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Effect of the $5HT_2$ Antagonist Ritanserin on Food Intake and on 5HT-Induced Anorexia in the Rat

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MASSI, M. AND S. MARINI. *Effect of the $5HT_2$ antagonist ritanserin on food intake and on 5HT-induced anorexia in the rat*. PHARMACOL BIOCHEM BEHAV 26(2) 333–340, 1987.—The present study investigated the effect on the rat's eating behavior of the new selective $5HT_2$ antagonist ritanserin. The results obtained indicate that: (a) single subcutaneous (SC) injection of ritanserin, at doses between 0.1 and 1 mg/kg b.wt., neither elicits food intake in sated rats, nor increases the intake induced by food deprivation; (b) subchronic SC treatment (15 days) with 0.1 mg/kg does not increase food intake nor body weight gain; (c) subchronic SC treatment with high doses, 1 or 10 mg/kg, produces small and transient increases in food intake without affecting body weight gain. When ritanserin was tested for its ability to block the anorectic effect of exogenous 5HT, it inhibited the effect of intraperitoneal (IP) 5HT, but proved to be completely inactive versus the effect of 5HT injected into the hypothalamic paraventricular nucleus, which is highly sensitive to this effect of 5HT. This last finding suggests that the anorectic action of central endogenous 5HT is also not blocked by ritanserin, thus proposing a reasonable explanation for the absence of orexigenic effect following its administration. Moreover, it suggests that in rats the hypothalamic receptors mediating the effect of 5HT on eating behavior are different from the $5HT_2$ of the frontal cortex which have been shown to be completely blocked by ritanserin under the experimental conditions employed in our study.

Ritanserin Food intake 5HT-Induced anorexia

A large body of evidence suggests that serotonin (5HT) inhibits eating behavior, both by a central and a peripheral mechanism of action [4–6, 8, 14, 17, 26, 27, 35, 37]. Consistent with this hypothesis, a number of studies have shown that the 5HT antagonists cyproheptadine and methysergide increase food intake as well as body weight gain in several animal species, including rats, cats and men [1, 3, 9, 18, 20, 24, 33, 38, 39, 42]. However, the pharmacological profiles of these drugs show that they are not selective agents. In fact, cyproheptadine shows equally potent interactions with histamine-H_1 and $5HT_2$ receptors and marked interactions have been reported both for cyproheptadine and for methysergide with other central receptor systems [21,30]. On the other hand, binding studies indicate that these drugs differentiate poorly between $5HT_1$ and $5HT_2$ sites [21, 30, 34]. Finally, methysergide and cyproheptadine display mixed agonist-antagonist properties [11,12]. These pharmacological profiles make the understanding of the mechanism of action of the above mentioned drugs difficult and raise the question of the effect on food intake of more selective 5HT antagonists.

Recently the new $5HT_2$ antagonist ritanserin has been synthesized. It has the following pharmacological properties [31]: first, it shows high selectivity for 5HT receptors, in fact, in *in vitro* binding assays the IC_{50} for brain histamine-H_1, dopamine-D_2 and adrenergic alpha$_1$ and alpha$_2$ sites are 39-, 77-, 107- and 166-times higher than for $5HT_2$ sites; second, ritanserin is very selective for different types of 5HT receptors. In fact, while showing high affinity binding to $5HT_2$ sites, it does not bind to the $5HT_1$ ones even at very high concentrations. Third, *ex vivo* experiments have shown that ritanserin easily crosses the blood-brain barrier, since subcutaneous (SC) injection of low doses (0.1 mg/kg) produces high occupation of frontal cortical $5HT_2$ sites both in rats and in guinea pigs. Finally, the occupation of these sites proves to be very long lasting, as shown by the fact that more than 70% occupation was detected at 48 hr following 2.5 mg/kg SC in rats.

Because of the above mentioned properties we thought it interesting to evaluate the effect of this new 5HT antagonist on the rat's eating behavior.

METHOD

Animals

Male Wistar rats (Charles River, Calco, Como, Italy) weighing 200–300 g were employed. Animals were kept in

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TABLE 1

FOOD SATED RATS, FOOD INTAKE (g/100 g b.wt.)

