Pharmacology Biochemistry & Behavior, Vol. 39, pp. 171–176. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Effect of the 5-HT_2 Antagonist Ketanserin on Salt Appetite in the Rat

L. GENTILI, A. SAIJA,* G. LUCHETTI† AND M. MASSI

Istituto di Farmacologia, Universitá di Camerino, 62032 Camerino, Italy
**Dipartimento Farmacobiologico, Universitá di Messina, 98100 Messina, Italy*
†Janssen Farmaceutici, 00138 Rome, Italy

Received 22 June 1990

GENTILI, L., A. SAIJA, G. LUCHETTI AND M. MASSI. *Effect of the 5-HT_2 antagonist ketanserin on salt appetite in the rat.* PHARMACOL BIOCHEM BEHAV **39**(1) 171–176, 1991.—The 5-HT_2 antagonist ketanserin inhibited salt appetite induced by subchronic deoxycorticosterone acetate (DOCA) treatment, as well as salt appetite induced by sodium depletion (which is governed by the synergy of aldosterone and angiotensin in the brain). The effect of ketanserin was more evident following intraperitoneal than intracerebroventricular injection. On the other hand, ketanserin did not inhibit either salt intake induced by intracranial renin injection, or the need-free salt intake of the multidepleted female rat, which is not dependent on the renin-angiotensin-aldosterone system. These findings suggest that the antinatriorexic action of ketanserin is selective for the mineralcorticoid mechanisms controlling salt appetite. Ritanserin, too, a potent 5-HT_2 antagonist showing a different receptor selectivity profile from that of ketanserin, suppressed DOCA-induced salt appetite, thus supporting the involvement of 5-HT receptors in the antinatriorexic action. DOCA treatment alters serotonin metabolism in the central nervous system and it has been proposed that changes in 5-HT metabolism may be important in the genesis of DOCA-induced hypertension. The present results indicate that ketanserin inhibits DOCA-induced salt appetite and suggest that serotoninergic mechanisms might be involved in the elicitation of mineralocorticoid-induced salt appetite.

Ketanserin Ritanserin Deoxycorticosterone acetate Renin Sodium depletion Salt appetite

DRUGS that modify serotoninergic transmission are known to affect the intake of hypertonic sodium chloride solutions in rats. 5-HT_{1A} receptor antagonists increase hypertonic saline consumption in rehydrating rats (5,6); this effect occurs at low doses and it is probably dependent on the stimulation of somatodendritic autoreceptors. On the other hand, 5-HT_{1C} agonists reduce hypertonic saline intake in the same experimental conditions (25). To our knowledge, there have been no reports on the effect on salt intake in the rat of agonists or antagonists endowed with potent activity at 5-HT_2 receptors.

The present study was aimed at evaluating the effects of the central and peripheral administration of the serotoninergic antagonist ketanserin. This is a serotoninergic antagonist endowed with high affinity for 5-HT_2 receptors (21,32). It has very low affinity for other 5-HT receptors (3,20), with the exception of the 5-HT_{1C} (19), which recent studies have shown to share similar structural and pharmacological characteristics with the 5-HT_2 ones (16, 18, 19). Ketanserin, however, shows pK_d values for the 5-HT_{1C} receptors about 2 orders of magnitude lower than those for 5-HT_2 receptors (19). In addition, ketanserin is also endowed with marked affinity for α_1-adrenergic and for histamine-H_1 receptors (4, 20, 22). The drug is employed as an antihypertensive agent (2, 8, 10, 26, 30). Our study was undertaken for 2 reasons: a) salt intake is known to play an important role in the pathogenesis and in the maintenance of hypertension (9), thus it is important to describe the effects of an antihypertensive agent on salt intake; b) the results obtained with ketanserin may give information on the role of 5-HT receptors in the control of salt intake. In relation to the latter point we thought it interesting to employ also ritanserin in some experiments. This antagonist shows higher affinity than ketanserin for 5-HT_2 and 5-HT_{1c} receptors (19,22), but lower affinity for α_1 adrenergic and histamine-H_1 receptors (22).

METHOD

Animals

Adult, male, albino Wistar rats (Charles River, Calco, Como, Italy; 350–400 g at the beginning of the experiments) were used. Animals were individually housed in a temperature-controlled room on a 12:12-h light-dark cycle. Food pellets (MILL, Morini, Reggio Emilia, Italy) and water (in graduated drinking tubes) were available ad lib except when noted. Sodium chloride (NaCl) solution (3%) was offered according to modalities reported in the experimental procedure.

Drugs

The following drugs were used: 1) ketanserin and 2) ritanserin (which were a gift of Janssen Farmaceutici, Rome, Italy);

[1]Requests for reprints should be addressed to Dr. Maurizio Massi, Istituto di Farmacologia, Universitá di Camerino, Via Scalzino 5, 62032 Camerino (MC) Italy.

3) deoxycorticosterone acetate (DOCA; Sigma, St. Louis, MO); 4) furosemide (Lasix; Hoechst, Milan, Italy); and 5) renin, which was a generous gift of Prof. Detlev Ganten of the German Institute for High Blood Pressure Research (17). It was their preparation, E III, which is purified from hog kidney by affinity chromatography.

