Pharmacology Biochemistry & Behavior, Vol. 3, pp. 985–992.

Natriuresis, Kaliuresis and Diuresis in the Rat Following Microinjections of Carbachol into the Septal Area

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(Received 1 October 1974)

SAAD, W. A., L. A. ARRUDA CAMARGO, C. R. SILVA NETTO, C. G. GENTIL, J. ANTUNES-RODRIGUES AND M. R. COVIAN. *Natriuresis, kaliuresis and diuresis in the rat following microinjections of carbachol into the septal area.* PHARMAC. BIOCHEM. BEHAV. 3(6) 985–992, 1975. – The effects of intraseptal injection of carbachol on natriuresis, kaliuresis and diuresis has been studied in conscious, unrestrained water-loaded male rats. Urinary sodium and potassium excretion increased following injections into the septal area. The intensity of the natriuresis and kaliuresis was dose-related. An antidiuretic effect was also observed. The Na^+/K^+ ratio increased with increasing doses of carbachol, indicating that the rise in urinary sodium exceeded that of potassium. Systematic mapping of the septal area yielded about the same results for all sites, excepting a zone located in the anterior-dorsal part of the medial nucleus which appeared more sensitive. The natriuretic effect of intraseptal carbachol in adrenalectomized rats demonstrated the secondary role played by the adrenals. Contrariwise the decrease of the natriuretic effect observed either in hypophysectomized rats or in rats bearing a median eminence lesion receiving intraseptal carbachol showed the important participation of these structures in urinary Na^+ excretion. Adrenalectomy or median eminence lesions did not modify the kaliuretic response while hypophysectomy produced a transitory diminution. This fact favours the hypothesis of different mechanisms involved in Na^+ and K^+ excretion following intraseptal carbachol. These results leave open the question as to mechanism of action but suggest a possible role of the pituitary in mediating the responses. Also, the possibility of a role played by hemodynamic shifts is suggested.

Septal area Urinary sodium output Urinary potassium output Carbachol intraseptal Urine excretion

A great number of consistent experiments have shown that the central nervous system regulates both the intake and urinary excretion of sodium. Regarding the latter the conclusions have been reached through application of different techniques: lesions [6, 15, 16, 22]; electrical stimulation [26]; intraventricular injection or infusion of hypertonic saline [1, 10, 11]; intraventricular and intra-hypothalamic injection of carbachol [5,9].

Since the septal area is one of the nervous structures implicated in the regulation of sodium chloride intake [3, 7, 13, 14, 20, 21], it seemed of interest to investigate whether this limbic structure also regulated the output of urine, sodium and potassium after chemical stimulation by carbachol.

METHOD

Animals

Adult male albino rats of the Holtzman strain, 250–300 g, were used. They were anesthetized with ether and a stainless-steel cannula (o.d. 0.71cm) was stereotaxically implanted in the septal area using the coordinates of de Groot's atlas [8]. Each cannula was provided with a mandrel to prevent its obstruction.

Procedure

To investigate if there were zones in the septal area with different sensitivities to carbachol, groups of 10 rats were implanted with cannulae at several points in the septum. The cannulae were placed either in the medial or lateral nuclei in different frontal and horizontal planes. The rats were kept in individual cages and during one week before the experimental procedure, they were handled daily and trained for the gavage to eliminate any emotional influence. The rats were fasted 14 hr before the experiment but still had free access to tap water. Each rat then received a stomach load of tepid tap water (5 percent of its body weight) and was placed into an individual metabolism cage without food and water. Voided urine passed through the funnel at the bottom of the cage into a graduated centrifuge tube. The first urine sample was collected 1 hr

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after the water load and then another 5 percent gavage of tepid water was given. The test solutions, consisting of different doses dissolved in 1 μl of saline (0.15 M NaCl), were injected into the septal area 20 min after the second water load. Injection was made through a dental stainless steel cannula (o.d. 0.31cm) connected to a 10 μl Hamilton microsyringe by means of a polyethylene tube. The dental cannula was placed inside the implanted one and pressed downward towards its tip. Subsequent urine collections were taken at 20 min intervals during 120 min. The experiment was stopped after the next sixth sample because by this time the changes induced had subsided. The test solutions were: NaCl (0.15 M), carbachol (carbamylcholine chloride, Sigma) and atropine (atropine sulfate).

Dose-response studies of carbachol as related to urinary excretion of Na^+ and K^+ were performed in 7 groups of rats. Each rat of every group received the complete series of doses (0.01, 0.02, 0.1, 0.2, 0.5, 1.0 and 2.0 μg) at 48 hr intervals. Atropine (50 μg) was injected through the septal connula 20 min after the first water load and 60 min later, that is 20 min after the second gavage, carbachol was administered through the same cannula. This interval between both injections was considered the most suitable after previous experiments at 15, 30, 60 and 80 min intervals.

