Pharmacology Biochemistry and Behavior, Vol. 45, pp. 593–596, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of *d*-Fenfluramine, MK-212, and Ondansetron on Saline Drinking in Two-Choice Tests in the Rehydrating Rat

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Received 16 October 1992

COOPER, S. J. AND D. J. BARBER. *Effects of d-fenfluramine, MK-212, and ondansetron on saline drinking in two-choice tests in the rehydrating rat.* PHARMACOL BIOCHEM BEHAV 45(3) 593–596, 1993.—The aim of the present studies was to investigate the effects of serotonergic compounds on preference for isotonic saline and aversion to hypertonic saline, respectively. Twenty-two-hour water-deprived rats were divided into two groups: The first was given a choice between 0.9% saline and water in a 30-min test; the second was given a choice between 1.8% saline and water. Animals were tested following administration of *d*-fenfluramine, the 5-HT_{1C} receptor agonist 6-chloro-2-(1-piperazinyl)pyrazine (MK-212), and the 5-HT_3 receptor antagonist ondansetron. *d*-Fenfluramine (0.3–3.0 mg/kg) did not reduce 0.9% saline preference; instead, at 0.3 mg/kg there was a significant increase in saline drinking. In contrast, MK-212 (0.3–3.0 mg/kg) abolished the preference for isotonic saline whereas ondansetron (10–100 μg/kg) had no effect. *d*-Fenfluramine and MK-212 reduced hypertonic saline drinking, although at the highest dose for each drug water drinking was also reduced. These data add further to the evidence for an important serotonergic involvement in the control of saline drinking and preference in the rat.

Saline drinking Thirst *d*-Fenfluramine MK-212 Ondansetron 5-HT receptor subtypes

THE theory of central serotonergic inhibition of appetite for food (2) is well grounded in a large experimental literature (5). One of the most intensively studied serotonergic compounds has been the clinically effective anorectic compound, fenfluramine, first in its racemic form and more recently as *d*-fenfluramine (13). At a theoretical level, it would be interesting to know if there is some form of specific serotonergic involvement in the control of food consumption or if there is a more generalised involvement that takes in other types of ingestion. In one recent study (12), the effects of *d*-fenfluramine and other serotonergic drugs on the drinking of three fluids (water, 0.9% saline, and 1.8% saline, respectively) were investigated in single-choice acceptance tests. *d*-Fenfluramine (1.0–3.0 mg/kg) did not reduce fluid intake nonspecifically; at 1.75 and 3.0 mg/kg, it significantly reduced hypertonic saline drinking but had no significant effect on isotonic saline consumption. Water intake was slightly reduced at 3.0 mg/kg (12). In the same study, the 5-hydroxytryptamine$_{1B/1C}$ (5-$HT_{1B/1C}$) receptor agonist mCPP (9,10) reduced hypertonic saline drinking at doses that did not significantly affect water consumption; a similar result was obtained for the 5-HT_{1C} receptor agonist 6-chloro-2-(1-piperazinyl)pyrazine (MK-212) (3). These data suggest that hypertonic saline ingestion may be sensitive to enhanced serotonergic activity, and may point to a 5-HT_{1C} receptor mediation (12).

In addition, it has been demonstrated that selective 5-HT_{1A} receptor agonists significantly increase the ingestion of hypertonic saline without affecting water consumption (6,7). These agonists may act presynaptically to inhibit central serotonergic activity and so raise hypertonic saline intake. 5-HT receptor subtypes (9,10) may therefore be an important factor in determining the direction of effect of 5-HT agonists on saline drinking.

