Am J Physiol Regul Integr Comp Physiol 290: R1136–R1141, 2006.
First published December 15, 2005; doi:10.1152/ajpregu.00075.2003.

Corticotropin-releasing hormone in the lateral parabrachial nucleus inhibits sodium appetite in rats

Emilio De Castro e Silva,[1] Josmara B. Fregoneze,[1] and Alan Kim Johnson[2]

[1]*Department of Physiology, Health Sciences Institute, Federal University of Bahia, Bahia, Brazil; and* [2]*Departments of Psychology and Pharmacology, and the Cardiovascular Center, University of Iowa, Iowa City, Iowa*

Submitted 7 February 2003; accepted in final form 8 December 2005

De Castro e Silva, Emilio, Josmara B. Fregoneze, and Alan Kim Johnson. Corticotropin-releasing hormone in the lateral parabrachial nucleus inhibits sodium appetite in rats. *Am J Physiol Regul Integr Comp Physiol* 290: R1136–R1141, 2006. First published December 15, 2005; doi:10.1152/ajpregu.00075.2003.—The present study investigated the role of corticotropin-releasing hormone (CRH) in the lateral parabrachial nucleus (LPBN) in the behavioral control of body fluid homeostasis by determining the effect of bilateral injections of the CRH receptor antagonist, α-helical corticotropin-releasing factor $(CRF)_{9-41}$, and the CRH receptor agonist, CRH, on sodium chloride (salt appetite) and water (thirst) intake. Groups of adult, male Sprague-Dawley rats had stainless-steel cannulas implanted bilaterally into the LPBN and were sodium depleted or water deprived. Bilateral injections of α-helical CRF_{9-41} into the LPBN significantly potentiated water and salt intake in the sodium-depleted rats when access to fluids was restored. Bilateral injections of α-helical CRF_{9-41} into the LPBN (1.0 μg) also increased sodium appetite in water-deprived rats. Conversely, in sodium-depleted animals, bilateral injections of CRH inhibited sodium chloride intake. These results suggest that there is an endogenous CRH inhibitory mechanism operating in the LPBN to modulate the intake of sodium (salt appetite). This mechanism may contribute to the behavioral control of restoration of body fluid homeostasis in sodium-deficient states.

water intake; α-helical corticotropin-releasing factor

THE PEPTIDE corticotropin-releasing hormone (CRH) is widely distributed in the mammalian central nervous system and has been implicated in many physiological and behavioral responses to physical and psychological stressors (see Ref. 35 for a review). CRH neurons in the hypothalamus projecting to the median eminence are classically recognized in the control of ACTH secretion from the anterior pituitary. In addition, other CRH pathways intrinsic to the brain are also activated by aversive stimulation or conditions (35). Stress-associated behaviors, including general arousal, emotional reactions, and reduced sexual activity to stressors (7, 20) are influenced by central CRH mechanisms. Brain pathways containing CRH are also involved in the activation of sympathetic nerve activity (9).

Brain CRH has been implicated in the modulation of several aspects of ingestive behavior. Food intake is enhanced following intracerebroventricular injections of CRH receptor antagonists, and appetite is inhibited after central CRH injections (11). However, the effects of CRH in the central control of body fluid balance appear somewhat equivocal. Pharmacological treatment to activate hypothalamic CRH receptors produced a significant inhibition of water and food intake in 24-h fasted rats (27). On the other hand, intracerebroventricular infusion of CRH in rabbits increased water and salt intake and increased renal sodium excretion (37, 38).

CRH-expressing neurons and receptors are present in many forebrain and hindbrain areas implicated in the control of water and salt intake (5, 19, 29, 39). One key brain structure associated with central CRH that has been implicated in the control of thirst and sodium appetite is the lateral parabrachial nucleus (LPBN). The LPBN receives ascending fibers from the nucleus of the solitary tract (NTS), which receives input from arterial and cardiopulmonary baroreceptors and from the area postrema (AP), which senses levels of circulating factors. The ascending pathways from the NTS and AP have been hypothesized to carry information reflecting the status of body fluid homeostasis (17). Extensive work has demonstrated that the LPBN exerts important modulatory influences on water and salt intake (4, 8, 22–24, 28). CRH containing soma and receptors are present in the LPBN (18), and CRH neurons are a component of a pathway linking the NTS to the LPBN (13). In addition, CRH has been shown to be present in descending projections from the central nucleus of the amygdala (CeA) (25), the bed nucleus of the stria terminalis (BNST) (26), and the lateral hypothalamic area (LHA) (19). Both the CeA and the BNST have been implicated in the control of sodium appetite (16, 42). Cellular dehydration increases CRH mRNA expression in the LHA-LPBN projecting cells (19).

The purpose of the present paper was to investigate the role of CRH in the LPBN on the control of water and salt intake in rats. The strategy employed in these studies was to determine whether the CRH receptor antagonist, α-helical corticotropin-releasing factor $(CRF)_{9-41}$, injected bilaterally into the LPBN altered hypertonic saline (2%) and water intake after treatments, which induce increased NaCl intake (i.e., salt appetite). After observing an increase in salt intake with this treatment, we evaluated the specificity of the effect by conducting a counter experiment using a CRH receptor agonist, that is, CRH itself.

