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DIFFERENT ELECTRICAL RESPONSES OF OUTER AND INNER MUSCLE OF RABBIT CAROTID ARTERY TO NORADRENALINE AND NERVES

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SUMMARY

1. Electrical responses of outer and inner muscle of the rabbit carotid artery to electrical stimulation and noradrenaline were investigated.

2. Mean values of the resting potential and space constant in the direction of the long axis of the cells were −47·3 mV and 1·61 mm for the inner muscle and −45·9 mV and 0·92 mm for the outer muscle. Both muscles showed strong outward-going rectification with no evoked action potential on continuous injection of depolarizing current.

3. Such current spread not only in the direction of the long axis of the smooth muscle cells but also to a lesser degree transverse to this axis (space constant approximately 0·55 mm). There was little or no spread of current from the outer muscle layer to the inner muscle layer.

4. At high frequencies of nerve stimulation (higher than 20 Hz), slow depolarizations representing very slow excitatory junction potentials (e.j.p.s) were recorded from the outer (innervated) muscle. However, e.j.p.s were not evoked from the inner (non-innervated) muscle at any rate of field stimulation. At low frequencies of stimulation (less than 5 Hz) no e.j.p. was observed in either the inner or outer muscle, although the muscle contracted.

5. Noradrenaline (10^{-6} M) depolarized inner but not outer muscle. High concentrations of noradrenaline (10^{-5} M to 2×10^{-4} M) caused large depolarization of the inner muscle, and also smaller depolarization of the outer muscle.

INTRODUCTION

In most large elastic arteries, including the rabbit common carotid (Rees, 1967), adrenergic nerves are confined to the adventitia and sometimes the outer part of the muscle layer, while the inner muscle is entirely free of nerves (see Keatinge & Harman, 1980; Vanhoutte, Verbeuren & Webb, 1981). Noradrenaline released by the nerves therefore reaches outer muscle in higher concentration than inner muscle (Bevan & Osher, 1970). Evidence that there is a difference in the mechanical response of the inner and outer smooth muscles to drugs was obtained first in the sheep carotid artery (Graham & Keatinge, 1972), and later in the coronary artery (Garland & Keatinge,

1982). Mekata & Nagatsu (1982) reported that there is a difference in electrical properties of inner and outer muscles in the thick-walled part of dog inferior vena cava, although not in the thin-walled part. Some difference in the properties of the inner and outer muscles seems to be a common feature in the thick-walled blood vessels. So far, systematic micro-electrode studies of the smooth muscle of large mammalian elastic arteries have been carried out only on the inner muscle, mainly because of difficulties in inserting micro-electrodes into outer muscle cells which are

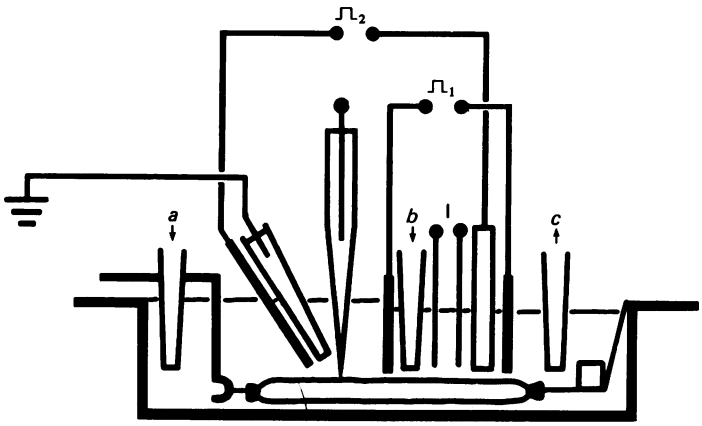

Fig. 1. Stimulating and recording arrangements. The chamber was irrigated with test solution from tube *a* to tube *c* (arrows indicate direction of the flow). Noradrenaline was applied from either tube *a* or tube *b*. External currents were passed into the muscle strip by means of Ag–AgCl plate electrodes. Relative intensity of externally applied current ($⎍_1$) was measured by a differential high input impedance amplifier (I). Short pulses ($⎍_2$) were applied via a second pair of electrodes to stimulate nerves.

protected by connective tissue (Mekata, 1971, 1974; Mekata & Niu, 1972). Thus the main aim of the present experiments was to investigate the electrical properties of both outer and inner muscle of the common carotid artery of the rabbit, and the responses of the muscles to application of noradrenaline and nerve stimulation. Furthermore, electrical transmission between smooth muscle cells in the directions along, around and through the vessel wall was investigated.