The concentration of sodium and potassium in the urine (expressed in μEq/min) was determined by flame photometry (instrumentation Laboratories, Model IL-143) in each sample. The cumulative value of the 6 samples following the injections was taken for the statistical evaluation of the results and expressed in μEq/120 min. The urine during the same period was measured and expressed in ml/min and the total volume in ml/120 min. Identical determinations were made following intraseptal injections of carbachol either in adrenalectomized or hypophysectomized animals or in rats bearing median eminence lesions.

Bilateral adrenalectomy was performed through a dorsal approach, the median eminence was destroyed by electrolysis and total transpharyngeal hypophysectomy was performed by suction under the light microscope. Twenty rats were used for each group. All the operations were carried out according to the technique described by Zarrow *et al.* [27].

Twenty-four hr after adrenalectomy, carbachol was again injected and substitution therapy started by daily intramuscular injection of 400 μg of DOCA and 1 mg of hydrocortisone. One week after adrenalectomy the effects of carbachol were tested again. Rats which underwent median eminence lesions were also tested at 24 hr and 7 days after the operation. Hypophysectomized rats were tested at 24 hr, 7 and 15 days after the operation and similar substitution therapy as in the adrenalectomized animals was started immediately after surgery. In a group of 10 rats blood pressure was determined. For this purpose one femoral artery was cannulated with polyethylene tubing (PE 50) according to Krieger's technique [17] under ether anesthesia. Carbachol was injected intraseptally 20 min after the second water load and its effects on pulsatile and mean blood pressure were measured with a pressure transducer and recorded on a physiograph in the conscious and unrestrained animal.

Routine histological procedures were done to localize the sites of the cannulae and the destruction of the median eminence. At autopsy hypophysectomy was varified by gross examination of the sella turcica and adrenalectomy was also checked. None of the rats showed evidence either of pituitary or of adrenal remnants.

The data obtained in these studies were analyzed statistically by means of a 1-way or 2-way analysis of variance [25].

RESULTS

Mapping Studies

At all points tested, changes in urinary output of sodium and potassium and urine volume following carbachol injection were elicited but the differences in responses were not significant ($p>0.05$). Nevertheless, in some rats a zone at F, 8.2; L, 0.0; H, +0.5 located in the anterior dorsal part of the medial nucleus showed a greater sensitivity. This zone was then chosen for our studies. Fig. 1 is a microphotograph of the septal area showing the location of the cannula at the parameters aforementioned.

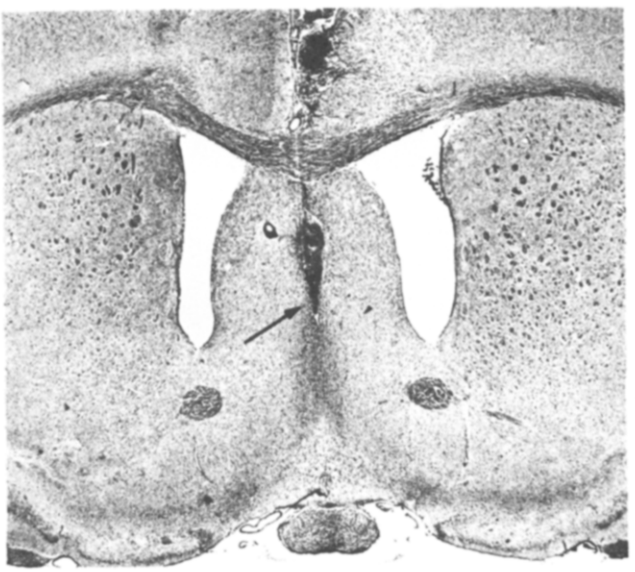

FIG. 1. Photomicrograph of a frontal section of one rat brain illustrating the placement (arrow) of the permanent cannula in the medial septal nucleus (F, 8.2; L, 0.0; H, +0.5).

Sodium and Potassium Excretion and Urinary Volume Following Injection of Carbachol into the Septal Area

Urinary sodium excretion increased following injection of carbachol into the medial septal nucleus. The intensity of natriuresis was dose-related as shown in Fig. 2A. Excretion increased with doses up to 0.5 μg when a plateau was reached. With 0.01 μg, natriuresis was 1.8 ± 0.34 μEq/min and with 0.5 μg it was 17.50 ± 1.92 μEq/min. Figure 2B shows the regression line obtained with the logarithm of the doses.

