Microelectrode studies, which are difficult to perform on mammalian blood vessels, have provided a few records of action potentials from mammalian arteries and veins. These studies indicated that epinephrine or sympathetic nerves could, at least on some occasions, induce action potentials in the smooth muscle cells. Two experiments had suggested that such electrical changes might play no part in causing the mechanical response of mammalian blood vessels to vasoconstrictor agents. Thus one group, using microelectrodes, was not able to record any change in the cell membrane potential of rabbit pulmonary arteries during contractions induced by noradrenaline, while purely mechanical studies suggested that canine mesenteric arteries gave the same contractile response to noradrenaline when in, and presumably depolarized by, potassium-rich solution as they did in sodium-based solution.

However, evidence that electrical changes did play a part in causing mechanical responses to vasoconstrictor agents was obtained from experiments in which a sucrose-gap technique was used to provide prolonged records of both electrical and mechanical activity of sheep carotid arteries. The results indicated that norepinephrine, epinephrine and histamine all caused depolarization which was often accompanied by a spike, indicating discharge of action potentials by many of the smooth muscle cells. The close association of electrical and mechanical activity, on rare occasions when repetitive discharges occurred, provided evidence that the electrical changes caused contraction; although they were not the sole cause because the hormones could contract, without electrical changes, arteries which were already depolarized by potassium-rich solution.

The present paper describes similar electrical and mechanical studies with vasodilator agents, including studies of the constrictor action that the vasodilator agents bradykinin and acetylcholine exert on some vessels, and studies with the constrictor hormone angiotensin which is chemically similar to bradykinin. Most of the studies were performed on common carotid arteries of sheep but a few were made on common carotid arteries of dogs, partly to see whether arteries of this species give any electrical responses at all to vasoactive hormones and partly to investigate the dilator action of acetylcholine which could be demonstrated in arteries of dogs but not of sheep.

**Methods**

Most experiments were performed on right common carotid arteries removed from sheep within 10 minutes after the animals had been killed at a slaughterhouse by a captive bolt. Others were performed on common carotid arteries taken from mongrel dogs killed by 100 to 200 mg/kg of pentobarbitone iv (Nembutal, Abbott). Arteries were cut into spiral strips 1 to 2 mm wide and the strip mounted for electrical and mechanical recording in the apparatus previously described; in the present experiments calomel electrodes were used instead of Ag-AgCl electrodes and KCl agar bridges. Contractions of the portion of the strip in the test solution, 8 to 12 mm long, were recorded isotonically at a tension of 2.5 ± 0.2 g, approximately the tension present in vivo, by paired silicone strain gauges. Some of the weaker mechanical effects were confirmed by additional experiments in which

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simple mechanical records were obtained from strips 25 mm long, by means of direct ink-writing isotonic levers (2.5 ± 0.2 g) and a kymograph. Except when otherwise stated experiments were done with the test portion of artery in solution at 35°C.

The standard saline solution contained (mm): NaCl 133; NaHCO₃ 16.3; NaH₂PO₄ 1.38; KCl 4.7; CaCl₂ 2.5; MgCl₂ 0.105, and dextrose 7.8. The potassium-rich solution contained (mm): NaCl 3.3; KHCO₃ 16.3; NaH₂PO₄ 1.38; K₂SO₄ 89.6; CaCl₂ 2.5; MgCl₂ 0.105 and dextrose 7.8 "Analar" reagents were used. Both solutions were equilibrated with a mixture of 5% CO₂ + 95% O₂.

The drugs used were acetylcholine chloride (L. Light and Company); synthetic bradykinin (Sandoz); synthetic angiotensin (valine-5-hypertensin amide, Ciba); amyl nitrite (B.D.H.); synthetic bradykinin (Sandoz); L-epinephrine; L-norepinephrine bitartrate; histamine acid phosphate (L. Light and Company); Hydergine (Sandoz), a mixture of dihydroergocornine, dihydroergocristine and dihydroergokryptine; phenolamine (Rogitine, Ciba) and hexamethonium bromide (Vegolysen, May and Baker). Apart from bradykinin, phenolamine and hexamethonium bromide which were obtained in solution, concentrated solutions (2.5 to 10 mg/ml) of the drugs were made up in distilled water on the day they were to be used.