METHODS

Adult rabbits of either sex and weighing 3–4 kg were used. Muscle strips were removed from one carotid artery after exsanguination from another carotid artery. Strips about 20 mm in length and 2 mm in width were spirally cut from the artery and mounted in an organ bath. The strips were kept in modified Krebs solution at 36–37 °C for 2–3 h before the start of the experiment.

The preparation was pulled through holes in the stimulating plates which divided the organ bath into recording and stimulating compartments, as shown in Fig. 1. With only minor modifications, the organ bath was as used in our previous experiments (Mekata, 1981). Normal Krebs or test solutions were perfused from tube *a* to tube *c* (Fig. 1). In experiments for studying conduction of noradrenaline-induced slow potentials, solutions containing noradrenaline were injected through tube *b* into the stimulating compartment in addition to normal perfusion. The end of the preparation in the recording bath was attached by a fine thread to a strain gauge; the other end, in the stimulating bath, was fixed. Extracellular polarizing currents were applied by the partitioned chamber method, and electrotonic potentials were recorded as described by Mekata (1974). To assess

electrical properties of outer and inner muscles, membrane potentials were recorded by inserting a micro-electrode from adventitial and intimal sides of the vessel wall, respectively. To produce a neuronal effect on the smooth muscle, field stimulation was applied to the tissue through one Ag–AgCl needle electrode (0·5 mm in diameter) insulated to near the tip with Araldite (CIBA Ltd) and placed at 5 mm from the insulating plate in the recording bath, and another Ag–AgCl plate electrode (3 mm in width) placed between the two insulating plates. The position of an indifferent electrode was adjusted carefully to minimize the field stimulation artifact. Intensity of field stimulation was limited to 30 V to protect the micro-electrode amplifier. In order to minimize direct smooth muscle stimulation (see Mekata, 1981), short-duration and relatively low-frequency electrical pulses (0·3 ms and less than 25 Hz) were used.

The solutions from tube *a* and *b* flowed through the organ bath at rates of 4 and 2 ml/min respectively at a temperature of 36–37 °C, and were aerated with 97 % O_2 and 3 % CO_2. Modified Krebs solution of the following composition was used (mM): Na^+, 137·4; K^+, 5·9; Mg^{2+}, 1·2; Ca^{2+}, 2·5; Cl^-, 134·0; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; glucose, 11·5.

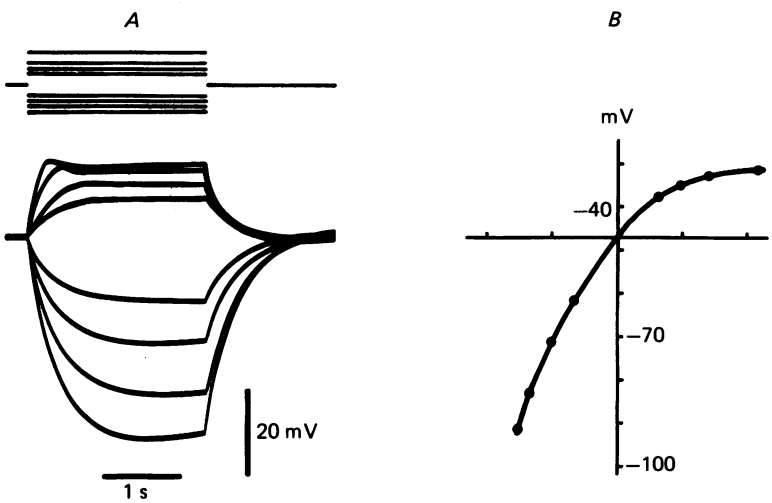

Fig. 2. Responses of the smooth muscle cell membrane of the outer layer of the rabbit carotid artery to external stimulation. *A*, intracellular records of electrotonic potentials (lower traces) produced by externally applied outward and inward currents of different intensities (upper traces). *B*, relation between externally applied current and intracellularly recorded potential. These were recorded at 0·3 mm from the stimulating partition. Depolarization is upward.

