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AN ELECTROPHYSIOLOGICAL INVESTIGATION OF THE ACTIONS OF SOME AUTONOMIC BLOCKING DRUGS ON TRANSMISSION IN THE GUINEA-PIG VAS DEFERENS

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Membrane potentials have been recorded from the guinea-pig isolated vas deferens with intracellular and sucrose-gap electrodes during stimulation of the hypogastric nerve and of intramural nerve fibres. Atropine had no detectable effect on the excitatory junction potentials in response to nerve stimulation or on the spontaneous discharge of small potentials. High concentrations of adrenolytic drugs, acting on α-receptors were needed to block the response to nerve stimulation and the spontaneous discharge. During the onset and recovery from yohimbine blockade, junction potentials in response to repetitive stimulation were not sustained. Bretylium initially reduced both the junction potentials and the spontaneous discharge. However, after 30 min exposure, the spontaneous discharge increased in frequency although the response to nerve stimulation was abolished. Block of the junction potentials by procaine was rapid in onset compared with that by bretylium and guanethidine, but the spontaneous discharge was not abolished. These results are discussed in relation to the mechanism of transmission from sympathetic nerve to smooth muscle.

The pharmacology of the isolated vas deferens-hypogastric nerve preparation of the guinea-pig has been widely discussed recently. This preparation has been considered by some workers to represent a typical sympathetic adrenergic junction (Huković, 1961); others (Chang & Rand, 1960; Jacobwitz & Koelle, 1963) have suggested that acetylcholine is involved in the transmission process according to the theory of Burn & Rand (1959); and, more recently, on the basis of electronmicroscope studies, Richardson (1964) has suggested that the longitudinal coat of the guinea-pig vas deferens may be innervated by cholinergic fibres. There is considerable evidence that most of the nerve fibres in the hypogastric trunk which supply the vas deferens are preganglionic (Sjöstrand, 1962; Burnstock & Holman, 1962b; Bentley & Sabine, 1963; Ferry, 1963; Kuriyama, 1963; Ohlin & Strömblad, 1963; Birmingham & Wilson, 1963).

Electrophysiological methods have provided a means of studying details of drug action at the skeletal neuromuscular junction (see Eccles, 1964). It is hoped that the application of similar techniques to the vas deferens may help to clarify some of the pharmacological problems that have arisen from studies of mechanical changes recorded from the whole organ.

The action of a number of ganglionic and sympathetic blocking agents on membrane potentials recorded from the vas deferens have been briefly described by Kuriyama (1963). The present work is mainly concerned with the actions of atropine, yohimbine, bretylium and procaine.

METHODS

For intracellular recording the vas deferens was mounted in a constant temperature bath at 35° C which was perfused continuously with Krebs bicarbonate-buffered saline. The electrodes, preamplifiers, etc., have been described previously (Burnstock & Holman, 1961).

For sucrose-gap recording, the vas deferens was dissected free of connective tissue, blood vessels, etc., and mounted in the apparatus as described previously (Burnstock, Holman & Kuriyama, 1964).

The following methods were used for nerve stimulation:

(1) Hypogastric nerve stimulation: the nerve trunk was passed through two platinum or silver rings, 2 mm apart and embedded in Araldite, placed at a distance of more than 1 cm from the muscle. (This method was only used with intracellular recording.) The stimulus duration was 0.05 to 0.5 msec.

(2) Intramural nerve stimulation: one electrode was inserted into the lumen of the vas deferens while the other made contact with the solution in the organ-bath (intracellular recording only), or the whole organ was passed through two silver rings, 3 mm apart and embedded in Araldite or mounted within the walls of the sucrose-gap (Bülbring & Burnstock, 1960). The stimulus duration was from 0.05 to 0.5 msec.

Stimulation of the hypogastric nerve, 1 cm. distant from the vas deferens, excites mainly preganglionic nerve fibres supplying the vas deferens. Intramural nerve stimulation probably excites only the postganglionic nerves (Kuriyama, 1963 ; Bentley & Sabine, 1963 ; Birmingham & Wilson, 1963).

The drugs used were: atropine sulphate (D.H.A.), bretylium tosylate (Darenthin, Wellcome Research Laboratories), guanethidine (pure substance, Ciba), acetylcholine chloride (Roche), noradrenaline (Levophed, Winthrop), yohimbine hydrochloride (N.B.C.), phentolamine methanesulphonate (Rogitine, Regitine, N.B.C.), ergotamine tartrate (Sandoz), phenoxybenzamine hydrochloride (Dibenzyline, S.K. & F.), piperoxan (933F), tolazoline hydrochloride (Priscol, Ciba), and procaine hydrochloride (Bull.).

