The Effects of Cardiac Denervation on Body Fluids[1] (37300)

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Maintaining a relative constancy of body fluid content is accomplished by a delicate balancing of intake and excretion. Sensing mechanisms are located in various organs which detect changes in the osmolality or volume of the circulating fluid and set into play reflexes aimed at restoring the internal environment to normal. One such critical area is the heart where receptors have been found which promote or inhibit a diuresis via a neurohumoral mechanism in response to left atrial distention (1, 2). Share and Levy (3) found that receptors in the carotid sinus responding to changes in blood volume also influence the excretion of urine by ultimately altering the plasma antidiuretic hormone (ADH) concentration. It is possible that similar receptors exist in other areas of the vascular tree as well. Share (4) ablated cardiac afferents by vagotomy and found that the response of plasma ADH to a severe hemorrhage was attenuated when compared with normal dogs. However, vagotomy as a method of afferent denervation is certainly not limited to the heart, and whether or not it is complete is also questionable.

Geis and associates (5) have recently devised a method of cardiac denervation in dogs which is complete and yet leaves nerves to other structures intact. In two stages the adventitia is stripped from the aorta and pulmonary artery in a circumferential band and the free walls of both atria and the interatrial septum are incised and reanastomosed without the use of cardiopulmonary bypass. Completeness of efferent denervation has been shown by an absence of change in heart rate and myocardial contractile force in response to stimulation of the vagus nerves and stellate ganglia. In view of demonstrated efferent denervation it is reasonable to assume that the afferent nerves have also been interrupted. This preparation was used to study some parameters of body fluids and the role of the heart in control of water metabolism.

Methods. Female mongrel dogs weighing 15 to 30 kg were trained to stand quietly on a table. A permanent cystostomy cannula was placed in the dome of the bladder to facilitate the collection of urine samples. Cephalic and saphenous veins were used for the infusion of solutions and sampling of blood. Control data assessing the volume of the body fluid compartments plasma osmolality, ADH levels, urine osmolality and volume were obtained during 2 days of testing without the use of anesthetics or sedatives. The two-stage denervation procedure as described by Geis *et al.* (5) was performed and the animals were allowed 1 wk following the second stage to recover from the effects of surgery. The same parameters were then evaluated under the same circumstances and the effects of cardiac denervation on body fluids were noted.

On the first day of testing following 16 hr of fasting with *ad libitum* access to water 1 mCi of tritiated water and 15 mg of Evans blue dye were injected intravenously in a total volume of 4 ml using a syringe buret, followed by a priming solution containing inulin, 40 mg/kg (3.3% solution). A maintenance solution composed of inulin, 37.5 mg/ml, was infused at a rate of 0.4 ml/min for 2 hr. Blood samples were taken before and at 30, 60, 90, and 120 min after the loading solutions were given and the concen-

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trations of Evans blue dye were estimated. Plasma volume (PV) was determined by indicator dilution technique and extracellular volume (ECF) was calculated by the formula (inulin infused — inulin excreted)/plasma inulin concn (6). Blood samples were taken at 5 and 7 hr after the initial infusion, tritiated water content of all six plasma samples was determined, and total body water was estimated by indicator dilution technique. All blood samples were collected in heparinized syringes under sterile conditions and the formed elements were resuspended in isotonic saline and reinfused into the animal.

Prior to the second day of testing, food and water were withheld for 25 hr. Four 15 min control urine samples were obtained without bladder irrigation, the volume of each sample was noted, and the osmolality was determined. During the last control period 50 ml of blood were drawn and the plasma osmolality and ADH concentration were determined. The plasma volume was then expanded 25% at a rate of 50 ml/min with 3% dextran in 0.9% saline. Initially 6% dextran was used but this was found to be hyperoncotic. Collection of urine samples every 15 min was continued for 5 hr after plasma volume expansion. At 37 min after infusion of dextran another plasma sample for osmolality and ADH concentration was obtained.

