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# SYMPATHETIC INNERVATION AND EXCITABILITY OF ARTERIOLES ORIGINATING FROM THE RAT MIDDLE CEREBRAL ARTERY

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SUMMARY

1. The densities of the adrenergic innervation of the internal carotid and middle cerebral arteries and their extracerebral branches have been determined using fluorescence histochemistry.

2. The density of the nerve plexus on the internal carotid artery was greater than that of the middle cerebral artery. The density of the plexus on the middle cerebral artery decreased with increasing distance from its origin.

3. The density and the peripheral extent of the nerve fibre plexus on the arterioles arising from the carotid artery were greater than those arising from the middle cerebral artery.

4. On any arteriole the density of innervation decreased with increasing distance from its origin.

5. The passive electrical properties of proximal and distal middle cerebral arteriolar segments were compared. Both proximal and distal arteriolar segments had similar resistances and time constants in the order of 100 MΩ and 250 ms respectively.

6. Small regenerative responses could be elicited in all proximal middle cerebral arteriolar segments but only in a proportion of corresponding distal segments.

7. The addition of external tetraethylammonium ions (TEA) provided much larger regenerative responses. Action potentials in proximal middle cerebral arteriolar segments had larger peak amplitudes and faster rise times than those of corresponding distal segments.

8. Distal carotid arteriolar segments had similar voltage-dependent excitability as proximal segments of middle cerebral arterioles but generated less inward current for a given voltage step.

9. There was a direct correlation between the density of innervation and the voltage-dependent excitability of arteriolar smooth muscle cells. The possibility that the presence of nerves is correlated with the density of calcium channels is discussed.

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## INTRODUCTION

Most arteries and arterioles have been shown by fluorescence microscopy to be innervated by nerves which contain catecholamines (Norberg & Hamberger, 1964). The major pial arteries have a dense network of fluorescent fibres (Falck, Mchedlishvili & Owman, 1965; Nielson & Owman, 1967) which originate in the superior cervical ganglion (Falck *et al.* 1965). In most vascular beds, the sympathetic nerves, when stimulated, cause vasoconstriction (Folkow & Neil, 1971). Electrophysiological recordings have indicated that such stimuli evoke excitatory junction potentials (e.j.p.s), these in turn initiate action potentials or local responses (Hirst, 1977; Holman & Suprenant, 1979). Arterial constriction appears to result not directly from the evoked junction potentials but rather from the membrane-potential-dependent conductance changes activated by the consequential membrane depolarization (Hirst, 1977; Holman & Suprenant, 1979).

While some cerebral arterioles possess a few non-myelinated fibres around them as they progress into neural tissue (Sato, 1966), others are devoid of nerve fibres (Samarasinghe, 1965). In this report, experiments are described which were designed to examine the differences between arterioles which possessed a catecholamine containing innervation and those which lacked such an innervation. It has been shown that the smooth muscle cells of cerebral arterioles which receive a sympathetic innervation generate, in response to membrane depolarization, two distinct calcium-dependent inward currents (Hirst, Silverberg & van Helden, 1986). The observations reported in this paper indicate that as the density of catecholamine-containing innervation received by cerebral arterioles falls, so does their ability to generate inward current in response to membrane depolarization.

## METHODS

Anatomical analysis of the innervation of the blood vessels arising from both the carotid and the middle cerebral arteries was carried out on thirteen preparations. These were dissected and stretched in the same way as those prepared for physiological recordings (Hirst *et al.* 1986). Arteries used for the physiological experiments were not included in the data, as preliminary studies showed that the nerve fibres were sometimes damaged and depleted of catecholamines by the razor cuts made in isolating short segments of vessels and so statements about the presence or absence of nerve fibres could not be made with confidence.

The stretched preparations for histology were fixed for 3 h in 0·5% (v/v) glutaraldehyde and 4% (w/v) formaldehyde in phosphate buffer pH 7·0 at 4 °C (Furness, Costa & Wilson, 1977), dried over phosphorous pentoxide, mounted in paraffin oil and viewed with a Zeiss IM35 microscope fitted with a 50 W mercury burner, bandpass filter 390–420 nm, dichroic mirror FT 425 nm and barrier filter 450 nm. The following measurements were made for each preparation: the distance of the origins of the branches off the middle cerebral artery from the junction of the middle cerebral with the internal carotid artery, the diameters at the origins of all the branches from both the carotid and the middle cerebral arteries and the extent of the nerve plexus along each branch. Each branch was photographed from its origin to the furthest extent of its innervation. From these montages, estimates of nerve fibre density over the surface of the vessels were made by measuring the intercepts of nerve fibres with a square grid over successive lengths of 400 $\mu$m to the peripheral limits of the nerve plexus (smallest grid size $20 \times 20$ $\mu$m; Hill, Hirst & van Helden, 1983). Nerve densities were calculated by multiplying the frequency of fibres across the vessel by the frequency of fibres along the vessel and expressed as the number of fibres/400 $\mu m^2$. Nerve densities were calculated over successive lengths of 400 $\mu$m up to 2 mm along all branches.

