Renal hemodynamics and antidiuretic hormone release associated with volume regulation

BARATZ, ROBERT A. AND RAYMOND C. INGRAHAM. Renal hemodynamics and antidiuretic hormone release associated with volume regulation. Am. J. Physiol. 198(3): 565-570. 1960.—Following the general reduction of extracellular fluid volume by hemorrhage, or the selective reduction of this volume in the thorax by positive pressure breathing, the volume regulating mechanism of the dog is implicated as part of the over-all compensatory mechanism to such stress situations, and appears to be dominant over any regulation via the osmoreceptors. Both these experimental procedures produce characteristic compensatory cardiovascular reactions, depressed renal function and highly significant increases in the circulating level of antidiuretic hormone. Following the isotonic expansion of the extracellular fluid volume with dextran, or selective expansion of the vascular capacity of the thorax by negative pressure breathing, the volume regulating mechanism appears to be subdominant to the control of body fluid volume via the osmoreceptors. In the case of an increase in extracellular fluid volume, reflex responses directed at volume regulation have no statistically significant action on renal hemodynamics, and any action on the release or inhibition of antidiuretic hormone could not be detected with certainty because of the limiting sensitivity of the bio-assay.

There exist at present two preponderant views whereby the homeostatic control of extracellular fluid volume is maintained. The first recognizes the existence of osmoreceptors which control the release of an antidiuretic hormone from the posterior pituitary gland. Alterations in the tonicity of the extracellular fluid elicit, or inhibit, the release of antidiuretic hormone (ADH) via these osmoreceptors, and thus by regulating water output serves to maintain tonicity at a constant level (1). The maintenance of a constant extracellular fluid volume has appeared to be of secondary importance in the over-all control of fluid balance as effected by the above mechanism. There has recently been demonstrated the existence of stretch receptors in the thoracic region which respond to changes in volume of the extracellular fluid, independent of changes in tonicity (2-4). There are two possible mechanisms whereby this volume control can be explained: 1) a neural or hormonal control of antidiuretic hormone release, or 2) a reflex effect on renal hemodynamics.

It is the purpose of these experiments to investigate simultaneously the changes occurring in antidiuretic hormone activity and renal hemodynamics under various conditions which activate the latter mechanism. It is necessary that the experimental conditions chosen be such that little, if any stimulation of the osmoreceptors occurs. In this way it can be determined if this type of direct volume regulation is effected by control of antidiuretic hormone release or by some direct action on renal hemodynamics.

METHODS

Female dogs (8.8-17.5 kg) served as the experimental animal, and water in all cases was withheld for a minimum of 3 days prior to being used for an experiment. On the experimental day all food was withheld, but, depending on the experiment, water was either allowed up to the time of the experiment or withheld for 18 hours prior to the experiment. The surface area in square meters was obtained for each animal from a nomogram.

The experiments were divided into four series:

a) Hemorrhage and subsequent reinfusion. Dogs were hemorrhaged 25% of their estimated blood volume (go cc/kg) to produce an isotonic decrease in the volume of total extracellular fluid. The subsequent reinfusion of all heparinized hemorrhaged blood was used to follow the course of recovery.

b) Plethora. Six percent dextran in isotonic saline was administered to produce an isotonic expansion of the extracellular fluid. The amount administered was 25% of the dog's estimated blood volume.

c) Positive pressure breathing. Dogs were subjected to positive pressure breathing of 11.2 mm Hg or 18.6 mm Hg using a pressure demand regulator (type A-16,
Bendix Aviation Corp.) attached to the dog's trachea, and a laboratory air pressure line. The procedure was designed to produce a selective isotonic decrease in the vascular volume of the thoracic region (5).

d) Negative pressure breathing. Dogs were subjected to average negative pressure breathing of −10 cm H₂O using a 20-liter reservoir container which was constantly at a pressure averaging 10.0 cm H₂O below atmospheric. This degree of negative pressure was maintained using a commercial tank vacuum cleaner which removed air from the reservoir container at the rate of 1500 l/min. The degree of negative pressure was controlled by an adjustable leak in the reservoir. As an added precaution, soda lime was placed in the bottom of the reservoir to absorb any carbon dioxide. This series of experiments was designed to produce a selective isotonic increase in the vascular volume of the thoracic region (6).

Animals which were to be hydrated were put on the following regimen. From 60 to 90 minutes prior to anesthesia the dog was given 60-70 cc/kg of tepid tap water by stomach tube. Immediately before anesthesia the animal was given an additional 20 cc/kg of water by the same route.