A dose-response curve for urinary potassium excretion after injecting the same doses of carbachol intraseptally to the same rats used for sodium determinations, was performed. It can be seen in Fig. 3A that the curve rises more quickly than that observed for sodium and reached its maximum, 6.76 ± 0.77 μEq/min, with 0.2 μg of carbachol while with the lowest dose, 0.01 μg, the excretion was 2.77 ± 0.20 μEq/min. At doses greater than 0.2 μg the potassium excretion reached a plateau. Figure 3B shows the linear

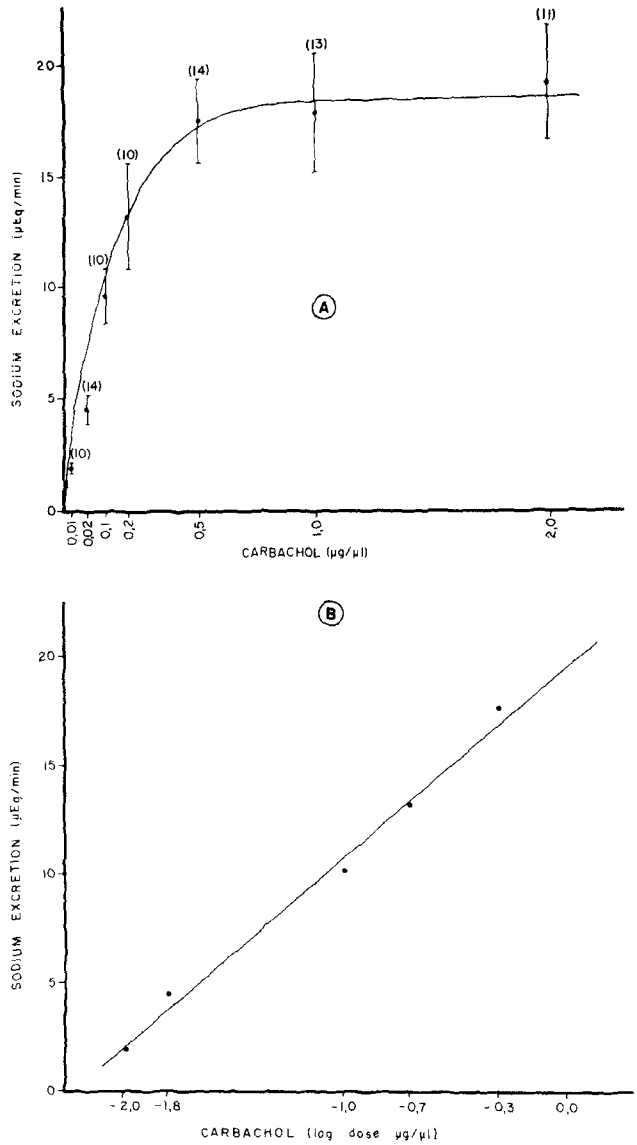

FIG. 2. (A) Dose-response curve of carbachol on urinary Na^+ excretion. (B) Regression line of the dose-response curve obtained with the logarithm of the doses. Points in A and B represent the mean Na^+ output during the 120 min period following the injection of different doses of carbachol into the medial septal area of the brain. Vertical lines in A represent the standard error of the mean. Each rat received the complete series of doses. The number of rats of every group is indicated in parentheses.

