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C. JUNCTION POTENTIALS AT ADRENERGIC SYNAPSES

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Electrophysiology has done much to clarify the mechanism of storage, release, action, and inactivation of acetylcholine at the skeletal neuromuscular junction and at autonomic ganglia (28, 40–42). In the first application of these methods to the study of adrenergic junctions (17), single smooth muscle cells of the guinea-pig vas deferens were impaled during stimulation of the hypogastric nerve. Stimulation of these excitatory nerves produced transient depolarisations of the smooth muscle membrane (excitatory junction potentials (EJP)) in every cell impaled. When the depolarisation produced by a single EJP, or by a train of EJPs, reached a critical level, an action potential was initiated and contraction occurred. Without nerve stimulation, a random discharge of spontaneous depolarisations [spontaneous excitatory junction potentials (SEJP)] was observed (18). Since these potentials were reduced by pretreatment of the guinea-pig with reserpine or by postsynaptic adrenergic blocking agents (19, 20), they probably represented the spontaneous release of packets of norepinephrine (NE) from nerves. Excitatory sympathetic transmission has been examined with the microelectrode method in the dog retractor penis (56–59) and in the guinea-pig mesenteric arteriole (70), both *in situ*. The observations made on these preparations were similar to those described for the vas deferens, although SEJPs were not observed in mesenteric arterioles. Further evidence for spontaneous release of NE was obtained when sympathetic denervation of the dog retractor penis was shown to abolish the SEJPs (59).

These results suggest that, despite the earlier, rather mysterious concepts of autonomic nervous control of smooth muscle (37, 60), the mechanism of storage and of release of NE from sympathetic nerves is much like the release of acetylcholine at cholinergic excitatory junctions (28). Yet several features of the relationship of sympathetic nerves to smooth muscle differ from those of other known junctions. These should be considered when interpreting the changes of membrane potential recorded from single smooth muscle cells during stimulation of the nerves. For example: 1) Smooth muscle cells are usually arranged in a functional syncytium with some degree of electrical coupling between neighbouring cells (1, 9, 22, 53, 71). 2) There is now strong evidence from electron microscopic studies (50, 53, 61–63) and from fluorescent histochemistry (29, 54) which supports and extends the earlier hypotheses of Rosenblueth (65) and Hillarp (37) about the nature of the autonomic innervation apparatus. It appears to consist of long varicose fibres (about 15 to 30 varicosities/100 μ) running in bundles in parallel with the longitudinal axis of the muscle fibres. The axons are for the most part enclosed by Schwann sheath, and usually terminate as swollen endings in shallow grooves in muscle cells. The varicosities contain numerous vesicles and mitochondria and high concentrations of transmitter. They may be sites of

release of transmitter (*en passage* junctions), as well as the true nerve endings. The separation of pre- and postjunctional membranes at nerve endings and sometimes at a small number of the *en passage* junctions is about 200 Å. The muscle membrane in these regions does not appear to have a specialised structure. There is considerable variation in the proportion of *en passage* junctions to nerve endings in different preparations. 3) There is evidence that the NE released from sympathetic nerves can be actively taken up again by the same nerves (see 7).

The implications of electrophysiological data on a number of problems concerning the storage, release and inactivation of NE at sympathetic junctions will now be examined.

MECHANISM OF RELEASE OF NE

Spontaneous release. A characteristic feature of the transmission of excitation at all nonautonomic junctions studied so far, is the packaging of transmitter into "quanta" and the random release of these from nerve endings, in the absence of stimulation (see 28). This gives rise to spontaneous miniature junction potentials at the postjunctional membrane. Packaging appears to be a means of building up a high local concentration of transmitter for a brief period of time in the vicinity of the postjunctional receptors (43). However, this would only be so if the separation between axon and postjunctional membrane was small. The larger the volume of extracellular space into which the contents of the packets are secreted, the smaller will be the concentration of transmitter at the postjunctional membrane. It is important to remember, however, that we do not know what fraction of the transmitter which is released spontaneously from the terminal is in the packaged form.