In the majority of experiments, recordings were made from segments of arterioles originating from the middle cerebral artery of rats. In a few experiments, segments of arteriole originating from the internal carotid were used. Special care was taken in this series of experiments to ensure that the arterioles were dissected from the surface of the brain until their point of entry into neural tissue.

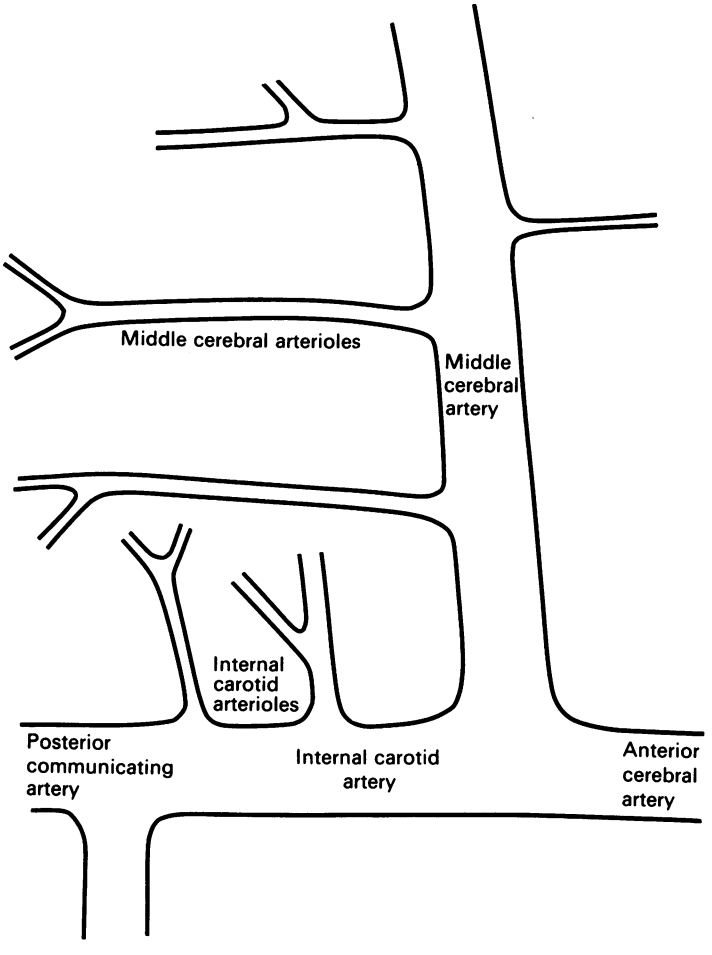


Fig. 1. Diagram of relations between the internal carotid artery, the middle cerebral artery and their arterioles in a typical preparation.

This meant that on most occasions arterioles of length 2–3 mm from their point of origination on their parent arteries were obtained. A schematic diagram of the preparation is shown in Fig. 1. Segments of arteriole were isolated using a fragment of razor blade; segments were cut to have lengths of 120–220 $\mu$m. The cutting procedure was carried out along the entire length of the arteriole. Segments were arbitrarily classified as proximal or distal if they were less than or more than 1 mm respectively from their parent arteries. Most proximal segments were less than 0·5 mm from the point of origination of the arteriole; most distal segments were more than 1·8 mm from the point of arteriolar origination.

Recording procedures and experimental conditions were as described previously (Hirst *et al.* 1986). In some experiments tetrodotoxin (Sigma) and tetraethylammonium chloride (Sigma) were used.

RESULTS

*General observations*

After pinning the preparations in the recording chamber, a few (seven from thirty-three) displayed myogenic activity which persisted for some 20–30 min before the arterioles became quiescent. The preparations then remained quiescent for the entire recording period (3–4 h) unless exposed to solutions containing tetraethyl-