RESULTS

Atropine

Atropine, in concentrations ranging from 10^{-7} to 10^{-4} g/ml., had no detectable effect on the amplitude, frequency or time course of the spontaneous potentials. Nor was there any clear change in the junction potentials in response to stimulation of the hypogastric nerve or to stimulation of intramural nerve fibres. Fig. 1 shows typical spontaneous potentials preceding a series of junction potentials in response to hypogastric nerve stimulation in the presence of atropine (10^{-6} g/ml.). Successive junction potentials summed with each other until sufficient depolarization had been generated to initiate an action potential. The muscle contracted at this point and the microelectrode was dislodged. This sequence of events in no way differed from that characteristic of isolated preparations in normal solution (Burnstock & Holman, 1961, 1962a ; Kuriyama, 1963).

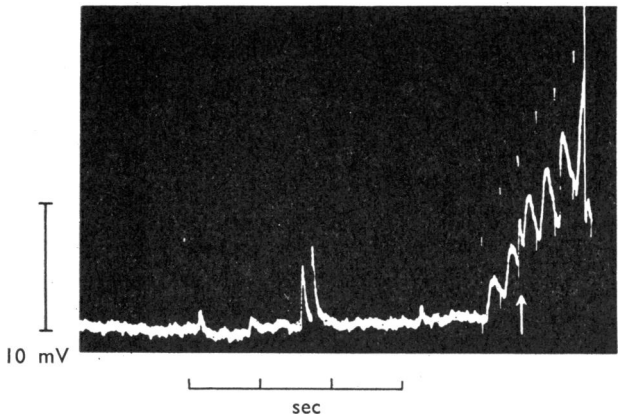

Fig. 1. Membrane potential of guinea-pig vas deferens recorded with an intracellular electrode in the presence of atropine (10^{-6} g/ml.). Stimulus artefacts precede junction potentials in response to stimulation of the hypogastric nerve. The arrow indicates a spontaneous potential occurring during nerve stimulation.

Our observations do not exclude the possibility that atropine may have caused small changes in the contractile response to nerve stimulation, especially if this had been recorded with a sensitive lever. Boyd, Chang & Rand (1960) and Birmingham & Wilson (1963) found that atropine (10^{-7} g/ml.) caused some decrease in the response to nerve stimulation. We also found a small and variable decrease in the contractile response recorded under both isotonic and isometric conditions. On the other hand, Ohlin & Strömblad (1963) reported an increase in the response to nerve stimulation in the presence of atropine. Changes in the normal sequence of events at the membrane level associated with small changes in the contractile response would not have been detectable in our electrophysiological experiments.

Bretylium

Bretylium, in concentrations ranging from 10^{-6} to 5×10^{-5} g/ml., blocked the response to stimulation of both the hypogastric nerve and the intramural nerve fibres (see also Bentley & Sabine, 1963 ; Kuriyama, 1963 ; Birmingham & Wilson, 1963). Some of our preparations showed a small increase in the response to nerve stimulation during the first 5 min exposure to the drug. In all experiments, 30 to 40 min were required for complete block. Fig. 2 illustrates the time course of action by bretylium and also shows that, as the nerve response decreased, there was an increase in the amplitude of contractions in response to added noradrenaline. In this experiment, there was a threefold increase in the response to noradrenaline, whereas the nerve response was reduced to one-quarter. Noradrenaline itself potentiates the nerve response (Huković, 1961 ; Sjöstrand, 1961) and tends to reverse the blocking action of bretylium (Bentley, 1962). We found that this effect persisted for some minutes after washing out and probably explains why the nerve response was not completely blocked in the experiment illustrated in Fig. 2. In ten experiments of this kind the response to added noradrenaline was always increased,

