Current uncertainty concerning the action of catecholamines on smooth muscle of coronary arterial vessels is convincingly documented by extensive controversial reports in the literature. Most authors now agree that dilatation is the final result of the action of these agents in vivo,\textsuperscript{1-6} as well as in the isolated perfused heart.\textsuperscript{7-10} Decrease of coronary flow, however, has been observed under many conditions.\textsuperscript{11-17} The physiological importance of this constrictor action is suggested by the observation that neurogenic sympathetic activity to the heart produces coronary vasoconstriction.\textsuperscript{18} It has been suggested that catecholamines have a direct constrictor action on coronary vessels\textsuperscript{12,15-17} and that the protracted dilatation usually seen after catecholamine administration is due to the overriding effects of increased activity of the myocardium.\textsuperscript{15-17,19,20} These observations raise the question of the relative magnitudes of a direct constrictor influence on catecholamines and an antagonistic dilator influence of the augmented myocardial metabolism. It would seem that such a clear-cut issue could be resolved by separating the coronary vessel from the myocardium and observing the direct effect of catecholamines on the vascular smooth muscle. However, results of such studies are contradictory. Some authors report constriction,\textsuperscript{21} some dilatation,\textsuperscript{22-28,31} and some report "ambiguous" action.\textsuperscript{18,20} Only large coronary arteries were used, however, and, as will be evident in the current report, the effects of catecholamines on smooth muscle from large coronary vessels are clearly different from their effects on that from small ones.

\textbf{Methods}

Mongrel dogs were anesthetized with pentobarbital, 30 mg/kg, and killed by intravenous injection of concentrated KCl. The hearts were removed immediately and stored at 4 C (never more than four days) in physiological salt solution (PSS). The composition of this PSS was, in mmol/liter: NaCl, 119; KCl, 4.7; KH\textsubscript{2}PO\textsubscript{4}, 1.18; MgSO\textsubscript{4}, 1.17; NaHCO\textsubscript{3}, 14.9; dextrose, 5.5; sucrose, 50; and CaCl\textsubscript{2}, 1.6. Segments of arteries were dissected from the myocardium and stripped of loose fat and connective tissue.

\textbf{ISOLATED MUSCLE STRIPS}

Helical strips were cut from the segments of small (250 to 500 \mu m diameter) and large (1.5 to 2.4 mm diameter) coronary arteries. Those from the small arteries were 2 mm long by 0.2 to 0.3 mm wide, those from the larger arteries were 8 mm long by 1 to 1.5 mm wide (all diameters given are O.D.). The strip, mounted in PSS in a muscle bath, had one end attached to an anchor point and the other end connected by means of a metal rod to a force transducer whose electrical activity, representing tension in the strip, was recorded by an ink-writing oscillograph. The muscle strip was stretched to a tension which would permit optimal response to stimulation, about 120 mg and 500 mg for small and large vessels, respectively. The muscle was allowed to equilibrate in the bath at 37 C for one to two hours, until it gave reproducible responses to a standard stimulation (50 mM KCl). Norepinephrine, which is a potent relaxant of this smooth muscle, was added to the bath to ascertain the degree of tone of the muscle. About one-third of the preparations developed sufficient spontaneous tone to permit study of the effects of relaxing agents. When this spontaneous contraction did not occur, the K concentration of the bath was increased to 15 or 20 mM to establish active tonic contraction. Norepinephrine to give concentrations of 0.01 to 1000 \mu g/liter, or epinephrine to give concentrations of 0.1 to 1000 \mu g/liter, was injected into the bath at 10 to 15-min intervals. The drug remained in the bath for 3 min, after which time it was removed by...
three flushings with PSS. Isoproterenol (0.01 to 100 μg/liter) and angiotensin (0.1 to 100 μg/liter) were also used. To simulate a physiological environment more closely, in a second group of four experiments, after the initial period of equilibration in PSS the strip was bathed with circulating blood from a donor dog, as shown in figure 1A. Test drugs were injected into the flowing bath.

In a third group, after equilibration the muscle was depolarized by replacing the PSS bath with one containing 100 mM K₂SO₄ (composition in mmoles/liter: K₂SO₄, 100; KH₂PO₄, 1.18; MgSO₄, 1.17; NaHCO₃, 14.9; dextrose, 5.5; and CaCl₂, variable, as mentioned below). This solution often caused a strong fixed contracture of the coronary smooth muscle. To avoid this the Ca in the solution was reduced to a concentration sufficient to maintain a moderate degree of tone, from which the muscle strip could be caused to relax. Catecholamines were injected as with the first group.

