Effect of Nicotinate Ester, Acetylcholine, and Other Vasodilating Agents on Cutaneous and Mesenteric Vascular Smooth Muscle

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ABSTRACT
Vasodilating agents were tested on smooth-muscle strips of small arterial blood vessels of dog and rabbit skin and mesentery, and two patterns of reaction were demonstrated. The type 1 vasodilating agents, for example nicotinate, caused relaxation of contracted strips or blocked their contraction. The type 2 vasodilating compounds, histamine, choline esters, and bradykinin, either caused contraction or had no effect on the vascular smooth-muscle preparation. Responses that were species specific and region specific characterize the action of type 2 vasodilators and must be accounted for in any in-vitro testing procedure for vasodilator drugs.

ADDITIONAL KEY WORDS
sodium nitrite epinephrine histamine bradykinin methacholine norepinephrine dog rabbit

Clinical vasodilation of the skin occurs with many physiologic and pharmacologic stimuli, and although the end result, that of vessel dilation, is the same, the mechanisms of its production and the rate of blood flow are varied. Clinical investigation of two vasodilators, nicotinic acid esters and methacholine, revealed two distinctive differences in erythema response to these agents. First, tetrahydrofurfuryl ester of nicotinic acid produces an erythema when applied to skin but not when applied to mucous membranes (1). This erythema is associated with dermographism and is blocked by oral administration of salicylates (2). Second, methacholine produces erythema of skin and mucous membranes and sweating of skin; these reactions are blocked by atropine.

Such biologic erythema responses can result from direct action of the drug on the cutaneous vessels, or the drug may induce formation of chemical mediators in the skin or blood which then react on the blood vessels. We have isolated the vascular smooth muscle of the skin and mesentery and tested the effect of these vasodilators on its contractile responses, demonstrating that there is a direct effect of one of these agents (nicotinate) on the vascular smooth muscle.

Materials and Methods
Small arterioles from the skin and mesentery, 200 to 500 μ, o.d., were dissected free from connective tissue in a Petri dish containing physiologic salt solution. Continuous helical strips of vascular smooth muscle were prepared from these vessels, as detailed by Bohr and co-workers (3) and by Sams and Winkelmann (4). These vessels were attached by 6-0 silk sutures to a fixed point and to the movable arm of a strain gauge (Grass). Contractions were recorded with a polygraph (Grass model 5, running at 0.25 mm/sec). The temperature of the preparation was maintained at 37°C. The physiologic salt solution had the following composition in millimoles per liter: NaCl 118.9, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, Na₂CO₃ 14.9, CaCl₂ 2.5, dextrose 5.5, and sucrose 50.0. Edetate calcium-disodium, 0.026 mM, was added to prevent the oxidation of epinephrine. The physiologic salt solution was oxygenated in a constant-temperature reservoir immediately before its introduction into the bath. The muscle strip was stretched by a
tension of 100 mg and permitted to equilibrate for 2 hours with washings of the physiologic salt solution every 15 minutes. After this period, a reproducible response to standard stimulation by epinephrine or potassium chloride was obtained. In most experiments, two muscle strips from mesentery or skin of dog or rabbit were compared in the same bath.

Twenty ear vessels of the dog and 25 ear vessels of the rabbit were studied. Ten mesenteric vessels of the rabbit and ten mesenteric vessels of the dog were used for comparison. Two vessels from the skin of the dog leg and two longitudinal muscle strips from the hepatic vein of the dog were also studied. Vasoconstricting and vasodilating agents were added directly to the bath and mixed rapidly by washing in and out of the delivery syringe three times. Contractions of all the muscle strips were produced by standard concentrations of potassium chloride (for example, 9.2 g/ml), epinephrine (for example, 10\(^{-8}\) g/ml), or norepinephrine (for example, 10\(^{-8}\) g/ml). The vasodilating agents were tested sequentially for their effect in separate tests upon each contraction-inducing agent at intervals of at least 5 minutes.

Tetrahydrofurfuryl nicotinate, acetylcholine, methacholine, histamine, and bradykinin were used as vasodilating agents. The sequence of testing used each of these agents separately in turn with each contractile stimulus in the order listed above. Care was taken to wash completely between trials. When the sequence was complete, the agents would be tried in turn before each contractile stimulus. Each agent could be used as outlined above, except for histamine and bradykinin, both of which produced contraction of some smooth muscle strips and became tachyphylactic. Repetition of trials with both agents was possible at the end of 2 hours. The effect of the other vasodilating agents on the histamine contractions was tested individually in turn, when such contractions were observed. The vasodilator agents were tested by progressive dilution to observe the dose-response relationship of the smooth-muscle strips. Acetylsalicylic acid and procaine also were tested for their effect on muscle responses. The studies were performed on responsive vessels at no less than 5-minute intervals. All quantities are expressed in terms of final concentrations in the bath.

