CANINE PULMONARY BLOOD VESSELS: PHARMACOLOGICAL AND IMMUNOLOGIC STUDIES

A THESIS

presented to the

University of Manitoba

in partial Fulfillment of the Requirements

for the Degree of

Master of Science

by

Siow-Kee KONG

April 1981

CANINE PULMONARY BLOOD VESSELS: PHARMACOLOGICAL AND IMMUNOLOGIC STUDIES

BY

SIOW-KEE KONG

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Though rare sudden death can occur on an acute asthmatic attack, its cause has not been elucidated, but quite apart from acute respiratory failure. The possibility exists that it could be cardiovascular. Because of these considerations, we undertook this study to determine whether anaphylaxis of pulmonary blood vessels developed in specific ovalbumen allergy. To this end, pharmacological and immunological studies were undertaken.

Pulmonary artery (PA) and vein (PV) from an ovalbumen-sensitized (S) canine model of allergic asthma showed hypersensitivity, a leftward shift of the dose-response curves, and hyper-reactivity (upward shift) to histamine, in in vitro study when compared to those from littermate control. Dose-response studies showed that sensitized pulmonary vein, but not artery, exhibit hypersensitivity but not hyper-reactivity to norepinephrine, when compared to the control. Both sensitized and control pulmonary arteries and veins displayed no difference in serotonin dose-response curves. The Schultz-Dale reaction was only observed in the sensitized pulmonary vein; it could be blocked (25-45%) by pyrilamine maleate (10^{-7}M) and 5-15% by phentolamine ($5x10^{-6}$M). We concluded that anaphylaxis develops only on the venous side of the pulmonary circulation but that increase in sensitivity or reactivity to agonists develops in both arteries and veins. Pretreated with pyrilamine maleate, histamine (10^{-6} to 10^{-5}M) can partly relax the active tension development of pyrilamine-pretreated pulmonary veins to high K^+ solution (48.2 mM). This suggests that H_2 receptors do exist in the pulmonary vein. Histamine appears to be the major mediator released and we have obtained evidence to suggest it exerts its contractile effect on the muscle via both the post-synaptic (directly) and the pre-synaptic (indirectly) membrane.

ACKNOWLEDGEMENTS

First of all the author would like to give gratitude to his mother, brothers, and sisters for their helping and supporting throughout his graduate studies in Canada, for the last two years.

The author is so deeply indebted to Professor N.L. Stephens (Steve), the best teacher he has every met, for supervision of his graduate student training, and more importantly for his understanding and friendship. The author is greatly influenced by Steve not only in science but also in philosophy. The voice of Luciano Pavarotti, and the summer fishing make them understand each other more and more....

The author would also like to thank Drs. E.A. Kroeger and C. Greenway for their aid and constructive criticisms throughout this research project as well. He is very appreciative of the friendship and helping from the members of "Profound Muscle Lab", they are Jerry, Richard, Blanca, Virgil, Subah, David, Edwin and Steve.

He would also like to say thanks to Cindy Wong and Janet Greer for the assistant of the computer program and the excellent typing of this dissertation, respectively.

Finally, the author would like to express his profound thanks to his girlfriend, Peng-Suat, for her encouragement and love. She is the most fascinated girl the author has ever seen.

This thesis is dedicated to the author's mother.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

CANINE PULMONARY BLOOD VESSELS: PHARMACOLOGICAL AND IMMUNOLOGICAL STUDIES

A) GENERAL INTRODUCTION

While considerable work has been done on the lung and airways in asthma relatively little has been done on the cardiovascular system. Though rare sudden death can occur on an acute asthmatic attack. Its cause has not been elucidated, but quite apart from acute respiratory failure. The possibility exists that it could be due to cardiovascular. Experiments carried out in intact sensitized dogs (Feigen et al, 1960), suggested that the documented reduction in coronary flow on systemic challenge with specific antigen is due to coronary vasoconstriction. Anaphylactic contraction of pulmonary blood vessels of chicken (Chand and Eyre, 1977) and bovine pulmonary vein (Eyre, 1971) have been shown in in vitro. This indicates that pulmonary arteries and pulmonary vein can be directly involved in the antigen-antibody reaction. However, physiologically, these animals are not so close to human being. In physiology, dog is often considered very close to humans especially in asthma, the antibody (IgE) which binds to the mast cell, and the allergic releasing mediators are same as in man. Becuase of this, the objective selected for this study was the delineation of the pharmacological properties of sensitized canine pulmonary blood vessels despite of what most researchers believe that airway smooth muscle is the primary target for asthmatic attack. The asthmatic airway smooth muscle is extremely sensitive to physical, chemical and pharmacological stimuli. The resulting reversible obstruction is associated with increased resistance to breathing, disturbance in distribution of ventilation and impairment of gas exchange.

In any sensitized tissue whether pulmonary airway or blood vessels, the classical thoery of antigen-antibody reaction states that in human patients, IgE antibodies are manufactured in response exposure to a specific antigen. These IgE molecules then migrate to mast cells distributed in the various portions of the pulmonary arteries and pulmonary veins, and attach to their surface via their FC endings. Repeated exposure results in antigens diffusing into the pulmonary tissue where they contact the antibodies. The IgE-antigen interaction on the mast cell surface activates internal enzyme system to release chemical mediators. The clear activities of this interaction are as follows: 1) Great specificity and sensitivity to sensitizing antigen is present 2) Between antigen challenge and appearance of the response there is a latent period of a few seconds to several minutes. This greatly varies from tissue to tissue, animal to animal and antigen to antigen. 3) A narrow dose-response relationship can be established on the same tissue, but usually two or three challenging doses of antigen given in close succession cause tachyphylaxis (desensitization) to antigen without altering the responsiveness of the smooth muscle preparation to histamine or other known spasmogens. If an animal has been sensitized to two or more different antigens, tachyphylaxis to one does not normally interfere with the responsiveness of the tissue to the other. However, cross tachyphylaxis among closely related sensitizing antigens has frequently been observed. 4) After desensitization allowing the tissue to "rest" for 1 to 3 hours results in partial to complete recovery of antigen response. 5) The sensitizing antigen itself should cause no direct pharmacological action on the smooth muscle over a wide dose range. 6) Tissues usually

exhibit slow relaxation from antigen-induced contraction, even with frequent washings.

Several things have been put forward to explain allergic bronchospasm. The irritant receptor: (IR). In asthmatic models, bronchial hyperirritability (Nadel, 1965) might be due to a lowering of the threshold, or an increase in the response of certain pulmonary vagal receptors or perhaps adrenergic receptors. Widdicombe (1977) suggests that the IR is stimulated by transmitters released as the result of IgE-mediated antibody-antigen reactions. It is hypothesized that the chemical mediators released by the mast cells act on the nervous receptors in the pulmonary tissue (Gold et al 1972). The smooth muscles of pulmonary blood vessels are richly supplied with adrenergic vasoconstrictor fibers. This involves large and small arteries, large arterioles, and the venous compartment, though the smooth muscle cover here is scant, and in some species almost absent. It is further known that the adrenergic transmitter, noradrenaline (norepinephrine) via α-receptor, can considerably enhance the flow resistance and decrease the blood volume of the lungs. It is possible that the adrenergic receptors of pulmonary blood vessels, sensitized to antigens, may increase their response during the antigen-antibody reaction.

Pharmacological studies in the airway smooth muscle (Antonissen et al 1980) have shown that sensitized tracheal smooth muscle is hypersensitive and hyper-reactive to histamine when compared to the control tracheal smooth muscle. The increased sensitivity of this sensitized tissue may be due to: 1) the presence of drug receptors which have the same affinity for the agonist but produce a greater response through a higher intrinsic activity of the agonist, 2) an increased number of drug receptors with

the same affinity and intrinsic agonist activity, so an additive cellular action (i.e., increased active tension development) is observed. The hyperreactivity phenomenon can be caused by 1) increasing the contractile protein, actin-myosin, so more cross-bridges will be formed 2) increase the calcium channels via the sarcolemma or the internal compartments eg. SR (sarcoplasmic reticulum).

Apart from these main avenues of research, a considerable amount of research has been performed to determine the role of genetic factors in the development of asthma. It has been generally concluded that the non-genetic factors discussed above, play a much greater role in the etiology of asthma than genetic factors; these perhaps being responsible more for the observed heterogeneity of the disease (Bias and Marsh, 1977).

B) THEORIES REGARDING MECHANISM OF ANTIGEN ACTION IN SMOOTH MUSCLE

1) Physical Theory:

A direct action of the specific antigen on the excitable membranes of sensitized smooth muscle cells induced contraction (Dale, 1920). This hypothesis was suggested by Dale (1952) who also indicated that smooth muscle cells are themselves sensitized. Note that antigen-antibody interaction are not involved.

Antigen - antibody reaction may produce alteration in the ionic permeability of the smooth muscle cell membrane and cause muscular contraction without pharmacological mediators necessarily being involved (Schild, 1956; Geiger, 1956; Alonsi-deflorida et al, 1968; Cirstea, 1970; Guschin, 1975).

On the basis of the "physical or membrane" or "biophysical" hypothesis of mode of action of antigen, the intensity and time (Cirstea, 1970) of

anaphylactic reaction is principally determined by the action of antigen-antibody complexes on the smooth muscle membrane which in turn depends upon the antigen binding capacity of the corresponding antibody. Guinea-pig taenia-coli which lacks mast cells shows anaphylactic contraction (Guschin, 1975) preceeded by depolarization and increased spontaneous activity. It has been pointed out that the presence of "local" mast cells is not an absolute prerequisite for the anaphylactic reaction of smooth muscle (Guschin, 1975; Katsch, 1958).

2) Chemical theory:

Release of pharmacologically active substances (principally histamine) from tissue-fixed mast cells by antigen-antibody reaction is generally considered to be the main initiating event for anaphylactic contraction of sensitized smooth muscle (Dale and Zilletti, 1970; Joiner et al, 1974). Histamine was the first mediator to be associated with anaphylaxis (Dale and Laidlaw, 1910). Bartosch et al (1932) demonstrated that isolated lungs of a sensitized guinea pig release histamine in anaphylaxis, with Schild et al (1951) demonstrating the same phenomenon in a human asthmatic lung. Experiments have shown that mast cells degranulate and release histamine in allergic dogs when challenged by antigen aerosols (Meyers et al, 1973; Gold et al, 1977). Histamine H_1 antagonists have been reported to inhibit partially (Joiner, et al, 1974; Hawkin and Rosa, 1956) or completely (Geiger et al, 1956; Dale and Zilletti, 1970; Eyre, 1971; Lulich et al, 1976) anaphylactic reactions in a number of tissues. Schild (1956) suggested that mast cell degranulation in smooth muscle by antigen-antibody reaction creates a histamine gradient and that the magnitude of anaphylactic muscular contraction may depend upon the number of smooth muscle cells reached

by effective concentrations of histamine released. Thus on the basis of these considerations it seems fairly certain that products released from the antigen-antibody reaction are responsible for allergic asthma.

Ash and Schild (1966) were the first to postulate the existence of more than one type of receptor for histamine. Eyre and Wells (1973) described H_1 contractile and H_2 relaxant receptors in the pulmonary circulation of the calf; the histamine H_2 receptors were able to antagonize the strong H_1 effect in systemic anaphylaxis. Histamine induced contractions of airway smooth muscle are antagonized by H_1 anti-histamines (Castillo et al, 1947). Eyre (1973) recently was also able to identify a specific relaxant H_2-receptor action of histamine on the isolated airway smooth muscle of cat and sheep. H_2 receptor blockers have successfully been used as well to potentiate IgE-mediated release of histamine and SRS-A by hindering the operation of an autoregulatory H_2-receptor in the immunologic release of mediators from sensitized mast cells. The H_2 receptor apparently stimulates adenylate cyclase in its action (Lichtenstein and Gillespie, 1973). Asthmatics may then differ from normals in that their H_1/H_2 receptor ratio may be larger, resulting in increased irritability to histamine stimuli in allergic asthma.

Other chemical mediators released from the sensitized lung tissues by antigen include prostaglandins. These appear to be synthesized, released and inactivated by the lung; they have been recovered from lung perfusates from sensitized and challenged guinea pigs (Piper and Vane, 1969). Apparently both PGE and PGF types are released (Gold, 1973).

PGE's generally dilated blood vessels and airways while PGF's constrict. However, PGE_2 has also occasionally been shown to be a bronchoconstrictor, particularly in asthmatic patients (Smith, 1974; Hedqvist and Mathe, 1977). Because of this discrepancy, it is now suggested that prostaglandins may play a messenger role between cells, the level of prostaglandins being related to cyclic GMP levels, as any concommitant rise in cyclic AMP (Stoner et al, 1977) can be blocked by indomethacin (Farmer et al., 1974).

PAF (platelet activating factor) is released from lung tissue during anaphylaxis in experimental animals and from passively sensitized human lung in vitro (Gold, 1976). PAF cause platelets to aggregate and release serotonin (5-HT), an airway smooth muscle and pulmonary vascular smooth muscle constrictor. Another chemotactic factor, NCF-A (neutrophil chemotactic factor of anaphylaxis), augments local inflammation, due to the activation of lysosomal enzymes as neutrophils are attracted to the antibody-antigen reaction site. There is some evidence as well for the release of prostaglandin endoperoxides or thromboxane A_2 from anaphylactic lung (Gryglewski et al., 1976).

Local eosinophil infiltration is common in asthmatic lungs, the cells being attracted to the site of allergic response by a substance called ECF-A (eosinophil chemotactic factor of anaphylaxis. Upon IgE interaction with antigen (Kay et al., 1971) histamine release from the target cells occurs as a preformed mediator. Eosinophil infiltration may represent a protective measure, since eosinophils contribute substances which eliminate products of the mast cell allergic response. Preliminary data suggest they

contain a histaminase which participates in histamine inactivation and an arylsulphatase which inactivates SRS-A (Gold, 1976).

SRS-A (slow reacting substance of anaphylaxis) is a low molecular weight, unsaturated acidic sulphate ester which is capable of contracting bronchial smooth muscle in very low concentrations. Its delayed bronchoconstrictor effect is a result of the fact that it must only be released by the mast cell but also be manufactured in response to the antigen-antibody reaction (Wasserman et al, 1973). The releasing of SRS-A is resistant to the effect of antihistamines, studies indicating that the release of SRS-A is modulated by levels of cyclic nucelotides (Orange et al, 1971; Kaliner et al, 1972).

3) Neuronal theory of anaphylaxis

The possible involvement of the cholinergic nerve in the anaphylactic response has been suggested (Geiger et al., 1956; Geiger and Alpers, 1957; Dale and Zilletti, 1970). Agents which interfere with the conduction of nerve impulses, eg. ethyl alcohol, n-butyl alcohol, urethane and nupercaine, block the Schultz-Dale anaphylactic contraction of guinea-pig ileum without interfering with contractions to histamine and acetylcholine (Geiger et al., 1956). Botulinum toxin blocks anaphylactic contraction. This mechanism may be by specifically paralysing the post-ganglionic cholinergic fibers (Geiger et al., 1956). However, resistance of anaphylactic contraction to ganglion blocking agents (hexamethonium and benzoquinonium) (Geiger et al., 1956) and to tetrodotoxin (Dale, and Zilletti, 1970) suggests that antigen does not directly stimulate ganglia (Geiger, et al., 1956) and plexises (Dale and Zilletti, 1970) of the guinea-pig ileum.

4) Lymphocyte theory of anaphylaxis

Katsh (1958) reported that guinea-pig vasa deferentia and seminal vesicles (devoid of lymphocytes and plasma cells) do not contract to specific antigen challenge, whereas ileum and uterus (rich in lymphoid tissue) exhibit strong Schultz-Dale reaction. On the basis of these findings, Katsh (1958) concluded that specific sensitizing antigen releases some factor(s) from the antibody producing cells (plasma cells and lymphyocytes) in the sensitized tissue which then stimulate(s) smooth muscle to contract. But it should be borne in mind that tissues such as uterus, seminal vesicle and vasa deferentia are also rich in mast cells.

None of the above mentioned hypotheses alone satisfactorily explains the exact mode of action of antigen in contracting sensitized smooth muscle. The mechanisms may have several component parts and may well vary between tissues and between species. However, today, most researchers believe the mediators, released during the Schultz-Dale reaction, cause the smooth muscle to contract.

C) MECHANISM OF MEDIATOR RELEASE IN THE SCHULTZ-DALE REACTION IN ALLERGIC ASTHMA

In allergic asthma, the immune response is mediated by IgE, an immunoglobin which is synthesized by the systemic immune system. In vitro studies (Ishizaka and Ishizaka, 1971) have established that IgE molecules have an affinity for tissue mast cells and basophil granulocytes, and combine with these target cells through the FC portion of the molecule. When the appropriate antigen is introduced, the antibody-antigen interaction triggers the secretion of anaphylactic mediators from the sensitized target cells. Mast cells in the bronchial epithelium of human

asthmatics, for example, have been shown to degranulate in the active phase of the disease (Salvato, 1976).

A variety of low molecular weight chemical mediators are released by antigen-antibody interaction, including histamine, SRS-A (slow reacting substance of anaphylaxis), ECF-A (eosinophil chemotactic factor of anaphylaxis), prostaglandins, thromboxanes, various kinins, 5-hydroxytryptamine and others. (Wilson and Galent, 1974; Eyre, 1972). The Schultz-Dale reaction has also been found to exist in bovine pulmonary smooth muscle (Eyre, 1970; Eyre, 1971; Eyre, 1972; Chand and Eyre, 1977). The secretory process involved a calcium dependent phase in which a diisopropylfluorophosphate sensitive esterase is activated, followed by a glycolytic energy-dependent stage, which is perhaps necessary for microtubular contractile proteins to extrude mediators. Exocytolysis of the chemical-containing granules occurs in the final calcium-dependent phase. The chemicals undergo ion exchange during release into the surrounding fluid.

