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Role Played by the Adenylcyclase–cAMP System of the Rat Septal Area on Na^+, K^+ and Water Renal Excretion

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CAMARGO, L. A. A., W. A. SAAD, F. G. GRAEFF, C. R. SILVA–NETO, J. ANTUNES–RODRIGUES AND M. R. COVIAN. *Role played by the adenylcyclase – cAMP system of the rat septal area on Na^+, K^+ and water renal excretion.* PHARMAC. BIOCHEM. BEHAV. 7(2) 93–97, 1977. – In this study, the participation of the adenylcyclase – cAMP system of the rat septal area in the mediation of the natriuretic, kaliuretic and diuretic effects of noradrenaline (NA) was investigated. The intraseptal injection of 20 nmol of NA caused a significant increase in the urinary excretion of Na^+ and K^+ as well as in the urinary volume during the 2 hr period following the intracerebral injection which was blocked by 40 nmol of phentolamine, locally injected, 30 min before the catecholamine. In contrast, pretreatment with propranolol (100 nmol) potentiated the effects of NA on salt and water renal excretion. The intraseptal injection of 3.12 to 50 nmol of dibutyryl cyclic adenosine monophosphate (db cAMP) caused dose-dependent increase in natriuresis and kaliuresis, but a decrease in urinary volume. Under the same experimental conditions, caffeine administration (6.25 to 100 nmol) also induced dose-dependent increases in Na^+ and K^+ urinary output. These results indicate that the saluretic effect of NA may be mediated by an alpha receptor-induced activation of the adenylcyclase – cAMP system in the septal area.

Noradrenaline and sodium excretion Septal area and electrolytes excretion
cAMP area and sodium excretion Catecholamines Adenylcyclase cAMP system

EARLIER results have shown that the septal area of the rat brain is involved in the regulation of sodium, potassium and water excretion by the kidneys [4]. It has also been suggested that cholinergic mechanisms in this area are involved in salt excretion, since local injection of the cholinergic agonist, carbachol, increases Na^+ and K^+ urinary output, through activation of both muscarinic and nicotinic receptors [13,14].

Adrenergic mechanisms in the septal area are also likely to play a role in the control of salt and water urinary excretion. It has been shown that intraseptal injection of noradrenaline (NA) or adrenaline (A) in the rat causes an increase in Na^+ and K^+ excretion, antagonized by pretreatment with the α-blocking agent, dibenamine, but not by a β-blocking agent like propranolol. Also, the β-agonist, isoproterenol, given before NA or A, counteracted the natriuretic and kaliuretic effect of the former catecholamines. In addition, the combination of NA and propranolol caused a diuretic effect. It has therefore been suggested that stimulation of α-adrenergic receptors in the septal area enhances salt and water urinary excretion, whereas β-receptor stimulation leads to Na^+ and K^+ retention by the kidneys [5]. In agreement, another study [14] has shown that the nicotinic component of carbachol saluretic action, which is probably mediated by the release of endogenous catecholamines, was antagonized by pretreatment with intraseptally injected dibenamine. Conversely, the β-blocker, propranolol, caused a small, but significant increase in Na^+ and K^+ renal excretion when injected into the same brain area and potentiated the effect of carbachol on Na^+ and K^+ excretion.

Several published reports give supportive evidence to the participation of cyclic 3', 5' -adenosine monophosphate (cAMP) in the central adrenergic mechanisms [2, 15, 18]. More specifically, Palmer [11] as well as Schultz and Daly [16] reported that the action of NA upon adrenergic receptors increases cAMP levels in several regions of the central nervous system (CNS). On the other hand, Butcher and Sutherland [3] have shown that two methylxanthines, caffeine and theophylline, in vitro, can increase the amounts of cAMP by competitively inhibiting its catabolic enzyme, phosphodiesterase. Therefore, the role of the adenylcylase-cAMP system in the mediation of the saluretic and diuretic actions of NA was investigated in the present study, by comparing the effects of the intraseptal injection of NA, on salt and water renal excretion with those of dibutryl cAMP (db cAMP), a cAMP analog that is more effective than the parent compound [17] and of the

