The role of the lateral parabrachial nucleus in the control of sodium appetite

Jose V. Menani, Laurival A. De Luca Jr. and Alan Kim Johnson

Dept of Physiology and Pathology, School of Dentistry, São Paulo State University, UNESP, Araraquara, SP, 14801-903, Brazil; Depts of Psychology, Pharmacology and Health and Human Physiology and the Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242–1407, USA.

Corresponding author
José Vanderlei Menani, Ph. D.
Department of Physiology and Pathology
School of Dentistry, São Paulo State University, UNESP
14801-903, Araraquara, SP, Brazil
Phone: +55 (16) 3301 6486
FAX: +55 (16) 3301 6488
E-mail: menani@foar.unesp.br
Abstract

In states of sodium deficiency many animals seek and consume salty solutions to restore body fluid homeostasis. These behaviors reflect the presence of sodium appetite which is a manifestation of the pattern of activity in central nervous system (CNS) circuitry that has both facilitatory and inhibitory components and is controlled by several neuro-humoral factors. The primary focus of this review is on one structure in this central system, the lateral parabrachial nucleus (LPBN). However, before turning to a more detailed discussion of the LPBN, a brief overview of body fluid balance-related body-to-brain signaling and the identification of the primary CNS structures and humoral factors involved in the control of sodium appetite is necessary. Angiotensin II, mineralocorticoids and extracellular osmotic changes act on forebrain areas to facilitate sodium appetite and thirst. In the hindbrain, the LPBN functions as a key integrative node with an ascending output that exerts inhibitory influences on forebrain regions. A nonspecific or general deactivation of LPBN-associated inhibition by GABA or opioid agonists produces NaCl intake in euhydrated rats without any other treatment. Selective LPBN manipulation of other neurotransmitter systems (e.g., serotonin, CCK, CRH, glutamate, ATP, or noradrenaline) greatly enhances NaCl intake when accompanied by additional treatments that induce either thirst or sodium appetite. The LPBN interacts with key forebrain areas that include the subfornical organ and central amygdala to determine sodium intake. To summarize, a model of LPBN inhibitory actions on forebrain facilitatory components for the control of sodium appetite is presented in this review.
Key words: sodium intake, angiotensin, electrolyte balance, thirst.
Introduction

Animals constantly lose water and electrolytes to the external environment, but specific autonomic, hormonal and behavioral mechanisms operate continuously to adjust and restore body fluid-electrolyte balance. The rate of loss from the kidneys is primarily controlled by neural and hormonal actions, but loss may also occur passively through the largely uncontrolled processes of respiration (evaporation), perspiration, transpiration and salivation. These later modes of loss are referred to as insensible loss because they cannot be easily measured. Severe loss of both sodium and water may also occur in disorders associated with emesis and diarrhea. However, in spite of such ongoing challenges, the osmolarity and volume of body fluids are maintained within reasonably narrow limits, thus allowing normal metabolic and cardiovascular functions. Although renal mechanisms can slow water and sodium loss, the restoration of fluid homeostasis is only made possible by the mobilization of behaviors that result in the ingestion of water and sodium (usually NaCl). The behavioral responses of seeking out and consuming water and salty substances involve the motivational states of thirst and salt appetite. Salt appetite, is also frequently referred to as sodium appetite, and salt or sodium hunger. The state of sodium appetite can be experimentally induced, tested and operationally defined by the ingestion of hypertonic NaCl solutions (in most cases 0.3 or 0.5 M NaCl) at concentrations that are usually aversive to rats in sodium balance (5, 50, 76, 77).

The behavioral, endocrine and autonomic mechanisms that control sodium balance involve an extensive central circuitry comprised of both forebrain and hindbrain structures (5, 50, 76, 77, 120). Within this brain circuitry, the lateral...
parabrachial nucleus (LPBN) stands as an important hindbrain region playing a pivotal role in the control of water and sodium intake.

This review begins by presenting an overview of the neural and humoral mechanisms controlling behaviors associated with body fluid homeostasis. It then turns to focus on 1) the role of the LPBN in the control of sodium intake by the rat, 2) how different neurotransmitters acting in the LPBN are related to behavioral inhibitory mechanisms, and 3) a current understanding of how LPBN-dependent inhibitory mechanisms interact with facilitatory processes to control sodium intake.

