J. Physiol. (1975), **247**, *pp.* 473–482
With 1 plate and 4 text-figures
Printed in Great Britain

RESPONSES OF INNER AND OUTER MUSCLE OF THE SHEEP CAROTID ARTERY TO INJURY

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(*Received 26 September 1974*)

SUMMARY

1. Direct injury to the smooth muscle of the sheep carotid artery *in vivo* caused large, persistent and sharply limited annular contraction, even with tetrodotoxin 10^{-5} M present to block nerves.

2. Sucrose gap records from artery strips showed that mechanical injury caused slow, prolonged depolarization of the smooth muscle that spread for a few millimetres in a circular direction in relation to the intact artery wall with an apparent space constant of 1·26–3·49 mm. In the longitudinal direction, no depolarization was recorded 1 mm from the site of injury. No spikes were recorded more than 1 mm in either direction from the site of injury except when procaine, which facilitates electrical activity in the smooth muscle, was present.

3. When responses of inner and outer muscle were recorded separately, injury caused comparable contraction of both parts.

4. Clotted blood caused large contractions of intact artery strips; it contracted inner much more than outer muscle.

5. The main factors in the intact vessel's response to injury therefore seem to be inner and outer muscles' direct response to injury, reinforced by spread of depolarization round the vessel wall, and inner muscle's response to vasoconstrictor agents released by clotting blood.

INTRODUCTION

It has long been realized that injury to arteries causes contraction (Hunter, 1786), sometimes of sufficient intensity to seal perforations of the wall (Magnus, 1923). Such responses occur even when vasoconstrictor nerves have been blocked by local anaesthetics (Cohen, 1944) or removed by chronic sympathectomy (Chen & Tsai, 1948) or by stripping of the adventitia (Kinmonth, Simeone & Perlow, 1949). They are due partly to direct reaction of arterial smooth muscle to injury since they have been observed even when any blood that leaks out is washed away continuously

(Chen & Tsai, 1948). These responses are normally reinforced by vasoconstrictor agents released in clotting blood (Stewart & Zucker, 1913), mainly from platelets (Zucker, 1947). The most important of these agents are 5-hydroxytryptamine, noradrenaline, histamine and vasoactive proteins (Kapp, Mahaley & Odom, 1968).

The present experiments were designed to show whether direct injury to the artery wall contracts only those smooth muscle cells injured, or whether depolarization spreads by passive means or by action potentials to cause more widespread contraction. They were also designed to show whether direct injury, and vasoconstrictor agents in clotting blood, act mainly on inner or outer muscle. Outer muscle of these arteries, which is the only part with a nerve supply (Keatinge, 1966), is much less sensitive than inner muscle to many vasoconstrictor agents (Graham & Keatinge, 1972). Responses of inner and outer muscle were separated in the present study, as in those experiments, by causing controlled heat damage to one or the other part. Electrical responses were again measured by the sucrose-gap method.

METHODS

All experiments were performed on the terminal 4–6 cm of the common carotid artery of sheep. *In vivo* studies were made on sheep anaesthetized with I.V. pentobarbitone (12 mg/kg). *In vitro* studies were made on arteries removed within 5 min of the animals being killed at a slaughterhouse.

Electrical and mechanical recording

Helical, and in a few cases, longitudinal strips 1–1·5 mm wide, were cut from the artery wall and mounted in a sucrose-gap apparatus described previously (Keatinge, 1964). A 20 mm length of the strip was in standard saline (test portion), and 10 mm in sucrose solution. The electrical output was amplified by a d.c. amplifier with high input impedance (500 MΩ) and displayed on a Devices heat stylus recorder (frequency response flat to 70 Hz). Contractions of the test portion of the strip were recorded by an isotonic ($2{\cdot}5 \pm 0{\cdot}2$g) strain gauge and displayed on the same recorder. Injury could be applied to the test portion of the strip through any one of four holes, 1 mm in diameter, drilled in the apparatus, centred 1, 4, 7 and 10 mm from the saline–sucrose junction. These were sealed by adhesive tape before each experiment. A blunt needle 1 mm in diameter with an insulated handle was pushed through to crush the strip when required.

Simple mechanical records were obtained, when required, from similar artery strips 1–1·5 mm wide and 20 mm long attached to isotonic ($2{\cdot}5 \pm 0{\cdot}2$ g) levers that recorded in ink on a kymograph.

