Water, Sodium Chloride, and Food Intake Induced by Injections of Cholinergic and Adrenergic Drugs into the Third Ventricle of the Rat Brain (34713)

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Previous studies suggest that the central nervous system is involved in the regulation of soduim chloride intake. Several regions have been extensively studied in this regard, among them the hypothalamus, septal area, and amygdaloid complex. Hypothalamic lesions can increase or decrease NaCl intake according to the area destroyed (1, 2). Similar results have been described after lesions of the amygdaloid complex (3), whereas, the destruction of the septal area always induced an increase in sodium chloride intake (4).

Grossman and others (5–10) have studied the effects of possible synaptic transmitters on food and water intake after their microinjection into a variety of loci in the hypothalamus, septum, and amygdaloid complex. With the use of this microinjection technique, cholinergic synapses seemed to be more involved in the elicitation of water intake, whereas adrenergic synapses were related to food intake. The effects of cholinergic and adrenergic agents were found to be blocked partially by systemic administration of the appropriate blocking drugs (9).

As an extension of our systematic studies on central nervous regulation of hydromineral metabolism, the influence of possible synaptic transmitters which might be involved in the regulation of sodium chloride intake was investigated. The drugs were microinjected into the third ventricle, and the effects of these agents was compared with that obtained with injection of isotonic and hypertonic NaCl solutions.

Methods. Adult Sprague-Dawley male rats, weighing 250–300 g, were kept in individual cages provided with a cup filled with dry food (a constant formula diet prepared in our animal facility) and two bottles, one filled with tap water and the other with 1.5% NaCl solution. Their normal food, water, and sodium chloride intake was studied for 1 week before the implantation of cannulas in the third ventricle.

Cannulas were prepared from 22-gauge, stainless steel tubing (Superior Tube Co., Norristown, Pa.). Each cannula was 15 mm in length, and had a flat tip with a beveled edge. Each cannula was provided with a mandril (28 gauge) to prevent its obstruction.

For cannulation the animals were anesthetized with ether, ear plugs were inserted, and the rat's head was fixed in a Krieg-Johnson stereotaxic instrument. The coordinates used for the third ventricle were the following: anteroposterior (AP) = 1.3 mm behind bregma; lateral = just on the midline (above the superior longitudinal sinus) and vertical = 0.3 mm above the base of the skull.

The cannula was mounted in the stereotaxic instrument with the aid of a stainless steel wire fitting exactly its inside diameter which served as the cannula guider. Two screws (2/56 × 3/16 in.) were screwed firmly into small holes drilled in the adjacent parietal and frontal bones to serve as an anchor for the dental cement. The cannula was introduced through a hole over the superior longitudinal sinus, after the sinus was pulled gently to the left side with a hypodermic needle (27 gauge). This procedure prevented rupture of the sinus with consequent hemor-

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rhage. The cannula was lowered to the skull base and then raised 0.3 mm. Its location in the third ventricle was confirmed when cerebrospinal fluid (CSF) flowed continuously from the cannula. This could be observed even with the guider in position. A small amount of dental cement was placed around the cannula and screws. Finally, after removing the guider mounted in the stereotaxic instrument, an additional amount of cement was added around the cannula and to cover the screws completely. The skin was left without suture. After surgery the animals were returned to their own individual cages until the day of the experiment. Once implanted, the mandril was removed every other day, rinsed with isotonic saline and returned to its original position, so as to maintain the cannula clean and to get the animal acquainted with the experimental procedure to be followed.

At the termination of the experiments histological sections were made in some animals to ascertain the cannula position in the third ventricle. It was observed that whenever CSF came out through the cannula during the implantation procedure the cannula was later found to be in the right position. Later on, leakage of CSF from the cannula was used as the criterion for proper placement of the cannula.

One week after the implantation the experiments were started by injecting different solutions, in minute amounts and volume, into the third ventricle. The following substances were applied in several doses to the hypothalamus: isotonic saline (0.15 *M* NaCl), hypertonic saline (0.86 *M*), acetylcholine, carbachol, *l*-noradrenaline, *l*-Adrenaline, dopamine, isoproterenol, and *l*-metaraminol.

