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ELECTROPHYSIOLOGICAL OBSERVATIONS ON THE MOTOR INNERVATION OF THE SMOOTH MUSCLE CELLS IN THE GUINEA-PIG VAS DEFERENS

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Many pharmacological studies have been made on the motor response of the isolated guinea-pig vas deferens to hypogastric nerve stimulation (Huković, 1961; Burn & Rand, 1962). The first electrophysiological studies of this preparation were made by Burnstock & Holman (1961, 1962*a*, *b*). They found that the changes in membrane potential following nerve stimulation (the 'junction potentials') resembled end-plate potentials of striated muscle. They assumed that they were due to the release of noradrenaline from the nerve terminals. Burnstock & Holman (1961) also measured the conduction velocity of the nerve, using pulses of more than 0·1 msec duration (Holman, personal communication) and found 0·9 m/sec. This value agrees well with that of post-ganglionic sympathetic fibres (Douglas & Ritchie, 1962).

Sjöstrand (1962) observed that ganglion-blocking agents inhibited the response to hypogastric nerve stimulation. He suggested that the action of the ganglion-blocking agents might be either on true ganglionic synapses in the course of the peripheral part of the nerve, or 'non-specific' on the nerve terminals, but probably not on chromaffin cells. He considered the complete noradrenaline depletion by reserpine treatment (Sjöstrand, 1963) as indicative that the storage of noradrenaline was in the nerve terminals rather than in chromaffin cells.

Ferry (1963), using the same tissue, reported that the compound action potential of the hypogastric nerve showed two groups of fibres: one conducting impulses at 3–6 m/sec, which triggered the junction potentials, and the other conducting impulses at less than 1 m/sec, which did not trigger junction potentials. He concluded that the fast fibres were preganglionic B fibres supplying ganglion cells situated peripherally to the site of stimulation of the hypogastric nerve trunk.

The present experiments were carried out in an attempt to clarify the nervous pathway and the site of action of ganglion-blocking and adrenergic-

blocking agents on this preparation. Hypogastric nerve stimulation and field stimulation of the muscle were used. Some of the results have been communicated to the Physiological Society (Kuriyama, 1963).

METHODS

The preparation used for all experiments was the hypogastric nerve–vas deferens of the guinea-pig, prepared according to the method described by Huković (1961) and Burnstock & Holman (1961); 2–3 cm of vas deferens with the attached 3–5 cm of hypogastric nerve being used. The muscle was mounted isometrically in a Perspex organ bath of 3 ml. volume, through which solution flowed continuously at the rate of 2–3 ml./min. The bath temperature was maintained at 35° C.

The membrane potentials were measured with intracellular electrodes, possessing a resistance of between 20 and 50 MΩ, by the 'floating' method described by Woodbury & Brady (1956). Tension was measured with a mechano-electronic transducer valve (RCA 5734) mounted in the manner described by Bülbring (1955). The upper surface of the vas deferens was kept less than 2 mm below the surface of the bathing solution. The normal Krebs's solution used in all experiments contained (mM): Na^+ 137·4, K^+ 5·9, Mg^{2+} 1·2, Ca^{2+} 2·5, Cl^- 134, $H_2PO_4^-$ 1·2, HCO_3^- 15·5, glucose 11·5; and was aerated with 97% O_2 + 3% CO_2.

The electrode used for stimulating the hypogastric nerve consisted of two silver–silver-chloride rings embedded 2 mm apart in insulating araldite, as described by Burn & Rand (1960) and Burnstock & Holman (1961). The nerve was lifted out of the fluid and the portion of the nerve fibres within the electrode was continuously irrigated with Krebs's solution from a fine plastic tube which was incorporated between the stimulating rings. The electrode used for stimulating the vas deferens was of the same type but with the rings spaced 5 mm apart.

A Nihon Kohden Ltd. pre-amplifier (negative capacitance amplifier) was used for amplifying the electrical activity, and a Grass Ltd. stimulator with isolation unit was used for stimulating the nerve and muscle fibres.

The concentrations of hexamethonium bromide, nicotine hydrochloride, bretylium tosylate and phentolamine are expressed as weight per volume.

RESULTS

The junction potential recorded from the muscle cells in response to stimulation of the hypogastric nerve and to field stimulation

Submaximal hypogastric nerve stimulation produces small changes of the membrane potential which Burnstock & Holman (1961) called junction potentials. The amplitude of the junction potentials varies according to the frequency, intensity and duration of the stimulus. When the junction potential reaches threshold it generates a spike. Repetitive stimulation enhances the amplitude of the junction potential. This facilitation occurs without any change of the resting membrane potential between successive junction potentials (Burnstock & Holman, 1961, 1962*a*, *b*; Burnstock, Holman & Kuriyama, 1963).

