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Direct Effects of Hypoosmolality on Vascular Resistance and Myocardial Contractile Force (38587)

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Hypoosmolality is frequently encountered clinically and is associated with important effects on the cardiovascular system. Acute salt depletion, for example, lowers plasma osmolality through loss of both sodium and chloride and this is associated with osmotic movement of water from the extracellular to the intracellular compartment. This in turn reduces plasma volume, venous return, stroke volume, cardiac output and hence blood pressure (1). These changes are apparently set in motion by the hypoosmolality rather than the reduced sodium chloride concentration per se since blood pressure does not fall during acute pure sodium chloride depletion (no water depletion) when plasma osmolality is held constant with sucrose (2).

Known compensatory events include increased heart rate, probably reflexly via the baroreceptors, and increased total peripheral resistance, in part via the same mechanism. Passive vasoconstriction, due to the fall in vascular transmural pressure, and increased blood viscosity, due to increased red cell concentration, size and rigidity, may also contribute to the increased resistance. Whether compensation also occurs via increased myocardial contractility has not been investigated but this would be the predicted effect of the baroreceptor reflex.

Recent studies suggest an additional type of compensation, namely increased vascular resistance due to direct effects of the hypoosmolality on vascular beds. Perfusion of peripheral vascular beds with hypoosmotic blood or perfusion fluids, systemic osmolality normal, is reported to increase resistance (3–8) and in one of the studies (3) the resistance increase appeared to result from the hypoosmolality rather than from reduced sodium chloride concentration per se. However, in all of these studies, the hypoosmolality was produced by dilutional techniques which also dilute many of the other components of blood. Thus a cause and effect relationship between the hypoosmolality and the resistance increase has been difficult to establish with certainty. Whether hypoosmolality has a direct effect on myocardial contractility *in vivo* has not been tested.

In this study we reduced the osmolality of the blood entering the intact dog gracilis and coronary vascular beds by a nondilutional technique; systemic osmolality was not altered. Effects on vascular resistance were studied. In the case of the coronary perfusions, we also measured left ventricular contractile force. Finally in the gracilis vascular bed we also reduced sodium chloride content at constant osmolality to see whether effects result from reduced sodium chloride *per se*.

The studies in the gracilis muscle have been summarized in a preliminary communication (9).

Methods. Mongrel dogs were anesthetized with sodium pentobarbital (33 mg/kg) and artificially ventilated. After anticoagulation with sodium heparin, the vascular bed of the gracilis muscle or heart was perfused alternately with normo and hypoosmotic arterial blood. Osmolality was controlled by dialyzing the blood against a Ringer's solution containing normal and reduced amounts of NaCl. Typical dialysates for an experiment were a control Ringer's solution at 300 mOsm/kg (for exact composition see Ref. 10) and two additional Ringer's solution at 250 and 200 mOsm/kg. The blood was maintained hyposmolal for 5 min. In the case of the gracilis muscle, the perfusing blood was also made NaCl deficient but isosmotic by adding sufficient mannitol to the NaCl deficient dialysates such that the blood was isoosmolal as it left the hemodialyzer.

Gracilis. The method for perfusing the gracilis muscle has been previously described

in detail (10, 11). In brief, the gracilis was surgically isolated except for the main artery, vein, and nerve. Left femoral artery blood was pumped at a constant rate through a hemodialyzer (10) and then into the gracilis artery. Initial flow rate was adjusted so that gracilis perfusion pressure was approximately equal to systemic pressure and this rate was maintained throughout the experiment. Thus changes in vascular resistance were reflected by changes in perfusion pressure. Systemic and gracilis perfusion pressures were continuously recorded. The gracilis arterial and venous blood were sampled during the control and experimental perfusions.

Heart. Blood from the left femoral artery was pumped through a hollow fiber hemodialyzer (Dow Chemical Company, Midland, MI) into the left common coronary artery, cannulated via the aorta as previously described (7). A strain gauge arch was attached directly to the surface of the left ventricle for recording contractile force.

In a first series of experiments, coronary flow rate was initially adjusted so that the coronary perfusion pressure was approximately equal to systemic pressure and this flow was then maintained. Left ventricular contractile force, coronary perfusion pressure, Lead II of the EKG, and systemic pressure were continuously recorded. Coronary arterial blood was sampled during control and hypoosmotic perfusion.

