Sodium-Induced Elevation of Blood Pressure in the Anephric State

Abstract. *Normotensive anephric rats infused with 2 milliliters of a hyperosmolar solution of either sodium chloride or mannitol showed an increase in arterial pressure that was very pronounced with the sodium chloride and that could be partly abolished by administration of an antagonist to the vasopressor action of antidiuretic hormone (ADH). Rats with congenital ADH deficiency subjected to the same treatment showed smaller increments in arterial pressure that remained unchanged after administration of the ADH antagonist. Expansion of intravascular fluid volume was similar in all four groups and bore no correlation to the change in arterial pressure. It is concluded that about half of the increase in blood pressure induced by saline was attributable to the vasopressor effect of stimulated ADH and the remainder to an additional sodium-related factor, since it was more pronounced in the saline-infused than in the mannitol-infused groups. Expansion of the intravascular volume per se could only account for a minimal part of the increment in pressure.*

Elevation of blood pressure has been attributed either to vasoconstriction induced primarily by substances such as angiotensin and catecholamines or to intravascular volume expansion (vasoconstriction-volume theory) (*1*) resulting from sodium and fluid overload. Hypertension in kidney-deprived animals is mostly attributed to the latter mechanism. A number of investigators, however, have challenged the concept of volume expansion as the prevailing mechanism in "volume-dependent" hypertension accompanied by low plasma renin activity. They have observed that in patients with suppressed renin activity blood volume is not necessarily increased (*2*); a sustained response in blood pressure after the administration of diuretics is not associated with intravascular volume contraction (*3*); and peripheral vascular resistance increases rather than decreases (*4*). These data suggest that arterioles are constricted rather than dilated as one would expect to find in a state of fluid volume overload. Such findings led to speculation that some humoral factors may be involved in salt-induced hypertension (*5*, *6*).

The possibility that vasopressin may play a role in blood pressure regulation has also been raised (*7*). Recently, a number of vasopressin analogs were developed that specifically antagonize the vasopressor action of antidiuretic hormone (ADH) without affecting its renal tubular action (*8*). These peptides can be used to investigate the possible contribution of vasopressin-induced vasoconstriction in various forms of hypertension. Indeed, in rats with mineralocorticoid-induced hypertension, which is the classic low-renin experimental model (*9*), it was shown (*10*) that vasopressin has a regulatory role. The following experiments were designed to investigate the relation between sodium loading, volume expansion, and vasopressin stimulation in kidney-deprived rats with hypertension.

Male Wistar rats (Charles River Breeding Laboratories) and rats with congenital antidiuretic hormone (ADH) deficiency (Brattleboro) were uninephrectomized under ether anesthesia and given free access to their usual diet of Purina rat chow and tap water for 2 weeks. At the time of the study the Wistar rats weighed 280 to 310 g and the ADH-deficient animals, 240 to 260 g. On the day of the study, under ether anesthesia the remaining kidney was removed. The right femoral vein was cannulated with a PE-10 catheter and the right external iliac artery with a PE-50 catheter. Arterial pressure was monitored with a Statham transducer and recorded on a Hewlett-Packard recorder (model 7702B). Upon awakening, the animals were maintained in a semirestrained position on a light mesh screen for 60 to 90 minutes until blood pressure rose to a steady baseline.

Solutions containing either 0.68 milliequivalents of NaCl per milliliter or 25 percent mannitol (1370 mosmole/liter) were used in the experiments. They were infused by a Harvard pump for 2 hours at a rate of 0.018 ml/min for a total of 2 ml. The peptide [1-deaminopenicillamine-2-(*O*-methyl)tyrosine]arginine-vasopressin, which is a competitive inhibitor of arginine-vasopressin at the vascular receptor level (*8*), was used as an ADH antagonist. A 2-mg amount of this compound was dissolved in a solution made from 10 ml of 0.9 percent NaCl, 10 mg of bovine serum albumin, and 3 μl of acetic acid, brought to a *p*H of 6.4 with NaOH. A dose of 0.15 ml of this solution containing 30 μg of the ADH antagonist was injected into the animals intravenously at the end of the 2-hour period.

Samples of 0.03 ml of blood were drawn before and after infusion of either solution for microhematocrit determination by centrifugation of blood in heparinized capillary tubes. Plasma volume was measured by the dilution principle with human serum albumin labeled with radioactive iodine (*11*). Changes in plasma volume were calculated from the difference in hematocrit before and after infusion.

Group A (N = 14) Wistar rats received the NaCl solution. At the end of the infusion, seven rats received an intravenous injection of ADH antagonist and seven rats had measurement of plasma volume. Group B (N = 15) Wistar rats received an infusion of mannitol for 2 hours, at the end of which nine animals received an intravenous injection of ADH antagonist and six had plasma volume determination. Group C (N = 7) and group D (N = 7) ADH-deficient rats received the saline and mannitol infusion, respectively, for 2 hours. At that time, plasma volume was determined and the ADH antagonist was injected.

Data are reported as means ± standard error. Student's t-test was used for paired and nonpaired data as appropriate and differences were considered significant if $P < .05$. Arterial pressures reported are mean blood pressures, as recorded directly during the experiments.

From Table 1 it is evident that infusion of either saline or mannitol significantly

Table 1. Changes in blood pressure and plasma volume induced by infusion of either hypertonic saline (groups A and C) or mannitol (groups B and D) in normotensive anephric rats.