**Body-brain signaling and the central nervous system (CNS) neural network maintaining body fluid homeostasis**

The subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT), located in the forebrain, and the area postrema (AP) and the nucleus of the solitary tract (NTS), located in the hindbrain, are sensory portals through which body fluid-related information enters the brain to be processed in order to coordinate mechanisms that control water and sodium intake. Areas comprising this neural network include the median preoptic nucleus (MnPO), septal area (SA), medial and lateral hypothalamus, bed nucleus of the stria terminalis (BNST), nucleus accumbens, parvocellular ventral posteromedial thalamic nucleus and parabrachial nucleus (PBN) (2-5, 27, 32-44, 75-77, 86, 88-97, 105, 106, 108, 113, 115-117, 120, 132, 133).

A decrease in blood volume releases humoral factors that act on the brain to facilitate sodium and water intake. Specifically, angiotensin II (ANG II) acts in conjunction with mineralocorticoids (aldosterone) to generate sodium intake (50,
76, 77) and hyperosmolality to produce thirst (76, 77). In contrast, blood volume expansion releases humoral factors, such as atrial natriuretic peptide (ANP), which inhibit salt and water intake (5, 6, 14, 49). Other agonists and receptor subtypes, such as oxytocin and $\alpha_2$-adrenoceptors, act in the brain to inhibit behavioral responses that expand body fluids (12, 13, 39, 40, 48, 93, 123, 125).

ANG II and hyperosmolality act on sites that function as sensory structures, specifically the OVLT, SFO and AP which are located outside the blood-brain barrier. In some cases they also act on structures located within the blood-brain barrier such as the supraoptic nucleus of the hypothalamus (25, 26, 75-77, 84, 86, 118, 119). ANG II acts on angiotensin type I receptors (AT$_1$-R) which are present in neurons and which by activating two different intracellular pathways have been proposed to lead to either water or sodium intake (31, 75-77, 86). Hyperosmolality activates nonselective cation channels (e.g. TRPV-related channels) and hypernatremia activates Na$_x$ channels (25, 26, 84, 118, 119).

The NTS contains the first synapse in the CNS that receives input from systemic visceral receptors. Signals that arise from peripheral high and low pressure baroreceptors (23, 30) can inhibit thirst and sodium appetite when blood volume is expanded or blood pressure is high and facilitate thirst and salt intake when pressure and volume are low (79, 112, 128, 129). Other systemic visceral receptors and afferents including osmoreceptors, taste receptors (36, 67, 76, 77) and possibly renal receptors (127) also influence thirst and sodium intake.

Mineralocorticoid receptors (MR) implicated in the control of sodium intake are located in both the forebrain and hindbrain at areas which have direct anatomical and physiological links with the LPBN (53, 60, 62-67, 102, 115, 117,
In the forebrain, electrolytic lesions or injections of antisense oligodeoxynucleotides against MR into components (medial or central nuclei) of the amygdala, abolish sodium appetite induced by the peripheral administration of aldosterone (ALDO) or deoxycorticosterone acetate (DOCA) (60, 102, 115, 117, 133). Direct administration of aldosterone or DOCA into the amygdala of sodium-replete rats also induces hypertonic NaCl intake (115). In addition, lesions of the BNST, an anatomical extension of central amygdala, reduce experimentally-induced sodium intake (108, 132).

In the hindbrain, ALDO-sensitive neurons of the NTS have been implicated in the control of sodium intake (62-67). These neurons, referred to as HSD2 neurons, co-express MR and the enzyme 11 β-hydroxysteroid dehydrogenase type 2 (HSD2) (62-67). The HSD2 enzyme inactivates endogenous glucocorticoids, and thus allows only ALDO to access the MR (104). HSD2 neurons connect with the LPBN and amygdala (64, 65) and are likely to play a role in sodium homeostasis because they are activated in sodium depleted rats and inactivated by sodium ingestion (62-67). Chronic infusions of ALDO into the 4th ventricle strongly increase daily sodium intake, whereas the injection of the mineralocorticoid antagonist RU 28318 into the 4th ventricle acutely reduces sodium depletion-induced NaCl intake (53). This suggests that ALDO acting on MR in the hindbrain, and most likely on cells containing HSD2 in the NTS, activates an important mechanism involved in the control of sodium appetite (53).

