Effect of Plasma Osmolality on Resistance to Blood Flow through Skeletal Muscle* (32998)

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Addition of solutes to the blood to change its osmolality results in a decrease in resistance to blood flow in various peripheral vascular beds (1–4). The mechanism of this response is essentially unknown, but it appears to operate locally in the peripheral tissues. The experiments reported here were performed to determine the effect of changes in the osmolality of plasma on flow through the isolated gastrocnemius–plantaris muscle group of the dog. When it was observed that the dilatation and increased flow in response to changes in osmolality were still present in the plasma-perfused preparation, experiments were performed to test the effect of changes in osmolality induced by a variety of different solutes on muscle flow. The results of these studies are reported below.

Methods. The muscle preparation used in this study was the left gastrocnemius–plantaris muscle group of 8–12 kg dogs anesthetized with sodium pentobarbital (30 mg/kg). The circulation to and from the muscle group was isolated, leaving intact only that entering and leaving by the popliteal vessels. The popliteal artery was cannulated and connected to the perfusion apparatus. Most vascular beds were denervated by cutting the sciatic nerve during the surgical isolation of the circulation. Mepesulfate (100 mg/kg) was used to prevent coagulation of blood. Booster doses of 100 mg were given every 0.5 hour.

The perfusion system (Fig. 1) was made of tygon and lucite and used a small Sigmamotor pump. When clamp A was opened, the muscle was perfused by means of the animal's own blood pressure. When the stopcock was closed to both reservoirs S_1 and S_2, and clamp B was opened and clamp A closed, perfusion was by pump using the animal's own blood. Other perfusates were placed in the polyethylene reservoirs S_1 and S_2 and pumped through the muscle after opening the stopcock to the desired reservoir and closing clamp B. Changing from one perfusate to another was accomplished by turning the stopcock from one reservoir to the other. Venous outflow through X was returned to the animal via the jugular vein during perfusion with the animal's own blood. Otherwise, the effluent was collected in a polyethylene bottle kept at the same level as the jugular vein. The blood pressure of the animal and the perfusion pressure of the muscle group were each measured by Statham transducers. Blood flow was measured by a rotameter. Both pressure and blood flow were recorded with a polygraph.

Donor dog plasma was used as the perfusate. The donor dog was anesthetized, anticoagulated, and bled during the time required for surgical isolation of the muscle of the recipient dog. The donor blood was centrifuged in polyethylene bottles and the plasma was separated into another polyethylene bottle. This plasma was then subdivided into a control sample and several other samples to which either water or osmotically active substances were added. The osmolality (milliosmols/kg) of the control plasma and all samples was measured by freezing point depression by one of us. The samples were given to the other with only the control plasma identified. All plasma samples were gassed before use for at least 20 min with 95% oxygen and 5% carbon dioxide in a water bath maintained at 38°C.

When the temperature of the samples reached 38°C and gassing was considered adequate, the experiment began and always followed the same protocol. The muscle perfusion fluid was switched to control plasma. The flow rate was then adjusted until the mean perfusion pressure was approximately 100 mm Hg after which the flow rate was kept constant. When a constant pressure trace

* This study was supported by National Institutes of Health Research Grant GM 06264.

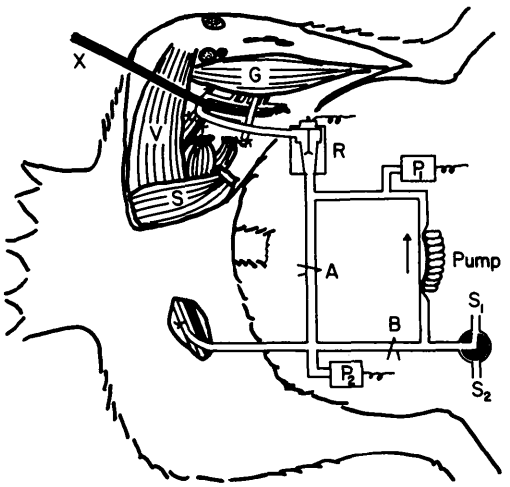

FIG. 1. Diagram of the perfusion circuit: P_1 and P_2 are pressure transducers; R is a recording rotameter; X is the venous outflow tube; A and B are clamps for directing the flow of blood; S_1 and S_2 are the reservoirs connected to a stopcock for plasma perfusion.

was recorded, the stopcock was turned so that a test sample perfused the muscle. The new perfusion pressure was recorded until it became constant or until the entire sample had been pumped through the muscle. Adequacy of oxygenation of the muscle was insured by the stability of the perfusion pressure. With an indaequate flow, progressive dilatation occurs in this preparation and pressure falls. This did not occur at the flows used in these studies.

