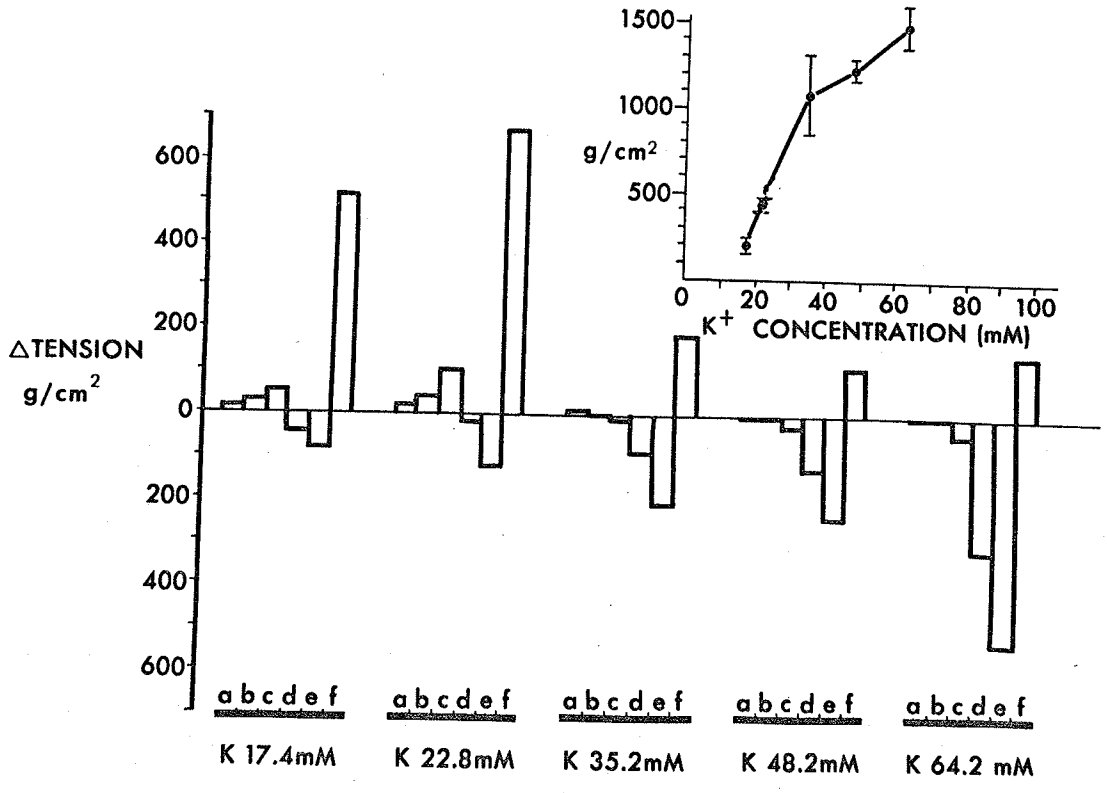


TABLE 1 Cumulative dose response relation to NE (10^{-8} to 10^{-7}M) following development of active tone (initial tension, g/cm^2). Values for NE and after propropranolol (PROP 10^{-5}M) addition are expressed as the percent of the initial contraction. All muscles which were contracted with potassium (K^+) were pretreated with atropine (10^{-6}M). Means ± SE for 7 muscles for each agonist.

AGONIST	INITIAL TENSION (g/cm^2)	% OF INITIAL TENSION					
		NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PROP 10^{-5}M
K^+ 17.4mM	187+55	107+4	118+6	124+16	80+15	66+14	494+125
K^+ 22.8mM	480±140	103±8	109±9	121±21	97±21	74±21	241±97
K^+ 35.2mM	1080±263	100±0	100±1	100±1	96±4	85±4	127±11
K^+ 48.2mM	1230±82	100±0	100±0	98±1	91±3	82±5	110±2
K^+ 64.2mM	1503±144	100±0	100±0	97±1	79±3	65±6	110±2
Histamine 10^6M	168±62	116±6	116±23	28±10	21±12	35±21	925±176
ACh 5x10 ^{8}M	449±80	102±1	106±2	93±9	65±18	59±19	166±29

K-induced tone both histamine (10^{-6}M) and ACh (5 x 10^{-8}M) were used. Table 1 indicates that the biphasic response is observed with all three agents and the maximum alpha-adrenoceptor mediated contraction is dependent on the amount of resting tone. Fig. 10 indicates that maximum alpha-mediated responses occur when tone is developed to a K^+ concentration of 22.8 mM. When the same responses are expressed as a percentage of the plateau tension developed to the agonist the maximum percentage increase occurs where active tone developed is least, (compare percentages in the presence of histamine and propranolol with those in high -K, 64.2mM and propranolol, Table 1).

Figures 11 and 13 illustrate the biphasic response to norepinephrine (NE 10^{-8} to 10^{-4}M) in TSM contracted with acetylcholine (ACh 5 x 10^{-8}M) and histamine (10^{-6}M) respectively. The change from contractile to relaxant responses however, occurs at a lower concentration of NE (10^{-6}M) in the presence of these two agonists, than that for an equivalent K-induced contraction (10^{-5}M NE, fig. 5). Addition of propranolol (10^{-5}M) reversed the relaxation in both cases (Figs. 11 and 13).

Whether the biphasic response developed with norepinephrine was in some way unique to K^+ stimulated contractions was further studied. Since a specific antagonist for potassium is not available, it was possible to test the effect of abolishing initial tone produced by acetylcholine (5 x 10^{-8}M) on the adrenoceptor mediated contraction. Addition of hyoscyamine (10^{-5}M) eliminated the contraction produced by acetylcholine (5 x 10^{-8}M) and NE (10^{-8} to 10^{-4}) in TSM pretreated with propranolol (10^{-5}M, Fig. 12) Addition of hyoscyamine (10^{-5}M) to histamine-pretreated (10^{-6}M) muscles in the presence of NE and prop

FIG. 11 Effect of norepinephrine (NE) on isometric tension developed with acetylcholine (ACh 5 x 10^{-8}M). The graph is based on means and standard errors for 11 muscles in which the dose-response relation of the muscle to NE was conducted at the plateau of a ACh-induced contracture. In seperate experiments the ACh contracture was found to be stable for the duration of the experiment. Propranolol (PROP, 10^{-5}M) was added following the NE dose-response determination. Significant ($p < 0.05$) differences from preceding response are indicated *. See table 15 appendix.

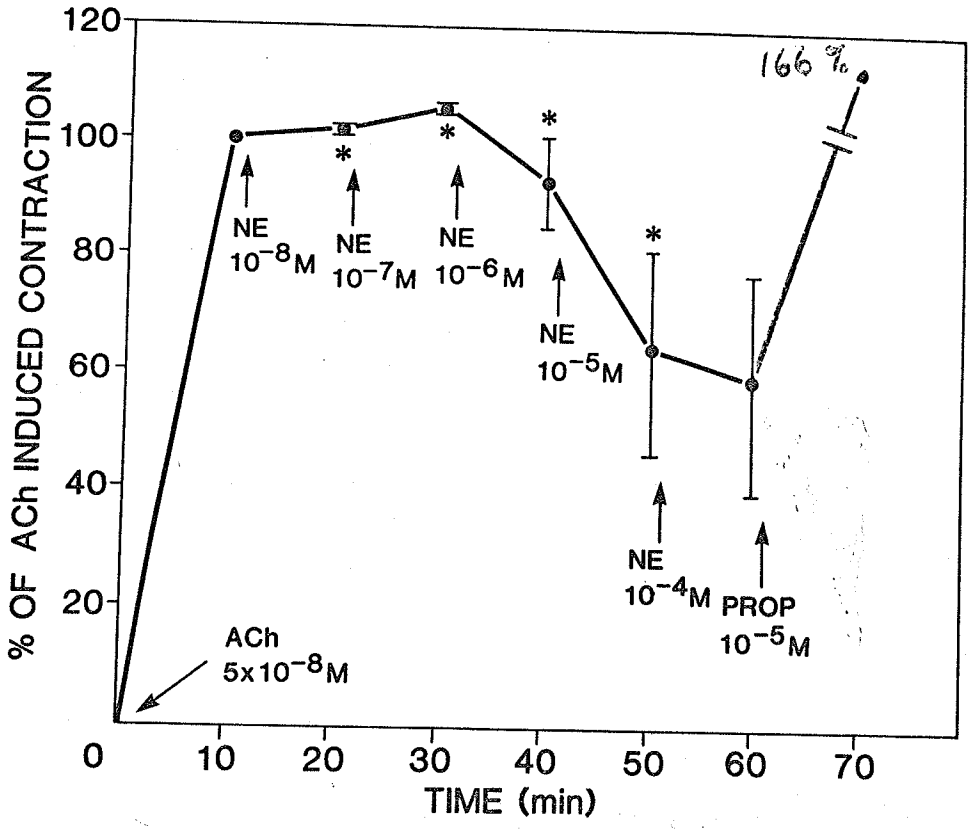


FIG. 12 Effect of norepinephrine (NE 10^{-8} to 10^{-4}M) on TSM (n=9) pretreated with propranolol (10^{-5}M) and contracted with acetylcholine (Ach 5 x 10^{-8}M). Hyoscyamine (HYO 10^{-5}M) was added at the end of the cumulative NE dose-response determination. Significant ($p < 0.05$) change from the preceding response is indicated *. Points indicate means and standard errors of data from experiments at the plateau of response following each drug addition. See table 16 appendix.

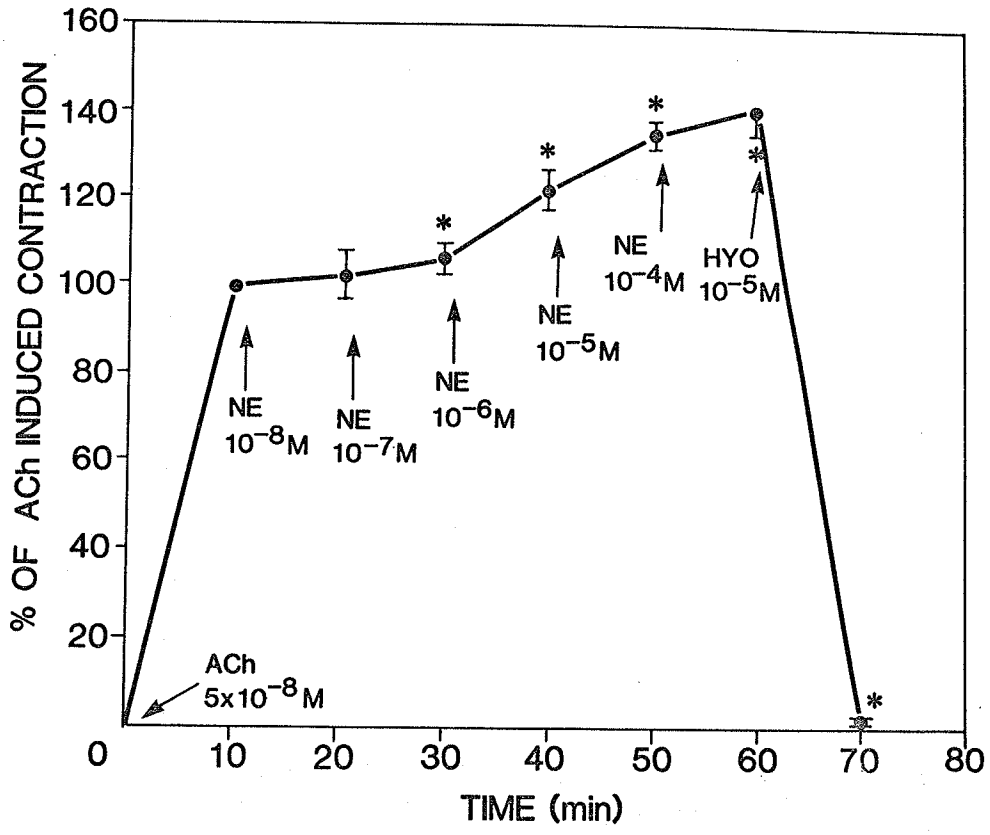


FIG. 13 Effect of norepinephrine (NE 10^{-8} to 10^{-4}M) on TSM (n=9) contracted isometrically with histamine (10^{-6}M). Propranolol (10^{-5}M) was added at the end of the cumulative NE dose-response determination. Significant ($p < 0.05$) change from the preceding response is indicated *. Values indicate means and standard errors of the plateau of the response following each drug addition. See table 17 appendix.

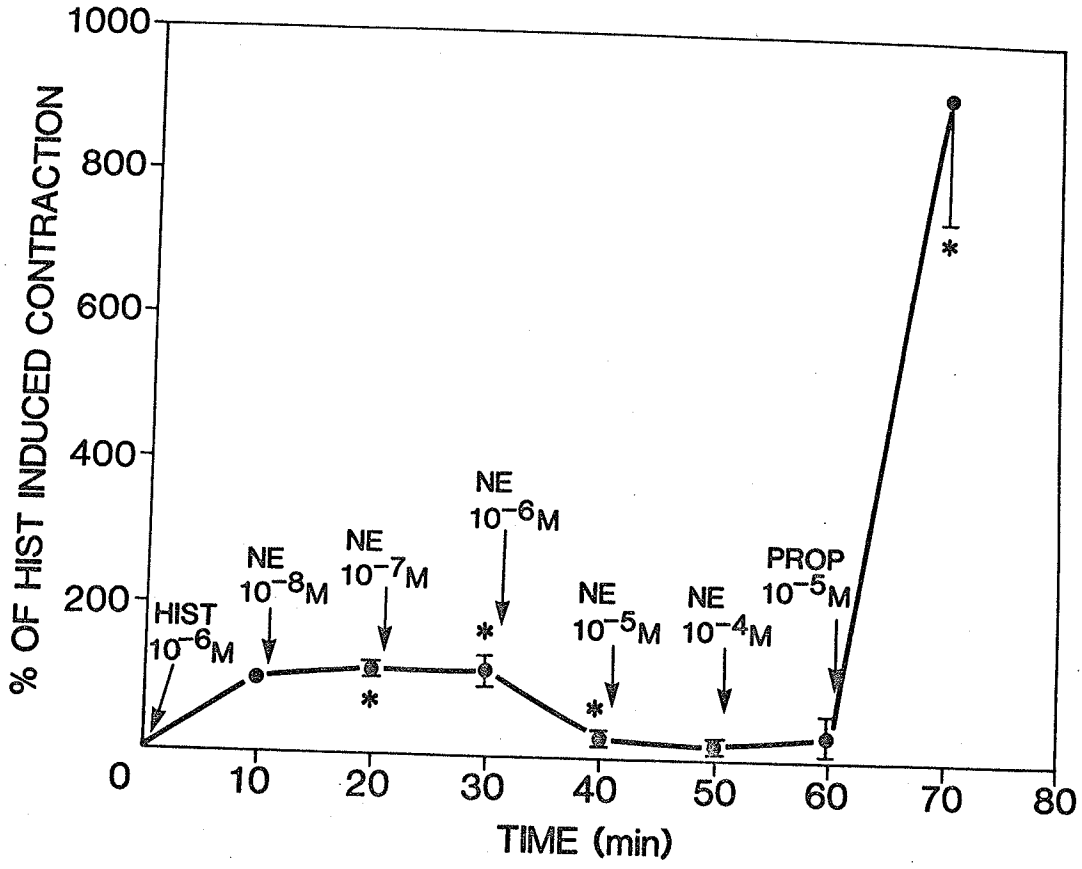
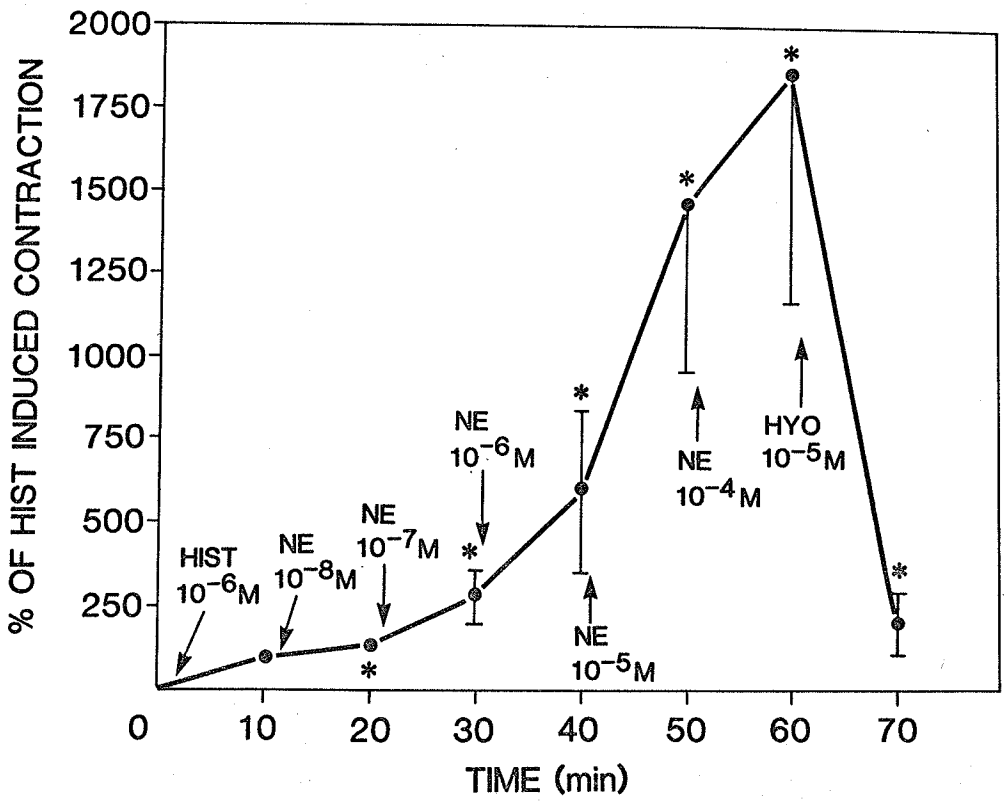


FIG. 14 Effect of norepinephrine (NE 10^{-8} to 10^{-4}M) on TSM (n=9) contracted isometrically with histamine (10^{-6}M) and pretreated with propranolol (10^{-5}M). Hyoscyamine (HYO, 10^{-5}M) was added following the cumulative NE dose-response determination. Significant ($p < 0.05$) change from the preceding response is indicated *. Values indicate means and standard errors of the plateau of the response following each drug addition. See table 18 appendix.



ranolol reduced the tension dramatically from 1864 ± 726% to 205 ± 83% of the initial histamine-induced (100%) contraction (Fig. 14). Table 18 (appendix) indicates a wide variability in the responses to histamine (10^{-6}M) and additions of NE (10^{-8} to 10^{-4}M) plus hyoscyamine. When expressed in g/cm^2 the results (table 18, appendix) indicate that hyoscyamine relaxed those muscles to the initial tension developed in the presence of histamine (10^{-6}M).

C) Adrenoceptor Mediated Responses in Canine TSM Sensitized to Ovalbumin (OA)

Studies of the biphasic response to norepinephrine (10^{-8} to 10^{-4}M) and its dependance on pre-existing tone were carried out in canine TSM from an allergic model of asthma. Responses of TSM muscle from dogs sensitized to ovalbumin (OA) were compared to those from control mongrel dogs. Since maximum alpha-adrenoceptor-mediated contractions were observed with a potassium concentration of 23mM prior to NE (10^{-8} to 10^{-4}M) exposure, the same protocol was used for sensitized tissue. The results reveal no significant differences in the biphasic response (Fig. 15), maximum alpha-adrenoceptor-mediated contraction following propranolol (10^{-5}M) exposure (Fig. 16), maximum beta-adrenoceptor-mediated relaxation (Fig. 17) or responses to tyramine (Figs. 18 and 19). Responses of a mongrel control population are similar quantitatively and qualitatively to those of OA-sensitized canine TSM with respect to adrenoceptor-mediated responses, when tone was first elevated with K^+ (23mM). Varying the concentration of K^+ to 48.2 mM resulted in a shift from alpha-adrenoceptor-mediated contraction to beta-adrenoceptor mediated relaxation; this shift was not significantly different from that in controls (Table 2).

FIG. 15 Effect of norepinephrine (NE 10^{-8} to 10^{-4}M) on isometric tension developed by TSM from control mongrel dogs (n=7) and dogs sensitized to ovalbumin, following induction of active tone with 23 mM K^+. Phentolamine (PHENT, 10^{-5}M) was added following the cumulative additions of NE.

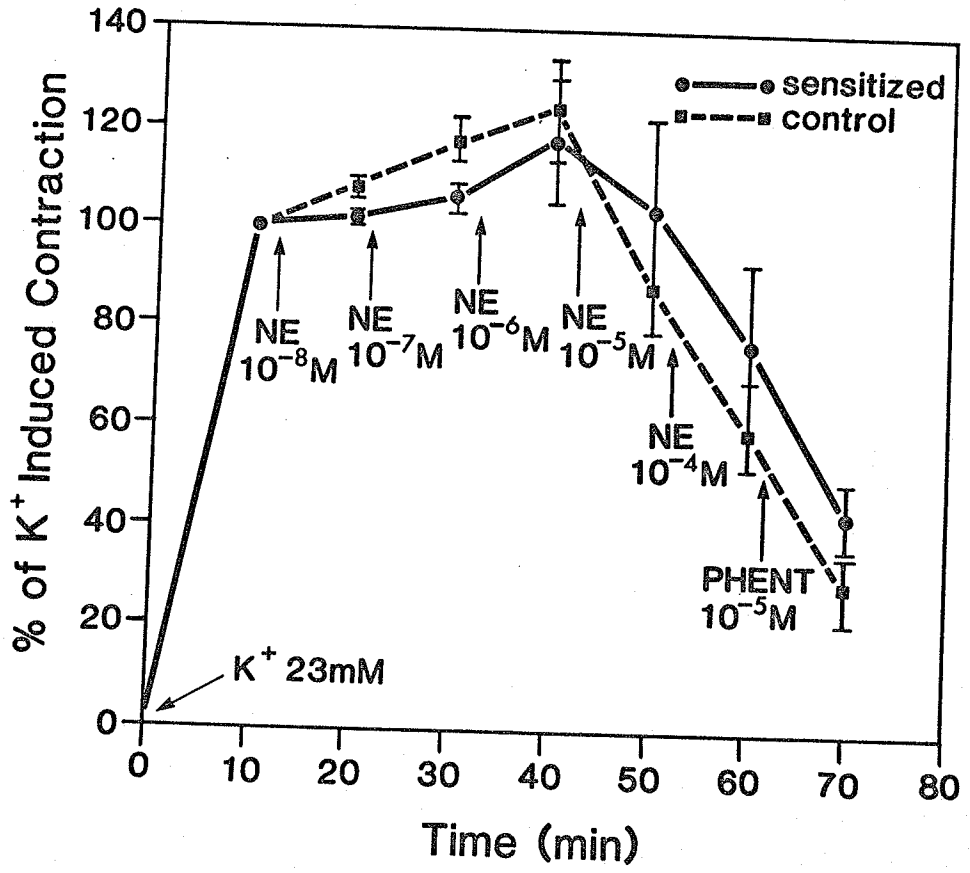


FIG. 16 Effect of atropine on the response of sensitized (n=10) and control (n=7) TSM to norepinephrine. Pre-existent tone was established with K^+(23mM) as in Fig. 15. The response to propranolol (PROP, 10^{-5}M) plateaued after 10 minutes.

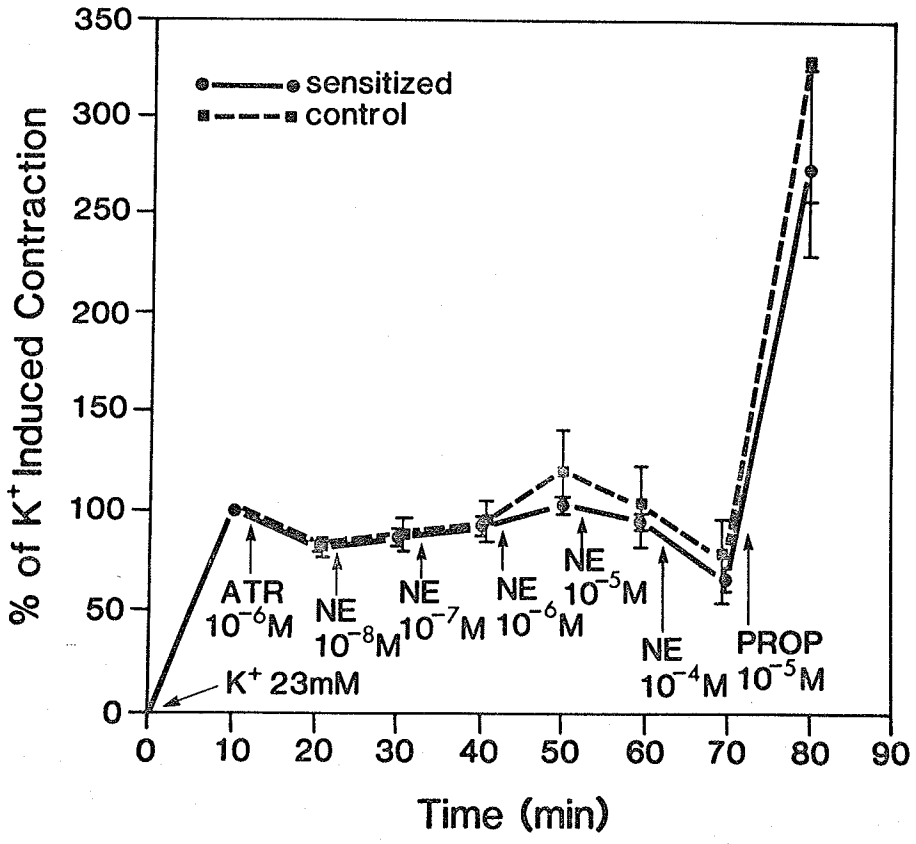


FIG. 17 Response of control (n=7) and sensitized (n=8) TSM strips to norepinephrine (10^{-8} to 10^{-4}M) at the plateau of tension developed in response to 23 mM K^+. Muscles were pretreated with phentolamine (10^{-5}M).