RESULTS

Responses of the outer and inner muscle to external current application, and electrotonic spread of current in three directions

When micro-electrodes were inserted into an artery strip from either its adventitial or intimal side, both outer and inner smooth muscle cells were found to be electrically quiescent, showing no spontaneous electrical activity. Mean values of membrane potential were $-45{\cdot}9 \pm 4{\cdot}3$ mV ($n = 88$) (mean ± standard deviation, number of penetrations) for outer muscle and $-47{\cdot}3 \pm 5{\cdot}4$ mV ($n = 73$) for inner muscle. There was no significant difference in response to direct current between the outer and inner muscle of these arteries. When outward pulses (1·5 ms duration) of increasing size were applied via the external electrodes, the membrane potential of both inner and outer muscle showed an initial hump followed by delayed rectification at the higher

intensities (Fig. 2). Increased intensities of stimulation enlarged the initial hump but this never grew to a spike. To application of inward current, the membrane behaved more like a constant electrical resistance, since the current–voltage relation was nearly linear. These properties are similar to those of other electrically quiescent vascular smooth muscles reported previously (inner muscle of rabbit aorta: Mekata, 1974; inner muscle of dog coronary artery: Mekata, 1980; outer muscle of guinea-pig pulmonary artery: Casteels, Kitamura, Kuriyama & Suzuki, 1977; outer and inner muscles of thin-walled part of dog inferior vena cava: Mekata & Nagatsu, 1982). The

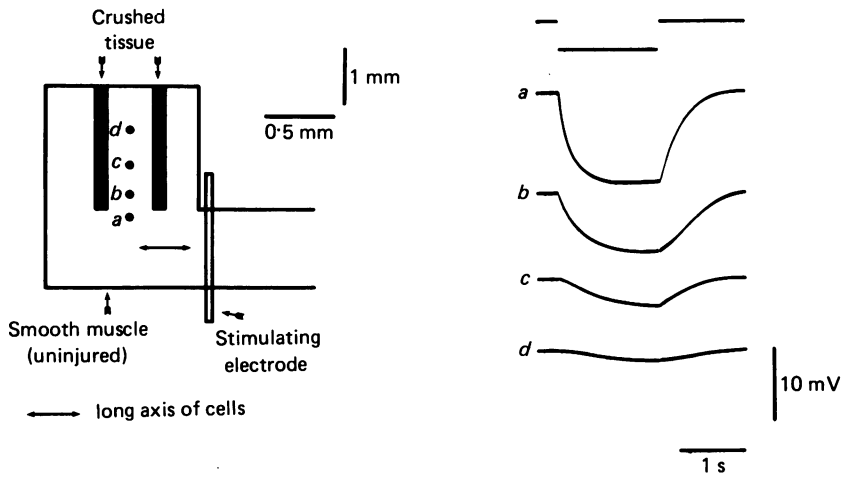

Fig. 3. Measurement of electrotonic spread of current transverse to the long axis of the cells. Electrotonic potentials evoked by the stimulating electrode were recorded by micro-electrodes from points *a*, *b*, *c* and *d*. Two parts of the tissue were crushed transversely to leave the bundle parallel to that in stimulating chamber. Points *b*, *c* and *d* are at 0·25, 0·70 and 1·35 mm from the border of the intact and damaged bundle.

space constant in the direction of the long axis of smooth muscle cells (around the vessel wall) was compared in inner and outer muscle by measuring with micro-electrodes the amplitude of electrotonic potential produced in cells at different distances from the stimulating partition used to apply hyperpolarizing current. A logarithmic relationship was observed between electrotonic potential and distance, in both inner and outer muscle. The space constant, calculated from the slope of this, was $0{\cdot}92 \pm 0{\cdot}32$ mm for outer muscle and $1{\cdot}61 \pm 0{\cdot}57$ mm for inner muscle (means $\pm$ S.D., $n = 8$).

