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Behavioural evidence for functional interactions between 5-HT-receptor subtypes in rats and mice

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1 Different 5-hydroxytryptamine (5-HT) receptor subtypes mediate different behavioural responses. Compounds acting at more than one 5-HT receptor exert behavioural effects which may be the result of response competition or a specific interaction between pathways within the CNS. Therefore the mutual interaction between different 5-HT receptor subtypes was studied.

2 Hypothermia and hypoactivity in mice induced by the 5-HT_{1A}-agonist 8-hydroxy-dipropylamino-tetralin (8-OH-DPAT) could be attenuated by the preferential 5-HT_{1C}-agonists MK 212, 1-(meta-chlorophenyl)-piperazine (mCPP) and m-trifluoromethyl phenyl piperazine (TFMPP), and by the mixed 5-$HT_{2/1C}$-agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). The mixed 5-$HT_{1A/1B}$-agonist CGS 12066B at 10 mg kg^{-1} potentiated hypothermia and had no effect on hypoactivity.

3 Forepaw treading in rats induced by the 5-HT_{1A}-agonist 8-OH-DPAT was attenuated by the 5-HT_{1C}-agonists MK 212 and mCPP. The 5-HT_{1C}-agonist TFMPP had a bimodal effect: at low doses (< 1 mg kg^{-1}) it potentiated, and at higher doses (> 2.2 mg kg^{-1}) it attenuated forepaw treading. the mixed 5-$HT_{2/1C}$-agonist DOI produced 5-HT_2-related behaviours and potentiated 8-OH-DPAT-induced forepaw treading. This indicates an attenuating effect of 5-HT_{1C}-receptor activation and a potentiating effect of 5-HT_2-receptor activation. CGS 12066B had no effect in this respect.

4 Head shakes in rats induced by DOI could be attenuated by 8-OH-DPAT, TFMPP, mCPP and MK 212. The ID_{50}s were 0.03, 0.7, 0.1 and 2 mg kg^{-1}, respectively. This suggests that a 5-HT_2-receptor-mediated effect may be attenuated by activation of 5-HT_{1A}- or 5-HT_{1C}-receptors. CGS 12066B attenuated the head shake response but only at 10 mg kg^{-1}.

5 The results suggest that interactions exist between the different 5-HT receptor subtype-mediated events. Therefore, care is needed in drawing conclusions from functional measurements when compounds have more or less equal affinities for more than one 5-HT-receptor subtype.

Introduction

Since Hess & Doepfner (1961) and later Grahame-Smith (1971) described the 5-hydroxytryptamine (5-HT) syndrome, consisting of forepaw treading, head weaving, flat body posture, head shakes, hind limb abduction and tremors, following injections of 5-hydroxytryptamine plus a monoamine oxidase inhibitor, this syndrome and elements of it have been seen after injection of a variety of 5-HT receptor agonists. The synthesis of 5-HT receptor subtype-selective agonists and/or antagonists has made it possible to study the functions of the different 5-HT receptors, and to ascribe some components of the 5-HT syndrome to selective activation of one of these 5-HT receptor subtypes.

At present there is evidence to suggest that drug-induced head shakes are the result of 5-HT_2-receptor activation (Yap & Taylor, 1983) and that stimulation of the presynaptic 5-HT_{1A}-receptor results in hypothermia (Goodwin *et al.*, 1985; Gudelsky *et al.*, 1986). Activation of the postsynaptic 5-HT_{1A}-receptor induces forepaw treading (Tricklebank *et al.*, 1984). Recently, we suggested that selective activation of the 5-HT_{1A}-receptors, possibly presynaptic, induces lower lip retraction and that the induction of penile erections is the result of 5-HT_{1C}-receptor activation (Berendsen *et al.*, 1989a; 1990).

