Asymmetry of Consequences of Drug Disposition Mechanisms in the Wall of the Rabbit Aorta

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SUMMARY The contraction elicited by norepinephrine (NE) and histamine (H) in the rabbit aortic strip occurs after a shorter latency and increases at a higher initial velocity to a greater steady state level when drug entry is limited to the intimal compared with the adventitial surface. Differences in the steady state contraction, but not in latency or initial velocity, disappear when intramural disposition pathways of the two agonists are blocked pharmacologically by deoxycorticosterone and a combination of iproniazid and 17β-estradiol, respectively. These and other observations are consistent with the hypothesis that inner vascular smooth muscle cells respond more than outer cells to submaximal concentrations of NE and H, an explanation that accounts for the persistence of differences in latency and initial velocity of response after disposition blockade. Although the effectiveness of disposition mechanisms in reducing agonist concentration may be uniform through the aortic medial thickness, because of differences in muscle sensitivity, the consequence of disposition on the contractile response to agonists entering through the adventitia would be greater than that through the intima. Thus, disposition mechanisms in the outer smooth muscle lamellae provide a functional barrier to susceptible agonists entering through the outside vessel wall surface.

The characteristics of the steady state contractile response of rabbit thoracic aortic strips to norepinephrine (NE) and to histamine (H) vary according to whether these drugs enter the media via the adventitia or the intima (Pascual and Bevan, 1978). Differences in the case of NE persist after the process of amine uptake into the neuronal plexus is blocked pharmacologically. They are not due to the presence of the tunica adventitia on the outer surface of the media in that the difference in response characteristics persists after physical removal of the adventitia (Pascual and Bevan, 1979). Furthermore, there is evidence that the adventitia does not constitute a significant barrier to the diffusion of NE (Bevan and Torok, 1970). This conclusion also is supported by the demonstration that 10 times as much NE and its metabolites overflow via the adventitia than via the intima on stimulation of the neuronal plexus, which is located between the tunica adventitia and the tunica media of the pulmonary artery. This vessel, like the aorta, is an elastic artery (Bevan and Su, 1974). The pattern of response of the strip to intimal entry of drug is essentially the same as that of the intact strip.

The difference in response to intimal compared to adventitial drug entry could be due to nonuniformity of muscle characteristics in the tunica media such as smooth muscle cell agonist sensitivity and contractility, cell density, or intramural drug disposition mechanisms. Differences in vascular smooth muscle sensitivity as a function of depth in the blood vessel wall have been described in the sheep carotid artery—the inner layer being more sensitive to NE and H than the outer (Graham and Keatinge, 1972). Extraneuronal uptake and metabolism are important in the disposition of NE by the rabbit thoracic aorta and influence the contractile response of the tissue (Kalsner and Nickerson, 1969;
Methods

New Zealand albino rabbits of either sex, weighing 2.3–2.6 kg, were stunned by a blow on the head and exsanguinated. The thoracic aorta between the aortic arch and diaphragm was removed rapidly, and helical aortic strips were prepared according to the method of Furchgott and Bhadrakom (1953). Two strips, each 20 mm long and 3–4 mm wide, were prepared, one strip acting as a control for the other. Ligatures were placed on both ends of the muscle strip and used to attach it to a holder and a Statham strain gauge [G 10B]. Changes in tension were registered either on a Grass polygraph or a strip chart recorder. The preparations were suspended vertically under a resting tension of 2 g in 50 ml tissue baths containing Krebs bicarbonate solution equilibrated with 95% O₂ and 5% CO₂ at 38°C.

A technique has been developed to restrict temporar­ily drug entry to one surface of an aortic strip. Silicone grease is applied to the surface of a strip of Saran wrap which is just larger than the aortic strips stretched in vitro. When these are placed against a temporarily dried surface of the vessel, they effectively prevent the entry of drug without damage to the tissue. The evidence that this procedure does not interfere with the contraction of the aortic strip has been presented previously (Pascual and Bevan, 1979).

Drugs were added directly to the tissue baths and expressed in molar concentration in bath fluid. They usually were left in contact with the tissue until an equilibration steady state response was obtained. Doses producing up to 70% of the final maximum contraction were added in random sequence, and an interval of 45–60 minutes was allowed between addition. Higher concentrations were added only at the end of the experiment.

Latency of contraction (measured using a stop watch) was the difference between the time of drug addition to the physiological saline in a tissue bath vigorously bubbled with gas mixture and a just­discernible increase in isometric tension. The initial velocity of contraction was derived from the tangent to the initial part of the tension record and was expressed in grams per second. The very considerable similarity in the size of the responses of different strips in this series of experiments precluded the necessity for correcting this measurement for individual variation.

