Biphasic Vasoconstriction of the Rabbit Ear Artery

By Odd S. Steinsland, Robert F. Furchgott, and Sadashiv M. Kirpekar

ABSTRACT

Sympathetic nerve stimulation and intraluminal norepinephrine infusion for more than 15 seconds produced a biphasic response in the isolated rabbit ear artery perfused with Krebs solution. This response consisted of an initial rapid constriction (phase A), which was followed by partial relaxation, and a final slowly developing constriction (phase B), which lasted for the duration of nerve stimulation or norepinephrine administration. Raising the potassium concentration of the Krebs solution to 12mM decreased the relaxation time between the two constrictor phases in response to norepinephrine; lowering the potassium concentration to 1.2 mM increased the relaxation time and decreased the degree of constriction of both phases. Biphasic vasoconstrictor responses were also elicited by the intraluminal infusion of phenylephrine, histamine, serotonin, or 35 mM potassium. When calcium was absent from the perfusing solution or when manganese sulfate (5w) was present, norepinephrine produced only a fast phase A constriction, with no subsequent slow phase B constriction. However, after treatment of the artery with ryanodine, the phase A constriction in response to norepinephrine was markedly inhibited, but the phase B constriction was not. We concluded that the fast phase A constriction depends on the release of calcium from an intracellular pool and that the slow phase B constriction depends on the influx of extracellular calcium.

KEY WORDS norepinephrine phenylephrine serotonin potassium manganese ryanodine calcium

A biphasic constriction of the perfused central ear artery of the rabbit in response to infused norepinephrine or periarterial nerve stimulation was noted by de la Lande et al. (1). This response was characterized by an initial rapid constriction, an intervening partial relaxation, and a final slowly developing constriction, which lasted for the duration of norepinephrine administration or nerve stimulation. More recently, Bevan and Watersoll (2) investigated the relationship between the diffusion of norepinephrine into the wall of the perfused artery and the time course of the biphasic response. During studies of the effect of cholinergic agents on adrenergic neurotransmission in the perfused rabbit ear artery (3, 4), we consistently observed the biphasic response whenever norepinephrine was administered or the periarterial nerves were stimulated for 15 seconds or longer.

Methods

Rabbits (2-4 kg) were killed by a blow to the head. According to the procedure of de la Lande and Rand (5), the proximal portion of the central ear artery (2-4 cm) was dissected free, cannulated at both ends, and mounted in a perfusion chamber 3 mm in diameter and 50 mm long. Two platinum electrodes were fixed near the bottom and the top of the chamber for the application of field stimulation (Fig. 1). The artery was perfused both intra- and extraluminally simultaneously, with both the inner and the outer flows being delivered at a constant rate (about 2 ml/min) by a polystaltic Four-channel pump (Buchler Instruments). In the present experiments, all of the outer flow was pumped from a common reservoir through the main superfusion channel and the minor superfusion channel; 80% of the inner flow was pumped from the same common reservoir through the main perfusion channel, and 20% of the inner flow was pumped from an auxiliary reservoir through the minor perfusion channel. Drugs were added as needed to this auxiliary reservoir. The perfusion temperature was 37°C. The intraluminal inflow perfusion pressure was measured with a Statham pressure transducer and recorded on a Grass polygraph. Since intraluminal flow rate was constant, changes in pressure reflected changes in vasoconstriction. Arterial constrictions were evoked either by field stimulation of the periartrial sympathetic nerves or by intraluminal administration of vasoconstrictor drugs through the...
Schematic diagram of the perfusion and superfusion system. The intraluminal perfusion fluid was pumped in through the main perfusion channel and the minor perfusion channel (A), and the extraluminal superfusion fluid was pumped in through the main superfusion channel and the minor superfusion channel (B). See text for further details.

RESULTS

BiPAsiC PRESSURE RESPONSES TO VARIOUS AGONISTS AND PERIArTERAL SYMPATHETIC NERVE STimulation

Figure 2 illustrates that both the stimulation of the periaortical sympathetic nerves and the intraluminal perfusion of norepinephrine for brief intermittent periods (5-10 seconds) produced a rapid, transient single constrictor response. Figure 2 also shows that, after the period of nerve stimulation or norepinephrine perfusion was extended to 3 minutes, the initial rapid constriction (phase A) was quickly followed by a partial relaxation and then by a slowly developing sustained constriction (phase B), which lasted for the duration of nerve stimulation or norepinephrine perfusion. In over 100 experiments of the type shown in Figure 2, the peak of the phase A response always occurred within 10 seconds of the beginning of nerve stimulation (4-8 Hz) or of the beginning of contact of norepinephrine (15-50 ng/ml) with the artery. The phase B response to nerve stimulation usually reached a maximum within 2 minutes and then exhibited a small decline during the remainder of the stimulation period. The phase B response to perfused norepinephrine usually reached a maximum or nearly a maximum level within 3 minutes of infusion.

