Distribution Theory of Resistance of Neurogenic Vasoconstriction to Alpha-Receptor Blockade in the Rabbit

By John A. Bevan and Che Su

ABSTRACT

The effects of α-receptor-blocking agents on the contractile responses of isolated rabbit arteries to sympathetic nerve stimulation and exogenous L-norepinephrine (L-NE) were compared. In the pulmonary artery and aorta, yohimbine, phentolamine, and phenoxybenzamine blocked the response to nerve stimulation less than that to an equipotent dose of L-NE. This resistance of neurogenic response was independent of the frequency and number of stimuli and persisted after inhibition of the nerve L-NE uptake by cocaine. The neurogenically released transmitter L-NE probably forms a high concentration near the adventitia-media junction, whereas the exogenous NE is distributed evenly throughout the thickness of media. Thus higher concentrations of α-receptor-blocking agents would be needed to block the effect of neurogenic L-NE than to block that of exogenous L-NE. This explanation of the resistance was thought to be more appropriate to the large vessels tested than that based on neuroeffector proximity.

KEY WORDS: distribution of NE phentolamine tunica media neuroeffector distance yohimbine smooth muscle phenoxybenzamine cocaine

In 1906, Dale (1) observed that ergot was less effective in blocking the effector response to sympathetic nervous activity than that to circulating epinephrine. Subsequently, parallel observations have been made with various α-receptor blocking agents at many adrenergically innervated sites. In his review, Nickerson (2) concluded that all α-receptor-blocking agents appear to be more effective against responses to circulating sympathomimetic agents than against responses to sympathetic nervous activity. All degrees of relative effectiveness of α-receptor-blocking agents against these two types of responses may be found at different sites in the body. With the exception of Levin and Beck (3) and Urquilla et al. (4), this conclusion is generally not questioned. Indeed it has been substantiated in recent studies with both vascular (5) and nonvascular tissues (6, 7).

The most generally accepted explanation of this type of phenomenon has been termed the proximity theory. This theory was originally proposed by Dale and Gaddum (8) to explain the resistance of a cholinergic synapse to atropine. They proposed “the liberation of choline ester in a relation of so much greater intimacy with the receptive mechanism that atropine cannot prevent its access to it.”

With our present knowledge of synaptic mechanism, the essential basis of the proximity theory might be restated in the following terms. Because of the narrowness of the synaptic cleft, the concentration of transmitter found between pre- and post synaptic membranes is not significantly affected by diffusion during the relative short time of transmission. In fact, diffusion barriers may exist which hinder transmitter egress from the cleft. This and the small spatial dimensions of the cleft...
result in extremely high local transmitter concentrations. For the same reasons, there is difficulty of access of blocking agents to the postsynaptic receptors acted upon by the neurogenically released transmitter. Presumably, both mechanisms contribute to the apparent resistance of the neurogenic response to α-receptor blockade.

On the other hand the receptors that are acted upon by exogenous agonists and are not necessarily confined to the cleft are those most "exposed" to these blocking agents in the extracellular space. Such receptors, other factors being equal, would be equally and readily accessible to both agonist and antagonist drugs.

Recent electron microscopic studies of large vessels have shown that the neuroeffector cleft in these structures is considerably wider than in many other adrenergic sites. The mean separation between bare nerve terminal elements and the closest smooth muscle plasma-lemma in the rabbit pulmonary artery, for example, was 19,000 Å (9). Furthermore, in this artery there is no clear organizational arrangement between the nodes on the terminal effector plexus and the subjacent individual smooth muscle lamellae. Since the effector innervation of this vessel in the adult is essentially restricted to the adventitia-media junction (10), there can be no close relationship between the terminal neural plexus and deeper medial cells.

These unique cytoarchitectural features of the large blood vessels afford an opportunity to test the proximity theory. It seems unlikely that a synaptic cleft of 1μ or more, which is greater than the mean intermembrane distance of the vascular muscle cells (11), could form a significant barrier to the entry of drugs. In such a tissue, the proximity theory would be untenable.

The sympathetic nerve-pulmonary artery preparation of the rabbit (12) or preparations derived from it were used. Experiments showed that the dose of α-receptor-blocking agent required to antagonize the response of the artery to neurogenic activity was significantly greater than that to an equipotent dose of l-norepinephrine (l-NE). It is proposed that the relative resistance to blockade of the sympathetic neurogenic response is the consequence of the nonuniform spatial distribution of transmitter within the media of the arterial wall. This hypothesis has been previously described in a preliminary communication (12).

