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Vascular Responses to Plasma Hypoosmolality in Man[1] (39030)

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Acute local increases in plasma osmolality decrease resistance in the limb of man (1, 2). Such responses may play a role in the hemodynamics of hyperosmolar states and in the mechanisms of exercise hyperemia (3, 4). The vascular effects of reductions in plasma osmolality, in contrast, have not been studied in man, although clinical states accompanied by plasma hypoosmolality, such as acute salt depletion or inappropriate secretion of antidiuretic hormone, are not rare. In animals several investigators have observed increased resistance in response to acute local reductions in plasma osmolality (5–11).

In the present study we measured the local vascular effects of acute plasma hypoosmolality in limbs of men. Our results indicate that in men, as in animals, this stimulus increases resistance. Because we have been interested in studying vascular responses to a number of agents in hypertensive patients, we additionally compared responses in normotensive patients with those in patients with essential hypertension.

Materials and methods. We studied limb vascular responses in 27 male inpatients (Table I) at the Veterans Administration Hospital, Saginaw, Michigan. Fourteen (12 Caucasian, 2 Black) had normotension documented by several normal casual blood pressure measurements during hospitalization. These convalescing patients had been hospitalized for mild chronic diseases not felt to affect vascular responses. No patient was febrile at time of study. Other than one patient on dilantin, no normotensive patients were receiving drugs that might affect vasoactivity.

The other 13 (7 Caucasian, 6 Black) patients had essential hypertension of mild to moderate severity documented by thorough study, including hospital diastolic blood pressures averaging above 90 mmHg during hospital days 4–6, normal rapid sequence intravenous pyelograms, normal 24 hr urine vanil mandelic acid (VMA) excretion and normal serum sodium and potassium concentrations. No hypertensives had retinal hemorrhages, exudates, or papilledema, elevated serum creatinine or blood urea nitrogen concentrations, or significant proteinuria. All antihypertensive drugs and diets were discontinued at least 4 weeks before the response study. Two hypertensive patients were receiving digitalis, but no patients had clinically discernible cardiac failure. No other hypertensive patients were receiving drugs felt to affect vasoactivity.

All subjects participating were fully informed by the investigators of the purposes, procedures, and hazards of the experiment; written consent was obtained. Procedures used were in accordance with institutional policies.

These volunteers were studied in the resting, postabsorptive state and supine position with laboratory temperature ranging from 26 to 27°. Prior to study, the volume of the arm distal to the level of the intercondylar line at the elbow was measured by water displacement; blood flows and resistances were accordingly calculated on a per volume basis. The procedures employed for infusions and measurement of limb intravascular pressures and calculation of blood flows by indicator-dilution have been described in detail (1, 12). Briefly, we infused 37° solutions at 8.2 ml/min, into the brachial arteries of these subjects. To improve mixing we used a jet-injection system, which has been described (12). Each infusion lasted about 16 min. All solutions contained indi-

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TABLE I. SUBJECTS.[a]

	Normotensives	Hypertensives
N	14	13
Age (yr)	43.1 ± 1.9	50.5 ± 2.5
Body weight (kg)	71.5 ± 2.2	84.2 ± 4.3
Limb volume (cc)	1534 ± 61	1750 ± 74
Hct (vol%)	45.0 ± 0.9	46.5 ± 0.8

[a] Means ± SEM.

cator (^{131}I-labeled human serum albumin in isotonic sodium chloride solution. Albumotope, Squibb, New Brunswick, N.J., or IHSA I 131, Mallinckrodt Chemical Works, St. Louis, Mo.), which, in all cases, was infused intraarterially at a rate of 0.5 μCi/min.