At adrenergic junctions in the vas deferens of guinea-pig, rat and mouse and in the dog retractor penis, a spontaneous discharge of small excitatory junction potentials has been recorded from smooth muscle cells in the absence of nerve stimulation. Figure 1 shows some examples of this discharge in the vas deferens. It is clear that the frequency of occurrence of SEJPs whose amplitude is greater than 2 mV, is much higher in mouse and rat than in guinea-pig. The catecholamine content of the vas deferens of these species is comparable (68; Solomon and Austin, personal communication). Electron microscopic studies have indicated, however, that in the rat there are more close contacts (200 Å) between nerve and muscle than in the guinea-pig (52, 61). This suggests that the larger SEJPs are due to the release of NE into the junctional cleft from regions of close contact.

The amplitudes of the SEJPs in the vas deferens of these species vary over a wide range between those just detectable above the noise level of the recording system (about 1 mV) and occasional giants of up to 22 mV. The large amplitude of some of the SEJPs may indicate that the packets of NE released from the nerve are bigger or that they are more effective in producing depolarisation of the smooth muscle membrane than packets of acetylcholine at cholinergic junctions. However, the input resistance of the smooth muscle cells of the guinea-

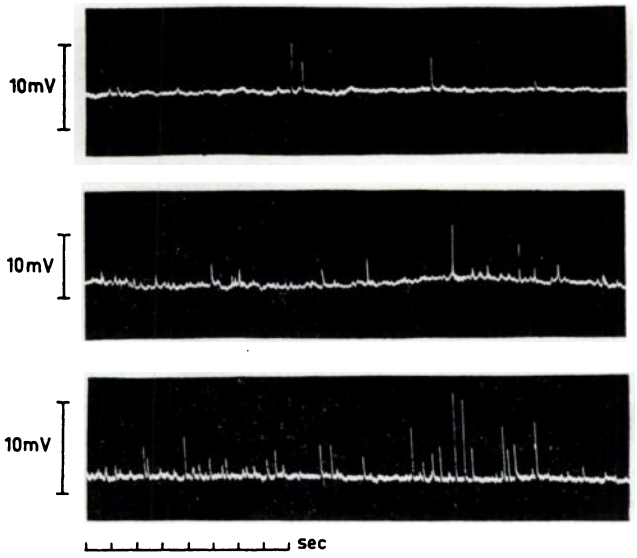

FIG. 1. Intracellular records from guinea-pig (upper record), rat (middle record), and mouse (lowest record) vas deferens, showing SEJPs in the absence of nerve stimulation.

pig vas deferens is high (10 to 30 MΩ) (39), and packets of transmitter of equal potency with those released at the skeletal neuromuscular junction would be expected to cause a larger depolarization (44). SEJPs of similar amplitude often occur in pairs or triplets. These observations may indicate an interaction of the release of packets of transmitter similar to that observed in autonomic ganglia (51).

When histograms showing the frequency distribution of SEJP amplitude recorded in any one cell are plotted, the skewed shape closely resembles those plotted for systems such as the slow fibres of the frog and chick which have multiterminal innervation (11, 36), in contrast to the bell-shaped histogram characteristic of the skeletal neuromuscular junction (30). There are three possible explanations for the skewed histogram seen in smooth muscle cells: 1) The packets of NE released from one or two nerve endings may vary in size. 2) Variable amplitude may be due to variable concentrations of NE reaching the smooth muscle membrane. Packets of equal content may be released from different varicosities in nerves at variable distances away, and into spaces of variable geometry. 3) The smaller SEJPs may represent activity spreading through the functional syncytium from neighbouring muscle cells.

An attempt to clarify this situation has been made by recording the spontaneous discharge of SEJPs from two cells simultaneously, with microelectrodes at a known distance apart. So far we have information about only eight pairs of cells from the guinea-pig vas deferens impaled by electrodes separated by 50 to 150 μ. In six experiments there was no correspondence between the SEJPs in the two cells, but in two, some, but not all, of the SEJPs occurred at the same moment in both cells (see fig. 2). The frequency of coincidence was too large to

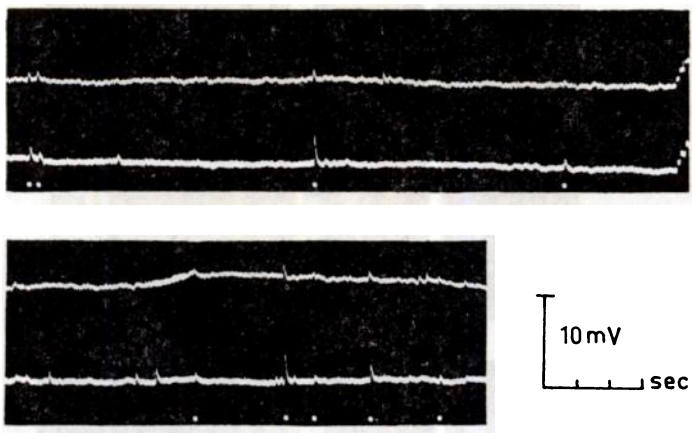

FIG. 2. Intracellular records from two cells of guinea-pig vas deferens, approximately 50 μ apart. Dots indicate coincidence of SEJPs in both cells.