The magnitude of phase A constriction was either equal to or greater than that of phase B when the infused norepinephrine concentration was low or when the sympathetic nerves were stimulated at low frequencies (Fig. 3). However, the phase B response became gradually greater than the phase A response as the norepinephrine concentration was increased. Table 1 shows the results of experiments on seven arteries which were first perfused with 10 ng/ml of norepinephrine and then with one or two higher concentrations. In each artery, the ratio of the height of the phase B response to that of the phase A response increased as the norepinephrine concentration was increased.

Histamine (two experiments), phenylephrine (two experiments), and serotonin (three experiments) caused biphasic pressure responses similar to those caused by norepinephrine and nerve stimulation (Fig. 4). However, the rate of relaxation after serotonin infusion was slower than that after infusion of norepinephrine, histamine, or phenylephrine. Also, a rapid increase in the potassium concentration of the intraluminal perfusion medium from the normal level of 5.9 mM to 35 mM caused a biphasic response, although the phase...
Comparison of pressure responses of a perfused rabbit ear artery to short and long periods of stimulation. A: Sympathetic nerve excitation by peritractor field stimulation for periods of 10 seconds and 3 minutes. B: Infusion of norepinephrine (NE) (0.05 μg/ml) for periods of 5 seconds and 3 minutes.

B constriction was less marked in this case than it was in the case of the other stimulating drugs (Fig. 4). The biphasic response to 35 mM KCl was obtained in arteries from reserpine-treated rabbits (four experiments) as well as in those from normal rabbits and therefore did not depend on the release of endogenous norepinephrine.

**TABLE 1**

<table>
<thead>
<tr>
<th>Norepinephrine concentration (μg/ml)</th>
<th>Peak of phase A response (mm Hg)</th>
<th>Ratio of phase B to phase A response</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>65.0 ± 5.3</td>
<td>1.00 ± 0.17</td>
</tr>
<tr>
<td>15</td>
<td>60.0 ± 6.5</td>
<td>1.14 ± 0.12</td>
</tr>
<tr>
<td>30</td>
<td>104.5 ± 6.5</td>
<td>1.52 ± 0.17</td>
</tr>
</tbody>
</table>

Means ± SE for seven experiments in all of which norepinephrine was perfused at 10 and 30 ng/ml and in four of which it was also perfused at 15 ng/ml. Each perfusion period was for 3-5 minutes. The ratio of phase B response to phase A response is the ratio of the increase in pressure 3 minutes after the start of the norepinephrine perfusion to the increase in pressure at the peak of the phase A response.

**EFFECT OF POTASSIUM CONCENTRATION ON THE BIPHASIC RESPONSE TO NOREPINEPHRINE**

Decreasing the potassium concentration of the Krebs solution in both the intra- and the extraluminal perfusates to 1.2 mM, or increasing it to 12 mM, did not by itself produce a response but did alter the relative magnitude and the degree of the separation of the two constrictor phases evoked by norepinephrine. When the potassium concentration was maintained at 1.2 mM, the degree of constriction of both phase A and phase B was depressed, and there was a greater separation between the phases (Fig. 5). However, at a concentration of 12 mM, the magnitude of phase B was enhanced, and the relaxation between the two phases was decreased to the extent that the two constrictor phases became almost continuous. Qualitatively similar results were obtained in five experiments of the type shown in Figure 5.

**EFFECTS OF CALCIUM AND MAGNESIUM ON THE BIPHASIC RESPONSE TO NOREPINEPHRINE**

Figure 6A illustrates that the phase B constrictor response to the administration of norepinephrine was selectively inhibited when calcium was removed from both the intra- and the extraluminal
Differences in the relative magnitude of phase A and phase B pressure responses with different intensities of stimulation. A: Continuous nerve stimulation for 3 minutes at different frequencies. B: Infusion of norepinephrine (NE) for 3 minutes at different concentrations. The same ear artery was used for both nerve stimulation and norepinephrine infusion.

perfusing media. Table 2 summarizes the results of six experiments on arteries when they were first exposed to norepinephrine in normal Krebs solution and then again 5 minutes after the beginning of perfusion with the calcium-free solution. On the average the phase B response was reduced by about 90%, and the phase A response was reduced by only about 20%. Manganese, which is known to block calcium permeability in numerous tissues (6-10), also caused selective inhibition of the phase B response. Figure 6B shows the record from an experiment in which 1 mM MnSO₄ was added to the perfusion medium; Table 2 summarizes the results of six experiments of this type.