Intracranial Surgery

All the rats employed were anaesthetized (Equithesin, 3 ml/kg b.wt.; intraperitoneally, IP) and fitted by stereotaxic surgery with a stainless-steel guide cannula aimed at the anteroventral third ventricle. The guide cannula was attached to the skull by stainless-steel screws and dental acrylic cement. Animals were allowed one week to recover from surgery before testing began.

Drug Injections

Ketanserin was dissolved in distilled water for both intracerebroventricular (ICV) and IP administration. Owing to its very low solubility in water, ritanserin was dissolved in a vehicle containing, in addition to a few drops of lactic acid, 20% propylene glycol in distilled water. After the drug was dissolved, the pH of the solution and of the vehicle was adjusted to 4.5 by adding 2 N NaOH. For ICV administration, ketanserin was given in a volume of 2 μl per rat by means of a 10 μl Hamilton microsyringe. For IP administration, both ketanserin and ritanserin were given in a volume of 1 ml/kg b.wt.

Experimental Procedures

Experiment 1: Effect of ketanserin on sodium depletion-induced salt appetite. Salt appetite was elicited by an adaptation of the method of Wolf (33), in which depletion is produced by combining pharmacological natriuresis with sodium-deficient diet. Natriuresis was produced by subcutaneous (SC) injection of furosemide (Lasiz; 2 injections of 5 mg/rat, separated by 2 h). At the time of the first injection, the pellets were replaced by a sodium-deficient powdered food (ICN Nutritional Biochemicals, Cleveland, OH, No. 902903) offered in a glass cup, 3% NaCl was removed from the cages, and the cages were washed to remove adherent salt. The animals were not deprived of water. Twenty-two to 24 h later they were injected either ICV (1 min before access to salt) or IP (10 min before access to salt) with ketanserin or with the vehicle used for drug solution (controls) and then they were offered 3% NaCl again. Consumption of 3% NaCl and water, as well as latency to drink each solution, was recorded at 15, 30, 60, 90 and 120 min. Each animal received different treatments at intervals of 7 days. Testing began with the third depletion, since the first 2 depletions produce a lower intake of salt than the subsequent ones (12).

Experiment 2: Effect of IP ketanserin on 10% sucrose intake in sodium-depleted rats. For seven days before the experiment, rats were offered a 10% sucrose solution 2 h a day, while they had water and food freely available throughout the day. On the seventh day, when the intake of sucrose solution was stable enough, rats were sodium depleted as in Experiment 1. Twenty-two to 24 h later, rats were IP injected with ketanserin 10 mg/kg or with simple vehicle. Ten min after the IP injection they were offered 10% sucrose solution and its intake was measured at 15, 30, 60 and 120 min. The rats employed had received two sodium depletions before the beginning of the experiment.

Experiment 3: Effect of ketanserin on renin-induced salt appetite. Renin, 200 ng/rat, was given by pulse ICV injection in a volume of 2 μl of isotonic saline. Animals were tested at intervals of 7 days. Testing began with the third renin injection, since repeated renin injections are known to produce a progressively larger salt intake that reaches a plateau beginning with the third administration (1). Immediately before the injection of renin, 3% NaCl was removed from the cage, but water and food pellets were freely available. One h after the injection of renin, rats were given access to 3% NaCl. The ICV ketanserin treatment took place 1 min before access to salt while IP ketanserin treatment was given 10 min before access to salt. This time schedule was adopted to be sure that ketanserin administration took place when the dipsogenic effect of renin was declining and after its natriorexigenic effect had already begun (23). Salt intake was measured over a 2-h period of observation.

Experiment 4: Effect of ketanserin and ritanserin on DOCA-induced salt appetite. Salt appetite was elicited by a daily SC injection of DOCA 2 mg/rat in 1 ml of sesame oil. The injection was given between 15.00 and 17.00 h. Animals had free access to food pellets and tap water. Three percent NaCl was offered 2 h/day, between 11.00 and 13.00 h. Testing began on the 9th day of DOCA treatment, when the daily intake of 3% NaCl was reliable and stable. Ketanserin or ritanserin were given IP 10 min before access to salt. Ketanserin was also tested by ICV injection, 1 min before salt was offered. Animals were tested at intervals of 3–4 days.

Experiment 5: Effect of IP ketanserin and ritanserin on 10% sucrose intake of SC DOCA-treated rats. As in Experiment 4, rats were given a daily SC injection of DOCA 2 mg/rat. During DOCA treatment they were given access to 3% NaCl 2 h a day between 11.00 and 13.00 h. Three h later, they were offered 10% sucrose for a period of 2 h. On the 9th day of DOCA treatment rats received the IP injection of the drug tested or of the relative vehicle at 10.50 a.m. Ten min later they were offered 10% sucrose to drink (instead of 3% NaCl), and its intake was measured over a 2-h period.

