Fundamental Difference in the Reactivity of the Blood Vessels in Skin Compared with those in Muscle

Blood Flow Response in these Two Beds to Ischemia, and to Intra-arterial Injections of Methacholine, Epinephrine and Noradrenalin before and after Administration of Antiadrenergic Drugs

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Blood flow was measured with the electromagnetic flowmeter in the saphenous artery bed (cutaneous) and in the distal femoral and popliteal beds (muscle). The effects of ischemia and of intra-arterial injection with methacholine, l-epinephrine and l-norepinephrine were studied before and again after each injection of progressively increased doses of antiadrenergic drugs. These studies indicate a fundamental difference between the reactivity of cutaneous and muscular vascular beds.

Blood flow responses in the total limb of the dog to vasodilator and vasoconstrictor drugs were studied before and after the administration of antiadrenergic drugs. It was felt that there might be some difference in the responses of the blood vessels supplying muscle and skin respectively. Hence, these studies were designed: (1) to determine a method of measuring muscle and skin flows separately; (2) to ascertain whether there is a difference in the responses in these two beds to ischemia, methacholine, l-epinephrine, and l-norepinephrine and (3) to determine the effects of antiadrenergic drugs on the above responses in the vascular beds supplying muscle and skin.

Methods

Thirty-five mongrel dogs of both sexes weighing from 7.5 Kg. to 28 Kg. were anesthetized with morphine 2.25 mg. per kilogram intramuscularly followed in a half to one hour by 0.25 ml. per kilogram intravenously of a solution containing 50 mg. of Dial, 200 mg. of urethane and 30 mg. of sodium pentobarbital per milliliter.* Anesthesia was maintained with additional doses of 1 to 2 ml. as needed. Clotting was controlled by the use of Treburon,† 20 mg. per kilogram intravenously initially, with an additional 10 mg. per kilogram every 30 minutes thereafter.

Thirteen dogs weighing 7.5 to 16 Kg. were used for muscle flow and 22 dogs weighing 15 to 28 Kg. were used for skin flow experiments. Of the 22 dogs used in skin flow experiments only nine were satisfactory; the flow could not be maintained at a high enough rate of flow to show accurate changes in 9 of the 22 animals and four were discarded because there was anatomic evidence that part of the measured flow was supplying muscle. A control rate of flow of 5 ml. per minute was set up as a minimum before the dog was used in the experiment. If this minimum control flow was not maintained the experiment was discontinued and not tabulated in the final results.

Two methods of cannulation were used for measuring skin flow. Between the twelfth and thirteenth experiments the system of cannulation was changed in an effort to improve the magnitude of flow. In the first system all muscle branches from the femoral artery were tied off, the artery was ligated distally to the origin of the saphenous artery and the femoral artery then cannulated proximally and distally.

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* The Dial Urethane solution was kindly supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

† The Treburon was kindly supplied by Hoffmann-La Roche, Inc., Nutley, N. J.
Several muscle branches from the saphenous were also tied off. The second method (fig. 1) involved cannulation of the femoral artery of the opposite leg proximally and the saphenous artery of the leg to be measured distally after first tying off all muscle branches from the saphenous artery. India ink was injected at the end of all experiments and the leg dissected. If any ink was found in muscle this method gave a pure muscle flow. India ink was injected at the end of each experiment to determine whether or not the flow had been purely to muscle.

The rate of blood flow in the femoral and saphenous arteries was measured with the electromagnetic flowmeter of Richardson, Denison and Green. This meter has a linear calibration for forward and backward flow with less than 5 per cent error. Lateral pressure was recorded just downstream from the flowmeter by means of a Statham P-23A strain gage and a Brush strain analyzer. The cannula and pressure gage were thoroughly flushed with water and then filled with sterile physiologic saline prior to cannulation. Flow and pressure were dampened electronically to give mean readings and were each recorded with Esterline-Angus 5 milliampere recorders. The position of the record at zero flow was determined before each drug injection by clamping off the proximal end of the cannula thus interrupting flow through the flowmeter. The deflection on the record was calibrated at various rates of flow at the beginning and end of each experiment by allowing blood to flow through the flowmeter and out a glass T-tube into a graduate for measured periods of time while recording the deflection on the recorder. The calibration at the end of the experiments did not vary more than 2 per cent from that at the beginning. The flow and simultaneous pressure measurements were used to calculate the resistance of the vascular bed in PRU units.

