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Role of α_2-adrenoceptors in the lateral parabrachial nucleus in the control of body fluid homeostasis

C.A.F. Andrade, G.M.F. Andrade-Franzé, P.M. De Paula, L.A. De Luca Jr. and J.V. Menani

Departamento de Fisiologia e Patologia, Faculdade de Odontologia, Universidade Estadual Paulista, Araraquara, SP, Brasil

Abstract

Central α_2-adrenoceptors and the pontine lateral parabrachial nucleus (LPBN) are involved in the control of sodium and water intake. Bilateral injections of moxonidine (α_2-adrenergic/imidazoline receptor agonist) or noradrenaline into the LPBN strongly increases 0.3 M NaCl intake induced by a combined treatment of furosemide plus captopril. Injection of moxonidine into the LPBN also increases hypertonic NaCl and water intake and reduces oxytocin secretion, urinary sodium, and water excreted by cell-dehydrated rats, causing a positive sodium and water balance, which suggests that moxonidine injected into the LPBN deactivates mechanisms that restrain body fluid volume expansion. Pretreatment with specific α_2-adrenoceptor antagonists injected into the LPBN abolishes the behavioral and renal effects of moxonidine or noradrenaline injected into the same area, suggesting that these effects depend on activation of LPBN α_2-adrenoceptors. In fluid-depleted rats, the palatability of sodium is reduced by ingestion of hypertonic NaCl, limiting intake. However, in rats treated with moxonidine injected into the LPBN, the NaCl palatability remains high, even after ingestion of significant amounts of 0.3 M NaCl. The changes in behavioral and renal responses produced by activation of α_2-adrenoceptors in the LPBN are probably a consequence of reduction of oxytocin secretion and blockade of inhibitory signals that affect sodium palatability. In this review, a model is proposed to show how activation of α_2-adrenoceptors in the LPBN may affect palatability and, consequently, ingestion of sodium as well as renal sodium excretion.

Key words: Parabrachial nucleus; Sodium; Natriuresis; Thirst; Hindbrain; Taste

Introduction

Noradrenaline is an important neurotransmitter involved in the essential control of body fluid balance. Its effects depend on the area of the encephalon studied, the type of treatment the animal receives, and the receptors involved (for a review see Refs. 1,2). Early studies using mixed α_2-adrenergic and imidazoline receptor agonists, like clonidine and moxonidine, injected into the lateral and third cerebral ventricles, septal area, lateral preoptic area, and lateral hypothalamus showed that they are effective in inhibiting water and sodium intake induced by different stimuli (1,3-10). The inhibition of either water or hypertonic NaCl intake with forebrain injections of moxonidine or clonidine was reduced by pretreatment with injections of α_2-adrenoceptor antagonists, like yohimbine or RX 821002 (1,9,11-13). Norepinephrine, the endogenous ligand with no imidazoline characteristics, injected intracerebroventricularly (*icv*), also inhibits water and NaCl intake, an effect antagonized by pretreatment with idazoxan, an α_2-adrenergic and imidazoline receptor antagonist (1). These results suggest that α_2-adrenoceptor activation in the forebrain inhibits water and NaCl intake.

In the hindbrain, important inhibitory mechanisms for the control of water and NaCl intake have been demonstrated in the lateral parabrachial nucleus (LPBN) (14-20), a pontine structure that lies dorsolateral to the superior cerebellar peduncle. Evidence for involvement of LPBN in the control of water intake arose from studies showing that electrolytic or chemical (ibotenic acid) lesions of the LPBN increased angiotensin II (ANG II)-induced water intake (15,21,22). Similar to the effects of the LPBN lesions, bilateral injections of lidocaine or methysergide (serotonergic antagonist) into the LPBN increased ANG II-induced water intake (19,23). Bilateral injections of methysergide into the LPBN also increased 0.3 M NaCl intake induced by ANG II, administered either *icv* or into the subfornical organ, or by subcutaneous (*sc*) injection of the diuretic furosemide (FURO) in combination with a low dose of the angiotensin-converting enzyme inhibitor,