Group	Baseline blood pressure (mm-Hg)	Blood pressure (mm-Hg)		Change in plasma volume after infusion (ml)
		After hypertonic solution infusion	After administration of ADH antagonist	
		Normal anephric rats		
A	109.71 ± 3.91	137.14 ± 2.72*	124.71 ± 3.42*‡	2.04 ± 0.27§
B	111.88 ± 2.75	128.66 ± 3.62*	121.66 ± 3.42†‡	1.95 ± 0.19§
		Anephric rats with congenital ADH deficiency		
C	104.14 ± 1.12	118.85 ± 2.12*	118.85 ± 2.12*	2.00 ± 0.13§
D	105.66 ± 2.3	110.8 ± 2.9	110.8 ± 2.9	2.05 ± 0.1§

*$P < .001$, compared to baseline blood pressure. †$P < .01$, compared to baseline blood pressure. ‡Significantly lower than pressure after infusion ($P < .01$). §Significantly higher than volume before infusion ($P < .001$).

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increased blood pressure in normal anephric rats but that the saline-induced increase was far more pronounced. Increments were 27.4 ± 2.46 mm-Hg as opposed to 16.8 ± 2.60 mm-Hg in the saline and mannitol groups, respectively ($P < .01$). A significant but lesser blood pressure increase was induced by infusion of saline hypertonic solution in the ADH-deficient rats, whereas the mannitol-induced increase in this model was not significant. Average increments in these two groups were 14.7 ± 1.50 mm-Hg and 5.16 ± 1.19 mm-Hg by saline and mannitol, respectively ($P < .01$). In all four groups the plasma volume expanded by approximately 21 percent. Administration of ADH-antagonist reduced blood pressure considerably in saline-infused normal rats and less but still significantly in mannitol-infused normal rats (by 12.4 ± 1.58 mm-Hg as opposed to 7.0 ± 0.83 mm-Hg, respectively, $P < .01$) but produced no change in ADH-deficient groups. It is noteworthy that a significant residual increase in arterial pressure remained after inhibition of ADH in groups A and B (15.0 ± 1.88 mm-Hg and 9.8 ± 2.23 mm-Hg, respectively, $P < .05$); this increase was of the same order as the increase in pressure caused by hypertonic infusion in ADH-deficient rats. In other words, after the blood pressure increment attributable to the vasopressor action of ADH had been abolished, there remained a residual increment in the saline-infused groups A and C and a similar but lesser increment in the mannitol-infused groups B and D. This increment could not be attributed to ADH.

There was no statistical correlation between the increment in plasma volume and the magnitude of blood pressure elevation.

It is well established that sodium overload induces elevation of blood pressure (*12*) and an increased sodium content has been found in the arteries of hypertensive subjects (*13*). However, some of the mechanisms incriminated for the hypertensive effect of sodium (for example, volume expansion, or increased vascular wall sensitivity to the pressor action of vasoconstricting substances such as angiotensin and norepinephrine) are mostly speculative. We chose the normotensive anephric rat as a model for this study because it lacks renal renin and cannot eliminate the load of fluid and sodium administered during this experiment. The amount of sodium infused was approximately equal to the average daily intake of a rat on a regular diet.

The prevailing theory regarding the regulation of ADH is that this hormone is stimulated through an osmoreceptor situated outside of the blood-brain barrier (*14*) and is therefore equally responsive to hyperosmolar stimuli in the form of either saline or mannitol solutions. However, the existence of a specific sodium-sensitive receptor has also been postulated (*15*). Nonosmotic stimuli of ADH have also been described, including angiotensin II and the automatic nervous system (*15*).

In our experiments infusion of 2 ml of a hyperosmolar solution of saline or mannitol led to intravascular volume expansion (by approximately 21 percent) and caused increments in blood pressure ranging from 5.2 to 27 mm-Hg. However, the expansion of the intravascular fluid volume per se could not account for the changes in blood pressure. In fact the increased blood pressure in these experiments appeared to be mostly the result of vasoconstriction due to stimulation of ADH. All four groups exhibited a similar fluid volume expansion, and the extensive network of small capacitance vessels could probably accommodate this increase in blood volume without noticeable change of arterial pressure. The lack of correlation between changes in blood volume and pressure corroborates the hypothesis that volume itself was not the determining factor in the pressure changes.

We conclude that the saline-induced increase in blood pressure was due mainly to vasoconstriction caused by the vasopressor action of ADH. The sodium ion per se appears to be a potent stimulator of ADH over and above its osmolar action, since hyperosmolality produced by mannitol had a less pronounced ADH-stimulatory action. The residual blood pressure elevation that was consistently observed after abolition of ADH and was more pronounced in the saline and less in the mannitol groups suggests that an additional sodium-sensitive factor exists, which cannot be defined from the present data. Fluid volume expansion might account for a minimal part of the observed increase in blood pressure.

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Male Contraception: Synergism of Gonadotropin-Releasing Hormone Analog and Testosterone in Suppressing Gonadotropin

Abstract. *Long-term administration of either superactive analog's of gonadotropin-releasing hormone or of testosterone suppresses gonadotropin secretion in male animals and humans. Testosterone administered in combination with gonadotropin-releasing hormone analog further suppresses serum gonadotropin levels in male rats. This observation indicates synergistic activity and suggests that the gonadotropin-releasing hormone analog and testosterone act at independent sites within the hypothalamic-pituitary axis. The primary actions of superactive analog are probably mediated by changes at a postreceptor site in the pituitary gonadotropin-secreting cells.*

Gonadal steroids, such as testosterone, and superactive synthetic analogs of gonadotropin-releasing hormone (GnRH) have been used in studies of reversible contraception in the human male. There is much information on the mechanism of action of testosterone, but little is known about the mechanism of action of GnRH analogs and their possible interactions with testosterone. Superactive GnRH analogs administered daily to male animals (*1*, *2*) or to human male subjects (*3*, *4*) led to initial stimulation and subsequent inhibition of gonadotropin secretion. When the superactive GnRH analog [D-Ala^6]des-Gly^{10}-GnRH

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