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Effects of serotonin$_{1/2}$ receptor agonists on dark-phase food and water intake in rats

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Abstract

The effects of serotonin (5-hydroxytryptamine, 5-HT)$_{1/2}$ receptor agonists for 5-HT$_1$ and 5-HT$_2$ receptors on dark-phase ingestive behavior were evaluated in 12-h food-deprived, female Wistar rats. The amount of food and water consumed after 1, 2, 6 and 12 h was measured. The following agonists were tested: ipsapirone [preferred 5-HT receptor(s) and dose range in mg/kg, IP: 5-HT$_{1A}$ and 3-30, respectively], CP-94,253 (5-HT$_{1B}$; 0.3–3), TFMPP (5-HT$_{1B/2C}$; 0.3–10), *m*-CPP (5-HT$_{2C/1B}$; 0.3–10), ORG 37684 (5-HT$_{2C}$; 0.3–10), BW 723C86 (5-HT$_{2B}$; 3–30) and DOI (5-HT$_{2A/2C}$; 0.3–3). Ipsapirone induced hyperphagia during the first hour of food access and hypophagia during the last interval. All other compounds induced dose- and time-dependent hypophagia. *m*-CPP and TFMPP induced the most marked reduction of food intake and were the only drugs inducing rebound hyperphagia. Except for *m*-CPP and TFMPP, effects on food intake could generally be dissociated from effects on water intake. The receptor profile of the compounds tested suggests that stimulation of 5-HT$_{1B}$, 5-HT$_{2C}$, 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors results in hypophagia. As the less selective agonists were the more potent anorexics, it is suggested that simultaneous activation of these receptors results in synergistic effects on ingestive behavior. Additional antagonism studies are required to ascertain the proposed role of particular 5-HT receptor subtypes in the hypophagic effects of the tested compounds.

Keywords: BW 723C86; CP-94,253; DOI; 5-Hydroxytryptamine (5-HT) receptors; 5-HT$_{1A}$; 5-HT$_{1B}$; 5-HT$_{1D}$; 5-HT$_{2A}$; 5-HT$_{2B}$; 5-HT$_{2C}$; Ingestive behavior; Ipsapirone; *m*-CPP; ORG 37684; TFMPP

Although serotonin (5-hydroxytryptamine, 5-HT) receptor agonists with differential selectivity for the various subtypes of the 5-HT$_1$ and 5-HT$_2$ receptor families can affect ingestive behavior [10,11,13–15,29], the specific role of the receptor subtypes involved remains unclear. For example, the suggested function of 5-HT$_{2C}$ and/or 5-HT$_{1B}$ receptors in the control of feeding behavior is largely based on the use of 5-HT receptor agonists with a limited degree of selectivity for these receptors, and their function has not yet been ascertained by means of antagonism studies with selective antagonists (for discussion, see Ref. [6]). There has also been a general lack of comparative studies assessing the potency, efficacy and time-dependency of the effects of 5-HT receptor agonists on ingestive behavior. Such studies, combined with appropriate antagonism studies, may be helpful in analyzing the role of the different 5-HT receptor subtypes in the control of food and water intake. In general, the effects of serotonergic compounds on ingestive behavior have typically been investigated over relatively short periods (mostly not longer than 1 h) and, therefore, information on the possible occurrence of rebound effects is generally lacking (but, see Refs. [3,28]). In addition, the majority of these studies have only assessed effects on food intake; whereas possible effects on water intake have not been reported. Although food and water intake are not functionally independent (e.g., prandial drinking), the *potential* relevance of additional measurements of water intake is that it can help in assessing the extent of behavioral specificity of drug-induced hypo- or hyperphagia.

In order to overcome some of these shortcomings, the present study compared the effects of a number of compounds that preferentially activate different receptor subtypes of the 5-HT$_1$ and 5-HT$_2$ receptor families on food and water intake in a standardized, 12-h food access paradigm. The selected compounds and their respective 5-HT receptor subtype(s) preference were: ipsapirone (5-

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HT_{1A}; [4,27]), CP-94,253 ({3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-b]pyridine}, 5-HT_{1B}; [18]), TFMPP ({1-[3-(trifluoromethyl)phenyl]piperazine}; 5-$HT_{1B/2C}$; [8]), *m*-CPP ({1-[3-chlorophenyl]piperazine}; 5-$HT_{2C/1B}$; [9]), ORG 37864 ({(*S*)-3-[(2,3-dihydro-5-methoxy-1*H*-inden-4-yl)oxy]-pyrrolidine hydrochloride}; 5-HT_{2C}; [24]); BW 723C86 ({1-[5-(2-thienylmethoxy)-1*H*-3-indoyl]propan-2-amine hydrochloride}; 5-HT_{2B}; [13]) and DOI ({1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane}; 5-$HT_{2A/2C}$; [25]). The compounds were selected for their relatively high receptor selectivity, or because they have been found to affect ingestive behavior in different paradigms [10,11,13–15,17,19,23,24,29]. Because rats are nocturnal animals, all compounds were given immediately before the onset of the dark-phase period. In addition, nocturnal assessment may be more sensitive to the hypophagic effects of 5-HT receptor agonists, as intrahypothalamic injection of 5-HT is most effective in suppressing food intake at the onset of the dark cycle [20]. Food and water intake was measured after 1, 2, 6 and 12 h in order to establish the time-dependency of the effects and the possible occurrence of rebound effects.

1. Material and methods

1.1. Animals

Female Wistar rats were purchased from Harlan-Winkelmann (Hsd/Win: WU, Borchen, Germany). Body weight upon arrival at the laboratory was around 200 g. Rats were individually housed in Makrolon® type 3 cages (37×25×16 cm), located in the experimental room under a reversed 12 h light/12 h dark regime (lights on at 09:00 PM). Ambient temperature and relative humidity were maintained at 22±1°C and at 55±5%, respectively. The procedure followed the guidelines for the use of animals, as given by the German government, and was approved by the local authorities.

1.2. Apparatus and experimental setting

Ingestive behavior was assessed in the individual home cage of each animal. Cages were bedded with sawdust and a metal grid covered the sawdust, preventing the animals from pushing sawdust against the drinking spouts or into the food cups. Two small shafts were fitted on the metal grid to keep the food cup in place near the center of the back wall. The food cup consisted of a cylindrical metal container (5 cm height, 10 cm) with a removable metal lid containing a central round opening (4 cm) in order to minimize spilling. One bottle (300 ml, filled with plain tap water) was placed on top of the cage, near the front wall. The drinking spout, fitted with double stoppers, protruded about 1 cm into the cage. An in-house constructed mechanical device, interfaced with an IBM PC, was mounted on top of the cage. This device automatically regulated the daily 12-h access to the food cup filled with vitamin-enriched, powdered lab chow (Ssniff Spezialdiäten, Soest, Germany). All sessions were conducted during the dark-phase of the day/night cycle under red light conditions.

1.3. Procedure

A maximum of four independent groups ($n = 8–10$/group) were tested in parallel, always including a control group treated with the corresponding vehicle of the particular drug(s) tested. That is, the animals of the different test groups did not serve as their own controls. Previous studies initially included baseline measurements for all groups. Since generally no differences were found between baseline and vehicle treatment the baseline measurements were abandoned. Rats were repeatedly tested (in general, one test per week), with a maximum of four tests. The rats received a single injection of a particular dose of a given drug, or its vehicle, 15 min before the respective test session. Subsequently, the amount of food and water consumed was measured 1, 2, 6 and 12 h following drug treatment by weighing food cups and water bottles.

1.4. Drugs

Ipsapirone (BAY q 7821), ORG 37864 and BW 723C86 were synthesized by the Chemistry Departments of Bayer; Leverkusen and Wuppertal, Germany, or Bayer, West Haven, CT. *m*-CPP, TFMPP and DOI were obtained from RBI, Natick, MA. CP-94,253 was kindly donated by Pfizer (Karlsruhe, Germany). Compounds were dissolved in the following vehicles: ipsapirone in distilled water, CP-94,253 in 0.9% saline, 5% solutol® HS 15 (12-hydroxystearic-acid ethoxilate (BASF, Ludwigshafen, FRG) and 5% ethanol, BW 723C86 in 0.9% saline, $NaHCO_3$ and a few drops of lactic acid, and the other compounds in 0.9% saline. All compounds were injected IP in a volume of 1 ml/kg body weight.