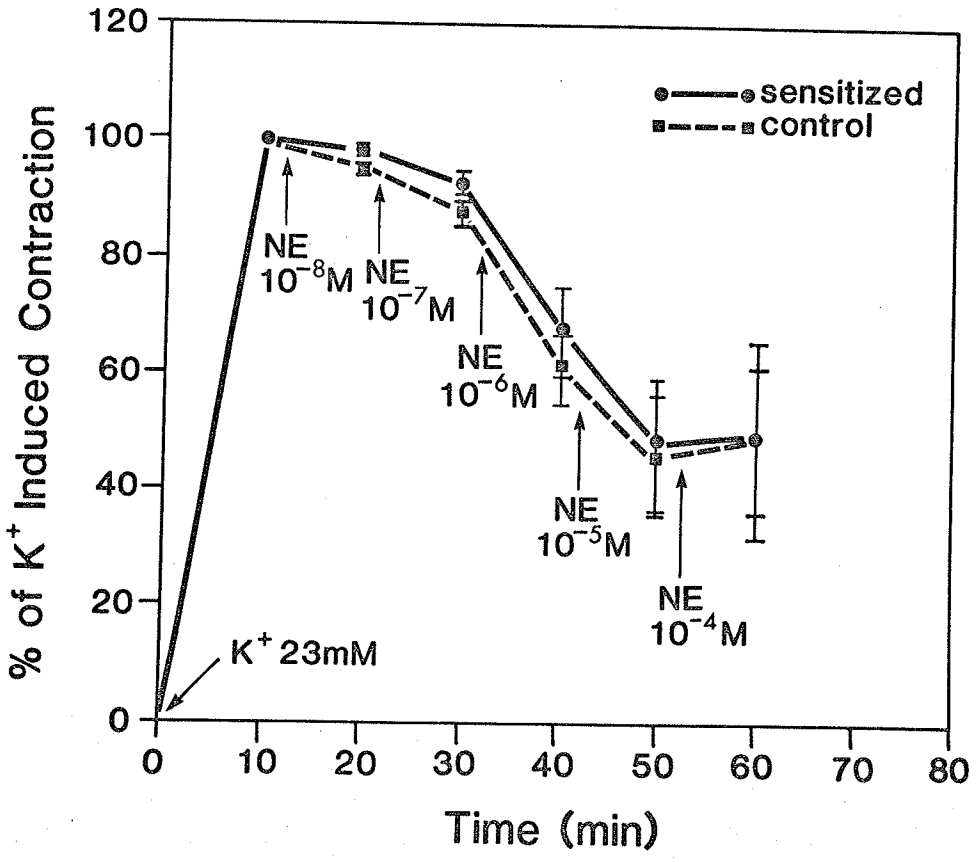


FIG. 18 Effect of atropine (ATR 10^{-6}M) and tyramine (TYR) on the K^+-contracture of TSM taken from sensitized (n=9) and mongrel control dogs (n=7). Propranolol (PROP, 10^{-5}M) was added as indicated.

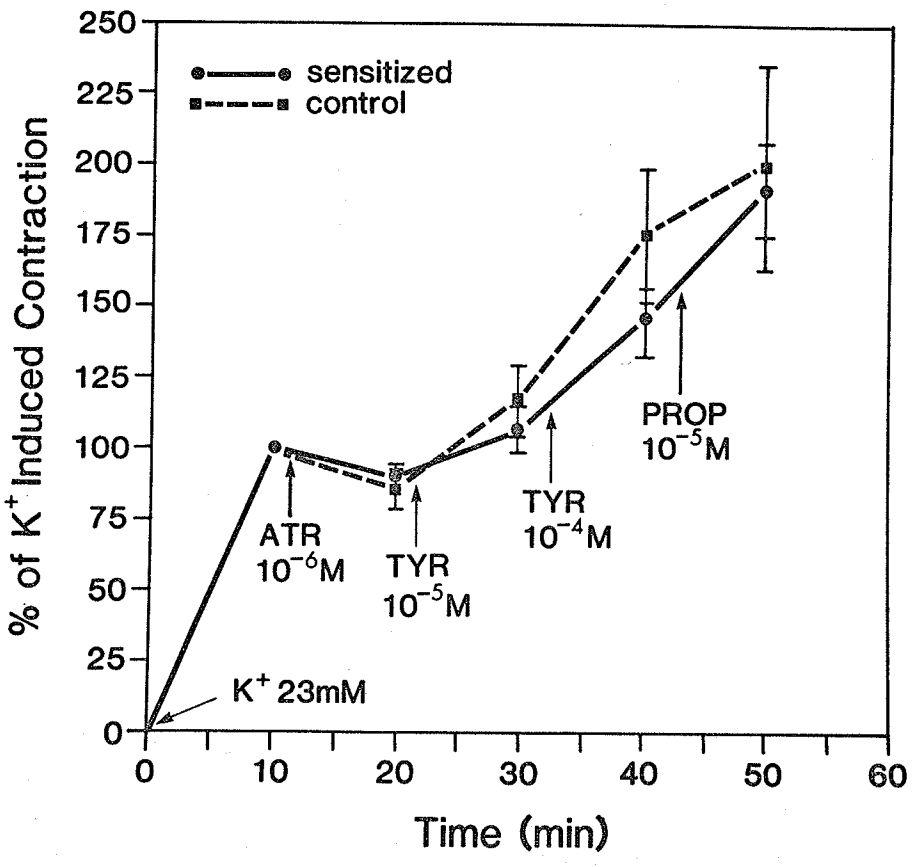


FIG. 19 Effect of tyramine (TYR 10^{-5} and 10^{-4}M) on atropine (10^{-6}M) pretreated TSM contracted initially with 23 mM K. Phentolamine (10^{-5}M) was added to sensitized (n=8) and control (n=7) muscles at the point indicated.

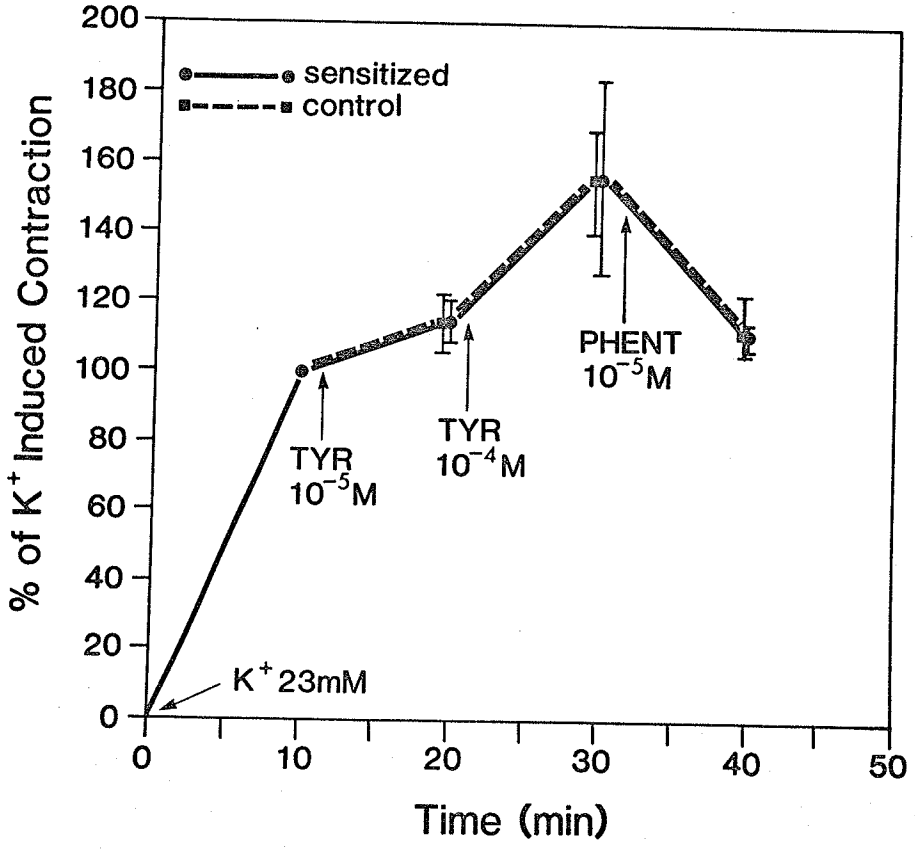
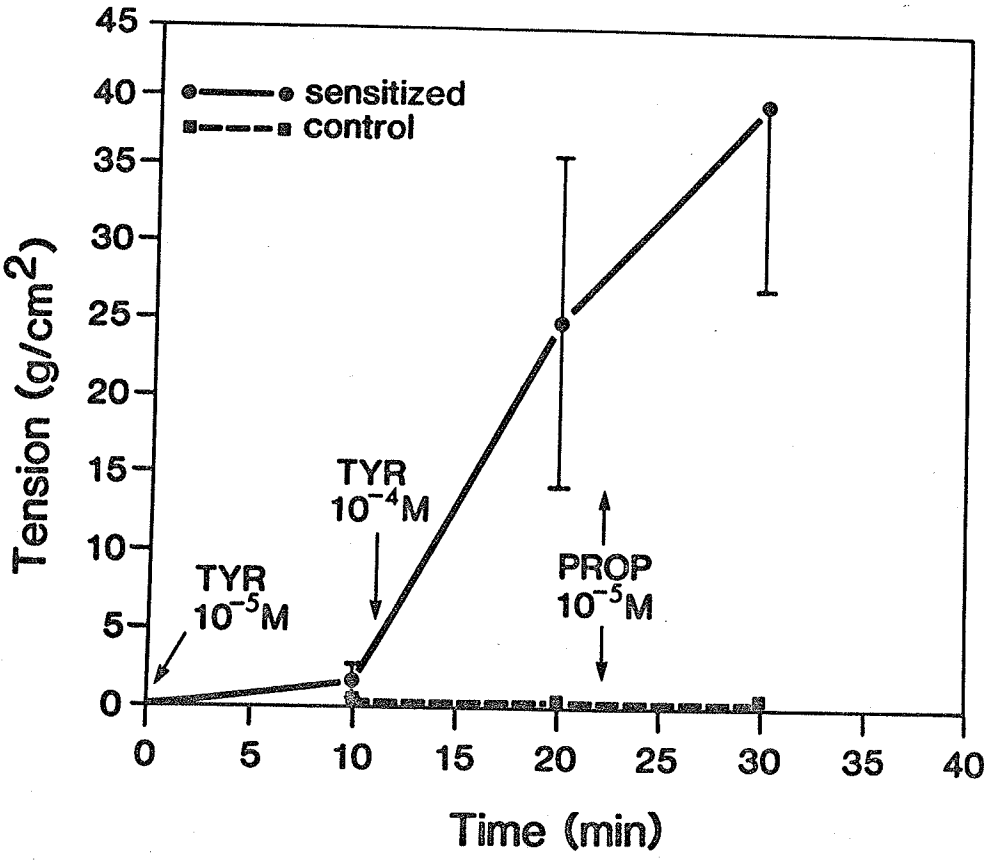


TABLE 2 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of K^+ (48mM), atropine ($10^{-6}M$) and norepinephrine (NE 10^{-8} to $10^{-4}M$) to OA sensitized TSM strips (n=3). A 5-minute time interval between drug additions allowed for each response to reach a stable plateau tension. Values in brackets are percentages of the initial tension (100%) developed following K^+ exposure.

	TREATMENT						
Muscle	K^+ 48mM	ATR $10^{-6}M$	NE $10^{-8}M$	NE $10^{-7}M$	NE $10^{-6}M$	NE $10^{-5}M$	NE $10^{-4}M$
1	1080 (100)	1080 (100)	1080 (100)	1080 (100)	1080 (100)	998 (92)	847 (78)
2	1093 (100)	1093 (100)	1093 (100)	1113 (101)	1171 (107)	1113 (101)	1015 (92)
3	2010 (100)	1671 (83)	1671 (83)	1671 (83)	1671 (83)	1609 (80)	1486 (73)
$\bar{X}$	1394	1281	1281	1288	1307	1240	1116
SE	308	195	195	192	184	187	191
$\bar{X}$	(100)	(94)	(94)	(95)	(97)	(91)	(81)
SE	0	6	6	6	7	6	6

FIG. 20 Effect of tyramine (TYR, 10^{-5} and 10^{-4}M) on TSM from control (n=7) and OA sensitized dogs (n=10). Propranolol (10^{-5}M) was added following plateau tension development in the presence of tyramine. See table 19, appendix for sensitized TSM data.



In unstimulated control TSM, tyramine (10^{-5} and 10^{-4}M) produced no response even with the further addition of propranolol (10^{-5}M, Fig. 20). Of the 10 sensitized TSM tissues 5 produced a small contractile response to tyramine 10^{-4}M (25 $\pm$ 11g/cm^2). This was enhanced by the addition of propranolol (10^{-5}M, Fig. 20 and table 19 appendix). Previous results demonstrated that control TSM responds to tyramine (10^{-5} and 10^{-4}M) when active tone has been increased by 22.8 mM K^+ (Fig. 8). As noted above, unstimulated control TSM did not respond to norepinephrine (10^{-8} to 10^{-4}M) even in the presence of beta-adrenoceptor blockade with propranolol (10^{-5}M, Fig. 21 and table 20 appendix). Addition of norepinephrine (10^{-6} to 10^{-4}M in a cumulative paradigm) to OA-sensitized TSM produced a contractile response with a mean of 183 $\pm$ 99 g/cm^2 (Fig. 21). Further addition of tyramine (10^{-5} and 10^{-4}M) increased the tension produced to a mean value of 402 $\pm$ 134 g/cm^2, which was abolished by phentolamine (10^{-5}M Fig. 21 and table 21 appendix).

D) Effects of Atropine and Phentolamine on Contractile Responses Produced by Histamine and Serotonin in Control and Ovalbumin-sensitized Canine TSM

The possible interaction of the antagonists atropine and phentolamine with histamine and serotonin (5-HT)-induced contractions of canine TSM were studied. A histamine (10^{-5}M)-induced contraction (100%) was partially antagonized by phentolamine (10^{-5}M) to 73 $\pm$ 6% of the initial contraction and plateaued 10 minutes following phentolamine administration at 78 $\pm$ 11% of the histamine induced active tone (Fig. 22). Pretreatment with phentolamine (10^{-5}M) also reduced the response to hista-

FIG. 21 Effect of cumulative addition of norepinephrine (NE 10^{-6} to 10^{-4}M), tyramine (10^{-5} and 10^{-4}M) and phentolamine (10^{-5}M) on OA-sensitized (n=9) and control (n=5) TSM. See table 21 appendix for data on sensitized TSM.

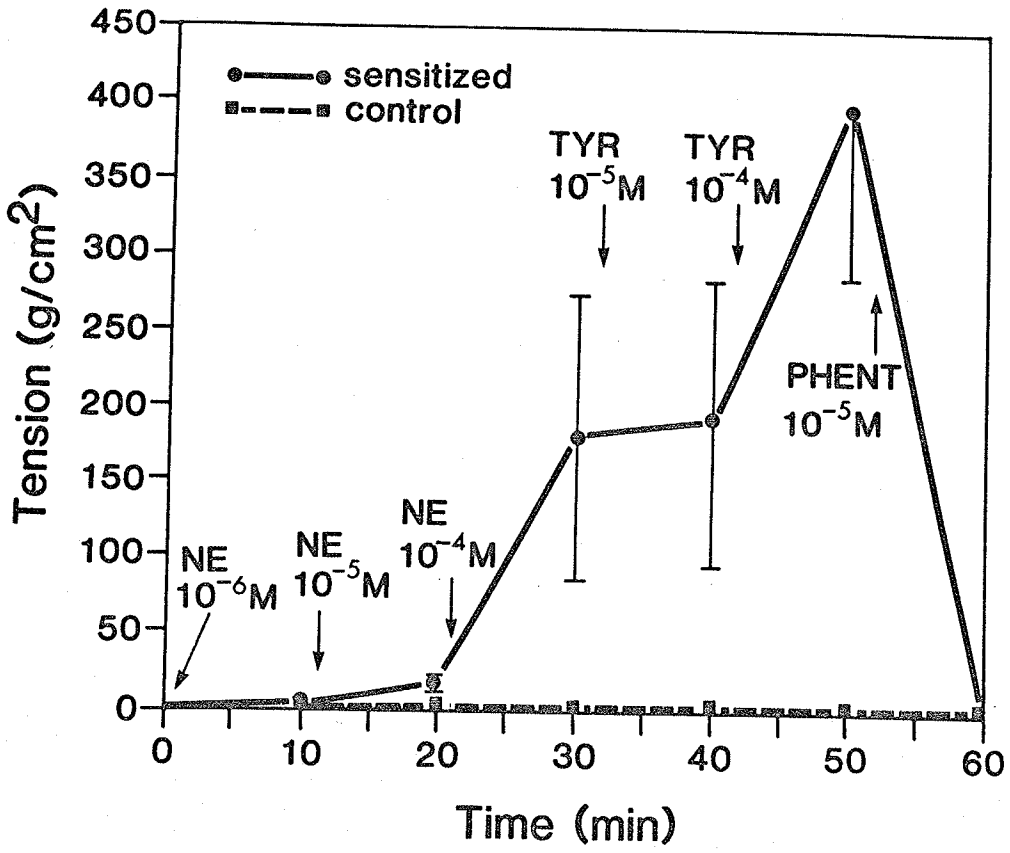
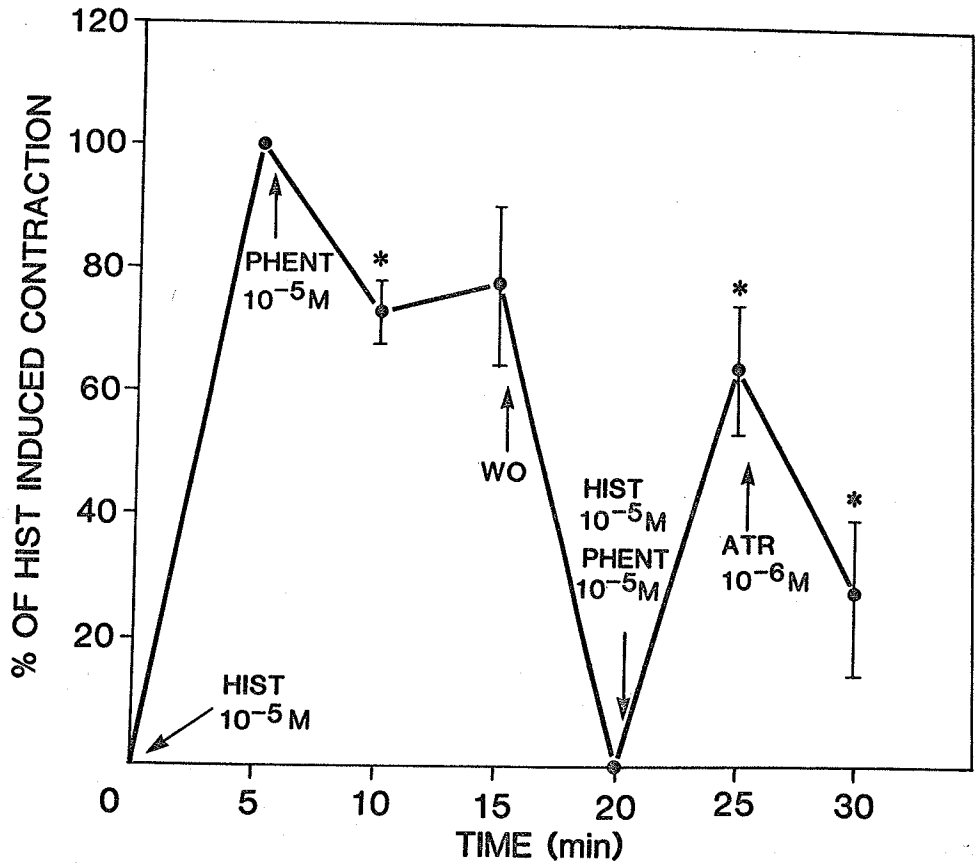


FIG. 22 Effect of sequential exposure to phentolamine (PHENT, 10^{-5}M) and atropine (ATR, 10^{-6}M) on isometric tension developed by histamine (HIST, 10^{-5}M) treated canine TSM (n=8). See table 22 appendix.



mine (10^{-5}M) such that only 65 $\pm$ 10% of the first histamine-induced contraction (100%) remained. This latter effect was not significantly different from that resulting when phentolamine (10^{-5}M) was added at the plateau of the histamine (10^{-5}M) induced contraction. Following the plateau of tension responses to histamine (10^{-5}M) in the presence of phentolamine (10^{-5}M), atropine (10^{-6}M) further reduced the contracture by 37% so that only 28 $\pm$ 12% of the first histamine contraction (100%) remained (Fig. 22 and table 22 appendix). When histamine (10^{-5}M)-stimulated TSM were treated with atropine (10^{-6}M) in the absence of phentolamine a dramatic 46% decline in active tone resulted (Fig. 23). Pretreating with atropine (10^{-6}M) resulted in a similar inhibition of histamine-induced tension development. In the previous study (Fig. 22) phentolamine significantly ($p < 0.05$) inhibited histamine-induced tension development, whereas in the present tissues which had been atropinized prior to phentolamine treatment, this inhibition was not observed (Fig. 23).

With serotonin (5-HT)-stimulated TSM the actions of the antagonists atropine and phentolamine were reversed as compared to their effects in histamine-treated tissues. Atropine (10^{-6}M) produced a small but significant ($p < 0.05$) inhibition of a 5-HT (10^{-6}M) induced contraction whether administered at the plateau of contraction or prior to 5-HT (10^{-6}M, Fig. 24 and table 24 appendix). This inhibition was not significant when the muscles had been blocked with phentolamine (10^{-5}M) prior to the addition of atropine (Fig. 25). These results indicate a potent inhibitory action of phentolamine (10^{-5}M) on contractions stimulated by 5-HT (10^{-6}M) in the presence of atropine (Fig. 24) or with 5-HT alone (Fig. 25, see also tables 24 and 25 appendix).

FIG. 23 Effect of atropine (ATR, 10^{-5}M) and phentolamine (PHENT, 10^{-5}M) on isometric tension stimulated by histamine (HIST, 10^{-5}M) in TSM strips (n=8). See table 23 appendix.

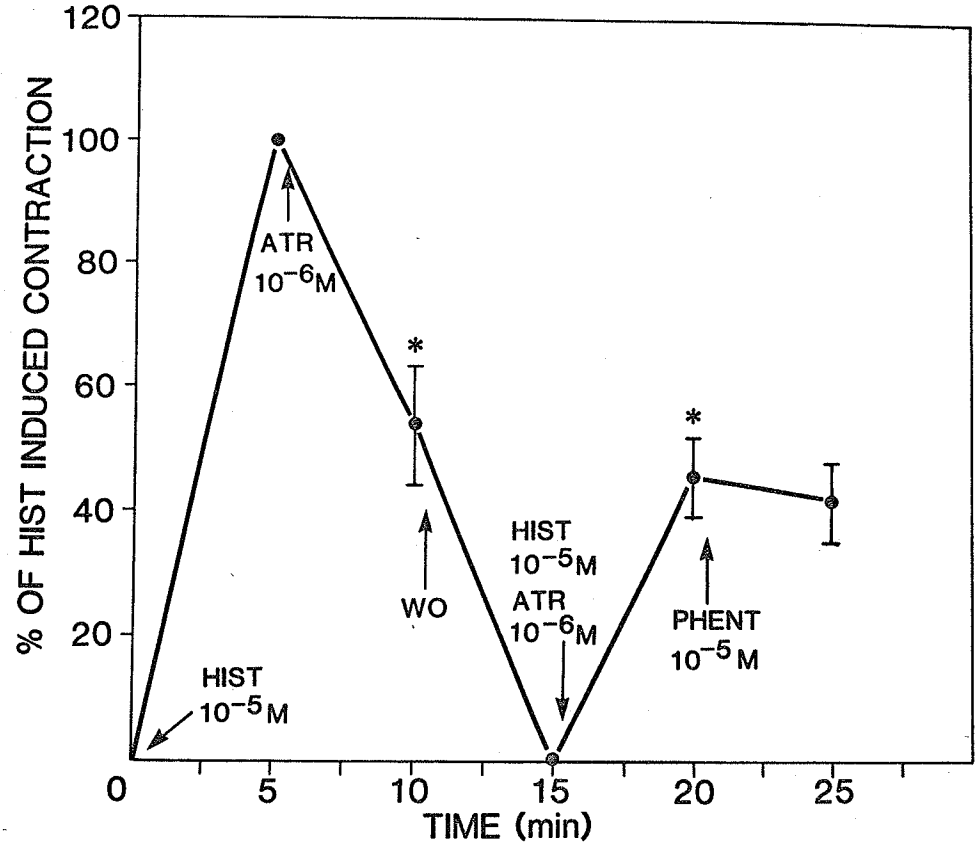


FIG. 24 Effect of atropine (ATR, 10^{-6}M) and phentolamine (PHENT, 10^{-5}M) on isometric tension stimulated by 5-HT (10^{-6}M) in TSM (n=7). Significant ($p < 0.05$) change from the previous value is indicated *. See table 24 appendix.