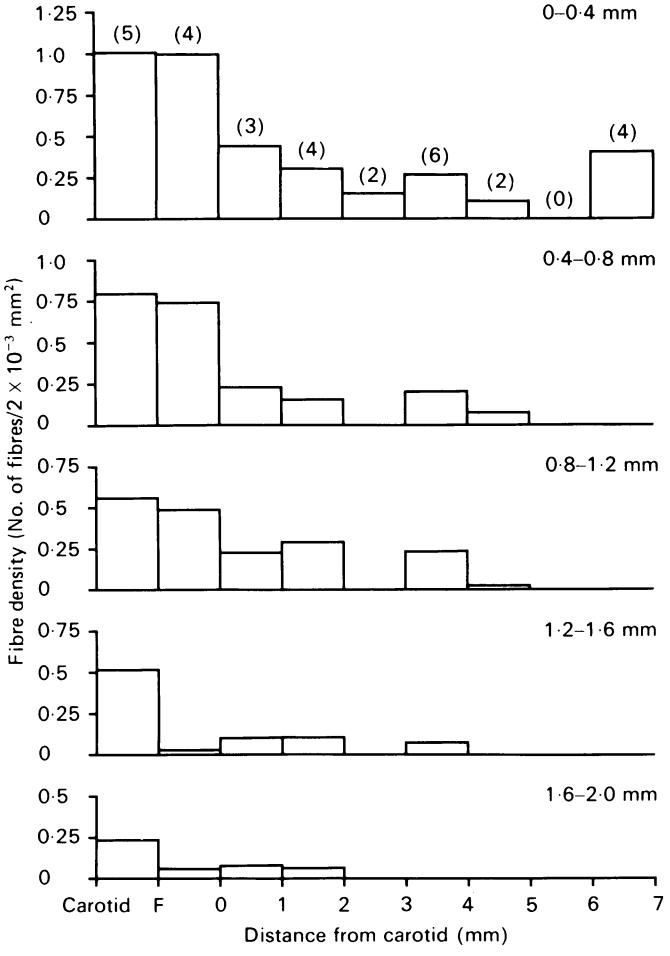


Fig. 2. Density of noradrenergic nerves along small arteries and arterioles from carotid artery (left column), from fenestration between carotid and middle cerebral arteries (F) and from middle cerebral artery at increasing distances from the carotid, cerebral artery junction (right columns). The figures at the top right of each histogram give the distance along the fine arteries from their points of origination on the major arteries. The fibre density is expressed as the number of fibres crossing a 400 $\mu m^2$ grid.

ammonium ions (TEA). A variable number of arterioles arose from both the internal and the middle cerebral arteries. The most common arrangement was that two arterioles arose from the internal carotid and three arose from the middle cerebral artery (Fig. 1). Occasionally a fenestration between the internal carotid and middle

cerebral arteries existed; this fenestration usually gave rise to one or two arterioles. Both the internal carotid and the middle cerebral arteries had similar diameters (range 270–390 $\mu$m) with wall thicknesses in the range 10–15 $\mu$m. The arterioles which originated from these arteries had diameters in the range 40–150 $\mu$m with wall thicknesses of 5–7 $\mu$m. The arterioles frequently branched at varying distances from their points of origination; this was accompanied with a reduction in external diameter but with little change in wall thickness. Intracellular recordings were made from preparations after they had been cut into segments, recordings being made from proximal segments ($<$ 1 mm from point of origination of arteriole) and from distal segments ($>$ 1 mm from origination).

*Density of fluorescent innervation to arterioles arising from carotid and middle cerebral arterioles of the rat*

As has been pointed out, the position and number of branches arising from both the internal carotid and the middle cerebral arteries varied from animal to animal. There was even variation between the two sides of any one animal. For this reason it was necessary to pool measurements from branches into groups according to the distance of their origin from the internal carotid artery rather than according to their positional number along the middle cerebral artery. Branches arising from fenestrations between the internal carotid and middle cerebral arteries formed a separate group as the density of innervation of such fenestrated vessels was less than that of either the carotid or the middle cerebral arteries.

The degree of innervation of the branches off the internal carotid and the middle cerebral arteries also varied from preparation to preparation. However, within any one preparation certain observations were consistent. The density of the nerve plexus on the internal carotid artery was greater than that on the middle cerebral artery (Pl. 1 *A* and *B*). Furthermore, the density of the innervation on the middle cerebral artery itself decreased with increasing distance from the internal carotid artery (Pl. 1 *C* and *D*), so that there were fewer fibres at the origin of peripheral, compared to proximal, branches. Both the density and the peripheral extent of the nerve fibre plexus on the branches arising from the carotid artery were greater than on those arising from the middle cerebral artery (Fig. 2; Pl. 2). Furthermore, for those branches arising from the middle cerebral artery, the plexuses were less dense and did not extend as far along the vessels as the distance of their origin along the middle cerebral artery increased from the carotid artery (Pl. 2). When the diameters of the various branches at their origins were plotted according to their position along the middle cerebral artery, it was found that there was a tendency for more peripheral vessels to be smaller. On any one branch, irrespective of its location, the density of the innervation decreased with increasing distance from the carotid or middle cerebral arteries (Fig. 2; Pl. 2). Frequently, on the most distal branches off the middle cerebral artery, there was only one fibre which ran along the vessel for a variable distance. In other preparations such a distal vessel was only contacted once or a couple of times by a fibre which ran nearby. These results suggest that the degree of innervation of the side branches emanating from the internal carotid and the middle cerebral arteries depends on both the density of the plexus on the parent blood vessel at the site of origin of the branch and also on the diameter of the branch itself. The results also indicate that invariably segments of arteriole within 1 mm of their

origination will receive an innervation, albeit scanty. Moreover, the likelihood that the arterioles which originate from the middle cerebral artery will receive an innervation at distances greater than 1 mm is slight. In contrast distal segments of arteriole originating from the carotid artery will have an innervation density similar to that of proximal segments of arterioles originating from the middle cerebral arteries.