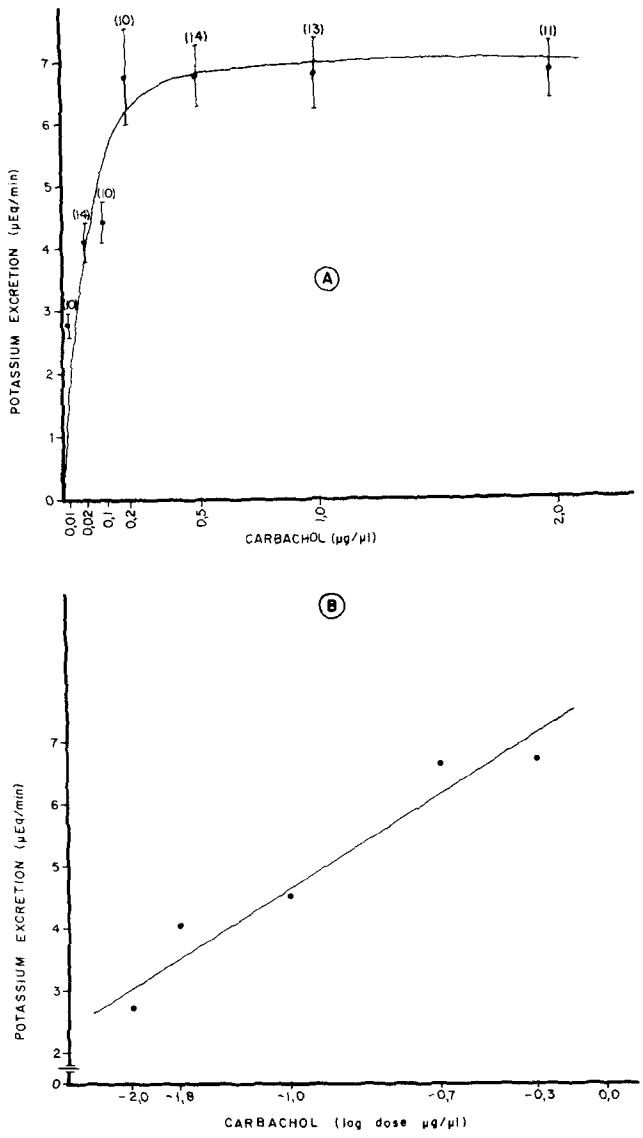

FIG. 3. (A) Dose-response curve of carbachol on urinary K^+ excretion. (B) Regression line of the dose-response curve obtained with the logarithm of the doses. Points in A and B represent the mean K^+ output during the 120 min period following the injection of different doses of carbachol into the medial septal area of the brain. Vertical lines in A represent the standard error of the mean. Each rat received the complete series of doses. The number of rats of every group is indicated in parentheses.

regression of the dose-response curve calculated using the logarithm of the doses.

Figure 4A shows the temporal course of sodium excretion values following the injection of the different doses of carbachol. It is observed that: (a) the excretion in response to the 0.01 µg of carbachol was not significantly different from the control (0.15 M saline) excretion; (b) the appearance of natriuresis started at a dose of 0.02 µg; (c) the maximum peak was reached with 0.5 µg at 40 min (6.12 µEq/min). The within-group variations between 0.01, 0.02, 0.1, 0.2 and 0.5 µg doses were statistically significant ($p<0.01$). Higher doses than 0.5 µg carbachol resulted in a slight reduction of the peak values of excretion.

Figure 4B shows the temporal course of potassium excretion values after injection of the different doses of carbachol. It can be seen that: (a) the excretion with the dose of 0.01 µg of carbachol was not significantly different from the control excretion values; (b) the appearance of kaliuresis started also at a dose of 0.02 µg; (c) the maximum peak was reached with 0.2 µg at 60 min (1.71 µEq/min). The within-group variations between 0.01, 0.02, 0.1 and 0.2 µg were statistically significant ($p<0.01$).