Major neurohumoral factors inhibiting water and/or sodium intake
Oxytocin

There is an inverse correlation between oxytocin secretion from the posterior pituitary and sodium appetite. Oxytocin release in the brain seems to parallel the secretion of this hormone into the blood, and oxytocin in both systems is activated by similar stimuli, including ANG II (12, 45, 46, 123). However, the oxytocin found and released in the CNS inhibits sodium appetite (12-14, 121-124). Intracerebroventricular (icv) injections of oxytocin reduce sodium intake (12-14), and central delivery of an oxytocin receptor antagonist, or the destruction of brain neurons containing oxytocin receptors by oxytocin conjugated to the cytotoxin ricin, increase hypertonic NaCl intake in rats treated with a central injection of ANG II (12, 123). The inactivation of central neurons containing oxytocin receptors also reduces hyperosmolarity-induced inhibition of sodium intake (13, 14). The central release of oxytocin by ANG II might explain why acute administration of ANG II immediately induces thirst, and only later when oxytocin release is reduced as a result of the dilution of body fluids by the ingested water, is sodium appetite expressed (12).

Atrial natriuretic peptide

ANP and oxytocin are both secreted under conditions of body fluid volume expansion. In addition to the inhibition of water and sodium intake, both peptides act on the kidney to increase diuresis and natriuresis (5). The icv injection of ANP reduces water and sodium intake induced by water deprivation, sodium depletion or central injections of ANG II (5, 6, 49). Central administration of antibodies against ANP or the inactivation of brain neurons containing ANP receptors with icv
injections of ANP conjugated to the cytotoxin ricin, increase hypertonic NaCl intake in response to ANG II or hypovolemia (14, 56).

Central $\alpha_2$ adrenergic mechanisms

Noradrenaline acting centrally has a dual role in the control of sodium and water intake. Acting on forebrain $\alpha_1$- or hindbrain $\alpha_2$-adrenoceptors, noradrenaline facilitates both behaviors. In contrast, acting on forebrain $\alpha_2$-adrenoceptors noradrenaline inhibits them (10, 11, 28, 40, 93, 125). Similar to noradrenaline, either icv or forebrain parenchymal injections into the lateral hypothalamus, medial septal area or preoptic area of $\alpha_2$-adrenoceptor agonists (e.g., $\alpha$-methylnoradrenaline) or of $\alpha_2$-adrenergic/imidazoline receptor agonists (e.g., clonidine or moxonidine) also inhibit water and sodium intake (39, 40, 47, 93, 125).

The effects of $\alpha_2$-adrenoceptor or $\alpha_2$-adreno/imidazoline receptor agonists on sodium and water intake are reversed by the pre-treatment with $\alpha_2$-adrenoceptor antagonists (e.g., yohimbine, RX 821002 or SK&F86466), which suggests that the activation of $\alpha_2$-adrenoceptors plays a role in the inhibition of water and sodium intake (40, 47, 93, 125). The increased efficacy provided by the imidazoline moiety to several $\alpha_2$-adrenoceptor agonists and antagonists suggests a synergic action between imidazoline receptors and $\alpha_2$-adrenoceptors acting to inhibit water and salt intake (40, 93, 125).

Peripheral administration of clonidine and moxonidine also inhibits water and sodium intake, whereas peripheral administration of yohimbine has the opposite effect (40, 57, 93, 132). Central antagonism of $\alpha_2$-adrenoceptors by yohimbine or RX 821002 reduces the antinatriorexigenic effects of
lipopolysaccharide which suggests that central adrenergic mechanisms are involved in the inhibition of sodium intake in pathological conditions such as bacterial or viral infections (1).