The membrane potential changes described above were also observed in response to field stimulation of the muscle with stimuli of short duration

(0·01 msec), provided the distance between the stimulating and recording electrodes did not exceed 6 mm. The following observations indicate that nerve fibres within the walls of the vas deferens were stimulated by this method and that the changes in membrane potential were not due to direct stimulation of the smooth muscle. In response to field stimulation the amplitude of the junction potential was enhanced, in the same way as in response to nerve stimulation, when the intensity or the duration of the stimulus was increased, or when a short stimulus (0·01 msec) was applied repetitively at a frequency of more than 0·25/sec. Furthermore, field

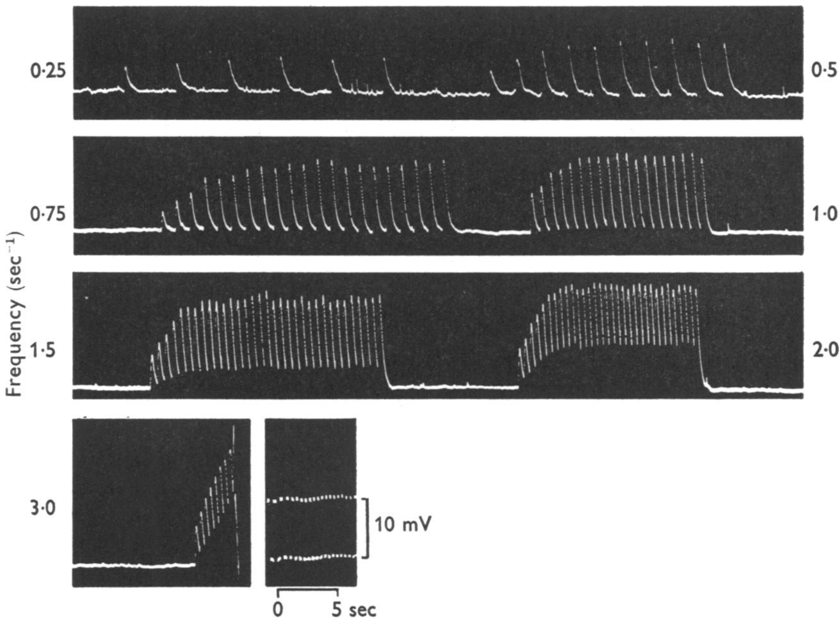

Fig. 1. Junction potentials recorded from a smooth muscle cell of guinea-pig vas deferens, evoked by repetitive hypogastric nerve stimulation at frequencies as indicated from 0·25 to 3·0/sec. Pulse 0·05 msec, 10 V. Note small brief depolarizations of the membrane between the junction potentials, so-called 'miniature junction potentials'.

stimulation of 0·01 msec had a latency of more than 5 msec for the generation of the junction potential. Figure 1 illustrates the effect of repetitive stimulation of the hypogastric nerve on the amplitude of the junction potential. The frequency of stimulation was varied from 0·25 to 30/sec, while the intensity (10 V) and pulse duration (0·05 msec) were constant. In comparison with the above experiment, Fig. 2 shows the effect of repetitive field stimulation of the muscle in conditions similar to those in Fig. 1.

Brief depolarizations of the membrane were observed between the junction potentials or superimposed on the repolarization phase of the junction potentials. Burnstock & Holman (1961) called these small spontaneous depolarizations 'miniature junction potentials'. Their frequency was increased during low-frequency stimulation (Fig. 1 at 0·25/sec). The amplitude was sometimes as high as that of the unfacilitated junction potentials (Fig. 2*A* at 0·1/sec).

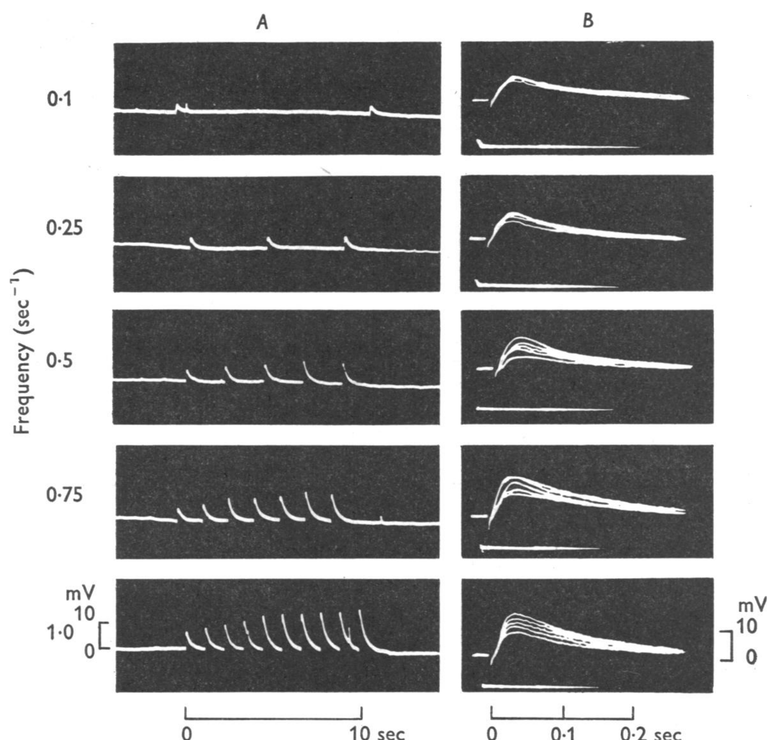

Fig. 2. Junction potentials recorded from smooth muscle cell evoked by repetitive field stimulation of the muscle, at frequencies as indicated from 0·1 to 1·0/sec. Pulses 0·05 msec, 10 V. *A*, trains of successive stimulation for 10 sec; *B*, five superimposed junction potentials at various frequencies. Note miniature junction potentials.