MATERIALS AND METHODS

General Methods

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–350 g were housed in individual wire cages with free access to Purina Rat Chow, tap water, and 2% NaCl. Room lights were on from 0600 until 1800 with room temperature maintained at 23°C. All experimental procedures were approved by the University of Iowa Animal Care and Use Committee.

Drugs. Furosemide (Abbott Laboratories, North Chicago, IL) was administered at 10 $mg \cdot kg^{-1} \cdot ml^{-1}$. The CRH antagonist α-helical

Address for reprint requests and other correspondence: A. K. Johnson, Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242–1407 (E-mail: alan-johnson@uiowa.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

CRF_{9-41} was acquired from Peninsula Laboratories (San Carlos, CA; catalog number 8565), and CRH was purchased from Sigma (St. Louis, MO; catalog number C 3042).

LPBN cannulation. After anesthesia with an Equithesin-like cocktail (composed of 0.97 g of pentobarbital sodium and 4.25 g of chloral hydrate/100 ml distilled water; 3 ml/kg ip; University of Iowa Hospital Pharmacy, Iowa City, IA), animals were placed in a Kopf stereotaxic instrument and stainless-steel cannulas (23 gauge) were implanted into each LPBN. The stereotaxic coordinates were 9.4 mm caudal to bregma, ±1.9 mm lateral to midline, and 6.0 mm below the skull. The tips of these guide cannulas were targeted to terminate 2 mm above the LPBN. The cannulas were cemented with dental acrylic resin to the skull and to jeweler's screws set into drilled holes in the skull. Each cannula was closed with an obturator (30 gauge). After the surgery, the animals were housed in individual cages for 7 days before the experiments.

Histology. At the end of the experiments, each animal was anesthetized with Equithesin, and both LPBN were injected with 0.2 μl of Evans Blue. The animals were then perfused transcardially with PBS followed by 10% formalin, and their brains were removed and fixed in 10% formalin. After fixation, the brains were frozen and cut into 40-μm coronal sections. To confirm the injection sites in relation to the LPBN, the slices were stained with cresyl violet and analyzed by light microscopy.

Experimental Protocols

Experiment 1: The effects of α-helical CRF_{9-41} injections into the LPBN on sodium and water intake in furosemide-treated sodium-depleted rats. To investigate the effects of bilateral LPBN injections of the CRH receptor antagonist on sodium appetite, the animals were sodium depleted. Beginning three days before tests of water and 2% saline intake, animals were given access to sodium-deficient chow (0.024% NaCl; ICN, Irvine, CA) and to bottles containing distilled water and 2% saline. Twenty-four hours before the fluid intake tests, the 2% saline solution was removed from the cage, and the animals received two subcutaneous injections of furosemide (10 $mg \cdot kg^{-1} \cdot ml^{-1}$) separated by a 1-h interval. Both sodium-deficient chow and water continued to be available. The next day, at the beginning of a 2% saline solution and water intake test, bilateral LPBN injections of vehicle (isotonic saline) or α-helical CRF_{9-41} (0.25, 0.50, or 1.0 μg) were administered at each site in 200-nl volume. After the injections, graduated bottles containing distilled water and 2% saline were placed on the cages. The intakes of water and 2% saline were then monitored for the next 120 min.

Experiment 2: The effects of α-helical CRF_{9-41} injections into the LPBN on sodium and water intake in water-deprived rats. To test for the generality of the effects of CRH receptor antagonist administered to the LPBN on the fluid intake in water- and sodium-deficient rats, bilateral injections of α-helical CRF_{9-41} were made into the LPBN in fluid-deprived animals. Rats that had been maintained on a normal sodium diet and given access to distilled water and 2% saline were subjected to complete fluid deprivation for the 24 h. After the deprivation period, bilateral injections of α-helical CRF_{9-41} (1.0 μg) or saline (controls) were made into each LPBN site in 200-nl volume. Graduated bottles containing distilled water and 2% saline were then introduced into the cages. The intakes of both solutions were then monitored for the next 120 min.

Experiment 3: Effects of CRH injections into the LPBN in furosemide-treated, sodium-depleted rats. To test the effects of bilateral LPBN administration of a CRH receptor agonist on sodium appetite and thirst, sodium- and water-depleted animals that were treated, as described above, received injections of CRH (100, 200, and 400 ng/200 nl), or isotonic saline (controls) into each LPBN. Immediately after these injections, access to 2% saline was restored, and the volumes of water and 2% saline consumed were recorded for the next 120 min.

Statistical analysis. SigmaStat for Windows (Jandel Scientific, San Rafael, CA) was used to analyze water and salt intake. A two-way ANOVA for repeated measures, followed by the post hoc Student-Newman-Keuls test was used to compare each dose of drug to its corresponding time in the control groups. The group means were considered significantly different when $P < 0.05$. The data are presented as means ± SE.

RESULTS

Histological analysis. Typical bilateral placements of LPBN cannulas are shown in Fig. 1. The sites were usually centered in the central lateral and dorsal lateral portions of the LPBN. However, we also included data from rats whose injection sites were located in both the ventrolateral and external lateral portions of the LPBN. Animals having injection sites within the Kölliker-Fuse nucleus were not included in the data analysis.

Of the 119 rats used in this study, 67 animals had bilateral LPBN injection sites (i.e., 52 rats had misplaced injection sites with one or both injection cannula tips located outside the LPBN). The results obtained from animals with misplaced injections are presented below in *Control for specificity of the injection site*.