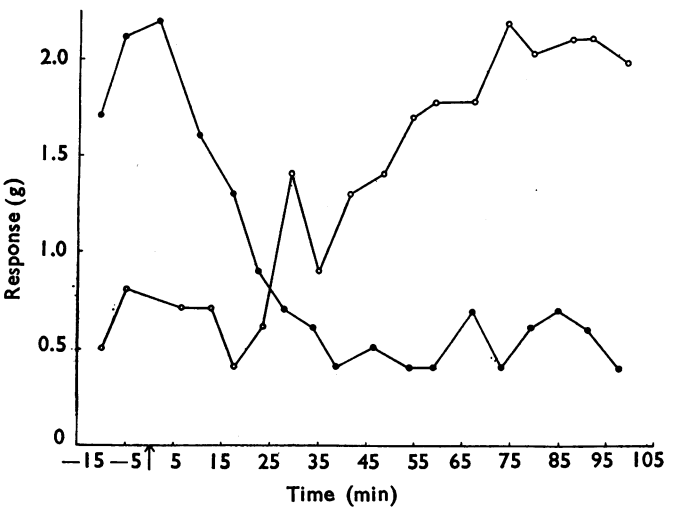

Fig. 2. Contractile response of the vas deferens to stimulation of the hypogastric nerve (●) and to noradrenaline (10^{-5} g/ml., ○). Contractions were recorded isometrically. The arrow indicates the time at which the preparation was exposed to bretylium (10^{-5} g/ml.).

the largest responses being obtained after 30 to 40 min exposure to the drug. In several experiments doses of noradrenaline (for example, 10^{-7} g/ml.) which were ineffective in the absence of bretylium caused a maximum contraction in its presence. Quantitative data for the degree of potentiation remain to be found.

The effect of bretylium on the response of the vas deferens to acetylcholine was studied in a similar series of experiments in which nerve stimulation was alternated with brief exposures to acetylcholine (10^{-6} to 10^{-5} g/ml.). In half of these experiments the response to acetylcholine was depressed initially but, in all experiments, prolonged exposure to bretylium increased the response to acetylcholine. Boyd, Burnstock, Campbell, Jowett, O'shea & Wood (1963) reported that bretylium could either potentiate or partially block the response to acetylcholine and suggested that this could be explained in terms of a competition between the antiacetylcholine and anticholinesterase actions of the drug.

The effect of bretylium on the membrane potential of the smooth muscle is shown in Fig. 3. Records (*a*) and (*b*) were taken in normal solution and show the spontaneous discharge of small potentials (*a*) followed by the response to submaximal stimulation of the hypogastric nerve (*b*). Records (*c*) and (*d*) were taken after 15 min exposure to bretylium (10^{-5} g/ml.). Both the spontaneous discharge (*c*) and the junction potentials in response to hypogastric nerve stimulation (*d*) were greatly depressed. Records (*e*) and (*f*) were taken from the same preparation after 40 min exposure to the drug. The spontaneous discharge (*e*) showed a great increase in frequency and amplitude, but the response to nerve stimulation was completely blocked (*f*). As shown in Fig. 3,*d*, junction potentials recorded just before bretylium had completely blocked the response to nerve stimulation were extremely variable in amplitude and configuration.

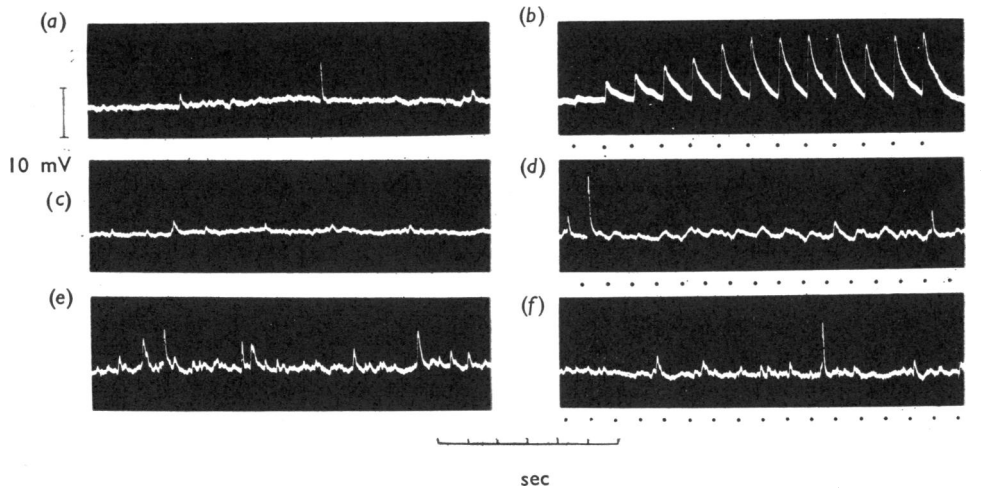

Fig. 3. The effect of bretylium (10^{-5} g/ml.) on the membrane potential of the vas deferens (intracellular recording). The tracings on the left show spontaneous potentials, and those on the right the response to stimulation of the hypogastric nerve as indicated by the dots. Tracings (*a*) and (*b*) are controls; tracings (*c*) and (*d*) were taken after 12 min; and records (*e*) and (*f*) after 30 min exposure to the drug.