1.5. Data analysis

For data presentation, amount of food and water consumed during the different time intervals (i.e., 0–1, 1–2, 2–6 and 6–12 h) were expressed in g/kg body weight and means and SEMs were calculated for all groups. For each compound, food and water intake were analyzed by a repeated measures-ANOVA with the factors TIME (four time intervals) and DOSE (four to five doses, dependent on the particular compound). The outcome of the analysis for the TIME factor was not presented in the Results section, as this factor was always affected in a statistically significant manner. Subsequently, one-way ANOVAs were performed for each time interval employing the factor DOSE and the dependent variables food and water intake. Post-hoc *t*-tests were done when appropriate and the outcomes of these

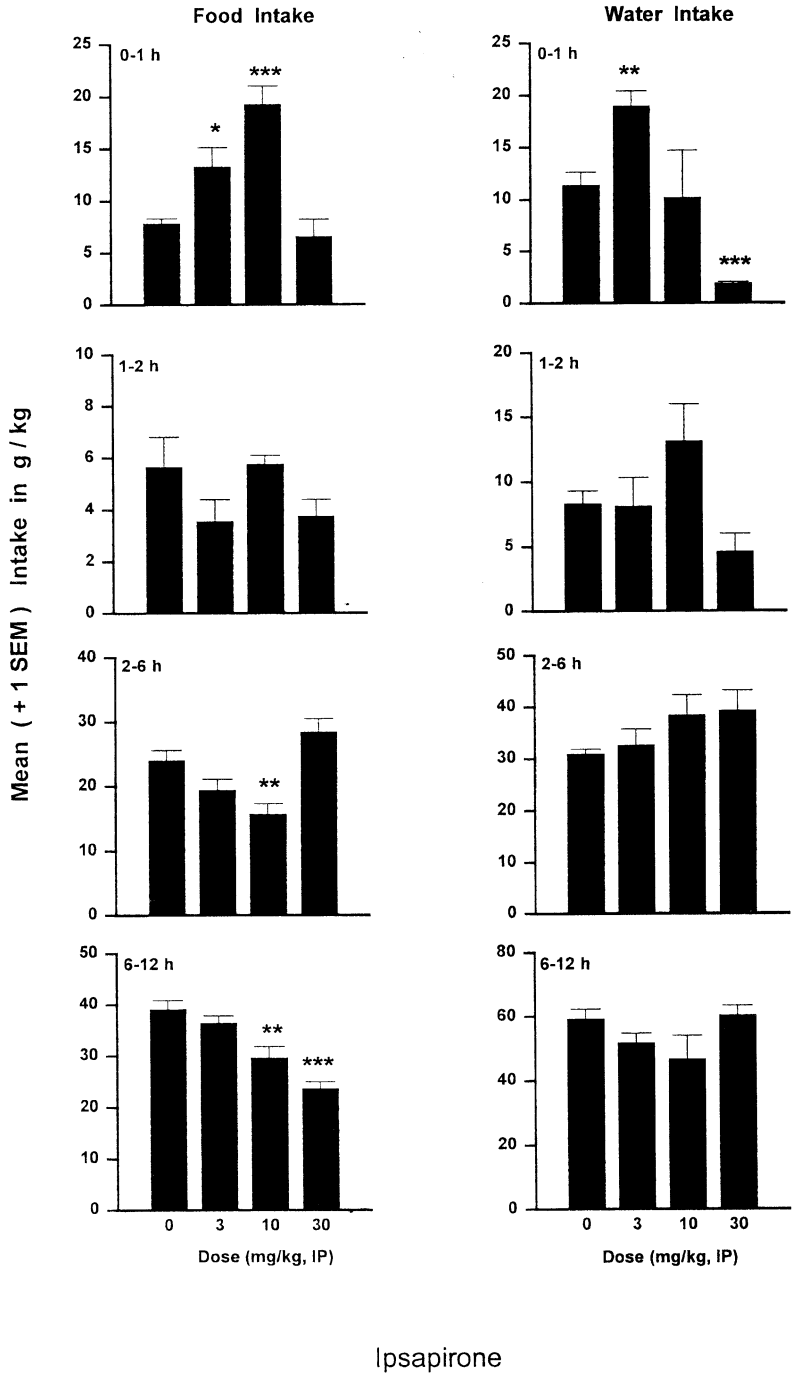

Fig. 1. Effects of the 5-HT_{1A} receptor agonist ipsapirone on food (left panels) and water (right panels) consumption during four consecutive time intervals in 12-h food-deprived rats (n = 10 per group). Data are presented as means ± SEM (g/kg). Results of two-way ANOVAs. Food intake, DOSE: $F(3,36) = 6.67$, $p < 0.001$ and DOSE × TIME: $F(9,108) = 10.51$, $p < 0.001$. DOSE × TIME for water intake: $F(9,108) = 4.03$, $p < 0.001$. Results of one-way ANOVAs. Food intake for 0–1 h: $F(3.36) = 12.27$, $p < 0.001$; 2–6 h: $F(3,36) = 7.73$, $p < 0.001$ and 6–12 h: $F(3,36) = 12.33$, $p < 0.001$. Water intake for the first hour: $F(3.36) = 7.23$, $p < 0.001$. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, as compared with the vehicle control group.

analyses were shown in the figures. In the case of TFMPP, *m*-CPP and ORG 37684, additional tests with particular doses were needed in order to obtain a complete dose–response curve. As there was in this case no evidence for a statistical difference between the first and second test with each particular vehicle, these data were pooled for data analysis and graphical presentation. The lowest dose that significantly ($p < 0.05$, as compared with vehicle) affected food or water intake was considered to be the minimal effective dose (MED). For estimation of ED_{50} values and

