NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Physiol Behav. Author manuscript; available in PMC 2015 September 01.

Published in final edited form as:
Physiol Behav. 2014 September ; 0: 91–96. doi:10.1016/j.physbeh.2014.03.027.

Acute hypernatremia promotes anxiolysis and attenuates stress-induced activation of the hypothalamic-pituitary-adrenal axis in male mice

Justin A. Smith[1], **Lei Wang**[1], **Helmut Hiller**[1], **Christopher T. Taylor**[1], **Annette D. de Kloet**[2], and **Eric G. Krause**[1]

Justin A. Smith: smith6jt@ufl.edu; Lei Wang: stone0338@ufl.edu; Helmut Hiller: hhiller@cop.ufl.edu; Christopher T. Taylor: gator.ctaylor@gmail.com; Annette D. de Kloet: adekloet@ufl.edu

[1]Department of Pharmacodynamics, College of Pharmacy, University of Florida, PO Box 100487, Gainesville, FL 32611

[2]Dept of Physiology and Functional Genomics, College of Medicine, University of Florida, PO Box 100274, Gainesville, FL 32610

Abstract

Previous investigation by our laboratory found that acute hypernatremia potentiates an oxytocinergic tone that inhibits parvocellular neurosecretory neurons in the paraventricular nucleus of the hypothalamus (PVN), attenuates restraint-induced surges in corticosterone (CORT), and reduces anxiety-like behavior in male rats. To investigate the neural mechanisms mediating these effects and extend our findings to a more versatile species, we repeated our studies using laboratory mice. In response to 2.0 M NaCl injections, mice had increased plasma sodium concentrations which were associated with a blunted rise in CORT subsequent to restraint challenge relative to 0.15 M NaCl injected controls. Immunofluorescent identification of the immediate early gene product Fos found that 2.0 M NaCl treatment increased the number of activated neurons producing oxytocin in the PVN. To evaluate the effect of acute hypernatremia on PVN neurons producing corticotropin-releasing hormone (CRH), we used the Cre-lox system to generate mice that produced the red fluorescent protein, tdTomato, in cells that had Cre-recombinase activity driven by CRH gene expression. Analysis of brain tissue from these CRH-reporter mice revealed 2.0 M NaCl treatment caused a dramatic reduction in Fos-positive nuclei specifically in CRH-producing PVN neurons. This altered pattern of activity was predictive of alleviated anxiety-like behavior as mice administered 2.0 M NaCl spent more time exploring the open arms of an elevated-plus maze than 0.15 M NaCl treated controls. Taken together, these results further implicate an oxytocin-dependent inhibition of CRH neurons in the PVN and

© 2014 Elsevier Inc. All rights reserved.

Corresponding author: Eric G. Krause, Department of Pharmacodynamics, College of Pharmacy, University of Florida, PO Box 100487, Gainesville, FL 32611, Phone: 1-352-273-6977, ekrause@cop.ufl.edu.

This manuscript is based on work presented during the 2013 Annual Meeting of the Society for the Study of Ingestive Behavior, July 30–August 3, 2013.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

demonstrate the impact that slight elevations in plasma sodium have on hypothalamic-pituitary-adrenocortical axis output and anxiety-like behavior.

Keywords

oxytocin; corticotropin-releasing-hormone; anxiety; depression; hypertension; hypernatremia

1. Introduction

In mammals the plasma sodium concentration (pNa^+) is regulated by neural, humoral and behavioral mechanisms that maintain blood tonicity at levels that allow normal physiological function. Sodium deficiency causes hyponatremia and increases circulating levels of angiotensin II (Ang-II) and aldosterone (ALDO) which activate receptors in the kidney and brain to restore the pNa^+ to homeostatic levels by promoting the retention and consumption of sodium. Conversely, excess sodium causes hypernatremia and suppresses Ang-II and ALDO but causes the secretion of vasopressin (AVP) and oxytocin (OT) into the systemic circulation which alleviates the elevated pNa^+ by promoting renal water retention and sodium excretion. Thus, the pNa^+ is tightly regulated by neurohumoral compensatory responses that act in the brain and periphery to maintain blood tonicity at homeostatic levels when challenged with sodium deficiency or excess.

The neuropeptides and hormones that maintain the pNa^+ are also known to influence mood, affect, and stress responsiveness. For example, studies conducted in humans and animals have found that elevated circulating levels of Ang-II and ALDO are predictive of affective disorder [1] [2] [3] [4], and that OT and AVP are mediators of stress responsiveness and anxiety [5] [6]. As mentioned, the secretion of Ang-II, ALDO, AVP, and OT are heavily influenced by the pNa^+, and therefore, it is possible that alterations in body sodium levels affect stress responding and anxiety-like behavior through manipulation of these neuroendocrine signals. In this regard, previous work from our group and others have found that sodium depletion is anxiogenic [7] but acute salt loading is anxiolytic and dampens stress responsiveness in laboratory rats [8] [9]. While the changes in the pNa^+ are found to influence mood and stress responding in rats, whether these effects generalize to mice, and therefore, allow the use of the genetic manipulations that mouse models afford to investigate central mechanism(s) underlying the stress limiting effects of acute hypernatremia has not been evaluated.

