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Structure and Electrical Properties of the Autonomic Neuromuscular Junction

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Structure and electrical properties of the autonomic neuromuscular junction

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A description is given of the autonomic neuromuscular junction and of the junction potentials which occur in smooth muscle bundles during transmission. The structural and electrical constants of these muscle bundles have been determined and used to develop an electrical model of the bundle. This has then enabled an analysis of the origin and distribution of current generated by transmitter released from autonomic nerves within the bundle.

Introduction

Sherrington (1906) defined a synapse as a specialized region between neurons or between a neuron and an effector, across which the action potential is conducted in a manner different from conduction within these cells. It is therefore necessary, in order to identify the autonomic neuromuscular synapse or junction, to first describe the effector unit in smooth muscle in which the propagating action potential is initiated and to then describe the innervation of this effector (Bennett 1972, chap. 1 and 2.1). A brief description of this junction is given below, followed by an analysis of the mechanisms responsible for the potential changes which occur in smooth muscle during neuromuscular transmission.

1. Muscle bundles

Smooth muscles are divided by perimysium into muscle bundles, which vary in outline from rectangular to circular (20 to 200 μm wide) and are composed of many thousands of individual smooth muscle cells which are only about 3 μm diameter (figure 1*a*). These muscle bundles are joined together at regions along their length by an anastomosis or by smaller bundles which leave one large bundle and join another. An exception to this geometry occurs in the muscular walls of arterioles, which are only 1–3 cell diameters thick and are therefore not organized into bundles. The geometrical packing of the bundles determines the shape of the muscle (figure 1*a*).

The spatial requirements for initiating a propagating action potential in smooth muscle are now established: it is necessary to depolarize simultaneously an area of tissue about 100 μm diameter, the size of a muscle bundle, to initiate an action potential which will propagate; smaller areas of depolarization, including that of the membrane of a single cell may initiate an action potential but this will not propagate. Thus the many smooth muscle cells in a transverse plane through a muscle bundle must be simultaneously depolarized in order to initiate a propagating action potential.

2. Nerve supply to the muscle bundle

Autonomic motor-nerves enter smooth muscles from the serosal surface in large bundles of axons (100 axons, 20 μm diameter) and split into a number of medium size bundles (10–20 axons, 10 μm diameter) which pass in the perimysium between the muscle bundles (figure 1*a*).

These axon bundles divide into small axon bundles (3–5 axons, 2 μm diameter) which enter the muscle bundles which they innervate. The diameter of individual axons within an axon bundle alternately increases and decreases every few micrometres, as the axon enters the smooth muscle, giving the axon a beaded appearance. These beads are called axon varicosities, and occur at the highest density in the terminal region of the axon within the muscle bundle. It is likely that transmitter is released from these varicosities during transmission, as they contain synaptic vesicles which store the transmitter.

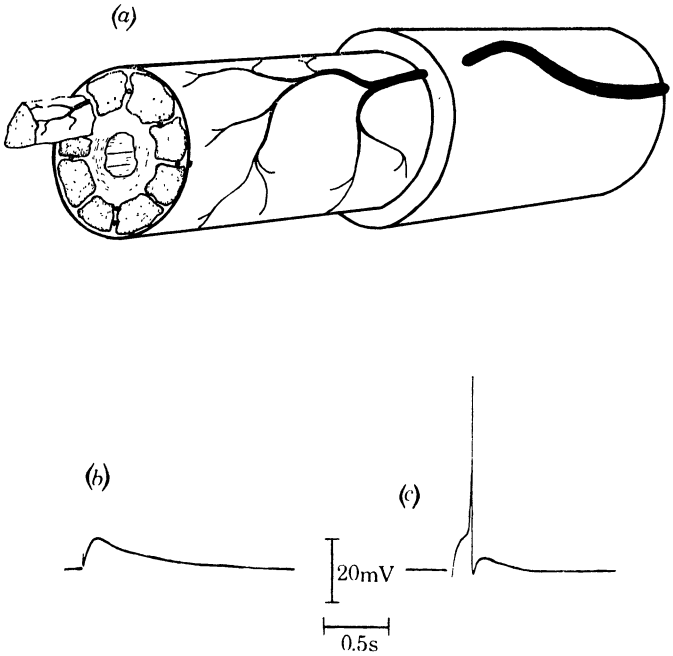

FIGURE 1. The structure and innervation of a smooth muscle. (*a*) The smooth muscle of this organ is divided into longitudinal and circular layers, which are in turn divided by a perimysium into muscle bundles; the nerve supply enters the serosal surface of the muscle as large bundles of axons which divide into smaller bundles that course between the muscle bundles. (*b*) Stimulation of a small percentage of the nerve supply gives a potential transient (the junction potential), which may be recorded in all the muscle cells of a bundle; (*c*) stimulation of a large percentage of the nerve supply gives a larger junction potential which initiates a propagating action potential.