When the junction potential reached threshold it triggered a spike. Figure 3 illustrates the generation of an action potential by a single stimulus, *A* with hypogastric nerve stimulation and *B* with field stimulation of the muscle. Though the threshold at which the junction potential triggered a spike differed between individual cells, it was consistently lower with field stimulation of the muscle than it was with hypogastric nerve stimulation. In five specimens at 35° C the mean membrane

potential of the muscle cells was 62 mV (50–73 mV), the mean overshoot potential of the spike was 15 mV (6–25 mV), the maximal rate of rise of the spike was 18 V/sec (11–32 V/sec) and the duration at 50 % height was 7·5 msec (4·5–11 msec).

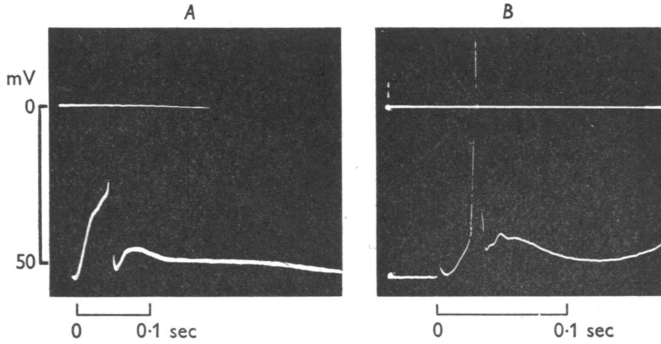

Fig. 3. Action potentials recorded from smooth muscle cells of the vas deferens elicited *A* by hypogastric nerve stimulation (5 V, 0·1 msec) and *B* by field stimulation of the muscle (10 V, 0·1 msec).

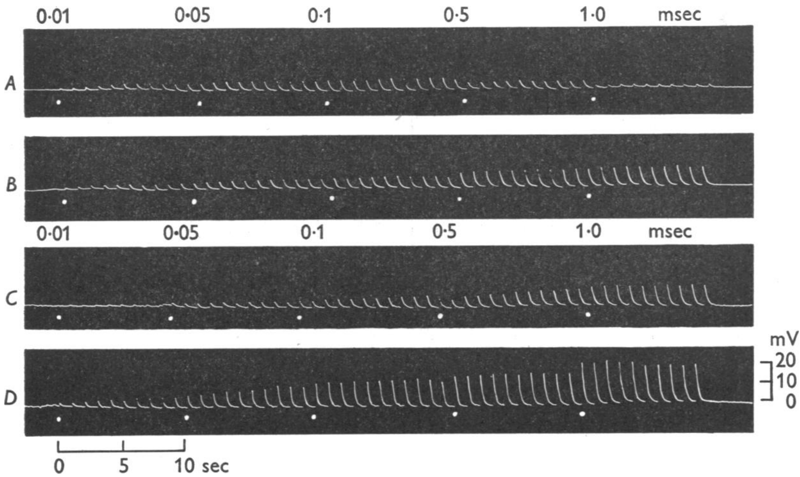

Fig. 4. Junction potentials produced by nerve (*A* and *B*) and field stimulation (*C* and *D*) recorded from the same cell. Constant intensity (10 V) and frequency (1/sec). The duration of the stimuli was increased stepwise from 0·01 to 1·0 msec as indicated. Ten pulses of each duration were applied. *A*, anodal electrode peripheral; *B*, cathodal electrode peripheral; *C*, anodal electrode, and *D* cathodal electrode closer to recording electrode.

Figure 4 shows junction potentials elicited from the same cell by both nerve and field stimulation. In this experiment, the frequency and the intensity of the stimulus were fixed at 1·0/sec and 10 V, respectively, and

the duration of the stimuli was increased stepwise from 0·01 to 1·0 msec. Ten stimuli were given at each step. Facilitation was observed with both types of stimulation. However, the polarity of the stimulating electrode affected the amplitudes of the junction potential elicited by nerve stimulations more than those produced by field stimulation. When the anodal electrode was placed peripherally on the nerve (Fig. 4*A*) pulses of more than 0·5 msec duration suppressed the amplitude of the junction potentials, but when the cathodal electrode was placed peripherally no suppression was seen. Instead, the amplitude of the junction potential continued to increase (Fig. 4*B*). The effect in Fig. 4 may be explained by an anodal block of excitation.