Two pairs of solutions were infused intrabrachial-arterially into each subject, each pair including first a control solution of isosmolar (290 mOsm/kg) NaCl solution, then the experimental solution. The first experimental solution contained isosmolar $MgSO_4$ to increase limb arterial plasma magnesium concentrations by approximately 3 mEq/l. The results have been reported (12); we found similar vasodilator responses to magnesium in hypertensives and normotensives. Following the magnesium infusion, we allowed a waiting period of 30 min. At the end of this waiting period, limb resistance had returned to or towards previous baseline levels. We then infused the second pair of solutions. The second experimental solution was hypoosmolar (145 mOsm/kg) NaCl. Infused at 8.2 ml/min into a limb blood flow of 100 ml/min it may be calculated that this infusion would decrease limb arterial plasma osmolality by about 20 mOsm/kg.

During infusions, we sampled ipsilateral cephalic venous, basilic venous, and contralateral brachial arterial blood simultaneously at the tenth and fifteenth minute of each infusion for determination of ^{131}I concentrations. We calculated limb blood flow from these isotope concentrations. We also recorded pressures in the ipsilateral cephalic and basilic veins and contralateral brachial artery, in turn, immediately after we sampled blood for ^{131}I concentrations. The equations for calculating limb blood flow and vascular resistance have been presented (1, 12). All experiments reported were considered technically satisfactory, because they met our defined criteria (1) for adequate mixing of infusate with limb blood, lack of hemolysis, restoration of steady state blood flow between vasoactive infusions and subject comfort and cooperation.

Hematocrit, serum sodium and potassium concentrations and osmolality of the cephalic venous and contralateral arterial samples taken at the tenth minute of the infusion in some patients were measured as previously described (1). Serum calcium and magnesium concentrations were measured on a Perkin-Elmer Atomic Absorption Spectrometer (Model 290).

For data analysis we used the paired Student's *t* test (13) to compare responses to control isosmolar NaCl infusions with those to the paired infusions of hypoosmolar NaCl, and to compare osmolality, hematocrit and serum electrolyte concentrations during saline and hypoosmolar infusions. The unpaired *t* test was used to compare pressures, flows, resistances, responses and concentrations in normotensive patients with values in hypertensive patients. We also calculated linear correlation coefficients to determine if there were significant relationships between limb initial resistance and magnitude of response to plasma hypoosmolality.

Results. We completed studies in 14 normotensive and 13 hypertensive patients. Clinical data are presented in Table I. As compared to normotensives, the hypertensive patients were somewhat older and heavier with slightly greater limb volumes. Plasma osmolality, serum sodium, potassium, calcium, and magnesium concentrations, and blood hematocrits in hypertensives were not significantly different from values in normotensives.

In these subjects the intrabrachial arterial infusions altered the hematocrit, osmolality, and electrolyte composition of ipsilateral limb blood without significantly changing these variables in systemic arterial blood. Measured changes induced in limb venous composition are indicated in Table II. The 8.2 ml/min control saline infusions reduced

TABLE II. LIMB ARTERIAL AND VENOUS CONCENTRATIONS DURING INFUSIONS.[a, b]

	Isosmolar NaCl infusion				Hypoosmolar NaCl infusion			
	N	Arterial	Venous	*d*	*N*	Arterial	Venous	*d*
Osmolality, mOsm/kg	11	285.3 ±3.4	285.3 ±3.4	0.0 ±1.0	11	284.5 ±3.3	272.5 ±3.9	−12.0 ±1.7**
[Na^+], mEq/l	11	151.5 ±2.0	151.6 ±1.7	+0.2 ±2.3	11	151.6 ±1.7	144.5 ±2.0	−7.2 ±2.0**
[K^+], mEq/l	11	4.1 ±0.1	3.5 ±0.2	−0.6 ±0.2**	11	4.1 ±0.1	3.7 ±0.1	−0.4 ±0.2*
[Ca^{2+}], mEq/l	10	4.1 ±0.2	3.4 ±0.2	−0.7 ±0.2**	10	4.1 ±0.2	3.5 ±0.2	−0.6 ±0.1**
[Mg^{2+}], mEq/l	10	2.14 ±0.12	2.00 ±0.12	−0.14 ±0.15	10	2.12 ±0.16	1.89 ±0.12	−0.23 ±0.05**
Hct, vol %	8	44.1 ±0.8	38.0 ±1.9	−6.1 ±1.8*	8	43.9 ±0.8	39.1 ±1.0	−4.8 ±0.9**

[a] Means ± SEM. Samples obtained from normotensive and hypertensive patients.
[b] $*P < 0.05$; $**P < 0.01$.