The anesthetic used was either sodium pentobarbital (35 mg/kg, i.v.) or a combination of morphine (15 mg, i.m.) and chloralose (8-10 cc/kg of 1% solution in 0.6% NaCl, i.v.), or chloralose alone. Maintenance doses (5 mg/kg plus enough isotonic sodium chloride to make the injection equal to 1 cc/kg) were given intravenously every 10 minutes. This injection was sufficient to maintain anesthesia as well as a proper degree of hydration. The chloralose was retained in solution in a constant temperature bath at 40°C.

Immediately following induction of anesthesia, the trachea was cannulated. The left external jugular vein was isolated high in the neck and by use of an x-ray catheter and fluoroscopy, the catheter was passed down via the external jugular vein into the superior vena cava to a point approximately one centimeter above the heart. This catheter was used to obtain all blood samples for chemical analyses as well as antidiuretic hormone assays. It was also used for the measurement of central venous pressure, while the right common carotid artery was used for measurement of mean arterial pressure.

To avoid errors in collecting urine and washing the bladder for the clearance studies, the ureters were cannulated with polyethylene tubing (P.E. 160) approximately at their entrance to the bladder and passed a distance of 4-5 cm up toward the renal pelvis. Urine was collected directly from the ureteral cannulas into a graduated cylinder.

Renal clearance studies were performed by standard techniques. The chemical determination of creatine was as described by Hoffman (7) and PAH determinations were by the method of Brun (8). The percentage of glomerular filtrate absorbed was calculated as follows: GFR (glomerular filtration rate) minus urine flow divided by GFR and multiplied by 100. Hematocrits were read in standard Wintrobe tubes and sodium determinations in blood and urine were done by flame photometry (9). Sodium concentrations in urine and serum are expressed as milliequivalents per liter, and the excretion fraction is determined from the expression:

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\text{Excretion Fraction} = \frac{\text{Conc. Na in urine} \times \text{urine flow}}{100 \times \text{conc. Na in plasma} \times \text{GFR}}
\]

Due to individual variation in many of the factors measured, all animals served as their own controls, and experimental procedures were instituted only after suitable control measurements. In the series of plethora, positive pressure breathing and negative pressure breathing, all renal clearance periods were 15 minutes in length with a blood sample being drawn midway in the period for the determination of plasma concentration, hematocrit, serum sodium concentration and ADH activity. In each of these series, three control renal clearance periods were determined consecutively on each dog, and then three consecutive clearance periods either immediately following the infusion of dextran, 10 minutes after the beginning of positive pressure breathing, or 15 minutes following the start of negative pressure breathing.

The series of hemorrhage-reinfusion were slightly different. All clearance periods were again 15 minutes in length except for the first experiment when 10-minute periods were used. Also, two control clearance periods were taken before the hemorrhage, two clearance periods immediately following the hemorrhage and two periods immediately following the reinfusion of all withdrawn blood.

The method used for the assay of the plasma level of antidiuretic hormone in the dog was a modification of
VOLUME REGULATING MECHANISM

were taken too soon following the hemorrhage to reflect the increase in circulating ADH. This bio-assay method is capable of detecting small dose changes of reference vasopressin, or endogenous circulating ADH.

RESULTS AND DISCUSSION

In the four experimental series, all results were subjected to an analysis of variance. In figures 1–5, one asterisk (*) indicates a significant difference to the 5 % level, two asterisks the 1 % level, and three asterisks the 0.1 % level.

A. Hemorrhage and reinfusion. The results of four experiments are summarized in Figure 1. The cardiovascular changes reported for these experiments are for the most part, characteristic responses which have been previously reported. The decrease in mean arterial pressure following hemorrhage and its return to normal levels after reinfusion are expected and consistent findings. The small decrease in heart rate observed following hemorrhage is not of statistical significance. The constant hematocrit values obtained throughout the experiment may be explained by the fact that either the samples were taken too soon following the hemorrhage to reflect any degree of hemodilution, or the hemorrhage was not of sufficient severity to promote any compensatory hemodilution.

The urine flow following hemorrhage was characteristically decreased as were the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). All these results were significant to the 0.1 % level. The decrease ERPF (less than 10 % of control), and the resulting tubular hypoxia may well depress the tubular transport mechanisms (12). Thus the decrease sensitivity of the tubules to ADH may explain the seeming paradox that a greater absorption of the glomerular filtrate occurred during control periods than following hemorrhage.

The circulating level of antidiuretic hormone showed a very large and very significant increase immediately following hemorrhage. The control level of about 100 μEq vasopressin/cc plasma rose to around 2000 μEq vasopressin following hemorrhage. The fact that ADH was not released by activation of the osmoreceptors is aptly shown by the relatively constant tonicity of the extracellular fluid as measured by serum sodium levels. Values for the excretion fraction of sodium were not included for these experiments as the low urine flow following hemorrhage, and the extent of dead space in the ureters and polyethylene catheters made these values invalid.