The records in Fig. 4 were obtained using the sucrose-gap technique and show the effect of bretylium on junction potentials in response to stimulation of intramural nerve fibres. The record taken after 30 min, when the blockade was almost complete, shows that successive junction potentials were still able to undergo facilitation. In this experiment responses to the first three stimuli were either absent or too small to be detected. Repeated stimulation, however, caused a progressive increase in response which eventually reached a steady level characteristic of that frequency of stimulation.

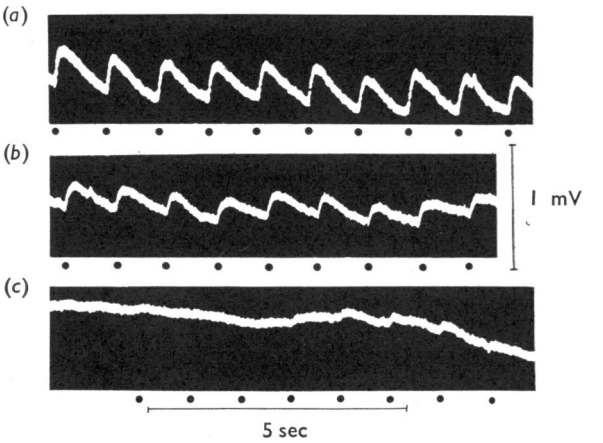

Fig. 4. Sucrose-gap records from the vas deferens showing the effect of bretylium (5×10^{-5} g/ml.) on junction potentials produced by stimulation (●) of the intramural nerve fibres (pulse duration, 0.08 msec). (*a*) control, (*b*) 15 min, and (*c*) 30 min after exposure to bretylium.

After blockade of the intramural motor nerve supply by bretylium or guanethidine it was usually possible to elicit a response which was probably due to direct stimulation of the smooth muscle (see also Bentley & Sabine, 1963). Stimuli whose duration was less than 1 msec were usually ineffective. Microelectrode recordings of the response to a stimulus of more than 1 msec duration showed an action potential, rising steeply from the resting potential, with a latency which varied from 200 to 500 msec (compare the latency of the junction potentials in response to intramural nerve stimulation, which was always less than 20 msec). Fig. 5,*b* shows sucrose-gap records of action potentials and contractions in the presence of guanethidine. A large increase in the latency of the response was apparent and junction potentials could no longer be seen to precede the spike.

It is interesting to consider the possibility that, under normal conditions, the smooth muscle might be able to respond directly to stimuli which were intended to excite only the intramural nerve fibres. The action potentials in Fig. 5,*a*, for

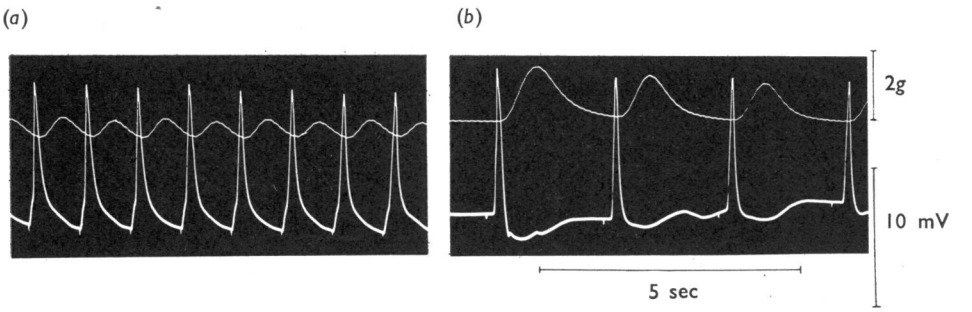

Fig. 5. Sucrose-gap records showing the effect of guanethedine (10^{-5} g/ml.) on the responses of the vas deferens to intramural stimulation. (*a*) Control responses to stimulation of the intramural nerve fibres (pulse duration, 0.05 msec). Note that the spikes arise from junction potentials which appear a few msec after the stimulus artefact. (*b*) Responses to stimuli (pulse duration, 1 msec) after 30 min exposure to guanethedine. Note that junction potentials no longer precede the spikes and that there is a great increase in latency.