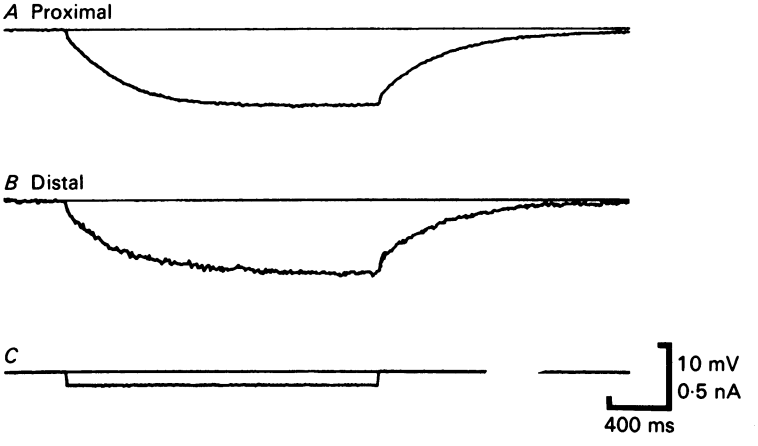


Fig. 3. Voltage response of a proximal and distal segment of middle cerebral arteriole to a current step. *A*, proximal response (average of twenty repeats; segment length 150 $\mu$m; diameter 75 $\mu$m) to hyperpolarizing current shown in *C*. *B*, distal response (average of ten repeats; segment length 200 $\mu$m; diameter 50 $\mu$m) to the same current.

*Passive membrane properties of proximal and distal segments of arterioles originating from rat middle cerebral artery*

Successful impalements of segments of arteriole of either location were associated with a negative voltage deflexion of about 40–50 mV. Over the next few minutes this resting membrane potential increased to lie in the range $-55$ to $-70$ mV. No differences between the final resting membrane potentials of segments of either location were detected. After stabilization of the resting membrane potential reading, the membrane potentials of all segments were adjusted to $-60$ mV by passing the appropriate steady current through the recording electrode. This same potential ($-60$ mV) was used as the holding potential in the voltage-clamp studies.

When hyperpolarizing current pulses were passed through the recording electrode (single-electrode, current-clamp mode) electrotonic potentials were recorded. With segments of either location, the electrotonic potentials had slow time courses, the onsets and offsets of which could be described by single exponentials (see Fig. 1, Hirst *et al.* 1986). Examples of electrotonic potentials from two segments, one proximal (Fig. 3*A*) and the other distal (Fig. 3*B*) are shown. It can be seen that their time courses are similar and that for a given current similar steady-state membrane potential changes occurred. This indicates that both the input resistances and time constants of segments of either location are similar. The mean values of input resistances for proximal and distal segments were not significantly different being $102 \pm 7$ MΩ (mean ± S.E. of mean, $n = 23$) and $120 \pm 15$ MΩ ($n = 9$) respectively; the

corresponding values of time constants were $265 \pm 15$ ms ($n = 23$) and $240 \pm 44$ ms ($n = 9$). On average the proximal segments were not different in length but had diameters $32 \pm 4\%$ ($n = 9$) greater than the distal segment. Therefore the distal segments would be expected to have about 30% higher input resistances. Unlike the input resistance, the segment time constant is independent of area. The lack of difference between proximal and distal membrane time constants suggests that the passive properties of the two locations are the same.

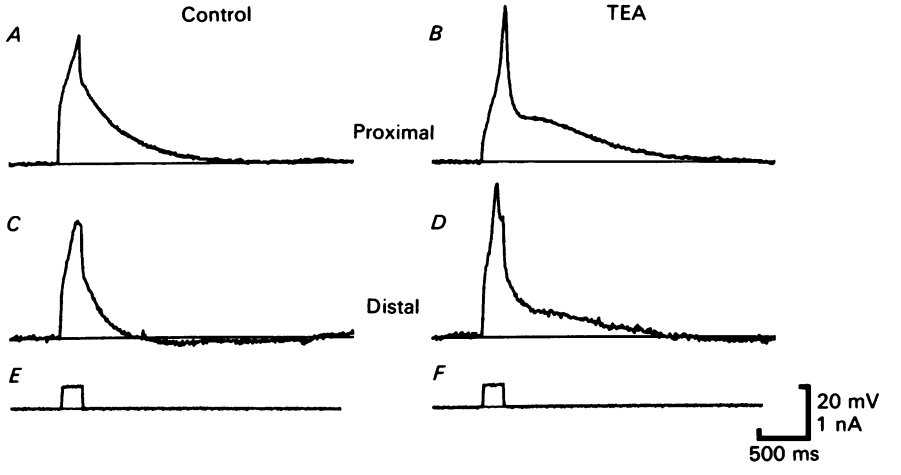


Fig. 4. Comparison of regenerative responses in a proximal and distal segment of the same middle cerebral artery. Voltage responses to depolarizing current (*E* and *F*) are presented in control solution and in the same solution with 10 mM-TEA present for the proximal segment (*A* and *B* respectively) and for the distal segment (*C* and *D* respectively).