Experiment 6: Effect of ketanserin on the need-free salt intake of the multidepleted female rat. Female rats with a history of repeated sodium depletions were employed in this experiment, since they show a "need-free" salt intake more pronounced and reliable than male rats (12,15). In response to the first 3 sodium depletions rats show an escalation of their daily salt intake to very high levels that remain elevated for months and probably for the life of the animals. This intake is referred to as "need-free," because it occurs in rats that are in positive sodium balance (28) and have continuous access to sodium-rich pellets, as well as to 3% NaCl. The rats employed in the present study were sodium depleted 3 times at intervals of 7 days, according to the method described for Experiment 1. In between depletions, they had free access to tap water, food pellets and 3% NaCl. After the third depletion, 3% NaCl was removed from the cage and animals had continuous access to 1.5% NaCl for 2 days. Subsequently, 1.5% NaCl was offered 2 h a day, between 11.00 and 13.00 h. Animals were allowed 10 days to get familiar with the 2-h schedule of access to 1.5% NaCl, before testing began. Again, the IP injection of ketanserin or of the vehicle took place 10 min before access to salt. Animals were tested once a week.

Validation of the ICV Injections

The ICV injection into the ventricle was firstly validated behaviorally by checking the dipsogenic response to the ICV injection of angiotensin II (10 ng/rat). Only animals that drank more than 5 ml in 15 min were employed in the following experiments. After completion of the experiments rats were given an ICV injection of 1 μl of India ink and immediately after-

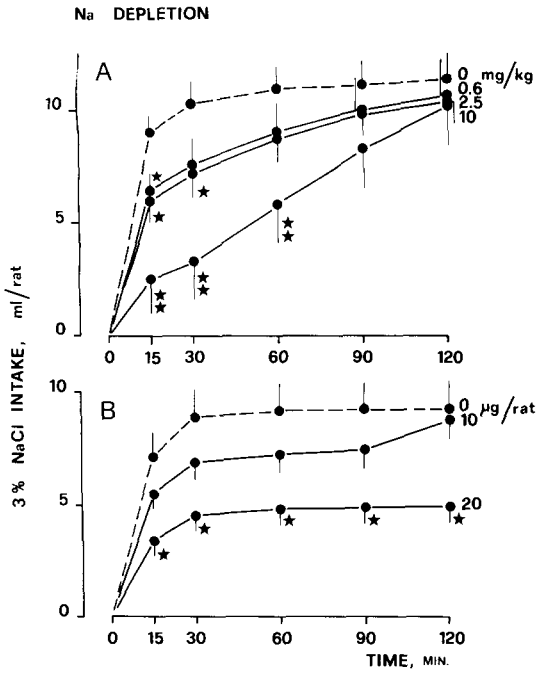

FIG. 1. Effect on salt intake induced by sodium depletion of: (A) IP injections of different doses of ketanserin (mg/kg b.wt.) or of isotonic saline (0) and (B) ICV injections of ketanserin (µg/rat) or of isotonic saline (0). Values are means ± S.E.M. of 12 subjects (panel A) and of 9 subjects (panel B). Difference from controls (0): *$p<0.05$; **$p<0.01$. Where not indicated, difference from controls was not statistically significant.

wards they were sacrificed with an overdose of anaesthetic. Diffusion of the dye into the brain ventricle was carefully evaluated.

Statistical Analysis

Data are presented as means ± S.E.M. Statistical analysis of data was performed by multifactorial analysis of variance to check the overall significance. Planned pairwise comparisons were made by means of *t*-tests. Statistical significance was set at $p<0.05$.

RESULTS

Experiment 1: Effect of Ketanserin on Sodium Depletion-Induced Salt Appetite

As shown in Fig. 1A, the IP injection of ketanserin produced a marked, dose-dependent decrease in 3% NaCl intake. The analysis of variance revealed a significant drug effect, $F(3,33)=3.726$, $p<0.05$, together with a potent drug-time interaction, $F(12,132)=7.158$, $p<0.001$. In response to 0.6 and 2.5 mg/kg b.wt., salt intake was slightly, but significantly reduced, respectively, for 15 and 30 min after access to salt. A more pronounced inhibition of salt intake was observed at the dose of 10 mg/kg. Following this dose the intake of treated rats was significantly lower than that of controls in the first 60 min of access to salt.

Ketanserin significantly inhibited depletion-induced salt appetite also by ICV administration, $F(3,24)=4.481$, $p<0.05$ (Fig. 1B). At the doses of 1 (data not shown) and 10 µg/rat, it produced only small, and statistically nonsignificant reductions in salt intake. In response to 20 µg/rat, the drug evoked a clear-cut suppression of salt intake. Planned pairwise comparisons showed that salt intake of ketanserin-treated rats was lower than that of controls during the entire 2-h period of observation. At 24 h, the intake of 3% NaCl of ketanserin-treated rats was nonsignificantly different from that of controls.