example, might have been conducted along the smooth muscle cells themselves, from the region of stimulation. If the conduction velocity of this smooth muscle is similar to that of the guinea-pig taenia coli, one would expect a delay of 100 to 200 msec after stimulation before a conducted action potential would be recorded in the sucrose-gap. Conduction velocity along the nonmyelinated intramural nerve fibres might well be slow but it is probably somewhat faster than along the smooth muscle. Stimuli of sufficient duration and intensity to excite the smooth muscle would probably produce junction potentials of sufficient amplitude to initiate an action potential. However, there would be a delay of at least 50 msec preceding the action potential due to the relatively slow rising-phase of the junction potential. Hence action potentials recorded within a few mm of the stimulating electrodes may be superimposed on junction potentials but not necessarily initiated by them.

The initial effect of the bretylium on the spontaneous potentials was always depression (as shown in Fig. 3). This was apparent before the nerve response was

completely blocked (after 10 to 15 min). After 30 to 40 min exposure, when the nerve response was abolished, there was a conspicuous but transient increase in the spontaneous discharge. Fig. 6 illustrates the effect of bretylium on the rate of the discharge. In this experiment the rate was considerably reduced during the first 15 min exposure but increased threefold 10 min later. In other experiments 30 to 40 min elapsed before the discharge was increased. In some preparations, spontaneous potentials occurred which were large enough to initiate action potentials and contractions. The apparent increase in rate of the discharge could have been due to an increase in the amplitude of the individual spontaneous potentials. In normal solution their amplitudes varied over a wide range and only those greater

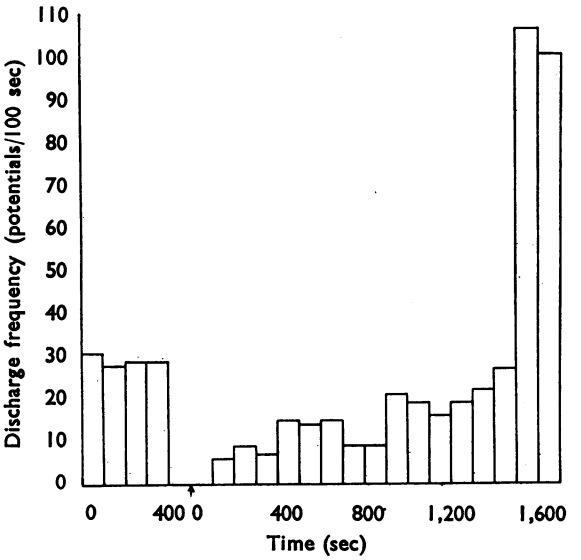

Fig. 6. The effect of bretylium (10^{-5} g/ml., at the arrow) on the frequency of the spontaneous discharge. Columns indicate the mean rates during periods of 100 sec.

than 1 mV could be resolved from the noise level of the recording system with any certainty. If the amplitude of the discharge was increased, it is possible that many of the smallest potentials previously lost in noise could now be resolved.

Yohimbine

The action of yohimbine on the intracellular response to hypogastric nerve stimulation is illustrated in Fig. 7. This drug blocked the response to nerve stimulation and also depressed the spontaneous discharge. In most experiments, however, the spontaneous potentials were not completely abolished. Potentials occurred from time to time even after prolonged soaking (2 hr) in yohimbine (10^{-4} g/ml.). Fig. 8 shows an example from such a preparation.

Fig. 9 consists of sucrose-gap records of junction potentials in response to intramural nerve stimulation during the onset of yohimbine blockade. Facilitation of successive junction potentials occurred in response to the initial stimuli of a train,

but this was followed by depression. In Fig. 9,*c*, for example, the first three junction potentials showed facilitation but the amplitude of the next two junction potentials was greatly reduced. This exaggerated " fatigue " effect occurred during both the onset of blockade and also during recovery after washing out the drug. This action of yohimbine is in marked contrast to that of bretylium and guanethidine which tended to prolong facilitation rather than reverse it.