Effect of α-helical CRF_{9-41} injected into the LPBN on sodium and water intake in furosemide-treated, sodium-depleted rats (experiment 1). Presented in Fig. 2, *A* and *B* are the effects of bilateral injections of α-helical CRF_{9-41} into the LPBN in sodium-depleted rats. At the highest dose of α-helical CRF_{9-41} (1.0 μg), 2.0% saline intake was significantly increased throughout the duration of the experiment, compared with vehicle-injected, sodium-depleted rats. At the lowest dose used (0.25 μg), bilateral injections of α-helical CRF_{9-41} into the LPBN did not modify NaCl intake. The 0.5 μg dose of α-helical CRF_{9-41} injected bilaterally into the LPBN significantly increased the amount of NaCl consumed after 90 and 120 min. There was no significant effect of α-helical CRF_{9-41} on water intake at any of the doses (0.25, 0.5, and 1.0 μg) studied (Fig. 2*B*).

Effects of α-helical CRF_{9-41} injected into the LPBN on sodium and water intake in water-deprived rats (experiment 2). Figure 3 shows that α-helical CRF_{9-41} (1.0 μg) bilaterally injected into the LPBN of water-deprived rats slightly, but significantly, increased 2% sodium solution intake compared with saline-treated controls. Bilateral injections of α-helical

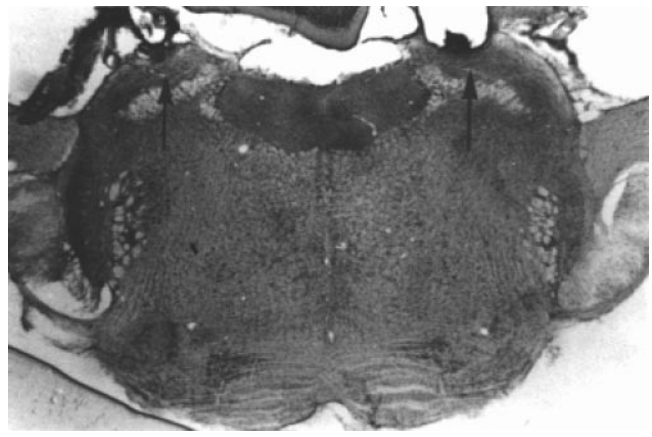

Fig. 1. Photomicrograph showing representative sites of lateral parabrachial nucleus injections (LPBN; arrows).

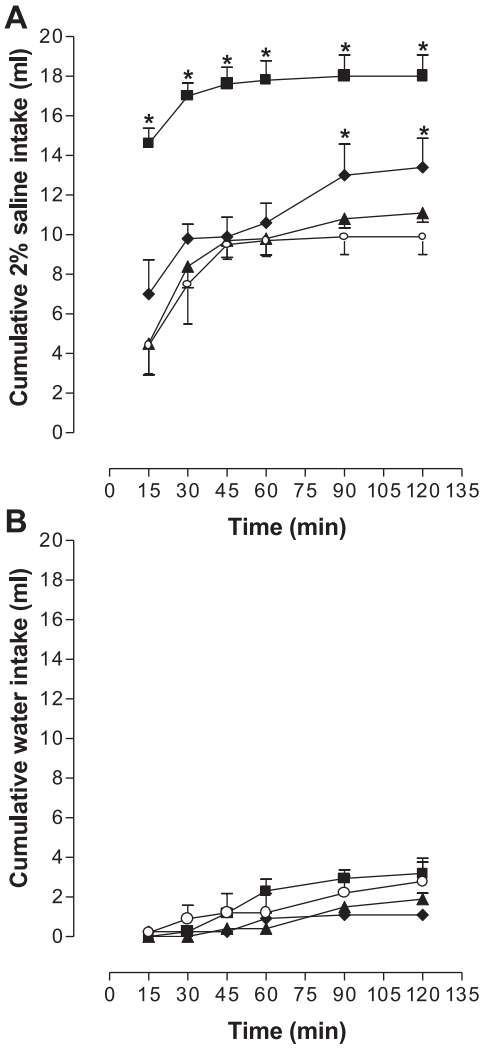

Fig. 2. Cumulative 2% saline intakes (*A*) and cumulative water intakes (*B*) in sodium-depleted rats receiving bilateral injections of α-helical corticotropin-releasing factor$_{9-41}$ (CRF$_{9-41}$), a corticotropin-releasing hormone (CRH) receptor antagonist, in different doses (■, 1.0 μg/200 nl, $n = 8$; ◆, 0.5 μg/200 nl, $n = 5$; ▲, 0.25 μg/200 nl, $n = 6$) or isotonic saline (○, $n = 5$). Values are presented as means ± SE. ANOVA for 2% saline intake indicated statistically significant treatment and time main effects and no significant treatment × time interaction [$F(3, 22) = 28.33$, $P < 0.0001$; $F(5, 15) = 25.23$, $P < 0.0001$; $F(15, 110) = 0.718$, $P = 0.7617$, respectively]. Bilateral injections of the lowest dose of α-helical CRF$_{9-41}$ (0.25 μg/200 nl) did not change 2% saline intakes compared with controls. The dose of 0.5 μg/200 nl α-helical CRF$_{9-41}$ significantly increased 2% saline intake only 90 and 120 min after treatment. At the highest dose (1.0 μg/200 nl) α-helical CRF$_{9-41}$ significantly increased 2% saline intakes during the entire duration of the experiment (*A*). ANOVA for water intake indicated statistically significant treatment and time main effects and a significant treatment × time interaction [$F(3, 22) = 5.82$, $P = 0.0044$; $F(5, 15) = 13.06$, $P < 0.0001$; $F(15, 110) = 4.21$, $P < 0.0001$, respectively]. However, the post hoc Student-Newman-Keuls test showed no statistically significant difference when saline-treated controls were compared with α-helical CRF$_{9-41}$-treated animals in any of the doses employed (*B*). *Statistically significant difference in 2% saline intake between animals treated with bilateral injections of saline (controls) and animals treated with bilateral injections of α-helical CRF$_{9-41}$.

