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# Adrenergic Stimulation of the Lateral Hypothalamic Area on Sodium and Potassium Excretion

ALICE X. PILLAR[1], CINCINATO R. SILVA-NETTO, LUIZ A. A. CAMARGO[2], WILSON A. SAAD[2], JOSE ANTUNES-RODRIGUES AND MIGUEL R. COVIAN.

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PILLAR, A. X., C. R. SILVA-NETTO, L. A. A. CAMARGO, W. A. SAAD, J. ANTUNES-RODRIGUES AND M. R. COVIAN. *Adrenergic stimulation of the lateral hypothalamic area on sodium and potassium excretion.* PHARMAC. BIOCHEM. BEHAV. 6(2) 145–149, 1977. – The effects of adrenergic stimulation of the lateral hypothalamic area on sodium and potassium excretion were studied in rats bearing implanted cannulae. When noradrenaline was injected into several points of the lateral hypothalamic area, a dose-related increase in natriuresis and kaliuresis was observed. Rats previously injected through the same cannulae with α (Regitine) or β (Propranolol) blocking agents showed different natriuretic responses when injected with noradrenaline. It was observed that the normal noradrenaline-induced natriuresis was abolished by the α-adrenergic blockers, while β-adrenergic blockers increased the response. Intrahypothalamic injection of Isoproterenol, an activator of the β-adrenergic receptor, induced a decrease in natriuresis, kaliuresis and urinary volume. In contrast, injection of Metaraminol, an α-adrenergic agonist, caused an increase in sodium and potassium excretion and a reduction of urinary volume. Drugs blocking the destruction of noradrenaline or its reuptake by the presynaptic nerve endings potentiated 2-fold the action of 20 nmol of noradrenaline. These experiments provide good evidence for the existence of an adrenergic mechanism consisting of α and β receptors which works antagonistically on the regulation of sodium and potassium excretion. The excretion of the two electrolytes is stimulated by the α-adrenergic system, and inhibited by the β-adrenergic system.

Adrenergic stimulation  Lateral hypothalamic area  Electrolytes excretion

A SERIES of investigations has been devoted to demonstrating the participation of the central nervous system in the regulation of the hydromineral equilibrium of the organism. Many authors have studied the effects produced by chemical or electric stimulation, or by electrolytic destruction of several areas in the limbic system on water, food and sodium intake. It has been reported that the preoptic lateral area plays a role in water equilibrium in the organism, when an alteration occurs in the osmolarity of the extracellular fluid [3,4]. Extensive research has been carried out recently with the purpose of proving a possible participation of the limbic area in the regulation of urinary excretion of sodium, potassium and water. Early works in this field have shown an increase in natriuresis after a lesion in the paraventricular nuclei [16], preoptic area [17], and the posterior hypothalamus [7]. Hypertonic solution of NaCl (0.58 M) injected into the third ventricle of goats also caused an increase in natriuresis [1]. Ventriculo cisternal perfusion with low-sodium solution decrease sodium excretion [19]. Cholinergic stimulation of various hypothalamic [6] and ventricular [11] areas, as well as of the septal area [22] in rats provoked an increase in the urinary excretion of sodium and potassium and a decrease in urinary volume. Silva-Netto *et al.* [23] demonstrated the relative participation of the hypothalamus-hypophysis system in the increase of $Na^+$ and $K^+$ excretion by injecting carbachol into the lateral hypothalamic area of hypophysectomized or median eminence lesioned rats. Camargo *et al.* [5] have studied the role played by the adrenergic pathways in the septal area in the regulation of electrolyte and water excretion, demonstrating that the stimulating and inhibiting effects are mediated, respectively, by the α and β adrenergic receptors. Considering these findings, it seemed of interest to us to investigate the possible participation of the adrenergic pathways in the hypothalamic area in the regulation of $Na^+$ and $K^+$ excretion and urinary volume, thus investigating the existence of a neural circuit in this regulatory mechanism similar to that already determined for sodium intake [8].