Other α-receptor blocking agents

High concentrations, at least 5×10^{-4} g/ml., of the drugs which block the adrenotropic α-receptor (phentolamine, ergotamine, tolazoline, phenoxybenzamine and piperoxan) were needed to decrease substantially the response of the guinea-pig vas deferens to hypogastric nerve stimulation. Reduction of the response to hypogastric nerve stimulation usually occurred at concentrations which had little or no effect on

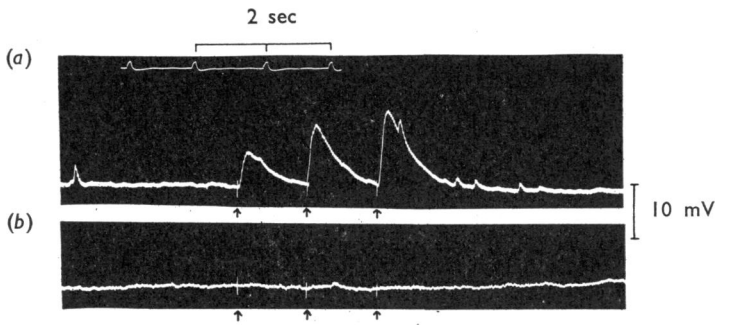

Fig. 7. The effect of yohimbine (10^{-4} g/ml.) on the membrane potential of the vas deferens (intracellular recording). The arrows indicate stimulations of the hypogastric nerve. Tracing (*a*) was taken in normal solution, and (*b*) 30 min after exposure to the drug.

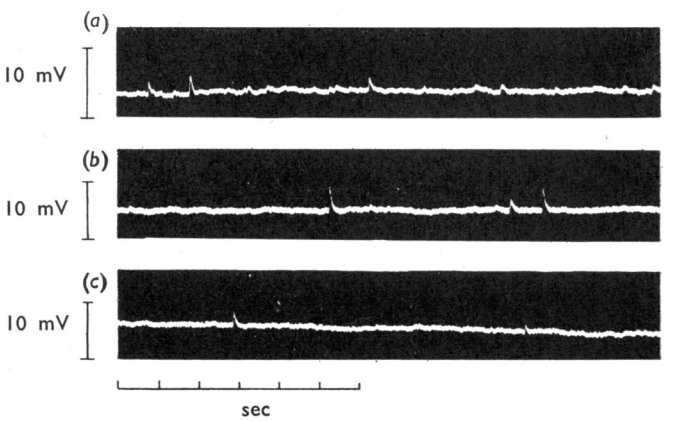

Fig. 8. Intracellular records from the vas deferens showing the persistence of spontaneous potentials in the presence of (*b*) phentolamine (5×10^{-4} g/ml.) and (*c*) yohimbine (5×10^{-4} g/ml.). Record (*a*) was taken in normal solution.

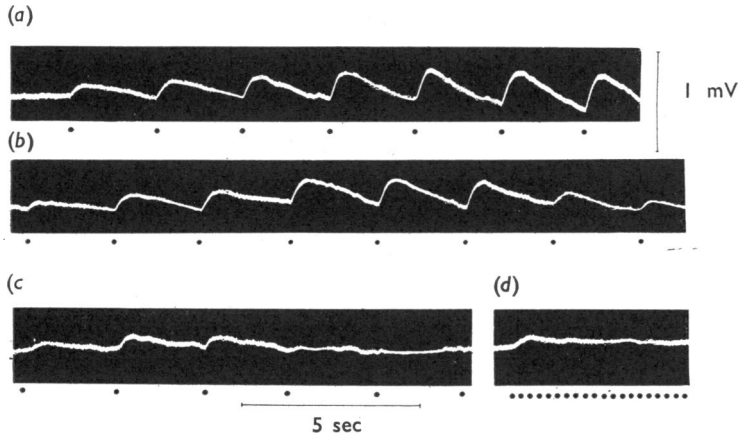

Fig. 9. Sucrose-gap records from the vas deferens showing the effect of yohimbine (5×10^{-5} g/ml.) on junction potentials produced by stimulation (●) of the intramural nerve fibres (pulse duration, 0.08 msec). (*a*) Control record showing normal facilitation of junction potentials; (*b*) 12 min, (*c*) 16 min and (*d*) 18 min after exposure to yohimbine. Note the progressive development of " fatigue " of junction potentials as blockade ensues.

the response to intramural nerve stimulation. For example, after prolonged exposure (1 to 2 hr) to phentolamine (10^{-4} g/ml.), small flat junction potentials could still be detected in response to intramural nerve stimulation and, although the spontaneous discharge was reduced, it was never abolished completely (see Fig. 8). Observations on the spontaneous discharge in the presence of high concentrations of α-receptor blocking agents were frequently complicated by contractions of the smooth muscle which became spontaneously active under these conditions (see also Boyd *et al.*, 1960).

