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EFFECTS OF TETRAETHYLAMMONIUM CHLORIDE ON SYMPATHETIC NEUROMUSCULAR TRANSMISSION IN SAPHENOUS ARTERY OF YOUNG RABBITS

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SUMMARY

1. Excitatory junction potentials and electrotonic potentials were recorded from the smooth muscle of the rabbit saphenous artery using intracellular electrodes.

2. Tetraethylammonium chloride (TEA) in concentrations greater than 2·5 mM caused depolarization. Concentrations greater than 5 mM caused spontaneous electrical activity in the form of excitatory junction potentials (e.j.p.s) and all-or-nothing action potentials which were associated with spontaneous mechanical activity.

3. Concentrations of TEA less than 2·5 mM did not alter the resting potential nor the passive membrane properties of the smooth muscle over a range of ± 15 mV.

4. The following effects were observed in 2·0 mM-TEA. (*a*) The minimum stimulus strength required for the initiation of an e.j.p. fell by three to fivefold. (*b*) Single stimuli that elicited only a small e.j.p. in normal solution evoked an all-or-nothing action potential of up to 70 mV amplitude. (*c*) Whereas in normal solution e.j.p.s could only be recorded up to 7 mm away from the perivascular stimulating electrode e.j.p.s could be recorded at distances of up to 13 mm. (*d*) The duration of the e.j.p. was prolonged.

5. Based on these results and the effects of TEA reported for other synapses it is proposed that TEA may act to increase the amount of transmitter released per axon, to increase the duration of release and to cause an increased invasion throughout the autonomic ground plexus by nerve impulses. This would imply that in normal solution, *in vitro*, the action potential may not propagate throughout the whole length of the terminal axon and its many branches due to failure of conduction at one or more points along the terminal portion of the axon.

INTRODUCTION

Recent studies on the membrane potentials of arterioles (Hirst, 1977), the saphenous artery of rabbits (Holman & Surprenant, 1979*a*) and the rabbit ear artery (Surprenant, 1979) have confirmed previous reports that neuromuscular transmission in small arteries is associated with the generation of excitatory junction potentials (e.j.p.s) (see Bell, 1969; Speden, 1970). In the rabbit ear artery repetitive stimulation causes facilitation and at frequencies greater than 1 Hz summation

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occurs until, at a critical level of depolarization, an action potential is initiated and the vessel contracts; this sequence of events is basically the same as that described by Burnstock & Holman (1961) for the guinea-pig vas deferens. In the rabbit saphenous artery, however, the depolarization produced by repetitive nerve stimulation usually failed to evoke an all-or-nothing action potential and contraction was associated with a more variable, graded type of 'active response' (Holman & Surprenant, 1979*a*). It is well known that tetraethylammonium ions (TEA) increases the amplitude of the action potentials of smooth muscles which are spontaneously active or electrically excitable (see Bolton, 1979). We therefore began this series of experiments in order to test whether TEA could convert active responses of the saphenous artery into more conventional action potentials.

We confirmed previous observations that relatively high concentrations of TEA (greater than 3–5 mM) caused depolarization, increased membrane resistance, and the initiation of spontaneous action potentials in vascular and other smooth muscles which are normally quiescent (guinea-pig stomach fundus, Osa & Kuriyama, 1970; Szurszewski, 1978; rabbit ear artery, Droogmans, Raeymaekers & Casteels, 1977; bovine trachea, Kirkpatrick, 1975; rabbit common carotid artery, Mekata, 1971; and pulmonary artery, Casteels, Kitamura, Kuriyama & Suzuki, 1977; Haeusler, 1978). These results are in accordance with the view that TEA acts to reduce the potassium permeability of the smooth muscle cells which increases during depolarization and tends to counteract the effects of any voltage dependent inward current.

We also found that TEA had marked effects on neuromuscular transmission, causing a great increase in the amplitude of the e.j.p. This effect, which was apparent at lower concentrations than those which appeared to affect the passive membrane properties of this smooth muscle, is the main concern of this report. As a result of these studies we conclude that the release sites of the terminal axons of the autonomic ground plexus in the saphenous artery fail to demonstrate their maximum capacity to release transmitter, *in vitro*. (A preliminary account of these observations has been reported to the Australian Physiological and Pharmacological Society (Holman & Surprenant, 1979*b*).)

METHODS

Young rabbits weighing between 800 and 1200 g were used in these experiments. Unless otherwise stated the saphenous artery and adjacent veins were removed and excess connective tissue dissected away leaving the veins on either side of the artery intact; these veins were used to pin the tissue securely to the silicone rubber base of the organ bath with minimal distortion of the artery itself.

In rabbits, the anterior tibial nerve runs with the saphenous artery and veins within the same connective tissue sheath from below the knee to the ankle. Along this length of the artery the tibial nerve sends off from two to six branches per mm. The number of branches increases progressively distalward. It has been shown that these branches supply all the noradrenergic innervation of this segment of the saphenous artery (Surprenant, 1978). In some experiments the anterior tibial nerve was left attached to the artery-vein sheath, care being taken not to place any undue stress on the connective tissue in which the small nerve branches were contained.

