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Effect of Cholinergic and Adrenergic Stimulation of the Subfornical Organ on Water Intake

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MENANI, J. V., W. A. SAAD, L. A. A. CAMARGO, J. ANTUNES-RODRIGUES, M. R. COVIAN AND W. A. SAAD. *Effect of cholinergic and adrenergic stimulation of the subfornical organ on water intake.* PHARMACOL BIOCHEM BEHAV **20**(2) 301–306, 1984.—Cholinergic and adrenergic agonists and antagonists were injected directly into the subfornical organ (SFO), via implanted cannulae, and the volume of water ingested was recorded over a period of 1 hour after injection. Application of 2 nmol carbachol caused intense water intake in 100% of the animals (8.78±0.61 ml), with a very short intake latency. When the 2 nmol carbachol dose was preceded by increased doses of atropine, a progressive reduction in water intake was observed, with complete blockage of the thirst-inducing response to carbachol at the 20 nmol dose level with atropine. Followed by several doses of hexamethonium, the water intake caused by application of 2 nmol carbachol was reduced, although the response was not totally blocked. Injection of 80 nmol of nicotine had a significant thirst-inducing effect in 50% of the animals studied (1.06±0.18 ml) and increase in water intake was further reduced by application of increased doses of hexamethonium. Raising the dose levels of noradrenaline into the SFO caused an increase in water intake although to a lesser degree than was observed after carbachol injection. When the 40 nmol dose of noradrenaline was preceded by increased doses of propranolol (5 to 40 nmol), there was a gradual reduction in water intake, with total blockage at the 40 nmol dose. Application of phentolamine in doses of 10 to 80 nmol caused no reduction in water intake after 40 nmol of noradrenaline. Application of isoproterenol at doses from 20 to 160 nmol into the SFO caused a dose-dependent increase in water intake which was blocked by previous applications of propranolol. These results support the hypothesis that the water intake caused by chemical stimulation of the SFO is mainly due to muscarinic cholinergic receptors, although the influence of nicotinic receptors or participation of adrenergic mediation should not be ruled out.

Water intake Subfornical organ Cholinergic mediation Adrenergic mediation

INTRACRANIAL application of both antiotensin II and carbachol has been shown to induce thirst [5, 6, 7, 15, 17, 25]. The independence of cholinergic and peptidergic pathways that induce drinking has been confirmed by intraventricular injections [8, 9, 11]. The water intake induced by cholinergic stimulation of the central nervous system is mainly due to muscarinic receptors, although Stein and Seifter [24] showed a small increase in water intake after application of nicotine into the central nervous system. Also others authors [19] demonstrated that cholinergic antagonist hexamethonium, decreased drinking when injected into brain ventricles.

The subfornical organ has been proposed as the main center for the thirst-inducing action of carbachol and angiotensin II. Ablation of this structure reduced or prevented drinking produced by third ventricular carbachol administration [20]. Central alpha- and beta-catecholamines also appear to participate of the control of drinking [2, 10, 14, 18]. The objective of the present research was to study the effect of the cholinergic and adrenergic system of the subfornical organ on water intake by direct injection of cholinergic and adrenergic agonist and antagonist drugs.

METHOD

Subjects

Male albino Holtzman rats weighing 250 to 300 g were used. The animals were maintained in individual cages 8 days before brain surgery, with free access to granular ration and tap water.

Brain Surgery

After adapting to laboratory conditions, the animals were

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anesthetized with ether and firmly restrained in a stereotaxic apparatus according to the technique of Krieg [12]. The coordinates for the approach to the SFO were obtained from the De Groot atlas [3]. A longitudinal incision was made on the animal's head, the subcutaneous cellular tissue was pulled back and the skull drilled with a spherical No. 7 drill. A 23-gauge stainless steel cannula was introduced into the brain through this orifice at a 10° angle to avoid damage to the venous sine. The tip of the cannula was left at 1 mm distance from the body of the SFO in order to avoid damage to this small organ. The cannula was secured to the skull with screws and acrylic resin. Prophylactic doses of intramuscular penicillin, 6000 U, were applied before and after surgery.