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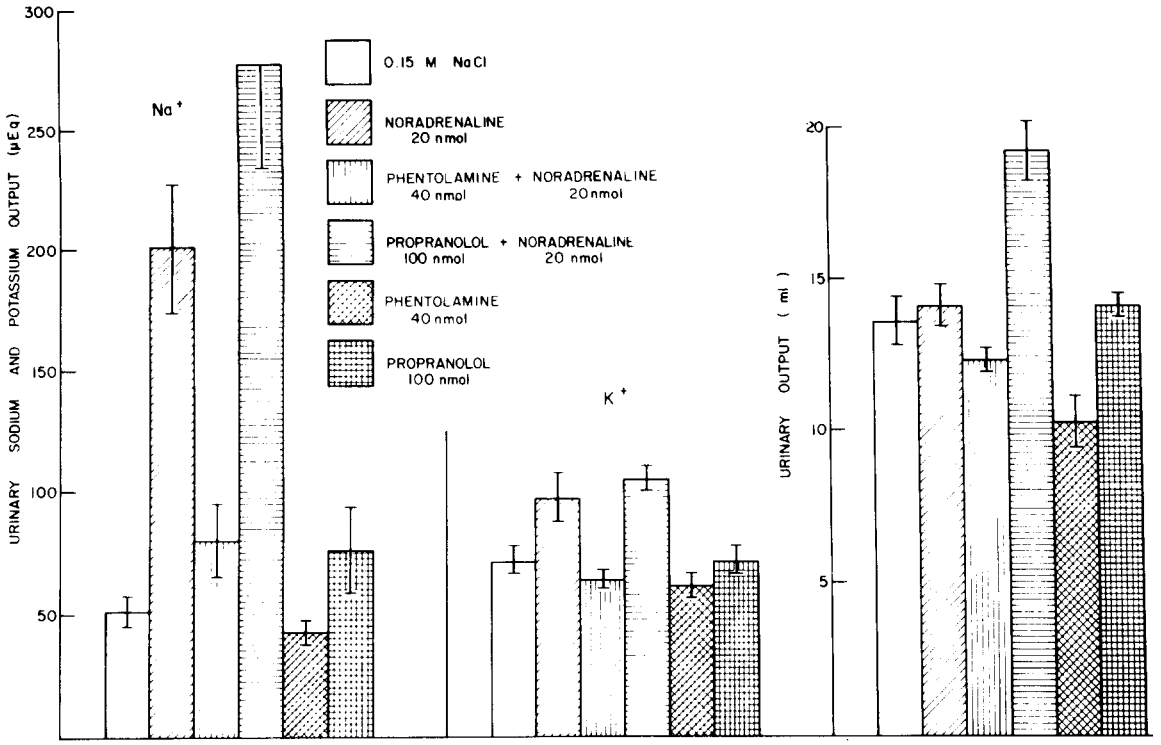

FIG. 1. Effect of phenotolamine and propranolol on noradrenaline-induced natriuresis, kaliuresis and on urinary volume. The columns represent the average cation and urine excretion in 11 rats during the 2 hr period following intraseptal drug injections. The vertical bars represent the mean ± SE. When combined with noradrenaline phentolamine and propranolol were injected 30 min prior to noradrenaline.

phosphodiesterase inhibitor, caffeine, both locally injected into the same brain site.

MATERIAL AND METHOD

Cannulation of the Medial Septal Area

Male, albino rats of the Holtzman strain, weighing 250 to 300 g, were used in all experiments. They were kept in individual metabolic cages, at room temperature (25–27°C), with free access to food and tap water. The animals were handled daily for 7 days prior to the experiment in order to avoid any emotional stress. After this adaptation period, a stainless steel cannula (O.D. 0.71) was implanted into the medial septal area of each animal, previously anesthetized with ether, according to the following coordinates of De Groot's rat brain atlas [7]. AP + 8.2; H + 0.5; L.0.0. The cannula was fixed to the skull with dental cement and jeweler screws. Prophylactic doses of penicillin and tetracycline were administered to the animals for 7 days following the operation. During this period, the cannula stopper was repeatedly removed and replaced, and a tube was introduced into the stomach, so that the animals would get used to the experimental conditions of intracerebral drug injection and gastric water overload.

Experimental Procedure

In order to obtain constant urinary volumes for each successive 20 min period during the experiment, the following procedure was used: after 14 hr of food deprivation, the animals received a water overload, equal to 5% of the body weight, at a temperature of 37°C and placed into individual metabolic cages without food and water. One hr later, the same volume of water was readministered. Twenty min thereafter, a control urine sample was collected, and 1 μl of saline (0.15 M NaCl) or drug solution was injected into the medial septal area. Subsequently, six urine samples were collected at 20 min intervals.