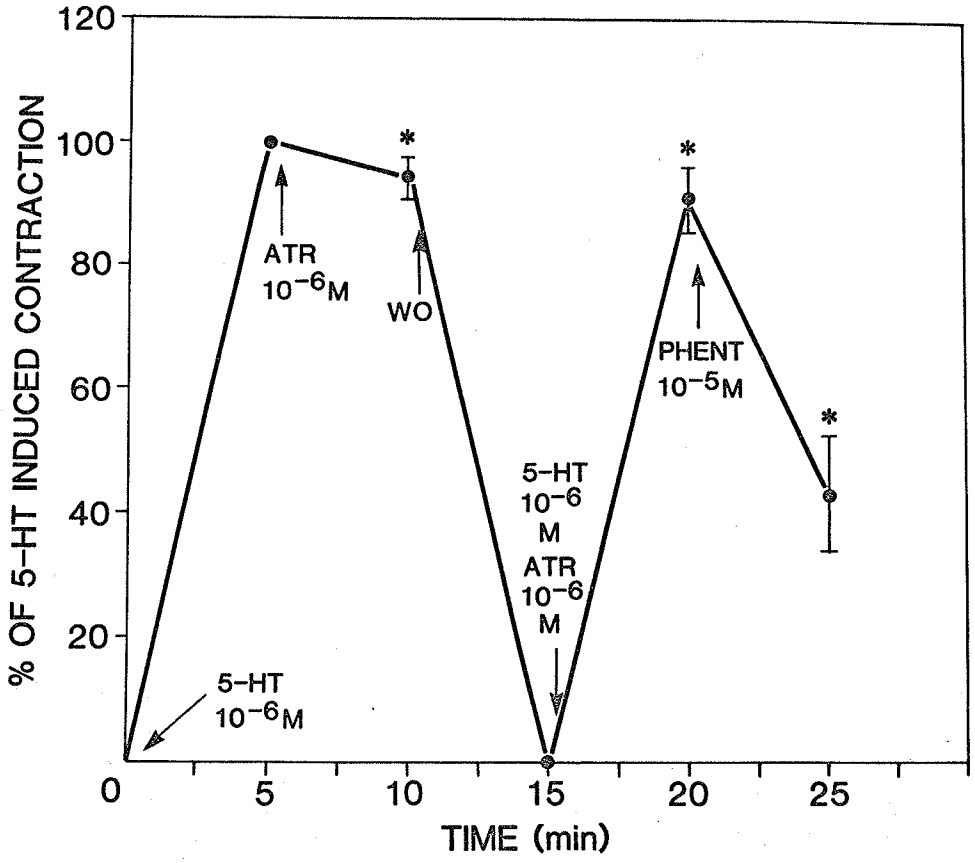


FIG. 25 Effect of phentolamine (PHENT, 10^{-5}M) and atropine (ATR 10^{-6}M) on isometric tension developed by 5-HT (10^{-6}M)-treated TSM (n=8). Significant ($p < 0.05$) change from previous value is indicated *. See table 25 appendix.

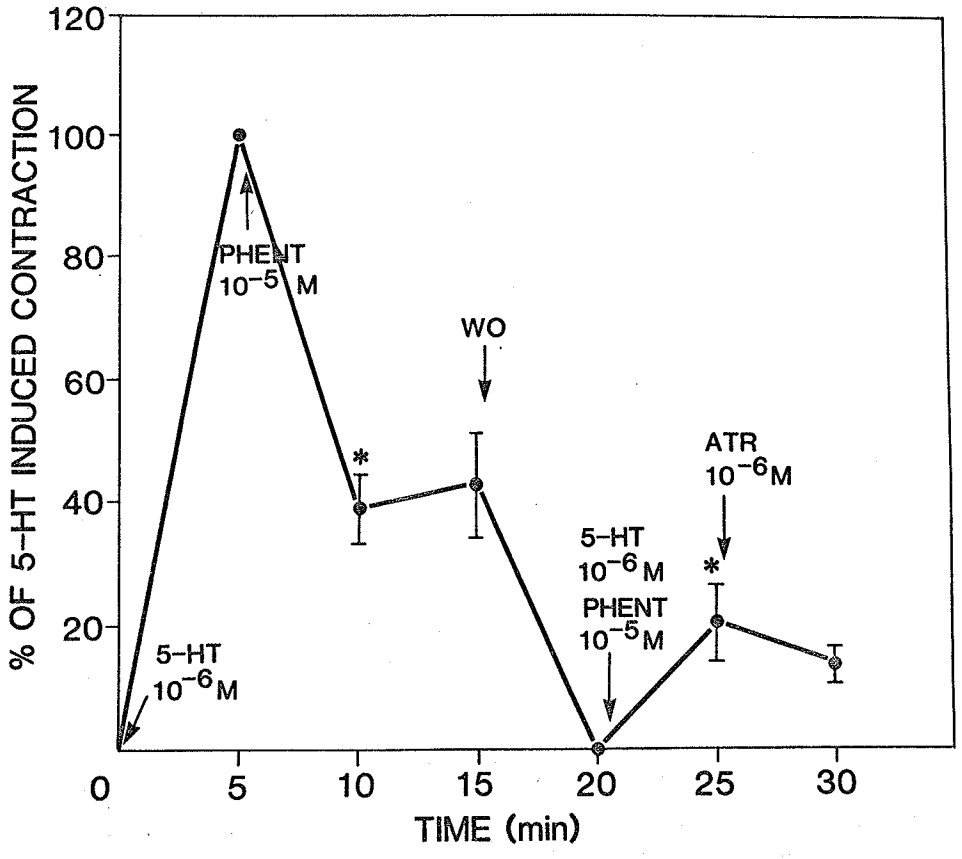


FIG. 26 Effect of phentolamine (PHENT 10^{-5}M) and atropine (ATR 10^{-6}M) on isometric tension developed by control (n=8) and OA sensitized (n=9) TSM contracted with histamine (10^{-5}M). After its introduction to the bath, phentolamine concentration was maintained throughout the remainder of the experiment.

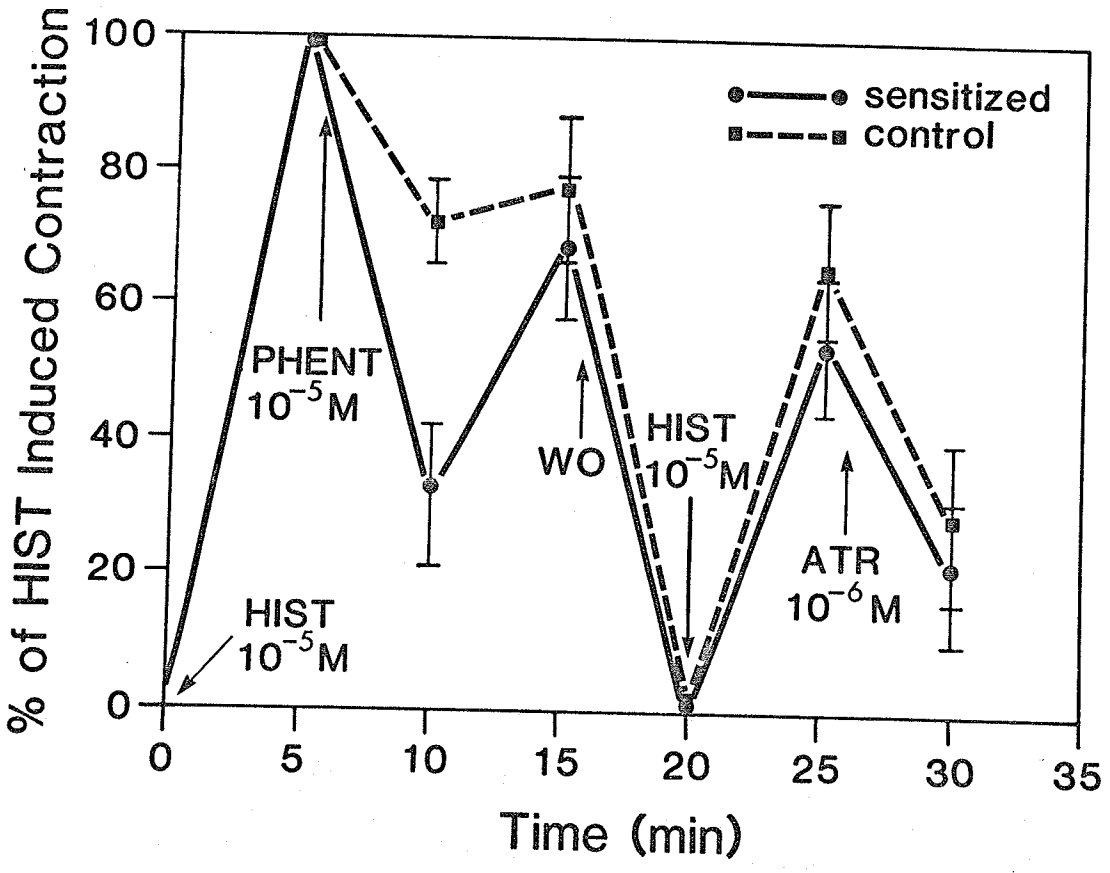


FIG. 27 Effect of atropine (ATR, 10^{-6}M) and phentolamine (PHENT, 10^{-5}M) on isometric tension developed by control (n=7) and OA sensitized (n=9) TSM contracted with 5-hydroxytryptamine (5-HT, 10^{-6}M).

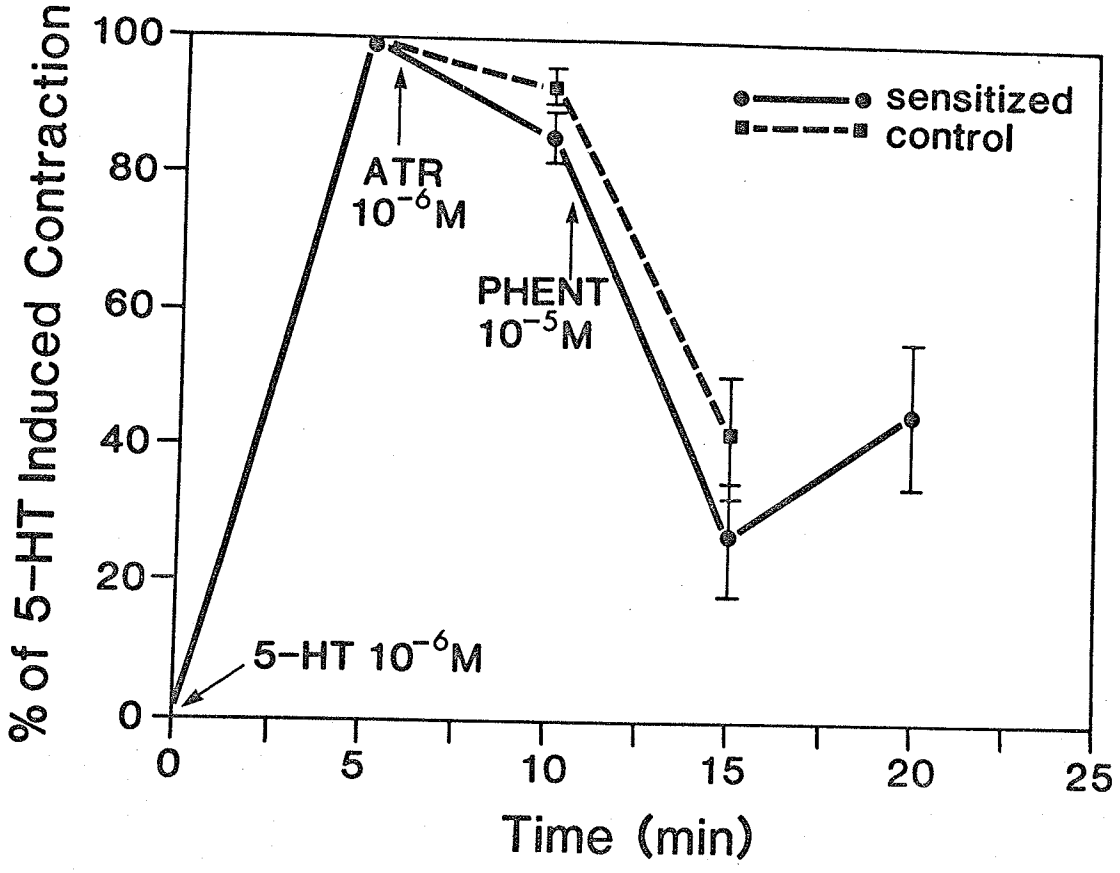


TABLE 3 Isometric tension (g/cm^2) stimulated in OA sensitized canine TSM (n=7) by 5-hydroxytyptamine (5-HT, $10^{-6}M$). Atropine (ATR, $10^{-6}M$) was added 5 minutes later. Muscles were pretreated (10 minutes prior) with phentolamine (PHENT, $10^{-5}M$).

	TREATMENT		
Muscle	PHENT $10^{-5}M$	5-HT $10^{-6}M$	ATR $10^{-6}M$
1	0 (0)	0 (100)	0 (100)
2	0 (0)	376 (100)	335 (89)
3	0 (0)	1078 (100)	1059 (98)
4	0 (0)	0 (100)	0 (100)
5	0 (0)	0 (100)	0 (100)
6	0 (0)	405 (100)	86 (21)
7	0 (0)	0 (100)	0 (100)
$\bar{X}$	0	266	211
SE	0	152	148
$\bar{X}$	(0)	(100)	(87)
SE	0	0	11

TABLE 4 Isometric tension (g/cm^2) stimulated by 5-hydroxytyptamine (5-HT $10^{-7}M$). After washout (WO) of the 5-HT the muscles were treated with eserine. Equillibration with eserine for 10 minutes was followed by sequential additions of 5-HT and propranolol (PROP. Values in brackets are percentages of the initial contraction (100%) stimulated by 5-HT in 5 muscles.

	TREATMENT			
Muscle	5-HT $10^{-7}M$	WO + ESERINE $10^{-6}M$	5-HT $10^{-7}M$	PROP $10^{-5}M$
1	984 (100)	0 (0)	2316 (235)	1920 (195)
2	522 (100)	0 (0)	1665 (319)	1478 (283)
3	340 (100)	0 (0)	578 (170)	367 (108)
4	674 (100)	0 (0)	816 (121)	497 (73)
5	651 (100)	0 (0)	2460 (378)	2406 (369)
X	634	0	1567	1334
SE	96	0	348	362
X	(100)	(0)	(245)	206
SE	0	0	43	50

Data obtained in tissues from OA-sensitized dogs showed both qualitative and quantitative similarity to controls. Figures 26 and 27 demonstrate that in histamine (10^{-5}M)- or 5-HT (10^{-6}M)-stimulated muscles the actions of phentolamine (10^{-5}M) and atropine (10^{-6}M) were similar for sensitized and control groups with the exception that a greater transient inhibitory action of phentolamine (10^{-5}M) on a histamine (10^{-5}M)-induced contraction in sensitized TSM was observed. Ten minutes following phentolamine exposure the stable plateau values for sensitized and control tissues were significantly different (Fig. 26).

In sensitized TSM pretreated with phentolamine (10^{-5}M) only 3 out of 7 muscles responded to the subsequent addition of 5-HT (10^{-6}M, Table 3). Although the mean contractile response (266 $\pm$ 152 g/cm^2) was not significantly different from the control response of 245 $\pm$ 66 g/cm^2 (Table 25 appendix), all the control muscles (n=8) contracted in response to 5-HT (10^{-5}M) exposure after phentolamine pretreatment.

E) Effects of Eserine, Propranolol and Phentolamine on the Contractile Responses to Histamine, 5-hydroxytryptamine, Acetylcholine and Potassium in Canine TSM <u>in vitro</u>

Further studies focussed on the possibility that 5-HT and histamine interact with cholinergic or adrenergically mediated responses. Eserine (physostigmine, 10^{-6}M) significantly potentiated contractions produced by 5-HT (10^{-7}M) to a mean of 245 $\pm$ 34% of the initial 5-HT (10^{-7}M)-induced contraction. Propranolol (10^{-5}M) reduced the tension to 206 $\pm$ 50% (Table 4). In eserine-pretreated muscles the response to histamine (10^{-6}M) rose to 703 $\pm$ 174% of the value produced by histamine alone (100%). Addition of hyoscyamine (10^{-6}M) reduced the response to histamine in the presence of eserine to 41 $\pm$ 32% of the initial histamine-induced contraction (Table 5).

Although atropine and hyoscyamine partially inhibit histamine-induced (10^{-6}M) contractions, histamine at higher concentration (10^{-4}M) is a potent stimulator of depolarized TSM, which has been blocked with atropine, propranolol and phentolamine. Histamine (10^{-4}M) under these pretreatment conditions produced a contraction of 280 $\pm$ 16% of that in the presence of K^+, 127 mM (Table 6).

In order to test for the specificity of eserine action on cholinergically mediated responses, muscarinic blockade with hysocyamine was used. The addition of eserine at the plateau of an acetylcholine-induced contraction produced an increase of 213 $\pm$ 18%, over the initial response (100%); the subsequent addition of hyoscyamine (10^{-5}M) abolished the response (Table 7). Phentolamine (10^{-5}M) reduced acetylcholine (10^{-8}M)-induced contraction (100%) to 57 $\pm$ 3% and the further addition of atropine (10^{-7}M) abolished the contraction completely (Table 8).

Potassium (K^+, 23 mM) induced contractions were also increased dramatically by eserine (10^{-6}M) and occurred to the same degree whether eserine was applied at the plateau of the K^+ induced contraction (Table 9) or prior to K^+ addition (Table 10). Although phentolamine (10^{-5}M) slightly reduced (Table 9) and propranolol (10^{-5}M) slightly augmented (Table 10) the tension stimulated by 23 mM K^+ in the presence of eserine, neither effect was significant ($p < 0.05$).

F) Factors Which May Modulate [^{3}H] Norepinephrine Overflow in Canine TSM *in vitro*

The previous studies demonstrate that phentolamine significantly reduced isometric tension developed by TSM in the presence of electrical field stimulation, (Fig. 2), tyramine (Fig. 8 and 21), histamine (Fig.

TABLE 5 Effect of eserine (10^{-6}M) on isometric tension (g/cm^2) produced by TSM strips following addition of histamine (10^{-6}M) before and after eserine treatment. Following washout (WO) and a second histamine-induced contraction muscles were exposed to hyoscyamine (10^{-6}M). An interval of 10 minutes was allowed between addition of drugs. Values in brackets are percentages of initial contraction (100%) developed following addition of histamine for 5 muscles.

	TREATMENT			
Muscle	HIST 10^{-6}M	WO + ESERINE 10^{-6}M	HIST 10^{-6}M	HYO 10^{-6}M
1	164 (100)	0 (0)	1875 (1142)	0 (0)
2	282 (100)	0 (0)	1490 (528)	0 (0)
3	82 (100)	0 (0)	573 (700)	136 (167)
4	163 (100)	0 (0)	225 (156)	41 (25)
5	221 (100)	0 (0)	2185 (990)	33 (15)
$\overline{X}$	182	0	1276	42
SE	33	0	372	25
$\overline{X}$	(100)	(0)	(703)	(41)
SE	0	0	174	32

TABLE 6 Effect of histamine (10^{-4}M) on isometric tension (g/cm^2) developed by TSM pretreated with atropine (10^{-7}M), propranolol (10^{-5}M) phentolamine (10^{-5}M) and contracted with exposure to 127 mM K^+. Values in brackets are percentages of contraction produced by 127 mM K^+ (100%) for 4 muscles.

	TREATMENT		
Muscle	Pretreat With	127mMK^+	Histamine 10^{-4}M
1	ATR 10^{-7}M	561 (100)	1532 (273)
2	PROP 10^{-5}M	417 (100)	1030 (246)
3	PHENT 10^{-5}M	446 (100)	1233 (276)
4		481 (100)	1563 (324)
$\overline{X}$		476	1339
SE		31	127
$\overline{X}$		(100)	(280)
SE		0	16

TABLE 7 Effect of eserine(10^{-6}M) and hyoscyamine (10^{-5}M) on isometric tension (g/cm^2) developed by TSM following exposure to acetylcholine (ACh 5 x 10^{-8}M). Values in brackets are percentages of initial contraction (100%) developed following exposure to ACh for 5 muscles.

	TREATMENT		
Muscle	ACH $5x10^{-8}$M	ESERINE 10^{-6}M	HYO 10^{-5}M
1	612 (100)	1645 (268)	0 (0)
2	1047 (100)	2537 (242)	0 (0)
3	1384 (100)	2500 (180)	0 (0)
4	1000 (100)	2018 (201)	0 (0)
5	483 (100)	843 (174)	0 (0)
$\overline{X}$	905	1909	0
SE	162	313	0
$\overline{X}$	(100)	(213)	0
SE	0	18	(0)

TABLE 8 Effect of phentolamine (10^{-5}M) and atropine (10^{-7}M) on isometric tension (g/cm^2) developed by TSM following exposure to acetylcholine (ACh 10^{-8}M). Values in brackets are percentages of initial contraction (100%) developed following exposure to ACh. (m=4).

	TREATMENT		
Muscle	ACH 10^{-8}M	PHENT 10^{-5}M	ATR 10^{-7}M
1	362	241	0
	(100)	(66)	(0)
2	201	105	0
	(100)	(52)	(0)
3	178	103	0
	(100)	(57)	(0)
4	198	108	0
	(100)	(54)	(0)
$\overline{X}$	235	139	0
SE	43	34	0
$\overline{X}$	(100)	(57)	0
SE	0	3	0

TABLE 9 Effect of eserine (10^{-6}M) and phentolamine (10^{-5}M) on isometric tension (g/cm^2) developed by TSM following active tension stimulated by K^+ 23 mM exposure. Values in brackets are percentages of initial contraction (100%) developed upon exposure to K^+. (n=5).

	TREATMENT		
Muscle	K+ 23mM	ESERINE 10^{-6}M	PHENT 10^{-5}M
1	558 (100)	949 (170)	692 (124)
2	609 (100)	1541 (253)	1182 (194)
3	588 (100)	712 (121)	712 (121)
4	86 (100)	1698 (1975)	1398 (1625)
5	279 (100)	1675 (600)	1454 (521)
$\overline{X}$	424	1315	1088
SE	104	203	164
$\overline{X}$	(100)	(624)	(517)
SE	0	348	287

TABLE 10 Effect of eserine (10^{-6}M) pretreatment and propranolol (10^{-5}M) on isometric tension (g/cm^2) developed by TSM following active tension produced by exposure to 23 mM K^+. Values in brackets are percentages of initial contraction (100%) developed upon exposure to K^+ prior to washout (WO).

	TREATMENT			
Muscle	23mMK^+	ESERINE 10^{-6}M+WO	23mMK^+	PROP 10^{-5}M
1	2069 (100)	0 (0)	3251 (157)	3251 (157)
2	452 (100)	0 (0)	1142 (253)	1142 (253)
3	561 (100)	0 (0)	656 (117)	916 (163)
4	108 (100)	0 (0)	1935 (1800)	1935 (1800)
5	952 (100)	0 (0)	2486 (261)	2605 (273)
$\overline{X}$	828	0	1894	1969
SE	338	0	463	438
$\overline{X}$	(100)	(0)	(518)	(529)
SE	0	0	322	319

22), serotonin (Fig. 25) acetylcholine (Table 8) and potassium in the presence of eserine, (although not significant, 4 of the 5 muscles responded with a decrease in tension, Table 9). The possibility that the above agonists act on adrenergic neural elements to increase norepinephrine release was studied by incubating muscles with [^{3}H]-norepinephrine and stimulating the preparation with electrical field stimulation (EFS) to promote NE turnover in nerves (see methods). Following the period of [^{3}H]- norepinephrine incubation, cocaine (10^{-7}M) was added to the bath to prevent NE neural uptake (Uptake 1) and was subsequently added to the bath following each sample collection throughout the experiment. Following a period of washout (24 minutes) an agonist was introduced for a 3-minute collection interval after which the muscle was exposed to Krebs-Henseleit solution alone for the duration of the experiment. Isometric tension was recorded simultaneously throughout the experiment. All of the studies involving labelled transmitter release were similar in their sampling of radioactivity overflow and all comparisons were made for the sample during which treatment was given (i.e. interval between 24 and 27 min.). Significant changes from the expected control value were determined for the values at time 27 minutes. Efflux curves (of rate coefficient) for unstimulated muscles demonstrate that the calculated expected values are an accurate representation of what actually occurs for control muscles (Fig. 28). The upper panel (Fig. 28) also indicates a stable resting tone for the entire period.

Electrical field stimulation (EFS) which is known to stimulate nerves, as seen by the sensitivity of EFS-induced tension responses to

tetrodotoxin (Fig. 2), was observed to increase active tension and tritium overflow significantly ($p < 0.05$). Histamine (10^{-5}M) which increased active tension did not change tritium overflow compared with the expected control values (Fig. 29). The change in rate coefficient was therefore not an artefact of tension-dependent alterations of the exchange kinetics in the extracellular space.