The goal of the present study was to determine whether acute modest increases in the pNa^+ affect anxiety-like behavior and hypothalamic-pituitary-adrenal (HPA) axis activation in laboratory mice. Mice were rendered mildly hypernatremic via systemic administration of 2.0 M NaCl, and subsequently, were subjected to psychogenic stress or tests of anxiety-like behavior. Administration of 2.0 M NaCl produced a modest but significant increase in the pNa^+ relative to control injection of 0.15 M NaCl. This modest rise in the pNa^+ was associated with attenuated anxiety-like behavior and decreased stress-induced HPA activation. Both quantitative and qualitative neuroanatomical studies were conducted to provide insight towards neural mechanisms contributing to the anxiolytic effects of acute mild hypernatremia. Collectively, the results demonstrate that the stress limiting and

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

anxiolytic effects of slight elevations in the pNa^+ also occur in mice. The implication is that acutely increasing the pNa^+ may trigger interactions between neurons expressing OT and corticotropin-releasing-hormone (CRH) to limit responding to psychological stress.

2. Materials and Methods

2.1 Animals

Studies examining the effects of acute hypernatremia on anxiety-like behavior and HPA activation used adult male C57BL/6 mice obtained from Harlan. Neuroanatomical studies utilized the Cre-LoxP system to generate male mice that express red fluorescent protein (tdTomato) in cells that produce CRH. Briefly, these CRH-reporter mice were generated by breeding mice that have Cre recombinase expression directed to CRH-producing cells (Jackson Laboratory Stock # 012704) to mice with a mutation of the Gt(ROSA)26Sor locus with a *loxP*-flanked STOP cassette preventing transcription of a CAG promoter-driven sequence coding for tdTomato (Jackson Laboratory Stock # 007914). All mice were 9–10 weeks old at the initiation of the study and were individually housed on a 12:12 h light/dark cycle in clear plastic ventilated cages with plumbed water supply. Standard mouse chow (Harlan) was suspended in a wire rack that also supported an accessory water bottle allowing *ad libitum* access to both food and water except where otherwise noted. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida.

2.2 Restraint Stress and Blood Sampling

Mice were injected subcutaneously with 0.1 mL of either 2.0 M (n=10) or 0.15 M NaCl (n=10) and returned to their home cages where water was made unavailable. Saline injections were preceded by 2% lidocaine (~0.01 mL) to minimize discomfort. Sixty-minutes after saline injections, mice were placed in clear plastic ventilated tubes to initiate a stress response in the context of normal or elevated pNa^+. Tail blood samples (~20 μL) were collected in chilled EDTA-coated plastic collection tubes immediately at the onset of restraint and again after 30 min of immobilization in plastic restrainers. Mice were then released and allowed to recover in their home cages where two more blood samples were taken at 60 min and 120 min relative to the initiation of restraint. Blood samples were kept on ice until centrifuging at 4° C at 6500 rpm for 15 min. Microcapillary samples were measured for hematocrit, and plasma was extracted and stored at −80° C until pNa^+, plasma proteins, and CORT analyses took place. Plasma sodium levels were determined for the blood sample taken at the onset of restraint using an auto flame photometer as previously described [9] (Instrumentation Laboratory, Lexington, Massachusetts). Plasma CORT was determined for each time point a blood sample was taken using an ^{125}I RIA kit (MP Biomedicals, Santa Ana, California) as previously described [9]. Plasma proteins and hematocrit were determined for the blood sample taken at the onset of restraint using a handheld refractometer (VET 360, Reichert) and microcapillary reader, respectively.

2.3 In situ hybridization

RNAscope *in situ* hybridization (ISH) was performed on brain tissue collected from CRH-reporter mice to determine the extent to which CRH mRNA co-localizes with tdTomato in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

the PVN. Mice were overdosed with sodium pentobarbital, transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). Subsequently, brains were extracted, coronally sectioned at 20 μm into 6 series and then immediately rinsed and mounted onto Superfrost Plus Gold slides. Tissue collection, sectioning and mounting of sections were performed in RNase-free conditions. Slides were allowed to air dry for 20–30 min and then were stored at −80°C until processing for *in situ* hybridization. Three slides containing separate series of sections through the PVN were allowed to reach room temperature for 30 min prior to performing the manufacturer's protocol (Advanced Cell Diagnostics; Hayward, CA). RNAscope ISH was performed using the following probes: (1) Negative Control, DapB, (2) Positive control, Ubc, (3) CRH. All images were captured at 40x magnification and the exposure time was adjusted for each image using the best-fit feature in Axiovision. Subsequently, the min-max feature was utilized to minimize background fluorescence and provide optimal visualization of RNA signal. All images were processed using the same automated parameters.

2.4 Immunohistochemistry

2.4.1—Two separate histological studies were performed: CRH-reporter mice (n = 6) and a separate group of CRH-reporter mice (n=8) were each further divided into groups given either 2.0 M NaCl or 0.15 M NaCl and then restrained 60 min later as described above. Mice were sacrificed 120 min after the onset of restraint (180 min after injections) and stimulated Fos induction, a marker of neuronal activity, is known to peak during this time [10]. Mice were overdosed with sodium pentobarbital inducing a level of anesthesia that rendered them unresponsive to toe-pinch before transcardial perfusion with 0.9% saline. Following the clearing of blood, mice were perfused with 4% PFA and brains were carefully extracted then post-fixed for 2 hours in 4% PFA before cryoprotection in 30% sucrose. Four series of coronal 30 μm brain sections were cut on a Leica CM3050 S cryostat (Leica, Buffalo Grove, Illinois) and then stored in cryoprotective solution at −20° C.