## METHOD

*Cannulae implanted into the lateral hypothalamic area (L.H.A.).* Holtzman albino rats weighing between 200 and 300 g were used in all experiments. Stainless steel cannulae were implanted into the LHA of all animals under ether anesthesia, according to the standard stereotaxic technique based on the coordinates of the De Groot [10] atlas. The

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cannulae were fixed to the skulls with dental cement and jeweler's screws. To prevent infection, a treatment with penicillin for 3 days before and after the operation was done. After cerebral surgery, the animals were returned to their own individual metabolic cages and fed with pellets and tap water until the day of the experiment (one week later). A polyethylene tube, to be used later for administering water loads, was placed daily into the stomach for 7 days, so that they would get accustomed to the experimental conditions.

*Experimental procedure.* After a 14 hr period of food deprivation, the animals were weighed and submitted to a first water load, with water at 37°C, and in a quantity equal to 5% of the animal's body weight. The animals were then returned to their cages and left without water and solid food. Voided urine passed through the funnil at the bottom of the cage into a graduate centrifuge tube. After 60 minutes, a similar second water load was administered and the first urine sample was collected and dismissed. Twenty minutes later, a control urine sample was collected, and 1 μl of saline solution (0.15 M NaCl) or the drug was injected into the LHA. After the injection, 6 more successive urine samples were collected at 20 min intervals for a period of 2 hr.

*Drugs.* Noradrenaline (Sigma Chemical Co.), Propranolol hydrochloride (Sigma Chemical Co.), Regitine (Ciba), Isoproterenol hydrochloride (Sigma Chemical Co.), Metaraminol (Winthrop Laboratories), Xilocaine – 2% (Astra Laboratories, Brazil – 1 μl) and Nialamide (Sigma Chemical Co.). For the intracerebral injections the drugs were dissolved into a 0.15M NaCl solution. The injections (all of them 1 μl in volume) were made through a microsyringe (Hamilton Co.) fitted with a 30 gauge needle, through PE 10 polyethylene tubing, over a period of 10 sec. A delay of at least 48 hr intervened between tests in a given rat. In occasional instances a drug was tested twice in the same animal. There was no detectable effect of prior treatments on the responses. All the drug doses used have been expressed in nmol, with the exception of Lidocaine, which was 1 μl of a 2% solution.

*Determination of $Na^+$ and $K^+$.* The sodium and potassium concentrations for each urine sample were determined with a IL-143 flame spectrophotometer (Instrumentation Laboratories).

*Histology.* After the experiments, the brains of all animals were removed under anesthesia and fixed in 10% formaldehyde solution. Later the brains were cut in 10μ sections and stained by the galocyanine and Pal-Weigert technique modified by Ehrart [12]. Only the animals whose cannulae were found to be placed in the LHA were used for analysis of the data.

*Statistics.* The Student's *t* test was applied. The dose-response curve was submitted to variance analysis and calculated linear regression. All data are expressed as average ± SEM.

## RESULTS

*Effects of the injection of various noradrenaline doses into the LHA on the urinary excretion of $Na^+$ and $K^+$. Dose-response curve.* The intracerebral injection of 2.5, 5.0, 10, 20, 40 and 80 nmol of noradrenaline provoked a dose-related increase in the urinary excretion of sodium and potassium in a group of rats (Fig. 1). Variance analysis showed a significant regression for sodium in relation to the different doses applied, but no significant deviation in linearity ($F_{11.76}$ for $Na^+$). The general regression equation for $Na^+$ was $y = 64.64 + 74.11_X$.

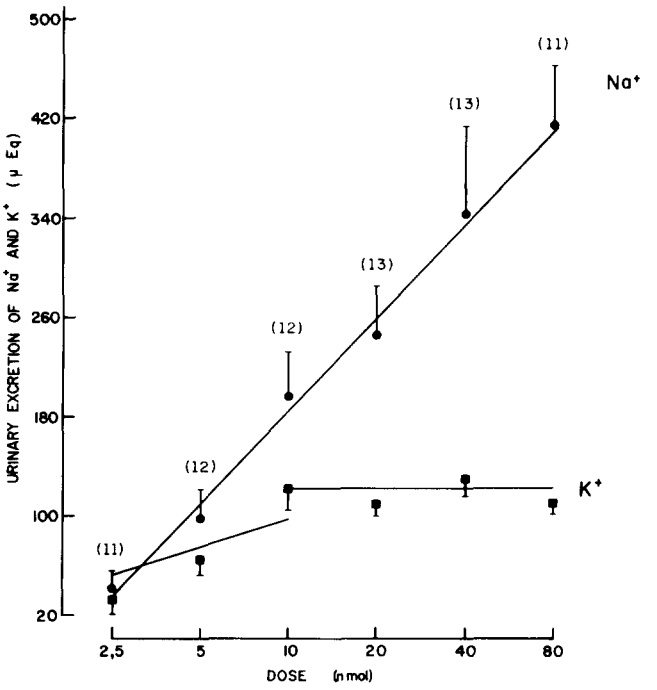


FIG. 1. Linear regression of the dose-response curve for the different doses of noradrenaline on sodium and potassium excretion. In parentheses, the number of rats.

