Resistance responses to local changes in plasma osmolality in three vascular beds

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GAZITIU, S., J. B. SCOTT, B. SWINDALL, AND F. J. HADDY. Resistance responses to local changes in plasma osmolality in three vascular beds. Am. J. Physiol. 220(2): 384-391. 1971.—The effects of local changes in plasma osmolality (durations of 3-10 min) on resistance to blood flow were compared in the forelimb, renal, and coronary circulations of anesthetized dogs. A 40- to 50-mOsm/kg increase in plasma osmolality with sodium chloride, dextrose, or urea initially lowered resistance in all three beds. The responses to dextrose and sodium chloride waned in the forelimb and kidney but not in the heart. The response to urea waned in each bed. Renal resistance rose above control on stopping the infusion of each agent, but this response was only seen on stopping urea in the forelimb and heart. A 40- to 50-mOsm/kg decrease in renal plasma osmolality caused a marked rise in renal resistance. Elevation of osmolality with dextrose or urea increased myocardial contractile force, whereas the same increase with sodium chloride caused an initial decrease in force followed by an increase. These studies show that the resistance response to osmolality depends not only on the bed but also on the agent used to alter osmolality.

renal resistance; forelimb resistance; coronary resistance; hyperosmotic urea; hyperosmotic dextrose; hyperosmotic sodium chloride; myocardial contractile force; hyposmotic sodium chloride

THE RESPONSE OF VASCULAR RESISTANCE TO LOCAL CHANGES IN PLASMA OSMOLALITY

Resistances responses to local plasma osmolality changes have been studied by many investigators under a variety of circumstances. Thus osmolality has been changed systematically and locally with a variety of substances in many different preparations and species. In general a slight to moderate local increase in plasma osmolality produces a decrease in resistance as an initial effect (see ref. in 2). However, preliminary studies (2) suggested that the response, in terms of time course and magnitude, differs somewhat in forelimb and kidney similarly prepared. Moreover, the dissimilarity appeared to depend to a certain degree on the agent used to alter the plasma osmolality. These preliminary findings stimulated a systematic comparison of the resistance responses to hyperosmotic solutions of sodium chloride, dextrose, and urea in the vascular beds of the forelimb, kidney, and heart. Hyperosmolarity has also been reported to influence myocardial activity (7, 9, 11, 13, 18), but the data are conflicting perhaps because experimental conditions varied. For this reason, measurements were also made of myocardial contractile force during intracoronary infusion of the three hyperosmotic solutions. Finally, attempts were made to further clarify the mechanisms of action on resistance and contractile force. While the mechanism of action of osmolality on contractile force has received little attention, previous studies suggest that the changes in resistance result, to a large extent, from active vasomotion, possibly due to osmotic changes in the water content of the smooth muscle cells (2, 6, 10).

METHODS

Mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg) and ventilated artificially. After injection of heparin, the vascular bed of the left heart, forelimb, or kidney was perfused at constant flow with a precalibrated blood pump. Solutions of different osmolalities were infused at a constant rate upstream to the blood pump in a random sequence. The solutions used were iso- and hyperosmotic sodium chloride, dextrose, and urea, and hyposmotic sodium chloride. Infusion periods were 3 min for the heart and kidney and 10 min for the forelimb. The solutions were infused at rates calculated to change local arterial plasma osmolality by 40-50 mOsm/kg. All solutions were infused at 37°C. Local arterial plasma osmolality during infusion was calculated from blood flow, infusion rate, and concentration of the infused solution by assuming that the control plasma osmolality was 300 mOsm/kg and the infused substance distributed equally in plasma and red cell water. Although these assumptions may not be entirely valid, experience indicates that infusion rates thus calculated in fact produce roughly the desired osmolalities. Osmolality and potassium concentration of the venous plasma were measured in some forelimb experiments by the freezing-point depression method and flame photometry, respectively. Aortic and perfusion pressures were monitored via resistance wire pressure transducers.

Heart. The method used to perfuse the left common coronary artery has been previously described (5). In brief, in 11 experiments, a curved metal cannula was inserted into the aorta via the left subclavian artery. The cannula was attached to an extracorporeal circuit which included a blood pump. With the pump delivering arterial blood, the cannula tip was manipulated down the ascending aorta into the mouth of the left common coronary artery and tied in place with a ligature previously placed around the artery. Contractile force of the left ventricle was monitored from a 120-ohm strain-gauge arch sutured to the venricular surface. Solutions studied included iso- and hyperosmotic sodium chloride, dextrose, and urea.

