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Title: Neural Control Mechanisms and Body Fluid Homeostasis

Summary of Research

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Project Aims:

The goal of the proposed research was to study the nature of afferent signals to the brain that reflect the status of body fluid balance and to investigate the central neural mechanisms that process this information for the activation of response systems which restore body fluid homeostasis. That is, in the face of loss of fluids from intracellular or extracellular fluid compartments, animals seek and ingest water and ionic solutions (particularly Na^+ solutions) to restore the intracellular and extracellular spaces. Over recent years, our laboratory has generated a substantial body of information indicating that 1) a fall in systemic arterial pressure facilitates the ingestion of rehydrating solutions and 2) that the actions of brain amine systems (e.g., norepinephrine; serotonin) are critical for precise correction of fluid losses. Because both acute and chronic dehydration are associated with physiological stresses, such as exercise and sustained exposure to microgravity, the present research will aid in achieving a better understanding of how vital information is handled by the nervous system for maintenance of the body's fluid matrix which is critical for health and well-being.

Studies and Results

I. Refinement and Application of Kainic Acid Neurotoxic Lesions to Destroy Neurons in the Nodose Ganglia and Functional Effects

One complication in identifying afferent pathways from systemic receptors sensing decreases in body fluids is that cutting the cervical vagus to remove afferent nerves also destroys vagal efferents. Destroying vagal efferents induces debilitation and a severely compromised preparation. A technique applied in our studies permits selective removal of vagal afferents while leaving efferent fibers intact. This method involves the application of kainic acid to the

nodose ganglia. Because of the pivotal nature of this technique, we have performed dose and validation studies to more thoroughly characterize the effects of kainic acid applied to the nodose ganglia. In these experiments, kainic acid was applied to either the right or left nodose ganglia (doses 0.8-2.0 μg). The opposite ganglion was injected with isotonic saline as a control. Rats were sacrificed two weeks later for examination of the ganglia. Kainic acid injections typically reduced the number of cell bodies by 80% in the treated side as compared to the control ganglia.

Because kainic acid administration does not completely eliminate the cell bodies in the nodose, a functional test of the remaining cells was performed. Active transport of the dye, True Blue, from nerve terminals to the chest or abdominal cavities to cell bodies in the nodose was used as a means to test the viability of cells. True Blue dye is actively taken up by nerve endings and transported to the soma of healthy cells. Microscopy allows visualization of True Blue in the ganglia. True Blue is not taken up by nerve endings of dying or dead cells and subsequently does not appear in cell bodies. In these experiments, nodose ganglia-kainic acid treated rats (n=5) were injected with 5% True Blue in either end of the chest or abdominal cavities. Seven days later, the rats were sacrificed and the nodose ganglia examined. From these experiments, it was found that there was approximately a 90% reduction in the number of viable cells in ganglia treated with neurotoxin.

As a further functional test of vagal deafferentation, we have employed the Bezold-Jarisch reflex which has been classically used to characterize removal of cardiopulmonary afferents immediately after vagotomy. One component of the Bezold-Jarisch reflex depends upon activation of low pressure baroreceptors in the cardiopulmonary circulation. When this component of the reflex is absent, the central nervous system does not receive input from low pressure baroreceptors. Therefore, in another series of experiments, we studied the Bezold-Jarisch reflex in nodose ganglia-kainic acid treated (2 μg to both nodose ganglia) rats and control rats. Bezold-Jarisch reflexes were accessed by examining the reductions in mean arterial pressure (MAP) and heart rate (HR) in response to the intravenous administration of serotonin (5-HT). The rats were studied 10 days after kainic acid treatment when they were vigorous and healthy. Baseline MAP and HR were equal between groups. However, kainic acid treated animals were significantly attenuated in their MAP and HR responses to each dose of 5-HT. Therefore, these data indicate that kainic acid treatment severely compromises the function of vagal afferents.