However, compounds with activity at more than one subtype of 5-HT receptor do not always induce the behaviour thought to be the result of activation of those receptors. For example, the compound RU 24969, (5-methoxy-3(1,2,3,6-tetrahydropiridin-4-yl) 1H indole) which binds to 5-HT_{1A}- and 5-HT_{1B}-receptors (Hoyer, 1988a) only induces the 5-HT_{1A}-related lower lip retraction. 5-Methoxy-N,N-dimethyl tryptamine (5-MeODMT), a compound that binds with high affinity to 5-HT_{1A}-, 5-HT_{1C}- and, with lower affinity to 5-HT_2-receptors (Hoyer, 1988b; Titeler *et al.*, 1988) only induces lower lip retraction after blockade of the 5-HT_{1C} receptors (Berendsen *et al.*, 1989a). (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), which binds to both 5-HT_2- and 5-HT_{1C}-receptors (Hoyer, 1988b; Titeler *et al.*, 1988), induces the 5-HT_2-related head shakes, but not 5-HT_{1C}-related penile erections. However, in rats pretreated with a 5-HT_2 antagonist, this compound induces penile erections while the head shakes disappear. It has also been shown that the potency to induce penile erections by compounds that have affinity for 5-HT_{1C}- and 5-HT_2-receptors, is dependent on their 5-HT_{1C}/5-HT_2 affinity ratio (Berendsen *et al.*, 1990). All this points to a functional interaction between the different receptor subtype-induced behaviours, in such a way that induction of a particular behaviour by activation of a certain receptor subtype can prevent or mask the behaviour induced by activation of another 5-HT-receptor subtype. Functional interactions in the penile erection test (5-HT_{1C}-receptor-induced) and in the lower lip retraction test (5-HT_{1A}-receptor-induced) have been described before (Berendsen *et al.*, 1989a; 1990). We have now studied more systematically how activation of one 5-HT-receptor subtype influences the expression of the behaviour induced by activation of another.

In this study, we have measured the 5-HT_{1A}-receptor- mediated forepaw treading and the 5-HT_2-receptor mediated head shake response in rats. Locomotor activity and body temperature in mice were included to show that the functional interaction of 5-HT-receptor subtypes also takes place in this species.

Animals

Mice Naïve male mice (Crl: CD-1(ICR)BR, from Charles River, Germany) weighing 24–26 g were used. The mice were housed in macrolon cages (40 × 23 × 15 cm) 20 animals per cage, under a controlled 12 h light-dark cycle (light on 6 h 00 min) and were allowed free access to standard food pellets and tap water.

Rats Naïve male Wistar rats (Cpb:WU, Harlan Sprague-Dawley, Zeist, The Netherlands) weighing 200–250 g were used. The animals were housed in white PVC cages (40 × 40 × 18 cm) with a wire mesh lid, 5 animals per cage, under controlled 12 h light-dark cycle (lights on 6 h 00 min) and were allowed free access to standard food pellets and tap water.

Procedures

Locomotor activity in mice The locomotor activity of the mice was measured in 20 small photocell cages (11 × 11 × 16 cm) (four rows of 5 connected cages). The lid and both side walls of the cages were made of grey PVC, the front and back walls of clear perspex and the base was a stainless steel grid floor. Each cage was supplied with 8 infra-red light beams connected to a counter for recording the light beam interruptions. Tests were done in blocks, consisting of 20 mice, with treatments randomized between all animals within the experiment. At least 10 animals per treatment were used. Immediately following administration of the compounds the mice were placed individually in the activity cages and the number of light beam interruptions was measured for 30 min.

Body temperature in mice The hypothermia tests were performed and replicated 3 times, each consisted of 3 mice from each treatment group. Within a block the various treatments were randomized between the cages, mice receiving the same treatment were placed within one cage. Ten min after treatment with a compound the rectal temperature of the mice was measured with an electrothermometer (Ellab TE3, Electrolaboriet, Copenhagen, Denmark). The lubricated probe was inserted about 2.5 cm. 8-Hydroxy-dipropylaminotetralin (8-OH-DPAT), 0.25 mg kg^{-1}, or placebo was then injected immediately and 10, 20, 30 and 40 min later rectal temperature was measured again. The room temperature was 21 ± 21 ± 1°1°C.

Forepaw treading Forepaw treading in rats was also measured three times. Within each block all treatments were randomized between animals. After the animals had been treated with the compound to be investigated, immediately followed by an injection of 8-OH-DPAT, 0.22 mg kg^{-1}, the rats were placed in small observation cages (7.5 × 18 × 30 cm) and forepaw treading was measured from 15 to 30 min after treatment by scoring the presence or absence of forepaw treading every 30 s. By use of this time sampling method a maximal score of 31 could be reached. Eight rats per treatment group were used.

Head shakes The head shake tests were run with blocks of eight animals, with each treatment present at least once in every block. Head shakes in rats were induced by DOI 0.22 mg kg^{-1}. Immediately after treatment with DOI and the agonist under study the rats were placed in small observation cages (7.5 × 18 × 30 cm) and the number of head shakes was counted during 30 min.