B. Positive pressure breathing (P.P.B.). The results are summarized as the average of five experiments in figure 2. The selective decrease in the vascular volume of the thoracic region by positive pressure breathing produced many of the cardiovascular changes which had been reported earlier by Fenn et al. (5) and Braunwald et al. (13). Primarily, there occurred a depression of mean arterial pressure and heart rate. The highly significant increase in central venous pressure can be explained by the increased extracardiac pressure caused by P.P.B. The increased hematocrit can possibly be explained by an increased transudation of plasma during the period of positive pressure breathing.

The highly significant decrease in urine flow, effective renal plasma flow and glomerular filtration rate reported in these experiments is in good agreement with the work of Drury, Henry and Goodman (14). It is noted that the degree of P.P.B. influences the degree of renal depression that positive pressure of 18.6 mm Hg usually causes anuria, whereas 11.4 mm Hg produces an oliguria. There is a suggestion that the reabsorption of the glomerular filtrate is greater during the periods of P.P.B. than during control periods. Assuming that the obligatory reabsorption remains constant, the over-all decrease in the reabsorption of the glomerular filtrate during P.P.B., is evidence for the fact that ADH is exerting a greater effect during P.P.B. than during control. Here also, tubular hypoxia due to decreased ERPF may have decreased the sensitivity of the tubules to ADH and thus the resulting increase in the absorption of glomerular filtrate is not as pronounced as might be expected.

The increase in the circulating level of antidiuretic
hormone observed in these experiments is not as pronounced as that produced in the hemorrhage experiments, but nevertheless is statistically significant to the 1% level. Serum sodium remains constant and therefore the increase in ADH is not occasioned by activation of the osmoreceptors. It thus appears that both the depressed renal hemodynamics and the increase in circulating level of antidiuretic hormone are instrumental in the production of the decreased urine flow during positive pressure breathing.

In both these series of experiments where either the entire extracellular fluid was decreased by hemorrhage, or selectively decreased in the thoracic region by positive pressure breathing, central circulatory depression can be considered to be a prime cause in the gross depression of renal function. The important question of how much of this renal depression is a compensatory response to the stress situation and how much can be attributed to a decreased activity of the volume receptor mechanism is impossible to define. The well-known hypothalamic pituitary interrelationships can account for the increased levels of circulating ADH following stress situations. Perhaps the most logical inference would be that the volume receptors themselves are part of the compensatory response to the imposed stress.

C. Plethora. The results are summarized as the average of five experiments in figure 3. Increasing the total extracellular fluid volume and thus distorting the relation between extra- and intracellular volume by infusion of isotonic dextran solution produced a slight increase in arterial blood pressure and the expected decrease in hematocrit due to the infusion of a cell-free solution. Urine flow increases progressively following the infusion of dextran and ERPF also exhibits a significant increase. This may simply be considered as due to the increase in effective circulating fluid volume. Autonomous control of the renal circulation and a relative constriction of the afferent arterioles is responsible for the stability of the GFR following plethora. It is interesting to note that there is a definite suggestion of a smaller percentage absorption of the glomerular filtrate following plethora than during control periods. Again assuming a constant obligatory absorption, the decreased over-all absorption of the glomerular filtrate suggests a decreased activity of antidiuretic hormone.

Concerning the circulating level of antidiuretic hormone, the results following plethora suggest an inhibition of ADH. The change from 37.5 μEq vasopressin/cc plasma to 17.6 μEq is not of statistical significance. There is however indirect evidence, as the smaller percentage absorption of glomerular filtrate following plethora than in control periods, as well as the diuresis without any change in GFR, which implicate a decreased circulating level of ADH even though actual measurements at these low plasma levels may be inconclusive.

In this series of experiments then, where a diuresis occurs upon the isotonic expansion of the extracellular fluid volume, it appears that both a small increase in glomerular filtration rate and a slight inhibition of antidiuretic hormone are instrumental in precipitating the diuresis.
An interesting result noted in the assay of ADH in the plethora series is that after infusion of dog plasma containing dextran, the rats exhibited a general anaphylactic reaction. It appeared 40–60 minutes following the injection and resulted in edematous jowls and paws and ultimately a complete anuria. This reaction of the injection and resulted in edematous jowls and paws and ultimately a complete anuria. This reaction of the injection and resulted in edematous jowls and paws and ultimately a complete anuria. This reaction of

The question continually arose throughout the course of these experiments if the apparatus used for the production of negative pressure breathing was actually transmitting the negative pressure to the dog. For this reason a side-arm on the tracheal cannula was connected to a water manometer and it was seen that the pressure at the trachea was the same as in the reservoir bottle. A cannula was placed between the ribs into the thorax and here also demonstrated that the negative pressure was transmitted.