Stimulation of the excitatory nerve supply to a smooth muscle gives rise to a transient depolarization of the muscle cells in a bundle, called an excitatory junction potential (figure 1*b*). This potential can be recorded undiminished in amplitude in any smooth muscle cell through the depth of a muscle bundle, and therefore satisfies the spatial criteria for initiating a propagating action potential. Thus, if the depolarization reached by the junction potential is suprathreshold, an action potential is initiated (figure 1*c*) and propagates along the muscle bundle.

3. CLASSES OF NEUROMUSCULAR JUNCTIONS

As the action potential is initiated by simultaneous depolarization of all the smooth muscle cells in a transverse section through the muscle bundle, the autonomic neuromuscular junction must consist of all the nerve elements and muscle cells in a transverse plane through the muscle bundle. On this basis, there are four different classes of neuromuscular junctions in the autonomic nervous system, corresponding to the four different ways in which a muscle bundle is

innervated. On entering the smooth muscle bundle, the small axon bundles may ramify parallel to and between individual smooth muscle cells, the individual axons more or less enclosed in Schwann cell sheath but never approaching the muscle cells closer than 100 to 200 nm (figure 2*a*; gastrointestinal tract and uterus). The small axon bundles on entering the muscle bundle may divide into single axons, which pass naked of Schwann cell covering between the muscle cells, and which frequently indent the surface of individual muscle cells; this indentation consists of an axon varicosity whose membrane is separated from the muscle membrane by a uniform gap of 20 nm, and is for this reason called a close-contact varicosity (figure 2*c*; mouse and rat vas deferens and rat seminal vesicles). Axon bundles on entering the muscle bundles of some organs innervate them with both small axon bundles as well as single axons which occasionally form close-contact varicosities on smooth muscle cells (figure 2*d*;

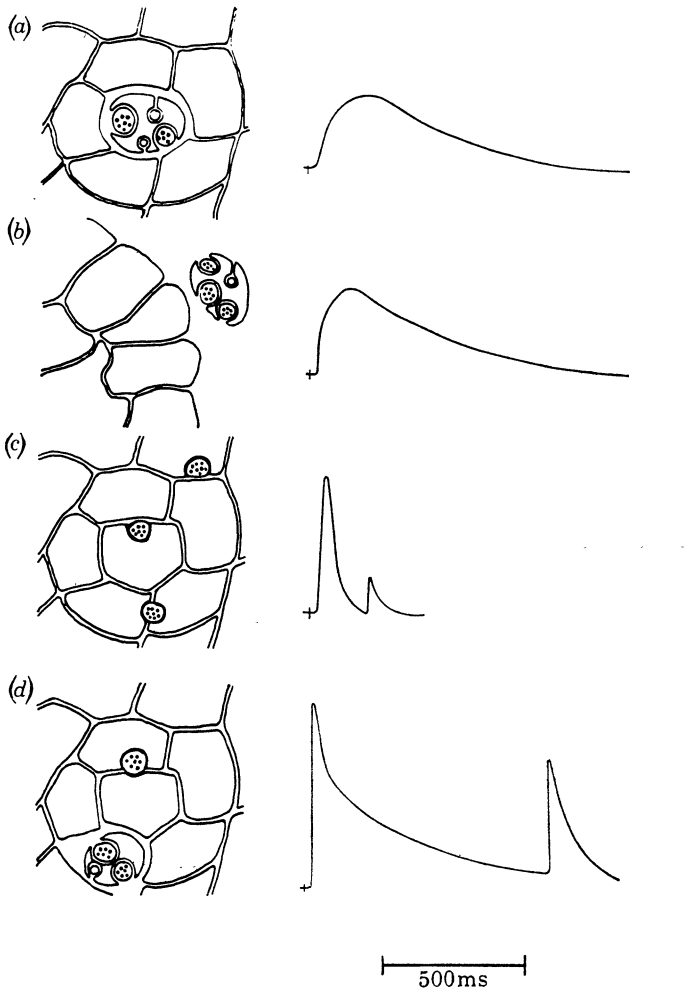

FIGURE 2. The four categories of innervation of a smooth muscle bundle. (*a*) Innervation by small axon bundles within the muscle bundle, stimulation of which gives a slow junction potential; no spontaneous junction potentials. (*b*) Innervation by small axon bundles confined to the medial-adventitial border of blood vessels, stimulation of which gives a slow junction potential; no spontaneous junction potentials. (*c*) Innervation by close-contact varicosities embedded in the surface of individual muscle cells, stimulation of which gives a fast junction potential; spontaneous junction potentials present. (*d*) Innervation by both small axon bundles and some individual cells by close-contact varicosities, stimulation of which gives slow or complex junction potentials depending on the cell impaled; spontaneous junction potentials present.