The methods of stimulation have been described previously (Holman & Surprenant, 1979*a*). A suction electrode around the proximal portion of the saphenous artery was used for the stimulation of perivascular nerves. When the anterior tibial nerve was left attached to the artery two suction electrodes were used, one around the proximal segment of the nerve and

the other around the proximal segment of the artery. The passive membrane properties of this smooth muscle were determined using the external stimulating partition method of Abe & Tomita (1968). A modified Krebs solution (concentrations in m-mole/l; NaCl 120; KCl 5; $CaCl_2$ 2·5; $MgSO_4$ 1; $NaHCO_3$ 25; NaH_2PO_4 1; glucose 11; equilibrated with 95% O_2: 5% CO_2) flowed continuously through the organ bath (volume 2 ml.) at a rate of 3–4 ml./min. Temperature was maintained at 35–36 °C.

Intracellular recordings were made from the smooth muscle cells using high resistance (100–200 MΩ) micro-electrodes filled with 2 M-KCl. Membrane potentials were recorded differentially between two such electrodes, one being intracellular and the other immediately outside the tissue. In many experiments averaged responses were obtained using a BIOMAC 1000 signal averager.

In some experiments both ends of the artery were cannulated and the distal end of the artery was connected, via a T-tap, to a Gould Statham P23 ID pressure transducer whose output was displayed on a separate channel of the oscilloscope. No attempt was made to perfuse the artery, which was distended at intervals by connecting its proximal end via a T-tap to a column of Krebs solution of up to 150 cm high. After a steady pressure was established the connection to the column of Krebs was closed off. Constriction of the artery in response to nerve stimulation resulted in an increase in the pressure recorded by the transducer. In a separate series of experiments spiral strips of the saphenous artery were prepared as previously described (Holman & Surprenant, 1979*a*) and longitudinal tension was recorded with a Grass FT 03 transducer and displayed on a Grass Polygraph pen recorder.

Tetraethylammonium chloride (Eastman–Kodak) was used in all these experiments. In some experiments tetrodotoxin (Sankyo), 10^{-7} g/ml., was used to prevent responses to nerve stimulation.

RESULTS

Control conditions

Intracellular recordings were made from the smooth muscle of the media of the rabbit saphenous artery; resting membrane potentials of -65 to -75 mV were observed and in the absence of stimulation these cells were electrically quiescent. Perivascular nerve stimulation at low frequencies evoked excitatory junction potentials (e.j.p.s) whose amplitudes were graded according to the stimulus strength (see Holman & Surprenant, 1979*a*).

The relation between stimulus strength and e.j.p. amplitude was determined by first finding the stimulus intensity giving rise to a maximal e.j.p. and then gradually reducing the intensity so as to avoid complications due to recruitment of unfacilitated terminals (Bell, 1969; Hirst, 1977). The preparation was stimulated repetitively at 0·45 Hz and the amplitude of an average of sixteen or thirty-two successive e.j.p.s determined for each new value of stimulus intensity. The results from one such experiment are shown in Fig. 1 (see also Fig. 6*C*). No steps or plateaux were apparent and the e.j.p.s gradually faded into baseline noise. These findings are similar to those obtained for the vas deferens (Burnstock & Holman, 1961) and indicate that the smooth muscle syncytium is functionally innervated by several axons and their terminal branches.

The amplitude and shape of e.j.p.s in response to a given stimulus strength, recorded from any cell, at any depth of penetration, within 3–5 mm from the perivascular stimulating electrode were virtually identical (Fig. 3*B*; see also Holman & Surprenant, 1979*a*). But beyond approximately 5 mm from the stimulating electrode the amplitude of the e.j.p. became smaller and no e.j.p.s could be recorded at a distance greater than 6–8 mm from the site of stimulation. The relationship

between the amplitude of the evoked e.j.p. and distance from the stimulating electrode is shown in Fig. 2 for three preparations. In each case stimulus strength was set to give a maximum amplitude e.j.p. close to the stimulating electrode. A frequency of 0·5 Hz was used and thirty-two or sixty-four e.j.p.s were averaged after each series had been recorded at random distances from the stimulating electrode.

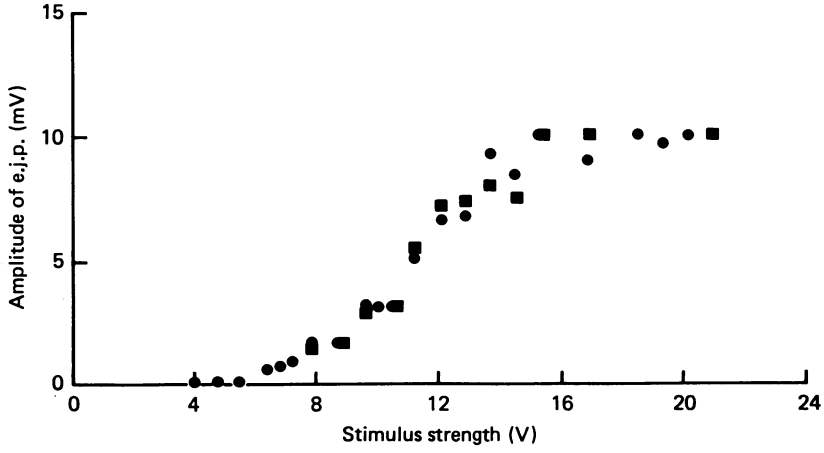

Fig. 1. Relationship between stimulus strength and amplitude of e.j.p. recorded from one cell of the saphenous artery. Each point is the averaged response of thirty-two successive stimuli at 0·45 Hz. The voltage scale is the output of a Grass S-88 stimulator. No discrete steps or plateaux are apparent. Squares represent values obtained after the first complete run (circles) had been accomplished. No differences in the amplitudes of the e.j.p.s were noted, and indicate that prolonged (greater than 30 min) stimulation at low frequencies does not cause any depression of the response. Stimulus duration 0·3 msec throughout.