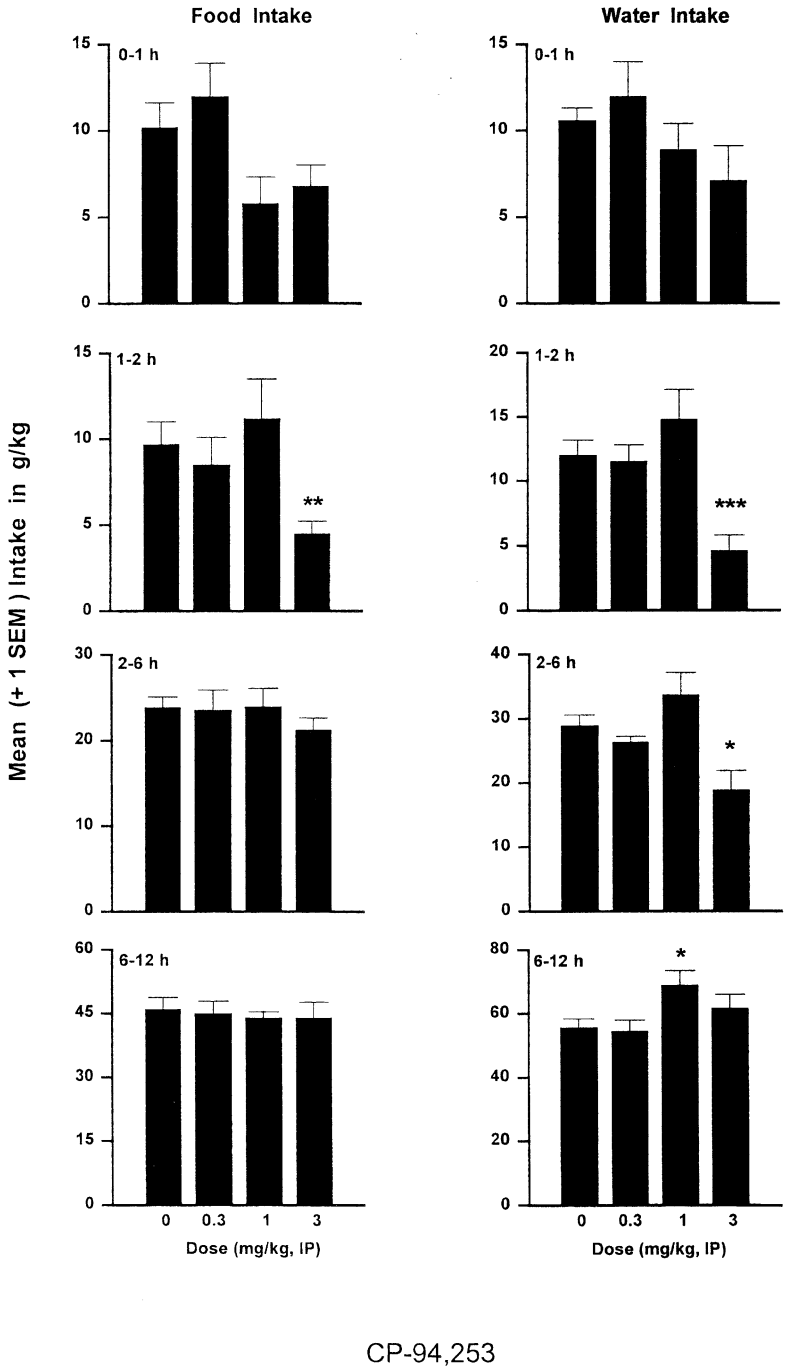

Fig. 2. Effects of the 5-HT_{1B} receptor agonist CP-94,253 on food (left panels) and water (right panels) consumption during four consecutive time intervals in 12-h food-deprived rats ($n = 8–10$ per group). Data are presented as means ± SEM (g/kg). Results of two-way ANOVAs. Food intake, DOSE: [$F(3,30) = 2.05$, $p = 0.13$]. Water intake, DOSE: $F(3,30) = 5.15$, $p < 0.01$ and DOSE × TIME: $F(9,90) = 2.52$, $p < 0.05$. Results of one-way ANOVAs. Food intake 0–1 h: $F(3,30) = 2.99$, $p < 0.05$ and 1–2 h: $F(3,30) = 2.95$, $p < 0.05$. Water intake 1–2 h: $F(3,30) = 6.80$, $p < 0.001$ and 2–6 h: $F(3,30) = 5.06$, $p < 0.01$. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, as compared with the vehicle control group.

the corresponding 95% confidence limits, data obtained with each dose of a particular test drug were expressed as percentage reduction as compared with vehicle control, and these percentages were transformed by log-probit analysis before submission to least-square analysis. ED_{50} values with non-overlapping 95% confidence limits were considered to be significantly different.

2. Results

Ipsapirone induced a biphasic effect on ingestive behavior, depending on dose and time interval (Fig. 1). Thus, during the first hour of food access, a marked hyperphagia occurred at 3 and 10 mg/kg, but not at 30 mg/kg; whereas, at later intervals, a decrease of food intake was observed at

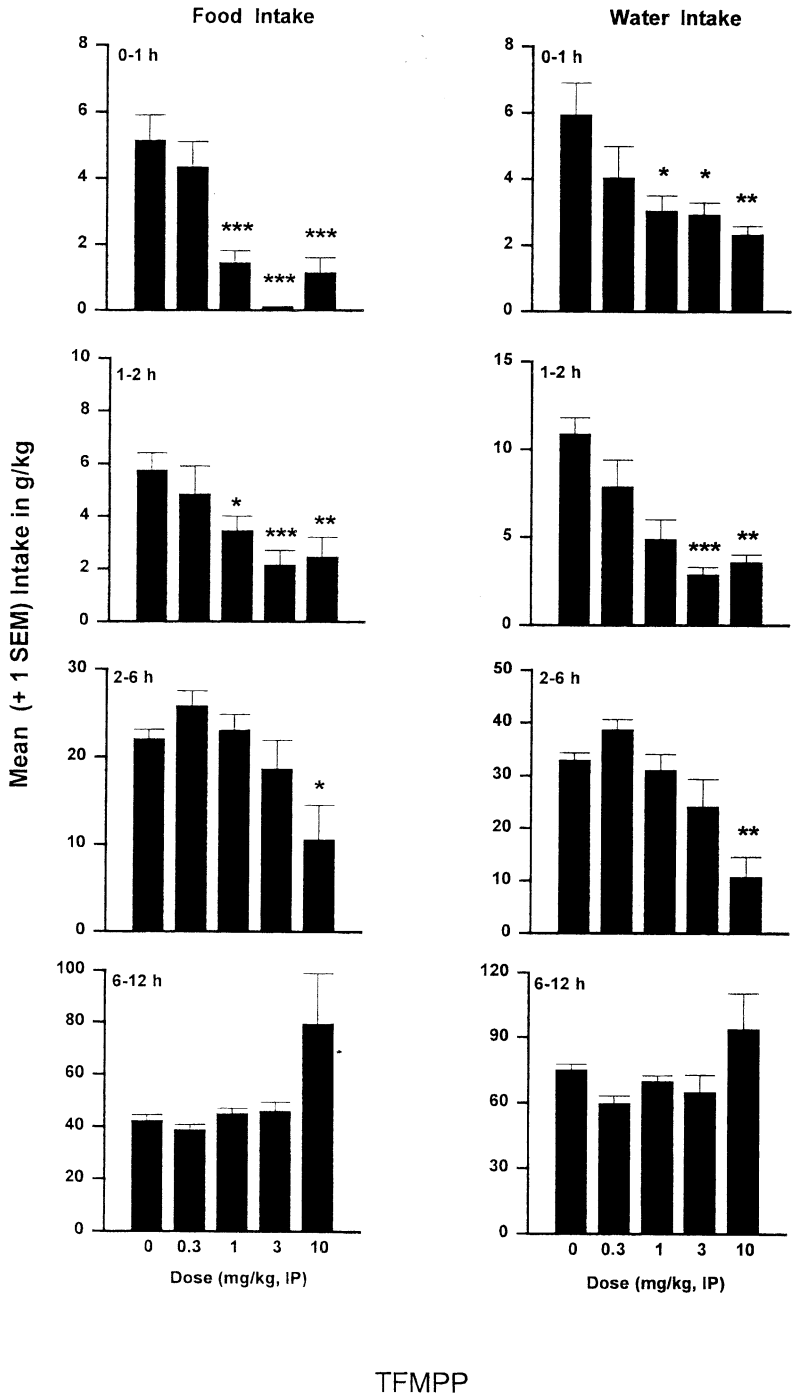

Fig. 3. Effects of the 5-$HT_{1B/2C}$ receptor agonist TFMPP on food (left panels) and water (right panels) consumption during four consecutive time intervals in 12-h food-deprived rats (n = 8–18 per group). Data are presented as means ± SEM (g/kg). Results of two-way ANOVAs. DOSE × TIME for food intake: $F(12,147) = 5.48$, $p < 0.001$. DOSE × TIME for water intake: $F(12,147) = 5.08$, $p < 0.001$. Results of one-way ANOVAs. Food intake for 0–1 h: $F(4,49) = 9.80$, $p < 0.001$; 1–2 h: $F(4,49) = 4.26$, $p < 0.01$; 2–6 h: $F(4,49) = 5.58$, $p < 0.001$ and 6–12 h: $F(4,49) = 4.30$, $p < 0.01$. Water intake for 0–1 h: $F(4,49) = 2.91$, $p < 0.05$; 1–2 h: $F(4,49) = 3.95$, $p < 0.01$; 2–6 h: $F(4,49) = 5.81$, $p < 0.001$ and 6–12 h: $F(4,49) = 2.86$, $p < 0.05$. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, as compared with the vehicle control group.

10 and 30 mg/kg. During the first hour, water intake was increased at 3 mg/kg, but decreased at 30 mg/kg.

CP-94,253 induced a relatively moderate and short-lasting reduction of food intake (Fig. 2); the effect being only present at the first and second time interval. Similar to its effect on food intake, CP-94,253 induced an equipotent reduction of water intake, but the effect appeared to be of longer duration, as it extended into the third time interval.