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guinea-pig vas deferens; sphincter and dilator of the rabbit iris; mouse urinary bladder and cat nictitating membrane). The final class of neuromuscular junction occurs in blood vessels; here the small axon bundles do not enter the muscle bundles, but ramify throughout the media-adventitia border of the vessels, approaching muscle cells no closer than about 100 nm (figure 2*b*).

The temporal characteristics of the junction potential evoked in the smooth muscle of an organ may be correlated with the type of neuromuscular junction occurring in the muscle but not with the excitatory or inhibitory nature of the transmission, or with the type of transmitter released. The junction potentials evoked in muscles by small axon bundles reach their peak in 100 to 150 ms, and decline with a time constant of 250 to 470 ms (figure 2*a*, *b*). The junction potentials evoked solely by varicosities forming close-contacts with muscle cells reach their peak in about 20 ms and decline with a time constant of about 30 ms (figure 2*c*). The junction potential in muscle bundles which receive an innervation of both kinds is generally of the slow type, but in some muscle cells junction potentials with both fast and slow components may be recorded (figure 2*d*). Furthermore, spontaneous miniature potentials with a time course similar to that of the fast junction potentials, are only recorded in those muscle bundles which have close-contact varicosities (figure 2*c*, *d*).

Thus the characteristics of the junction potential are not dependent on the electrical constants of different muscles, as complex junction potentials consisting of both fast and slow components may be recorded in the same muscle.

4. A hypothesis concerning the origin and distribution of current during transmission

This correlation between the structure of junctions and the temporal characteristics of the junction potential suggests the hypothesis that the focal application of transmitter on muscle cells throughout a bundle from close-contact varicosities gives rise to fast electrical transients, whereas the diffuse application of transmitter on cells from varicosities in axon bundles gives rise to slow potential transients. If the innervation of the bundle is mixed, some cells receiving only diffusely applied transmitter and others both diffuse and focal, then the characteristics of the electrical transient in a particular cell in the bundle will be dependent on the spatial distribution of the different kinds of transmitter application to cells throughout the muscle bundle. This hypothesis has been studied by developing a model of the smooth muscle electrical syncytium, and then applying this to the smooth muscle of the guinea-pig vas deferens to analyse the origin and distribution of current spread in the muscle bundle during transmission.

5. An electrical model of a smooth muscle bundle

The model of the smooth muscle bundle has been developed from an examination of the electrical transients which occur in smooth muscle to intracellular and extracellular current injection and from an analysis of transverse serial sections through a muscle bundle, examined with the electron microscope (Bennett 1972): in this model each cell is coupled to at least six other cells, two which overlap by 50% and maintain electrical continuity in the longitudinal direction, and four which completely overlap, and maintain electrical continuity in the radial direction. The electrical model of the smooth muscle cell bundle consists of the coupling of an

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impedance made up of the parallel resistance and capacitance of a muscle cell membrane by resistances representing the junctions between the cells, in the configuration shown in figure 3.

This electrical model of the muscle cell bundle responds in a similar way to intracellular or extracellular current as does the actual muscle bundle. Figure 4 shows the spatial and temporal distributions of the potentials in the syncytium for different kinds of current injection, determined by numerically solving the simultaneous differential equations describing the model. The experimental values obtained are compared with the numerical solution and are of similar time course and spatial distribution.

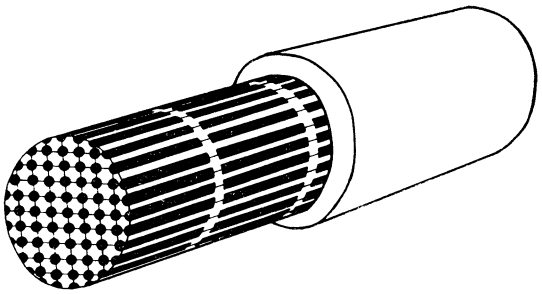

FIGURE 3. A model of a smooth muscle bundle. The bundle is delimited by a connective tissue sheath (the perimysium), in which each smooth muscle cell (black cylinder) is surrounded by a number of cells, to some of which it is coupled (lines). In the electrical model, each cell had an impedance made up of the parallel resistance (r_m) and capacitance (c_m) of the cell membrane ($r_m = 3.6 \times 10^9\ \Omega$; $c_m = 2.8 \times 10^{-11}$ F) and each coupling was represented by a simple resistance ($r_b = 7 \times 10^7\ \Omega$); each cell coupled with four cells in the transverse (or radial) plane through the bundle and with two cells in the longitudinal direction along the bundle.

