The relation between the structure and innervation of small arteries and arterioles and the smooth muscle membrane potential changes expected at different levels of sympathetic nerve activity

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The membrane potential changes in arterial smooth muscle due to natural sympathetic nerve activity have been calculated. The electrical properties of the smooth muscle syncytium were taken into account and various assumptions made concerning the release of noradrenaline by the perivascular nerves. The depolarization that would result from asynchronous nerve activity at various mean frequencies was calculated for arterioles and small arteries of various diameters up to 150 μm. The calculations suggested that the depolarization would be irregular and that discrete excitatory junction potentials as evoked by synchronous nerve stimulation would not be recorded during natural nerve activity. The irregularity of the depolarization would be greater in small arterioles and would cause them to reach threshold for action potential generation at lower frequencies of nerve activity than larger arteries.

Introduction

Numerous electrophysiological studies of arterial and arteriolar smooth muscle have shown that stimulation of the periartrial sympathetic nerves produces excitatory junction potentials (e.j.ps) (Speden 1964; Hirst 1977; Surprenant 1980; Keef & Neild 1982). However, the few recordings that have been made from arteries in living animals have mostly not shown changes in membrane potential resembling e.j.ps except when the perivascular nerves were stimulated. Speden (1964), recording from the mesenteric arteries of anaesthetized guinea-pigs, could evoke e.j.ps by stimulating the splanchnic nerve but recorded no spontaneous e.j.ps. Steedman (1966) recorded from the mesenteric arteries of rats, and although she observed membrane potential changes that probably were due to alterations in the level of sympathetic nerve activity they did not resemble e.j.ps. Similarly, Neild & Keef (1983) recorded no recognizable e.j.ps from arteries in the ears of anaesthetized rabbits or guinea-pigs except when the nerves were stimulated.

One obvious difference between natural nerve activity and that evoked by the electrical stimulation is that stimulation will produce synchronous action potentials in all axons, whereas natural firing of post ganglionic sympathetic axons is not synchronized. It is possible to calculate what effect this difference will have on the membrane potential of the arterial smooth muscle, and these calculations are presented below. They show that because of the long membrane time constant and
strong intercellular coupling of arterial smooth muscle, natural activity in perivascular nerves will produce an irregular depolarization but no recognizable e.j.ps.

**Basis for the calculations**

**Cable properties of arteries**

The smooth muscle layer of an artery is a network of electrically connected cells. In all cases in which the electrical coupling has been measured (for example, Hirst & Neild 1978; Holman & Surprenant 1979; Kajiwara *et al.* 1981) it has been found to be so strong that the difference in membrane potential between adjacent cells will be small; small enough to allow a mathematical treatment based on the assumption that the smooth muscle layer is a homogeneous mass of low resistance substance bounded by a high resistance membrane (Bennett 1972, p. 21 *et seq.*).

The most convenient equations describing such a system are the cable equations that have been applied extensively to cylindrical nerve and muscle cells (Jack *et al.* 1975).

The cable equations describe the properties of a thin cylinder of membrane filled with cytoplasm, whereas the smooth muscle layer in an artery is topologically a tube with membrane on both the outer and inner surfaces. Figure 1 shows why this situation can be represented in some cases by a simple cylinder. If current flows into the smooth muscle at one point, for instance because of the action of noradrenaline there, most will flow through the muscle before crossing the membrane. There will always be, however, a plane somewhere opposite the point of current entry across which no current flows (figure 1a). If the muscle were cut along this plane and the cut edges sealed it would not alter the membrane potential change caused by the current. Therefore with respect to point sources of current the smooth muscle can be represented by a sheet of finite width (figure 1b). If the width of the sheet is sufficiently small and the overall electrical resistance inside the sheet in the side-to-side direction sufficiently low then the shape of the cross-section of the sheet no longer matters, and the cylindrical cable model can...
be used (figure 1c). The coupling between the cells must be such that all cells in a ring at any point along the length of the artery must be isopotential.