The excretion of $K^+$ increased in direct proportion to the amounts of noradrenaline up to 10 nmol. Beyond this dose the excretion remained constant, showing that 10 nmol are sufficient to activate all the receptors connected with $K^+$ excretion.

Variance analysis of regression for $K^+$ showed significant regression up to the 10 nmol dose ($y = 21.59 + 22.97_X$). Beyond this dose the regression was not significant ($y = 124.51 - 0.52_X$).

Table 1 shows the time course and magnitude of the typical noradrenaline-induced natriuresis and kaliuresis by intervals of twenty minutes in comparison with results obtained after isotonic saline injection into the LHA.

After injection of noradrenaline (20 nmol) into the LHA the natriuresis rose rapidly, reached a maximum at 60 min later, then declined. When compared with baseline levels of 0.42 ± 0.05 μEq/min, this represents about a 8-fold increase in urinary sodium, while $K^+$ increases was of less intensity. Isotonic saline did not induce any change.

*Effects of sympatholytic drugs on the increase of urinary excretion of $Na^+$ and $K^+$ induced by injection of noradrenaline into the LHA metaraminol and isoproterenol agonism.* The injection of 20 nmol of noradrenaline into the LHA induced a marked increase in the renal excretion of $Na^+$ and $K^+$ during a period of 2 hours after the drug was administered, an effect similar to that obtained by injecting noradrenaline into the septal area [5].

The intrahypothalamic injection of 100 nmol of Propranolol 30 min before intracranial injection of noradrenaline caused a significant increase in the natriuretic effect provoked by administering 20 nmol of noradrenaline into the LHA. In contrast, intrahypothalamic injection of 20

TABLE 1

SODIUM AND POTASSIUM EXCRETION AND URINE VOLUME PLOTTED AS FUNCTION OF TIME, BY 20 MIN INTERVALS, BEFORE AND AFTER INJECTIONS OF THE TEST SOLUTIONS, ISOTONIC SALINE (0.15 M) OR NORADRENALINE (20 NMOL), INTO THE LATERAL HYPOTHALAMIC AREA

| | −20 ↓ 20 | 40 | 60 | 80 | 100 | 120 min |
|---|---|---|---|---|---|---|
| Isotonic saline | | | | | | |
| $Na^+$ ( $\mu$Eq/min) | 0.26 ± 0.02 | 0.26 ± 0.03 | 0.27 ± 0.02 | 0.32 ± 0.01 | 0.25 ± 0.01 | 0.23 ± 0.02 | 0.22 ± 0.02 |
| $K^+$ ( $\mu$Eq/min) | 0.50 ± 0.03 | 0.44 ± 0.03 | 0.47 ± 0.03 | 0.50 ± 0.03 | 0.43 ± 0.03 | 0.39 ± 0.03 | 0.34 ± 0.03 |
| Urine vol. (ml/20 min) | 1.75 ± 0.10 | 2.05 ± 0.13 | 2.51 ± 0.13 | 2.92 ± 0.12 | 2.52 ± 0.13 | 2.04 ± 0.13 | 1.84 ± 0.11 |
| Noradrenaline | | | | | | | |
| $Na^+$ ( $\mu$Eq/min) | 0.42 ± 0.05 | 1.78 ± 0.23 | 2.78 ± 0.23 | 3.39 ± 1.24 | 1.25 ±0.14 | 0.64 ± 0.05 | 0.60 ± 0.07 |
| $K^+$( $\mu$Eq/min) | 0.69 ± 0.05 | 0.74 ± 0.09 | 1.14 ± 0.10 | 1.11 ± 0.08 | 0.86 ± 0.09 | 0.61 ± 0.05 | 0.75 ± 0.04 |
| Urine vol. (ml/20 min) | 1.94 ± 0.12 | 1.58 ± 0.16 | 1.74 ± 0.18 | 2.30 ± 0.14 | 2.24 ± 0.16 | 2.25 ± 0.14 | 2.01 ± 0.14 |

Each value represents the mean ± SEM, obtained in 20 rats. Baseline values are those obtained at −20 min relative to injection of test solutions (arrow). Noradrenaline was injected 48 hr after isotonic saline.