Correspondence: C.A.F. Andrade, Rua Humaitá, 1680, 14801-903 Araraquara, SP, Brasil. Fax: +55-16-3301-6488. E-mail: carina.andrade@foar.unesp.br

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captopril (CAP), whereas 2,5-dimethoxy-4-iodoamphetamine hydrobromide (a serotonergic 5-$HT_{2A/2C}$ receptor agonist) injected into the LPBN reduced NaCl intake induced by FURO + CAP (14,20). Treatment with FURO + CAP elicited significant decreases in serotonin and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the LPBN, under the condition that 0.3 M NaCl and water are not available for drinking, and enhanced serotonin and 5-HIAA levels in the LPBN if rats had ingested water and 0.3 M NaCl (24). These results suggested that serotonergic mechanisms in the LPBN play an important inhibitory role in modulation of sodium appetite.

The LPBN is reciprocally connected to forebrain areas implicated in the maintenance of blood pressure and body fluid homeostasis, such as the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala, and the median preoptic nucleus. The LPBN is also richly interconnected with medullary regions, which include the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS) (25-32). Therefore, the LPBN may integrate and relay taste and visceral signals that ascend from the AP/mNTS to the forebrain areas involved in the control of fluid and electrolyte balance (16,17,20,33,34). Furthermore, α_2-adrenoceptors are present in the LPBN (35,36). Most α_2-adrenoceptors are located in neurons of the external LPBN and the waist area of the parabrachial nucleus (35), which correlates well with the pattern of ascending axons from the AP/mNTS terminating in the parabrachial nucleus (26). Injections of the α_2-adrenoceptor antagonist yohimbine into the LPBN resulted in a 77% inhibition of spontaneous activity of visceral-responsive neurons in the ventral basal thalamus (37), suggesting that α_2-adrenoceptors in the LPBN may modulate the visceral sensory information that ascends from AP/mNTS to the visceral forebrain.

Contrary to α_2-adrenoceptors located in the forebrain, the activation of α_2-adrenoceptors in the LPBN increases sodium and water intake and reduces renal excretion, without changing arterial pressure (2,38,39). The present review summarizes the current state of knowledge about the importance of α_2-adrenergic mechanisms of the LPBN in the control of body fluid and electrolyte balance. Such control involves behavioral, endocrine, and renal responses that converge for the acquisition and conservation of sodium in the body.

Role of LPBN α_2-adrenoceptors on sodium appetite

The activation of α_2-adrenoceptors with bilateral injections of moxonidine into the LPBN strongly increases 0.3 M NaCl intake induced by *sc* FURO + CAP treatment (38). The enhancement produced by moxonidine (up to 10-fold the amount ingested by controls treated with FURO + CAP *sc* and vehicle injected into the LPBN) was completely suppressed by RX 821002, an α_2-adrenoceptor antagonist (38). Furthermore, FURO + CAP-induced 0.3 M NaCl intake strongly increased after bilateral injections of noradrenaline or the specific α_2-adrenoceptor agonist α-methylnoradrenaline into the LPBN. Bilateral injections of RX 821002 into the LPBN abolished the effects of noradrenaline and α-methylnoradrenaline in the same area on 0.3 M NaCl (2,39). The increase in sodium intake after LPBN moxonidine or noradrenaline injection in fluid-depleted rats (2,38,39) sharply contrasts with the inhibitory effect that α_2-adrenoceptor activation in the forebrain has on sodium appetite and thirst (1,9,11-13). Therefore, forebrain and hindbrain (or more specifically LPBN) α_2-adrenergic mechanisms play opposite roles in water and sodium intake (38,39).