The time course of the smaller amplitude e.j.p.s was different from the e.j.p.s recorded close to the stimulating electrode. Those e.j.p.s whose amplitude was about 5 mV occasionally had a faster time course associated with a decrease in rise time and in the rate of the initial part of the decay phase (Fig. 3*C*). This change in shape may be taken to indicate that this region of the artery was not undergoing a uniform change in membrane potential as the result of the synaptic current.

An extreme case of a non-uniform change in membrane potential occurs when current is injected at a point into a three dimensional syncytium. Purves (1976) calculated the response of a syncytium to the injection of a current wave form whose time course resembled that of the spontaneous e.j.p. of the guinea pig vas deferens. (The reasons for the choice of such a wave form are discussed by Purves (1976).) The time to peak amplitude of the voltage response recorded close to a point source of current (distance = 0·1 λ where λ is the length constant) was less than half that calculated for uniform current injection (see Purves, 1976; Fig. 3). In the present experiments the decrease in time course of the smaller amplitude e.j.p.s was less dramatic than this and it seems likely that the smaller faster e.j.p.s were due to an uneven distribution of current rather than a single point source.

The smallest e.j.p.s (1–4 mV) had a slower time course than those recorded close to the stimulating electrodes. Although their shape was not analysed in detail their half-duration was 30–40 % longer than that of the e.j.p.s recorded close to the

stimulating electrode. This would be expected to occur if the smallest e.j.p.s were due to passive depolarization of a region beyond the action of the transmitter.

When the anterior tibial nerve trunk was stimulated e.j.p.s were recorded along the entire length of the saphenous artery. The e.j.p.s recorded in all cells in response to low frequency (0·45 Hz) supramaximal stimulation of the tibial nerve were similar in amplitude and time course. The time course of the e.j.p.s recorded using this method of stimulation was identical to that of the e.j.p.s recorded within 2–4 mm from the perivascular stimulating electrode.

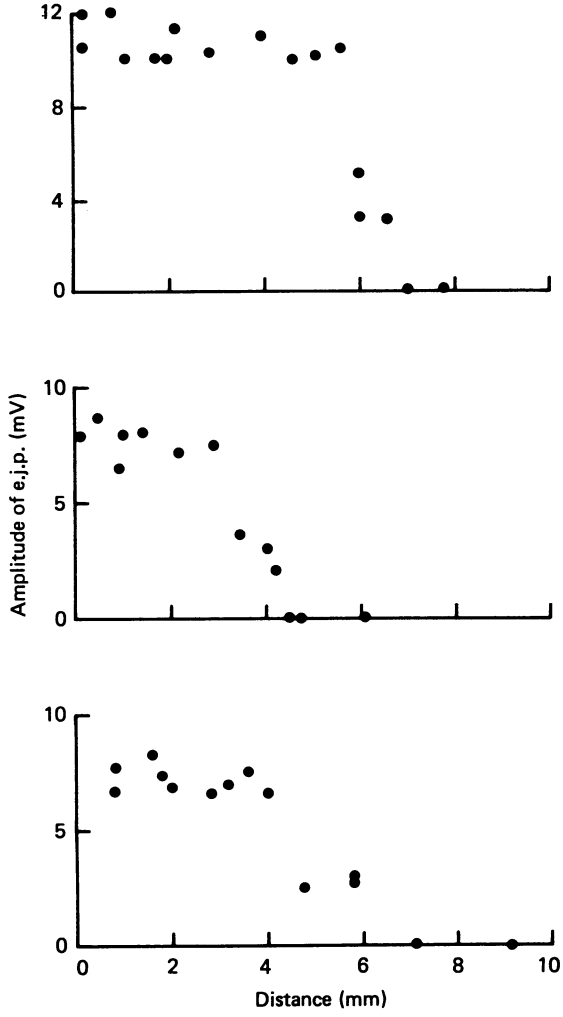

Fig. 2. Relationship between amplitude of e.j.p. and distance from proximal perivascular stimulating electrode for three different preparations. Each point is the averaged response from thirty-two stimuli (stimulation frequency 0·5 Hz). The stimulus strength was maintained constant. The amplitudes of the e.j.p.s recorded in any cell within 3–5 mm were similar; but beyond this distance the amplitudes decreased progressively and no e.j.p. could be elicited beyond approximately 6–7 mm from the stimulating electrode.

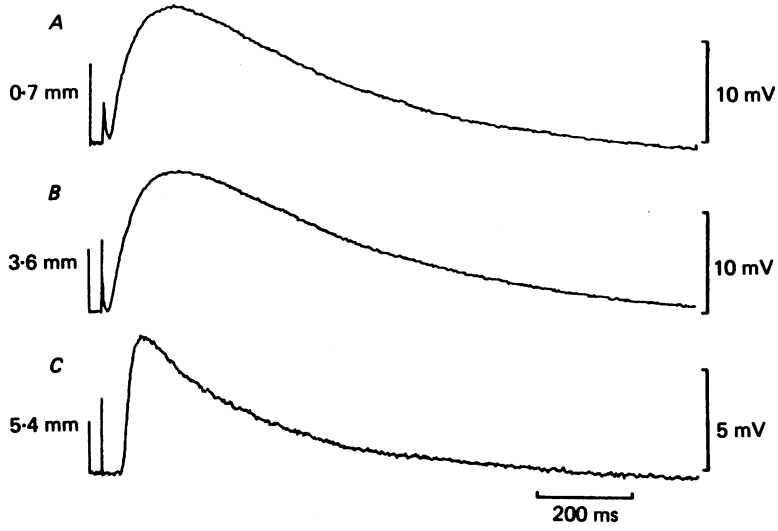

Fig. 3. Averaged e.j.p.s recorded in three cells from the same preparation at distances (0·7, 3·6 and 5·4 mm) away from the stimulating electrode. The shapes and amplitudes of the e.j.p.s recorded up to 4 mm from the stimulating electrode were virtually identical (*A* and *B*). The e.j.p. recorded 5·4 mm away (*C*) showed a smaller amplitude and a faster time-to-peak, as well as a faster decay phase. Stimulus strength was constant throughout the experiment.