Results

EFFECT OF AMYL NITRITE AND SODIUM NITRITE (SHEEP CAROTID ARTERIES)

The effects of dilator agents were studied first on arteries which had been stimulated by a constrictor agent. When arteries had been partly contracted and depolarised by norepinephrine (0.25 mg/100 ml) or histamine (2.5 mg/100 ml), either amyl nitrite or sodium nitrite (10 mg/100 ml) caused repolarisation followed by mechanical relaxation. The amount of repolarisation varied between 0.5 and 2.5 mv (5 experiments with each drug) and the mechanical relaxation between 2 and 4 mm. The only difference between the actions of the two drugs was that amyl nitrite caused more abrupt and often larger effects than sodium nitrite. Even with amyl nitrite, neither the electrical nor the mechanical changes were as abrupt as the depolarisation and contraction produced by constrictor agents. Figure 1 shows a typical experiment. Norepinephrine produced a spike of depolarisation followed by a lower level of sustained depolarisation, and amyl nitrite, 10 minutes later, produced repolarisation which preceded the onset of me-

![Figure 1](https://circres.ahajournals.org/)

**FIGURE 1**

*Electrical and mechanical response of sheep carotid artery to norepinephrine 0.25 mg/100 ml followed by amyl nitrite 10 mg/100 ml and then by reduction in concentration of amyl nitrite with norepinephrine still present. NADR: norepinephrine. Lower trace is electrical, upper is mechanical.*

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mechanical relaxation by several seconds. The concentration of amyl nitrite was then reduced since it was steadily carried away as vapour by the gas mixture oxygenating the solution, while the norepinephrine remained; 9 minutes after the addition of amyl nitrite depolarisation returned with two spike discharges, each of which was followed by contraction.

Neither amyl nitrite nor sodium nitrite produced significant hyperpolarisation (<0.25 mV) or relaxation (<0.5 mm) in arteries which had been left to relax for two hours in standard saline, and had not been exposed to any constrictor agent (5 experiments). Figure 2 gives an example. It shows that although sodium nitrite caused repolarisation and relaxation of an arterial strip contracted by norepinephrine it had no effect on the same strip in the absence of norepinephrine.

Both amyl nitrite and sodium nitrite relaxed arterial strips which had been contracted and completely depolarised by potassium-rich solution. These relaxations took place without any detectable electrical change (2 experiments with each drug). Figure 2 gives an example.

The nitrites had no appreciable electrical or mechanical action at 5°C, even on arteries which had been partly depolarised and contracted by histamine before they were cooled (2 experiments with each drug).

In order to see whether the nitrites were producing their actions by stimulating inhibitory nervous structures in the vessel wall the actions of nitrites were studied in the
presence of agents which would be expected to block nervous activity or neurohormones. Both amyl nitrite and sodium nitrite (one experiment in each case) were found to produce their usual effects of repolarisation and relaxation on arteries which had been exposed for 30 minutes to nicotine (2.5 mg/100 ml), hexamethonium bromide (1 mg/100 ml), Hydergine (1 mg/100 ml) and atropine (1 mg/100 ml), and which had then been partly contracted by histamine 2.5 mg/100 ml. It is therefore highly probable that the nitrites exerted their effects directly on the vascular smooth muscle cells.

**ACTION OF EPINEPHRINE AND NORADRENERGIC BLOCKING AGENTS (SHEEP CAROTID ARTERIES)**

When arteries were exposed to phentolamine (10 mg/100 ml) or Hydergine (1 mg/100 ml) for 10 to 30 minutes in order to block the stimulant action of catecholamines, and were then contracted and depolarised by histamine (2.5 mg/100 ml), epinephrine or noradrenaline caused relaxation of up to 4 mm. The relaxation was accompanied usually (5 out of 7 experiments) by a small repolarisation (up to 1.5 mv). Both the electrical and the mechanical changes were weaker than with the nitrates, and the electrical changes were barely outside the random fluctuations of the base line (fig. 3). Epinephrine was rather more effective than norepinephrine. Epinephrine in the presence of phenotolamine, like the nitrates, relaxed arteries in potassium-rich solution without electrical changes (2 experiments), and at 5°C had no demonstrable electrical or mechanical effect on arteries previously contracted and depolarised by histamine at 35°C (2 experiments).

