DENERVATION INDUCED MECHANICAL RHYTHMICITY IN THE MULTIUNIT CANINE TRACHEAL SMOOTH MUSCLE

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Abstract

Cholinergic denervation, but not sympathetic denervation was shown to induce changes in the canine trachealis smooth muscle similar to that observed in the presence of tetraethylammonium or after metabolic depletion. Surgical inhibition of the motor supply resulted in oscillatory contractions of the muscle when stimulated with carbachol ($2X10^{-7}M$) or histamine ($10^{-7}M$) but not potassium chloride.

The phasic activity of the muscle continued in the presence of tetrodotoxin but was acutely sensitive to alterations in the calcium concentration of the bathing medium Mechanical oscillations were reduced and abolished in the presence of a calcium EGTA buffer or 0-Ca while the tonic component of the contraction was reduced only after larger doses were employed.

Differentiation of the mechanical tracing showed that the oscillatory behavior of the muscle was biphasic, consisting of short rapid oscilations superimposed on the slow mechanical contractions. Further investigation gave evidence which suggested that the two components might be due to different pools of calcium or that calcium might work in conjunction with chloride to produce the biphasic response.

The myogenic response observed upon quick stretch of the denervated tracheal muscle suggests that the surgical

treatment of the muscle sets up the conditions necessary for this phenomenon. Such a response is observed only after the denervated muscle has been stimulated with the appropriate agonist and was never observed in the innervated or unstimulated denervated preparation. It would appear that surgical denervation of the motor supply is responsible for a conversion from a multiunit type to a single unit type muscle.

Fluctuations in ATP have been implicated in the phasic activity of some muscles, (Westfall, 1975) and it has been suggested that variations in the ATP content may be, at least partially, responsible for observed alterations in ionic fluxes (Gradman and Slayman, 1975; Sperelakis and Schneider, 1976). Evidence of a decreased ATP content in the denervated tracheal muscle was found, however, the correlation between this decrease in ATP content and the phasic activity of the muscle can only be surmised.

Inhibition of the active pumping processes within the muscle, by cooling or through the administration of ouabain, had an inhibitory effect on the oscillatory nature of the denervated preparation. It is unlikely, however, that the electrogenic sodium pump is entirely responsible for the rhythmicity since the peak tension observed, after cooling or the administration of ouabain, does not reach the peak tension observed while the muscle is rhythmic.

Many of the changes observed in tracheal smooth muscle following motor but not inhibitory denervation are suggestive of a conversion from a multi to a single unit type muscle. These changes are similar to those seen in tracheal smooth muscle made rhythmic by substrate deprivation. The precise mechanism responsible for the oscillatory nature of this preparation remains to be elucidated.

Chapter I

INTRODUCTION AND HISTORICAL REVIEW

Fibrillatory or rhythmic behavior of muscle (striated or smooth muscle) is not a recently observed phenomenon. Such fibrillatory activity in striated muscle was first observed by Schiff in 1851 after motor denervation. He observed that the onset of rhythmic activity was variable, depending on the muscle and species studied and that this activity could be recorded for up to a year after denervation (Cannon, W.B. and Rosenblueth, A., 1949). Similar accounts of rhythmicity in striated muscle have been reported by Philipeaux and Vulpian, 1863, Heidenhain, 1883, Tower, 1939, Eccles, 1941, Purves, 1974, Thessleff, 1975, Camerino and Bryant, 1976 (Cannon, and Rosenblueth, 1949; Purves and Sakmann, 1974; Camerino and Bryant, 1976a, 1976b).

The intrinsic spontaneous activity of the smooth muscle of the alimentary canal has been observed for some time. Detailed descriptions of the mechanical and electrical behavior of the rhythmically active gut have been published in essays by Klee (1927), Dietlen (1913), Kaufmann and Kienbock (1911), Trendelenburg (1917), and more recently by Prosser (1974) and El-Sharkawy and Daniel (1975). The mechanical oscillatory nature of many vascular smooth muscle preparations is a well known phenomenon and is best described in fairly recent reviews by Somlyo and Somlyo (1968 and 1970).