The release of mediators, particularly histamine and SRS-A from mast cells and basophils, appears to be modulated by the level of cyclic nucleotides. Orange et al (1971) showed that increased levels of cyclic AMP suppressed IgE-mediated release of both histamine and SRS-A from sensitized monkey lung, while beta-adrenergic blockade (decrease cyclic AMP levels) enhanced the release of both mediators. Bergstrom (1967) showed that prostaglandins (notably PGE1 and PGE_2) are the activators of adenylate cyclase in a number of in vitro systems. In many situations elevation of cyclic AMP by adenylate cyclase activation is associated with

suppression of secretion. Prostaglandin E_1 and prostaglandin E_2 decrease secretion of anaphylactic mediators in a dose-related manner and this action is mirrored by changes in intracellular cyclic AMP (Lichtenstein 1973; Tauber et al, 1973). There is a good correlation between the rank order of potency of prostaglandins A,B,E and F as inhibitors of histamine release from Leukocytes and as stimulators of cyclic AMP production, (Lichtenstein, 1973). As a consequence of this it is easy to see why indomethacin enhances allergic mediator secretion in a number of experimental models of anapylactic response (Walker, 1972; Engineer et al, 1978; Marone et al., 1979). Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) were shown to be potent inhibitors of the enzyme cyclo-oxygenase, the pivotal enzyme for production of various prostaglandins from arachidonic acid (Vane, 1971). Thus, aspirin-sensitive asthma (ASA) is a well defined clinical entity in which bronchospasm can be induced by ingestion or inhalation of aspirin or other NSAIDs, and that their rank efficacy in inducing bronchospasm corresponds to their potency as inhibitors of prostaglandin synthesis (Szczeklik et al., 1975). Indirect evidence also suggests that increased cyclic guanosyl-monophosphate (cyclic GMP) augments antigen-induced secretion of mediators. Observations are, in general, consistent with the view that relaxation of smooth muscle is associated with an elevation of the cyclic AMP content of the tissue; a relationship between cyclic GMP and contraction has also been suggested (Shultz et al., 1973). Pulmonary vascular smooth muscle contraction and relaxation may be initiated and/or modulated by altered concentrations of these cyclic nucleotides.

D) THEORIES OF DESENSITIZATION AND RECOVERY OF ANTIGEN-ANTIBODY REACTION

There are several factors which can cause the vascular smooth muscle to become desensitized to the antigen-antibody reaction: 1) antigen-antibody complexes are biologically active only for a brief time although they are structurally intact for long periods. Thus, subsequent antigenic challenge results in the formation of biologically inactive antigen-antibody complexes of alternatively complexes which are already formed may interfere with subsequent interaction of antigen with the tissue fixed antibodies (Dale and Okpako , 1969). 2) occupancy of all the antigen-combining sites on the corresponding antibody leads to desensitization to second antibody challenge. Katsch (1958) showed that guinea-pig ileum, rich in antibody forming cells - lymphoid tissues, plasma cells and lymphocytes - needs several frequent antigen challenges or prolonged antigen exposure before desensitization takes place, while guinea-pig uterus which is poor in lymphoid tissues, is desensitized easily compared to the ileum. 3) Temporary exhaustion of the preformed or stored mediators (biologically active substances) from the target cells (mast cells) in vascular smooth muscle may also lead to densensitization to subsequent anaphylactic reaction (Feldberg, 1961). 4) Antigen-antibody combination results in the activation of an enzyme system which acts as a catalyst in this reaction leading to the release of histamine and muscular contraction. The active enzyme is short-lived (Mongar, and . Schild, 1957) resulting in a desensitization to subsequent antigen challenge. 5) Release of some inhibitory substance(s) upon second antigen challenge may be responsible for biphasic (relaxant-contraction)

or relaxant response in guinea-pig trachea and ileum (Alonso-Deflorida, F. and F. Cordoba, 1965).

Dale (1965a, 1965b), Okpako (1970), Eyre (1971), Chand and Eyre (1976), and Swineford and Reynolds (1951) have reported that desensitization is reversible, but it is also not an absolute phenomenon (Dale, 1965a; Okpako, D.T., 1970; McEwen, L.M., 1959; S. Wineford and Raynolds, 1951). However, if uterine, intestinal, and vascular smooth muscle (Swineford and Raynolds, 1951; McEwan, 1958; Dale, 1965a,b; Dale and Okpako, 1969; Eyre, 1971; Eyre and Change, 1976, 1977) are allowed to 'rest' for one to three house (Dale and Okpako, 1969) with frequent washings, after desensitization, partial or complete recovery of the anaphylaxis occurs.

Based on the above, one can speculate on mechanisms to explain the 'recovery' of the anaphylactic response; the exact one is not known. Some of these are 1) restoration of ionic permeability changes in the membrane of the smooth muscle cells, or 2) replenishment of the exhausted store of pharmacologically active substance (eg, SRS-A, prostaglandin) in the mast cell or lymphocytes.

E. SMOOTH MUSCLE MECHANICS - ISOMETRIC CONTRACTION

When striated muscle contracts isometrically, the total tension (TP) is equal to the sum of the resting tension (RP) and active tension (AP). The maximum active tension (Po) is a measure of the number of actomyosin bridges formed during contraction and represents the sum of the tensions developed at each cross-bridge. More bridges per unit cross-sectional area result in a higher Po. The maximum isometric force developed per unit cross-sectional area of muscle is a common and desirable normalized index of the contractile function of muscle. Smooth muscle behaves qualitatively

in the same way but since sarcomeres have not been clearly identified, it is not exactly correct to try to explain force-development by actomyosin in such muscle on the basis of sliding filament cross-bridge theory of contraction. However because of the striking similarities in the length-tension and force velocity curves of the two muscles it is not unlikely that a similar system of force generating sites also present in smooth muscle.

In studies where the effect of drugs on the mechanical function of muscle is to be compared, a convenient and commonly used test parameter is isometric tension. Differences in the dose-response curves elicited provide information as to differences in mechanical function of muscle and afford insight into the mechanics responsible.

In carrying out such dose-response studies, attention must be paid to holding all variables constant except the one under study. For example, it is important that muscles being compared should be at comparable lengths. A convenient length is the optimal length of the muscle(Lo)which can be easily determined by carrying out a limited length-tension manouevre.

Hargraves and Weiss (1977) have suggested that comparing dose-response data obtained from muscle at different lengths may not be valid. This is so because of variable effects of stretch on membrane stability. The deformation of the muscle may also affect the availability of calcium pool target sites to agonists. This is even more critical in comparing different drugs, since they often differ in their mechanisms of action. The Lo length (that length at which maximal contractile response develops), used for studies of the effects of various agents on isometric tetanic

tension, however, is the most consistently identifiable muscle length on the length-tension curve. It provides a more sensitive preparation for studying muscle function under varying conditions, as the contractile response is at a maximum.

F. BIOCHEMISTRY OF SMOOTH MUSCLE

Evaluation of changes in mechanical properties of sensitized smooth muscle requires some understanding of the underlying biochemical processes. It is for this reason that a brief description of smooth biochemistry now follows. It deals with the biochemistry of excitation-contraction coupling and of actomyosin interaction.

It has been well established that the mechanical activities of muscle cells, including the vascular smooth muscle cell, are controlled by myoplasmic calcium concentration (Ebashi and Endo, 1968). At rest, intracellular calcium is maintained at an extremely low level and upon stimulation it is introduced into the cell from various sources (Van Breemen et al, 1973), such as: 1) external medium through the cell membrane and, 2) mobilization of membrane bound calcium. Yamashita et al (1977) found at least three kinds of such calcium binding sites on the external and internal surface of the cell membrane, a) loosely and b) tightly bound calcium on the external surface the cell membrane, c) calcium localized on the internal surface of the cell membrane; 3) release from an internal storage site (e.g., sarcoplasmic reticulum, SR) (Devine et al, 1972). Regardless of the source, once the intracellular Ca^{2+} concentration rises to an adequate level the smooth muscle contracts. The utilization mechanism of activator calcium might be complicated because the rate of calcium release from the source may depend on the nature of stimulus as well as the kind of muscle (Gabella, 1973; Disalvo and Schmidt, 1976;

Hudgins and Weiss 1968); moreover, these calcium stores seem to be functionally interrelated with each other.

Actin and myosin are present in smooth muscle as in striated muscle in the form of thin and thick myofilaments lined up with each other and roughly parallel to the longitudinal axis of the cell (Somlyo and Somlyo, 1975). The actin filaments in smooth muscle insert on so-called dense bodies which are analogous to the Z disc of striated muscle (Ashton, et al., 1975). Smooth muscle actin is a globular protein with a molecular weight of 42,000. In the contractile apparatus actin exists as long filaments composed of double stranded helical polymars of globular subunits. In its filamentous form actin is associated with a fibrous protein, tropomyosin, which has a molecular weight of 66,000 and is composed of two subunits. Tropomyosin molecules align themselves end to end in the groove of the actin helix. At present no specific function has been assigned to tropomyosin in smooth muscle, although in striated muscle it acts with troponin to mediate calcium regulation of the actin-myosin interaction.

The myosin filaments in vascular smooth muscle are longer than in striated muscle, and in cross-sections they are often seen to be surrounded by an array of 15-18 thin filaments (Ashton, et al., 1975). Whereas striated muscle shows a regular hexagonal arrangement, many actin filaments in smooth muscle appear not to come near any thick filament along their entire length. These ultrastructural features fit with biochemical data which show that arterial smooth muscle contains more actin but considerably less myosin per unit cell weight than striated muscle (Murphy et al, 1974).

Smooth muscle myosin has a molecular weight of 460,000 and is composed

of three pairs of polypeptide chain: one pair of heavy chains with molecular weights of 200,000 and two pairs of light chains with molecular weights of 20,000 and 15,000. In muscle cells the individual myosin molecules are aligned into filaments arranged so that a portion of the molecule (called the head) extends toward the actin filaments and is available for interaction.

Myosin is also an enzyme that catalyzes the hydrolysis of ATP to ADP and Pi with liberation of heat and mechanical energy. In the absence of actin, hydrolysis proceeds very slowly, but in the presence of actin, under certain conditions, the hydrolysis of ATP increases 100-fold. The energy liberated is used to slide actin filaments along myosin filaments, thereby developing shortening. Calcium is required to initiate the interaction of actin and myosin in smooth muscle and striated muscle, although by different mechanisms.

A fundamental difference between striated and smooth muscle myosins is that smooth muscle myosin requires a modification of one pair of its light chains, the 20,000 dalton light chain, in order for actin- activation of myosin ATPase activity to occur (Adelstein, 1978). This modification, which is under enzymatic control, consists of the addition of phosphate (donated by ATP - Adenosine triphosphate) to a particular series residue on the myosin molecule. Myosin phosphorylation is a prerequisite for actin activation of smooth muscle myosin ATPase activity (Gorecka et al., 1976; Chacko et al., 1977; Sobieszek and Small, 1977).

Phosphorylation of myosin is catalyzed by a specific enzyme, myosin light chain kinase, and the reverse reaction, which restores myosin to its unphosphorylated form, is controlled by the enzyme myosin light chain phosphatase (Gorecka et al, 1976; Chacko et al., 1977; Sharry et al., 1978).

In smooth muscle, where myosin phosphorylation is required for interaction with actin, calcium initiates contraction by activating the enzyme myosin light chain kinase (Sobieszek and Small, 1977). The mechanism by which calcium regulates smooth muscle myosin kinase has recently been defined (Dabrowska et al., 1978); it requires the interaction of two proteins, one with a molecular weight (Adelstein et al., 1978) of 125,000 and a smaller protein of 16,000 daltons. Whereas the larger protein governs the specificity and unique ability of the enzyme to catalyze the transfer of phosphate from ATP to myosin, the smaller protein, named calmodulin, mediates the effect of calcium.

In smooth muscle, when the intracellular calcium level is 10^{-8} molar or less, calmodulin may not bind to the myosin light chain kinase and the enzyme is inactive. When calcium level rises to 10^{-6} molar, calcium binds to calmodulin, and the calcium-calmodulin complex binds to myosin kinase, resulting in activation of the enzyme. The activated enzyme phosphorylates the 20,000 dalton light chain of myosin, and finally actin and myosin interact to produce contraction. When the calcium concentration is lowered to resting levels, calmodulin dissociates from myosin kinase and myosin phosphorylation ceases.

Cycle AMP (adenosine 3',5' cyclic monophosphate) may enhance calcium uptake by membrane fractions, accelerate calcium efflux from smooth muscle cells and prevent transmembrane influx of calcium (Anderson and Nilsson, 1977). The cyclic AMP increase can be due to the stimulation of adenylate cyclase, the enzyme that converts ATP to cyclic AMP, or to inhibition of phosphodiesterase. Myosin light chain kinase, which regulates phosphorylation of myosin,

itself by phosphorylated by another kinase, regulated by cyclic AMP phosphorylation. The intracellular calcium level increase will cause more calcium binding to the calmodulin, and this could trigger the adenylate cyclase to increase the cyclic AMP level in the cell. Myosin light chain kinase, which regulates phosphorylation of myosin, can itself be phosphorylated by another kinase, regulated by cyclic AMP. Phosphorylation of myosin light chain kinase results in two to three fold decrease in the activity of this enzyme (Adelstein et al., 1978). The net effect of phosphorylating myosin light chain kinase is to prevent phosphorylation of the 20,000 dalton light chain of myosin. Because unphosphorylated myosin cannot interact with actin, smooth muscle relaxation ensues.

G. DEFINITION OF THE PROBLEM

The postulation that there may be a single primary defect to explain the pathophysiology of asthma has led to considerable investigation of the pathogenic role the immune and nervous systems may play in this disease. Evidence implicating any one such factor is lacking, however. While conceding the possibility that the pulmonary vascular smooth muscle of asthmatics may be abnormal in the pharmacological sense (Widdicombe, 1977), investigators still concentrate upon the trigger mechanism in allergen induced attacks. The hypotheses just described illustrate this fact.

As a result, very little is known about the pulmonary vascular smooth muscle in asthma-surprising when one considers that it is the tissue mobilized during as asthmatic shock. The few in vitro studies of pulmonary vascular smooth muscle in asthma have dealt with the effects of drug or other agents-neural and anaphylactic - on the smooth muscle, illustrating the preoccupation with investigation of triggering mechanism. It therefore

becomes important to determine if a specific vascular smooth muscle defect is present in asthma. In studying the way the pathological condition of asthma affects pulmonary vascular smooth muscle physiology, investigations logically should be conducted in terms of the muscle's function and the mechanism by which the control of that function is normally effected. Muscle function includes, first of all, the ability to stiffen and bear weight, and secondly to shorten and move a load through a distance.

Pulmonary vascular smooth muscle contractility depends upon the interrelationship of local chemical and reflex effects. A hypersensitivity of asthmatic pulmonary vascular smooth muscle to various mediators could result from a defect in processes such as membrane excitability, excitation-contraction coupling, the contractile machinery with its associated energy utilizing reaction, or one of the components of the pharmacologic receptor systems. Various sites of defect of dysfunction are possible. At the membrane level, hyperexcitabilities, a reduced threshold for the opening of sodium/calcium channels, or a greater number of channels engaged in excitation may be responsible. Thus a muscle could become hypersensitive and/or hyperreactive to various stimuli. Excitation-contraction coupling changes could result in greater amounts of calcium released after excitation, such that active tension is increased. Another possibility is that a defect in calcium sequestration systems might terminate in a muscle which displays relaxation abilities. Abnormalities in the contractile proteins and the associated enzymes may result in altered active tensions or shortening velocities, as well as shortening capacities. For example, an increase in the number of cross-bridges would result in higher active tensions developed and the presence of abnormal enzymes might result in

altered velocities of shortening. The structure of the contractile element and other cellular aspects associated with contraction may change to affect shortening capacities.

If all the mediators and other agents which induce pulmonary blood vessels constriction under normal circumstances, demonstrate increased activity during an asthmatic attack, the smooth muscle itself must be hyperexcitable. Presumably, if the defect lies in the contractile element above, than all agents (including anaphylactic mediators) which stimulate, would as well produce a hypersensitive response in the pulmonary vascular smooth muscle. However, the possibility also exists that the defect lies in only one of the systems mentioned and thus exhibits a great deal of specificity (Fleisch, 1973). This might be the case if the abnormality existed only in a specific receptor system. One must also keep in mind that an alteration in the mechanical properties of the muscle could stem from alterations in its intrinsic properties or from variations in control systems. It is not impossible that the changes in muscle activity are secondary to changes occurring in the nerve supply. The present studies will attempt to show that asthmatic canine pulmonary blood vessels are more sensitive and/or reactive in response to certain drugs (natural mediators) when compared to control canine pulmonary blood vessels.

H. THE EXPERIMENTAL MODEL

A canine model of allergic asthma (Kepron et al, 1977) was chosen to study the pharmacologic properties of pulmonary vascular smooth muscle and reveal its possible role in the pathogenesis of the circulatory changes occurring in asthma. Over the last few years our laboratory has been working with a canine model of hypersensitivity and completed studies of

Sphene occurs as very fine grained accessory xenoblastic grains.

Zircon occurs as very fine grained accessory xenoblastic grains.

5.2 OXIDIZED MINERALS

The oxidized minerals were identified by optics and hand specimen tests. They are present as coatings in fractures, along foliation direction, mineral grain boundaries, and in box works.