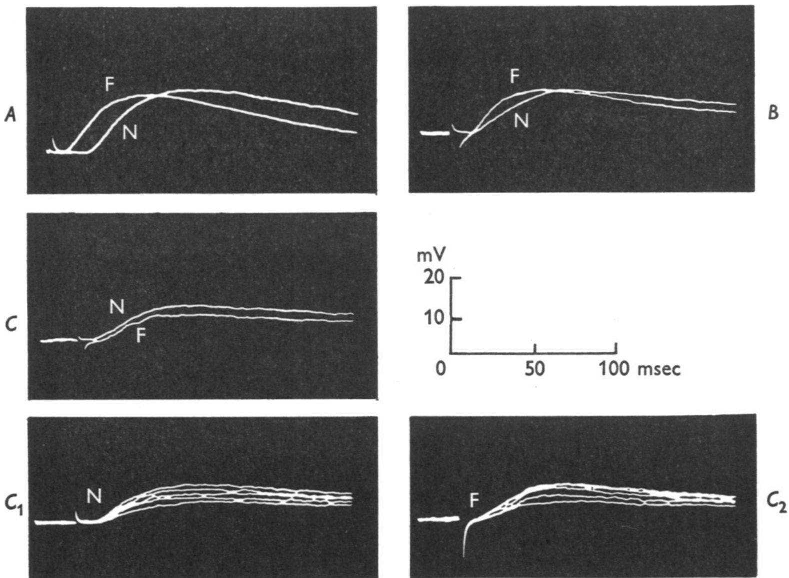

Fig. 5. Latencies of the junction potentials evoked by nerve (N) and field (F) stimulation measured from the same cell. The nerve stimulating electrodes were fixed at 10 mm distance from the recording electrode. The distance of the field stimulation electrode was changed from 1 mm at *A*, to 3 mm at *B*, and 5 mm at *C*. C_1 and C_2 show facilitation by repetitive nerve and field stimulation recorded from the same cell as *C*.

When the junction potentials in response to the two different methods of stimulation were recorded from the same cell differences in latency and amplitude were seen which could be partly attributed to the distance between the stimulating electrodes and the recording cell, partly to different conduction velocities. Figure 5 illustrates the junction potentials which were triggered when the nerve-stimulating electrodes were fixed at 10 mm distance from the muscle, while the electrodes for field stimulation

were moved to three different positions on the muscle. When the junction potential was recorded at a distance less than 1 mm from the field-stimulating electrodes (F) a short latency of 6 msec was recorded (Fig. 5*A*). The latency of the response to stimulation of the nerve (N) 10 mm from the muscle was 25 msec. This difference in latency is too great to be explained by conduction along the extra length of nerve fibres, but it could be the result of a synaptic delay. Similarly, the latency following field stimulation may be the delay due to the release of the chemical transmitter. When the field-stimulating electrodes were moved farther from the micro-electrode the latency increased and at a distance of 3 mm the latency for field stimulation was the same as that for nerve stimulation at a distance of 10 mm (Fig. 5*B*). On further increasing the distance to 5 mm (Fig. 5*C*) the latency for field stimulation became longer than that for nerve stimulation, but the amplitude of the junction potential was less. The rapidly increasing latency as the field electrode was shifted away may be due to the decremental propagation of excitation along fine terminal nerve branches. It is unlikely that the long latency is due to excitation of the muscle and cell-to-cell propagation, or to passive electrotonic spread, because the duration of each pulse was too short (0·01 msec). Decremental conduction would also explain the smaller amplitude of the junction potential as the electrode separation was increased and its final disappearance when the distance exceeded 7 mm. Further evidence for the delay being due to propagation along terminal nerves which were stimulated by field stimulation is shown in Fig. 5C_1 and C_2. These potential changes were recorded in the same cell and at the same electrode position as those in Fig. 5*C*, but repetitive responses were superimposed and these showed facilitation both with nerve stimulation (C_1) and with field stimulation (C_2).

The conduction velocity of the nerve fibre was measured from the latency of the junction potential by using two pairs of stimulating electrodes placed on the nerve trunk at 10 mm distance from each other. The conduction velocities varied from 0·7 to 2·8 m/sec and the mean value was 1·8 m/sec ± 0·38 (n = 15). However, the conduction velocity along the nerve trunk (i.e. between the two pairs of stimulating electrodes) was not the same as that in the terminal part of the nerve where conduction was delayed. Figure 6 illustrates the measuring of the latencies of the junction potentials with three equally spaced pairs of stimulating electrodes (electrodes 1 and 2 were on the nerve, and 3 was on the muscle, the distance between 3 and 2 being the same as that between 1 and 2). The difference between the latencies of the two nerve stimuli (1 and 2) was about 10 msec, leaving a latency of about 20 msec unaccounted for. After subtracting 6 msec latency for the field stimulation (3), the latency which may be

required for the release of transmitter, there remained 14 msec delay. If we assume that the conduction velocity along the nerve trunk was not decremental, this gap might be due either to the delay along the small nerve endings which are widely distributed throughout the smooth muscle (Richardson, 1962) or to a synaptic delay in a ganglion.

