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ACTION OF SYMPATHETIC NERVES ON INNER AND OUTER MUSCLE OF SHEEP CAROTID ARTERY, AND EFFECT OF PRESSURE ON NERVE DISTRIBUTION

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SUMMARY

1. The direction of torsion produced during active shortening of helical strips of sheep carotid arteries was measured to assess whether inner or outer muscle was contracting.

2. Noradrenaline contracted inner (non-innervated) muscle in lower concentrations than were needed to contract outer (innervated) muscle, even with desipramine present to prevent uptake of noradrenaline by the nerves and with enough cyanide present to raise the normally low O_2 tension of inner muscle to that of outer muscle.

3. Activation of sympathetic nerves in the outer part of the artery by nicotine caused almost evenly balanced contraction of both parts of the wall, with slight bias to outer contraction.

4. Moderate external constriction of the artery *in vivo* for 10–17 days, in order to raise pressure throughout the wall to intraluminal pressure, made the entire wall nerve-free.

5. The results provide evidence that the nerves can induce substantial activation of inner muscle, which is highly sensitive to noradrenaline, and that the absence of nerves from inner muscle can be explained by the high pressure there.

INTRODUCTION

Sympathetic nerve fibres are restricted to the outer half to three-quarters of the smooth muscle of sheep carotid arteries (Keatinge, 1966). Inner smooth muscle responds to lower concentrations of vasoconstrictor hormones than outer muscle does (Graham & Keatinge, 1972). In those studies the responses of the two regions were separated by destroying one or other by heat. For the present study we have assessed relative contractions of inner and outer muscle by measuring the direction of torsion

produced during contractions of helical strips. This made it possible to test the previous conclusions by a different method, and to see if activity of the nerves in outer muscle caused any contraction of inner muscle, which could not be tested in partially killed strips.

Little of the noradrenaline released by arterial nerves seems to reach the intimal surface (Bevan & Osher, 1970), but it seemed possible that the high sensitivity of inner muscle to noradrenaline, or electrical transmission from outer smooth muscle, might enable it to respond to the nerves. We have also increased the low O_2 tension that normally exists in inner muscle (Niinikoski, Heughan & Hunt, 1973) to see if this affected its sensitivity.

The cause of the arteries' nerve supply being restricted to outer muscle was also investigated. Pressure within the artery wall varies from arterial blood pressure in the innermost tissue to near atmospheric pressure in the outermost tissue. It is well known that pressure can cause degeneration of somatic nerves (Denny-Brown & Brenner, 1944; Danta, Fowler & Gilliat, 1971) and it seemed possible that the high pressure in inner tissue of arteries excludes nerves from this region. Cylindrical metal clips were therefore applied to the outside of arteries of anaesthetized sheep, tight enough to reduce the artery diameter but not tight enough to occlude the lumen, in order to raise tissue pressure throughout the artery wall to intraluminal pressure. The effect of this on nerve distribution 10–17 days later was compared with the effect of control clips placed loosely over the artery.

METHODS

Arteries. Sheep carotid arteries were obtained from a slaughter-house immediately after the animals (Welsh Mountain sheep aged 6–24 months) had been killed by exsanguination. As much adventitia as possible was removed and a helical strip 4–5 mm wide and 50–55 mm long was cut at an angle of 45° to the long axis of the vessel. The strip was mounted vertically. Its upper end was perforated by two hooks, at the same level and 2·5 mm apart from each other. They were attached to calibrated, isometric strain gauges (Devices, range ± 100 g) to record lateral, but not vertical pull; the gauges were directed so as to record force in opposite directions if the strip developed torsion. The output of each gauge was displayed on a Devices heat stylus recorder (frequency response flat to 50 Hz). The summed output of the two gauges was displayed on a third channel. Torsion was calculated from summed output (y) as $Dy/2$, where D is the distance between the hooks.

The lower end of the strip was attached by hooks to a hinged lever that was counterweighted to provide a tension of 15·0 g ± 0·5 g, approximately the physiological tension on the intact artery wall. It thus allowed isotonic vertical movement of the lower end of the strip but not lateral movement or rotation. The lever activated a further Devices strain gauge; the signal from this was displayed on a fourth channel to give a measure of longitudinal shortening of the artery strip. The strip was mounted so as to contain one-and-a-half turns, approximately the number that were present in this portion of the intact artery.

When set up, the strip was allowed to equilibrate for 90 min in standard saline (see below) at $37 \pm 0{\cdot}5°$ C. The solution was stirred and oxygenated by bubbling continuously with a mixture of 5% CO_2 and 95% O_2. Concentrated solutions of drugs (2 ml. of each) were added to the bath as required.