A high-potassium (23 mM) solution increased tension development to 669 ± 85 g/cm^2, which was accompanied by a significant increase in rate coefficient to 2.34 ± 0.45 (Fig. 30). Both K^+ and EFS depolarize nerves, although by different methods, and were expected to increase tritium overflow. Phentolamine (10^{-5}M) however produced a significant ($p < 0.05$) increase in rate coefficient in the absence of any mechanical effects (Fig. 31). Norepinephrine (10^{-6}M) also produced a significant increase in tritium overflow without active tension development (Fig. 32). Acetylcholine (10^{-8}M) produced a contraction which was partially phentolamine-sensitive (Table 8), and ACh 10^{-6}M was observed to increase [^{3}H] norepinephrine overflow significantly (Fig. 33). Previous experiments demonstrated a marked sensitivity of 5-HT (10^{-6}M)-induced contractions (Fig. 25) to phentolamine, while the present results (Fig. 34) do not demonstrate a significant increase in tritium overflow accompanying the development of isometric tension.

Figure 35 summarizes the results for overflow of tritium and compares each with the control value expected. A significant ($p < 0.05$) overflow of tritium was produced in TSM following exposure to EFS, phentolamine, K^+ 23 mM, acetylcholine and norepinephrine. Both histamine and 5-HT were without significant effect. Although significant

FIG. 28 Effect of time alone on active tension (upper panel) and rate coefficient of efflux (lower panel) in TSM (n=8) previously incubated with [^{3}H]-norepinephrine. The dashed line represents the means and standard errors for 8 control strips of TSM compared with the rate coefficient and 95% confidence limits calculated from all the TSM strips (n=44) in this experiment. For calculation of rate coefficient, see methods.

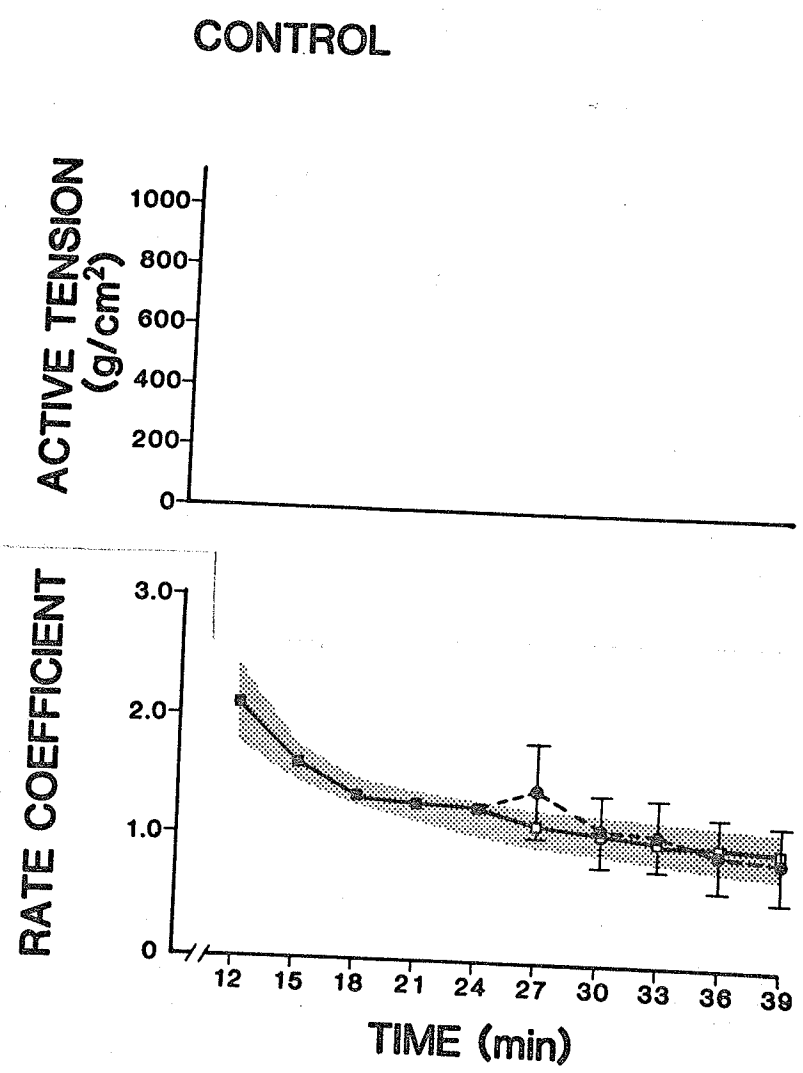


FIG. 29 Effect of histamine (HIST 10^{-5}M) and electrical field stimulation (EFS, 15V, 60HZ, AC, 3 minute duration) on tension (upper graph) and rate coefficient of efflux (lower graph) for TSM (n=8) previously incubated with [^{3}H] norepinephrine. Efflux curve of the rate coefficient and 95% confidence limits were extrapolated by obtaining expected values indicated by empty squares. Solid symbols indicate means with accompanying standard errors. Significant ($p < 0.05$) differences in rate coefficient of treatment response at time 27 minute versus expected value are indicated by asterisk *.

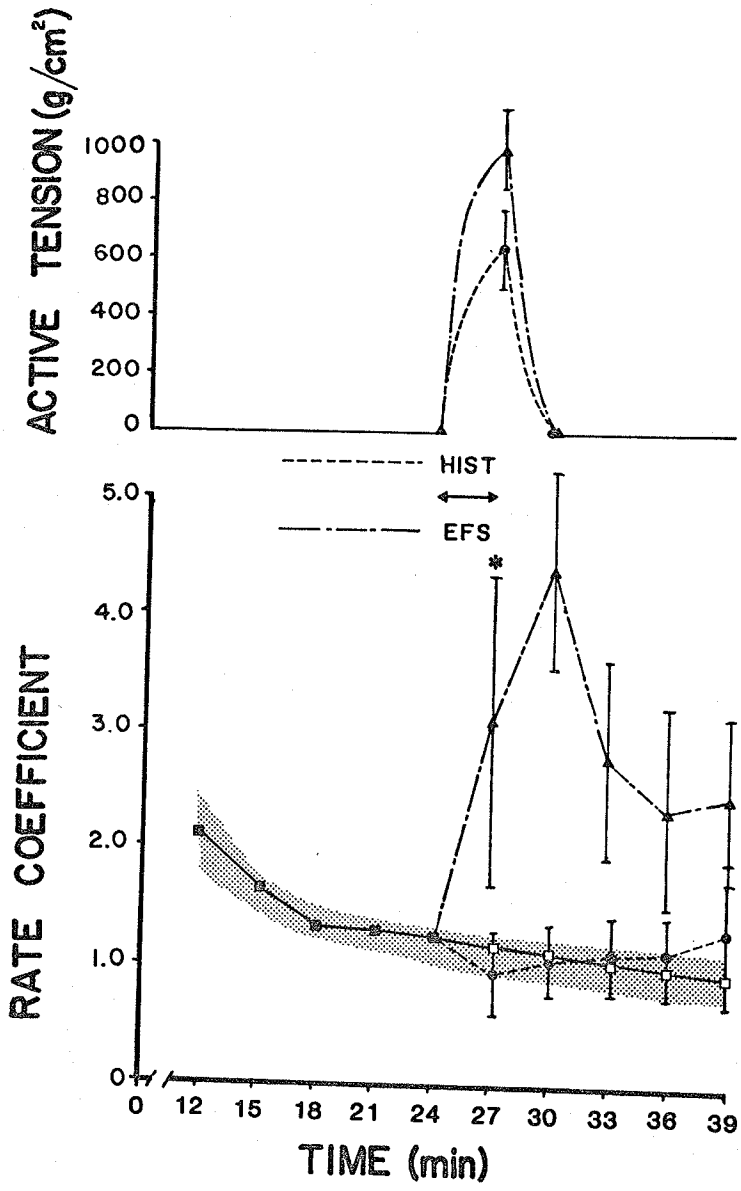


FIG. 30 Effect of 23 mM K^+ on active tension (upper graph) and rate coefficient (lower graph) for TSM (n=5), previously incubated with [^{3}H]-norepinephrine. Means, standard errors, 95% confidence limits of efflux curve expected values and significance ($p < 0.05$) are as for Fig. 28.

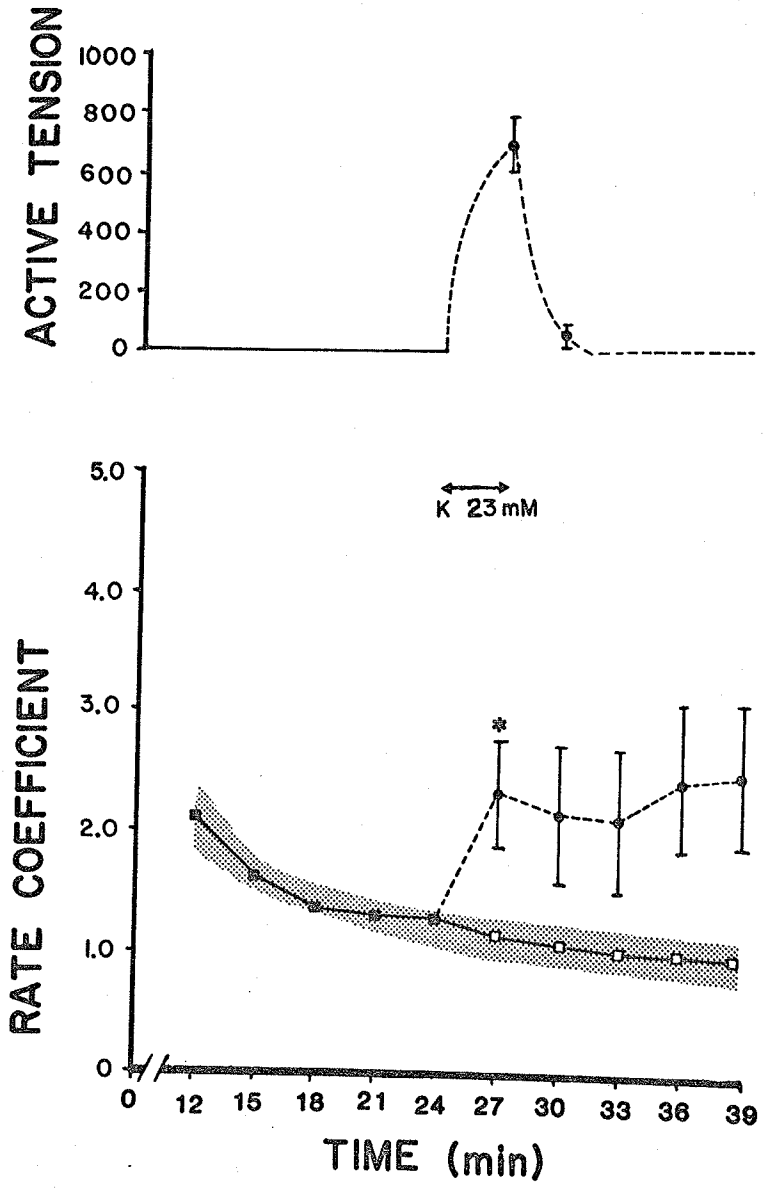


FIG. 31 Effect of phentolamine (PHENT 10^{-5}M) on active tension (upper graph) and rate coefficient (lower panel) for TSM (n=5), previously incubated with [^{3}H]-norepinephrine. Significance ($p < 0.05$) and other details are as for Fig. 28.

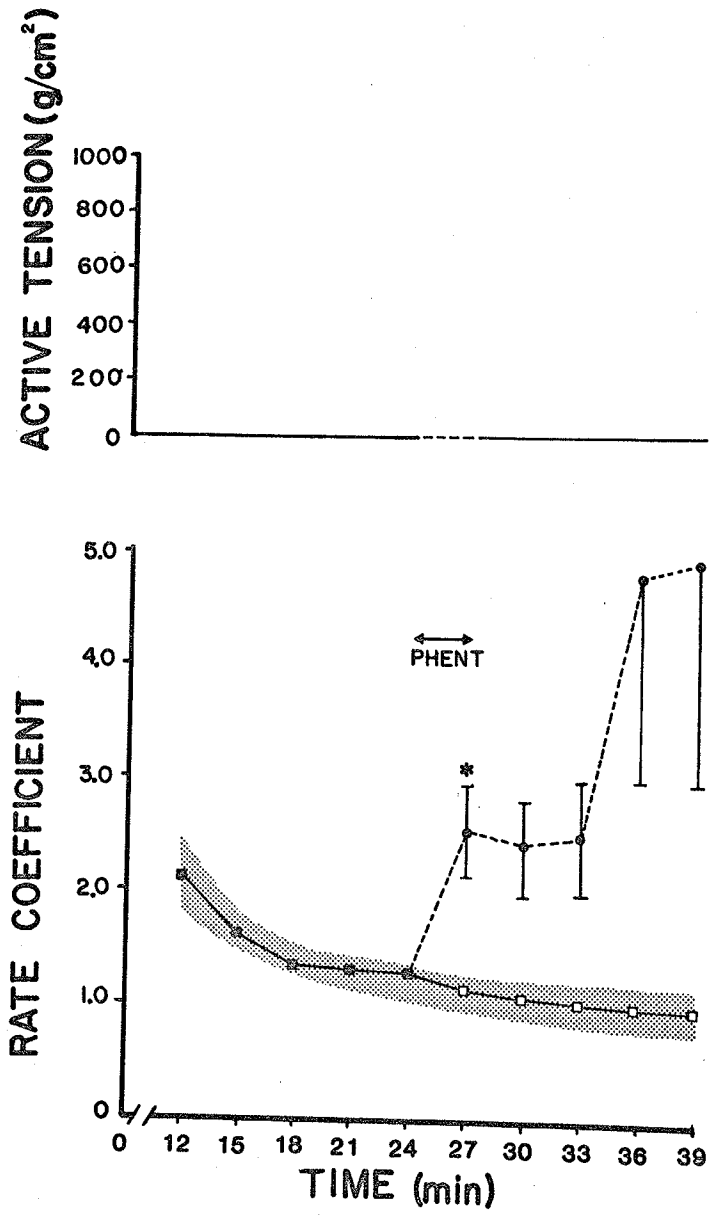


FIG. 32 Effect of norepinephrine (NE 10^{-6}M) on active tension and rate coefficient for TSM (n=5). Muscles were previously incubated with [^{3}H]-norepinephrine as in Fig. 28.

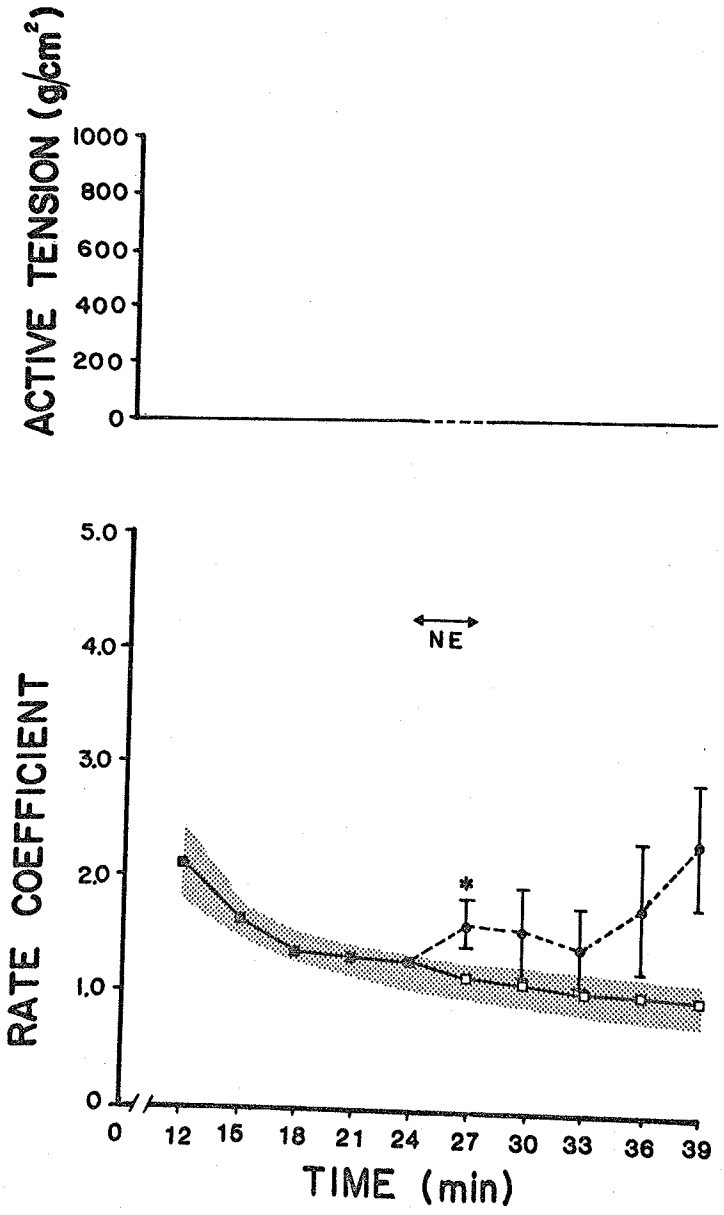


FIG. 33 Effect of acetylcholine (ACh, 10^{-6}M) on active tension (upper graph) and rate coefficient (lower panel) for TSM (n=5), previously incubated with [^{3}H]-norepinephrine. Significance ($p < 0.05$) and other details are as for Fig. 28.

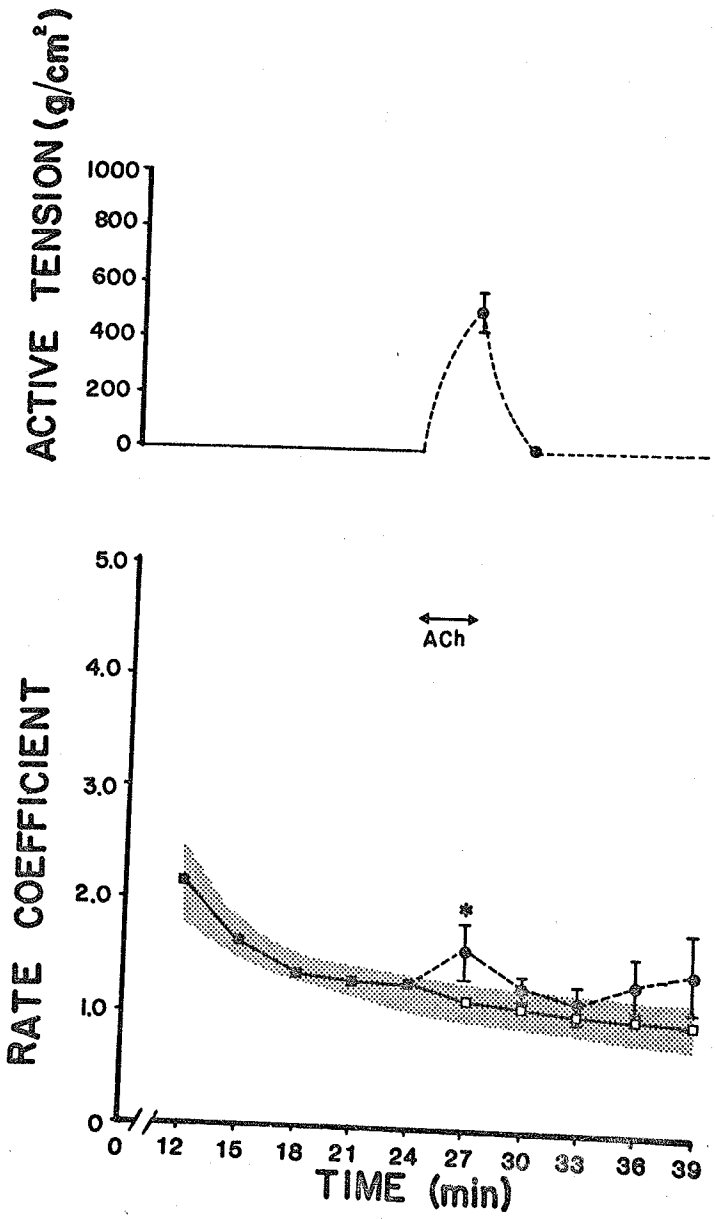
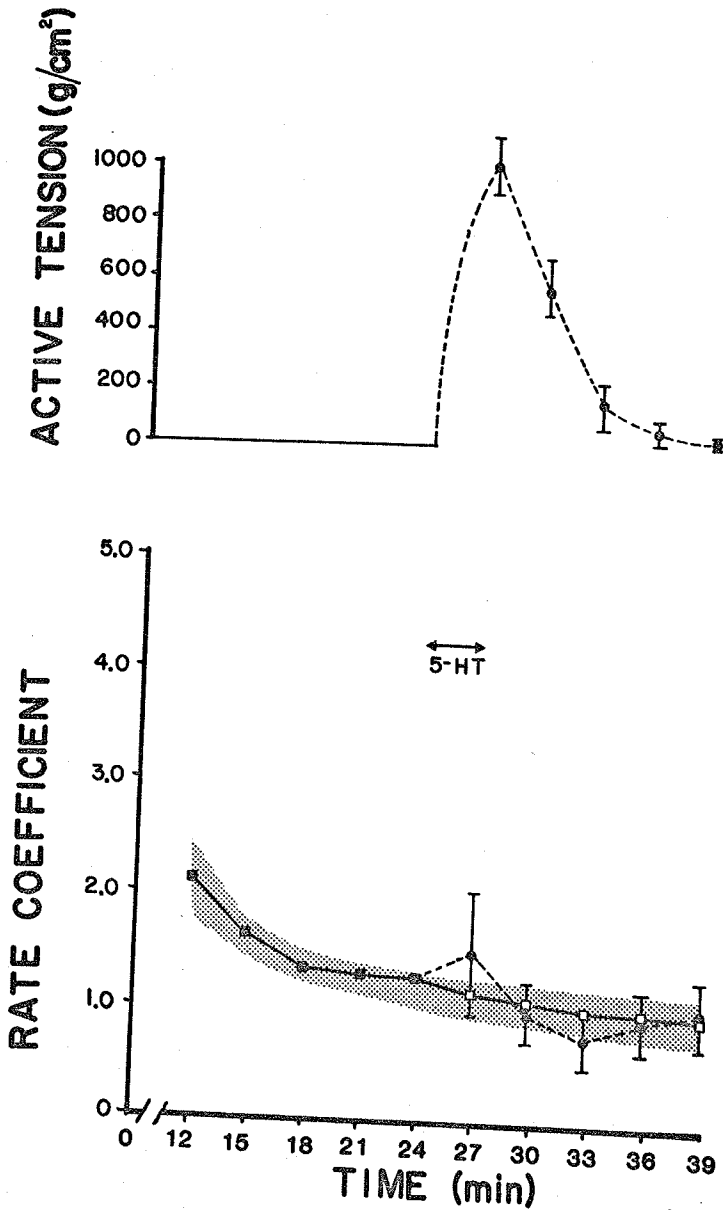


FIG. 34 Effect of 5-hydroxytryptamine (5-HT, 10^{-6}M) on active tension and rate coefficient for TSM (n=8). Muscles were previously incubated with [^{3}H]-norepinephrine as in Fig. 28.



change in rate coefficient was not observed it was noted that 6 of 8 muscles demonstrated an increase in rate coefficient following the addition of 5-HT (10^{-6}M). Since the method used is not intended for quantitative estimation of transmiter release but rather to indicate qualitative changes, the present data suggest that 5-HT (10^{-6}M) may also promote tritium overflow.

In a separate study, the effect of tyramine (10^{-4}M) was observed to be without significant ($p > 0.05$) effect on tritium efflux or active tension development in canine TSM (n=7). The experiments were carried out in the presence of TTX (10^{-6}M) and cocaine (10^{-7}M); the latter may account for this negative result.

G) Neuromodulating Influence of Several Factors on the Cholinergic Nervous System in Canine TSM <u>in vitro</u>

Experiments were carried out to determine the effects of 5-HT (10^{-6}M), K^+ (23 mM), EFS (15V, 60 HZ, AC, 3 minute duration), histamine (10^{-5}M), and norepinephrine (10^{-7} and 10^{-4}M) on the efflux of ^{14}C- choline. Following an incubation period to load the nerves with radioactivity, a washout curve was obtained similar to the above procedure for [^3H]-norepinephrine studies. The first set of experiments described was carried out without eserine or TTX present.

Figure 36 illustrates that a stable basal resting tension in untreated muscles was maintained for the duration of the experiment and that the actual experimental values for the rate coefficient were similar to the values predicted by extrapolation. Electrical field stimulation increased tension and ^{14}C-efflux significantly ($p < 0.05$) while

FIG. 35 Effect of various experimental conditions on the efflux of radioactivity from ^{3}H-NE equilibrated TSM. Rate coefficients at time 27 minutes are compared with expected calculated control value (H). Significant ($p < 0.05$) differences from control are indicated by asterisk, and were determined by students t-test. Stimuli used are as follows; electrical field stimulation (EFS, 15 V, 60 HZ, AC, 3 minute duration), phentolamine (PHENT, 10^{-5}M), K^{+} (23mM), acetylcholine (ACh, 10^{-6}M), norepinephrine (NE, 10^{-6} M), 5-hydroxytryptamine (5-HT, 10^{-6} M) and histamine (HIST, 10^{-5} M).