In spite of the strong effect on FURO + CAP-induced NaCl intake, moxonidine, like methysergide or the cholecystokinin antagonist proglumide (20,40), produced no effect on water or NaCl intake when injected alone into the LPBN in satiated animals not treated with FURO + CAP (38). Therefore, activation of α_2-adrenoceptors in the LPBN increases NaCl intake if the excitatory mechanisms are simultaneously activated by treatments like FURO + CAP, whereas activation of only these receptors in the LPBN induces no NaCl intake in satiated, normohydrated rats.

Bilateral injections of moxonidine into the LPBN also produced no change in the ingestion of 0.06 M sucrose (38) or food intake induced by 14 or 24 h of food deprivation (41), suggesting that the activation of α_2-adrenoceptors in the LPBN modulates specifically sodium intake. This specificity is reinforced by results showing that, without changing food intake, moxonidine injected into the LPBN increased meal-associated 0.3 M NaCl intake in rats submitted to 14 or 24 h of food deprivation (41). Signals produced by a meal, like ANG II and hyperosmolarity, usually stimulate water intake (42), and some may also facilitate hypertonic NaCl intake. However, some of these signals, like hyperosmolarity, activate LPBN-inhibitory mechanisms restraining sodium intake. Moxonidine deactivates LPBN-inhibitory mechanisms and releases the influence of facilitatory signals activated by a meal to induce hypertonic NaCl intake. According to these results, activation of LPBN-inhibitory mechanisms seems necessary to curb sodium intake during a meal.

LPBN α_2-adrenoceptor activation facilitates sodium intake in hyperosmotic rats

Although moxonidine injected into the LPBN of satiated rats produces no effect on water or 0.3 M NaCl intake (38), α_2-adrenoceptor activation with moxonidine injections into the LPBN induces an unexpectedly strong ingestion of 0.3 M NaCl in addition to water in a two-bottle test in rats with an increase in plasma osmolarity [induced by intragastric (*ig*) load of 2 M NaCl as 2 mL/rat] (43).

Prior injections of the α_2-adrenoceptor antagonist RX 821002 into the LPBN almost abolished the effects of moxonidine on 0.3 M NaCl intake in hyperosmotic rats (43). Ingestion of hypertonic NaCl occurred in spite of hyperosmolality, hypernatremia, reduction of plasma renin activity, and normovolemia present in animals that received an *ig* load of 2 M NaCl (44).

The effects of α_2-adrenoceptor activation with moxonidine injections into the LPBN on sodium intake in hyperosmotic rats are similar to those of a previous study (45) that showed that bilateral injections of the serotonergic receptor antagonist methysergide into the LPBN, combined with an increase in plasma osmolarity, also induced ingestion of hypertonic NaCl in a two-bottle test. According to De Luca Jr. et al. (45), these results suggest that the increase in plasma osmolarity may also be an excitatory stimulus for sodium intake, activating brain circuits that facilitate sodium appetite, in addition to those subserving thirst. However, simultaneously with the activation of facilitatory mechanisms, increased activity of osmoreceptors produced by hyperosmolarity also activates LPBN-inhibitory mechanisms, which strongly curb sodium appetite (43,45) (Figure 1). Activation of α_2-adrenoceptors in the LPBN, similar to the blockade of serotonergic receptors in this area, deactivates inhibitory mechanisms and releases sodium appetite in a condition of high osmolarity.

Sodium taste reactivity in rats treated with moxonidine injected into the LPBN

In order to test if LPBN-inhibitory mechanisms restrain sodium intake during the consumatory phase of the behavior, we investigated whether moxonidine injected into the LPBN alters the hedonic value or palatability of sodium during a taste reactivity test.