Such data strongly suggest that there may be important serotonergic involvement not only in appetite for food but also in sodium appetite and in salt taste preference/aversion. In support of these new hypotheses, it has recently been demonstrated that the 5-HT_{1C}/5-HT_2 receptor antagonists ketanserin and ritanserin inhibited the salt appetite induced by deoxycorticosterone acetate (DOCA) (9). Moreover, we recently found that the putative 5-$HT_{1B/1C}$ agonists, mCPP and TFMPP, abolished the preference for a 0.9% salt solution and enhanced the aversion shown to a hypertonic (1.8% NaCl) solution (Cooper and Ciccocioppo, submitted for publication). In the present series of experiments, we sought to extend our previous work. Water-deprived animals were given the

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choice between water or 0.9% saline, on the one hand, and between water and 1.8% saline, on the other. We chose three serotonergic drugs for testing: a) *d*-fenfluramine, the prototype serotonergic anorectic compound; b) the selective 5-HT_{1C} receptor agonist MK-212; c) ondansetron, a selective 5-HT_3 receptor antagonist that can be used to test for potential 5-HT_3 receptor involvement in ingestion (1,4). The data from these studies add to the growing evidence for serotonergic modulation of sodium appetite and salt preference/aversion.

METHOD

Animals

Subjects were 40 adult, male rats (hooded General strain), which were bred in our laboratory. They were housed individually in stainless steel cages with ad lib access to food pellets (modified Diet 41B, Heygate & Sons). They were maintained under a 12 L : 12 D cycle (light on at 7:00 a.m.) and the room temperature was maintained at 20–21 °C. Animals were accustomed to being handled and weighed 400–450 g.

Drugs

d-Fenfluramine HCl was kindly supplied by Institut de Recherches Internationales Servier (Neuilly-sur-Seine, France); MK-212 was obtained from Merck Sharp & Dohme (Harlow, UK); ondansetron was obtained from Glaxo Group Research (Ware, UK). All drugs were dissolved in isotonic saline and injected SC in a volume of 1 ml/kg 20 min before the start of the drinking tests. Doses for each drug were chosen on the basis of previous work (13,16).

Procedure

Animals were first divided at random between 2 groups of 20 rats each. Over a 7-day period, they were adapted to a 22-h water-deprivation schedule, with water available in home cages for 2 h each day. The first group was then trained in a two-choice test and had access to 1.8% saline and water provided in two 50-ml calibrated tubes, respectively. The second group was trained with a choice between 0.9% saline and water. Animals were tested daily for 30 min immediately after the period of water deprivation. After each test, the home cage water supply was restored for a further 90 min. During this adaptation period, animals also received injections of the drug vehicle on three occasions to familiarize them with the injection procedure. Throughout all the fluid preference tests, the positions of the drinking tubes were regularly interchanged to avoid the development of strong position preferences.

For each drug that was tested, 9–10 animals were selected from each group (1.8 and 0.9% saline groups). Each animal then received each dose of the drug being tested, as well as the vehicle injection. The sequence of injections was counterbalanced across subjects within each group. At least 48 h separated successive injections of the same drug. During the intervals between drug testing, the water deprivation schedule was maintained and animals continued to receive daily 30-min test sessions. One week was allowed between successive drug treatments, and each animal was tested with no more than two drugs.

For each test session, the volume of fluid consumed in the 30-min period from each cylinder was measured to the nearest 0.5 ml. Data were analysed using a two-way analysis of variance (ANOVA), with repeated measures on both factors (fluid choice; drug doses). Dunnett's *t*-test was used to compare individual dose conditions against the vehicle control.

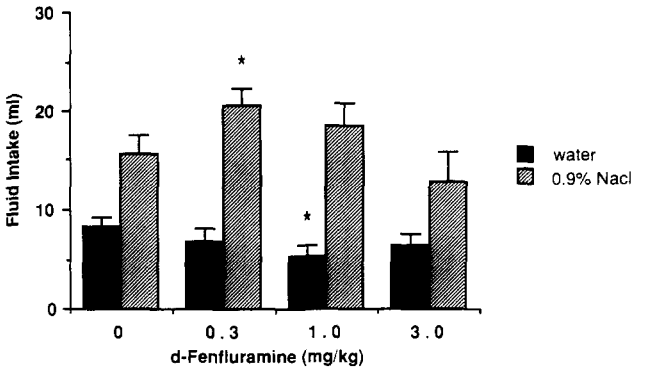

FIG. 1. Effects of *d*-fenfluramine (0.3–3.0 mg/kg) on isotonic saline preference in a two-choice test. Rats were 22-h water deprived prior to the 30-min choice test. Data are shown as mean intake (ml) ± SEM. $n = 10$ per group. Levels of significance for comparisons between individual group means and the vehicle control: $*p < 0.05$; $**p < 0.01$ (Dunnett's *t*-test).