**ISOLATED PERFUSED CORONARY ARTERIES**

In another approach to this study a coronary artery 1 mm in diameter, with its branches, was dissected free from myocardium, cannulated with a polyethylene catheter, and perfused with PSS at constant pulsatile flow. Large branches were tied off so that the entire outflow emerged through the free ends of branches of about 100 μ diameter. This emerging fluid filled and overflowed the 30 ml muscle chamber in which the vessel was mounted. Perfusion pressure was measured by a strain gauge transducer and was monitored by an ink-writing oscillograph. The pump rate was adjusted to give a pressure of about 70 mm Hg; this required a volume flow of 4 to 5 ml/min. Drugs were injected into a section of rubber

**FIGURE 1**

A: Method for circulating blood from a donor dog through a 3 ml muscle chamber, and responses of strip (from 350 μ vessel) in such a chamber to norepinephrine and epinephrine. Catecholamines were injected directly into the bath; rate of blood flow was approximately 10 ml/min. B: Responses of strip (from 500 μ vessel) similarly mounted but in a circulating K₂SO₄ solution. In both the most and the least physiological environments studied, catecholamines caused relaxation and norepinephrine was more potent than epinephrine.
tubing near the tip of the catheter, in volumes usually not exceeding 0.02 cc. After a period of equilibration the PSS perfusion fluid was replaced by one in which the K concentration was raised to 20 mM and the osmolarity kept constant by reducing sucrose proportionately.

**Results**

**ISOLATED STRIPS FROM SMALL CORONARY VESSELS**

Catecholamines caused relaxation of smooth muscle of small coronary arteries. This was seen consistently with over 100 muscle strips. The magnitude of the relaxation was limited mainly by the amount of active tone initially present in the strip. Figure 2 shows responses of a strip from a 400 μ coronary artery to graded concentrations of epinephrine and norepinephrine. In this case the threshold concentration for norepinephrine was 0.01 μg/liter; for epinephrine it was over 10 times this concentration. The average threshold for relaxation with norepinephrine, determined from 24 observations, was 0.08 μg/liter, and for epinephrine, from 18 observations, 1.0 μg/liter. These thresholds were similar when relaxation was elicited in a muscle exhibiting spontaneous tone and in one whose tone was augmented by increased K concentration in the bath. It is noteworthy that norepinephrine at the normal plasma level of 1 μg/liter had a definite relaxing effect. The threshold concentration for relaxation by isoproterenol was not determined. However, in 13 of 15 experiments, at a given concentration it produced greater relaxation than did norepinephrine.

Administration of 1 mg/liter of nethalide (a-isopropylamino-1-[2-naphthyl] ethanol HCl), a specific beta adrenergic blocker  before addition of the catecholamine, blocked relaxation (fig. 3), and in some cases the catecholamine then caused a slight contraction. Normal relaxation in response to catecholamines returned 10 to 15 minutes after the nethalide was flushed out.

Angiotensin, in over 30 coronary strips, always caused contraction, but the muscle rapidly developed tachyphylaxis to this polypeptide. The threshold concentration for angiotensin contraction was between 0.1 and 0.5 μg/liter. Catecholamines added during an angiotensin response produced relaxation.

A shift from PSS to blood as the bathing fluid in four experiments produced a marked increase in tension in the strip, and this increased tension was maintained to a greater or lesser degree throughout the exposure to blood. Epinephrine and norepinephrine in amounts as low as 0.01 μg in the flowing bath caused the muscle to relax (fig. 1A).

In six experiments catecholamines also caused relaxation of coronary smooth muscle depolarized by K2SO4 (fig. 1B). This relaxation was blocked by nethalide.