Drugs

Noradrenaline (Sigma Chemical Co.), phentolamine (Ciba), propranolol hydrochloride (Sigma Chemical Co.), N^6, O^2-dibutyryl adenosine 3', 5'-cyclic monophosphoric acid (Sigma Chemical Co.) and caffeine sulphate (Merck Lab.) were used. For the intraseptal injections, the drugs were dissolved in 0.15 M NaCl solution. A volume of 1 μl of drug solution was injected, during 10 sec by means of 10 μl microsyringe (Hamilton Co.), connected to a 30 gauge needle by a PE 10 polyethylene tubing. Doses are expressed in nmol. When combined with NA, phentolamine and propranolol were injected 30 min prior to NA. A delay of at least 48 hr intervened between tests in a given rat. Occasionally, the drugs were reinjected into the same animal; under these conditions, no significant effect of the prior treatment was detected.

Na^+ and K^+ Determination

The concentration of sodium and potassium in urine samples was determined by means of an IL–143 flame photometer (Instrumentation Laboratories).

Histology

After the end of the experiments, rats were sacrificed under ether anesthesia and their brain removed and fixed in

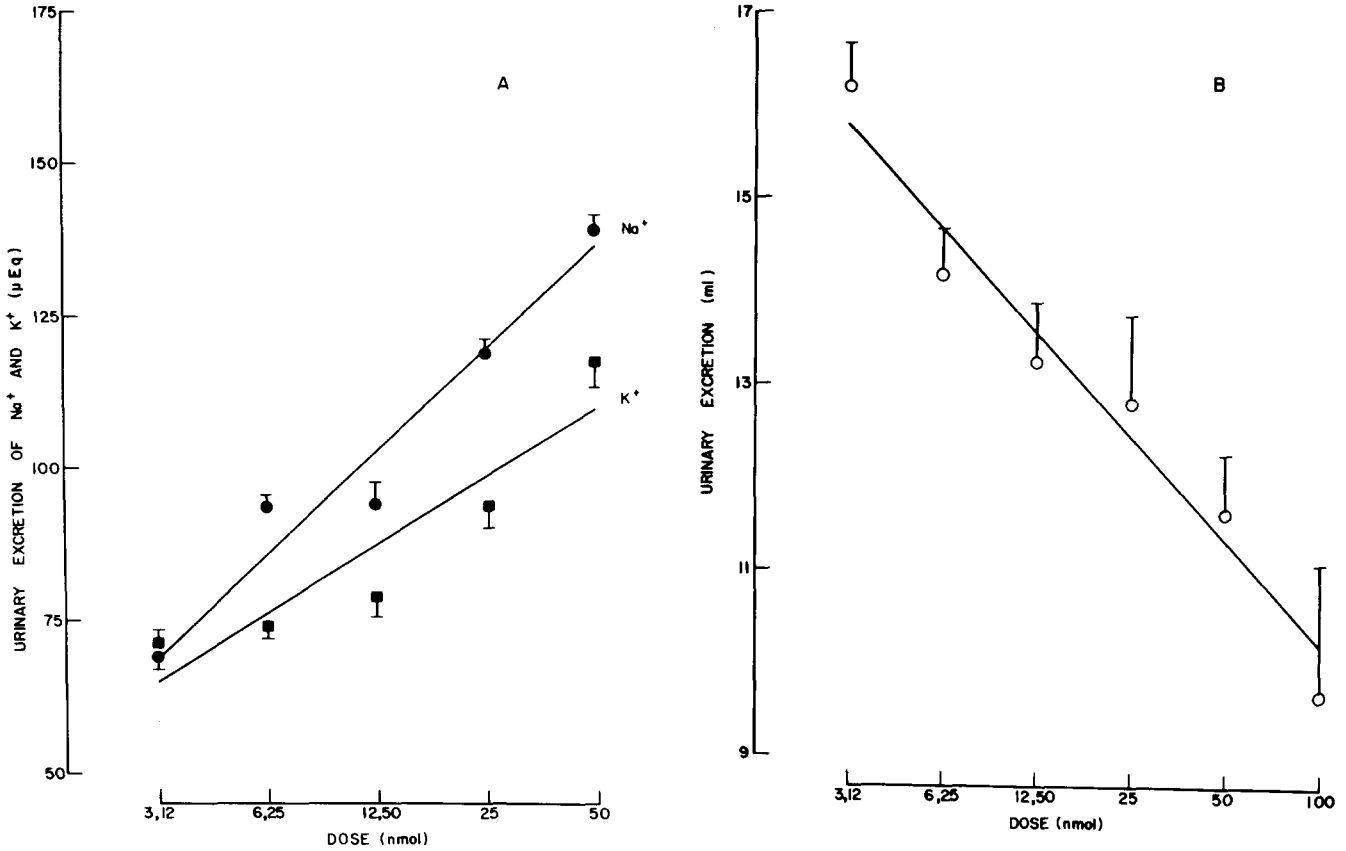

FIG. 2. Dose-effect relationships of N^6, 0^2 – dibutyryl adenosine 3',5'-cyclic monophosphoric acid on sodium (•) and potassium (▪) excretion (2A) and on urinary volume (○) – (2B). The dots represent the average of total cation excretion in 11 animals, for 2 hr following injection of different doses db cAMP into the medial septal area of the rat brain. Vertical bars represent ± SE.