TABLE 1

EFFECT OF IP KETANSERIN ON 10% SUCROSE INTAKE OF SODIUM-DEPLETED RATS

	15 Min	30 Min	60 Min	120 Min
Controls	11.4 ± 1.5	12.0 ± 1.5	13.4 ± 1.3	18.7 ± 2.9
IP KETA	9.9 ± 1.6	11.2 ± 1.4	13.1 ± 1.4	18.6 ± 2.4

Ten percent sucrose intake (ml/rat) of sodium-depleted rats following IP injection of ketanserin (KETA; 10 mg/kg b.wt.) or of isotonic saline (Controls). Values are means ± S.E.M. of 7 subjects. Difference from controls was never statistically significant.

Experiment 2: Effect of IP Ketanserin on 10% Sucrose Intake in Sodium-Depleted Rats

To evaluate the selectivity of the inhibitory effect of IP ketanserin 10 mg/kg, the same dose of the drug was administered to sodium-depleted rats, which were offered 10% sucrose solution and their intake of this solution was measured. As shown in Table 1, 10% sucrose intake of treated rats was very similar and statistically indistinguishable from that of controls, which received only IP distilled water. Accordingly, the analysis of variance revealed the absence of drug effect, $F(1,12)=0.076$, $p>0.05$, and of drug-time interaction, $F(3,36)=0.222$, $p>0.05$.

Experiment 3: Effect of Ketanserin on Renin-Induced Salt Appetite

The IP injection of ketanserin, 2.5 or 10 mg/rat, did not significantly affect salt intake in response to intracranial renin injection (Fig. 2A).

Also the ICV injection of ketanserin 10 or 20 µg/rat did not significantly modify the intake of 3% NaCl induced by central renin injection (Fig. 2B).

Experiment 4: Effect of Ketanserin and Ritanserin on DOCA-Induced Salt Appetite

Ketanserin. The IP injection of ketanserin, 2.5 or 10 mg/rat, significantly suppressed DOCA-induced salt appetite (Fig. 3A). The analysis of variance revealed a highly significant drug effect, $F(2,16)=14.516$, $p<0.001$, as well as a potent drug-time interaction, $F(8,64)=4.156$, $p<0.001$. In response to 2.5 mg/kg the intake of treated rats was significantly lower than that of controls in the first 60 min of access to salt. The inhibitory effect of IP ketanserin was very pronounced at the dose of 10 mg/rat, which significantly inhibited salt intake of treated rats for 120 min after access to it.

As shown in Fig. 3B, the ICV administration of ketanserin, 10 or 20 µg/rat, did not significantly modify salt intake induced by SC subchronic DOCA administration.

Ritanserin. Similar results were obtained with the IP injection of ritanserin. The overall analysis of variance revealed a highly significant drug effect, $F(3,33)=12.540$, $p<0.001$. No statistically significant effect was elicited at 1 mg/kg. On the other hand, the IP administration of ritanserin at doses of 2.5 and 10 mg/kg produced significant reductions of DOCA-induced salt intake (Fig.4). Following the dose of 2.5 mg/kg a signif-

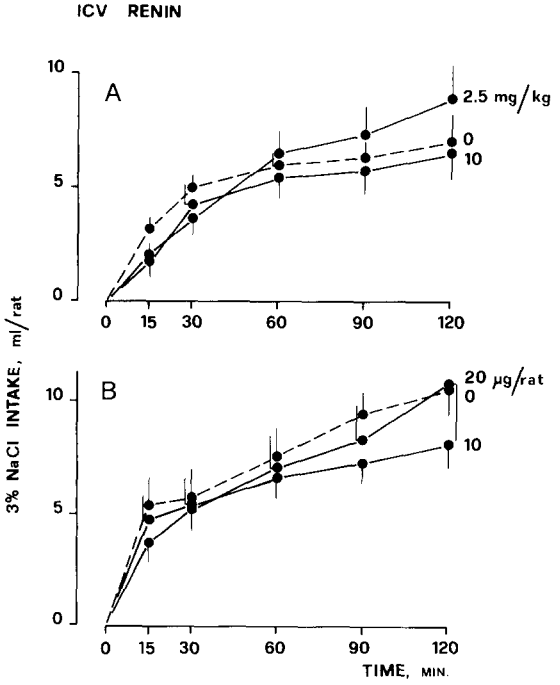

FIG. 2. Effect on salt intake induced by ICV renin of: (A) IP injections of different doses of ketanserin (mg/kg b.wt.) or of isotonic saline (0) and (B) ICV injections of ketanserin (µg/rat) or of isotonic saline (0). Values are means ± S.E.M. of 8 subjects. Difference from controls was never statistically significant.

