# ADRENERGIC RECEPTORS IN CORONARY ARTERIES*

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Teleologically, the activity of vascular smooth muscle is directed toward two distinct and sometimes conflicting goals. The first is the control of total peripheral resistance so that arterial pressure will be sufficient to maintain adequate perfusion of all of our parts, yet not so great that it will blow out our tubes. This system operates for the welfare of the whole organism. The second goal is concerned with the needs of the individual organs. Distribution of blood flow must be adjusted to meet fluctuations in the requirements of our various parts. These two goals find a clear parallelism in those of our Democratic and Republican parties, respectively. It is the "Republican" focus on individuality which forms the context of the studies that I will describe.

Blood vessels are equipped with three distinct control systems which are capable of local regulation of flow to meet the individual requirements of specific regions:

1. *Neurogenic control* may be a highly individualized phenomenon. Not only does the total amount of innervation vary from tissue to tissue[1] but the pattern of neurogenic influence on the different organs is specific for the type of situation to which the whole organism is responding.[2]

2. *Local humoral regulation* is an exceptionally effective mechanism for individualization of flow since, by virtue of the vasodilator response of resistance vessels to local metabolites[3] and local $pO_2$,[4] each tissue can regulate its own blood flow.

3. Finally, individuality of control is effected through differences in behavior among *vascular smooth muscles* in different parts of the body. Smooth muscles from various sites differ in: (a) degree of spontaneous myogenic tone, (b) capability for autoregulation, and (c) individuality of "receptors." The observation that cerebral resistance vessels fail to respond to reasonable concentrations of catecholamines, whereas renal resistance vessels are highly responsive to these neurohumoral agents,[5] is interpreted as an indication that there is a marked disparity in the populations of adrenergic receptors in vascular smooth muscle at these two sites. A final basis for individuality, which is emphasized in the current study, is that, in addition to differences in populations of adrenergic receptors in various sites of the body, there are qualitative differences in these receptors in vascular smooth muscle from various tissues.

In the present study the relative sensitivity of *alpha* and *beta* adrenergic receptors to epinephrine and to norephinephrine was evaluated in vascular smooth muscle from resistance vessels in coronary and skeletal muscle.

*Supported by National Institutes of Health Grant HE 03756-08.

## *Methods*

Procedures used in the current study were those employed in a recent study of the responses of coronary smooth muscle to catecholamines.[6] Isolated smooth muscle preparations were obtained from coronary arteries (250$\mu$ to 2.4 mm o.d.) of dogs, rabbits, monkeys and humans, and small arteries (400–800 $\mu$ o.d.) from skeletal muscle of the dog. Isolated helical strips of these vessels were prepared[5] for tension recording in a bath of physiological salt solution (PSS) aerated with 95% $O_2$, 5% $CO_2$, and maintained at 38°C. Isometric tension was monitored by means of a force transducer recording on an ink-writing oscillograph. Occasionally, in the isolated bath, these strips of smooth muscle develop "spontaneous myogenic tone" (FIGURE 2) so that the relaxing influence of *beta* adrenergic activation can be studied directly. However, in normal PSS, most of the smooth muscle preparations remain completely relaxed, so that in order to study a relaxing influence the muscle has to be stimulated artificially. The stimulus used in the current study was KCl at a concentration which would give approximately 1/3 maximum contraction. This usually required about 20 mmole KCl added/l of PSS. Epinephrine (E), norepinephrine (NE), or isoproterenol (ISO) were added to give final bath concentrations of .0001 to 1.0 $\mu$M. Where blocking agents were used, they were added to the bath at least four minutes prior to the injection of the catecholamine. Agents used for *beta* adrenergic blockade were pronethalol, propranolol, and MJ-1999 (.01 to 10 $\mu$M; for *alpha* adrenergic blockade, phentolamine or phenoxybenzamine (.01 to 10 $\mu$M) was used.