be explained on a random basis and it seems likely that both potentials were caused by the same event. There was no obvious relationship between the amplitude of coincident SEJPs, so that it may be possible for the same packet of transmitter to give rise to SEJPs of differing amplitudes in different cells. This result appears to favor explanation 2), but further analysis is needed.

It is unfortunately not possible to calculate with any certainty the minimum distance away from a muscle cell that release of a packet of NE would still give rise to detectable SEJPs. Such factors as the size of a packet of NE released, the rate of its inactivation, the barriers to diffusion between the cells and the time for the action of NE on the postjunctional membrane are unknown. However, it is likely to be more than 0.1 μ. It is also not known how many different sources of transmitter release contribute to the SEJPs recorded in a cell.

The frequency of occurrence of SEJPs depends on the history of the preparation. If isolated preparations of the vas deferens are stimulated rarely, the frequency of the SEJPs may become very low, whereas if they are stimulated at regular intervals every 2 or 3 min, the frequency of the discharge is increased. Furthermore a single or repetitive stimulus, of the nerve is followed by a transient, but marked increase in the discharge of SEJPs (fig. 3). At the skeletal neuromuscular junction the probability of quantal release of acetylcholine has also been shown to depend on the previous history of the preparation (26).

High calcium and low magnesium solutions, which increase the release of acetylcholine from cholinergic nerve endings (25, 42), produce complex effects on the discharge of SEJPs recorded in muscle cells of the vas deferens (48). The evidence suggests that these ions are involved in the mechanism of release of NE, but interpretation is complicated by simultaneous effects of these ions on the smooth muscle membrane (23, 38).

At the skeletal neuromuscular junction the miniature end plate potentials (MEPPs) have a fast time course (duration about 1.5 msec). The rising phase

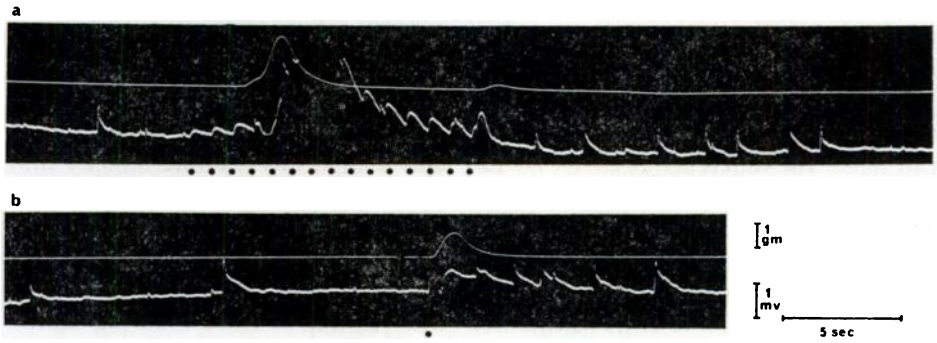

FIG. 3. Sucrose gap record (lower trace) from guinea-pig vas deferens showing SEJPs and EJPs in response to intramural nerve stimulation (indicated by dots). Upper trace shows tension. Note increased frequency of SEJPs following nerve stimulation.

of the MEPP has been taken to indicate that a packet of acetylcholine released from the nerve terminal acts very rapidly at the postsynaptic membrane and that most of the time taken is due to diffusion of the acetylcholine molecules. The falling phase of the MEPP is determined by the time constant of the muscle membrane.

The time course of the SEJPs of the vas deferens is much longer than that of the MEPP. The briefest SEJPs observed in rat and mouse were 30 to 50 msec in duration while those of guinea-pig were about 100 msec. The time taken for diffusion of transmitter across regions of close contact (200 to 500 Å) is likely, by analogy with the skeletal neuromuscular junction, to be less than a millisecond. The membrane time constant of the guinea-pig vas deferens ranges from 2 to 7 msec and it seems unlikely that the time constants of rat and mouse vas deferens are of a different order of magnitude. Thus, the time relationships suggest that the rising phase depends on the reaction of NE with the smooth muscle membrane.