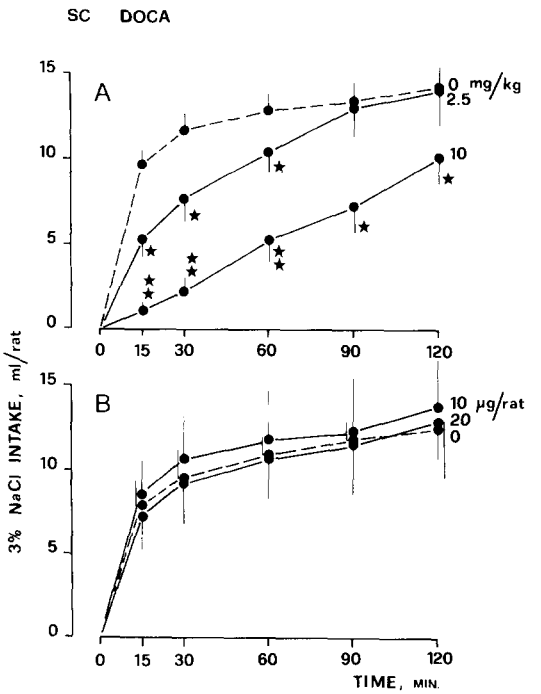

FIG. 3. Effect on salt intake induced by SC DOCA of: (A) IP injections of different doses of ketanserin (mg/kg b.wt.) or of isotonic saline (0) and (B) ICV injections of ketanserin (µg/rat) or of isotonic saline (0). Values are means ± S.E.M. of 9 subjects (panel A) and 7 subjects (panel B). Difference from controls as in Fig. 1.

icant reduction of salt intake was observed only in the first 15 min after access to salt. At the dose of 10 mg/kg, the effect was statistically significant up to 120 min after access to salt.

Experiment 5: Effect of IP Ketanserin and Ritanserin on 10% Sucrose Intake of SC DOCA-Treated Rats

This experiment was carried out to evaluate the selectivity of the inhibitory effect of IP ketanserin and ritanserin on DOCA-induced salt appetite. As shown in Table 2, 10% sucrose intake following ketanserin, 10 mg/kg, was strictly similar to that following vehicle administration. The analysis of variance revealed the absence of drug effect, $F(1,10)=0.242$, $p>0.05$, and of drug-time interaction, $F(3,30)=2.86$, $p>0.05$.

On the other hand, ritanserin, 10 mg/kg, evoked a statistically significant inhibition of sucrose intake, $F(1,14)=6.54$, $p<0.05$. The analysis of variance revealed also a statistically significant drug-time interaction, $F(3,42)=14.17$, $p<0.001$. Planned pairwise comparisons showed that difference from controls was significant only at 15 and 30 min after drug administration (Table 2).

Experiment 6: Effect of Ketanserin on the Need-Free Salt Intake of the Multidepleted Female Rat

As shown in Fig. 5, the IP administration of ketanserin, 10 mg/kg, slightly reduced the intake of 1.5% NaCl, however, the analysis of variance revealed the absence of a significant drug effect, $F(1,7)=0.903$, $p>0.05$.

DISCUSSION

The results of the present study show that the 5-HT_2 antagonist ketanserin is able to inhibit salt appetite in the rat, and that its inhibitory effect clearly depends on the natriorexic determinant employed.

Our first finding was the observation that ketanserin, given by IP injection, markedly suppressed salt appetite induced by sodium depletion. The suppression of salt appetite was very pronounced at the dose of 10 mg/kg b.wt. However, the same dose, given by IP administration, was completely ineffective in inhibiting 10% sucrose intake of sodium-depleted rats. We interpret these

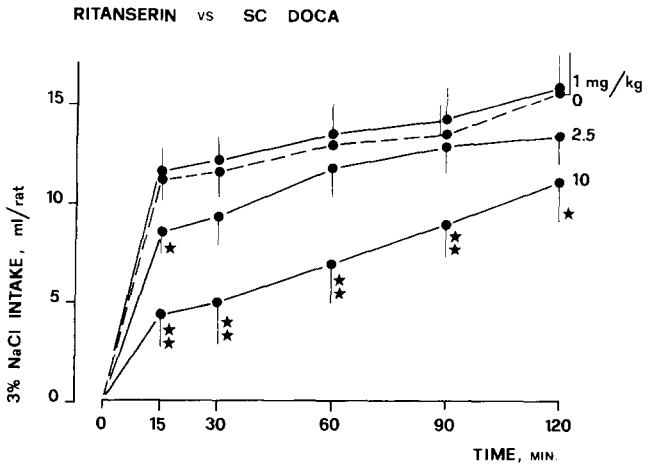

FIG. 4. Effect on salt intake induced by SC DOCA of IP injections of different doses of ritanserin (mg/kg b.wt.) or of isotonic saline (0). Values are means ± S.E.M. of 12 subjects. Difference from controls (0) as in Fig. 1.

TABLE 2

EFFECT OF IP KETANSERIN OR RITANSERIN ON 10% SUCROSE INTAKE IN DOCA-TREATED RATS

	15 Min	30 Min	60 Min	120 Min
Controls	13.9 ± 0.7	14.8 ± 0.4	15.9 ± 0.9	17.7 ± 1.3
IP KETA	11.1 ± 0.5	13.0 ± 0.6	15.9 ± 0.9	20.3 ± 2.1
Controls	13.2 ± 0.9	14.2 ± 0.7	15.2 ± 1.0	17.1 ± 1.2
IP RITA	6.2 ± 1.3*	8.6 ± 1.4*	13.6 ± 0.9	15.8 ± 1.0

Ten percent sucrose intake (ml/rat) of DOCA-treated rats following IP injection of ketanserin (KETA, 10 mg/kg), of ritanserin (RITA, 10 mg/kg b.wt.) and of the vehicles used for the 2 drugs (Controls). Values are means ± S.E.M. of 6 subjects for ketanserin and its vehicle, of 9 subjects for ritanserin and of 7 subjects for its vehicle. Difference from controls: *$p<0.01$; where not indicated, difference from controls was not statistically significant.

findings as evidence that the inhibitory effect of IP ketanserin on depletion-induced salt appetite is not related to general impairment of the behavior, but is rather the expression of a selective effect of the drug on salt appetite.

