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Effect of Beta-Adrenergic Stimulation of the Septal Area on Renal Excretion of Electrolytes and Water in the Rat

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CAMARGO, L. A. A., W. A. SAAD, C. R. SILVA NETO, J. ANTUNES-RODRIGUES AND M. R. COVIAN. *Effect of beta-adrenergic stimulation of the septal area on renal excretion of electrolytes and water in the rat.* PHARMAC. BIOCHEM. BEHAV. 11(2) 141–144, 1979.—In order to evaluate the relative roles played by the central beta-receptors in the regulation of renal excretion of Na^+, K^+ and urine volume a study was carried out on the effect of intraseptal injections of isoproterenol on untreated rats and on rats pretreated with locally injected butoxamine and practolol. Injection of 20–160 nmol isoproterenol into the median septal area elicited a dose dependent decrease in Na^+, K^+ and urinary volume. Intraseptal injection of 40 nmol isoproterenol elicited a significant decrease in Na^+ urinary excretion and in urinary volume during the 2 hr period immediately following injection. This effect was blocked by 160 nmol of locally injected butoxamine administered before isoproterenol. In contrast, pretreatment with practolol (160 nmol) did not alter the effect induced by isoproterenol. Pretreatment with these $beta_1$ and $beta_2$ antagonists did not abolish the effect of isoproterenol on K^+ urinary excretion. The $beta_2$ agonist agents, terbutaline and salbutamol, elicited a significant decrease in renal excretion of water and of the two salts. These results indicate that activation of $beta_2$ receptors in the septal area elicits a decrease in Na^+ and urinary volume excretion, while the same effect on K^+ cannot be described as being due to type 2 beta-receptors.

Isoproterenol and sodium excretion Septal area and electrolytes excretion Beta-receptors and sodium excretion $Beta_1$ and $beta_2$ antagonists $Beta_2$ agonists

SEVERAL areas in the central nervous system play a role in the regulation of Na^+ and K^+ urinary excretion [13]. The septal area was shown to be one of the nervous centers involved in the control of Na^+, K^+ and urine excretion [9], with cholinergic and adrenergic neurotransmitting mechanisms also involved in this regulation. Injection of the cholinergic agonist, carbachol, increases Na^+ and K^+ excretion through the activation of muscarinic and nicotinic receptors, however the muscarinic activation shows a decrease in urinary volume while the nicotinic activation showed an increase [24,25]. In turn, the adrenergic mechanism of the septal area elicit two types of effects: intraseptal injection of noradrenaline or adrenaline causes an increase in the excretion of Na^+ and K^+, which is antagonized by treatment with the alpha-blocking agents, dibenamine and phentolamine, but not by the beta-blocking agent, propranolol. Propranolol injected before adrenaline causes a diuretic effect. Thus, it has been suggested that the alpha-adrenergic receptors of the septal area increase Na^+, K^+ and urine excretion, while stimulation of beta-adrenergic receptors elicits salt and urine retention by the kidneys. The different beta-agonist and antagonists have relative specificities, and the existence of two types of beta-adrenoceptors has been postulated [2, 3, 17, 18]. Those which regulate effects on the heart, on lipolysis and the intestines were called $beta_1$, and those which regulate bronchodilatation, vasodilatation, effects on the uterus and muscular glycogenolysis were called $beta_2$. Isoproterenol and the antagonist propranolol are not specific in their reaction with beta-adrenoceptors [1,14]. Salbutamol [10] and the antagonist butoxamine [6,19] shown higher specificity for tissues having $beta_2$ adrenoceptors. Practolol (ICI 50172) shows affinity for $beta_1$ adrenoceptors, but very little for those of $beta_2$ [4,14]

In this paper, the role of beta-receptors in the adrenergic mediation regulating natriuresis, kaliuresis and diuresis has been investigated and a comparison made on the effect of $beta_1$ and $beta_2$ adrenergic blocking agents on intraseptal injections of isoproterenol in terms of excretion.

METHOD

Cannulation of the Medial Septal Area

Male albino Holtzman rats, weighing 250–300 g, were used in the experiments. The animals were kept in individual metabolic cages, room temperature (25–27°C), with free access to food and tap water. To avoid emotional stress, the

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animals were handled daily for 7 days prior to the experiment. After this adaptation period, a stainless steel cannula (O.D. 0.71) was implanted into the median septal area of each animal (previously anesthetized with ether), according to the following coordinates of the De Groot atlas for rat brain [12]: AP + 8.2; H + 0.5; L 0.0. The cannula was secured to the skull with dental cement and jeweler's screws. Prophylactic doses of penicillin and tetracyclin were administered to the animals for 7 days, following the operation. During this time stopper of the cannula was removed and replaced repeatedly, and a tube was inserted into the rat's stomach, so that the animal could get used to the experimental conditions of drug injection and intragastric water overload. One week after the prophylactic treatment the experiments were started.