There was an increase of 25% or more in urine flow due to N.P.B. in 6 of the 31 experiments (19.4%). In 4 of the 31 experiments (12.9%) there was a decrease in urine flow of 25% or more, and in the remaining 21 experiments (67.8%) the change in urine flow amounted to less than 25%. The anesthetic, degree of hydration or position of the animal had no consistent effect on the diuresis or antidiuresis due to N.P.B.

The arbitrary division of the results of the negative pressure breathing experiments into those producing a diuresis of 25% or more over control flows and those producing an antidiuresis of 25% or more was necessitated by the irregular response to N.P.B. in all the above experimental groups. Even with this division, the only statistically significant change in either the experiments showing a diuresis or antidiuresis was a decrease in central venous pressure, which in itself is an indication that the negative pressure was being transmitted.

A careful study of the six negative pressure breathing experiments in which a diuresis was obtained (fig. 4), shows there is a consistent, but small, increase in GFR. Contrariwise, the GFR and ERPF for the four negative pressure breathing experiments in which an antidiuresis was obtained (fig. 5), generally show no change or a decrease in each individual experiment. In this group of N.P.B. experiments, there was greater percentage absorption of the glomerular filtrate during N.P.B. which tends to imply a decreased activity of antidiuretic hormone. Contrariwise, the GFR and ERPF for the four negative pressure breathing experiments in which an antidiuresis was obtained (fig. 5), generally show no change or a decrease in each individual experiment. In this group of N.P.B. experiments, there was greater percentage absorption of the glomerular filtrate during N.P.B. periods than during control periods. This is consistent with an increased level of antidiuretic hormone, although the mechanism of this release is not clear. Considering the fact that the circulating level of ADH exhibits no consistent pattern in these two groups of N.P.B. experiments, it may be reasonable to attribute the changes in urine flow to the small changes in renal hemodynamics, or to unmeasurable changes in the circulating level of ADH.

The experimental results of the remaining 21 experiments on N.P.B. where the change in urine flow is less than 25% are not tabulated. All values measured had insignificant changes during N.P.B. except for central venous pressure which showed the characteristic increased negativity.

It is interesting to note that the control urine flows in
those N.P.B. experiments exhibiting a diuresis (mean 0.444 ml/min.) are much lower than the control urine flows in experiments where an antidiuresis was obtained (mean 1.247 ml/min.). It could be postulated that in the latter group where control urine flows were higher, the existing inhibition of ADH was at such a level that the activation of any mechanism which would further inhibit ADH would essentially be ineffective.

The finding that negative pressure breathing has no statistically significant effect on glomerular filtration rate or the excretion fraction of sodium is in agreement with the reports of Sieker et al. (17) and Surtshin et al. (20). Thus, activation of the volume receptors by N.P.B. does not appear to have any significant action on renal hemodynamics, and the sensitivity limits of the bio-assay were such that no direct effect of the volume receptors on antidiuretic hormone release could be exhibited.

In those experiments employing negative pressure breathing where a diuresis did occur, it was noted that a latent period of about 15–30 minutes elapsed before the onset of the diuresis. The increase in urine flow may persist as long as 2 hours after ambient pressure replaced the negative pressure. This would indicate that stimulation of the volume receptors is not the continuing controlling factor in the diuresis. This accommodation to continued N.P.B. is in agreement with the findings of Sieker et al. (17, 18) and Surtshin et al. (20).

The real question arising then from these experiments is whether there is a specific receptor which responds indirectly to changes in volume or a volume sensitive area per se. The excellent work of Sieker et al. (17, 18), Gauer et al. (3) and Henry et al. (4) is the best available evidence for the existence of such an area. It is however interesting to note, that these authors do not, in any of their papers, refer to the percentage of negative pressure breathing experiments in which a diuresis was obtained. Only Surtshin and co-workers (20) allude to the fact that specific conditions must be imposed (i.e. a low control urine flow) before a consistent diuresis to N.P.B. will be obtained. Also, the extensive surgery needed to place a balloon in the left atrium may have, in some fashion influenced the results of Henry et al. (4). It would be interesting to see if the same transient diuresis occasioned by inflation of a balloon in the left atrium could be obtained by a less traumatic, constriction of the ascending aorta which would produce a similar stretch of the postulated volume receptors.

It thus appears that in those instances where the extracellular fluid is either increased by generalized plethora or selectively increased in the thorax by negative pressure breathing, the volume receptor mechanism is not of great importance in the regulation of body fluid volume. It appears to be a subdominant mechanism with the majority of control carried out by alterations in the tonicity of the extracellular fluid which elicits, or inhibits, the release of antidiuretic hormone via the osmoreceptors. However, in situations where the extracellular fluid is seriously decreased as in severe hemorrhage, depletion of extracellular electrolytes or in untreated Addison’s disease, the volume receptor mechanism appears to be part of the general compensatory reaction to the imposed stress.

REFERENCES