Following ICV injection ketanserin inhibited salt intake only at the dose of 20 μg/rat, but not at lower doses. The low solubility in water of the drug prevented the use of larger doses.

However, ketanserin administration (both IP and ICV) produced no significant suppression of renin-induced salt appetite. The drug proved to be ineffective on renin-induced salt appetite even at the IP dose of 10 mg/kg, which had proven to be very potent in suppressing depletion-induced salt appetite.

On the other hand, the drug markedly inhibited salt appetite evoked by SC DOCA administration, following IP injection, but not after the ICV administration of 10 or 20 μg/rat. Again, the IP injection of ketanserin, 10 mg/kg, did not modify 10% sucrose intake of DOCA-treated rats, thus providing evidence for a behaviorally selective inhibitory effect of ketanserin on DOCA-induced salt appetite.

It is well known that both angiotensin and aldosterone, given separately, are able to raise salt appetite, acting through different brain mechanisms [see for review (9)]. Moreover, Epstein and co-workers (11, 14, 24, 27, 29, 34) showed that salt appetite following sodium depletion is elicited by the synergistic action of angiotension II and of aldosterone. Large doses of the two hormones given separately are necessary to raise the appetite; however, when they are coadministered they can evoke the appetite at far lower doses. Evidence in favor of the synergism of the hormones comes also from the observation that pharmacological blockade of the receptors for both hormones completely suppresses the appetite.

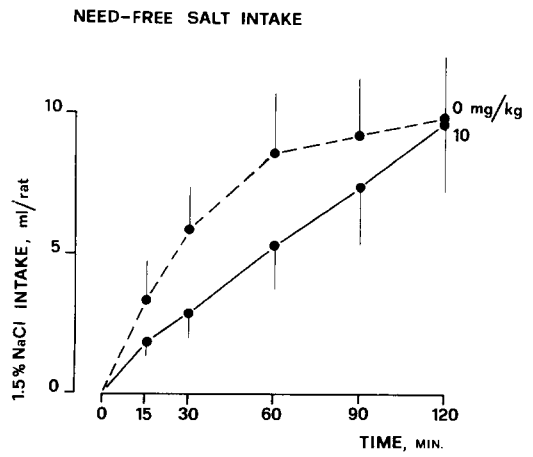

FIG. 5. Effect of IP ketanserin, 10 mg/kg, on the need-free salt intake of multidepleted female rats. Values are means ± S.E.M. of 8 subjects. Difference from controls was never statistically significant.

On the basis of these concepts regarding the mechanisms controlling salt intake, our findings apparently indicate that the inhibitory effect of ketanserin is directed towards the mineralocorticoid component of the central mechanisms for salt appetite. The observed suppression of depletion-induced salt appetite could be accounted for by the action of ketanserin on the mineralocorticoid mechanisms involved in depletion-induced salt appetite. The selectivity of the inhibitory effect towards the mineralocorticoid component of the appetite is suggested by the fact that ketanserin is completely ineffective on renin-induced salt appetite and on the "need-free" salt intake of the multidepleted female rat, which is not dependent on the renin-angiotensin-aldosterone system (13).

The lack of effect of the ICV treatment with ketanserin on DOCA-induced salt appetite probably does not allow the conclusion that the drug acts on a peripheral site of action. In fact, the ICV doses tested were far lower than the IP ones. The low potency of the ICV treatment might be related, on the other hand, to the fact that the site of action for ketanserin is not located into or near the anteroventral third ventricle, where the drug was injected. In relation to this, it is interesting to note that a site for the natriorexic action of mineralocorticoid in the brain is the medial amygdala (31), which is away from the anteroventral third ventricle.

As mentioned in the Introduction, ketanserin shows affinity also for receptors different from those for 5-HT. Therefore, the results obtained with ketanserin might be due either to its action on 5-HT receptors, or on other receptors. In relation to this, also ritanserin was tested in the present study; this drug is a potent and long-acting antagonist at 5-HT_2 and 5-HT_{1C} receptors (19), while having lower affinity than ketanserin for α_1 adrenergic and for histamine-H_1 receptors (22). The data obtained with ritanserin indicate that a marked suppression of DOCA-induced salt appetite can be obtained also with ritanserin. This drug inhibited also 10% sucrose intake in DOCA-treated rats. However, the inhibitory effect of ritanserin on sucrose intake was lower than that observed on DOCA-induced salt intake, and lasted only for 30 min while the effect on salt intake was still statistically significant at the end of the 2-h observation period. Immediately after ritanserin rats were clearly sedated and rather immobile, thus the drug might have induced a rather nonselective suppression of the ingestive behavior, at the beginning. However, our results show that by 1 h after injection the effect of ritanserin was clearly a selective effect on the rat ingestive behavior, since salt intake but not sucrose intake was inhibited. In keeping with these findings, previous unpublished experiments of our group showed that ritanserin, 10 mg/kg, suppresses water intake induced by water deprivation, but only for the first 15–30 min after drug administration.