Serotonin

Serotonin receives particular attention here because it is associated with the initial findings and subsequent work that focuses research on the role of the LPBN in the control of salt and water intake. Several pharmacological studies indicate that endogenous serotonin acts on different receptor sub-types (e.g. 5HT$_1$, 5HT$_2$, 5HT$_3$), particularly in the forebrain, to inhibit sodium appetite (19, 29, 52, 58, 114). Drinking studies in the pigeon suggest that an homologous serotonergic system inhibits fluid intake in both birds and mammals (18). Consistent with the inhibitory effect that serotonin receptor activation has on sodium appetite, early studies also showed that when given systemically, agonists that reduce serotonin release or antagonists that act on 5HT$_2$ receptors increase sodium intake (29, 114).

Studies showing enhanced sodium intake in rats with electrolytic or ibotenic acid lesions in the dorsal raphe nucleus (DRN), suggest that the DRN is a source of endogenous serotonin that inhibits salt appetite (20, 21). Such inhibition is possibly linked to reduced ANP release and modulation of forebrain activity in circumventricular organs (9, 87, 109, 110). Moreover, in sodium depleted rats, the ingestion of hypertonic or isotonic saline results in reduced activity in serotonin neurons of the DRN (54, 69). Together these results suggest that DRN serotonergic neurons are associated with the process of satiating sodium appetite.
The importance of the PBN for the control of water and sodium intake

The PBN is a pontine structure composed of several cell groups located around the superior cerebellar peduncle which is also known as the brachium. In the human brain, the PBN is divided into the medial and lateral parabrachial nuclei (MPBN and LPBN, respectively) according to its position in relation to the brachium. According to Paxinos and Watson (107), the same divisions also apply for the rat brain. However, due to the position of the brachium in the rat brain, the MPBN and LPBN lie ventromedially and dorsolaterally to the brachium, respectively (see Figure 1 for the position of these structures in the rat brain).

The PBN receives projections from the NTS and AP. These inputs convey information arising from peripheral receptors, and the PBN in turn projects to other hindbrain and forebrain areas controlling water and sodium intake (76, 77). The PBN is reciprocally connected with forebrain structures, such as the paraventricular nucleus of the hypothalamus, the central nucleus of amygdala (CeA) and MnPO, and with medullary regions, including the AP and the medial portion of the NTS (24, 59, 71, 73). Therefore, the PBN is uniquely positioned to integrate and convey signals generated in the peripheral viscera that then enter the CNS through the AP and the medial NTS.

Both PBN nuclei are involved in the control of sodium intake. After passing through the AP and the NTS, the LPBN receives information about the status of the systemic viscera that arise from circulating factors, cardiovascular receptors, peripheral osmoreceptors and perhaps gastrointestinal receptors, all of which influence the amount of sodium and water ingested (36, 38, 55, 81, 89-92, 94-97, 131). Cells in the LPBN are activated after ingestion of sodium solutions by
dehydrated rats or rats that received intragastric loads of hypertonic NaCl (55, 81, 131). The distension of an intravascular balloon positioned at the superior vena cava and right atrial junction increases the expression of Fos protein in the LPBN, suggesting that the LPBN is also activated by signals from cardiovascular receptors (36).

Another portion of the PBN, often referred to as the gustatory PBN, includes medial portions of the anatomically defined MPBN and LPBN (32, 51, 103, 116). Neurons in the gustatory PBN are activated by applying 0.3 M NaCl to the anterior tongue indicating that this portion of the PBN receives signals related to salty taste (32, 51, 103, 116). Bilateral lesions in the gustatory PBN abolish sodium appetite (32, 51, 116).

**Inhibitory mechanisms of the LPBN involved in the control of water and sodium intake**

*Neurotransmitter and receptor distribution in the LPBN: the basis for neuropharmacological studies*

Serotonergic, adrenergic and noradrenergic neurons are present in projections from the AP/NTS to the LPBN, and binding and immunohistochemical studies indicate the presence of α₂-adrenergic sites in the LPBN (70, 83, 98). There is evidence from both receptor binding and *in situ* hybridization studies indicating that there are GABA receptors in the PBN (7, 16). Opioid receptors and enkephalin are present in the LPBN, and the activation of GABA and μ-opioid receptors in LPBN increases potassium conductance, inhibiting neural activity as a
result of hyperpolarization (22, 130). Whole cell electrophysiological recordings in rat brain slices also showed that most (62%) LPBN neurons tested responded to GABA superfusion, and in most cases this response was attenuated by the GABA<sub>A</sub> antagonist bicuculline (80). Glutamate, cholecystocinin (CCK) and corticotrophin-releasing factor (CRF) have also been identified in the parabrachial nucleus (15, 68, 78).