	1 hr	2 hr	3 hr	4 hr	24 hr
Vehicle	0.10 ± 0.09	0.10 ± 0.09	0.12 ± 0.11	0.12 ± 0.11	9.77 ± 0.15
Ritanserin 0.01 mg/kg	0.06 ± 0.03	0.16 ± 0.11	0.16 ± 0.11	0.19 ± 0.11	9.55 ± 0.32
Ritanserin 0.1 mg/kg	0.06 ± 0.06	0.16 ± 0.14	0.16 ± 0.14	0.16 ± 0.14	10.04 ± 0.26
Ritanserin 1 mg/kg	0.01 ± 0.01	0.09 ± 0.06	0.09 ± 0.06	0.09 ± 0.06	10.21 ± 0.42

Food intake (g/100 g b.wt.) in food sated rats, following SC ritanserin injection. Values, determined at different times after drug or vehicle administration, are means ± SEM of 6–7 data.

TABLE 2

FOOD SATED RATS, WATER INTAKE (ml/100 g b.wt.)

	1 hr	2 hr	3 hr	4 hr	24 hr
Vehicle	0.02 ± 0.02	0.08 ± 0.02	0.13 ± 0.04	0.17 ± 0.04	11.04 ± 0.29
Ritanserin 0.01 mg/kg	0.10 ± 0.09	0.19 ± 0.15	0.30 ± 0.15	0.44 ± 0.18	10.61 ± 0.48
Ritanserin 0.1 mg/kg	0.16 ± 0.13	0.20 ± 0.16	0.24 ± 0.17	0.32 ± 0.17	11.27 ± 0.50
Ritanserin 1 mg/kg	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	11.05 ± 0.50

Water intake (ml/100 g b.wt.). in food sated rats, following SC ritanserin injection. Values, determined at different times after drug or vehicle administration, are means ± SEM of 6–7 data.

individual cages on a 12:12 hr light-dark schedule (lights on at 7:30 a.m.).

Drugs

Ritanserin (Janssen Pharmaceutica, Beerse, Belgium) and 5HT creatinine sulphate (Fluka, Buchs, Switzerland) were employed.

Drug 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 dissolution of the drug the pH of the solution was adjusted to 5 by adding 2 N NaOH. When ritanserin was used at the highest concentration (10 mg/ml) the pH of the solution was 4.

In all the experiments ritanserin was given SC in a volume of 1 ml/kg b.wt. Control animals received a SC administration (1 ml/kg) of the vehicle. When ritanserin was tested in animals whose eating behavior was stimulated (by 15 hr deprivation or by time-scheduled access to food) the drug was administered SC 1 hr before food presentation. This interval was adopted to assure the pharmacological action since the beginning of the behavioral test. On the other hand, in conditions of basal, unstimulated eating behavior the drug was given immediately before food presentation, so as to detect its effect, if any, also in the first hr following drug administration.

5HT creatinine sulphate dissolved in sterile isotonic saline was given intraperitoneally (IP) or intracranially (IC) into the nucleus paraventricularis hypothalami (PVN).

Food and Water

Except when noted, animals had continuous access to food in pellets (Mill, Morini, Reggio Emilia, Italy) containing: 17% protein, 6% fat, 59% nitrogen free extracts (carbohydrates), 9% fiber and 9% ash. For subchronic experiments, in which animals were kept in metabolic cages, the same pellets were powdered.

Food intake was determined by weighing the pellets or the container with the powdered food to the nearest 0.01 g. Tap water was always available in graduated drinking tubes (or in 200 ml bottles when metabolic cages were employed).

Effect of Ritanserin on Eating Behavior

The effect of ritanserin on the rat's eating behavior was investigated in the following conditions: (1) acute administration in sated rats, (b) acute administration in food deprived rats, (c) subchronic administration (15 days) in animals with food always available during the day, (d) subchronic administration (15 days) in rats on a 6 hr/day schedule of access to food (10:00 a.m. to 4:00 p.m.).

Acute administration in sated rats. Ritanserin was given SC at the doses of 0.01, 0.1 and 1 mg/kg. The administration was made between 10:00 and 10:30 a.m. when control intake

TABLE 3

FOOD DEPRIVED RATS, FOOD INTAKE (g/100 g b.wt.)