Control experiments were done on two animals. Control values for blood volume, plasma volume and body weight were obtained. Following these determinations a left thoracotomy was made and the left atrium was injured by making a small incision in the atrial appendage which was quickly sutured closed. Four days later blood and plasma volumes were again measured.

Alkali stable inulin was determined by the method of Wasler, Davidson, and Orloff (7). Tritiated water was measured in a liquid scintillation counter with the efficiency of each sample determined by comparing the channel ratio obtained with the ratios of four quenched standards. The concentration of Evans blue dye was obtained by measuring optical density at 610 nm on a spectrophotometer. Osmolality was estimated using an Advanced osmometer and plasma ADH was ascertained by bioassay using ethanol-anesthetized rats (8).

Results. Of the nine dogs from which preoperative data were obtained eight survived the two stage denervation procedure and were tested at least once postoperatively. Three dogs died of postoperative complications before testing could be completed, but each of the parameters was evaluated in at least five dogs.

Table I shows that the mean weight of

TABLE I. Effects of Cardiac Denervation on Body Water.[a]

	Control	Denervated	Mean change	p	N
Body wt (kg)	22.9 ± 1.7	21.4 ± 1.4	−1.5	<.05	7
Hematocrit (%)	40.7 ± .9	36.1 ± 1.7	−4.6	.02	7
Plasma vol (ml)	1400 ± 117	1592 ± 168	+192	.03	7
(ml/kg)	61.0 ± 1.4	73.8 ± 4.0	+12.8	.02	7
Blood vol (ml)	2359 ± 194	2495 ± 207	+136	.02	7
(ml/kg)	103 ± 3	116 ± 3	+13	.01	7
ECF (ml)	5618 ± 184	5732 ± 218	+114	>.6	5
(ml/kg)	227 ± 16	258 ± 25	+30	.07	5
Total body water (ml)	16,190 ± 737	14,520 ± 601	−1670	<.01	5
(ml/kg)	631 ± 10	643 ± 17	+11	.8	5
ISF (ml)	4092 ± 193	3929 ± 358	−163	>.5	5
(ml/kg)	167 ± 16	178 ± 25	+11	.37	5
Intracellular water (ml)	10,572 ± 747	8788 ± 197	−1784	<.01	5
(ml/kg)	404 ± 11	385 ± 18	−19	.14	5

[a] ECF = extracellular fluid volume (inulin space); ISF = interstitial volume (inulin space—Evans blue space).

the dogs decreased from 22.9 to 21.4 kg between denervation surgery and the onset of testing. In spite of this both blood volume and plasma volume were significantly greater. The extracellular fluid volume appeared to be slightly increased as a percentage of body weight following denervation. On the other hand, total body water decreased significantly following denervation. The mean loss of 1670 ml of total body water (TBW) roughly approximates the weight loss of 1.5 kg which occurred over the same interval. This loss of total body water appears to be due entirely from loss of intracellular water; there was no significant loss of interstitial fluid.

In the two dogs with sham surgery similar results were not obtained. Body weight increased 0.7 kg, plasma volume increased by only 43 ml and blood volume decreased 105 ml. In terms of body weight, plasma volume increased 0.3 ml/kg and blood volume decreased 6.4 ml/kg. None of the measured parameters in these dogs changed in a manner similar to that of the dogs with denervated hearts.

The urinary response to plasma volume expansion was compared in control dogs to that seen in the same dogs following cardiac denervation. In Fig. 1 these data are shown graphically. The denervated animals not only had higher control urine flow rates, but also responded to plasma volume expansion with a greater diuresis. The increase in this parameter focuses one's attention on the ADH system which has an afferent limb leaving the left atrium. Control levels of this hormone following 24 hr of water deprivation