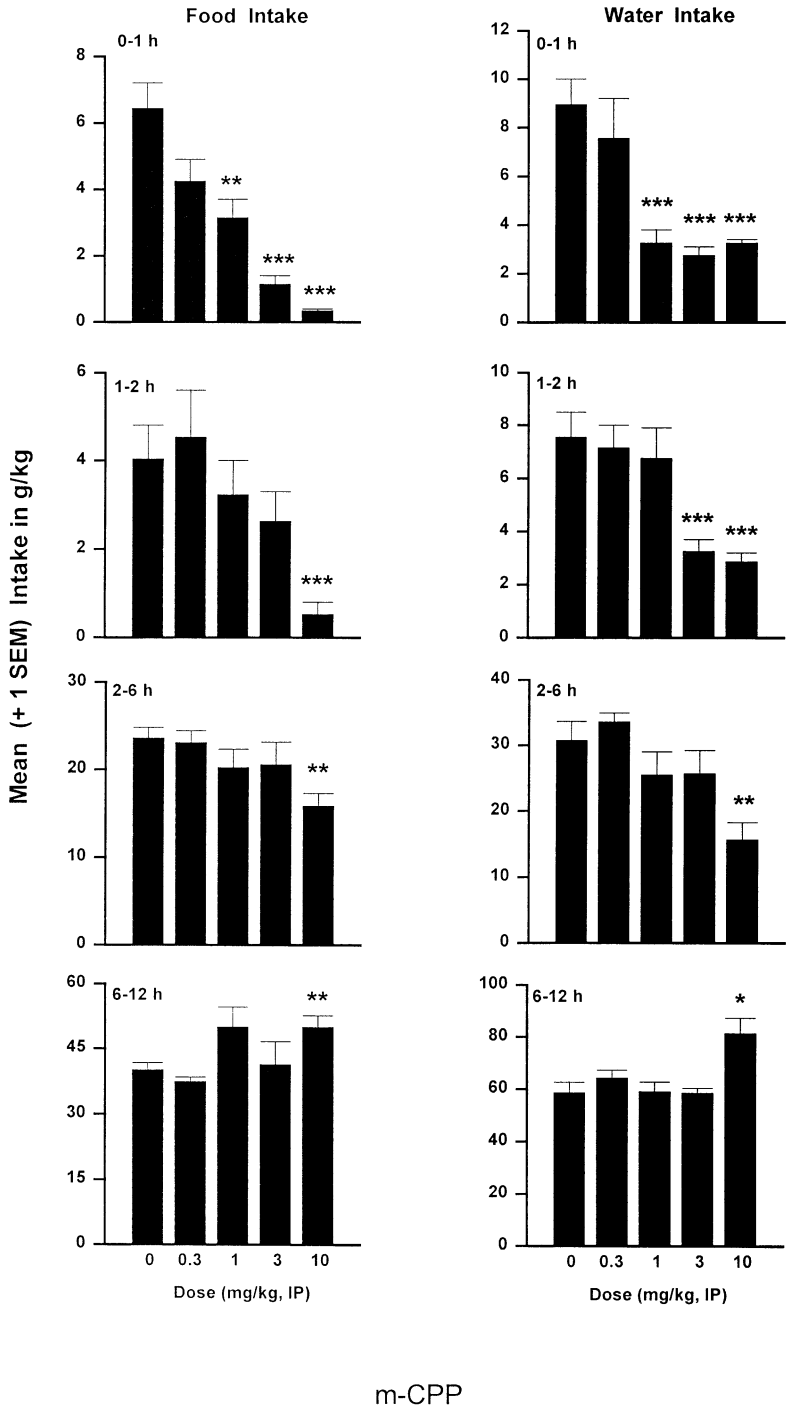

Fig. 4. Effects of the 5-$HT_{2C/1B}$ receptor agonist *m*-CPP on food (left panels) and water (right panels) consumption during four consecutive time intervals in 12-h food-deprived rats ($n = 8–19$ per group). Data are presented as means ± SEM (g/kg). Results of two-way ANOVAs. DOSE × TIME for food intake: $F(12,153) = 3.66$, $p < 0.001$. DOSE × TIME for water intake: $F(12,147) = 5.37$, $p < 0.001$. Results of one-way ANOVAs. Food intake for 0–1 h: $F(4,51) = 13.28$, $p < 0.001$; 1–2 h: $F(4,51) = 3.00$, $p < 0.05$; 2–6 h: $F(4,51) = 2.33$, $p = 0.068$ and 6–12 h: $F(4,51) = 2.61$, $p < 0.05$. Water intake for 0–1 h: $F(4,51) = 7.56$, $p < 0.001$; 1–2 h: $F(4,51) = 5.18$, $p < 0.001$; 2–6 h: $F(4,51) = 3.54$, $p < 0.05$ and 6–12 h: $F(4,51) = 3.53$, $p < 0.05$. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, as compared with the vehicle control group.

TFMPP induced a potent and pronounced reduction of ingestive behavior (Fig. 3). The effect was relatively long lasting, but tended to be biphasic in nature, equipotent, dose-dependent hypophagic and hypodipsic effect was observed at the first three time intervals; whereas an increase in ingestive behavior occurred during the last interval.

The profile of effects induced by *m*-CPP was very similar to that of TFMPP (Fig. 4). Again, the effects were biphasic in nature; with hypophagic and hypodipsic effects

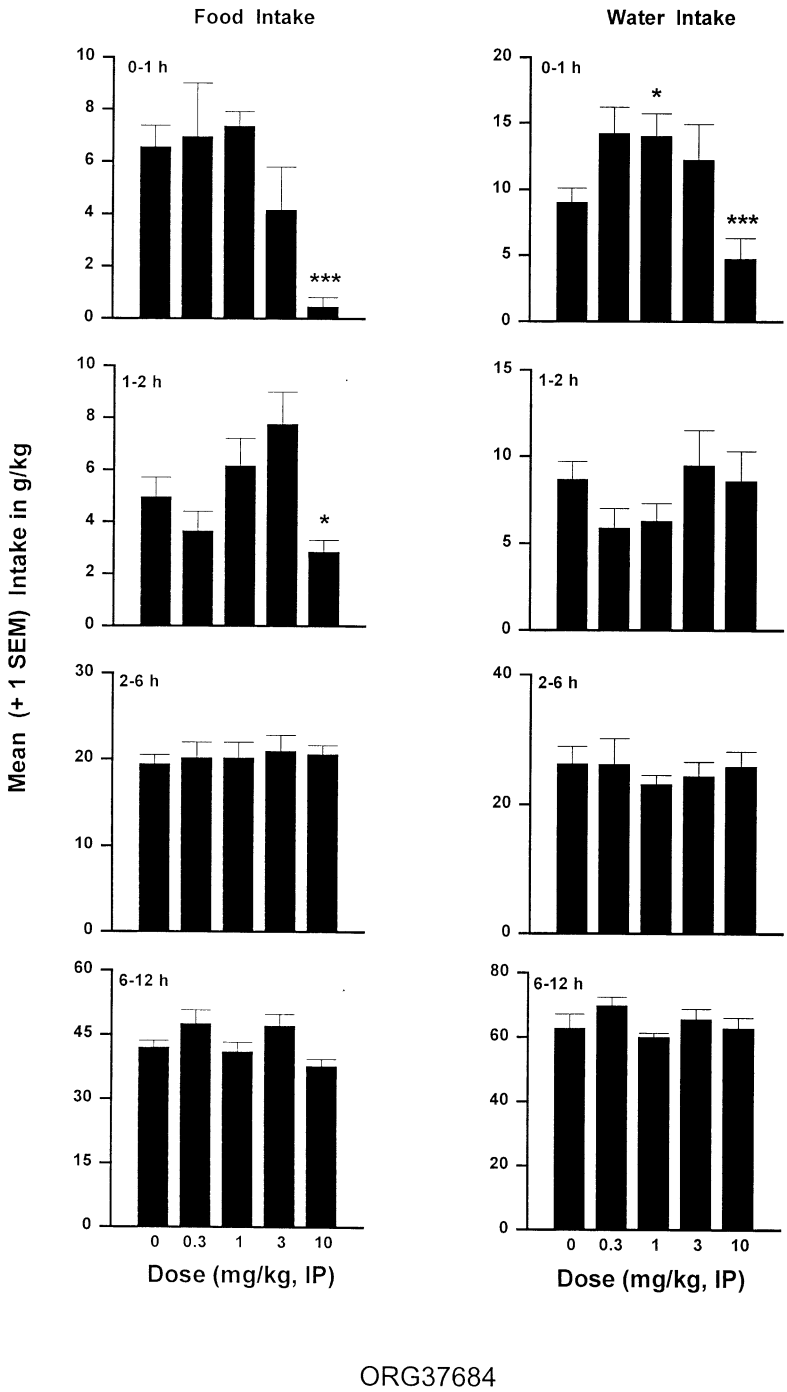

Fig. 5. Effects of the 5-HT_{2C} receptor agonist ORG 37684 on food (left panels) and water (right panels) consumption during four consecutive time intervals in 12-h food-deprived rats (n = 9–19 per group). Data are presented as means ± SEM (g/kg). Results of two-way ANOVA. Food intake, DOSE: $F(4,51) = 2.70$, $p < 0.05$; TIME × DOSE: $F(12,15) = 2.10$, $p < 0.05$. Results of one-way ANOVAs. Food intake for 0–1 h: $F(4,51) = 5.37$, $p < 0.001$ and 1–2 h: $F(4,51) = 3.67$, $p < 0.05$. Water intake for 0–1 h: $F(4,51) = 4.29$, $p < 0.01$. * $p < 0.05$ and * * * $p < 0.001$, as compared with the vehicle control group.

obtained during the first three intervals, and hyperphagic and hyperdipsic effects at the last interval.