Analysis of the time course of the SEJPs from mouse and rat has shown that the total duration of the rising phase is independent of amplitude. This may also be true of many of the SEJPs recorded from guinea-pig vas deferens. It seems that the time course of depolarization must be dictated by a different process from that which is responsible for the variation in amplitude.

The falling phase of the SEJP is so slow that, unlike the corresponding potential in skeletal neuromuscular junctions, it cannot be attributed to the passive properties of the postjunctional membrane. The slow falling phase may indicate a slow rate of inactivation of transmitter.

Release of NE after nerve stimulation. In the guinea-pig vas deferens, the EJPs generated in any one cell in response to increasing strength of stimulation of the intramural nerves are of increasing amplitude, which is graded between about 1 mV and 35 mV in a stepwise manner. Furthermore the amplitude of EJPs is remarkably constant from one cell to another and reduction in the number of nerve fibres stimulated in the hypogastric trunk leads to a reduction in ampli-

tude (17, 47). These observations are strong evidence for the influence of a number of different nerve fibres on the response of a single muscle cell.

In the rat vas deferens it is much more difficult to obtain a graded subthreshold response. For example, an increase in pulse duration of less than 0.03 msec can make the difference between no EJP and a large EJP leading directly and often indistinguishably into the rising phase of a spike. Stimulation with a single supramaximal pulse causes maximal contraction of the rat vas deferens, whereas several successive pulses are required for maximal contraction of the guinea-pig vas deferens. Thus the innervation apparatus of the rat vas deferens appears to be geared for fast all-or-none contractions whereas that of the guinea-pig allows graded responses. These results are consistent with the morphological evidence that a high proportion of the muscle cells in rat and mouse vas deferens have nerve endings, compared to the sparsely innervated muscle cells in the guinea-pig vas deferens.

The amplitude of the EJP which is just sufficient to generate an action potential varies from one smooth muscle to another. In the vas deferens, which is not normally spontaneously active, EJPs of 25 to 35 mV are needed for excitation. Spontaneously active smooth muscles like the guinea-pig mesenteric artery and dog retractor penis undergo rhythmic fluctuations of membrane potential and excitability, so that the amplitude of the EJP needed varies according to the state of the membrane.

The time course of the EJP recorded in the muscle cells of the guinea pig vas deferens during stimulation of the hypogastric nerves or during transmural stimulation at low pulse durations is extremely long, up to 10 times longer than the time course of the SEJP. The maximal rate of depolarisation of EJPs occurring in response to stimulation was often lower than that of the fastest SEJPs, but for the largest EJPs it was about the same. The falling phase of the EJP was generally much longer than that of the SEJP. These results could again be explained in terms of the release of transmitter from a number of sources at variable distances from the cell. The long falling phase of the EJP could be due to the successively weaker effects of packets of transmitter diffusing from progressively longer distances away. Sequential transmitter release due to slow conduction of the action potential down the terminal varicose portions of the postganglionic fibres may also be a contributory factor. The time course of the EJPs recorded from the mouse (see fig. 4) and the rat vas deferens is considerably shorter than in the guinea-pig. This would be expected from a system where release of transmitter from close junctions dominated.

The delay (or latency) between stimulation of sympathetic nerve fibres and the onset of the EJP varies considerably in different preparations. The delay between stimulation of the distal end of the hypogastric nerve (mainly preganglionic fibres) is about 20 msec (17). Kuriyama (47) found a minimum latency of 6 msec for EJPs in response to stimulation of intramural nerve fibres when the distance between recording and stimulating electrodes was 1 mm. Separation of electrodes by 3 mm gave a delay of 25 msec. These results suggest that conduction along the fine varicose sympathetic nerve fibres is probably slow (about 0.1 mm/sec) and may also be decremental.