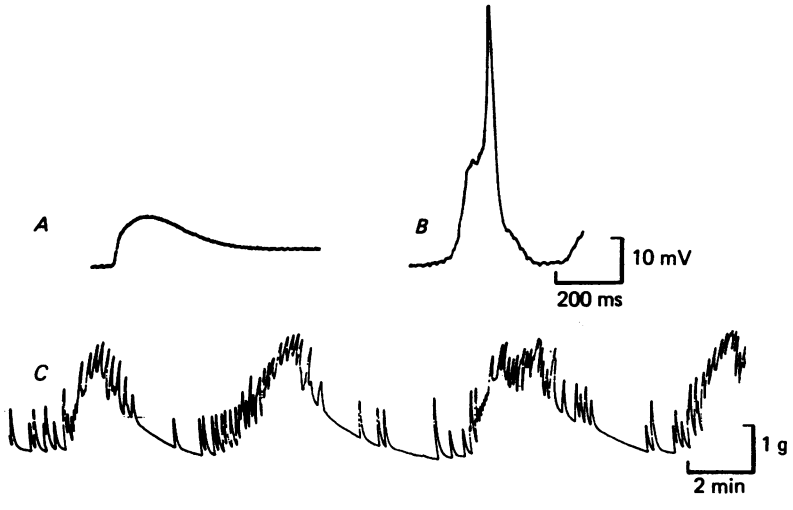

Fig. 4. Examples of spontaneous electrical activity (*A* and *B*) and mechanical activity (*C*) in higher concentrations of TEA. *A*, spontaneous excitatory junction potential recorded in 5 mM-TEA. This was felt to be due to a spontaneous action potential in a preterminal axon as such e.j.p.s were never seen in the presence of TTX. *B*, spontaneous action potential recorded in 10 mM-TEA. Such action potentials were always associated with dislodgement of the micro-electrode. *C*, mechanical activity recorded in spiral strip of the saphenous artery exposed to 15 mM-TEA. Note the 'bursts' of contractile activity and the phasic nature of the contractions.

Effects of TEA on membrane potential and spontaneous activity

Concentrations of TEA greater than 2·5 mM depolarized the muscle membrane of the saphenous artery in a dose-dependent way as described previously by Droogmans *et al.* (1977). In 5 mM-TEA occasional spontaneous e.j.p.s were observed (Fig. 4*A*). These were probably due to spontaneous action potentials in the nerve fibres because they were never seen when tetrodotoxin (10^{-7} g/ml.) was present in the bathing solution. In concentrations of TEA between 5 and 15 mM occasional spontaneous muscle action potentials of 40–55 mV amplitude were recorded (Fig. 4*B*) which always caused dislodgement of the micro-electrode and were considered to be associated with a contraction of the artery. Spontaneous action potentials were recorded in higher concentrations of TEA (greater than 5 mM) even when tetrodotoxin was present.

Concentrations of 10–15 mM resulted in spontaneous bursts of contractions of the spiral strip preparations of the saphenous artery. At these concentrations it was not possible to maintain an impalement in the intact segment of the artery for more than about 30 sec due to the mechanical activity of the artery. The phasic nature of the TEA-induced spontaneous contractions is illustrated in Fig. 4*C* which shows the mechanical response from a spiral strip of the saphenous artery exposed to 15 mM-TEA. These findings are similar to those of Droogmans *et al.* (1977) in the rabbit ear artery, in which spontaneous action potentials with bursts of contractions were seen to occur in 10–15 mM-TEA which depolarized the membrane to about −35 mV. All the effects of TEA were readily reversible within 10 min of washout.

Effects of TEA on excitatory junction potentials

(*a*) *The amplitude of the e.j.p. was increased.* In normal solution the largest response to a single stimulus of maximum intensity applied to the nerve supply of the saphenous artery was an e.j.p. whose amplitude was 14–15 mV. This was the maximum amplitude observed when e.j.p.s were recorded close to the perivascular electrodes or in response to stimulation of the tibial nerve. Large all-or-nothing action potentials were never recorded in normal solution, although frequencies of 5 Hz or greater produced a brief peak of depolarization ('active responses') of up to 52 mV amplitude (see Holman & Surprenant, 1979*a*). A single, supramaximal stimulus applied to the nerve supply in the presence of 2·0 mM-TEA evoked an all-or-nothing action potential associated with a strong contraction. This is shown in Fig. 5*A* and *B* in which the pressure change was recorded at the same time as the electrical response. In control solution a typical e.j.p. (Fig. 5*A*) was recorded which failed to cause any detectable change in pressure.

