ELECTRICAL AND MECHANICAL RESPONSES OF CORONARY ARTERY SMOOTH MUSCLE TO CATECHOLAMINES

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There are conflicting reports concerning the actions of adrenaline and noradrenaline on smooth muscle of coronary artery. Some authors have observed that both adrenaline and noradrenaline decrease the coronary flow.1,2) But most workers have reported that in most coronary vessel preparations, including the fibrillating heart, heart-lung preparation, the open-chest animal and the unanesthetized animal, dilatation is the final result of the action of these drugs.3,4,5,6) Others have, however, suggested that their primary action on coronary smooth muscle is vasoconstriction and the increased coronary flow due secondarily to effects of metabolites produced by increased activity of the myocardium with these drugs.7) It would seem that such a clear cut issue could be resolved by separating the coronary vessel from the myocardium and observing the direct effects of catecholamines on the vascular smooth muscle. However such studies have indicated contradictory results as well as in the unisolated perfused heart.8,9,10,11) It is interesting that some among these reports indicated that the effects of adrenaline and noradrenaline on isolated strips from large coronary vessels are clearly different from their effects on small ones.12)

Most current works on the electrophysiology of the visceral smooth muscle have suggested that change in spike activity or membrane potential is normally a necessary prelude to change in tension induced with adrenaline.13,14) It has recently been reported that contraction of some vascular preparations elicited by adrenaline or noradrenaline also was accompanied by depolarization or an increase in spike activity of the cell membrane.15,16,17,18) Su et al. however found that in pulmonary artery, sympathetic nerve stimulation and 1-noradrenaline cause contraction without changes in the membrane potential and the absence of action potential.19) We designed the present experiment in order to observe mechanical responses of the coronary artery
METHOD

Mongrel dogs were killed by beating. The hearts were removed immediately and stored (never than 5 hours) in Krebs Ringer solution at 1°C. The segments of the left descending coronary arteries were dissected from myocardium and stripped of loose fat and connective tissue. Ring strips isolated from the segments of small (0.9 to 1.5 mm outside diameter) and large (2.0 to 4.0 mm O.D.) coronary arteries were used for experiments on the mechanical activity. These strips were about 2.0 mm wide. The strips were completely immersed in Krebs Ringer solution which was kept at 37°C and equilibrated with 95% O₂ and 5% CO₂. The artery ring was mounted almost horizontally between a rigid metal support and a cotton wire loop connected to a force-displacement transducer. Muscle strips were stretched twice as wide as the normal strip. The artery rings isolated from the segments of the small coronary arteries were used for simultaneous recording of the electrical and mechanical responses. Conventional KCl-filled rigidly mounted glass microelectrodes of 30 to 70 megohms were used to impale single muscle cells. Tip potential of microelectrodes was 5 to 20 mV. The electrical and mechanical changes were simultaneously recorded on a cathode ray or pen writing oscilloscopes.

The composition of salt solutions used in present experiments was in m moles/liter;

- Normal Krebs Ringer solution:  NaCl. 120.7, KCl. 5.9, CaCl₂. 2.5, MgCl₂. 1.2, NaHCO₃. 15.5, NaH₂PO₄. 1.2, Dextrose. 11.5.
- K₂SO₄-Krebs Ringer solution: K₂SO₄. 84.4, CaCl₂. 2.5, MgCl₂. 1.2, KHCO₃. 15.5, KH₂PO₄. 1.2, Dextrose. 11.5.
- 3×K⁺-Krebs Ringer solution and 5×K⁺-Krebs Ringer solution were prepared by replacing 17.7 and 29.5 mM NaCl of the normal Krebs Ringer solution by equimolar amounts of KCl respectively.

RESULTS

Mechanical Responses

Isolated Strips from Large Coronary Vessels

In experiments on strips cut from the large coronary arteries both adrenaline and noradrenaline (10⁻⁸ g/ml—10⁻⁵ g/ml) caused various responses, which were contraction, relaxation or biphasic responses i.e. initial contraction followed by relaxation. (Fig. 1, 2) Changes in concentration of these drugs (10⁻⁸ g/ml—10⁻⁵ g/ml) did not produce qualitative changes of responses. Isoprenaline (10⁻⁶ g/ml—10⁻⁵ g/ml) caused relaxation of the smooth muscle of large coronary arteries.