The injections of these substances were performed according to the following procedure: the mandril was removed and an inner cannula of the same length was introduced. This cannula was connected by a polyethylene plastic tube to a 10-μl syringe filled with the chemical to be injected. After 60 sec, with the inner cannula in its position inside the animal's brain, the chemical solution was injected slowly, with the animal free in its cage. The injection time was approximately 60 sec.

Two types of controls were used: (i) animals injected with same volume of isotonic saline (2–5 μl); and (ii) rats with permanent cannulas, in which the only treatment was to remove and clean the mandril the day of the experiment.

The drugs were applied randomly when tested in the same animal in order to avoid the possible interaction of successive treatments. 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. Papers beneath the cage were carefully checked during and after each experiment for possible spillage of fluids or food. None of the treatments was followed by increased spillage of liquid or food. Water, sodium chloride, and food intake were measured 1 and 6 hr after the injection. The mean responses presented represent the arithmetic mean of all trials with a particular treatment. Statistical analysis was performed using an analysis of variance and Duncan's multiple range test.

Results. One hundred forty-eight experiments were performed with rats under various treatments. Control animals injected with isotonic saline exhibited no significant alteration in their intake of water, hypertonic salt solution, or food on comparison with rats receiving no treatment except for removal and replacement of the mandril in the cannula. In the analysis of subsequent experiments all statistical comparisons were made using the isotonic saline-injected group as the control.

Water intake. Of all substances injected only carbachol (2 μg) consistently modified water intake during the first hour after its intraventricular injection. Drinking began within 3 min of injection and lasted 15–20 min, so that water intake was increased from 1 ml in the saline-injected controls to 15 ml during the first hour after carbachol, a highly significant effect ($p < .01$) (Table I). No significant additional stimulation of drinking was observed during the remaining 5 hr, but at the end of 6 hr the total intake by this

TABLE I. Water Intake in Satiated Rats 1 and 6 hr After Third Ventricular Injection of Adrenergic and Cholinergic Drugs and Hyper- and Isotonic Saline.

Treatment	Dose	Vol (μl)	No. of rats	Water intake (ml); time after injection: 1 hr	6 hr
No injection	—	—	27	1.37 ± 0.29[a]	3.33 ± 0.46[a]
Isot. saline	0.15 *M*	2	30	0.90 ± 0.20	2.80 ± 0.41
Hypertonic saline	0.86 *M*	2	8	2.56 ± 0.85	9.25 ± 1.64[b]
l-Epinephrine	5 μg	5	9	3.89 ± 0.87	7.33 ± 1.85
l-Norepinephrine	5 μg	5	15	3.60 ± 0.77	7.06 ± 1.02
Dopamine	2 μg	5	5	0.80 ± 0.37	2.50 ± 0.67
	5 μg	5	5	1.20 ± 0.37	2.80 ± 0.72
	10 μg	5	5	0.50 ± 0.32	2.90 ± 0.36
Isoproterenol	1 μg	5	5	0.10 ± 0.10	3.10 ± 1.80
	5 μg	5	5	0.30 ± 0.30	4.90 ± 0.78
	10 μg	2	7	0.93 ± 0.60	4.00 ± 1.21
Metaraminol	11 μg	2	6	4.67 ± 2.14	6.25 ± 2.06
	22 μg	4	7	2.36 ± 0.74	6.07 ± 1.07
Acetylcholine	5 μg	5	5	2.00 ± 0.32	2.80 ± 0.58
Carbachol	2 μg	2	9	15.33 ± 3.27[b]	17.33 ± 3.07[b]

[a] Mean ± SEM.

[b] $p < 0.01$ versus isotonic saline control.

group still greatly exceeded that of the controls ($p < .01$). Acetylcholine injection produced no significant alteration in water intake.

Several of the adrenergic drugs injected produced an increase in water intake in some individual animals during the first hour; however, the responses were not consistent, so that the mean responses were not significantly different from the control. Both epinephrine and norepinephrine and *l*-metaraminol, a stimulator of α-adrenergic receptors, produced this tendency to increased water intake; whereas, isoproterenol, a stimulator of β receptors, produced either no change or a tendency to reduced water intake apparent only in the first hour after injection of lower doses (1 or 5 μg).