CRF$_{9-41}$ into the LPBN (1.0 μg) did not significantly change water intake in water-deprived rats.

Effects of injection of CRH into the LPBN on sodium and water intake in furosemide-treated sodium-depleted rats (experiment 3). Bilateral injections of the highest dose (400 ng) of CRH into the LPBN produced a significant reduction in salt intake throughout the entire duration of the experiment (Fig. 4*A*). A dose of 200 ng of CRH injected bilaterally into the LPBN produced a significant decrease in salt intake beginning at 45 min and all time points that followed. The lowest dose studied (100 ng) did not significantly reduce hypertonic NaCl intake. Water intake in furosemide-treated, sodium-depleted

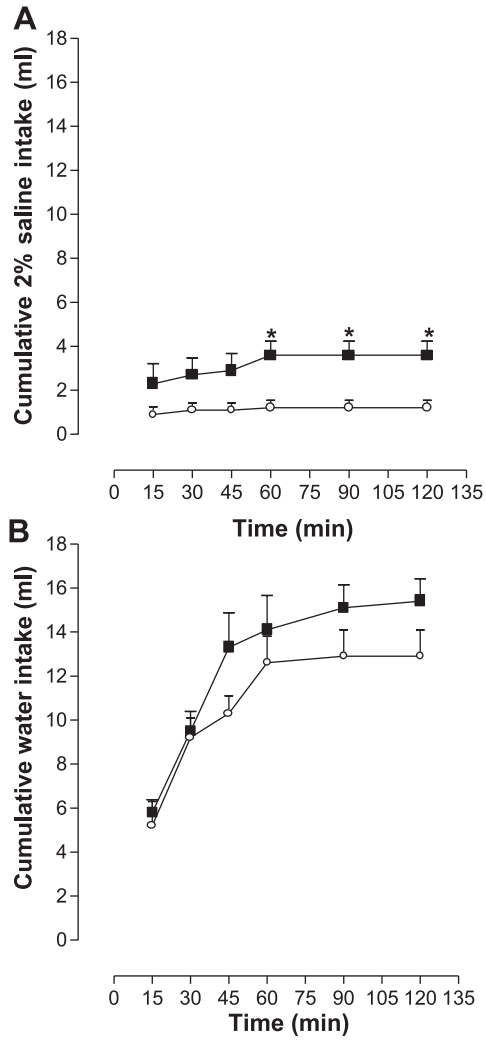

Fig. 3. Cumulative 2% saline intakes (*A*) and cumulative water intakes (*B*) in fluid-deprived rats receiving bilateral injections of α-helical CRF$_{9-41}$, a CRH receptor antagonist, in the dose of 1 μg/200 nl (■, $n = 7$) or isotonic saline (○, $n = 7$). The animals were fluid deprived for 24 h before the experiment. Values are presented as means ± SE. ANOVA for 2% saline intake indicated a significant effect of time but no significant treatment effect and no significant treatment × time interaction. [$F(5, 5) = 6.432$, $P < 0.0001$; $F(1, 14) = 0.65$, $P = 0.4335$; $F(5,70) = 2.17$, $P = 0.0672$, respectively]. The post hoc Student-Newman-Keuls test showed that bilateral injections of α-helical CRF$_{9-41}$ in the dose of 1 μg/200 nl produced a statistically significant increase in 2% saline intake after 60 min of treatment compared with controls (*A*). ANOVA for water intake indicated that there was no statistically significant treatment effect, but there was a significant effect of time [$F(1,14) = 4.291$, $P = 0.573$; $F(5,5) = 22.55$, $P < 0.001$]. There was no significant treatment × time interaction [$F(5,70) = 0.97$, $P = 0.439$]. The post hoc Student-Newman-Keuls test showed no statistically significant difference in water intake when saline-treated controls were compared with α-helical CRF$_{9-41}$-treated animals (*B*). *Statistically significant difference in 2% saline intake between animals treated with bilateral injections of saline (controls) and animals treated with bilateral injections of α-helical CRF$_{9-41}$ after analysis with the post hoc Student-Newman-Keuls test.