## *Results*

*Coronary Arteries.* When E, NE, or ISO in adequate concentration is added to the solution bathing a strip of smooth muscle from a small coronary artery (250–500 $\mu$ diameter) the muscle always relaxes. This is true, of course, only when the muscle is contracted prior to the addition of the catecholamine. Occasionally, these isolated strips have spontaneous myogenic tone in a bath of PSS, but, for the current study it was found advantageous to maintain a degree of artificial tone by stimulation with KCl. In a previous study,[6] it had been observed that the relaxing effect of the catecholamine was not influenced by the agent used to produce contraction, since the relaxation was approximately the same whether contraction was produced by spontaneous tone, elevated KCl concentration, or addition of plasma or whole blood to the bath. The degree of relaxation was, however, highly dependent on the magnitude of the contraction. When contraction was minimal, the possible relaxation was also small; when vigorous contraction was produced, as by depolarization with high concentrations of $K_2SO_4$, the catecholamines could not overcome the contraction unless the calcium concentration of the bath was reduced to about

10% of its physiological level. Relaxation could be studied optimally when about 1/3 max contraction of the smooth muscle was effected.

The relative potencies of E, NE, and ISO are illustrated in FIGURE 1. The current study corroborates our earlier observation[6] that NE is approximately ten times as potent as E both in producing a threshold response and in producing equivalent relaxations. In the present study, ISO was found to be three times as potent as NE in producing this relaxation.

Relaxation produced by all three agents was equivalently eliminated by *beta* adrenergic agents (FIGURE 1). After complete *beta* adrenergic blockade, addition of NE or ISO to the bath, even in high concentrations, failed to produce contraction; on the other hand, E commonly did produce an increase in tension. The relative potency of the three blocking agents studied was propranolol > pronethalol > MJ-1999.

*Alpha* adrenergic blockade with either phentolamine or phenoxybenzamine produced little or no change in the relaxation brought about by any of the three catecholamines.

Exploratory studies on other species indicate that strips from small coronary arteries of humans, rabbits, and monkeys (FIGURE 2) also show predominantly or exclusively *beta* adrenergic activity, and that the relative potency of the three catecholamines is roughly parallel to that studied in detail in strips prepared from dog coronaries.

Responses of large coronary arteries differ from those of small coronary arteries only in the relative *alpha* and *beta* adrenergic activity. Previous

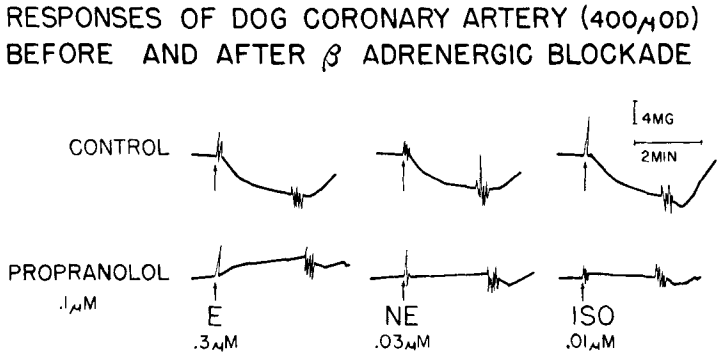


FIGURE 1. Responses of isolated small coronary artery (400 μ o.d.) of the dog before and after *beta* adrenergic blockade. Basal tone of approximately 1/3 maximum contraction was established by increasing the concentration of KCl in the bath to 25 mmoles/l. Concentrations of the three catecholamines were then found which would give approximately equivalent degrees of relaxation, in this case ±7mg. The ratio of the concentrations required for E:NE:ISO was 30:3:1. Propranolol was then added to the PSS to give a concentration of 0.1 μmole/l and the lower row of tracings was obtained. E caused a contraction of approximately 4 mg; NE and ISO failed to alter the basal tension. Injection and rinse artifacts indicate the beginning and end of exposure to the catecholamines.