Microinjection of 2 μl of hypertonic saline (0.86 *M*) produced behavioral changes followed by an increase in water intake. Just after injection of this solution the rats exhibited increased locomotor activity for around 10 min which was followed by somnolence. Possibly because of these additional behavioral changes, water intake was increased only slightly and not significantly during the initial hour after injection. A definite ($p<.01$) increase of nearly threefold was observed 6 hr after injection.

Hypertonic (1.5%) NaCl intake. Only two drugs had a significant effect on the intake of this solution (Table II). Carbachol increased NaCl intake 14.5-fold above control levels obtained with injection of isotonic saline solution ($p<.01$). Again, carbachol acted within minutes and the effect lasted around 20 min. There was no additional effect but the increase in salt intake was still significant ($p<.05$) at 6 hr.

The increase in salt intake evoked by isoproterenol (10 μg) was slightly smaller (12.5-fold) ($p<.05$) but the drinking lasted longer. Consequently, the total intake at 6 hr was actually greater than that produced by carbachol at 6 hr. The difference from control intake at this time was highly significant ($p<.01$). Lower doses of isoproterenol (1 or 5 μg) were ineffective. Rather surprising in view of the response to isoproterenol was the failure of either epinephrine or norepinephrine to produce a significant alteration of

TABLE II. Sodium Chloride Intake in Satiated Rats 1 and 6 hr After Third Ventricular Injection of Adrenergic and Cholinergic Drugs and Hyper- and Isotonic Saline.

Treatment	Dose	Vol (μl)	No. of rats	Sodium chloride intake (ml); time after injection: 1 hr	6 hr
No injection	—	—	27	0.40 ± 0.11[a]	1.26 ± 0.25[a]
Isot. saline	0.9%	2	30	0.17 ± 0.07	0.98 ± 0.10
Hypertonic saline	0.86 *M*	2	8	0.12 ± 0.12	0.75 ± 0.40
l-Epinephrine	5 μg	5	9	0.61 ± 0.23	1.33 ± 0.53
l-Norepinephrine	5 μg	5	15	0.87 ± 0.27	1.77 ± 0.47
Dopamine	2 μg	5	5	0.00 ± 0.00	0.70 ± 0.12
	5 μg	5	5	0.00 ± 0.00	1.00 ± 0.35
	10 μg	5	5	0.16 ± 0.10	1.90 ± 0.56
Isoproterenol	1 μg	5	5	0.10 ± 0.10	0.50 ± 0.15
	5 μg	5	5	0.18 ± 0.09	0.80 ± 0.12
	10 μg	2	7	2.14 ± 1.16[b]	4.28 ± 1.67[c]
Metaraminol	11 μg	2	6	1.00 ± 0.34	1.83 ± 0.70
	22 μg	4	7	0.21 ± 0.15	0.43 ± 0.27
Acetylcholine	5 μg	5	5	0.40 ± 0.24	1.00 ± 0.32
Carbachol	2 μg	2	9	2.44 ± 1.30[c]	3.44 ± 1.19[b]

[a] Mean ± SEM.

[b] $p < 0.05$ versus isotonic saline control; [c] $p < 0.01$.

saline intake although both did produce some increased consumption during the first hour. Dopamine appeared to inhibit salt intake since intake ceased entirely during the first hour after injection of both lower doses (2 and 5 μg). This apparent inhibition was not observed after the 10-μg dose, and intake was back to normal at 6 hr after all three doses.

Food intake. This was modified by the intraventricular injection of both adrenergic and cholinergic drugs (Table III). During the first hour after injection increased feeding was observed ($p < .01$) with both epinephrine and norepinephrine (5-μg doses). This effect was rather prolonged, and the animals ate almost continuously during the first hour. No additional increase in intake was noted during the remainder of the experiment, and in fact the total intake over the 6 hr period was significantly increased only with norepinephrine ($p < .01$). The α-receptor stimulator, metaraminol, also induced an increase in food intake which was significant ($p < .05$) at the end of the first hour with the higher dose (22 μg). At the end of 6 hr, significant ($p < .05$) increases were observed with the lower dose (11 μg) as well. Isoproterenol, a drug which effects β receptors, was active in increasing food intake at 6 hr but had no significant effect during the first hour. Dopamine similarly increased food intake at 6 ($p < .05$) but not at 1 hr. Even carbachol was effective at this later time ($p < .01$). Hypertonic saline also increased food intake at 6 hr ($p < .01$) although the slight augmentation of intake obtained at 1 hr was not significant.