Solutions and drugs. The standard saline used in all experiments contained (in mM): NaCl, 133; $NaHCO_3$, 15·3; NaH_2PO_4, 1·38; KCl, 4·7; $CaCl_2$, 1·25; and dextrose 7·8. Analar reagents were used.

Pentobarbitone (Nembutal veterinary, Abbot) in 60 mg/ml. solution was used for anaesthesia. Other drugs used, noradrenaline bitartrate (Koch-Light), sodium cyanide (B.D.H.), nicotine hydrogen tartrate (B.D.H.), and Desipramine (Geigy) were obtained as solids. Concentrated solutions of these drugs were made up in standard saline shortly before they were to be used.

Measurement of O_2 tension across the artery wall. Oxygen tension (P_{O_2}) within the artery wall was measured by a miniature O_2 electrode fitted with a polytetrafluoroethylene (Teflon) membrane. Tip diameter was 0·5 mm. The calibration was found not to be affected by cyanide 10 mM.

A track 10 mm long was made diagonally from the inner to outer surface, using a needle 1 mm in diameter. The needle was removed and the O_2 electrode inserted so that its tip protruded from the far end of the track. With the artery strip under the usual tension in oxygenated saline, the electrode was withdrawn through the track in steps of a quarter of the track length. Stable O_2 tension readings were made at each step. The track closed as the electrode was withdrawn. The distance of the electrode tip from inner and outer surfaces at each reading was calculated on the assumption that the track was a straight diagonal between the two. In order to correct for O_2 tension depression caused by O_2 utilization of the electrode, each experiment was repeated with the artery killed by boiling. The small O_2 tension depression recorded at each point in these controls was added to the reading made at the same point in the main experiments, to give O_2 tension in these.

Prolonged compression of arteries in vivo. Sheep were anaesthetized with I.V. pentobarbitone 30–50 mg kg^{-1}. A paramedian incision was made in the neck and one common carotid artery with the adjacent vagus and sympathetic nerve was exposed and separated from surrounding tissues. A cylindrical steel clip of sufficient diameter (approximately 4 mm) to constrict but not occlude the carotid artery was selected and fixed around the artery and nerves. The carotid artery and nerves of the other side were then similarly exposed and a clip of larger diameter, just too large to constrict the vessel, was placed around them as a control. The animals were allowed to recover and after 10–17 days they were again anaesthetized; both carotid arteries were removed and the portions within the clips were snap frozen in isopentane at $-45°$ C. Additional experiments were made in which the clips were placed round the artery but not round the vagus and sympathetic trunk. These are not described in detail as control experiments for these, using a loose clip, produced considerable denervation of the vessel, presumably by severing fibres running from the trunk to the vessel.

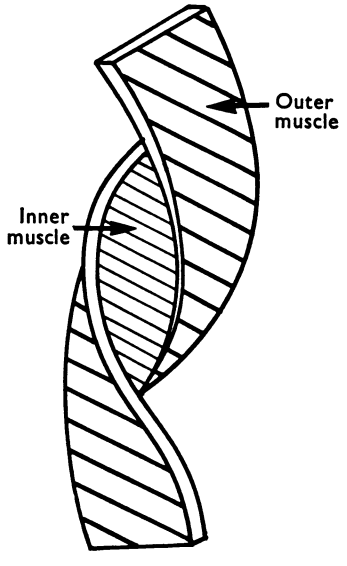
Microscopy. Frozen arteries were examined for catecholamine-containing nerves by the method of Falck (1962). They were dried *in vacuo* at $-40°$ C for 3 days, exposed to formaldehyde vapour at 80° C for 1 hr and embedded in paraffin wax. Sections 7 μm thick were cut, mounted in liquid paraffin and examined by fluorescence microscopy with incident light of wave-length 400 nm.

For histological studies of cells in intact arteries, vessels were fixed in formaldehyde solution (10%, w/v, in water) and embedded in paraffin wax at 56° C. Serial sections, 7 μm wide, were cut tangentially through the artery wall, stained with haematoxylin and eosin and examined microscopically.