Results. Portions of the record from two such plasma samples are shown in Fig. 2. The upper half of the record shows the vascular response to a plasma sample which was hypoosmotic relative to control plasma. The lower half shows the vascular response to plasma which was hyperosmotic relative to control plasma. The magnitude of the pressure change was proportional to the difference in osmolality between the control and test plasma samples. The relationship between osmolality (X) and change in perfusion pressure (Y) is shown graphically in Fig. 3. It is linear over the range of plasma osmolalities examined. The equation for the line shown is $y = -0.6X + 181.5$ and the correlation coefficient, $r = 0.931$, indicates that the relationship is highly significant; ($n = 37$).

A number of osmotically active substances were tested and were all found to be equally effective at the same osmolar concentration. Substances known to cause vascular activity directly were avoided. The substances tested were sodium chloride, glucose, mannitol, sucrose, low-molecular-weight dextrans, PVP high-molecular-weight dextrans, and urea. The initial response to all these substances was the same. A few of these substances are marked in Fig. 3. Except for urea, the change in resistance appeared to persist as long as perfusion was continued (up to 25 min). The vascular response to urea was definitely transient. The peak response which lasted only 2 or 3 min fell on the graph as shown. In about 10 min the response had nearly completely disappeared. When perfusion with urea-containing plasma ended and perfusion with control plasma or blood began, a second vascular response occurred, similar to the first one but in the opposite direction. This response also disappeared in about 10 min.

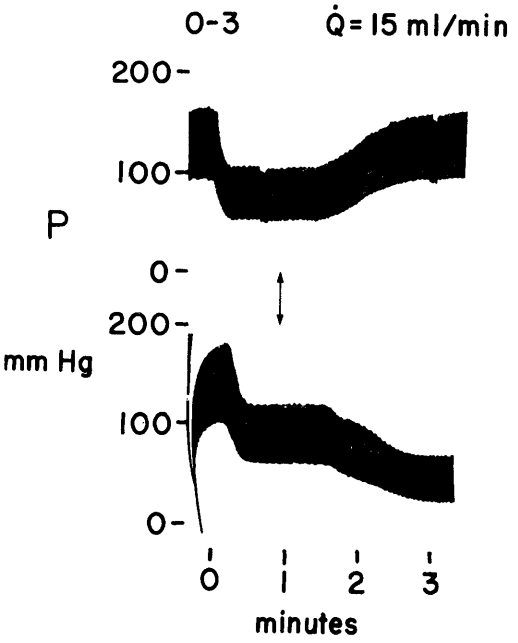

FIG. 2. Sample records showing the pressure response to perfusion with plasma of low osmolality, upper trace, and high osmolality, lower trace. Control plasma perfusion began at the start of the record; sample plasma perfusion began at the arrow; flow was constant.

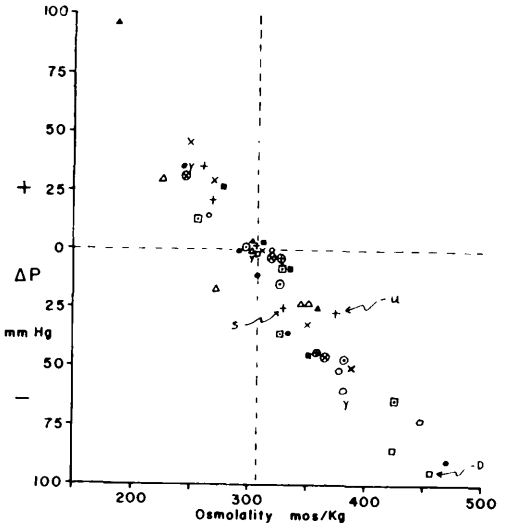

FIG. 3. Relationship between change in pressure, at constant flow, and plasma osmolality for 13 dogs. Control plasma averaged 323.9 milliosmols/kg. Increased osmolality resulted in decreased perfusion pressure. Marked points indicate a few of the plasma samples in which different substances were used to increase plasma osmolality; S = sodium chloride, U = urea, D = dextran. Decreased osmolality resulted in increased pressure.

Discussion. The vascular resistance decreased when the osmolality of the plasma was increased. The decrease in resistance was the same for a given change in osmolality regardless of the solute tested. The solutes with higher molecular weights, such as the dextrans, presumably remained mainly in the plasma and did not achieve significant concentrations in the interstitial space. Sodium chloride, on the other hand, should have entered the interstitial space fairly rapidly. The similarity of the two responses suggests that differences of osmolality between the plasma and the interstitial space does not explain the results. The initial response to urea was like that of the other substances tested, but after about 10 min of continuous perfusion, the response was much reduced, and a reverse response occurred when urea–plasma perfusion was switched subsequently to control plasma perfusion. The time of the decay of the response seems consistent with entry of the urea into cells surrounding the vessels. The results with urea suggest the possibility of a cellular "osmometer" as part of the mechanism of this response; that is, the response appears to be related both to the presence and the maintenance of an intracellular to extracellular osmotic gradient. Beyond this, little is known.