Forelimb. The effects of osmolality on the forelimb were
studied in a total of 23 animals. In 10 of these animals, the right brachial artery, forelimb nerves, and brachial and cephalic veins were dissected free at a level 3–5 cm above the elbow. Collateral flow to the limb was abolished by including all other structures in tourniquets. The humerus was sectioned and bone wax was applied to the exposed ends. A finger-type blood pump was interposed between the right femoral artery and the distal segment of the exposed brachial artery. The main anastomotic branch between the cephalic and brachial veins at the elbow (median cubital vein) was ligated, and the two ends were cannulated to measure the brachial and cephalic venous pressures. The brachial and cephalic veins were transected 3–5 cm downstream to the sites of pressure measurement. The distal end of each vein was cannulated and the outflows directed into a reservoir. With the limb prepared in this manner, brachial flow is mainly from muscle and cephalic flow from skin. Blood was returned to the animal at the rate of its appearance with a second pump. The forelimb was placed on a wire-mesh pan suspended from a strain-gauge torsion beam balance modified slightly from that described by Stish et al. (16). The scale was calibrated by placing known weights on the pan before and at various intervals during each experiment. Solutions studied were iso-, hyper- and hyposmotic sodium chloride and hyperosmotic dextrose and urea. In 7 of these 10 experiments, venous plasma osmolality was also determined before and at the 1st and 10th min of each infusion. In 3 of the 10 experiments, the cephalic and brachial outflows were measured with cylinders and a stopwatch before and periodically during the infusion.

In 10 other animals, the major forelimb vessels were exposed as described above and collateral flow excluded by tourniquets. A blood pump was again interposed between the femoral and brachial arteries but the bone and brachial and cephalic veins were left intact. Pressure was also measured in a peripherally cannulated small artery on the ventral surface of the footpad. Solutions studied were isosmotic sodium chloride and hyperosmotic sodium chloride, dextrose, and urea. In 5 of these 10 experiments, osmolality and potassium concentration of the venous plasma were determined before and at the 1st and 10th min of infusion. In 3 of the 10 experiments, the cephalic and brachial outflows were measured with cylinders and a stopwatch before and periodically during the infusion.

Kidney. The left kidney was approached through a flank incision and isolated from the body except for the ureter, renal artery, and renal vein. The ureter was cannulated and heparin was administered. A blood pump was then interposed between the femoral and renal arteries. The renal vein was ligated close to the vena cava and the kidney segment of the vein was opened to the atmosphere. The kidney was removed from the body (with the collateral vessels open) and placed in a wire-mesh box suspended from a strain-gauge torsion beam balance. The blood pump was then started. During the isolation procedure, the kidney was without blood flow for less than 3 min. The temperature of the box was maintained at 36–38 °C. The venous effluent from the isolated kidney was collected and returned to the animal via the right femoral vein. Solutions studied in the kidney were iso-, hyper-, and hyposmotic sodium chloride and hyperosmotic dextrose and urea. In four preliminary experiments it appeared that the magnitude of the response of renal resistance to changes in osmolality was related to the initial level of perfusion pressure. For this reason, in six animals, the effects of changes in renal plasma osmolality on vascular resistance and weight were systematically investigated at two levels of perfusion pressure, i.e., ~60 mm Hg (low) and ~80 mm Hg (high). Urine flow was not seen at the low perfusion pressure but was seen at the high. In each experiment the solutions were first administered at the low pressure level. Perfusion pressure was then elevated by increasing renal blood flow and the infusions repeated.

The effect of papaverine (3 mg/min) on the response to hyperosmotic urea was determined in four of these kidneys. In these experiments, renal resistance was initially low and spontaneously rose after completing the infusion sequence. Papaverine administration at this time fortuitously returned pressure to approximately the level observed before the spontaneous increase. Thus, it was possible to compare the effects of hyperosmotic urea on renal resistance and weight before and during papaverine administration at the same blood flow and pressure.

Hyperosmotic urea and hyposmotic sodium chloride were studied before and during papaverine infusion in six additional experiments. In these experiments, papaverine reduced perfusion pressure below the control level. In order to study the effects of osmolality at the same pressure level, renal blood flow was increased with the pump.

Data analysis. All data on perfusion pressure, contractile force, and weight were evaluated by the Student t test modified for paired replicates. The value at the end of the 1st min of infusion was compared with the control value and designated the initial response. In addition, the value at the end of the 1st min of infusion was compared with the value during the last minute of infusion, thereby distinguishing a sustained response from an increasing initial response or a decreasing (waning) initial response. Finally, both the control value and the value during the last minute of the infusion were compared to the maximal or minimal value 40–80 sec after stopping the infusion, thereby characterizing the off response (overshoot, undershoot, return, control value). All changes referred to under results are significant with a confidence level of <0.05.