Drugs and solutions

The following drugs were used: 2 chloro-6-(1-piperazinyl) pyrazine monohydrochloride (MK 212; Merck, Sharpe and Dome); 1-(meta-chlorophenyl)-piperazine 2HCl (mCPP; EGA-chemie); (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), (±)-8-hydroxy-dipropylaminotetralin HBr (8-OH-DPAT) and 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrolo[1,2-a] quinoxaline dimaleate (CGS 12066B) all from Research Biochemicals Inc. (RBI) and m-trifluoromethyl phenyl piperazine HCl (TFMPP; Duphar). All compounds were dissolved in sterile saline solution and solutions were freshly prepared. Injections were made subcutaneously into the loose skin at the back of the neck. In rats a dose volume of 5 ml per kg body weight was used and in mice this dose volume was 10 ml per kg body weight. Control animals were injected with an equivalent volume of vehicle.

Statistics

Locomotor activity test The total test period of 30 min was divided into 3 periods of 10 min each. The results of the first 10 min period are presented as mean photocell interruptions ± s.e.mean. Statistical comparisons were made by the Mann-Whitney U test.

Hypothermia test The temperature changes are given in degrees centigrade compared to control groups and expressed as means per treatment group ± s.e. Statistical comparisons were made by use of an analysis of variance for a completely randomized design.

Forepaw treading and head shakes tests The results are expressed as the mean number of scores ± s.e.mean. Statistical comparisons were made by comparing the results of each group with the results of the control group by use of the Mann-Whitney U test.

Results

Locomotor activity in mice

Locomotor activity in mice was measured for 30 min which was divided into periods of 10 min each. The activity score of the placebo-treated mice was highest during the first 10 min and decreased during the following 10 min periods. Suppression of locomotor activity by 8-OH-DPAT (0.5 mg kg^{-1}) was strong and highly significant during the first 10 min. In the next two 10 min periods this effect weakened. In the third period the effect was no longer different from placebo (Figure 1). For the interaction studies of 8-OH-DPAT with other agonists only the first 10 min periods are presented (Figure 2).

When given alone the compunds DOI (0.046–0.22 mg kg^{-1}) and TFMPP (0.22–1.0 mg kg^{-1}) did not significantly change activity in any of the 3 periods. In the third period these compounds tended to increase locomotor activity, but this effect was never significant. The lowest and highest doses of mCPP (0.22 and 1.0 mg kg^{-1}) and MK 212 (0.22 and 1.0 mg kg^{-1}) significantly inhibited locomotor activity of the mice but only in the first 10 min period. CGS 12066B (2.2 and 10 mg kg^{-1}) significantly inhibited the locomotor activity in the same period. But in the second and third period there was no difference compared with placebo.

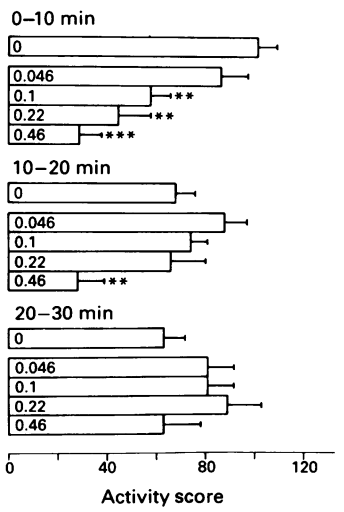

Figure 1 Effect of 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) on locomotor activity in mice. Measurement started immediately after s.c. injection of 8-OH-DPAT. Columns represent the mean activity score of at least 10 animals per group. Horizontal bars show the s.e.mean. ** $P < 0.01$; *** $P < 0.001$ when compared with the placebo group.