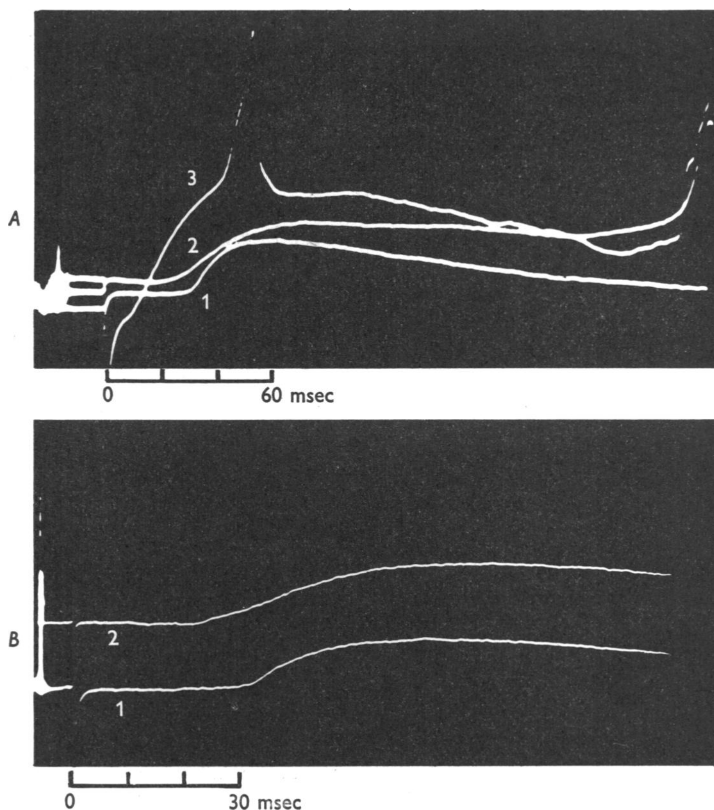

Fig. 6. Conduction velocities were measured with three pairs of stimulating electrodes. *A*, two pairs of electrodes were placed on the hypogastric nerve at 20 mm (1) and 10 mm (2) from the recording micro-electrode. The electrode for field stimulation (3) was placed less than 1 mm from the recording electrode. Note: field stimulation produced abortive spike on the junction potential. *B*, effect of nerve stimulation recorded on faster time base.

Figure 7 illustrates the facilitation of the junction potentials evoked from the same cell by field (*Aa*) and nerve stimulation (*Ab*). Each was applied 10 times at 0·01 msec, 10 V and 1/sec. The junction potentials produced by nerve stimulation had no influence on those elicited by field stimulation in the first cell (*A*); i.e. preceding repetitive field stimulation did not affect facilitation of the junction potential elicited by nerve

stimula ion (*A c*) nor vice versa (*A d*). In another cell, shown in *B a* and *b*, there was some interrelation, i.e. preceding field stimulation caused already some facilitation in the subsequent nerve stimulation (*B a*) and vice versa (*B b*). In a third cell no more facilitation was observed when nerve stimulation followed field stimulation (*C a*), presumably because the

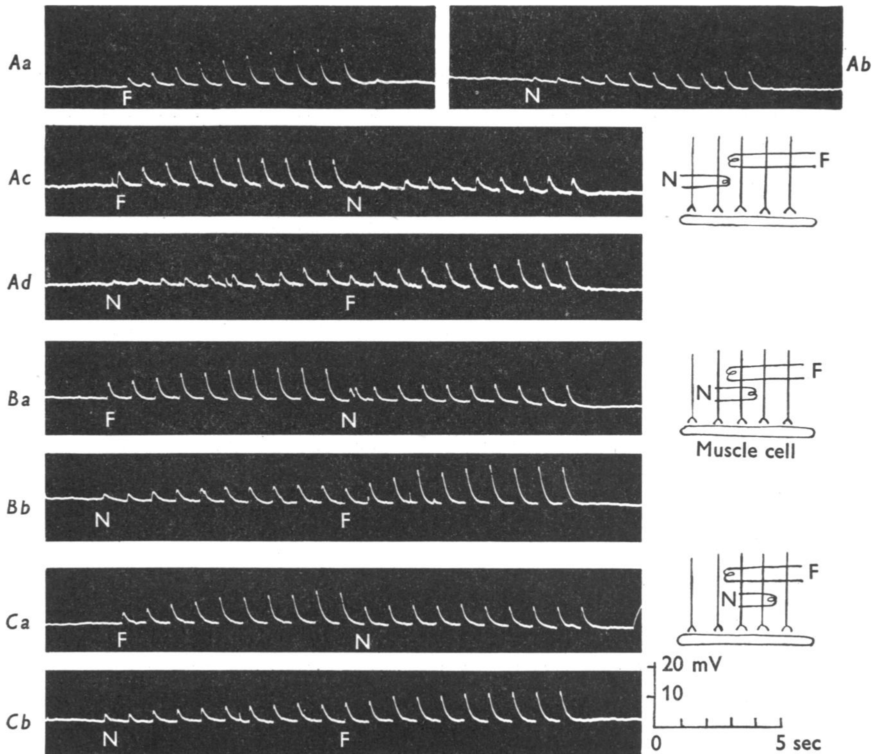

Fig. 7. Facilitation of the junction potentials evoked by field (F) and nerve (N) stimulation recorded from the same cell; 10 V, 0·01 msec, 1/sec. Ten stimuli were applied each time. Between *A a* and *b* was an interval of 2 min which was not allowed in the other records. *A*, *B* and *C* represent different cells; for explanation see text.

amplitude of the junction potentials had already reached the maximum. However, when the sequence was reversed (*C b*) some facilitation of field stimulation still occurred. These findings suggest a multiple innervation of individual muscle cells and a variable degree of overlap. This conclusion may agree with other electrophysiological and anatomical observations (Boeke, 1949; Stöhr, 1954; Hillarp, 1959; Richardson, 1960, 1962; Burnstock & Holman, 1961).

The effect of ganglion-blocking agents on the junction potentials

Nicotine hydrochloride. Nicotine hydrochloride (10^{-5}) blocked the generation of junction potentials in response to nerve stimulation, but had no effect on those evoked by field stimulation. Figure 8*A* shows the junction potential triggered by 0·01 msec nerve stimulation (N) and by field stimulation (F) recorded from the same cell before nicotine treatment. In the presence of nicotine (*B*) the junction potential elicited by nerve stimulation was abolished. Five repetitive stimuli (1/sec) to the nerve had no effect (*C*), but with five field stimuli normal facilitation was observed (*D*).