There is no doubt that the cable equations give a good description of the properties of small arteries. Hirst & Neild (1978, 1980a) found that in arterioles up to 90 μm diameter with a single layer of smooth muscle cells the voltage changes caused by intracellular injection of current could be reproduced exactly. For large arteries with smooth muscle layers more than one cell thick the cable approximation must cease to be valid as the vessel gets very large, but the upper size limit for the useful application of the cable equations is not known. (It could be found by comparing cable calculations with calculations for a thin sheet of finite width, but the equations for this are not available.) The most important factor is the electrical resistance through the muscle in the circumferential direction. This is probably lower than in the longitudinal direction because the cells are oriented circumferentially and the resistance of the cytoplasm is less than the coupling resistance (Hirst & Neild 1978; also Tomita 1969 and Nagai & Prosser 1963, for intestinal smooth muscle). The calculations in this paper were carried out for arteries up to 150 μm diameter with an average wall thickness of 1.5 cells of smooth muscle, and resistance in the radial direction was ignored.

Branching of arteries

The cable equations in their simplest form apply to unbranched cylinders of constant diameter. Rall (1959) investigated the effects of branching, and found that in the special case of a cable splitting into two branches of equal diameter the equations for unbranched cables could be used if the ratio of the diameter of the cable before and after branching was 2:1. Where branching occurs in blood vessels it usually does not fulfil this condition, and the ratio of artery lumen diameter tends to be closer to 2:1 (Sherman 1981). This will be the same as the ratio of the diameter of the equivalent cable for thin walled arteries, and as wall thickness increases relative to the lumen the ratio will be closer to unity. The simple cable equations therefore cannot be applied to highly branched arterial systems but fortunately there are areas where arteries run for many millimetres without branching and there the cable equations can be applied.

Amplitude of voltage changes

To apply the cable equations to arterial smooth muscle certain parameters of the muscle must be known. The membrane time constant (τ) and cable space constant (λ) must be known before any calculations can be made. These two parameters can be measured relatively easily (Bywater & Taylor 1980; Holman & Neild 1979) and values from several different arteries are available (Hirst & Neild 1978; Holman & Surprenant 1980; Kajiwara et al. 1981). With this information it is possible to calculate the time course of membrane potential changes, but to find their amplitude values are needed for one of the three basic parameters: specific membrane resistance (Rm), specific membrane capacitance (Cm) and internal resistivity (Ri). These are much more difficult to measure than τ and λ, and have only once been measured in arteriolar smooth muscle (Hirst & Neild 1978).

The values of τ and λ for a variety of different arteries fall into a fairly narrow
range, $r$ being between 250 and 400 ms and $\lambda$ between 1 and 1.5 mm. If it is true, as generally assumed (Cole 1968), that specific membrane capacitance varies little from one tissue to another then the consistency of $r$ implies that all arterial smooth muscle has about the same specific membrane resistance. It follows from this that as $\lambda$ is relatively constant $R_I$ must not vary greatly between tissues, and the results obtained from submucous arterioles (Hirst & Neild 1978, 1980a) can be used for simulations of changes in other arteries. The absolute values of $R_m$, $R_I$ and $C_m$ were not used because their determination involved assumptions about cell membrane surface area that may lessen their validity. Instead the calculations were based on the fact that in short pieces of submucous arteriole the average value of the amplitude of the voltage change caused by the release of one quantum of noradrenaline was 1.5 mV (Hirst & Neild 1980a). This situation was simulated and a scaling factor derived that gave the amplitude of the voltage changes, based only on the assumption that $r$ and $\lambda$ are similar for all arterial smooth muscle. To allow for arteries of different diameter it was assumed that the number of smooth muscle cells per unit length of artery was directly proportional to diameter, and so the scaling factor was reduced proportionately as diameter increased. If the smooth muscle layer was more than one cell thick the scaling factor was divided by the average wall thickness expressed in number of smooth muscle cells.

**Pattern and density of perivascular innervation**

The depolarization of smooth muscle during perivascular nerve activity will depend on the number of nerve fibres innervating the muscle and the amount of noradrenaline that each releases when an action potential travels along it. Information about the density of innervation is available for several different arteries. The two most common methods of quantitating a perivascular innervation have been to measure nerve varicosity density (number of varicosities per mm$^2$ of arterial surface) and intercept density (number of nerve fibres intersecting an imaginary line 1 mm long running in a circumferential direction on the artery surface). Table 1 lists intercept densities and varicosity densities for some arteries.