RESULTS

0.9% NaCl vs. Water

Figure 1 indicates that rats showed a preference for the 0.9% NaCl solution over water in terms of fluid consumed in the test period, $F(1, 9) = 21.41$, $p < 0.005$. There was a significant *d*-fenfluramine main effect, $F(3, 27) = 3.98$, $p < 0.05$, and a significant drug × fluid interaction, $F(3, 27) = 3.95$, $p < 0.05$. Most interestingly, *d*-fenfluramine did not reduce the preference for 0.9% saline. Two significant effects, shown in Fig. 1, were actually in the direction of increased preference. Thus, at 0.3 mg/kg *d*-fenfluramine there was a significant increase in saline drinking. At 1.0 mg/kg *d*-fenfluramine, there was a significant suppression of water drinking without reduction in saline drinking. Over a range of doses that produce marked anorexia, therefore (11), *d*-fenfluramine did not reduce fluid ingestion in a two-choice test and did not reduce 0.9% saline preference.

The selective 5-HT_{1C} receptor agonist MK-212, however, affected saline preference differently. It had a main effect to reduce fluid consumption, $F(3, 27) = 8.60$, $p < 0.001$, and there was a significant drug × fluid interaction, $F(3, 27) =$

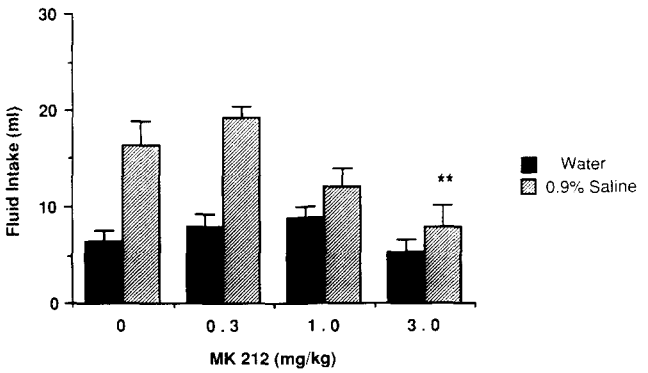

FIG. 2. The 5-hydroxytryptamine$_{1C}$ (5-HT_{1C}) receptor agonist MK-212 (0.3–3.0 mg/kg) abolished the preference for the 0.9% NaCl solution. Other details are as described in Fig. 1 legend.

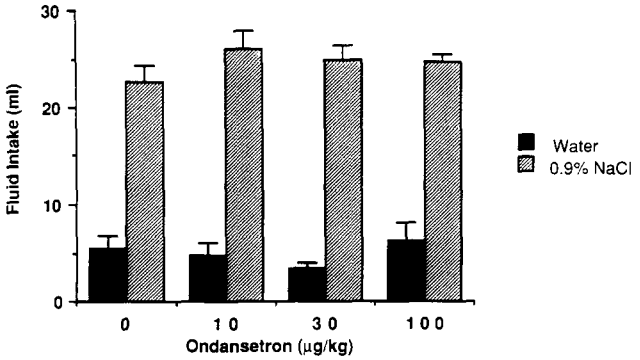

FIG. 3. The selective 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonist ondansetron (10–100 μ/kg) had no effect on the intake of either water or 0.9% NaCl solution. Other details are as described in Fig. 1 legend.