When 1·5–2·0 mM-TEA was added to the perfusion fluid the increase in the amplitude of the e.j.p. was associated with a decrease in the threshold strength for initiation of an e.j.p. This is shown in Fig. 5*C* in which the relation between stimulus strength and amplitude of response is plotted from the same cell before and 20–40 min after the addition of 2·0 mM-TEA to the bathing solution. In normal solution each point was obtained in the manner described above, whereas during exposure to TEA stimulus intensity was set at minimum and gradually increased. This was necessary in order to prevent dislodgement of the microelectrode due to contraction

associated with action potential initiation by the larger e.j.p.s. It can be seen that the threshold strength for initiation of an observable e.j.p. was greatly reduced in the presence of low concentrations of TEA. In seven cells, from seven arteries, in which an impalement was maintained for over one hour after the addition of TEA,

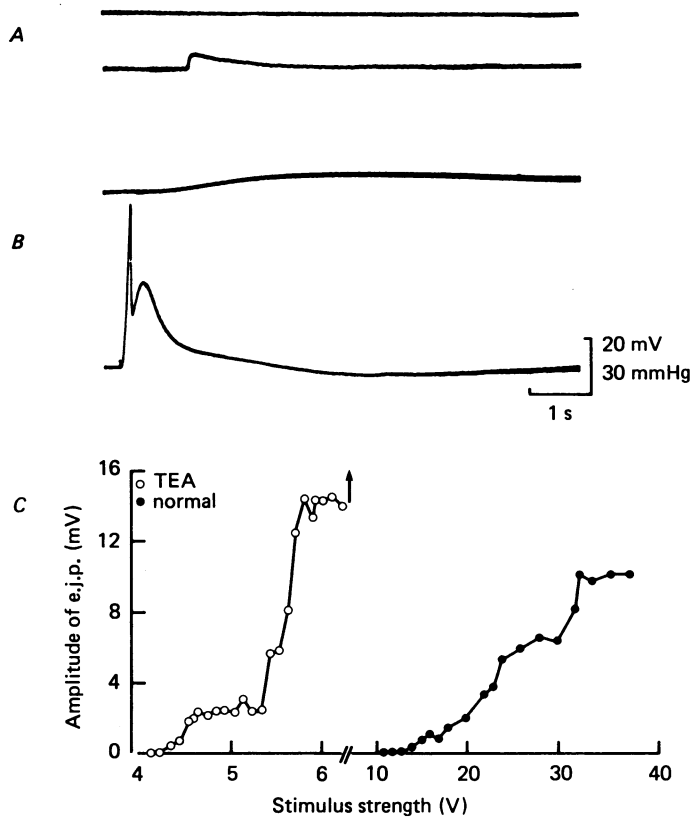

Fig. 5. *A* and *B*, pressure (upper traces) and electrical (lower traces) recordings from the same cell before (*A*) and 15 min after exposure to 2·0 mM-TEA (*B*). A supramaximal stimulus applied in normal solution evoked an e.j.p. of 10 mV amplitude while the same stimulus evoked an action potential of 55 mV amplitude in the presence of 2·0 mM-TEA. No change in resting membrane potential was observed during exposure of this concentration of TEA. *C*, stimulus strength–response curve obtained in the same cell before and during exposure to 2·0 mM-TEA. Each point represents the averaged e.j.p. obtained from thirty-two or sixty-four stimuli at 0·45 Hz. Filled circles refer to normal solution and open circles to TEA. The threshold stimulus strength for initiation of an e.j.p. was always reduced by TEA. Note also the presence of 'steps' in TEA but not in normal solution. Arrow marks dislodgement of the micro-electrode, which was associated with an action potential.

the final value for the threshold stimulus strength was found to be decreased by 2·5–5 times that found for the same cell in normal solution.

(*b*) *The latency of onset of the e.j.p. was increased.* At the skeletal neuromuscular junction as well as the squid stellate ganglion the latency of onset of the post-synaptic potential is increased by TEA (Koketsu, 1958; Kusano, Livengood &

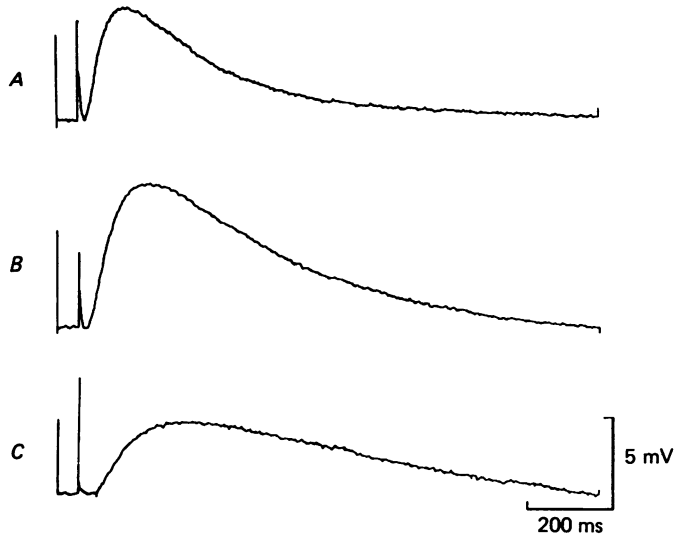

Fig. 6. Averaged e.j.p.s in normal solution (*A*) and 2·0 mM-TEA (*B*, *C*). *A* and *B* were obtained from the same cell 0·8 mm from the stimulating electrode; the total duration of the e.j.p. was prolonged by 62% (*B*) in the presence of TEA. *C*, record obtained 6·4 mm away from the stimulated end; the duration of the e.j.p. was increased by 125% over that in *A*. Sixty-four responses averaged at 0·45 Hz in each case. Stimulus strength was the same for *B* and *C*.