The oxidized minerals are: azurite, calcite, hematite, jarosite, limonite, malachite, and a fibrous white mineral either smithsonite or hemimorphite.

demonstrated that neither the acute nor the chronic lung volume changes in allergic guinea pigs reproduce changes observed in human asthma. The immunologic class of antibodies involved may differ as well - this particular issue has not been adequately resolved at this time.

While our model, at best, could serve as one for extrinsic asthma, if the fundamental defect is proven to be at muscle level and if the vascular manifestations of clinical asthma are merely due to differences in precipitating stimuli, then the canine pulmonary vascular smooth muscle model of extrinsic asthma will be of more general application.

I. EXPERIMENTAL PLAN AND SPECIFIC AIMS

Mongrel dogs were sensitized to ovalbumin (OA) and pulmonary artery (PA) and vein (PV) isolated from sensitized animals and their littermate controls. The following pharmacological studies were carried out on PA and PV strips.

1) Determination of whether the Schultz-Dale reaction occurs on specific challenge in sensitized PA and PV.

2) Elucidation of what mediator(s) were released during the antigen-antibody reaction.

3) Examination of the sensitivity of control and sensitized PA and PV to histamine and other agonists (eg, NE and 5-HT).

4) Determination of whether H_1 and H_2 receptor exist in the PV and PA and whether the H_1/H_2 ratio between the sensitized and control tissue was different.

5) Determination of whether desensitization to specific antigen occurred and if so whether it was possible to reverse it.

METHODS

METHODS

A SMOOTH MUSCLE SENSITIZATION PROCEDURE

The method developed by Pinckard et al (1972) for the induction of IgE antibody response and anaphylactic sensitivity to ovalbumin in rabbits was adapted to a canine model. For the induction of IgE antibody production, mongrel dogs received intraperitoneal immunization with 10 ug of a conjugate dinitrophenol and ovalbumin (DNP_2-OA) mixed with 30 mg of $Al(OH)_3$, within 24 hours of birth. Booster injections, consisting of the same dose, were repeated at weekly intervals for eight weeks, thereafter every two weeks. This regimen of immunization has been shown to induce prolonged IgE antibody production of high titers against both the DNP and OA determinants (Kepron, W. et al., 1977). Littermates of sensitized dogs were given injections of the adjuvant, $Al(OH)_3$, only (without DNP_2-OA) using the identical regimen, and these animals were used as controls. Pulmonary artery (PA) and vein (PV) were obtained from sensitized dogs whose PCA (passive cutaneous anaphylaxis) titers were at or above 128, in non-sensitized dogs the PCA titers were far below 64. In all experiments , the dogs were generally about 3 months old at this stage, this development of specific sensitivity was tested for by eliciting a Schultz-Dale response to specific antigen challenge.

B DISSECTION AND MOUNTING OF TISSUES

Mongrel dogs (both sensitized and control) were anaesthetized by intravenous injections of 30 mg/kg body weight sodium pentobarbitol (Nembutal, Abbott), followed by an intracardiac injection of saturated potassium chloride solution to kill the animal. The lungs together with the heart

were removed from the dogs, and placed immediately in a beaker of ice-cold Krebs-Henseleit solution. Excision usually was completed within five to ten minutes. Intrapulmonary artery and vein were dissected from the lung which was placed in cold Krebs-Henseleit solution.

The vascular strips were cut from the vessels at angle of 90^{o} relative to the long axis of the vessels (Herlihy, 1980). The 2 mm wide strips used for study were obtained from intrapulmonary vessels of 2-3 mm diameter. The strips were mounted in a muscle bath containing mammalian Krebs-Henseliet solution, the composition of which is given in Table 1. The solution was equilibrated with a 95% O_2 - 5% CO_2 mixture which maintained a PO_2 of 600 Torr, a PCO_2 of 40 Torr and a pH of 7.4, at a temperature of $37^{o}C$. For isometric studies, the lower ends of the muscle strips were attached by a short loop of 000 braided surgical silk to the hook at the end of the rigidly clamped aerating tube of the muscle bath (Fig. 1). The upper end was fastened to a Grass FT .03 force transducer mounted on a rack and pinion, enabling the muscle to be stretched to any desired length and held there isometrically. Output from the force transducer was amplified and recorded on a 4-channel Gould 2400 Brush recorder. Compliance of the system was negligible. The strips were equilibrated in the bath without any resting tension for two hours.

C PHARMACOLOGICAL STUDIES

It was necessary to first carry out a stimulus-response (Electrical stimuli, for PA and isosmotic KCL solution for PV so as to identify an appropriate supramaximal stimulus: Using this stimulus in a length-tension

Table 1: Composition of Krebs-Henseleit Solution

	mM	g/L
NaCl	115	6.72
$NaHCO_3$	25	2.10
NaH_2PO_4	1.38	0.167
KCl	2.51	0.187
$MgCO_4 \cdot 7H_2O$	2.46	0.296
$CaCl_2$	1.91	0.145
dextrose	5.56	1.00
osmolarity = 304 mOsM		

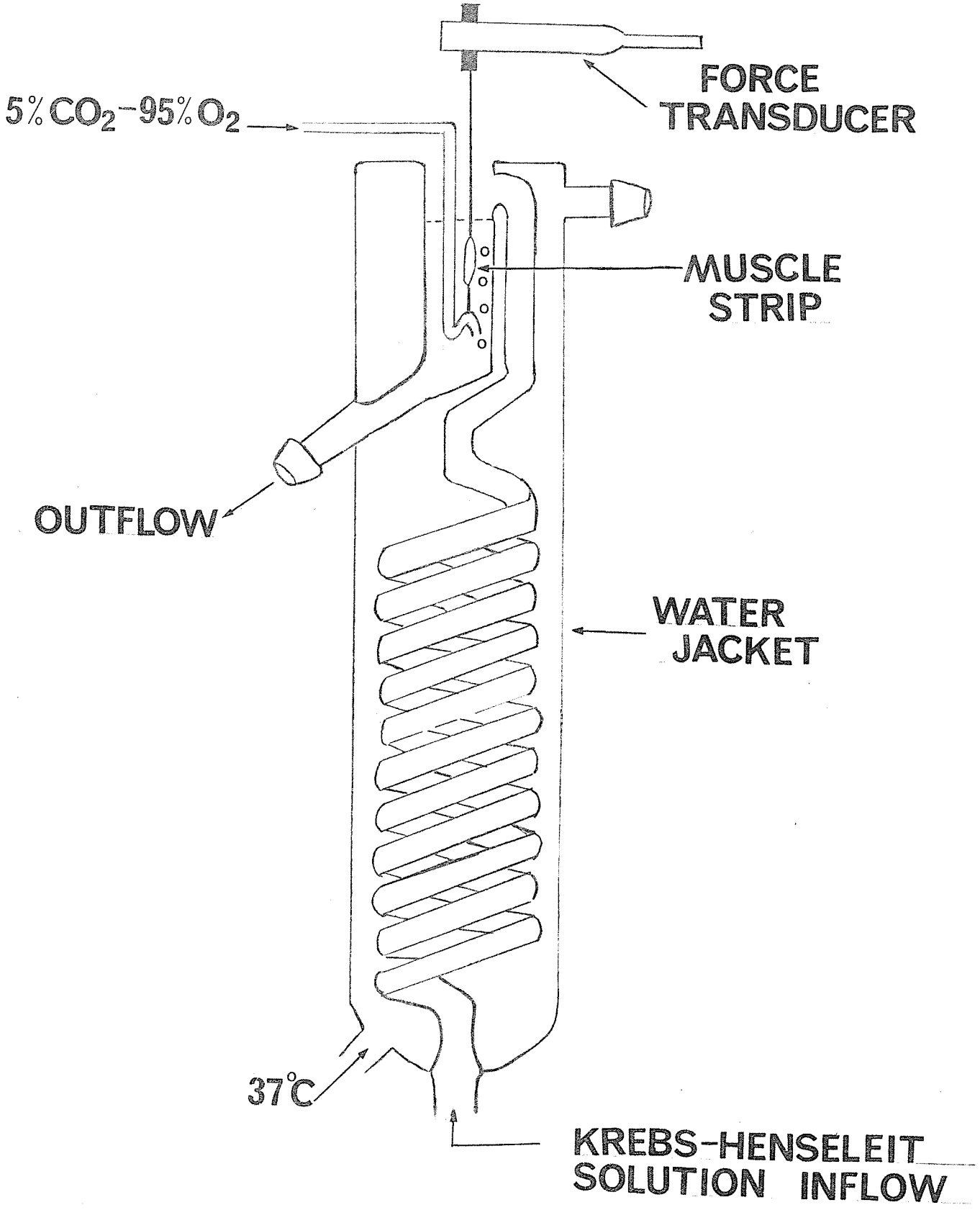

Fig. 1

Simplified schematic diagram of the apparatus used in isometric experiments, which allowed recording of isometric tension development under various stimuli.

study enabled determination of L_o for each muscle. All further immunologic and pharmacological studies were conducted at this length.

Histamine, norepinephrine and 5-hydroxytryptamine dose-response curves for sensitized and control PA and PV were obtained by adding successively higher half-log doses of the drugs to the muscle bath. Three to four rinses of fresh 37^{o}C Krebs-Henseleit solution followed the moment active tension reached a plateau for each dose. Contact time with the agonist was thus reduced. This diminished the development of tachyphylaxis.15 to 20 minutes elapsed before the introduction of the next dose.

The cross-sectional area of the muscle was estimated at the end of the experiment using the length of the tissue and its blotted wet weight, and assuming the density of the tissue to be one.

The maximum active tensions developed were recorded for each concentration and after appropriate normalization (maximum isometric tension in gm/cm^2 against the log of the dose), plotted to obtain dose-response curves.

The agonists and antagonists listed in table 2 was used for characterizing the histamine receptors and their properties.

Determination of the mediators released from the mast cells during antigen-antibody reaction was achieved by using the blockers like pyrilamine maleate and phentolamine.

TABLE 2: Histamine agonists and antagonists

Compound	Action	Stock Solution	Source
histamine dihydrochloride		10^{-3}, 10^{-2}, 10^{-1}M in .1 N HCl	Sigma
2 - methyl histamine dihydrochloride	H_1 agonist	10^{-2} M in .1 HCl	+Smith, Kline & French
2-(2-pyridyl)-ethylamine dihydrochloride	H_1 agonist	10^{-3}M in .1 N HCl	+Smith, Kline & French
pyrilamine maleate (mepyramine)	H_1 antagonist	1 mg/ml in DDW*	Sigma
4 - methyl histamine dihydrochloride	H_2 agonist	10^{-1}M in .1 N HCl	+Smith, Kline & French
metiamide	H_2 antagonist	10^{-3}M in .1 N HCl	+Smith, Kline & French

* double distilled deionized water

\+ generously donated by Smith, Kline and French Laboratories Ltd., England

RESULTS

1) ANTIGEN-ANTIBODY REACTION

In order to demonstrate specific sensitization in our canine model of allergic bronchospasm, the pulmonary blood vessels must display a "vascular spasm" when challenged with specific antigen. In all experiments reported in this thesis, such a study was carried out. Both sensitized and control, pulmonary blood vessel strips were challenged with ovalbumin (OA) by adding it to the muscle bath to a final concentration of 0.3 mg/ml of Krebs-Henseleit solution. Only the sensitized pulmonary vein, developed active tension (168.2 g/cm^2 ± 51.4, n = 7) but not sensitized pulmonary artery (0 g/cm^2 ± 0, n = 7) in response to contact with OA; control tissues were always quiescent (Fig. 2). The in vitro response to OA could only be elicited once, the sensitized pulmonary vein becoming desensitized to further challenge.

The specificity of the antigen-antibody reaction was demonstrated by the inability to produce any tension (0 g/cm ± 0, n = 6) in vitro response to bovine albumin (BA), in Fig. 2. However, pretreatment of the sensitized pulmonary vein with histamine did not modify or abolish the Schultz-Dale responses. Aerosal challenge with OA in vivo in sensitized dogs which were littermates of the animals used in the in vitro studies reported a significant and large decrease in specific airway conductance (Kepron et al., 1977).

2) HISTAMINE DOSE-RESPONSE STUDIES

Histamine was the first mediator associated with allergic disease processes and, in many respects, remains the most important (Dale and Laidlaw, 1910). Therefore, dose-response studies were carried out in an

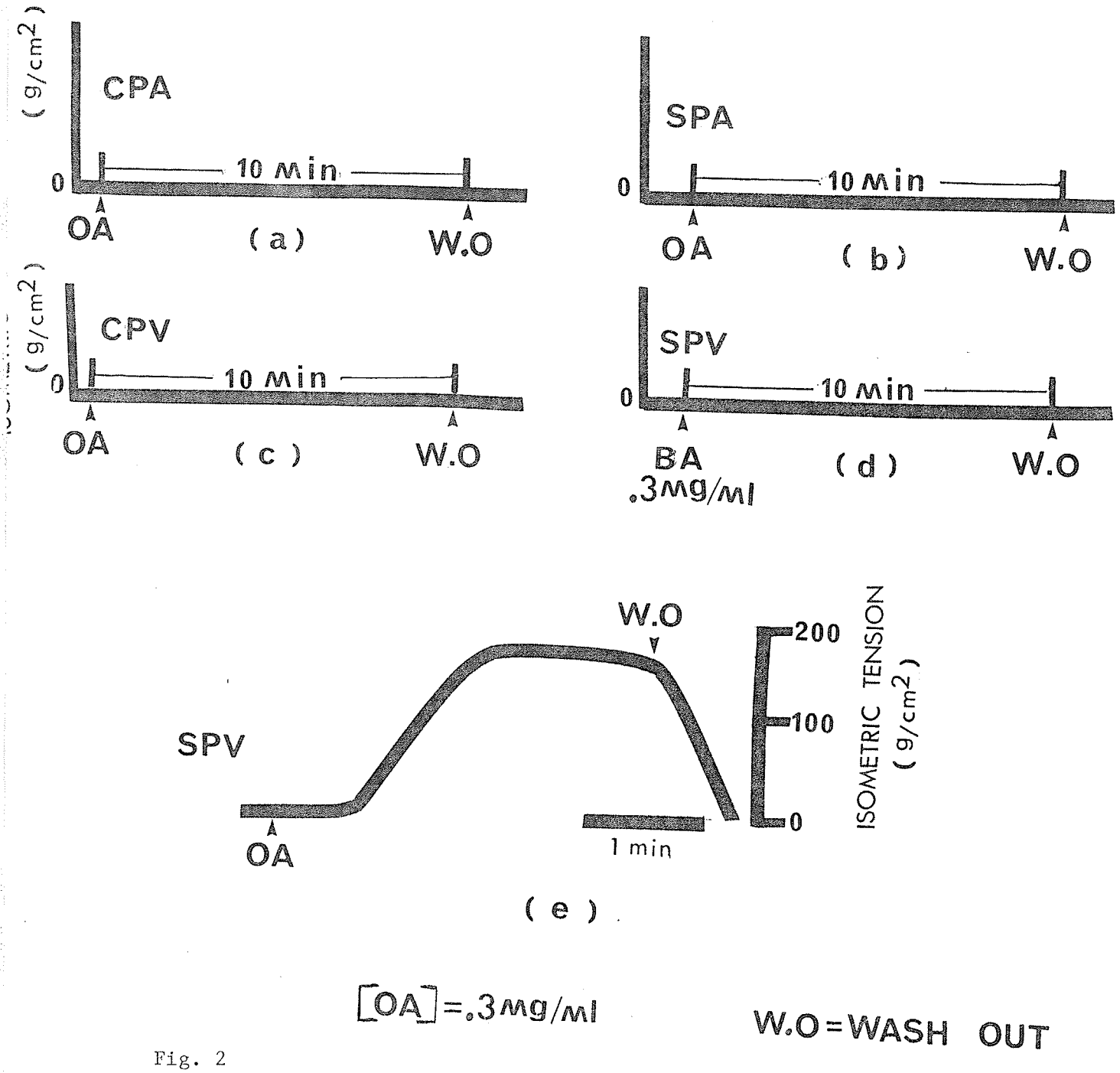

Fig. 2

Effect of ovalbumin to sensitized and control pulmonary blood vessels: Sensitized and control pulmonary blood vessels strips were challenged with ovalbumin (OA). (a), (b) and (c) showed that control pulmonary artery (CPA), sensitized pulmonary artery (SPA), and control pulmonary vein (CPV) did not develop any active tension in response to OA (0.3 mg/ml) challenge, even after 10 minutes of incubation with OA, (d) sensitized pulmonary vein (SPV) had no response to bovine albumin (BA) challenge, (e) sensitized pulmonary vein displayed the Schultz-Dale reaction but only restricted to the specific antigen - OA - challenge.

attempt to define demonstrable differences between sensitized and control pulmonary blood vessels reacting to the compound. The sensitized pulmonary arteries and veins exhibited statistically significant hyper-sensitivity (shift to the left of the curve for the sensitized muscle vis-a-vis that of the control, in figs. 3 and 4) and hyper-reactivity (upward shift) to histamine (figs. 5 and 6). The digit from which figure 3 was derived are shown in Table 3. Histamine doses administered ranged from 10^{-6}M to 10^{-3}M for both sensitized and control pulmonary arteries, and 5 x 10^{-8}M to 5 x 10^{-4}M for the sensitized and control veins; the standard errors for the means of the active tensions developed at each dose are shown. Concentrated stock solution (table 2) of histamine were used so that the pH and the ionic strength of the bathing medium were not significantly affected.