	1 hr	2 hr	3 hr	4 hr	24 hr
Vehicle	3.69 ± 0.21	4.57 ± 0.20	4.76 ± 0.20	5.10 ± 0.23	13.57 ± 0.58
Ritanserin 0.01 mg/kg	3.67 ± 0.16	4.08 ± 0.20	4.38 ± 0.25	4.60 ± 0.17	14.03 ± 0.48
Ritanserin 0.1 mg/kg	3.79 ± 0.15	4.45 ± 0.30	4.47 ± 0.30	4.65 ± 0.30	13.78 ± 0.16
Ritanserin 1 mg/kg	3.80 ± 0.17	4.32 ± 0.24	4.35 ± 0.24	4.73 ± 0.09	13.47 ± 0.34

Food intake (g/100 g b.wt.) in food deprived rats following SC ritanserin administration. Drug or vehicle injection was given 1 hr before food presentation. Values, means ± SEM of 6–7 data, were determined at different times after food presentation.

TABLE 4

FOOD DEPRIVED RATS, WATER INTAKE (ml/100 g b.wt.)

	1 hr	2 hr	3 hr	4 hr	24 hr
Vehicle	3.07 ± 0.42	4.49 ± 0.53	4.79 ± 0.51	5.43 ± 0.54	16.33 ± 0.85
Ritanserin 0.01 mg/kg	2.72 ± 0.35	3.41 ± 0.32	3.74 ± 0.29	4.36 ± 0.41	16.15 ± 0.62
Ritanserin 0.1 mg/kg	2.10 ± 0.22	3.21 ± 0.38	3.35 ± 0.33*	3.60 ± 0.28*	15.53 ± 0.30
Ritanserin 1 mg/kg	2.19 ± 0.24	3.09 ± 0.30*	3.17 ± 0.30*	3.65 ± 0.10*	15.88 ± 0.50

Water intake (ml/100 g b.wt.) in food deprived rats following SC ritanserin administration. Drug or vehicle injection was given 1 hr before food presentation. Values, means ± SEM of 6–7 data, were determined at different times after food presentation. Difference from controls (vehicle): *$p<0.05$; where not indicated, difference was not statistically significant.

was as low as possible. Immediately after injection, rats were put back in their cages with food available. Food intake was determined at 1, 2, 3, 4 and 24 hr after injection.

Acute administration in food deprived rats. The drug was tested in animals deprived of food, but not of water, for 15 hr (from 8:00 p.m. to 11:00 a.m. of the following day). Sixty min after drug administration, rats had access to food. All the animals had already experienced a 15 hr deprivation of food twice before being tested. Food, as well as water intake, were determined at 1, 2, 3, 4 and 24 hr after food presentation.

Subchronic experiments in rats with continuous access to food. For these experiments rats were kept in individual metabolic cages (Morini, Reggio Emilia, Italy) in which powdered food was available in containers designed to avoid spillage. Tap water was provided in 200 ml bottles. These cages also allowed the collection of urine and feces separately. Baseline values for food and water intake, as well as for body weight gain, were determined over a period of 5 days before treatments began. On the basis of these data, animals were assigned to control or ritanserin treated groups so as to have mean food intake of the 2 groups as similar as possible. Drug solutions were administered each day for 15 days between 12:00 and 1:00 p.m. Immediately before administration the following parameters were determined: (1) daily food intake, (2) daily water intake, (3) body weight, (4) daily urine excretion and (5) daily feces elimination (feces were weighed after being kept for 23 hr at 80°C). Three different doses (0.1, 1 and 10 mg/kg/day) of ritanserin were tested in three different experiments. In each experiment 6 rats were employed as controls and 6 were tested with ritanserin.

Subchronic administration in rats on a 6 hr schedule of access to food. A single daily injection of ritanserin, 1 mg/kg b.wt., was given for 15 days in rats trained over the previous 20 days to eat between 10:00 a.m. and 4:00 p.m. The drug was given SC 60 min before access to food. In this, as well as in other subchronic experiments, baseline values for food and water intakes and for body weight gain were determined in the 5 days before the experiment. On the basis of these data the animals were divided in two groups that were as homogeneous as possible for daily food intake. Immediately before drug injection, daily water intake, daily urine and feces elimination and body weight gain were determined. Food and water intakes were measured at intervals of 1 hr after food presentation.

Effect of Ritanserin on the Anorectic Action of 5HT

The effect of ritanserin on the anorectic action evoked (a) by IP injection of 5HT and (b) by administration of 5HT into the PVN was investigated. In both instances the rats em-

ployed had been trained for at least 15 days to a 6 hr schedule of access to food (10:00 a.m.–4:00 p.m.).