**Results**

All smooth-muscle strips of cutaneous and mesenteric vessels of the dog and rabbit were consistently and reproducibly contracted by epinephrine, norepinephrine, or potassium chloride. Rabbit tissue was more responsive to the vasoconstrictors than was dog tissue. The vessel strips of the rabbit skin and mesentery were contracted by histamine but not by bradykinin. The vessel strips of the dog skin and mesentery gave contractile responses to bradykinin but not to histamine. These "species specific" contractile responses to histamine and to bradykinin were tachyphylactic.

Tetrahydrofurfuryl nicotinate (5.0 \(\times\) 10\(^{-4}\) g/ml or 2.5 \(\times\) 10\(^{-4}\) M) caused a dramatic relaxation of the vascular smooth-muscle strips of the 25 rabbit and 20 dog ears; these strips first had been contracted by potassium chlo-
Nicotinate relaxed vascular smooth-muscle strips from the 10 mesentery vessels and skin vessels equally, and rabbit and dog tissues were similarly affected. Lower concentrations produced progressively less effect, until no change was noted at $2.5 \times 10^{-6}$M. When nicotinate was added to the bath first, it almost or completely inhibited contraction from subsequently applied pharmacologic stimuli (Fig. 1). The nicotinate effect could be washed away readily so that, after the washing, the responsiveness to epinephrine would return. Acetylsalicylic acid ($1.8 \times 10^{-3}$ g/ml or 0.01M) did not block the direct effect of nicotinate on five vascular smooth-muscle strips. Procaine ($2.7 \times 10^{-4}$ g/ml or 0.025M) caused a similar relaxation of vascular strips in four experiments with skin and mesentery vessels of the dog and rabbit (Fig. 2). The magnitude of the relaxation was dose dependent and decreased with dilution.

Sodium nitrite ($1.7 \times 10^{-3}$ g/ml or 0.025M) caused relaxation of rabbit vascular smooth muscle (20 muscle strips) from skin and mesentery after this muscle had been made to contract by potassium chloride or epinephrine (Fig. 3). When a lower concentration of nitrite ($2.5 \times 10^{-5}$M) was used, a slow relaxation occurred in the rabbit. Sodium nitrite ($1.7 \times 10^{-3}$ g/ml or 0.025M) caused a slight additive contraction to that of catecholamine or potassium chloride in 20 preparations of the dog skin and mesentery vascular smooth muscle (Fig. 3). Dilution did not change the response, but a tenfold increase in concentration caused slight relaxation of the epinephrine contraction of vascular muscle strips from dog skin.

Methacholine and acetylcholine used in concentration of $10^{-3}$ g/ml to $10^{-4}$ g/ml did not produce a consistent response of vascular smooth-muscle strips from the skin or mesentery of the rabbit or dog. Four of 20 cutaneous and mesentery vessel strips of the rabbit did give minimal contractile responses to methacholine, but such small contractile responsiveness would not last. No relaxation of any contractile response to epinephrine or potassium chloride was seen with methacholine. On the contrary, acetylcholine often caused increased contraction when the muscle had been previously stimulated with epinephrine or norepinephrine. In summary, methacholine and acetylcholine did not produce any consistent response in vascular smooth muscle from skin or mesentery and did not specifically or constantly alter epinephrine, norepinephrine, or potassium chloride contractions of smooth muscle when given before or after these stimuli.

Histamine in concentrations of $3 \times 10^{-6}$ g/ml histamine base produced prompt contractions of 20 cutaneous and 10 mesentery vascular smooth-muscle strips of the rabbit.
(Fig. 4). The threshold or lowest concentration that produced contraction was $1.1 \times 10^{-9}$ g/ml. The responses were tachyphylactic in both the cutaneous and the mesenteric strips. The contraction was blocked or relaxed by nicotinate in five experiments and by sodium nitrite ($1.73 \times 10^{-3}$ g/ml or 0.025M) and procaine ($0.67 \times 10^{-5}$ g/ml or 0.001M) in two experiments. Strips of dog vessel from ear or leg skin or mesentery in the same bath as the rabbit vessels did not respond. Concentrations as high as $1 \times 10^{-3}$ g/ml histamine base were used without success in dog strips. No relaxation or inhibitory effect of histamine at any concentration was observed on the contractile responses of the vessel strips from dog or rabbit skin or mesentery. Two longitudinal strips from the hepatic vein of the dog were used in one experiment and contracted to the same concentrations of histamine capable of contracting vascular smooth muscle of the rabbit.