Rats which receive bilateral removal of vagal afferents combined with unilateral removal of efferents recover and remain quite viable. Consequently, we have studied the effects of unilateral complete vagotomy (i.e., afferent and efferent) combined with unilateral kainic injections into the nodose on both thirst and salt appetite (i.e., BVUNG treated animals). Control and BVUNG animals treated with s.c. injections of furosemide (10 mg/kg) and 5 min later with captopril (4 mg/kg) were given 1.8% NaCl and water to drink 1 hr later. Over a 2 hr drinking test, the two groups drank equivalent amounts of both water (10.2 ± 1.3 ml vs. 8.5 ± 1.3 ml; $p > 0.05$) and hypertonic saline (2.8 ± 1.4 ml; vs. 4.0 ± 1.8 ml; $p > 0.05$) control vs. BVUNG, respectively.

In contrast to the null effects seen in the test involving sodium depletion, there was a reliable effect of BVUNG treatment on thirst induced by s.c. (2 ml) administration of 6% NaCl.

In a 1 hr test after administration of the dipsogen, control animals drank 11.8 ± 1.5 ml, whereas BVUNG animals drank significantly less 5.4 ± 1.8 ml ($p < 0.05$). Taken together, these results suggest that interruption of vagal efferents may disrupt important input from systemic osmoreceptors which mediate thirst, but that systemic vagal receptors do play a role in the control of salt appetite.

II. Central Biogenic Amines and the Control of Extracellular Fluid Volume

A. Noradrenergic Mechanisms

In previous work, we discovered that systemic administration of the α_2-adrenergic receptor antagonist, yohimbine (3 to 9 mg/kg s.c.), produces both vigorous water and concentrated NaCl intake in rats. Yohimbine is known to cross the blood-brain barrier. Therefore, we wished to determine whether intracerebroventricular infusions of yohimbine would induce thirst and sodium intake. Yohimbine was infused intraventricularly (1 µl/min) in doses of 0 (vehicle control), 3.5 (LO), 7.5 (MED), and 9.5 (HI) µg/µl for 1 hr. The animals had access to both water and 2% NaCl solutions from graduated burettes for a total of 3 hrs. At the conclusion of a 1 hr infusion period, access to both NaCl and water continued for an additional 2 hrs. Yohimbine infusion in a dose-related manner significantly increased the intake of both 2% NaCl and water. Water intake for the LO, MED and HI groups was significantly greater than intakes shown by vehicle treated animals after 120 min. The HI and MED experimental groups showed dose-related increases in 2% NaCl intake after 105 and 75 min respectively. The results of these experiments suggest that blockade of noradrenergic action on α_2 receptors at a brain site(s) accessed from the ventricles induces thirst and sodium appetite which collectively serve to expand extracellular fluid volume. Further studies aimed at testing the role of a specific brain site for yohimbine action in mediating salt appetite have indicated that the central nucleus of the amygdala is probably not involved.

B. Serotonergic Mechanisms

In work completed prior to initiation of the present NASA grant, we had observed that manipulations that increase synaptic 5-HT in the brain produce a pattern of systemic sympathetic withdrawal which resembles that present during cardiovascular shock and/or orthostatic hypotension (i.e., orthostatic intolerance). Consequently, we were motivated to determine whether blockade of central 5-HT receptors would reduce the fall in sympathetic outflow to hemorrhage. In these experiments, mean arterial pressure, heart rate (HR) and renal sympathetic nerve activity (RSNA) were measured in conscious rats during hemorrhage after intracerebroventricular injection of the 5-HT_1/5-HT_2-receptor antagonist, methysergide (40 µg). Progressive hemorrhage caused an initial rise (109 ± 33%), followed by a fall in RSNA (-60 ± 7%) and a fall in HR (-126 ± 7 bpm). Methysergide delayed the hypotension and prevented both the sympathoinhibitory and bradycardic responses to hemorrhage. Systemic 5-HT_3-receptor blockade did not influence responses to hemorrhage. These data indicate that a central serotonergic receptor-mediated component contributes to the sympathoinhibitory and bradycardic response to hypotensive hemorrhage in conscious rats. These results are especially promising in that they suggest that blockade of central 5-HT receptors (most likely 5-HT_1 or 5-HT_2) may have salutary effects in reducing orthostatic intolerance.

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