Activity and Thermosensitivity of Canine Cutaneous Veins after Inhibition of Monoamine Oxidase and Catechol-O-Methyl Transferase

By Paul M. Vanhoutte, M.D., and John T. Shepherd, M.D., D.Sc.

ABSTRACT
The lateral saphenous vein of the dog was perfused with homologous blood at constant flow. Changes in driving pressure were used to measure active changes in venous tone. Administration of tranylcypromine greatly reduced the activity of monoamine oxidase in the vein and increased its response to electric stimulation of the lumbar sympathetic chain and to norepinephrine infusions. Administration of pyrogallol (a catechol-O-methyl transferase inhibitor) also increased the response of the vein to adrenergic stimulation. These chemicals did not alter the increase in the sensitivity of the venous smooth muscle to sympathetic nerve impulses and infused norepinephrine produced by cooling the perfusate. Thus, the potentiating effect of local cooling on the response of the cutaneous veins to adrenergic stimulation cannot be explained by interference with the local enzymatic inactivation of catecholamines.

ADDITIONAL KEY WORDS tranylcypromine pyrogallol
catecholamine inactivation local temperature and venous reactions
increased venous response to adrenergic stimulation
monoamine oxidase in veins

Webb-Peploe and Shepherd (1-3) have shown that the cutaneous veins of the intact dog are highly sensitive to local changes in temperature: cooling the blood perfusing them augmented the venomotor response to a constant adrenergic stimulation and warming depressed the response. It appeared that an alteration in the sensitivity of the venous smooth muscle to both endogenous catecholamines released by sympathetic nerve stimulation and exogenous infused norepinephrine accounted for the venous responses to local temperature changes (3). A logical explanation for this sensitizing effect could be that the local inactivation of catecholamines is influenced by temperature, as suggested by the longer-lasting phase of increase in perfusion pressure and the delayed relaxation observed with local cooling. However, the potentiation by cold could not be explained by inhibition of catecholamine reuptake at the nerve terminals (4).

Another possible pathway for local inactivation of catecholamines, at different levels of the release-reuptake process, is enzymatic degradation by monoamine oxidase and catechol-O-methyl transferase (5-10), so that one could consider the hypothesis that local temperature changes may interfere with the local inactivation of catecholamines. Indeed, in different sympathetically innervated systems it appeared that inhibition of one or both of those enzymes resulted in an increase of the response to adrenergic stimulation (11-22). Arteries and veins are reported to contain both monoamine oxidase (11, 23) and catechol-O-methyl transferase (24), and there is evidence that at least monoamine oxidase is present (25) and effective (26) in the
sympathetic nerve supply of the cutaneous blood vessels. While the actual role played by these enzymes in the sympathetic system is still a matter of discussion (5, 12, 27, 28), their importance for the inactivation of catecholamines in vascular smooth muscle has recently been emphasized (29-31).

In this study we investigated the effect of tranylcypromine1 and pyrogallol, potent inhibitors of monoamine oxidase (32, 33) and catechol-O-methyl transferase (8), respectively, on the response of the canine saphenous vein to adrenergic stimulation and on the pattern of venomotor responses to local temperature changes. The data suggest that the effect of local temperature changes cannot be explained by interference with the local action of the enzymes because, after inhibition of these enzymes, the dependence of the venomotor reactions on the local temperature was unchanged.