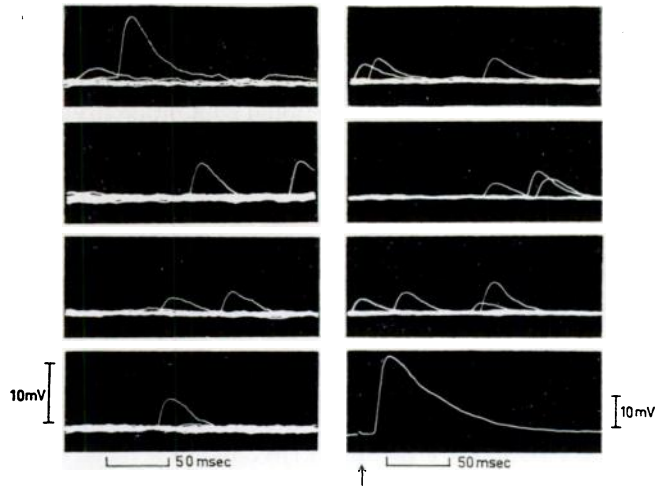

FIG. 4. Intracellular records from mouse vas deferens. SEJPs recorded by superimposing up to 10 sweeps. Record bottom right shows EJP in response to hypogastric nerve stimulation (indicated by arrow).

The minimum latency of 6 msec recorded in the guinea-pig vas deferens is slow compared to cholinergic junctions in skeletal muscle, but fast compared to other known autonomic junctions. Only a fraction of a millisecond is likely to be taken up by diffusion across distances of 200 Å. By analogy with the latency at a cholinergic junction (41), it may be that the time taken for the release of NE from the nerve accounts for most of the latency. However, there is no information yet about the time taken for NE to produce current flow at the postjunctional membrane and this may be much longer than the almost instantaneous effect of acetycholine at the motor end plate.

The long delays observed for transmission to cells in the mesenteric artery (70) are comparable with those observed at several other autonomic nerve-smooth muscle junctions including the transmission from inhibitory sympathetic nerves to intestinal smooth muscle (3, 33), from intramural inhibitory nerves to the taenia coli (4, 15), and from cholinergic excitatory nerves to the intestine (16, 34). Since electron microscopic studies of intestinal preparations reveal few, if any, close junctions (200 Å) between nerve and muscle (60, 64, 72), it may be that the long latencies can be accounted for largely in terms of time for diffusion of transmitter from distant sources of release. Diffusion time increases greatly with increasing distances and the movement of transmitter across a 10 μ space may take several tens of milliseconds (27).

Facilitation of the release of acetylcholine in the presence of high magnesium with successive pulses at the skeletal neuromuscular junction is well known (26). Similarly, in the guinea-pig vas deferens, at stimulation frequencies from 0.2 to 3 pulses per sec, there is as much as a 6-fold increase in amplitude of successive EJPs (17, 21). This facilitation does not normally occur beyond the first 6 to 8 pulses of a train. At low frequencies facilitation occurs without any change in resting potential, while at frequencies greater than about 2 per sec, summation

as well as facilitation occurs. Evidence has been presented (21) which strongly suggests that, as in cholinergic junctions, this facilitation is due to the successive increase in NE released from prejunctional sites with each pulse, rather than to an increase in sensitivity of the postjunctional membrane to successive releases of the same concentration of NE. For example, the amplitude and configuration of SEJPs remain unaltered during low frequency facilitation.

There still remains the question of whether the successive increase in NE release is due to the recruitment of more prejunctional sites of release or to an increase in NE released from the same number of sites. The fact that the time course of successive EJPs remains constant despite the increase in amplitude would support the latter hypothesis.

Facilitation of EJPs in response to repetitive low-frequency stimulation was less marked in the mouse and there was little or none in the rat.

EFFECT OF DEPLETION OF NE

In considering the effects on the transmission process of various methods of depleting NE from stores in sympathetic terminations, it must be realised that these treatments may also affect the muscle cells. SEJPs recorded from vas deferens muscle cells from chronically reserpinised guinea-pigs (5 to 10 mg/kg per day for 3 days) were reduced in both frequency and amplitude (19). SEJPs recorded in the dog retractor penis were also reduced after reserpine (59). With guinea-pig EJPs, facilitation was slower, so that more than 25 stimulating pulses were sometimes required before a spike was initiated (19, 21). Another result of the depletion of NE was the more rapid decrease in amplitude or fatigue of the EJPs in cells from reserpinised animals. Despite the 90% depletion of NE in vas deferens from reserpine-treated guinea-pigs (68), transmission was still possible.