There is qualitative evidence that current can spread electrotonically along the rabbit aorta to some extent, in the direction transverse to the long axis of the smooth muscle cells (Mekata, 1974). In order to measure the space constant for such transverse electrotonic spread in the carotid artery, two parts of the preparation (as shown in Fig. 3) were transversely crushed, at distances of about 0·3 and 0·7 mm from the stimulating partition. Electrotonic potentials evoked by external stimulation were recorded intracellularly both from cells in line with those exposed to the stimulation (point *a* in Fig. 3) and from those which electrotonic current could only

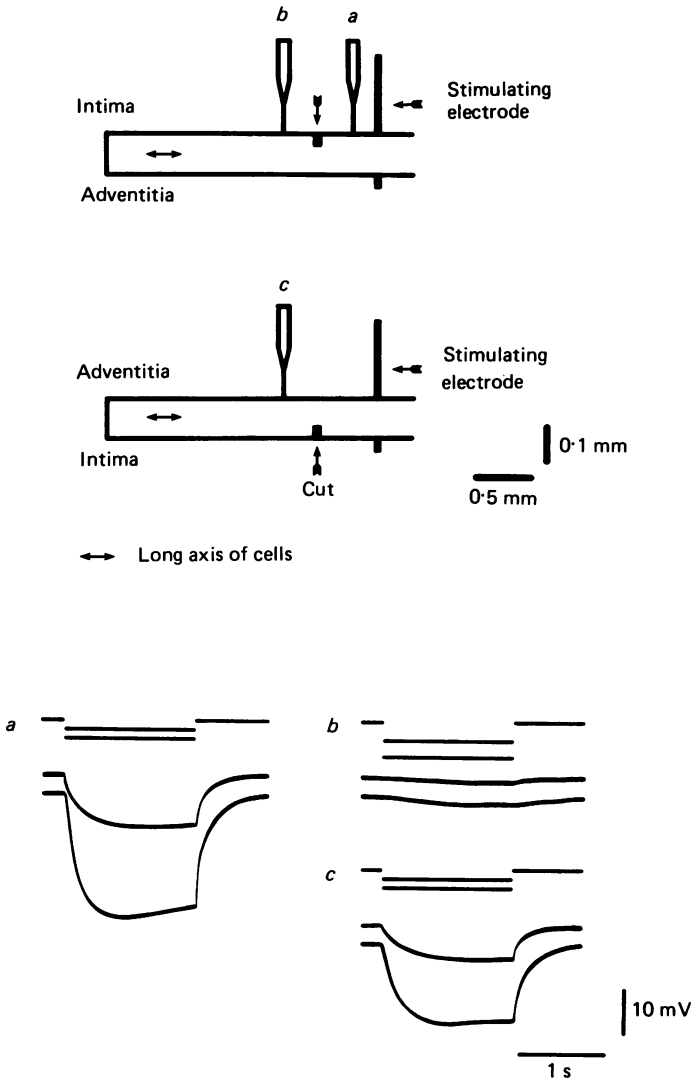

Fig. 4. Measurement of electrotonic spread of current in the direction from outer to inner muscle. The traces show that large electrotonic potentials evoked by the stimulating electrode were recorded at points *a* and *c*, but very little at point *b*, in which spread along the long axis of cells was prevented. Electrotonic current spread from outer to inner muscle was therefore slight or absent.

reach by transverse spread (points *b*, *c* and *d* in Fig. 3), all at a distance of 0·5 mm from the stimulating partition. When the external stimulating currents were applied, electrotonic potentials could be recorded from all four points, although their amplitudes decayed with distance from the border of the intact and crushed bundle (Fig. 3). The space constants calculated from this decay were 0·44, 0·58 and 0·62 mm in three preparations. When all of the tissue between the stimulating and recording electrodes was crushed, no electrotonic potential was ever recorded. It can be

concluded that intercellular electrical connexions exist in the direction transverse as well as longitudinal to the long axis of the cells.