(a) 5HT was given IP at the dose of 6 mg (15 μmoles)/kg b.wt. 20 min before food presentation. Forty min before 5HT injection (i.e., 60 min before food was offered) rats were pretreated with SC ritanserin 0.01, 0.1 and 1 mg/kg. Food intake was determined at 30 min, 1, 2, 3, 4 and 6 hr after food presentation.

(b) 5HT was injected at the dose of 25 nmoles/rat into the PVN in a volume of 0.4 μl. Immediately afterwards the animals were put back in their cages in which food and water were available. Sixty min before IC injection, rats received a SC administration of ritanserin, 0.1 and 1 mg/kg b.wt., or of simple vehicle. For this experiment 11 animals weighing between 270 and 285 g were employed. Under equithesin anaesthesia (3 ml/kg b.wt.) a unilateral guide cannula was implanted into the PVN. Drug injections were made by means of a stainless steel injector temporarily inserted into the guide cannula and protruding 2 mm beyond the cannula tip. The stereotaxic coordinates for the PVN, from the König and Klippel atlas [22], were: A=6.5 mm, L=0.3 mm, V=1.6 mm up from the interaural line. During the postoperative week, animals were handled and mock-injected to adapt them to the testing procedure.

Each rat was tested with the 2 SC doses of ritanserin, as well as with vehicle administration. Testing was conducted every 4 days. In the first 2 tests all the animals received SC vehicle and IC injection of saline or of 5HT in counterbalanced order. In the following 2 tests, animals received IC 5HT and the 2 SC doses of ritanserin, again in counterbalanced order. This sequence was adopted in relation to the long lasting activity of the drug, so as to be sure that previous drug administration could not affect control tests.

Upon completion of testing, animals were sacrificed and histology was performed to evaluate their cannula placements. Frozen brain sections (50 μ) were cut and stained with cresyl violet.

Statistical Analysis

For experiments evaluating the effect of ritanserin on eating behavior statistical analysis of data was performed by split-plot analysis of variance with between group comparisons for drug treatment and within group comparisons for time (hr after injection in acute experiments or day of treatment in subchronic experiments). Planned comparisons between controls and treated animals were carried out by *t*-tests.

For experiments evaluating the effect of ritanserin on 5HT-induced anorexia one way ANOVA, followed by Newman-Keuls multiple range tests, was used. Statistical significance was set at $p<0.05$.

RESULTS

Effect of Ritanserin on Eating Behavior

Acute administration in sated rats. Food intake following vehicle or ritanserin SC administration in sated rats in reported in Table 1. At the 3 doses tested, the drug induced no statistically significant modification of food intake at the different times of observation.

Water intake of the same animals is reported in Table 2. Again, the overall analysis showed that no significant effect on water intake was elicited by the different doses of ritanserin employed.

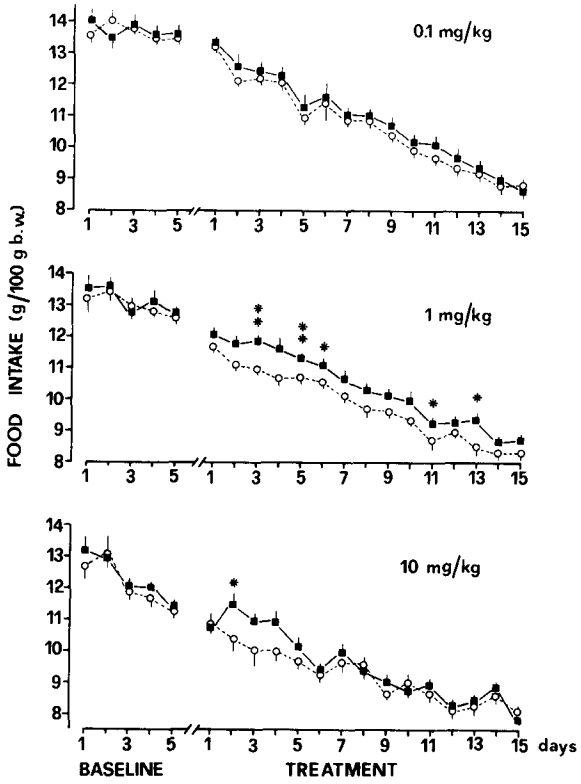

FIG. 1. Daily food intake (g/100 g b.wt.) before (BASELINE) and during a 15 day SC treatment with 0.1, 1 or 10 mg/kg/day of ritanserin (■) or with simple vehicle (○) in rats having continuous access to food. Values are means±SEM of 6 data. Difference from controls: *$p<0.05$; **$p<0.01$; where not indicated, difference was not statistically significant.

