Response of large and small coronary arteries to nitroglycerin, NaNO₂, and adenosine

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SCHNAAR, RONALD L., AND HARVEY V. SPARKS. Response of large and small coronary arteries to nitroglycerin, NaNO₂, and adenosine. Am. J. Physiol. 223(1): 223-228. 1972.—Large (2 mm od) and small (5 mm od) coronary artery strips were mounted side by side in a muscle bath. KCl concentration was brought to 35 mm to produce tone and isometric tension was measured. Responses were expressed as the percent of the maximum relaxation caused by calcium free physiological salt solution. NaNO₂ and nitroglycerin caused greater relaxation of large vessels than small (P < 0.05). Adenosine caused greater relaxation of small vessels than large vessels (P < 0.05). Depolarization with an isotonic high potassium (100 mm K₂SO₄) solution did not reduce the relaxation in response to nitroglycerin or adenosine, suggesting that relaxation can occur without changes in the electrical state of the cell membrane. The effects of adenosine and nitroglycerin on Ca²⁺-induced contraction suggest that the differential effect of adenosine on large and small arteries may be explained by different effects on Ca⁺⁺ flux, but that the differential effect of nitroglycerin may not be explained in this way. This study supports in situ work suggesting differences in the response of vascular smooth muscle of large and small coronary arteries to nitroglycerin.

vascular smooth muscle; vasodilators; coronary; helical arterial strips; nitrate vasodilators

METHODS

Twenty mongrel dogs were anesthetized with pentobarbital (30 mg/kg) and sacrificed using an intravenous injection of saturated KCl. The heart of each dog was removed and stored at 4°C for not more than 2 days in physiological salt solution (PSS) containing the following constituents, in millimoles per liter: NaCl, 119; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄, 1.17; NaHCO₃, 14.9; dextrose, 5.5; sucrose, 50; and CaCl₂, 1.6. Storage of the coronary arteries for 2 days did not result in a change in the response to the agents used in this study.

Large, (2 mm od) and small, (550 μ od) arteries were dissected from the myocardium and stripped of fat and connective tissue. Helical strips cut from large arteries were 1 cm long by 1.5-2 mm wide; those cut from the small arteries were 2 mm long by 0.3-0.5 mm wide. The strips were mounted side by side in the same muscle bath in PSS with the potassium ion concentration brought to 35 mm to produce resting "tone." Although the effect of the resulting increase in osmolarity of 70 mOsm may favor relaxation, the net effect of 35 mm KCl was an increase in active tension. This potassium concentration produces about 60% of the maximum response of coronary artery strips to potassium (Fig. 1). The strips were anchored at one end and the other end was attached to a Grass force transducer, the output of which was recorded by a Grass polygraph. The tension on the strips was adjusted to 1.6 g on the large vessel and 0.12 g on the small vessel, which are estimates of the in vivo tensions on such vessels assuming a transmural pressure equal to 100 mm Hg and the dimensions given above. The bath was kept at 37°C and aerated with 95% O₂ and 5% CO₂. The vessels were allowed to equilibrate for 2 hr after mounting and before the first experimental maneuver was performed. The student t test for paired observations was used to determine statistical significance of differences observed in the various experiments.

Relaxation in response to nitroglycerin, sodium nitrite, and adenosine. Nitroglycerin, sodium nitrite, and adenosine were administered in the following concentration ranges: nitroglycerin, 4.40 × 10⁻⁹ to 1.38 × 10⁻⁴ M; sodium nitrite, 1.45 × 10⁻⁷ to 4.53 × 10⁻⁴ M; and adenosine, 3.74 × 10⁻⁸ to 1.17 × 10⁻⁴ M, and at fivefold increments in between. An agent was injected into the bath every 12-15 min. Each

MEASUREMENT OF large and small coronary artery pressures before and after administration of nitrate vasodilators suggests that this class of drugs acts preferentially on large coronary arteries (14, 15, 16). Winbury (14) and Winbury, Howe, and Hefner (15), as well as Fam and McGregor (4), have suggested that this results in increased blood flow to ischemic areas and so may be related to the antianginal effect of nitrates. The most likely explanation of the greater effect on large arteries is that nitrates cause greater relaxation of the vascular smooth muscle of the large than of the small arteries. The observation that there are differences in the response of vascular smooth muscle of large and small artery strips to catecholamines (17) gives support to the idea that the smooth muscle of large and small coronary arteries could have different responses to nitrates. The current study directly tests this possibility by examining the effect of nitroglycerin, sodium nitrite, and adenosine on isolated vascular smooth muscle from large and small coronary arteries. Adenosine has been included since it is a suggested coronary metabolic vasodilator (1, 2, 3, 9) which might be expected to affect small resistance arteries at least as much as large coronary arteries. In addition, we have performed experiments designed to determine whether the effects of nitroglycerin and adenosine are related to membrane excitation or excitation-contraction coupling.
dose remained in the bath for 3 min, after which it was removed by three rinses with PSS (35 mM KCl). After the last dose of a drug was administered, the contribution of passive tension at the particular length of a strip was determined by bathing the strip in calcium-free PSS plus 4 mM EGTA. In all except six experiments, tissue preparations were used to test only one drug. In six experiments, the nitroglycerin dose-response curves were obtained after observing the adenosine dose-response curves of the same tissues. These nitroglycerin dose-response curves were in no way different from other curves of tissues which had not been previously exposed to adenosine.