2.4.2—Rinses and solutions were made using 50 mM potassium phosphate buffered saline (KPBS) and took place at room temperature on an orbital shaker unless otherwise noted. Immunofluorescent labeling of Fos in brain sections from CRH-reporter mice began by rinsing free-floating sections 5 × 5 min to remove cryoprotectant. Blocking consisted of 2% normal donkey serum (Jackson ImmunoResearch, West Grove, Pennsylvania) with 0.2% Triton-X (Sigma) for 1 h followed by primary antibody incubation with rabbit anti-Fos (sc-52 1:1000; Santa Cruz) in blocking solution overnight at 4° C. The second day consisted of rinses 5 × 5 min and incubation for 2 h in blocking solution with donkey anti-rabbit Alexa-Fluor 647 (1:500; Jackson ImmunoResearch, West Grove, Pennsylvania). After a final series of rinses, sections were mounted on Superfrost Plus slides (Fisher) in KPB, allowed to air dry, and then coverslipped using polyvinyl alcohol with DABCO (Sigma).

2.4.3—CRH-reporter mice were used for double-immunofluorescent labeling of OT and Fos. The protocol used was identical to the one used above (2.4.2) except for the addition of primary antibody (mouse anti-oxytocin neurophysin, PS-38 1:400; generously provided by Dr. Gainer, NIH) [11] to the blocking solution containing Fos antibody on day one. Additionally, the secondary antibody, donkey anti-mouse Alexa-Fluor 488 (1:500; Jackson

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

ImmunoResearch, West Grove, Pennsylvania), was mixed with donkey anti-rabbit Alexa-Fluor 647 in blocking solution on day two.

2.4.4—Following perfusion fixation as detailed above (2.4.1), pituitary glands from CRH-reporter mice were extracted and cryoprotected with brains in 30% sucrose. 15 μm sections were thaw-mounted on slides and processed for immunofluorescent identification of proopiomelanocortin (POMC) at room temperature in a humidified chamber. Following rinses in KPBS, sections were blocked with a 2% NDS and 0.2% Triton X-100 KPBS solution and then incubated overnight with primary antibody (Rabbit anti-POMC; 1:1000, Phoenix Pharmaceuticals) in blocking solution. The following day, slides were rinsed and sections were incubated for 2 hours in secondary antibody (Alexa 488 Donkey anti-Rabbit; 1:500, Jackson), rinsed, and coverslipped.

2.5 Image capture and analysis

All images were captured using an AxioImager M.2 fluorescent microscope (Carl Zeiss, Thornwood, New York) connected to a PC running Axiovision 4.8. Using a Plan-Apochromat 10x/0.45 M27 objective, z-stacks of tdTomato and Fos expression were captured through the PVN (from bregma −0.46mm to −1.22mm) using anatomical landmarks found in *The Mouse Brain in Stereotaxic Coordinates 3rd Ed.*[12]. Image size was 1388 × 1040 pixels and each z-step was 1 μm with an average of 20 optical sections per PVN image. Excitation/emission spectra used to image tdTomato and Fos were 540/580 nm and 649/670 nm, respectively. Exposure time was automatically set by the software and varied from 400–600 ms for the channel capturing tdTomato images and 1–3 s for the channel capturing Fos images. Quantification of Fos positive tdTomato neurons took place by first opening each z-stack in Axiovision 4.8 and then marking co-labeled neurons using an event marker function. To verify that Fos-positive nuclei were within tdTomato neurons, z-stacks were scrolled through and channels were turned on and off as needed. Fos counts were performed by personnel blind to treatment conditions on matched sections from both sides of the PVN and then averaged for animals injected with 0.15 M NaCl and 2.0 M NaCl. Image capture and analysis of Fos positive OT neurons was performed using the same methods. Visualization of OT used an excitation/emission of 470/509 and exposure of ~700 ms while parameters for Fos were similar to those described above.

2.6 Behavioral Testing

Naïve C57BL/6 mice were injected with 2.0 M NaCl (n=13) or 0.15 M NaCl (n=12) and water deprived for 60 min before assessment of anxiety-like behavior on an EPM. During the light phase, mice were brought one at a time into a procedure room with a black curtain which separated the EPM from the experimenters. Testing began by placing a mouse in the center of an orthogonally-oriented two plank maze (62 cm × 62 cm) facing an open arm. A 5 min period of exploration was recorded by a ceiling-mounted video camera connected to a PC running TopScan software (TopScan; CleverSys, Reston, Virginia). Simultaneous video tracking of the mouse's position allowed for the automated scoring of time spent in the open arms and total distance traveled. The testing apparatus was cleaned with 30% ethanol between subjects.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

2.7 Statistics

All data presented as mean +/− SEM. Plasma sodium, hematocrit, plasma proteins, EPM data, Fos positive OT cells and Fos positive CRH cells were assessed with a 2-tailed *t* test. Plasma CORT was analyzed using a 2-factor ANOVA using GraphPad Prism version 5.04 for Windows (GraphPad Software; San Diego, California).

3. Results

3.1 Subcutaneous delivery of 2.0 M NaCl modestly increases pNa^+ but blunts restraint-induced elevations in plasma CORT

3.1.1—Analysis of blood samples taken from mice given injections of 2.0 M NaCl found a slight but significant increase in pNa^+ relative to controls injected with 0.15 M NaCl (Figure 1A). Hematocrit and plasma protein measurements were similar between hypernatremic and control mice.