Acute Administration in Food-Deprived Rats

As shown in Table 3, food intake of treated animals was strictly similar to that of controls and no significant drug effect was detected over the whole period of observation. Moreover, the latency to eat at the 3 doses tested was never statistically different from that of control rats (47±12 sec).

Food associated drinking in the same animals is reported in Table 4. A statistically significant effect of the drug was observed in these conditions, $F(3,22)=3.049$, $p<0.05$. Water intake of treated rats was statistically lower than that of controls at 3 and 4 hr following ritanserin 0.1 mg/kg and at 2, 3 and 4 hr following 1 mg/kg. However, the 24 hr intake of treated and of control rats was statistically indistinguishable.

Subchronic Administration in Rats With Food Always Available

As shown in Fig. 1, daily food intake (expressed as g/100 g b.wt.) declined during the baseline period, as well as during the 15 days of the treatment. Within group analysis for day of treatment showed a statistically significant ($p<0.001$) decrease in % food intake for each of the three subchronic treatments. This was related to the fact that the absolute food intake (g/rat) of ritanserin treated rats and of controls remained stable during the 15 days of the treatment, while a progressive increase in body weight was observed at the same time. For instance, in the experiment with ritanserin 10

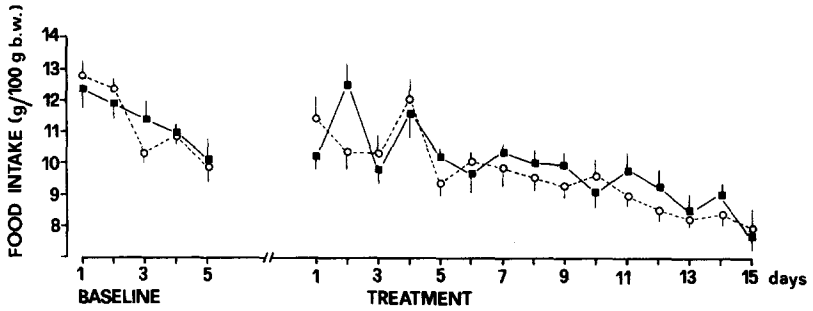

FIG. 2. Food intake (g/100 g b.wt.) before (BASELINE) and during a 15 day SC treatment with 1 mg/kg/day of ritanserin (■) or with simple vehicle (○) in rats on a 6 hr schedule of access to food. Ritanserin or vehicle injection was given 1 hr before food presentation. Values, cumulative 6 hr intakes, are means±SEM of 6 data.

mg/kg/day, on the 1st day of the treatment food intake was 22.92±1.61 g/rat in treated animals and 24.76±0.94 g/rat in their controls, and on the 15th day treated animals took 23.34±0.32 g/rat, while controls took 24.59±1.46 g/rat. In the same period the mean body weight rose in treated animals from 225.83±3.72 to 303.66±4.32 and in controls from 228.66±3.27 to 315.66±12.07.

Between group analysis showed that at the dose of 0.1 mg/kg b.wt., ritanserin produced no significant effect on food intake. Moreover, the other parameters determined (daily water intake, daily urine and feces elimination and daily body weight gain) were also never statistically different in the two groups of animals.

On the other hand, at the dose of 1 mg/kg the analysis of variance revealed a significant drug effect, $F(1,10)=9.166$, $p<0.05$. A small but consistent increase in food intake was observed beginning on the 2nd day of treatment. The difference between the 2 groups reached a maximum on the 4th day of treatment when ritanserin treated rats ate 8.29% more than controls. Statistically significant differences between groups were detected on the 3rd, 5th, 6th, 11th and 13th day. Also feces elimination of treated rats was higher than in controls during the 15 days of treatment; statistically significant differences were, however, detected only on the 12th, 13th and 15th day (+16.7%, +14.4% and +10.63%, respectively).

On the other hand, during the 15 days of the treatment, water intake, urine output and body weight gain of the 2 groups were essentially identical.