RESULTS

Alignment of smooth muscle cells

Provided that both inner and outer muscle are arranged circularly, with neither showing any systematic tendency to helical arrangement, inner contraction will tend to tighten and outer contraction to unwind the helical strips (see Text-fig. 1). Tangential sections through six arteries



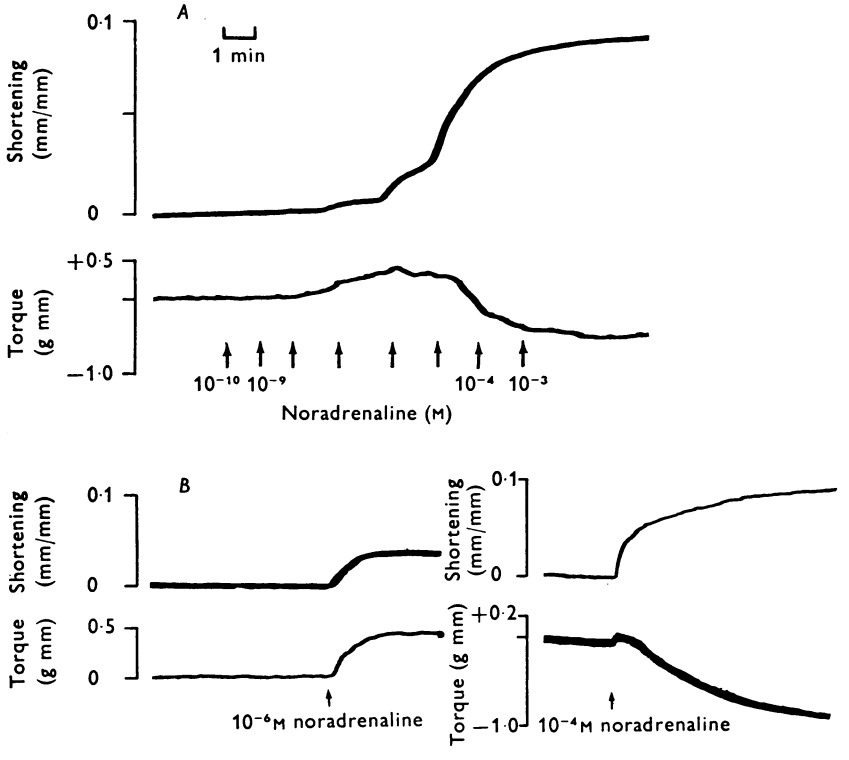
Text-fig. 1. Diagram to show average alignment of inner and outer muscle cells in helical artery strip.

showed that the precision of circular alignment was very high in inner muscle cells. The deviation of outer muscle bundles from circular alignment was greater but was random in direction, and no systematic deviation from circular alignment was observed in either region.

Changes of torque induced by noradrenaline

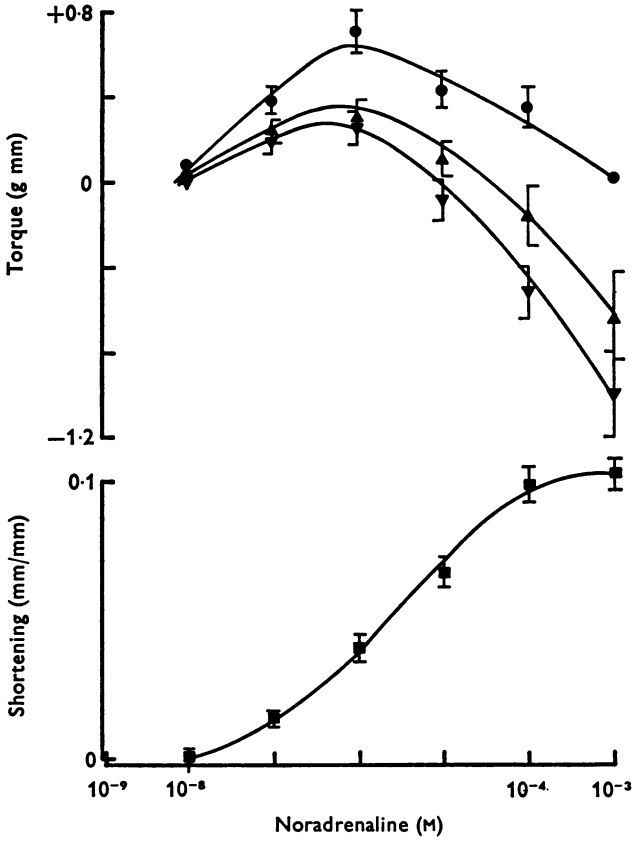
Text-fig. 2*A* shows the response of a helical strip of artery to increasing concentrations of noradrenaline from 10^{-10} to 10^{-3} M. Each concentration was allowed to produce its maximum effect before the next, ten times greater, was added. It can be seen that noradrenaline, 10^{-8} and 10^{-7} M, caused shortening and produced torque in the direction representing a tendency to tighten the helix, indicative of inner muscle contraction. Concentrations of 10^{-6} M and greater caused further shortening, but while

the initial change in torque with each concentration was generally in the direction of further tightening, this was followed by a change of torque in the reverse direction. The final effect of the highest concentrations was to cause net torque in the unwinding direction with respect to the unstimulated preparation. The tendency for low concentrations of noradren-