In a second series of heart experiments, the left common coronary artery was perfused at constant pressure (approximately 100 mmHg) and coronary flow was measured. This was accomplished by pumping approximately twice the normal coronary flow through the dialyzer and inserting a Y-tube between the dialyzer and left common coronary artery. One branch of the Y supplied blood to the coronary artery and the other branch, which contained a Starling resistor to maintain pressure constant, diverted blood to a reservoir. The reservoir blood was returned to the femoral vein via gravity feed. Flow shunted through the variable resistor was measured with graduate and stopwatch every minute and total pump flow was measured at the end of the experiment. Thus coronary blood flow at any time was equal to total flow minus the shunt flow. A second extracorporeal circuit containing a macroelectrode for measuring oxygen tension was inserted between the coronary sinus and left jugular vein. Systemic pressure, left ventricular contractile force, coronary perfusion pressure, and coronary sinus oxygen tension were continuously recorded. Blood samples were taken from the coronary artery inflow and coronary sinus outflow tubings during control and hypoosmotic perfusion.

Blood samples were analyzed for plasma $[Na^+]$ and $[K^+]$ by flame photometry, plasma $[Ca^{2+}]$ by atomic absorption, hematocrit by microcentrifugation, and plasma osmolality by freezing point depression. The data were statistically analyzed using Student's t test modified for paired replicates. All changes are referred to as significant if $P < 0.05$.

Results. Figure 1 shows typical responses of two gracilis muscles to two different levels of hypoosmolality. When the dialysate was changed from normo to hypoosmotic, gracilis artery perfusion pressure at constant flow gradually increased after a time lag and reached a steady state within 4–5 min. Upon returning the dialysate to control, perfusion pressure promptly returned to the initial value. The more severe hypoosmolality caused the greater increase in perfusion pressure.

Figure 2 shows that the relationship between plasma osmolality and perfusion pressure was linear over the range of 300–225 mOsm/kg. The change in gracilis artery perfusion pressure shown is the average of the "on" (steady state change elicited by switching from control to experimental dialysate) and "off" responses. There were no significant differences between the two responses. The regression analysis relationship between percent change in vascular resistance (y) and osmolality (x) is $y = -2.0x$ with a correlation coefficient (r) equal to 0.93.

The effect on gracilis artery perfusion pressure of partial replacement of plasma NaCl with mannitol is shown in Fig. 3. As shown by the closed circles, reducing the plasma $[Na^+]$ and $[Cl^-]$ while maintaining the blood isoosmolal always produced an increase in resistance to flow (a linear fit yielded a correlation coefficient of 0.79). However returning the dialysate to the control produced variable results (open circles). In these experi-

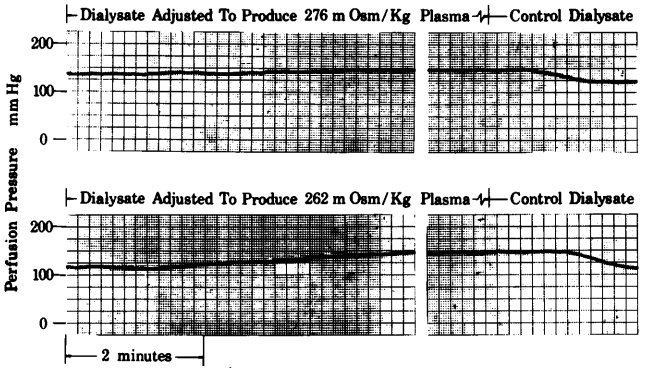

FIG. 1. Typical responses of gracilis perfusion pressure at constant flow to hypoosmolal perfusion. Two different levels of hypoosmolality in two gracilis muscles.

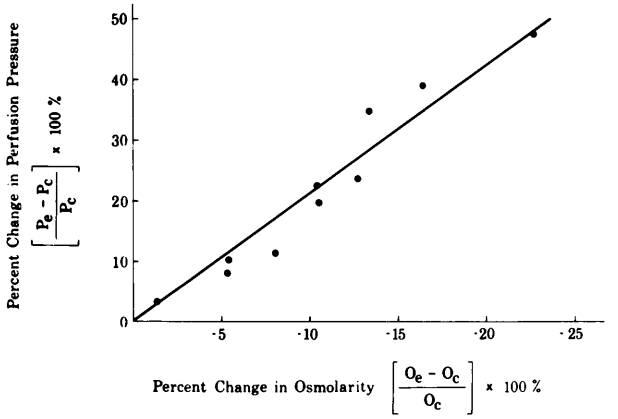

FIG. 2. Relationship between gracilis perfusion pressure at constant flow and plasma osmolality over the range of 300–225 mOsm/kg, in ten different preparations. P_e = experimental perfusion pressure; P_c = control perfusion pressure; O_e = experimental osmolality; O_c = control osmolality.

ments the dialyzer produced no measurable change in blood hematocrit and pH, or in plasma $[K^+]$ and $[Ca^{2+}]$.