The analysis of variance for the dose of 10 mg/kg revealed no significant treatment effect, but a significant treatment-day interaction was detected, $F(14,140)=2.981$, $p<0.001$, indicating a drug effect for part of the test period. Treated animals showed a food intake slightly larger than controls between the 2nd and the 5th day of treatment, the maximum difference (10%) occurring on the 2nd day ($p<0.05$). On this day also feces elimination of treated rats (3.06±0.11 g/100 g b.wt.) was higher than in controls (2.68±0.08 g/100 g b.wt.), $p<0.05$. Again no significant difference was ever detected for the other parameters studied.

Subchronic Treatment in Rats on a 6 hr Schedule of Access to Food

This experiment was designed to evaluate whether the effect on food intake of daily injections of ritanserin was increased by administering the drug just before eating. In this experiment we employed the dose of 1 mg/kg, which, as reported above, in experiments with continuous access to food produced some increase in the intake. However, no significant treatment effect was detected in these conditions and, as reported in Fig. 2, the intake of treated rats was strictly similar to that of controls.

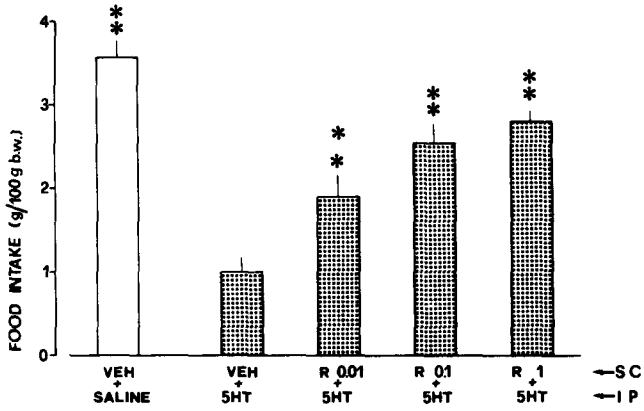

FIG. 3. Effect of ritanserin (R) (0.01–1 mg/kg b.wt.) on the anorectic action of IP 5HT (6 mg/kg) in rats on a 6 hr schedule of access to food. Values reported are mean food intake (g/100 g b.wt.) ±SEM in the first hr following food presentation. VEH=Vehicle used for ritanserin injection (1 ml/kg, SC); SALINE=isotonic NaCl solution (1 ml/kg, IP). Difference from SC VEH + IP 5HT: $^{**}p<0.01$.

Effect of Ritanserin on the Anorectic Action of 5HT

IP 5HT administration (Fig. 3). In these experimental conditions 5HT evoked a clear anorectic effect, which was particularly pronounced during the first hr following food presentation (% food intake inhibition=71.6%, $p<0.01$). Ritanserin, given SC, produced a dose-dependent inhibition of the anorectic action of 5HT, the effect being statistically significant even at the dose of 0.01 mg/kg ($p<0.01$). However, in the range of doses tested ritanserin never completely blocked the anorectic action of IP 5HT. In fact, even following 1 mg/kg of ritanserin, the intake of treated animals (2.79±0.15 g/100 g b.wt.) was clearly lower than that of controls (3.56±0.18; $p<0.05$).

5HT given into the PVN. Histological analysis of the brain cannula placements revealed that 7 of the 11 rats employed in this experiment had an "on target" cannula aimed at the PVN. Four rats had cannulae on miscellaneous hypo-