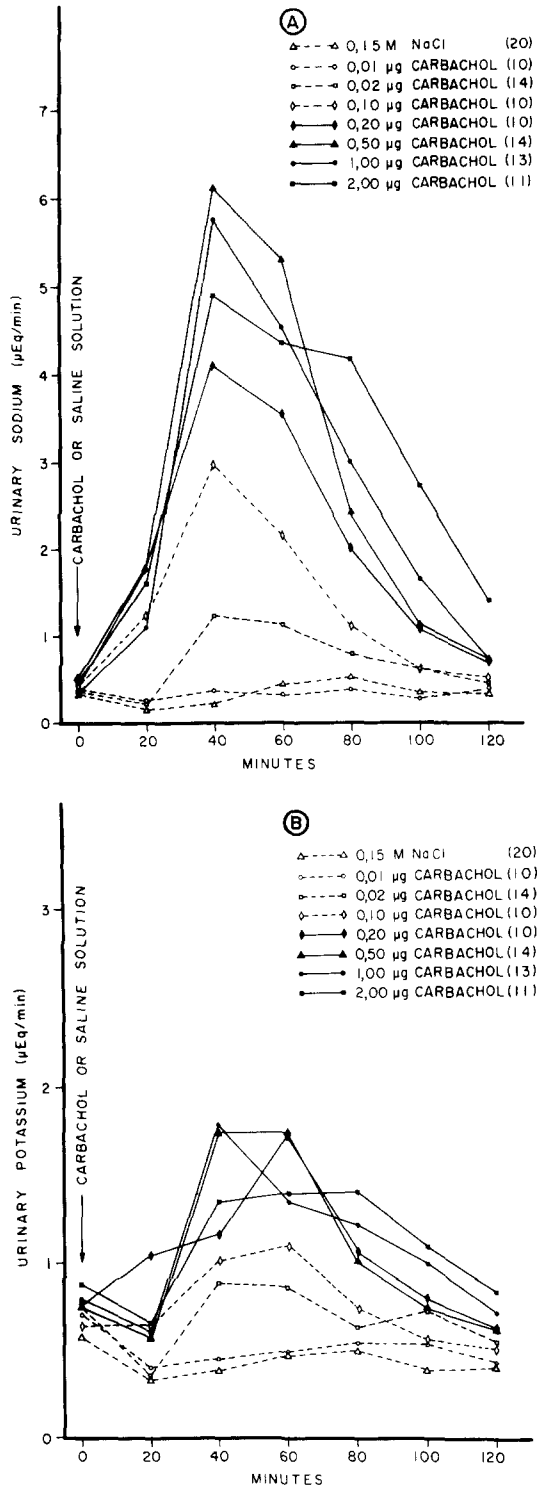

FIG. 4. Sodium (A) and Potassium (B) excretion plotted as a function of time after the injection of NaCl (0.15M) or 0.01, 0.02, 0.1, 0.2, 0.5, 1.0 and 2.0 µg of carbachol into the medial septal area. Number of animals in every group is indicated in parentheses. Each point represents the mean value of each group during a 20 min interval. Arrow marks the baseline values at 0 min relative to injection of test solutions.

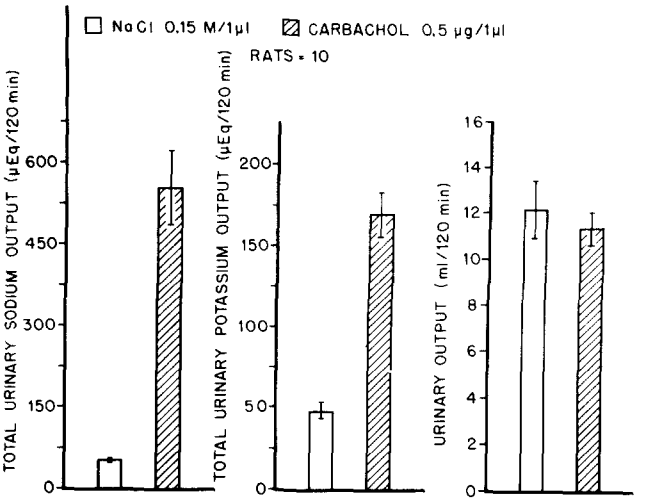

FIG. 5. Urinary Na^+ and K^+ output and volume of urine during the 120 min period following the injection into the medial septal area of NaCl (0.15M) or 0.5 µg carbachol. Each bar represents mean of 10 rats; the standard error of the mean is indicated by vertical lines.

Taking into account the above results, only the dose of 0.5 µg of carbachol was used in the subsequent studies. Control values for sodium and potassium excretion and for urine volume were obtained by analyzing the total output of both electrolytes and the volume of urine excreted after the intraseptal injection of 0.15 M NaCl solution. The results observed following the injection of carbachol (0.5 µg) into the septal locus were related to the control values shown in Fig. 5. It is seen that there was approximately a tenfold and a threefold increase in Na^+ and K^+ excretion respectively over the control saline-injected rats. On the contrary, there was no difference in the total volume of urine. It should be noted that, when the temporal sequence of urine excretion was considered, as seen below (Fig. 6C), an antidiuresis appeared at some intervals.

The same doses of carbachol in the lateral ventricle also determined similar changes in natriuresis, kaliuresis and diuresis. The response was greater than that obtained after intraseptal injections with doses of 0.1 and 0.2 µg/µl, but with 0.01, 0.02, 0.5 µg/µl the difference was not significant.

Urinary Na^+/K^+ Ratio Following Intraseptal Injections of Carbachol

The Na^+/K^+ ratio increased with the dose of carbachol indicating that the increases in urinary sodium exceeded those of potassium. The correlation coefficient (r) for the excretion of both electrolytes was +0.86.

Effect of Atropine Sulfate

In order to investigate the possible participation of cholinergic synapses in the effect elicited by carbachol, pharmacological blockade by atropine was tested. Atropine sulfate (50 µg) was injected through the septal cannula into a group of 10 rats, and 60 min later, carbachol (0.5 µg) was administered through the same cannula. The results, shown in Fig. 6A and B show the blocking effect of atropine on natriuresis and kaliuresis induced by carbachol. The values