10% formol. After one week, they were cut into 10 μm sections and stained according to the Pal-Weigert technique, as modified by Ehrart [8]. Only the animals with brain cannulae localized inside the medial and dorsal region of the anterior septum were used for data analysis.

Statistics

The student's *t*-test was used. Dose-response curves were submitted to variance analysis and linear regressions were calculated.

RESULTS

Effect of Intraseptal Injection of NA, Phentolamine, and Propranolol on Na^+ and K^+ Excretion and on Urinary Volume

As previously reported [5], the intraseptal injection of 20 nmol of NA markedly increased the urinary excretion of Na^+ and K^+, during the 120 min period following the injection ($p<0.0005$ and $p<0.025$). The concentration of Na^+ and K^+ in 11 rats rose rapidly following NA injection, reached a maximum after a 40–60 min and then declined. No changes were observed in the urinary volume (Fig. 1).

Pretreatment with 40 nmol of phentolamine, given intraseptally 30 min before NA, antagonized the saluretic ($p<0.05$ for Na^+ and $p<0.005$ for K^+) effect of the catecholamines (Fig. 1). Phentolamine, alone, caused no change in Na^+ and K^+ urinary excretion, but significantly decreased urinary volume ($p<0.005$).

In contrast, under the same experimental conditions, pretreatment with 100 nmol of propranolol, potentiated the natriuretic ($p<0.025$) but not the kaliuretic effect of intraseptal NA. Also a significant increase in urinary volume ($p<0.005$) was observed. Propranolol, alone caused a significant increase in Na^+ urinary excretion ($p<0.05$), but no significant change in K^+ excretion or urinary volume.

Effect of Intraseptal Injection of Dibutyryl cAMP on Na^+ and K^+ Excretion and on Urinary Volume Dose-Response Curve

The intracerebral injection of 3.12 to 50 nmol of db cAMP into the medial septal area caused a dose-dependent increase in Na^+ and K^+ urinary excretion in a group of 11 rats (Fig. 2A). Variance analysis of the log-dose response relationships showed a significant regression and negligible deviations from linearity (F 6.41 and F 5.15 for Na^+ and K^+, respectively). The general equations for the regressions were $y = 41.17 + 16.83\ x$, for Na^+ and $y = 46.54 + 11.21\ x$, for K^+. Doses of db cAMP ranging from 3.12 to 100 nmol determined dose-dependent decreases in urinary volume, in the same animal group; the analysis of variance showed a significant regression (F 15.87) with the following general equation: $y = 17.47 - 1.11\ x$ (Fig. 2B).

Effect of Intraseptal Injection of Caffeine on Na^+ and K^+ Excretion and on Urinary Volume. Dose-Response Curve