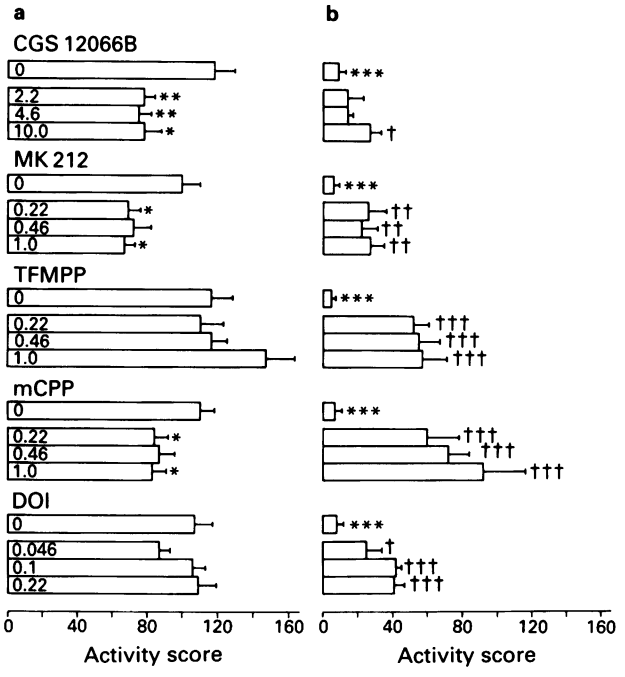

Figure 2 Effects of drug interactions on locomotor activity during the first 10 min after treatment. 8-Hydroxy-dipropylaminotetralin (8-OH-DPAT, 0.5 mg kg^{-1}) was injected immediately after an injection with one of the other compounds or placebo. In (a) the columns represent the mean activity scores after placebo + placebo or 5-hydroxytryptamine (5-HT)-agonists + placebo treatment. In (b) the columns represent the mean activities after 8-OH-DPAT + placebo or 8-OH-DPAT + 5-HT-agonist pretreatment. Horizontal bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared with placebo + placebo-treated group. † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$ when compared with placebo + 8-OH-DPAT-treated group. TFMPP = m-trifluoromethyl phenyl piperazine, mCPP = 1-(meta-chlorophenyl)-piperazine, DOI = 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane.

When combined treatments were given, DOI partly attenuated the 8-OH-DPAT-induced hypoactivity in the first 10 min. In the second period the activity of the animals, given the combined treatment, was no longer significantly different from the placebo group and in the third period their activity was significantly higher than that of the placebo group. TFMPP and mCPP at all doses tested markedly attenuated 8-OH-DPAT-induced hypoactivity. In the third period the activity of the combined treatment groups was significantly higher than that of the control groups. MK 212 attenuated 8-OH-DPAT-induced hypoactivity in the first and second period. In the third period no significant differences existed between the groups. Only a dose of 10 mg kg^{-1} CGS 12066B slightly attenuated 8-OH-DPAT-induced hypoactivity in the first 10 min period.

Body temperature in mice

8-OH-DPAT, 0.25 mg kg^{-1}, consistently induced a hypothermic response which was maximal at 10 min after treatment. Therefore, in Figure 3 the responses at this time point only are given. DOI, TFMPP, mCPP and MK 212 all dose-dependently attenuated 8-OH-DPAT-induced hypothermia. When given alone, none of these compounds significantly changed the body temperature compared with the placebo groups. CGS 12066B 10 mg kg^{-1} induced a weak but not significant hypothermia by itself. Given in combination with 8-OH-DPAT this compound at 10 mg kg^{-1} potentiated 8-OH-DPAT-induced hypothermia significantly.

Forepaw treading in rats

8-OH-DPAT, 0.22 mg kg^{-1}, induced forepaw treading with a mean score varying from 13.5 to 19.5 (maximal possible score was 31) (Figure 4). Forepaw treading induced by 8-OH-DPAT was dose-dependently potentiated by DOI (ED_{150} =