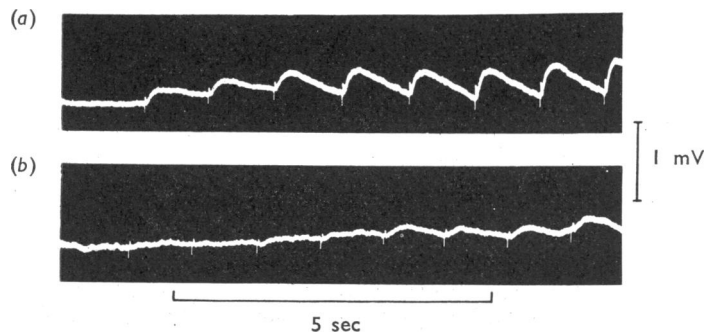

Fig. 10. Sucrose-gap records from the vas deferens showing the effect of procaine (5×10^{-4} g/ml.) on junction potentials produced by stimulation of the intramural nerve fibres (pulse duration, 0.08 msec). (*a*) Control record showing normal facilitation of successive junction potentials; (*b*) 6 min after exposure to procaine. Note the rapid onset of blockade and the delayed appearance of facilitating junction potentials.

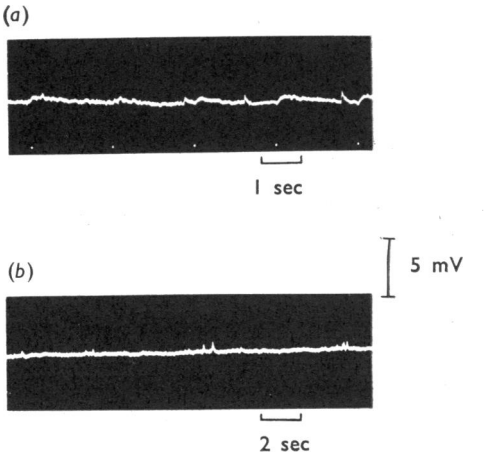

Fig. 11. Intracellular records from a vas deferens showing the effect of procaine on junction potentials in response to intramural nerve stimulation (*a*) at white dots and spontaneous potentials (*b*). (Pulse duration, 0.1 msec.) Record (*a*) was taken after 15 min exposure to procaine and record (*b*) after 20 min exposure when the nerve response was abolished.

Procaine

The effect of procaine (5×10^{-4} g/ml.) on the response to intramural nerve stimulation is shown in Fig. 10. Procaine blockade was characteristically rapid in onset. Most preparations were completely blocked 5 min after the junction potentials began to decrease in amplitude, that is about 10 min after exposure to the drug. Spontaneous potentials occurred in the presence of procaine, but after prolonged exposure appeared to be somewhat reduced in frequency (Fig. 11). Junction potentials returned rapidly upon washing out the drug. Procaine had no effect on the facilitation of successive junction potentials. There was no suggestion of the exaggerated fatigue effect which occurred in the presence of yohimbine.

DISCUSSION

A spontaneous release of transmitter from prejunctional nerve terminals appears to be characteristic of both central and peripheral synapses where chemical transmission takes place. This has been demonstrated using bioassay methods for isolated ganglia (Birks & MacIntosh, 1961) and for rat diaphragm (Mitchell & Silver, 1963). The release of transmitter in uniformly sized packets gives rise to miniature junction potentials at the postjunctional membrane and this discharge has been studied extensively with a variety of electrophysiological methods in skeletal muscle, ganglion cells and neurones (see Eccles, 1964). The spontaneous discharge of small potentials in the vas deferens shows similarities with miniature junction potentials, especially those recorded from muscle fibres which have multiple innervation. It has been suggested that the discharge is partly or wholly due to the release of transmitter from the axons of the autonomic ground plexus (Burnstock & Holman, 1962a). However, this interpretation has yet to be confirmed. Some uncertainty regarding

the origin of the spontaneous potentials in the vas deferens must be kept in mind in trying to interpret the effects of drugs on this preparation.