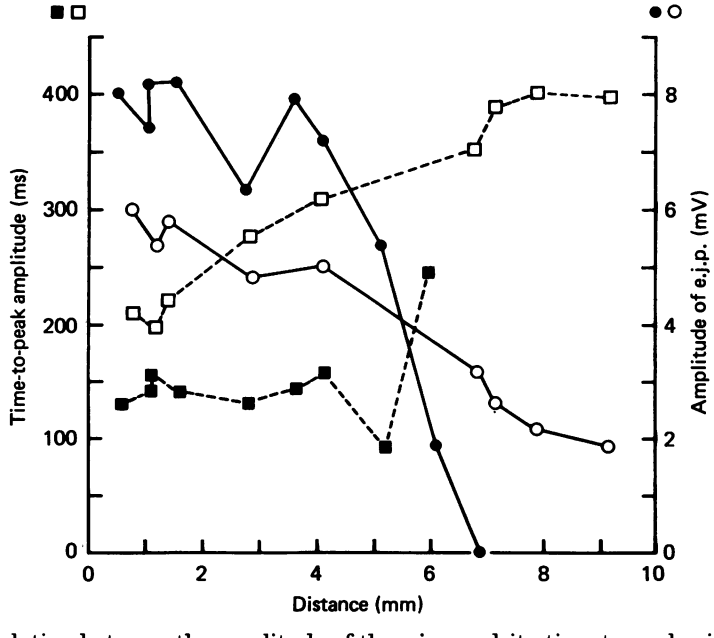

Fig. 7. Relation between the amplitude of the e.j.p. and its time-to-peak with distance from the stimulating electrode obtained in one artery. Circles represent amplitude of the e.j.p., squares represent time-to-peak (filled = normal solution; open = 2·0 mM-TEA). It can be seen that the amplitude of the e.j.p. in TEA decreases gradually with distance and the time-to-peak increases with distance for the same stimulus strength. Each point is the averaged response from thirty-two e.j.p.s.

Werman, 1967; Benoit & Mambrini, 1970). The mechanism of this TEA-induced latency shift has not been elucidated. In the saphenous artery the latency of onset of the e.j.p. was also increased in the presence of TEA. In normal solution the time from the onset of the stimulus artifact to the beginning of the e.j.p. ranged from 10 to 14 msec when records were made within 1–2 mm from the stimulating electrode whereas in TEA latencies ranged from 15·5 to 33 msec over the same distance.

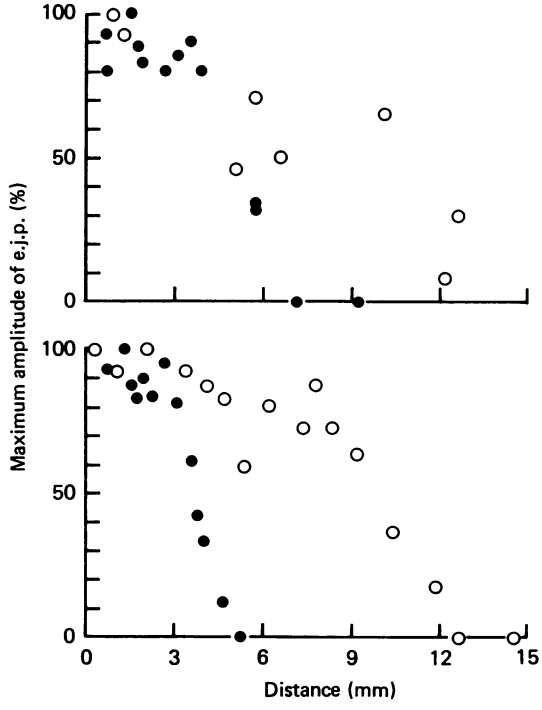

Fig. 8. Amplitude of averaged e.j.p.s (thirty-two or sixty-four averaged at each distance) obtained at different distances from the stimulating electrode. Graphs from two preparations are shown. Filled circles = normal solution; open circles = 2·0 mM-TEA. Stimulus intensity constant for all points obtained in normal solution. Intensity also constant, at a lower voltage for all points obtained in TEA.

(c) *The duration of the e.j.p. was prolonged.* Within 5–7 min after TEA was added to the bath the duration of the majority of the e.j.p.s began to increase. During the next 20–25 min such e.j.p.s became progressively lengthened and thereafter remained relatively constant. The degree by which the e.j.p. was prolonged varied among preparations but was consistent for any single preparation. The time-to-peak increased up to fourfold compared to the control e.j.p. and the time to decay from peak to half maximum amplitude increased by as much as two or threefold. It was noted that the increase in the duration of the e.j.p. was related to the distance from the stimulating electrode. An example is shown in Fig. 6 which shows averaged e.j.p.s before and during the addition of 2·0 mM-TEA. Fig. 6*A* and *B* were obtained from the same cell at a distance of 0·8 mm from the stimulated end while the record

of Fig. 6*C* was made 6·4 mm away. The relationship between time-to-peak and amplitude as a function of distance from the stimulating electrode is shown in Fig. 7 for one preparation.