*Action potentials recorded from proximal and distal segments of arteriole*

When depolarizing current pulses of appropriate intensity were passed through the recording electrode, segments of arteriole which lay close to their points of origination from the middle cerebral artery, initiated regenerative responses and constricted (see Hirst *et al.* 1986). In contrast, many distal segments failed to initiate detectable regenerative responses and failed to constrict in response to membrane depolarization (up to 60 mV from rest). In other preparations distal segments generated small amplitude regenerative membrane potential changes in response to membrane depolarization. An example is shown in Fig. 4 where recordings were made from proximal and distal segments of the same arteriole. With the recording from the proximal segment, a membrane depolarization of about 30 mV initiated a local response of about 15 mV amplitude (Fig. 4*A*). In the distal segment the peak local response was smaller and broader during application of depolarizing current; the duration of the regenerative response which followed the depolarizing current was also less (Fig. 4*C*). Such differences would occur either if the properties or numbers of voltage-dependent inward current channels or, if the properties of voltage-dependent outward current channels differed in the two segments. TEA ions have

been shown to largely suppress voltage-dependent potassium rectification in rat cerebral arterioles (Hirst *et al.* 1986). When TEA (10 mM) was added to the perfusion fluid, the amplitudes of the regenerative responses evoked by the depolarization in either segment increased; regeneration responses of varying size could now be recorded in all distal segments. However, as shown in Fig. 4, the effect was no more marked in distal segments than in proximal segments. In TEA the amplitudes of

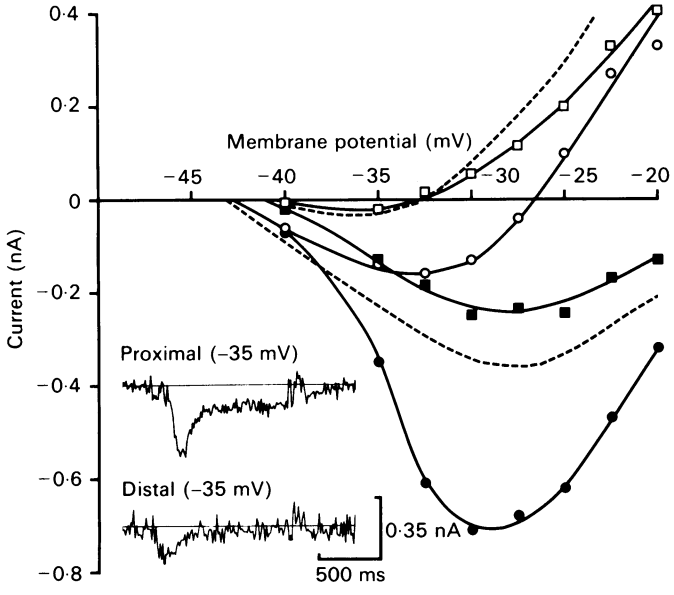


Fig. 5. Comparison between peak and plateau inward currents recorded from a proximal (circles) and a distal (squares) segment from the same middle cerebral arteriole. Curves have been fitted by eye. The dashed curves are the fits to the peak and plateau currents obtained in the distal segment but scaled by a factor of 1·5 to allow for differences in segment dimensions where the length and diameter were 190 $\mu$m and 90 $\mu$m respectively for the proximal segment compared to 170 $\mu$m and 70 $\mu$m respectively for the distal segment; both segments had similar wall thicknesses ($\approx 5$ $\mu$m). The distance from the parent middle cerebral artery was 0·8 mm and 3 mm respectively for the proximal and distal segments. Current records obtained for a depolarization to −35 mV (holding potential −60 mV) for the proximal (upper) and distal segments are included as insets.

action potentials of the proximal segments had peaks of $56 \pm 3$ mV ($n = 10$) and those of the distal segments had peak amplitudes of $46 \pm 4$ mV ($n = 5$) respectively. Moreover, the maximum rates of rise of the action potentials in the proximal segments were faster than those of the distal segments with values of $1{\cdot}4 \pm 0{\cdot}2$ V/s ($n = 10$) and $0{\cdot}4 \pm 0{\cdot}1$ V/s ($n = 5$) respectively. The after-depolarization observed for action potentials recorded in the presence of TEA was also consistently larger in proximal than in distal segments (see Fig. 4). The amplitude of the after depolarization recorded with 10 mM-TEA present was $13 \pm 2$ mV ($n = 10$) in proximal segments and $4 \pm 1$ mV ($n = 5$) in distal segments (all values expressed as mean $\pm 1$ S.E. of mean).