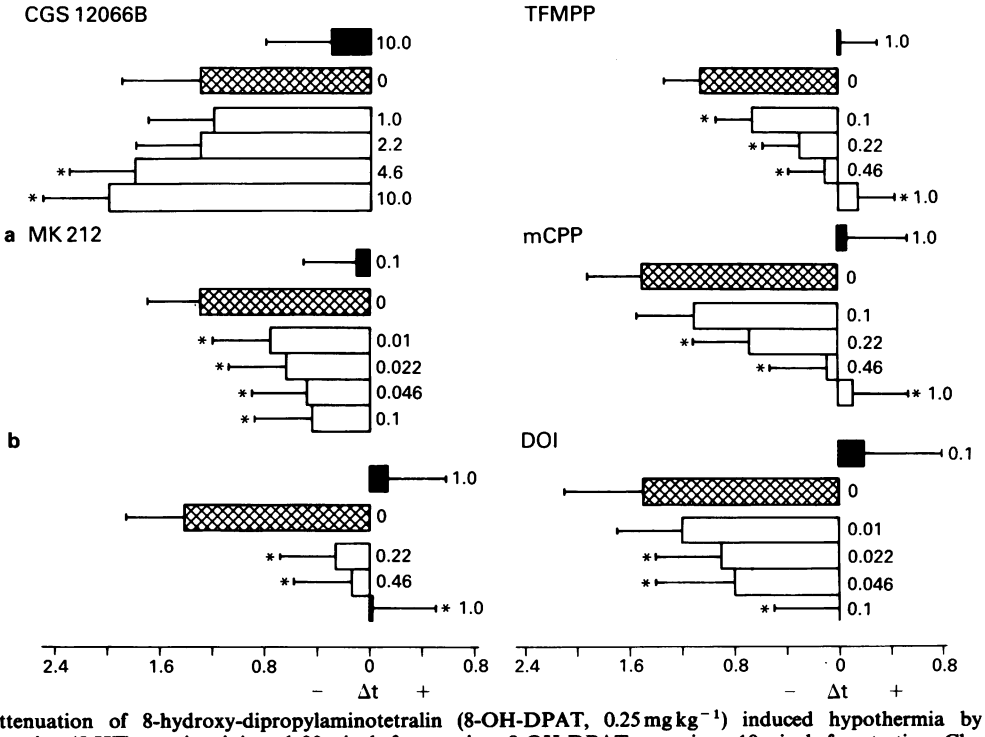

Figure 3 Attenuation of 8-hydroxy-dipropylaminotetralin (8-OH-DPAT, 0.25 mg kg^{-1}) induced hypothermia by various 5-hydroxytryptamine (5-HT)-agonists injected 20 min before testing. 8-OH-DPAT was given 10 min before testing. Changes in temperature (Δt) compared with the placebo + placebo-treated group are shown. The solid columns represent the 5-HT-agonist at the highest dose + placebo treated groups; the hatched columns represent the placebo + 8-OH-DPAT-treated groups; the open columns represent the 5-HT-agonist + 8-OH-DPAT-treated groups. Horizontal bars show s.e.mean. All values are the mean of 9 animals. (a) and (b) are data from 2 separate experiments with MK 212. * $P < 0.05$ when compared with placebo + 8-OH-DPAT group. The effect of 8-OH-DPAT was significantly different from placebo treatment in each test. For key to abbreviations used see legend of Figure 2

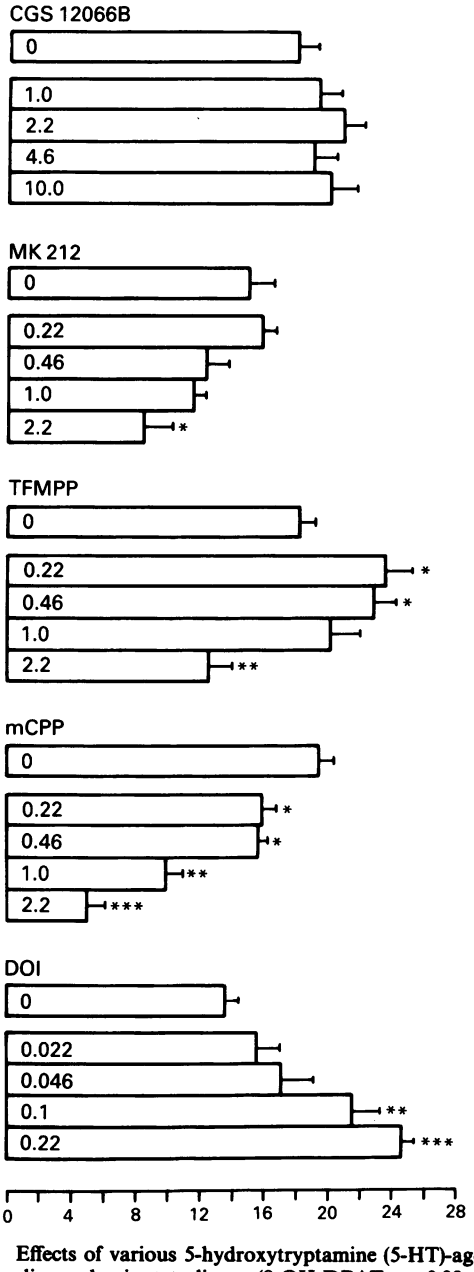

Figure 4 Effects of various 5-hydroxytryptamine (5-HT)-agonists on 8-hydroxy-dipropylaminotetralin (8-OH-DPAT, 0.22 mg kg^{-1})-induced forepaw treading in rats. The 5-HT-agonists were injected immediately before 8-OH-DPAT. The columns represent the mean forepaw treading scores for groups of 8 animals from 15–30 min after 8-OH-DPAT. The horizontal bars show the s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared with the control groups. For key to abbreviations used see legend of Figure 2.