**ELECTRICAL AND MECHANICAL RESPONSE TO BRADYKININ AND ANGIOTENSIN (SHEEP CAROTID ARTERIES)**

Bradykinin either caused contraction or had no effect on sheep carotid arteries, whether the arteries were initially relaxed or whether they were contracted by norepinephrine. In a concentration of 0.01 mg/100 ml bradykinin had no detectable electrical or mechanical effect in 6 out of 15 relaxed arteries, but in the other 9 it caused contraction of 0.3 to 1.5 mm accompanied by depolarisation of 1 to 8 mv (fig. 4 top, left). The electrical and mechanical responses to bradykinin differed from those to norepinephrine, epinephrine and histamine in dying away although exposure to the drug continued. With bradykinin in 0.01 mg/100 ml, the mechanical response largely died away within 10 minutes; higher concentrations of bradykinin caused responses of sharper onset but shorter duration, and lower concentrations caused smaller but more prolonged depolarisation and contraction.

Angiotensin (0.05 mg/100 ml) like bradykinin caused depolarisation (up to 9 mv) and contraction (up to 5 mm) (fig. 4, bottom left). It produced these effects on all of 11 arteries, and as with bradykinin the responses died away in spite of the continued presence of the hormone, though less rapidly and less completely, taking 15 to 20 minutes to decline to 50% of their initial height. Arteries which had relaxed and repolarised during prolonged exposure to one of these hormones would not respond to increased concentration of the same hormone but they could still be depolarised and contracted by the other hormone, so that bradykinin and angiotensin clearly acted on...
Electrical and mechanical response of sheep carotid artery to bradykinin 0.01 mg/100 ml and to angiotensin 0.05 mg/100 ml in standard saline (left) and in potassium-rich solution after partial relaxation by sodium nitrite, 10 mg/100 ml (right). Lower traces are electrical on left hand records, mechanical on right hand records. N.B. Lower left record was obtained 15 minutes after upper left record, in the presence of bradykinin, 0.06 mg/100 ml. Lower right record was obtained 15 minutes after upper right record in the presence of bradykinin, 0.08 mg/100 ml. For explanation see text.

Different receptor sites. Figure 4, bottom left, shows an example; this response to angiotensin was obtained from an arterial strip which had responded to bradykinin 0.01 mg/100 ml (fig. 4, top left), had then been left for 10 minutes until the contraction had died away, had failed to respond to the addition of a further 0.05 mg/100 ml of bradykinin, and was in the presence of 0.06 mg/100 ml of bradykinin when the angiotensin was applied.

Unmistakable evidence of action potentials in the form of large discrete spikes was not obtained with bradykinin and angiotensin as it has been with epinephrine, norepinephrine and histamine. However, the abruptness of depolarisation at the beginning of responses to bradykinin and angiotensin (fig. 4, left) suggests that there was propagation of action potentials through a limited number of the smooth muscle cells of the arterial strip.

It may be noted that the contractions induced by both hormones continued after repolarisation had started. This might be due to the fact that the cell membrane at this time was still depolarised compared to its resting potential, and in consequence of this was still inducing contraction. Alternatively, it might have been due to the ability of the hormones to induce contraction by nonelectrical means (see below). Continued contraction during repolarisation was often seen in responses to all the constrictor agents used in these experiments.

When arteries were depolarised by exposure to potassium-rich solution at 35°C for 20 minutes, and were then partially relaxed by amyl nitrite (10 mg/100 ml), bradykinin, and angiotensin, like other constrictor agents, caused contraction but without any detectable electrical changes (fig. 4, top and bottom right). Again, the contractile response to bradykinin 0.01 mg/100 ml died away within 10 minutes and addition of a further 0.05 mg/100 ml of bradykinin had no effect, but angiotensin 0.05 mg/100 ml caused contraction. Bradykinin and angiotensin resembled other constrictor and dilator agents in failing to produce any significant depolarisation or contraction at 5°C (5 experiments).