Methods
Experiments described in this paper were carried out with the standard helical strip preparation of the rabbit aorta (13), the isolated right recurrent cardiac nerve-pulmonary artery preparation (14), and the superfused, transmurally stimulated vessel strip (15). All preparations were immersed in Krebs bicarbonate saline solution equilibrated with 95% O₂ and 5% CO₂ at 38°C.

Isolated Right Recurrent Cardiac Nerve-Pulmonary Artery Preparation.—The cardiac nerve-pulmonary artery ring was isolated from the rabbit and set up in an organ bath containing 50 ml Krebs bicarbonate solution. Stimulation was applied at the nerve with a pair of ring electrodes through which the nerve was passed. Trains of 200 rectangular pulses of 2-msec duration, supramaximal voltage, frequency 10/sec, were applied at 4-minute intervals. The contractile responses were recorded with an isometric strain gauge and a pen recorder. The contractile responses to cumulative doses of l-NE were ascertained by leaving each dose in the bath until the response attained a plateau before the subsequent dose was added. Doses which caused responses both smaller and larger than the neurogenic response were selected.

Superfused and Transmurally Stimulated Aorta Strip.—The rabbit thoracic aorta was helically cut into 4 × 30 mm strips and superfused with Krebs bicarbonate solution at 37°C. Nerve stimulation was achieved by applying pulses transmurally, and contraction was recorded isometrically (16). The stimulation consisted of rectangular pulses of 0.3 msec, supramaximal voltage, varying frequencies, given in trains of 100, 200, or 500 pulses, or as long as needed to produce a steady-state contraction. After the exposure of the artery to the α-receptor-blocking agent, it was stimulated with only one train of pulses before it was discarded.

Nature of Yohimbine-l-Norepinephrine Antagonism.—Characteristics of the antagonism between l-NE and yohimbine were determined by using four rings from each rabbit aorta simultaneously. Each ring was suspended in an organ bath containing 50 ml Krebs bicarbonate solution. The
response was recorded isometrically. After 1 hour of equilibration, contractions to 0.02 μg/ml L-NE were elicited. After washing several times, varying doses of yohimbine were added to three baths; the fourth was used as control. After 30-minute exposure to yohimbine, responses to cumulative doses of L-NE were elicited in the presence of the blocking agent. Each dose of L-NE was added to the bath for 5 to 10 minutes, during which the response attained a plateau. The expected maximal responses for the treated tissues were calculated from the proportion of their response to the response of control tissue initially obtained with 0.02 μg/ml L-NE, this proportion was multiplied by the ratio of the responses of control tissue to the maximal dose and initial 0.02 μg/ml L-NE. Each response was plotted as percent of the expected maximum against dosage of L-NE.

**Results**

1. Cardiac Nerve-Pulmonary Artery Preparation
   - Equivalent responses to nerve stimulation and L-NE were determined before and 30 minutes after addition of a submaximal blocking dose of an α-receptor-blocking agent to the tissue bath. After addition of this blocking agent, doses of L-NE higher than those used in the control were invariably required to span the neurogenic responses. Doses of α-receptor-blocking agent were selected to reduce the neurogenic response by more than 70%. Since phenoxybenzamine does not produce an equilibrium blockade within the permissible duration of an experiment, the blocking effects of a single dose of this drug were studied 2 hours after its addition to the tissue bath.

2. Superfused Transmurally Stimulated Aorta
   - There is evidence that all nerve endings in the arterial wall can be excited by transmural electrical stimulation during superfusion without concomitant excitation of vascular muscle cells.
   - The α-receptor-blocking drug yohimbine was used extensively in this study. Up to a dose of 30 μg/ml, yohimbine caused no depression of the maximum response of the aorta to L-NE. The mean ratio of experimental to expected maximal L-NE responses was 1.08 ± 0.031 (n = 5). The dose-response curve was displaced in parallel and to the right of the control curve. Furthermore, the plot of dose of antagonist against that of agonist to produce a standard response (19) was linear up to a yohimbine concentration of 30 μg/ml.