Text-fig. 2. Response of a helical strip to: *A*, increasing concentrations of noradrenaline; *B*, separate applications of noradrenaline, 10^{-6} and 10^{-4} M. In each case upper trace shows longitudinal shortening (mm/mm artery length), lower trace torque (g mm); upward deflexion indicates tightening of helix (inner muscle contraction), downward deflexion indicates unwinding torque (outer muscle contraction).

aline to cause tightening and higher concentrations to cause unwinding was seen in all of ten such experiments. Text-fig. 3 summarizes these and gives values for corrected torque, calculated as described below. Text-fig. 2*B* shows that application of noradrenaline, 10^{-6} M, to a strip caused tightening torque. After the drug had been washed out, noradrenaline, 10^{-4} M, caused tightening followed by unwinding torque.



Text-fig. 3. Effect of noradrenaline on helical artery strip. Torque: ●—● corrected index of inner/outer contraction (see text); ▲—▲, corrected for passive torque due to tightening (see text); ▼—▼, measured. Upward deflexion represents inner, downwards outer contraction. ■—■, shortening of strip. Values are means ± S.E. of mean of ten experiments.

Correction of torque to indicate relative inner and outer muscle contraction

The above findings give an immediate indication that inner muscle responded to the lower concentrations of noradrenaline, and that outer muscle responded to the higher concentrations and at a slower rate. This agrees with the previous findings, but the indication given by torque of relative inner and outer contraction is distorted to some extent. Isotonic shortening of twisted strips increases the angle of the twist, which itself creates an unwinding torque analogous to that in a twisted elastic band under longitudinal tension. The size of this effect can be calculated as follows

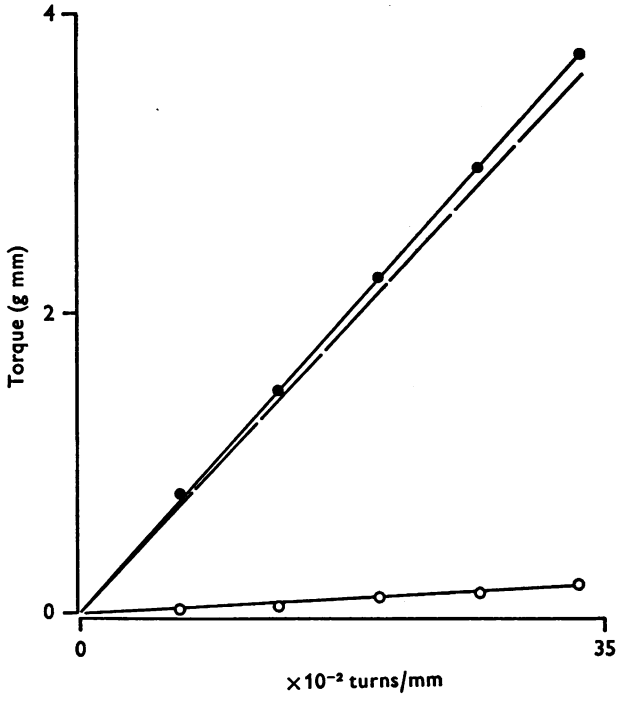
for a strip whose thickness is negligible relative to its width under constant longitudinal tension. Torque is produced by angulation of the twisted strip from the vertical at points away from its mid line. Vertical force is assumed to be evenly distributed across the width of the strip. For a vertically mounted strip of width $2X$ under total vertical tension F, the vertical tension per unit horizontal distance x from the vertical axis of the strip $(dF) = Fdx/2X$. The horizontal component of this force at 90° to the broad axis of the strip is $dF\tan\theta$ where θ is the angle of the strip from the vertical axis at this point. Since $\tan\theta$ is $2\pi tx$ where t is the number of turns of the strip per unit vertical length, the horizontal force is $\pi F dx tx/X$ at this point and the angular moment about the vertical axis at this point is $\pi F t x^2 dx/X$. Integration of the last expression for $x = -X$ to $+X$, gives the torque as $2\pi FtX^2/3 = 2{\cdot}1FtX^2$.