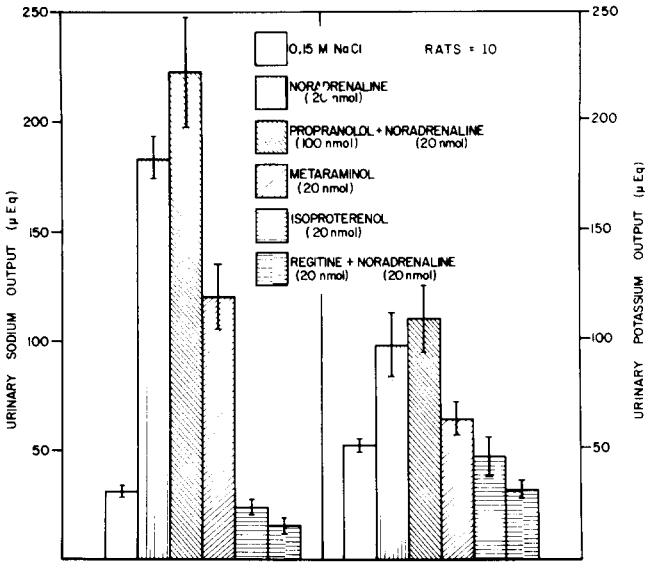


FIG. 2. Effects of adrenergic stimulation and adrenergic blocking of the hypothalamus on sodium and potassium excretion. The values represent the mean ± SEM.

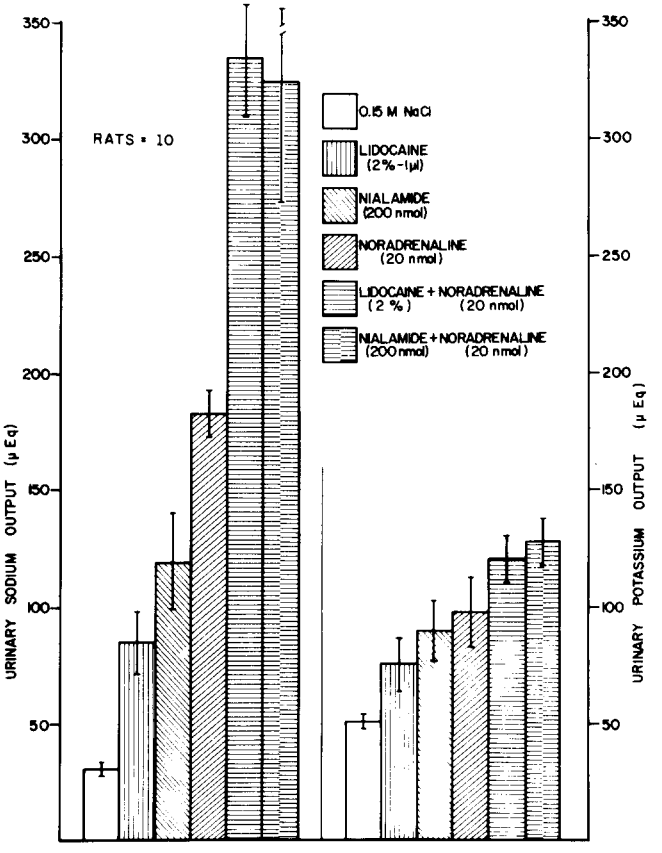


FIG. 3. Effects on sodium and potassium excretion of intrahypothalamic injections of the agents acting on noradrenaline metabolism and recaptation. The values represent the mean ± SEM.

nmol of Regitine before administering the agonist provoked a marked decrease in the natriuretic effect.

The injection of 20 nmol of Isoproterenol, a $\beta$-adrenergic stimulant, into the LHA caused a significant decrease in renal excretion of $Na^+$ and $K^+$ in comparison with the effects of both saline and noradrenaline injections. In contrast, the injection of 20 nmol of Metaraminol, an $\alpha$-adrenergic stimulant, caused an increase in the excretion of $Na^+$ and $K^+$ (Fig. 2).

