Relation Between Membrane Potential and Contractile Force in Smooth Muscle of the Rat Tail Artery During Stimulation by Norepinephrine, 5-Hydroxytryptamine, and Potassium

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The relation between smooth muscle membrane potential and contractile force was investigated in the rat tail artery to assess the importance of smooth muscle depolarization in the control of smooth muscle tone. Smooth muscle membrane potential and contractile force were measured simultaneously in isolated pieces of rat tail artery exposed to a range of concentrations of norepinephrine, 5-hydroxytryptamine, or raised external potassium. Potassium caused depolarization and contraction when the membrane was depolarized beyond $-40 \text{ mV}$. Maximum contraction occurred at $-19 \text{ mV}$, and further depolarization gave no increase in contraction. Both norepinephrine and 5-hydroxytryptamine caused contraction and depolarization, but the relation between depolarization and contraction was not the same as when potassium was used. There was significant contraction when the membrane potential was more negative than $-50 \text{ mV}$, and the membrane potential was around $-30 \text{ mV}$ during maximal contractions. Although they acted on pharmacologically different membrane receptors, the relation between membrane potential and contraction was the same for norepinephrine and 5-hydroxytryptamine. Prazosin reduced the responses to norepinephrine but did not change the relation between membrane potential and contractile force. These results indicated that norepinephrine and 5-hydroxytryptamine binding to their respective receptors might activate the same sets of intracellular processes that subsequently caused both depolarization and contraction.

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relation between contractile force and depolarization for 2 different agonists (NE and 5HT) that cause contraction by pharmacomechanical coupling and to compare this with the relation when potassium was used to cause contraction by depolarization alone. It was found that the relation was the same for both NE and 5HT, even though they acted on different membrane receptors. Partial block of the NE receptors by prazosin also did not affect the relation. This suggests that stimulation of either receptor activates the same intracellular processes or membrane channels.

Materials and Methods
Wistar rats of either sex and weighing 230–300 g were killed by stunning and decapitation. The central tail artery was dissected out and a piece 2–2.2 mm long was taken from a region 80–90 mm from the base of the tail. The piece of artery was mounted on an apparatus that permitted the simultaneous recording of smooth muscle force in the circumferential direction and smooth muscle membrane potential. The artery was held in place by 2 parallel tungsten wires stretched tightly and threaded through the lumen. The separation between the wires was adjusted to 800 μm, which gave a resting force reading of 10–15 mN. A pair of platinum wires were positioned one on either side of the artery and used to stimulate the perivascular nerves. The artery was superfused with a physiological saline solution containing a mmol/l concentration of Na 145, K 5, Ca 2.5, Mg 2, Cl 134, HCO3 25, H2PO4 1, and dextrose 11. The solution was equilibrated with 95% O2–5% CO2. Solutions with raised potassium concentration were made by replacing sodium chloride with an equimolar amount of potassium chloride. It was warmed so that the temperature at the artery was 32°C, a physiological temperature for this artery. This had been determined in preliminary experiments in which a thermocouple was implanted in the tail of anesthetized rats.

Membrane potentials were recorded using glass microelectrodes rigidly attached to a micromanipulator. They were made from 1.5 mm o.d. borosilicate glass with an internal fiber to facilitate filling (Clark Electro medical Instruments Model GC150F-15, Reading, U.K.). They were filled with 2 M KCl and had resistances in the range 80–120 MΩ.

Criteria for Accepting a Recording of Membrane Potential
A recording of membrane potential in the presence of a constrictor agonist was accepted only if 1) the microelectrode remained in the cell throughout the contraction or 2) the microelectrode was dislodged during the onset of contraction but could be reinserted during the plateau of the contraction, and after removal of the agonist it gave a reading of resting membrane potential that was the same as before the agonist was applied. These criteria were not fulfilled in many experiments, but when they were also noted that the resting membrane potential was particularly stable and consistent if repeated penetrations were made. The data in this paper come from 22 arteries in which a full range of concentrations of one agonist was tested. Additional data have also been included from other experiments in which one or two concentrations of one of the agonists was used in the course of the experiments designed to investigate other matters, and where the recording conditions were good enough for the above criteria to be fulfilled.

Drugs used were norepinephrine bitartrate (Sigma), 5-hydroxytryptamine creatinine sulphate complex (Sigma), prazosin hydrochloride (Pfizer) and phentolamine mesylate (Regitine, Ciba).

Results
Effects of Norepinephrine and 5-Hydroxytryptamine
The arteries were exposed to various concentrations of either NE or 5HT. The order in which different concentrations were used was random, and a recovery period was allowed between each application. Both drugs caused constriction and depolarization; some typical experimental records are shown in Figure 1.

The depolarization caused by either drug was usually steady, as shown in Figure 1, but occasionally the membrane potential would show oscillations of a few millivolts amplitude at 0.3–1 Hz. These sometimes gave rise to action potentials in the smooth muscle and small increments of contractile force associated with each action potential. The relation between the concentration of NE or 5HT and membrane potential and contractile force is summarized in Figure 2.

The maximum force produced by the NE and 5HT was the same (p > 0.2, paired t test, n = 15). The EC50 for contraction, determined for each artery by the method of Nakashima et al, was 1.03 μM ± 0.023 (SEM, n = 6) for NE and 58.9 nM ± 1.1 (SEM, n = 6) for 5HT.