It is not clear what the relation is between density of the perivascular nerve plexus and the extent of innervation of the smooth muscle beneath, but there does seem to be some relation as the strength of contraction that can be elicited by nerve stimulation is correlated with intercept density (Gillespie & Rae 1972) or varicosity density (Griffith et al. 1982). It is not possible to determine which nerves form junctions with the smooth muscle because there seems to be no visible post-junctional specialization in smooth muscle. The presence of varicosities along the axons is no guide, because the axons become varicose long before they reach their target organ (Hillarp 1959; see also Neild & Zelcer 1982). However, it has been possible to find the number of quanta of noradrenaline released following a stimulus to the nerves and relate this to the number of varicosities to find the fraction of varicosities from which release occurred. Results vary from 1 in 7 or 8 (Bevan et al. 1972) to 1 in 60 (Hirst & Neild 1980a), with 1 in 30 regarded as a typical value (Bevan et al. 1980).

From the number of varicosities around an artery and the fraction of those varicosities that release, it is possible to find the rate at which quanta of
Noradrenaline are released during various levels of nerve activity. The number of quanta released by a single stimulus always greatly exceeds the number of axons present as estimated from the intercept density, indicating that one axon releases many quanta.

### Table 1. Innervation density of various arteries

<table>
<thead>
<tr>
<th>Artery</th>
<th>Varicosity density (varicosities/mm²)</th>
<th>Intercept density (axons/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig carotid</td>
<td>1283</td>
<td>25</td>
</tr>
<tr>
<td>Superior mesenteric</td>
<td>2371</td>
<td>20</td>
</tr>
<tr>
<td>Submucous arteriole</td>
<td>5350</td>
<td>86</td>
</tr>
<tr>
<td>Rabbit central ear artery</td>
<td>35,000</td>
<td>—</td>
</tr>
<tr>
<td>Rabbit central ear artery</td>
<td>—</td>
<td>156</td>
</tr>
<tr>
<td>Rabbit central ear proximal</td>
<td>10,561</td>
<td>67</td>
</tr>
<tr>
<td>Rabbit central ear middle</td>
<td>8,293</td>
<td>51</td>
</tr>
<tr>
<td>Rabbit central ear distal</td>
<td>6,537</td>
<td>38</td>
</tr>
<tr>
<td>Rabbit ear tip</td>
<td>4,120</td>
<td>64</td>
</tr>
</tbody>
</table>

Cowen & Burnstock 1980
Cowen & Burnstock 1980
Gillespie & Rae 1972
T. O. Neild, unpublished
Bevan et al. 1972
Gillespie & Rae 1972
Griffith et al. 1982
Griffith et al. 1982
Neild & Keef 1983

### Summary of the simulation procedure

All calculations were carried out to simulate the membrane potential change that would be recorded in the smooth muscle of a small artery when nerve activity released noradrenaline over a region of five space constants on either side of the recording point. Contributions from greater distances would have been negligible (see Jack et al. 1975, p. 53 et seq.). The type of artery was chosen and the number of varicosities in the region of simulation was calculated from the varicosity density and the external diameter. Two situations have been simulated: in the first, the axons were assumed to release their quota of noradrenaline quanta evenly spaced along their length and in the second all quanta were released over a region 400 µm in length. The number of axons was calculated from the intercept density and the circumference. Action potentials in individual axons were assumed to occur randomly at a given mean rate, not correlated with those of other axons. Noradrenaline release was made proportional to nerve activity. Membrane properties of the smooth muscle were assumed to be linear, which constrained the simulations to small depolarizations only. A scaling factor was derived taking into account artery diameter and thickness of the smooth muscle layer, and the absolute amplitude of the voltage changes was found.