Drugs

Carbachol hydrochloride, nicotine, hexamethonium bromide, norepinephrine bitartarate, isoproterenol hidrochloride, and propranolol hydrochloride were obtained from Sigma (USA). Atropine sulfate was purchased from Merk (GFR), and phentolamine mesilate (regitine) from Ciba (USA).

All drugs to be used for injection were dissolved in physiological saline solution (0.15 M NaCl). Pure saline solution (0.15 M) was injected into the controls.

Experimental Design

Six experimental groups were used. Each group was composed of between 13 and 38 rats. The animals in each group received injections into the subfornical organ using the agonist and an isotonic saline solution. The agonist injection was preceded by different doses of the antagonist. Each animal was intracranially injected at 48 hour minimum intervals between experiments. The first group received 2 nmol of carbachol preceded by 5 doses of atropine (1.25, 2.5, 5.0, 10.0, 20.0 nmol). The second group received 2 nmol of carbachol preceded by 4 doses of hexamethonium (5.0, 15.0, 30.0, 50.0 nmol). The third group received 80 nmol of nicotine preceded by 5 doses of hexamethonium (5.0, 10.0, 20.0, 40.0, 80.0 nmol). In the fourth group different doses of noradrenaline (20.0, 40.0, 80.0, 160.0 nmol) were first injected and after 48 hours noradrenaline was injected in a dose of 40 nmol preceded by 4 doses of propranolol (5.0, 10.0, 20.0, 40.0 nmol). A fifth group was injected with noradrenaline using a 40 nmol dose preceded by 10 to 80 nmol of phentolamine. In the last and sixth group of animals the isoproterenol was first injected in varying doses (20.0, 40.0, 80.0, 160.0 nmol) and after 48 hours a dose of 80 nmol of isoproterenol was injected preceded by 5 doses of propranolol (10.0, 20.0, 40.0, 80.0, 160.0 nmol).

Behavioral Testing

After a 7 day period of recovery the animals were submitted to the experimental sessions. A 10-μl Hamilton syringe graduated with 0.1-μl marks was used for drug application to the SFO. A piece of PE 10 polyethylene tubing having a 34-gauge needle at one end, i.e., 2 mm larger than the guiding cannula implanted in the animal's head, was adapted to the syringe. All drugs were injected in 0.5-μl volumes at 30–60 second intervals. The antagonists were injected 20 minutes before the agonists.

After drug application, the animals were returned to their cages and water intake was measured at 30-minute intervals for 1 hour. Latency of water ingestion was also recorded. Water was offered in burettes graduated with 1-ml marks to which metal spouts had been provided. No solid food was offered during the experiments.

Histology

After the experiments, the animals were anesthetized with ether, perfused through the heart with physiological saline, and perfused once more with 10% formalin in saline solution. The brains were removed, placed in formalin and cut into 10 μ sections for routine histological processing using the technique of Pal Weigert, modified by Ehrart [4].

Three hundred sixty-five rats were used in these experiments. In 175 of the subjects, the cannula reached the subfornical organ, thus only the analyses from these animals were evaluated. Among the 190 in which the subfornical organ was not localized, there was no evidence of any difference in water intake when compared with the control experiments.

Statistical Analysis

For individual comparisons the mean square within groups was used as the error term for t-test.

The dose-effect relationship was submitted to analysis of variance. All data are expressed as means±SE.

RESULTS

Location of the Cannula in the Subfornical Organ

Figure 1 is a photomicrograph of a frontal section of one rat brain showing cannula positions in the subfornical organ.

Effect of Carbachol Injection into the Subfornical Organ on Water Intake. Antagonistic Action of Atropine (Fig. 2).

Injection of 2 nmol carbachol into the subfornical organ caused copious water intake by all animals. When different doses of atropine (1.25, 2.5, 5.0, 10.0 and 20.0 nmol) were injected into the subfornical organ 20 minutes before carbachol (2 nmol), a significant reduction in water intake occurred at all dose levels ($p<0.01$). A 20 nmol dose of atropine caused 100% blockage of the thirst-inducing response to carbachol, whereas the 1.25 nmol dose reduced the response by 38%. When water intake in the control experiments was compared to water intake after injections of carbachol plus 20 nmol atropine there was no noticeable difference ($p>0.05$). However there was a significant difference when the control values were compared after injection of carbachol plus 1.25, 2.5 and 5.0 nmol of atropine ($p<0.01$). Analysis of variance showed that water intake values after increased doses of atropine plus 2 nmol of carbachol differed in a statistically significant manner, $F(4,80)=13.73$, $p<0.01$.