In rat, serotonergic, catecholaminergic, glutamatergic, cholecystokinerergic, opioid and CRF immunoreactive neurons are found in the pathway from the AP/NTS to the LPBN (68, 72, 83, 85, 98, 111). GABAergic and CRF immunoreactive neurons are present in the projections from the CeA to the LPBN (74, 99). It is also likely that there are GABAergic interneurons in the LPBN. Among other neuropeptides, enkephalin- and CRF-like immunoreactive neurons are particularly numerous within areas of the BNST that project to the PBN (100). Enkephalin-, dynorphin- and CRF- immunoreactive neurons are also described in the projections from several hypothalamic nuclei to the PBN (101).

**LPBN and thirst**

Initial studies on the role of the LPBN in the control of fluid and electrolyte balance were directed at investigating hindbrain control of water intake. Results from these experiments showed that electrolytic or neurotoxic lesions of the LPBN increased thirst induced by manipulations that are related to the depletion of extracellular fluid, but not to the reduction of intracellular volume (44, 105, 106). Also the inactivation of LPBN neurons with bilateral injections of lidocaine or by non-selective antagonism of serotonin receptors by injecting methysergide into the
LPBN increased ANG II-induced water intake (94). Such results suggested that inhibitory mechanisms in the LPBN are involved in the control of the magnitude of drinking induced by ANG II (94) and paved the way for studies that demonstrated an inhibitory role for the LPBN in the control of sodium appetite.

LPBN and sodium appetite: enhanced sodium intake associated with accompanying dipsogenic or natriorexigenic challenges

When the LPBN was injected with methysergide at the same time ANG II was injected icv, rats with access to both water and 0.3 M NaCl showed remarkably large intakes of normally aversive hypertonic NaCl (96). Usually, acute icv injections of dipsogenic doses of ANG II produce only modest, if any, intake of 0.3 or 0.5 M NaCl. However, ANG II (50 ng/μl) injected icv 15 min after bilateral LPBN injections of methysergide (4 μg/0.2 μl) induces a robust ingestion of 0.3 M NaCl that is accompanied by increased water intake (Figure 2; 96). These results suggest that serotonin acts in the LPBN to inhibit both ANG II-induced sodium appetite and thirst. The typical sites of bilateral LPBN injections are illustrated in Figure 1. Besides increasing icv ANG II-induced water and 0.3 M NaCl intake, bilateral LPBN injections of methysergide also increase water and/or 0.3 M NaCl intake induced by several other natriorexigenic and dipsogenic treatments (Table 1).

Methysergide injections into the LPBN increase sodium appetite induced by ANG II administered into the SFO or by different treatments that activate the circulating renin-angiotensin system such as sodium depletion, water deprivation, acute sc injection of the diuretic furosemide (FURO), or a combined treatment of sc
FURO with a low sc dose of the angiotensin converting enzyme inhibitor captopril (CAP) (27, 90-92, 94, 96). In contrast to the effects of methysergide, LPBN injections of the 5HT2 receptor agonist 2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) reduces FURO + CAP-induced 0.3 M NaCl intake (96).

Consistent with these functional results, the release of serotonin and its metabolite 5-hydroxyindoleacetic acid is reduced in the PBN after FURO + CAP treatment and enhanced by 0.3 M NaCl intake (126).

Further investigation also showed that the enhancing effects of methysergide are not restricted to the effects of ANG II on hypertonic sodium chloride intake. Methysergide injected into the LPBN also increases sodium appetite in rats treated with sc DOCA (34). Interestingly, the combination of methysergide injected into the LPBN along with intragastric 2 M NaCl also results in what seems a paradoxical ingestion of 0.3 M NaCl (38). Treatments that induce cellular dehydration typically only produce water intake. Although icv carbachol, sc isoproterenol or icv relaxin usually only elicit water intake, they induce a significant sodium appetite when combined with LPBN methysergide injections (88, 89, 92).