ORG 37684 induced a pronounced, but relatively short-lasting reduction of food intake (Fig. 5). Water intake was also affected by ORG 37684, but the effect was only observed during the first interval. Moreover, effects on water intake were biphasic, with an increase in consumption at low to moderate doses and a decrease at the highest dose.

BW 723C86 induced a pronounced decrease of food intake (Fig. 6). The hypophagic effect was long lasting, as it

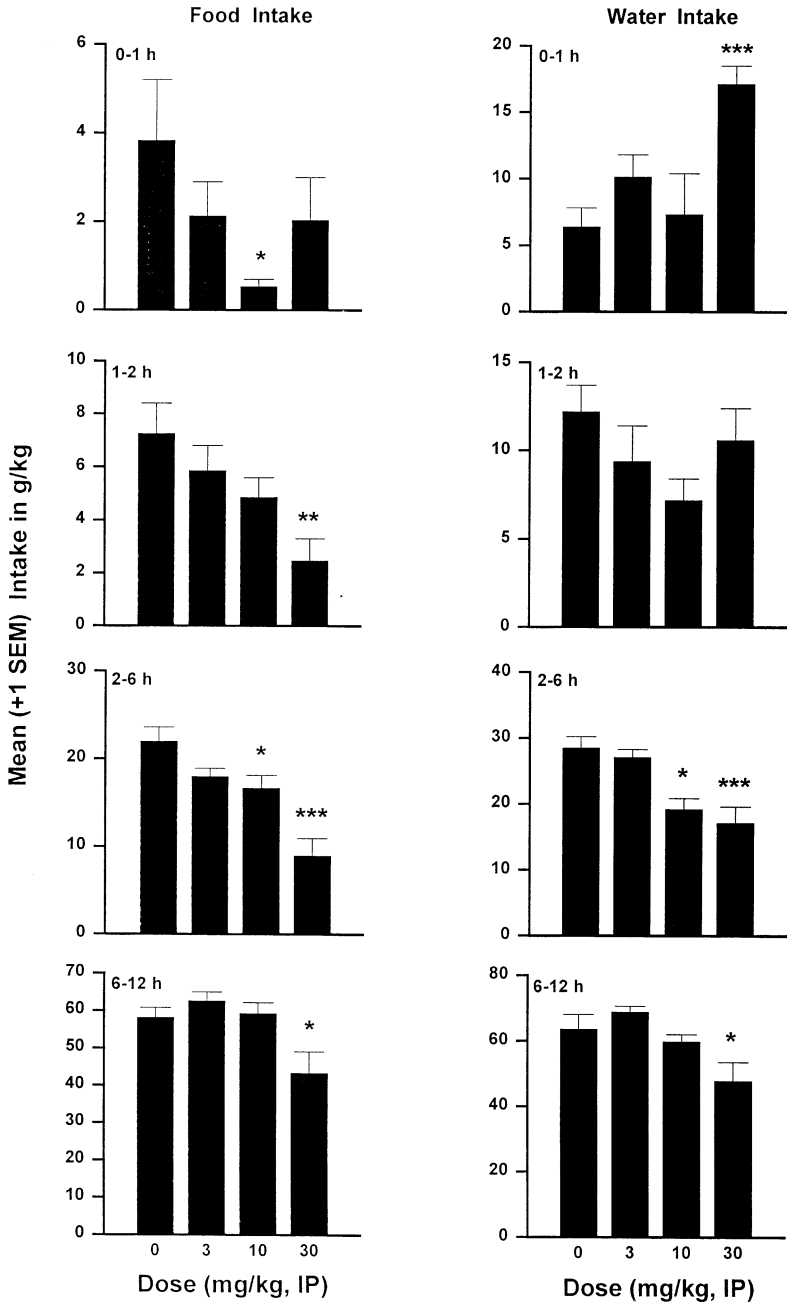

Fig. 6. Effects of the 5-HT_{2B} receptor agonist BW 723C86 on food (left panels) and water (right panels) consumption during four consecutive time intervals in 12-h food-deprived rats (n = 10 per group). Data are presented as means ± SEM (g/kg). Results of two-way ANOVA. Food intake, DOSE: $F(3,36) = 6.18$, $p < 0.01$ and TIME × DOSE: $F(9,108) = 4.36$, $p < 0.001$. Water intake, DOSE: $F(3,36) = 2.92$, $p < 0.05$ and TIME × DOSE: $F(9,108) = 5.50$, $p < 0.001$. Results of one-way ANOVAs. Food intake for 6–12 h: $F(3,36) = 4.60$, $p < 0.01$. Water intake for 0–1 h: $F(3.36) = 5.02$, $p < 0.01$; 2–6 h: $F(3,36) = 7.59$, $p < 0.001$ and 6–12 h: $F(3.36) = 4.24$, $p < 0.05$. * $p < 0.05$, * * $p < 0.01$ and * * * $p < 0.001$, as compared with the vehicle control group.

extended into the last interval. The compound had also a pronounced and long-lasting effect on water intake, but the effect was biphasic in nature. Thus, a hyperdipsic effect was obtained during the first interval followed by a hypodipsic effect at later intervals.

DOI induced a pronounced and dose-dependent hypophagia but the effect was short lasting, as it was only obtained during the first interval (Fig. 7). In addition, the compound tended to reduce water intake, but the effect appeared to be less potent.