Analysis of the latency data was often difficult, since many animals that were induced to drink by action of carbachol did not ingest water when given atropine. Thus latency was recorded as 30 minutes for these animals, i.e., the maximum time taken by all animals before drinking.

The results also showed a significant difference in water intake latency after application of increased doses of atropine followed by 2 nmol of carbachol, $F(4,80)=17.7$, $p<0.01$.

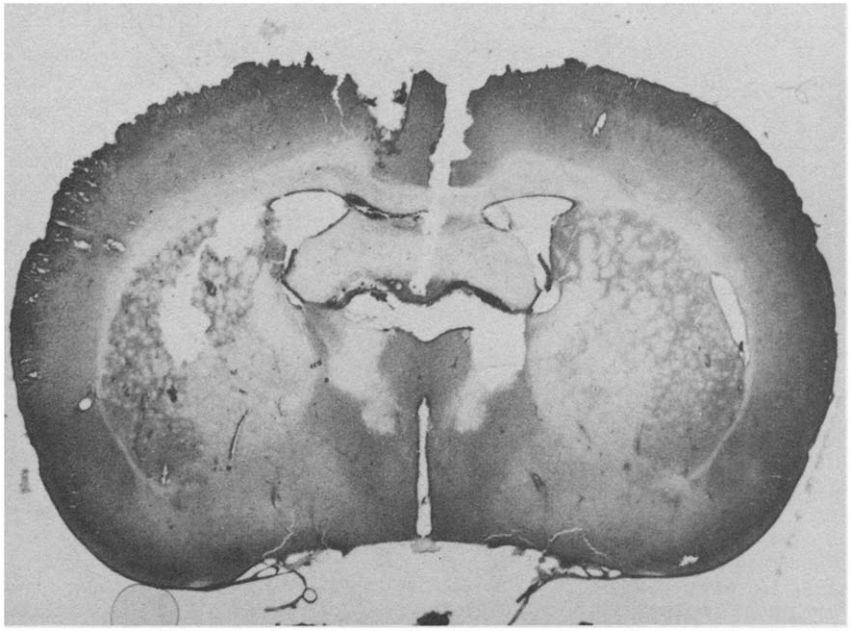

FIG. 1. Micrograph of a rat brain showing the cannula pathway reaching the subfornical organ.

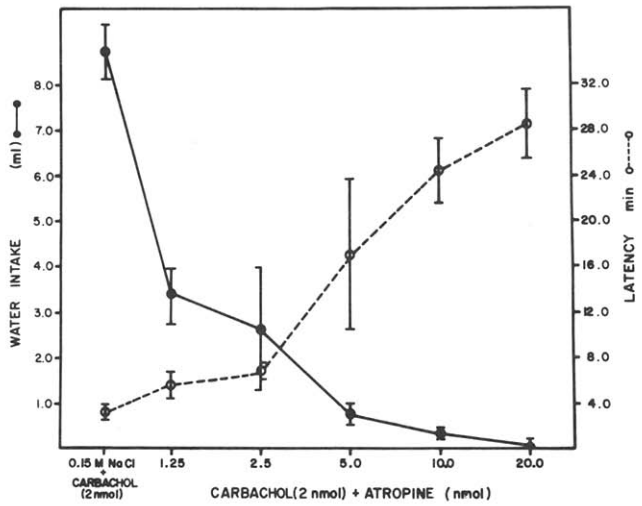

FIG. 2. Dose-response curve for the water volume drunk after injection of 2.0 nmol carbachol into the SFO preceded by 0.15 M NaCl and increasing doses of atropine (left ordinate), and respective latencies (right ordinate). The values represent the means ± SE for 26 rats, each submitted to an average of 3 experiments.