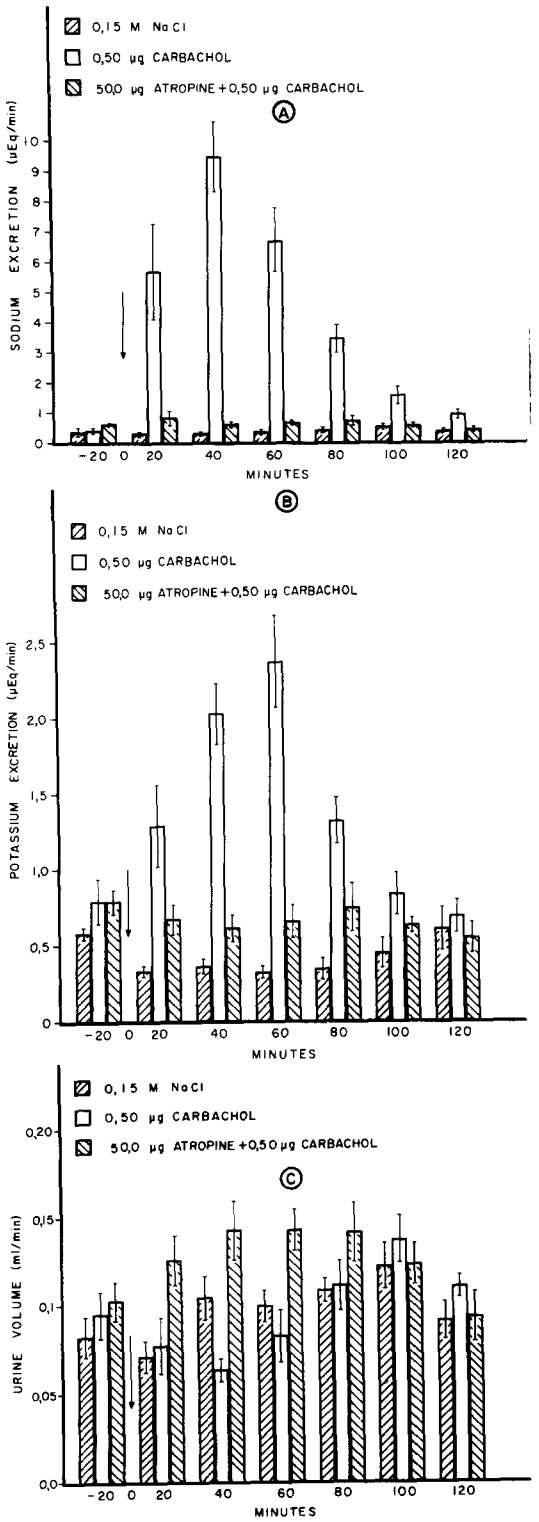

FIG. 6. Sodium (A), potassium (B) excretion and urine volume (C) plotted as a function of time after injection into the medial septal area of NaCl (0.15 M), or carbachol (0.5 µg) or atropine sulfate (50.0 µg) given 60 min before carbachol through the same cannula. Baseline values are those obtained at −20 min relative to injection (arrow) of test solutions, which were given at 48 hr interval. Each bar represents mean of 10 rats during a 20 min interval. The standard error of the mean is indicated by vertical lines.

for sodium and potassium excretion following the injection of atropine and carbachol were not significantly different from the controls. The correlation coefficient (r) for the excretion of Na^+ and K^+ was +0.71.

Figure 6C shows the results obtained by measuring the urinary volumes. The following facts must be emphasized: (a) a significant decrease was induced by carbachol at 40 min ($p<0.01$); (b) urine volume was significantly increased after administration of atropine-carbachol at 20, 40 and 60 min ($p<0.01$) in comparison with saline or carbachol excretion values.

Effects of Adrenalectomy

In order to ascertain the role played by the adrenals in the above described effects, cannulae were implanted in the septal area of 20 rats. The effects of carbachol were studied before adrenalectomy and 24 hr and 7 days afterwards. The results presented in Table 1 show a significant decrease in Na^+ excretion ($p<0.01$) only at 24 hr after operation. On the contrary, K^+ excretion did not show any significant change.

Effects of Median Eminence Lesions

The same experimental design was followed in 20 rats which underwent median eminence lesions, and the results are also shown in Table 1. It is seen that Na^+ excretion diminished significantly ($p<0.01$) at 24 hr but it regained the preoperative value at 7 days after the operation. Regarding K^+ excretion and urinary volume no significant changes could be observed. Figure 7 shows the microphotograph of the median eminence lesion of one rat of this group.

Effects of Hypophysectomy

To investigate the role of the hypophysis in the observed effects, 20 rats with cannulae implanted in the medial septal nucleus were tested with 0.15 M NaCl and carbachol, then hypophysectomized and tested again with carbachol 24 hr, 7 and 15 days afterwards. In Fig. 8A is depicted the significant decrease ($p<0.01$) of Na^+ excretion during the 3 postoperative periods. Twenty-four hr after the operation the lowest excretion value was reached, ($p<0.01$) which then increased significantly to values higher than that obtained by the injection of NaCl 0.15 M in the preoperative period. Regarding potassium excretion a transitory but significant diminution ($p<0.01$) 24 hr and 7 days after hypophysectomy was observed (Fig. 8B); these values were not statistically different from that obtained with NaCl 0.15 M. Fifteen days afterwards the output regained its preoperative value. Diuresis (Fig. 8C) diminished significantly during the three postoperative periods ($p<0.01$).