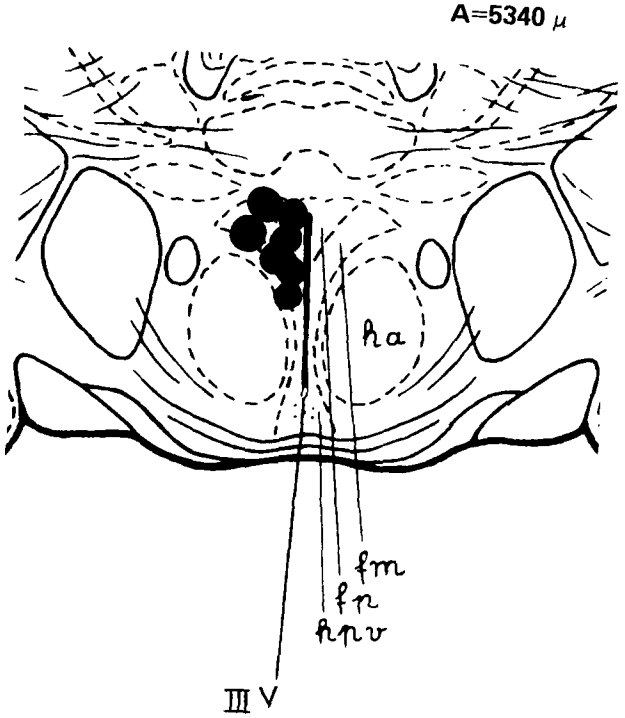

FIG. 4. Histological analysis of rat brains showing cannula placement into the PVN. Results of the histological analysis are illustrated on a coronal drawing of the rat brain, taken from König and Klippel atlas [22]. Abbreviations: fm=nucleus paraventricularis, pars magnocellularis, fp=nucleus paraventricularis, pars parvocellularis, hpv=nucleus periventricularis hypothalami, ha=nucleus anterior hypothalami, IIIV=third ventricle.

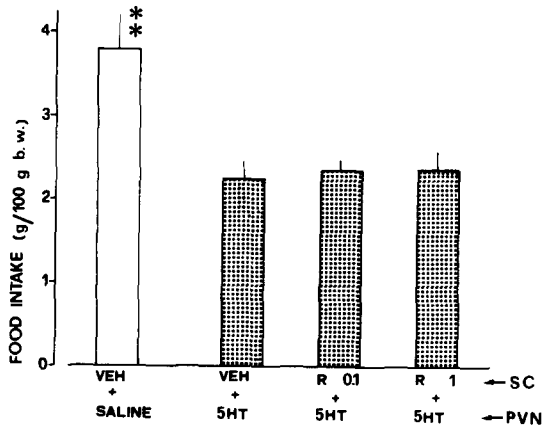

FIG. 5. Effect of SC ritanserin (R) (0.1, 1 mg/kg) on the anorectic action of 5HT (25 nmoles/rat) given into the PVN in rats on a 6 hr schedule of access to food. Values reported are mean food intake (g/100 g b.wt.) ±SEM in the first hr following food presentation. VEH=vehicle used for ritanserin injection (1 mg/kg, SC); SALINE=isotonic NaCl solution (0.4 μl/rat into the PVN). Difference from SC VEH + PVN 5HT: $^{**}p<0.01$; where not indicated, difference was not statistically significant.

thalamic sites outside the PVN and were therefore eliminated from the study. The precise cannula placements for the 7 animals with PVN cannulae are reported in Fig. 4.

5HT produced a marked suppression of food intake following injection into the PVN at the dose of 25 nmoles/rat. The inhibition at 30 and 60 min after injection was 57.7 and 40.36% ($p<0.001$ and $p<0.01$, respectively). At 2 hr after drug administration the intake of treated animals (4.23±0.31 g/100 g b.wt.) was still lower than that of controls (5.22±0.34 g/100 g b.wt.), although the difference was not statistically significant.

As shown in Fig. 5, at 1 hr after food presentation ritanserin 0.1 and 1 mg/kg did not antagonize at all the anorectic effect of IC 5HT. The intake following SC ritanserin was in fact statistically indistinguishable from that of control animals which received only SC vehicle injection.

DISCUSSION

The results of the present study show that acute administration of the new $5HT_2$ antagonist ritanserin neither elicits food intake in sated rats, nor increases food deprivation-induced eating. In this respect it is important to emphasize that in the range of doses employed (0.01–1 mg/kg, SC) the drug did not produce any sign of malaise, sedation, discomfort, nor other behavioral alterations that might hypothetically prevent the expression of an orexigenic action. In accordance with these observations are the findings of Colpaert *et al.* [10] who indeed showed that in the same range of doses ritanserin releases the behavior of rats exposed to an anxiogenic environment.

On the other hand, pharmacokinetic factors cannot easily account for the lack of orexigenic effect, since ritanserin has been clearly shown to readily cross the blood-brain barrier. In fact, in rats treated with ritanserin 0.63 mg/kg, $5HT_2$ receptor sites in the frontal cortex have been reported to be occupied by more than 80% for 6 hr and after 48 hr 30% of them were still occupied [31].