RESULTS

Figure 1 presents the time course of the changes in venous plasma osmolality and compares these values with those calculated for arterial plasma. In the forelimb, venous plasma osmolality was elevated by the 1st min but not to the extent seen by the 10th min (Fig. 1A); the latter value was similar to that calculated for perfusing blood. In addition, the level of venous osmolality during the 4th min of the infusion was significantly lower than that calculated for arterial blood (Fig. 1B). In the kidney (Fig. 1C), venous osmolality rose more promptly, achieving a level close to or equal to the calculated value in arterial plasma during the
produced by intracoronary infusion of 0.1-0.5 mg/min of norepinephrine (0.5 pg as a bolus) on contractile force was tested before and during beta-adrenergic blockade. Although the effect of norepinephrine on contractile force was blocked by both agents, the effects of hyperosmolality on contractile force and vascular resistance were unaffected.

Heart. Pressure and weight responses to iso-, hyper-, and hyposmotic solutions are shown in Figs. 4 and 5. A sustained fall in pressure was seen in response to isosmotic sodium chloride (Fig. 5A). Pressure fell initially with all the hyperosmotic agents to a significantly lower level than that observed with isosmotic sodium chloride. Pressure increased toward the control level during all infusions, increased above the control level on stopping urea, and decreased for a short period of time on stopping dextrose and, in some cases, on stopping sodium chloride. The response waned the most during infusion of urea and the least during infusion of dextrose. Forelimb weight decreased progressively in response to hyperosmolality. The weight loss was significantly greater with dextrose than with sodium chloride or urea; the latter caused the smallest weight loss of the three solutions. On stopping the infusion, weight abruptly increased and then returned slowly toward the control level.

Figure 5B illustrates responses of pressure and weight to iso- and hyposmotic sodium chloride. Pressure fell in both cases during the 1st min; it then continued to fall slowly during infusion of isosmotic sodium chloride but was not further affected by hyposmotic sodium chloride. The fall in pressure by the 10th min was less with hyposmotic sodium chloride than with isosmotic sodium chloride. Weight gradually rose during infusion of both solutions; the greatest rise occurred during the infusion of hyposmotic sodium chloride.

Brachial and cephalic outflows did not change in the three animals in which they were measured. Neither were
there significant changes in brachial and cephalic venous pressures (measured in all animals).

In those experiments in which it was measured, small-artery pressure changed in the same direction as perfusion pressure and calculations indicated that the resistance changes occurred mainly distal to the site of pressure measurement in the small artery. Venous plasma potassium concentration was equally reduced at the 1st and 9th min by isosmotic sodium chloride, hyperosmotic dextrose, and hyperosmotic sodium chloride, but was unaffected by hyperosmotic urea. The fall in potassium concentration ranged from 0.2 to 0.3 mEq/liter.

Figure 6 presents pressure responses to infusion of hyperosmotic urea before and during papaverine administration in one of the three experiments. The infusion rate of urea was adjusted to produce the same change in arterial plasma osmolality in both instances. Papaverine reduced the initial pressure fall and completely abolished the wane and overshoot. Infusion of isosmotic sodium chloride at the same rate as urea showed that the residual fall in pressure was to a large extent the result of dilution.

Kidney. Pressure responses in the in situ constantly perfused kidney to iso-, hyper-, and hyposmotic solutions have been previously reported (2), and are similar to those described herein for the isolated kidney.

Figure 7 shows tracings from a representative experiment. Figure 8 shows that the hyperosmotic solutions, infused at the high perfusion pressure level, produced a fall in resistance followed by a wane and overshoot. The fall in resistance was similar with each agent but the wane and overshoot were most pronounced with urea. In contrast to the findings in the forelimb, the weight changes were generally in the same direction as the changes in pressure. The magnitude of the fall in weight was independent of the agent used but urea produced the greatest increases in weight after the infusion. Iso- and hyposmotic sodium chloride, infused at the high perfusion pressure level, produced a transient fall in pressure followed by a rise above the control level, most pronounced with hyposmotic sodium chloride. Weight increased with both solutions.