Preparation of strips with only inner or outer muscle alive

Helical strips of artery wall 2–3 mm wide and 25–35 mm long were cut. The inner half or the outer two-thirds of the wall was killed by heat as described previously (Graham & Keatinge, 1972) by placing the strips between metal foil windows, held

at 90 and 0° C or 27 and 90° C respectively by vigorously stirred water on the other side.

The standard saline solution used in all experiments contained (mM): NaCl 133; $NaHCO_3$ 15; KCl 4·7; $CaCl_2$ 1·25 and dextrose 7·8. Analar reagents were used. The solution was equilibrated with a mixture of 5% CO_2 + 95% O_2 and kept at 36 ± 0·5° C. Artery strips were always left in this solution, under 2·5 g tension, for 90 min before an experiment was started.

Pentobarbitone in 60 mg/ml. solution was used for anaesthesia. Heparin (Weddell) was obtained in solution in ampoules. The other drugs used: L-noradrenaline bitartrate (Koch-Light); tetrodotoxin (Sankyo); procaine hydrochloride (Pharmaceutical Manufacturing Co.); were obtained as solids. Concentrated solutions of these drugs were made up in standard saline on the day that they were used.

Statistical analysis

Responses of strips to drugs and to traumatic stimulation *in vivo* were compared by the *t* test. Responses of inner strips from given arteries were paired with responses of other strips from the same arteries.

RESULTS

Mechanical response to injury in vivo

When a blunt needle 1 mm in diameter was pushed into the muscle coat of a carotid artery and rotated, an annular band of contraction approximately 2 mm wide appeared at the level of the puncture and persisted for more than 10 min (Pl. 1). Blood that leaked from damaged vasa vasorum in small amounts during and after the needle puncture was carefully re-removed by gauze soaked in saline. At the deepest point of the band of contraction artery diameter was reduced by 1·9 ± 0·1 mm, from an initial value of 7·3 ± 0·5 mm (means ± S.E., five experiments). Similar needle puncture 5 min after the injection of 2 ml. tetrodotoxin 10^{-5} M in saline around the artery produced a similar annular contraction that reduced artery diameter by 1·8 ± 0·3 mm, from an initial value of 6·8 ± 0·4 mm (means ± S.E., five experiments).

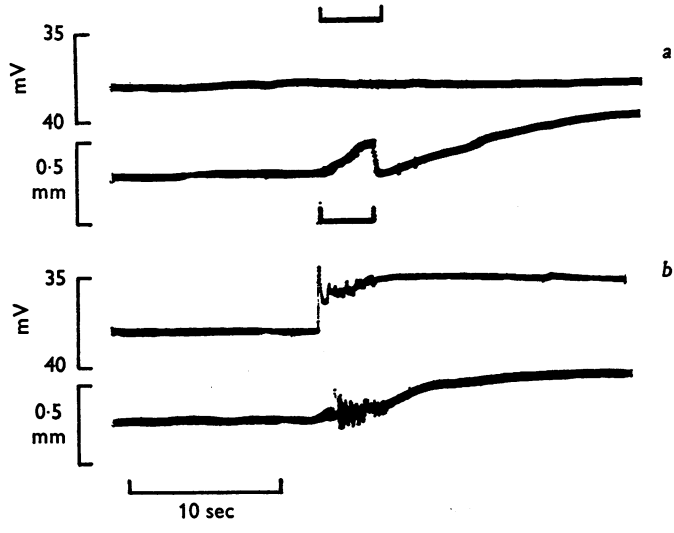
Electrical and mechanical responses of isolated artery strips to injury

Helical strips

Helical strips 2–3 mm wide were mounted in the sucrose gap and allowed to recover in standard saline at 36° C for 1 hr. A 1 mm diameter probe was pushed lightly against the strip and then rotated with firm pressure for 3–4 sec to crush the strip. The light pressure was never followed by clear electrical changes, but the crush injury applied at positions centred successively 10, 7, 4 and 1 mm from the saline-sucrose junction often caused depolarization.