3.1.2—Plasma CORT analysis revealed a significant time X condition interaction [Figure 2, $F(3,39) = 3.48$, ($P<0.05$)]. Restraint increased CORT at 30 min compared to baseline concentrations in both groups; however, mice injected with 2.0 M NaCl had an attenuated rise at 30 and 60 min compared with mice injected with 0.15 M NaCl. This attenuation was specific to these time points as both baseline and 120 min CORT concentrations were not significantly different between groups. With respect to interpolated time points, the area under the curve was also decreased relative to controls [Figure 2 inset, ($P<0.05$)].

3.2 tdTomato expressing neurons are consistent with CRH expression in the hypothalamus and pituitary

Figure 3 (A–C) depicts a unilateral PVN fluorescent photomicrograph from a coronal section taken from the brain of a CRH-reporter mouse and processed for *in situ* hybridization. Highly specific single-strand fluorescent labeling of CRH mRNA revealed consistent co-localization of CRH probe and tdTomato fluorescence (Figure 3C). Figure 3D shows a qualitative image of the ventral portion of a coronal brain section taken from the hypothalamus of a CRH-reporter mouse and processed for immunofluorescent labeling of OT. Consistent with hypothalamic-pituitary axonal projections, green OT fibers and red tdTomato fibers are seen in the internal and external zones of the median eminence, respectively. Figure 3E depicts a coronal section through the anterior pituitary of a CRH-reporter mouse processed for immunofluorescent labeling of POMC. POMC and tdTomato colocalize (inset) in pituicytes consistent with CRH activation of corticotrophs.

3.3 Acute hypernatremia inversely affects restraint-induced activation of CRH and OT neurons in the PVN

Figure 4 shows photomicrographs of atlas matched sections through the PVN containing tdTomato reporting of CRH positive neurons and immunofluorescent labeling of Fos. Whereas control mice (Figure 4A) expressed robust restraint-induced Fos expression in CRH neurons, this same region displayed fewer activated CRH neurons following 2.0 M NaCl injection (Figure 4B). Compared with mice injected with 0.15 M NaCl, mice injected with 2.0 M NaCl had significantly less ($P<0.05$) restraint-induced Fos positive CRH neurons

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

(Figure 4C). Conversely, mice injected with 2.0 M NaCl and restrained (Figure 5B) exhibited a significant [Figure 5C, ($P<0.05$)] increase in Fos induction in OT neurons relative to controls (Figure 5A).

3.4 Slight elevations in the pNa^+ are associated with decreased anxiety-like behavior in the EPM

Mice injected with 2.0 M NaCl spent more time exploring the open arms of an EPM than mice injected with 0.15 M NaCl [Figure 6A, ($P<0.05$)]. This increased exploration was not due to an overall increase in locomotion as the total distances traveled were similar between groups (Figure 6B).

4. Discussion

The goal of the current study was to determine whether the anxiolytic and stress dampening effects of acute mild hypernatremia occur in laboratory mice. To this end, mice systemically delivered 2.0 M NaCl had a modest but significant increase in the pNa^+ relative to control mice treated with 0.15 M NaCl. The slight rise in the pNa^+ that was observed in mice administered 2.0 M NaCl was associated with an attenuated restraint-induced activation of the HPA axis and increased time spent in the open arms of an EPM. We conducted neuroanatomical studies to evaluate how acute hypernatremia may interact with restraint-stress to affect Fos induction, a marker of neuronal activation, in PVN neurons expressing OT or CRH. Acute hypernatremia elicited robust Fos induction within OT neurons but significantly decreased Fos within CRH neurons. Our results, in conjunction with previous studies, suggest that acute hypernatremia dampens stress responsiveness, in part, by promoting OT-mediated inhibition of CRH neurons. The implication is that increased salt intake may be used as a coping strategy to alleviate the impact of psychological stressors.

Mice rendered hypernatremic via systemic administration of 2.0 M NaCl had a slight (≈2%) but significant increase in the pNa^+ relative to control mice delivered 0.15 M NaCl. While this increase stimulates OT and AVP release as well as osmotically driven water intake [13] [14], the elevation in the pNa^+ observed in our study is modest relative to the order of magnitude (10% increase) that is required to increase brain osmolytes to defend the CNS against the damaging effects of severe hypernatremia [15]. Our experimental paradigm, does however, produce an increase in the pNa^+ that is strikingly similar to that which occurs in humans and animals given excess dietary sodium to cause increases in blood pressure or the development of salt-sensitive hypertension, respectively [16] [17] [18] [19] [20]. Thus, our experimental model of hypernatremia produces a modest increase in the pNa^+ that likely affects cardiovascular function as well as endocrine and behavioral responses to psychogenic stress.

Activation of the HPA axis is an endocrine measure of stress responsiveness that is initiated by excitation of CRH neurons in the PVN which stimulate ACTH secretion from the anterior pituitary, which in turn, promotes CORT release from the adrenal cortex. Relative to isonatremic controls, mice rendered mildly hypernatremic had decreased plasma CORT subsequent to the onset of restraint. These results are in agreement with our previous studies demonstrating that rats subjected to a similar degree of hypernatremia have decreased

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

plasma CORT subsequent to a restraint challenge [8] [9]. Our previous study also determined that acute hypernatremia decreases the secretion of adrenocorticotropic-releasing-hormone (ACTH) into the systemic circulation [8]. Because the release of ACTH is controlled by excitation of CRH neurons in the PVN we hypothesized that acute hypernatremia may attenuate Fos induction in PVN neurons producing tdTomato, a marker for CRH expression. Importantly, mRNA for CRH was present in $\approx 95\%$ of all tdTomato producing neurons in the PVN, thereby demonstrating the validity of CRH-reporter mice. Consistent with our hypothesis, restraint-evoked Fos induction of tdTomato producing neurons was significantly decreased in mice subjected to acute hypernatremia relative to controls. Taken together, these results suggest that acute hypernatremia dampens stress-induced activation of the HPA axis by inhibiting CRH neurons in the PVN.