The anticoagulant and anesthesia were injected intravenously through a cannula in the external jugular vein and washed in by a reservoir of sterile, pyrogen-free saline. All other injections were made intra-arterially by means of a needle inserted through a piece of rubber tubing downstream from the flowmeter cannula. The dead space from the site of injection to the artery contained 0.3 to 0.5 ml. for the skin flow and 0.5 to 0.8 ml. for the muscle flow experiments. Before each experiment all syringes were washed for 24 hours with pyrogen-free water, wrapped and autoclaved at 15 pounds pressure for 30 minutes.

Solutions for injection were made up daily using sterile pyrogen-free saline as a diluent. Commercial epinephrine 1:1000 in 1 ml. ampules was diluted to a concentration of 1:100,000. Stock solutions of 1-norepinephrine were made up in concentrations of 1:1000 every five days. These were diluted to 1:100,000 for injection. One microgram injections of epinephrine and 1-norepinephrine were given in 0.1 ml. volume. Methacholine was given in doses of 0.08 micrograms contained in 0.4 ml. saline. Regi-

* One milliliter ampule of adrenaline chloride lepinephrine solution, 1:1000, Parke, Davis and Co., Detroit, Mich.

† The 1-norepinephrine was Levophed® Bitartrate, monohydrate and was kindly supplied by Winthrop-Stearns, Inc., New York City.
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Tine,* Priscoline* and Ilidar† were given in increasing doses from 0.01 mg. per kilogram to 30 mg. per kilogram. All injections were made over a period of 5 to 10 seconds except for the higher concentrations of the blocking drugs.

At least two experiments were carried out with each blocking drug on skin and muscle. The results indicated there was no qualitative difference between them. The structural formulas for these drugs are as follows:

**PRISCOLINE**

**REGITINE**

**ILIDAR**

The effect of ischemia was determined in both muscle and skin flow by clamping off the blood flow for periods of 20 seconds to one minute, usually the latter.

Responses to the various vasoconstrictor and vasodilator drugs, given before the blocking drugs, served as the control data for each experiment. In all but five experiments both 1-epinephrine and 1-nor-epinephrine were used and these drugs were given one or more times after each injection of the blocking drug. Only one of the blocking drugs was given in any one experiment.

**RESULTS**

In all experiments a one to two minute period of control flow was recorded prior to injection of the drug. During this period three types of rhythmic variation in control flow were noted: (1) small pulsations synchronous with the heart beat (see fig. 3), (2) larger, slower fluctuations synchronous with the respiration, and (3) fluctuations of about one every 30 seconds to one minute (see figs. 3, 8 and 11) which may represent Traube-Herring waves.

**Fig. 3.** Blood flow records from a cutaneous vascular bed in response to ischemia. Solid line at bottom of each record indicates duration of period of ischemia; Segment A = control response before administration of antiadrenergic drug; B = responses after the intra-arterial injection of 0.3 mg. per kilogram dose of the antiadrenergic drug, Ilidar; C = responses after 3 mg. per kilogram of Ilidar; zero flow in each case was recorded on the second line up from the bottom—indicated by the word Zero; figures adjacent to record give the rates of flow in milliliters per minute; Time = 7.5 seconds between each vertical ordinate; the figures at the bottom of the records give the time in minutes from the beginning of the ischemia (or drug injection). The numbers in the left lower corner of each segment of record is the serial number of the record. Each period of ischemia, drug injection, etc., was numbered consecutively; approximately 4 to 10 minutes elapsed between each record. The number in the left lower corner of the figure (BJ-6) indicates the number of the experiment.

**Fig. 4.** Responses to ischemia in blood vessels supplying muscle (see legend to figure 3 for explanation of lettering).

To shorten the record for publication most of the control segment of record has been eliminated (see figs. 4, 6–12), however, in all cases...
an adequate length of relatively constant control has been recorded which was essentially identical with the portion of the record reproduced in the illustrations just prior to the drug injections.

In all cases a segment of zero flow was recorded before and at the end of the drug injection. In many instances the period of drug injection was long enough to permit a complete recovery of vasomotor tone. In some cases, however, it was necessary to read off the flow rate on a zero-check curve, as in the experiments shown in figures 3, 4, and 10. When such zero-check curves were used, the control and test periods in the records were extended to provide a sufficient length of zero flow for accurate measurement.