Both sensitized and control pulmonary arteries developed their maximal tensions (380.5 g/cm^2 and 231 g/cm^2 respectively, table 3), at a histamine concentration of 5 x 10^{-4}M. Sensitized pulmonary veins developed mean maximal tensions (329.9 gm/cm^2) correspond to vein 239.5 gm/cm^2 at 5 x 10^{-5}M (table 4). The ED_{50}'s (the doses which produce 50% of the maximal contractile responses) of the sensitized artery and vein were 3.0 x 10^{-5} ± 0.56 x 10^{-5} and 2 x 10^{-6} ± 0.5 x 10^{-6}; the control artery and vein 7.4 x 10^{-4} ± 0.60 x 10^{-4} and 3.4 x 10^{-6} ± 0.5 x 10^{-5}. This showed that sensitized pulmonary vein and control pulmonary vein were highly sensitive ($P < 0.01$) to histamine than their arterial counterparts, but developed similar ($p > 0.10$) significantly tensions.

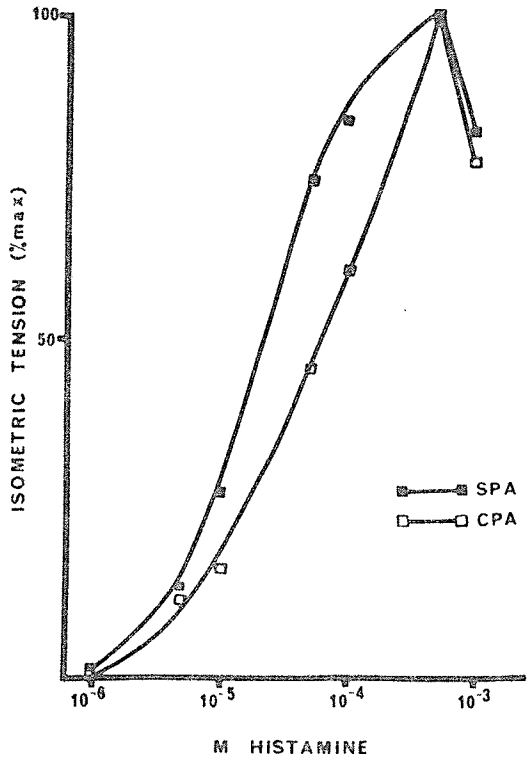

Fig. 3

Histamine dose-response curves of sensitized and littermate control canine pulmonary arteries. Isometric tension developed expressed as a percentage of the maximum tension and plotted against the log dose histamine. SPA = sensitized pulmonary artery; CPA = control pulmonary artery. (Mean Curves)

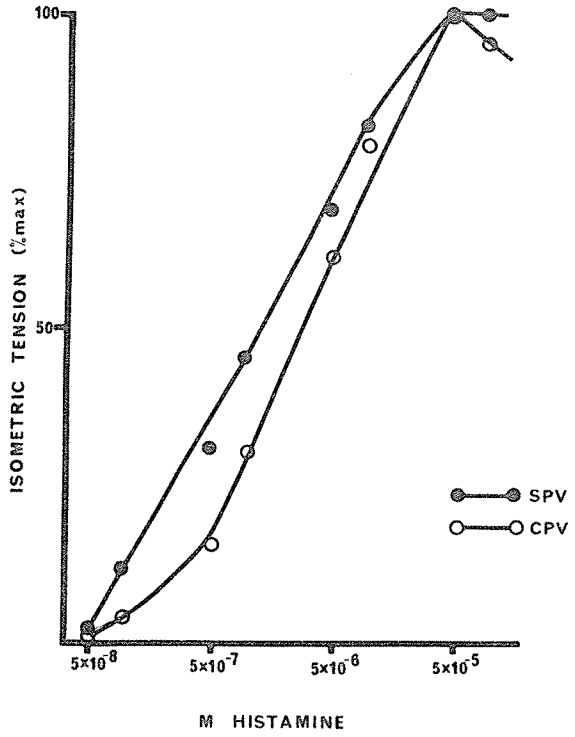

Fig. 4

Histamine dose-response curves of sensitized and littermate control canine pulmonary veins (SPV and CPV). Isometric tension expressed as a percentage of the maximum tension and plotted against the log dose of histamine. (Mean Curves)

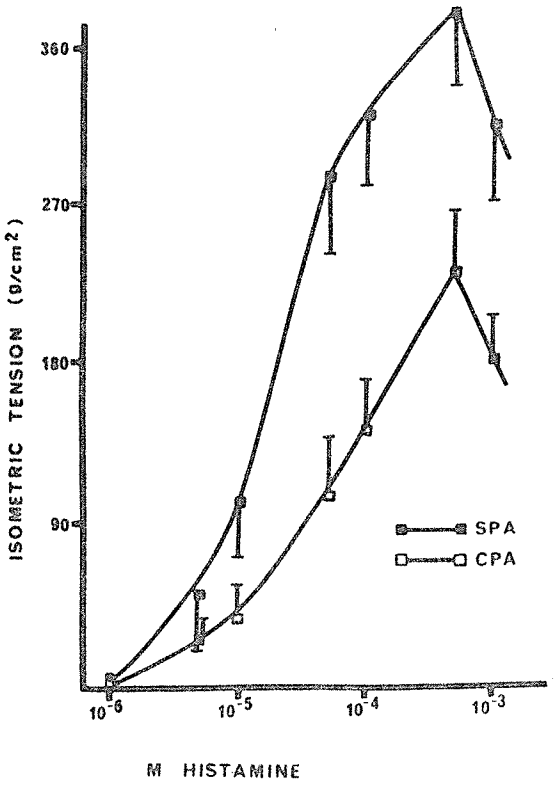

Fig. 5

Histamine dose-response curves of sensitized and littermate control canine pulmonary arteries. Isometric tension developed (in grams per centimeter^2) plotted against the log dose of histamine. SPA = sensitized pulmonary artery (n=10); CPA = control pulmonary artery (n=9). S.E. bars are shown

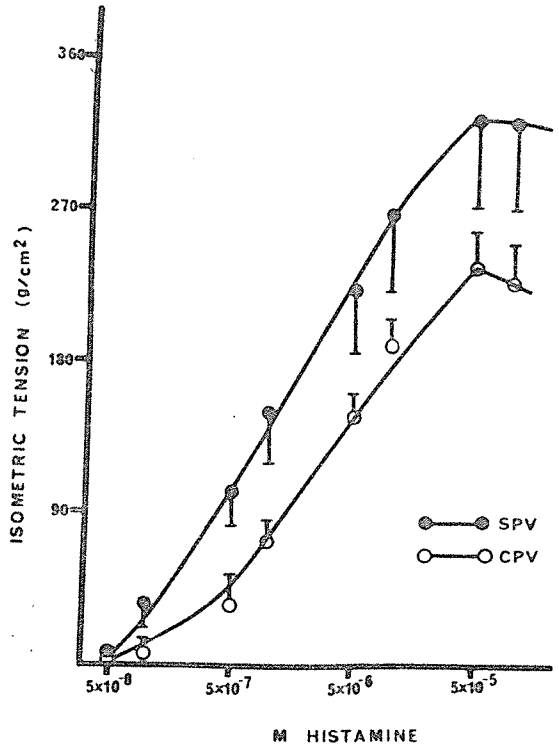

Fig. 6

Histamine dose-response curves of sensitized and littermate control canine pulmonary veins (SPV and CPV). Isometric tension developed (in grams per square centimeter) plotted against the log dose of histamine. For SPV n = 7; for CPV n = 11. S.E. bars are shown.

TABLE 3: HISTAMINE DOSE-RESPONSE FOR CANINE PA

DOSE HISTAMINE (M)	Tension (gm/cm^2) Control Group	Sensitized Group
No. of Animal	9	10
5×10^{-6}	25.67 ± 13.08	50.90 ± 28.59
1×10^{-5}	36.56 ± 17.50	102.80 ± 31.24
5×10^{-5}	105.56 ± 31.80	285.70 ± 43.31
1×10^{-4}	140.89 ± 28.22	320.10 ± 39.32
5×10^{-4}	231.00 ± 34.73	380.50 ± 40.50
10^{-3}	180.56 ± 24.53	314.00 ± 43.81

TABLE 4: HISTAMINE DOSE-RESPONSE FOR CANINE PV

DOSE HISTAMINE (M)	Tension (gm/cm^2)	
	Control Group	Sensitized Group
No. of Animals	11	7
1×10^{-7}	6.0 ± 3.05	37.4 ± 12.28
5×10^{-7}	35.64 ± 9.75	100.86 ± 18.73
1×10^{-6}	73.09 ± 10.5	147.57 ± 26.84
5×10^{-6}	146.0 ± 14.01	222.57 ± 38.28
1×10^{-5}	190.64 ± 16.37	267.14 ± 45.05
5×10^{-5}	239.46 ± 21.06	325.86 ± 51.64
1×10^{-4}	226.09 ± 21.79	323.29 ± 51.26
5×10^{-4}	216.09 ± 21.61	319.86 ± 51.51

3) NOREPINEPHRINE DOSE-RESPONSE STUDIES

The pulmonary vessels are fairly richly supplied with adrenergic vasoconstrictor fibers (Folkow and Neil, 1971). It is known that the adrenergic transmitter, norepinephrine, via α-receptors, can considerably enhance the flow resistance. Studies showed no left-ward and up-ward shift in the dose-response to NE curves of sensitized pulmonary artery when compared to these of controls. Both sensitized and control arteries developed maximal tension 863.9 gm/cm^2 and 840.25 gm/cm^2 (table 5) at 10^{-5}M NE. However, the SPV showed left-ward, but no up-ward shift (fig. 7 and 8), compared to control value. ED 50 of sensitized pulmonary vein was 1.5×10^{-7}M ± 0.7 X 10^{-7}; control 4.0 10^{-7}M ± 1.1 x 10^{-7}. Although at 5 X 10^{-6}M the sensitized vein developed 318 gm/cm^2 average tension when compared to the control mean value of 244.9 gm/cm^2 (Table 6), it was not statistically significant. The dose-response curves were plotted expressing active tension developed for each dose as a percentage of the maximal control P_o (fig. 7).

4) SEROTONIN DOSE RESPONSE

PAF (platelet activating factor) is released from tissue during anaphylaxis in experimental animals and from passively sensitized human lung in vitro (Gold, 1976). PAF causes platelets to aggregate and release serotonin. Since serotonin could therefore be a possible mediator for the anaphylactic response in pulmonary vessels, serotonin dose-response curve were studied to determine whether any difference between sensitized and control vessels existed. Neither the sensitized artery nor the vein showed any left-ward or up-ward shift of the serotonin dose response curve, when compared to the control. The maximal tensions (table 7 and 8)

TABLE 5: NE DOSE-RESPONSE FOR CANINE PA

DOSE NE (M)	Tension (gm/cm^2) Control Group	Sensitized Group
No. of Animal	8	10
1×10^{-8}	0 ± 0	3.50 ± 2.59
5×10^{-8}	92.25 ± 45.66	104.00 ± 38.03
1×10^{-7}	324.00 ± 73.96	301.60 ± 66.77
5×10^{-7}	552.13 ± 77.04	577.80 ± 66.73
1×10^{-6}	651.38 ± 73.90	720.70 ± 69.10
5×10^{-6}	795.25 ± 83.34	843.40 ± 88.25
1×10^{-5}	840.25 ± 86.46	863.90 ± 96.96
5×10^{-5}	837.25 ± 88.90	860.7 ± 94.13

TABLE 6: NE DOSE-RESPONSE FOR CANINE PV

DOSE NE (M)	Tension (gm/cm^2)	
	Control Group	Sensitized Group
No. of Animals	7	8
1×10^{-8}	12.86 ± 4.39	34.88 ± 16.53
5×10^{-8}	26.43 ± 8.91	89.63 ± 21.18*
1×10^{-7}	66.86 ± 14.13	168.00 ± 34.49*
5×10^{-7}	140.43 ± 24.50	222.38 ± 36.54*
1×10^{-6}	173.71 ± 37.00	267.38 ± 36.68*
5×10^{-6}	244.86 ± 32.10	318.12 ± 50.00
1×10^{-5}	208.86 ± 35.53	287.13 ± 45.86
5×10^{-5}	209.43 ± 36.55	271.25 ± 50.18

* $P < 0.05$

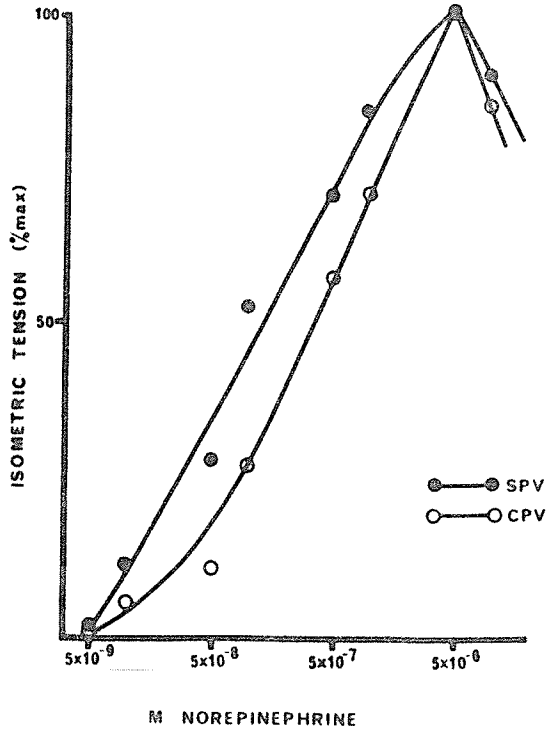

Fig. 7

Norepinephrine (NE) dose-response curves of sensitized and littermate control canine pulmonary veins (SPV and CPV). Isometric tension expressed as percentage of the maximum tension and plotted against the log dose of NE. (Mean curves)

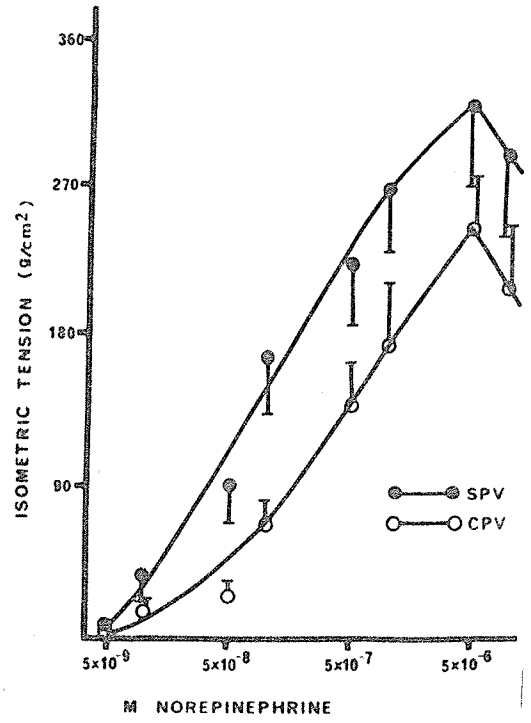

Fig. 8

Norepinephrine (NE) dose-response curves of sensitized and littermate control canine pulmonary veins (SPV and CPV). Isometric tension developed (in grams per square centimeter) plotted against the log dose of NE. For SPV n = 8; for CPV n = 7. Standard error bars are shown.

for both sensitized and control artery and vein are very close to the maximal tension developed by norepinephrine. The ED_{50}'s of the sensitized artery and vein were 2-3 x 10^{-7}M ± 0.88 x 10^{-7} and 1.77 x 10^{-7}M ± 0.5 x 10^{-7}; control 1.96 x 10^{-7}M ± 1.0 x 10^{-7}, 2.0 x 10^{-7}M ± 0.8 x 10^{-7}. (n=8 for all experiments). Also by using analysis variance for repeated measures method, both CPV and SPV showed no significant difference over each dosage of response.

5) DETERMINATION OF PRESENCE OF PRE-SYNAPTIC HISTAMINE RECEPTORS IN PULMONARY VEIN

Since a positive Schultz-Dale response was seen in the sensitized pulmonary vein, it became necessary to determine whether pre-synaptic histamine receptors existed in the vein. Such receptors have been shown to exist in control (Kroeger and Bergen, 1980) and sensitized airway smooth muscle (Mitchell et al, 1980). Fig. 9 shows that at the plateau of a histamine induced (5 X 10^{-5}M contraction, 5 x 10^{-6}M phentolamine caused the pulmonary vein to relax 20 to 40%. Histamine dose-response studies (Fig. 10 and 11) indicated that curves obtained from pulmonary veins which are pretreated with phentolamine and propranolol were displaced right-ward and down-ward with respect to those obtained from control pulmonary vein. The maximal tension and the ED_{50} of the control was 228 gm/cm^2 ± 18.07 gm/cm^2 and 4.43 x 10^{-6}M ± 0.93 10^{-6}M and 192.5 gm/cm^2 ± 12.86 gm/cm^2 and 7.77x10^{-6}M ± 2.0 x 10^{-6}M for the phentolamine and propranolol treated muscle. (n=6 paired t-test $p < 0.05$) these suggest that histamine could induce contraction via direct stimulation of the muscle cell histamine receptors and cause norepinephrine release due to the stimulation of the histamine pre-synaptic receptors in the nerve endings.