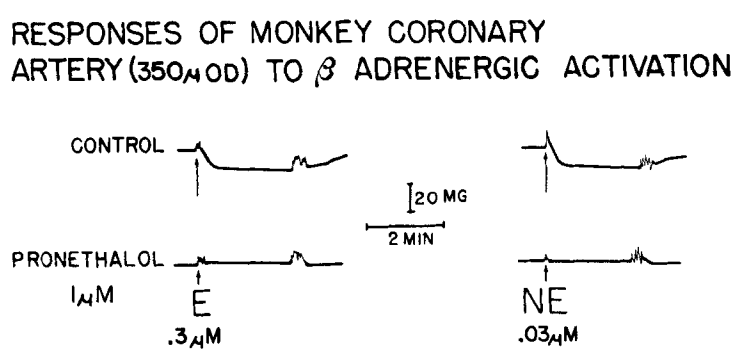


FIGURE 2. Responses of monkey coronary artery (350 $\mu$ o.d.) to *beta* adrenergic activation. The upper row of tracings demonstrates that the ratio of the concentration of E:NE required to produce equivalent relaxations from spontaneously developed tone, was 10:1. Pronethalol (1 $\mu$M) blocked this beta adrenergic relaxation.

studies have indicated that the closer to the aorta the strip is obtained, the higher will be the ratio of *alpha* to *beta* adrenergic activity.[6] In the dog, smooth muscle from coronary arteries of approximately 1.5 mm diameter show a predominance of contraction over relaxation, especially when E is used as the stimulating agent.

When smooth muscle of the large coronary is stimulated with increased concentrations of E, the first response, at low concentrations, may be only contraction; a moderate concentration of E produces initial contraction with subsequent relaxation; high concentrations of E may produce only relaxation.[6] This observation has been interpreted as an indication that the time course for *alpha* receptor activation is shorter than that for *beta* receptor activation.

After complete *beta* adrenergic blockade, E produces a greater contraction than does NE, when equal concentrations of the agents are used. In this *beta*-blocked condition, ISO, even in high concentrations, has no influence on smooth muscle tension. When smooth muscle preparations from these larger coronaries are blocked with phentolamine or phenoxybenzamine, only relaxation is observed, and the relative potency of E, NE and ISO is similar to that in the small coronaries.

*Skeletal Muscle Artery.* In the isolated bath, smooth muscle from small arteries supplying skeletal muscle shows a predominantly constrictor response to E and NE. However, in low concentrations, E produces only relaxation; in moderate concentrations, relaxation preceeding contraction; and in high concentrations, only contraction (FIGURE 3). NE, on the other hand, has almost a pure constrictor effect at all concentrations, and ISO produces only relaxation.

After *alpha* adrenergic blockade (FIGURE 4), all three catecholamines produce relaxation, that of ISO being greatest; that produced by E is usually slightly greater than that produced by NE. When pure *alpha* adrenergic receptor activity is unmasked by blockade with propranolol, only contraction