Experimental Procedure

The animals were deprived of food for 14 hr before the morning of the experiment. A water overload equal to 5% body weight was injected through the intragastric tube in order to obtain constant urinary volumes. One hr later, a new overload was administered, and 20 min later a urine sample was collected. All the animals were intraseptally injected with an isotonic saline solution, before the experiments with drugs were begun. After the injection, 6 successive urine samples were collected from the metabolic cage in graduated ml tubes, every 20 min over a 2 hr period.

Drugs

The drugs used were: dl—isoproterenol hydrochloride (Sigma Chemical, USA), butoxamine hydrochloride (Burroughs Wellcome, U.K.), practolol base (Imperial Chemical Industries, U.K.), terbutaline sulfate (Astra Lab., Brazil) and salbutamol sulfate (Glaxo Lab., Brazil).

The drugs were dissolved in a 0.15 M NaCl solution and 1 μl volumes were injected intraseptally over a period of 10 sec with a microssyringe (Hamilton Co., USA) connected to a 30 ga needle by means of a PE 10 polyethylene tube. The doses are expressed in nmol. When the experiment was made in combination with isoproterenol, butoxamine and practolol were injected 30 min before isoproterenol. An interval of at least 48 hr between tests was allowed for each rat. Sodium and potassium concentrations in the urine samples were determined with an IL-143 flame photometer (Instrumentation Laboratories, USA).

Histology

At the end of the experimental period, the rats were sacrificed under ether anesthesia and the brains removed and fixed in 10% formol. One week later, the brains were cut into 10 μm sections and fixed so that the path of the cannula could be located. Only the animals whose cannulae turned out to be located in the median and dorsal region of the anterior septum were used for the study.

Statistics

The dose-response curves were submitted to variance analysis, and linear regression was calculated.

The data of the effects of the isoproterenol, beta$_1$ and beta$_2$ agonists and antagonists were analyzed using one-way analysis of variance and for individual comparison we used