### Methods

Voltage transients caused by a given current waveform in cable-like structures were computed by first obtaining the voltage change caused by a brief current pulse (impulse function) and convolving this with the current waveform. For infinitely
long cables the response to an impulse of current applied at a distance $X$ from the recording point is given by Hodgkin (in Fatt & Katz 1951)

$$V = C_0 \frac{1}{\sqrt{T}} \exp \left\{ \frac{-X^2 - 4T^2}{4T} \right\}, \tag{1}$$

where $X$ is the distance in cable length constants. $T$ is the time after the impulse in membrane time constants. $C_0 = Q_0/2cmA/\pi$ where $Q_0$ is the charge in the impulse, $c_m$ is the capacitance per unit length of cable, and $\lambda$ is the cable space constant. For cables of finite length terminated by an open circuit the impulse response at a point $X$ space constants from one end is

$$V = C_0 e^{-T} \left\{ \exp\left(\frac{-(X - Y)^2}{4T}\right) + \exp\left(\frac{(X + Y)^2}{4T}\right) \right\} + \sum_{n=2,4,6,...}^{\infty} \left[ \exp\left(\frac{-(nL - Y - X)^2}{4T}\right) + \exp\left(\frac{(nL - Y + X)^2}{4T}\right) \right] + \exp\left(\frac{-(nL + Y - X)^2}{4T}\right) + \exp\left(\frac{(nL + Y + X)^2}{4T}\right) \right\}. \tag{2}$$

where $Y$ is the distance of current injection from the same end. $L$ is the length of the cable. $C_0$ is the same as in equation (1). Successively higher terms of the series were evaluated and compared with the existing value of the function. The calculation was stopped when the value of a term was less than 0.01% of the current value of the function.

The function chosen to represent the current was:

$$I = Te^{-17T}, \tag{3}$$

when $T$ is time in time constants. This expression gives a good approximation of the current caused in arteriolar smooth muscle by the release of noradrenaline from perivascular nerves (Hirst & Neild 1980a). Convolutions were carried out by the method described by Jack & Redman (1971).

All calculations were made using normalized time ($\tau = 1$) and distance ($\lambda = 1$). The time interval between points was 0.002 time constants. The derivation of the scaling factor used to find the amplitude of the depolarization in millivolts implied that the values of $\tau$ and $\lambda$ were the same as for arterioles of the guinea-pigs submucous plexus, i.e. $\tau = 341$ ms and $\lambda = 1.5$ mm (Hirst and Neild 1980a).

**Results**

**Effect of one action potential in a single axon**

When an action potential is conducted along a perivascular nerve it may release noradrenaline at many points along its length or it may release only from its terminal region or the terminations of branches. There is no experimental evidence to indicate which scheme is the correct one, and so two extreme situations have been considered. Firstly that the axon will release noradrenaline with equal probability along the whole length of the artery that is included in the calculation, i.e. five space constants on either side of the recording point. A single action
potential in a nerve would then give rise to an excitatory junction potential (e.j.p.) of the shape shown in figure 2a. Its amplitude would depend on the number of quanta of noradrenaline released and the diameter of the artery. For this calculation the conduction velocity in the nerve was taken to be $0.21 \, \text{m s}^{-1}$ (Keef & Neild 1982). It makes very little difference if conduction is ignored and release is assumed to be instantaneous along the whole region of calculation (figure 2b), because the conduction time along ten length constants ($= 15 \, \text{mm}$) is only 71 ms, which is short compared to the membrane time constant of 350 ms.

![Figure 2](image)

**Figure 2.** (a) The membrane potential change due to the release of neurotransmitter from many points along a piece of artery as an action potential travels along a single nerve axon. (b) as (a), but conduction along the axon is ignored so that release occurs at all points at the same time. (c) Potential changes due to neurotransmitter release over regions of artery 400 μm ($0.27\lambda$) long at various distances from the recording point. For the largest potential change the region was centred $0.2\lambda$ from the recording point, and distance was increased by $0.5\lambda$ for each successive calculation up to $3.2\lambda$. All calculations are for a period of 3τ.