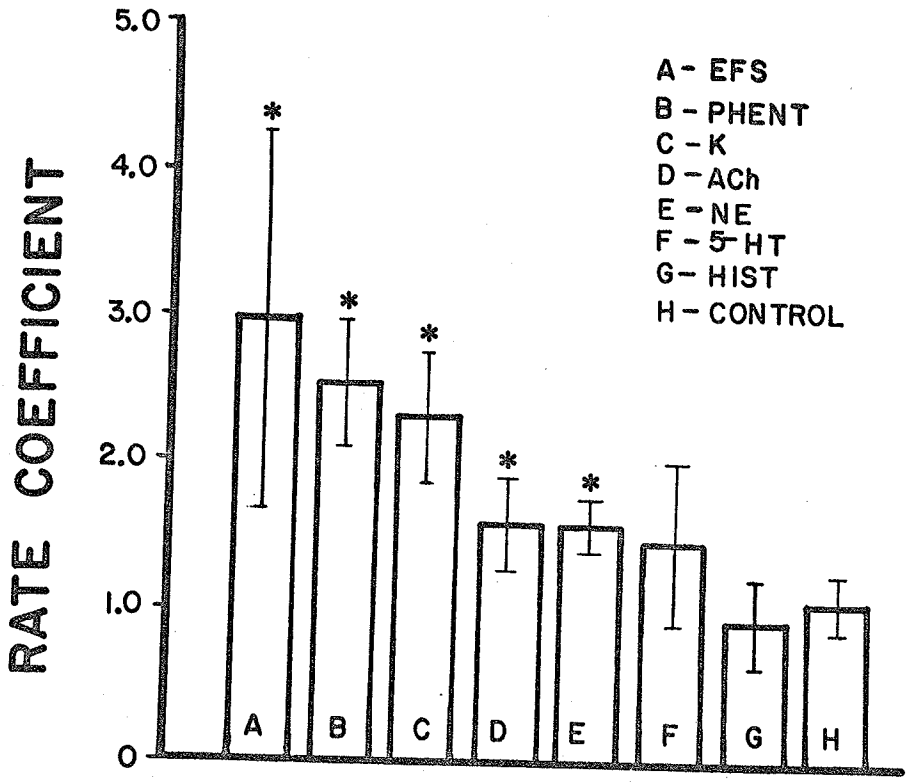
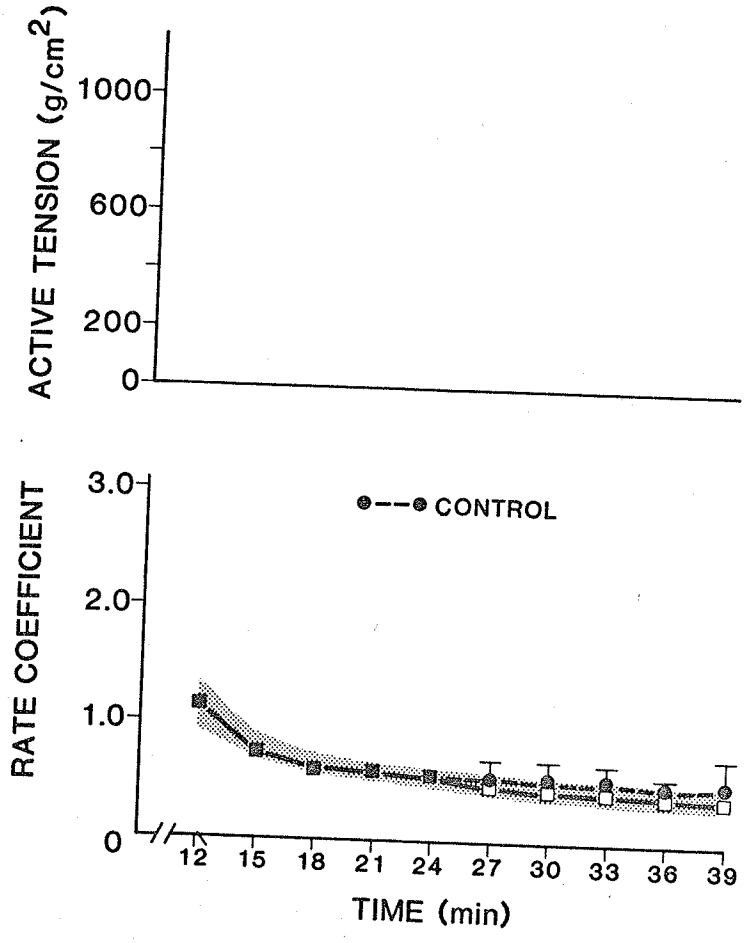


FIG. 36 Effect of time on active tension (upper graph) and efflux of radio- activity into the bathing solution (expressed as a rate coefficient; lower graph) in TSM (n=6), previously incubated with [^{14}C]-choline. The rate coefficient and 95% confidence limits are shown by shaded area, and expected values by empty squares. Solid symbols indicate observed means with standard errors.



histamine stimulated isometric tension development without changing the rate coefficient of ^{14}C-efflux, thus demonstrating, as for tritiated norepinephrine, that contraction alone does not stimulate or increase acetylcholine release (Fig. 37). A similar result was found when high-potassium and 5-HT were tested. Figure 38 illustrates the significant increase of isometric tension and ^{14}C-efflux accompanying a K^+-induced contracture and the contractile effect of 5-HT without a concomitant change in rate coefficient.

Norepinephrine, for which indirect evidence suggests an inhibitory effect on acetylcholine release (Vermiere and Vanhoutte, 1979) was tested at two concentrations in the present studies. Figure 39 demonstrates that both concentrations of norepinephrine (10^{-7} and 10^{-4}M) were without significant effect ($p > 0.05$) on resting tension or ^{14}Cefflux.

The second and third types of experiments on factors which may modify acetylcholine efflux were carried out in the presence of eserine (10^{-6}M), an acetylcholinesterase inhibitor, or with eserine (10^{-6}M) and tetrodotoxin (10^{-6}M) throughout the experiment. The anti-acetylcholinesterase effect of eserine was expected to enhance the stimulated overflow of acetylcholine by various substances. TTX was used to inhibit ganglion-initiated action potentials in nerves. When eserine was present, histamine significantly ($p < 0.05$) increased ^{14}C-efflux and this effect was eliminated by TTX (Fig. 40). In the previous experiment (Fig. 37) no increase of ^{14}C overflow was observed.

The effect of 5-HT on ^{14}C-efflux was similar to that of histamine but the amount of the increase (6 of the 8 muscles increased

FIG. 37 Effect of electrical field stimulation (EFS, 15V, 60HZ, AC, 3 minute duration) and histamine (HIST 10^{-5}M) on active tension of (upper graph) and rate coefficient from (lower graph) TSM (n=6). Confidence limits and expected values are as for Fig. 36. Significant ($p < 0.05$) change in efflux is indicated (*) for the treatment interval 24-27 minutes.

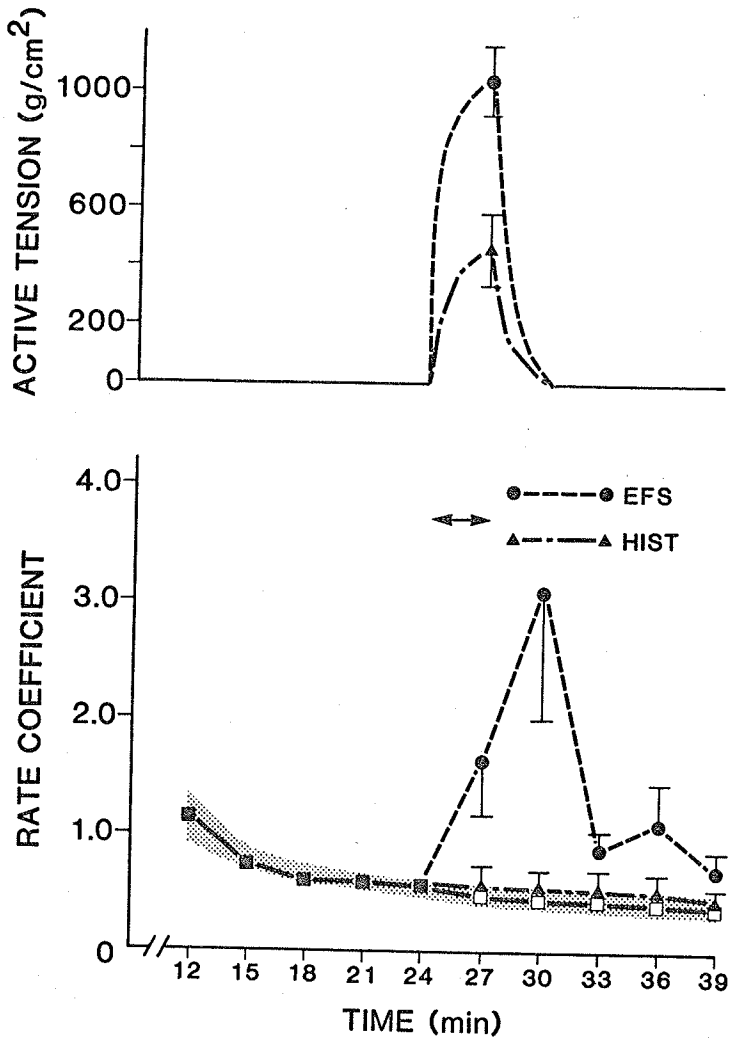


FIG. 38 Effect of 5-hydroxytryptamine (5-HT, 10^{-6}M) and K^+ (23 mM) on active tension (upper panel) and rate coefficient (lower panel) following incubation with [^{14}C] choline. Significant ($p < 0.05$) change for the treatment interval is shown by asterisk * n=6.

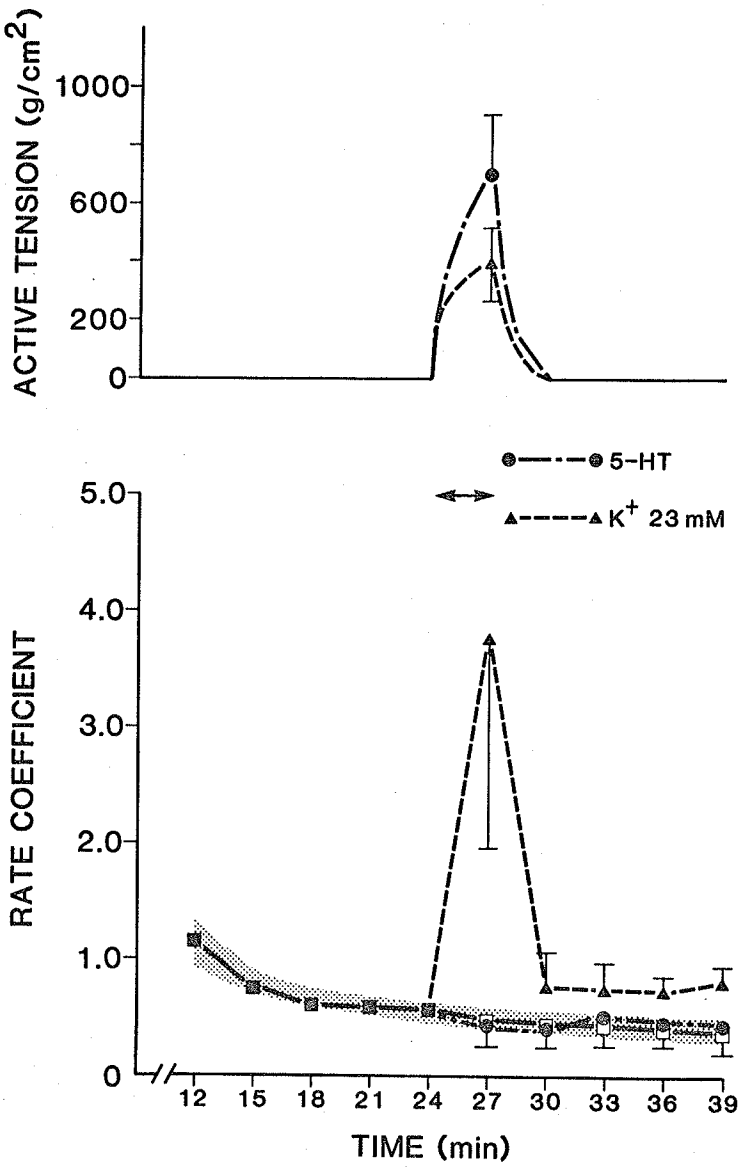


FIG. 39 Effect of norepinephrine (10^{-7} and 10^{-4}M) on active tension (upper panel) and rate coefficient (lower panel) of ^{14}C-efflux (n=6).

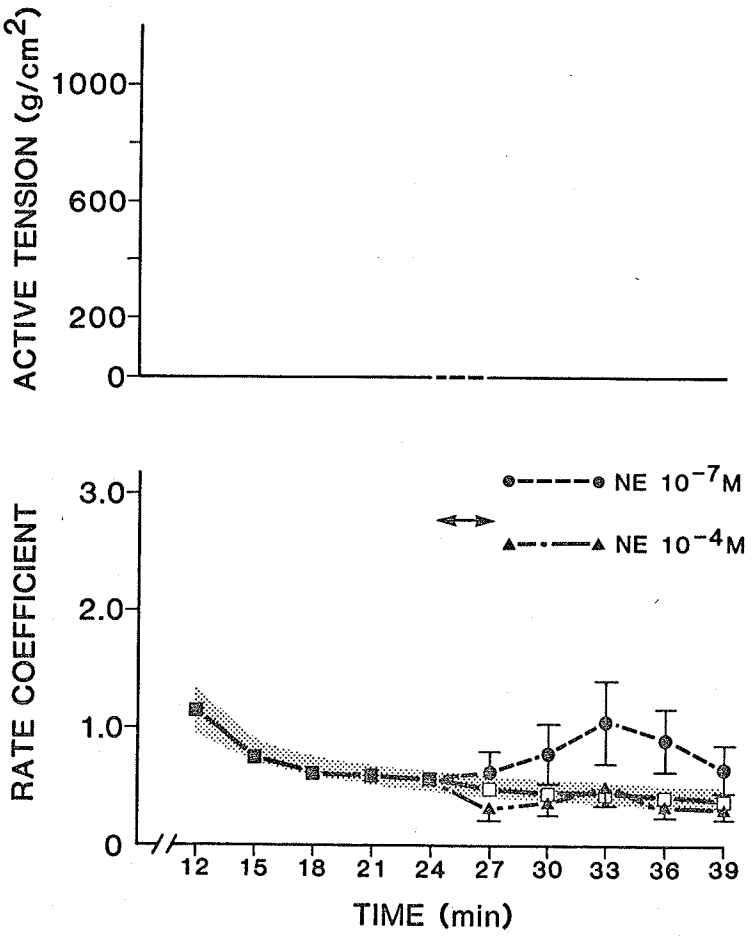
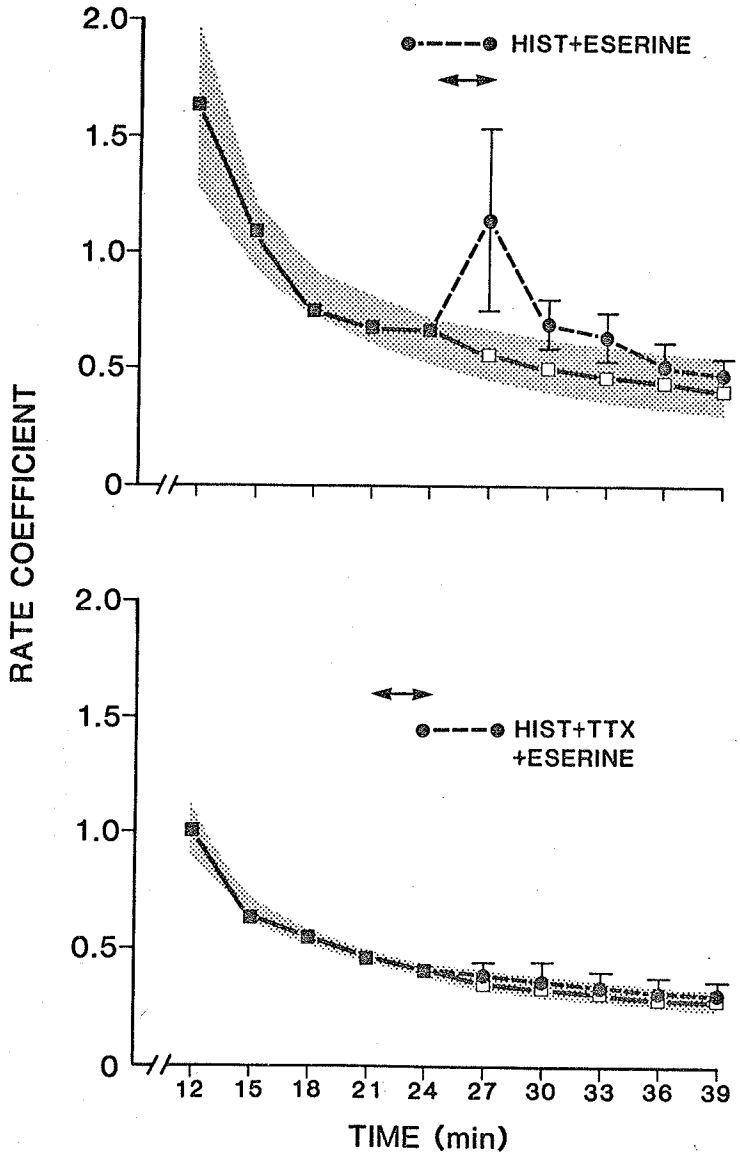


FIG. 40 Effect of histamine (HIST, 10^{-5}M) on the rate coefficient for [^{14}C]-acetylcholine efflux when eserine (10^{-6}M) was present throughout the experiment (upper panel) or when eserine (10^{-6}M) and tetrodotoxin (TTX, 10^{-6}M) were present throughout the experiment. Upper panel is based on n=8 and lower panel n=6. Significant change ($p < 0.05$) for treatment interval is indicated.



^{14}C-efflux after 5-HT) did not reach statistical significance ($p < 0.05$). The action of TTX was to inhibit the response entirely (Fig. 41).

In order to test for possible autoregulation of acetylcholine release in a resting state, both acetylcholine and atropine were tested in the presence of eserine and TTX. The results indicate that neither was effective in altering ^{14}C-efflux (Fig. 42).

Previously (Fig. 39) a trend for norepinephrine ($10^{-7}M$) to enhance ^{14}C-efflux was observed and this effect was enhanced, though non-significantly ($p > 0.05$) by eserine (Fig. 43 upper panel). A higher concentration of norepinephrine ($10^{-4}M$) was observed to depress the efflux of ^{14}C in the presence of eserine; when eserine and TTX were present throughout the experiment (Fig. 44), the effect was significant ($p < 0.05$). An attempt to demonstrate the relevance of this finding by using tyramine ($10^{-4}M$), an agent which releases norepinephrine from adrenergic nerves, was unsuccessful (Fig. 43, lower panel).

Since nerves are thought to release transmitter at a basal rate, two antagonists of norepinephrine actions were used. The effect of phentolamine, an alpha-adrenergic antagonist was without effect on ^{14}C overflow, whereas the beta-adrenoceptor antagonist propranolol depressed the efflux of ^{14}C significantly in the presence of eserine and TTX, (Fig. 45). This effect was similar to that observed for a high concentration ($10^{-4}M$) of norepinephrine (Fig. 44).

FIG. 41 Effect of 5-hydroxytryptamine (5-HT 10^{-6}M) on rate coefficient of ^{14}C efflux in the presence of eserine (n=8) throughout the experiment (upper panel) or when eserine (10^{-6}M) and TTX (10^{-6}M) were present throughout the experiment (lower panel; n=6).

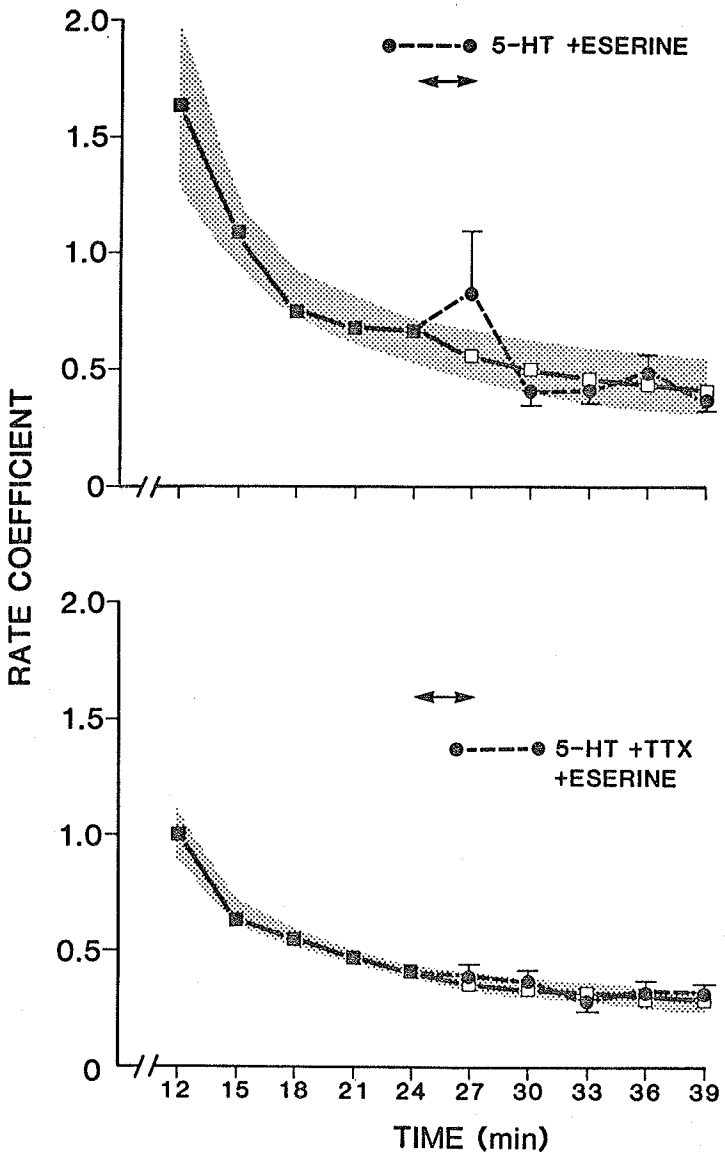
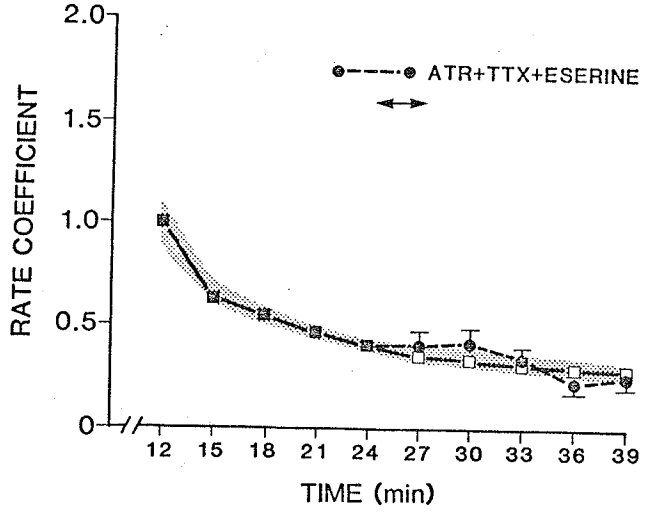


FIG. 42 Effect of atropine (ATR, 10^{-6}M upper panel) and acetylcholine (ACh, 10^{-6}M, lower panel) on the rate coefficient of ^{14}C-efflux when eserine (10^{-6}M) and TTX (10^{-6}M) were present throughout the experiment on canine TSM (n=6 for each).



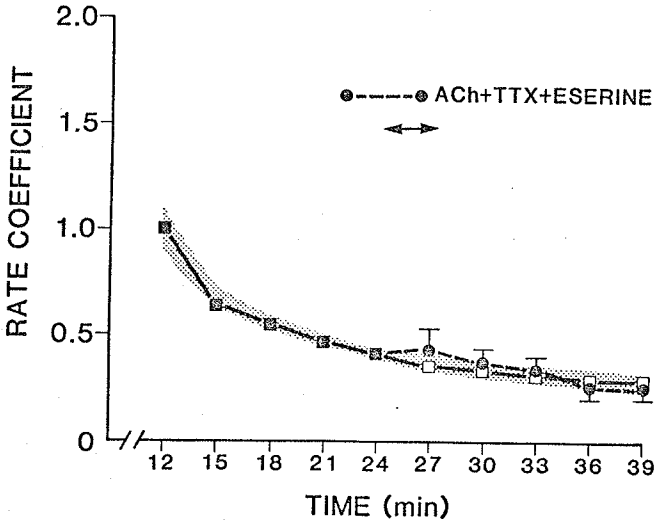


FIG. 43 Effect of norepinephrine (NE, 10^{-7}M) and tyramine (10^{-4}M) on the rate coefficient of ^{14}C-efflux when eserine was present throughout the experiment. The tyramine study also included TTX (10^{-6}M) throughout. N=6 for each experiment.