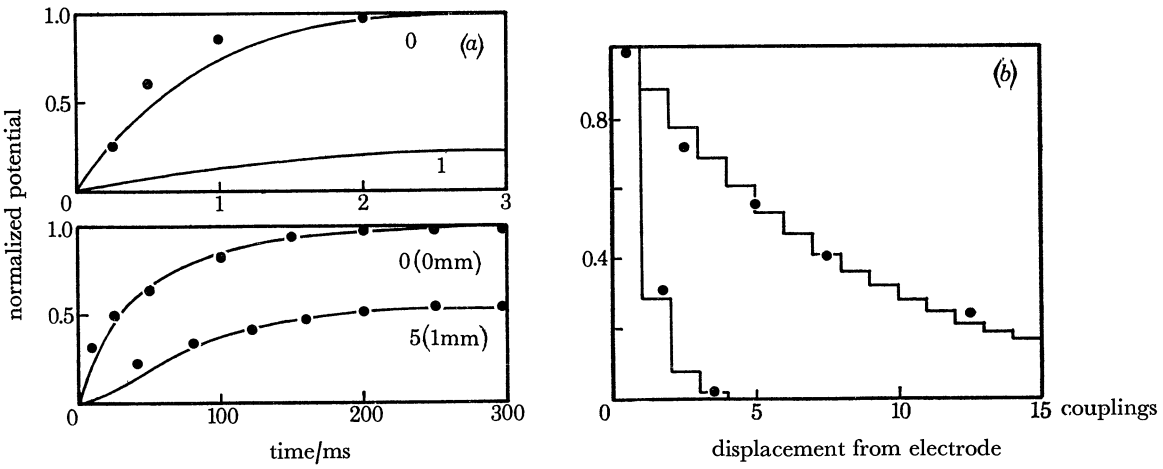

FIGURE 4. Response of an electrical model of the smooth muscle cell bundle to different patterns of current injection. (*a*) Temporal distribution of potential in the model due to current injection from either an intracellular electrode (upper curves) or an extracellular ring electrode (lower curves); the experimental results for these kinds of current injection in the guinea-pig vas deferens are given by the filled circles; the number of couplings distant from the point of current injection is given for each curve. (*b*) Spatial distribution of potential in the model due to current injection from either an intracellular electrode (short curve) or an extracellular ring electrode (long curve); the experimental results for extracellular current injection in the guinea-pig vas deferens are given by the filled circles; the experimental results for intracellular current injection are for the cat duodenum, assuming a cell diameter of 5 μm. (Data from fig. 11, Kobayashi, Prosser & Nagai 1967.)

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6. The mechanism of transmitter action on muscle cells

This model of the smooth muscle bundles allows a determination of both the mechanism and duration of transmitter action on the muscle cells in the syncytium. If the transmitter acts on the muscle cells throughout the bundle to increase the permeability to certain ion species during the junction potential, then it should be possible to alter the amplitude of this potential with simultaneous current injection into a large number of cells, but not by current injection into individual cells. This is what is observed in the guinea-pig vas deferens (figure 5*a*, *b*) and suggests that the current generating the junction potential in any particular cell mostly originates from the action of transmitter in increasing the membrane permeability of surrounding cells, rather than directly on the cell in question. In contrast, the amplitude of the spontaneous junction potential is completely under the control of current injection into a single cell (figure 5*c*), indicating that this potential is generated by the action of transmitter to increase the permeability of the impaled cell or a closely neighbouring cell.