The second possibility that was considered was that an axon released all its noradrenaline over an area of artery 400 μm in length. The value of 400 μm was chosen because this was the length covered by a single axon innervating an arteriole in the partially denervated iris (Malmfors & Sachs 1965); it is not necessarily typical of all arteries. It can be seen from figure 2c that the amplitude and time course of the e.j.ps would vary considerably with distance between the recording point and area of release. The amplitude will also depend on the amount of noradrenaline released and the artery diameter.

**Effect of continuous activity in many axons**

The membrane potential changes caused by continuous nerve activity can be found by adding the effects of action potentials in single axons shifted appropriately
in time. This procedure is valid only so long as the membrane properties do not change. As soon as the smooth muscle membrane is depolarized sufficiently to generate action potentials the simulation technique used here cannot be applied. In some of the following figures depolarizations of up to 40 mV are shown, but these are included only to give a complete range of calculations.

![Figure 3](http://rspb.royalsocietypublishing.org/) Membrane potential changes in the smooth muscle of arteries of various outside diameters following the onset of perivascular nerve activity at a mean rate of 2 Hz. In this and subsequent figures the calculations were made for a period of 10 s with nerve activity beginning after 1 s.

Figure 3 shows the effects of perivascular nerve activity at a mean frequency of 2 Hz on the membrane potential of the smooth muscle of ‘typical’ arteries of various diameters. Innervation density was characterized as a varicosity density of 4000 mm\(^{-2}\) and an intercept density of 50 mm\(^{-1}\). Release was assumed to occur from 1 in 30 of the varicosities for each action potential, with equal probability anywhere in the simulated region. There was no correlation between the time of occurrence of action potentials either in the same axon or different axons. Calculations were performed for artery diameters of 25, 50, 100 and 150 µm. All were assumed to have a smooth muscle layer one cell thick except for the 150 µm artery where the average thickness was taken to be 1.5 cells. It can be seen that the average depolarization produced by the nerve activity is the same in all arteries except the one of 150 µm diameter. This is a direct consequence of the assumption that the innervation density was the same in all the arteries. There are fewer nerves on the smaller arteries but there is also less smooth muscle so that a given amount of noradrenaline causes a greater depolarization. For the 150 µm artery it was assumed that the muscle layer was thicker, averaging 1.5 cells thick and so the depolarization was less.

The most striking feature of the results shown in figure 3 is that the fluctuations of the membrane potential increase greatly as vessel diameter is reduced. This occurs because the effect of a single action potential in a nerve is much greater on a small artery than on a large one. The amplitude of the fluctuations also increases as the mean rate of nerve activity increases (figure 6), as would be expected for a system in which many identical discreet events add to give the depolarization (Rice 1954).

An important consequence of the larger membrane potential fluctuations in small vessels is that they should reach threshold for action potential generation.
Nerves on smooth muscle membrane potential

25 μm diameter

50 μm

100 μm

150 μm

20 mV threshold

10 mV

1 s

Figure 4. The effect of 5 Hz nerve activity on arteries of various diameters. When the depolarization exceeded 20 mV a spike was drawn on the record to represent an action potential, provided that no action potential had occurred in the previous second. The smaller arteries were more likely to produce action potentials.

at lower frequencies of nerve activity. The action potential threshold in arterial smooth muscle is usually about 15–30 mV from the resting potential recorded in vitro. Figure 4 shows the effect of assuming a threshold of 20 mV from resting potential on the effect of 5 Hz nerve activity on various arteries. When the membrane potential exceeded threshold a spike was drawn on the record to

Figure 5. The effect of 2 Hz nerve activity but on the assumption that an axon releases all its neurotransmitter in one region over a 400 μm length of artery. The membrane potential fluctuations are much greater than in figure 3 for which it was assumed that release occurred along the whole length of the axon in the region used for calculation.
represent an action potential, provided that no spike had been drawn in the previous second. It is clear that the smaller arteries generate more action potentials.

For the above calculations it was assumed that release from one axon could occur along the whole length of the region of artery used for the calculation (i.e. 15 mm). If it was assumed that each axon released only onto a region 400 μm long as

\[
\text{(a) 150 μm diameter rabbit ear tip 2,4,8,12 Hz nerve activity}
\]

\[
\text{(b) 50 μm diameter guineapig submucous arteriole}
\]

discussed above, the membrane potential fluctuations were larger and more irregular (figure 5). This is because in effect fewer axons influence the membrane potential at the recording point, but those whose 400 μm region falls close to the recording point cause a large transient e.j.p. Those more than 3 mm away have little effect (see figure 2c). The effects of different artery diameters or of changing the rate of nerve activity are qualitatively the same as when release can occur all along the axon.