Doses ranging from 6.25 to 100 nmol caused dose-

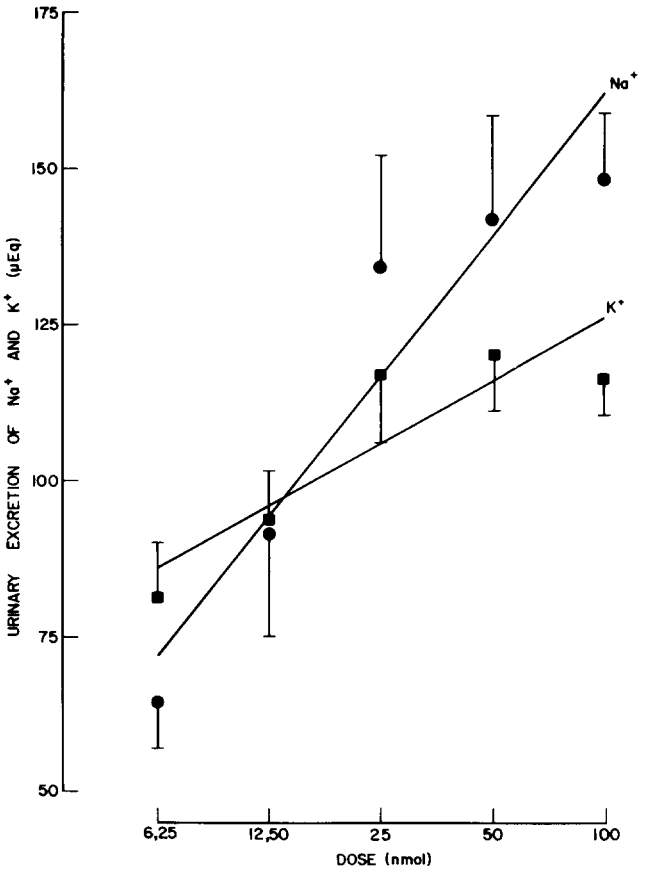

FIG. 3. Dose-effect relationships of caffeine on urinary sodium (•) and potassium (▪) excretion. The dots represent the average of total cation excretion in 12 animals, for a 2 hr period following injection of different doses of caffeine into the medial septal area of the rat brain. Vertical bars represent ± SE.

dependent increases in Na^+ and K^+ excretion, in a group of 12 animals (Fig. 3). Variance analysis of the log-dose response relationships evidenced a significant regression (F 5.57 and F 4.09 for Na^+ and K^+, respectively). The general equations for the regressions were: $y = 13.53 + 22.24\ x$, for Na^+ and $y = 59.64 + 9.94\ x$, for K^+.

The 2 hr urinary volumes following a control injection of 0.15 M NaCl or 6.25 to 100 nmol of caffeine were 13.60 ± 0.82 ml and 13.70 ± 0.80 to 16.08 ± 0.54 ml, respectively. The analysis of variance showed no significant regression (F 1.869) between the doses of caffeine injected and the urinary volume collected during 2 hr period.

DISCUSSION

In agreement with previously reported observations [5], the present results show that the injection of appropriate doses of NA into the medial septal area of the rat brain increases urinary excretion of Na^+ and K^+. It has also been reported [5] that the α-receptor blocking agent, dibenamine, antagonized these effects. Present results extend this observation to phentolamine, a more specific α-blocker than dibenamine. Our results additionally shown that local pretreatment with the β-blocking agent, propranolol, potentiates the saluretic responses to intraseptal NA. Conversely, the β-adrenergic agonist, isoproterenol, has been shown to counteract the saluretic effect of intraseptal NA [5]. Therefore, the hypothesis put forward by Camargo *et al.* [5] that stimulation of α-adrenergic receptors in the medial septal area leads to increased Na^+ and K^+ renal excretion whereas β-receptor stimulation causes salt retention by the kidneys, gains further support from the present results.

The α-induced saluretic response to intraseptal NA may be mediated by an increase in brain cAMP formation. It is known that adrenergic α-receptor stimulation activates the adenylcyclase-cAMP system in several regions of the rat brain [16] and present results indicate that the injection of db cAMP into the medial septal area leads to increases in urinary cation output, an α-like effect. Therefore, it is reasonable to conclude that NA injected into the medial septal area could stimulate α-adrenergic receptors in neuronal membranes that in turn induce intracellular formation of cAMP, activating neuronal systems that promote Na^+ and K^+ excretion by the kidneys. The present evidence that the phosphodiesterase inhibitor, caffeine, causes saluresis when injected into the medial septal area supports this interpretation.

Our results have shown that the combination of NA and propranolol increase urinary volume, confirming previous observations [5] and suggesting that α-receptor stimulation in the septal area causes diuresis. In contrast, local injection of db cAMP caused antidiuresis, an effect similar to that reported for the β-adrenergic agonist, isoproterenol or the α-blocking agent phentolamine, or the association of NA plus phentolamine, as presently shown. Since β-receptor stimulation is also known to result in activation of the adenylcyclase – cAMP system in brain [16], as well as in peripheral organs [17], it may be suggested that the antidiuretic response to intraseptal db cAMP could be due to the stimulation of a β-sensitive neuron system in which cAMP would play a mediator role. The anatomical background of this possibility is supported by the study of Powell and Rorie [12] which demonstrate the intimate connections of the septal area with the paraventricular and supraotic nuclei. In this way, the present finding that caffeine or NA did not significantly affect urinary volume could be explained by simultaneous activation of two cAMP mediated systems, α and β-adrenergic, exerting opposite influences on renal water excretion. It is known that the effects of caffeine in central adrenergic mechanisms may involve not only phosphodiesterase inhibition, but also the release of catecholamines from presynaptic nerve terminals [1,6]. Since the rat septal area is likely to be involved in the regulaton of salt and water balance [4] and has been reported to contain dense catecholamine innervation [10], it is possible that the adenylcyclase - cAMP system in the septal area play a physiological role as mediator of catecholamine-induced changes in salt and water renal excretion.

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