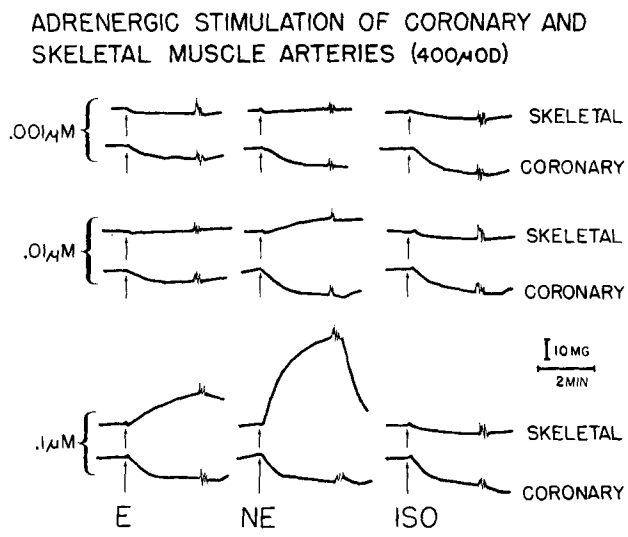


FIGURE 3. Responses of coronary and skeletal muscle arteries (400 $\mu$ o.d.) to increased concentrations (.001, .01 and .1 $\mu$M) of E, NE and ISO. Two strips, one coronary and one skeletal muscle, were mounted in the same PSS bath and tension of the two was recorded simultaneously. The smooth muscle from the coronary relaxed in response to all three agents; E was less potent than NE or ISO, the latter two appear approximately equipotent. ISO at all concentrations produced a slight relaxation of the artery strip from skeletal muscle. E, .001 $\mu$M, produced only relaxation, at .01 $\mu$M the response was ambivalent and at .1 $\mu$M it produced only contraction. NE in skeletal muscle produced a minimal ambivalent effect at the lowest concentration, at the higher concentrations it produced only contraction. In the small artery from skeletal muscle NE was a more potent activator of *alpha* receptors than was E.

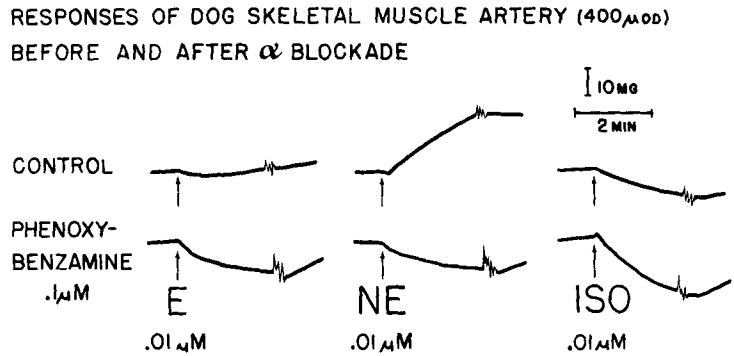


FIGURE 4. Responses of dog skeletal muscle artery (400 $\mu$ o.d.) before and after *alpha* adrenergic blockade. *Upper row,* responses to equimolar concentrations of E, NE and ISO. Response to E was weakly ambivalent whereas that to NE was clearly contraction and that to ISO clearly relaxation. *Lower row, alpha* adrenergic blockade (phenoxybenzamine, .1$\mu$M) unmasked the pure *beta* receptor activity of all three catecholamines. In this experiment ISO was more potent than E or NE, the latter two appear equipotent in producing relaxation.

is observed in response to both E and NE; that which results from NE is significantly greater; ISO causes no response.

## *Discussion*

Two types of receptor differences have been demonstrated in the current experiments. These provide mechanisms through which individualization of vascular control is effected:

1. There is a clear *difference between the ratio of alpha to beta receptors* in small coronary arteries and that in large coronary arteries. The *alpha* receptors at these two sites appear to be similar, as do also the *beta* receptors; comparable receptors from the two sites are alike in relative responsiveness to E, NE and ISO.

2. *Alpha* and *beta* adrenergic receptors of small arteries in skeletal muscle, however, appear to differ from their counterparts in coronary vascular smooth muscle in that the *relative responsiveness to E and to NE is reversed*. These two types of differences are illustrated in FIGURE 5.