Bradykinin ($1.25 \times 10^{-3}$ g/ml or 0.0012M) caused contraction of all of 10 mesenteric and 10 cutaneous vascular smooth-muscle strips from the dog. No rabbit vascular strips in the bath at the same time responded (Fig. 4). This response was tachyphylactic and could be demonstrated again after a 1- to 2-hour rest period. The reaction was a sharp immediate contraction followed by immediate relaxation. There was no sustained contractile phase. Acetylsalicylic acid in $1.8 \times 10^{-3}$ g/ml or 0.01M concentration had no effect on the bradykinin response. Bradykinin had no relaxing or inhibitory effect on the norepinephrine or other contraction of dog mesentery or cutaneous vessels.

**Discussion**

Our observations indicate that the cutaneous vasodilating agents may be broadly divided into two categories based on their effect on isolated vascular smooth-muscle strips (Table 1). Type 1 vasodilating agents (nicotinate and procaine) cause relaxation of smooth-muscle contraction—whether these contractions are produced by catecholamines, potassium, or type 2 agents—and affect the skin and mesenteric smooth-muscle strips equally. Type 1 agents also block the contraction responses of smooth-muscle strips. The type 2 vasodilating agents (histamine, bradykinin, methacholine, and acetylcholine) either cause contraction or have no effect on isolated vascular smooth-muscle strips. Type 2 agents...
TABLE 1
Action of Vasodilating Agents on Contractions of Cutaneous Vascular Smooth-Muscle Strip of the Rabbit and Dog

<table>
<thead>
<tr>
<th>Type</th>
<th>Agent</th>
<th>Concentration (g/ml)</th>
<th>Rabbit Response</th>
<th>Dog Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nicotinate</td>
<td>$5 \times 10^{-4}$</td>
<td>relaxation</td>
<td>relaxation</td>
</tr>
<tr>
<td></td>
<td>Procaine</td>
<td>$2.7 \times 10^{-4}$</td>
<td>relaxation</td>
<td>relaxation</td>
</tr>
<tr>
<td>2</td>
<td>Histamine</td>
<td>$3 \times 10^{-4}$</td>
<td>occasional</td>
<td>contraction</td>
</tr>
<tr>
<td></td>
<td>Bradykinin</td>
<td>$1.25 \times 10^{-2}$</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>Methacholine</td>
<td>$1 \times 10^{-3}$</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>Acetylcholine</td>
<td>$1 \times 10^{-2}$</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

have no relaxing effect on the contracted smooth-muscle strip. These agents also cause contraction of the isolated smooth muscle of intestine and uterus of various species. Nitrites have a type 1 vasodilating action at most concentrations, but changes in concentration of nitrite may alter its capacity to augment or diminish a contractile response, unlike nicotinate or procaine.

De la Lande and Rand (5) showed that chemical stimulation of intact blood vessels may produce vasoconstriction; but if the associated nerve is electrically stimulated, the same chemical can produce vasodilation in the same vessel. Starr and West (6) confirmed these findings, showing that bradykinin constricted rabbit pulmonary vessels. However, if the vessels had previously been constricted by an electric stimulus, then bradykinin would cause vasodilation. This indicates that under normal circumstances vascular tone created by chemical or neural stimulation is important to the activity of vasodilating agents.

Studies by Bohr and associates (3) and by Starr and West (6) have demonstrated the unique nature of the response of the cerebral, pulmonary, coronary, mesentery, and cutaneous vascular beds. Lack of response of the lung and cerebral vessel smooth muscle to epinephrine is one example of such variability. Constrictor or dilator response of vascular smooth muscle can vary with the size of the vessels, as in the coronary vessel response to epinephrine observed by Zuberbuhler and Bohr (7). Our present study shows that the response to vasodilator chemicals may be different in two species. Failure to respond to histamine by canine cutaneous and mesentery vascular smooth-muscle strips is an example of such species specificity. To demonstrate lack of response, we first used the large, unphysiologic doses of histamine and kinin. From our observations and those of others, it is apparent that variations in size of vessels, variations among species and regional vascular beds, and dose-dependent variations make it imperative to use a standard vessel preparation for any comparative study of chemical stimulation of vascular smooth muscle.

Erythema of the skin is not a definitive means of classifying vasodilating agents nor does it correlate with blood flow. The vasodilating agents may work on vessels of different size from those studied here. The two kinds of vasodilating activity demonstrated in this study may relate to the differences between types of cutaneous erythema responses caused by nicotinate and by choline esters, which have been observed in earlier clinical investigations (1). Preparations such as those used in this experiment provide a standard means of assessing threshold responses of isolated vascular smooth muscle to drugs. We plan to extend this experience to search for and recognize vasodilators by their activity in vascular smooth-muscle preparations.

References


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