In order to see whether electrotonic current can flow from outer to inner muscle cells, a preparation was made as shown in Fig. 4, in which a thin muscle layer on the intimal surface was transversely cut to a depth of approximately 30 μm, at a distance of about 0·5 mm from the stimulating partition. Before the cut was made, large electrotonic potentials of similar size, evoked by external stimulation, were recorded intracellularly from both intimal and adventitial surfaces 0·2 mm from the stimulating partition. Substantial though rather smaller electrotonic potentials were recorded at a distance of 0·8 mm from the stimulating electrode, both from outer and inner muscle. After the cut was made to separate the inner muscle 0·8 mm from the stimulating electrode from direct transmission of electrotonic current along the long axis of the cells, virtually no electrotonic hyperpolarization could be recorded from the inner muscle at that point, although Fig. 4 shows that large hyperpolarizations were still recorded from outer muscle opposite that point, and from inner muscle between the stimulating electrode and the cut. There was therefore little electrotonic spread of current from the outer to inner muscle. Similar results were obtained in ten further experiments of this kind; in eight of these no electrotonic hyperpolarization was recorded from inner muscle at 0·8 mm from the stimulating electrode after the cut was made. A very small hyperpolarization, like that in Fig. 4, was seen in two experiments. It may be concluded that electrical connexions between the outer side and the inner side of the artery are absent or very poorly developed.

Effects of nerve stimulation

Responses of outer muscle. In no preparations used for the present experiments were there any fluctuations in membrane potential in the absence of stimulation. Field stimulation to activate nerves, with a large single brief (0·3 ms) pulse by Π_2 shown in Fig. 1, caused no recordable electrical or mechanical responses. Trains of stimuli at higher frequencies (more than 20 Hz, 3–20 shocks and 0·3 ms pulse duration) evoked a slow membrane depolarization with a time-to-peak of some 0·5–2 s. The membrane potential then returned towards its resting value. Similar membrane potential changes, termed excitatory junction potentials (e.j.p.s), have been recorded from many muscular arteries or arterioles. The time course of those in the rabbit carotid were much slower than those recorded in most such blood vessels (Bell, 1969; Hirst, 1977; Holman & Surprenant, 1979; Cheung, 1982) but was similar to those of the coronary artery (Mekata, 1980). As in many other blood vessels, the amplitude and time-to-peak of the e.j.p. were graded with the number and frequency of stimulating pulses, presumably due to increasing concentrations of noradrenaline released from nerve terminals with increasing pulse trains. Examples of this are shown in Fig. 5. No action potentials like those which have been observed in many visceral and vascular smooth muscles were ever recorded. The initiation of an e.j.p. was invariably associated with contraction. Contraction was also seen in the absence of an e.j.p.; the outer muscles failed to respond electrically to trains of low-frequency stimuli (less than 5 Hz, 10–300 shocks and 0·3 ms pulse duration), but at frequencies as low as 1 Hz these stimuli caused a slowly developing increase in tension. Fig. 6 illustrates some of these results. They suggest that the threshold for a mechanical

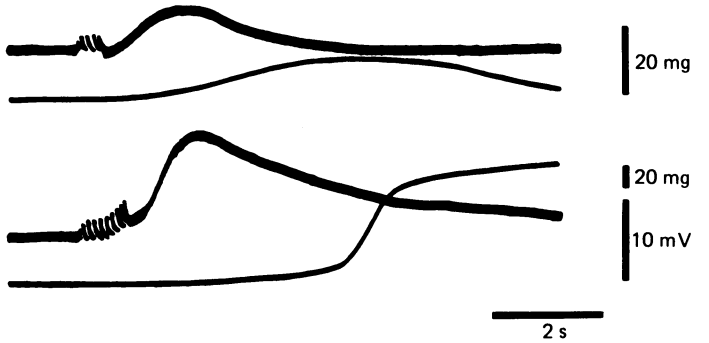

Fig. 5. Excitatory junction potentials (upper traces) recorded from the outer muscle of the carotid artery and tension (lower traces) during repetitive stimulation. Upper record, five shocks, 20 Hz; lower record, ten shocks, 20 Hz.

