Volume Receptors and Post-prandial Diuresis in the Seal (*Phoca vitulina* L.)* (26831)

H. V. MURDAUGH JR., W. L. MITCHELL, J. C. SEABURY AND H. O. SIEKER
Mount Desert Island Biological Laboratory, Salisbury Cove, Maine, Depts. of Medicine, University of Alabama and Duke University School of Medicine, and Birmingham Veterans Administration Hospital

Acute changes in urine flow in the seal have interested physiologists since Hiatt reported the post-prandial diuresis which occurs 2 to 4 hours after feeding. This diuresis is accompanied by hypertonic urine and an increase in glomerular filtration rate(1). Schmidt-Nielsen and co-workers confirmed these findings(2).

Attempts to reproduce this pattern of change with diuresis in the seal using various types of osmotic diuresis or hydration failed unless the seal had been fed(1,2). This unexplained form of diuresis stimulated this inquiry into the possible existence of a volume receptor in the seal.

The results of this study show that plasma volume expansion produces the pattern of diuresis seen post-prandially and suggest the presence of a volume receptor in the seal.

Methods. Four young female harbor seals, weighing 20 to 25 kg, were studied at the Mount Desert Island Biological Laboratory after a short period of adjustive training. They were maintained on a diet of fresh herring, lived in wooden enclosures floated in the bay and appeared in good health throughout the studies. Because seals in captivity frequently develop subcutaneous infections, Bicillin® was administered to each seal every 2 weeks. No anesthesia was used except local anesthesia for venipunctures.

The seals were studied in the fasting state comfortably laced into a canvas sling suspended by ropes. A gentle swinging motion of the sling and soft music were used to calm the seal. The seal was periodically wetted with water to prevent dehydration.

Three types of experiments were conducted: continuous negative pressure breathing, expansion of plasma volume, and water infusion studies.

The continuous negative pressure breathing studies were performed using a plastic box with a hole to fit the neck of the seal snugly when its head was inserted into the box. Suction was applied to the box and maintained at minus 20 cm water using a water trap. Soda lime containers were placed in the box to prevent carbon dioxide accumulation. Plasma volume expansion was produced using 400 ml 1% gelatin in saline in each seal, and 400 ml fresh seal plasma in one seal, administered intravenously over a 50 minute period.† On a different day each seal was given by intravenous route 400 ml 5% dextrose in water over a 50 minute interval. Studies were preceded by a 2 to 4 hour observation period to assure a stable state and urine flow.

Intravenous infusions contained creatinine and were administered through an intravenous cannula in a fore-flipper using a constant infusion pump. The infusion rate during control observation periods was 0.6 ml/min which is less than the seal's normal fluid intake(2,3). Creatinine concentration in the test infusion fluids (gelatin, plasma, and water) was adjusted to obtain the same minute creatinine administration given during the control observation periods.

Urines were collected at 20 to 30 minute intervals *via* an inlying catheter, and heparinized blood samples were obtained at 30 to 60 minute intervals. Urine and plasma samples were analyzed for sodium and potassium content using a flame photometer, creatinine concentration by Hare's method without Lloyd's reagent(4), and osmolality using a

* Supported by grants from Alabama Heart Assn. and U.S.P.H.S.

† Dextran was not used because the seal excretes it in a quantity sufficient to produce a viscous urine.

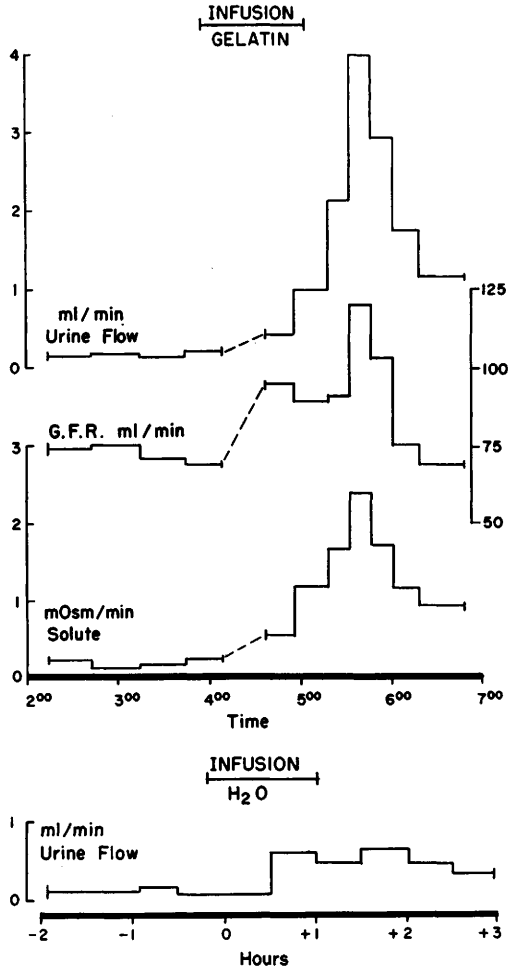

FIG. 1. Effect of infusion of 400 ml 1% gelatin in 0.9% NaCl in a harbor seal (above). Change in urine flow in response to the same volume of water infused in the same seal (below).

Fiske osmometer. Urines were checked for gelatin during the experiment using silver nitrate, and total urine protein determinations were performed later by Nesslerization.

Results. Continuous negative pressure breathing for periods up to one hour did not cause an increase in rate of urine flow in any of the 4 seals. Infusion of 5% dextrose in water did cause increase in rate of urine flow, but this increase was small in comparison to the changes found with expansion of plasma volume (Fig. 1). No change in glomerular filtration rate was caused by water administration.