Methods

The material and methods used in this study have been described by Webb-Peploe and Shepherd (34). Mongrel dogs weighing 15 to 25 kg were anesthetized with thiopental (15 mg/kg iv) and chloralose (80 mg/kg iv) and artificially ventilated with oxygen. Additional amounts of chloralose were given as required to maintain the proper level of anesthesia. The left lateral saphenous vein was cannulated at the ankle and, after heparinization of the animal, perfused at constant flow (100 ml/min) with autologous blood from the median sacral artery. The perfusion circuit consisted of a roller pump, proper level of anesthesia. The left lateral saphenous vein was dissected free, all the collaterals were tied off, and as long a segment as possible (weight, 300 to 500 mg) was removed. The segment was cut into small pieces and homogenized in 10 volumes of isotonic KCl at 0° to 4°C, with addition of washed glass powder, using a pestle and mortar. 5-Hydroxytryptamine-3,3'-14C creatinine sulfate (specific activity, 40 mc/m mole, obtained from Amersham-Searle) was dissolved in water and stored at —1°C. In a typical assay, 100 or 200 μliters of homogenate, 25 μliters (5.834 nmoles, 12,000 counts/min) of 5-hydroxytryptamine-14C, and 250 μliters 0.5M phosphate buffer (pH 7.4) were mixed in a glass-stoppered centrifuge tube and incubated at 37°C for 20 minutes. The reaction was stopped by the addition of 0.2 ml of 2N HC1, and the deaminated radioactive material, 5-hydroxyindoleacetic acid, was extracted by shaking with 6 ml of ether after the addition of NaCl to saturation. After centrifugation, a 4-ml sample of the ether layer was transferred to a vial containing a 2:1 mixture of toluene-PPO-DMPOP (p-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene) and Triton X-100 (36), and the radioactivity was measured in a liquid scintillation counter (Packard).

Results

MONOAMINE OXIDASE INHIBITION WITH TRANYLCYPROMINE

In seven dogs the response to adrenergic stimulation and to local temperature changes was compared before and after intravenous administration of tranylcypromine. Three dogs received an intravenous injection of 1 mg/kg and four dogs received 10 mg/kg.

1Provided by Smith Kline & French Laboratories.
infused over 25 minutes. In four other dogs, the effects of the same doses of tranylcypromine on the monoamine oxidase activity of the saphenous vein were investigated.

Tranylcypromine caused a transient increase in mean aortic blood pressure (range, 25 to 65 mm Hg) and in saphenous driving pressure (mean, 23 mm Hg; range, 5 to 70 mm Hg). No tests of the response of the saphenous vein to adrenergic stimuli or to local temperature changes were made until these pressures had returned to the preinfusion levels.

Effect on Response of Saphenous Vein to Sympathetic Nerve Stimulation.—In three dogs the injection of tranylcypromine (1 mg/kg) markedly augmented the response of the cutaneous veins to electric stimulation of the lumbar sympathetic nerves, as compared to that obtained for an identical stimulus under control conditions. The potentiation was maximal 10 to 20 minutes after the injection. The saphenous driving pressure during sympathetic nerve stimulation reached 215%, 300%, and 365% of the control response.

In three other dogs the reaction to an identical electric stimulation of the lumbar sympathetic chain was compared before and after an infusion of tranylcypromine at a dose of 10 mg/kg. As shown in Figure 1, in a typical experiment, the response was progressively potentiated after the infusion and reached a maximum within 30 to 50 minutes. During identical sympathetic nerve stimulation, the maximal saphenous driving pressures reached 150%, 354%, and 365% of the control reaction. In the first dog, in which a smaller degree of potentiation was observed, additional amounts of chloralose had been adminis-

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Effect of Tranylcypromine on Monoamine Oxidase Activity in Lateral Saphenous Veins of Four Dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Vein sample (mg)</th>
<th>Controls</th>
<th>After tranylcypromine</th>
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<tbody>
<tr>
<td></td>
<td>5-HIAA (counts/min)</td>
<td>5-HIAA-14C/g vein (nmoles)</td>
<td>Dose (mg/kg)</td>
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<tr>
<td>1</td>
<td>9.3</td>
<td>276</td>
<td>21.8</td>
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<td>3</td>
<td>18.6</td>
<td>213</td>
<td>8.4</td>
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<tr>
<td>4</td>
<td>18.6</td>
<td>110</td>
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Data were corrected for blank values (27 counts/min) obtained by incubating 5-hydroxytryptamine-14C with boiled enzyme but not for recovery of 5-hydroxyindoleacetic acid (5-HIAA) through the ether extraction. Radioactivity (counts/min) is expressed as mean of two duplicate samples.

tered, and this is known to depress the reactivity of the preparation (1).