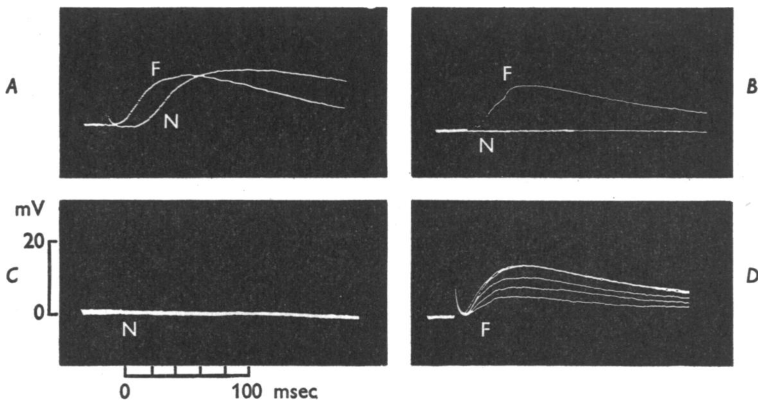

Fig. 8. Effect of nicotine (10^{-5}) on the junction potential. The junction potentials evoked by both types of stimulation were recorded from the same cell (N, nerve stimulation; F, field stimulation). *A*, before applying nicotine; *B*, after nicotine; *C*, repetitive stimulation of the nerve, and *D*, repetitive field stimuli, in the presence of nicotine. (10 V, 0·01 msec, 1·0/sec).

When the duration of the nerve stimulus was increased to 0·1 msec a small junction potential was generated, as is shown in Fig. 9*B*. When a stimulus was applied 5 times, some facilitation was observed. The amplitude became larger when the stimulus duration was further increased to 1·0 and 10·0 msec (Fig. 9*C*, *D*). These potential changes produced by nerve stimulation in the presence of nicotine had, however, different properties from the potential changes in normal conditions. First, the latency was much longer. With the same electrode separation the latency was 20 msec before and 45 msec after applying nicotine. Secondly, the amplitude of the junction potentials was much smaller. The increase of stimulus duration enhanced the amplitude of the junction potential but it did not reduce its latency. The junction potentials triggered by field stimulation were not abolished by nicotine. On the contrary, they were sometimes enhanced and their latency remained unchanged.

Hexamethonium. The concentration of hexamethonium required to block the generation of junction potentials in response to nerve stimulation of 0·01–0·1 msec duration was 10^{-4}. Lower concentrations had no effect. The generation of junction potentials in response to field stimulation was not affected in the presence of hexamethonium. Often, during the exposure to hexamethonium, the frequency of spontaneous miniature junction potentials was increased and the amplitudes exceeded 7 mV. In every respect hexamethonium bromide (10^{-4}) had the same effect as nicotine (10^{-5}).

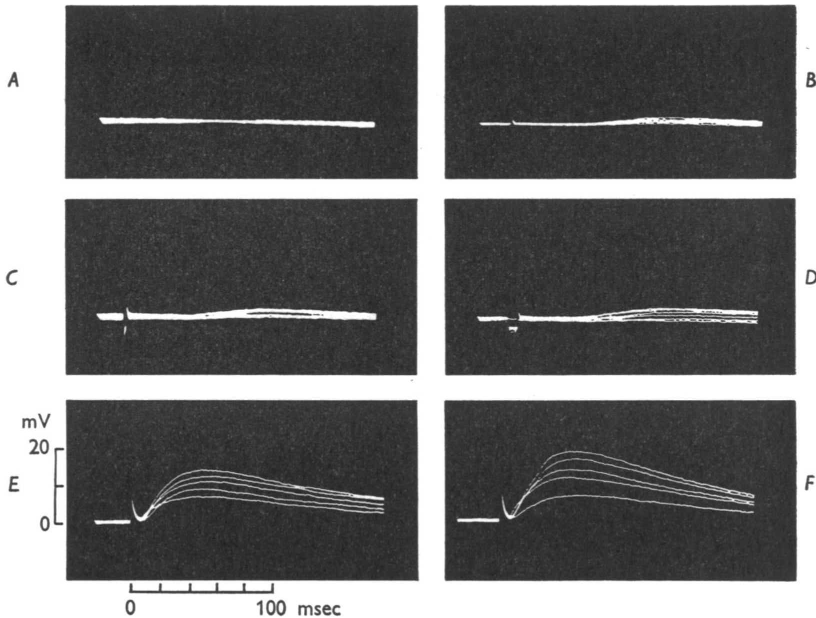

Fig. 9. Junction potentials evoked by nerve (*A–D*) and field (*E, F*) stimulation recorded from the same cell in the presence of nicotine (10^{-5}). (*A*) Five successive stimuli of 10 V, 1·0/sec, 0·01 msec; (*B*) 0·1 msec; (*C*) 1 msec; (*D*) 10 msec; (*E*) 0·01 msec, and (*F*) 0·1 msec; see text.