The validity of this formula in predicting the torque produced passively by tension on twisted artery strips was rested with strips of artery that had been killed and relaxed by immersion for 12 hr in $MgCl_2$, 1·25 mM. They were mounted under the usual tension of 15 g and initially each strip was straight. The lower end was then rotated through five successive steps of 180°. Text-fig. 4 shows that when this was done with one strip 75 mm long and 3·8 mm wide, torque increased linearly with degree of rotation, in close agreement with the predicted increase. Similar twisting of the strip with no longitudinal tension produced virtually no torque. Mean and S.E. of torque produced in six such experiments by two-and-a-half rotations was $4{\cdot}29 \pm 0{\cdot}20$ g mm under tension compared to a theoretical value of of $3{\cdot}92 \pm 0{\cdot}12$ g mm; without tension similar twisting of the same strips produced only $0{\cdot}025 \pm 0{\cdot}002$ g mm. The formula therefore predicted passive torque due to twisting with considerable accuracy.

Text-fig. 3 shows that when the torque produced during noradrenaline contractions was corrected by subtraction of passive torque calculated by the formula, some net unwinding torque was still induced by the higher concentrations of noradrenaline. This implies that under maximal stimulation outer muscle generated rather more torque than inner muscle, presumably because of minor differences in arrangement of muscle cells and strength of contraction. In order to allow for this effect, a simple empirical adjustment was made to each torque measurement by subtracting from it the unwinding torque under maximal stimulation multiplied by the fraction of maximal longitudinal contraction present at the time the torque measurement being adjusted was made:

$$\text{Inner/outer index} = \text{Actual torque} - \text{measured torque at maximum contraction} \times \frac{\text{Actual contraction}}{\text{Maximum contraction}}.$$

The effect of this correction is to provide an index of inner to outer contraction, each being expressed as a proportion of their maximum. Text-fig. 3 shows that after torque correction by this index, low concentrations of noradrenaline are still seen to contract mainly inner and high concentrations mainly outer muscle, with the relative effect of low concentrations on inner muscle being more important than the raw torque values would suggest.

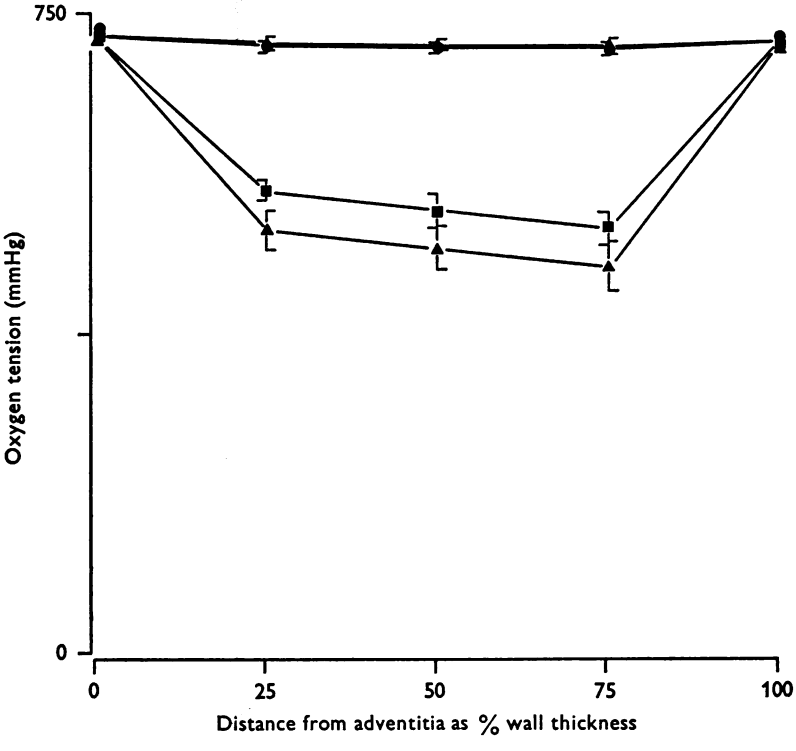


Text-fig. 4. Torque produced by twisting a dead artery strip under 15 g tension. ●—●, strip under 15 g tension, observed values; — —, strip under 15 g tension, predicted values for passive torque; ○—○, strip under no tension, observed values.