FIG. 5. Responses of a cutaneous vascular bed to intra-arterial injection of methacholine (Mecholyl). (See figure 3 for explanation.) These records are from same experiment as figure 3.

FIG. 6. Responses of the blood vessels supplying a muscular vascular bed to intra-arterial injection of methacholine. (See figure 3 and first paragraph of results in the text for explanation.) These records are from same experiment as figure 4.

In most instances we have reproduced these zero checks (see figs. 6–12). In the muscle flow records a period of reactive hyperemia usually followed these zero checks (see fig. 6), but in all cases the flow had stabilized before the test substance was injected (see fig. 8C).

FIG. 7. Responses of a cutaneous vascular bed to intra-arterial injections of 1-epinephrine. (See figure 3 for explanation.) This is the same experiment as figure 3. There is an artefact in the last part of segment A (above the word "control"); the flow should have returned to control rates analogous to that in segment A of figure 10. This record was nevertheless chosen for publication because it was from the same experiment as figures 3, 5 and 10.

FIG. 8. Responses in a vascular bed of muscle (hyporeactor group) to intra-arterial injections of 1-epinephrine. (See legend for figure 3 for explanation.)

FIG. 9. Responses in a vascular bed of muscle (hyper-reactive group) to intra-arterial injections of 1-epinephrine. (See legend for figure 3 for explanation.) These records are from same experiment as figure 4.
Reactivity of Blood Vessels in Skin and Muscle

Injection, indicated by the solid line at the bottom of the records, caused little disturbance in the recorded rate of flow; however, in some experiments the flow appeared to be reduced during the drug injection (see fig. 5A) due to the volume of fluid injected.

**Control**

Ischemia. The skin showed little or no reaction to ischemia (see fig. 3A) which produced very slight dilation or constriction with the average response being zero (see table 1, I, A, a). In muscle there was a marked dilation in response to ischemia (see figure 4A and table 1, II and III, A, a). The average period of dilation in muscle following ischemia was 30 seconds.

Methacholine. Methacholine was less active in producing dilation in the skin (fig. 5A, and table 1, I, A, b) and more active in muscle (fig. 6A, and table 1, II and III, A, b).

L-norepinephrine. L-norepinephrine caused constriction with no or very little secondary vasodilation and was equally effective in all three groups (figs. 10A and 11A and table 1, I, II and III, A, d). The degree of constriction was very similar to that seen with epinephrine in both skin and muscle.

**Fig. 10.** Responses in a cutaneous vascular bed to intra-arterial injections of L-norepinephrine. (See legend for figure 3 for explanation.) Same experiment as figure 3.

**Fig. 11.** Response in a muscular vascular bed (hyporeactor group) to intra-arterial injections of L-norepinephrine. (See legend for figure 3 for explanation.) Same experiment as figure 8.

**Fig. 12.** Responses in a muscular vascular bed (hyper-reactive group) to intra-arterial injections of L-norepinephrine. (See legend for figure 3 for explanation.) Same experiment as figure 4.
Responses after a Reversal Dose (for Muscle) of the Blocking Agents

In both the hypo- and hyper-reactor muscle groups the constrictor effect of epinephrine was blocked by doses of 0.05 mg. per kilogram of blocking agents that caused an epinephrine reversal in muscle had little effect on the epinephrine responses in skin (see fig. 7B and table 1, I, B, g). There was no dilation following epinephrine but the constrictor response was not quite as great as that seen in the controls. The vasodilation due to methacholine was slightly reduced in skin, but unchanged in muscle after this dose of the blocking drugs (figs. 5 and 65 and table 1, I, II and III, B, f).