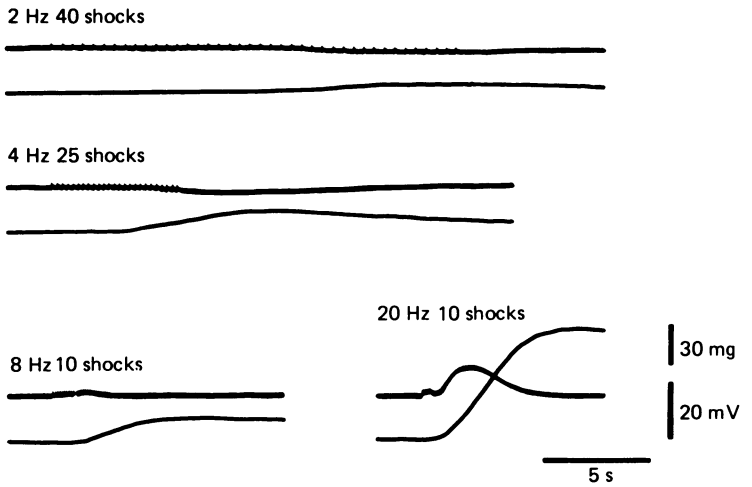

Fig. 6. Excitatory junction potentials (upper traces) in the outer muscle of a carotid artery and tension development (lower traces) in response to increasing frequencies of stimulation.

response to the transmitter is lower than the threshold for a measurable electrical response. Electrical (e.j.p.s) and mechanical responses evoked by field stimulation were blocked by tetrodotoxin at a concentration of 3×10^{-6} M (six experiments). Phentolamine (10^{-5} M) strongly depressed these responses (eight experiments).

Responses of inner muscle. Micro-electrode studies on inner smooth muscle revealed no detectable potential change during trains of repetitive stimulation (25 Hz, 10–30 shocks and 0·3 ms pulse duration, thirteen experiments) which were enough to evoke an e.j.p. from the outer muscle and large contraction of the muscle strip. Nor did lower frequencies evoke electrical responses from inner muscle.

Effects of noradrenaline on electrical properties of the outer and inner muscle

Noradrenaline at 10^{-6} M depolarized the membrane of inner muscle by 5–8 mV ($n = 4$) and at 10^{-5} M by 12–15 mV ($n = 6$). Repetitive slow waves of potential were

often superimposed on the depolarization (in two experiments out of four at 10^{-6} M and three of six at 10^{-5} M). Noradrenaline at both 10^{-6} and 10^{-5} M produced strong contraction. These results were similar to the response of the carotid artery to adrenaline reported previously by Mekata & Niu (1972). However, these concentrations of noradrenaline (10^{-6} and 10^{-5} M) did not significantly modify the membrane potential, membrane resistance or current–voltage curve of outer muscle. Higher concentrations of noradrenaline (5×10^{-5} and 2×10^{-4} M) did elicit depolarizations, of about 4–8 mV ($n = 6$) and 6–12 mV ($n = 10$) respectively, of the outer muscle. No fluctuations of the membrane potential were recorded from the outer muscle during these responses (sixteen experiments) although inner muscle often produced these. This gives further evidence of poor electrical connexion between outer and inner muscle cells. Electrical and mechanical responses of outer and inner muscles to noradrenaline were prevented by phentolamine at ten times the concentration of noradrenaline applied. Fig. 7 gives some of these results.

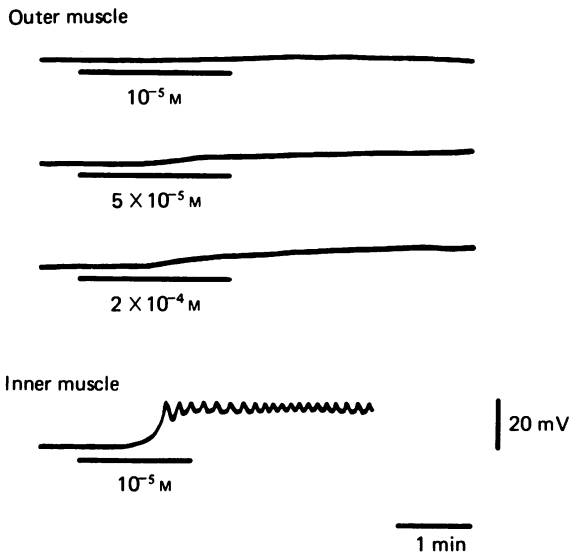

Fig. 7. Electrical responses of the outer and inner muscle to noradrenaline. The drug was applied from tube *a* shown in Fig. 1. Bars under membrane potential recordings indicate the period of noradrenaline applications.