Hypernatremic mice had strong Fos induction in OT neurons in the PVN relative to isonatremic controls and these results are consistent with those from our previous research using rats to demonstrate that acute hypernatremia combined with restraint more than tripled the number of Fos positive OT neurons in the PVN compared to restraint alone [8]. Work conducted by Ludwig and colleagues established that systemic hypernatremia triggers the dendritic release of OT, which produces robust and sustained elevations in central levels of this peptide [21]. Given that exogenous administration of OT decreases stress-induced activation of the HPA axis [22] [23], it is possible that acute hypernatremia triggers endogenous release of OT within the CNS, which inhibits activation of CRH neurons, and consequently, blunts HPA activity. In support of this, electrophysiological studies conducted by Frazier and colleagues [9] revealed that acute hypernatremia created an inhibitory tone on putative CRH neurons that was dependent on activation of oxytocin receptors. Therefore, elevating the pNa^+ may cause activation of osmosensitive OT neurons in the hypothalamus, which influence excitation of neurons responsive to psychogenic stress by releasing OT into the CNS.

Hypernatremia and restraint likely utilize different neural circuits to activate the PVN and both are known to elicit robust Fos induction in this nucleus [24, 25]. Our previous study found that hypernatremia followed by restraint significantly increased Fos in OT and AVP containing neurons; however, the total number of cells expressing Fos within the PVN was similar to that of isonatremic animals subjected to restraint [8]. These results suggested that the effects of hypernatremia and restraint were not additive. Given that hypernatremia blunted restraint-induced activation of the HPA axis [8], we hypothesized that hypernatremia activated OT and AVP containing neurons, but inhibited CRH containing neurons in the PVN. Consistent with this hypothesis, the present study determined that hypernatremia followed by restraint significantly decreased Fos induction in tdTomato expressing neurons in the PVN. Taken together, the results suggest that hypernatremia selectively activates OT and AVP containing neurons in the PVN but inhibits those that express CRH, which accounts for the similarities in total Fos induction observed between hypernatremic and isonatremic animals subjected to restraint.

In addition to attenuating stress-induced activation of the HPA axis, the present study found that acute hypernatremia decreased anxiety-like behavior in the EPM relative to that of isonatremic controls. Once again, these results are consistent with those from our previous

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

studies using rats that found that slight elevations in the pNa^+ are anxiolytic, especially in social situations [8] [9]. Central administration of OT decreases anxiety-like behavior in the EPM [26] and OT efferents are found in limbic brain regions [27] like the amygdala, that are heavily implicated in the expression of fear and anxiety-like behavior [28]. Of relevance, some of the OT efferents in the amygdala arise from magnocellular oxytocinergic neurons in the PVN and supraoptic nucleus [27]. Magnocellular oxytocinergic neurons are osmosensitive and become excited by elevations in the pNa^+ [29]. Therefore, it is possible that the anxiolytic effects of acute hypernatremia result from excitation of osmosensitive OT neurons with axonal projections to limbic brain nuclei controlling the expression of fear and anxiety-like behavior.

Collectively our results demonstrate that the anxiolytic and stress dampening effects of acute hypernatremia extend to mice. Consequently, these results allow the use of mice and the genetic manipulations that this animal model affords to further investigate the neural and humoral mechanism(s) underlying the stress limiting effects of acute hypernatremia. From a broader perspective, our results may provide insight as to why some patients with salt-sensitive hypertension and high levels of life-stress [30] [31] [32] also have difficulties complying with restricted dietary sodium intake [33].

Acknowledgments

Support: NIH HL096830 (EGK), HL116074 (ADdK)

References

1. Häfner S, Baumert J, Emeny RT, Lacruz ME, Bidlingmaier M, Reincke M, et al. Hypertension and depressed symptomatology: a cluster related to the activation of the renin-angiotensin-aldosterone system (RAAS). Findings from population based KORA F4 study. Psychoneuroendocrinology. Oct. 2013 38:2065–74. [PubMed: 23608138]
2. Murck H, Held K, Ziegenbein M, Künzel H, Koch K, Steiger A. The renin-angiotensin-aldosterone system in patients with depression compared to controls--a sleep endocrine study. BMC Psychiatry. Oct.2003 3:15. [PubMed: 14585110]
3. Grippo AJ, Francis J, Beltz TG, Felder RB, Johnson AK. Neuroendocrine and cytokine profile of chronic mild stress-induced anhedonia. Physiol Behav. Apr.2005 84:697–706. [PubMed: 15885245]
4. Niebylski A, Boccolini A, Bensi N, Binotti S, Hansen C, Yaciuk R, et al. Neuroendocrine Changes and Natriuresis in Response to Social Stress in Rats. Stress and Health. Aug.2012 28:179–185. [PubMed: 22282077]
5. Neumann ID, Landgraf R. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends in Neurosciences. 2012; 35:649–659. [PubMed: 22974560]
6. Benarroch EE. Oxytocin and vasopressin Social neuropeptides with complex neuromodulatory functions. Neurology. Apr.2013 80:1521–1528. [PubMed: 23589638]
7. Leshem M. Low dietary sodium is anxiogenic in rats. Physiology & Behavior. Jul.2011 103:453–458. [PubMed: 21453714]
8. Krause EG, de Kloet AD, Flak JN, Smeltzer MD, Solomon MB, Evanson NK, et al. Hydration state controls stress responsiveness and social behavior. J Neurosci. Apr.2011 31:5470–6. [PubMed: 21471383]
9. Frazier CJ, Pati D, Hiller H, Nguyen D, Wang L, Smith JA, et al. Acute Hypernatremia Exerts an Inhibitory Oxytocinergic Tone That Is Associated With Anxiolytic Mood in Male Rats. Endocrinology. Jul.2013 154:2457–2467. [PubMed: 23653461]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