Effect on Response of Saphenous Vein to Norepinephrine Infusion.—The maximal increase in saphenous driving pressure caused by an infusion of norepinephrine was compared under control conditions and 30 to 60 minutes after either an injection of 1 mg/kg (two dogs) or an infusion of 10 mg/kg (four dogs) of tranylcypromine. In every case the response was markedly increased and averaged 304% of the control response (Fig. 2). As already observed for the response to sympathetic nerve stimulation, no significant difference in potentiation could be shown between animals which received the lower dose of tranylcypromine and those which received the higher dose.

Effect on Monoamine Oxidase Activity.—Monoamine oxidase activity was detected in veins from five of six dogs. In four of these dogs, the enzyme activity of the heterolateral veins 60 minutes after administration of tranylcypromine (either 1 mg/kg or 10 mg/kg) was greatly reduced (Table 1).

Effect on Response of Saphenous Vein to Local Temperature Changes.—In the absence of external stimulation, cooling or warming the perfusate had minimal effects on the saphenous driving pressure, as reported previously (1, 3). The reactions to changes in perfusate temperature were therefore investigated during a sustained venoconstriction caused either by a norepinephrine infusion or by continuous electric stimulation of the lumbar sympathetic chain. When the venoconstriction reached its plateau, the perfusate was warmed from 37° to 43°C and then gradually cooled. At each temperature (43°, 37°, 30°, 25°, and 20°C) the perfusion pressure was allowed to stabilize, so that a temperature-response curve was obtained. This procedure was repeated several times in control conditions and after administration of tranylcypromine.

1. Norepinephrine venoconstriction. The effects of identical changes in perfusate temperature during a sustained venoconstriction caused by an infusion of norepinephrine were compared in six dogs before and after tranylcypromine. Two animals received 1 mg/kg, and four, 10 mg/kg. Figure 3 compares, for each dog, the temperature-response curves determined before and approximately 60 minutes after administration of tranylcypromine. In each experiment, except one, the same doses of norepinephrine were used before and during the monoamine oxidase inhibition. In one experiment, the dose had to be reduced to obtain a comparable reaction at 37°C. In every experiment, similar temperature-response relationships were observed before and after administration of tranylcypromine.
2. Sympathetic nerve stimulation. In five dogs, the effects of identical changes in perfusate temperatures were tested during a sustained venoconstriction induced by electric stimulation of the lumbar sympathetic chain (Fig. 4). After treatment with tranylcypromine the responses were potentiated, and in one experiment it was necessary to decrease the stimulation frequency to obtain a reaction comparable to that obtained under control conditions. Similar temperature-response relationships were observed before and after administration of tranylcypromine.

The results shown in Figures 3 and 4 were obtained approximately 60 minutes after administration of tranylcypromine. Curves obtained earlier or later (90 to 120 minutes) showed a similar temperature-response relationship.

CATECHOL-O-METHYL TRANSFERASE INHIBITION WITH PYROGALLOL

These experiments were performed on seven dogs. Two had previously received tranylcypromine at a dose of 1 mg/kg and three at a dose of 10 mg/kg. The responses in these dogs and in those which had not received tranylcypromine were similar. All of them received two intravenous injections (37.5 mg/kg) of pyrogallol 15 minutes apart, according to the schedule proposed by Crout (14). The activity of the preparation and its thermosensitivity were tested before the first injection of pyrogallol and 5 to 10 minutes after the second injection.

The injections of pyrogallol caused either an immediate slight increase in aortic blood pressure (five dogs) followed by a rapid decrease to control levels or an immediate decrease followed by a stabilization slightly above control levels (two dogs). Except for two dogs that reacted with a very slight transient venodilatation, the injection of pyrogallol into the perfusion circuit resulted in a slight transient venoconstriction (maximal reaction, 10 mm Hg). These changes occurred within the first 5 minutes after the injection.