Calculations for specific arteries

The preceding calculations were performed for hypothetical arteries with typical properties. For the arterioles of the guinea-pigs submucous plexus all the information required for the calculations is known. The arterioles have one layer of smooth muscle cells (Hua & Cragg 1980). The electrical properties of the muscle are known, and the one-dimensional cable equations can be applied (Hirst & Neild 1978). The innervation density has been determined (table 1) and the fraction of varicosities releasing was found to be 1 in 60 (Hirst & Neild 1980 a). From this data the effects of nerve activity on a 50 μm external diameter arteriole were calculated and they are shown in figure 6b. A mean rate of nerve activity of 4 Hz produced a depolarization that varied between 15 and 20 mV which is about the threshold of the muscle for action potential generation. At this frequency the contractile mechanism of the muscle would certainly be activated by action potentials, but probably not to the maximum extent. At higher frequency the continuous activation of voltage-dependent membrane conductances would pro-
probably mean that the calculations shown here would not be valid. However, some higher frequencies are included because substances such as prazosin and phentolamine (Hirst & Neild 1981; Neild & Zelcer 1982) do raise the muscle threshold for action potential generation and presumably the membrane conductances are insensitive to depolarization over a greater range in the presence of these drugs.

The structure and innervation density of the small arteries in the tip of the rabbit ear is also known, and these vessels are of particular interest because the membrane potential of the smooth muscle has been recorded in the intact animal (Neild & Keef 1983). Unfortunately no information is available as to the fraction of varicosities that release transmitter during the passage of an action potential along the perivascular nerves, and a value of 1 in 30 has been assumed. An even more serious limitation is that these arteries may be too large for the one-dimensional cable approximation to be valid. If the calculation is valid it illustrates why Neild & Keef (1983) recorded no excitatory junction potentials from these arteries even though there was evidence of continuous perivascular nerve activity. At the frequencies of nerve activity expected, about 1–2 Hz, there would have been an irregular depolarization of a few millivolts (figure 6a) but nothing resembling an excitatory junction potential.

**Discussion**

There have been few successful recordings of the membrane potential of mammalian smooth muscle under the influence of natural nerve activity (Speden 1964; Steedman 1966; Neild & Keef 1983) and they resemble the simulations shown in figures 3 and 4. No large e.j.ps were recorded except after nerve stimulation. There was less variation of membrane potential than in the simulations shown in figure 5, suggesting that individual axons were not releasing their share of noradrenaline over a single small region.

The calculations here suggest that the smaller arterioles will generate action potentials in their smooth muscle and constrict at lower frequencies of nerve activity than large arteries. This has often been observed when using synchronous electrical nerve stimulation (for example Marshall 1982), although experiments of the same precision have not been performed using natural stimulation.

It should be pointed out that action potentials are not the only cause of contraction of arterial smooth muscle. Contraction resulting from the stimulation of alpha receptors by noradrenaline occurs without action potentials (Somlyo & Somlyo 1968; Hirst & Neild 1980b; Neild & Zelcer 1982) and there is evidence that nerve-released noradrenaline can reach alpha receptors on arteries (Cheung 1982).

In small arteries of the rabbit ear Neild & Keef (1983) showed that the resting arterial tone did not depend on action potentials. Action potentials are probably involved only in intense vasoconstrictions after sudden increases in sympathetic activity. Gregor & Janig (1977) showed that there was a significant increase in vascular resistance in skeletal muscle when the firing rate of its sympathetic vasoconstrictor nerves increased from 2 to 6 Hz. Vascular resistance continued to
rise as the nerve activity increased to 13 Hz. The calculations presented here, which suggest that action potential generation might start at about 3 Hz in the smallest arterioles, are in remarkably good agreement with this observation.

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References
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