10. Hoffman GE, Smith MS, Verbalis JG. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. Front Neuroendocrinol. Jul.1993 14:173–213. [PubMed: 8349003]
11. Ben-Barak Y, Russell JT, Whitnall MH, Ozato K, Gainer H. Neurophysin in the hypothalamo-neurohypophysial system. I. Production and characterization of monoclonal antibodies. J Neurosci. Jan.1985 5:81–97. [PubMed: 3880813]
12. Franklin, KBJ.; Paxinos, G. The Mouse Brain in Stereotaxic Coordinates, Compact Third Edition. 3. 2008.
13. Stricker EM, Verbalis JG. Interaction of osmotic and volume stimuli in regulation of neurohypophyseal secretion in rats. Am J Physiol. Feb.1986 250:R267–75. [PubMed: 3946641]
14. Krause EG, Melhorn SJ, Davis JF, Scott KA, Ma LY, de Kloet AD, et al. Angiotensin Type 1 Receptors in the Subfornical Organ Mediate the Drinking and Hypothalamic-Pituitary-Adrenal Response to Systemic Isoproterenol. Endocrinology. Dec.2008 149:6416–6424. [PubMed: 18687780]
15. Heilig CW, Stromski ME, Blumenfeld JD, Lee JP, Gullans SR. Characterization of the major brain osmolytes that accumulate in salt-loaded rats. Am J Physiol. Dec.1989 257:F1108–16. [PubMed: 2603957]
16. He FJ, Markandu ND, MacGregor GA. Modest salt reduction lowers blood pressure in isolated systolic hypertension and combined hypertension. Hypertension. Jul.2005 46:66–70. [PubMed: 15956111]
17. de Wardener HE, He FJ, MacGregor GA. Plasma sodium and hypertension. Kidney Int. Dec.2004 66:2454–66. [PubMed: 15569339]
18. Fang Z, Carlson SH, Peng N, Wyss JM. Circadian rhythm of plasma sodium is disrupted in spontaneously hypertensive rats fed a high-NaCl diet. Am J Physiol Regul Integr Comp Physiol. Jun.2000 278:R1490–5. [PubMed: 10848515]
19. O'Donaughy TL, Qi Y, Brooks VL. Central action of increased osmolality to support blood pressure in deoxycorticosterone acetate-salt rats. Hypertension. Oct.2006 48:658–63. [PubMed: 16966581]
20. O'Donaughy TL, Brooks VL. Deoxycorticosterone acetate-salt rats: hypertension and sympathoexcitation driven by increased NaCl levels. Hypertension. Apr.2006 47:680–5. [PubMed: 16520400]
21. Ludwig M, Leng G. Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci. Feb.2006 7:126–36. [PubMed: 16429122]
22. Windle RJ, Shanks N, Lightman SL, Ingram CD. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology. Jul.1997 138:2829–34. [PubMed: 9202224]
23. Windle RJ, Kershaw YM, Shanks N, Wood SA, Lightman SL, Ingram CD. Oxytocin attenuates stress-induced c-fos mRNA expression in specific forebrain regions associated with modulation of hypothalamo-pituitary-adrenal activity. J Neurosci. Mar.2004 24:2974–82. [PubMed: 15044536]
24. Sharp FR, Sagar SM, Hicks K, Lowenstein D, Hisanaga K. C-FOS MESSENGER-RNA, FOS, AND FOS-RELATED ANTIGEN INDUCTION BY HYPERTONIC SALINE AND STRESS. Journal of Neuroscience. Aug.1991 11:2321–2331. [PubMed: 1908006]
25. Herman JP, Cullinan WE. Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. Trends in Neurosciences. Feb.1997 20:78–84. [PubMed: 9023876]
26. Mak P, Broussard C, Vacy K, Broadbear JH. Modulation of anxiety behavior in the elevated plus maze using peptidic oxytocin and vasopressin receptor ligands in the rat. J Psychopharmacol. Apr. 2012 26:532–42. [PubMed: 21890582]
27. Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, et al. Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron. Feb.2012 73:553–66. [PubMed: 22325206]
28. Davis M, Rainnie D, Cassell M. Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci. May.1994 17:208–14. [PubMed: 7520203]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

29. Hattori T, Morris M, Alexander N, Sundberg DK. Extracellular oxytocin in the paraventricular nucleus: hyperosmotic stimulation by in vivo microdialysis. Brain Research. Jan 1.1990 506:169–171. [PubMed: 2105821]
30. Calhoun DA. Hypertension in blacks: socioeconomic stress and sympathetic nervous system activity. Am J Med Sci. Nov.1992 304:306–11. [PubMed: 1442872]
31. Calhoun DA, Oparil S. Racial differences in the pathogenesis of hypertension. Am J Med Sci. Dec; 1995 310(Suppl 1):S86–90. [PubMed: 7503132]
32. Barnes V, Schneider R, Alexander C, Staggers F. Stress, stress reduction, and hypertension in African Americans: an updated review. J Natl Med Assoc. Jul.1997 89:464–76. [PubMed: 9220696]
33. Epstein DE, Sherwood A, Smith PJ, Craighead L, Caccia C, Lin PH, et al. Determinants and consequences of adherence to the dietary approaches to stop hypertension diet in African-American and white adults with high blood pressure: results from the ENCORE trial. J Acad Nutr Diet. Nov.2012 112:1763–73. [PubMed: 23000025]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Highlights