(*d*) *The effective distance of transmitter action was increased.* After a cell was impaled close to the stimulating electrode a stimulus strength was selected to give

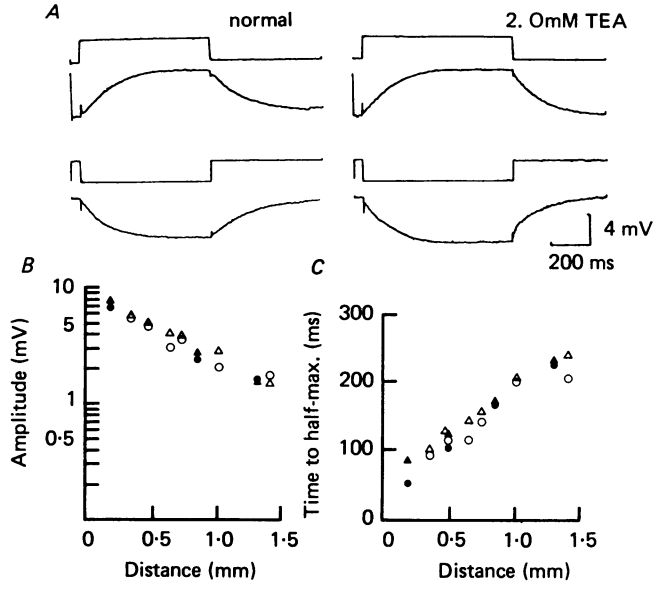

Fig. 9. *A*, averaged electrotonic potentials produced by constant current pulses recorded in the same cell in normal (left) and during exposure to 2·0 mM-TEA (right). *B*, semilogarithmic plot of steady-state potential *vs.* distance; points in *B* and *C* obtained from records as in *A*. In both *B* and *C* triangles represent responses to depolarization; circles represent responses to hyperpolarization (filled symbols = normal solution; open symbols = TEA). *C*, graph of time to reach half-maximum steady-state amplitude *vs.* distance. In four arteries there were no significant differences in the amplitude or time course of the electrotonic potential recorded at any distance in the presence of 2·0 mM-TEA compared to that recorded in normal solution. The values of λ and τ_m calculated from these relationships were similar in normal and 2·0 mM-TEA.

e.j.p.s of constant mean amplitude, thus ensuring that the same number of axons would be stimulated on each occasion. The stimulation strength and frequency (0·5 Hz) were then kept constant and an average of thirty-two or sixty-four e.j.p.s obtained at several distances along the artery. The results from two such experiments are shown in Fig. 8. Whereas in normal solution no e.j.p.s could be elicited beyond approximately 7 mm from the stimulating electrode, in TEA (2 mM) e.j.p.s could be recorded up to 10–13 mm from the electrode. This was a consistent finding in all arteries (24) in which this experiment was carried out.

Effects of low doses of TEA on passive membrane properties

Concentrations of TEA less than 2·5 mM did not alter the resting membrane potential. In these lower concentrations of TEA depolarization of the muscle in the

presence of tetrodotoxin, by the application of long duration, low intensity current pulses failed to evoke any active response or action potential although action potentials could be evoked by depolarization of the muscle when higher concentrations were used (see Holman & Surprenant, 1979*a*).

Values of the membrane time constant (τ_m) and space constant (λ) were determined both in normal solution and after exposure to 2·0 mM-TEA. Electrotonic potentials produced by a constant voltage gradient were recorded at four to eight different distances from the stimulating plates. In each cell electrotonic potentials were recorded from sixteen alternating hyperpolarizing and depolarizing current pulses and the responses averaged. Results from one such experiment are shown in Fig. 9. In Fig. 9*A* averaged records of electrotonic potentials recorded from the same cell in normal solution and in 2·0 mM-TEA are shown. In Fig. 9*B* the semi-logarithmic graph of the steady-state potential versus distance from the stimulating partition is plotted; and in Fig. 9*C* the time to reach half-maximum amplitude is plotted against distance. It can be seen that in both cases the decay of the electrotonic potential with distance was exponential for hyperpolarizing as well as depolarizing pulses and reached 1/e (λ) at approximately the same distance. The slopes of the lines for the time to half-maximum amplitude *vs.* distance were nearly identical and τ_m values calculated by this method were not significantly different. In four preparations the values of λ and τ_m in normal solution were $0{\cdot}84 \pm 0{\cdot}04$ mm (S.E. of mean) and 229 ± 12 msec respectively and in 2·0 mM-TEA were $0{\cdot}8 \pm 0{\cdot}1$ mm and 230 ± 18 msec.

Since the passive electrical properties of the artery did not show any detectable change in the values under these conditions we can only conclude that the changes in the properties of the e.j.p. described above must have been due either to a different pattern of release of transmitter resulting in non-uniformity of synaptic current and subsequent changes in membrane potential; to a much more prolonged time course of transmitter action; or to a change in the kinetics of the action of the transmitter in the presence of TEA.

DISCUSSION

The experiments described in this report were mainly concerned with the action of TEA on sympathetic neuromuscular transmission. This was possible since we found that concentrations of 2·5 mM or less had no effect on the resting membrane potential of the smooth muscle nor its passive electrical properties over the range of values of membrane potential which permitted us to record subthreshold e.j.p.s. The possibility that these low doses of TEA may have increased the excitability of the smooth muscle membrane in response to larger depolarizations cannot be ruled out. However, we would like to suggest that the large 'all-or-nothing' action potentials which were recorded in response to stronger stimuli were due, at least in part, to the rapid rate of depolarization associated with a suprathreshold e.j.p. We have previously suggested that the rate of depolarization is an important factor in determining whether or not the 'active responses' of the rabbit saphenous artery have the characteristics generally attributed to action potentials (Holman & Surprenant, 1979*a*). There did not appear to be any obvious differences between the

threshold membrane potential at which active responses occurred in normal solution during repetitive stimulation and action potentials in TEA in response to a single stimulus. However it should be emphasized that we have not attempted to explore the effects of very high frequencies of stimulation which might be able to depolarize the membrane at the same rate as that associated with a suprathreshold e.j.p. in the presence of TEA. It will continue to be difficult to resolve this question as the long time constant of the membrane of the saphenous artery (about 250 msec) and the syncytial nature of this muscle make it difficult to find a method which can cause rapid changes in membrane potential comparable with those due to the release of large amounts of excitatory transmitter.