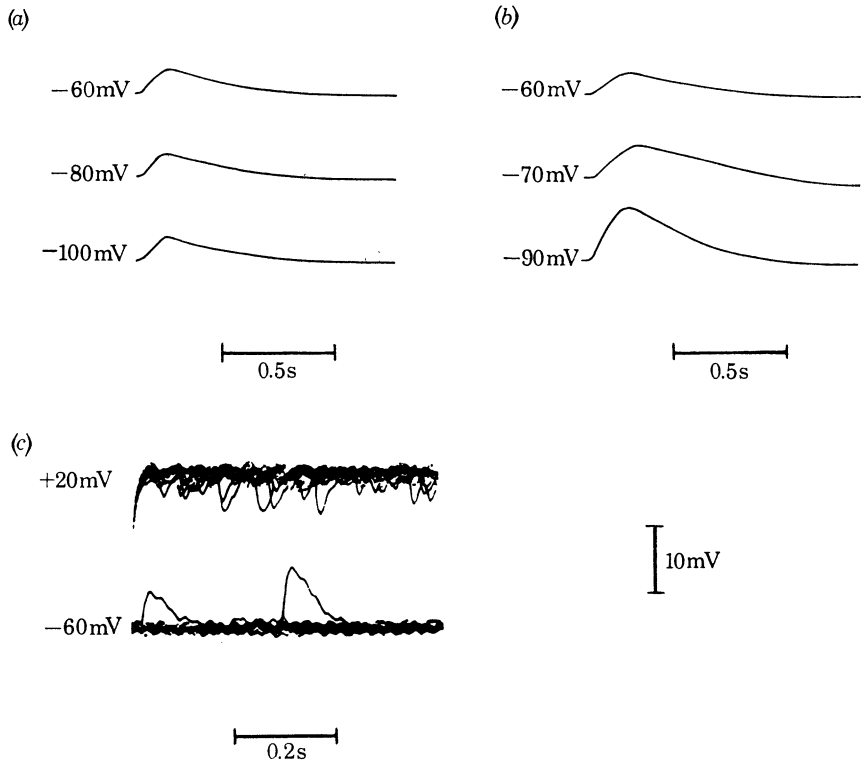

Figure 5. The effect of current injection from intracellular and extracellular electrodes on the amplitude of the junction potentials evoked in a muscle bundle of the vas deferens. (*a*) Hyperpolarizing the membrane of a cell by current injection into that cell from an intracellular electrode during a slow junction potential does not alter the amplitude of the junction potential. (*b*) Hyperpolarizing the membrane of a cell by current injection from extracellular ring electrodes increases the amplitude of the junction potential. (*c*) Depolarizing the membrane of a cell by current injection into that cell from an intracellular electrode during spontaneous junction potentials, reverses their polarity (from Holman 1970).

If the action of the transmitter is to increase the membrane permeability in this way, and if it is very brief compared with the time constants of the syncytium, then it would be expected that the electrical transient generated by transmitter acting simultaneously on all cells in the syncytium should decay with a time constant of 100 ms. In contrast, the transient generated by transmitter acting on a single cell in the syncytium should decay with a time constant of

2 ms. However, both the slow junction potential and the spontaneous junction potential, which are generated in this way, decay almost an order of magnitude more slowly than predicted. This suggests that the main factor controlling the time course of these transients is the time course of transmitter action on the receptors and not the electrical properties of the syncytium.

7. The characteristics of the conductance change during transmission

The electrical model of the smooth muscle syncytium allows the time course of transmitter action during the junction potential to be determined. Slow junction potentials can be recorded in all the smooth muscle cells, and the conductance change which occurs in each of the cells in the syncytium, on the assumption that each cell is affected to the same degree by the transmitter, is shown in figure 6*b*. In contrast to this, the conductance change which occurs in a single cell of the syncytium during a spontaneous junction potential is much faster (figure 6). Complex junction potentials may be analysed into both slow and fast components as shown in figure 7: the conductance change responsible for the slow component is determined as before, whereas that for the fast component is determined on the assumption that it is due either to transmitter acting only on the impaled cell (if the time constant is approximately 30 ms) or to transmitter acting on a number of cells in the vicinity of the impaled cell (if the time constant is approximately 100 ms).