TABLE III. LIMB HEMODYNAMIC RESPONSES TO INTRAARTERIAL INFUSIONS.[a, b, c]

Infusion	Limb blood flow (ml/min)	Limb blood flow (ml/min/ 100 cc)	Change in limb blood flow (ml/min/ 100 cc)	$\bar{P}_A$ (mmHg)	$\bar{P}_V$ (mmHg)	Vascular resistance (mm/Hg/ ml/min/ 100cc)	Change in resistance (mm/Hg/ ml/min/ 100 cc)
Normotensives (*N* = 14)							
Isosmolar NaCl	75.4 ±10.3	4.80 ±0.54		98.6 ±2.6	9.8 ±0.5	21.60 ±2.32	
Hypoosmolar NaCl	53.6 ±5.5	3.44 ±0.26	−1.39 ±0.29‡	98.9 ±2.8	10.6 ±0.6	27.54 ±2.07	+5.94 ±1.76‡
Hypertensives (*N* = 13)							
Isosmolar NaCl	110.8 ±17.5	6.19 ±0.87		129.7 ±3.7**	11.7 ±0.7*	27.44 ±5.62	
Hypoosmolar NaCl	86.0 ±15.0	4.80 ±0.74	−1.36 ±0.31‡	130.4 ±4.0**	12.3 ±1.0	34.50 ±6.22	+7.06 ±2.46†

[a] Means ± SEM. $\bar{P}_A$ = mean brachial arterial pressure. $\bar{P}_V$ = mean basilic or cephalic venous pressure.
[b] †$P < 0.05$; ‡$P < 0.01$, significance values for comparison of variables during control and hypoosmolar infusions (paired Student's *t* test).
[c] * $P < 0.05$. ** $P < 0.01$. significance values for comparison of variables in hypertensives with those in normotensives.

limb venous hematocrit, and serum concentrations of potassium and calcium, without significantly changing limb venous concentrations of magnesium, sodium, or osmolality. As compared to venous serum concentrations during these control saline infusions, the hypoosmolar solutions decreased limb venous plasma osmolality on the average by 12 mOsm/kg, and limb venous serum sodium concentrations on the average by 7 mEq/l. In contrast there were no significant differences between limb venous serum concentrations of potassium, calcium, magnesium, and blood hematocrit during the hypoosmolar infusions and values during the paired control saline infusions. Concentrations in hypertensives did not differ significantly from those in normotensives.

In many subjects during the hypoosmolar infusions we noted blanching of forearm

and hand skin and, during the following isosmolar infusion we noted transient hyperemia. During the hypoosmolar infusions some subjects noted mild but not not uncomfortable sensations in the ipsilateral hand. They described these sensations as "warmth", "tingling", "pressure", or "numbness". Skeletal muscle contractions or limb edema were not noted in any subjects.

As Table III indicates, there were highly significant differences between the mean arterial pressures of hypertensives and normotensives, directly measured at the time of the response study. The infusions did not change systemic arterial or venous blood pressures. We found no significant differences in resting resistances or blood flows between normotensive and hypertensive patients, although there was a trend towards higher resistance and blood flows in the hypertensives (in an enlarged study that included some of these same patients we found significantly elevated limb resistances in the hypertensives, but there were still no significant differences in blood flow (14)). The hypoosmolar infusions evoked decreases in limb blood flow ($P < 0.01$) and increases in limb vascular resistance ($P < 0.01$) in normotensives. Similar changes ($P < 0.001$ and $P < 0.02$, respectively) occurred in hypertensives. We detected no significant differences in resistance or flow responses between normotensives and hypertensives ($P > 0.5$).