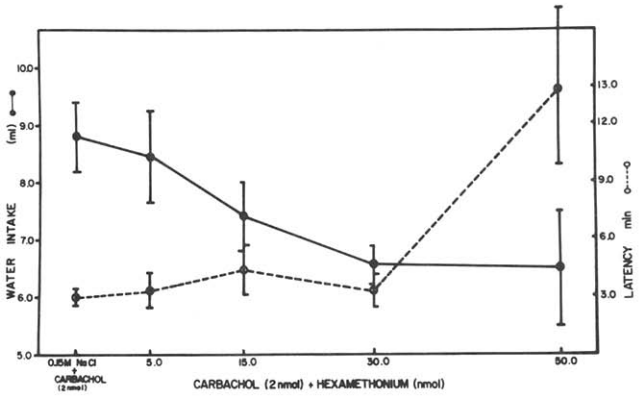

FIG. 3. Dose-response curve for the water volume drunk after injection of 2.0 nmol of carbachol into the SFO preceded by 0.15 M NaCl and increasing doses of hexamethonium (left ordinate), and respective latencies (right ordinate). The values represent the means ± SE for 24 rats, each submitted to an average of 3 experiments.

Effect of Carbachol Injection into the Subfornical Organ on Water Intake. Antagonistic Action of Hexamethonium (Fig. 3).

In this experiment, the sharp rise in water intake caused by 2 nmol carbachol was reduced by injection of 5.0, 15.0, 30.0, and 50.0 nmol of hexamethonium 20 minutes prior to carbachol injection, although the water intake response was not totally blocked. The 50 nmol dose of hexamethonium caused the greatest reduction of response. There were significant differences in water intake when the values obtained from experiments using pure carbachol or isotonic saline solution were compared to those after application of several doses of hexamethonium followed by carbachol ($p<0.01$).

Analysis of variance showed that water intake values differed in a statistically significant manner, $F(3,62)=8.57$, $p<0.01$, after increased doses of hexamethonium were followed by 2 nmol carbachol.

The 15 and 50 nmol doses of hexamethonium caused

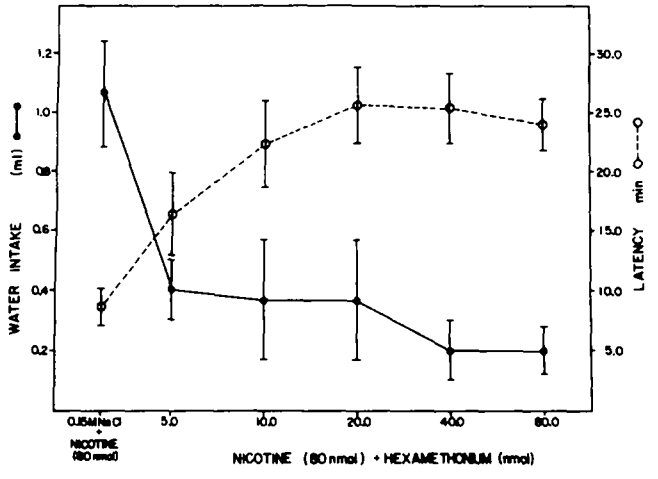

FIG. 4. Dose-response curve for the water volume drunk after injection of 80.0 nmol of nicotine into the SFO preceded by 0.15 M NaCl and increasing doses of hexamethonium (left ordinate), and respective latencies (right ordinate). The values represent the means ±SE for 28 rats, each submitted to an average of 3 experiments.