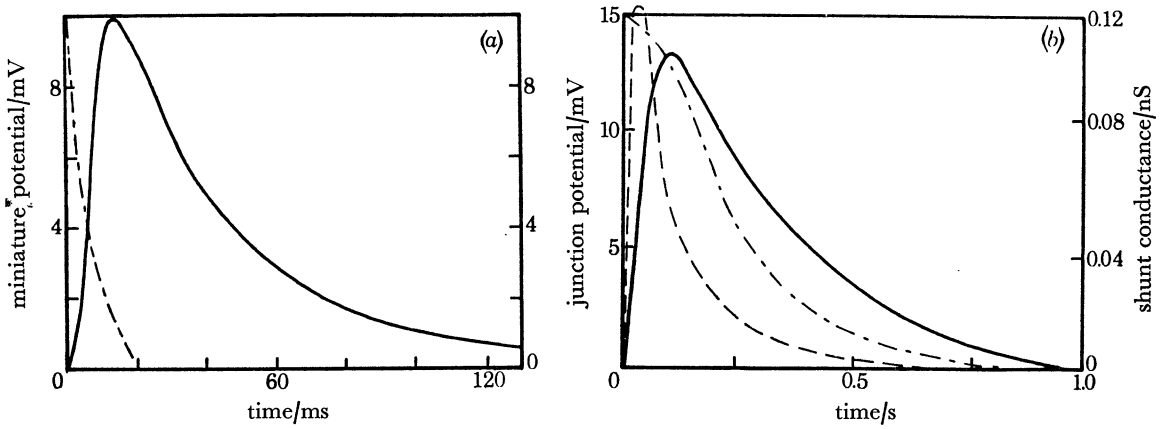

Figure 6. Comparison of the time course of the conductance change during a spontaneous junction potential and an evoked slow junction potential with the theoretical time for diffusion of transmitter. (*a*) Continuous line gives both the time course of a spontaneous junction potential and the conductance change which generates it in a single cell; the dot-and-dashed line gives the theoretical time for diffusion of transmitter out of a cylinder of radius 2 μm, representing a large axon varicosity (diffusion constant, 7.6×10^{-6} cm^2 s^{-1}). (*b*) Continuous line gives the time course of a slow junction potential due to the conductance change in all the cells of the bundle indicated by the dashed line; the dot-and-dashed line gives the theoretical time course for the decrease in concentration of transmitter – at the centre of a muscle bundle 40 μm diameter, as the transmitter diffuses from the bundle into the perimysium after its release from nerves (diffusion constant, 3×10^{-6} cm^2 s^{-1}).

If these assumptions as to the spatial distribution of transmitter action on the cells in the muscle bundle during junction potentials are correct, then the junction potentials are generated by conductance changes with two quite different time courses (figure 7). Furthermore, the faster of these conductance changes is associated with innervation by close-contact varicosities and the slower with innervation by other varicosities (figure 2*c*, *d*).

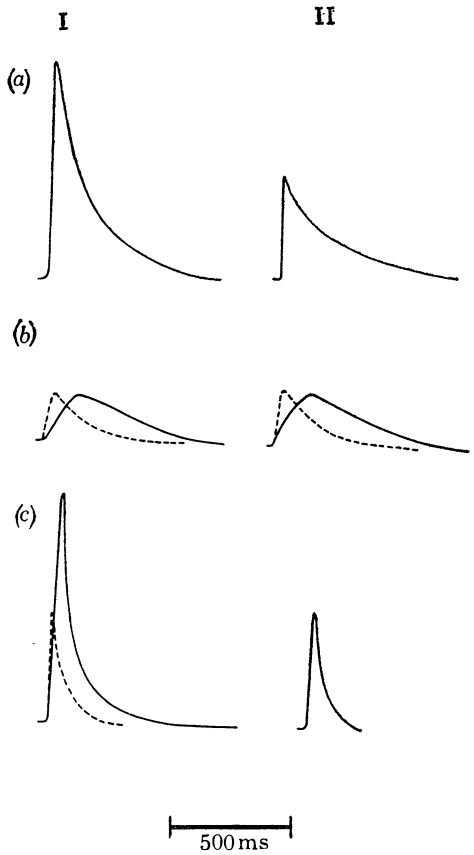

FIGURE 7. Evoked junction potentials recorded in two smooth muscle cells in the guinea-pig vas deferens. (I) this cell possessed occasional complex junction potentials (*a*), interspersed with slow junction potentials (*b*) (τ decay of 380 ms); subtraction of the slow potential from the complex potential revealed a fast potential transient (*c*) (τ decay of 100 ms). (II) this cell also possessed occasional complex junction potentials (*a*), interspersed with slow junction potentials (*b*) (τ decay of 380 ms); subtraction of the slow potential from the complex potential revealed a very fast potential transient (*c*) (τ decay of 30 ms). The conductance changes responsible for these transients, based on the assumptions given in the text, are shown by the broken lines.

8. FACTORS DETERMINING THE CHARACTERISTICS OF THE CONDUCTANCE CHANGE

These time-course differences are unlikely to be due to differences in the time course of release of transmitter at varicosities which form close-contacts from those which do not. The time course of action of the transmitter is probably determined either by the time for removal of the transmitter from the vicinity of the receptors or by the kinetics of transmitter–receptor interaction.