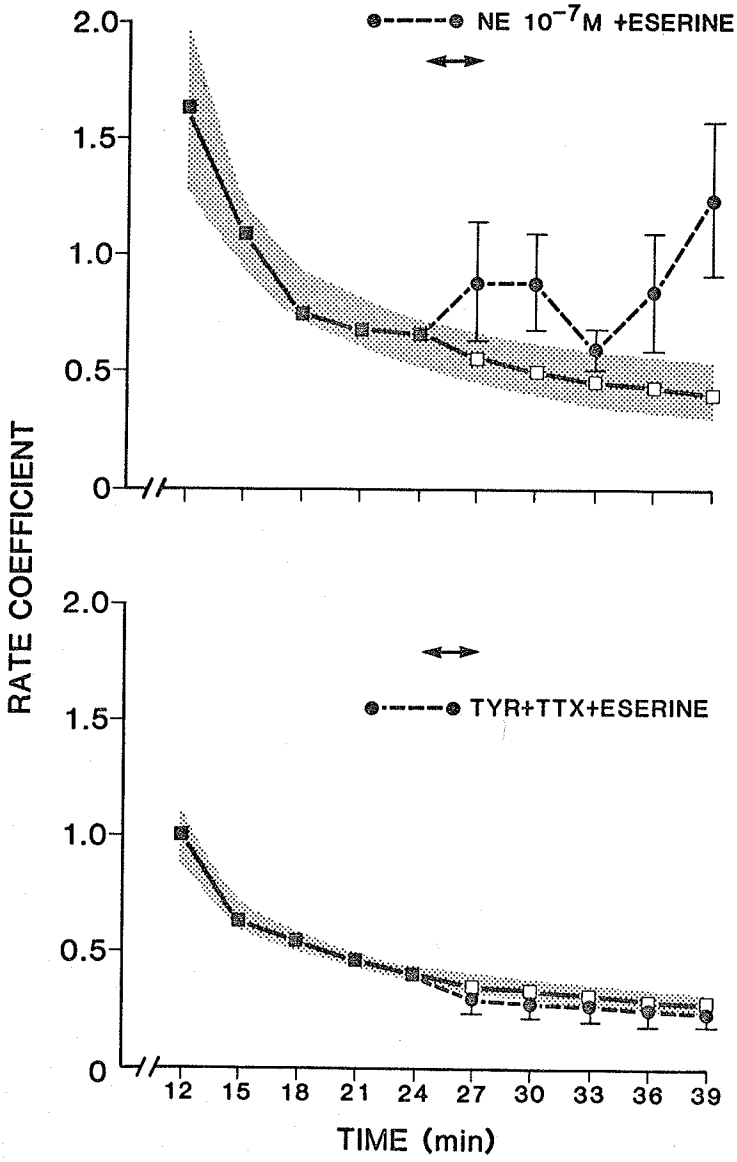


FIG. 44 Effect of norepinephrine (NE 10^{-4}M) on the rate coefficient of ^{14}C-efflux when eserine (10^{-6}M) was present throughout the experiment (upper panel) or when eserine (10^{-6}M) and TTX (10^{-6}M) were present throughout. N=6 for each study.

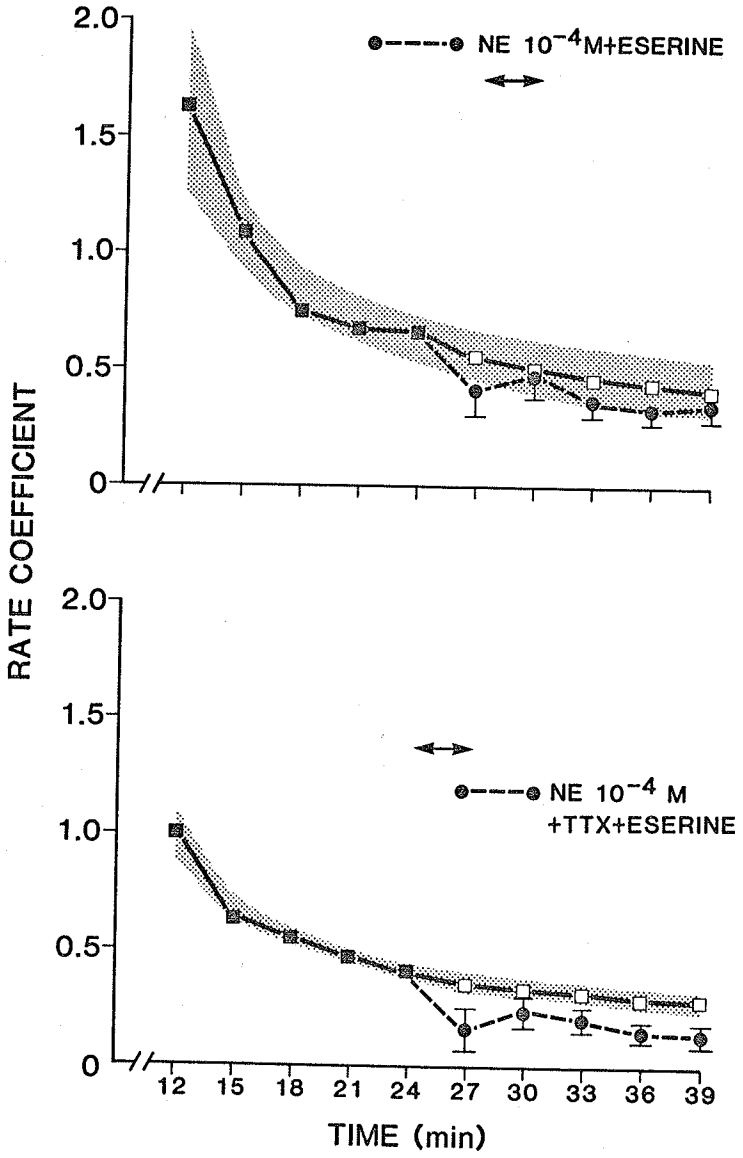
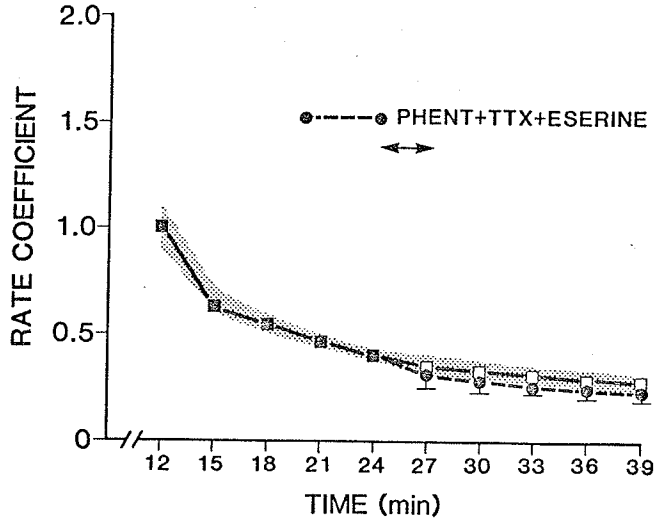
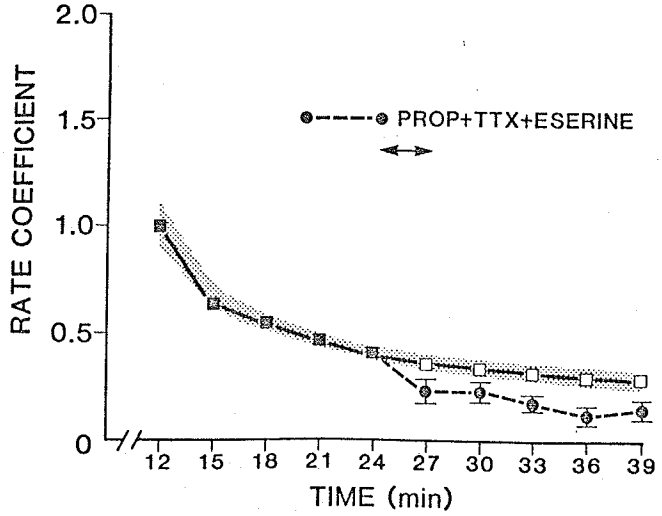


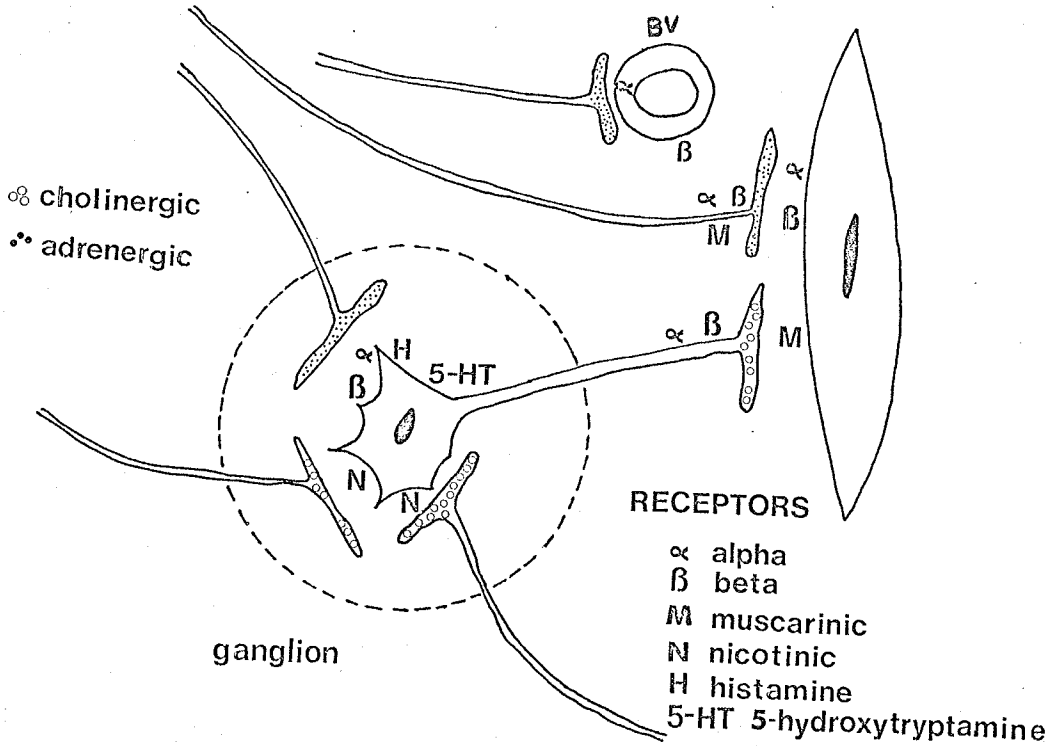
FIG. 45 Effect of phentolamine (PHENT 10^{-5}M, upper panel) and propranolol (PROP, 10^{-5}M, lower panel) on rate coefficient of ^{14}C-efflux when eserine (10^{-6}M) and TTX (10^{-6}M) were present throughout the experiment. N=6 for each experiment.





DISCUSSION

Fig. 46 Model of Innervation of Canine Tracheal Smooth Muscle. The following model of innervation of canine tracheal smooth muscle (represented by a single spindle shaped cell) an associated blood vessel (BV) and a ganglion may serve to provide a working framework for the discussion.



DISCUSSION

A) Electrical Field Stimulation and Isometric Tension Development

Electrical field stimulation of canine tracheal smooth muscle *in vitro* was used to demonstrate the stimulatory effect of nerves in the preparation. Previous studies on dog trachealis *in vitro* (Russell, 1978) and *in vivo* (Brown et. al., 1980; Brown, et. al., 1982) have shown the parasympathetic system to be the mediator of contractile responses, while the role of the adrenergic system is controversial (Nadel, 1980). Our studies indicate that EFS stimulates cholinergic nerves, the response (isometric tension) being enhanced by eserine and blocked by tetrodotoxin which has a selective action on nerves (Bolton, 1979; Narahashi, 1972), or hyoscyamine which blocks muscarinic receptors. Similar results were obtained by Colebatch and Halmagyi (1963) and Olsen, et. al. (1965) who demonstrated that atropine abolished isometric tension following EFS stimulation, thereby indicating that muscle stimulation occurs via postganglionic vagal fibres.

In vivo studies have shown a functional adrenergic neural innervation of canine trachealis and suggested that the major role was to promote relaxation (Russell, 1980; Brown et. al., 1980). Others (Beinfield and Seifter, 1980) have demonstrated an alpha-adrenoceptor mediated contractile response to low doses (3×10^{-9} to 2.4×10^{-8} mol/kg) of exogenous norepinephrine administered intravenously in the absence of beta-adrenoceptor blockade. However when Cabezas et. al., (1971) electrically stimulated the sympathetic nerves in vagotomized dogs no contractile activity was apparent and they interpreted these findings in terms

of an absence of alpha-adrenoceptors in canine airways. Our studies of TSM *in vitro* show a significant inhibition of the electrically stimulated isometric tension by the alpha-adrenoceptor antagonist phentolamine. When a beta-adrenoceptor antagonist propranolol was used isometric tension was reduced in all muscle strips. This unexpected decline may be due to the anesthetic action of high concentrations (> 10^{-5}M) of propranolol. The addition of norepinephrine (10^{-6}M) was without effect on electrically stimulated tension development whereas a higher concentration (10^{-4}M) non-significantly reduced tension in all muscle strips. An attempt to block the relaxant effects of norepinephrine with propranolol resulted in a further reduction in isometric tension. We attribute this unexpected decline to the possible anesthetic action of propranolol (10^{-5}M).

Conclusions from these studies are that canine TSM is predominantly innervated by cholinergic fibers, which when stimulated electrically mediate contractile responses by releasing acetylcholine which acts on post-junctional muscarinic receptors. Adrenergic fibers are also stimulated by EFS and the resulting release of norepinephrine may act on post-junctional alpha- or beta-adrenoceptors to mediate contractile (phentolamine sensitive) or relaxant (propranolol sensitive) responses, respectively. It is likely that an alpha-adrenoceptor-mediated contraction, following treatment with hyoscyamine, is not observed with EFS due to its dependance on development of adequate active tone prior to stimulation. The small amount of EFS-induced tone remaining following hyoscyamine may be due to stimulation of muscle directly or another, as yet unidentified, phentolamine-insensitive stimulatory system.

B) Effect of Tone on Adrenoceptor Mediated Responses in Canine TSM *in vitro*

The present studies investigated the effect of tonus on adrenoceptor-mediated responses in TSM. While controversy surrounds a functional role of the adrenergic system in human airways (Stone et. al., 1973) it is generally agreed that beta-adrenoceptors have a functional role postjunctionally in both human (Jack, 1973; Fleisch, 1980) and canine airway smooth muscle (Beinfield and Seifter, 1980; Bergen and Kroeger, 1979). The existence of alpha-adrenoceptors mediating contractile responses is less clear with some studies supporting their presence (Beinfield and Seifter, 1980; Kneussl and Richardson, 1978) and others finding no evidence for their presence in airway smooth muscle (Foster, 1966; Stone et. al., 1973; Cabezas, et. al., 1971). The reasons for this discrepancy may be attributed to differences in the tonus of the respective muscles at the time the alpha-adrenoceptor agonists were applied. Several recent studies (Bergen and Kroeger, 1980; Kneussl and Richardson, 1978; Ohno et. al., 1981) have suggested that for canine TSM *in vitro* tone developed prior to alpha-adrenoceptor stimulation is required to demonstrate a response.

Norepinephrine (10^{-8} to 10^{-4}M) was without mechanical effect in resting TSM *in vitro* even in the presence of propranolol. Unlike the findings of Leff, et. al., (1981) an independant effect of alpha-adrenoceptor activation was not gradually lost but was absent from the earliest time at which the muscle was equilibrated (one hour) and throughout the duration of the experiment (up to 6 hours).

Since NE activates both alpha- and beta-adrenoceptors, the complex responses seen are not surprising. The tone-dependence of the alpha-

mediated component and the relative dominance seen at various concentrations of NE, however, is novel. The same muscles which had shown no response to NE alone produced a contractile response to NE (10^{-8} to 10^{-6}M) after tension was augmented with K^+ (23 mM). Further increases in NE (10^{-5} and 10^{-4}M) produced a shift to relaxation (Fig. 5). The contraction was mediated by alpha-adrenoceptors, relaxation by beta-adrenoceptors as evidenced by the sensitivity to phentolamine and propranolol respectively. In considering the basis for this pattern of response we considered the possibility that the respective receptors might have different thresholds for response. Figure 5, however, demonstrates similar thresholds for alpha- or beta-adrenoceptor mediated responses in the presence of propranolol and phentolomine, respectively. The finding of Vermiere and Vanhoutte (1979) that NE may interact with cholinergic nerves via pre-junctional receptors provided another possible explanation. While this interaction is of importance it appears that the biphasic response is independent of the cholinergic system since it is unaffected by atropine (Fig. 6). With reference to the alpha-adrenoceptor mediated contraction its magnitude is striking as seen by the rise to 223% above the initial plateau following K^+ (22.4 mM) exposure in the presence of NE 10^{-4} and propranolol (Fig. 6). Of special interest is the finding that while beta-adrenoceptor blockade greatly enhances alpha-adrenoceptor-dediated contractions the antagonist is not required to unmask the receptors as others have found for canine TSM *in vivo* (Beinfield and Siefter, 1980). When muscles were pretreated with propranolol all concentrations of norepinephrine $\geq$ 10^{-8}M produced contractions which were antagonized by phentolamine (Fig. 7).

Our attention then turned to the functional role of these receptors. The addition of tyramine (10^{-5} to 10^{-4}M) to 23 mM K-stimulated muscles resulted in contractile responses which were augmented by propranolol (10^{-5}M) and diminished by phentolamine (10^{-5}M). These results suggested the functional importance of the adrenergic nerves and the respective alpha- and beta-adrenoceptor-mediated responses to their neurotransmitter release. Atropine did not alter the qualitative aspect of this response (Fig. 8). Since the possibility of a direct effect of tyramine should not be ignored, muscles were stored for 3 days at low temperature (4°C), a procedure known to destroy adrenergic nerves (Hattori et. al., 1972); in these muscles the response to tyramine was lost but the biphasic response to NE remained. These findings therefore demonstrate the adrenergic neural component of canine TSM and the probable innervation of alpha-adrenoceptors. Stimulation of nerves to airways in vivo has demonstrated a function role of adrenergic nerves in the dog (Russell, 1980; Suzuki, et. al., 1976).

The nature of the alpha-adrenoceptor-mediated component and biphasic response was found to be influenced by the pre-existent tone in the preparation, the use of adrenergic antagonists and the specific agonists used to induce tone. With reference to the level of pre-existent tone a reduction of both contractile and relaxant responses (as a % of the K^+-induced contraction) following addition of norepinephrine was observed in 48 mM K-stimulated muscles. The alpha-adrenoceptor mediated response was demonstrated only in propranolol pretreated muscles, while a reduced but atropine-sensitive component of the total contraction was still evident (Fig. 9).

Figure 10 demonstrates that a maximum alpha-adrenoceptor mediated contraction is produced when initial tone is developed with 22.8 mM K^+. This contractile response to NE was maximal at a concentration of 10^{-6}M and greatly augmented by the addition of propranolol (10^{-5}M). As basal tension was increased by increasing concentrations of K^+ the alpha-mediated repsonse (g/cm^2) declined and the beta-adrenoceptor-mediated relaxant response increased in absolute terms.

Although the dose-response relation to NE (10^{-8} to 10^{-4}M) was biphasic, the shift from alpha- to beta-adrenoceptor-mediated responses occurred at a lower concentration (10^{-6}M NE) when histamine or acetylcholine were used to initiate tone (Fig. 11 and 13), than when K^+ solution producing an equivalent amount of initial active tone was used. The reason for this difference in a shift of the biphasic response is not known.

The dependence of the alpha-adrenoceptor-mediated contractile response on tone is further supported by the finding that hyoscyamine which eliminated tension stimulated by acetylcholine, also eliminated the existing alpha-adrenoceptor mediated contraction. This suggests that not only is tone required to unmask an alpha-adrenoceptor response but that tone is required to maintain it even when a substantial alpha-adrenoceptor-mediated active tone is already present. When histamine was used to develop tone initially hyoscyamine also reduced the adrenoceptor-stimulated contraction to near initial tonus levels, but the contraction was not abolished as for acetylcholine. The fact that alpha-adrenoceptor mediated contractions are not altered by atropine as was demonstrated earlier suggests that hyoscyamine which has the same mode of action as

atropine inhibits a component of the histamine response. The proportion of the remaining contraction which was produced by alpha-adrenoceptor stimulation was not determined.

Thus, these studies provide further evidence for an adrenergic innervation of canine TSM and the functional presence of both alpha and beta adrenoceptors. While others have demonstrated that elevation of tone may unmask alpha-adrenoceptor responses in human (Kneussl and Richardson, 1979) and dog TSM *in vitro* (Ohno et. al., 1981; Leff et. al., 1982), the relationship of the response to varying the active tone has not been studied previously. The present work, as previously reported (Bergen and Kroeger, 1980), demonstrates a relationship of initial active tone of the muscle to the biphasic dose-response relation seen with increasing NE concentrations (10^{-8} to 10^{-4}M). While the role of the adrenergic system in producing relaxant responses is widely accepted for canine airways the contractile response has been questioned. Whether a contractile response is observed is likely dependent on the resting or active tone of the preparation. Cabezas et. al. (1971) were unable to demonstrate an alpha-adrenoceptor mediated contractile response to electrical stimulation of sympathetic nerves in vagotomized dogs treated with propranolol *in vivo*. It is likely that active tone or resting tone following vagotomy was reduced and therefore alpha-adrenoceptor mediated contractions were not produced as we have seen for canine TSM *in vitro*.

C) Adrenoceptor Mediated Responses in Sensitized Canine TSM

Interest in the role of alpha-adrenoceptors mediating airway narrowing merits evaluation in the light of recent findings demonstrating an

increased contractile response of diseased human airways in vitro (Kneussl and Richardson, 1978) and in asthmatics (Henderson et. al., 1979; Patel and Kerr, 1975) following stimulation of alpha-adrenoceptors. The role of adrenoceptors in airway hyperreactivity is of interest from several perspectives. Changes in the number or activity of beta-adrenoceptors may occur (Szentivanyi, 1968; Szentivanyi; 1979). Recently Barnes et. al. (1980) demonstrated an increase of alpha-adrenoceptor-agonist binding in allergic guinea pigs. Changes in basal tone were observed in allergic dogs (Antonissen et. al., 1979) and may also contribute to altered adrenoceptor responses in the light of the study discussed above. The present study examined the adrenoceptor-mediated responses in dogs sensitized to ovalbumin and compared the findings to those of a non-sensitized mongrel dog population. The results indicate similar responses, both qualitatively and quantitatively for alpha-mediated contractions and beta-mediated relaxant responses in sensitized and control TSM. The biphasic response was unaltered for sensitized when compared to control responses.

When resting muscle strips from sensitized dogs were exposed to tyramine 10^{-4}M they contracted while control strips did not respond even in the presence of propranolol. Propranolol enhanced the contractile responses to tyramine and norepinephrine in sensitized muscle strips and the alpha-adrenoceptor nature of the contractile response was shown by its sensitivity to phentolamine. In order to develop a contractile response to norepinephrine, muscles from OA sensitized dogs were pretreated with propranolol; tyramine-mediated contractions, however, were produced in unblocked resting sensitized TSM.

We conclude by attributing increased alpha-adrenoceptor-mediated responsiveness of sensitized TSM to an increased resting basal tone in these muscles although we did not measure the latter directly. In view of the similar responses to norepinephrine and tyramine observed when tone was increased, it is unlikely that the sensitization of dogs to ovalbumin changes adrenoceptor status qualitatively, although these results do not suggest that altered adrenoceptor status is improbable in other situations. The possibility that disease states alter basal tone of airway smooth muscle require further examination. The factors which may alter resting tone and hence subsequent responses to adrenergic stimulation or circulating catecholamines are not well understood. Cholinergic overactivity may contribute to increased resting tone in vivo but this is an unlikely explanation for data obtained in vitro. Resting tone may be due to increased "leakiness" of the membrane to calcium, and if this occurs in sensitized canine TSM, we would expect increased responsiveness to alpha-adrenoceptor stimulation due to the dependance of the alpha-mediated contraction on tone. The increased tone may also result from an intracellular change which regulates the contractile elements of TSM.

D) Actions of Histamine and Serotonin on Canine TSM

Much experimental interest has centered on airway responsiveness to histamine in normal and disease states such as asthma. Histamine plays a major role in allergic asthma but the precise location of its action is not known. Several sites of action are suggested: post junctional H_1 receptors on smooth muscle (Antonissen, et. al., 1980; Himori and Taisa, 1978; Nathan et. al., 1979); an irritant receptor in the epithelium of

the airways (Kessler et. al., 1973; Wasserman, 1975; Yanta et. al., 1981) and at an unspecified site interacting with the cholinergic efferents to airway smooth muscle, perhaps in ganglia (Loring et. al., 1978). Less is known of the role of serotonin but it also likely acts at several sites to produce airway constriction. These include 1) receptors on airway smooth muscle (Hahn et. al., 1978; Offermeier and Ariens, 1966) the adrenergic or cholinergic system at the level of the ganglia (Hahn et. al., 1978; Dixon et. al., 1980; Sheller et. al., 1982) and 3) the vagus nerve to promote constrictor activity (Sheller et. al., 1982).

The present studies have focussed on the interaction of 5-HT and histamine on the cholinergic and adrenergic systems in canine TSM from animals sensitized to ovalbumin, their litter mates and a mongrel control population.