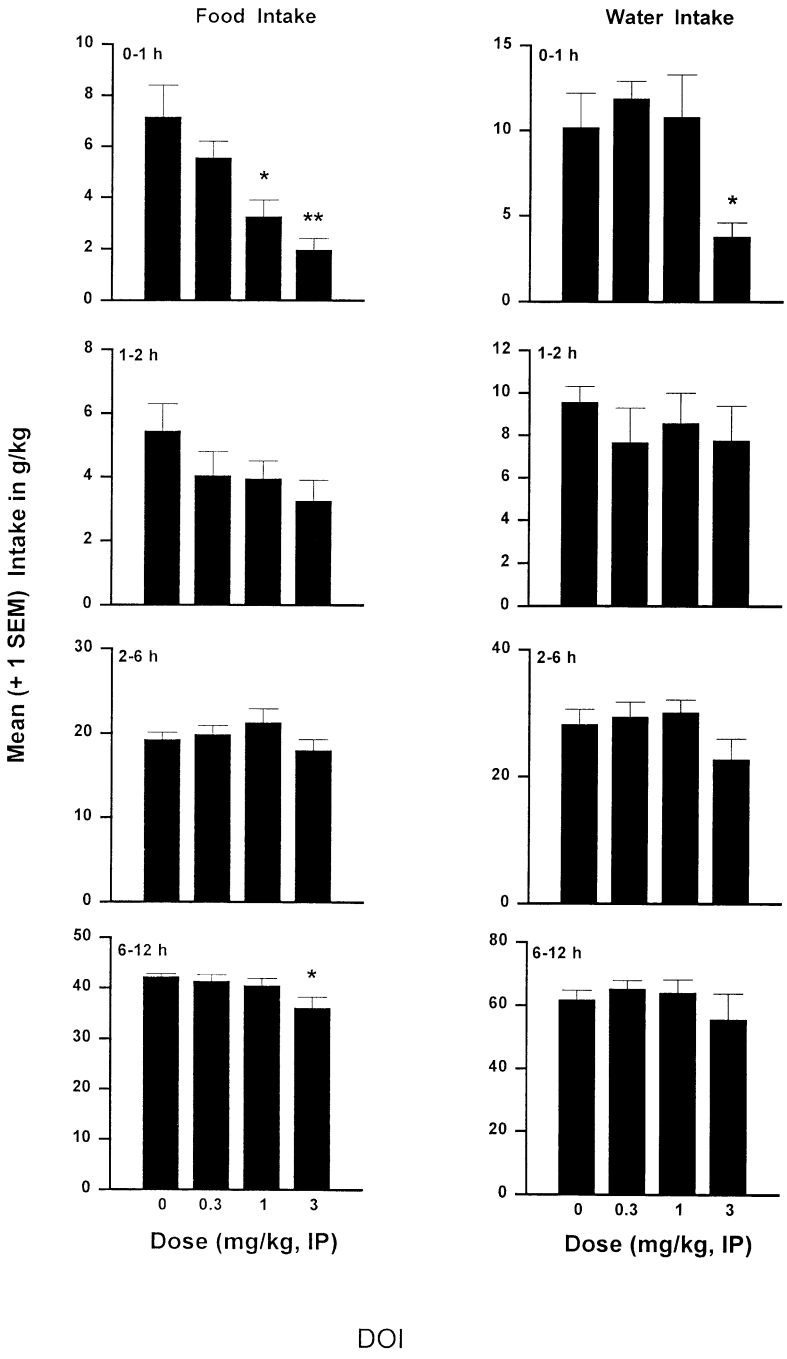

Fig. 7. Effects of the 5-$HT_{2A/2C}$ receptor agonist DOI on food (left panels) and water (right panels) consumption during four consecutive time intervals in 12-h food-deprived rats (n = 10 per group). Data are presented as means ± SEM (g/kg). Results of two-way ANOVA. Food intake, DOSE: $F(3,36) = 5.79$, $p < 0.01$. Water intake, DOSE: $F(3,36) = 2.40$, $p = 0.084$. Results of one-way ANOVA. Food intake 0–1 h: $F(3,36) = 7.80$, $p < 0.001$. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, as compared with the vehicle control group.

CP-94,253, ORG 37684 and BW723C86 reduced total 12-h food intake in this order of potency (Fig. 8). The rebound feeding response in *m*-CPP and TFMPP-treated rats completely reversed the hypophagic response in the early time intervals. Twelve-hour water intake was only affected by CP-94,253 (Fig. 9).

In order to further assess the relative order of the compounds, dose–response curves were established for the hypophagic and hypodipsic effects (calculated as percentage reduction in intake, as compared with vehicle treatment; Fig. 10), and MED and ED_{50} values were estimated as calculated for the first hour of the test session

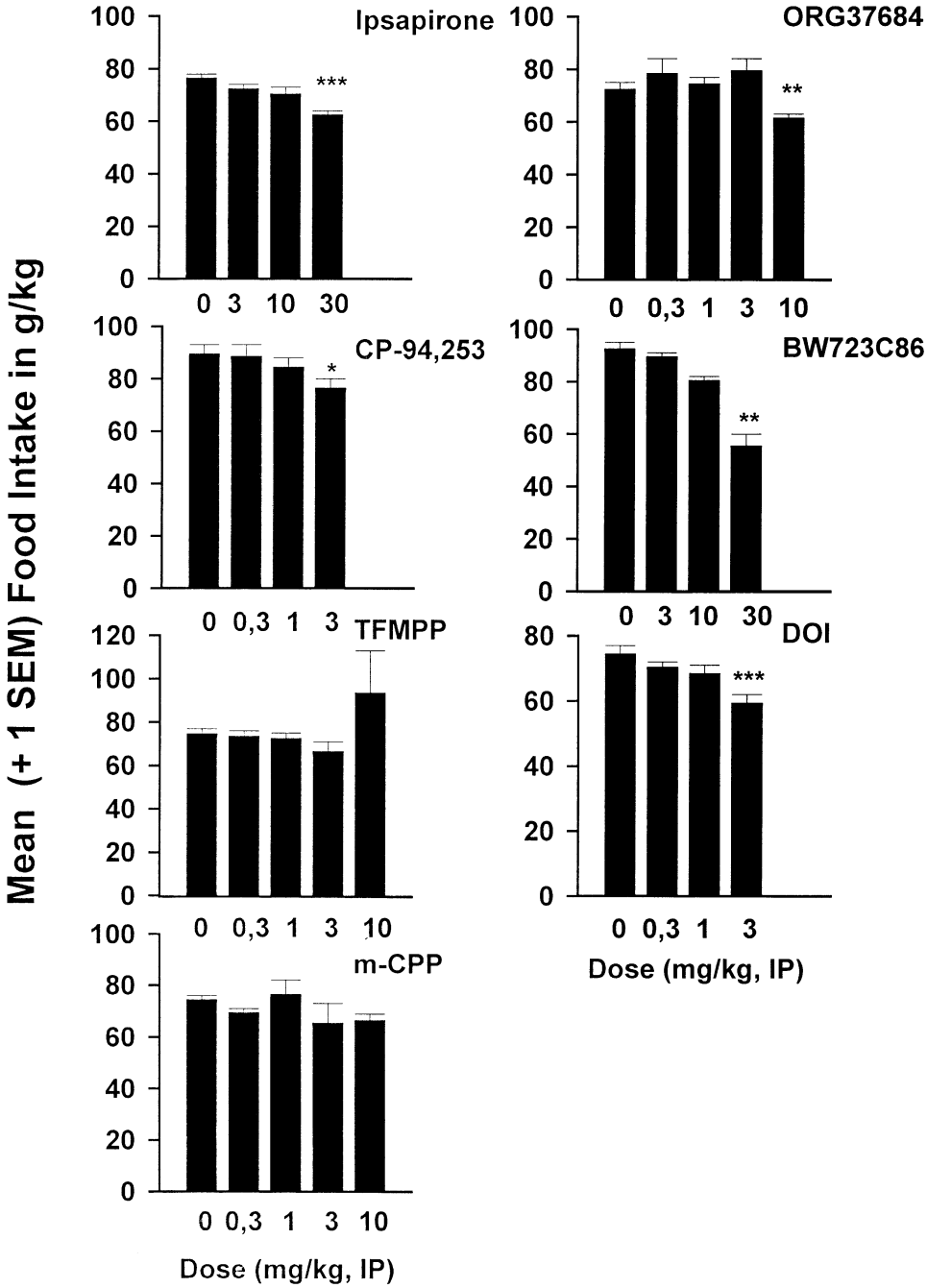

Fig. 8. Comparison of the effects of 5-HT receptor agonists with differential selectivity for the different subtypes of 5-HT_1 and 5-HT_2 receptors on food intake during the 12 h following administration in 12-h food-deprived rats (n = 8–19 per group). Data are presented as means ± SEM (g/kg). Results of one-way ANOVAs for the factor DOSE. Ipsapirone: $F(3,36) = 6.67$, $p < 0.001$; ORG 37684: $F(4,51) = 4,29$, $p < 0.01$ and DOI $F(3,36) = 5.75$, $p < 0.01$. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, as compared with the vehicle control group.

(Table 1). Comparison of the relative potencies was restricted to the first time intervals because the majority of compounds showed only effects at these intervals. With respect to food intake, the following order of potency was obtained: TFMPP = *m*-CPP ≥ DOI > CP-94,253 ≥ BW 723C86>ORG 37684>ipsapirone. In general, water intake was less potently affected, but the order of potency remained relatively similar: *m*-CPP ≥ TFMPP ≥ DOI ≥ CP-94,253>ipsapirone>BW 723C86.

3. Discussion

The present study compared the effects of a number of 5-HT receptor agonists with different degrees of selectivity for the receptor subtypes of the 5-HT_1 and 5-HT_2 receptor families, on food and water intake in a standardized 12-h, dark-phase paradigm using female rats. The compounds were selected for their particular receptor selectivity, or their reported efficacy on ingestive behavior of animals.