Kuriyama (1963) found that the junction potentials in response to nerve stimulation were blocked by bretylium (10^{-5} g/ml.) and phentolamine (10^{-4} g/ml.). We have also found that high concentrations of the commonly used α-receptor blocking agents were needed to block completely the response to nerve stimulation. Even when drugs were injected into the aorta, in order to perfuse the preparation *in situ* it was not possible to block the response to hypogastric nerve stimulation with phentolamine although the response was abolished by hexamethonium (Bentley, personal communication). The spontaneous potentials were never completely abolished by the α-receptor blocking drugs. These results may be considered to indicate that the transmitter involved here is not noradrenaline. On the other hand, it seems unlikely that this is a cholinergic junction. The guinea-pig vas deferens has a high noradrenaline content [2.3 μg/g wet weight (Ohlin & Strömblad, 1963); 10 μg/g (Sjöstrand, 1962)], and histochemical fluorescent staining methods indicate a high concentration of noradrenaline associated with the autonomic ground plexus (Falck, 1962). Thus it seems likely that the transmitter *is* noradrenaline and that there is some other explanation for the pharmacological results. In order to explain the atropine-resistant contractions of the bladder in response to pelvic nerve stimulation, Ursillo (1961) suggested that diffusion barriers may exist which prevent access of drugs to receptors associated with nerve terminals. Electronmicroscope studies of the vas deferens (Richardson, 1962; Merrillees, Burnstock & Holman, 1963) show that many of the axons of the ground plexus are deeply embedded in narrow spaces between closely packed smooth muscle cells. Some of the narrow gaps between the cells may be obstructed by processes of Schwann cells in which the axons are partially invaginated. Occasionally an axon makes close contact with the smooth muscle membrane (200 Å) but no specialization of the " postjunctional " smooth muscle membrane has been observed in this tissue. Emmelin & MacIntosh (1956) suggested that the release of transmitter from nerve terminals might lead to a high local concentration in the vicinity of postjunctional receptors. A high local concentration of antagonist drug might be needed to counteract this. Experiments involving the ionophoretic application of drugs may be helpful in testing this hypothesis.

Our observation that bretylium blocks the nerve response without blocking the spontaneous potentials accords with the widely accepted hypothesis that bretylium blocks transmission at a prejunctional site. The initial decrease in the frequency of the spontaneous potentials suggests that bretylium may temporarily reduce the spontaneous output of transmitter. During the same period a suppression of the spontaneous release of noradrenaline from the cat spleen has been demonstrated after exposure to bretylium (Hertting, Axelrod & Patrick, 1962). The *increase* in frequency of the discharge of small potentials which occurs after exposure for about 30 min may be due to an increase in the sensitivity of the smooth muscle to noradrenaline or it may be that bretylium causes transient increases in output of transmitter during prolonged exposure. It should be pointed out that the rate of spontaneous potentials might not be directly correlated with the amount of trans-

mitter which is collected in the venous effluent. At the neuromuscular junction the spontaneous output of acetylcholine is much larger than that responsible for the miniature endplate potentials (Mitchell & Silver, 1963).

Procaine blocked the junction potentials without blocking the spontaneous discharge and in this way resembled very closely the initial phase of bretylium blockade. Furthermore procaine, like bretylium, potentiates the response to noradrenaline (Bentley & Sabine, 1963). However, as already noted by Chang & Rand (1960), the onset of the block by procaine was abrupt and there was no sign of an increase in the spontaneous discharge during prolonged exposure. These results indicate that drugs may act at several different sites on the prejunctional axons of the autonomic ground plexus.

The action of yohimbine in blocking responses to repeated stimulation before blocking the response to the first few stimuli of a train resembles its effects on amphibian nerve and skeletal muscle (Shaw, Holman & McKenzie, 1955). This suggests that the blocking action of yohimbine may be partly prejunctional in origin. If successive action potentials in the prejunctional nerve fibres were reduced in amplitude as a result of blockade by yohimbine this would explain the successive decreases in output of transmitter and the decrease in the amplitude of the junction potentials.

This work was undertaken in the hope that electrophysiological studies might help to clarify some of the problems concerning the action of drugs on transmission from sympathetic nerve to smooth muscle. It is clear that much work remains to be done on the mechanism of transmission in normal solution before any speculations about drug action can be verified. However, the observation that both bretylium and procaine block the response to nerve stimulation, but do not block the response to added noradrenaline or the spontaneous discharge, accords with the view that the spontaneous discharge is due to the release of noradrenaline from the axons of the ground plexus. Several of the questions raised in this paper are under investigation at the present time.

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