If the perfusion fluid was changed from regular Krebs solution to a calcium-free solution and norepinephrine (3 x 10⁻⁸ g/ml) was then tested only two or three times for 1-minute periods during the following hour, the phase A constriction response after 1 hour was still appreciable (Fig. 7). In nine experiments of this type, the phase A response after 1 hour of calcium deprivation averaged 54.2 ± 8.7% of the control phase A response obtained immediately before removal of calcium, and the phase B response was completely suppressed. The complete inhibition of the phase B response and the moderate depression of the phase A response produced by perfusion with a calcium-free solution could be readily reversed by perfusing again with normal Krebs solution.

EFFECT OF RYANODINE ON THE BIPHASIC RESPONSE TO NOREPINEPHRINE

The alkaloid ryanodine selectively inhibited the fast phase A constriction. Figure 8 shows a record of the pressure response to a 3-minute infusion of norepinephrine before and after a 30-minute exposure to ryanodine (3 x 10⁻⁸ g/ml). The results of six experiments with ryanodine are summarized in Table 2. On the average, the phase A response was reduced by over 80%, and the phase B response (measured after 3 minutes of norepinephrine perfusion) was not significantly altered. In some experiments (Fig. 8), ryanodine treatment appeared to potentiate somewhat the phase B
Biphatic pressure responses of perfused rabbit ear arteries to nerve stimulation and to intraluminal infusion of norepinephrine, histamine, phenylephrine, serotonin, and high potassium concentrations.

In every experiment, the rate of relaxation of the artery after removal of norepinephrine at the end of a perfusion period was slower after ryanodine treatment than it was before treatment.

Effects of different concentrations of potassium in the perfusion fluid on the biphasic pressure response of an ear artery to norepinephrine (NE). A: Low potassium. B: Normal potassium. C: High potassium. The concentration of infused norepinephrine was 0.03 μg/ml.
Effects of removal of calcium and of addition of manganese on the biphasic pressure responses to norepinephrine (NE). A: Marked depression of the phase B response to norepinephrine 3 minutes after the perfusate was changed to calcium-free Krebs solution. B: Depression of the phase B response by manganese sulfate (1 mM) added to modified Krebs solution (see Methods) containing the normal concentration of calcium. The concentration of infused norepinephrine was 0.03 μg/ml.

Discussion

The present experiments confirm the findings (1, 2) that norepinephrine, either infused or released by periartrial nerve stimulation, produces a biphasic constriction of the perfused rabbit ear artery. Bevan and Waterson (2) attributed the phase A constriction to a myogenic propagation of excitation into the wall of the artery following

Effect of prolonged perfusion with calcium-free Krebs solution on the phase A pressure response to norepinephrine (NE). Norepinephrine (0.03 μg/ml) was administered at 30-minute intervals for either 1 minute (first three periods) or 2 minutes (last period).
**Biphasic Vasodilation**

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase A</th>
<th>Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca deprivation*</td>
<td>0.93 ± 0.002</td>
<td>0.176 ± 0.034</td>
</tr>
<tr>
<td>MnSO₄ (1 mM)</td>
<td>0.731 ± 0.065</td>
<td>0.030 ± 0.026</td>
</tr>
<tr>
<td>Ryanodine</td>
<td>0.177 ± 0.070</td>
<td>1.073 ± 0.190</td>
</tr>
</tbody>
</table>

Ratio values are means ± SE for six experiments. Phase A response was the pressure increase at the peak of that phase, and phase B response was the pressure increase present after 5 minutes of perfusion with norepinephrine. The concentration of norepinephrine used ranged from 20 to 50 nM.

*Calcium deprivation (perfusion of Krebs solution without calcium) was initiated 5 minutes before perfusion with norepinephrine.

MnSO₄ (1 mM) was added to a tris-hydroxymethylaminomethane-buffered, modified Krebs solution (see Methods) 5 minutes before perfusion with norepinephrine. In four experiments with added manganese 95% O₂-5% CO₂ was used for perfusion, and in two experiments 100% O₂ was used. Results were similar with both gases and were therefore pooled.

Ryanodine (10 μg/ml) was perfused for 10-30 minutes, and norepinephrine was perfused 10 minutes after termination of perfusion with ryanodine.

**Figure 6**

Effect of ryanodine on the biphasic response to norepinephrine (NE). The concentration of infused norepinephrine was 0.03 μg/ml. Ryanodine (5 μg/ml) was infused for 30 minutes before the second norepinephrine infusion. Phase A was markedly depressed after ryanodine treatment. Note the delayed relaxation after norepinephrine washout.