Effect of cyanide on O_2 tension and sensitivity to noradrenaline of inner and outer muscle

Text-fig. 5 shows O_2 tension measured by an O_2 electrode in the wall of six artery strips in oxygenated standard saline. The O_2 tension was lowest in the inner part of the wall, probably due to the intima providing a partial barrier to diffusion. Similar but rather lower values were obtained when the strips were contracted by noradrenaline. No value lower than 485 mmHg was ever observed. None of the wall was therefore hypoxic,

but it remained possible that the O_2 gradient between the inner and outer parts of the wall might affect their responses. The Text-figure also shows that sodium cyanide 10^{-3} M eliminated the O_2 gradients. Text-fig. 6 shows that even with this concentration of cyanide present as well as desipramine, 10^{-6} M, to block nerve uptake of noradrenaline, low concentrations of nor-

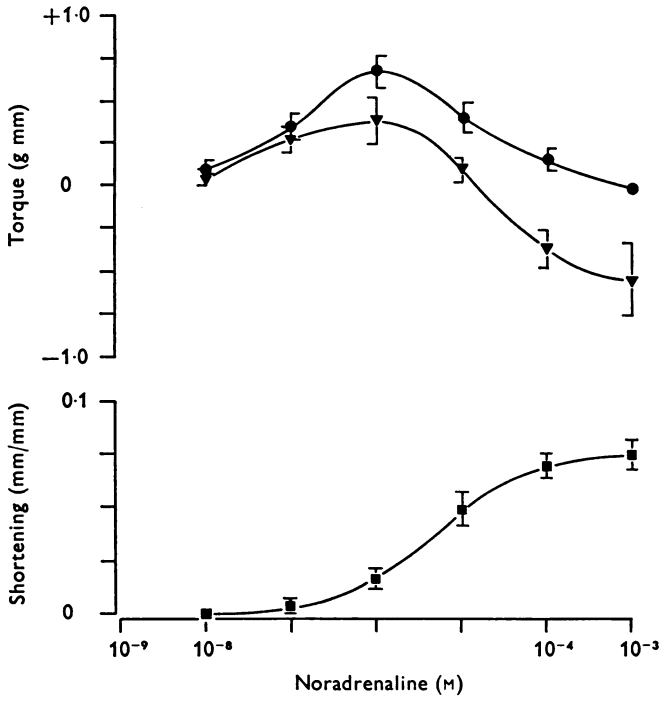


Text-fig. 5. Oxygen tension in artery wall. Sodium cyanide, 10^{-3} M, present: ●—●, no noradrenaline; ○—○, noradrenaline, 10^{-3} M, present. No sodium cyanide present: ■—■, no noradrenaline; ▲—▲, noradrenaline, 10^{-3} M, present. Means ± S.E. of mean of six experiments.

adrenaline (10^{-8}–10^{-6} M) caused predominant torque in the tightening direction and higher concentrations (10^{-5}–10^{-3} M) caused predominant torque in the unwinding direction indicating that inner muscle still responded to lower concentrations of noradrenaline than outer muscle.