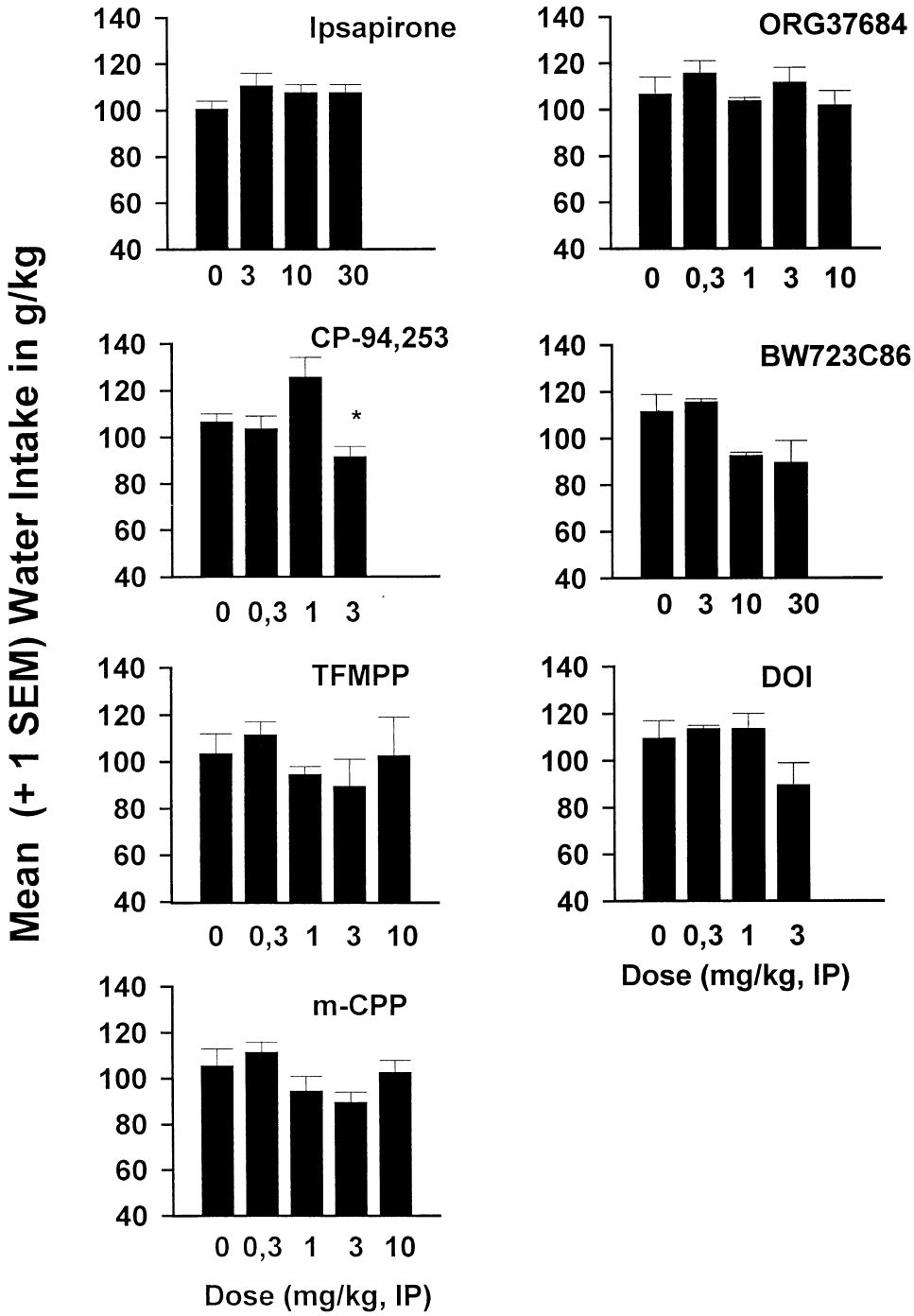

Fig. 9. Comparison of the effects of 5-HT receptor agonists with differential selectivity for the different subtypes of 5-HT_1 and 5-HT_2 receptors on water intake during the 12 h following administration in 12-h food-deprived rats (n = 8–19 per group). Data are presented as means ± SEM (g/kg). Results of one-way ANOVA for the effect of CP-94,253: DOSE, $F(3,30) = 5.15$, $p < 0.01$. * $p < 0.05$ as compared with the vehicle control group.

The selective 5-HT_{1A} receptor agonist ipsapirone induced a biphasic effect on food intake, consistent with the profile previously obtained with this class of compounds (e.g., Refs. [10,29]). It is likely that the biphasic effects of ipsapirone on ingestive behavior involve different mechanisms. Thus, it has been proposed that hyperphagia induced by 5-HT_{1A} receptor agonists results from stimulation of somatodendritic 5-HT_{1A} autoreceptors [10]. The progressive loss of hyperphagia and the occurrence of hypophagia at later time intervals or at higher doses may be the result of a compensatory process (rebound hypophagia). Similar to its effects on food intake, ipsapirone produced biphasic effects on water intake, but the latter effects could be dissociated from the former effects, as they were restricted

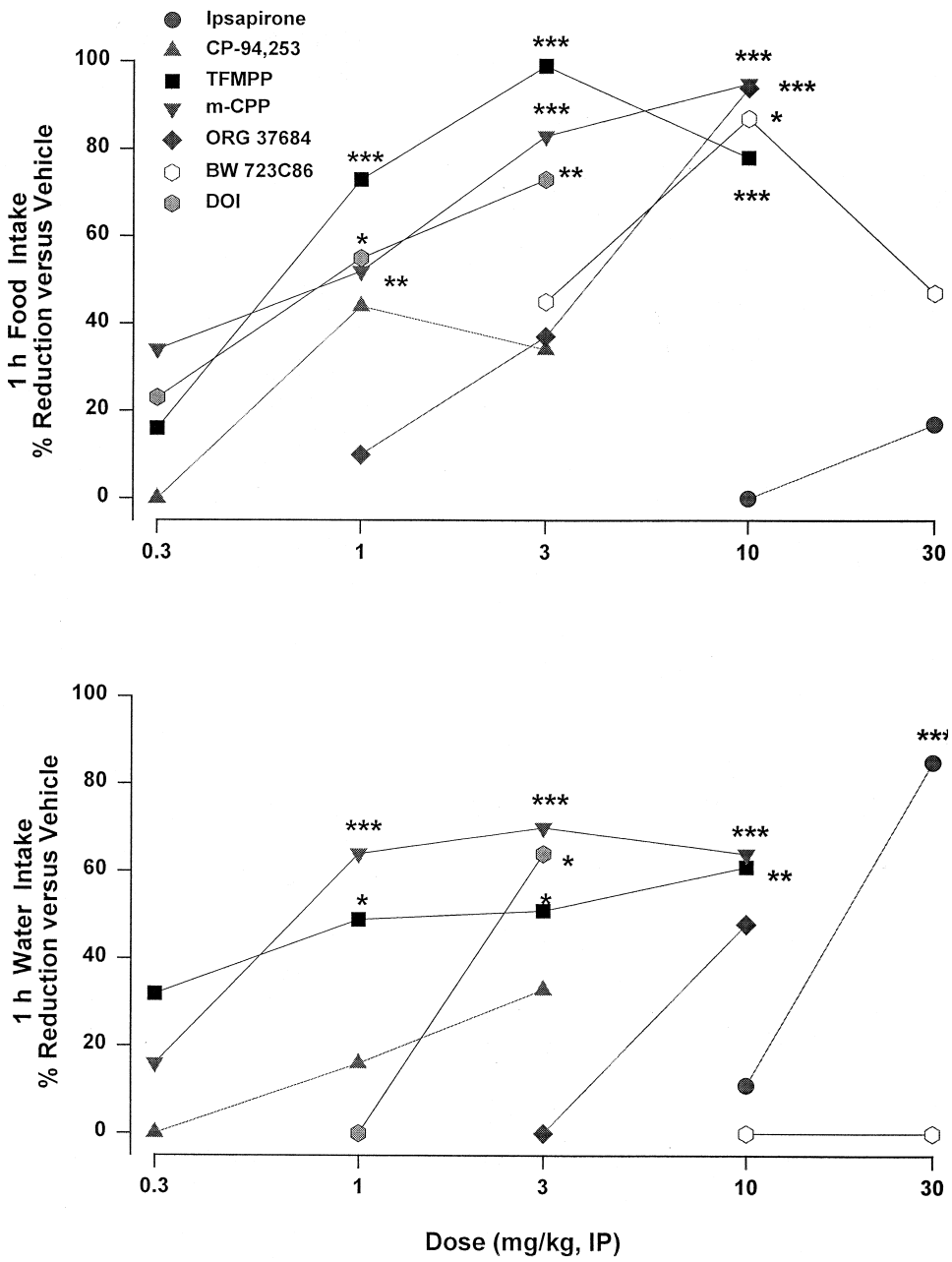

Fig. 10. Comparison of the effects of 5-HT receptor agonists with differential selectivity for the different subtypes of 5-HT_1 and 5-HT_2 receptors on food (upper panel) and water (lower panel) intake during the first hour following administration in 12-h food-deprived rats ($n = 8–19$ per group). Data are presented as percentages from vehicle values. $*p<0.05$, $**p<0.01$ and $***p<0.001$, as compared with the vehicle control group.

to the first hour and appeared to be only dependent on the dose of the compound.