Figure 9 shows the effects of papaverine on the responses of the renal vascular bed to hyperosmotic urea and hyposmotic sodium chloride. Kidneys were studied at a similar flow before and during papaverine in group A, but at a higher flow during papaverine in groups B and C. In groups B and C, the responses of the vascular bed to norepinephrine and acetylcholine were tested before and during papaverine administration. It was found that papaverine blocked ~80% of the response to either agent. The control level of pressure was similar before and during papaverine in each group. The initial fall in pressure in response to hyper-
FIG. 4. Representative tracings from one experiment showing changes of brachial perfusion pressure (upper trace in each panel) and forelimb weight (lower trace in each panel) during and after infusion of iso- and hyperosmotic solutions. Arrows below the tracings indicate beginning (0), 5th min, and 10th min of a 10-min infusion period. The 2nd min after stopping infusion (12) is also indicated. Blood flow, 60 ml/min; infusion rate, 1.91 ml/min. Calculated blood osmolality during infusion of hyperosmotic solutions, 338 mOsm/kg; forelimb weight at end of experiment, 344 g.

osmotic urea or hypotonic sodium chloride was not affected by papaverine. However, the subsequent wane of the pressure response with all solutions as well as the overshoot on stopping hyperosmotic urea was reduced by papaverine. The weight pattern was similarly affected; the initial fall in weight was similar before and during drug administration, but the subsequent weight gain was significantly reduced by papaverine. It is apparent that the resistance response to osmolality is more effectively blocked by papaverine in the forelimb than in the kidney.

FIG. 5. Average changes in brachial perfusion pressure and forelimb weight during and after infusion of iso-, hyper-, and hypotonic solutions. Solutions infused (mOsm/kg)—A: (O—O) sodium chloride, 300; (■—■) urea, 1,500; (●) sodium chloride, 1,500; (□) dextrose, 1,500; B: ▲ sodium chloride, 300; △ sodium chloride, 200. Ten dogs were studied in A and 7 of these in B. Horizontal bar indicates infusion period. Mean blood flow (ml/min)—A: 75; B: 71. Mean infusion rate (ml/min)—A: 2.52, B: 2.3. Calculated mean blood osmolality (mOsm/kg): during hyperosmotic solutions, 340; during hypotonic sodium chloride, 267. Mean forelimb weight (g)—A: 391; B: 395.

FIG. 6. Representative tracings from one experiment showing effects of a hyperosmotic urea solution (1,500 mOsm/kg) on brachial pressure before (A) and during (B) papaverine hydrochloride infusion (3 mg/min). Arrows below tracing indicate beginning (0), 5th min (5), and 10th min (10) of a 10-min infusion period. Blood flow (ml/min)—A: 80; B: 180. Urea infusion rate (ml/min)—A: 3.15; B: 6.29. Calculated blood osmolality during infusion (mOsm/kg)—A: 347; B: 342. Forelimb weight, 410 g.

DISCUSSION

These studies show that the patterns of the resistance responses to 3-min intra-arterial infusions of hyperosmotic solutions in forelimb, kidney, and heart are similar but differ in some respects depending on the agent infused and the bed under study. The resistance decrease in response to dextrose wanes in the forelimb and kidney but does not wane appreciably in the heart. On stopping the infusion, resistance increases above the control value in the kidney but not in the heart (this overshoot also fails to occur in the forelimb at the end of a 10-min infusion). With sodium chloride, the response wanes in the kidney and forelimb but does not wane appreciably in the heart and an overshoot is seen only in the
kidney. The pattern of the response to urea is similar in the three beds but differs in magnitude. Dependence on the agent is also seen in contractile force; hyperosmotic sodium chloride produces a decrease in force followed by a wane and overshoot whereas dextrose and urea produce only increases in force of different magnitudes. The specificity of the bed is dramatically revealed during infusion of hyperosmotic sodium chloride. After 3 min of infusion, resistance is still below the control value in the forelimb but well above this value in the kidney.

Blood viscosity (changes in cell concentration, size, deformability, and aggregation), passive vasomotion (changes in caliber subsequent to alterations in vascular transmural pressure and wall hydration), and active vasomotion (changes in caliber subsequent to alterations in vascular smooth muscle activity) all must be considered in attempting to explain the resistance responses to local hyper- and hypoosmolality (2, 3, 4, 10, 14). The fall in resistance seen in the 1st min of infusion of all solutions undoubtedly results, at least in part, from a fall in blood viscosity subsequent to dilution of red cells by the infusate. The contribution of this factor is obviously greater during infusion of iso- and hypotonic solutions at the rapid rate. It is possible that changes in viscosity other than those produced by dilution also participate in the responses but these changes appear to be relatively unimportant since responses in the kidney still occur during perfusion with cell-free fluids (2). Furthermore, other vascular beds also respond during perfusion with cell-free fluids. Hindlimb (8, 12), forelimb (3), and skeletal muscle (15) resistances still fall with hyperosmolality; forelimb (3), lung (3), and skeletal muscle (15) resistances still rise with hypoosmolality. In skeletal muscle, the fall in resistance seen during infusion of hyperosmotic urea even wanes with time and then transiently rises above the control value on stopping the infusion (15). Finally, as will be discussed below, the responses during blood perfusion are greatly influenced by agents which change the activity of vascular smooth muscle.