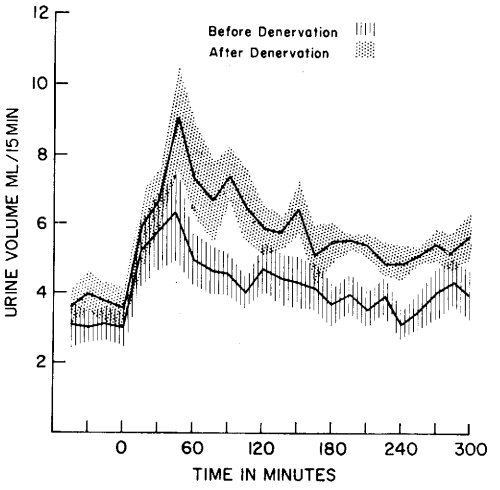

FIG. 1. The effect of cardiac denervation on urine volume, $n = 6$. The mean of each of the 15 min samples is plotted, and the shaded areas represent the SEM. Plasma volume was rapidly expanded at time = 0. The volumes before and after denervation were compared using Hotelling's T^2 test with grouping of intervals (12), $p < 0.05$.

were significantly lower after denervation than beforehand (Table II). Associated with this finding was the observation that the urine osmolality measured simultaneously was also depressed following surgery (Fig. 2). Following plasma expansion both before and after denervation, all animals were able to lower urine osmolality. In addition, during the predenervation period, volume expansion resulted in a decreased ADH concentration of plasma (Table II). However, animals with denervated hearts were unable to lower the circulating level of ADH significantly.

Discussion. The etiology of the increase in

TABLE II. Effects of Cardiac Denervation and Volume Expansion on the Concentration of ADH in Plasma and Plasma Osmolality.

	Control		Denervated		
	Before expansion	After expansion	Before expansion	After expansion	N
$[ADH]_p$ (μU/ml)	4.3 ± 1.8	2.7 ± 1	1.8 ± 0.4	1.3 ± 0.3	7
p^a	$<.05$		$<.02$	$>.1$	
P_{osm} (mOsm/kg H_2O)	297 ± 3	297 ± 3	292 ± 2	293 ± 1	7
	NS		NS	NS	

[a] The three p values are comparisons between columns 1 and 2, 1 and 3, 3 and 4. Because of the analytical variation the Wilcoxon's Signed rank test was used.

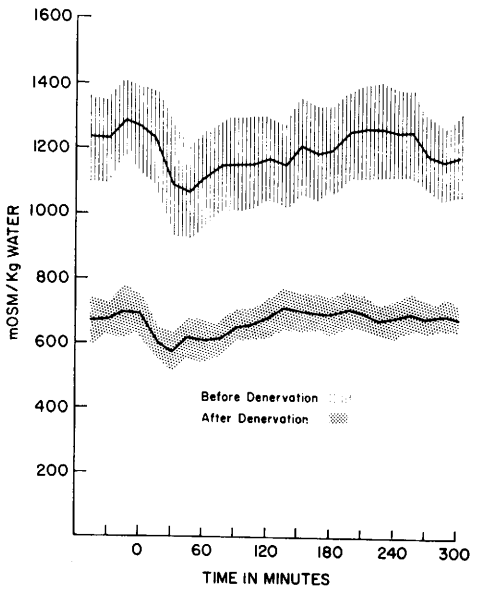

FIG. 2. The effect of cardiac denervation on urine osmolality, $n = 6$. The mean of each of the 15 min samples is plotted, and the shaded areas represent the SEM. Plasma volume was rapidly expanded at time = 0. Osmolalities before and after denervation were compared using Hotelling's T^2 test with grouping of intervals (12), $p < 0.05$.