There is evidence from experiments in dogs (10) that the magnitude of vascular response to plasma hypoosmolality is a positive function of the level of limb "baseline" or "initial" resistance; such a relationship might obscure a real difference between responses in normotensives and hypertensives. Therefore, we calculated linear correlation coefficients for resistance during control infusion vs. change in resistance in response to hypoosmolar infusions, but found no significant correlation in either group.

Discussion. Our present experiments in man corroborate and extend previous findings by us and by other investigators in animals (5–11); in man, as in animals, decreases in limb plasma osmolality within ranges seen in life elevate limb vascular resistance. In these men a slight (4%) decrease in plasma osmolality caused an impressively large (26–28%) increase in limb resistance. Thus our present experiments in conscious men support the suggestion (11) that increases in vascular resistance may play a role in the hemodynamics of certain clinical states accompanied by decreased plasma osmolality. Such states include acute salt depletion or inappropriate secretion of antidiuretic hormone.

As in our previous experiments in animals, the vascular responses we observed in man were local effects of hypoosmolality within the limb, because the intraarterially administered infusions did not change systemic plasma osmolality, electrolyte concentrations, or blood pressure. The results of our experiments might be attributable in part to other local effects of the dilutional techniques we used. In this regard, however, the limb levels of potassium, calcium, or magnesium were not changed by the hypoosmolar infusions, if compared to the effects of our control infusions. Thus we cannot attribute the observed increases in resistance in our experiments to the effects of these vasoactive ions. It is of note here that Brace *et al.* (11) decreased local plasma osmolality by a nondilutional technique (hemodialysis) and observed increased resistance in vascular beds of the dog. These same investigators further concluded that only a small portion of the changes in resistance evoked by hypoosmolality could be attributed to associated changes in sodium and chloride. Our previous observations in dogs (5) would also argue against an important role for sodium and chloride in the response to plasma hypoosmolality.

Elevation of limb viscosity due to increases in erythrocyte concentration or size or to decreases in erythrocyte deformability contribute to the increased resistance evoked by plasma hypoosmolality (15). In this regard, there were no significant increases in limb venous blood hematocrit during our hypoosmolar infusions. Contraction of vascular smooth muscle in response to plasma hypoosmolality probably played an impor-

tant role in the responses we observed (8, 16, 17). The several mechanisms proposed to explain the excitatory effects of hypoosmolality on vascular smooth muscle have been recently discussed (8, 9, 11).

Passive mechanisms changing vessel lumen not involving vascular smooth muscle activity are also felt to play a role in vascular responses to changes in plasma osmolality and have also been recently discussed (11). The notion that such passive constriction of vessels may occur in hypertensives has received special attention since Tobian and Binion (18) noted that arterial walls of hypertensive animals and men contain increased amounts of water. Tobian has also found increases in water content in the walls of arterioles in hypertensive animals (19) and has therefore suggested that the increased arteriolar resistance in hypertensives might be attributable to vascular wall "waterlogging".

Considering Tobian's hypothesis, we felt that demonstration of altered vascular responses to changes in plasma osmolality might be further evidence suggesting that abnormal movement of water into vascular walls plays a significant role in the pathogenesis of hypertension. In renal hypertensive dogs (10), however, we found no real evidence to suggest that responses to either hypoosmolality or hyperosmolality were altered. The results of our present experiments in essential hypertensive men are similar in that we found no evidence for abnormal responses in hypertensives to plasma hypoosmolality.

In these same essential hypertensive men, we also found no abnormalities in limb vascular responses to intrabrachial arterial infusions of magnesium (12) and of calcium (20). In contrast, we have observed attenuated responses to intrabrachial arterial infusions of the potassium ion (14). Thus the present experiments add to the evidence suggesting that the attenuated response we observed to potassium in essential hypertensives may be a specific abnormality, perhaps indicating an underlying defect in vascular smooth muscle K^+ metabolism (10, 14).