In an attempt to explain why ritanserin does not stimulate food intake, we thought it interesting to evaluate the effect of this drug on the anorectic action of exogenously administered 5HT. Indeed, a very interesting finding resulted from this study; that is ritanserin did not antagonize at all the anorectic action of 5HT given into the PVN. This nucleus has been shown to play an important role in the regulation of eating behavior in the rat [25, 27, 28, 40] and proves to be a brain structure particularly sensitive to the anorectic action of 5HT [27, 29, 41] and of various serotoninergic compounds [43]. Clearly this finding suggests that the anorectic action of central endogenous 5HT is also not blocked by ritanserin, thus proposing a reasonable explanation for the absence of orexigenic effect following its administration. It is interesting to note in this regard that cyproheptadine, which has been reported to stimulate food intake in rats, completely blocks the anorectic action of 5HT given into the lateral ventricles [23].

Indeed, our results show that ritanserin markedly reduces, although it does not completely block, the anorectic effect of IP 5HT; however, in the absence of central antagonism, the inhibition of the peripheral anorectic action of endogenous 5HT is likely not enough to initiate food intake.

On the other hand, the results of the present experiments apparently suggest that the hypothalamic receptors, which mediate the effect of 5HT on eating behavior are different from the $5HT_2$ receptors of the frontal cortex, which have been shown to be powerfully bound by ritanserin. At the moment it is not possible, using only the evidence gained with the use of this selective antagonist, to put forward a more detailed hypothesis concerning the type of 5HT receptors involved in food intake control.

As far as subchronic experiments are concerned, it is interesting to note that in the first day of treatment no increase

in food intake was observed, even at the maximum dose of 10 mg/kg. These data are in keeping with those obtained in acute experiments. On the other hand, in the following days animals treated with high doses of ritanserin (1 and 10 mg/kg) but not with the dose of 0.1 mg/kg, showed an increased food intake. This increase was, however, small in magnitude (never larger than 10% of the control intake) and transient (only for some days during the treatment).

Indeed it is not easy, on the basis of the results of the acute experiments, to explain the occasional increases observed in subchronic experiments. The fact that the increased intake in subchronic experiments took place only at high doses that give a maximal and prolonged binding to central $5HT_2$ receptor sites, apparently rules out the possibility that the effect might be simply due to a gradual pharmacological accumulation leading to effective doses of the drug. An interesting hypothesis to explain these findings might be derived from the studies of Fuller *et al.* [16]. They found that the 5HT agonist quipazine produces an increase in the levels of brain 5HT as well as a decrease in brain 5-hydroxyindoleacetic acid levels, which was attributed to a compensatory decrease in 5HT release following stimulation of 5HT receptors. The 5HT antagonist ritanserin might block this feed-back mechanism thus producing massive 5HT release and a partial 5HT depletion in the brain which could account for the increases in food intake. In fact, several experimental conditions that produce 5HT depletion [7,36] or that reduce 5HT release through autoreceptor stimulation [2, 13, 19, 32] have been reported to stimulate food intake. However, whether this explanation applies to the high ritanserin doses employed in this study remains speculative, since published biochemical studies of the effect of ritanserin on 5HT levels in the central nervous system did not employ the experimental conditions used in our study. Indeed, the finding reported by Leysen *et al.* [31], that oral administration of 160 mg/kg of ritanserin produces about 40% reduction in striatal 5HT as early as 2 hr after administration does not exclude this possibility.

Because ritanserin appears to antagonize the anorectic effect of peripheral 5HT, the possibility that prolonged inhibition of the peripheral 5HT system might be responsible for the observed increase in food intake should also be considered.

As far as water intake is concerned, our results show that ritanserin significantly inhibits food associated drinking at 3 and 4 hr after drug administration. Since 5HT has been shown to possess a dipsogenic action following both central and peripheral administration (see for review [15]), the inhibition observed could be reasonably explained on the basis of a specific pharmacological antagonism. However, more detailed studies are required to evaluate the effect of ritanserin on water intake induced by other dipsogenic determinants.

In conclusion, the results of our study show that ritanserin neither stimulates food intake in sated rats, nor increases the intake induced by food deprivation. In subchronic experiments it does not increase food intake at a daily dose of 0.1 mg/kg, but produces some small and transient increases at high doses (1 and 10 mg/kg). However it seems possible that at these doses a more general modification of the central serotoninergic system might occur.

When ritanserin was tested for its ability to block the anorectic action of 5HT it antagonized the action of IP 5HT, but was completely ineffective versus that of 5HT injected into the PVN. These findings apparently suggest that the hypothalamic receptors mediating the effect of 5HT on eating behavior are different from those of the frontal cortex which have been shown to be completely blocked by ritanserin.

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