The first of these bases for individuality probably represents merely differences in numbers of active sites of alpha and beta receptors. The latter suggests a more subtle difference, whereby the receptors are able to differentiate between E and NE. *Alpha* receptors of coronary vascular smooth muscle are

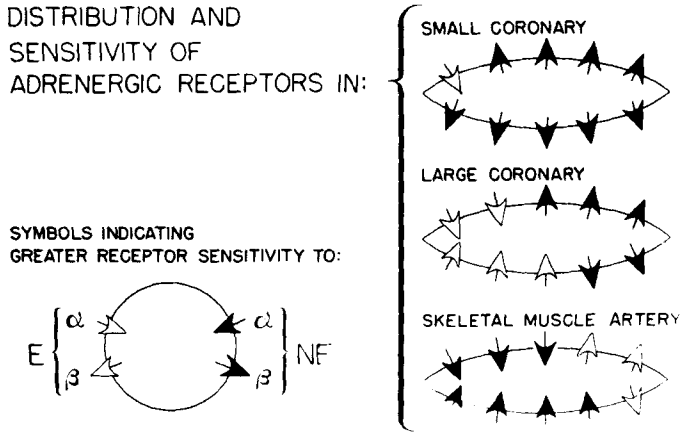


FIGURE 5. Representation of *alpha* and *beta* adrenergic receptors in vascular smooth muscle from three different sites in the dog. Smooth muscle from small coronary arteries (100–500 $\mu$ o.d.) exhibits almost negligible *alpha* adrenergic activity and is shown as having one *alpha* receptor. Smooth muscle from coronary arteries closer to the aorta (greater than 1mm diameter) evidences a greater amount of *alpha* adrenergic activity and less *beta* activity. Smooth muscle from small skeletal muscle arteries (300–500 $\mu$ o.d.) shows an excess of *alpha* adrenergic activity over *beta* adrenergic activity. In smooth muscle from coronary artery the *alpha* receptors are more sensitive to E than to NE; the converse sensitivity is observed for *alpha* receptors of skeletal muscle artery. *Beta* receptors of the coronary vessels are more sensitive to NE than to E, by a factor of 10 to 1; *beta* receptors in the skeletal muscle artery are more sensitive to E than to NE, but the difference is not as marked as in coronary vessels.

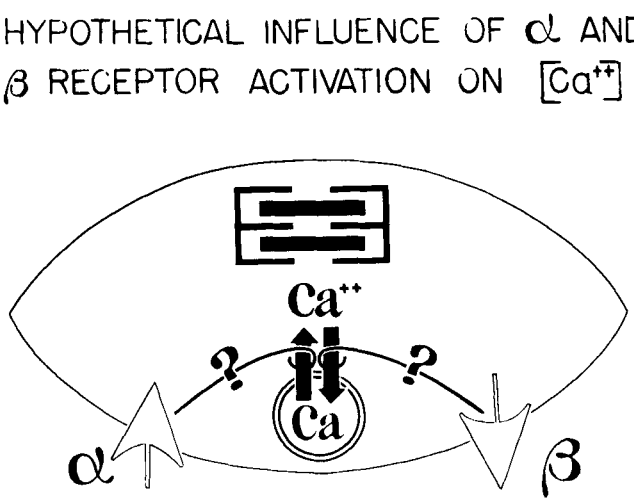


FIGURE 6. Schematic model showing the probable influence of *alpha* and *beta* receptor activity on the concentration of ionized calcium in the environment of the myofilaments. Assuming that the degree of tension developed by actomyosin is a direct function of the concentration of ionized calcium in its environment, then *alpha* adrenergic activity must cause an increase in this concentration and *beta* adrenergic activity, a decrease. The mechanisms through which these receptor activations produce the changes in concentration of ionized calcium are not clear.

more responsive to E than to NE; those of small arteries in the skeletal muscle are more responsive to NE than to E. The converse is true for the responsiveness of *beta* receptors at these two sites. The most unexpected finding in this series is the marked sensitivity of the *beta* receptors in the coronary vessels to NE. The observation that in each tissue the *beta* receptor is more responsive to one of the physiological catecholamines—whereas the *alpha* receptor is more responsive to the other—raises the question of some reciprocal artifact, such as masking of activity of one receptor by that of the other. This possibility would seem to be obviated, however, by the fact that after blockade of one receptor, there persists in the remaining receptor the same ratio of sensitivities that was observed prior to blockade.