**ISOLATED STRIPS FROM LARGE CORONARY ARTERIES**

In 16 experiments on strips cut from the larger coronary arteries, epinephrine and norepinephrine caused either contraction or relaxation. Three factors determine which of these responses will develop: 1) Smooth muscle taken from the major coronary artery within 3 or 4 cm of aorta, with a diameter of ± 2.3 mm, contracts; smooth muscle from coronaries of 1.7 mm diameter or less relaxes. 2) Low concentrations of these catecholamines cause predominantly contraction; high
concentrations accentuate relaxation. 3) Epinephrine is the more potent contractor; norepinephrine is the more potent relaxer. Figure 4 shows responses of two coronary strips mounted in a common bath, to norepinephrine, epinephrine, and isoproterenol. The strip from the larger artery was contracted by epinephrine and norepinephrine, the strip from the small one was relaxed; both were relaxed by isoproterenol. Other strips of large coronaries were initially contracted and then relaxed by epinephrine or norepinephrine (fig. 5); the higher the concentration of the catecholamine the smaller was the contraction and the greater the relaxation. At high concentrations the response began as a rapid contraction which was reversed by a relaxation response which had a slower time course. Isoproterenol caused relaxation at any active dose. Contraction and relaxation were selectively blocked by Dibenzyline and nethalide (fig. 3); blockade of either action greatly enhanced the

Effect of beta and alpha adrenergic blocking agents on responses of strips from large and small vessels. Bath was physiological salt solution containing 20 mM KCl. Large vessels apparently have about equal numbers of alpha and beta receptors; small vessels have more beta than alpha receptors.

Comparison of responses of strips from large and small coronary arteries, mounted in 15 mM KCl in a common bath. The large coronary artery is contracted by epinephrine and norepinephrine, while the small one is relaxed. Epinephrine was a more potent constrictor than norepinephrine. Isoproterenol caused relaxation of both large and small vessels.
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EPINEPHRINE

40 mg

1 μg/l

10 μg/l

100 μg/l

NOREPINEPHRINE

ISOPROTERENOL

1 μg/l

2 min

FIGURE 5

Responses of strip from large coronary artery (2.0 mm diameter) to catecholamines. With epinephrine and norepinephrine an initial contraction is followed by relaxation, and relaxation is accentuated at high concentrations; with isoproterenol there is only relaxation.

opposite effect. As may be seen in figure 3, top row, a concentration of epinephrine that caused a strong contraction when beta receptors were blocked, caused relaxation greater than that of the unblocked strip when alpha receptors were blocked.

ISOLATED PERFUSED CORONARY ARTERIES

Ten isolated segments of coronary arteries having outflow branches of approximately 100 μ were perfused with PSS. They remained maximally dilated when perfused with PSS of normal K concentration. In order to increase the tone of the segments, after the period of equilibration the perfusion fluid was replaced by one in which the K concentration was increased to 20 mM. This usually induced a sustained vasoconstriction that persisted throughout the experiment, lasting two to three hours. Norepinephrine injected into the perfusing fluid in amounts as low as 0.003 μg produced vasodilatation (fig. 6). Epinephrine also produced vasodilatation but was less potent than norepinephrine.

Discussion

That catecholamines relax isolated coronary vessels of most mammals has been known for a long time. Drugs tested in the early experiments were not pure, so we cannot know the actual concentrations used. However, in more recent papers only high concentrations of these drugs have been reported to be effective. Lundholm and Mohme-Lundholm found that epinephrine and norepinephrine at a concentration of 500 μg/liter relaxed about half of the strips from bovine coronary vessels, but were ineffective in the remainder. Smith and Coxe, using a plethysmographic technique, found coronary arteries of swine were relaxed with epineph-
rine and norepinephrine in concentrations of 10 μg/liter. Kovalčík obtained relaxation of coronary strips from dogs and sheep only when using 100 μg/liter of epinephrine or norepinephrine. Most studies on isolated human coronary arteries indicate that catecholamines produce contraction. However, Kountz, reported that human coronary rings were contracted by low concentrations of epinephrine and relaxed by high concentrations. In all these studies on isolated coronary arteries only large vessels were used.

In our experiments with small coronary arteries of dogs, the average threshold concentration for norepinephrine relaxation was about 0.08 μg/liter, a figure well below the normal blood concentration of the hormone, 1 μg/liter. The threshold concentration for epinephrine relaxation was over 10 times that for norepinephrine. This relative sensitivity of the coronaries to epinephrine and norepinephrine is in accord with the data of Smith and Coxe, who found that at a given concentration, norepinephrine was 2.5 times as potent as epinephrine in producing coronary dilatation. Lewis et al. have reported coronary blood flow studies in the intact heart which also indicate that norepinephrine has a more potent dilator action than does epinephrine. In their studies isoproterenol was more potent in this respect than either of the two physiologically occurring catecholamines.