The response to changes in osmotic pressure was equally good in both innervated and denervated preparations. It is, therefore, a local or autoregulated response. Except when the vascular bed was maximally dilated while perfusing with a plasma of very high osmolality, other autoregulated vascular responses such as active and reactive hyperemia and resistance change in response to changes in blood flow, were all present. Apparently the osmotic response does not alter the more usual autoregulated responses. The question comes to mind whether this response to change in plasma osmolality may operate by the same mechanism as the other autoregulated responses which do not appear to be metabolically linked. The vascular response to changes in blood flow, increased arterial pressure and increased venous pressure would all be likely to increase capillary hydrostatic pressure and incease filtration of water into interstitial spaces. All three result in vasoconstriction as an autoregulated response. Decreased plasma osmolality, which also causes vasoconstriction, should also tend to increase water movement out of the capillary. The reverse water movement occurs with both decreased capillary hydrostatic pressure and plasma hyperosmolality and vasodilatation occurs.

Although it is possible that such a common mechanism for regulation of transcapillary water exchange could exist, the results of these experiments do not prove the point. In particular it should be emphasized that the osmotic response seems much less sensitive than the hydrostatic pressure response. If one assumes a change in plasma osmolality of 1 milliosmole as equivalent to a change of about 19 mm Hg pressure, then the vascular response to change in osmolality is relatively small. In addition, the response was the same whether the osmotically active substance was large enough to remain in the capillary or small enough to diffuse into interstitial space.

Further, the response to plasma containing urea was transient with a reverse response when control plasma perfusion was resumed. It would seem that simple movement of water across the capillary wall is not a primary factor in the osmotic response. Although it would appear that cell membranes are involved in the response, the mechanism remains to be elucidated.

Another general mechanism suggested for autoregulation in response to arterial and venous pressure changes is that of compression of the resistance vessels by tissue pressure secondary to transcapillary water movement. The osmotic response would fit this model in general. However, the osmotic response is complete in 1–2 min and is quite stable. For the tissue-pressure, vascular compression model to reach equilibrium so soon would require that the involved extracapillary space be very small.

It is also possible that the response is simply the result of swelling by hydration or shrinking by dehydration of the endothelium or some other part of the vascular bed, swelling being at least partly into the lumen of the vessels, thereby decreasing luminal cross section and increasing resistance to flow. This hypothesis seems the least favorable of those suggested but evidence to support or refute it awaits further study.

Summary. The gastrocnemious–plantaris muscle group of the dog was pump-perfused at constant flow with fresh plasma from a donor dog. When the osmolality of the perfusing plasma was increased by adding sodium chloride, glucose, sucrose, mannitol, dextran, or urea, the resistance to flow through the muscle decreased. The decrease in resistance was proportional to the increase in plasma osmolality. When distilled water was added to the plasma to decrease the osmolality, the resistance to flow through the muscle increased in proportion to the decrease in osmolality. The response to most test substances persisted for at least 20 min. These data suggest a mechanism regulating blood flow in resting muscle that is sensitive to osmolar concentration differences.

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Received Dec. 20, 1967. P.S.E.B.M., 1968, Vol. 128.

Purification of a Horse Placental Inhibitor to Hemagglutination by H-1 or HB Viruses* (32999)

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The H-viruses are a group of minute DNA agents, approximately 200 Å in diameter, that have been found associated with rapidly proliferating tissues such as tumors and embryos (1). H-1 and HB have been isolated from human tissues, H-3 from a transplantable human tumor carried in rats, and RV from rat tumors only. All of the agents, which can be distinguished from one another serologically and by their hemagglutination patterns, produce a "mongoloid-type" deformity in newborn hamsters. In 1964, Toolan (2) reported that fluid expressed from human placentas inhibited the hemagglutination (HA) of guinea pig red cells by H-1 and HB viruses but did not affect HA by the closely related agents, H-3 or RV. Human maternal or fetal sera, on the other hand, did not prevent HA by any of these viruses.

Recent studies (3,4) have shown that the inhibitor in the human placental fluids is apparently a glycoprotein that sediments as a

* Aided by Grant E-343B from Am. Cancer Soc., Grant CA-07826-04 from Nat. Cancer Inst., N.I.H., USPHS and a generous gift from the Given Foundation.