Effect on Response of Saphenous Vein to Sympathetic Nerve Stimulation.—In three dogs, the response to the same electric stimulation of the lumbar sympathetic chain was compared before and after the two injections of pyrogallol. After pyrogallol, the responses were potentiated and reached 184%, 260%, and 390% of the control reaction.
Effect on Response of Saphenous Vein to Norepinephrine Infusion.—In seven dogs, the maximal reaction to an identical norepinephrine infusion was compared before and after pyrogallol injections. The results are shown in Figure 5. In one dog, no potentiation was observed. In the others the response reached 125%, 160%, 217%, 244%, 247%, and 270% of that to the same norepinephrine doses before the pyrogallol injections.

Effect on Response of Saphenous Vein to Local Temperature Changes.—These studies were carried out before the first and after the second injection of pyrogallol and were conducted like those before and after administration of tranylcypromine.

1. Norepinephrine venoconstriction. The effects of identical changes in perfusate temperature imposed during a sustained norepinephrine contraction were compared in seven dogs before and during catechol-O-methyl transferase inhibition (Fig. 6). In two animals, a smaller dose of norepinephrine was used after the pyrogallol to obtain comparable reactions at 37°C. From those experiments it appears that the response to changes in perfusate temperature is not depressed by pyrogallol; the same temperature dependency of the norepinephrine venoconstriction was observed before and after the two injections of the drug.

2. Sympathetic nerve stimulation. In four dogs, the effect of identical changes in local temperature was investigated during a sustained venoconstriction induced by continuous electric stimulation of the lumbar sympathetic chain. After the two injections of pyrogallol,
Individual responses in 7 dogs of lateral saphenous vein to changes in temperature of blood perfusing vein during continuous infusion of norepinephrine. Left, control; right, after two injections (37.5 mg/kg each) of pyrogallol.

Individual responses in 4 dogs of lateral saphenous vein during electric stimulation of lumbar sympathetic trunk. Left, control; right, after two intravenous injections (37.5 mg/kg each) of pyrogallol.

Discussion

Evidence in the literature indicates that monoamine oxidase acts as an intraneuronal stabilizer of the norepinephrine stores (6, 8, 10, 19, 22, 37) by deaminating the catecholamines which slowly diffuse out of the storage sites. Different arteries and veins have been observed to have different responses to changes in temperature. The response to sympathetic nerve stimulation was so potentiated that in three of the animals it was necessary to decrease the stimulation frequency to obtain comparable responses at 37°C. Temperature-response relationships before and after the two injections (Fig. 7) were similar.

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shown histologically (38) and biochemically (11, 23) to possess monoamine oxidase activity. In arterial smooth muscle, monoamine oxidase has been shown to be an important alternate pathway of inactivation of adrenergic amines (29, 30). In particular, Jenkinson and associates (25) described a dense network of monoamine oxidase-containing nerve fibers around both cutaneous veins and arteries. In the dog, the venous effluent from the skin appeared to contain more catecholamines during sympathetic nerve stimulation after inhibition of monoamine oxidase than it did before inhibition (26).

These observations suggest that monoamine oxidase plays a role in the nervous control of skin blood vessels. Our data, which show the presence of monoamine oxidase in the saphenous vein of the dog, support this view.

The increased response to adrenergic stimuli following administration of known inhibitors of monoamine oxidase is believed (33, 39) to be due mainly to inhibition of the enzyme rather than to other effects of the inhibitors. With the enzyme inhibited, the consequent leakage of norepinephrine out of the tissue stores could increase the concentration of transmitter at the receptors and, by the resulting partial activation, facilitate the action of locally liberated or exogenously added catecholamines (19, 22). The increased response of the saphenous vein to sympathetic nerve stimulation after inhibition of monoamine oxidase was observed in the present experiments can be explained by depression of monoamine oxidase activity, since the doses used were sufficient to inhibit the enzyme. Part of the potentiation might be attributed to interference by tranylcypromine with norepinephrine reuptake mechanisms (5, 22, 40) or to some direct effect of the drug on smooth muscle; such an effect may explain the increase in aortic blood pressure and saphenous vein driving pressure during the period of the drug infusion (33).