Histamine stimulates contraction of TSM. The partial (50%) atropine-sensitivity of this response (Fig. 23), suggests an action of histamine on the cholinergic system to promote acetylcholine release. Besides atropine sensitivity, several other of our studies support this view. Eserine effects a hyoscyamine-sensitive enhancement of the histamine-induced contraction (Table 5). Other studies using histamine aerosol (Drazen and Austen, 1975) or a specific antigen thought to release histamine from mast cells (Gold, 1975; Snow et. al., 1979) demonstrated a marked inhibition of the constrictor response when atropine was administered prior to challenge. These studies suggested that histamine was stimulating a vagal reflex by acting on the irritant receptor (Mortola et. al., 1975; Vidruk et. al., 1976; Gold et. al., 1972). In the present studies using TSM <u>in vitro</u> from which the mucosa has been removed, it is

unlikely that either a reflex or irritant receptors remain. However the ganglia present near or in the outer layer of smooth muscle may be intact providing a possible site of histamine interaction with the cholinergic efferents in vitro. Further evidence in the present studies demonstrated directly a release of ^{14}C- choline from cholinergic nerves (Fig. 40). This measure of overflow of radioactivity, which was interpreted in terms of neural release of intact acetylcholine, was not significantly elevated in the absence of eserine (Fig. 37) or the presence of tetrodotoxin (Fig. 40 lower panel). Thus it appears that histamine promotes the release of acetylcholine from cholinergic nerves. The inhibitory effect of TTX suggests that histamine acts at the level of the ganglia to enhance ACh efflux by increasing the action potential frequency or that the stimulatory action of histamine on the nerve, at the ganglia or varicosities, requires a TTX-sensitive influx of sodium ions. The finding that eserine is required in order to measure an increase in overflow suggests that neural elements efficiently take up the choline product of ACh hydrolysis, and that the effect is not as large as that seen following electrical field stimulated overflow in the absence of eserine (Fig. 37).

Histamine induced contractions were also partially inhibited by phentolamine although this effect was smaller than that following atropine (Fig. 22). While this finding suggested that histamine may stimulate neural NE release the overflow studies using ^{3}H-NE did not support this possibility (Fig. 29).

The Burn-Rand hypothesis (Burn and Rand, 1965) suggested a link of cholinergic influence on adrenergic nerves and visa versa, based on the close anatomical association seen for these two systems in the intestine.

Manber and Gershon (1979) in fact demonstrated that such an interaction occurs in intestinal smooth muscle. A similar close anatomical relationship has been described for cholinergic and adrenergic nerves in airway smooth muscle, and it is conceivable that interaction between these nerves could occur. Thus the ACh released by histamine stimulation of cholinergic nerves (Fig. 41) may promote norepinephrine release from nearby adrenergic neurons and serve to explain the phentolamine sensitivity of a histamine-mediated contraction (Fig. 22). These studies indicate a significant release of [^{3}H]-norepinephrine stimulated by exogenously applied acetylcholine. The norepinephrine thus released may bind to postjunctional alpha, or beta$_2$ receptors which could promote contractile or relaxant responses depending on the active tone of the muscle and concentration of norepinephrine at receptor sites. Another possibility is that any NE released may further promote ACh release forming a positive feedback loop. Others have suggested that as NE levels increase in the space around the nerve they may inhibit ACh release and promote some reciprocal inhibition via this mechanism in canine trachealis (Vermiere and Vanhoutte, 1979). Our studies examined the actions of norepinephrine at two concentrations (10^{-7} and 10^{-4}M) on ACh overflow. Norepinephrine (10^{-7}M) produced a small enhancement of the release of ^{14}C-ACh overflow, in the presence of eserine (Fig. 39,43). At a higher concentration, NE (10^{-4}M) inhibited choline overflow (Fig. 39). Addition of eserine did not qualitatively alter this effect (Fig. 44 upper panel) but the further addition of TTX resulted in a significant depression of [^{14}C]-acetylcholine efflux (Fig. 44 lower panel). We have no explanation for this action of TTX since the latter is present throughout the

experiment. The action of TTX on a lower concentration of NE was not investigated. An attempt to release [^{3}H]-norepinephrine with tyramine was unsuccessful, likely due to the presence of cocaine which may inhibit the uptake of tyramine (Fig. 43, lower panel). Therefore the effect of tyramine (in the presence of TTX) on acetylcholine release measured direct effects of tyramine on cholinergic nerves rather than the indirect effects produced by the release of norepinephrine. It was expected that the release of endogenous norepinephrine might have a neuromodulating effect on acetylcholine release. The studies however do support a stimulatory effect of lower concentration and an inhibitory action of higher concentrations (NE 10^{-4}M) of norepinephrine on [^{14}C]-acetylcholine overflow. When propranolol was added an inhibition of ACh efflux was observed (Fig. 45 lower panel) suggesting that if cholinergic nerves have both alpha- and beta-adrenoceptors prejunctionally it is likely that alpha-stimulation inhibits ACh overflow at resting levels. Phentolamine, which blocks alpha-adrenoceptors pre and postjunctionally should unmask prejunctional beta-receptors and thus enhance acetylcholine overflow in response to locally released norepinephrine or circulating catecholamines. The present studies did not demonstrate an effect of phentolamine in the presence of TTX and eserine (Fig. 45). The results of figure 45 may be explained in terms of a lack of endogenous norepinephrine reaching cholinergic nerves when the preparation is unstimulated. Although a low basal efflux of norepinephrine is likely, as suggested by the steady efflux of radioactivity in the present experiments, this low level of norepinephrine may be insufficient or too distant to act on cholinergic nerves. Propranolol may depress ^{14}C-acetylcholine overflow by a nonspecific anesthetic action on nerves.

These studies, including the actions of histamine pre-junctionally on the cholinergic system suggest that 1) histamine increases basal efflux of acetylcholine from cholinergic nerves by acting at a site on the postganglionic efferent to the smooth muscle in canine trachea and 2) acetylcholine increases the basal efflux of norepinephrine from postganglionic adrenergic nerves and it is by this action that histamine contractions show partial inhibition by phentolamine. When an atropinized muscle was stimulated with histamine the resulting tension was not significantly altered by phentolamine, suggesting that the ACh action to promote NE efflux occurs at a muscarinic site which is blocked by atropine (Fig. 23). When phentolamine was added at the plateau of a histamine induced contraction prior to atropine treatment a significant inhibition of isometric tension was observed (Fig. 22). Histamine (10^{-4}M) was also shown to produce a further contraction in a fully depolarized muscle (127mM K^+) which had been pretreated with atropine, phentolamine and propranolol. This pharmacomechanical coupling demonstrates a direct action of histamine on canine TSM, and has been shown to be mediated by H_1, receptors (Antonissen et. al., 1980).

A histamine-induced contraction is therefore composed of three separate entities; 1) postjunctional H_1 receptors on the airway smooth muscle cells 2) a stimulation of acetylcholine efflux which has postjunctional muscarinic effects and 3) an enhancement of NE release (by histamine stimulated ACh overflow) which mediates contraction by alpha-adrenoceptor stimulation. The latter is further supported by the finding that phentolamine significantly reduced isometric tension developed by a low concentration (10^{-8}M) of ACh (Table 8).

The action of 5-HT on canine TSM is to induce contraction which is partly (10%) atropine-sensitive (Fig. 24). Sheller et. al., (1982) noted an enhancement of vagally stimulated contractions in the presence of 5-HT in canine airways in vivo and suggested an interaction between 5-HT and efferent cholinergic fibers to airway smooth muscle. The isometric contractile response following 5-HT was increased dramatically by eserine, following which propranolol did not further enhance the contraction (Table 4). In fact all muscles showed a reduction in tension following propranolol administration although the response was not statistically significant. These results suggest a possible enhancing action of 5-HT on acetylcholine release from unstimulated (i.e. electrically) nerves. Serotonin, however, was without significant effect on ^{14}C-acetylcholine overflow normally (Fig. 38), in the presence of eserine (Fig. 41) or TTX (Fig. 41, lower panel). The finding that eserine enhanced 5-HT-stimulated contractions may be due to a non-specific action of eserine on the muscle in addition to its inhibition of acetylcholinesterase. Table 7 documents an increase of tension development following eserine administration in muscles already contracted by ACh. The observation that hyoscyamine eliminated the entire contraction argues strongly against a nonspecific contractile effect of eserine on tracheal smooth muscle. Perhaps the inhibitory effect of atropine on 5-HT induced contractions was due to an indirect effect through a 5-HT-stimulated release of norepinephrine acting on cholinergic nerves to release ACh. This would be analagous to the situation suggested previously for the action of histamine on the cholinergic system except in reverse. The potent inhibitory action of phentolamine on 5-HT induced isometric tension

responses (Fig. 25) suggested a stimulatory action of 5-HT on basal NE release. However 5-HT was found to be without significant effect on [^{3}H]-norepinephrine overflow. It is therefore concluded that phentolamine and perhaps atropine have nonspecific inhibitory actions on 5-HT induced contractions perhaps by interferring with the binding of 5-HT to specific serotonergic receptors on tracheal smooth muscle. It is also possible that 5-HT, which is somewhat similar in structure to norepinephrine, may interact with postjunctional alpha receptors which would explain the potent inhibitory action of phentolamine on 5-HT contractions. The inhibitory actions of phentolamine as an antiserotonergic compound have been demonstrated to occur in other systems (Garattini and Samanin, 1978).

These studies are not inconsistent with an effect of 5-HT on neurotransmitter overflow since only non-stimulated nerve preparations were studied. Early studies have demonstrated an excitatory effect of 5-HT on smooth muscle directly and via increasing the activity of intramural ganglion cells in isolated guinea pig ileum (Gaddum and Picarelli, 1957). An excitatory action of 5-HT is observed for ganglion cells which have an inhibitory action in the guinea pig stomach (Bülbring and Gershon, 1968). The present studies do not permit a prediction of the action of 5-HT on the neural components of airway smooth muscle when action potential frequency is increased. Serotonin may enhance neurotransmitter release as in the cholinergic nerves of the small intestine (Adam-Vizi and Vizi, 1978) or inhibit release as for adrenergic nerves to blood vessels (McGrath, 1977).

The lack of a demonstrable increase of either [^{3}H]-norepinephrine

or [^{14}C] acetylcholine efflux following treatment with 5-HT may be due to the small amount of labelled transmitter in the nerve varicosity in relation to the unlabelled pool. Such an effect would decrease the sensitivity of the technique and allow the demonstration of increased transmitter overflow only for those substances having a strong stimulatory action on nerves such as electrical field stimulation or potassium induced depolarization. The effects of these two stimuli were studied and did indeed dramatically increase [^{3}H]- norepinephrine overflow (Figs. 29 and 30) and [^{14}C]- acetylcholine overflow (Figs. 37 and 38), the latter in the absence of eserine. Histamine however, did not measureably stimulate [^{14}C]- acetylcholine efflux (Fig. 37) in the absence of eserine but when eserine was added a significant stimulation of efflux was observed (Fig. 40, upper panel). Although the efflux data are not useful for accurate quantitative measurements of transmitter release, it is likely that histamine stimulates a greater acetylcholine release than does 5-HT as judged by the greater effect of both atropine and eserine on histamine induced contractions. Since histamine stimulated efflux of [^{14}C]-acetylcholine was weak relative to electrical field stimulation or K^+ stimulated efflux, it is not surprising that the effect of 5-HT was not found to be significant.

The release of acetylcholine stimulated by high-K^+ is supported by an increase of K^+-induced contraction in the presence of eserine (Table 9) and a reduction in tension developed in the presence of atropine (Fig. 6). A reduction of tension, developed upon exposure to high-K^+ and eserine was observed in the presence of phentolamine (Table 9), supporting the relevance of K^+-stimulated [^{3}H]-norepinephrine overflow.

Since the high K solution also increased [^{14}C]- acetylcholine overflow it is likely that the ACh released also enhanced [^{3}H]-norepinephrine efflux in addition to the direct effect of K^+ on adrenergic nerves. The effect of propranolol on isometric tension developed in response to potassium and eserine was negligible, probably due to the high level of tone produced following eserine (Table 10) (note our finding above that the unmasked alpha-adrenoceptor response is very small when tone is high, Table 1).

Isometric tension developed following exposure to 5-HT or histamine was similar when comparing TSM from OA sensitized dogs to mongrel controls. The effects of the antagonists phentolamine and atropine on these isometric responses were also similar (Figs. 26 and 27, Table 3). The greater transient inhibition by phentolamine of a histamine-induced contraction in sensitized tissue is not explained (Fig. 26). These studies suggest that release of the neurotransmitters norepinephrine and acetylcholine by histamine and 5-HT are similar to that of control tissues and therefore labelled transmitter efflux studies on OA sensitized TSM were not carried out.

E) Autoregulation of Transmitter Overflow

Previous studies have suggested that prejunctional receptors on both adrenergic and cholinergic nerves may regulate or modulate transmitter release (Westfall, 1977; Langer, 1977; Weiner, 1979). It has been shown that adrenergic nerves possess prejunctional alpha$_2$-adrenoceptors which when stimulated inhibit stimulated norepinephrine overflow and when blocked by phentolamine or phenoxybenzamine enhance electrically stimu-

lated norepinephrine release (Langer, 1977). Dixon et. al., (1979) suggested a feedback inhibition of norepinephrine on its own further release which was dependent on norepinephrine concentration in the junctional cleft and therefore on the action potential frequency. At low concentrations (not known, but likely 10^{-7}M or less) norepinephrine may enhance its release, an effect blocked by propranolol (Langer, 1977) while at higher concentrations inhibition of norepinephrine efflux occurs.

The present studies have shown that for canine TSM phentolamine enhanced [^{3}H]-norepinephrine overflow and norepinephrine (10^{-6}M) also enhanced overflow (Figs. 31 and 32). While further studies were not carried out we conclude that alpha-adrenoceptor blockade enhances norepinephrine release as in other tissues even in the absence of electrical stimulation. The action of norepinephrine to increase basal norepinephrine release may occur by stimulation of prejunctional beta-adrenoceptors although the evidence at present is preliminary. The fact that neuromodulation occurs in adrenergic nerves in canine trachealis was previously not known and our present results serve as a starting point for further investigations designed to characterize the receptor set involved.

The present studies were unable to demonstrate an autoregulatory effect of acetylcholine on basal [^{14}C]- acetylcholine efflux. The addition of atropine did not alter the response (Fig. 42). Since both experiments were carried out in the presence of TTX it is possible that acetylcholine feedback on further acetylcholine release may require action potential discharge.

All of the experiments on radioactive acetylcholine or norepine-

phrine efflux were intended to determine whether several factors, especially histamine and 5-HT, had effects on the nerve network in canine trachealis at normal resting tone. Previous studies on other tissues have examined the effects of these substances on electrically-or K^+-stimulated transmitter overflow. Further studies of this nature are also required for canine and human airways. Changes in transmitter release by local factors (neurotransmitters, prostaglandins, leukotrienes, pH, hypoxia, etc.) nerve interaction, circulating substances (5-HT, catecholamines) or therapeutic agents may all influence the contractile state of airway smooth muscle. Only when the nature of these interactions have been elucidated can changes which may occur in diseases such as asthma be interpreted with confidence.

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APPENDIX

TABLE 1 Isometric tension developed (g/cm^2), following electrical field stimulation (ELEC) (15 V, 60 HZ, AC, 15 sec. duration), by tracheal smooth muscle. Stimuli were given at 10 minute intervals when electrical alone and 20 minutes after addition of eserine (10^{-7} M) or hyoscyamine (10^{-5} M). Values in brackets are percentages of the initial electrical induced contraction (100%). Each muscle strip was rinsed with fresh Krebs-Henseleit solution between each stimulus.

	TREATMENT						
Muscle	ELEC	ELEC	ELEC	ELEC	ELEC	ESERINE +ELEC	HYO +ELEC
1	1676	1725	1774	1823	1823	2661	24
	(100)	(102)	(105)	(108)	(108)	(158)	(1)
2	1674	1674	1707	1741	1741	2277	16
	(100)	(100)	(102)	(104)	(104)	(136)	(1)
3	1440	1453	1481	1481	1453	1782	82
	(100)	(101)	(102)	(102)	(100)	(123)	(5)
4	850	874	874	850	826	1148	47
	(100)	(102)	(102)	(100)	(97)	(139)	(5)
5	1815	1815	1815	1815	1815	2360	165
	(100)	(100)	(100)	(100)	(100)	(130)	(9)
6	600	600	576	517	505	835	23
	(100)	(100)	(96)	(86)	(84)	(139)	(3)
7	1377	1437	1456	1456	1456	1712	78
	(100)	(104)	(105)	(105)	(105)	(124)	(5)
$\bar{X}$	1347	1368	1383	1383	1374	1825	62
SE	172	173	180	192	195	307	20
$\bar{X}$	(100)	(101)	(102)	(101)	(99)	(136)	(4)
SE	0	1	1	3	3	6	1

TABLE 2 Isometric tension developed (g/cm^2), following electrical field stimulation (ELEC) (15 V, 60 HZ AC, 15 sec. duration), by tracheal smooth muscle. Stimuli were given at 10 minute intervals when electrical alone and 20 minutes after addition of phentolamine (10^{-5} M) or tetrodotoxin (10^{-6} M). Values in brackets are percentages of the initial electrical induced contraction (100%). Each muscle strip was rinsed with fresh Krebs-Henseleit solution between each stimulus.

	TREATMENT						
Muscle	ELEC	ELEC	ELEC	ELEC	ELEC	PHENT + ELEC	TTX + ELEC
1	1209	1251	1272	1315	1315	1187	0
	(100)	(103)	(105)	(108)	(108)	(98)	(0)
2	1429	1457	1486	1500	1500	997	0
	(100)	(101)	(103)	(104)	(104)	(69)	(0)
3	1537	1558	1558	1579	1537	1558	83
	(100)	(101)	(101)	(102)	(100)	(101)	(5)
4	1017	1017	1017	990	962	797	0
	(100)	(100)	(100)	(97)	(94)	(78)	(0)
5	618	603	618	633	633	--	0
	(100)	(97)	(100)	(102)	(102)	--	(0)
6	1287	1287	1287	1287	1287	154	--
	(100)	(100)	(100)	(100)	(100)	(12)	--
7	1225	1277	1277	1303	1329	1212	77
	(100)	(104)	(104)	(106)	(108)	(98)	(6)
8	1428	1466	1466	1485	1485	1371	19
	(100)	(102)	(102)	(104)	(104)	(96)	(1)
$\bar{X}$	1218	1239	1247	1261	1256	1039	25
SE	103	108	108	110	109	174	14
$\bar{X}$	(100)	(101)	(102)	(103)	(103)	(79)	(2)
SE	0	1	1	1	2	12	1

TABLE 3 Isometric tension developed (g/cm^2), following electrical field stimulation (ELEC) (15 V, 60 HZ AC, 15 sec. duration), by tracheal smooth muscle. Stimuli were given at 10 minute intervals when electrical alone and 20 minutes after addition of propranolol (PROP 10^{-5} M) or phentolamine (Phent (10^{-5} M). Values in brackets are percentages of the initial electrical induced contraction (100%). Each muscle strip was rinsed with fresh Krebs-Henseleit solution between each stimulus.

	TREATMENT						
Muscle	ELEC	ELEC	ELEC	ELEC	ELEC	PROP 10^{-5}M +ELEC	PROP 10^{-5}M +PHENT 10^{-5}M +ELEC
1	1372	1618	1667	1667	1716	1470	833
	(100)	(117)	(121)	(121)	(125)	(107)	(60)
2	1502	1554	1571	1589	1537	1381	639
	(100)	(103)	(104)	(105)	(102)	(91)	(42)
3	1215	1250	1250	1232	1250	1197	880
	(100)	(102)	(102)	(101)	(102)	(98)	(72)
4	1067	1057	1057	1037	1016	838	737
	(100)	(99)	(99)	(97)	(95)	(78)	(69)
5	618	618	633	633	633	618	--
	(100)	(100)	(102)	(102)	(102)	(100)	--
6	1040	960	960	1040	1040	960	800
	(100)	(92)	(92)	(100)	(100)	(92)	(76)
7	795	795	795	795	795	681	113
	(100)	(100)	(100)	(100)	(100)	(85)	(14)
8	984	1151	1227	1227	1242	1212	939
	(100)	(116)	(124)	(124)	(126)	(123)	(95)
9	1146	1146	1175	1189	1189	1117	943
	(100)	(100)	(102)	(103)	(103)	(97)	(82)
$\bar{X}$	1082	1127	1148	1156	1157	1052	735
SE	90	108	111	111	112	99	96
$\bar{X}$	(100)	(103)	(105)	(106)	(106)	(97)	(64)
SE	0	3	4	3	4	4	9

TABLE 4 Isometric tension developed (g/cm^2), following electrical field stimulation (ELEC) (15 V, 60 HZ AC, 15 sec. duration), by tracheal smooth muscle. Stimuli were given at 10 minute intervals when electrical alone or norepinephrine (NE 10^{-6} or 10^{-4} M) and 20 minutes after the addition of propranolol (PROP 10^{-5} M). Values in brackets are percentages of the initial electrical induced contraction (100%). Each muscle strip was rinsed with fresh Krebs-Henseleit solution between each stimulus.

	TREATMENT							
Muscle	ELEC	ELEC	ELEC	ELEC	ELEC	NE 10^{-6}M + ELEC	NE 10^{-4}M + ELEC	PROP 10^{-5}M + NE 10^{-4}M + ELEC
1	1188	1280	1316	1353	1371	1353	1280	950
	(100)	(107)	(110)	(113)	(115)	(107)	(107)	(80)
2	1593	1593	1667	1667	1593	1618	1323	1103
	(100)	(100)	(104)	(104)	(100)	(101)	(83)	(69)
3	1254	1275	1317	1254	1275	1254	1003	1129
	(100)	(101)	(105)	(100)	(101)	(100)	(80)	(90)
4	821	919	955	955	955	991	955	883
	(100)	(111)	(116)	(116)	(116)	(120)	(116)	(107)
5	1665	1710	1687	1665	1665	1687	1530	1305
	(100)	(102)	(101)	(100)	(100)	(101)	(91)	(78)
$\bar{X}$	1304	1355	1388	1378	1371	1380	1218	1074
SE	152	138	135	134	126	126	106	73
$\bar{X}$	(100)	(104)	(107)	(107)	(106)	(107)	(95)	(85)
SE	0	2	3	3	4	4	7	6

TABLE 5 Isometric tension (g/cm^2), developed following addition of potassium (K^+ 23 mM), norepinephrine (NE 10^{-8} to 10^{-4} M) and phentolamine (10^{-5} M) cumulatively. Values in brackets are percentages of the initial tension developed following K^+ exposure (100%).

	TREATMENT						
Muscle	23 mM K^+	NE 10^{-8} M	NE 10^{-7} M	NE 10^{-6} M	NE 10^{-5} M	NE 10^{-4} M	PHENT 10^{-5} M
1	507	571	660	622	330	203	51
	(100)	(112)	(130)	(123)	(65)	(40)	(10)
2	854	880	920	998	880	657	341
	(100)	(103)	(108)	(117)	(103)	(77)	(40)
3	163	163	169	136	109	87	71
	(100)	(100)	(103)	(83)	(67)	(53)	(43)
4	191	210	268	344	153	77	38
	(100)	(110)	(140)	(180)	(80)	(40)	(20)
5	416	456	473	529	489	244	33
	(100)	(110)	(114)	(127)	(118)	(59)	(8)
6	232	250	267	285	178	98	54
	(100)	(108)	(115)	(123)	(77)	(42)	(23)
7	1195	1247	1318	1423	1423	1370	703
	(100)	(104)	(110)	(119)	(119)	(115)	(59)
$\bar{X}$	507	539	381	619	508	390	183
SE	146	151	157	170	182	180	96
$\bar{X}$	(100)	(107)	(117)	(125)	(90)	(61)	(29)
SE	0	2	5	11	9	10	7

TABLE 6 Isometric tension (g/cm^2), developed in a cumulative dose response fashion, in muscles pretreated with phentolamine (10^{-5} M). Values in brackets are percentages of the initial response following exposure to K^+ (23 mM) (100%).

	TREATMENT					
Muscle	23 mM K^+	NE 10^{-8} M	NE 10^{-7} M	NE 10^{-6} M	NE 10^{-5} M	NE 10^{-4} M
1	838	800	711	356	127	51
	(100)	(96)	(85)	(42)	(15)	(6)
2	657	644	604	368	158	53
	(100)	(98)	(92)	(56)	(24)	(8)
3	196	185	152	114	98	76
	(100)	(94)	(78)	(58)	(50)	(39)
4	229	210	191	96	57	57
	(100)	(92)	(83)	(42)	(25)	(25)
5	196	187	179	130	82	82
	(100)	(96)	(92)	(67)	(42)	(42)
6	383	365	339	303	348	482
	(100)	(95)	(88)	(79)	(91)	(126)
7	1405	1370	1318	1142	966	1230
	(100)	(98)	(94)	(81)	(69)	(88)
$\bar{X}$	557	537	499	358	262	289
SE	169	166	159	138	123	167
$\bar{X}$	(100)	(96)	(87)	(61)	(45)	48
SE	0	1	2	6	10	17

TABLE 7 Isometric tension (g/cm^2), developed in a cumulative dose response fashion following addition of potassium solution (23 mM K^+), atropine (10^{-6} M), norepirephrine (10^{-8} to 10^{-4} M) and propranolol (10^{-5} M). Values in brackets are percentages of the initial tension developed following K^+ exposure (100%).