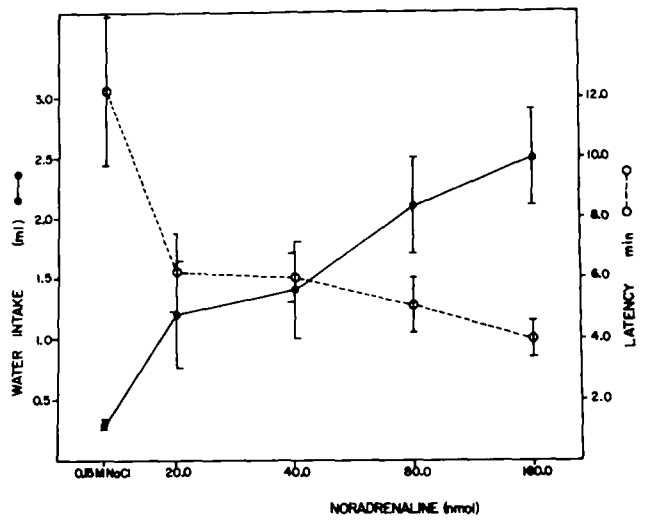

FIG. 5. Dose-response curve for the volume of water drunk after injection of varying doses of noradrenaline into the SFO preceded by 0.15 M NaCl (left ordinate), and respective latencies (right ordinate). The results represent the means±SE for 21 rats, each submitted to an average of 3 experiments.

longer latency in the thirst-inducing response to carbachol. Analysis of variance indicated that latencies in water intake caused by carbachol preceded by increased doses of hexamethonium showed a wide variation, $F(3,62)=5.89$, $p<0.01$. These results indicate that the thirst-inducing action of carbachol may be partially due to stimulation of nicotinic receptors in the subfornical organ.

Effect of Nicotine Injection into the Subfornical Organ on Water Intake. Antagonistic Action of Hexamethonium (Fig. 4).

Injection of 80 nmol of nicotine caused no significant

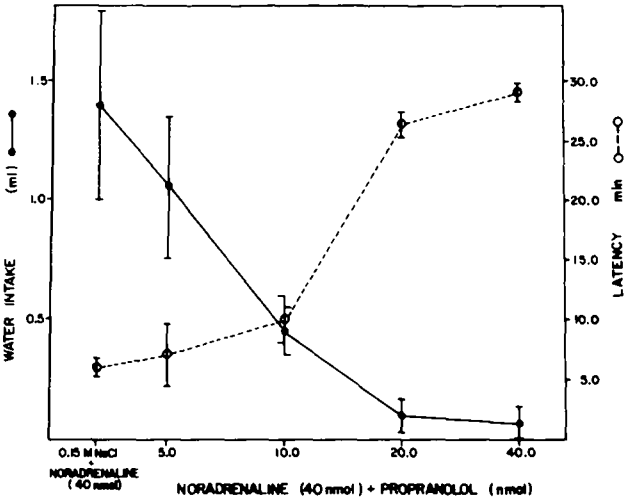

FIG. 6. Dose-response curve for the water volume drunk after injection of 40.0 nmol of noradrenaline into the SFO preceded by 0.15 M NaCl and increasing doses of propranolol (left ordinate) and respective latencies (right ordinate). The values represent the means ±SE for 24 rats, each submitted to an average of 3 experiments.

changes in water intake in 50% of the animals. In the remaining 50%, nicotine caused an increase of water intake when compared with the controls, $F(1,75)=18.74$, $p<0.01$.

The effect of previous applications of increased doses of hexamethonium (5.0, 10.0, 20.0, 40.0 and 80.0 nmol), induced a reduction in the water intake caused by 80 nmol nicotine but were not statistically different, $F(4,84)=0.90$, $p>0.05$.

Latency was correlated with water intake levels: the animals that ingested lower amounts of water did so with longer latency, and the animals which ingested a higher amount had a shorter latency. The experiment showed that nicotinic receptors are involved, although minimally so, in the regulation of water intake by the subfornical organ.

Effect of Injection of Several Doses of Noradrenaline into the Subfornical Organ on Water Intake. Effect of Propranolol (Figs. 5 and 6).

Injection of increased doses of noradrenaline (20.0, 40.0, 80.0 and 160.0 nmol) into the subfornical organ caused a rise in water intake that was not dose dependent. Analysis of variance showed no significant differences in water intake or latency at the various dose levels, $F(3,47)=1.41$, $p>0.05$, but the difference was significant in relation to water intake after injection of isotonic solution (Fig. 5).