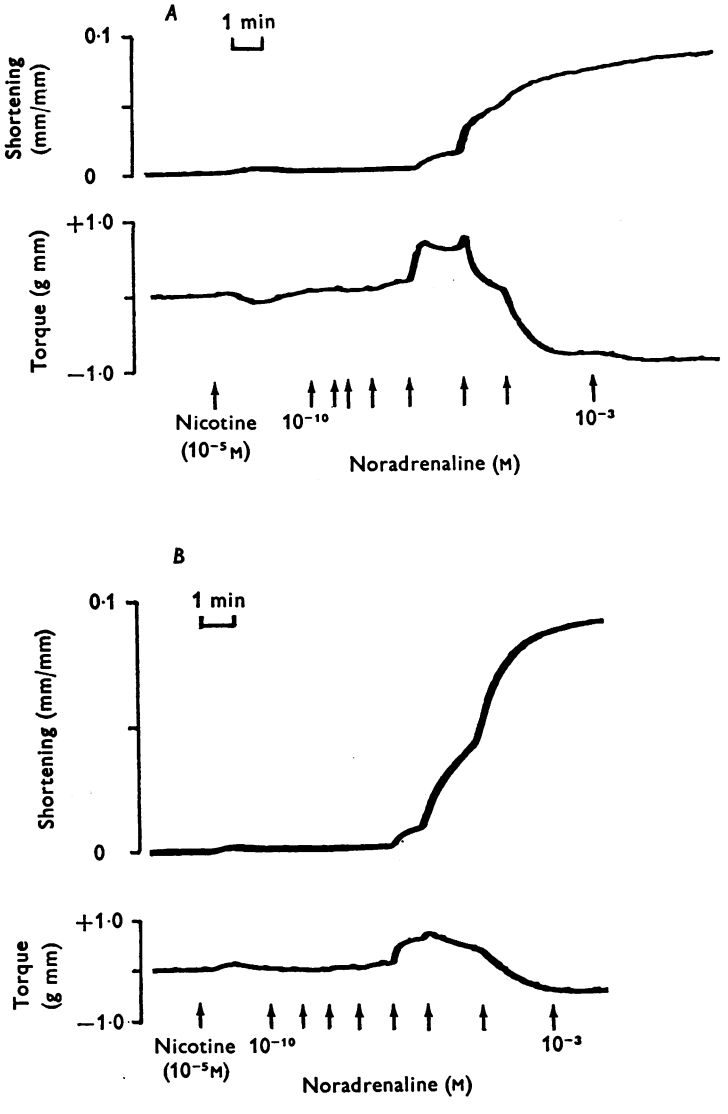
Response to nerve stimulation

The sympathetic nerves in the outer part of the media were stimulated by nicotine, 10^{-5} M, which induces action potentials in the nerves without affecting the smooth muscle directly (Keatinge, 1966). Twenty out of



Text-fig. 6. Effect of noradrenaline on helical strips in the presence of desipramine, 10^{-6} M, and sodium cyanide 10^{-3} M. Torque: ●—●, corrected index of inner/outer contraction (see text); ▼—▼, measured. Means ± S.E. of six experiments.

sixty-seven strips stimulated gave clear responses. Longitudinal shortening in these twenty was 0·010 ± 0·002 mm/mm artery length (mean ± S.E. of mean). The crude change in torque produced by nicotine varied in direction. It was usually in the unwinding direction; Text-fig. 7*A* shows an example, followed by the usual pattern of response to increasing concentrations of noradrenaline. However, in other cases there was little change in torque after nicotine, and in some the change in torque was in the tightening direction (Text-fig. 7*B*). The responses to noradrenaline, 10^{-3} M, allowed the index of inner/outer muscle response to nicotine to be calculated for each strip as described above. Measured torque in response to nicotine was 0·092 ± 0·034 g mm (mean ± S.E. of mean of twenty experiments) in the unwinding direction, and the index of inner to outer contraction was only 0·018 ± 0·032 g mm in the same (outer) direction, which does not differ significantly from zero.



Text-fig. 7. Effect of nerve activation by nicotine, and of noradrenaline, on helical strips from two different arteries. *A*, showing net unwinding torque ('outer contraction') produced by nerve activation; *B*, showing net tightening torque ('inner contraction') produced by nerve activation. Upward deflexion represents tightening torque, downward unwinding torque.

Effect of prolonged external pressure in vivo *on distribution of nerves in the artery wall*

Pl. 1*A* shows a fluorescence micrograph of an artery that had been compressed by a clip for 10 days in life, removed under anaesthesia and prepared by the Falck method to demonstrate monoamines. Pl. 1*B* shows the control artery, treated in the same way except that the stainless steel clip placed around it was too large to compress the artery. No sympathetic nerve fibres are present in the compressed artery, although they are present as usual in the outer half to three-quarter of the wall in the control artery. In all of six such pairs of arteries the control vessels contained nerve fibres in their outer regions. No nerve fibres could be identified in any of the arteries that had been compressed. Histological observations of compressed and control arteries confirmed that the smooth muscle cells throughout the tissue were of normal appearance. Three additional compressed arteries in which the blood in the lumen of the artery was found to have thrombosed were not included in the series; they showed degeneration of smooth muscle as well as nerves.

DISCUSSION

The conclusion that inner muscle was more sensitive than outer muscle to noradrenaline confirms the previous findings (Graham & Keatinge, 1972) in which heat damage to inner or outer muscle was used to separate responses of the two regions. The present studies show that the difference in sensitivity was not due to a difference in O_2 tension that existed between inner and outer muscle, since it persisted in the presence of enough cyanide to eliminate O_2 gradients, as well as desipramine to eliminate nerve uptake of noradrenaline.

The method used in the present study, which left both inner and outer parts of the wall alive, made it possible to obtain evidence that activation of nerves caused contraction of inner as well as outer muscle. It seems to be a general rule in systemic arteries of mammals that inner muscle is free of nerves, while nerves generally penetrate outer muscle to a slight extent in small arteries and to a greater extent in large ones (Falck, 1962; Keatinge, 1966; Ehinger, Falck & Sporrong, 1967; Mohri, Ohgushi, Ikeda, Yamamoto & Tsunekawa, 1969; Bevan & Purdy, 1973). It has been suggested (e.g. Folkow, Oberg & Rubenstein, 1964) that only outer muscle responds when the nerves are activated. Gillespie & Rae (1972), using a variety of arteries, found that nerve stimulation failed to increase wall stiffness as much as noradrenaline did. This would fit the view that nerves only activated outer muscle, but alternatively might represent failure of