The current study indicates that coronary relaxation by catecholamines is mediated through beta receptors. Evidence for this is that the response was blocked by nethalide. It is noteworthy, however, that the “classical” relative potency of these agents in stimulating beta receptors, i.e., isoproterenol > epinephrine > norepinephrine, does not hold for coronary receptors, where norepinephrine > epinephrine.

Catecholamines were effective in causing relaxation of smooth muscle from small coronary artery regardless of the stimulus responsible for its contraction. This is true whether the contraction was spontaneous in PSS or induced by angiotensin, KCl, plasma, or whole blood.

Although the relaxation was not influenced by the particular stimulus responsible for contraction, the amount of the relaxation did depend on the magnitude of the existing contraction. When contraction was minimal, relaxation was, of course, limited by the amount of this existing active tone. On the other hand, when the smooth muscle was made to contract maximally, the catecholamine proved ineffective in overriding the contraction. In the presence of such a strong stimulus, if the vigor of the contraction was reduced by lowering the Ca concentration in the PSS the relaxing action of catecholamines could again be demonstrated.

Large and small coronary arteries do not react alike to catecholamines. Smooth muscle of small coronary vessels is always relaxed by these agents, that of large vessels is either contracted or, after an initial transient contraction, relaxed. During beta adrenergic receptor blockade, the small vessels either do not react to catecholamines or contract slightly, whereas large coronary vessels contract strongly (fig. 3). On the other hand, alpha blockade greatly enhances the catecholamine-produced relaxation of large coronary vessels but has a much smaller effect on the response of the small vessels. These differences of behavior can be explained by assuming that there are different relative populations of alpha and beta receptors at different levels of the coronary tree. Assuming that the large vessels have about equal populations of alpha and beta receptors, a slight predominance of one would determine whether contraction or relaxation would occur. The time required for activation of contraction through the alpha receptors is less than that required for activation of relaxation through the beta receptors. The “ambivalent” effect of catecholamines on large vessels may account for some of the contradictions found in the literature. The small vessels of 100 to 500 μ diameter, however, are regulated almost exclusively by beta receptors. They have not previously been studied after isolation from the myocardium.

The mechanism by which catecholamines produce coronary relaxation remains unde-
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fined. Bulbring proposed for the smooth muscle of the taenia coli that epinephrine may stimulate the production of energy-rich compounds which then are available for more effective membrane stabilization. The resulting hyperpolarization of the cell membrane and inhibition of spike discharge would produce relaxation. Mohme-Lundholm, on the other hand, showed that epinephrine and norepinephrine increase the lactic acid content of coronary smooth muscle. Since this metabolic product was found to be a potent relaxing agent, she concluded that catecholamines relax coronary smooth muscle by stimulating lactic acid production in the cell. Furthermore, beta receptors, which mediate coronary relaxation, also control myocardial metabolism, either directly or indirectly by transmitting the positive inotropic action of catecholamines. Therefore, although this indirect evidence is in agreement with the possibility that catecholamine relaxation of coronary smooth muscle is brought about through the stimulation of smooth muscle cell metabolism, evidence as to how this increased cell metabolism causes relaxation of this particular smooth muscle is lacking. Apparently, it is not through hyperpolarization of the cell membrane because strips whose muscle cells were depolarized by 100 mM K2SO4 were relaxed by catecholamines. It seems improbable that increased lactic acid production is the cause of coronary relaxation, since this increase occurs also in mesenteric smooth muscle which is contracted by catecholamines. Rather, lactic acid production may be a nonspecific result of the action of catecholamines on vascular smooth muscle mounted in PSS.

That myocardial metabolism greatly influences coronary vascular tone is well established. Siegel et al. showed, in the open chest dog, that after dichloroisoproterenol, which blocks the positive inotropic effect of catecholamines, epinephrine, and norepinephrine produce constriction of the coronary vessels, and concluded that the direct action of catecholamines on these vessels is constriction. Our results, however, indicate that the use of beta adrenergic blockers would not only block the positive inotropic effect on the myocardium, but would also abolish any direct relaxing action of catecholamines. Therefore, constriction due to stimulation of the alpha receptors present in the larger vessels would be evident. This explanation can also be applied to the report by Hashimoto et al. that, in the fibrillating heart, blockade with dichloroisoproterenol reversed the vasodilatation produced by catecholamines.