*Effects of intrahypothalamic injection of drugs influencing the metabolism and recaptation of noradrenaline in nerve endings on the renal elimination of $Na^+$ and $K^+$.* The injection of 1 $\mu$l of Lidocaine at 2% into the LHA caused a significant increase in the urinary excretion of $Na^+$ and $K^+$ in relation to the control levels obtained with saline injection.

Lidocaine injected 50 min before the injection of 20 nmol of noradrenaline provoke a significant increase in the natriuretic and kaliuretic effect of the adrenergic agonist.

The injection of 200 nmol of nialamide determined an increase in the urinary excretion of the two electrolytes when compared with saline injection. Nialamide given 50 min prior to intrahypothalamic injection of 20 nmol noradrenaline once more provoked a marked increase in the urinary excretion of $Na^+$ and $K^+$, when these effects are

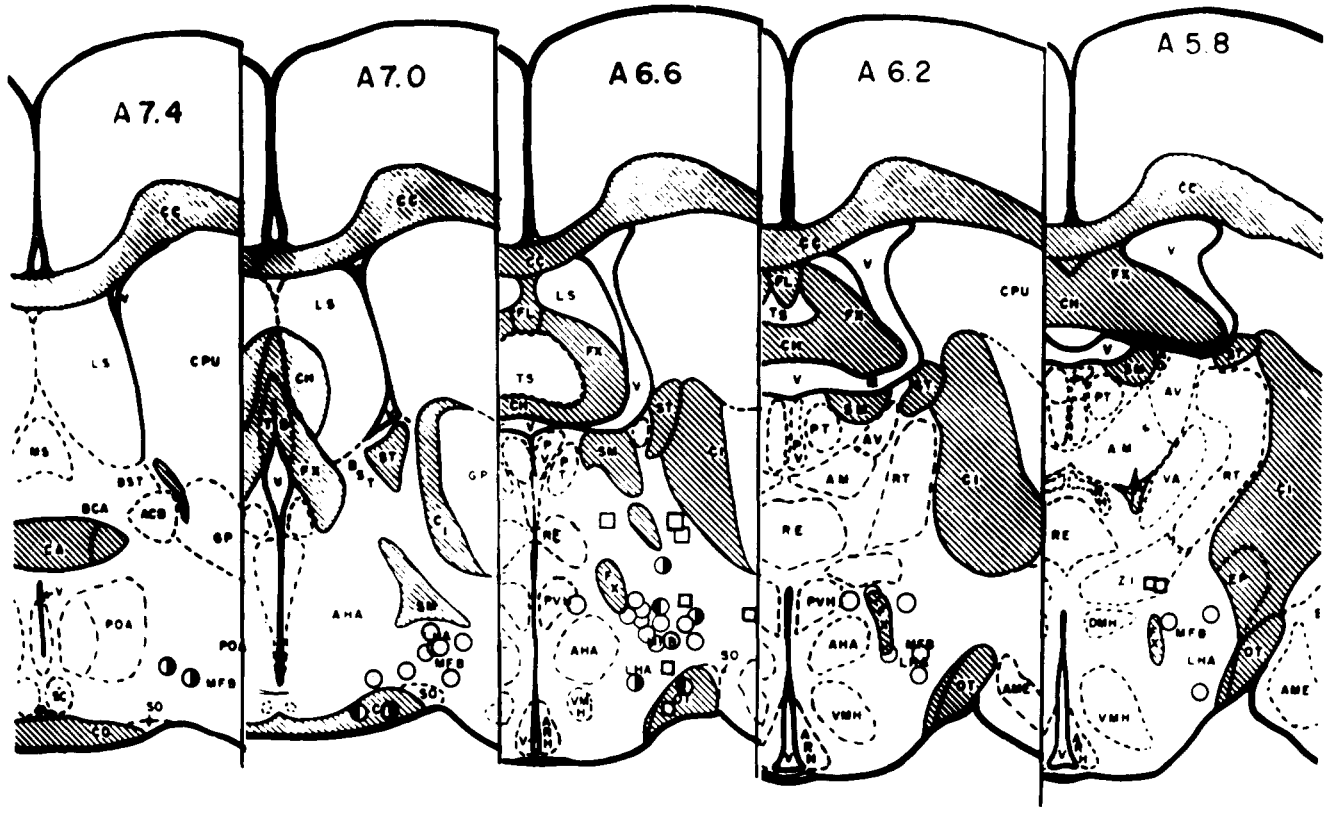


FIG. 4. Loci of stimulation shown on sections from the rat brain, based on the De Groot's atlas [10]. The open circles indicate consistently excellent effects of adrenergic stimulation on the increase of sodium and potassium excretion; the half-filled circles show the placements with some effects; the open squares indicate negative placements.

compared with those of saline or noradrenaline alone (Fig. 3).