TABLE 7: 5HT DOSE-RESPONSE FOR CANINE PA

DOSE 5HT (M)	Tension (gm/cm^2) Control Group	Sensitized Group
No. of Animals	8	9
1×10^{-8}	58.63 ± 29.11	67.89 ± 46.21
5×10^{-8}	288.63 ± 59.20	266.22 ± 75.81
1×10^{-7}	543.63 ± 60.31	470.00 ± 60.83
5×10^{-7}	838.63 ± 76.05	857.89 ± 58.58
1×10^{-6}	893.38 ± 74.21	983.56 ± 115.47
5×10^{-6}	913.38 ± 73.80	1012.44 ± 121.38

Table 8: 5-HT DOSE-RESPONSE FOR CANINE PV

Dose 5-HT (M)	Tension (g/cm^2)	
	Control Group	Sensitized Group
No. of Animals	7	6
1×10^{-8}	16.3 ± 8.9	15.5 ± 6.94
5×10^{-8}	62.9 ± 28.3	86.5 ± 26.0
1×10^{-7}	139.7 ± 37.7	130.5 ± 52.5
5×10^{-7}	234 ± 53.3	233 ± 79.4
1×10^{-6}	252.6 ± 45.7	273 ± 81.8
5×10^{-6}	255.6 ± 47.9	296 ± 84.2
1×10^{-5}	254.8 ± 46.8	286 ± 82.3

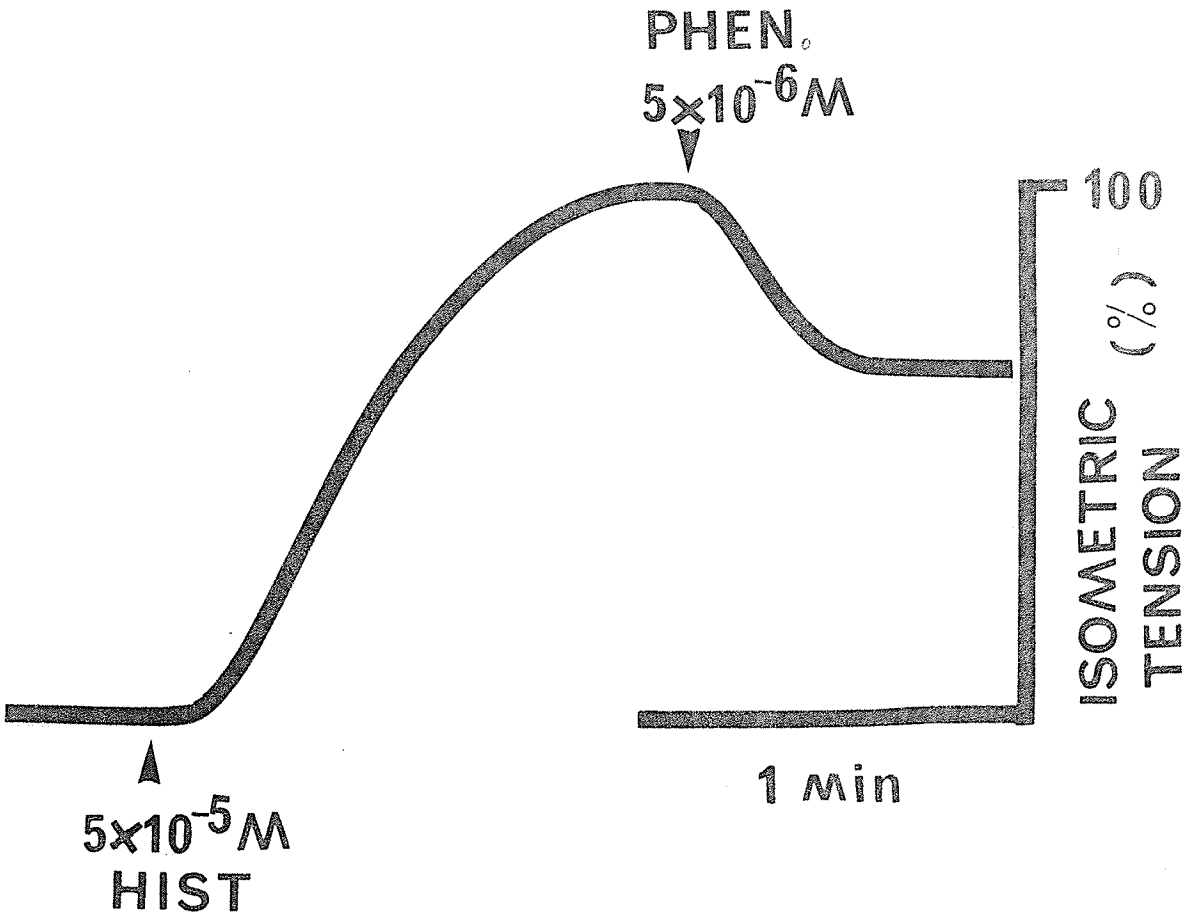

Fig. 9

Effect of phentolamine at the plateau of histamine:
At the plateau of histamine (HIST) (5×10^{-5}M) contraction, 5×10^{-6}M of phentolamine (PHEN) cause the pulmonary vein to relax 20 to 40% (n = 7).

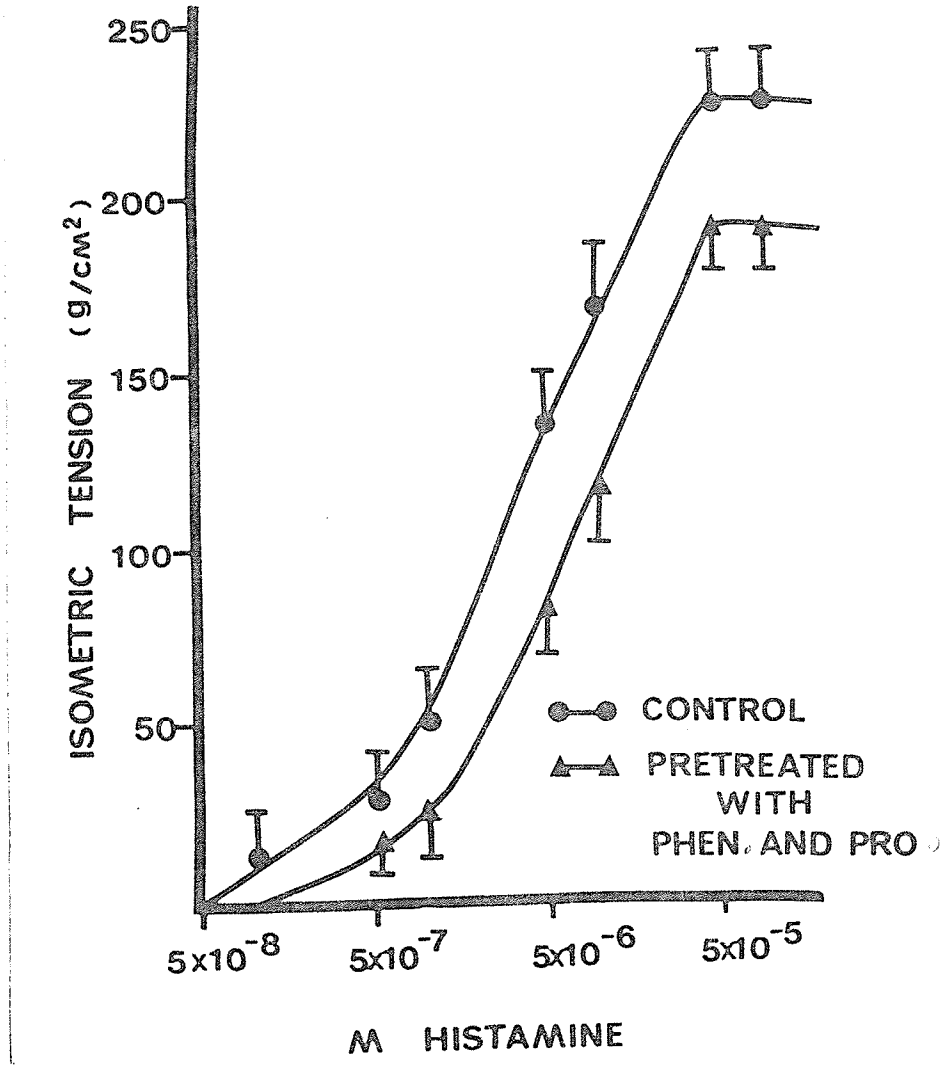

Fig. 10

Histamine dose-response curves of control and test pulmonary veins which pretreated with 5 x 10^{-6}M of phentolamine (PHEN) and 5 x 10^{-6}M of propranolol (PRO). Isometric tension developed (in gram per square centimeter) plotted against the log dose of histamine. n = 6 for both the control and test group. S.E. bars are shown.

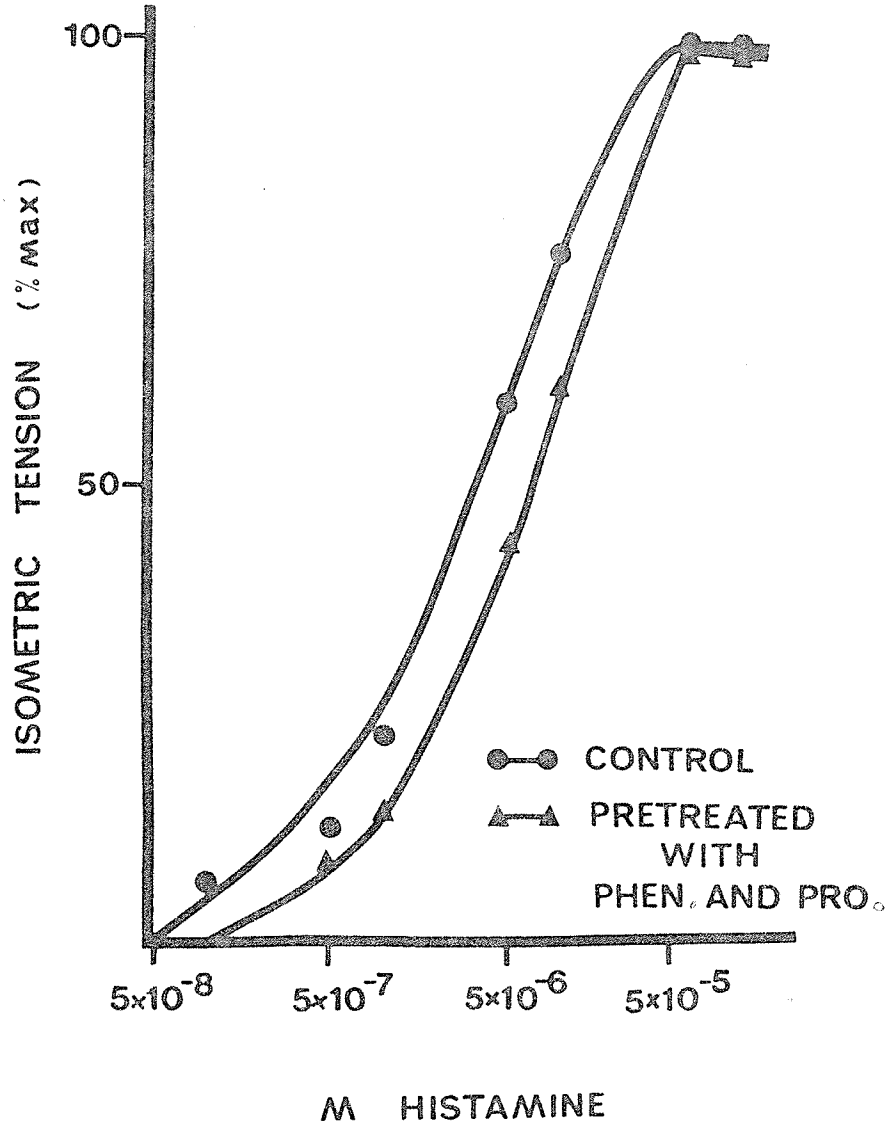

Fig. 11

Histamine dose-response curves of control and test pulmonary veins which pretreated with 5 x 10^{-6}M of phentolamine (PHEN) and 5 x 10^{-6}M of propranolol (PRO). Isometric tension expressed as percentage of the maximum tension and plotted against the log dose of histamine. (Mean curves)

6 DO H_2 RECEPTORS EXIST IN PULMONARY BLOOD VESSELS ?

Pyrilamine (H_1 antagonist) 10^{-6}M was added prior to addition of isosmotic high potassium (48.2 mM) solution to the pulmonary vein bath. At the plateau of contraction, histamine was added in graded doses ranging from 10^{-7}M to 10^{-3}M. At 10^{-6}M histamine, the tension developed by high K^+ was reduced by 6.12%± 0.77 (n=12). 10^{-5}M histamine caused a further 11.76% ± 1.15 (n=12) relaxation, (fig. 12a). These differences were statistically significant. This showed that H_2 receptors do exist in the pulmonary vein. Sensitized and control vein showed no significant difference. However, fig. 12b shows that metiamide (H_2 antagonist) did not potentiate the pulmonary pressure response to histamine.

7 NATURE OF MEDIATOR INVOLVED IN SCHULTZ-DALE RESPONSE OF SENSITIZED PULMONARY VEIN

Antonissen et al (1979) showed that 2 x 10^{-7}M pyrilamine can block the antigen-antibody reaction in sensitized canine tracheal smooth muscle. However, in sensitized canine pulmonary vein, 10^{-7}M and 10^{-6}M of pyrilamine blocked only 25 to 45% of the antigen-antibody reaction (Fig. 14 a). Phentolamine, 5 x 10^{-6}M blocked calculate (fig. 14b). The tension developed by the antigen-antibody reaction in the individual pulmonary vein was equal to the tension developed by 1.4 x 10^{-6}M (± 0.3 x 10^{-6}) of histamine.

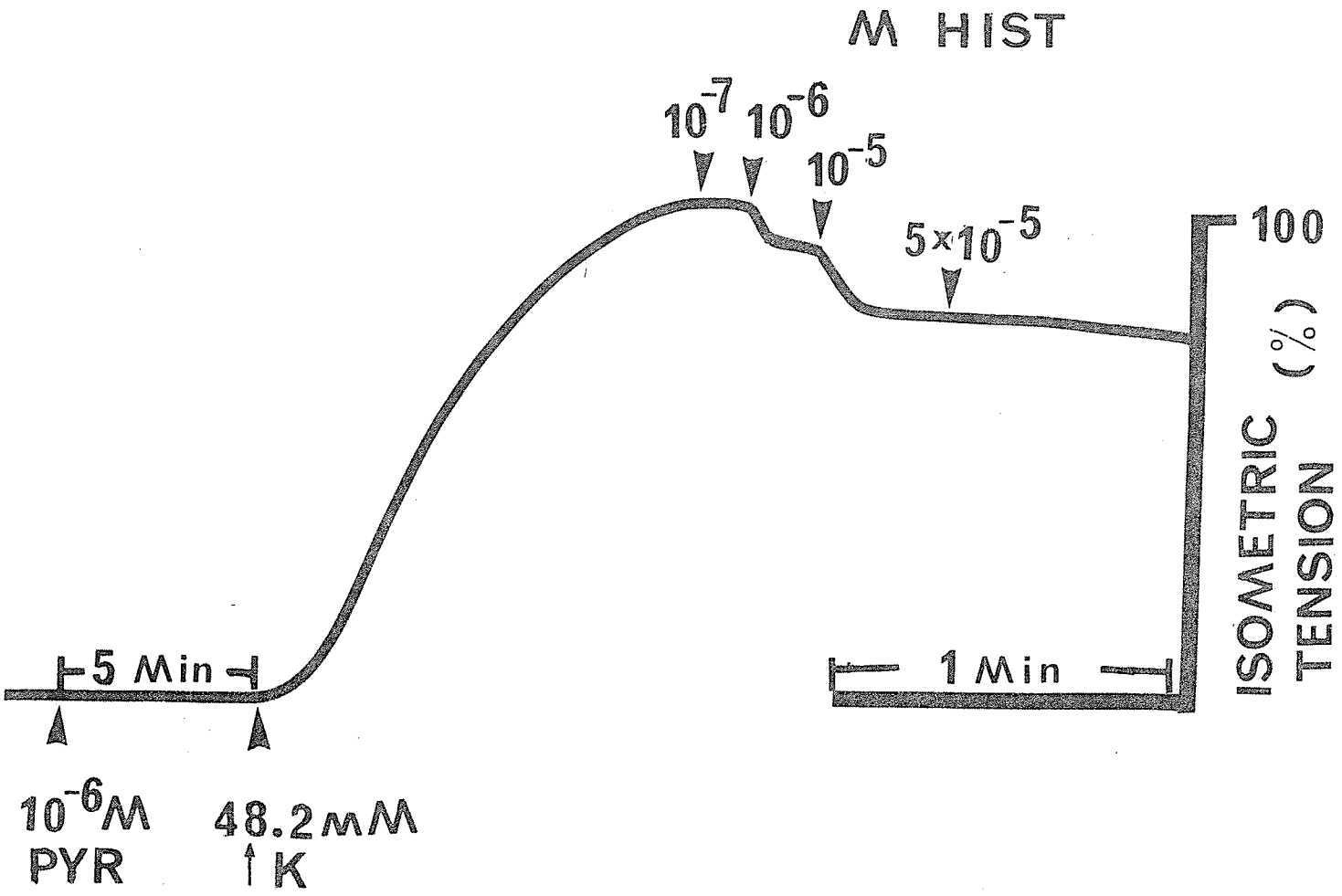

Fig. 12

Effect of histamine to high K^+ solution, after pretreated of pyrilamine: 10^{-6}M of pyrilamine was added 5 minutes prior to addition of isosmotic high potassium (48.2 mM) solution to the pulmonary vein bath. At the plateau of contraction, histamine was added in graded doses ranging from 10^{-7}M to 10^{-3}M. At 10^{-6}M histamine, the tension developed by K^+ was reduced by 6.12 % ± 0.77 (n = 12). 10^{-5} M of histamined caused a further 11.76% ± 1.15 (n = 12) relaxation.