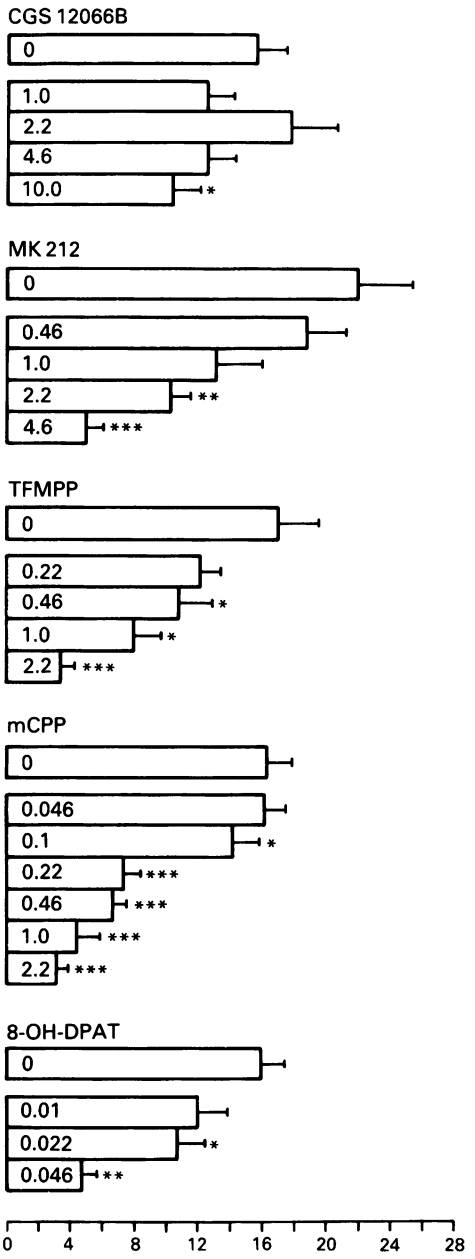

Figure 5 Effect of various 5-hydroxytryptamine (5-HT)-agonists on 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 0.22 mg kg^{-1})-induced head shakes in rats. The 5-HT-agonists were injected immediately before DOI. The columns represent the mean number of head shakes of at least 8 animals measured for 30 min after DOI. The horizontal bars show the s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared with control group. For key to abbreviations used see legend of Figure 2.

0.08 mg kg^{-1}). TFMPP, at lower doses potentiated and at higher doses inhibited forepaw treading. mCPP and MK 212 dose-dependently inhibited forepaw treading (ID_{50} = 1 mg kg^{-1} for mCPP). However, MK 212 had less inhibitory activity than mCPP, at 2.2 mg kg^{-1} the inhibition was 43%. CGS 12066B had no effect.

Head shakes in rats

The results of these experiments are given in Figure 5. Head shakes induced by DOI 0.22 mg kg^{-1} were dose-dependently inhibited by 8-OH-DPAT, mCPP, TFMP and MK 212. Their ID_{50} values were 0.03, 0.1, 0.7 and 2 mg kg^{-1} respectively. CGS 12066B significantly inhibited DOI-induced head shakes only at the highest dose tested (10 mg kg^{-1}).

Discussion

The main finding of the present experiments was that behavioural responses elicited by various 5-HT-receptor subtypes can be modified by co-activation of other 5-HT-receptor subtypes. A key element in interpreting these findings is the affinities of each agonist for the different receptor subtypes. These are summarized in Table 1. The table also contains the selectivity-ratios of these compounds for 5-HT_{1C} over 5-HT_2 receptors. These data show that very selective agonists or antagonists for these receptors do not exist. We realize that the lack of selectivity of these compounds for the 5-HT-receptor subtypes seriously hinders the interpretation of the data obtained in the present study. Also, binding data, on which we have to rely extensively, do not distinguish agonist and antagonist properties of the compounds. Nevertheless, our

Table 1 Affinity values of 5-hydroxytryptamine (5-HT)-agonists for the various 5-HT receptor subtypes

Compound	*Affinity values (pK_d) for* $5\text{-}HT_{1A}$-receptor[1]	$5\text{-}HT_{1B}$-receptor[1]	$5\text{-}HT_{1C}$-receptor[1]	$5\text{-}HT_2$-receptor[1]	*Preference ratio for* $5\text{-}HT_{1C}$-receptor[5]
8-OH-DPAT	8.74	4.22	5.24	5.94	0.2
5MeODMT	7.9[3]	6.2[3]	7.06[2]	6.21[2]	7.1
CGS 12066B	7.19[4]	6.94[4]	4.89[4]	—	—
MK 212	5.32	5.03	6.16	4.76	25
mCPP-	6.49	6.58	7.68	6.70	10
TFMPP	6.34	6.36	7.21	6.57	4.4
DOI	5.6[3]	5.9[3]	7.73[2]	7.84[2]	0.8

[1] The data are from several sources: pKd values from Hoyer 1988a; [2] from Hoyer (1988b); [3] calculated from Titeler *et al.* (1988); [4] from Schoeffter & Hoyer (1989); [5] the $5\text{-}HT_{1C}$ preference ratio represents the ratio of dissociation constants for $5\text{-}HT_2$- and $5\text{-}HT_{1C}$-receptors. Dissociation constants were the antilogs of the pK_d values as given in the table.