During constant flow perfusion of the left common coronary artery, hypoosmolality always increased coronary vascular resistance, as seen in Fig. 4. The data are the average of the "on" and "off" responses in each animal. As in the gracilis muscle, there were no significant differences between the two responses. In eight of the nine animals, the greater reduction in plasma osmolality produced the larger increase in resistance. On the average, coronary vascular resistance increased by 33% in response to a 7.5% decrease in plasma osmolality. However, the responses did not correlate well (r = 0.50).

Associated with the increased vascular resistance was an increased contractile force in each animal which persisted for the 5-min period of hypoosmolal perfusion. In six of the eight animals, the increase in contractile force was greater with the more severe hypoosmolality. On the average (n = 9), a 7.5% decrease in osmolality produced a 20% increase in contractile force. However, the correlation between change in contractile force and osmolality was also poor (r = 0.51). There were also simultaneous decreases in the QT interval. On the average (n = 7), the QT interval decreased by 12%. Heart rate did not change.

The effects of reducing plasma osmolality by an average of 20 mOsm/kg while perfusing the left common coronary artery at constant pressure are shown in Fig. 5. Hypoosmolality significantly increased contractile force, but the initial increase waned with

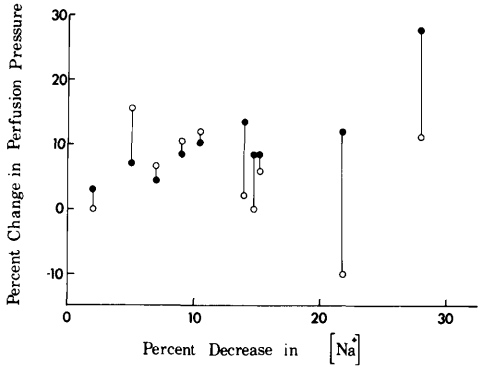

FIG. 3. Relationship between gracilis perfusion pressure at constant flow and plasma sodium chloride concentration (osmolality constant) in ten preparations. Closed circles = response to switching from normal dialysate to low sodium dialysate; open circles = response to switching from low sodium chloride dialysate to normal dialysate.

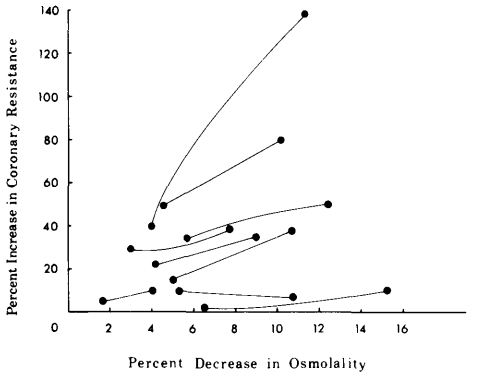

FIG. 4. Effects of hypoosmolal perfusion on coronary resistance at constant flow. Two different levels of osmolality in nine different preparations. Lines curved to avoid intervening points.

time. Associated with the rise in contractile force were falls in left common coronary flow ($P < 0.0001$) and coronary sinus oxygen tension ($P < 0.005$). When the coronary artery was again perfused with normoosmotic blood, contractile force and coronary sinus Po_2 returned to their initial values. Coronary flow increased but failed to return to the control value.

Discussion. These studies show that acute local decreases in plasma osmolality produced by partial removal of NaCl from blood increases skeletal muscle and coronary vascular resistances and myocardial contractile force. Furthermore, in the gracilis muscle, vascular resistance increased linearly as plasma osmolality decreased.