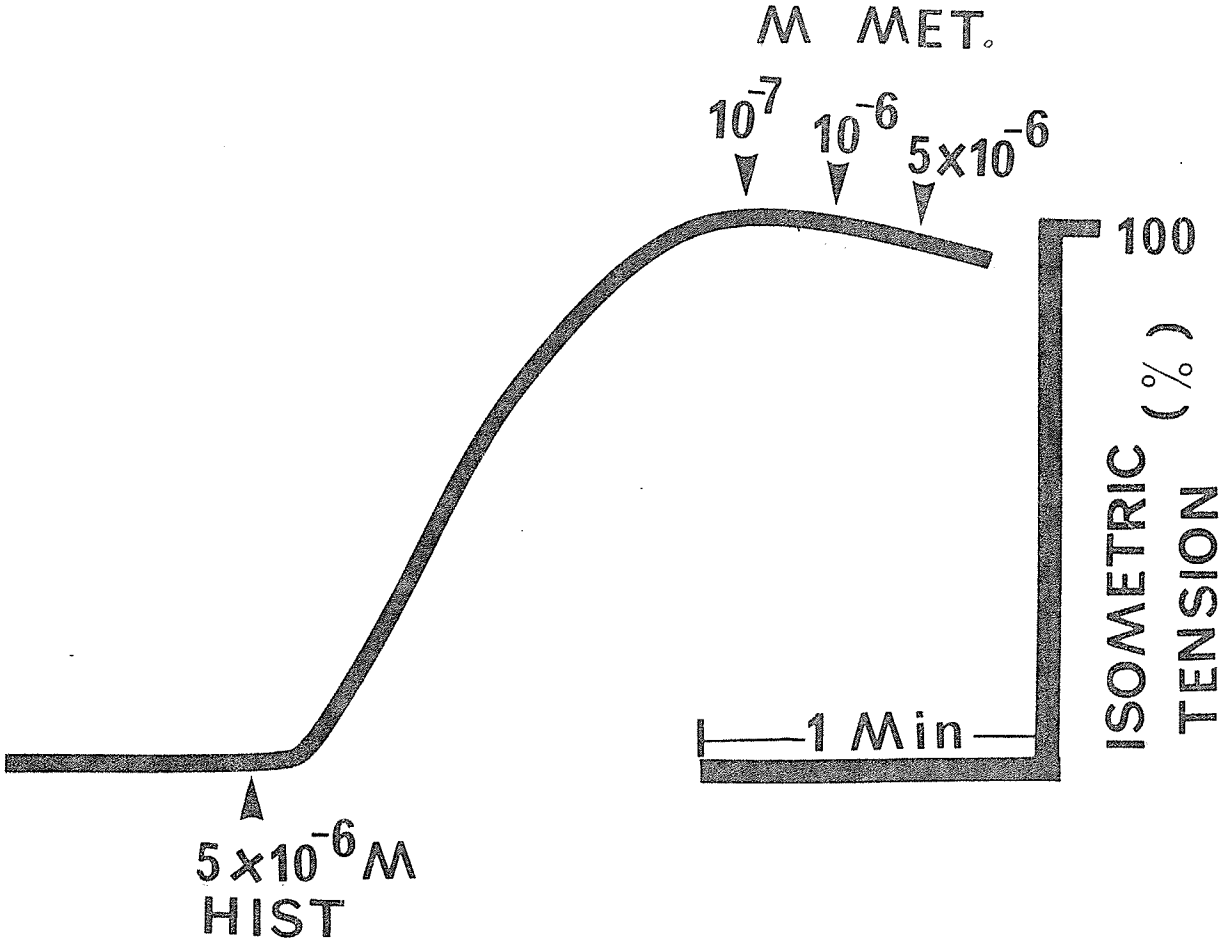

Fig. 13

Effect of metiamide at the plateau of histamine:
At the plateau of 5 x 10^{-6}M of histamine (HIST) contraction, metiamide (MET) was added from 10^{-7}M to 5 x 10^{-6}M and causes no further additional contraction (n=7).

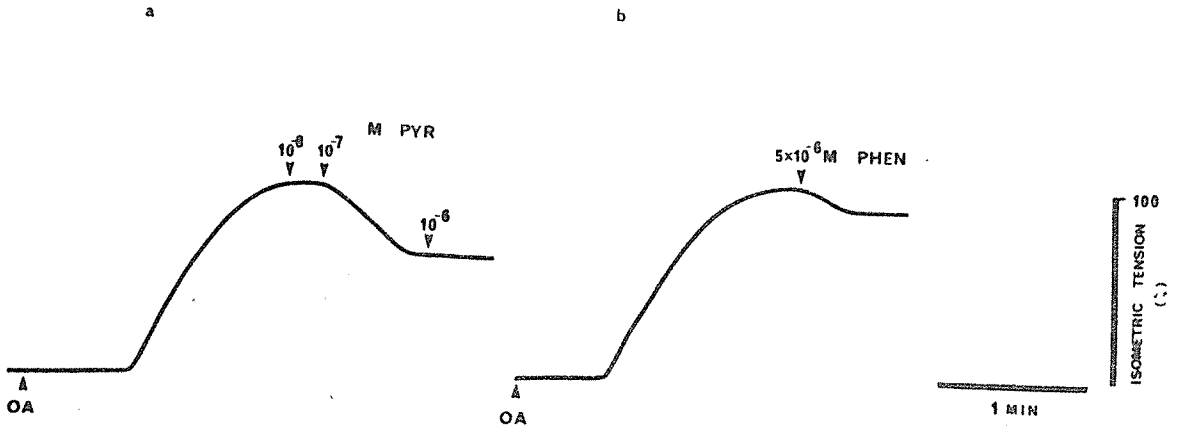

Fig. 14

In sensitized pulmonary veins (SPV) the Schultz-Dale reaction occured within 1 minute after challenge with OA (0.3 mg/ml final bath concentration).
a - At plateau of isometric contraction, 10^{-7}M pyrilamine maleate (PYR) blocked 25 - 45% of the reaction. Higher dose of pyrilamine (eg. 10^{-6}M) had no further blocking effect (n=7).

b - At plateau of isometric contraction of the Schultz-Dale reaction 5 x 10^{-6}M of phentolamine (PHEN) produced 5 - 15% relaxation (n=7).

DISCUSSION

ANTIGEN-ANTIBODY REACTION

The Schultz-Dale reaction was only seen in the sensitized pulmonary vein (Fig. 2c, 2d) when challenged with the specific antigen; the sensitized pulmonary artery showed no such response, even after 8 to 10 minutes of incubation with antigen. This result is very similar to that of Chand and Eyre (1979), who showed that the sensitized dog pulmonary artery had no response. This could be due to the sensitized pulmonary artery having very few mast cells in its tissues or an adequate number of such cells which however released only a low concentration of mediators such as histamine. Histamine dose response curves (Fig. 4, 6) and ED 50 values showed that the sensitized vein was hypersensitive and hyperreactive to histamine. Since it is known that mast cells are plentiful in lung (Wilhelm et al., 1978; Guerzon et al., 1979), careful electronmicroscopic and histochemical studies will be needed in the future to prove that there is paucity of mast cells in the pulmonary artery. However even granting that there is a paucity, there are other factors that must be considered. One such factor stems from Dale's (1920) suggestion of a direct action of the specific antigen on the excitable membrane of sensitized smooth muscle. Studies on electrophysiological characteristics of the Schultz-Dale reaction, (Guschin, 1975) strongly supported the theory of direct mode of action of antigen on smooth muscle. Guschin (1975) and Katsch (1958), even suggested the presence of "local" mast cells is not an absolute prequisite for anaphylaxis. However, in our preparation, this mechanism seems very unlikely. The in vitro response to OA could only be elicited once; the pulmonary vein failed to develop a second reaction even after careful wash-out and incubation of the tissue for 6 hours in fresh solution. The

conclusion appears to be that the specific antigen can not directly cause the smooth muscle to contract.

The Schultz-Dale reaction could only be partly blocked by pyrilamine (H_1 antagonist) and phentolamine (α-adrenoreceptor antagonist); this is shown in fig. 13. This is not unique since histamine H_1 antagonists have been reported to partially inhibit (Hawkins and Rosa, 1956; Joiner et al., 1974) anaphylactic reactions in a number of tissues. These reports suggested that mast cell degranulation in sensitized smooth muscle by antigen-antibody reaction releases additional chemical mediators which along with histamine are responsible for the total mechanical response. But the absence of any response to the second challenge suggests that tachyphylaxis developed to all the mediators. These reports and mine were different from those of Chand et al (1979) who showed tachyphylaxis can be partially recovered from in the sensitized lung strip. The reasons for the tachyphylaxis to antigen could be: a) occupancy of all the antigen-combining sites on the corresponding antibody leads to desensitization to second antigen challenge, b) Exhaustion of the preformed or stored mediators (biologically active substance) in the target cells (mast cells) in the smooth muscles (Feldberg, 1961), c) antigen-antibody combination results in the activation of an enzyme system which acts as a catalyst in this reaction leading to the release of mediators and muscular contraction. The activated enzyme system could be short-lived (Mongar and Schild, 1957) resulting in a desensitization to subsequent antigen challenge. Pretreatment of the sensitized pulmonary vein with histamine did not modify or abolish the Schultz-Dale response. This result contrasts to Antonissen et al (1980) who reported that Schultz-Dale reaction of sensitized tracheal smooth muscle does not occur after exposure to histamine. This could be due to the

tachyphylaxis to histamine can be completely or mostly recovered in the vein; or other mediators release from the mast cells.

In addition to the Schultz-Dale reaction that we saw in the sensitized pulmonary vein, it has also been reported in the canine lung strip (Chand et al., 1979), bovine pulmonary vein (Eyre, 1970; Eyre, 1971), horse lung (Burka et al., 1976), pulmonary blood vessels of chicken (Chand and Eyre, 1977) and human lung (Morr, 1979). It has been known for many years that anaphylaxis or the intravenous infusion of histamine in a variety of different species may be accompanied by pulmonary vasoconstriction, especially in the veins (Lecomte, 1956; Karczewski and Widdicombe, 1969; Eyre et al., 1973; Eyre and Lewis, 1973). With respect to the in vivo significance of the Schultz-Dale reaction of the sensitized pulmonary vein it may be worth recalling that histamine and other vasoactive substances constrict pulmonary venules. One can therefore speculate that anaphylaxis in the pulmonary vein could lead to pulmonary venospasm and development of pulmonary edema (Eyre, 1977).

HISTAMINE DOSE-RESPONSE STUDIES

Fig. 13 showed that pyrilamine, the H_1 antagonist can block 25 to 45% of the Schultz-Dale reaction in the sensitized pulmonary vein of our canine model of allergic bronchospasm, therefore, it was necessary to compare histamine dose response curves for sensitized and control pulmonary blood vessels, in order to obtain an insight into the mechanism of the anaphylactic response of the pulmonary vein. Although the sensitized pulmonary artery showed no Shultz-Dale reaction in my study, it seemed to be still of interest to find out if it had developed any charge in sensitivity to agonists

such as histamine. Experimental ED_{50} values indicated that the sensitized pulmonary vein and artery were hypersensitive to a histamine stimulus when compared to controls (Fig. 3 to 6). In addition, the sensitized pulmonary vein and artery were hyperreactive, the sensitized vein producing 40% and the sensitized artery 64.7% more isometric tension to a supra-maximal dose of histamine. This result resembles that recently reported by Antonissen et al (1980) for the sensitized airway smooth muscle. However, it is in conflict with the findings of Souhrada et al (1977) who found normal reactivity to histamine in sensitized guinea pig tracheal smooth muscle. The reason for this difference may be due to the difference in animal species. It has been suggested that sensitized guinea pigs are not a reliable model of allergic asthma (Pare et al., 1977).

A pharmacological approach, involving classic receptor theory, was employed to interpret our experimental data. According to Van Rossum (1968), dose response curves reflect both receptor affinity and the intrinsic activity of a drug. The process of receptor occupancy is reflected by the curve's shape, assuming the concentration of the drug in the direct vicinity of the receptor is directly proportional to the dose, and the intensity of the drug effect is directly proportional to the quantity of occupied receptors. These assumptions are more easily satisfied by in vitro studies than by in vivo studies (Van Rossum, 1968).

Dose-response curves elicited for a particular group of receptor agonists which display similar slopes and maximal responses, but which are displaced laterally with respect to each other are classically interpreted to mean the drugs have the same intrinsic activities but differ in their affinities for the receptor sites. Similarly, if two tissues, stimulated by the same drug are compared, exhibit displaced curves with equal slopes and

maximal response, then the receptor-agonist binding must be capable of similar cellular effectuation, but the receptor affinities for the agonist molecules must be different. Unfortunately the results obtained in the present studies do not reveal such a parallel displacement between dose-response curves for the sensitized and control veins; this prevents application of classical theory to interpret results. For the pulmonary artery, the dose-response curves were not statistically different. Even though the curves for the control and sensitized pulmonary veins were not parallel, the difference in their ED_{50}'s can be validly interpreted to mean that the tissues have different responses (Goldstein, 1964).

The sensitized tissues do show hyperreactivity when compared to the control. This can be due to: a) a higher intrinsic activity of the histamine receptors in the sensitized tissues, b) an increased number of histamine receptors with the same intrinsic activity, resulting in an additive cellular action, that is higher active tension development. The higher intrinsic activity could be due to: a) An increase in the number of calcium channels through the plasma membrane or, sarcoplasmic reticulum. b) An increase in the number of cross-bridges or force-generating sites (per cross-sectional area) in the tissue.

The hypersensitivity and hyperreactivity of the sensitized pulmonary vein to histamine suggests that histamine release during the antigen-antibody reaction may be responsible both directly and indirectly for vasospasm in vivo.

With respect to indirect contraction of the venous smooth muscle by histamine a mechanism that must be considered is the release of norepinephrine from sympathetic nerve filaments and stimulation of α adrenoceptors in the

muscle cell membrane. The possibility exists that the receptors exist pre-synaptically and their stimulation by histamine results in NE release. This is discussed below.

NOREPINEPHRINE DOSE-RESPONSE STUDIES

Since the pulmonary vessels are fairly richly supplied with adrenergic vasoconstrictor fibers (Folkow and Neil, 1971) which release norepinephrine during stimulation by the nervous impulse and presynaptic regulation of adrenergic neurotransmitter release by histamine and serotonin had been reported by Kroeger and Bergen (1980), it had seemed of interest to determine whether any change in sensitivity to norepinephrine had developed in sensitized pulmonary blood vessels. Furthermore since sensitized airway smooth muscle shows increased sensitivity to a variety of agonists it seemed that a similar situation could exist for pulmonary vascular smooth muscle. Positive responses to both histamine and norepinephrine would then enable us to conclude that the increased sensitivity was of a non-specific type.

Experimental ED_{50} values showed that only the sensitized vein was hypersensitive to norepinephrine when compared to control. Neither the sensitized vein nor the artery were hyperreactive to it. Invoking the receptor theory mentioned previously the leftward, parallel displacement of the dose-response curve of the sensitized pulmonary vein with respect to the control may be ascribed to a higher affinity for norepinephrine. The absence of hyper-reactivity would suggest that efficacy (Van Rossum, 1968) was unchanged. The excitation-contraction coupling and the contractile machinery were normal.

SEROTONIN DOSE RESPONSE STUDY

Serotonin (5-hydroxytryptamine) release from mast cells has been only reported in rat tissues (Benditt et al., 1955). In tissues from other species, including dog and human, serotonin is lacking from mast cells (Sjoerdsma et al., 1957; West, 1959), although Eyre (1972) has reported that it may be released from sensitized calf lung. However, in dogs during anaphylactic shock, PAF is released from the lung, which causes platelet to aggregate and release serotonin. Hence an indirect mechanism for serotonin release exists in canine tissues. Therefore it was important to determine whether increased sensitivity to this agent existed in sensitized blood vessels. However, dose-response curves for sensitized and control pulmonary blood vessels were not different from each other. Hence it may be concluded that altered sensitivity to serotonin does not exist.

PRE-SYNAPTIC HISTAMINE RECEPTORS

Histamine is known to be a powerful stimulant of "irritant" pulmonary receptors (Karczewski and Widdicombe, 1969) which give rise to vagal reflex bronchomotor effects, in addition to direct tracheobronchoconstrictor. The respiratory effects of histamine may be effectively blocked by vagotomy (Martinez et al., 1961; Karczewski and Widdicombe, 1969), or by medication with hexamethonium (Bouhuys et al., 1960) or atropine (Drazen and Austen, 1975). Recently, Kroeger and Bergen (1980) and Mitchell et al., (1980) have shown that pre-synaptic histamine receptors do exist in the control and sensitized canine airway smooth muscle. These results led us to determine whether any pre-synaptic histamine receptors also exist in the canine pulmonary vein in which histamine is released during the anaphylactic shock.

Fig. 9 shows that at the plateau of histamine (5×10^{-5}M) contraction, 5×10^{-6}M of phentolamine caused the pulmonary vein to relax 20 to 40%. This observation suggests that pre-synaptic histamine receptors do exist in the pulmonary vein. Fig. 10 and 11, show histamine dose response curves for a control pulmonary vein and are pretreated with phentolamine (5×10^{-6}M) and propranolol (5×10^{-6}M). The latter shows a leftward and upward shift with respect to the former. Fig. 13 also shows that the Scultz-Dale reaction can be partly blocked by pyrilamine and phentolamine. These results confirm the presence of pre-synaptic histamine receptors in the pulmonary vein.

During antigen-antibody reaction, histamine released from the target cell (mast cell) can act directly on the pulmonary venous smooth muscle H_1 receptor and cause contraction. However, histamine also can act on the pre-synaptic H_1 receptor and cause the adrenergic nerve to release norepinephrine (Kroeger and Bergen, 1980). Therefore, the smooth muscle will have further contraction via the α receptors.

DO H_2 RECEPTORS EXIST IN THE PULMONARY BLOOD VESSELS ?