Table 2 Proposed effects of activation of 5-hydroxytryptamine (5-HT) receptor subtype on the effect induced by activation of another 5-HT-receptor subtype

	Response	*Effect of activation of* $5\text{-}HT_{1A}$	$5\text{-}HT_{1B}$	$5\text{-}HT_{1C}$	$5\text{-}HT_2$ *receptors*
$5\text{-}HT_{1A}$-induced	Hypoactivity in mice		0	–	–
	Hypothermia in mice		(+)	–	–
	Lower lip retraction in rats[a]		0	–	–
	Forepaw treading in rats		0	–	+
$5\text{-}HT_{1C}$-induced	Penile erections in rats[b]	–	0		–
$5\text{-}HT_2$-induced	Head shakes in rats	–	(–)	–	

0 = no effect; – = inhibition; + = potentiation; (–) = inhibition only at a high dose; (+) = potentiation only at a high dose; [a] Berendsen *et al.* (1989a); [b] Berendsen *et al.* (1990).

data require a tentative interpretation in order to provide a working hypothesis for future experiments. Furthermore, the interactions that we have observed should be taken into account when interpreting behavioural changes induced by drugs acting on 5-HT receptors. The proposed interactions between the 5-HT receptor subtype responses are summarized in Table 2.

The effect of 8-OH-DPAT on locomotor activity in rats is controversial, increased locomotor activity was found by Tricklebank *et al.* (1984) and Broekkamp *et al.* (1989) whereas Hillegaart *et al.* (1989) found decreased activity. In mice we found a hypoactivity, at least during the first 20 min, after treatment with 0.5 mg kg^{-1}. The hypothermic effect on 8-OH-DPAT in mice is consistent with other studies in mice and rats (Goodwin & Green, 1985; Hjorth, 1985; Goodwin *et al.*, 1987). In contrast to our results in mice, TFMPP, mCPP and MK 212 have been shown to induce hyperthermia in rats (Maj & Lewandowska, 1980; Yamawaki *et al.*, 1983; Pawlowski, 1984; Gudelski *et al.*, 1986). However, these authors gave a higher dose of the compounds and/or did the experiments in heat adapted rats. Both 8-OH-DPAT-induced hypoactivity and hypothermia in mice could be attenuated by DOI, TFMPP, mCPP and MK 212. These compounds bind preferentially to the $5\text{-}HT_{1C}$- and/or $5\text{-}HT_2$-receptors, suggesting that both activation of $5\text{-}HT_{1C}$- and/or $5\text{-}HT_2$-receptors can functionally inhibit these $5\text{-}HT_{1A}$-induced effects. The mixed $5\text{-}HT_{1A/1B}$ agonist CGS 12066B (Neale *et al.*, 1987; Schoeffter & Hoyer, 1989) had no effect on 8-OH-DPAT-induced hypolocomotion. However, hypothermia induced by 8-OH-DPAT was potentiated by a high dose of CGS 12066B. Previously, we demonstrated that lower lip retraction induced by 8-OH-DPAT is not influenced by CGS 12066B, whereas activation of $5\text{-}HT_{1C}$- and/or $5\text{-}HT_2$-receptors attenuated this effect (Berendsen *et al.*, 1989a). This might point to a lack of interaction between the $5\text{-}HT_{1A}$- and $5\text{-}HT_{1B}$-receptor-induced behaviours. It is also possible that the penetration of CGS 12066B into the brain is poor. A lack of interaction with the $5\text{-}HT_{1A}$- and $5\text{-}HT_{1B}$-receptors might also be inferred from the ability of the mixed $5\text{-}HT_{1A}/5\text{-}HT_{1B}$-agonist RU24969 to induce the $5\text{-}HT_{1A}$-related lower lip retraction (Berendsen *et al.*, 1989a). One might argue that the increased locomotor activity after DOI, TFMPP and mCPP contributes to the attenuation of 8-OH-DPAT-induced hypothermia. However, the effect of these compounds on hypothermia was strongest after 10 min, whereas the increase in locomotor activity occurred no earlier than 20–30 min after treatment. The forepaw treading induced by 8-OH-DPAT was influenced differently by the agonists: DOI dose-dependently potentiated the response, while TFMPP potentiated it at low doses but attenuated it at higher doses. mCPP and MK 212 both attenuated forepaw treading. These compounds bind to $5\text{-}HT_{1C}$ and $5\text{-}HT_2$ sites, but if the selectivity-ratio of these compounds for the $5\text{-}HT_{1C}$- over $5\text{-}HT_2$-receptors is calculated the sequence is MK 212 > mCPP > TFMPP > DOI (Table 1). It thus seems that as the preference for $5\text{-}HT_{1C}$-receptors over $5\text{-}HT_2$-receptors decreases the effect on forepaw treading changes from an inhibition to a potentiation. We have seen the same order of potency with these compounds for their ability to induce penile erections (Berendsen *et al.*, 1990). The potentiating effect of DOI and TFMPP on forepaw treading confirms previous findings (Berendsen *et al.*, 1989b; Arnt & Hyttel, 1989). Among the symptoms induced by 8-OH-DPAT, hypothermia and lower lip retraction are thought to be presynaptically mediated (Goodwin *et al.*, 1987; Wozniak *et al.*, 1988; Berendsen *et al.*, 1989a), whereas forepaw treading is thought to be a postsynaptically-mediated effect (Tricklebank *et al.*, 1985). The data thus suggest that presynaptically-induced $5\text{-}HT_{1A}$ effects are attenuated by both $5\text{-}HT_{1C}$- and $5\text{-}HT_2$-receptor activation, whereas postsynaptically-mediated $5\text{-}HT_{1A}$-effects are potentiated by concomitant activation of $5\text{-}HT_2$ and attenuated by concomitant activation of $5\text{-}HT_{1C}$-receptors. Injection of CGS 12066B did not change the 8-OH-DPAT-induced hypoactivity or forepaw treading response. Therefore, there is no evidence as yet to suggest an influence of $5\text{-}HT_{1B}$-receptor activation on $5\text{-}HT_{1A}$-receptor-mediated effects.

