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 12 mM 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 (LMW) 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, histamine, 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, cumulated 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 constractions were evoked either by field stimulation of the periarterial 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.

Figure 2 illustrates that both the stimulation of the periarterial sympathetic nerves and the intraluminal perfusion of norepinephrine for brief intermittent periods (5–10 seconds) produced a rapid, transient constriction. Figure 2 also shows that, after the period of nerve stimulation or superfusion 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 superfusion. In over 100 experiments of the type shown in Figure 2, the peak of the phase A response usually occurred within 10 seconds of the beginning of nerve stimulation (4–6 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 superfused 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 or as the frequency of nerve stimulation 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 peritractorial field stimulation for periods of 10 seconds and 3 minutes. B: Injections 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 (mm Hg)</th>
<th>Ratio of phase B to phase A response</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>45.0 ± 5.5</td>
<td>1.00 ± 0.17</td>
</tr>
<tr>
<td>15</td>
<td>66.0 ± 6.5</td>
<td>1.14 ± 0.12</td>
</tr>
<tr>
<td>20</td>
<td>104.5 ± 4.5</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 µg/ml and in four of which it was also perfused at 15 µg/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 CALCIUM AND MANGANESE ON THE BIPHASIC RESPONSE TO NOREPIHNEPHINE

Figure 6A illustrates that the phase B constriction 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.

EFFECT OF RYANObine 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 response.
response. 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 ng/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 periarterial 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 VASOCONSTRICTION

EFFECTS OF CALCIUM DEPRIVATION, MANGANESE, AND RYANODINE ON THE TWO PHASES OF THE VASOCONSTRICTOR RESPONSE OF THE RABBIT EAR ARTERY TO NOREPIENEPHRINE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase A</th>
<th>Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca deprivation*</td>
<td>0.900 ± 0.002</td>
<td>0.726 ± 0.004</td>
</tr>
<tr>
<td>MnSO₄ (1 mM)</td>
<td>0.731 ± 0.065</td>
<td>0.093 ± 0.026</td>
</tr>
<tr>
<td>Ryanodine</td>
<td>0.177 ± 0.070</td>
<td>1.071 ± 0.100</td>
</tr>
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</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 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 aeration and in two experiments 100% O₂ was used. Results were similar with both gases and were therefore pooled.

Ryanodine (1-10 μg/ml) was perfused for 30-60 minutes, and norepinephrine was perfused 10 minutes after termination of perfusion with manganese.

The biphasic constrictor response of the rabbit ear artery is not restricted to stimulation of that preparation by norepinephrine. Phenylephrine, which like norepinephrine acts on alpha receptors, and histamine and serotonin, which act on other kinds of receptors, also produced a similar biphasic response (Fig. 4). Even a sudden increase in the potassium concentration of the perfusion solution to 35 mM (about six times normal) produced a biphasic response, although in this case the magnitude of the phase B constriction was distinctly less than it was in the case of the other stimulating agents.

Reducing the potassium concentration in the perfusion fluid to about a fifth of normal or raising it to two times normal caused no significant alterations in basal perfusion pressure of the artery.
but both changes did produce striking modifications in the pattern of the biphasic constrictor response to norepinephrine (Fig. 5). It is likely that these modifications are related to changes in membrane potential caused by the changes in extracellular potassium concentration, but speculation about detailed mechanisms is not justified at this time.

Our experiments with calcium-free perfusion medium and with manganous (Fig. 6, Table 2) indicate that the calcium ions which are involved in producing the total constrictor response to norepinephrine reach the contractile proteins from two separate sources. Since the slow phase B response was selectively inhibited after a short perfusion with calcium-free solution or in the presence of manganous ions, which reduce permeability to calcium ions in numerous tissues (6-10), the source of the calcium ions mediating the phase B response appears to be the calcium of the extracellular fluid. However, the fast phase A response was little affected by the time the phase B response had been markedly or completely inhibited by the removal of extracellular calcium or the addition of manganous; even after the 1-hour perfusion with calcium-free solution, the phase A response was only moderately reduced in magnitude (Fig. 7). Therefore, an intracellular pool of calcium that does not readily equilibrate with extracellular calcium appears to supply the calcium ions mediating the phase A response.

If Bevan and Waterson (2) are correct in concluding that an initial transient myogenic propagation of excitation is responsible for the phase A response, then this propagated excitation could be the stimulus for release of the intracellular calcium mediating the response. Reports from other laboratories have also indicated that the calcium ions which activate contractile elements in some smooth muscle cells may arise, in part, from a reservoir of calcium which is associated with specific intracellular components (11-14). Thus, some smooth muscle cells, including those of the rabbit ear artery, may resemble skeletal muscle cells in that they have an intracellular store of activator calcium. In skeletal muscle, this store is in subsarcolemmal structures that form part of the sarcoplasmic reticulum and accounts for the persistence of vigorous contractile responses to electrical stimulation even after prolonged exposure to calcium-free solution (15).

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 (19). The exact mechanism 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 in the perfused artery (Fig. 8, Table 2) provides additional supporting evidence that the fast constriction is activated by calcium ions released from an intracellular pool and the slow phase B constriction is activated by calcium ions entering the muscle from the extracellular fluid. Our results do not show whether activation of the phase B constriction results directly from a greater influx of calcium ions from the extracellular fluid or from an 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 reaches the contractile proteins from two separate sources. Since the slow phase B response was selectively inhibited after a short perfusion with calcium-free solution or in the presence of manganous ions, which reduce permeability to calcium ions in numerous tissues (6-10), the source of the calcium ions mediating the phase B response appears to be the calcium of the extracellular fluid. However, the fast phase A response was little affected by the time the phase B response had been markedly or completely inhibited by the removal of extracellular calcium or the addition of manganous; even after the 1-hour perfusion with calcium-free solution, the phase A response was only moderately reduced in magnitude (Fig. 7). Therefore, an intracellular pool of calcium that does not readily equilibrate with extracellular calcium appears to supply the calcium ions mediating the phase A response.

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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 epinephrine-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, Stritir 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 epinephrine. 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).

References


References:

1. STEINSLAND, FURCHGOTT, KIRPEKAR
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