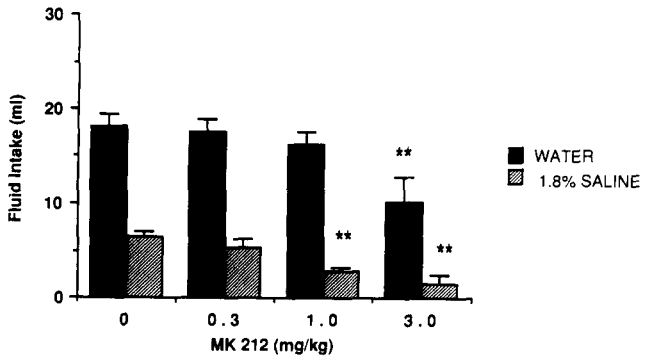

FIG. 5. The 5-hydroxytryptamine$_{1C}$ (5-HT$_{1C}$) receptor agonist MK-212 (0.3–3.0 mg/kg) reduced 1.8% saline consumption in a two-choice test. n = 9 per group. Other details are as described in Fig. 1 legend.

3.84, $p < 0.05$. The effect of MK-212 could be accounted for in terms of a selective decrease in saline drinking (Fig. 2). At 1.0 and 3.0 mg/kg, MK-212 abolished isotonic saline preference.

The selective 5-HT$_3$ receptor antagonist, ondansetron, had no significant effects on water or 0.9% saline consumption (Fig. 3).

1.8% NaCl vs. Water

Figure 4 confirms that rats showed a relative aversion to the 1.8% NaCl solution, $F(1, 8) = 149.6$, $p < 0.001$. There was a significant main effect of *d*-fenfluramine to reduce fluid intake, $F(3, 24) = 11.83$, $p < 0.001$, but there was no significant drug × fluid interaction. As Fig. 4 indicates, *d*-fenfluramine significantly reduced hypertonic saline drinking at 1.0 mg/kg but reduced both water and hypertonic saline drinking at 3.0 mg/kg. Under control conditions, the level of 1.8% saline consumption was 24.2% of the total fluid intake; at 1.0 mg/kg *d*-fenfluramine, it was reduced to 17.5% of the total, and at 3.0 mg/kg *d*-fenfluramine it was reduced still further to 12.8% of the total.

MK-212 produced a profile that was similar to that of *d*-fenfluramine (Fig. 5). It had a significant main effect to reduce fluid intake, $F(3, 24) = 23.1$, $p < 0.001$, but there was no significant drug × fluid interaction. Figure 5 shows that at 1.0 mg/kg MK-212 significantly reduced hypertonic saline drinking and at 3.0 mg/kg reduced both water and hypertonic saline drinking. Under control conditions, the level of 1.8% saline consumption was 26% of the total fluid intake. It reduced to 14.9% at 1.0 mg/kg MK-212 and to 12.8% at 3.0 mg/kg MK-212.

The selective 5-HT$_3$ receptor antagonist, ondansetron, had no significant effects on water or 1.8% saline consumption (Fig. 6).

DISCUSSION

The present data provide further evidence for serotonergic involvement in the control of salt drinking in water-deprived rats. In the case of both *d*-fenfluramine and MK-212, a dose of 1 mg/kg significantly reduced hypertonic saline drinking without significantly affecting concurrent water drinking. At the higher dose of 3 mg/kg, both drugs reduced both water and hypertonic saline drinking, although the effect on the salt drinking was proportionately greater in each case. These data for hypertonic saline ingestion are broadly consistent with the previous acceptance data obtained for these two drugs at comparable doses (13). Moreover, we also observed that the 5-

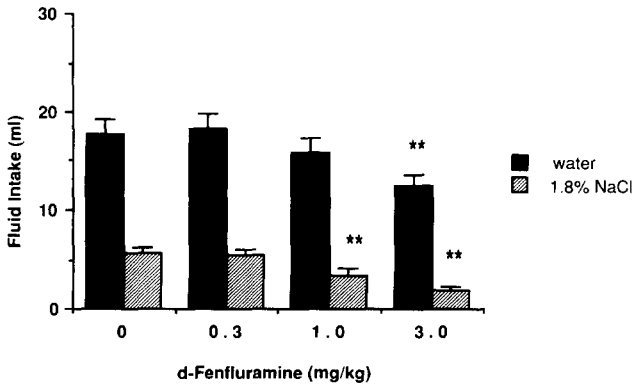

FIG. 4. Effects of *d*-fenfluramine (0.3–3.0 mg/kg) on the relative aversion for 1.8% NaCl solution in a two-choice test. n = 9 per group. Other details are as described in Fig. 1 legend.