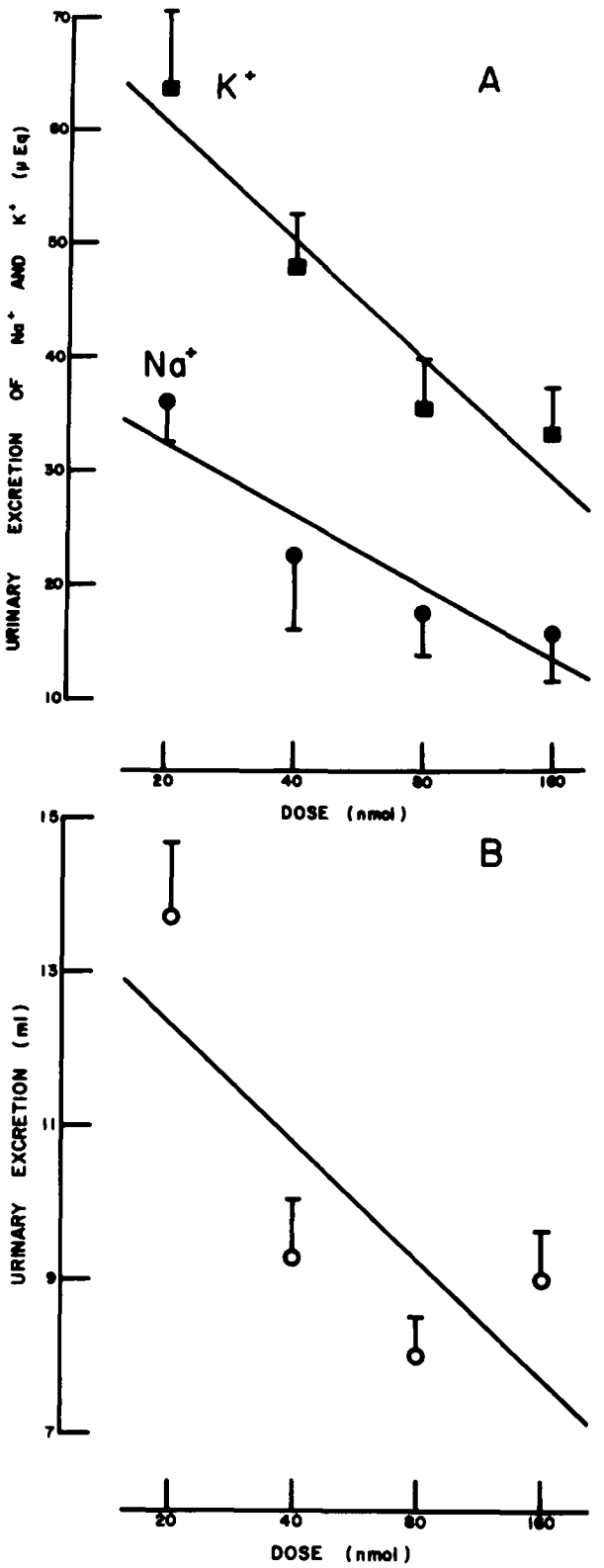

FIG. 1. Dose-effect relationship of isoproterenol on sodium (●) and potassium (■) excretion 1 (A) and on urinary volume (○) 1 (B). The symbols represent average total excretion to the two cations in 10 animals during a 2 hr period following injection of different doses of isoproterenol into the rat median septal area. The vertical bars represent the mean ± S.E.

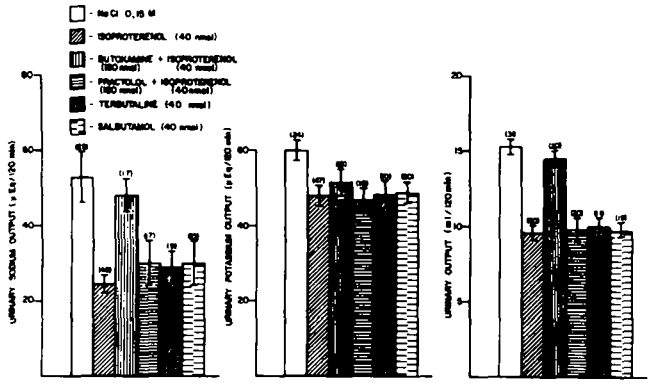

FIG. 2. Sodium, potassium and water excretion during a 2 hr period following injection into the median septal area of: NaCl (0.15 M), isoproterenol (40 nmol), butoxamine (160 nmol), practolol (160 nmol), terbutaline (40 nmol) and salbutamol (40 nmol). Butoxamine and practolol were administered 30 min before isoproterenol through the same cannula. The vertical lines represent the S.E. and the tops of the bars the means.

the mean square within groups as the error term for the t-test.

RESULTS

Effect of intraseptal injection of isoproterenol on Na^+ and K^+ excretion and on urine volume. Dose-response curve. Intracerebral injection of 20 to 160 nmol isoproterenol into the median septal area elicited a dose-dependent decrease in Na^+ and K^+ urinary excretion in a group of 10 rats (Fig. 1A). Variance analysis of the log-dose response relationship showed significant regression and negligible deviations from linearity, $F(3,36)=3.98$, $p<0.05$ and $F(3,36)=8.08$, $p<0.01$ for Na^+ and K^+, respectively. The general equations for the regression were $Y=58.70-6.17x$ for Na^+, and $Y=105.34-10.38x$ for K^+. Doses of isoproterenol varying between 20 and 160 nmol determined a dose-dependent decrease in urinary volume for the same group of animals. Variance analysis showed significant regression, $F(3,36)=11.9$, $p<0.01$ with the following general equation $Y=10.03-1.55x$ (Fig. 1B).

Excretion of Na^+, K^+ and urinary volume after intraseptal injection of isoproterenol, terbutaline and salbutamol. Effects of the beta blocking agents. Figure 2 shows Na^+ urinary excretions following intraseptal injection of 1 μl isotonic saline, 40 nmol isoproterenol, terbutaline and salbutamol. Also shown in this figure is the effect of 160 nmol of a $beta_2$ blocking agent (butoxamine) and 160 nmol of a $beta_1$ blocking agent (practolol) on the effect of isoproterenol. The isoproterenol dose was based on the dose-response curve and considered to be close to the average dose. The same criteria was used in the choice of terbutaline and salbutamol which showed a dose-response curve similar to isoproterenol.