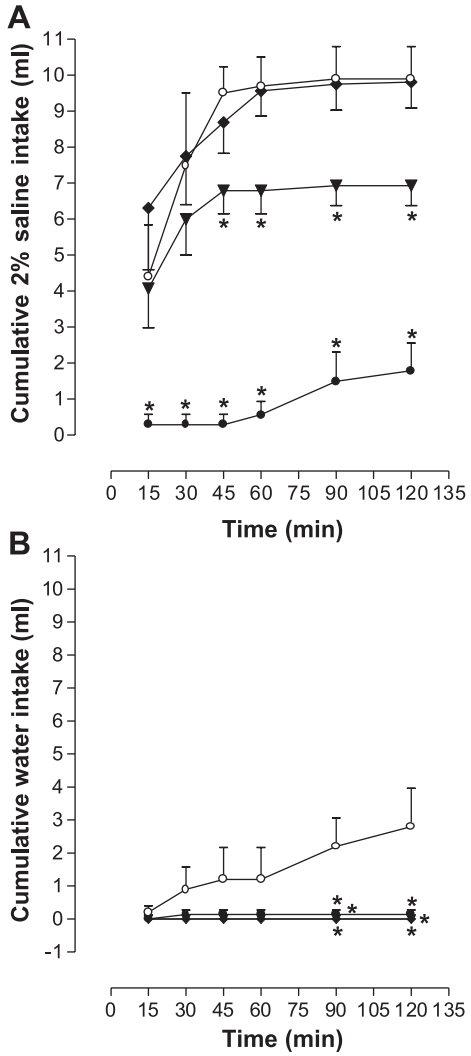

Fig. 4. Cumulative 2% saline intakes (*A*) and cumulative water intakes (*B*) in sodium-depleted rats receiving bilateral injections of CRH (● 400 ng/200 nl, $n = 7$; ▼, 200 ng/200 nl, $n = 7$; ◆ 100 ng/200 nl, $n = 8$) or isotonic saline (○, $n = 5$). Values are presented as means ± SE. ANOVA for water intake indicated statistically significant treatment and time main effects and significant treatment × time interaction [$F(3,23) = 6.24$, $P = 0.0029$; $F(5,15) = 4.96$, $P = 0.0004$; $F(15,115) = 3.72$, $P < 0.0001$, respectively]. Bilateral LPBN injections of CRH in the doses of 200 and 400 ng significantly reduced 2% saline intakes in sodium-depleted animals compared with controls (*A*). Bilateral LPBN injections of CRH in the dose of 100 ng were unable to modify the high 2% saline intake observed in sodium depleted animals treated with bilateral injections of saline (controls) (*A*). All doses of bilateral LPBN CRH injections reduced water intakes in sodium-depleted animals after 90 and 120 min after treatment (*B*). ANOVA for 2% saline intake indicated statistically significant treatment and time main effects and no significant treatment × time interaction [$F(3,23) = 24,23$, $P < 0.0001$; $F(5,15) = 18,56$, $P < 0.0001$; $F(15,115) = 1.72$, $P = 0.056$, respectively]. *Statistically significant difference in water or 2% saline intakes between animals treated with bilateral injections of saline (controls) and animals treated with bilateral injections of CRH after analysis with the post hoc Student-Newman-Keuls test.

rats (those receiving bilateral injections of isotonic saline into the LBPN) was relatively small (2.8 ± 1.2 ml after 120 min) and may have been due partially to cellular dehydration as a result of the intake of 2% saline. Bilateral injections of CRH at all doses studied (100, 200, and 400 ng) significantly inhibited water intake in sodium-depleted rats after the 90-min time point (see Fig. 4*B*).

Control for specificity of the injection site. The analysis of data from the animals with cannulas located outside the LPBN confirms that bilateral application of CRH receptor agonist and antagonist to the LPBN is necessary for the alterations in salt appetite and thirst observed here. Table 1 shows that CRH (400 ng) and α-helical CRF_{9-41} (1.0 μg) when injected outside the LPBN did not modify either water or salt intake in sodium-depleted rats. In addition, Table 2 shows that fluid-deprived animals receiving α-helical CRF_{9-41} injections (1.0 μg) that did not reach the LPBN have water and salt intakes that did not differ from those exhibited by saline-treated controls.

DISCUSSION

The major finding of the present studies demonstrates that antagonism of the actions of CRH in the LPBN significantly enhances the intake of hypertonic saline. This result suggests that an endogenous action of CRH in the LPBN acts to limit the amount of hypertonic NaCl consumed by animals displaying a salt appetite. The results of the studies also provide evidence that *1*) the sodium intake-enhancing effects of the CRH receptor antagonist are not merely the result of a nonspecific, pharmacological action of α-helical CRF_{9-41} and *2*) the effects of α-helical CRF_{9-41} and CRH are due to action within the LPBN, and the action must be bilateral to be effective. Taken together, these results suggest that there may be a CRH mechanism associated with the LPBN that acts to inhibit sodium appetite in states of sodium depletion.

CRH-containing neurons in the central nervous system comprise an extensive interconnected network (5, 19, 25, 26, 29, 36, 39) mediating neuroendocrine events and modulating a variety of behavioral responses related to psychological and physiological stressors or associated with the correction of autonomic disturbances (20). The data presented here add new evidence that indicates that brain CRH also participates in the regulation of salt intake, a specific behavior that contributes to the maintenance of body fluid homeostasis.