*Localization of the implanted cannulae.* Figure 4 shows the placement of the cannulae in frontal section. The open circles indicate the points where the adrenergic stimulation was effective in stimulating sodium and potassium excretion. It may be noted that most of the loci are concentrated into the lateral hypothalamic area between the anterior and the median hypothalamus.

## DISCUSSION

The present results indicate that the LHA participates in the neural regulatory control of urinary sodium and potassium excretion when its adrenergic pathways are stimulated. The administration of noradrenaline into this area provoked a progressive, dose-dependent increase in the urinary output of sodium and potassium. The effects of noradrenaline on potassium excretion are more rapid and less intense, with a plateau being reached earlier than for sodium. The 20 nmol dose chosen for sodium excretion is a maximum dose for potassium excretion, which probably prevents any possible potentiation of its effects. On the other hand, knowing that noradrenaline acts through $\alpha$ and $\beta$ adrenergic receptors, we attempted to verify whether its effects were mediated preferentially by one or the other of the two pathways. The injection of noradrenaline into animals previously injected with the $\beta$-adrenergic antagonist Propranolol determined a potentiation of effects. However, when an animal previously treated with Regitine (an $\alpha$-adrenergic blocking agent) was injected with catecholamine an intense decrease of the effects normally obtained with noradrenaline was observed. These data are in agreement with those of Camargo *et al.* [5], showing the existence, within the septal area adrenergic system, of an $\alpha$-adrenergic pathway which stimulates the excretion of sodium and potassium and a $\beta$-adrenergic pathway that inhibits it. These effects were confirmed with the use of the specific stimulating agonist of the $\alpha$ and $\beta$ adrenergic receptors (Metaraminol and Isoproterenol, respectively). Isoproterenol injected into the LHA inhibits sodium and potassium excretion, while Metaraminol stimulates it. Thus, it would seem that the $\alpha$-adrenergic pathway stimulates excretion, while the $\beta$-adrenergic one inhibits it. These results indicate the possibility of an interaction between the adrenergic pathways of the septal area with those of the lateral hypothalamic area, because, as shown in the work of Papez [21], MacLean [18] and Nauta [21], the hypothalamus is a structure constantly influenced by stimuli coming from other structures, such as the cerebral cortex, basal nuclei, hippocampus, amygdaloid complex and septal area.

The permanence of noradrenaline near the receptors depends on its reuptake by the nerve endings towards the granule, or on its destruction by the specific enzymes COMT and MAO. When an inhibitor of noradrenaline reuptake (lidocaine) is administered [13, 15, 24], an increase in the renal elimination of sodium and potassium occurs; similar effects are obtained with nialamide, an inhibitor of monoamine oxidase activity [2, 9, 14]. In the experiments performed to verify the possibility of synergism between endogenous and exogenous noradrenaline, a summation of effects was obtained. These results demon-

strate that when noradrenaline is allowed to remain for a longer time, or at higher concentrations at the level of the synaptic cleft, in contact with the receptors, a potentiation of the natriuretic and kaliuretic response is obtained as compared with the normal action of noradrenaline.

When the LHA is stimulated with noradrenaline, a reduction in urinary volume occurs, concomitant with natriuresis. This reduction is very well defined at the 20–40 min interval and disappears later, suggesting that this response is mediated in part by the liberation of ADH and/or oxytocin. However, when Regitine was given prior to noradrenaline stimulation, a blocking of the natriuretic response occurred with no interference on the anti-diuretic response.

In summary, the present results allow us to conclude that in the lateral hypothalamus the adrenergic system is involved in the regulatory mechanism of sodium and potassium excretion, acting antagonistically through its $\alpha$ (stimulating) and $\beta$ (inhibiting) receptors.

Further investigations will be necessary for the study of the possible mechanisms involved in the mediation of these responses.

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