The theoretical diffusion of transmitter from beneath a close-contact varicosity occurs much faster than that of the conductance change (figure 6*a*) indicating that the kinetics of the transmitter–receptor interaction probably govern the duration of the membrane conductance change. However, the theoretical rate of diffusion of transmitter out of a muscle bundle into the perimysium surrounding the bundle, after release from all the axon varicosities, is of the right time course to generate the slow junction potential (figure 6*b*). It is therefore uncertain if the kinetics of transmitter–receptor interaction determine these potential transients or not.

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The kinetics of the reaction between the transmitter and receptors near the varicosities in smooth muscle cannot be easily studied. We have therefore examined the potential transient due to the iontophoretic application of transmitter to the receptors of a striated muscle which receives an innervation from varicose axons similar to the innervation of smooth muscle. The slow avian muscle fibres are innervated at intervals of 650 μm along their length by bunches of varicose nerve terminals forming close-contacts with the muscle (figure 8*a*). The iontophoretic application of acetylcholine to the receptors at the close-contact varicosities gives rise to large and fast potential transients in the muscle cells (figure 8*b*), whereas the potential transient due to application of acetylcholine to receptors at regions which do not receive an innervation is small and very slow (figure 8*c*) (see also Feltz & Mallart 1971).

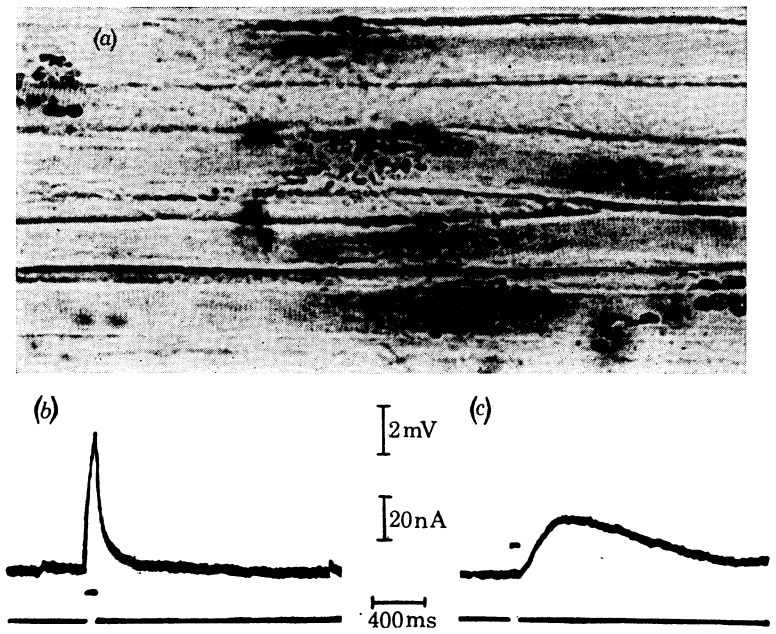

FIGURE 8. Comparison of the time course of the potential transients evoked in a muscle fibre by the iontophoretic application of transmitter on to receptors at close-contact varicosities and elsewhere. (*a*) Cholinesterase stain showing the distribution of close-contact varicosities on the surface of three muscle fibres in the avian anterior latissimus dorsi muscle. (*b*) Fast electrical transient due to the iontophoretic application of acetylcholine on the receptors at the close-contact varicosities. (*c*) Slow electrical transient due to the iontophoretic application of acetylcholine on the receptors 300 μm from the close-contact varicosities. (Courtesy, A. Pettigrew.)

These observations suggest that the kinetics of the reaction between transmitter and receptors at close-contact varicosities on smooth muscle may give rise to fast potential transients; in contrast, the kinetics of the reaction between transmitter and receptors located elsewhere over the surface of the smooth muscle cells may give rise to slow potential transients. According to this explanation then, neural release of transmitter onto receptors at close-contact varicosities generates potential transients in the muscle which are an order of magnitude faster than those due to the neural release of transmitter onto receptors located over the remainder of the muscle cell surface.

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9. Summary

The autonomic neuromuscular junction consists of an effector unit, generally the muscle bundle, together with its nerve supply. The innervation of the muscle bundle varies between organs; in some, bundles of axon varicosities run parallel to and at a considerable distance from individual muscle fibres giving a diffuse application of transmitter onto cells; in other organs, single nerve varicosities are indented into the surface of individual muscle fibres, giving a focal application of transmitter to individual cells. It is suggested that the time course and intensity of transmitter action on the receptors, and the resultant conductance changes, are dependent on whether the transmitter application is diffuse or focal. The time course of the junction potentials in a particular cell in the muscle bundle syncytium is then dependent on the spatial distribution of these conductance changes due to the diffuse and focal application of transmitter onto cells throughout the muscle bundle.