When similar experiments were carried out on distal segments of arteriole which originated from the internal carotid artery, i.e. an adjacent distal arteriole but one which received an innervation of similar density to proximal segments of middle

cerebral arteriole (Fig. 2), action potentials were readily initiated in TEA-containing solutions. In three experiments the maximum amplitude of the action potential recorded in TEA from an arteriole branch of the internal carotid (each more than 1·5 mm distal from the point of origination) was $61 \pm 6$ mV ($n = 3$) and the maximum rate of rise was $1{\cdot}1 \pm 0{\cdot}3$ V/s ($n = 3$). These values are very similar to those of proximal segments of the middle cerebral arteriole. Thus it would appear that the reduced ability of distal segments of arterioles originating from the middle cerebral artery to generate action potentials is more closely linked with the reduction in sympathetic innervation which occurs naturally along these branches than to their distal location *per se*.

*Membrane currents recorded from proximal and distal segments of arteriole originating from the rat middle cerebral artery*

Proximal segments of middle cerebral arterioles generate two calcium-dependent inward currents in response to membrane depolarization. There is a low-threshold sustained inward current and a higher-threshold, time-inactivating inward current (Hirst *et al.* 1986). When membrane currents were recorded from paired distal and proximal segments of the same arteriole (10 mM-TEA present) both currents could be detected in segments of either location (see insets, Fig. 5). However, the currents recorded from the distal segments were consistently smaller than those recorded from the proximal segments at any given level of membrane depolarization. Both currents were masked at similar levels of depolarization by the onset of a TEA resistant rectifier (Hirst *et al.* 1986). In the experiment shown in Fig. 5, the amplitudes of the plateau currents and the peak currents for each segment are plotted as a function of the membrane potential at which the currents were measured. Comparison of the proximal current amplitudes to the distal current amplitudes (standardized to allow for differences in segment dimensions) demonstrates that both inward currents are substantially larger in the proximal segment. Inward currents in either segment were abolished by substituting cobalt for calcium in the extracellular fluid.

These observations would suggest that the properties of the calcium channels in either segment had similar voltage sensitivities. Evidently, either the conductances of the channels activated vary with location or, alternatively, the number of channels per unit area of membrane is reduced at distal locations.

## DISCUSSION

The histological studies on the innervation of arterioles which originate from the rat middle cerebral artery have indicated that such arterioles receive only a scanty catecholamine-containing innervation (Fig. 2, Pl. 2). These results are consistent with the ultrastructural study of Sato (1966) who described that central branches of the middle cerebral artery of the rat possessed only a few non-myelinated fibres around them. Fibres described in the latter study would include cholinergic nerves which also innervate pial arteries (Florence & Bevan, 1979; Duckles, 1981); about 50% of the axons on the anterior cerebral artery remain after removal of the superior cervical ganglion (Iwayama, Furness & Burnstock, 1970). Close to their origination from the parent artery most arterioles were innervated by more than two fibres. At more distal

locations however, the likelihood of these arterioles even receiving innervation from a single catecholamine-containing fibre is slight. This fall in density of innervation along individual arterioles was not correlated with a change in arteriole diameter; indeed in many preparations branchings along the first few millimetres of arteriole were not detected. In contrast to the arterioles which originated from the middle cerebral artery, those which originated from the more densely innervated carotid artery had detectable fluorescent nerve fibres along their length, at least until they entered neural tissue. Non-myelinated axons around branches from the carotid artery have also been observed even after the branches have penetrated the brain (Sato, 1966).

The gradient of innervation density along 'middle cerebral arterioles' was not associated with systematic differences in the passive properties of the arteriolar smooth muscle cells. Segments of arteriole from proximal (innervated) and distal (non-innervated) locations had similar resting potentials and membrane time constants. In contrast the ability to produce regenerative responses during direct membrane depolarizations did vary with location. Segments of a more distal location consistently generated a smaller, slower regenerative response than did those of a proximal location. These differences were still detectable, and even exaggerated in the presence of TEA, suggesting that they did not result from variations in the ability of the segments to exhibit delayed rectification. When the membrane currents generated by proximal and distal segments of arteriole were measured using a voltage-clamp technique (Finkel, Hirst & van Helden, 1984; Hirst *et al.* 1986), inward currents were obtained over similar membrane potential ranges. Yet those obtained at any given depolarized potential from a distal segment had smaller amplitudes than those from the corresponding proximal segment. Evidently the ability to support voltage-dependent inward currents varies along those arteriolar branches. In the previous report evidence was given which suggested that such inward currents are carried by calcium ions (Hirst *et al.* 1986).