   In Table 1, the effect of yohimbine on the neurogenic response of rabbit aorta to trains of 100, 200, and 500 pulses which result in progressively greater responses is shown. The
Effect of Yohimbine on the Neurogenic Contractile Response

<table>
<thead>
<tr>
<th>Pulses in train</th>
<th>No. of expts</th>
<th>Yohimbine (µg/ml)</th>
<th>Percent blockade of neurogenic response</th>
<th>EQUIV. CONCN. 1-NE after blockade/before blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3</td>
<td>1.1 ± 0.2</td>
<td>85.3 ± 6.1</td>
<td>5.3 ± 1.85</td>
</tr>
<tr>
<td>200</td>
<td>5</td>
<td>1.32 ± 0.27</td>
<td>74.7 ± 1.9</td>
<td>6.45 ± 1.54</td>
</tr>
<tr>
<td>500</td>
<td>3</td>
<td>1.8 ± 0.16</td>
<td>91.0 ± 3.05</td>
<td>63.2 ± 2.17</td>
</tr>
<tr>
<td>Steady state</td>
<td>4</td>
<td>21.33 ± 1.1</td>
<td>89.75 ± 2.2</td>
<td>28.4 ± 3.75</td>
</tr>
<tr>
<td>200*</td>
<td>4</td>
<td>1.33 ± 0.12</td>
<td>82.75 ± 4.26</td>
<td>7.73 ± 1.28</td>
</tr>
</tbody>
</table>

*Experiments carried out in the presence of cocaine 3 µg/ml.

Pulse trains were delivered at 10/sec. EQUIV. CONCN is concentration of 1-NE required to produce an equivalent response (all values are means ± SE).

TABLE 2

Effect of Yohimbine on Steady-State Neurogenic Contractile Response at Different Frequencies

<table>
<thead>
<tr>
<th>Pulses trains/sec</th>
<th>No. of expts</th>
<th>Yohimbine (µg/ml)</th>
<th>Percent of neurogenic blockade</th>
<th>EQUIV. CONCN. 1-NE after/ before blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>4.8 ± 0.19</td>
<td>90.3 ± 2.96</td>
<td>5.07 ± 0.54</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10 ± 0.0</td>
<td>88.3 ± 3.74</td>
<td>11.83 ± 2.17</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>21.25 ± 1.1</td>
<td>89.75 ± 2.2</td>
<td>28.4 ± 3.75</td>
</tr>
</tbody>
</table>

See footnote to Table 1.

FIGURE 1

The effect of submaximal blocking dose of yohimbine on the contractile response of the transversely stimulated superfused rabbit aorta strip to repetitive nerve stimulation (n. St.) (200 pulses, 10/sec, duration 0.3 msec) and to cumulative doses of 1-NE expressed as µg/50 ml bath solution.

steady-state response to continuous nerve stimulation at various frequencies and the doses of 1-NE that elicit an equivalent response are shown in Table 2 (for example, Fig. 1). Doses of yohimbine were chosen which caused between 70 and 95% blockade of the neurogenic response. Under all conditions, the response of the vessel to nerve stimulation appears considerably more resistant to blockade than that to 1-NE.
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B. Influence of Transmitter Uptake Inhibition

The main route of disposition of neurogenically released adrenergically transmitter in the elastic artery is reuptake into terminal nerve endings (20). If this route is blocked, the neurogenic response will be potentiated, since released transmitter is not diverted to neural sites. Alpha-receptor-blocking agents inhibit catecholamine reuptake (21, 22). Yohimbine is no exception. The mean uptake of $^3$H-NE (0.5 μL) into four control aorta strips after 45 minutes was 18.86 ± 4.07 ml/g wet weight (mean ± SE). Uptake in the presence of yohimbine (20 μg/ml) was 9.10 ± 1.20 in four other strips. These values are significantly different ($P < 0.05$). If the neurogenic response was potentiated by uptake inhibition more than the response to exogenous Z-NE, this might account for the apparent resistance of the neurogenic response to α-receptor-blocking agents.

This possibility was tested by comparing the relative sensitivities to yohimbine of the neurogenic and Z-NE responses matched after treatment with cocaine, a drug that inhibits neural uptake of Z-NE (Table 1). Tissues were first treated with cocaine (3 μg/ml) for 30 minutes. This concentration maximally potentiates the neurogenic response of the blood vessel (10) and greatly inhibits Z-NE uptake into the sympathetic nerve terminals (23). It is clear that previous treatment with cocaine does not significantly reduce the relative concentration of NE that matches the neurogenic response before and after blockade. There is evidence that cocaine exerts an action at postsynaptic sites in vascular muscle (10). However, the latter effect should be common to both responses and would not negate the validity of the conclusion.