Examination of muscle cells from the guinea-pig vas deferens 8 to 13 days after section of the hypogastric nerve showed only a small decrease in frequency of SEJPs and in amplitude of EJPs (19). This was attributed to only partial denervation because of the existence of postganglionic neurones in the pelvic plexus (5, 6, 31, 55, 68). Complete sympathetic denervation was achieved with the dog retractor penis and both SEJPs and EJPs were abolished (59).

In one experiment (21a), after intraperitoneal injection of sympathetic anti-nerve growth factor (ANF, Abbott Labs.) into newborn guinea-pigs for 13 days, the most obvious effect on transmission in the vas deferens was the absence of facilitation of the first four EJPs of a train. Nevertheless a spike was initiated, mainly because of summation, and a contraction ensued. A surprising effect was a 5-fold increase in the time course of contraction of the muscle.

EFFECT OF AUTONOMIC DRUGS ON SYMPATHETIC TRANSMISSION

The actions of a number of drugs on EJPs and SEJPs recorded in cells of the guinea-pig vas deferens have been described (20, 47). High concentrations of α-receptor blocking agents such as phenoxybenzamine, yohimbine, phentolamine, ergotamine, piperoxane and tolazoline blocked the EJPs, but never com-

pletely abolished the discharge of SEJPs. During the onset and recovery from yohimbine blockade, the EJPs showed a marked fatigue effect, *i.e.*, the first one to four EJPs showed facilitation, but the amplitudes of the EJPs which followed were rapidly reduced to zero. This effect suggests that the blocking action of yohimbine at sympathetic terminations may be partly prejunctional.

Bretylium initially reduced both EJPs and SEJPs, but after 30 min exposure, the SEJP frequency increased, although the response to nerve stimulation was abolished (20). Thus it is possible to block release of NE mediated by nerve without preventing its spontaneous release. Guanethidine also blocked the EJPs but not the SEJPs. With procaine, block of EJPs was rapid and after prolonged exposure, the SEJPs were reduced in frequency (20). With nicotine (10^{-5} g/ml), the discharge of SEJPs increased (22a). This result supports the view that nicotine can act by releasing NE from postganglionic portions of sympathetic nerves (12, 35). Atropine had no detectable effect on either EJPs or SEJPs (20). Hexamethonium did not reduce EJPs in response to stimulation of intramural nerve fibres (47).

Few studies have been made of drug action on the electrical events taking place at sympathetic junctions in the guinea-pig mesenteric artery or dog retractor penis. Deep urethane-chloralose anaesthesia reduced the amplitude of the EJPs recorded in cells of the mesenteric artery (70). Injection of adrenaline (E) into anaesthetised dogs increased the amplitude of EJPs recorded in the retractor penis (57), whereas the amplitude of EJPs was reduced in smooth muscle cells from dogs adrenalectomised 7 to 9 days previously. SEJPs recorded in the retractor penis were also reduced after adrenalectomy (59). These results were attributed to postjunctional effects of these treatments on the resting potentials of the smooth muscle cells.

RELEASE OF NE FROM INTESTINAL INHIBITORY SYMPATHETIC NERVES

With the rabbit sympathetic nerve-distal colon preparation, Gillespie (33) could not detect any membrane potential changes in single smooth muscle cells until the nerves were stimulated at frequencies greater than 10 pulses per sec. In stretched preparations, stimulation at high frequencies caused hyperpolarisation of the membrane and suppression of both action potentials and slow waves, so that relaxation resulted.

A recent electrophysiological study of transmission of inhibition from perivascular nerves to the taenia coli has confirmed and extended this result (3). Membrane potential changes were not observed in response to single stimuli, in marked contrast to all the known excitatory junctions. The probable reason for this is that the concentration of inhibitory transmitter reaching a muscle cell after a single stimulus is too low. This may be because of various factors, including the amount of transmitter released, the number and distance of sources of transmitter release from nerves influencing the muscle cell, and the rate of inactivation of the transmitter. The inhibitory system is unlike excitatory junctions, in that the concentration of transmitter reaching the muscle cells must be increased by repetitive stimulation at frequencies greater than 5 to 10 pulses

per sec before there is any detectable change in the membrane potential after long latencies of up to 270 msec.