Central nervous system regulation of body fluid and blood pressure involves interconnected areas modulating behavioral and physiological responses that collectively regulate osmolarity, blood volume, cardiac output, and vascular tone. The subfornical organ, the periventricular tissue surrounding the anteroventral third cerebral ventricle (AV3V), amygdala, BNST, paraventricular hypothalamic nucleus, supraoptic nucleus, and the perifornical hypothalamic region comprise a forebrain neural network that acts to expand blood volume and increase arterial blood pressure (17). In contrast, the AP, the NTS, and the LPBN are portions of a hindbrain system that appears to be largely inhibitory in nature (17). Previously (e.g., see Ref. 17 for discussion), it has been suggested that humoral input from the AP and/or arterial blood pressure or blood volume conveyed by visceral afferent input via the NTS may reach the LPBN to inhibit activity when blood pressure or blood volume is increased. CRH may be one of the neurotransmitters or neuromodulators that act within the LPBN to inhibit sodium intake, thereby preventing an excessive increase in blood volume or blood pressure.

The LPBN sends many projections to the forebrain areas associated with hydromineral and cardiovascular homeostasis. Fibers originating in the LPBN project to the hypothalamic

Table 1. *Cumulative water and 2% saline intakes in sodium-depleted rats after bilateral injections of CRH, α-helical CRF_{9-41}, or isotonic saline with at least one of the sites in an area outside the LPBN*

	Cumulative Fluid Intake, ml					
	Saline		CRH, 400 ng		α-$hCRF_{9-41}$, 1 μg	
Time, min	Water	2% saline	Water	2% saline	Water	2% saline
15	0.17±0.17	5.25±1.67	0.00±0.00	6.14±1.22	0.00±0.00	5.00±1.38
30	0.17±0.17	7.33±1.50	0.25±0.25	7.29±1.19	0.00±0.00	6.80±0.92
45	0.83±0.65	9.08±1.37	0.50±0.27	7.36±1.15	0.00±0.00	11.00±0.76
60	0.83±0.65	9.92±1.42	0.63±0.26	7.50±1.09	0.40±0.40	11.50±0.55
90	1.17±0.65	9.92±1.42	0.88±0.30	7.93±0.96	1.10±0.51	11.90±0.40
120	1.17±0.65	10.40±1.59	1.00±0.38	9.29±0.94	1.10±0.51	11.90±0.40

Results are expressed as means ± SE; $n = 6$ for the saline group; $n = 8$ for the corticotropin-releasing hormone (CRH) group; $n = 5$ for the α-$hCRF_{9-41}$ group.

paraventricular nucleus, the amygdala, the BNST, and the median preoptic nucleus (10, 15, 21, 32). Forebrain and brain stem areas involved in body fluid and cardiovascular regulation send projections back to the LPBN (1, 12, 25, 26). Dense concentrations of CRH receptors are found within the LPBN (18). It is notable that immunocytochemistry has identified CRH-like material in the pathways projecting from the CeA (25), the BNST (26), the LHA (19), and the AP/NTS complex (13). It is possible to speculate that ascending hindbrain projections may limit volume expansion but that descending CRH pathways from the forebrain may play a role in controlling excessive hypertonic saline intake in the face of accumulating hypertonicity. Watts (39) has demonstrated that CRH mRNA is enhanced in the LHA but decreased in the amygdala when animals are given 2.5% NaCl as a sole drinking fluid overnight.

A commonly employed operational definition for sodium appetite is a significant intake of a hypertonic saline solution, which rats normally avoid or find aversive. In the present studies, we used two methods to induce a sodium appetite. The first was to employ a pharmacological treatment, the diuretic furosemide followed by 24 h of exposure to a sodium-free diet with water as the only available fluid. This protocol has been used extensively (14, 41) to study sodium appetite. The second method used to induce enhanced hypertonic NaCl consumption was water deprivation. Water deprivation induces a Na^+ deficit (40) and a sodium appetite, as evidenced by enhanced hypertonic saline intake after restoration of access to water (6, 33, 40). In the present experiments, rats exhibited enhanced hypertonic NaCl intake regardless of the method used to induce the initial sodium deficit. Specific blockade of CRH receptors in the LPBN by the selective CRH receptor antagonist α-helical CRF_{9-41} potentiates salt intake in rats depleted of sodium by either pharmacological (furosemide) or more natural (water deprivation) methods. These observations strongly suggest that there is a role for endogenous CRH acting in the LPBN in mediating the ingestion of hypertonic NaCl (salt appetite) that is induced by sodium deficiency.

Table 2. *Cumulative water and 2% saline intakes in fluid-deprived rats after bilateral injections of α-helical CRF_{9-41} or isotonic saline with at least one of the sites in an area outside the LPBN*

	Cumulative Fluid Intake, ml			
	Saline		α-$hCRF_{9-41}$, 1 μg	
Time, min	Water	2% saline	Water	2% saline
15	2.00±0.90	0.10±0.10	4.40±0.75	0.40±0.24
30	6.10±1.70	1.10±0.51	9.30±0.66	0.50±0.22
45	10.40±1.60	1.60±0.43	12.80±1.07	0.70±0.20
60	12.70±1.30	2.00±0.57	14.10±0.51	1.20±0.34
90	14.00±0.80	2.00±0.57	14.60±0.73	1.40±0.37
120	14.00±0.80	2.00±0.57	15.50±0.74	1.40±0.37

Results are expressed as means ± SE; $n = 5$ for saline group and α-$hCRF_{9-41}$ group.