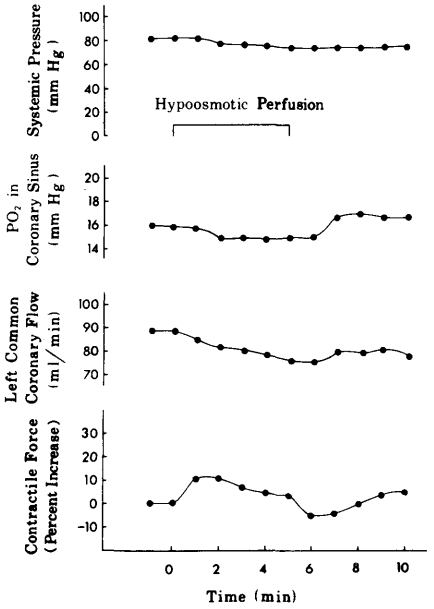

FIG. 5. Average effects of hypoosmotic perfusion at constant pressure on mean arterial blood pressure, oxygen tension of coronary sinus blood, left common coronary artery flow and left ventricular contractile force. Plasma osmolality reduced an average of 20 mOsm/kg in eleven preparations.

Stainsby and Fregly (5) also found that skeletal muscle vascular resistance was linearly related to osmolality over the range of approximately 200–450 mOsm/kg. The slope of our line relating resistance to osmolality is slightly greater than theirs (0.67 compared to 0.60). It is difficult to compare our data with theirs since they perfused with cell-free plasma and produced hypoosmolality by addition of water to the normal plasma. However, it appears that the secondary blood changes produced with their dilutional technique were of little consequence in comparison with the effects of the change in plasma osmolality. There are no previous *in vivo* studies on the effects of hypoosmolality on myocardial contractile force and coronary vascular resistance to compare with the present study.

The increase in coronary and skeletal muscle vascular resistance which occurred during the hypoosmolal perfusion most likely resulted from both passive and active

decreases in vessel caliber and because of an increased blood viscosity subsequent to red cell swelling. The passive effects have been considered in previous communications (6, 7). In brief, passive changes in vessel caliber may result from (a) cellular (endothelial, smooth muscle, etc.) swelling reducing lumenal diameter, and (b) in the case of the coronary vascular bed, decreased transmural pressure due to the effects of increased contractile force. Active vasoconstriction also contributes to the increase in resistance. Gazitua *et al.* (6) concluded from their data, as well as from the data of others, that in the kidney the increase in vascular resistance produced by hypoosmotic perfusion resulted, to a large extent, from active vasoconstriction subsequent to osmotic shift of water into the vascular smooth muscle cells.

Studies in isolated tissues show that hypoosmolality produces decreased intracellular concentrations of Na^+ and K^+ (12) and partial depolarization (13) as the cells gain water. It has been suggested that the depolarization most likely results from the reduced $[K^+]_i$ decreasing the passive K^+ efflux (6, 7). In addition, the electrogenic Na^+–K^+ pump (14) would be slowed by the decreased $[Na^+]_i$ (15). Thus, both intracellular Na^+ and K^+ appear to be important in the change in membrane potential and muscle activity. In this regard, calculation with a previously developed computer model which predicts transient changes in resting membrane potential (16) indicates that hypoosmolality does indeed reduce resting potential for the above reasons. Furthermore, actomyosin isolated from hog carotid arteries shows an increase in the rate of ATPase activity as the ionic strength of the medium is reduced (17). Thus osmotic influx of water may lead to contraction by decreasing total intracellular ionic strength. In addition, reduced sodium concentration might lead to greater calcium binding to the sarcolemma and therefore greater calcium influx with each depolarization. Each of these effects may contribute to the active vasoconstriction.

Clearly, acute local hypoosmolality produces a significant increase in myocardial contractile force. However, the detailed mechanism(s) involved is not well defined. It is generally accepted that an increase in contractile force is produced by an increase in the free $[Ca^{2+}]$ within the myocardial cells. Perhaps the initial increase in contractile force was produced by the effect of the change in the extracellular $[Na^+]$; the decreased $[Na^+]_e$ increases the $[Ca^{2+}]/[Na^+]^2$ ratio and thus increases Ca^{2+} binding to the cell surface, permitting more calcium to enter the myocardial cell during the action potential (18–20). The gradual waning of contractile force during constant pressure perfusion to the coronary artery may be due to the effects of Na^+ loss from the myocardial cells. The fact that coronary sinus oxygen tension fell and then remained constant suggests that the gradual waning of contractile force is not due to increasing hypoxia.