It is important to emphasize that bilateral LPBN injections of methysergide by itself in fluid replete rats produces no water or hypertonic NaCl intake. This indicates that only blocking the action of serotonin in the LPBN without the presence of some type of facilitating factor is not sufficient to induce sodium or water intake. On the other hand, sodium intake is easily generated when attenuation of LPBN serotonergic inhibitory mechanisms is combined with either a dipsogenic or a natriorexigenic stimulus. This suggests that the LPBN acts strongly to inhibit sodium intake (27, 90-92, 94, 96).
Other neurotransmitters in addition to serotonin act to inhibit sodium intake under dipsogenic or natriorexigenic conditions. Similar to methysergide, blockade of cholecystokinin (CCK), corticotrophin-releasing factor (CRF) or glutamate receptors, or activation of either α₂-adrenoceptors or purinergic P2 receptors in the LPBN, increases 0.3 M NaCl intake induced by FURO + CAP or 24 h sodium depletion (2, 33-35, 61, 95, 97). Just like methysergide, LPBN injections of these agents have no effect on sodium appetite or thirst in rats unless they are accompanied by a dipsogenic or natriorexigenic treatment (2, 33-35, 61, 95, 97).

LPBN and sodium appetite: “de novo” sodium intake under sodium-replete and euhydrated conditions

In contrast to the effect that serotonin, CCK, CRF or glutamate antagonists have in the presence of a dipsogenic or natriorexigenic signal, LPBN injections of GABA_A, GABA_B, or opioid receptor agonists produce sustained salt and water intakes lasting three to four hours without the need for any concomitant treatment (3, 17, 41, 42, Figure 3). These results suggest that blocking LPBN neuronal activity with the injections of GABA or opioid receptor agonists attenuates a tonic inhibitory mechanism that normally restrains sodium appetite and thirst.

The significance of multiple neurotransmitter receptors in the control of sodium appetite

The various neurotransmitter/neuromodulators involved in inhibitory mechanisms acting within the LPBN can be grouped under those that act in the
presence of a dipsogenic/natriorexigenic signal and those that act in the absence of such signals. Whereas the deactivation of LPBN inhibitory mechanisms produced by the blockade of serotonin, CCK, CRF or glutamate receptors, or the activation of $\alpha_2$-adrenoceptors or purinergic receptors, requires the presence of additional signals associated with sodium and water deficits, GABAergic or opioid activation in the LPBN is sufficient to drive fluid replete rats to ingest hypertonic saline in quantities that easily surpass the amount ingested by sodium depleted animals.

It is possible that the manipulation of those neurotransmitters/receptors which require the synergistic assistance of accompanying dehydration or sodium deficiency does not elicit sodium intake in hydrated animals because, in contrast to GABAergic activation, it does not remove a sufficient amount of tonic inhibition. It seems reasonable to speculate that the manipulation of those neurotransmitters/receptors which require the synergistic assistance of accompanying dehydration or sodium deficiency would be effective in fluid replete rats if a critical number of other restraining inhibitory influences were also blocked. Without sufficient suppression of multiple inhibitory influences that converge on the LPBN, the threshold for removing the tonic inhibitory influence projected to the forebrain is not reached and sodium intake is not released in fluid replete rats.

A possibility to consider is that the various types of inhibitory input that converge on the LPBN use different neurochemical codes. For example, input from arterial baroreceptors might use serotonin as an inhibitory signal, whereas inhibition generated by the taste of a highly concentrated salt solution might use
CCK as an inhibitory signal. Experiments testing such a hypothesis have yet to be conducted.

Interaction between different neurotransmitters/receptors is also possible as suggested by preliminary results showing that the increased sodium intake produced by the activation of $\alpha_2$-adrenoceptors in the LPBN depends at least partially on GABA$_A$, opioid and purinergic receptor activation. In addition, the effects of GABA$_A$ receptor activation may depend partially on opioid receptor activation.

Some neurotransmitters like serotonin and CCK may act in the LPBN based on a model of cooperativity and interdependence (35). According to this model, elevated serotonin release and action tend to increase the release and effects of CCK and vice versa. The interdependence assumption is that both 5-HT and CCK acting at their respective receptors is a necessary condition for the normal inhibition of ingestive behaviors.