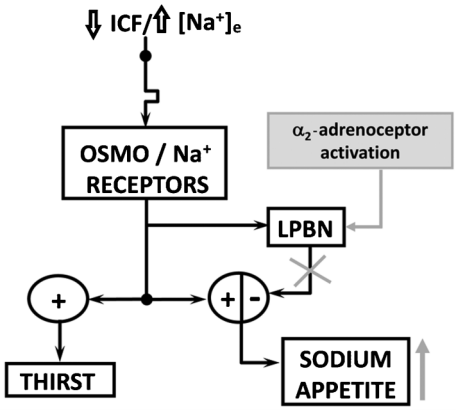

Figure 1. Diagram showing the possible involvement of the lateral parabrachial nucleus (LPBN) in the control of thirst and sodium appetite during cell dehydration. Osmoreceptors or sodium receptors activate circuits that subserve thirst and sodium appetite, and a parallel inhibitory circuit of sodium appetite involves the LPBN. The α_2-adrenoceptor activation in the LPBN blocks this LPBN-inhibitory mechanism. ICF: intracellular fluid; $[Na^+]_e$: extracellular sodium concentration. Adapted from Ref. 45, with permission.

Using a taste reactivity test that determines the frequency of ingestive and aversive behavioral reactions in response to intraorally delivered solutions (46,47), it was demonstrated that sodium depletion (induced by furosemide followed by 18 to 24 h of restricted dietary sodium) increases the palatability of sodium, producing a strong enhancement of ingestive reactions, and reduced aversive reactions, to a concentrated salty taste, in rats (48,49). FURO + CAP-treated rats that received injections of vehicle or moxonidine into the LPBN showed similar enhanced ingestive responses and decreased aversive responses to intraoral 0.3 M NaCl if they have no access to water and saline (50). These responses are consistent with previous results demonstrating that animals with an experimentally induced salt appetite show enhanced ingestive and reduced negative orofacial and body behaviors (48,49). Although LPBN moxonidine treatment failed to change taste reactivity in FURO + CAP-treated rats that had no access to water and NaCl, taste reactivity to intraoral 0.3 M NaCl in FURO + CAP-treated rats after fixed periods of 0.3 M NaCl and water consumption was totally different if rats were treated with vehicle or moxonidine injected into the LPBN. Vehicle-treated rats that ingested water and 0.3 M NaCl showed a progressive reduction in ingestive responses and an increase in aversive behaviors over the course of a 1-h test period. In contrast, rats treated with moxonidine injections into the LPBN maintained a high level of ingestive responses and a low level of aversive reactions to 0.3 M NaCl throughout the entire course of the 60-min test period of free access to water and sodium, in spite of ingesting significant amounts of 0.3 M NaCl and water (50). These results suggest that moxonidine injected into the LPBN possibly reduces some type of inhibitory signals produced as a consequence of the ingestion of NaCl and water. The interactions of taste and inhibitory signals that normally limit excess salt and water consumption may occur at one or more central nervous system sites where information from gustatory and visceral sensory systems converge. The parabrachial nucleus is potentially one of these places.

As sodium is ingested, neural and humoral postingestive signals from the gut and the blood reach the NTS and AP (for a review see Ref. 51). Viscerosensory afferent fibers in the vagus nerve are stimulated by gut distention or hypertonicity and activate neurons in the NTS, whereas cells in the AP may directly detect plasma osmolarity (52). The AP/mNTS also receives afferent projections from volume receptors (arterial baroreceptors and cardiopulmonary receptors), and these receptors can influence the ingestion of water and sodium (31,33,34). Many neurons in the caudal or mNTS and AP project to nuclei within the LPBN (26). In turn, neurons in these LPBN nuclei release

this information to limbic and hypothalamic targets in the forebrain (53,54), providing negative feedback signals that inhibit ingestive behaviors (17,20,33,38,40,43,55,56). Moxonidine injections into the LPBN change the pattern of taste reactivity to 0.3 M NaCl by maintaining ingestive reactions and reducing aversive responses after free access to water and 0.3 M NaCl intake. Therefore, it seems that moxonidine, acting in the LPBN, reduces activity of the pathways that convey signals produced by sodium ingestion, which are important for controlling taste reactivity to sodium and, consequently, the amount of sodium ingested (50).