Depolarization experiments. To determine the effect of potassium depolarization on the responses to nitroglycerin and adenosine, we used the following procedure. After equilibration, the response to nitroglycerin (bath concentration of \(5.50 \times 10^{-6}\) M) or adenosine (\(2.34 \times 10^{-5}\) M) was measured as before. These doses were chosen because they caused opposite differential effects (Figs. 3 and 5). The muscle was then placed in isotonic PSS solution containing, in millimoles per liter: \(K_2SO_4\), 100; \(KH_2PO_4\), 1.18; \(MgSO_4\), 1.17; \(NaHCO_3\), 14.9; dextrose, 5.5; and \(CaCl_2\), 1.0. This potassium ion concentration (200 mM) is above that necessary for a maximum response (Fig. 1). Calcium ion concentration (200 mM) is above that necessary for a maximum response (Fig. 1). Calcium ion concentration was reduced to 1 mM in order to minimize changes in active tension. When a steady tension was reached in the high potassium solution, the same concentration of the drug was again introduced, and then rinsed out after 3 min.

Calcium ion induced contraction. After equilibration, PSS was replaced with calcium-free PSS and the strips were allowed to come to a steady tension. A bolus of \(CaCl_2\) was then injected into the bath to bring the calcium ion concentration to 1.6 mM, which produced a rapid increase in tension. The procedure was then repeated with bath concentrations of either \(5.50 \times 10^{-6}\) M nitroglycerin, or \(2.34 \times 10^{-5}\) M adenosine introduced into the bath 3 min before the \(CaCl_2\) injection.

Action of adenosine in the presence of dipyridamole. After equilibration of large vessels, adenosine was introduced into the bath (final concentration, \(1.25 \times 10^{-6}\) M) for 3 min, and was then removed with three rinses. This was repeated 2–3 times. Adenosine was administered in a similar fashion 30 min after the introduction of 0.1 ml propylene glycol, and also 30 min after the injection of dipyridamole (bath concentration \(10^{-6}\) M) dissolved in 0.1 ml propylene glycol.

Then, the adenosine response after propylene glycol alone was observed again.

RESULTS

Response to nitroglycerin, sodium nitrite, and adenosine. Responses of large and small vessels to the vasodilators could not be directly compared because the small vessel, having less cross-sectional area, could not exhibit absolute tension changes as great as those of the large vessel. The responses, therefore, were expressed as the percent of the vessel’s relaxation in calcium-free PSS. The difference between the resting tension before each injection and the calcium-free tension level was measured, and the responses to that injection was expressed as the percent of this “maximum” relaxation. Figure 2 illustrates this procedure using typical responses to a dose of adenosine.

We have compared the response of large and small vessels to nitroglycerin by administering each of the doses indicated in Fig. 3 in nine experiments. This drug caused the large vessels to relax significantly more (\(P < 0.05\)) than the small vessels at all except one dose level (\(2.75 \times 10^{-5}\) M) which did not result in a significant difference. At the highest dose level the large vessel percent relaxation was nearly 1.5 times that of the small vessel. At this dose the relaxation of the large vessel was 73.5 ± 8.6 % of the relaxation in response to calcium-free PSS, whereas the relaxation of the small vessel was only 51.1 ± 6.5 %. Sodium nitrite, in seven experiments, gave similar results. At dose levels above \(3.62 \times 10^{-6}\) M, sodium nitrite (Fig. 4) caused a significantly greater percent relaxation of the large vessels (\(P < 0.05\)). At the highest dose level the large-vessel percent relaxation was nearly twice that of the small vessel: 78.6 ± 7.4 % as compared to 40.7 ± 13.4 %.

Adenosine caused less relaxation of both vessels, and the opposite differential effect. In each of six experiments adenosine caused a greater percent relaxation of the small vessels at all dose levels (\(P < 0.05\)). At the \(2.34 \times 10^{-5}\) M dose level the small-vessel percent relaxation (31.4 ± 8.3 %) was over 3 times that of the large vessel (8.6 ± 2.5 %), as shown in Fig. 5.

Effect of potassium ion depolarization. To determine if the difference in the response of large and small vessels could be
related to the difference in the effect of nitroglycerin or adenosine on the membrane excitation of these two tissues, we studied the effect of potassium ion depolarization on the vessels' responses to these drugs. The relative enhancement or diminution of the control-drug response in depolarizing solution is expressed as:

relative enhancement
\[
= \left( \frac{\text{response in depolarizing solution}}{\text{control response}} \right) - 1
\]

relative diminution
\[
= \left( \frac{-1 \times \text{control response}}{\text{response in depolarizing solution}} \right) + 1
\]

The normalizing factor of one was subtracted from the relative enhancement and added to the relative diminution to make the scale continuous through zero. Zero represents a response that is not measurably different from control response. Figure 6 demonstrates that the mean response to nitroglycerin or adenosine was not significantly reduced by the depolarizing solution in 16 experiments.