- Hypernatremia decreased activation of the HPA axis and anxiety-like behavior
- Hypernatremia activated OT neurons but inhibited CRH neurons.
- Excess dietary sodium intake may alleviate the impact of psychological stress.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

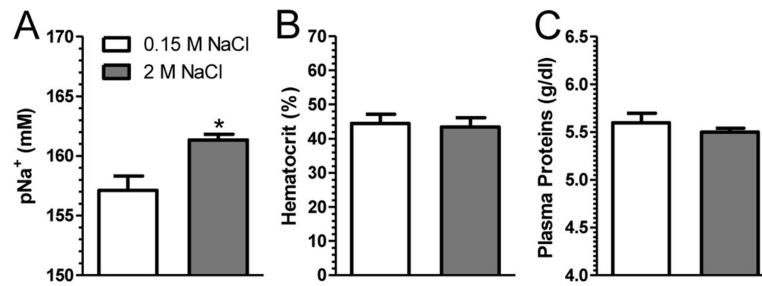

Fig. 1.

Plasma measurements from mice 60 min after 2.0 M or 0.15 M NaCl injections. (A) Injections of 2.0 M NaCl significantly increased pNa^+ relative to control injections of 0.15 M NaCl, but had no effect on (B) hematocrit or (C) plasma proteins. *$p<0.05$ Error bars indicate SEM.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

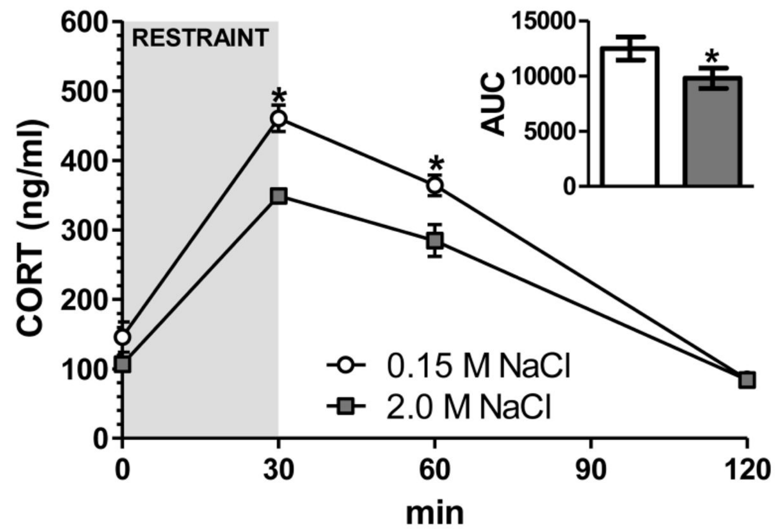

Fig. 2.
2.0 M NaCl administration reduces the CORT response to restraint stress relative to 0.15 M NaCl. The integrated CORT response was also significantly reduced. *$p<0.05$ 0.15 M NaCl vs 2.0 M NaCl. AUC=Area Under the Curve. Error bars indicate SEM.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

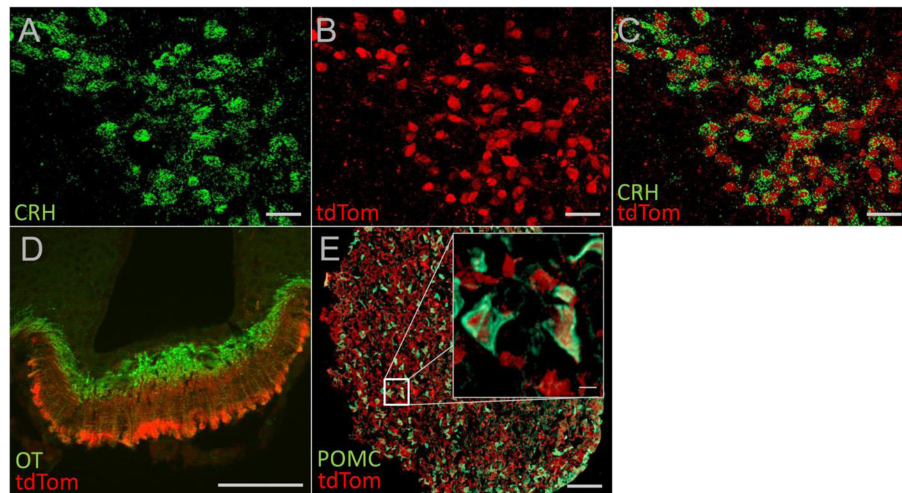

Fig. 3.