Blood Pressure Effect of Intraseptal Carbachol

In 10 rats in which 0.5 µg of carbachol elicited a clearcut natriuretic and kaliuretic response, its effect on blood pressure of the unanesthetized animals was studied, to investigate a possible hemodynamic influence. In all animals the blood pressure increased significantly outlasting the chemical application by 50–60 min. The average value of the median arterial pressure rose from 110 ± 5 mm Hg to 128 ± 5 mm Hg. Atropine blocked this effect and the injection of NaCl 0.15 M (1 µl) did not elicit any change.

TABLE 1

EFFECT OF INTRASEPTAL CARBACHOL (0.5 μg) ON URINARY Na^+ AND K^+ EXCRETION (μEq/min) AND URINE VOLUME (ml/120 min) IN TWO GROUPS OF 20 RATS EACH, BEFORE AND 24 HR AND 7 DAYS AFTER EITHER ADRENALECTOMY OR MEDIAN EMINENCE LESION. BASELINE VALUES WERE OBTAINED BY INJECTING NaCl (0.15 M) BEFORE OPERATIONS. MEAN VALUE AND STANDARD ERROR OF THE MEAN ARE SHOWN.

Excretion	Adrenalectomy				Median Eminence Lesion			
	Control (NaCl 0.15M)	Carbachol	24 hr	7 days	Control (NaCl 0.15M)	Carbachol	24 hr	7 days
Na^+	2.8 ± 0.1	23.3 ± 1.0	17.2 ± 0.7	29.1 ± 2.4	2.1 ± 0.2	19.6 ± 2.3	5.6 ± 1.0	19.6 ± 2.4
K^+	2.1 ± 0.3	10.3 ± 0.5	8.5 ± 0.6	9.1 ± 0.6	2.4 ± 0.2	6.5 ± 0.5	5.6 ± 0.3	6.4 ± 0.4
Urine	11.9 ± 0.7	11.5 ± 0.7	9.2 ± 0.5	12.6 ± 0.7	15.1 ± 0.5	13.2 ± 0.7	11.0 ± 0.6	10.8 ± 0.7

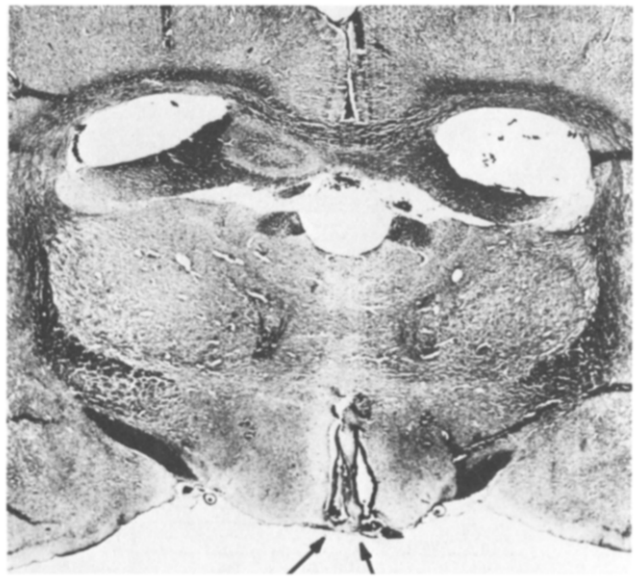

FIG. 7. Photomicrograph of frontal section of one rat brain showing the median eminence lesions, indicated by arrows.