Since monoamine oxidase is present in the saphenous vein and since its action was inhibited by the dose of tranylcypromine that was used, it was possible to test the hypothesis that the potentiation of adrenergic stimuli by local cooling of the vein might be due to an alteration in the enzyme kinetics of monoamine oxidase. Although the activity of this enzyme is dependent on temperature (11), its inhibition by tranylcypromine did not prevent the potentiating effect of local cooling on the response of the vein to adrenergic stimulation.

Blood vessels, and in particular veins, contain catechol-O-methyl transferase (24). It is suggested that this enzyme aids in the inactivation of norepinephrine released locally and of circulating catecholamines (27, 28). Recent studies on isolated vascular smooth muscle have provided evidence that O-methylation is a primary mechanism for inactivation of epinephrine and norepinephrine and may be even more important than the binding and storage mechanism (30, 31).

After the intravenous injections of pyrogallol in a dose which has been shown to block the activity of catechol-O-methyltransferase rapidly in the dog (14), the saphenous vein showed increased response to sympathetic nerve stimulation and infusion of norepinephrine. While the latter may be explained in part by decreased inactivation of circulating catecholamines in other parts of the body, especially the liver (8), the potentiation of the reaction to sympathetic nerve stimulation indicates the local origin of the effect.

One could question whether the potentiated response is due to the inhibition of catechol-O-methyl transferase by pyrogallol or to some other side effect of the drug. After pyrogallol injections, the $P_{O_2}$ of the blood is decreased and its hydrogen ion concentration is increased, as demonstrated in experiments in which we measured pH and $P_{O_2}$ in the blood perfusing the vein. Since the animal was artificially ventilated with oxygen, the oxygen saturation of the blood (range, 65% to 72%) was not markedly different from the saturation of the venous blood in normal conditions. To make sure that this decrease in saturation did not account for the potentiation observed after pyrogallol, in two experiments we compared the response of the saphenous vein to lumbar sympathetic chain stimulation when
perfused with blood taken from either the median sacral artery or the right atrium. Perfusing the saphenous vein with venous blood did not increase its response to sympathetic nerve impulses. Thus, the decrease in Po2 after pyrogallol does not explain the increased response of the cutaneous veins. Indeed, if a decrease of Po2 affected the response, one would expect a depression, as shown for other vascular smooth muscles (41).

After the pyrogallol injections, the pH of the arterial blood decreased by 0.25 pH unit. However, acidosis has been shown (42, 43) to depress the reaction of the cutaneous veins to adrenergic stimulation.

Other investigators have observed a potentiation or prolongation of the adrenergic reactions by catechol derivatives in variously sympathetically innervated systems or in intact animals (7, 8, 12-16, 19-21, 30, 31). While we cannot conclude that the increased response to catecholamines is solely a consequence of the action of pyrogallol on catechol-O-methyl transferase, it would seem reasonable to assume that this played a major role.

As with monoamine oxidase, the inhibition of catechol-O-methyl transferase by pyrogallol did not prevent the potentiating effect of local cooling on the response of the vein to adrenergic stimulation. The present results, along with those of Webb-Peploe (4), demonstrate that the effect of local temperature changes on the response of the cutaneous veins to adrenergic stimulation is not due to interference with local inactivation of catecholamines. The mechanism of this thermo-sensitivity has still to be elucidated.

Acknowledgment

We thank Dr. Gertrude M. Tyce for her help and advice in assaying monoamine oxidase activity and Robert R. Lorenz for his advice and technical assistance.

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doi: 10.1161/01.RES.25.5.607

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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