The story of airway smooth muscle rhythmicity or peristaltic movement is certainly one of interest, especially

when one considers that tracheal smooth muscle is generally classified as a tonic muscle that rarely responds to a stimulus with phasic contractions. Early reports of tracheal and bronchial rhythmicity, in both whole animal and isolated tissue preparations, would tend to disagree with this general assumption. Rhythmic behavior, in tracheal and bronchial smooth muscle, was recorded by a plethysmographic technique in 1903 by Dixon et al. It is especially interesting to note that agents like pilocarpine and muscarine were observed to potentiate the oscillatory nature of this smooth muscle after vagotomy (Dixon and Brodie, 1903). Examination of the trachea and large bronchi, with the help of a bronchoscope, enabled Jackson in 1917 to see the rhythmic behavior of airway smooth muscle. He described in some detail the various oscillatory movements, the major type being a to-and-fro motion toward and from the bronchoscope as well as a number of lateral movements of the airway muscle. Much, if not all, of this rhythmic activity was attributed to extrinsic mechanical forces exerted on the bronchi and trachea via the lungs during inspiration and expiration and the cardiovascular system (Ellis, 1936). This is refuted by early X-ray studies by Bullowa (1920) and Reinburg (1925), who described the peristaltic activity in both man and dogs. Both investigators describe three types of movement in the trachea. The first is a lateral movement and the second is

a "bellows-like" expansion and contraction. These are attributed to purely mechanical forces from the heart and the inspiratory and expiratory movements of the lungs. The third type of movement i.e. peristaltic waves of low amplitude, were observed to travel up the trachea and reported to be independant of coughing, respiration and swallowing (Bullowa and Gottlieb, 1920; Reinberg, 1925). Such movements are claimed to be slower than those observed in the cough reflex yet too rapid for ciliary action. This peristaltic action has on occasion been shown to be quite pronounced, even to the point of being termed "tracheal vomiting" (Reinberg, 1925). A number of investigators have attributed this rhythmic behavior in the trachea to pathological conditions. Reinberg's observations were based on a patient diagnosed as having "paralysis of the recurrent nerves" (Reinberg, 1925), while Macklin claims that the peristaltic activity in the tracheas that he had observed were probably due to pathological conditions (Macklin, 1929). Along with these pathological conditions various changes in the physiological state of the preparation were observed to enhance the spontaneous contractions. Asphyxia, changes in temperature, bilateral vagotomy, histamine and cholinergic agents initiated or potentiated the rhythmic response (Ellis, 1936; Sollmann and Gilbert, 1937; Wish, 1952; Loofbourrow, et al, 1957).

Although little is known about the cause(s) or mechanism(s) of action underlying the peristaltic activity of the trachea, it has been suggested that the oscillatory behavior of the airway smooth muscle should not be too surprising, since it has a common derivation with the intestinal tract. It is also proposed that the motor innervation, by way of the vagus, and the presence of ganglia within the smooth muscle may possibly correspond to the ganglia of Auerbach and Meissner in the gut (Macklin, 1929; Sollmann and Gilbert, 1937). Although this is a pleasing theoretical possibility it fails to take into account the specialization of tissues during embryological development.

The functional significance of tracheal rhythmicity is still unknown. It is doubtful that it is of any benefit in expectorating substances from the airways since these oscillations are usually not powerful enough and the cough reflex appears to satisfy this function adequately (Widdicombe, 1963).

In 1948 Bozler subdivided smooth muscle preparations into two types, single unit and multiunit. Single unit muscles were those that had low electrical resistance between adjacent cells, propogated electrical activity within the tissue, spontaneous rhythmicity and myogenic contractions in response to stretch; while single unit muscles are sparsely innervated yet have a relatively large number of nexuses or tight-junctions (Westfall, et al, 1975). It has been pro-

posed that these nexuses represent low resistant pathways for the propagation of electrical activity (Somlyo and Somlyo, 1968a and 1970; Prosser, 1974; Westfall, et al, 1975). Singleunit muscles on the other hand are usually more densely innervated and reported to have fewer nexuses (Mekata, 1971; Westfall, et al, 1975). If each cell or group of cells has it's own innervation then there would be little need for the propagation of electrical activity. This may account for the graded response seen in multiunit muscle.