Expansion of plasma volume by gelatin infusion or by plasma infusion provided a pronounced increase in rate of urine flow (Fig. 1). With this diuresis, there occurred an increase in glomerular filtration rate as measured by exogenous creatinine clearance, concentrated urines with osmolal U/P greater than 2 at peak of diuresis, and a solute diuresis without change in plasma osmolality. No gelatin was found in the urine during diuresis in response to gelatin infusion.

Discussion. Man's response to an increase in plasma volume is a water diuresis without change in glomerular filtration rate(5), but the response of the seal to the same stimulus is a solute diuresis with hypertonic urine and an increase in glomerular filtration rate. This diuresis indicates the presence of a volume receptor in the seal but implies that it is of a different mechanism than that of man.

This pattern of diuresis in response to increase in plasma volume in the seal is like that seen in the post-prandial diuresis. It would therefore appear likely that the post-prandial diuresis is mediated *via* a volume receptor in response to increase in plasma or blood volume after feeding.

A seal swimming with its head above water breathes against a relative negative pressure comparable to a man breathing while immersed in water to his neck. In such a situation man experiences a water diuresis(6). Continuous negative pressure breathing, another situation where pressure against the exterior of the body is greater than the pressure respired against, causes a water diuresis in dog(7) and man(8,9). These findings have been interpreted as evidence for intrathoracic volume receptors which initiate changes in rate of urine flow with changes in thoracic blood volume.

The finding that the seal will tolerate prolonged periods of continuous negative pressure breathing without change in urine flow indicates that the animal does not have atrial stretch receptors or that he has some adaptive mechanism to prevent displacement of blood into the thorax. The seal's inferior vena cava valve may be such an adaptive mechanism.

Summary. The effect of continuous negative pressure breathing and of plasma volume

expansion was studied in young harbor seals. Negative pressure breathing did not increase rate of urine flow. Expansion of plasma volume with seal plasma or with 1% gelatin in saline caused a solute diuresis with increase in glomerular filtration rate similar to the changes seen in the post-prandial diuresis of the seal. It is suggested that the post-prandial diuresis of the seal is mediated *via* a volume receptor.

1. Hiatt, E. P., Hiatt, R. B., *J. Cell & Comp. Physiol.*, 1942, v19, 221.

2. Schmidt-Nielsen, B., Murdaugh, H. V., Jr., O'Dell, R., Bacsanyi, J., *ibid.*, 1959, v53, 393.

3. Irving, L., Fisher, K. C., MacIntosh, F. C., *ibid.*, 1935, v6, 387.

4. Hare, R. S., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 148.

5. Welt, L. G., Orloff, J., *J. Clin. Invest.*, 1951, v30, 751.

6. Bazell, H. C., Thurlow, S., Crowell, C., Steward, W., *Am. J. Physiol.*, 1924, v70, 430.

7. Gauer, O. H., Henry, J. P., Sieker, H. O., Wendt, W. E., *J. Clin. Invest.*, 1954, v33, 287.

8. Sieker, H. O., Gauer, O. H., Henry, J. P., *ibid.*, 1954, v33, 572.

9. Murdaugh, H. V., Jr., Sieker, H. O., Manfredi, F., *ibid.*, 1959, v38, 834.

Received May 4, 1961. P.S.E.B.M., 1961, v108.

Adrenocortical Response to Stress in Rats with Lesions in Hippocampus and Amygdala.* (26832)

KARL M. KNIGGE

Department of Anatomy, University of Cincinnati, Cincinnati, Ohio

A continually mounting volume of research, summarized regularly(1-3), has explored mechanisms of neural regulation of ACTH secretion. The most intense interest is centered about isolation and characterization of the ACTH-regulating neurohumor(4-7) and identification of the hypothalamic area(s) in the region of the median eminence which represent the terminal link(s) between the central nervous system and the adenohypophysis(8-11). More recently attention has been directed also to other regions of the brain which may have direct connections with the final hypothalamic effector sites or which may influence indirectly the amount of ACTH neurohumor produced there. Among these areas are the cerebral cortex(12,13), limbic system(14,15), brain stem and reticular formation(12,16-18). The present report describes alterations in basal levels of plasma free corticoids (PFC) and in the pattern of PFC response to stress occurring in rats with lesions of hippocampus or amygdala.

Materials and methods. Bilateral electrolytic lesions (3 ma, 30 sec) were placed by stereotaxic means in hippocampus, amygdala and habenular complex of adult male rats. The insulated stainless steel needle used for lesioning was bared approximately 1 mm at its tip for placement of lesions in amygdala and habenula; for hippocampus, the needle tip was bared 1.5-2 mm. Using a stereotaxic instrument with reference planes adjusted to the atlas of de Groot(19), the anterior vertical and lateral coordinates used for lesioning amygdala, hippocampus and habenula were (+5.4, −2.8, ± 4.5), (+3.0, −2.5, ± 5.0) and (+3.4, +0.8, ±0.5) respectively. Two months after lesioning, I^{131} uptake was measured by counting the external neck activity 18-24 hrs after intraperitoneal administration of 5 μc carrier-free NaI^{131}; a region of the hind limb was counted as a measure of the non-thyroidal neck activity. After one additional month, peripheral PFC levels were determined. Basal PFC levels and PFC response to 30 min and 4 hrs of continuous physical immobilization (paws bound with adhesive tape) were examined in both control and lesioned animals. Animals were sacrificed by decapitation, blood collected in heparinized beakers and PFC determined fluorometrically according to Guillemin *et al.*

* Supported by U.S.P.H.S. and N.S.F. grants.