Distal segments of the innervated arterioles which originated from the internal carotid artery were however able to generate local responses similar to those of proximal segments of 'middle cerebral arterioles'. This would suggest that the change in ability of middle cerebral arterioles to support inward calcium movements was not simply related to distance from the parent artery. Rather it would appear that change in excitability was correlated with changes in the density of fluorescent innervation. It may be of interest to note that in a similar voltage-clamp study of calcium currents in arterioles of ileal submucosa, which receive a denser sympathetic innervation than any of the cerebral arterioles studied here, the intensities of the calcium currents detected during smaller voltage-clamp steps were greater than any detected in cerebral arterioles (G. D. S. Hirst & D. F. van Helden, unpublished observations).

Thus the suggestion is made that in some way the density of sympathetic innervation to an arteriole in part determines the ability of that arteriole to generate inward calcium movements. Quite clearly this does not necessarily imply that the presumptive sympathetic transmitter, noradrenaline, is responsible for this phenomenon. Sympathetic nerves are known to contain a number of peptides, some of which, for example neuropeptide Y, appear to be restricted in some species to nerve fibres innervating arterial smooth muscle (Furness, Costa, Emson, Häkanson, Moghimzadeh,

Seendler, Taylor & Chance, 1983). Such peptides may have a more regulatory role on, for example, calcium-channel density than a more conventional transmitter role. Alternatively, of course, sympathetic nerves may secrete an as yet unknown trophic agent.

The observations given in this report also suggest that arteriole tone in distal 'middle cerebral arterioles' is unlikely to be under the influence of membrane potential changes. This may be in accord with the view that the finer branches of cerebral vasculature have their control exerted by local metabolic products (Folkow & Neil, 1971).

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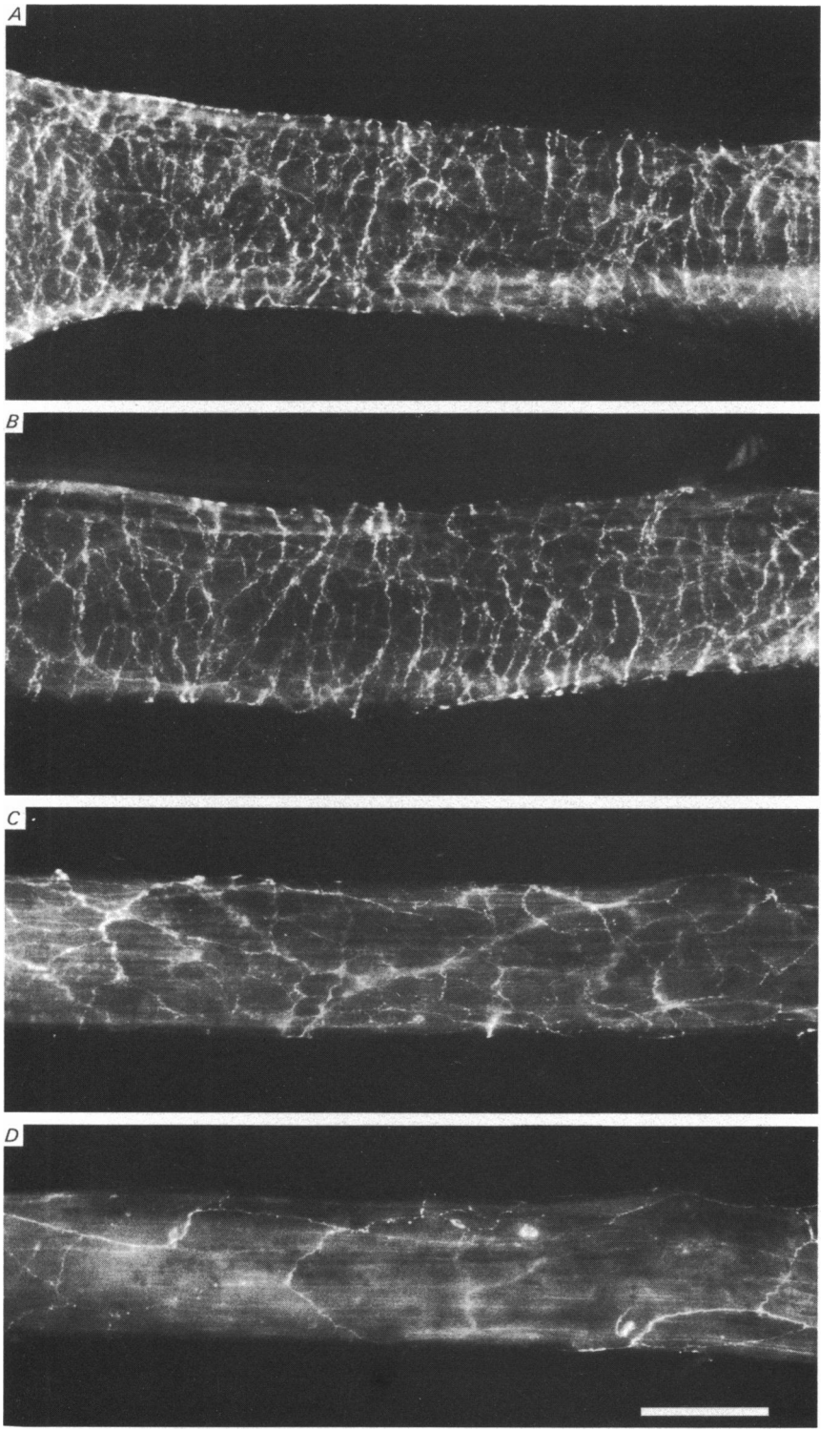
## EXPLANATION OF PLATES