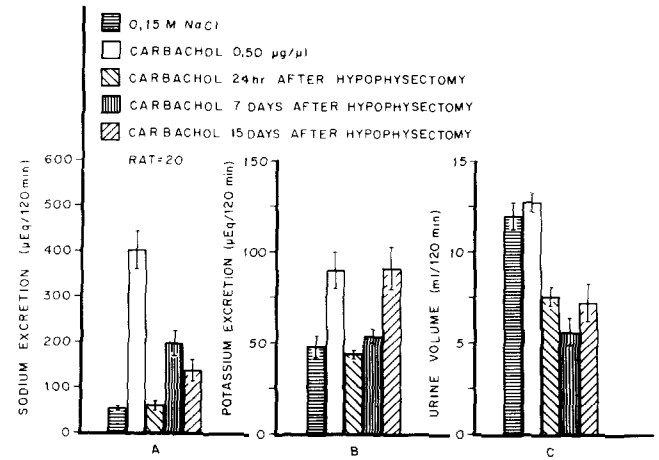

FIG. 8. Urinary Na^+ (A) and K^+ (B) output and volume of urine (C) during the 120 min period following the injection into the medial septal area of NaCl (0.15 M) or carbachol (0.50 μg). The tests were performed before hypophysectomy and 24 hr, 7–15 days after operation. Each bar represents mean of 20 rats; the standard error of the mean is indicated by vertical lines. Substitution therapy (DOCA and hydrocortisone) was started immediately after operation.

DISCUSSION

The results here presented show that septal stimulation by carbachol modifies natriuresis, kaliuresis and the urine output in conscious, unrestrained water-loaded male rats, induces also a hypertensive reaction. All these modifications were blocked by atropine indicating that the action of carbachol is a muscarinic one, and suggesting the existence of a cholinergic pathway mediating the responses in the septal area. It should be remembered that intraseptal carbachol induces water ingestion in the rat [2]. The systematic mapping of the septal area yielded about the same results for all sites, excepting a zone located in the anterior dorsal part of the medial nucleus which appeared more sensitive. There was a close relationship between the dose of intraseptal carbachol and Na^+ and K^+ urinary excretion with a greater sensitivity for the rise in K^+. The hyperbolic shape of the dose-response curve, transformed by a logarithmic plot suggests that an interaction takes place between carbachol and pharmacological receptors present in the septal area.

The fact that similar changes were obtained when carbachol was injected in the lateral ventricle indicates that structures responsive to it are likewise located within the ependymal lining of the lateral ventricle. The possibility of a diffusion from the septal area into the ventricle exists and has not been investigated in our work but it is worthy to remember that the most sensitive septal zone was not lateral but medial.

Adrenalectomy interferes to a small extent with urinary sodium excretion but no significant changes occurred in the excretion of potassium. It should be noted that if there was a diminution of sodium output following adrenalectomy this excretion was always significantly higher than the control. This fact and the absence of change in potassium output is evidence for the subsidiary influence of adrenals. This influence can not be explained by the lack of aldosterone, a circumstance which might be anticipated to lead to an augmented excretion of sodium and not to a decrease as was shown in our experiments. Maybe the withdrawal of the glucocorticoids induced a decrease in the glomerular filtration rate and renal plasma flow and consequently a smaller presentation of sodium to the kidney.

Lesion of the median eminence did not interfere with K^+ and urine excretion and its effects on Na^+ excretion disappeared 7 days after the operation. It is difficult to explain this transitory diminution unless an action through the hypophysis is considered. The interruption of the vascular connection between both structures and its subsequent regeneration would explain the transitoriness of the effect.

The role played by the hypophysis, can be attributed to its release of hormones with known natriuretic effects: vasopressin, oxytocin and the melanocyte stimulating hormone (MSH) which has been shown to elicit an intense urinary sodium output in the water-loaded rat. The diminution of urine output in our rats suggest an increased secretion of antidiuretic hormone by the neurohypophysis.

The fact that adrenalectomy or median eminence lesions did not modify the kaliuretic response and the transitory decrease observed after hypophysectomy as well as the differences in the dose-response curves favours the hypothesis of different mechanisms involved in Na^+ and K^+ excretion due to intraseptal carbachol injection.

The mechanism whereby carbachol injected into the septal area determines an increase in the renal excretion of sodium and potassium remains to be elucidated. In our experiments using conscious and unrestrained rats intraseptal carbachol raised the blood pressure for 40–60 min. It has been reported that hemodynamic factors, such as changes in renal capillary pressure which influence epithelial transport rates [12,18] or changes in renal arteriolar tone can influence the level of urinary sodium [19]. It is possible that the increased blood pressure in our rats played a role in the natriuretic and kaliuretic effects of intraseptal carbachol. It has been shown recently that natriuresis of central origin appears secondary to increased blood pressure [4]. Our present experiments have not determined the mechanism of the induced natriuretic and kaliuretic effect of carbachol but research is being conducted to clear up this problem by correlating the blood pressure changes with changes in renal plasma flow and inulin clearance.

Our results leave open the question as to mechanism of action but suggest a possible role of the hypophyseal hormones in mediating the Na^+ and urine excretion after intraseptal carbachol injection and also that factors as hemodynamic changes could contribute to some extent to modulate that excretion. For K^+ excretion induced by carbachol injected into the septal area, a different mechanism might be involved.

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