Further differences between relative *alpha* and *beta* adrenergic activity in coronaries, compared with that in the skeletal muscle vessels, is observed in the time course of activity of the two systems. Using moderate doses of catecholamines in large coronary arteries, a biphasic response is obtained in which contraction is followed by relaxation. Conversely, in smooth muscle from skeletal muscle arteries, the initial process is always relaxation, and this is followed by contraction. It would appear that in the coronary system the time course or threshold for activity of the *alpha* receptors is lower than that for the *beta* receptors; whereas in the arteries from skeletal muscle, the time course or threshold for activity of the *beta* receptors is lower.

One observation has been made which suggests a final common pathway

for the action of these two antagonistic receptor systems in vascular smooth muscle. During extreme contracture of smooth muscle from small coronary arteries, *beta* adrenergic activity failed to produce relaxation. However, when the normal physiological salt solution in which the muscle was bathed was replaced with one containing approximately 10% of the normal concentration of calcium chloride, the *beta* adrenergic activity was capable of producing relaxation. Since tension development by contractile protein of vascular smooth muscle is dependent upon the calcium concentration in the environment of the myofibril,[7] this suggests that relaxation can be effected by *beta* receptor activity if the concentration of calcium in the environment of the myofibrils is not excessive. It can be hypothesized that *alpha* receptor activity normally causes an increase in ionized calcium in the environment of the myofilaments while *beta* receptor activity causes a sequestration of ionized calcium (FIGURE 6).

## *Summary*

1. Smooth muscle from small coronary arteries has little or no *alpha* receptor activity. The relative potency of E:NE:ISO in activating *beta* receptors of small coronary arteries is 1:10:30.

2. Large coronary arteries demonstrate both *alpha* and *beta* adrenergic activity; the *beta* receptors have the same relative sensitivity to the three catecholamines as the small coronary arteries. *Alpha* receptors of large coronary arteries are more responsive to E than to NE.

3. Isolated smooth muscle from small skeletal muscle arteries contains both *alpha* and *beta* receptors. The relative potency of the three catecholamines in activating the *beta* receptors is ISO$\gg$E$>$NE. Their relative potency in activating the *alpha* receptors is NE$>$E$\gg$ISO.

## *Acknowledgment*

The author gratefully acknowledges the able technical assistance of Miss Judith Coy of this laboratory.

## *References*

1. CELANDER, O. & B. FOLKOW. 1953. A comparison of the sympathetic vasomotor fiber control of the vessels within the skin and the muscles. Acta Physiol. Scand. **29:** 241–250.
2. JOHANSSON, B. & J. B. LANGSTON. 1964. Reflex influence of mesenteric afferents on renal, intestinal and muscle blood flow and on intestinal motility. Acta Physiol. Scand. **61:** 400–412.
3. STAINSBY, W. N. 1964. Autoregulation in skeletal muscle. *In* Autoregulation of Blood Flow. Proceedings of an International Symposium, June, 1963, Indianapolis. Am. Heart Assn. Monograph **8:** 1–39 to 1–45.
4. CARRIER, O., JR., J. R. WALKER & A. C. GUYTON. 1964. Role of oxygen in autoregulation of blood flow in isolated vessels. Am. J. Physiol. **206:** 951–954.
5. BOHR, D. F., P. L. GOULET & A. C. TAQUINI, JR. 1961. Direct tension recording from smooth muscle of resistance vessels from various organs. Angiology **12:** 478–485.

6. ZUBERBUHLER, R. C. & D. F. BOHR. 1965. Responses of coronary smooth muscle to catecholamines. Circulation Res. **16:** 431-440.
7. FILO, R. S., D. F. BOHR & J. C. RUEGG. 1965. Glycerinated skeletal and smooth muscle: calcium and magnesium dependence. Science **147:** 1581-1583.