Black et al (1972) not only defined H_2 receptors but also demonstrated their presence in lung, kidney, intestine, and blood vessels. Eyre (1969) and Maegwyn-Davies (1968) were the first to demonstrate histamine reactions in the lung which could not be explained on the basis of H_1-receptor activity. Subsequently, Eyre (1973) was able to identify specific H_2 receptor action of histamine isolated airway smooth muscle of cat and sheep using the H_2 antagonist, burimamide. Others had shown in the guinea pigs that burimamide potentiated the pulmonary pressor response to histamine and abolished the depressor action of histamine after mepyramine (Goadby and Phillips, 1973;

Tucker, 1973; Okpako, 1974). A comparable study of the intact dog was reported by Tucker et al (1975). Okpako (1974) further demonstrated in the guinea pig that 4 MH could relax the muscle contracted by histamine. This was evidently due to an H_2 agonist effect. The effect of 4 MH could be blocked by burimamide a specific H_2 antagonist. These results all suggest that the vasodepressor effects of histamine on the pulmonary vascular bed are mediated via the H_2 receptor.

Burimamide was the first H_2 antagonist to be synthesized (Black et al., 1972), but this agent proves to have other pharmacological actions in addition to histamine antagonism. Subsequently, metiamide was synthesized and proved to be a more specific and potent antagonist of H_2 receptors (Black et al., 1973). Therefore, in the present study of H_2 receptors in pulmonary blood vessels metiamide was selected as the H_2 antagonist. Fig. 12b shows that metiamide did not potentiate the pulmonary pressor response to histamine. However, fig. 12a shows that H_2 receptor seem to exist. Contrariwise, Antonissen et al (1980) showed that H_2 receptors do not exist in canine tracheal smooth muscle. These suggest that the H_2 receptor distribution is very variable even between organs in the same animal.

MEDIATORS RELEASED DURING THE SCHULTZ-DALE REACTION

Fig. 12 shows that pyrilamine (10^{-7}M) and phentolamine (5×10^{-6}M) can block 25 to 45% and 5 to 15% of the Schultz-Dale reaction. This proves that histamine is rdleased from the mast cells during specific antigen challenge, and directly causes the smooth muscle to contract. However, histamine also can bind to the presynaptic H_1 receptor, and trigger norepinephrine release which produces additional contraction. These results contrast with those of Antonissen et al (1980) wherein pyrilamine (5×10^{-7}M)

was shown to entirely block the Schultz-Dale reaction in the sensitized tracheal smooth muscle. Other reports (Castillo, 1948; Schild et al., 1951; Hawkins and Rosa, 1956; Cirstea and Suhaciu, 1968; Chand and Eyre, 1976; Dunlop and Smith, 1977) also showed that the anaphylactic Schultz-Dale contraction is either resistance or partially susceptible to H_1-antagonist Schild et al (1951) had observed in in vitro experiments with human bronchial smooth muscle, that anti-histamines were 10,000-fold more active in inhibiting the bronchospasm elicited by exogenous histamine than by release from tissue mast cells following an antigen-antibody response (Schild et al., 1951). The most likely explanation, is that anti histamine doses used exogenously are too low to block the high concentration of histamine released from the mast cells immediately adjacent to bronchial smooth muscle. Unfortunately high doses of anti histamine can not be applied because of their non specific effects. Besides histamine, other agonistic mediators which may be released from sensitized pulmonary vein during antigen-antibody reaction, are slow-reacting substances of anaphylaxis (SRS-A), and prostaglandins.

SRS-A, is released by mast cells and basophils, following an antigen-IgE interaction, though these cells seem not the only cellular source of SRS-A (Kaliner and Austen, 1975). IgE-mast cell initiated SRS-A generation has been recognized in the rat peritoneal cavity (Orange et al., 1970; Stechschulte et al., 1970) and in human lung fragments and dispersed cell suspensions (Paterson et al., 1976a,b; Lewis et al., 1974). Recently, the contractile actions of partially purified rat SRS-A on the guinea pig peripheral airway smooth muscle have been confirmed by showing dose-related loss of activities after treatment of smooth muscle with the antagonist FPL 55712 (Drazen et al., 1979). Other studies have been carried out by Adams and Lichtenstein (1979) on guinea pig trachea and human bronchus which

provide indirect evidence that histamine is responsible for the initial phase of airways contraction and that SRS-A causes the delayed phase.

Prostaglandins of the E and F series are normally present in animal and human lungs (Anggard, 1965; Karim et al., 1967) and are known to be released by a variety of stimuli (Piper and Vane, 1971) including anaphylaxis. Histamine, SRS-A, ECF-A and PFA are regarded as "primary" mediators of the antigen-antibody reaction, and it has been hypothesized that the release of prostaglandins in anaphylaxis is dependent upon these mediators (Kaliner and Austen, 1975). The increased release of prostaglandins may well be a secondary event in the acute allergic reaction since the increase in synthesis of $PGF_{2\alpha}$ is blocked by H_1 type histamine blocking agents whereas the release of PGE_2 is blocked by H_2 type histamine blocking agents (Mathe, 1976).

Human bronchial muscle was relaxed by PGE_1 and PGE_2 but was contracted by $PGF_{2\alpha}$ (Horton, 1969; Mathe et al., 1971). Mathe and co-workers have shown that patients with bronchial asthma are exquisitely sensitized to the bronchoconstrictor effect of $PGF_{2\alpha}$ (Mathe et al., 1973; Mathe and Hedquist, 1975). With the exception of PGE_1, all the prostaglandins tested contracted canine intrapulmonary vein. (Kadowitz et al., 1975). The effects of PGE_1 and $PGF_{2\alpha}$ were studied by Joiner et al (1975) on isolated strips of intrapulmonary arteries and veins from dog, man, and other species. $PGF_{2\alpha}$ contracted human arterial strips in a dose-dependent fashion. Canine and human venous strips were contracted by $PGF_{2\alpha}$. PGE_1 slightly relaxed both veins and arteries from dog. Human arteries usually contracted slightly and human veins usually relaxed slightly to PGE_1. All these reports indicate that SRS-A and prostaglandins could

be involved in the Schultz-Dale reaction in the sensitized pulmonary vein.

In future, more studies (including dose-response curve delineation) will be conducted to determine all the mediators released in the course of the antigen-antibody reaction in the sensitized pulmonary and their mechanism of action. Other mediators such as bradykinin arachnoids, acetylcholine and serotonin will also be considered.

GENERAL CONCLUSION

In sensitized canine pulmonary blood vessels, the Schultz-Dale reaction can only be observed in the vein and only the specific antigen can trigger the antigen-antibody reaction. The significance of this is that during anaphylactic shock, veno-constriction could lead to capillary pressure increase and pulmonary oedema.

Not unexpectedly, histamine is the mediator released in anaphylaxis, and the sensitized arterial and venous smooth muscle are hypersensitive and hyperreactive to it. The pulmonary artery ED_{50} values of the control and sensitized tissues are different ($p < 0.05$). Most attention has focussed on the pulmonary vein, since a Schultz-Dale reaction was seen to only occur in the sensitized vein. With respect to histamine receptor status both H_1 and H_2 receptors have been shown to exist in both sensitized and control vein. Presynaptic histamine receptors have also been found in the pulmonary vein as evidenced by the observation that phentolamine can block 5 to 15% of the Schultz-Dale reaction. Since pyrilamine can not abolish the Schultz-Dale reaction entirely, other mediators such as SRS-A and prostaglandin should be considered.

REFERENCES

Adam, G.K. and L.M. Lichtenstein: In vitro studies of antigen-induced bronchospasm: effect of anti-histamine and SRS-A antagonist on response of sensitized guinea pig and human airways to antigen. J. Immunol. 122(2):555-562, 1979.

Adelstein, R.S.: Myosin phosphorylation, cell motility and smooth muscle contraction. Trends Biochem. Sci. 3:27-30, 1978.

Adelstein, R.S., M.A. Conti, D.R. Hathaway, C.B. Klee: Phosphorylation of smooth muscle myosin light chain kinase by the catalytic subunit of adenosine 3':5'-monophosphoate-dependent protein kinase. J. Biol. Chem. 253:8347-8350, 1978.

Alonso-DeFlorida, F. and F. Cordoba: The anaphylactic relaxation of the smooth muscle of the guinea-pig. Arch. Int. Pharmacodyn. 158:24-29, 1965.

Alonso-DeFlorida, F., J. Delcastillo, X. Garcia and E. Guon: Mechanism of the Schultz-Dale reaction in the denervated diaphragmatic muscle of the guinea-pig. J. Gen. Physiol. 51:577-693, 1968.

Anderson, R.G.G., K.B. Nilsson: Role of cyclic nucleotide metabolism and mechanical activity in smooth muscle. In: The Biochemistry of Smooth Muscle. ed. N.L. Stephens, Baltimore, University Park Press, pp. 261-291, 1977.

Anggard, E.: The isolation and determination of prostaglandins in lungs of sheep, guinea-pig, monkey and man. Biochem. Pharm. 14:1507-1516, 1965.

Antonissen, L.A., R.W. Mitchell, E.A. Kroeger, W. Kepron, K.S. Tse and N.L. Stephens: Mechanical alterations of airway smooth muscle in a canine asthmatic model. J. Appl. Physiol. 46:681-687, 1979.

Antonissen, L.A., R.W. Mitchell, E.A. Kroeger, W. Kepron, N.L. Stephens and J. Bergen: Histamine pharmacology in airway smooth muscle from a canine model of asthma. J. Pharmacol. Exp. Ther. 213: 150-155, 1980.

Ash, A.S.F. and H.O. Schild: Receptors mediating some actions of histamine. Br. J. Pharmac. Chem. 27:427-439, 1966.

Ashton, F.T., A.V. Somlyo and A.P. Somlyo: The contractile apparatus of vascular smooth muscle: Intermediate high voltage stereo electron microscopy. J. Mol. Biol. 98:17-29, 1975.

Bartosch, R., W. Feldberg and E. Nagel: Das Freiwerden eines histamin-ahulichen stoffer bei der Anaphylaxie des Meerschweinchens (Mit 11, Textabbildungen). Plfug. Arch. ges Physiol. 230:120, 1932.

Bergstrom, S.: Prostaglandins: Members of a new hormonal system. Science (New York) 157:382, 1967.

Benditt, E.P., R.L. Wong, M. Arase and E. Roeper: 5-Hydroxyptamine in Mast cells. Proc. Soc. Exp. Biol. Med. 90:303-304, 1955.

Bias, W. and D. Cmarsh: The Genetic Base of Asthma. In: Asthma: physiology, immunopharmacology and treatment ed: L. Lichtenstein and K.F. Austen, Acard. Press. pp. 21-26, 1977.

Black, J.W., W.A. Duncan, C.J. Durant, C.R. Ganellin and E.M. Parsons: Definition and antagonism of histamine H_2-receptors. Nature, Lond. 236:385-390, 1972.

Black, J.W., W.A. Duncan, J.C. Emmentt, C.R. Ganellin, T. Hesselbo, E.M. Parsons and J.H. Wyllie: Metiamide-an orally active histamine H_2-receptor antagonist. Apent Action, 23:133-137, 1973.

Bouhuys, A., R. Jonson, S. Lichtneckert, S.E. Lindell, C. Lundgren, G. Lundin, and T.R. Ringuist: Effect of histamine on pulmonary ventilation in man. Clin. Sci. 19:79-94, 1960.

Burka, J.F., T.R. Deline, M.C. Holroyde and P. Eyre: Chemical mediators of anaphylaxis (Histamine, 5-Ht and SRS-A) released from horse lung and leukocytes in vitro. Res. Commun. Chem. Path. Pharmac. 13:379-388 1976.

Castillo, J.C. and E.J. deBeer: The tracheal chain I. A preparation for the study of antispasmodics with particular reference to broncho-dilator drugs. J. Pharm. Exp. Ther. 90:104-109, 1947.

Castillo, J.C.: The anaphylactic guinea-pig trachea and its response to antihistamine and bronchodilators drugs. J. Pharm. Expt. Ther. 94:412-415, 1948.

Chacko, S., M.A. Conti and R.S. Adelstein: Effect of phosphorylation of smooth muscle myosin on actin activation and on Ca^{++} regulation. Proc. Natl. Acad. Sci. U.S.A. 74:129-133, 1977.

Chand, N. and P. Eyre: Pharmacology of anaphylaxis in chicken intestine. Br. J. Pharmac. 57:399-408, 1976.

Chand, N. and P. Eyre: Anaphylactic contraction of pulmonary blood vessels of chicken. Br. J. Pharmac. 59:201-208, 1977.

Chand, N., L. Deroth and P. Eyre: Pharmacology of Schultz-Dale reaction in canine lung strip in vitro: possible model for allergic asthma. Br. J. Pharmacol. 66:511-516, 1979.

Cirstea, M. and G. Suhaciu: Role of histamine substance P, slow-reacting substance of anaphylaxis (SRS-A) in the Schultz-Dale reaction of the guinea-pig ileum. Arch. Int. Physiol. Biochem. 76:344-362, 1968.

Cirstea, M.: Discrepancies between systemic anaphylaxis and the Scultz-Dale reaction. Int. Arch. Allergy 38:113-118, 1970.

Dabrowska, R., J.M.F. Sherry, D.K. Aromatorio and D.J. Hartshorne: Modulator protein as a component of the myosin light chain kinase from chicken gizzard. Biochemistry 17:253-258, 1978.

Dain, D. and W.M. Gold: Mechanical properties of the lungs and experimental asthma in conscious allergic dogs. J. Appl. Physiol. 38:96, 1975.

Dale, M.M.: The applicability of anaphylactic tests in studies of antigen mixtures I. The sensitivity of the tests. Immunology 8:435-443, 1965a.

Dale, M.M.: The applicability of anaphylactic tests in studies of antigen mixtures. II. The discriminatory capacity of the test. Immunology 8:444-453, 1965b.

Dale, M.M. and D.T. Okpako: Recovery of anaphylactic sensitivity in the guinea-pig ileum after desensitization. Immunology 17:653-663, 1969.

Devine, C.E., A.V. Somlyo and A.P. Somlyo: Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscles. J. Cell. Biol., 52:690-718, 1972.

Disalvo, J. and C. Schmidt: Contractile response to epinephrine, serotonin and potassium in arterial smooth muscle. Proc. Soc. Exp. Biol. Med., 151:478-483, 1976.

Dale, M.M. and L. Zilletti: The Schultz-Dale response of the longitudinal muscle strip preparation of guinea-pig ileum. Br. J. Pharmac. 39: 542-555, 1970.

Dale, H.H. and P.P. Laidlaw: The physiological action of B-imina-
zolylethylamine. J. Physiol. (London) 41:318, 1910.

Dale, H.H.: The biological significance of anaphylaxis. Proc. Roy.
Soc. B. 91:126-146, 1920.

Dale, H.H.: The mechanism of anaphylaxis. Acta. Allergy KBH 5,191-206,
1952.

Drazen, J.M. and K.F. Austen: Atropine modification of the pulmonary
effects of chemical mediators in the guinea pig. J. Appl. Physiol.
38:834-838, 1975.

Drazen, J.M., R.A. Lewis, S.I. Wasserman, R.P. Orange and X.F. Austen:
Differential effects of partially purified preparation of SRS-A
on guinea pig tracheal spirals and parenchymal strips. J. Clin.
Invest. 63:1-5, 1979.

Dunlop, L.S. and A.P. Smith: Reduction of antigen induced contraction
of sensitized human bronchus in vitro by indomethacin. Br. J.
Pharmacol. 59:475-497, 1977.

Ebashi, S. and M. Endo: Calcium and muscle contraction. Prog.
Biophys. Mol. Biol., 18:123-183, 1968.

Engineer, D.M., U. Neiderhauser, P.J. Piper and P. Sirois.

Br. J. Pharma. 62:61-66, 1978.

Eyre. P.: The pharmacology of sheep trachebronchial muscle: The relevant
effect of histamine on the isolated bronchi. Br. J. Pharmacol.
30:409-417, 1969.

Eyre, P.: The Schultz-Dale reaction in bovine pulmonary smooth muscles.
Br. J. Pharmac. 40:166P-167P, 1970.

Eyre, P.: Pharmacology of bovine pulmonary vein anaphylaxis in vitro. Br. J. Pharmac. 43:302-311, 1971.

Eyre, P.: Release of 5-hydroxyptamine from sensitized calf lung by antigen and compound 48/80. Arch. int. pharmacodyn 199:245-252, 1972.

Eyre, P.: Histamine H_2-receptors in the sheep bronchus and cat trachea: the action of burimamide. Br. J. Pharmac. 48:321-323, 1973.

Eyre, P. and A.J. Lewis: Acute systemic anaphylaxis in the horse. Br. J. Pharmac. 48:426-437, 1973.

Eyre, P., A.J. Lewis and P.W. Wells: Acute systemic anaphylaxis in the calf. Br. J. Pharmac. 47:504-516, 1973.

Eyre, P. and P.W. Wells: Histamine H_2-receptors modulate systemic anaphylaxis: a duel cardiovascular action of histamine in calves. Br. J. Pharmac. 49:364-367, 1973.

Eyre, P. Pulmonary histamine H_1 and H_2 receptor studies. In: Asthma, physiology, immunopharmacology and treatment. ed. L.M. Lichtenstein and K.F. Austen. pp. 169-180. Academic press. New York, 1977.

Farmer, J.B., D.G. Farrar and J. Wilson: Antagonism of tone and prostaglandin mediated response in a tracheal preparation by indomethacin and SC-19220. Br. J. Pharmac. 52:559-565, 1974.

Feigen, G.A., E.M. Vaughan Williams, J.K. Peterson and L.B. Nielson: Histamine release and intracellular potentials during anaphylaxis in the isolated heart. Circ. Res. 8:713-723, 1960.