Drugs used were: desmethyli mipramine (DMI) (USV Pharmaceutical Corporation), deoxytocsterolone (DOC) (Aldrich Chemical Co.), 17-β-estradiol (Calbiochem), L-norepinephrine HCl (Calbiochem), histamine hydrochloride (Pfamstiehl Chemical Co.), iproniazid phosphate (Sigma Chemical Co.), oxytetracycline (Calbiochem), quinidine hydrochloride (Calbiochem), serotonin creatine sulfate (Calbiochem), and U-0521 (3′, 4′-dihydroxy-2-methyl propiophenone) (Upjohn). Data were statistically evaluated by Student's t-test for paired samples. A 0.05 level of probability was accepted as significant.

Results

Effect of Blockade of Intramural Disposition on the Steady State Response to Norepinephrine and Histamine Entering Through Only One Surface of Aortic Strip

Figures 1 and 2 show the steady state or equilibrium concentration-response curves from paired aortic strips to NE and H entering exclusively through either the intimal or adventitial surface. NE was added in the presence of DMI (10⁻⁸ M) to block its neuronal uptake. The same figures also show the effect of DOC (6 X 10⁻³ M) on the dose-response curve to NE and of combined iproniazid (2 X 10⁻⁴ M) and 17-β-estradiol (2 X 10⁻³ M) on those for H. Corticosteroids impede the entry of NE into extraneuronal sites of inactivation (Kalsner and Nickerson, 1969; Levin and Furchgott, 1970; de la Lande, 1973); iproniazid and 17-β-estradiol block major inactivation pathways for H in the thoracic aorta (Zeller, 1956; Kalsner, 1970).

The concentration-response curves for entry of NE (in the presence of DMI) and H through the intima were placed to the left of those for entry of the same drug via the adventitia. However, after treatment with the appropriate disposition blocking drugs, the concentration-response curves for adventitial and intimal entry were superimposed and were both displaced to the left of that for the drug effect via the inside of the non-pretreated strips. Similar results were found when DOC was substituted by the COMT inhibitor U-0521 (10⁻⁶ M) in the case of NE (see, for example, Trendelenburg et al. (1971) and 17-β-estradiol by quinidine (10⁻⁴ M) for the H responses (Kalsner, 1970; Cohn, 1965).

These observations, in the case of NE, are illustrated in Figure 3 in which the records of three
strips with similar response characteristics are shown. The contraction of NE entering via the intima was increased only a small extent by the addition of DOC. By comparison, the same dose of NE entering the aortic strip via the adventitia in the DMI-pretreated tissue caused a much smaller equilibrium response. However, in contrast, the addition of DOC resulted in a relatively dramatic increase in tension to a level that was indistinguishable from that when NE entered via the intima after the addition of DMI. The third strip showed only a small response to adventitial NE (after DMI), which increased immediately upon removal of the barrier to intimal entry to the same level as when NE entered via the intimal only. The additional effect of DOC in the intact strip was the same as in the strip with the adventitial barrier. The relative magnitudes of these various responses at any concentration level and the size of the potentiation by DOC can be deduced from Figures 1 and 2. A similar pattern of responses to H before and after iproniazid (2 × 10⁻⁴ M) and 17-β-estradiol (2 × 10⁻⁴ M) was obtained.

**Effect of Blockade of Intramural Disposition on the Latency and Initial Velocity of Contraction to Norepinephrine and Histamine Entering Only Through One Surface of Aortic Strip**

The latency and initial velocity of the contractions of untreated aortic strips to both NE and H were shorter and faster, respectively, for entry via the intimal compared with the adventitial surface. After tissue pretreatment with DOC for NE and with iproniazid and 17-β-estradiol for H, such differences in latency and initial velocity remained (Figs. 4 and 5; Table 1). Although iproniazid plus 17-β-estradiol caused a reduction in mean latency to H entering via the adventitia but not the intima, and DOC appeared to prolong mean latency to intimal NE, such alterations were not statistically significant.

It has been claimed by Powis (1973) that oxytetracycline prevents extraneuronal binding of NE to collagen and elastin. If DOC-insensitive extraneuronal uptake or binding were responsible for these differences, then they should disappear after this drug. However, neither parameter was influenced by oxytetracycline (10⁻⁴ M) applied 30 minutes before test responses were elicited.

**The Contractile Response to KC1 Entering Either via the Adventitial or Intimal Surface**

Contractions to KC1 (60 mM) were elicited in strips with either adventitial or intimal surface covered. In view of the fact that K⁺ will release NE...
from storage in the nerve terminal, experiments were conducted in the presence of phentolamine ($10^{-5}$ M). Peak response of paired strips to a given concentration of K⁺ was the same. However, the shape of the response differed with surface of entry. The isometric tension record for intimal entry rose abruptly after a short latency, and then after a time the rate of increase fell off toward an equilibrium peak tension. For adventitial entry, after a much longer latency, the velocity of contraction rose slowly at first and then with increasing velocity. Eventually, the rate of tension increase fell off toward a peak equilibrium level.