Bradykinin and angiotensin can release catecholamines from the adrenal medulla and it seemed possible that they acted on the arteries, as nicotine does, by releasing norepinephrine from sympathetic nerves. The effects of bradykinin and angiotensin were therefore tested again in two arteries after exposing the arteries from 20 minutes to Hydergine (1 mg/100 ml) to block the action of norepinephrine released by sympathetic nerves. The hormones still produced large depolarisations and contractions, indicating that at least the greater part of the action of the hormones was a direct one on the vascular smooth muscle cells. Further evidence that the major part of the action of bradykinin and angiotensin was not exerted via nerves was
obtained from an experiment in which large electrical and mechanical responses to both hormones were obtained from an artery which had been exposed to nicotine (2.5 mg/100 ml), hexamethonium bromide (1 mg/100 ml), Hydergine (1 mg/100 ml), and atropine (1 mg/100 ml) for 30 minutes before the hormones were applied (fig. 5).

**ACTION OF ACETYLCHOLINE (SHEEP CAROTID ARTERIES)**

Acetylcholine, like bradykinin, was previously shown to cause depolarisation and contraction of sheep carotid arteries even after its action via sympathetic nerves had been blocked by nicotine. Its action has now been tested on three arteries depolarised by exposure for 30 minutes to potassium-rich solution, partly relaxed by sodium nitrite (10 mg/100 ml) and exposed to nicotine (2.5 mg/100 ml) to block any action via nerves. Acetylcholine (2.5 mg/100 ml) then produced small contractions (approximately 0.25 mm) without any electrical changes.

Acetylcholine (2.5 mg/100 ml) had no electrical or mechanical effect on three arteries in standard saline at 5°C.

In order to see if acetylcholine could exert any relaxant action on these arteries when they were initially contracted, three strips were contracted by norepinephrine (0.25 mg/100 ml), and nicotine (2.5 mg/100 ml) was added to block any action on nerves. The addition of acetylcholine (2.5 mg/100 ml) then produced a small contraction followed in each case by relaxation to a length just greater than that before the acetylcholine was added. With the exception of the late phase of this biphasic effect, no relaxant action of acetylcholine was obtained in the sheep arteries.

**ACTION OF NOREPINEPHRINE AND ACETYLCHOLINE ON CANINE CAROTID ARTERIES**

When norepinephrine (2.5 mg/100 ml) was applied to three canine carotid arteries it produced depolarisation and contraction (fig. 6). The electrical and mechanical responses were not so large (<2 mv) or abrupt as...
responses of sheep carotid arteries to nor-
epinephrine and there were no spike discharges. 
Nicotine (2.5 mg/100 ml) was then added to 
block any stimulation of nerves. Acetylcholine 
(2.5 mg/100 ml) then caused a slight 
relaxation in two, and slight contraction in 
the third artery. Bradykinin caused small 
contractions (2 experiments). Relaxation pro-
duced by acetylcholine appeared to be ac-
companied by repolarisation and the contrac-
tion produced by acetylcholine or bradykinin 
by depolarisation, but these electrical changes, 
being within the limits of random fluctuations 
of the base line, were too small for confidence.

Discussion

The finding that the relaxant action of the 
nitrites on sheep carotid arteries was accom-
panied by and presumably in part due to an 
increase in membrane potential is in keeping 
with the known effects of relaxant agents on 
intestinal smooth muscle; epinephrine is 
known, for instance, to cause hyperpolarisa-
tion and relaxation of taenia coli. The 
ability of the nitrites to relax arteries depo-
larised by potassium-rich solution, and to 
do so without electrical changes also paral-
lels results obtained from other smooth muscle, 
since epinephrine can relax rat uterus in 
potassium-rich solution. However, the elec-
trical effects of relaxant agents on these 
arteries, as well as their mechanical effects, 
could be demonstrated only in arteries which 
had been contracted by some stimulant agent. 
This contrasts with the ability of epinephrine to 
produce hyperpolarisation and relaxation in 
unstimulated taenia coli, and suggests that 
the smooth muscle cells of unstimulated ar-
teries, unlike those of some smooth muscles, 
were electrically very stable and were me-
chanically almost fully relaxed. These arteries 
are probably representative of other mam-
malian arteries in these respects, since other 
arteries have little spontaneous mechanical 
tone.