	TREATMENT							
Muscle	23 m MK+	ATR 10^{-6}M	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PROP 10^{-5}M
1	1123	986	1014	1069	1206	548	192	1671
	(100)	(88)	(90)	(95)	(107)	(49)	(17)	(149)
2	859	848	837	837	794	576	413	1000
	(100)	(99)	(98)	(98)	(92)	(67)	(48)	(117)
3	464	448	448	456	407	228	122	709
	(100)	(97)	(97)	(98)	(88)	(49)	(26)	(153)
4	90	68	90	113	203	136	68	700
	(100)	(75)	(100)	(125)	(225)	(150)	(75)	(775)
5	478	165	198	214	264	429	379	1533
	(100)	(35)	(41)	(45)	(55)	(89)	(79)	(321)
6	170	144	162	187	263	340	306	979
	(100)	(85)	(95)	(110)	(155)	(200)	(180)	(575)
7	886	702	725	794	920	1001	1012	1496
	(100)	(79)	(82)	(90)	(104)	(113)	(114)	(169)
	$\bar{X}$ 581	480	496	524	579	465	356	1155
	SE 146	140	138	142	148	107	119	153
	$\bar{X}$ (100)	(80)	(86)	(94)	(115)	(102)	(77)	(323)
	SE 0	8	8	9	21	21	21	97

TABLE 8 Isometric tension (g/cm^2), developed in a cumulative dose response fashion following addition of potassium solution (23 mM K^+), atropine (10^{-6} M), norepinephrine (NE 10^{-8} to 10^{-4} M) and phentolamine (10^{-5} M) to TSM strips pretreated with propranolol (10^{-5} M). Values in brackets are percentages of the initial tension developed following K^+ exposure (100%).

	TREATMENT							
Muscle	23 mMK+	ATR 10^{-6}M	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PHENT 10^{-5}M
1	1370	1315	1369	1424	1616	1945	1972	657
	(100)	(96)	(100)	(104)	(118)	(142)	(144)	(48)
2	261	250	260	271	347	445	489	282
	(100)	(95)	(100)	(104)	(133)	(171)	(187)	(108)
3	203	197	209	228	318	457	559	413
	(100)	(97)	(103)	(112)	(156)	(225)	(275)	(203)
4	158	135	158	225	587	903	948	812
	(100)	(86)	(100)	(143)	(371)	(571)	(600)	(514)
5	197	197	230	263	544	1137	1351	692
	(100)	(100)	(117)	(133)	(275)	(575)	(683)	(350)
6	459	459	468	493	638	1012	1353	1157
	(100)	(100)	(101)	(107)	(139)	(220)	(294)	(252)
7	598	598	598	655	897	1587	1898	1610
	(100)	(100)	(100)	(109)	(150)	(265)	(317)	(269)
$\bar{X}$	463	450	470	508	707	1069	1224	803
SE	163	157	161	164	168	209	223	171
$\bar{X}$	(100)	(96)	(103)	(116)	(192)	(310)	(357)	(249)
SE	0	2	2	6	36	70	77	58

TABLE 9 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of potassium solution (23 mM K^+), atropine (10^{-6} M), tyramine (10^{-5} and 10^{-4} M) and propranolol (10^{-5} M) to TSM strips. Values in brackets are percentages of initial tension developed following K^+ exposure (100%).

	TREATMENT				
Muscle	23 mM K+	ATR 10^{-6}M	TYR 10^{-5}M	TYR 10^{-4}M	PROP 10^{-5}M
1	989	949	1266	1701	1820
	(100)	(96)	(128)	(172)	(184)
2	744	720	886	1146	1205
	(100)	(97)	(119)	(154)	(162)
3	220	198	220	330	385
	(100)	(90)	(100)	(150)	(175)
4	102	102	183	326	387
	(100)	(100)	(180)	(320)	(380)
5	346	159	317	620	923
	(100)	(46)	(92)	(179)	(267)
6	290	215	262	336	318
	(100)	(74)	(90)	(116)	(109)
7	706	682	753	917	941
	(100)	(97)	(107)	(130)	(133)
$\bar{X}$	485	432	555	768	853
SE	124	129	158	197	206
$\bar{X}$	(100)	(86)	(117)	(174)	(201)
SE	0	3	12	26	35

TABLE 10 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of potassium solution (23 mM K^+), atropine (10^{-6} M), tyramine (10^{-5} and 10^{-4} M) and phentolamine in atropine (10^{-6} M) pretreated TSM strips. Values in brackets are percentages of initial tension developed following K^+ exposure (100%).

	TREATMENT				
Muscle	23 mMk+	ATR 10^{-6}M	TYR 10^{-5}M	TYR 10^{-4}M	PHENT 10^{-5}M
1	1681	1642	1681	1978	1484
	(100)	(98)	(100)	(117)	(88)
2	650	650	744	993	650
	(100)	(100)	(114)	(153)	(100)
3	220	225	247	330	302
	(100)	(102)	(112)	(150)	(137)
4	142	142	183	346	203
	(100)	(100)	(128)	(243)	(143)
5	461	461	548	721	433
	(100)	(100)	(119)	(156)	(94)
6	374	374	393	439	421
	(100)	(100)	(105)	(118)	(113)
7	788	788	835	1012	906
	(100)	(100)	(106)	(128)	(115)
$\bar{X}$	617	612	662	831	628
SE	197	192	193	219	167
$\bar{X}$	(100)	(100)	(112)	(152)	(113)
SE	0	1	4	16	8

TABLE 11 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of potassium solution (17.4 mM K^+), norepinephrine (NE 10^{-8} to 10^{-4} M), and propranolol (10^{-5}) to TSM pretreated with atropine (10^{-6} M). Values in brackets are percentages of initial tension developed following K^+ exposure (100%).

	TREATMENT						
Muscle	17.4 mMK+	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PROP 10^{-5}M
1	469	480	539	527	304	211	1289
	(100)	(102)	(115)	(113)	(65)	(45)	(275)
2	213	213	213	142	59	59	818
	(100)	(100)	(100)	(67)	(28)	(28)	(383)
3	50	50	56	44	39	33	144
	(100)	(100)	(111)	(89)	(78)	(66)	(289)
4	21	21	21	21	21	21	256
	(100)	(100)	(100)	(100)	(100)	(100)	(1200)
5	204	265	286	316	92	61	1082
	(100)	(130)	(140)	(155)	(45)	(30)	(530)
6	167	183	200	250	175	100	875
	(100)	(110)	(120)	(150)	(105)	(60)	(525)
7	186	200	264	357	264	242	479
	(100)	(108)	(142)	(192)	(142)	(131)	(258)
$\bar{X}$	187	202	225	237	136	104	706
SE	55	57	65	69	43	33	161
$\bar{X}$	(100)	(93)	(118)	(124)	(80)	(66)	(494)
SE	0	15	6	16	15	14	125

TABLE 12 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of potassium solution (35.2 mM K^+), norepinephrine (10^{-8} to 10^{-4} M), and propranolol (10^{-5}) to TSM pretreated with atropine (10^{-6} M). Values in brackets are percentages of initial tension developed following K^+ exposure (100%).

	TREATMENT						
Muscle	35.2 mMK+	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PROP 10^{-5}M
1	2011	2011	1977	1943	1841	1670	2080
	(100)	(100)	(98)	(97)	(92)	(83)	(103)
2	1943	1968	1968	1917	1662	1432	2377
	(100)	(101)	(101)	(99)	(86)	(74)	(122)
3	913	913	913	875	812	763	1013
	(100)	(100)	(100)	(96)	(89)	(84)	(111)
4	630	630	630	630	610	540	650
	(100)	(100)	(100)	(100)	(97)	(86)	(103)
5	1326	1326	1326	1351	1224	969	1606
	(100)	(100)	(100)	(102)	(92)	(73)	(121)
6	419	419	419	432	439	412	611
	(100)	(100)	(100)	(103)	(105)	(98)	(146)
7	320	320	320	340	360	320	580
	(100)	(100)	(100)	(106)	(113)	(100)	(181)
$\bar{X}$	1080	1084	1079	1070	992	872	1274
SE	263	266	263	255	224	195	283
$\bar{X}$	(100)	(100)	(100)	(100)	(96)	(85)	(127)
SE	0	0	1	1	4	4	11

TABLE 13 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of potassium solution (48.2 mM K^+), norepinephrine (10^{-8} to 10^{-4} M), and propranolol (10^{-5}) to TSM pretreated with atropine (10^{-6} M). Values in brackets are percentages of initial tension developed following K^+ exposure (100%).

	TREATMENT						
Muscle	48.2 mMK+	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PROP 10^{-5}M
1	1352	1328	1328	1303	1183	1134	1376
	(100)	(98)	(98)	(96)	(88)	(84)	(102)
2	1522	1544	1544	1433	1167	944	1611
	(100)	(102)	(102)	(94)	(77)	(62)	(106)
3	974	974	974	932	870	712	1037
	(100)	(100)	(100)	(96)	(89)	(73)	(107)
4	951	963	963	963	938	901	1074
	(100)	(101)	(101)	(101)	(99)	(95)	(113)
5	1404	1404	1404	1427	1288	1058	1634
	(100)	(100)	(100)	(102)	(92)	(75)	(116)
6	1255	1255	1255	1255	1200	1090	1400
	(100)	(100)	(100)	(100)	(96)	(87)	(112)
7	1154	1154	1154	1154	1138	1122	1285
	(100)	(100)	(100)	(100)	(99)	(97)	(111)
$\bar{X}$	1230	1232	1232	1210	1112	994	1345
SE	82	82	82	77	57	58	89
$\bar{X}$	(100)	(100)	(100)	(98)	(91)	(82)	(110)
SE	0	0	0	1	3	5	2

TABLE 14 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of potassium solution (64.2 mM K^+), norepinephrine (10^{-8} to 10^{-4} M), and propranolol (10^{-5}) to TSM pretreated with atropine (10^{-6} M). Values in brackets are percentages of initial tension developed following K^+ exposure (100%).

	TREATMENT						
Muscle	64.2mMk+	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PROP 10^{-5}
1	1675	1675	1675	1625	1475	1325	1700
	(100)	(100)	(100)	(97)	(88)	(79)	(102)
2	1813	1813	1813	1694	1217	1026	2099
	(100)	(100)	(100)	(93)	(67)	(57)	(116)
3	829	829	829	754	628	578	930
	(100)	(100)	(100)	(91)	(76)	(70)	(112)
4	1100	1100	1100	1080	920	840	1200
	(100)	(100)	(100)	(98)	(84)	(76)	(109)
5	1638	1638	1638	1638	1311	596	1906
	(100)	(100)	(100)	(100)	(80)	(36)	(116)
6	1771	1771	1771	1771	1344	1131	1899
	(100)	(100)	(100)	(100)	(76)	(64)	(107)
7	1696	1696	1696	1696	1438	1291	1844
	(100)	(100)	(100)	(100)	(85)	(76)	(109)
$\bar{X}$	1503	1503	1503	1465	1190	969	1654
SE	144	144	144	147	117	116	161
$\bar{X}$	(100)	(100)	(100)	(97)	(79)	(65)	(110)
SE	0	0	0	1	3	6	2

TABLE 15 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of acetylcholine (ACh 5 x 10^{-8} M), norepinephrine (10^{-8} to 10^{-4} M), and propranolol (10^{-5}) to TSM strips. Values in brackets are percentages of initial tension (100%) developed following ACh exposure.

	TREATMENT						
Muscle	ACH $5X10^{-8}M$	NE $10^{-8}M$	NE $10^{-7}M$	NE $10^{-6}M$	NE $10^{-5}M$	NE $10^{-4}M$	PROP $10^{-5}M$
1	217	229	241	221	88	80	281
	(100)	(105)	(111)	(101)	(40)	(37)	(129)
2	157	157	175	210	350	368	630
	(100)	(100)	(111)	(133)	(222)	(233)	(400)
3	525	543	560	310	129	129	1094
	(100)	(103)	(106)	(59)	(24)	(24)	(208)
4	144	144	153	158	81	48	--
	(100)	(100)	(106)	(110)	(56)	(33)	--
5	1043	1075	1106	1233	1169	1011	1296
	(100)	(103)	(106)	(118)	(112)	(96)	(124)
6	240	248	260	268	184	152	276
	(100)	(103)	(108)	(111)	(76)	(63)	(115)
7	553	553	553	444	284	255	568
	(100)	(100)	(100)	(80)	(51)	(46)	(102)
8	557	557	518	286	87	95	621
	(100)	(100)	(92)	(51)	(15)	(17)	(111)
9	678	678	678	546	281	264	711
	(100)	(100)	(100)	(80)	(41)	(39)	(104)
10	414	423	482	558	304	270	846
	(100)	(102)	(116)	(134)	(73)	(65)	(204)
11	408	433	450	200	16	0	666
	(100)	(106)	(110)	(48)	(4)	(0)	(163)
	$\bar{X}$ 449	458	470	403	270	242	698
	SE 80	82	83	93	96	84	101
	$\bar{X}$ (100)	(102)	(106)	(93)	(65)	(59)	(166)
	SE 0	1	2	9	18	19	29

TABLE 16 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of acetylcholine (5×10^{-8} M), norepinephrine (10^{-8} to 10^{-4} M), and hyoscyamine (10^{-5}) to TSM strips pretreated with propranolol (10^{-5} M). Values in brackets are percentages of initial tension (100%) developed following ACh exposure.

	TREATMENT						
Muscle	ACH $5X10^{-8}$M	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	HYO 10^{-5}M
1	241	257	269	318	342	334	0
	(100)	(106)	(111)	(131)	(141)	(138)	(0)
2	1155	1189	1206	1293	1431	1500	0
	(100)	(102)	(104)	(111)	(123)	(129)	(0)
3	144	144	153	192	211	220	--
	(100)	(100)	(106)	(133)	(146)	(153)	--
4	288	296	308	337	365	373	0
	(100)	(102)	(106)	(116)	(126)	(129)	(0)
5	626	641	648	699	786	786	0
	(100)	(102)	(103)	(111)	(125)	(125)	(0)
6	638	646	654	755	923	1024	8
	(100)	(101)	(102)	(118)	(144)	(160)	(2)
7	811	827	827	893	993	1042	0
	(100)	(102)	(102)	(110)	(122)	(128)	(0)
8	660	660	685	803	994	1121	0
	(100)	(100)	(103)	(121)	(150)	(169)	(0)
9	650	683	716	816	950	950	0
	(100)	(105)	(110)	(125)	(146)	(146)	(0)
$\bar{X}$	579	594	607	678	777	817	0
SE	105	107	115	132	142	142	0
$\bar{X}$	(100)	(102)	(105)	(120)	(136)	(142)	(0)
SE	0	1	1	3	4	5	0

TABLE 17 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of histamine (10^{-6} M), norepinephrine (10^{-8} to 10^{-4} M), and propranolol (10^{-5}) to TSM strips. Values in brackets are percentages of initial tension (100%) developed following exposure to histamine.

	TREATMENT						
Muscle	HIST 10^{-6}M	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	PROP 10^{-5}M
1	15	20	25	15	15	15	168
	(100)	(133)	(166)	(100)	(100)	(100)	(1100)
2	128	157	171	14	0	0	1171
	(100)	(122)	(133)	(11)	(0)	(0)	(911)
3	40	56	72	8	24	72	475
	(100)	(140)	(180)	(20)	(60)	(180)	(1180)
4	25	32	45	6	0	0	270
	(100)	(125)	(175)	(25)	(0)	(0)	(1050)
5	39	39	19	9	0	0	585
	(100)	(100)	(50)	(25)	(0)	(0)	(1475)
6	361	312	98	0	0	0	1479
	(100)	(100)	(27)	(0)	(0)	(0)	(409)
7	334	281	114	0	0	0	1214
	(100)	(84)	(34)	(0)	(0)	(0)	(363)
8	53	71	107	26	17	17	920
	(100)	(133)	(200)	(50)	(33)	(33)	(1708)
9	518	536	402	89	0	0	679
	(100)	(103)	(77)	(17)	(0)	(0)	(131)
	$\bar{X}$ 168	167	117	19	6	12	773
	SE 62	59	39	9	3	8	150
	$\bar{X}$ (100)	116	116	28	21	35	925
	SE 0	6	23	10	12	21	176

TABLE 18 Isometric tension (g/cm^2) developed in a cumulative dose response fashion following addition of histamine (10^{-6} M), norepinephrine (10^{-8} to 10^{-4} M), and hyoscyamine (10^{-5}) to TSM strips pretreated with propranolol (10^{-5} M). Values in brackets are percentages of initial tension (100%) developed following exposure to histamine.

	TREATMENT						
Muscle	HIST 10^{-6}M	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	HYO 10^{-5}M
1	50	61	96	188	280	310	170
	(100)	(120)	(190)	(370)	(550)	(610)	(350)
2	19	19	29	38	582	932	--
	(100	(100)	(150)	(200)	(3000)	(4800)	--
3	128	142	264	521	921	1235	821
	(100)	(111)	(205)	(405)	(716)	(961)	(638)
4	19	38	129	335	522	606	77
	(100)	(200)	(666)	(1733)	(2700)	(3133)	(400)
5	119	129	198	406	635	744	0
	(100)	(108)	(166)	(341)	(533)	(625)	(0)
6	493	591	887	1249	1578	1775	263
	(100)	(120)	(180)	(253)	(320)	(360)	(53)
7	545	563	633	821	1214	1355	88
	(100)	(103)	(116)	(151)	(222)	(248)	(16)
8	17	44	134	323	898	1055	26
	(100)	(250)	(750)	(1800)	(5000)	(5875)	(150)
9	429	572	572	617	706	724	125
	(100)	(133)	(133)	(143)	(164)	(168)	(29)
$\bar{X}$	202	239	326	500	815	970	197
SE	74	85	99	121	131	147	94
$\bar{X}$	(100)	(138)	(284)	(599)	(1467)	(1864)	(205)
SE	0	17	81	223	567	726	83

TABLE 19 Isometric tension (g/cm^2) developed in TSM from dogs (n = 10) sensitized to ovalbumin. Drugs tyramine (TYR 10^{-5} and 10^{-4} M) and propranolol (10^{-5} M) were added cumulatively.

	TREATMENT		
Muscle	TYR 10^{-5}M	TYR 10^{-4}M	PROP 10^{-5}M
1	9	36	63
2	0	0	0
3	0	0	0
4	0	21	86
5	8	66	99
6	0	107	109
7	0	0	0
8	0	0	0
9	0	0	0
10	0	22	23
X	2	25	39
SE	1	11	15

TABLE 20 Isometric tension (g/cm^2) developed in unstimulated TSM from mongrel control dogs (n = 10), following cumulative dose addition of norepinephrine (NE 10^{-8} to 10^{-4} M). Similar results were obtained when muscles in the same protocol were pretreated with propranolol (10^{-5} M).

	TREATMENT				
Muscle	NE 10^{-8}M	NE 10^{-7}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M
1	0	0	0	9	9
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	55
9	0	0	0	0	0
10	0	0	0	0	0
X	0	0	0	1	6
SE	0	0	0	0	1

TABLE 21 Isometric tension (g/cm^2) developed in OA sensitized, propranolol (10^{-5} M) pretreated TSM strips (n = 9) following cumulative dose addition of norepinephrine (NE 10^{-8} to 10^{-4} M), then tyramine (10^{-5} and 10^{-4} M) and finally phentolamine (10^{-5} M).

	TREATMENT						
Muscle	PROP 10^{-5}M	NE 10^{-6}M	NE 10^{-5}M	NE 10^{-4}M	TYR 10^{-5}M	TYR 10^{-4}M	PHENT 10^{-5}M
1	0	9	18	72	81	154	0
2	0	0	0	0	0	114	0
3	0	9	19	19	19	28	0
4	0	0	21	64	64	365	0
5	0	0	0	182	-	639	0
6	0	0	15	950	980	1225	0
7	0	25	50	175	200	750	0
8	0	0	44	155	177	311	0
9	0	0	0	28	28	28	0
$\bar{X}$	0	5	19	183	192	402	0
SE	0	3	6	99	102	134	0

TABLE 22 Isometric tension (g/cm^2) developed following cumulative dose addition of histamine (10^{-5} M), and phentolamine (10^{-5} M) followed by a washout. The response to phentolamine was transient at 5 minutes but a stable plateau tension was maintained at 10 minutes following exposure. Following the washout the same muscles pretreated with phentolamine were exposed to histamine (10^{-5} M) followed 5 minutes later by atropine, then finally (5 min. later) 127 mM k^+. Values in brackets are percentages of the initial contraction (100%) produced by exposure to histamine.

	TREATMENT						
Muscle	HIST 10^{-5}M	PHENT 10^{-5}M 5 MIN	PHENT 10^{-5}M 10 MIN	WO	HIST 10^{-5}M	ATR 10^{-6}M	127 mM K^+
1	810	641	742		418	20	1282
	(100)	(79)	(91)		(51)	(2)	(158)
2	253	134	253		168	25	421
	(100)	(53)	(100)		(66)	(10)	(166)
3	1571	1042	1242		971	871	2000
	(100)	(66)	(79)		(61)	(55)	(127)
4	777	427	58		486	87	1419
	(100)	(55)	(8)		(63)	(11)	(183)
5	1066	644	620		99	99	1984
	(100)	(60)	(58)		(9)	(9)	(186)
6	1533	1533	1500		1266	66	1566
	(100)	(100)	(97)		(82)	(4)	(102)
7	943	811	943		698	301	943
	(100)	(86)	(100)		(74)	(32)	(100)
8	829	695	743		926	792	1853
	(100)	(83)	(89)		(111)	(96)	(223)
$\bar{X}$	972	740	762		629	289	1433
SE	151	147	168		144	123	193
$\bar{X}$	(100)	(73)	(78)		(65)	(28)	(156)
SE	(0)	6	11		10	12	15

TABLE 23 Isometric tension (g/cm^2) developed following cumulative dose addition of histamine (10^{-5} M), atropine (10^{-6} M) and a washout (WO). This washout was followed by pretreating the muscle strips with atropine (10^{-5} M) then adding histamine (10^{-5} M), phentolamine (10^{-5} M) and 127 mM K^+. A time interval of 5 minutes was allowed between drug additions to allow for stable plateau tension development. Values in brackets are percentages of the initial contraction (100%) produced by exposure to histamine (10^{-5} M).

	TREATMENT				
Muscle	HIST 10^{-5}M	ATR 10^{-6}M	WO	HIST 10^{-5}M	PHENT 10^{-5}M	127 mM K^+
1	390	122	122	66	1047
	(100)	(31)	(31)	(17)	(268)
2	249	168	160	88	964
	(100)	(67)	(64)	(35)	(387)
3	2142	1885	1307	1242	2850
	(100)	(88)	(61)	(58)	(133)
4	515	73	147	147	1363
	(100)	(14)	(28)	(28)	(264)
5	739	403	268	313	1500
	(100)	(54)	(36)	(42)	(203)
6	800	622	408	408	1208
	(100)	(77)	(51)	(51)	(151)
7	307	97	97	97	657
	(100)	(31)	(31)	(31)	(213)
8	632	385	428	471	1714
	(100)	(61)	(68)	(75)	(271)
$\bar{X}$	721	469	367	354	1412
SE	214	213	141	138	235
$\bar{X}$	(100)	(54)	(47)	(42)	(236)
SE	0	9	6	6	28

TABLE 24 Isometric tension (g/cm^2) developed following a cummulative dose addition as in Table 23 appendix except initial agonist used is 5-hydroxytryptamine (5-HT 10^{-6} M). Values in brackets are percentages of the initial contraction (100%) produced by exposure to 5-HT (10^{-6} M).

	TREATMENT				
Muscle	5-HT 10^{-6}M	ATR 10^{-6}M WO	5-HT 10^{-6}M	PHENT 10^{-5}M	127 mM K^+
1	474	377	401	192	1108
	(100)	(79)	(84)	(40)	(233)
2	2466	2466	2250	1416	1166
	(100)	(100)	(91)	(57)	(47)
3	1363	1258	1195	734	1279
	(100)	(92)	(87)	(53)	(93)
4	1400	1373	1346	538	1965
	(100)	(98)	(96)	(38)	(140)
5	1114	1114	1114	131	1269
	(100)	(100)	(100)	(11)	(113)
6	738	649	531	125	1092
	(100)	(88)	(72)	(17)	(148)
7	1451	1463	1609	1219	1463
	(100)	(100)	(110)	(84)	(100)
$\bar{X}$	1286	1242	1206	622	1334
SE	240	252	238	199	115
$\bar{X}$	(100)	(94)	(92)	(43)	(125)
SE	0	2	5	9	22

TABLE 25 Isometric tension (g/cm^2) developed in a cumulative dose addition as in Table 22 appendix, except the initial agonist used is 5-hydroxytryptamine (5-HT 10^{-6} M). Values in brackets are percentages of the initial contraction (100%) produced by exposure to 5-HT (10^{-6} M).

	TREATMENT					
Muscle	5-HT 10^{-6}M	PHENT 10^{-5}M	PHENT 10 min 10^{-5}M	WO	5-HT 10^{-6}M	ATR 10^{-6}M	127 mM K^+
1	478	168	260		232	105	1231
	(100)	(35)	(54)		(48)	(22)	(257)
2	1537	225	806		656	431	2156
	(100)	(14)	(52)		(42)	(28)	(140)
3	2557	852	681		149	149	2855
	(100)	(33)	(26)		(5)	(5)	(111)
4	1394	435	1024		174	163	1525
	(100)	(31)	(73)		(12)	(11)	(109)
5	1509	581	1054		336	127	1600
	(100)	(38)	(69)		(22)	(8)	(106)
6	821	443	197		115	106	1183
	(100)	(54)	(24)		(14)	(13)	(144)
7	1942	1311	252		252	252	1917
	(100)	(67)	(12)		(12)	(12)	(98)
8	901	300	240		52	52	1006
	(100)	(33)	(26)		(5)	(5)	(111)
$\bar{X}$	1392	539	564		245	190	1562
SE	235	134	130		66	44	277
$\bar{X}$	(100)	(38)	(43)		(21)	(14)	(135)
SE	0	6	8		6	2	18