The classification of smooth muscle into spike generating (phasic) and gradedly responsive (tonic), is not based solely on electrophysiological studies. The potassium contractions and apparent membrane permeabilities to calcium (Ca^{++}) of the phasic muscles and the tonic muscles, are reported to be quite different (Somlyo and Somlyo, 1968a). Clearly, some smooth muscles would not conform completely to either one or the other category. It is reasonable to assume, therefore, that some smooth muscles may exhibit various shades of single or multiunit behavior (Somlyo and Somlyo, 1968a; Somlyo and Somlyo, 1968b).

Ultrastructure

Microscopic examination of smooth muscle cells reveals a distinct membrane, about 80Å thick, surrounding each cell

with no evidence of direct cytoplasmic continuity between adjacent cells. Each cell has a central elongated, ellipsoid nucleus, often containing one or two nucleoli (Somlyo and Somlyo, 1968a). In areas of close apposition or nexus the fused membrane of adjacent cells lack a basement membrane. These intercellular connections provide a means of direct transfer of information from one member of a given cell population to another of the same or to a different cell population. Since electrical transmission would be impossible over large intercellular distances due to short circuiting by the extracellular fluid (ECF), the nexuses would provide a low resistance pathway for conducting action potentials (AP), (Somlyo and Somlyo, 1968a; Prosser, 1974; Daniel, 1978). Available evidence indicates that there is a good positive correlation between the presence of nexuses and conducted action potentials. In addition to these nexal regions there are also peg and socket type of interdigitations and gap-junctions found along the membrane. These may serve to greatly increase the surface area of the cell and possibly aid in cell to cell communication (Somlyo and Somlyo, 1968a; Bose and Innes, 1974).

It has been reported that these structures are found in far greater numbers in single unit muscles (Somlyo and Somlyo, 1968a) and may be only sparsely distributed or even lacking in some multiunit muscles (Mekata, 1971).

Small vesicles are found in large numbers along the inner side of the smooth muscle membrane. These small bodies are known to accumulate Ca^{++} in concentrations equal to that of the ECF and, therefore, several times greater than that of the myoplasm. They appear to be rich in ATPase and may be involved in the relaxation process by means of removing Ca from the myoplasm, (Somlyo and Somlyo, 1968a; Prosser, 1974). Because these vesicles greatly increase the surface area of the membrane they could provide binding sites for both the release of calcium into and the reuptake of calcium from the cytoplasm (Van Breemen, et al, 1972). It has been suggested that these vesicles may be smooth or rough endoplasmic reticulum or even pinocytic vesicles detached from the muscle membrane (Somlyo and Somlyo, 1968a). Because they are not fixed structures, but communicate freely with the ECF, it is possible that they are concerned with the maintenance of intracellular calcium levels. The mitochondria in vascular smooth muscles are often in close apposition to the surface vesicles, and it has been suggested that cations accumulated by mitochondria may be extruded into the extracellular space through the surface vesicle-mitochondrial contacts (Somlyo, et al, 1974; Somlyo and Somlyo, 1976).

In addition to the round vesicles communicating with the ECF there is also a "closed vascular-tubular system", at least in some of the spike generating smooth muscles.

Some of these closed tubules and vesicles appear to make contact with the open pinocytotic vesicles. It has been suggested that they function analogously to the triads of skeletal muscle and are involved in intracellular translocation of calcium (Somlyo and Somlyo, 1970). It should be mentioned too, that the number of these vesicles can be easily overestimated. During histological preparation of the tissue, sections may be taken transversely through membrane invaginations or sockets, thus giving false estimates of the number and location of these vesicles.