Both Celander (24) and Kock (46) have suggested that many perivascular sympathetic nerve fibres are restricted to supplying vascular smooth muscle, and that the transmitter reaches the general musculature of the innervated organ only by diffusion after high-frequency stimulation. Furthermore Schofield (67), using a degeneration technique, and Norberg and Hamberger (54), using the fluorescent histochemical staining method, showed that the perivascular sympathetic nerves to the intestine appear to innervate the blood vessels and some ganglion cells in Auerbach's plexus, but not the muscle coats. Thus, stimulation of the perivascular nerves with one pulse, although affecting the vascular smooth muscle, may have little effect on the intestinal muscle cells. With higher frequencies of stimulation, the quantity of transmitter released may be sufficient to diffuse to and affect muscle cells which are not influenced by single pulses. Similar theories of diffusion of transmitter occurring with high frequency stimulation of nerves have been proposed by Rosenblueth and Rioch (66), Klopp (45) and Folkow (32).

SENSITIVITY OF THE POSTJUNCTIONAL MEMBRANE TO TRANSMITTER

E acts on smooth muscle systems supplied by inhibitory sympathetic nerves by causing hyperpolarisation of the muscle membrane and reduction of cessation of spike activity (8, 10, 13). When E is applied to smooth muscle systems innervated by excitatory sympathetic nerves it produces depolarisation and initiation or increase in frequency of spike activity which is indistinguishable from the excitatory action of acetycholine (14, 57).

It is usually assumed that drugs applied directly to isolated smooth muscles act uniformly on all the cells in the system and probably on the whole of the smooth muscle membrane. This assumption might be questioned on several grounds. For example, by analogy with the skeletal neuromuscular junction, it seems likely that the sensitivity of the smooth muscle membrane in the region of a close sympathetic nerve-smooth muscle junction (200 Å) may be considerably higher than the membrane of the rest of the cell. In contrast, the sensitivity of the membrane of muscle cells receiving transmitter by diffusion from many distant sources may be homogeneous.

Some recent results might be relevant to this question. The guinea-pig taenia coli preparation has been used to examine the mechanism of transmission not only from sympathetic inhibitory nerves (3), but also from intramural inhibitory nerves (4, 15), and from excitatory cholinergic nerves (2, 16). Bennett (2a) has recently shown that upon transmural stimulation of the taenia, most cells gave an inhibitory response, but some less than 0.5 mm away gave an excitatory response. Perhaps this result is due to the position of the muscle cells in relation to the concentrations of different transmitters reaching them from sources at variable distances. Spatial asymmetry might also lead to a differential sensitivity of different smooth muscle cells to different transmitters.

Experiments are also in progress to examine whether α- and β-receptors are located in the same smooth muscle membranes or on separate cell populations.

SUMMARY

The mechanism of transmission of excitation from sympathetic nerves to smooth muscle appears to be much like transmission at cholinergic junctions. 1) There is a spontaneous release of packets of NE from sympathetic nerves. 2) On nerve stimulation, many packets of NE reach the effector cells. 3) Stimulation of the nerves leads to increase of spontaneous release of NE. 4) NE released from the nerves depolarises the postjunctional smooth muscle membrane, and this leads to a spike and contraction. 5) The prejunctional terminations of the sympathetic nerves are packed with vesicles and mitochondria. The main difference from the skeletal neuromuscular junction is the slow time course of the junction potentials and the long delay before their appearance after stimulation.

In the vas deferens, where there are junctions with separations of only 200 Å between nerve and muscle, the minimum delay recorded is 6 msec and the duration of SEJPs may be less than 100 msec. It is possible that these delays are due to the reaction of NE with the postjunctional receptors.

In the mesenteric artery and gut, the delay is much longer, of the order of 150 msec. Few, if any, junctions with nerve-muscle separation of 200 Å have been observed in these preparations and the majority of varicose fibres run in bundles with wide separations from muscle membranes. These long delays might therefore be due to diffusion of NE over considerable distances. That NE can diffuse over long distances without being inactivated is another point of difference from cholinergic transmission.

The sensitivity of different smooth muscle cells, or even of different regions of the muscle membrane, to NE might be influenced by the nature of the sympathetic end apparatus in that it is likely to differ in those cells receiving transmitter primarily from localised close junctions compared with cells receiving transmitter by diffusion from many sources at variable distances.

The variations in the geometry of the end apparatus may account for the variation in functional organisation of different organs. For example, close junction on most muscle cells is correlated with fast, coordinated contraction (*e.g.*, rat vas deferens), whereas wide separation of nerves from muscle, arranged so that different cells receive different amounts of transmitter, is correlated with graded and regional differentiation of contraction and relaxation (*e.g.*, intestine).

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