Representative images of tdTomato expression. TOP: A unilateral coronal section through the PVN depicting two images of the same set of neurons labeled for (A) CRH mRNA probe amplification (green) and (B) tdTomato (red soma). A merged image (C) illustrates a high degree of CRH mRNA and tdTomato co-localization. Scale bars = 20 μm. BOTTOM: tdTomato expression adjacent to (D) OT in the median eminence and (E) colocalized with POMC in the anterior pituitary. Scale bars = 100 μm. Inset scale bar = 5 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

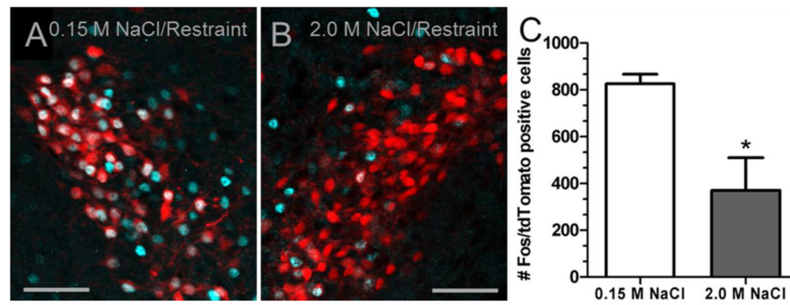

Fig. 4.

2.0 M NaCl injection attenuates restraint-induced activation of CRH neurons. (A) Representative photomicrograph of a unilateral coronal section through the PVN depicting Fos induction (cyan nuclei) in tdTomato (red soma) containing neurons following 0.15 M NaCl and restraint. (B) Representative photomicrograph of a unilateral coronal section through the PVN depicting Fos induction in tdTomato containing neurons following 2.0M NaCl and restraint. (C) The group mean for Fos induction in tdTomato containing neurons was significantly less for mice subjected to 2.0 M NaCl injection and restraint relative to control. *$p<0.05$ Scale bars = 50 μm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

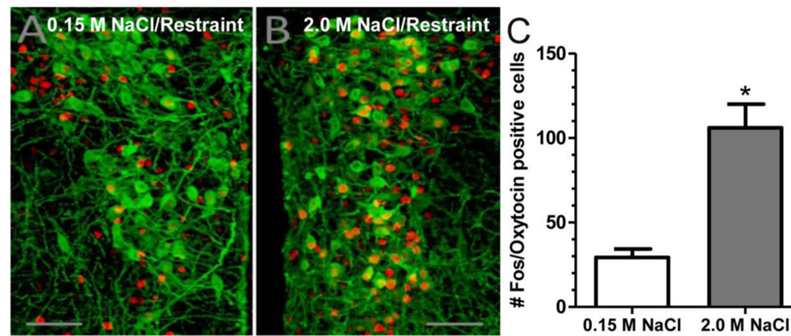

Fig. 5.

2.0 M NaCl administration and restraint increases activation of OT-producing cells in the PVN. (A) Representative photomicro- graph of a unilateral coronal section depicting Fos induction (red nuclei) in OT (green cell bodies) containing neurons following 0.15 M NaCl and restraint. (B) Representative photomicrograph of a unilateral coronal section depicting Fos induction in OT neurons following 2.0 M NaCl and restraint. (C) The group mean for Fos induction in OT-producing cells was significantly more for mice subjected to 2.0 M NaCl injection and restraint relative to control. *$p<0.05$ Scale bars = 50 μm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

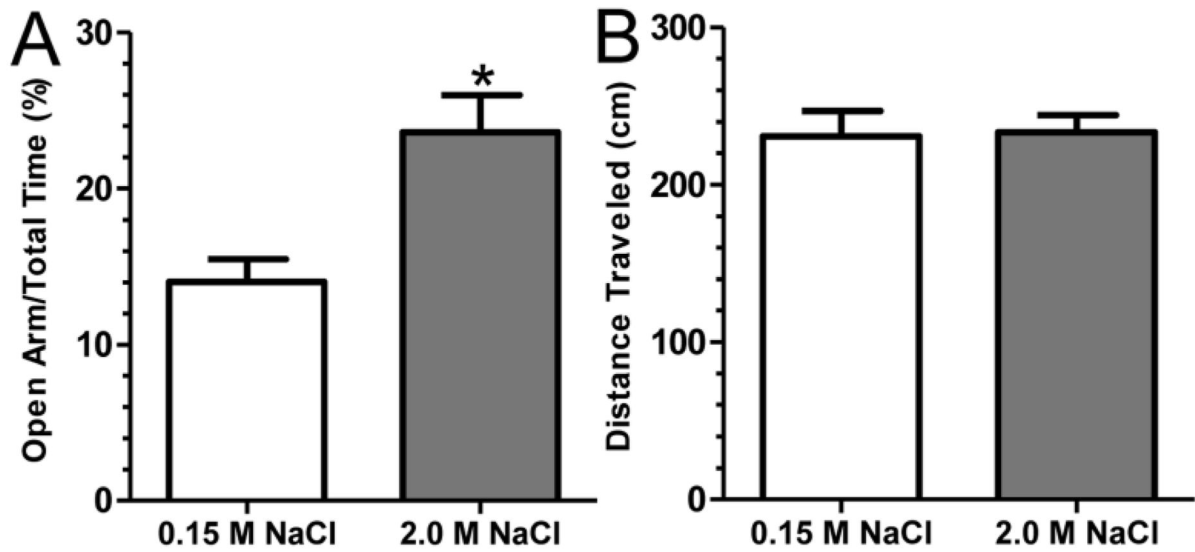

Fig. 6.

Acute hypernatremia attenuates anxiety-like behavior. (A) Mice treated with 2.0 M NaCl spent a greater proportion of time exploring the open arms of an EPM than mice treated with 0.15 M NaCl. (B) Overall locomotion was unaffected by 2.0 M NaCl as total distance traveled was similar for each group. * $p < 0.05$. Error bars indicate SEM.