The sarcoplasmic reticulum (SR), is a system of tubules present in most, and probably all, smooth muscles. The amount of SR is relatively small in comparison with that found in striated muscles. Tubules of the sarcoplasmic reticulum lie very near the inner cell membrane, separated by a gap of only a few nanometers (Somlyo and Somlyo, 1976). It has been suggested that these are sites where action potentials may release calcium activating twitch contractions (Somlyo and Somlyo, 1971). The volume of sarcoplasmic reticulum differs in different types of smooth muscle and it's extent correlates with the ability of a given smooth muscle to contract in the absence of extracellular calcium. Even in calcium free solutions drugs can elicit relatively large contractions in the smooth muscle of large elastic arteries in which the SR occupies approximately 5-7.5% of the cyto-

plasmic volume. Portal-anterior mesenteric vein and taenia coli smooth muscle contain an SR of only 1-2% of the cytoplasmic volume and such muscles generally fail to contract in calcium-free medium (Somlyo and Somlyo, 1970; Devine, et al, 1972; Popescu, et al, 1974; Somlyo and Somlyo, 1976).

The contractile mechanism in smooth muscle appears to be based on the sliding of filaments in a manner fundamentally similar to that of striated muscle (Somlyo and Somlyo, 1970; Somlyo and Somlyo, 1976). Although it has been difficult to isolate and identify these filaments, recent work by Somlyo and Somlyo, 1976, has shown that a thin actin, a thick myosin and an intermediate filament are present in most if not all smooth muscles. The myosin filaments, which are in parallel and arranged in contractile units, are longer than those in striated muscle. This may be why smooth muscle can develop tension at least equivalent to that developed by skeletal muscle, in spite of the relatively low concentrations of myosin in smooth muscle. The thin (actin) filaments are observed to be inserted into the cell membrane and in the dense bodies which are believed to act as anchor points for these filaments. The average ratio of thin to thick filaments is approximately 16:1. In the best organized examples of cross sections the thin filaments form a rosette surrounding a central thick filament (Somlyo and Somlyo, 1976). A third type of filament, frequently asso-

ciated with the dense bodies, has been described in a variety of smooth muscles. These structures are composed of neither actin nor myosin and their function is basically unknown (Somlyo, et al, 1971; Cooke and Chase, 1971; Somlyo, et al, 1973). In abnormal smooth muscle fibers these intermediate filaments may replace large proportions of the myofilament latice (Somlyo, et al, 1973).

Dense bodies are dark-staining spindle-shaped areas which vary in length from 4000 to 9000 Å and in width from 2000 to 5000 Å. They are often found close to or adhering to the cytoplasmic side of the membrane or dispersed within the membrane (Somlyo and Somlyo, 1968a; Somlyo and Somlyo, 1976). A large proportion of these structures appear to have a function similar to that of the Z-line of striated muscle (Somlyo and Somlyo, 1976).

Tracheal Smooth Muscle

The primary function of the smooth muscle of the lung is to control the distribution of inspired gas entering the lung. Smooth muscle is distributed from the trachea downwards as far as the respiratory bronchioles but it's attachments vary, so that the effects of muscle contraction are different in the largest airways from the effects in the smallest ones. The mass of muscle, in proportion to the diameter of the airways, increases as one goes distally

from the larynx to the lungs (Kamburoff, 1976).

In the trachea and main bronchi, which have complete cartilagenous rings, the muscle is arranged circularly being attached to the outer aspects of the tips of the cartilage. Contraction of this part of the muscle can, therefore, dramatically reduce the diameter of the trachea and main bronchi. Changes in tracheal and bronchial diameter occur during the respiratory cycle, dilating during inspiration and contracting during expiration.

The muscle attachment to the cartilage disappears at the level of the hilum of the lung and from then onwards due to the direction of the muscle contraction, produces not only narrowing of the bronchi but also longitudinal shortening (Kamburoff, 1976).

Tracheal Innervation

The vagus nerves, in the dog, carry both sympathetic and parasympathetic fibers and are ultimately responsible for both the afferent and efferent impulses to and from the trachea. The recurrent laryngeal nerves which arise from the vagi, supply the parasympathetic or motor control, while collaterals from the sympathetic trunk travelling with the vagi provide the inhibitory control over the tracheal smooth muscle.

There are parasympathetic ganglia throughout the