Text-fig. 1 shows the response of a strip to the first and the last of these crush injuries. Crush 10 mm from the junction caused contraction of

0·56 mm but no electrical response was recorded (Text-fig. 1*a*). Text-fig. 1*b* shows that the final crush injury 1 mm from the junction was followed by a wave of depolarization which persisted after the period of stimulation and preceded the mechanical response. There was an initial



Text-fig. 1. Sucrose-gap record of electrical and mechanical responses of artery strip to injury applied (*a*) 10 mm and (*b*) 1 mm from the sucrose–saline junction. Horizontal bars indicate duration of stimulation.

TABLE 1. Depolarization (mV) produced by injury applied to helical strips of artery at varying distances from the recording point (saline–sucrose junction)

Distance from the saline–sucrose junction (mm)				Apparent space constant (mm)
1	4	7	10	
6·59	1·36	0·91	0·13	2·66
14·55	0	0	0	Not calculable
4·78	3·48	1·74	0·43	3·49
6·12	1·02	0·40	0·12	1·26
6·36	0·68	0·45	0·23	2·28
2·73	1·82	0	0	Not calculable
3·10	0·69	0·52	0	3·07

spike of depolarization which may be artifact, as the injury was applied. Such spikes were recorded during crush injury 1 mm from the junction in three out of seven experiments but never at 10, 7 or 4 mm from the junction. Table 1 gives the electrical response of seven strips to crush injury at all four positions, measured at the highest point of the wave of depolariza-

tion after injury. The space constants of each strip, given in the Table, were obtained as follows.

If the portion of artery strip in saline is treated as a blind-ended cable and voltage distribution is assumed to be passive, depolarization at the site of injury (V_m) will be $V_0 \cosh x/\lambda$ (Adrian & Freygang, 1962), where V_0 is the voltage recorded at the saline–sucrose junction, x is the distance of the junction from the site of injury and λ is the space constant. Cosh y can be expanded to

$$1+\frac{y^2}{2!}+\frac{y^4}{4!}+\frac{y^6}{6!}+\ldots.$$

Therefore for small values of x,

$$V_m = V_0\left(1+\frac{x^2}{2\lambda^2}\right)$$

and

$$\frac{1}{V_0} = \frac{1}{V_m}+\frac{x^2}{2V_m\lambda^2}.$$

Text-fig. 2 shows that a plot of $1/V_0$ against x^2 for one artery strip gives a linear relationship for values of x of 7 mm and below. This regression line was calculated, and its intercept ($1/V_m$) gave V_m as 3·86 mV, and its slope ($\frac{1}{2}V_m\lambda^2$), with V_m gave the space constant as 2·66 mm.

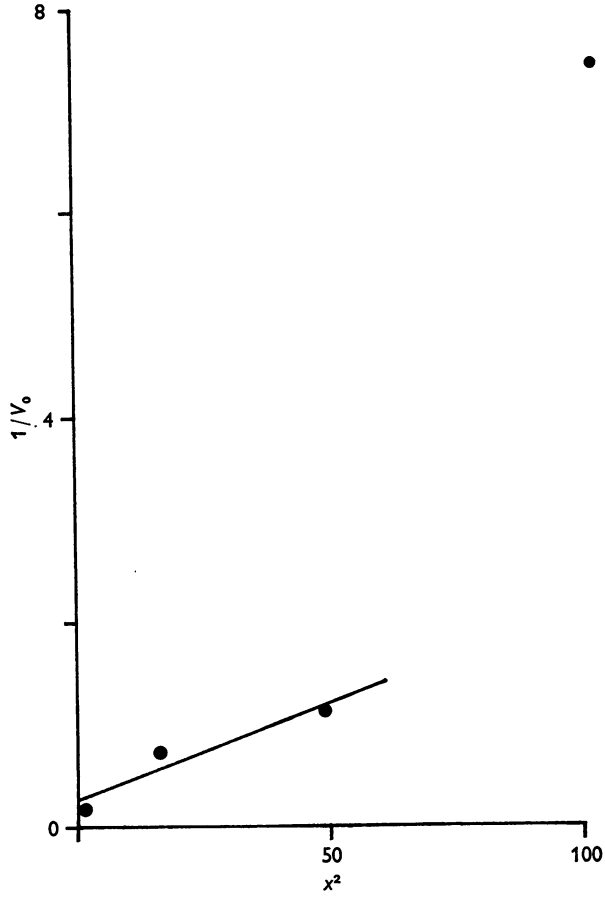
These crush injuries always caused contraction. The first, applied 10 mm from the junction, caused contraction of $0{\cdot}52 \pm 0{\cdot}11$ mm (mean ± S.E., seven experiments). The mean time taken to develop 50 % of total response was $7{\cdot}9 \pm 1{\cdot}0$ sec (mean ± S.E.). The duration of the contractions was variable but in three cases they persisted without substantial decrement for more than 15 min. The subsequent crush injuries nearer the junction caused similar though smaller contractions.