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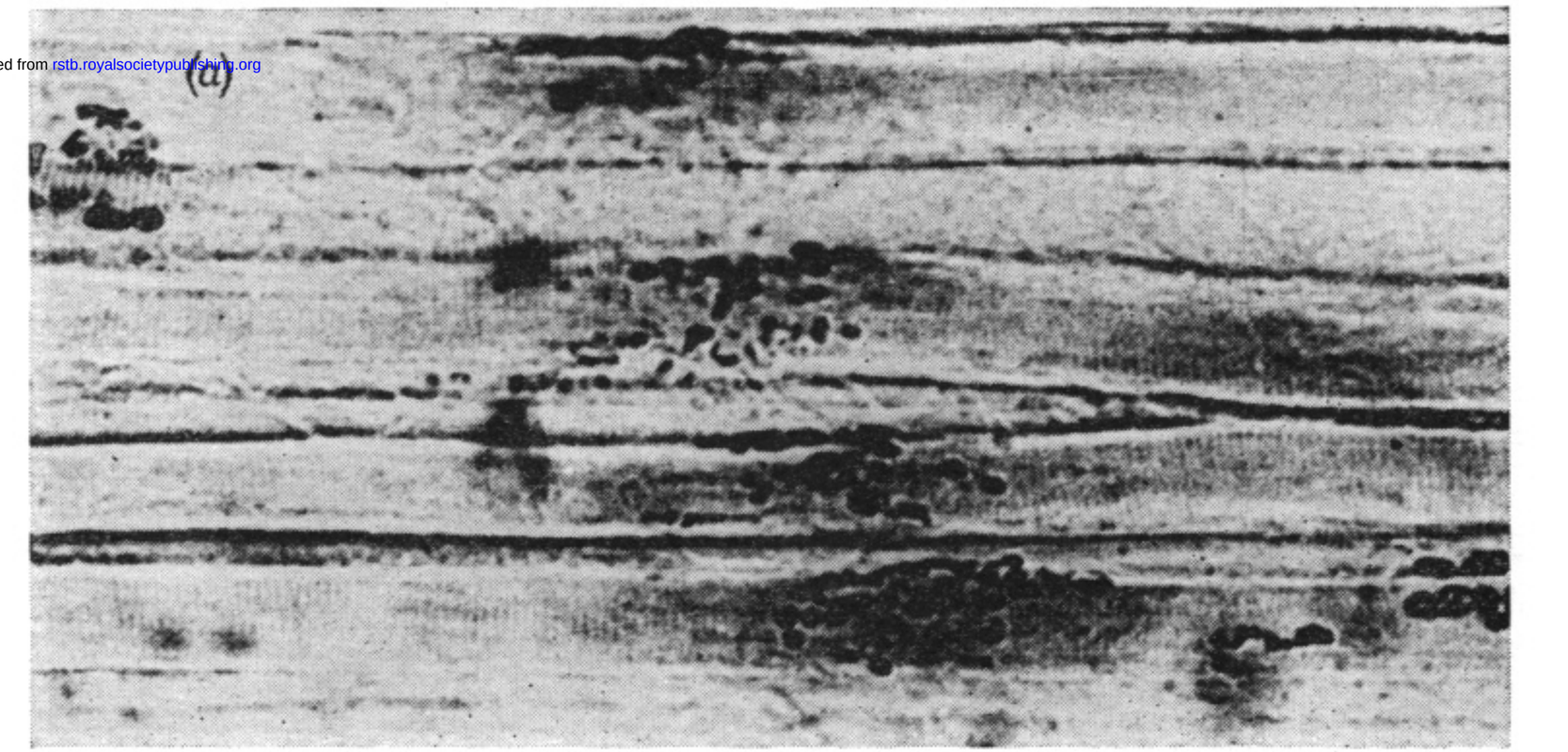

FIGURE 8. Comparison of the time course of the potential transients evoked in a muscle fibre by the iontophoretic application of transmitter on to receptors at close-contact varicosities and elsewhere. (*a*) Cholinesterase stain showing the distribution of close-contact varicosities on the surface of three muscle fibres in the avian anterior latissimus dorsi muscle. (*b*) Fast electrical transient due to the iontophoretic application of acetylcholine on the receptors at the close-contact varicosities. (*c*) Slow electrical transient due to the iontophoretic application of acetylcholine on the receptors 300 μm from the close-contact varicosities. (Courtesy, A. Pettigrew.)