The gracilis muscle responded uniformly to a decrease in plasma $[Na^+]$ and $[Cl^-]$ with an increase in vascular resistance even though plasma osmolality, $[K^+]$, $[Ca^{2+}]$, hematocrit, and pH did not change. From Fig. 2, it is seen that a 20% reduction in osmolality increases resistance by 40%, whereas, as seen in Fig. 3, a 20% reduction in $[Na^+]$ and $[Cl^-]$ increased resistance by only 10%. The effect of the Na ion is complicated by the fact that resistance did not always return to control when the normal $[Na^+]$ was reinstituted. In any event, these changes in resistance are small and show that in the osmolality experiments, the changes in gracilis vascular resistance were produced primarily by the changes in osmolality and not by the change in $[Na^+]$ and $[Cl^-]$.

Regardless of the mechanisms of the increases in resistance and contractile force, it is clear that they increase substantially in response to acute changes in osmolality which can occur naturally. It is therefore possible that direct effects of osmolality on vascular and cardiac muscle participate in the mechanisms which compensate blood pressure in hypoosmolal states, such as acute salt depletion.

Summary. Hemodialysis was used to study the direct effects of acute hypoosmolality upon the resistance to blood flow through the gracilis and coronary vascular beds and upon

left ventricular contractile force in the dog. When sodium chloride was removed from the blood perfusing the gracilis vascular bed, resistance increased linearly over the range 300–225 mOsm/kg. A 10% decrease in osmolality produces a 20% increase in resistance. When the removed sodium chloride was replaced with mannitol such that plasma osmolality did not change, resistance rose slightly but failed to regularly return to the control value. Hypoosmolality also raised coronary vascular resistance and this was associated with an increase in left ventricular force. Thus direct effects of hypoosmolality on peripheral vascular beds and perhaps myocardium may participate in the compensatory mechanisms that tend to stabilize blood pressure during hypoosmotic states such as acute salt depletion.

1. Elkinton, J. R., Danowski, T. S., and Winkler, A. W., J. Clin. Invest. **25,** 120 (1946).
2. Haddy, F. J., and Scott, J. B., Proc. Soc. Exp. Biol. Med. **136,** 551 (1971).
3. Overbeck, H. W., Molnar, J. I., and Haddy, F. J., Amer. J. Cardiol. **8,** 533 (1961).
4. Overbeck, H. W., Daugherty, R. M., and Haddy, F. J., Ann. Rev. Physiol. **9,** 258 (1966).
5. Stainsby, W. N., and Fregly, M. J., Proc. Soc. Exp. Biol. Med. **128,** 284 (1968).
6. Gazitúa, S., Scott, J. B., Chou, C. C., and Haddy, F. J., Amer. J. Physiol. **217,** 1216 (1969).
7. Gazitúa, S., Scott, J. B., Swindall, B., and Haddy, F. J., Amer. J. Physiol. **220,** 384 (1971).
8. Overbeck, H. W., Amer. J. Physiol. **233,** 1358 (1972).
9. Scott, J. B., Brace, R. A., Anderson, D. K., and Haddy, F. J., Physiologist **14,** 226 (1971).
10. Anderson, D. K., Roth, S. A., Brace, R. A., Radawski, D., Haddy, F. J., and Scott, J. B., Circ. Res. **31,** 165 (1972).
11. Nagle, F. J., Scott, J. B., Swindall, B. T., and Haddy, F. J., Amer. J. Physiol. **214,** 885 (1968).
12. Brading, A. F., and Setekleiv, J., J. Physiol. **195,** 107 (1968).
13. Johansson, B., and Jonsson, O., Acta Physiol. Scand. **72,** 456 (1968).
14. Thomas, R. C., Physiol. Rev. **52,** 563 (1972).
15. Whittam, R., and Ager, M. E., Biochem. J. **97,** 214 (1965).
16. Brace, R. A., and Anderson, D. K., J. Appl. Physiol. **35,** 90 (1973).
17. Maxwell, L. C., Bohr, D. F., and Murphy, R. A., Amer. J. Physiol. **220,** 1871 (1971).
18. Chapman, J. P., Gibbs, C. L., and Gibson, W. R., Circ. Res. **27,** 601 (1970).
19. Luttgau, H. C., and Niedergerke, R., J. Physiol. **143,** 486 (1958).
20. Niedergerke, R., J. Physiol. **167,** 515 (1963).

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