In order to confirm that these were active responses of smooth muscle cells and not electrical and mechanical artifacts, similar experiments were carried out on four artery strips that had been killed by heating to 90° C for 8 min; no changes in length greater than 0·05 mm, or changes in potential greater than 1 mV after the end of stimulation, were produced by crushing at any of the positions.

Spike discharges were recorded with the sucrose junction 10 mm from the site of injury when procaine was present to facilitate electrical activity in the smooth muscle, even when pressure of 50 ± 10 g/mm^2, insufficient to crush the tissue, was used as the stimulus and was applied for only 1 sec. Fresh helical strips were exposed to procaine 5 mM in standard saline for 10–20 min. Text-fig. 3 shows that pressure 10 mm from the sucrose junction was then followed by two spike discharges. A similar experiment on a second strip also produced two discharges, and a third

produced one discharge. No definite electrical discharge were recorded in two other experiments.

Longitudinal strips. Crush injury applied to five longitudinally cut artery strips in saline (without procaine present) produced no detectable depolarization (< 1 mV) even when applied 1 mm from the sucrose junction.

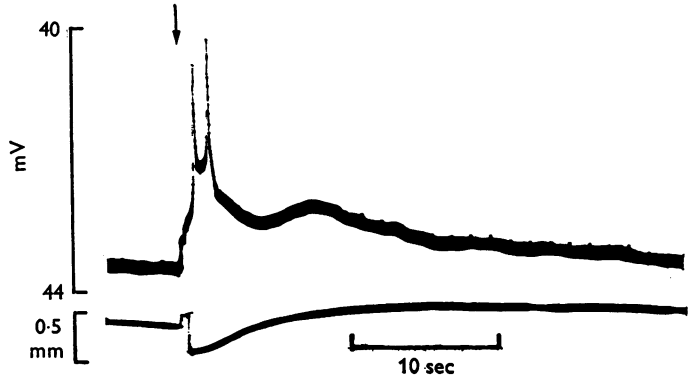


Text-fig. 2. Relationship of reciprocal of change in potential (V_0) in mV recorded after injury, to the square of the distance of the injury from the sucrose–saline junction (x) in mm.

Mechanical responses of helical stips of inner and outer smooth muscle to trauma

Crush injury in the sucrose-gap apparatus, without procaine present, contracted helical strips that contained only live inner muscle or only live outer muscle, the rest of the wall having been killed by heat. Crush injury

of inner muscle caused shortening of $0{\cdot}38 \pm 0{\cdot}10$ mm and outer $0{\cdot}32 \pm 0{\cdot}10$ mm (means ± S.E., five experiments). Inner muscle gave 50 % maximal response in $10{\cdot}10 \pm 1{\cdot}65$ sec and outer muscle in $7{\cdot}10 \pm 1{\cdot}38$ sec. There was therefore no significant difference in response to injury between the two tissues, but great individual variation was present within each. No electrical responses to crush injury greater than 2 mV were recorded in these strips even with injury 1 mm from the sucrose junction, perhaps because of conduction breaks in the tissue in the sucrose section.

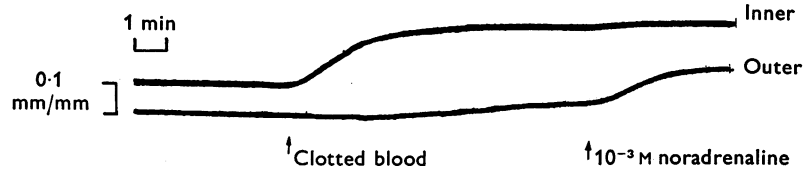


Text-fig. 3. Sucrose-gap recording of electrical and mechanical responses of an artery strip to brief injury 10 mm from the sucrose–saline junction in the presence of procaine 5 mM. Arrow indicates injury.