To exclude a possible action of bromide present in such a high concentration of hexamethonium bromide, the effect of sodium bromide $5{\cdot}6 \times 10^{-6}$ g/ml. (equivalent to the bromide content in 10^{-4} hexamethonium bromide) on the junction potential was examined. No effect on the amplitude or the shape of the junction potentials triggered both by nerve and field stimulation could be observed.

Effect of ganglion-blocking agents on the tension development

In order to produce a tension response to hypogastric nerve and field stimulation the frequency was increased to 10/sec and the intensity fixed at 10 V. Figure 10 (taken from the same experiment as Fig. 7) shows the

tension produced by trains of stimuli when the pulse duration was varied from 0·01 to 5 msec. With short pulse duration (from 0·01 to 0·1 msec) a higher tension development was produced by nerve stimulation than by field stimulation. This was reversed when pulses of longer duration were applied (0·5–1·0 msec). In the presence of hexamethonium (10^{-4}) or nicotine (10^{-5}) the tension response evoked by nerve stimuli of short duration was abolished, while the response to field stimulation with a pulse of 0·01 msec was not.

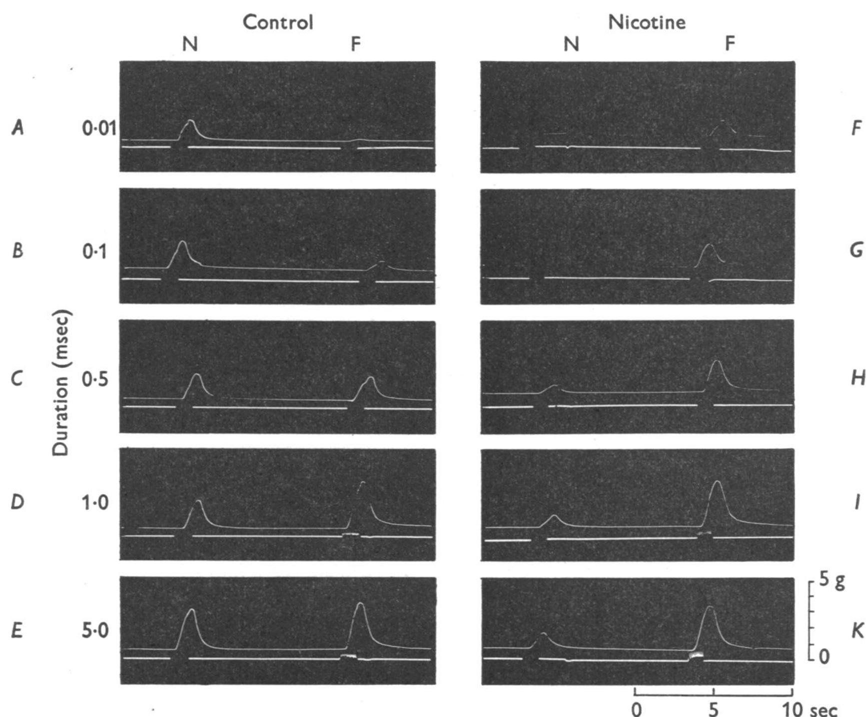

Fig. 10. Effect of nicotine (10^{-5}) on the tension development produced by nerve (N) and field (F) stimulation. Each repetitive stimulation was applied for 1 sec at 10/sec 10 V. The duration of the pulses was increased from 0·01 msec (*A*), to 0·1 msec (*B*), 0·5 msec (*C*), 1 msec (*D*) and 5 msec (*E*). Left-hand side, before applying nicotine; right-hand side, after nicotine. Gap of continuous line under the tension curve indicates the time for the stimulus. For description see text.

There were two striking effects of nicotine on the tension development and these are shown in Fig. 10. First, nicotine increased the tension evoked by field stimulation. Secondly, nicotine abolished the tension response to nerve stimulation when the pulse duration was short. But when the pulse duration was increased it became effective also in the presence of nicotine. However, the absolute tension development was smaller than

in the absence of nicotine. When the tension after treatment was subtracted from the normal one, the difference remained the same (i.e. for 0·01, 0·1, 0·5, 1·0 and 5·0 msec the values were 1·4, 1·3, 1·2, 1·2 and 1·4 g, respectively). This applied to nerve stimulation only.

These observations and the above-mentioned electrophysiological evidences suggest that two kinds of nerve fibres are mixed in the hypogastric nerve trunk. Both fibres trigger junction potentials as well as contractions. From Fig. 10 it appears that the contraction in (*A*) is elicited only by the fast fibres and any increment is due to progressive recruitment of slow fibres as shown in the records *G–K*.

Effect of adrenergic blocking agents on the generation of the junction potentials

Bretylium (10^{-6}) reduced the amplitude of the junction potentials elicited by nerve and field stimulation alike. The maximal response resulting from facilitation was less, but the latency was not affected. Bretylium 10^{-5} abolished the junction potentials within 30 min.

Phentolamine 3×10^{-5} decreased the amplitude of the junction potentials and the degree of facilitation; 10^{-4} blocked it completely. Nerve and field stimulation were equally affected. This observation provides additional support for the assumption that field stimulation activated small adrenergic nerve fibres within the muscle. Moreover, stimuli of 5 msec duration triggered spikes and contraction of the tissue. This was probably due to direct stimulation of the muscle cells.