The functional effect of $5\text{-}HT_{1A}$-, $5\text{-}HT_{1B}$- and $5\text{-}HT_2$-receptor activation on a $5\text{-}HT_{1C}$-receptor-mediated effect has been discussed before (Berendsen *et al.*, 1990). It has been shown that activation of the $5\text{-}HT_{1A}$-receptor attenuated

the 5-HT_{1C}-mediated induction of penile erections (PE) and that induction of PE by mixed 5-HT_{1C}/5-HT_2 agonists depends on the selectivity of these compounds for 5-HT_{1C}- over 5-HT_2-receptors. This suggests that activation of the 5-HT_2-receptor inhibits or prevents expression of 5-HT_{1C}-receptor-mediated PE. DOI binds with similar affinity to 5-HT_2 and 5-HT_{1C} sites but induces 5-HT_2-mediated behaviours. This indicates that for a mixed 5-HT_{1C}/5-HT_2 agonist the behaviour related to activation of the 5-HT_2-receptor prevails over the behaviour induced by 5-HT_{1C}-receptor activation. The head shake response induced by DOI, could be attenuated by 8-OH-DPAT, TFMPP, mCPP and MK 212 suggesting that activation of both 5-HT_{1A}- and 5-HT_{1C}-receptors functionally inhibits this 5-HT_2-mediated response.

Functional interactions between the different 5-HT receptor subtype-mediated effects does not seem to be restricted to the behavioural expression of activation of these receptors. It has been shown that mCPP and TFMPP inhibit 5-HT_2-receptor-mediated vascular contractions (Cohen & Fuller, 1983) and that mCPP antagonizes cortical 5-HT_2-receptors that are linked to phosphoinositide turnover (Conn & Sanders-Bush, 1987). Moreover, it was shown in electrophysiological experiments that 5-HT_{1A} and 5-HT_{1B}/5-HT_{1C} agonists have different effects on neuronal cell firing in different brain regions (Sprouse & Aghajanian, 1988). Opposite effects of 5-HT_{1A}- and non-5-HT_{1A} (5-HT_{1B} or 5-HT_{1C})-receptor activation have been found in several experiments. For example, in the startle response in rats (Davis *et al.*, 1986), in aversive responding seen after electrical stimulation of the periaqueductal grey (Jenck *et al.*, 1989) and in several models of animal anxiety (Broekkamp & Jenck, 1989).

The observed functional interactions of 5-HT-receptor subtype-mediated effects may occur at different levels, such as a molecular allosteric mutual influence, opposing effects on second messengers or more broadly via presynaptic-postsynaptic locations or functional influences from different brain structures.

The data emphasize the difficulty of drawing conclusions from functional measurements when the compounds that are being investigated have mixed receptor affinities and efficacies. The availability of selective antagonists for the different 5-HT receptor subtypes would greatly facilitate the interpretation of such data.

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