### Table 1.—Responses to one-minute periods of ischemia and intra-arterial injections of 0.8 μg. of methacholine, of 1 μg. of epinephrine and of 1 ng. of l-norepinephrine expressed as per cent of control resistance to flow

<table>
<thead>
<tr>
<th>Table 1</th>
<th>A. Control</th>
<th>B. After epinephrine reversal dose of antiadrenergic drug</th>
<th>C. After blocking dose of antiadrenergic drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>No.</td>
<td>7</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Avg.</td>
<td>105</td>
<td>67</td>
<td>497</td>
</tr>
<tr>
<td>S.e.</td>
<td>4.4</td>
<td>6.5</td>
<td>70</td>
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I. Skin flow—Hyporeactors

<table>
<thead>
<tr>
<th>Table 1</th>
<th>A. Control</th>
<th>B. After epinephrine reversal dose of antiadrenergic drug</th>
<th>C. After blocking dose of antiadrenergic drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>No.</td>
<td>2</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Avg.</td>
<td>58</td>
<td>37</td>
<td>521</td>
</tr>
<tr>
<td>S.e.</td>
<td>2.7</td>
<td>125</td>
<td>140</td>
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</tbody>
</table>

II. Muscle flow—Hyporeactors

<table>
<thead>
<tr>
<th>Table 1</th>
<th>A. Control</th>
<th>B. After epinephrine reversal dose of antiadrenergic drug</th>
<th>C. After blocking dose of antiadrenergic drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>No.</td>
<td>10</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Avg.</td>
<td>56</td>
<td>30</td>
<td>414</td>
</tr>
<tr>
<td>S.e.</td>
<td>8.7</td>
<td>2.0</td>
<td>60</td>
</tr>
</tbody>
</table>

A, control—before any antiadrenergic drug was given; B, after a dose of antiadrenergic drug sufficient to convert the constrictor response in muscle to epinephrine to a dilator response (the approximate doses were Priscoline or Ilidar, 0.3 to 1 mg./Kg., Regitine 0.05 mg./Kg., given intra-arterially); C, after a dose of the antiadrenergic drug sufficiently large to block completely the constrictor response in muscle to l-norepinephrine (the approximate doses were Ilidar or Priscoline 3 to 30 mg./Kg., Regitine 15 to 3 mg./Kg.).
In muscle the reaction to ischemia was somewhat diminished but was unchanged in skin (figs. 3 and 4B, and table 1, I, II and III, B, e). After this dose of the blocking drug l-norepinephrine caused less constriction in the hyporeactors but in the hyper-reactive group we found an initial period of vasoconstriction lasting one to two minutes followed by a period of vasodilation lasting one to five minutes.

**Responses after a Blocking Dose of the Blocking Agents**

When the doses of the blocking drugs were increased to about 10 times that required to convert the epinephrine response in muscle to vasodilation, the dilation due to methacholine (fig. 6C and table 1, I, C, j) and constriction due to epinephrine and l-norepinephrine (figs. 7C and 9C and table 1, I, C, k and l) in skin were all blocked. Ischemia again caused little or no reaction in the skin (fig. 3C and table 1, I, C, i). However, in muscle, ischemia and methacholine still caused vasodilation although the degree of dilation was diminished (figs. 4C and 6C and table 1, I, II and III, C, i and j). Epinephrine still caused vasodilation but again this was diminished (figs. 8C and 9C and table 1, I, II and III, C, k). L-norepinephrine constriction was blocked by this dose of the blocking drugs in both muscle groups and did not cause any secondary dilation (figs. 11C and 12C, and table 1, II and III, C, l).

**Discussion**

In no instance did we find a very definite increase in flow following ischemia in the skin. The greatest reduction in resistance we saw was to 74 per cent of control PRU. In this case the control flow was 7 ml. per minute with a response after one minute of ischemia of 9 ml. per minute. However we also saw slight constriction after the same period of ischemia in the skin. The largest constriction following ischemia increased resistance to 130 per cent of control PRU. Here the control flow was 13 ml. per minute and decreased to 10 ml. per minute. In these two examples the change is too small to be really significant, since the control flow itself may vary as much as 3 or 4 ml. per minute over a period of one or two minutes. Also when the flow was this small the sensitivity of our flowmeter had to be increased so much that the zero flow line could drift up or down enough to give changes of 1 or 2 ml. per minute. These two were the only experiments with flows this small, the control flows for the rest of our experiments ranged between 15 ml. per minute and 55 ml. per minute with an average of 32 ml. per minute. From these results it would appear that, if the results in the dog can be translated to man, the reactive hyperemia which is evidenced in man by a flushing of the skin after period of ischemia is not due to an increase of blood flow in the skin but probably represents capillary dilation with pooling.

In muscle the response to ischemia was marked, the flow increasing to as much as five times the control flows; the average increase in muscle flow was up to three times the control flow with an average control flow of 38 ml. per minute.