The experiments using NE were repeated in the presence of a moderate concentration (10 nM) of the α-adrenoceptor antagonist prazosin. This reduced both the contraction and depolarization produced by NE, but near maximal responses could still be obtained (Figure 2, open circles). Prazosin or phentolamine in concentrations up to 1 μM had no effect on the responses to 5HT, showing that its actions did not involve α-adrenoceptors.

Effects of Extracellular Potassium
Raising extracellular potassium concentration caused depolarization and contraction of the smooth muscle. Prazosin (200 nM) was added to the solutions in these experiments to block the effects of any NE that might be released from the perivascular nerves. If no drugs were added to block α-adrenoceptors, the depolarization and tension produced by raised potassium was greater and less reproducible. The addition of 200 nM prazosin was sufficient to block all responses to NE in concentrations up to 1 μM.

The artery was exposed to a range of extracellular potassium concentrations from the normal value of 5 mM up to 100 mM. The order was randomized, with a
return to normal between each elevated concentration. Raised potassium caused a sustained depolarization; action potentials were sometimes recorded on the rising phase of the depolarization as potassium was being increased, but none were recorded once the depolarization approached its final level, nor were there any oscillations of membrane potential such as those caused by NE or 5HT.

Potassium concentrations up to 20 mM caused no contraction. At higher concentrations, there was a dose-dependent contraction that reached a maximum at 60 mM potassium. Higher concentrations produced the same contractile force, although they caused greater depolarization. The maximum force produced by potassium was 62.4% ± 2.1 (SEM, n ± SEM) of the maximum force produced in the same artery by 5HT.

The mean depolarization and contraction of 6 arteries are plotted in Figure 3.

**Discussion**

The results presented above are summarized in Figure 4, in which contractile force is plotted against membrane potential. The most striking feature is that the relation was the same when NE and 5HT were used, despite the fact that these agonists act on different membrane receptors. (NE on α-adrenoceptors that are mainly α₁ and 5HT on 5HT₂ receptors.¹³) The results for these 2 agonists are shown on separate graphs; the 2 sets of points would overlap throughout their range if plotted on the same graph.

When NE was used, partial block of the α-receptors reduced both the depolarization and the contractile force but did not change the relation between them. This suggested that both depolarization and contraction were due to activation of the same receptor and that it was an α₁-receptor.

These results are compatible with the idea that activation of an α₁ or 5HT receptor changes the level of a common intracellular messenger that influences both contractile activity and membrane conductances. The identical relation between membrane potential and contractile force following activation of either receptor is a strong quantitative indication that they are coupled to the same process. In other tissues, α₁-receptor stimulation has been shown to increase the activity of a phospholipase C in the membrane, leading to increased levels of inositol triphosphates, diacylglycerols, protein kinase C activity, and intracellular calcium.¹⁴ Complete data are not available for vascular smooth muscle, although it is clear that inositol triphosphate releases calcium from the sarcoplasmic reticulum.¹⁵ In the rat tail artery, contraction evoked by either α₁-receptor stimulation or increased protein kinase C activity share a common biochemical pathway.¹⁶

When potassium was raised, the smooth muscle had
to be depolarized to $-50$ mV before any contractile force was produced, as reported previously for this artery by Cheung.\textsuperscript{17} However, the force produced was very small, and as seen in Figure 4, significant force was associated with depolarization beyond $-40$ mV. This agrees with the results of previous work\textsuperscript{2} and coincides with the level at which voltage dependent calcium channels in arterial smooth muscle begin to be activated.\textsuperscript{3} Therefore, the contraction in raised potassium was probably due to a calcium influx through voltage-dependent membrane channels. The contraction reached a maximum when the depolarization was $-19$ mV, and further depolarization produced no increase in force. This may correspond to full activation of the calcium channels.

NE and SHT in concentrations that caused contraction greater than 20% of maximum depolarized the smooth muscle beyond threshold for contraction as determined from the experiments with potassium. The depolarization could therefore contribute an additional component to the contraction independent of the pharmacomechanical coupling, and any hyperpolarization that effectively removed the depolarization-dependent component would cause a partial relaxation. Relaxation associated with hyperpolarization has been observed following electrical stimulation\textsuperscript{18} or release of endothelium-derived relaxing factor.\textsuperscript{19} It appears that depolarization may play a significant part in contraction generated by higher concentrations of NE.

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**References**


**Figure 3.** Graphs showing the relation between external potassium concentration (log scale), contractile force (left panel), and membrane potential (right panel). Averaged data from 6 arteries, bars show SEM. Contractile force has been normalized to the maximum force produced by SHT. The dotted line on the right hand graph has a slope of RT/ZF = 60.5 mV/decade potassium: the points would fall on this line if the membrane potential was determined only by potassium.

**Figure 4.** Graphs of membrane potential against contractile force. Left panel: contraction caused by NE (○), NE + 10 nM prazosin (□), and potassium (□). Right panel: contraction caused by SHT (●), and potassium (○, same data as left). Contractile force has been normalized to maximum force produced by NE or SHT. Bars show SEM. Data for potassium was obtained from 6 arteries, and was also used for Figure 3. Data for NE and SHT was the same used for Figure 2.
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