Isolated Strips from Small Coronary Vessels

Adrenaline, noradrenaline and isoprenaline always caused relaxation of small coronary artery at the concentration between 10⁻⁸ g/ml and 10⁻⁵ g/ml. (Fig. 3, 4, 5)

Catecholamine-induced relaxation of both large and small coronary arte-
FIG. 1. Responses of strips from large coronary arteries (2.0–4.0 mm outside diameter) to adrenaline.

FIG. 2. Effects of noradrenaline on isolated strips from large coronary arteries.

FIG. 3. Effects of adrenaline on isolated strips from small coronary arteries.
Fig. 4. Effects on noradrenaline on isolated strips from small coronary arteries.

Fig. 5. Effects of beta adrenergic blocking agents on responses of the coronary smooth muscle. (upper) Effects of nethalide on isoprenaline-induced relaxation of the large coronary artery. (lower) Effects of nethalide on noradrenaline-induced relaxation of small coronary artery. Nethalide was added under administration of isoprenaline (upper) or noradrenaline (lower).

Fig. 6. Effects of nethalide on responses of adrenaline on the small coronary artery. Adrenaline was added under administration of nethalide.
after preadministration of dibenamine and dibenzyline, "specific alpha adrenergic blockade", and inversely produced a slight relaxation of the vessels. (Fig. 7) This observation may indicate that there are some $\beta$-receptor even in large vessels.

![Fig. 7. Effects of alpha adrenergic blocking agents on responses of large coronary smooth muscle. (upper) Responses of adrenaline on large coronary artery. (lower) Dibenzyline was applied 20 minutes after adrenaline (upper) was washed away and then adrenaline (lower) was again added under administration of Dibenzyline. Upper and lower trace is a continuous experiment.](image)

**Electrical responses**

The electrical and mechanical changes were simultaneously recorded on cathode ray or pen writing oscilloscopes. Since the adventitia contains an abundance of connective tissue, a microelectrode was inserted into the arterial wall from its intimal surface. As the electrode was slowly advanced, a series of sudden well-defined potential changes was seen, presumably associated with successful penetration of the intimal cell and some smooth muscle cells.

The mean resting potential was $47 \pm SE$ 10 mV (number of observation=82). The distribution of the resting potentials is shown in Fig. 8. In normal

![Fig. 8. Distribution of values for resting membrane potential of small coronary artery smooth muscle.](image)
Krebs Ringer solution, action potential was never recorded in the small coronary artery and the membrane potential was stable. Even in Ca\textsuperscript{++}-free Krebs Ringer solution, membrane potential of coronary smooth muscle was stable and the initiation of action potential was never observed.

Adrenaline (10\textsuperscript{-7} g/ml), adrenaline (10\textsuperscript{-5} g/ml) and KCl (30 mM) were applied in turn. Microelectrodes were kept in a stable state during the three tests.

In 6 experiments, during relaxation in small coronary artery following application of adrenaline (both 10\textsuperscript{-7} g/ml and 10\textsuperscript{-5} g/ml), detectable changes (more than 2 mV) in membrane potential were not recorded. (FIG. 9) How-
ever high K (30 mM) caused depolarization of 19±2 mV. In another 5 experiments when the potassium chloride in the bathing solution was increased to three and five times its normal concentration (5.9 mM), microelectrodes were impaled in each solutions with different potassium concentrations, over and over again, the membrane potential decreased from 64±6 mV to 50±7 mV and 43±6 mV respectively.