DISCUSSION

The most interesting findings of the present studies were that the outer muscle of the carotid artery was much less sensitive than inner muscle in giving an electrical response to noradrenaline, paralleling the difference in mechanical sensitivity of inner and outer muscle reported in other arteries (Graham & Keatinge, 1972; Garland & Keatinge, 1982). Hirst & Neild (1981) obtained evidence that electrical responses of the guinea-pig mesenteric artery were mediated only by high-threshold receptors present only at neuromuscular junctions. The present findings show that electrical responses of the rabbit carotid artery to noradrenaline have low threshold in inner

muscle, far from neuromuscular junctions, and high threshold in the outer muscle, near neuromuscular junctions.

There have been varied reports about the electrical effects of noradrenaline and nerve activity on other vascular smooth muscles. Some negative reports may simply reflect technical problems, but it seems clear that considerable variability exists between different arteries. Some intracellular records, made largely by micro-electrodes inserted from the outer surface of the vessel – for example on rabbit pulmonary arteries (Su, Bevan & Ursillo, 1964) and rabbit saphenous arteries (Holman & Surprenant, 1979) – have recorded no electrical changes of any kind during contraction induced by high concentrations of noradrenaline. At the other extreme, rat aorta developed electrical discharges and depolarization in response to even a low concentration of noradrenaline (6×10^{-9} M) (Biamino & Krukenberg, 1969). Other studies, based on micro-electrode records from inner muscle or on sucrose-gap records, revealed little or no depolarization with low concentrations of noradrenaline, but clear depolarization with higher concentrations (Mekata & Niu, 1972; Mekata, 1976; Keatinge & Harman, 1980).

Stimulation of adrenergic nerves to small arteries and arterioles often produces e.j.p.s which may summate to trigger action potentials (e.g. Bell, 1969; Holman & Surprenant, 1979; Surprenant, 1980; Mekata, 1980; Hirst & Neild, 1980; Cheung, 1982). In large arteries, which tend to have wider neuromuscular gaps, e.j.p.s and action potentials have not been reported before in micro-electrode studies, although sucrose-gap studies on the sheep carotid artery showed that activation of sympathetic nerves could produce irregular spike discharges (Keatinge, 1966). The present experiments suggest that the low electrical sensitivity of the innervated outer muscle to noradrenaline is a factor reducing the ability of nerves to induce e.j.p.s in large arteries; repetitive stimulation of the nerves was required to produce e.j.p.s in these rabbit carotid arteries.

Some of the low responsiveness to noradrenaline of the outer part of the carotid artery in these experiments may be due to uptake of noradrenaline by sympathetic nerve terminals in the outer part of the vessel, but this is not likely to be a major factor during prolonged application of noradrenaline, as was used in these experiments. Also, more noradrenaline may be released from nerves *in vivo* and may produce larger electrical responses than seen in these isolated preparations; adrenergic nerves in pig uterine artery can produce a brief, local, post-junctional concentration of 4×10^{-4} M noradrenaline (Bell & Vogt, 1971), enough to produce large electrical depolarization in our experiments. In addition, the present experiments strengthen the view that much of the response of such arteries to nerves is brought about by low concentrations of noradrenaline diffusing to stimulate distant cells by non-electrical means (cf. Keatinge & Torrie, 1976). Our electrophysiological findings suggest that diffusion of transmitter provides the only obvious means by which the nerves in the outer part of the vessel wall might induce contraction in the inner part of the vessel wall, since electrical transmission from the outer part to the inner part was slight or absent.

In dog inferior vena cava, the space constants of smooth muscle of the infrarenal segment and the segment between the liver and renal veins, in which noradrenergic nerve terminals were widely distributed, were small compared with those of the smooth muscle of the non-innervated supradiaphragmatic segments (Mekata &

Nagatsu, 1982). The low space constant found also for innervated outer muscle in the present study suggests that poor cell-to-cell conduction may possibly be induced by prolonged influence of noradrenaline released from nerves.

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