The administration of CRH into the LPBN had the opposite effect of α-helical CRF_{9-41} treatment. That is, bilateral LPBN injection of CRH reduced hypertonic NaCl intake. The inhibition of sodium appetite by substances acting on the LPBN is consistent with other findings demonstrating that increased serotonin (5-HT) and CCK in the LPBN inhibits salt and water ingestion in rats (4, 8, 22–24). It is possible that CRH administration in the LPBN facilitates the release of 5-HT or CCK. Interestingly, CRH fibers appear to be associated with serotonergic cell bodies in raphe structures (5, 31). CRH type 1 and type 2 receptors present on serotonergic neurons have been suggested to mediate serotonergic actions (2, 3). Stress-related stimuli, as well as intracerebroventricular injections of CRH, alter brain 5-HT metabolism (30, 34).

Intracerebroventricular infusions of CRH in rabbits have been reported to increase water and salt intake and enhance renal sodium excretion (37, 38). This suggests that CRH acting on circumventricular structures has effects that are opposite to those observed when the peptide is injected into the LPBN. Alternatively, the rabbit is a different species whose mechanisms regulating body fluid homeostasis may differ from those of the rat.

The data presented here add new information to identified actions of brain CRH and provides additional evidence that the LPBN is a hindbrain area involved in the control of behaviors associated with the maintenance of body fluid homeostasis. Future work will be necessary to *1*) explore the relationship between LPBN CRH mechanisms and other agents, such as 5-HT acting in the LPBN to modify sodium appetite, *2*) test the inhibitory effect of CRH in the LPBN on salt intake using other experimental models of sodium appetite, and *3*) investigate the CRH receptor subtypes involved in the LPBN-related inhibition of sodium appetite.

GRANTS

The work was supported in part by the National Heart, Lung, and Blood Institute Grants HL-14388 and HL-57472, National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-066086, the Office of Naval Research Grant N00014–97-1–0145, the Brazilian Council of Research (CNPq) Grant 200343/89–0, and the Brazilian Ministry of Education (CAPES) Grant BEX 1554/99–9.