Feldberg, W.: Allergische Rektions Mechanismen. Arch. Klin. Exp. Derm. 213:343-381, 1961.

Fleisch, J.H. In: Asthma, physiology, immunopharmacology and treatment. ed. K.F. Austen and L.M. Lichtenstein. Academic press. New York and London. pp. 304, 1973.

Folkow, B. and E. Neil: Pulmonary circulation In: Circulation Oxford University Press. London and Toronto, pp. 379-398, 1971.

Gabella, G.: Cellular structures and electrophysiology behaviour - Fine Structure - Philos. Trans. R. Soc. London 265:7-16, 1973.

Geiger, W.B., E.M. Hill and M. Thompson: A study of the mechanism of the Schultz-Dale reaction. Proc. Soc. Exp. Biol. Med. 92: 793-796, 1956.

Geiger, W.B. and H.S. Alpers: Mode of action of antigen and other smooth muscle stimulants. Science, 125: 1141- , 1957.

Goodby, P. and E.A. Phillips: Some effects of burimamide on the isolated perfused pulmonary circulation of the guinea pig. Br. J. Pharmacol. 49:368-369, 1973.

Gold, W.M., G.F. Kessler, D.Y.C. Yu, and O.L. Frick: Pulmonary physiologic abnormalities in experimental asthma in dogs. J. Appl. Physiol. 33:496- , 1972.

Gold, W.M.: Cholinergic pharmacology in asthma. In: Asthma, physiology, immunopharmacology and Treatment. ed K.F. Austen and L.M. Lichtenstein. Academic Press, New York and London. pp. 324, 1973.

Gold, W.M.: Asthma In: Basic of RD. American Thoracic Society, ed. A.F. Pierce. American Lung Association. Vol. 4, No. 3, 1976.

Gold, W.M., G.L. Meyer, D.S. Dain, R.S. Miller and H.R. Bourne: Changes in airway mast cells and histamine caused by antigen aerosol in allergic dogs. J. Appl. Physiol. 43(2):271-275, 1977.

Goldstein, A.: Biostatistics: An Introductory Text. ed. by the MacMillan Co., New York, pp. 147-154, 1964.

Gorecka, A., M.O. Aksoy and D.J. Hartshorne: The effect of phosphorylation of gizzard myosin on actin activation. Biochem. Biophys. Res. Commun. 71:325-331, 1976.

Gryglewski, R.J., A. Dembinska-Kiec, L. Grodzinska and B. Panczenko: Differential generation of substances with prostaglandin-like and thromboxane-like activities by guinea-pig trachea and lung strips. In: Lung Cells in disease. ed. A. Bouhuys, Elsevier/North Holland Biomedical Press, 1976.

Guerzon, G.M., P.D. Pare, M-C. Michoud and J.C. Hogg: The number and distribution of mast cell in monkey lungs. Am. Rev. Respir. Dis. 119(1):59-66, 1979.

Guschin, I.S.: Electrophysiological characteristics of anaphylaxis of isolated smooth muscle in: Physiology of smooth muscle. ed. E. Bulbing and M.F. Shuba, Raven Press, New York, pp. 419-425, 1975.

Hargraves, W.A. and E.B. Weiss: Effect of histamine on the length-tension properties of guinea pig trachealis muscle. Respir. Physiol. 29(2):211-224, 1977.

Hawkins, D.F. and I.M. Rosa : Some discrepancies in the histamine theory of anaphylaxis in smooth muscle. In: Histamine, CIBA Foundation symposium (Eds. G.E.W. Wolstenholme and C.M. O'Connor; J. and A. Churchill Ltd. London, pp. 180-182, 1956.

Hedqvist, P. and A.A. Mathe: Lung function and the role of prostaglandins. In: Asthma, physiology, immunopharmacology and treatment. Ed. L.W. Lichtenstein and K.F. Austen. Academic Press, New York. pp. 131-146, 1977.

Herlihy, J.T.: Helically cut vascular strip preparation: geometrical considerations. Amer. J. Physiol. 238:H107-H109, 1980.

Horton, E.W.: Hypotheses on physiological roles of prostaglandins. Physiol. Rev. 49:122-161, 1969.

Hudgins, P.M. and G.B. Weiss: Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and K^+. J. Pharmacol. Exp. Ther. 159:91-97, 1968.

Ishizaka, K. and T. Ishizaka: Mechanisms of reaginic hypersensitivity. Clin. Allergy, 1:9, 1971.

Joiner, P.D., M. Wall, I.B. Davis and F. Hahn: Role of amines in anaphylactic contraction of guinea pig isolated smooth muscle. J. Allergy and Clin. Immun. 53:261-270, 1974.

Joiner, P.D., P.J. Kadowitz, J.P. Hughes and A.L. Hyman: Action of prostaglandins E_1 and F_{22} on isolated intrapulmonary vascular smooth muscle. Pro. Soc. Exp. Biol. Med. 150:414-421, 1975.

Kadowitz, P.J., P.D. Joiner, and A.L. Hyman: Physiological and pharmacological roles of prostaglandins. Annu. Rev. Pharmacol. 15:285-303, 1975.

Kaliner, M., R.P. Orange and K.F. Austen: Immunological release of histamine and slow reacting substance from human lung in enhancement by cholinergic and alpha adrenergic stimulation. J. Exp. Med. 136:556-567, 1972.

Kaliner, M. and K.F. Austen: Immunologic release of chemical mediators from human tissues. Am. Rev. Pharmacol. 15:177-189, 1975.

Karczewski, W. and J.G. Widdicombe: The role of the vagus nerves in the respiratory and circulatory reaction to anaphylaxis in rabbits. J. Physiol. (Lond.) 201:293-304, 1969.

Karim, S.M.M., M. Sandler, and E.D. Williams: Distribution of prostaglandin in human tissues. Br. J. Pharmac. 31:340-344, 1967.

Katsch, S.: In vitro anaphylactic and non-anaphylactic responses of organs of immunized guinea-pigs. J. Pharm. Exp. Ther. 124: 86-93, 1958.

Kay, A.B., D.J. Stechschulte, and K.F. Austen: Eosinophil chemotactic factor in anaphylaxis. J. Exp. Med. 133:602-619, 1971.

Kepron, W., J.M. James, B. Kirk, A.H. Sehon, and K.S. Tse: A canine model for reaginic hypersensitivity and allergic bronchoconstriction. J. Allergy. Clin. Immunol. 59:64-69, 1977.

Kessler, G.F., O.L. Frick, and W.M. Gold: Immunologic and physiologic characterication of the role of reaginic antibodies in experimental asthma in dogs. In: Arch. Allergy. Appl. Immunol. 47:313- , 1974.

Kroeger, E.A. and J. Bergen: Presynaptic regulation of cholinergic and adrenergic neurotransmitter release by histamine and serotonin in airway smooth muscle (abstract). Fed. Proc. 39:1176, 1980.

Lecomte, J.: Les substances histamino-liberatrices sont imcapables de modifier le choc anaphylactique. Arch. In. Physiol. Biochem. 64: 302-308, 1956.

Lewis, R.A., S.I. Wasserman, E.J. Goetzl and K.F. Austen: Formation of slow-reacting substance of anaphylaxis in human lung tissue and cells before release. J. Exp. Med. 140:1133-1146, 1974.

Lichtenstein, L.M. In: Asthma, physiology, immunopharmacology and treatment. ed. K.F. Austen and L.M. Lichtenstein, Academic Press, London; pp. 91-104, 1973.

Lichtenstein, L.M. and E. Gillespie: Histamine receptors inhibition of histamine release is controlled by an H_2-receptor. Nature (Lond.) 244:287-288, 1973.

Lulich, K.M., H.W. Mitchell and M.P. Sparrows: The cat lung strip as an in vitro preparation of peripheral airways: A comparison of beta-adrenoceptor agonists, antacoid and anaphylactic challenge on the lung strip and trachea. Br. J. Pharmac. 58:71-79, 1976.

Maengwyn-Davies: The dual mode of action of histamine in the cat isolated tracheal chain. J. Pharm. Pharmacol. 20:572-573, 1968.

Marone, G., A. Kagey-Sobotka and L.M. Lichtenstein: Effects of arachiodonic acid and its metabolites on antigen-induced histamine release from human basophils in vitro. J. Immunology 123:1669-1677, 1979.

Martinez, de Letonia, J., D.L.M. Ramiro Castro and M.A. Domingo: Local and reflex effects of bronchial arterial injection of drugs. J. Pharmacol. Exp. Ther. 133:295-303, 1961.

Mathe, A.A., A. Astrom and N.A. Persson: Some bronchoconstricting and bronchodilating response of human isolated bronchi: evidence for the existence of -adrenoceptors. J. Pharm. Pharmacol. 23:905-910, 1971.

Mathe, A.A., P. Hedqvist, A. Holmgren and N. Svanborg: Bronchial hyper-reactivity to prostaglandin F_2 and histamine in patients with asthma. Br. Med. J. 1:193-196, 1973.

Mathe, A.A. and P. Hedqvist: Effect of prostaglandins F_2 and E_2 on airway conductance in healthy subjects and asthmatic patients. Am. Rev. Resp. Dis. 111:313-320, 1975.

Mathe, A.A.: Studies on actions of prostaglandins in the lung. Acta. Physiol. Scand. Suppl. 441, 1976.

McEwen, L.M.: The Schultz-Dale anaphylactic test for carcinoma antigen. Br. J. Med. ii, 615-617, 1959.

Meyers, G.I., D. Dain, W.M. Gold, R.L. Miller and H.R. Bourne: Bronchial mast cells and histamine in experimental canine asthma. The physiologist 16:396, 1973.

Mitchell, R.W., L. Antonissen, J. Bergen and N.L. Stephens: Atropine sensitivity of K^+ and histamine response in airway smooth muscle from a canine model of asthma. Fed. Proc. 39:1175, 1980 (abstract).

Mongar, J.L. and H.O. Schild: Inhibition of the anaphylactic reaction. J. Physiol. 135:301-319, 1957.

Moor, H.: Immunological release of histamine from human lung. Respiration, 38:273-279, 1979.

Murphy, R.A., F.T. Herlihy, and J. Megerman: Force-generating capacity and contractile protein content of arterial smooth muscle. J. Gen. Physiol. 64:691-705, 1974.

Nadel, J.A.: Structure-function relationships in the airways: broncho-constriction mediated via vagus nerves or bronchial arteries; peripheral lung constriction mediated via pulmonary arteries. Med. Thorac. 22:231, 1965.

Okazaki, T., T.F. Johnson, R.E. Reisman, C.E. Arbesman and E. Middleton, Jr.: Plasma prostaglandin concentration in a allergic bronchial asthma. Int. Archs. Allergy appl. Immun 57:279-281, 1978.

Okpako, D.T.: Antigen-dose response relationships in guinea-pig ileum anaphylaxis. Int. Arch. Allergy 38:474-481, 1970.

Okpako, D.T.: A vasodepressor action of histamine mediated by H_2-receptor activation. Eur. J. Pharmacol. 29:10-14, 1974.

Orange, R.P., D.J. Strechshulte and K.F. Austen: Immunochemical biological properties of rat IgE II. Capacity of mediate the immunologic release of histamine and SRS-A. J. Immunol. 105:1087-1095, 1970.

Orange, R.P., W.G. Austen, and K.F. Austen: The immunological release of histamine and SRS-A from human lung. I. Modulation by agents influencing cellular levels of cyclic 3',5' adenosine monophosphate. J. Exp. Med. 134: Suppl. 1365, 1971.

Pare, P.D., M.C. Michaud, R. Boucher and J.C. Hogg: Lung volumes after acute and chronic antigen exposure of immunized guinea pigs. The Physiologist 20(4): 71, 1977.

Paterson, N.A.M., R.W. Leid, J.W. Said, S.I. Wasserman, and K.F. Austen: In: "Lung Cell in Disease" ed. A. Bouhuys, pp. 223 North Holland Publish. Amsterdam, 1976a.

Paterson, N.A.M., S.I. Wasserman, J.W. Said, and K.F. Austen: Release of chemical mediators from partially purified human lung mast cells. J. Immunol. 117:1356-1362, 1976b.

Patterson, R.: Investigation of spontaneous hypersensitivity of the dog. J. Allergy, 31:351-363, 1960.

Patterson, R.: Laboratory models of reaginic allergy. Prog. Allergy, 13:322- , 1969.

Pinckard, P.R.N., M. Halonen and A.L. Meng: Preferential expression of antibovine serum albumin IgE homocytotropic antibody synthesis and anaphylactic sensitivity in the neonatal rabbit. J. Allergy Clin. Immunol. 49:301-310, 1972.

Piper, B.P.J. and J.R. Vane: Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. Nature (Lond.) 223:29-35, 1969.

Piper, P.J. and J.R. Vane: The release of prostaglandins from lung and other tissues. Ann. N.Y. Acad. Sci. 180:363-385, 1971.

Salvato, G.: Mechanism of the asthma attack. Brit. Med. J. 2:179- 1976.

Schild, H.O., D.F. Hawkins, J.L. Monger and H. Herxheimer: Reactions of isolated human asthmatic lung and bronchial tissue to specific antigen: Histamine release and muscular contraction. Lancet 2: 376, 1951.

Schild, H.O.: Histamine release and anaphylaxis, in: Histamine, CIBA Foundation Symposium (Eds. G.E.W. Wolstemholme and C.M. O'Connor; J and A Churchill Ltd., London) pp. 139-149, 1956.

Sharry, J.M.F., A. Gorecka, M.O. Aksoy, R. Dabrowska, D.J. Hartshorne: Role of calcium and phosphorylation in the regulation of the activity of gizzard myosin. Biochemistry 17:4411-4418, 1978.

Shultz, G., J.G. Hardman and W. Sutherland: In: Asthma: physiology, immunopharmacology and treatment, Academic Press, New York, pp. 123, 1979.

Sjoerdsma, A., T.P. Waalkes, and H. Weissbach. Serotonin and histamine in mast cells. Science, 125:1202-1203, 1957.

Smith, A.P.: A comparison of the effects of prostaglandin E_2 and salbutainol by intravenous infusion on the airways obstruction of patients with asthma. Br. J. Clin. Pharmac. 1:399-404, 1974.

Sobieszek, A., J.V. Small: Regulation of the actin-myosin interaction in vertebrate smooth muscle: activation via a myosin light chain kinase and the effect of tropomyosin. J. Molec. Biol. 112:559-576, 1977.

Somlyo, A.P. and A.V. Somlyo: Ultrastructure of smooth muscle. In: Methods in pharmacology, Vol. 3, ed. E.E. Daniel, D.M. Paton, New York, Plenum Press, pp. 3-45, 1975.

Souhrada, J.F., G. Kaub, and J. Loader: Physiological and biochemical characteristics of asthmatic airway smooth muscle. The Physiologist, 20(4):90, 1977.

Stoner, J., V.C. Manganiello, and M. Vanghan: Effects of bradykinin and indomethacin on cyclic GMP and cyclic AMP in lung slices. Proc. Nat. Acad. Sci. 70:3830-3833, 1973.

Strechschulte, D.J., R.P. Orange and K.F. Austen: Immunochemical and biological properties of rat IgE: I. Immunochemical Identification of Rat IgE, J. Immunol. 105:1082-1086, 1970.

Swineford, O. and R.J. Reynolds: Studies in bacterial allergy. In: Transitory nature of desensitization of passively sensitized guinea-pig uterus. J. Allergy 22:156-160, 1951.

Szczeklik, A. D.J. Gryglewski and G. Mysik-Czerneioska:

Br. Med. J. i:67-69, 1975.

Tauber, A.I., M. Kaliner, D.J. Stechschulte and K.F. Austen:

Journal of Immunology 111:27-32, 1973.

Tucker, A., E.K. Weir, J.T. Reeves and R.F. Grover: Histamine H_1 and H_2 receptors in pulmonary and systemic vasculature of the dog. Am. J. Physiol. 229(4):1008-1013, 1975.

Turker, R.K.: Presence of histamine H_2-receptors in the guinea-pig pulmonary vascular bed. Pharmacology, 9:306-311, 1973.

Vane, J.R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (Lond.), New Biol. 231: 232-235, 1971.

Van Breemen, C., B.R. Farinas, R. Casteels, P. Gerga, F. Wuytack, and P. Deth: Factors controlling cytoplasmic calcium concentration. Philos Trans. R. Soc. London, 265:57-71, 1973.

Van Rossum, J.M.: Biological drug parameters for studies for the relationship between structure and activity. In: Physio-chemical aspect of drug action. ed. E.J. Ariens, Vol. 7. Pergamon Press, New York, 1968.

Walker, J.L.: In: Advances in Biosciences, vol. 9, ed. S. Bergstiom and S. Bernhard. Pergamon Press, Oxford, pp. 235-240, 1972.

Wasserman, S.I., E.J. Goetzl, and K.F. Austen: Immunologic release of preformed eosinophil chemotactic factor of anaphylaxis (ECF-A) from isolated mast cells. Fed. Proc. 32:819, 1973 (abstract).

West, G.B.: Tissue mast cells and tissue amines, J. Pharm. Pharmacol., 11:513-534, 1959.

Widdicombe, J.G.: Some experimental models of acute asthma. J.R. Coll. Physicians London 11(2):141-155, 1977.

Wilhelm, D.L., L.C.J. Yong and S.G. Watkins: The mast cell: distribution and maturation in the rat. Agents Actions 8(1-2): 146-152, 1978.

Wilson, A.F. and S.P. Galant: Recent advances in the pathology of asthma. West. J. Med. 120:463, 1976.

Yamashita, K., T. Takagi, and K. Hotta: Mobilization of cellular calcium and contraction-relaxation of vascular smooth muscle. Jap. J. Physiol. 27:551-564, 1977.