Berne showed that, in the beating and fibrillating heart in situ, the administration of catecholamines produced an initial decrease of coronary blood flow and then, secondarily, increased flow over control values. Oxygen consumption increased concomitantly with the secondary increase of coronary flow. In the heart arrested by KCl infusion, catecholamines caused only coronary vasoconstriction. Berne concluded that the direct coronary action of these agents was constrictor, and that the usual relaxation seen was due to the increased metabolic activity of the myocardium. It is clear from our experiments that isolated coronary smooth muscle from vessels of 100 to 500 \( \mu \) diameter, unlike most other vascular smooth muscle, is relaxed by catecholamines. The consistent relaxation observed by us is not due to the experimental procedure used in cutting the strips since intact perfused coronary vessels of 100 \( \mu \) diameter relaxed when exposed to catecholamines (fig. 6), whereas helical strips of small mesenteric vessels were contracted by them. The relaxing effect is not related to the artificial environment since coronary strips bathed in circulating arterial blood from a donor dog were also relaxed by these drugs (fig. 1A).

Since many investigators have observed that epinephrine and norepinephrine produce an initial increase in coronary vascular resistance, it is relevant to inquire about the contribution made to vascular resistance by vessels of the size currently studied. Catecholamines produced only relaxation of the small coronary vessels in this study. Vessels used for helical sections were between 250 and 500 \( \mu \) diameter, and in the perfusion studies the diameter of the outflow end of the isolated
arterial segment was approximately 100 μ. Both techniques study responses of muscle from vessels smaller than the 500 μ vessels catheterized by Haddy et al. in their studies of segmental vascular resistance in the dog.40 These investigators observed that over one quarter of the resistance of the vascular tree was overcome proximal to vessels of this size. Beyond this point pressure fell more precipitately.

There is no anatomical division between vessels having resistance and conduit functions. Actually all vessels serve both functions. Any predominantly "conduit" vessel may become a "resistance" vessel if it constricts adequately, and it is probable that the actual level of the arterial tree which makes the major contribution to resistance to flow differs from organ to organ and varies with the physiological state of the animal, e.g., exercise, erect position, etc. This interpretation would reconcile our observations, that smooth muscle from smaller segments of the coronary arterial tree relax in response to epinephrine and norepinephrine, with those of Berne, and others, indicating that these agents increase coronary vascular resistance in the perfused heart. Possible explanations of this paradox are that: 1) an intense constriction of the large conduit vessels may dominate flow initially in the perfused heart, 2) very small coronary vessels and/or capillary sphincters may constrict in response to catecholamines, and 3) normal myocardial metabolites may in some way change the response of coronary resistance vessels to catecholamines so that a constrictor component of the response develops.

A general examination of the evidence concerning the action of catecholamines on coronary arteries indicates that in situ coronary resistance is increased in transient fashion before being reduced under the influence of increased myocardial metabolism. Isolated coronary smooth muscle from large coronary vessels can either contract or relax in response to catecholamines; in low concentrations contraction predominates, in high concentrations relaxation supersedes. Smooth muscle from vessels ranging in size from 100 to 500 μ only relaxed in response to catecholamines in any effective concentration. Teleologically, this direct relaxing action of catecholamines on coronary vascular smooth muscle would be appropriate to provide the increased circulation required by the increased metabolic demands imposed by these agents.

**Summary**

Responses to catecholamines were studied in isolated helical muscle strips of large and small coronary vessels and in isolated perfused coronary arteries from the dog. Epinephrine and norepinephrine in concentrations well below those normally present in the blood uniformly caused relaxation of small coronary vessels. Norepinephrine was much more potent than epinephrine. The relaxation was reversibly blocked by the beta adrenergic blocker, nethalide. During this blockade, catecholamines either were inactive or produced a slight contraction. Strips contracted by angiotensin, blood or KCl, as well as those completely depolarized by K2SO4, were relaxed by catecholamines.

Strips taken from large coronary vessels, unlike those from small vessels, were, in some cases, contracted by catecholamines; in others, after a transient contraction, they were relaxed. Contraction was blocked by Dibenzyline. This difference in behavior of large and small vessels may account for some of the contradictions found in the literature concerning the response of coronary vessels to catecholamines.

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