DISCUSSION

The discussion of this paper is based on the assumption that the junction potentials triggered by field stimulation of the muscle are due to stimulation of terminal nerve fibres in the wall of the vas deferens, and not to direct stimulation of the muscle cells. This may be assumed because (1) a stimulus of 0·01 msec had more than 5 msec latency, (2) facilitation was observed without change of membrane potential during repetitive stimulation and (3) the junction potential disappeared in the presence of adrenergic blocking agents, while stimuli of more than 5 msec duration still triggered spikes.

The hypogastric nerve appears to contain two groups of nerve fibres. One has a low threshold, fast conduction velocity and triggers junction potentials of high amplitude which are blocked by ganglion-blocking agents. The other fibre group has a high threshold, slow conduction velocity and triggers junction potentials of low amplitude which are resistant to the action of ganglion-blocking agents.

Sjöstrand (1962) attributed the effect of ganglion-blocking agents to

one of three possibilities: (1) a block at true ganglionic synapses; (2) a block at peripherally located chromaffin cells; and (3) an 'unspecific' blocking action at the delicate nerve terminals. On the basis of subsequent observations on the noradrenaline content of the vas deferens after reserpine treatment and after hypogastric denervation Sjöstrand (1963) suggested the probable existence of peripheral synapses in the sympathetic innervation. In the present experiments 5×10^{-6} hexamethonium, the blocking concentration used by Sjöstrand (1962), did not block the propagation of excitation in the nerve, but block occurred with 10^{-4} hexamethonium and 10^{-5} nicotine, and these findings agreed with Holman's observations (personal communication).

Ferry (1963) observed that maximal sizes of junction potentials and of contractions were recorded when the fast fibres (3–6 m/sec) only were excited and that the slow fibres (conducting at less than 1 m/sec) had no effect on the size or on the rate of rise of the junction potential, nor on the tension developed. He postulated that the fast fibres were preganglionic B fibres supplying ganglion cells peripheral to the point at which the hypogastric nerve trunk was stimulated, and that the slow fibres were post-ganglionic C fibres.

In the present experiments the conduction velocity was not measured from the nerve action potentials, but from the latencies of the junction potentials obtained by stimulating the nerve fibre at two different points. The observed values varied in different experiments from 0·7 to 2·8 m/sec. The wide range of variation may be due to a variable location of ganglion cells within the hypogastric nerve. Vogt (1963) found that the hypogastric nerve of the dog contained scattered ganglion cells which were aggregated in larger clusters at the central end of the nerve. If this also applies to the guinea-pig the position of the stimulating electrodes would be important in determining the proportion of pre- and post-ganglionic fibres which are stimulated, and also the proportion of the post-ganglionic fibres actually supplying the muscle fibres in the vas deferens. The maximal electrode distance in the present experiments was 20 mm from the muscle, but Ferry (personal communication) stimulated farther away. This may also explain another difference in results. I found that not only the fast fibres, presumably preganglionic, but also the slow fibres, presumably post-ganglionic, could generate junction potentials and a tension response of the muscle. When two pairs of electrodes were used for nerve stimulation the delay of conduction between the nearest electrode and the muscle cell suggested the existence of ganglia somewhere very near in the peripheral part of the hypogastric nerve. However, we do not know whether ganglion-blocking agents also act non-specifically on the very fine peripheral nerve fibres.

When the junction potentials were recorded from the same cell, triggered by nerve or field stimulation, it was observed in some cases that stimulation of the one did not cause facilitation of the other, but more frequently they overlapped. This suggests that a single cell of the vas deferens is innervated by several nerve terminals of at least more than one nerve fibre. This agrees with the histological observations made by Richardson (1962), and may be a specific property of the innervation of the vas deferens by the hypogastric nerve.

SUMMARY

1. Neuromuscular junction potentials, action potentials and tension, evoked by nerve stimulation and field stimulation of the muscle, were recorded from single cells in the hypogastric nerve–vas deferens preparation of the guinea-pig.

2. The junction potentials elicited by short pulses (0·01 msec) with either type of stimulation and recorded from the same muscle cell were qualitatively alike although not always of the same amplitude.

3. Facilitation was seen with both types of stimulation, either independent or mutually influencing each other, the overlap being variable in different cells.

4. The minimal latency of junction potentials produced by field stimulation was 6 msec, presumably due to the transmission delay at nerve terminals.

5. The conduction velocity of the nerve fibres measured along the last 10–20 mm of the nerve trunk varied from 0·7 to 2·8 m/sec, but along the portion where the nerve entered the muscle there was a considerable delay.

6. Ganglion-blocking agents (10^{-4} hexamethonium bromide and 10^{-5} nicotine hydrochloride) blocked the generation of junction potentials evoked by short pulses (0·01 msec) to the nerve. Pulses of longer duration triggered junction potentials in the presence of nicotine which were smaller and had a much longer latency. Similarly, the tension response elicited by short pulses to the nerve was abolished, while the part of the tension attributable to the long pulses remained constant in the presence of ganglion-blocking agents. These observations indicate the presence of both pre- and post-ganglionic fibres in the hypogastric nerve.

7. Ganglion-blocking agents did not block the generation of junction potentials nor the tension produced by field stimulation.

8. Adrenergic-blocking agents (phentolamine 10^{-4} g/ml. and bretylium tosylate 10^{-5} g/ml.) abolished the generation of junction potentials for both types of stimulation.

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