Since the relative sensitivities of the two responses to yohimbine are unchanged by cocaine in doses that potentiate the magnitude of the neurogenic response, transmitter uptake inhibition is not an important factor determining the relative resistance of the neurogenic response to α-receptor blockade.

C. TRANSMITTER DISTRIBUTION HYPOTHESIS

Adrenergic transmitter liberated in the region of the adventitia-media junction subsequently emerges from both adventitial and intimal surfaces of the arterial wall (20). Consequently, a concentration gradient in the extracellular space should be highest near the site of release and lowest near the intima and the outer edge of adventitia. Experimental evidence in support of this has been obtained (24).

In contrast, exogenous Z-NE would probably be distributed evenly throughout the thickness of the vessel wall. If these considerations are true, for equivalent responses the peak concentration of transmitter in the media would be higher during the neurogenic than during the exogenous Z-NE response. Furthermore, higher doses of α-receptor-blocking agents would be needed to block the former than to block the latter.

The distribution of exogenous NE in the blood vessel wall was determined in helical strips of rabbit aorta by the isotope-frozen section technique after exposure of both surfaces to $^3$H-NE. The tissues were first treated with cocaine (3 μg/ml) for 30 minutes and then exposed to $^3$H-NE for 10 minutes, the time to equilibrium contraction of an equivalent amount of Z-NE. This concentration of cocaine will block specific uptake of NE into nerve but not muscle tissue (23, 24). Only after the inhibition of nerve uptake can medial and adventitial distribution of NE be determined by this technique.

In Figure 2 the distribution of radioactivity throughout the thickness of the wall of the vessel is shown. The amount of radioactivity in the media is fairly constant throughout its thickness and higher than that expected from occupation by the bath solution of the extracellular space. This space, which is approximately 0.40 ml/g, is evenly distributed throughout the media (25). Thus the tritiated material must include $^3$H-NE bound to tissue elements.

Discussion

Since the extraordinarily large nerve-muscle junction in the large blood vessels used in this study renders the proximity theory untenable, other possible explanations of the resistance of
the neurogenic response to α-receptor-blocking agents must be entertained. Explanations based upon the existence of junctional and extrajunctional receptors proposed for other tissues (6, 7) are not applicable to large blood vessels. Electron microscope studies show absence of neuromuscular juxtaposition and specialized areas in the vascular muscle cells. Because of this, it is difficult not to reach the conclusion that l-NE, whether neurogenic or exogenous, would have equal access to all aspects of a smooth muscle cell (9, 11).

The present work demonstrates unequivocally that the phenomenon of resistance of neurogenic response to blockade exists in the sympathetically innervated large blood vessels of the rabbit. It has been demonstrated by yohimbine, phenolamine, and phenoxybenzamine in the cardiac nerve-pulmonary artery preparation of the rabbit, and by yohimbine, which is a reversible competitive α-receptor antagonist, in the superfused, transmurally stimulated aorta. Its presence is independent of the number and frequency of impulses in the train of nerve stimulation. Furthermore, it is not associated with the uptake-blocking action of the blocking agent, since the effect is still present to the same degree in tissues in which the reuptake or neurogenic NE has been depressed by cocaine.

One possible explanation of the neurogenic resistance might be termed the mixed transmitter hypothesis. If a second substance in addition to l-NE were released during neuromuscular transmission and made a significant contribution to the transference of excitation, this could account for resistance to blockade.

L-Norepinephrine is generally believed to be the postganglionic sympathetic neurotransmitter in mammals (for review see ref. 20). The characteristics of the rabbit pulmonary artery are uniformly consistent with this concept. Thus the contractile response of this artery to sympathetic nerve stimulation was blocked by yohimbine, phenoxybenzamine, bretylium, and reserpine and potentiated by cocaine, whereas hexamethonium and atropine were without effect (14, 18). Use of physostigmine, hemicholinium, and bretylium gave no evidence for the involvement of acetylcholine in the sympathetic neuroeffector transmission (27). Histological and electron microscopic studies demonstrated structures which could be identified with the adrenergic nerve terminal plexus and catecholamine storage vesicles (9, 11). The vasoconstrictor substance released from the pulmonary artery by sympathetic nerve stimulation was recently assayed and identified with NE or epinephrine by blocking its vasoconstrictor action with yohimbine and by its spectrofluorometric spectrum (28). Even if a small amount of epinephrine is contained in the neurotransmitter, it is as susceptible to α-receptor-blocking agents as l-NE in rabbit aorta strips (29), and its presence cannot account for the resistance under discussion. Although many other naturally occurring vasoconstrictor substances exist, there is insufficient evidence, as far as

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we are aware, to warrant serious consideration of these substances as sympathetic transmitters in the pulmonary artery.