Recent studies in kidney (2) and skeletal muscle (10) are consistent with the hypothesis that the changes in resistance produced by hyperosmolality in these organs result in a large part from active vasomotion. Certain findings in the present study also suggest an important role for active vasomotion. In the forelimb, papaverine essentially abolishes the response to hyperosmotic urea. This finding is consistent with observations in skeletal muscle. In this tissue, papaverine blocks the fall in resistance seen during infusion of hyperosmotic sucrose and xylose (10). Furthermore, the maximally dilated (acetylcholine) forelimb, either blood or dextran perfused, does not respond to hyperosmotic dextrose (3). Papaverine also influences the responses in the kidney. The wane and overshoot are attenuated in the case of hyperosmotic urea, as is the rise in resistance in the case of hypotonic sodium chloride.

On the other hand, papaverine has no effect on the initial fall in renal resistance in response to hyperosmotic urea even though the response to acetylcholine is greatly attenuated. Furthermore, the subsequent wane and overshoot appear to be blocked to a lesser extent than the response to norepinephrine. Moreover papaverine does not completely abolish the response to hypotonic sodium chloride. These findings coupled with the observed weight changes suggest a role for one or both of the other two possible mechanisms, namely viscosity and passive vasomotion, at least in the kidney.

With respect to the mechanism of the active vasomotion, both indirect and direct actions must be considered. Thus changes in osmolality might act indirectly through release of vasoactive agents from the red cells, nerves, or parenchyma. This seems unlikely because 1) responses still occur in the absence of red cells (see above), 2) bioassay studies...
It has been proposed that changes in plasma osmolality induce active vasomotion by altering intracellular ionic concentrations in vascular smooth muscle cells subsequent to osmotic movement of water (2, 6, 10). For example, osmotic dehydration of the cell would increase the intracellular potassium concentration and produce hyperpolarization. The reverse would occur in overhydration. In vitro studies of smooth muscle (vascular and intestinal) support this hypothesis (1, 6, 10, 17). Thus it appears that osmolality influences the activity of vascular smooth muscle through some direct mechanism.

Various vascular beds to a given agent might also be, in part, related to differences in permeability of the vascular smooth muscle cells to the agent, but perhaps more important are differences in basal vasomotor activity, compliance of the extravascular compartment, and metabolic response of the parenchymal cells.

The effects of hyperosmolality on myocardial contractile force deserve special consideration. Clearly, the hyperosmotic solutions produce immediate and striking changes in this parameter. In this regard there are apparently no in vivo studies dealing with the effects of local (intracoronary) increases in osmolality. However, bolus intracoronary injections of hyperosmotic glucose (1,200–2,400 mOsm/liter) and sodium chloride (1,800–3,000 mOsm/liter) have been reported to lower blood pressure (11). The effects of generalized changes in plasma osmolality on myocardial activity have been investigated but the findings are contradictory. In agreement with the present studies is the finding of Wildenthal et al. (18) that progressive increases in systemic plasma osmolality from normal to 400 mOsm/kg, produced by either sucrose or urea, enhanced left ventricular dp/dt. On the other hand, in a preliminary study, Regan et al. (19) reported that elevation of plasma osmolality by as little as 30 mOsm/liter with an intravenous infusion of...
sucrose is associated with a fall in stroke work in the face of an elevation in end-diastolic pressure. Data were obtained, however, 1 hr after beginning the infusion of sucrose.

In vitro data from papillary muscle and atrial and ventricular strips are similar to the in vivo findings in the present study. Tension of isolated papillary muscle and atrial strips increases when the osmolality of the bathing medium is raised with sucrose or mannitol over the range investigated herein (7). Above 385 mOsm/liter, papillary muscle tension begins to return to the control level and reaches it at 485 mOsm/liter. Further increases in osmolality decrease tension below the control level. Isolated atrial strips also respond with an increase in tension when the bathing medium is made hyperosmotic by addition of urea (7). Furthermore, a strip of right ventricle from the rat has been reported to respond with an initial decrease in contractility when the bathing medium is made hyperosmotic with sodium chloride (9). Within a short time the contractility recovers slightly even though hyperosmolality is maintained. These in vitro studies, as well as the findings with propranolol and AY 21,011 in the present study, indicate that catecholamines are not importantly involved in the response of contractile force to small local increases in osmolality. Thus, the changes in myocardial contractile force, like the changes in coronary vascular resistance, seem to result from a more direct effect on myocardial cells.

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