Adrenaline (10^-5 g/ml) also caused relaxation of small coronary arteries depolarized by K_2SO_4 Krebs Ringer solution. (FIG. 10)

**DISCUSSION**

**Mechanical Responses**

In previous papers, it has been reported that adrenaline and noradrenaline cause either relaxation or constriction of isolated coronary arteries of various mammals. Most studies on isolated human, monkey, and horse coronary arteries indicated that there catecholamines produce contraction. Conversely it has been known for a long time that the catecholamines relaxed coronary strips isolated from sheep, pigs, and oxen. Studies on the catecholamine effects on coronary strips from dog have not revealed definite results. Most of these papers have not indicated what part of coronary artery preparations were isolated from. In our experiments with the large coronary arteries of the dog, we found that adrenaline and noradrenaline cause either constriction or dilatation of ring strips. The strips isolated segments of small coronary arteries were always relaxed with the catecholamines. Ours are approximately the same as Zuberbuhler and Bohr's found in helical strips of the isolated coronary artery of the dog. Both relaxation of the small coronary artery with catecholamines and contraction of large coronary with adrenaline or noradrenaline were blocked by nethalide, dibenamine or dibenzyline respectively. Addition of adrenaline after alpha blockade produced an initial small relaxation of the large coronary artery while after beta, an initial slight contraction of the small coronary vessels. This behavior of the coronary artery can be explained by assuming that coronary contraction and relaxation with catecholamines is mediated through alpha and beta receptors respectively, at different levels of coronary tree. Relative population of alpha and beta receptors in the large coronary artery is remarkably different among each strip. Therefore a predominance of one or the other would determine whether contraction or relaxation would occur or not.

**Electrical Responses**

Most current works of the electrophysiology of the vascular smooth muscle have suggested that a change in spike activity or membrane potential are normally a necessary prelude to change in tension induced with catecholamine. But it is also considered that catecholamine and others
drugs could produce a change in tension without electrical change in the cell membrane. The idea might be received on considering the fact, that preparation of smooth muscle immersed in K-rich solution caused tension development with catecholamine and other drugs. Su, et al. showed that in the pulmonary artery, sympathetic nerve stimulation and 1-noradrenaline caused contraction without changes in the membrane potential and, the action potential was absent. In the present experiments, relaxation of small coronary artery without electrical changes in cell membrane was recorded. Our experimental results and data of Su, et al. obtained in the normal physiological solution may emphasize the consideration that mechanical changes of vascular smooth muscle were not always dependant on electrical changes. In our experiments it is doubtful whether microelectrode was inserted in single muscle cell. But we will disperse this doubt by these facts:

1. As the electrode was advanced, a series of potential changes was observed. It may be a reasonable consideration that these potential changes are associated with successful penetration of an endotherial cell and some smooth muscle cells.
2. The magnitude of the resting transmembrane potential of the dog coronary artery is comparable to that of the well-investigated vascular smooth muscles.15,19
3. The depolarizing effects of potassium chloride could be roughly explained by value calculated from Nernst equilibrium.

Relaxation of small coronary artery immersed in K$_2$SO$_4$ Krebs Ringer solution with adrenaline may support the consideration that not only contraction but also relaxation of smooth muscle occur without electrical changes. Relaxation of guinea-pig's taenia coli with adrenaline is caused by decreasing of electrical activity induced by activation of a sodium pump or increasing of potassium permeability by the drug.20 But since the small coronary artery relaxed with adrenaline without electrical change in muscle membrane, adrenaline must directly cause a decrease in calcium permeability or an increase in bound calcium in the smooth muscle of coronary artery regardless of electrical phenomenon in cell membrane.

**SUMMARY**

1. Electrical and mechanical responses to catecholamines were studied in isolated ring muscle strips of the coronary artery of the dog.
2. Adrenaline and noradrenaline caused various responses of large coronary artery and relaxation of the small coronary artery. Isoprenaline caused relaxation of both large and small coronary artery.
3. Catecholamine-induced relaxation or contraction was blocked by beta or alpha adrenergic blockade respectively.
4. The mean resting membrane potential of small coronary artery recorded intracellularly was -47 mV.
5. Adrenaline caused relaxation without changes in the membrane potential and in the absence of action potentials.
6. Increase in extracellular potassium initiated depolarization of cell membrane.
7. Dissociation in smooth muscle between relaxation and membrane potential change were discussed.

REFERENCES

17) SOMLYO, A.V. and SOMLYO, A.P.: Electromechanical and pharmacomechanical