Discussion. Previous studies indicate that there is a hypothalamic regulation of food, water, and sodium chloride intake and that a circuit involving hypothalamus, septal area and amygdaloid nuclei may also be involved in the regulation of salt intake.

Our results confirm the earlier findings of Grossman (5–9) that carbachol can increase water intake by producing a dramatic and rapid stimulation of drinking. Since none of the other drugs were effective, the response to carbachol would appear to be quite specific. The failure of acetylcholine to induce drinking is explained by its rapid destruction by choline esterase. Thus, the current work lends strong support to the concept of a cholinerg-

TABLE III. Food Intake in Satiated Rats 1 and 6 hr After Third Ventricular Injection of Adrenergic and Cholinergic Drugs and Hyper- and Isotonic Saline.

Treatment	Dose	Vol (μl)	No. of rats	Food intake (g); time after injection: 1 hr	6 hr
No injection	—	—	27	0.60 ± 0.13[a]	3.39 ± 0.26[a]
Isot. saline	0.9%	2	30	0.62 ± 0.16	2.70 ± 0.31
Hypertonic saline	0.86 *M*	2	8	1.22 ± 0.45	6.92 ± 0.91[c]
l-Epinephrine	5 μg	5	9	2.83 ± 0.59[c]	4.16 ± 0.70
l-Norepinephrine	5 μg	5	15	2.66 ± 0.17[c]	5.56 ± 0.46[c]
Dopamine	2 μg	5	5	0.56 ± 0.24	4.74 ± 0.46[b]
	5 μg	5	5	1.04 ± 0.28	5.00 ± 0.89[b]
	10 μg	5	5	0.92 ± 0.21	4.70 ± 0.56
Isoproterenol	1 μg	5	5	0.98 ± 0.26	5.12 ± 1.17
	5 μg	5	5	0.92 ± 0.15	4.62 ± 0.92
	10 μg	2	7	0.08 ± 0.08	5.85 ± 0.86[b]
Metaraminol	11 μg	2	6	1.73 ± 0.55	4.97 ± 0.84[b]
	22 μg	4	7	2.02 ± 0.52[b]	7.03 ± 0.69[b]
Acetylcholine	5 μg	5	5	0.20 ± 0.20	2.80 ± 0.92
Carbachol	2 μg	2	9	1.44 ± 0.32	5.85 ± 0.72[c]

[a] Mean ± SEM.

[b] $p < 0.05$ versus isotonic saline control; [c] $p < 0.01$.

ic synapse in the pathways regulating water intake.

Microinjection of hypertonic saline into the ventricle also produced a delayed increase in water intake. The delay in drinking may be ascribed to the initial hyperactivity followed by somnolence which was induced by the saline. These results are the first to our knowledge to show that third ventricular injection of hypertonic saline can increase water intake in rats. This phenomenon is well documented in the goat after injection of hypertonic saline into either hypothalamic tissue (11–12) or into the third ventricle (13) and has provided evidence for the existence of osmoreceptors in this part of the brain which are involved in the regulation of water intake.

Our data demonstrate that intraventricular carbachol induces an increase in salt as well as water intake which suggests that a cholinergic synapse may also lie in the pathways that mediate this behavior. The only other drug which significantly modified salt intake was isoproterenol, a drug which stimulates β-adrenergic receptors. This produced a highly significant effect at the high dose (10 μg). Metaraminol, which affects α-adrenergic receptors, was without effect as were epinephrine and norepinephrine. The results suggest the presence of a β-adrenergic synapse in the salt-regulating mechanism, although one would expect a positive response to epinephrine and norepinephrine. Possibly epinephrine and norepinephrine were metabolized too rapidly by monoamine oxidase for the effects to become manifest at the doses used. Both did produce an increase in salt intake which did not achieve statistical significance.