The 40 nmol dose of noradrenaline was selected for the subsequent experiments, in which noradrenaline and beta antagonist were utilized. Injection of 5.0, 10.0, 20.0, and 40.0 nmol propranolol, a beta antagonist, 20 minutes before the use of noradrenaline caused a reduction in the thirst-inducing effect of noradrenaline and total blockage of the effect at the 40 nmol dose (Fig. 6). The water intake values obtained in the experiments with propranolol and noradrenaline were statistically different from those obtained in the experiments in which only noradrenaline ($p<0.01$) was used. However these results were not statistically different from those in the experiments in which pure saline solution was used

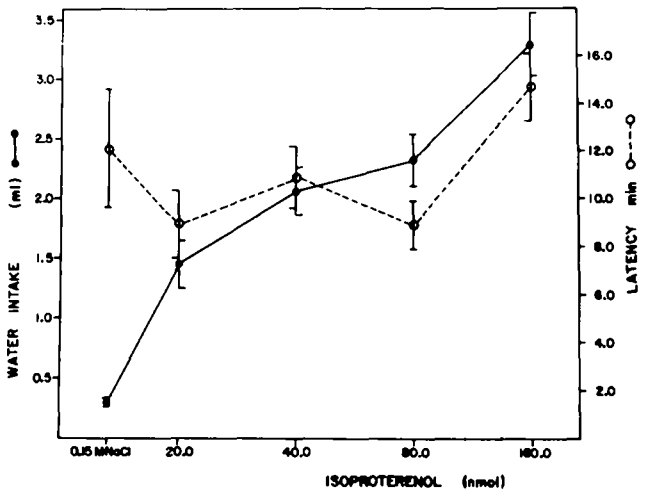

FIG. 7. Dose-response curve for the water volume drunk after injection of varying doses of isoproterenol into the SFO preceded by 0.15 M NaCl (left ordinate) and respective latencies (right ordinate). The results represent the means±SE for 25 rats, each submitted to an average of 3 experiments.

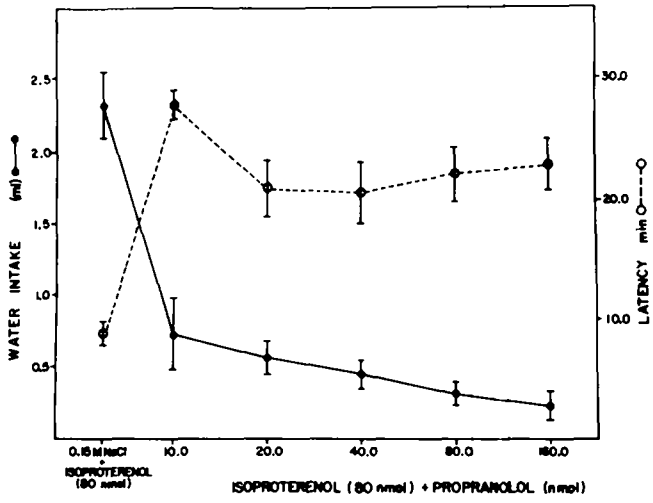

FIG. 8. Dose-response curve for the water volume drunk after injection of 80.0 nmol of isoproterenol into the SFO preceded by 0.15 M NaCl and increasing doses of propranolol (left ordinate) and respective latencies (right ordinate). The values represent the means ±SE for 27 rats, each submitted to an average of 3 experiments.

($p>0.05$). Analysis of variance showed that the values on water intake after increased doses of propranolol followed by 40 nmol noradrenaline, were significantly different from one another, $F(3,60)=9.50$, $p<0.01$. A significant change occurred in latency, $F(3,60)=17.82$, $p<0.01$.

Effect of Noradrenaline Injection into the Subfornical Organ on Water Intake. Effect of Phentolamine.

Application of 10 of 80 nmol of phentolamine before noradrenaline had no reducing effect on water intake caused by 40 nmol of noradrenaline ($p>0.05$). Mean water intake caused by noradrenaline was 1.4±0.5 ml, while mean intake after 80 nmol of phentolamine followed by noradrenaline was 0.8±0.3 ml.

Effect of Isoproterenol Injection into the Subfornical Organ on Water Intake. Dose-Response Curve and Antagonistic Action of Propranolol (Figs. 7 and 8).

Injection of increased doses of isoproterenol (20.0, 40.0, 80.0 and 160.0 nmol) into the subfornical organ caused a dose-dependent increase in water intake. There were statistically significant differences in water intake in relation to the various doses, $F(3,72)=9.24$, $p<0.01$. Latency values also differed, as shown by analysis of variance, $F(3,72)=3.13$, $p<0.05$ (Fig. 7). There was also a latency increase after higher dose levels.