blood volume following cardiac denervation is obscure and has been noted in at least two other series of experiments (9, 10). Whether it is due to the ablation of afferents from the heart or to the effects of denervation on hemodynamics cannot be ascertained from our experiments. However, it appears that the average weight loss of 1.5 kg following denervation is due almost entirely to shrinkage of the intracellular compartment, while the osmolality and volume of the interstitial space remained relatively unchanged. Solute and water seem to have left the cells to replace urinary losses. The cause of this intriguing aspect of cardiac denervation is difficult to determine. It appears that intracellular water and solute are lost to the interstitial space in exact quantities to make up for the solute and water lost in the urine. The cause for the diuresis must in part be due to lowered levels of ADH resulting from disruption of cardiac afferents. The loss of solute is more difficult to explain, although it has been demonstrated the atria contain afferents which control, in part, sodium excretion (11). The increase in solute excretion which must have occurred may have resulted in part from the increase in blood volume which is known to cause natriuresis. Perhaps liberation of a natriuretic hormone is involved in this response. It should be pointed out that it is unlikely that these results are a nonspecific result of surgery. The two dogs which had a thoracotomy and atrial damage without denervation underwent surgery as traumatic as the denervated group. However, neither of these dogs showed a significant alteration in plasma or blood volume.

Under the same conditions of fluid restriction plasma ADH was lower in all dogs after denervation ($p < 0.02$) compared to that before cardiac denervation. Undoubtedly afferents from left atrial receptors regulating ADH secretion in response to changes in blood volume had been severed during surgery. However receptors in other areas such as the great veins and carotid sinus were left intact. It is possible that these receptors responded to the increase in blood volume seen following denervation by sending impulses to the neurohypophysis which ultimately resulted in a decrease in ADH secretion. It is also possible that interruption of afferents from the heart is directly responsible for the lowered concentrations of ADH. This would be the case if volume expansion and left atrial stretch reduced the afferent firing rate and so caused reduced secretion of ADH, rather than increased the firing rate of inhibitory neurons. Unfortunately it is not possible to decide between these two alternatives.

In accord with lower circulating level of ADH, urine osmolality was depressed and volume was increased. Acute plasma volume expansion in normal dogs lowered the urine osmolality from 1250 to 1050 mOsm/kg water, a decrease of 16%. Likewise a 15% decrease from 650 to 550 mOsm/kg water was seen under the same conditions following denervation. It would appear that volume receptors, other than those located in the atria are responsible for this effect.

Summary. Evaluation of certain parameters of body fluids was carried out before and 1

wk following total denervation of the heart by a method which leaves nerves to other organs intact. In comparison with the normal state, dogs with denervated hearts showed an elevation of blood volume due almost entirely to an increase in the plasma component, little change in the volume of the interstitial space, and a large deficit of intracellular water. A loss of solute and water in the urine with solute leaving the cells to replace urinary losses could account for the decrease in the volume of the intracellular compartment. In the face of an elevated blood volume the concentration of ADH in plasma was diminished which was associated with a decreased urinary osmolality and increased urine volume following surgery.

1. Henry, J. P., Gauer, O. H., and Reeves, J. L., Circ. Res. 4, 85 (1956).

2. Henry, J. P., and Pearce, J. W., J. Physiol. (London) **131**, 572 (1956).

3. Share, L., and Levy, M. N., Amer. J. Physiol. **211**, 721 (1966).

4. Share, L., Amer. J. Physiol. **215**, 1384 (1968).

5. Geis, W. P., Tatooles, C. J., Kaye, M. P., and Randall, W. C., J. Appl. Physiol. **30**, 289 (1971).

6. Gaudino, M., and Levitt, M., Amer. J. Physiol. **157**, 387 (1949).

7. Wasler, M., Davidson, D., and Orloff, J., J. Clin. Invest. **34**, 1520 (1955).

8. Bonjour, J. P., and Malvin, R. L., Amer. J. Physiol. **218**, 1128 (1970).

9. Thames, M. D., Hassan, Z. U., Brackett, N. C., Jr., Lower, R. R., and Kontos, H. A., Amer. J. Physiol. **221**, 1115 (1971).

10. William, V. L., Merjavy, J. P., Pennell, R., and Hanlon, C. R., Ann. Surg. **166**, 513 (1967).

11. Goetz, K., Hermreck, A. S., Slick, G. L., and Stark, H. S., Amer. J. Physiol. **219**, 1417 (1970).

12. Hotelling, H., Proc. Berkeley Symp. Math. Statist. and Prob., 2nd, p. 23 (1951).

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