**Interaction between forebrain facilitatory and LPBN inhibitory mechanisms controlling sodium intake**

Early indications that the LPBN interacts with forebrain mechanisms derived from functional studies where angiotensinergic antagonism in the forebrain was combined with methysergide injection into the LPBN (27, 90). This type of interaction between LPBN and angiotensinergic mechanisms has been recently explored in relation to the effects of the GABA$_A$ agonist muscimol on hydrated rats.
Muscimol injected into the LPBN induces large intakes of water and 0.3 M NaCl in fluid replete rats (8, 113). This effect is almost abolished by the blockade of either AT₁ or muscarinic receptors with icv injections of losartan or atropine (8, 113). This suggests that the increase in water and hypertonic saline intake produced by LPBN muscimol injections must depend on a combination of blockade of LPBN inhibitory mechanisms while at the same time there must be facilitation that arises from tone generated by angiotensinergic and cholinergic mechanisms (8, 113). However, at the present time it is not known whether that tone is tonic or phasic in nature. That is, whether the tone is always present or is actually produced by deactivation of a LPBN inhibitory mechanism.

The importance of forebrain facilitatory mechanisms for the ingestion of 0.3 M NaCl and water induced by deactivation of LPBN inhibitory mechanisms has also been demonstrated by studies that tested the interaction between the LPBN and the CeA for the control of sodium intake. The ingestion of hypertonic NaCl induced by different treatments such as sodium depletion, icv injection of renin, sc DOCA or sc yohimbine is significantly reduced by bilateral electrolytic lesions of the CeA. This suggests that important facilitatory mechanisms for sodium intake are present in the CeA (60, 102, 117, 132, 133). There are reciprocal connections between the amygdala and the LPBN (73, 103). Bilateral electrolytic lesions of the CeA abolishes 0.3 M NaCl intake produced by bilateral LPBN injections of muscimol in fluid replete rats (3) and by bilateral LPBN injections of moxonidine or methysergide in rats previously treated with s.c. FURO + CAP (4). Therefore, similar to the need for a functional central angiotensinergic pathway, CeA
facilitatory activity is also essential for enhanced natriorexigenic responses induced by deactivation of the inhibition produced by the LPBN (3, 4).

The marked reduction of sodium and water intakes with the blockade of central AT$_1$-R and muscarinic receptors or CeA lesions suggests that activity of facilitatory mechanisms produces signals that drive normovolemic rats to ingest sodium and water when treated with LPBN injections of muscimol. If under basal conditions, LPBN mechanisms are normally active, facilitatory mechanisms are inhibited by signals from the LPBN to restrain the ingestion of water and NaCl. The inactivation of LPBN inhibition releases forebrain facilitatory mechanisms resulting in increased water and sodium intake. In other words, the behavioral systems that defend blood volume and blood pressure are tonically “armed” to respond rapidly when homeostasis is challenged, but these behaviors are restrained by LPBN-associated inhibitory mechanisms. Such a “primed for action” intake system can in some ways be viewed as analogous to the control of heart rate and blood pressure by arterial baroreceptors. When afferent inputs from high pressure baroreceptors are interrupted, the tonic inhibitory control of sympathetic outflow is immediately released to produce the acute onset of hypertension (43, 82).

**Multiple neurotransmitter mechanisms in the LPBN: a model for the control of sodium appetite**

The previously described mechanisms involved in the control of sodium intake in rats can be summarized in a model represented by Figure 4. In such model, the LPBN belongs to a brain circuit whose primary purpose is to inhibit sodium intake. The core of the model accounts for two key aspects of the LPBN
inhibitory mechanism, that is, its modulation and interaction with the facilitatory mechanisms. Modulation of the LPBN inhibitory mechanism depends on ascending visceral or humoral signals generated through primary relays located in the hindbrain (AP, NTS). Those signals modulate the release of two sets of neurotransmitters, some (serotonin, CCK, CRF and glutamate) which increase the inhibitory signals and others (GABA, opioids, noradrenaline and ATP) which reduce them. Reduction of inhibitory signals generated in the LPBN can occur by direct inhibition of output neurons or reduced release from interneurons of neurotransmitters such as serotonin, CCK, CRF and glutamate. Interaction between the LPBN inhibitory mechanism and facilitatory mechanisms depends on signals sent to brain integrative areas. A candidate area to integrate those signals is the amygdala, particularly the CeA. There, signals from LPBN inhibit facilitation produced, for example, by ANG II or hyperosmolality acting on the SFO and/or OVLT.