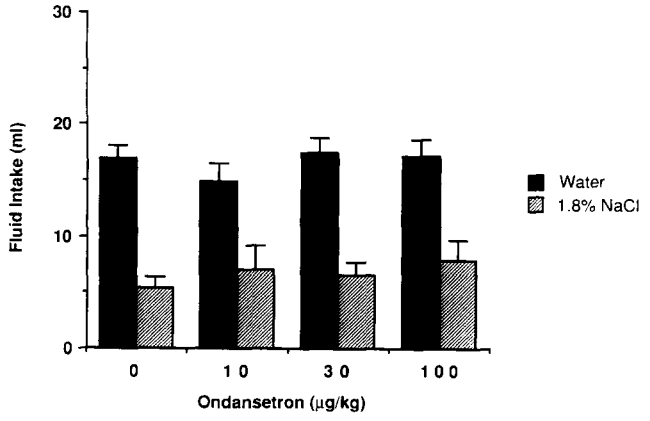

FIG. 6. Ondansetron (10–100 μg/kg) had no effect on the intake of either water or 1.8% saline. n = 9 per group. Other details are as described in Fig. 1 legend.

$HT_{1B/1C}$ receptor agonists, mCPP and TFMPP, similarly produce selective reductions in 1.8% NaCl solution intake in a two-choice test at 0.3 mg/kg in both cases (Cooper and Ciccocioppo, submitted). Hence, there is consistent evidence for a greater sensitivity of hypertonic saline ingestion to the suppressant effects of serotonergic agonists (excluding 5-HT_{1A} receptor agonists, which stimulate hypertonic saline ingestion selectively).

In contrast to the general resemblance of the results for *d*-fenfluramine and MK-212 in the hypertonic saline consumption test, the effects of the two drugs were dissimilar in the 0.9% saline preference test. MK-212 was effective in abolishing the preference for 0.9% saline, whereas *d*-fenfluramine not only did not abolish the salt preference but had effects that tended in the direction of increasing the preference. Earlier, we found that the putative 5-$HT_{1B/1C}$ receptor agonists, mCPP and TFMPP, abolished 0.9% saline preference (Cooper and Ciccocioppo, submitted for publication). This contrast between *d*-fenfluramine, on the one hand, and 5-HT receptor agonists like MK-212, mCPP, and TFMPP, on the other, is striking. It emphasises that small modifications to the test conditions can uncover striking differences in drug effects. Although *d*-fenfluramine had effects on hypertonic saline consumption that bore a family resemblance to the effects of 5-$HT_{1B/1C}$ or 5-HT_{1C} receptor stimulation, it did not maintain this resemblance in the case of isotonic saline preference. It is interesting to note that Fletcher found no evidence that *d*-fenfluramine reduced sweet taste preference (8); it would be instructive to investigate effects of selective 5-$HT_{1B/1C}$ receptor agonists on sweet taste preference. It would be intriguing if they abolished this form of preference, as they do salt taste preference. If they did, while *d*-fenfluramine does not (8), then some mechanism other than stimulation of 5-HT_{1B} and/or 5-HT_{1C} receptors will be needed to account for *d*-fenfluramine's effects in taste preference tests (e.g., Fig. 1).