### PLATE 1

Fluorescence photomicrographs of the internal carotid artery (*A*) and the middle cerebral artery (*B–D*) near its origin from the internal carotid (*B*) and at 6 mm (*C*) and 8 mm (*D*) from its origin. Note that the density of the nerve plexus is greater on the internal carotid than on the middle cerebral and on the latter artery, decreases in density with increasing distance from the carotid. Bar represents 100 $\mu$m.

### PLATE 2

Fluorescence histochemistry of one branch from the carotid (*A–C*) and two branches from the middle cerebral arteries (*D–F* and *G–I*) photographed at their origins (*A*, *D*, *G*, respectively), and 1 mm (*B*, *E*, *H*) and 2 mm (*C*, *F*, *I*) from their origins. The origin of the branch shown in *D–F* was located 1·8 mm from the internal carotid and the origin of the branch shown in *G–I* was 6·9 mm from the internal carotid artery. Bar represents 100 $\mu$m.



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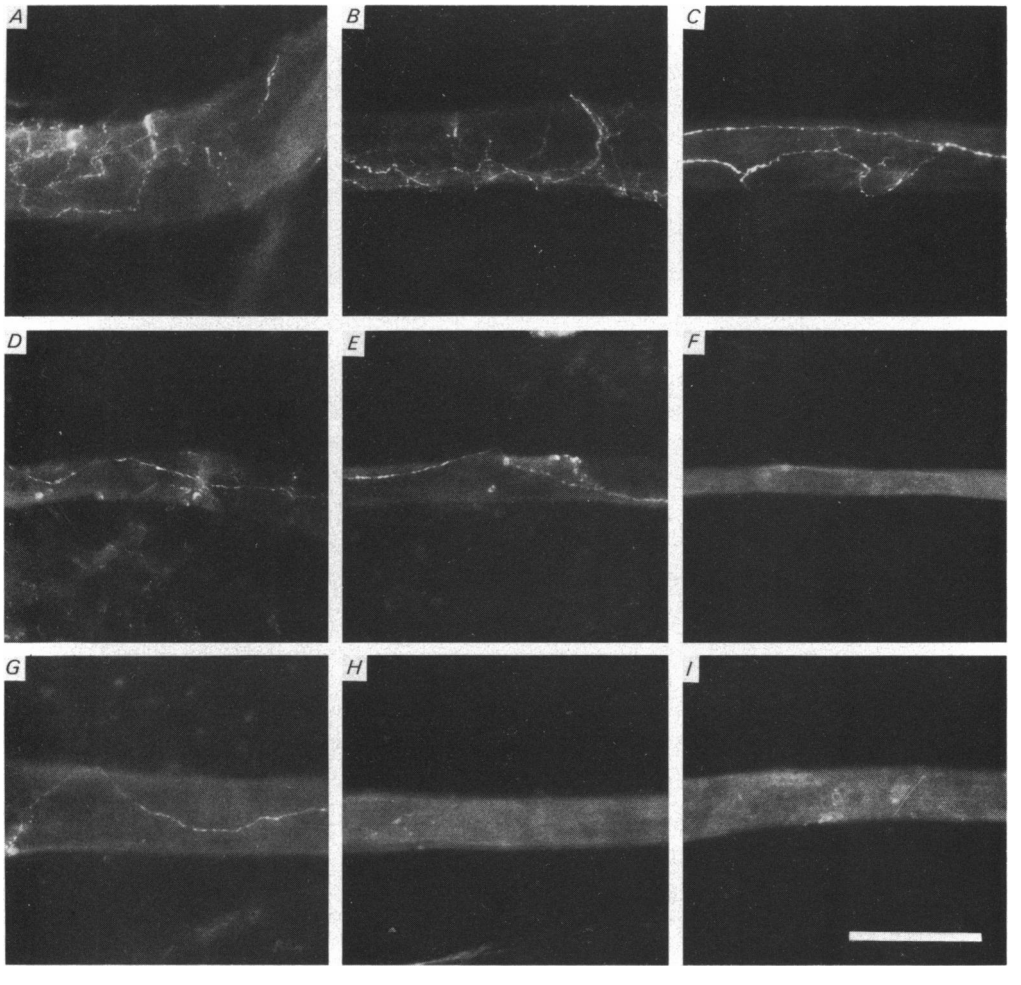
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