Responses of inner and outer parts of the media to vasoconstrictor materials present in clotted blood

Text-fig. 4 shows that application of clotted sheep blood caused powerful contraction of inner muscle but had little effect on outer muscle. Noradrenaline 10^{-3} M, however, did cause a large contraction of outer muscle; the maximum response of outer muscle to clotted blood and noradrenaline together was similar to that of inner muscle. In five such experiments clotted blood caused inner muscle to contract by $0{\cdot}19 \pm 0{\cdot}05$ mm/mm while noradrenaline 10^{-3} M caused a little further contraction to $0{\cdot}22 \pm 0{\cdot}06$ mm/mm (means ± S.E.); clotted blood caused outer muscle to contract by $0{\cdot}02 \pm 0{\cdot}01$ mm/mm, while noradrenaline caused further contraction to a total of $0{\cdot}12 \pm 0{\cdot}03$ mm/mm (means ± S.E.).

Sheep blood in which clotting had been prevented by heparin 10 i.u./ml. did not contract either inner or outer muscle (two experiments).



Text-fig. 4. Responses of an inner and an outer strip to clotted sheep's blood and to noradrenaline 10^{-3} M.

DISCUSSION

Preliminary experiments showed that dissection of connective tissue around these arteries can cause some diffuse, transient contraction by irritating sympathetic nerves, but the large, persistent annular contractions induced by direct arterial damage in the present study were not prevented by the nerve-blocking agent tetrodotoxin. Since any blood that extravasated was removed immediately, the contractions must have been largely a direct response of the smooth muscle of the artery wall to injury. The size of the contraction produced by localized injury 1 mm in diameter was unexpectedly large. The mean reduction in diameter, 1·8 cm, represents a reduction in circumference of 5·7 mm. Since the smooth muscle cells are only approximately 100 μm long (Keatinge, 1966), this reduction in circumference could not be accounted for by shortening of only those cells directly injured, and suggests that there had been electrical transmission of excitation from damaged to undamaged cells. The narrowness of the band of contraction suggests that there was little conduction longitudinally, at right angles to the circular alignment of the smooth muscle of this vessel.

The sucrose-gap studies on isolated strips showed that depolarization was in fact transmitted from the site of injury for a few millimetres in a circular direction, but not in a longitudinal direction. Sympathetic nerve activity induces brief, irregular action potentials in the smooth muscle of these arteries (Keatinge, 1966) but crush injury of the smooth muscle in the present study caused a slow wave of depolarization without widely conducted action potentials. Action potentials may occasionally have been present 1 mm from the site of injury, but were never found at greater distances in normal solution. The apparent space constant for circular conduction indicated by the spatial decay of the slow wave of depolarization varied considerably between 1·26 and 3·49 mm. This was rather longer than the value of 1·13 mm obtained by Mekata (1971) from decay of hyperpolarizing pulses in strips of rabbit carotid artery, perhaps because some regenerative depolarization assisted spread in the present study. It is interesting that procaine, which facilitates electrical activity

in these arteries (Keatinge, 1974) greatly altered the pattern of response to injury, enabling even mild injury to induce widely conducted action potentials and contraction.

Although both inner and outer muscle was shown to respond to direct injury, only inner muscle gave large responses to the constrictor agents of clotted blood. Apart from the vasoactive proteins present in clotting blood, the platelets release amines that they contain. These include 5-hydroxytryptamine (Rand & Reid, 1951; Born & Bricknell, 1959), adrenaline and noradrenaline (Weissbach, Bogdanski & Udenfriend, 1958; Sano, Kakimoto, Taniguchi & Takesada, 1959), and in some species histamine (Humphrey & Jaques, 1954). 5-hydroxytryptamine is the principal amine in human platelets (Weissbach & Redfield, 1961). The relative sensitivity of inner muscle to clotted blood in the present study implies that a given amount of platelet thrombus and blood clot forming on the inside of the vessel as a result of intimal damage will cause more spasm of the vessel wall than will blood clot outside the vessel.

This work was supported by a grant from the Medical Research Council.

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EXPLANATION OF PLATE

Carotid artery. Arrow shows band of contraction caused by puncture of the vessel wall with a needle. Horizontal bar equals 1 cm.