Drug effects on calcium ion induced contraction. To determine whether a drug effect on net Ca\(^{++}\) flux could be ruled out, we observed the effect of nitroglycerin and adenosine on the contraction of the vascular smooth muscle strips in response to increased calcium ion concentration. Two variables, the rate of tension development (mg/min) and the final tension developed (mg) were expressed as the percent of control response:

\[
\text{% control response} = 100 \times \left( \frac{\text{response with drug present}}{\text{response without drug present}} \right)
\]

Figure 7 shows that in the presence of adenosine, the initial rate of tension development by the small vessel was reduced...
vasodilators act preferentially on small coronary arteries. The differential drug effects on the vascular smooth muscle of the two vessel sizes observed in the current study (Figs. 3, 4, and 7) support this hypothesis. Using the relaxation in calcium-free PSS to normalize the responses, nitroglycerin caused preferential dilation of large coronary arteries, as did sodium nitrite. Adenosine, however, preferentially relaxed small coronary arteries. Greater differential effects might have been observed if we had been able to examine the responses of vessels smaller than the small 0.5-mm-id vessels used in this study.

It has been proposed (1, 2, 3, 9) that adenosine may be involved in the local metabolic regulation of blood flow. It is interesting in the light of this hypothesis, that adenosine has a preferential effect on small coronary arteries, since local regulation probably involves effects on the smaller intramural resistance vessels.

Other studies have suggested differences in the pharmacology of vascular smooth muscle of large and small arteries. Zuberbuhler and Bohr (17) showed that large coronary vessels (2 mm od) from the dog may contract in response to a dose of catecholamine, whereas small coronary vessels (0.4 mm od) dilate in response to the same dose. In vivo studies on the dog forelimb (5), measuring pressures of large and small arteries, suggest that the phosphorylated derivatives of adenosine act primarily on small resistance vessels. Norton and Detar (8) have observed differences similar to those reported here in the response of large and small rabbit coronary arteries to adenosine.

To investigate the mechanism of the differential effects, we observed the effects of membrane depolarization on responses to representative doses of nitroglycerin and adenosine. The potassium ion concentration used to cause depolarization (200 mM) is higher than that which causes maximal potassium effect (Fig. 1) and is therefore likely to maximally depolarize the tissue. If either drug has its effect on membrane electrical activity, this high concentration of potassium ion should greatly reduce or abolish the drug’s effect. However this was not the case, since depolarization as often enhanced as diminished the drug effect. These data suggest that both drugs can exert their effect without altering membrane potassium in the presence of drug (Fig. 7) suggests that adenosine inhibits net calcium ion influx of the small vessel. Thus this experiment does not eliminate alteration.
to block adenosine uptake by smooth muscle cells in large vessels. Dipyridamole has been shown to inhibit the breakdown of adenine nucleotides to inosine by up to 80% in the anoxic myocardium of the dog. Concentrations of $10^{-6}$ M have been shown to block the uptake of adenosine in human erythrocytes by 80% and into blood cells of the dog by 30% (7). Assuming that dipyridamole acts on vascular smooth muscle cells as it does on red blood cells, the addition of dipyridamole should decrease uptake of adenosine by the outer layer of cells of the large vessels, allowing more cells to respond to adenosine. The mean percent relaxation to adenosine was increased only 0.74 over the control values in the presence of dipyridamole. In 10 experiments this difference was not significant ($P > 0.22$) and, even if larger numbers of experiments would make this difference significant, the increase in percent relaxation is not large enough to explain the threefold larger percent relaxation of small vessels over large vessels (Fig. 5). These data suggest that adenosine uptake is not a significant factor in explaining the difference in the effect of adenosine on large and small vessels.

In summary, the results of our experiments with isolated vascular smooth muscle support in situ studies which suggest that nitrate and non-nitrate vasodilators have opposite differential effects on the vascular smooth muscle of large and small coronary arteries. Nitroglycerin and sodium nitrite, established antianginal agents, relax the large vessels preferentially, whereas adenosine, a suggested coronary metabolic vasodilator, relaxes the small vessels preferentially. Potassium depolarization studies suggest that the drugs do not act by affecting the electrical state of the cell membrane. Calcium ion contraction studies do not rule out the possibility that adenosine affects the regulation of net calcium ion flux in the small vessel smooth muscle cells while having little or no effect on the large vessel net flux. Adenosine uptake by an outer cell layer of large vessels does not appear to play a major role in the differential effect of this agent. Nitroglycerin affects net calcium ion flux of both large and small vessels; however, it seems unlikely that the greater effect of nitroglycerin on large vessels can be explained by a greater effect on net calcium ion flux.

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