Taken together, the results obtained with ketanserin and ritanserin apparently suggest that the antinatriorexic action observed might be related to 5-HT receptor blockade, and not to blockade of other receptor systems, including that of α_1-adrenergic receptors.

It has been reported that DOCA treatment is able to alter serotonin metabolism in the central nervous system (7); increased brain levels of serotonin and of its metabolite 5-hydroxyindoleacetic acid are found in many brain areas. Accordingly, it

has been proposed that changes in 5-HT metabolism may be important in the genesis of DOCA-induced hypertension. The results of our study suggest that serotoninergic mechanisms might be involved in the elicitation of salt appetite induced by DOCA treatment. Ketanserin shows much higher affinity for 5-HT_2 than for 5-HT_{1C} receptors (19), therefore, it seems reasonable to hypothesize that 5-HT_2 receptors might be the ones involved in the effect of ketanserin. However, further studies with agonists and, hopefully, with even more selective antagonists are required to definitely establish the receptor subtype involved in the effect of ketanserin on salt intake.

ACKNOWLEDGEMENTS

The authors wish to thank E. Caraffa, A. Fiorelli and M. Cucculelli for skillful technical assistance. The authors are grateful to Prof. Detlev Ganten of the German Institute for High Blood Pressure for the generous gift of renin, and to Janssen Farmaceutici, Italia, for the gift of ketanserin and ritanserin.