Summary. To study limb vascular responses to plasma hypoosmolality in man, we infused test solutions of hypoosmolar NaCl (145 mOsm/kg) and control solutions of isosmolar NaCl (290 mOsm/kg) into the brachial arteries of 14 normotensive and 13 essential hypertensive patients. Limb blood pressures were monitored, limb blood flow was measured by indicator-dilution, and limb vascular resistance was calculated as mmHg/ml flow/min/100 cm^3 limb volume. The infusions did not significantly change systemic plasma osmolality, sodium concentration, or blood pressure. Compared to control infusions, the hypoosmolar infusions decreased limb venous plasma osmolality and serum sodium concentrations by an average of 12 mOsm/kg and 7 mEq/l, respectively. Compared to control infusions, limb venous serum concentrations of potassium, calcium, magnesium, or blood hematocrit were not altered by the hypoosmolar infusions. In response to the hypoosmolar infusions, limb resistance increased by 28% in normotensives and by 26% in hypertensives. We conclude that the acute local vascular response to a small reduction in plasma osmolality in the limb of man is a large increase in vascular resistance. We found no evidence for abnormal responses to plasma hypoosmolality in essential hypertensives.

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1. Overbeck, H. W., and Grega, G. J., Circ. Res. **26,** 717 (1970).
2. Lundvall, J., Mellander, S., and White, T., Acta Physiol. Scand. **77,** 224 (1969).
3. Mellander, S., Johansson, B., Gray, S., Jonsson, O., Lundvall, J., and Ljung, B., Angiologica **4,** 310 (1967).
4. Scott, J. B., and Radawski, D., Circ. Res. Suppl. I **28** and **29,** I–26 (1971).
5. Overbeck, H. W., Molnar, J. I., and Haddy, F. J., Amer. J. Cardiol. **8,** 533 (1961).
6. Haddy, F. J., and Scott, J. B., *in* "Electrolytes and Cardiovascular Diseases" (E. Bajusz and S. Karger, eds.), p. 383. Basel, New York (1965).

7. Stainsby, W. N., and Fregly, M. J., Proc. Soc. Exp. Biol. Med. **128,** 284 (1968).
8. Gazitúa, S., Scott, J. B., Chou, C. C., and Haddy, F. J., Amer. J. Physiol. **217,** 1216 (1969).
9. Gazitúa, S., Scott, J. B., Swindall, B., and Haddy, F. J., Amer. J. Physiol. **220,** 384 (1971).
10. Overbeck, H. W., Amer. J. Physiol. **223,** 1358 (1972).
11. Brace, R. A., Scott, J. B., Chen, W. T., Anderson, D. K., and Haddy, F. J., Proc. Soc. Exp. Biol. Med. **148,** 578 (1975).
12. Overbeck, H. W., Daugherty, R. M., Jr., Haddy, F. J., J. Clin. Invest. **48,** 1944 (1969).
13. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," Chap. 5, 10, McGraw-Hill, New York (1960).
14. Overbeck, H. W., Derifield, R. S., Pamnani, M. B., Sözen, T., J. Clin. Invest. **53,** 678 (1974).
15. Braasch, D., Physiol. Rev. **51,** 679 (1971).
16. Johansson, B., and Jonsson, O., Acta Physiol. Scand. **72,** 456 (1968).
17. Jonsson, O., Acta Physiol. Scand. **77,** 191 (1969).
18. Tobian, L., and Binion, J. T., Circulation **5,** 754 (1952).
19. Tobian, L., Olson, R., and Chesley, G., Amer. J. Physiol. **216,** 22 (1969).
20. Overbeck, H. W., Pamnani, M. B., and R. S. Derifield., Proc. Soc. Exp. Biol. Med. **149,** 519 (1975).

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