Injections of 80 nmol of isoproterenol were selected for the experiments in which propranolol was also used. When doses of propranolol (10.0, 20.0, 40.0, 80.0 and 160.0 nmol) were injected 20 minutes before 80 nmol of isoproterenol, there was a reduction in water intake which was almost completely blocked by the 160 nmol dose. Analysis of variance showed a significant difference in water intake after increased doses of propranolol, $F(4,110)=39.50$, $p<0.01$ (Fig. 8).

As seen in the noradrenaline experiments, results of investigations with isoproterenol and propranolol were shown to be statistically different from those in which pure isoproterenol was used ($p<0.05$). There was, however, no difference when isoproterenol and propranolol values were compared with those of the saline solution ($p>0.05$).

Because of the similarity in results, the values from all animals injected with saline solution (0.15 M) including those in which the cannulae did not reach the SFO, were totalled and an average was made. The means for 132 animals were 0.32±0.04 ml for water intake, and 12.2±2.55 min for latency.

DISCUSSION

Our results indicate that the application of carbachol to the subfornical organ results in increased water intake with short latency. Prior application of atropine caused a near-total blockage of this effect, and the latency data showed that atropine had a dose-dependent effect on water intake. When the atropine dose was changed there was a small but definite thirst-inducing effect caused by carbachol which still persisted. A previous injection of hexamethonium also reduced the thirst-inducing effect of carbachol, although to a much lower degree than atropine. The data obtained after injection of nicotine into the subfornical organ confirmed the results reported above, i.e., nicotine produced increased water intake at a much lower degree than carbachol. This effect was blocked by a previous injection of hexamethonium. Mangiapane and Simpson [16] demonstrated that pretreatment with atropine abolished carbachol induced drinking while nicotine antagonists had no effect. In the present study, the latencies obtained after nicotine and carbachol and the antagonism of hexamethonium showed a dose-dependent effect. Thus, present data are consistent with the results of others authors [1, 19, 20, 21, 24], and together suggest that a population of specifically cholinoceptive neurons in subfornical organ mediates the drinking response to carbachol.

A second group of experiments in the present study showed that adrenergic stimulation of the subfornical organ induced water intake. These results were based on the fact that noradrenaline, as well as isoproterenol, cause increased water intake when applied to the subfornical organ; an effect that was blocked by the administration of propranolol, but not by phentolamine when applied prior to noradrenaline. Isoproterenol, predominantly a beta agonist, caused a more intense response than noradrenaline, which acts both on alpha- and beta-receptors. Because of the blockage by propranolol, we conclude that beta-receptors of the subfornical organ participate in the regulation of water intake. Latency after noradrenaline and noradrenaline plus propranolol was also dose dependent, i.e., high doses that caused increased water consumption did so in a shorter period of time and vice versa. It was only after isoproterenol injection that this correlation was not observed. The effect obtained by isoproterenol could be due to a greater sensitivity of the beta adrenergic receptors to isoproterenol, than to noradrenaline; presumably lower doses than those tested would elicit less water intake. The present findings are thus consistent with other reports which demonstrated that noradrenaline [2, 10, 13, 18, 23], and in particular beta-adrenergic agonists [18] can cause water intake in satiated rats.

It should be pointed out that the subfornical organ also has receptors that are sensitive to the action of angiotensin II and are closely related to water intake [22]. Setler [18] showed the partial noradrenaline and dopamine depletion resulted in decreased water intake in response to angiotensin, but not to carbachol. Fitzsimons and Setler [8] showed that the dopamine antagonist haloperidol blocked the thirst-inducing effect of angiotensin but not of carbachol. Also destruction of catecholaminergic neurons markedly reduced water intake induced by angiotensin, but not the thirst-inducing effect of carbachol [10]. Therefore, our results support the hypothesis that water intake induced by chemical stimulation of the subfornical organ is mainly due to cholinergic muscarinic receptors. The influence of nicotinic receptors and a catecholaminergic mediation, nevertheless, cannot be definitely discarded.

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