Acknowledgments

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Legends of figures

Figure 1: Photomicrograph of a coronal section of a rat brain showing the sites of injections into the LPBN. SCP, superior cerebellar peduncle (outlined); MPBN, medial parabrachial nucleus; LPBN, lateral parabrachial nucleus.

Figure 2: Ingestion of (A) 0.3 M NaCl and (B) water induced by icv injection of ANG II (50 ng/1 μl) in rats pre-treated with bilateral injections of vehicle (polyethylene glycol/water 2:1) or methysergide (4 μg/0.2 μl) into the LPBN. Results are represented as means ± SEM. n = number of rats. * different from vehicle. From Menani et al., 1996 (96).

Figure 3: Ingestion of (A) 0.3 M NaCl and (B) water by fluid replete rats treated with bilateral injections of muscimol (0.5 nmol/0.2 μl) or saline combined with injections of vehicle (polyethylene glycol/water 2:1) or bicuculline (GABA<sub>A</sub> antagonist, 1.6 nmol/0.2 μl) into the LPBN. Results are represented as means ± SEM. n = number of rats. From Callera et al., 2005 (17).

Figure 4: Schematic diagram based on studies in rats showing the modulation of the LPBN inhibitory mechanism by different neurotransmitters and the interaction between the LPBN inhibitory mechanisms and forebrain facilitatory mechanisms involved in the control of sodium intake.
Figure 2

A

Cumulative 0.3M NaCl intake (ml)

- ANG II + vehicle
- ANG II + methysergide

B

Cumulative water intake (ml)

Minutes

0 15 30 45 60
Figure 3

- Vehicle + saline
- Vehicle + muscimol
- Bicuculline + saline
- Bicuculline + muscimol

* Different from vehicle + saline

(A) Cumulative 0.3 M NaCl intake (ml)
(B) Cumulative water intake (ml)

(n=5)
Figure 4

Body Fluid Balance - Blood Pressure

SODIUM INTAKE

INTEGRATIVE AREA
(amygdala or other)

ANG II
SFO/OVL

MINERALOCORTICOIDS

5-HT
CCK
CRH
Glutamate

LPBN

OPIOIDS
Noradrenaline
ATP
GABA

NTS

Taste receptors
Baroreceptors
Cardiopulmonary and other visceral receptors
Humoral signals

△ AT1 RECEPTORS

FACILITATION
INHIBITION
DIRECT CONNECTIONS
INDIRECT CONNECTIONS
**Table 1:** Changes in water and hypertonic NaCl intake in rats induced by different natriorexigenic/dipsogenic stimuli combined with different treatments in the LPBN

<table>
<thead>
<tr>
<th>Pharm. class</th>
<th>Drug injected LPBN</th>
<th>Natriorexigenic/dipsogenic stimulus</th>
<th>Water intake</th>
<th>Hypertonic NaCl intake</th>
<th>Reference number</th>
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<td>FURO + CAP</td>
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αβmATP, αβ methyl adenosine tri-phosphate; α₂, α₂ adrenoceptor; Agon., agonist; ANG II, angiotensin II; ant., antagonist; β endorph, β endorphin; CAP, captopril; CCK, colecystokinin; CRH, corticotrophin releasing factor; DNQX, 6,7-dinitroquinoxaline-2,3(1-H,4H)-dione; DOCA, deoxycorticosterone; DOI, 2,5-dimethoxy-4-iodoamphetamine; FURO, furosemide; Glut., glutamate; 5-HT, serotonin; icv, intracerebroventricular; Methy, methysergide; Moxo, moxonidin; Noradr, noradrenalina; Pharm. class, pharmacological class; Proglu, proglumide; Pur., purinergic; Hypertonic NaCl, 0.3 or 0.5 M NaCl; SFO, subfornical organ.