Roberts, Richardson and Green have reported an epinephrine response in their controls in which the flow decreased and returned to slightly higher levels. This would correspond to our hyper-reactive muscle group of dogs. They did not see as high a rise in flow in response to epinephrine as we did but this probably was due to the fact that they were using the whole leg; the simultaneous reduction in flow in the skin, which showed only constriction with no secondary dilation, would, in their experiments, have masked the secondary rise in flow in the muscle. In our experiments with pure muscle flow we saw the full dilator response. Since this secondary dilation was often seen in dogs that showed the greatest dilation following ischemia, we thought that it might be on the basis of ischemia. The constrictor response lasted about as long as our period of ischemia, one minute, but the secondary dilation lasted one to five minutes with a slow rise and a slow fall. Dilation following ischemia lasted only for a period of 30 seconds with a rapid rise and fall, hence, the secondary response to epinephrine must not be on the basis
of ischemia. In no case was a dilator effect due to epinephrine seen in the skin, either before or after blocking drugs.

L-epinephrine and L-norepinephrine both caused a vasoconstriction of about the same magnitude in skin. The constriction to each was blocked by the same doses of blocking agents mentioned above. There was never any reversal of the epinephrine effect in skin following these agents. A secondary vasodilation was also never seen in the skin with epinephrine. L-norepinephrine appeared to act the same as epinephrine in four animals after small doses of antiadrenergic drugs. But in five animals there was secondary vasodilation after small doses of blocking agents, the dilation was, however, blocked before the constriction in two animals and the dilation and constriction were blocked at the same time in three animals. The secondary dilation was never as prominent as that seen in muscle with L-norepinephrine.

In muscle epinephrine given after any of the blocking agents caused a very potent vasodilation. At this dose of the blocking drug L-norepinephrine still caused a simple constriction except in the hyper-reactive group where a small secondary dilation was seen. When progressively higher doses of the blocking agent were given both the constrictor and dilator effects of L-norepinephrine were blocked, the dilator part being blocked completely before the constrictor response (see fig. 12B').

The dilation in muscle following epinephrine was always diminished but never completely blocked by this dose of blocking agents that just blocked the constrictor action of epinephrine and norepinephrine in skin, and norepinephrine in muscle.

The presence of active vasodilation in response to ischemia, to methacholine, and to epinephrine in muscle, and the absence of significant dilation in response to these in skin suggests a fundamental difference in the reactivity of these two vascular beds. This difference may in some way serve to maintain muscle flow, by a cholinergic mechanism, during periods of stress when cutaneous flow may be sacrificed. The marked similarity in the reactivity to L-epinephrine and L-norepinephrine in skin and in muscle in the absence of a blocking drug, and their marked difference in muscle after a blocking drug makes one wonder if there may not be some etiologic reason for such difference and particularly, if there may not be elaborated in the body some humoral substance with epinephrine reversing properties for muscle blood vessels.

**Summary**

1. The vascular bed in skin in general was found to be less reactive to dilator effects than muscle. Skin flow did not show any response to ischemia and a poor response to methacholine, while muscle flow showed a marked vasodilation following corresponding periods of ischemia or methacholine.

2. Control L-epinephrine injections in skin and the hyporeactive muscle group caused only a constriction while the hyper-reactive muscle group showed initial constriction followed by a secondary dilation.

3. Following antiadrenergic drugs epinephrine injections caused a reversal of the L-epinephrine effect, that is, dilation in muscle but only constriction or no response was seen.

4. Following small doses of antiadrenergic drugs in five dogs used in skin flow experiments and the hyper-reactive muscle group a small secondary dilation occurred in response to L-norepinephrine. Antiadrenergic drugs in higher doses blocked both the constrictor and dilator effect of L-norepinephrine in muscle and skin and epinephrine constriction was blocked in skin, but epinephrine still caused dilation in muscle.

5. Poor or no dilation in response to methacholine, to ischemia and to epinephrine after the blocking drugs are associated together in the response of the blood vessels in cutaneous vascular beds. Good dilation to ischemia, to methacholine and to L-epinephrine after the blocking drugs are associated together in the vascular bed supplying muscle. These differences seem to be fundamental, but a possible causal relationship between them has not yet been established.
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