Conclusions are summarized at the end of the document.
but both changes did produce striking modifications in the pattern of the biphasic vasoconstrictor response to norepinephrine (Fig. 5). It is likely that these modifications are related to changes in intracellular calcium, which readily equilibrates with extracellular calcium. In skeletal muscle, this store is in extracellular fluid. The fact that ryanodine selectively inhibits the phase A constriction (10-18), although ryanodine produces rigor in stimulated skeletal muscle in a calcium-containing medium, it inhibits the contractile response of skeletal muscle in a calcium-free medium (18). The effect of ryanodine action is not understood, but it appears that this agent interferes in some way with the intracellular calcium binding in muscle (19). The fact that ryanodine selectively inhibits the phase A constriction is due to the intracellular release of membrane-bound calcium which readily equilibrates with extracellular calcium.

To explain the transiency of the phase A constriction, Bevan and Waterson (2) proposed that excitation in the ear artery may be related more to the rate of change of norepinephrine concentration than to the absolute concentration of norepinephrine, so that only when the rate of rise of norepinephrine concentration near the smooth muscle cell is very rapid will the necessary rapid depolarization to produce a myogenically propagated excitation be possible. The characteristics of diffusion into the muscle wall, the necessary rapid depolarization to produce a myogenically propagated excitation, Bevan and Waterson (2) proposed that excitation in the ear artery may be related more to the rate of change of norepinephrine concentration than to the absolute concentration of norepinephrine, so that only when the rate of rise of norepinephrine concentration near the smooth muscle cell is very rapid will the necessary rapid depolarization to produce a myogenically propagated excitation.

Electrical stimulation even after prolonged exposure to calcium-free solution (15). Although ryanodine produces rigor in stimulated skeletal muscle in a calcium-containing medium, it inhibits the contractile response of skeletal muscle in a calcium-free medium (18). The effect of ryanodine action is not understood, but it appears that this agent interferes in some way with the intracellular calcium binding in muscle (19). The fact that ryanodine selectively inhibits the phase A constriction is due to the intracellular release of membrane-bound calcium which readily equilibrates with extracellular calcium.

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The plant alkaloid, ryanodine, inhibits the contractile response of cardiac muscle to electrical stimulation (16-18). Although ryanodine produces rigor in stimulated skeletal muscle in a calcium-containing medium, it inhibits the contractile response of skeletal muscle in a calcium-free medium (18). The effect of ryanodine action is not understood, but it appears that this agent interferes in some way with the intracellular calcium binding in muscle (19). The fact that ryanodine selectively inhibits the phase A constriction is due to the intracellular release of membrane-bound calcium which readily equilibrates with extracellular calcium.

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If depolarization of cells within the muscle increased simultaneously with the rise in norepinephrine concentration in the extracellular space, then, possibly by making the cells more permeable to extracellular calcium or by releasing membrane-bound calcium intracellularly, the depolarization might partially account for the slowly developing phase B constriction. However, the phase B constriction might also partially result from an action of norepinephrine independent of depolarization. This latter possibility is favored because many isolated arteries, when brought to complete depolarization in solutions in which all sodium has been replaced by potassium, can still give additional contraction in response to norepinephrine and other stimulating drugs (20).

Bohr (21) showed that the total norepinephrine-stimulated contraction of the rabbit aortic strip could be separated into an initial fast and a final slow component. The fast component was completed within 45-60 seconds and, without any intervening relaxation, was followed by the slow component, which often required many minutes for completion. The slow component was usually abolished at a calcium concentration below 0.3 mm, but the fast component was often enhanced by lower-than-normal calcium concentrations and depressed by higher-than-normal concentrations. Bohr (21) suggested that the fast contractile component depended on membrane excitability which increased as extracellular calcium concentration was decreased. More recently, Stratton and Bohr (22), by modifying calcium and sodium concentrations of the bathing solution, were able to differentiate the fast and slow components of the contractile response of dog mesenteric artery strips to norepinephrine. They suggested that cellular-bound calcium was the activator of the fast component, and that extracellular calcium was the activator of the slow component. It is not clear at present whether the two components of contraction observed in the rabbit aortic strip and the dog mesenteric artery strip are similar in nature to the two distinct phases of contraction exhibited by the rabbit ear artery.

However, it appears very unlikely that the fast component of contraction of the rabbit aortic strip is similar in nature to the phase A constriction of the ear artery, because it has a much longer time course than the phase A constriction, is not followed by a relaxation phase, and is not altered by treatment with ryanodine (unpublished observations).


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Circ Res. 1973;32:49-58
doi: 10.1161/01.RES.32.1.49

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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