REFERENCES

1. Avrith, D. B.; Fitzsimons, J. T. Renin-induced sodium appetite: effects on sodium balance and mediation by angiotensin in the rat. J. Physiol. (Lond.) 337:479–496; 1983.
2. Basso, N.; Kurnjek, M. L.; Mikulic, L.; Trolliet, M. R. Chronic administration of ketanserin and the development of two-kidney-two-clip goldblatt renovascular hypertension in the rat. J. Cardiovasc. Pharmacol. 10(Suppl.3):S62–S64; 1987.
3. Bradley, P. B.; Engel, G.; Feniuk, W.; Fozaed, J. R.; Humphrey, P. P. A.; Middlemiss, D. N.; Mylecharane, E. J.; Richardson, B. P.; Saxena, P. R. Proposal for the classification and nomenclature of functional receptors for the 5-hydroxytryptamine. Neuropharmacology 25:563–576; 1986.
4. Cohen, M. L.; Schenk, K. W.; Kurz, K. D. 5-HT_2 receptor antagonists: α_1 vs 5-HT_2 receptor blocking properties in blood vessels. J. Cardiovasc. Pharmacol. 11(Suppl.)1:S25–S29; 1988.
5. Cooper, S. J.; Desa, A. Benzodiazepines and putative 5-HT_{1A} agonists increase hypertonic saline consumption in rehydrating rats. Pharmacol. Biochem. Behav. 28:187–191; 1987.
6. Cooper, S. J.; Fryer, M. J.; Neill, J. C. Specific effect of putative 5-HT_{1A} agonists, 8-OH-DPAT and gepirone, to increase hypertonic saline consumption in the rat: evidence against a general hyperdipsic action. Physiol. Behav. 43:533–537; 1988.
7. Dawson, R.; Nagamhama, S., Jr.; Oparil, S. Central serotoninergic alterations in deoxycorticosterone acetate/NaCl (DOCA/NaCl)-induced hypertension. Neuropharmacology 27:417–425; 1988.
8. De Cree, J.; Leempoels, J.; De Cock, W.; Geukens, H.; Verhaegen, H. The antihypertensive effects of a pure selective serotonin-receptor blocking agent (R 41468) in elderly patients. Angiology 32:137–144; 1981.
9. Denton, D. The hunger for salt. New York: Springer-Verlag; 1982.
10. Docherty, J. R.; Warnock, P. Effects of antagonists of 5-hydroxytryptamine on blood pressure in the anaesthetised spontaneously hypertensive rat. J. Cardiovasc. Pharmacol. 10(Suppl. 3):S59–S61; 1987.
11. Epstein, A. N. Mineralocorticoids and cerebral angiotensin may act together to produce sodium appetite. Peptides 3:493–494; 1982.
12. Epstein, A. N.; Sakai, R. R. Angiotensin-aldosterone synergy and salt intake. In: Buckley, J. P.; Ferrario, C. M., eds. Brain peptides and catecholamines in cardiovascular regulation in normal and diabetes status. New York: Raven Press; 1986:58–64.
13. Epstein, A. N.; Sakai, R. R.; Stamoutsos, B. Changes in plasma ANG and ALDO do not account for the enhanced salt appetite of the multidepleted rat. Physiologist 29:117; 1986.
14. Fluharty, S. J.; Epstein, A. N. Sodium appetite elicited by intracerebroventricular infusion of angiotensin II in the rat: II. Synergistic interaction with systemic mineralocorticoids. Behav. Neurosci. 97: 746–758; 1983.
15. Frankmann, S. P.; Dorsa, D. M.; Sakai, R. R.; Simpson, J. B. A single experience with hyperoncotic colloid dialysis persistently alters water and sodium intake. In: de Caro, G.; Epstein, A. N.; Massi, M., eds. The physiology of thirst and sodium appetite. New York: Plenum Press; 1986:115–121.
16. Frazer, A.; Maayani, S.; Wolfe, B. B. Subtypes of receptors for serotonin. Annu. Rev. Pharmacol. Toxicol. 30:307–348; 1990.
17. Ganten, D.; Speck, G.; Meyer, D.; Loos, H. E.; Schelling, P.; Rettig, R.; Unger, T. Biochemical and functional aspects of brain renin. In: Gross, T.; Vagel, G., eds. Enzymatic release of vasoactive peptides. New York: Raven Press; 1980:31–48.
18. Hartig, P. R. Molecular biology of 5-HT receptors. Trends Pharmacol. Sci. 10:64–69; 1989.
19. Hoyer, D. Molecular pharmacology and biology of 5-HT_{1C} receptors. Trends Pharmacol. Sci. 9:89–94; 1988.
20. Janssen, P. A. J. 5-HT_2 receptor blockade to study serotonin-induced pathology. Trends Pharmacol. Sci. 4:198–206; 1983.
21. Leysen, J. E.; Awouters, F.; Kenis, L.; Laduron, P. M.; Vanderberk, J.; Janssen, P. A. J. Receptor binding profile of R 41468, a novel antagonist at 5-HT_2 receptors. Life Sci. 28:1015–1022; 1981.
22. Leysen, J. E.; Gommeren, W.; Van Gompel, P.; Wynants, J.; Janssen, P. A. J.; Laduron, P. M. Receptor-binding properties in vitro and in vivo of ritanserin, a very potent and long acting serotonin-S_2 antagonist. Mol. Pharmacol. 27:600–611; 1985.
23. Massi, M.; Perfumi, M.; de Caro, G.; Epstein, A. N. Inhibitory effect of kassinin on salt intake induced by different natriorexigenic treatments in the rat. Brain Res. 440:232–242; 1988.
24. Moe, K. E.; Weiss, M. L.; Epstein, A. N. Sodium appetite during captopril blockade of endogenous angiotensin II formation. Am. J. Physiol. 247:R356–R365; 1984.
25. Neill, J. C.; Cooper, S. J. Selective reduction by serotoninergic agents of hypertonic saline consumption in rats: evidence for possible 5-HT_{1C} receptor mediation. Psychopharmacology (Berlin) 99: 196–201; 1989.
26. Persson, B.; Gradin, K.; Pettersson, A.; Hedner, T. Antihypertensive effects of ketanserin and ritanserin in the spontaneously hypertensive rat. J. Cardiovasc. Pharmacol. 11(Suppl.1):S22–S24; 1988.
27. Sakai, R. R. The hormones of sodium conservation act synergistically to arouse a sodium appetite in the rat. In: de Caro, G.; Epstein, A. N.; Massi, M., eds. The physiology of thirst and sodium appetite. New York: Plenum Press; 1986:425–430.
28. Sakai, R. R.; Frankmann, S. P.; Epstein, A. N. Natriuresis does not cause the enhanced salt appetite of multidepleted rats. Physiologist 29:117; 1986.
29. Sakai, R. R.; Nicolaidis, S.; Epstein, A. N. Salt appetite is suppressed by interference with angiotensin II and aldosterone. Am. J. Physiol. 251:R762–768; 1986.
30. Saxena, P. R.; Bolt, G. R.; Dhasmana, K. M. Serotonin agonists and antagonists in experimental hypertension. J. Cardiovasc. Pharmacol. 10(Suppl. 3): S12–S18; 1987.
31. Schulkin, J.; Marini, J.; Epstein, A. N. A role for the medial region of the amygdala in mineralocorticoid-induced salt hunger. Behav. Neurosci. 103:178–185; 1989.
32. Van Nueten, J. M.; Janssen, P. A. J.; Van Beek, J.; Xhonneux, R.; Verbeuren, T. J.; Vanhoutte, P. M. Vascular effects of ketanserin (R41468), a novel antagonist of 5-HT_2 serotoninergic receptors. J. Pharmacol. Exp. Ther. 218:217–230; 1981.
33. Wolf, G. Refined salt appetite methodology for rats demonstrated by assessing sex differences. J. Comp. Physiol. Psychol. 36:1012–1021; 1982.
34. Zhang, D. M.; Stellar, E.; Epstein, A. N. Together intracranial angiotensin and systemic mineralocorticoids produce avidity for salt in the rat. Physiol. Behav. 32:677–681; 1984.