The lack of effect of ondansetron in the two test situations suggests that 5-HT_3 receptor blockade has little consequence for either saline or water drinking. There is some suggestion, however, that 5-HT_3 receptor antagonists have some effect to reduce ethanol preference in rats (15). In summary, therefore, the selective 5-HT_{1C} receptor agonist MK-212 abolished the preference for a 0.9% salt solution but enhanced the aversion to a hypertonic (1.8%) salt solution. The results for this compound are consistent with data we previously obtained with the 5-$HT_{1B/1C}$ receptor agonists, mCPP and TFMPP (Cooper and Ciccocioppo, submitted for publication). Hence, selective stimulation of 5-HT_{1C} receptors (and possibly 5-HT_{1B} receptors, too) causes marked alterations in the choices that water-deprived rats make with salt solutions. To a certain degree, the indirectly acting serotonergic agonist, *d*-fenfluramine, shared effects with these more selective drugs. It also tended to suppress hypertonic saline drinking. However, it departed markedly in its effects on the preference for the 0.9% salt solution. There was no indication that it reduced the salt preference; if anything, it tended to enhance the preference. At a more general theoretical lever, these results add weight to the view that the investigation of serotonergic involvement in ingestional processes cannot be limited to appetite for food and must also incorporate controls relevant to sodium appetite and salt preference/aversion.

ACKNOWLEDGEMENT

The authors thank Dorothy Trinder for preparation of the manuscript.

REFERENCES

1. Barnes, J. M.; Barnes, N. M.; Cooper, S. J. Behavioural pharmacology of 5-HT_3 receptor ligands. Neurosci. Biobehav. Rev. 16: 107–113; 1992.
2. Blundell, J. E. Serotonin and appetite. Neuropharmacology 23: 1537–1551; 1984.
3. Conn, P. J.; Sanders-Bush, E. Relative efficacies of piperazines at the phosphoinositide hydrolysis-linked serotonergic (5-HT_2 and 5-HT_{1C}) receptor. J. Pharmacol. Exp. Ther. 242:552–557; 1987.
4. Cooper, S. J. Ingestional responses following benzodiazepine receptor ligands, selective 5-HTA agonists and selective 5-HT_3 receptor antagonists. In: Rodgers, R. J.; Cooper, S. J., eds. 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: Their comparative behavioural pharmacology. Chichester, UK: John Wiley; 1991:233–265.
5. Cooper, S. J. 5-HT and ingestive behaviour. In: Marsden C.; Heal, D., eds. Central serotonin receptors and psychotropic drugs. Oxford: Blackwell Scientific; 1992:260–291.
6. 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.
7. Cooper, S. J.; Fryer, M. J.; Neill, J. C. Specific effect of putative 5-HT_{1A} agonist, 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.
8. Fletcher, P. J. The effects of *d*-fenfluramine on saccharin intake and preference, and on food and water intake. Pharmacol. Biochem. Behav. 29:687–691; 1988.
9. Gentili, L.; Saija, A.; Luchetti, G.; Massi, M. Effect of the 5-HT_2 antagonist ketanserin on salt appetite in the rat. Pharmacol. Biochem. Behav. 39:171–176; 1991.
10. Hoyer, D. The 5-HT receptor family: Ligands, distribution and receptor-effector coupling. In: Rodgers, R. J.; Cooper, S. J., eds. 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: Their comparative behavioural pharmacology. Chichester, UK: John Wiley; 1991:31–57.
11. Middlemiss, D. N.; Tricklebank, M. D. Centrally active 5-HT receptor agonists and antagonists. Neurosci. Biobehav. Rev. 16: 75–82; 1992.
12. Neill, J. C.; Cooper, S. J. Evidence that *d*-fenfluramine anorexia is mediated by 5-HT_1 receptors. Psychopharmacology (Berl.) 97: 213–218; 1989.
13. Neill, J. C.; Cooper, S. J. Selective reduction by serotonergic agents of hypertonic saline consumption in rats: Evidence for possible 5-HT_{1C} receptor mediation. Psychopharmacology (Berl.) 99:196–201; 1989.
14. Rowland, N. E.; Carlton, J. Neurobiology of an anorectic drug: Fenfluramine. Prog. Neurobiol. 27:13–62; 1986.
15. Sellers, E. M.; Higgins, G. A.; Sobell, M. B. 5-HT and alcohol abuse. Trends Pharmacol. Sci. 13:69–75; 1992.
16. van der Hoek, G. A.; Cooper, S. J. Microstructural analysis of the effects of the selective 5-HT_3 antagonists, ondansetron, or feeding and other behavioural responses. Br. J. Pharmacol. 99: 237P; 1990.