REFERENCES

1. **Cechetto DF and Calaresu FR.** Parabrachial units responding to stimulation of buffer nerves and forebrain in the cat. *Am J Physiol Regul Integr Comp Physiol* 245: R811–R819, 1983.
2. **Chalmers DT, Lovenberg TW, and De Souza EB.** Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. *J Neurosci* 15: 6340–6350, 1995.
3. **Chen Y, Brunson KL, Muller MB, Cariaga W, and Baram TZ.** Immunocytochemical distribution of corticotropin-releasing hormone receptor type-1 (CRF(1))-like immunoreactivity in the mouse brain: light microscopy analysis using an antibody directed against the C-terminus. *J Comp Neurol* 420: 305–323, 2000.
4. **Colombari DS, Menani JV, and Johnson AK.** Forebrain angiotensin type 1 receptors and parabrachial serotonin in the control of NaCl and water intake. *Am J Physiol Regul Integr Comp Physiol* 271: R1470–R1476, 1996.
5. **Cummings S, Elde R, Ells J, and Lindall A.** Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: an immunohistochemical study. *J Neurosci* 3: 1355–1368, 1983.
6. **De Luca LA Jr, Xu Z, Schoorlemmer GH, Thunhorst RL, Beltz TG, Menani JV and Johnson AK.** Water deprivation-induced sodium appetite: humoral and cardiovascular mediators and immediate early genes. *Am J Physiol Regul Integr Comp Physiol* 282: R552–R559, 2002.
7. **De Souza EB.** Corticotropin-releasing factor receptors: physiology, pharmacology, biochemistry and role in central nervous system and immune disorders. *Psychoneuroendocrinology* 20: 789–819, 1995.
8. **Edwards GL and Johnson AK.** Enhanced drinking after excitotoxic lesions of the parabrachial nucleus in the rat. *Am J Physiol Regul Integr Comp Physiol* 261: R1039–R1044, 1991.
9. **Fisher LA.** Corticotropin-releasing factor: endocrine and autonomic integration of responses to stress. *Trends Pharmacol Sci* 10: 189–193, 1989.
10. **Fulwiler CE and Saper CB.** Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain Res* 319: 229–259, 1984.
11. **Heinrichs SC and Richard D.** The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. *Neuropeptides* 33: 350–359, 1999.
12. **Herbert H, Moga MM, and Saper CB.** Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. *J Comp Neurol* 293: 540–580, 1990.
13. **Herbert H and Saper CB.** Cholecystokinin-, galanin-, and corticotropin-releasing factor-like immunoreactive projections from the nucleus of the solitary tract to the parabrachial nucleus in the rat. *J Comp Neurol* 293: 581–598, 1990.
14. **Jalowiec JE.** Sodium appetite elicited by furosemide: effects of differential dietary maintenance. *Behav Biol* 10: 313–327, 1974.
15. **Jhamandas JH, Harris KH, Petrov T, and Krukoff TL.** Characterization of the parabrachial input to the hypothalamic paraventricular nucleus in the rat. *J Neuroendocrinol* 4: 461–471, 1992.
16. **Johnson AK, de Olmos J, Pastuskovas CV, Zardetto-Smith AM, and Vivas L.** The extended amygdala and salt appetite. *Ann NY Acad Sci* 877: 258–280, 1999.
17. **Johnson AK and Thunhorst RL.** The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. *Front Neuroendocrinol* 18: 292–353, 1997.
18. **Kainu T, Honkaniemi J, Gustafsson JA, Rechardt L, and Pelto-Huikko M.** Co-localization of peptide-like immunoreactivities with glucocorticoid receptor- and Fos-like immunoreactivities in the rat parabrachial nucleus. *Brain Res* 615: 245–251, 1993.
19. **Kelly AB and Watts AG.** The region of the pontine parabrachial nucleus is a major target of dehydration-sensitive CRH neurons in the rat lateral hypothalamic area. *J Comp Neurol* 394: 48–63, 1998.
20. **Koob GF and Heinrichs SC.** A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. *Brain Res* 848: 141–152, 1999.
21. **Krukoff TL, Harris KH, and Jhamandas JH.** Efferent projections from the parabrachial nucleus demonstrated with the anterograde tracer *Phaseolus vulgaris* leucoagglutinin. *Brain Res Bull* 30: 163–172, 1993.
22. **Menani JV, De Luca LA Jr., and Johnson AK.** Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion. *Am J Physiol Regul Integr Comp Physiol* 274: R555–R560, 1998.
23. **Menani JV and Johnson AK.** Lateral parabrachial serotonergic mechanisms: angiotensin-induced pressor and drinking responses. *Am J Physiol Regul Integr Comp Physiol* 269: R1044–R1049, 1995.
24. **Menani JV and Johnson AK.** Cholecystokinin actions in the parabrachial nucleus: effects on thirst and salt appetite. *Am J Physiol Regul Integr Comp Physiol* 275: R1431–R1437, 1998.
25. **Moga MM and Gray TS.** Evidence for corticotropin-releasing factor, neurotensin, and somatostatin in the neural pathway from the central nucleus of the amygdala to the parabrachial nucleus. *J Comp Neurol* 241: 275–284, 1985.
26. **Moga MM, Saper CB, and Gray TS.** Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. *J Comp Neurol* 283: 315–332, 1989.
27. **Ohata H, Suzuki K, Oki Y, and Shibasaki T.** Urocortin in the ventromedial hypothalamic nucleus acts as an inhibitor of feeding behavior in rats. *Brain Res* 861: 1–7, 2000.
28. **Ohman LE and Johnson AK.** Lesions in lateral parabrachial nucleus enhance drinking to angiotensin II and isoproterenol. *Am J Physiol Regul Integr Comp Physiol* 251: R504–R509, 1986.
29. **Olschowka JA, O'Donohue TL, Mueller GP, and Jacobowitz DM.** The distribution of corticotropin releasing factor-like immunoreactive neurons in rat brain. *Peptides* 3: 995–1015, 1982.
30. **Price ML, Curtis AL, Kirby LG, Valentino RJ, and Lucki I.** Effects of corticotropin-releasing factor on brain serotonergic activity. *Neuropsychopharmacology* 18: 492–502, 1998.
31. **Ruggiero DA, Underwood MD, Rice PM, Mann JJ, and Arango V.** Corticotropic-releasing hormone and serotonin interact in the human brainstem: behavioral implications. *Neuroscience* 91: 1343–1354, 1999.
32. **Saper CB and Loewy AD.** Efferent connections of the parabrachial nucleus in the rat. *Brain Res* 197: 291–317, 1980.
33. **Sato MA, Yada MM, and DeLuca LA Jr.** Antagonism of the renin-angiotensin system and water deprivation-induced NaCl intake in rats. *Physiol Behav* 60: 1099–1104, 1996.
34. **Singh VB, Hao-Phan T, Corley KC, and Boadle-Biber MC.** Increase in cortical and midbrain tryptophan hydroxylase activity by intracerebroventricular administration of corticotropin releasing factor: block by adrenalectomy, by RU 38486 and by bilateral lesions to the central nucleus of the amygdala. *Neurochem Int* 20: 81–92, 1992.
35. **Strand FL.** Neuropeptides: *Regulators of Physiological Processes*. Cambridge, MA: MIT Press, 1999.
36. **Swanson LW, Sawchenko PE, Rivier J, and Vale WW.** Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology* 36: 165–186, 1983.
37. **Tarjan E and Denton DA.** Sodium/water intake of rabbits following administration of hormones of stress. *Brain Res Bull* 26: 133–136, 1991.
38. **Tarjan E, Denton DA, and Weisinger RS.** Corticotropin-releasing factor enhances sodium and water intake/excretion in rabbits. *Brain Res* 542: 219–224, 1991.
39. **Watts AG.** Osmotic stimulation differentially affects cellular levels of corticotropin-releasing hormone and neurotensin/neuromedin N mRNAs in the lateral hypothalamic area and central nucleus of the amygdala. *Brain Res* 581: 208–216, 1992.
40. **Weisinger RS, Denton DA, McKinley MJ, and Nelson JF.** Dehydration-induced sodium appetite in rats. *Physiol Behav* 34: 45–50, 1985.
41. **Wolf G, McGovern JF, and Dicara LV.** Sodium appetite: some conceptual and methodologic aspects of a model drive system. *Behav Biol* 10: 27–42, 1974.
42. **Zardetto-Smith AM, Beltz TG, and Johnson AK.** Role of the central nucleus of the amygdala and bed nucleus of the stria terminalis in experimentally-induced salt appetite. *Brain Res* 645: 123–134, 1994.