The finding that the stimulus strength required for the initiation of an e.j.p. was greatly reduced by TEA may be taken as indirect evidence that the threshold for initiation of an action potential in sympathetic axons was decreased. Alternatively, it may be that TEA increased the output of transmitter per axon, making it possible to detect an e.j.p. from a site at which the effects of transmitter action in normal solution were insufficient to evoke an e.j.p. discernible above the noise level. Although this possibility cannot be discounted there are reasons for considering it unlikely. In normal solution, at a stimulus strength which failed to evoke an observable e.j.p. on individual traces, e.j.p.s could often be distinguished using the averaging procedure. However, when the stimulus strength was reduced to that which evoked a large e.j.p. in the presence of TEA, no responses were observed in normal solution even after averaging more than 250 stimuli at a low frequency (0·5 Hz) or high frequency (5 Hz).

Plots of e.j.p. amplitude against stimulus intensity obtained in normal solution did not show any evidence of distinct 'steps' or plateaux but resembled the gradual increase in the amplitude of the e.j.p. with an increase in stimulus intensity as observed in the guinea-pig vas deferens. It may be concluded that as the e.j.p.s gradually faded into the noise, the output of transmitter from one or a few axons must be relatively ineffective in producing a change in membrane potential. As can be seen in Fig. 5, besides lowering the threshold strength for initiation of an e.j.p. TEA resulted in the appearance of discrete steps on stimulus–response graphs. If each plateau represents the activation of a separate axon, then it would appear that in the presence of TEA the output of transmitter per axon must be quite considerable.

The innervation of the isolated saphenous artery is such that in control solution e.j.p.s could only be recorded at relatively short distances from a perivascular (suction) stimulating electrode (up to 6 mm). Sympathetic axons leave the anterior tibial nerve at sites about 2–4 mm apart to contribute to the autonomic ground plexus. Perhaps the most remarkable effect of TEA observed in these experiments was to increase the distance from the suction electrode over which it was possible to record e.j.p.s. We would like to suggest that TEA increases the maximum distance between the stimulating and recording electrode at which an e.j.p. can be recorded because it enables the presynaptic action potential to invade the furthermost extensions of the axons of the ground plexus. This implies that action potentials fail to reach all of the terminal varicosities in the absence of TEA *in vitro*. A further possible explanation for this effect would be to assume that TEA caused an increase in the excitability of the autonomic ground plexus which enabled the stimulus to

spread to axons with release sites closer to the recording intracellular electrode. This seems unlikely in view of the increase in latency of the e.j.p.s recorded at the same distance from the stimulating electrode. However, this cannot be ruled out since one of the well documented (but unexplained) effects of TEA is to prolong the latency of the post-synaptic response. We found that the latency increased progressively with distance from the stimulating electrode as would be expected if the action potential were propagating throughout the axon and its branches and not originating at variable sites of excitation.

The effect of low doses of TEA in prolonging the time course of the e.j.p.s is also difficult to explain. If TEA increases the invasion of certain individual terminal branches of the ground plexus it is possible that the release of transmitter may become non-uniform and 'patchy'. In such circumstances it would be very difficult to predict the time course of transmitter action from a knowledge of the passive properties of the muscle according to any simple assumptions about the distribution of release of transmitter. We cannot rule out the possibility that TEA may have a postsynaptic effect which alters the kinetics of the reaction of noradrenaline with the receptors or the ionophores activated by this reaction. However, preliminary experiments on the guinea pig vas deferens failed to reveal any obvious effects of TEA on the time course of spontaneous e.j.p.s. It is difficult to avoid the conclusion that TEA causes a marked increase in the duration of transmitter release.

Our results suggest that under normal conditions, *in vitro*, the autonomic ground plexus of the saphenous artery releases transmitter in amounts which are well below its maximal capacity. We have suggested that one of the reasons why this should be so is the lack of invasion by nerve impulses of all the terminal varicosities of the autonomic ground plexus. It also seems likely that TEA increases transmitter release from those sites which *are* invaded by nerve impulses. Recent experiments by Hirst & Neild (1980) have suggested that the probability of release of a quantum of transmitter in response to field stimulation of the varicosities surrounding a small isolated segment of mesenteric arteriole was such that an average of 3–4 quanta were released from some 100–200 varicosities. Thus it seems possible that there is a great deal of reserve functional capacity in the autonomic ground plexus in terms of release of transmitter. One can only speculate what this may imply in terms of other, more long term actions; for example, the reuptake of transmitter by high affinity amine pumps.

As pointed out by Speden (1970), vascular smooth muscle and its nerve supply may be very sensitive to its environment. We doubt that inadequate oxygenation of the autonomic ground plexus could explain our observations since these are in the adventitia and are exposed to an abnormally high partial pressure of oxygen (95% O_2:5% CO_2). However it is clear that the physiology of small arteries must be examined under more physiological conditions, *in vivo*, before any further attempts are made to speculate on the significance of these observations.

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