The fact that the nitrites could increase the 
membrane potential of arteries depolarised 
by stimulant hormones, but not that of un-
stimulated arteries, suggests that they acted 
by increasing the permeability of the cell 
membrane to potassium or chloride ions in 
the same way that inhibitory agents are known 
to do in other tissues. Acetylcholine for in-
stance produces its inhibitory effect on the 
electrical activity of cardiac muscle mainly by 
increasing permeability to potassium and 
an inhibitory neurohormone depresses moto-
neurones mainly by increasing their permeability to chloride. An increase in the permeabil-
ity of the arterial cell membrane to either of 
these ions would be expected to repolarise 
cells whose membrane potential had been 
lowered by stimulant hormones but would 
not be expected to have much effect on the 
cells of resting arteries, whose membrane 
potential was high and presumably close to 
the potassium and chloride equilibrium value.

These results provide no evidence about the 
means by which the stimulant agents caused 
contraction and the relaxant agents caused 
relaxation of arteries whose cells were already 
de polarised by potassium-rich solution. Since 
there is some evidence that carbachol, an 
adrenaline analogue, increases the permea-
bility of intestinal smooth muscle to calcium 
as well as to other ions the stimulant agents 
might possibly act by increasing the mem-
brane permeability of the arterial smooth 

muscle cells, allowing calcium to enter the 
cells and cause contraction, and relaxant hor-
mones might produce the reverse effect. An 
alternative or additional hypothesis is that 
stimulant hormones may cause contraction by 
releasing bound calcium in smooth muscle 
cells; again, relaxant agents might produce 
the reverse effect. This latter possibility is a 
particularly attractive one for sheep carotid 
arteries since they can contract and relax in 
the presence of very low extracellular concen-
trations of calcium.

It may seem surprising that bradykinin and 
acetylcholine caused depolarisation and con-
traction of the sheep arteries, since it is well 
known that both these hormones increase limb 
blood flow in cats and in man. However acetylcholine has been shown to cause contraction in vitro of several large 
blood vessels of sheep, cattle, pigs, and
rabbits and bradykinin of at least one, the coronary artery of sheep. These apparent contradictions might be due in part to differences in behaviour under in vitro and in vivo conditions, but must be due in part to species differences since both hormones dilate canine coronary arteries in vitro, and a dilator action of acetylcholine on canine carotid arteries was sometimes demonstrated in vitro in the present experiments.

The inability of either constrictor or relaxant agents to produce electrical or mechanical effects at 5°C indicates that an early step in their interaction with the cell membrane, possibly a step common to all the agents, failed at low temperature. Evidence of a similar failure in the constrictor action of noradrenaline at low temperatures, which is the probable cause of cold vasodilatation, has been presented previously. The present findings have the practical implication that administration of vasodilator agents is unlikely to increase blood flow in cold extremities and so is unlikely to be of value in averting the risk of frostbite.

**Summary**

Amyl nitrite or sodium nitrite repolarised and relaxed sheep carotid arteries which had been depolarised and contracted by norepinephrine or histamine. These agents had little electrical or mechanical effect on unstimulated arteries. Epinephrine in the presence of phentolamine or Hydergine produced similar effects but the electrical changes were smaller and more variable than with the nitrates. All these agents could cause some relaxation, without electrical changes, of arteries which had been completely depolarised by potassium-rich solution.

Bradykinin and angiotensin, like acetylcholine, depolarised and contracted the arteries even after actions via nerves had been blocked by nicotine and other agents. The effects that they produced when in sustained high concentration were limited in duration. All three agents could contract arteries which were completely depolarised by potassium-rich solution, without any electrical change.

None of these relaxant or stimulant agents had any significant effect at 5°C.

In canine carotid arteries, norepinephrine produced depolarisation and contraction as it does in sheep carotid arteries, but acetylcholine had variable electrical and mechanical effects.

The results suggest that all these vasodilator and vasoconstrictor agents produce their mechanical effects on the arteries partly by changing the cell membrane potential and partly by other means.

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Electrical and Mechanical Responses of Vascular Smooth Muscle to Vasodilator Agents and Vasoactive Polypeptides
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