Role of LPBN α_2-adrenoceptors on sodium balance

Besides increasing water and sodium intake, bilateral injections of moxonidine into the LPBN reduce the diuretic and natriuretic responses to increased plasma osmolality produced by *ig* 2 M NaCl. These effects were also abolished by pretreatment of the LPBN with the α_2-adrenoceptor antagonist RX 821002, suggesting that α_2-adrenoceptor activation in the LPBN also reduces renal responses to increases in plasma osmolarity (57), producing a positive sodium balance in hyperosmotic rats that have free access to water and NaCl (57).

Bilateral injections of moxonidine into the LPBN also reduce the increase in plasma oxytocin (OT) and arginine vasopressin levels produced by *ig* 2 M NaCl, without changing plasma levels of these hormones in rats that receive an *ig* isotonic NaCl load (57). Oxytocin facilitates renal sodium excretion (for a review, see Ref. 58) and, therefore, reduction in plasma levels of this hormone might be the reason for reduced natriuresis to *ig* 2 M NaCl in rats treated with moxonidine injected into the LPBN (57). In spite of some controversies (59,60), OT centrally may also activate inhibitory mechanisms for sodium intake (61,62). Therefore, reduction in OT secretion by LPBN moxonidine injections may also contribute to the release of sodium intake.

Concluding remarks

Table 1 summarizes the effects of α_2-adrenoceptor activation in the LPBN on behavioral, renal, and hormonal responses, showing that this activation in the LPBN increases hypertonic NaCl intake and reduces urinary sodium and water excretion causing positive sodium and water balance, suggesting that α_2-adrenoceptor activation in the LPBN deactivates mechanisms that restrain body fluid volume expansion. Figure 2 presents a schematic model showing the control of sodium appetite, sodium palatability, and renal and hormonal responses by α_2-adrenergic mechanisms in the LPBN. Ingestion of water and sodium is affected by signals from baroreceptors, cardiopulmonary receptors, and taste and other visceral receptors, as well as humoral signals that reach the central nervous system through the NTS/AP (31,33,34). The LPBN is richly interconnected with medullary regions, including the AP and mNTS, and with forebrain areas implicated in the maintenance of blood pressure and body fluid homeostasis, such as the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala (CeA), and the median preoptic nucleus (25-32). Therefore, the LPBN may integrate and relay taste and visceral signals that ascend from AP/mNTS to the forebrain areas involved in the control of fluid and electrolyte balance (16,17,20,33,34). At least one forebrain area that is directly or indirectly inhibited by the

Table 1. Effects of α_2-adrenoceptor activation in the LPBN on different ingestive, behavioral, renal and hormonal responses.

Responses	Effect	References
0.3 M NaCl intake in fluid-depleted rats	↑	(38)
Water intake in fluid-depleted rats (when only water is available)	no change	(38)
2% sucrose intake (“dessert test”)	no change	(38)
0.3 M NaCl intake in satiated rats	no change	(38)
Mean arterial pressure in satiated or fluid-depleted rats	no change	(38)
0.3 M NaCl intake in cell-dehydrated rats	↑	(43)
Water intake associated with food intake in food-deprivated rats	no change	(41)
Food intake in food-deprivated rats	no change	(41)
0.3 M NaCl intake associated with food intake in food-deprivated rats	↑	(41)
Ingestive reactions to an intraoral infusion of 0.3 M NaCl	Maintenance in high levels	(50)
Aversive responses to an intraoral infusion of 0.3 M NaCl	Maintenance in low levels	(50)
Diuresis in cell-dehydrated rats	↓	(57)
Natriuresis in cell-dehydrated rats	↓	(57)
Oxytocin plasma levels in cell-dehydrated rats	↓	(57)
Sodium and water balance in cell-dehydrated rats	↑	(57)

↑ : increase; ↓ : decrease.