Certain temporal factors should be considered. In this study, comparisons are made between a state in which the concentration of l-NE in the region of the muscle cells is presumed to be essentially steady (that following exogenous NE) and the state following nerve stimulation in which the concentration is not steady. Presumably, the transmitter is discharged intermittently by nerve impulses, and, particularly in relation to those cells close to the nerve plexus, the local concentration of l-NE might oscillate at a rate which is some function of nervous activity (28). Nothing is known about the time relationships of NE concentration changes in the extracellular space and their quantitative relationship to contraction. At present, this mechanism remains a possible contributory factor to the blockade resistance.

There is very considerable evidence that myogenic propagation of excitation is of little consequence in the media of the rabbit aorta (30, 31). This being the case, the distribution of l-NE in the media, and consequently the distribution of its concentration at receptors, throughout the vessel wall becomes of importance in determining the magnitude of the total response of the media.

There is experimental evidence that, whereas the distribution of exogenous l-NE at equilibrium is essentially uniform throughout the media (Fig. 2), the distribution of l-NE during nervous activity is not (24). During steady-state contraction established by continuous nervous activity, the extracellular concentration of liberated transmitter is high near the adventitia-media junction and falls off toward the intima to concentrations similar to those in the lumen. Since the distribution of exogenous l-NE was determined in the presence of cocaine, a drug which blocks uptake by the nerves, it might be argued that this experimental condition is inappropriate to the argument. Previous studies (10), however, have shown that the sensitivity of the aorta to exogenous l-NE is independent of the presence of the nerve plexus. Thus nerve structures do not significantly influence the distribution of exogenous l-NE in the media. If we assume homogeneity of muscle cell sensitivity, contractility and density throughout medial thickness, a uniform distribution of exogenous and a nonuniform distribution of neurogenic l-NE, for equal responses to neurogenic and exogenous l-NE, the peak concentration for the neurogenic must be higher than for the exogenous (Fig. 3). The concentration of an α-receptor-blocking agent that would just block a response to l-NE is obviously a function of l-NE concentration at the receptor: the higher the concentration of l-NE, the higher the concentration of blocking agent. Thus a dose of blocking agent that would just obliterate or antagonize exogenous l-NE throughout medial thickness would reduce but not eliminate that to neurogenic l-NE near the adventitia-media junction, where it forms a peak concentration.

Two recent studies do not support the concept of a relative resistance of the neurogenic response to α-receptor blockade. Levin and Beck (3), using the perfused extremity of the dog, found that phenoxybenzamine produced a significantly greater reduction in the response to pre- and postganglionic stimulation than to intraarterially injected NE. It is of note that the vascular response measured by these authors reflects, by and
large, changes in diameter of the small resistance blood vessels. Compared with the elastic arteries, the media of these vessels is thin and the innervation relatively dense. As a consequence, differences in distribution of endogenous and exogenous NE in these vessels would be minimized and other considerations would become important.

The findings of Urquilla et al. (4) are less satisfactorily explainable. They studied the effect of phentolamine on the responses of rabbit aorta strips to nerve stimulation and exogenous NE and found that the response to exogenous NE more resistant to blockade than the response to nerve stimulation. There are important technical differences between the two studies. Urquilla et al. (4) used only propranolol-blocked preparations, and these were exposed to the blocking drug too briefly to reach equilibrium. Furthermore, the mode of electrical excitation of intramural nerve elements produced concurrent direct excitation of smooth muscle elements, and pulse trains were restricted to 10 seconds. Without further experimentation, it is impossible to assess the importance of these considerations. Ljung (5) has pointed out that when the concentration of exogenous NE is below that found at local muscle sites during nerve activity, as it is under most circumstances, an alpha-receptor-blocking agent would block the former more readily than the latter. On the other hand, if exogenous NE levels exceeded the levels found during nerve activity, the response to exogenous NE levels would be the more resistant to blockade of the two. By the design of our experiments, this cannot occur. Although this is a possible explanation of our differences with Urquilla, on the basis of the limited evidence available, we do not consider it adequate.

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References
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