Variation in Effect of Drug Washout 30 Seconds after Exposure with Surface of Drug Entry

Aortic strips were exposed either via their adventitial or intimal surfaces to NE or H at their respective EC₅₀ levels. As previously described, responses to drug entry through the intima were faster and greater than via the adventitia. After 30 seconds of exposure (the strip normally takes 5–10 minutes to reach an equilibrium contraction to NE or H), the drug-containing tissue bath fluid was exchanged for physiological saline previously equilibrated with

### Table 1  Latency and Initial Velocity of Contraction of Rabbit Aortic Strips to NE and H Entering via the Adventitial or Intimal Surfaces in the Presence and Absence of Drugs that Inhibit Intramural Disposition

<table>
<thead>
<tr>
<th>Drugs and concentrations (M)</th>
<th>Via adventitia</th>
<th>Via intima</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latency (sec)</td>
<td>Initial velocity (g/sec)</td>
</tr>
<tr>
<td>NE*</td>
<td>10⁻⁶</td>
<td>2.35 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>16.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>8.2 ± 1.0</td>
</tr>
<tr>
<td>NE after DOC (6 x 10⁻⁵ M)</td>
<td>10⁻⁶</td>
<td>24 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>12 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>6.5 ± 1.1</td>
</tr>
<tr>
<td>H</td>
<td>10⁻⁶</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>15 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>10.5 ± 1.8</td>
</tr>
<tr>
<td>H after iproniazid (2 x 10⁻⁵ M) and 17-β-estradiol (2 x 10⁻⁴ M)</td>
<td>10⁻⁶</td>
<td>12 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>4.9 ± 1.0</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SE; n varied between 7 and 12. In each case when compared using the paired t-test, the latency via the adventitia is significantly longer and the initial velocity significantly slower than via the intima.

* In the presence of desmethylimipramine (2 X 10⁻⁵ M).
† Could not be measured.
Discussion

One of the differences in the contractile responses to NE and H consequent upon their entry via either the adventitial or intimal surface, the magnitude of the steady state contractile response, disappeared when the strips were pretreated with drugs that prevented agonist intramural disposition. It follows that the relative effect of these agents was much greater on the agonist response to adventitial compared to intimal entry (Figs. 1 and 2). The functional consequence of these observations is that probably the response of the rabbit thoracic aorta to circulating NE or H is mediated primarily by the drug that enters into the tissue via the intima. This conclusion is strengthened by the observation that the pattern of response of the intact aortic strip to agonists and its alteration by agents that modify their disposition is not different from that observed with exclusively intimal entry (Pascual and Bevan, 1979).

Although there are a number of explanations for such differences, only two hypotheses will be advanced. First, there may be an unequal distribution of the disposition capacity—in particular, the metabolic enzymes through the thickness of the blood vessel wall. For example, if in the case of NE there was a higher concentration of COMT activity in cells of the outer compared with the inner media, then the response to intimal entry might be expected to be different from adventitial entry.

This, however, seems not to be the case. Verity et al. (1972) showed that, when adventitia and media were separated by the method of Maxwell et al. (1968), COMT enzyme activity in the adventitia was only 5% of that in the media: this is consistent with the observation that removal of adventitia or COMT block has no effect on the initial features of the response to adventitial drug entry. The transmural distribution profile of COMT activity derived from a study of sequential frozen sections through the entire thickness of the thoracic aorta parallel to the intima showed negligible activity in the adventitia and an abrupt rise at the adventitiomedial junction leading to essentially a plateau across the thickness of the muscle-containing tunica. There was an apparent drop at the intimal/subintimal region, which is to be expected in view of the decreased density of smooth muscle cells in this region. The distribution of the extracellular space through the media uniform (Torok et al., 1971). The available evidence suggests that extraneuronal binding of NE is also fairly uniform across the tunica media (Bevan et al., 1972; Bevan and Su, 1971). These several observations, although limited, suggest the apparent uniformity of disposition mechanisms through the thickness of the media. Thus, it is unlikely that differences in transmitter diffusion capacity across the vessel wall provide an explanation of the differences in contraction characteristics with drug entry from the inside and from the outside. In any case, differences in disposition would not explain the results of the washout experiments illustrated in Figure 6.