Since the completion of this study, an important paper by Chiaraviglio and Taleisnik (10) has appeared in which they showed that implantation of crystalline norepinephrine directly into the medial hypothalamus or third ventricle would induce an increase in salt intake (1% NaCl). In the present study, norepinephrine did not produce a significant effect on salt intake. The difference may be related to the use of crystalline norepinephrine in their study to provide a higher concentration of the drug than the aqueous solution used here.

They further showed that dibenamine, an α-adrenergic blocking agent, and reserpine, a compound which depletes brain catecholamine, would block the increased salt intake in salt-depleted rats, whereas dichloroisoproterenol, a β blocker, was ineffective. The results with the α and β blocker are puzzling in view of our finding that a β-receptor stimulator, isoproterenol, would increase salt intake, whereas the α-receptor stimulator, metaraminol, was ineffective. It would appear that an adrenergic synapse is involved in the pathway mediating salt intake. The receptor involved may not satisfy completely the criteria for either a β or α receptor.

Chiaraviglio and Taleisnik also reported that crystalline acetylcholine stimulated water, but not saline drinking, and concluded that a cholinergic synapse was involved in the mediation of water but not salt intake. In our experiments, carbachol clearly stimulated salt as well as water intake; whereas, acetylcholine failed to modify either behavior. The discrepancy may be related to the fact that carbachol is a more potent cholinergic drug than acetylcholine which is rapidly inactivated by cholinesterase. Their finding that atropine reduced the salt intake of salt-depleted rats would fit with our conclusion that a cholinergic synapse also lies in the pathways that mediate salt intake.

With regard to food intake, the present results agree with those already reported by Grossman (6, 7) and by Coury (1). It seems that even when applied through the third ventricle adrenergic drugs can stimulate selectively the hypothalamic mechanism which regulates food intake. The new findings presented are those concerned with the increase in food intake promoted either by adrenergic or cholinergic drugs and even by hypertonic saline. In the case of adrenergic stimulation, the ability of both isoproterenol and metaraminol to stimulate food intake within 6 hr after their injection suggests that both α- and β-adrenergic receptors are involved in the positive responses to *l*-norepinephrine and epinephrine.

Injections into the third ventricle probably spread considerably from the site of injection. Therefore, it is difficult to determine the exact locus of drug action. Since hypothalamic structures are known to be involved in the regulation of these types of behavior, it is reasonable to assume that the observed results after injection of drugs into the third ventricle are due to stimulation of these hypothalamic regions; however, effects on structures at a greater distance from the ventricle, such as the mid- or hindbrain cannot be ruled out from our experiments. Therefore, it would be interesting to make a survey of the hypothalamus using these chemicals in order to localize more precisely their sites of action, especially in the case of carbachol and isoproterenol which produced increases in salt intake after intraventricular injection.

One could ask whether or not the intake of water, salt, and food stimulated by the various drugs were interdependent in these experiments. Presumably they were independent in the case of drugs which modified only one behavior. In the case of drugs which altered more than one of these appetitive behaviors this question could only be answered conclusively by allowing only a single choice to the animal. Thus, it is impossible to be certain that the intake of saline induced by carbachol was unrelated to the concomitant intake of water which also occurred at the same time.

Summary. The effects of microinjection of adrenergic and cholinergic drugs and hypertonic saline into the third ventricle on the intake of water, hypertonic salt (NaCl) solution, and food were studied. Carbachol induced a dramatic, rapid, 15-fold increase in water intake, whereas none of the other drugs were active. Both carbachol and isoproterenol evoked large increases in salt intake. Again, all other drugs failed to produce significant effects. Food intake was increased by the following adrenergic compounds: epinephrine, norepinephrine, metaraminol, isoproterenol, and dopamine. Carbachol was also effective in augmenting food intake but the effect was delayed. Hypertonic saline produced a delayed increase in both water and food intake but did not alter salt intake. The results are interpreted to mean that a cholinergic synapse lies in the pathways which mediate water intake, whereas both

cholinergic and adrenergic synapses may be involved in the mediation of salt and food intake.

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