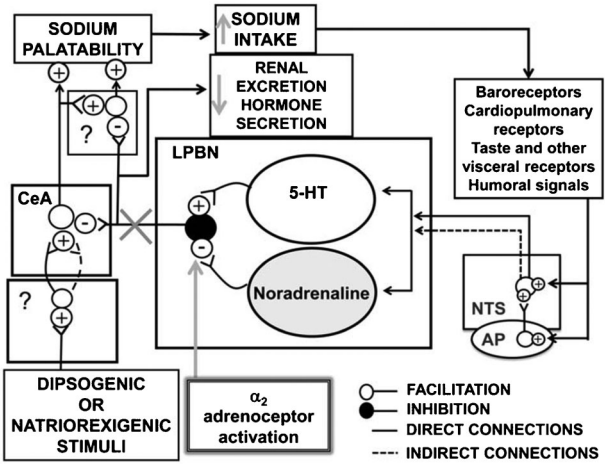

Figure 2. Schematic diagram showing how α_2-adrenoceptors in the lateral parabrachial nucleus (LPBN) affect the control of sodium intake, sodium palatability, renal excretion, and hormonal secretion. 5-HT: serotonin; NTS: nucleus of solitary tract; AP: area postrema; CeA: central nucleus of the amygdala.

LPBN is the CeA. Bilateral electrolytic lesions of the CeA blocked the increase in sodium intake produced by moxonidine injections into the LPBN of fluid-depleted rats, suggesting that LPBN mechanisms inhibit CeA-facilitatory mechanisms for sodium intake (63,64).

The taste reactivity test clearly showed that activation of α_2-adrenoceptors in the LPBN removes inhibitory signals that affect the palatability of NaCl. As a fluid-depleted animal ingests sodium, signals activated as a consequence of the ingestion of hypertonic NaCl solution increase the activity of LPBN-inhibitory mechanisms, which reduces the palatability of NaCl, limiting the amount of sodium ingested. Activation of α_2-adrenoceptors in the LPBN in this condition impairs the action of the LPBN-inhibitory mechanisms, keeping high the palatability of sodium and increasing sodium intake. Therefore, an increased activity of α_2-adrenergic mechanisms in the LPBN in fluid-depleted animals might be one of the mechanisms that act to release sodium intake. On the other hand, deactivation of α_2-adrenergic mechanisms in the LPBN as the animal ingests sodium produces opposite effects. Future studies are necessary to investigate changes in neurotransmission in the LPBN under different physiological conditions, to determine the relative importance of α_2-adrenergic mechanisms for the control of LPBN-inhibitory mechanisms. It is also important to remember that, besides α_2-adrenergic mechanisms, different neurotransmitters like serotonin, cholecystokinin, glutamate, and corticotrophin-releasing factor activate LPBN-inhibitory mechanisms, whereas GABAergic, opioid, or purinergic receptors deactivate LPBN-inhibitory mechanisms for water and NaCl intake (14,16-20,65-70). Serotonin was included in Figure 2 as an example of a neurotransmitter that, contrary to α_2-adrenoceptors, activates LPBN-inhibitory mechanisms reducing sodium and water intake and increasing renal excretion (14,16-20,71). The relative importance of each one of these neurotransmitters/receptors, or how they interact with each other in the LPBN to control the inhibitory mechanisms for sodium and water intake, is still unknown and should be investigated in future studies. Parallel to control of sodium intake by affecting sodium palatability, signals from the LPBN modulated by α_2-adrenergic mechanisms probably reach forebrain areas, like the paraventricular nucleus and supra-optic nucleus, reducing the release of OT, and thus reducing sodium excretion. Increased sodium and water intake and reduced renal excretion produced by α_2-adrenoceptor activation in the LPBN are all responses to expand body fluid volume, which suggests that α_2-adrenergic mechanisms in the LPBN are important for the control of behavioral, renal, and hormonal responses that affect body fluid volume.

Increased sodium and water intake and reduced renal excretion produced by activation of α_2-adrenergic mechanisms in the LPBN suggest an important hindbrain system for the control of behavioral, renal, and hormonal responses that expand body fluid volume.

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