An alternative hypothesis is that the inner vascular smooth muscle cells are more sensitive, or at least contract more, to NE and H than the outer. Thus, the initial phase of the contraction to drug entering via the intima is due in the main to the response of the inner more sensitive cells; that to drug entering from the outside, to the outer less sensitive cells. Thus, latency would be less and initial velocity greater for intimal entry. The final steady state response in the absence of agonist disposition block is the composite of the response of both inner and outer layers of smooth muscle—the inner contributing a greater proportion of the tension change than the outer. However, drug metabolism by COMT does occur within the wall of the aorta. Drug entering exclusively via the adventitia causes a relatively modest contraction from the outer cells because of their relatively low sensitivity: the contribution from the inner smooth muscle layers to the drug is potentially greater than from the outer were it not that drug concentration is reduced by metabolic attrition as the drug passes through the outer to the inner layers. In contrast, drug entering via the intima would significantly contract inner cells—an effect that would be influenced by metabolism to only a small extent. This process would have a relatively smaller effect on the contraction elicited from the outer smooth mus-
cles since these contribute relatively less to the overall response of the aortic strip. Such an argument would explain both the larger initial contraction from the intimal surface and the greater increment with metabolism block seen with adventitial entry.

This possibility is consistent with the literature. Graham and Keatinge (1972) compared the contractile response of inner and outer layers of sheep carotid artery. After DMI, the cells from the inner smooth muscle layer were approximately 2–3 times more sensitive to NE and H than were the outer. De la Lande and Johnson (1973) perfused ear arteries from reserpinized rabbits pretreated with MAO inhibitors. In contrast to the rapid restoration achieved by extraluminal NE, intraluminal NE failed to restore monoamine fluorescence in the nerve terminals at the adventitio-medial junction. However, some recovery occurred after additional metanephrine treatment. The inference is that COMT tends to limit the diffusion of NE across the media from the intima, but not across the adventitia.

These various studies favor the conclusion that COMT limits NE movement across the media in the steady state and that probably it is distributed uniformly. They support the hypothesis that the inner vascular smooth muscle cells are more sensitive to drugs than are the outer. This latter likelihood is consistent with the shorter latency and greater initial velocity of contraction seen with intimal entry, even after disposition block. It must be borne in mind, however, that these latter observations may in part the consequence of limited myogenic propagation from the intima. (Keatinge and Graham, 1974).

This proposal is consistent with the results of the washout experiments. After 30 seconds, NE has only partly penetrated the vessel wall. When the bath solution is changed, drug near the surface of entry will diffuse immediately toward the bath solution, i.e., will reverse direction. However, since the original concentration gradient within the depth of the vessel still persists for some time, drug in the substance of the vessel will continue to enter more deeply. This will occur at a progressively slower rate until it too reverses and moves en masse toward the surface of entry. When entry is via the adventitia, movement of the drug that is within the wall is toward smooth muscle cells of increasing sensitivity. Thus, contraction does not reverse immediately and indeed may increase. When entry is via the intima, continued movement of the deeply penetrated drug is toward less sensitive cells. Contraction reverses rapidly because of this and because most of the contraction originates from cells near the tunica intima.

Supporting this conclusion are the observations with potassium. This ion, when the adventitia is covered, causes a rapid brisk response, but when the intima is covered, elicits an initially slow response, which subsequently accelerates temporally. As K⁺ diffuses rapidly, the initial part of the response would be due to the less sensitive outer muscle followed by the contribution of the inner, more sensitive muscle. The observation that oxytetracycline failed to influence the difference between intimal and adventitial entry after DOC and DMI emphasizes that differences between the outside and inside reside in vascular smooth muscle cells not in extracellular elements.

The proposed hypothesis may be restated as follows (see also Fig. 7). If the inner smooth muscle cells are more sensitive to agonists than are the outer, in the steady state after blockade of all disposition routes, the inner cells provide the greatest contribution to the developed tension to a given concentration of agonist. Thus, drug entering via the intima causes a greater response and one less influenced by COMT than does drug entering via the adventitia. If disposition mechanisms are uniform through the media, the influence of COMT on the concentration of NE in the biophase of cells at a distance from the surface of entry can be considered to be the same for either surface. Because the response of distant cells to agonist is less for intimal compared with adventitial entry—their sensitivity being lower—the consequence of COMT blockade is less for intimal compared with adventitial entry. Thus, although the effect of COMT on NE concentration may have been the same for each direction of entry—transmitter concentration profiles through the wall may have mirrored each other—the consequences of such degradation on the contractile response would be different. It is as though COMT forms a functional metabolic barrier to adventitial NE. Similar arguments could be made to explain the basis of the responses to H with a removal system for H taking the place of COMT in the tunica media.
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Asymmetry of consequences of drug disposition mechanisms in the wall of the rabbit aorta.
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Circ Res. 1980;46:22-28
doi: 10.1161/01.RES.46.1.22

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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