the nerves to activate any part of the wall fully. Bevan & Osher (1970), using rabbit aorta, showed that much less of the noradrenaline released from sympathetic nerves emerged from the intimal than from the adventitial surface. However, the high sensitivity of inner muscle might allow it to respond even to the small amounts of noradrenaline diffusing to it. Inner muscle might also be activated by spread of action potentials from outer muscle; sucrose-gap records indicate that a sympathetic nerve can activate 1200 smooth muscle cells in sheep carotid arteries (Keatinge, 1966), and it is likely that some of these are activated by electrical transmission from cell to cell. Whichever of these means of activation was responsible, the present results indicate that inner muscle's response to nerves was on average not significantly less, and was sometimes greater, than outer muscle's. It should be noted that these findings apply to initially relaxed arteries; in an artery already stimulated by a circulating vasoconstrictor agent in sufficient concentration to contract the more sensitive inner muscle fully, only the outer muscle could respond to any further stimulus.

The finding that prolonged elevation of pressure throughout the wall caused disappearance of nerves from it supports the view that the normal exclusion of nerves from inner muscle is due to the high pressure there. Pressure-induced degeneration of somatic nerves starts at the point of greatest pressure gradient rather than greatest absolute pressure (Ochoa, Fowler & Gilliat, 1972). Pressure in the artery wall will vary from arterial blood pressure at the innermost part of the wall to atmospheric pressure at the outermost part. Distribution of pressure within the wall will depend on the relative degree of contraction of inner and outer muscle at the time, and inner muscle with its ability to respond both to nerves and to moderate concentrations of circulating vasoconstrictor agents is likely to have high tone and therefore a steep pressure gradient more often than outer muscle. Whatever the relative importance of local gradients of this kind, and of total pressure change, vasomotor nerves in the present studies were apparently not able to tolerate localized application of a pressure of approximately 100 mmHg, the pressure in the lumen of this artery (Keatinge, 1966).

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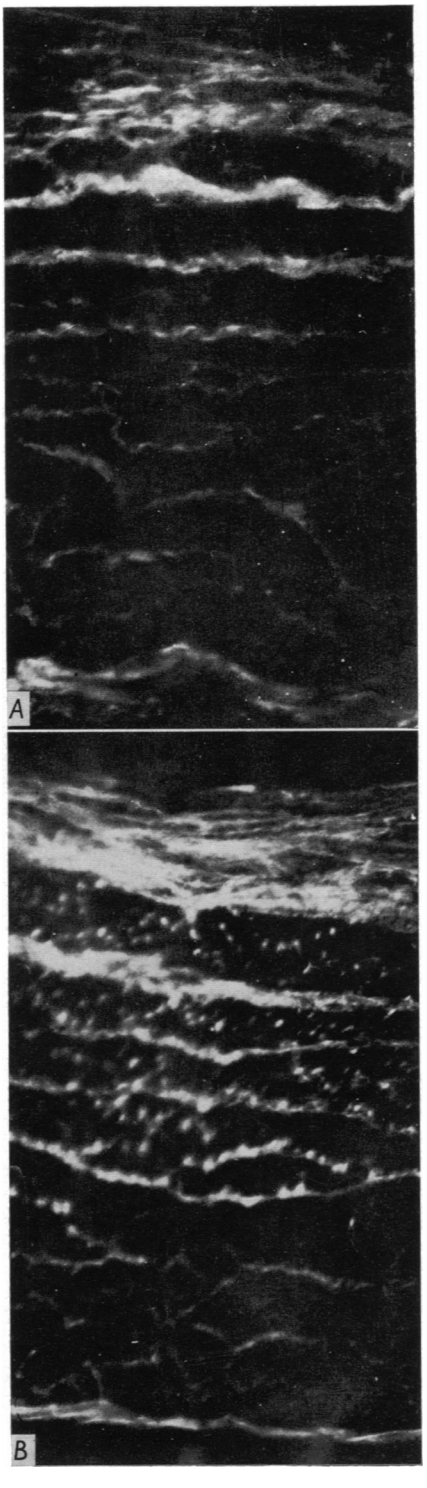
EXPLANATION OF PLATE

A, fluorescence micrograph of artery that had been compressed for 10 days in life to raise pressure throughout the wall to intraluminal pressure. Sympathetic nerve fibres are absent. *B*, fluorescence micrograph of control (sham operated) artery from same sheep. Normal distribution of sympathetic nerve fibres. Longitudinal sections × 300.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