The reduction of food intake by the 5-HT_{1B} receptor agonist CP-94,253 [18] suggests that activation of 5-HT_{1B} receptors is sufficient to induce hypophagia (e.g., Refs. [11,22,26]). In particular, it appears that 5-HT_{1B} receptor activation results in a general inhibition of ingestive behavior, as hypophagia coincided with hypodipsia (see also: Ref. [19]). The effect of CP-94,253 on food intake was relatively mild and short lasting, although complete suppression feeding behavior was obtained at a dose of 10 mg/kg (Schreiber et al., manuscript in preparation). The magnitude of its hypophagic effect was comparable with those obtained in studies using the behavioral satiety sequence paradigm [11,19]. Although CP-94,253 markedly suppressed 24-h diurnal feeding, this occurred after oral administration of more than 10-fold higher doses [18].

Analogous to the results obtained with CP-94,253, the finding that the preferential 5-HT_{2C} receptor agonist ORG 37684 [21] reduced food intake in a marked, but relatively

Table 1
Estimated potency values for the hypophagic and hypodipsic effects of 5-$HT_{1/2}$ receptor agonists as calculated for the first hour of the 12-h access period

Compound	5-HT receptor[a]	Doses[b]		Food (0–1 h)	Water (0–1 h)
Ipsapirone	5-HT_{1A}	3–30	MED	>30	30
			ED_{50}	>30	16.93 (10.66–26.89)
CP-94,253	5-HT_{1B}	0.3–3	MED	$\geq$ 3	>3
			ED_{30}	3.21 (1.24–8.28)	>3
TFMPP	5-$HT_{1B/2C}$	0.3–10	MED	1	1
			ED_{50}	0.62 (0.34–1.14)	2.14 (0.19–23.70)
m-CPP	5-$HT_{2C/1B}$	0.3–10	MED	1	1
			ED_{50}	0.68 (0.31–1.53)	1.02 (0.47–2.20)
ORG 37684	5-HT_{2C}	0.3–10	MED	10	10
			ED_{50}	3.87 (2.30–6.49)	>10
BW 723C86	5-HT_{2B}	3–30	MED	10	>30
			ED_{50}	3.39 (1.47–7.82)	>30
DOI	5-$HT_{2A/2C}$	0.3–3	MED	1	3
			ED_{50}	1.41 (0.50–3.96)	2.62 (1.83–3.75)

[a] 5-HT receptor subtype(s) at which the compound shows highest binding affinity.
[b] Tested dose range in mg/kg IP, ED_{50} (95% confidence limits) in mg/kg IP, MED in mg/kg IP.

short-lasting manner, is consistent with the suggestion that stimulation of 5-HT_{2C} receptors is sufficient to induce hypophagia (e.g., Ref. [16]). But, in contrast to 5-HT_{1B} receptor activation, stimulation of 5-HT_{2C} receptors may lead to a more specific effect on food intake, as the latter effect could be dissociated in dose and time from effects on water intake.

The mixed 5-$HT_{1B/2C}$ receptor agonists *m*-CPP and TFMPP induced a more potent, pronounced and longer-acting hypophagic effect than CP-94,253 and ORG 37684. Their effects were comparable in potency and magnitude as described in the numerous reports published previously (e.g., Refs. [14,15,23]; for review, see Ref. [6]). As TFMPP and *m*-CPP bind with similar affinity to 5-HT_{1B} and 5-HT_{2C} receptors, and both receptors appear to play a role in the control of food intake, the relative contribution of these receptors to the hypophagic effects of these compounds is still unclear (for discussion, see Ref. [6]). In addition, TFMPP and *m*-CPP possess moderate affinity for 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A} and 5-HT_{2B} receptors [2], and as some of these receptors are also thought to be involved in the control of ingestive behavior, it cannot be excluded that they contribute to the mechanism underlying the hypophagic effects of these compounds.

The relatively potent and pronounced hypophagic effect of *m*-CPP or TFMPP may be a consequence of simultaneous activation of 5-HT_{1B} *and* 5-HT_{2C} receptors, resulting in a synergistic effect on ingestive behavior (but, see Refs. [15,16]). Simansky [26] has suggested that 5-HT_{1B} receptors are more involved in the regulation of meal size; whereas 5-HT_{2C} receptors are more involved in the regulation of eating rate, and therefore it can be expected that a compound that activates both receptor subtypes will induce a more dramatic effect on ingestive behavior. Indeed, *m*-CPP was reported to produce full expression of satiety [3], whereas CP-94,253 reduced meal size but not local eating rate [11].

In general, the effects obtained with the mixed 5-$HT_{2A/2C}$ receptor agonist DOI were comparable in potency and magnitude with those previously reported in a variety of paradigms [1,12,17,24]. Results from meal pattern studies suggest that the hypophagic effects of DOI are behaviorally nonspecific [12,17], and results from antagonism studies support involvement of 5-HT_{2A} receptors [24]. However, it was found in the present study that the compound affected water intake less potently than food intake, suggesting that the effects on ingestive behavior are not entirely nonspecific. DOI possesses, besides 5-HT_{2A} receptor affinity, high affinity for 5-HT_{2C} receptors and moderate affinity for 5-HT_{2B} receptors, and it is unclear to what extent activation of these latter receptors contributes to the hypophagic effects of DOI. Indeed, using the same dark-phase feeding procedure, it was found that pretreatment with a highly selective 5-HT_{2A} antagonist failed to completely block the hypophagic effects of DOI [6].

BW 723C86, a relatively selective 5-HT_{2B} receptor agonist, induced a marked and long-lasting decrease of food intake, which could be partially dissociated from a short-lasting increase in water intake. These results are in apparent contrast with the short-lasting increase of food intake previously reported at a similar dose range (administered SC; [13]). However, in the latter study a marked (>50%), but statistically non-significant, reduction of food intake was found at relatively low doses. Although we have no clear explanation for this discrepancy, it is possible that the particular experimental conditions (e.g., nocturnal feeding in 12-h food-deprived female rats versus diurnal feeding in non-deprived male rats) may affect the outcome of 5-HT_{2B} receptor stimulation on ingestive behavior. Since the hypophagic effects occurred at relatively high doses, it cannot be ruled out that they are partly the result of activation of 5-HT_{2A} and/or 5-HT_{2C} receptors, or due to behavioral side-effects. Interestingly, we found recently that the compound was also able to induce a very long-lasting (1

week) reduction of food intake, as assessed in an operant paradigm [5].

In contrast to the other compounds tested, the hypophagic effects obtained with TFMPP and *m*-CPP were followed by rebound hyperphagia. Although most previous studies did not report rebound hyperphagia (presumably because access periods of insufficient duration were used), a similar finding was reported with *m*-CPP [3,28]. The occurrence of rebound hyperphagia may be related to the relatively short half-life time of *m*-CPP (1 h in the brain; [9]), as it was not observed following sustained release of a comparable dose [7]. Alternatively, it may be related to the magnitude of the hypophagic effect. Thus, rebound hyperphagia was only obtained for those compounds that induced a very marked initial hypophagic effect (>80% reduction of food intake during the first 2 h of the access period).

In conclusion, the present study demonstrated that 5-HT receptor agonists with different degrees of selectivity for the receptor subtypes of the 5-HT_1 and 5-HT_2 receptor families affect ingestive behavior differentially. Thus, although all compounds were able to reduce food intake, the dose and time dependency of this effect varied among the compounds tested and some compounds induced biphasic effects, either as a function of dose, or a function of time (or both). Furthermore, although drug-induced hypophagia coincided to a certain extent with hypodipsia, the effects on water intake were generally less potent and pronounced, and in some instances could be completely dissociated from effects on food intake. Based on the presumed binding affinities and the degree of selectivity for the diverse 5-HT receptor subtypes, it appears that compounds with a less selective binding profile, such as *m*-CPP and TFMPP, induce a more potent effect on ingestive behavior, as compared with compounds that show a more selective binding profile. On the other hand, some of the latter compounds, such as the 5-HT_{2C} receptor agonist ORG 37684, the 5-HT_{1B} receptor agonist CP-94,253 and the 5-HT_{2B} receptor agonist BW 723C86, were able to induce a considerable level of hypophagia, which, in contrast to *m*-CPP and TFMPP, was not followed by rebound hyperphagia within the 12-h observation period. Appropriate antagonism studies with selective receptor antagonists are needed to ascertain the proposed role of particular 5-HT receptor subtypes in the control of ingestive behavior.

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