Analysis of variance of the urinary sodium output was performed for cumulative sodium excretion at 120 min following intraseptal injections. It was found that some drug injections affected the sodium excretion, $F(5,131)=6.60$, $p<0.01$. This effect was further analyzed and it was found that the isoproterenol group decreased the excretion of urinary Na^+ when compared with the levels determined by application of control saline ($p<0.01$). Pretreatment with 160 nmol butoxamine, applied intraseptally 30 min before isoproterenol, antagonized the antinatriuretic effect, this group did not differ in cumulative Na^+ excretion when compared with the control saline group ($p>0.01$). However, pretreatment with 160 nmol practolol did not abolish the antinatriuretic effect ($p<0.01$). A significant decrease in natriuresis was also observed upon injection of terbutaline and salbutamol ($p<0.01$). Figure 2 also shows the effect of the $beta_1$ and $beta_2$ blocking agents on the decrease in K^+ urinary excretion elicited by intraseptal injections of isoproterenol, and by the $beta_2$ agonists. Analysis of variance on these data, indicated that the urinary K^+ excretion was affected, $F(5,131)=2.45$, $p<0.05$. Cumulative K^+ excretion for all five groups with drugs treatment were nearly identical. All showed a decrease in K^+ urinary excretion when compared with the results of the saline group ($p<0.05$). Analysis of variance was performed on the 120 min cumulative urine output. Urine excretion had the same characteristics as Na^+ excretion, $F(5,115)=11.66$, $p<0.01$. These effects were further analyzed with the t test, as described above, and it was found that isoproterenol group had an antidiuretic effect ($p<0.01$) which was abolished by butoxamine ($p>0.01$), but maintained by a previous injection of practolol ($p<0.01$), when these values were compared with the saline group. Centrally injected terbutaline and salbutamol produced the same effect as isoproterenol when compared with saline control ($p<0.01$).

DISCUSSION

In agreement with previous observations [7] these results show that injection of appropriate doses of isoproterenol into the median septal area of the rat brain produces a decrease in urinary excretion of Na^+, K^+ and in urine volume. It has already been reported that alpha-receptors blocking agents, such as dibenamine and phentolamine, disguise this effect, and that pretreatment with the beta-blocking agent, propranolol, potentiates the saluretic response elicited by intraseptally injected noradrenaline [8]. The results presented here show that there is a strict relationship between the intraseptal dose of isoproterenol and the decrease in renal excretion of Na^+, K^+ and water. The regression line of the dose-response curve suggests the existence of a monomolecular interaction between the isoproterenol molecules and the beta receptors in the median septal area. The antisaluretic and antidiuretic responses induced by intraseptal injection of isoproterenol indicate a possible mediation by beta receptors. Our results show that a combination of isoproterenol and butoxamine did not elicit any significant alterations when compared with control levels of Na^+ and urinary volume excretion obtained with intraseptal injection of isotonic saline. In contrast, previous injection of the $beta_2$ agonist agent, practolol, shows effects similar to those obtained with isoproterenol alone. These data are confirmed by injection of the $beta_2$ agonists, terbutaline and salbutamol.

The possible efferent pathways which may be responsible for alterations in the excretion of the two cations and in diuresis are still a matter for discussion. One suggestion is that the antidiuretic responses to intraseptal injection of isoproterenol and beta agonist agents are due to the stimulation of a $beta_2$ sensitive neuronal system. The existence of intimate connections between the septal area and paraventricular and supraoptic nuclei has already been demonstrated anatomically [23]. Urinary levels of sodium may be influenced by hemodynamic factors [15,20] or by changes in ar-

teriole tonus [22]. The existence of a natriuretic hormone ('third factor') has also been suggested [11,21].

One possibility is that beta central pathways may interfere with the bladder. Elmer [16] demonstrated that isoprenaline and the $beta_2$ stimulating agents, terbutaline and salbutamol, induce a relaxation of the detrusing muscle with a consequent decrease in the rat intravesicular pressure.

Butoxamine and practolol were unable to block the effect of reduced K^+ excretion induced by isoproterenol. Thus, it is not possible to classify these effects according to the two types of beta-receptors, since the classification into $beta_1$ and $beta_2$ by Lands possibly represents two extremes of a variable spectrum of isoreceptors [5].

In conclusion, the results presented here indicate that the beta-receptors of the median septal area inducing a decrease in Na^+ excretion and antidiuresis may be of the $beta_2$ type, while the effects on potassium do not permit a characterization of these receptors as being typically $beta_2$.

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