Pharmacology Biochemistry and Behavior, Vol. 45, pp. 777–783, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Chronic, Combined Treatment With Desipramine and Mianserin: Enhanced 5-HT_{1A} Receptor Function and Altered 5-HT_{1A}/5-HT_2 Receptor Interaction in Rats

ANDERS LUND[1] AND NORMA MJELLEM

Department of Physiology, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway

Received 15 June 1992

LUND, A. AND N. MJELLEM. *Chronic, combined treatment with desipramine and mianserin: Enhanced 5-HT_{1A} receptor function and altered 5-HT_{1A}/5-HT_2 receptor interaction in rats.* PHARMACOL BIOCHEM BEHAV **45**(4) 777–783, 1993.—A greater percentage of depressed patients respond to combined treatment with a tricyclic antidepressant and the tetracyclic antidepressant mianserin than to treatment with these drugs given alone. The functional sensitivity of the 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptor, and the functional interaction between the 5-HT_{1A} and the 5-HT_2 receptors were investigated after treatment with desipramine and mianserin either alone or combined for 21–28 days. Pretreatment with desipramine and mianserin in combination induced the most intense 5-HT syndrome and the greatest fall in colonic temperature after injection of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT). The rats pretreated with desipramine alone had the largest elevation of the response temperature in the increasing temperature hot-plate test after injection of 8-OH-DPAT . After the combined pretreatment with desipramine and mianserin, no enhanced functional response in these tests was found when the 5-HT_{1A} and the 5-HT_2 receptors were stimulated simultaneously using 8-OH-DPAT and the 5-HT_2 agonist, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), contrasting the findings for desipramine or mianserin treatments given alone, where an increased functional response was found for the colonic temperature and the response temperature in the increasing temperature hot-plate test. In vitro receptor binding using [^{3}H]-8-OH-DPAT as ligand revealed an increase in K_d and B_{max} in the spinal cord after chronic treatment with the combination of desipramine and mianserin. In the hippocampus and the frontal cortex the changes were small. Thus, the combination of desipramine and mianserin increased the functional response to 5-HT_{1A} receptor stimulation, and decreased the response to simultaneous stimulation of the 5-HT_{1A} and 5-HT_2 receptors, when compared to treatments with either one of the antidepressants alone, or controls. These rather large functional changes were not clearly reflected in the receptor binding study, indicating that changes in the postreceptor signal transduction may be of importance.

Desipramine Mianserin 5-HT_{1A} receptor 5-HT_2 receptor Behavior Rats

MOST types of antidepressant treatments seem to lead to the same long-term result, namely an increased sensitivity of postsynaptic neurons to 5-hydroxytryptamine (5-HT) (4,7), and a downregulation of the β-adrenoceptors in the brain (36). Several 5-HT receptor subtypes are described (30). In the human brain, 5-HT_{1A}, 5-HT_{1C}, and 5-HT_2 receptors mediate the postsynaptic effects of 5-HT, while the 5-HT_{1A} receptor also functions as autoreceptor on the 5-HT cell bodies in the raphe nuclei (20). The 5-HT_{1A} receptor has been localized with high concentration in the limbic regions and in the dorsal raphe nucleus, and is also found in the spinal cord and the frontal cortex, while the highest level of 5-HT_2 receptors is in layer IV of the cerebral cortex (18,30).

The details of the modulation of serotonergic systems by antidepressants are contentious, but several studies indicate that the 5-HT_{1A} and 5-HT_2 receptor subtypes are of special importance (9,10,13–16,21,22,26,33,34,37,40,41). It has been proposed that the 5-HT_{1A} receptor may be downregulated and the 5-HT_2 receptor may be upregulated in depression, and that antidepressants reverse this imbalance (9).

Opposing effects of 5-HT_1 and 5-HT_2 receptors in different behaviors (sleep, sexual behavior, temperature regulation) are often reported (13), but a potentiation of behavior has also been reported after simultaneous stimulation of these two receptor subtypes in rats: the ability of the 5-HT_{1A} agonist, 8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT) to induce forepaw treading was increased twentyfold after cotreatment with 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane

[1] To whom requests for reprints should be addressed.

(DOI), which is predominantly a 5-HT_2 agonist (1). As far as we know, no investigations on this interaction have been conducted after antidepressant treatments.

The acute effect of tricyclic antidepressants (TCAs) is mainly to inhibit the reuptake of noradrenaline or 5-HT (3), while mianserin is an antagonist both of the 5-HT_2 receptor (35) and of the presynaptic α_2 receptor (31). There is some evidence indicating that chronic treatment with TCAs may have a different mode of action on the 5-HT receptors in humans than chronic treatment with mianserin (5), and a difference may also be found in rats (11).

Resistance to antidepressant treatment is a well-known phenomenon (28). Lithium has been reported to increase the antidepressant effect of TCAs, due to a synergistic action enhancing some aspects of brain 5-HT function in humans (6). It has also been reported that a greater percentage of depressed patients respond to antidepressant treatment when a TCA is combined with a tetracyclic antidepressant (2). The combination of different TCAs and mianserin has been investigated specifically, and relief of depression is found in a greater percentage of patients receiving this combination than those receiving either treatment given alone (27). In this preliminary study, five treatment-resistant patients are described in detail, all with a remarkable antidepressant effect of the combination therapy after a few days, and it is stated that 40 other patients have received the same treatment, mainly with a favorable outcome.

In the present study, a combined desipramine/mianserin treatment was compared to these treatments given alone in different behavioral tests, reflecting the functional sensitivity of the 5-HT_{1A} receptor. The functional interaction between this receptor and the 5-HT_2 receptor was also studied. In vitro receptor binding studies of the 5-HT_{1A} receptor was performed in the hippocampus, the frontal cortex, and the spinal cord in rats after these treatments.

METHOD

Drugs

Desipramine HCl (DMI, gift from Ciba-Geigy, Switzerland) and mianserin HCl (MIAN, gift from Organon, The Netherlands) were dissolved in tap water in a concentration of 0.15 mg/ml. A combination of the two drugs was administered in the same way in a concentration of 0.075 mg/ml of each. This was the only source of drinking water in the three treatment groups, yielding a dose of DMI of approximately 8 mg/kg/24 h (23), of MIAN about 12 mg/kg/24 h (a larger volume was drunk in this group), and of approximately 5 mg/kg/24 h of each of the two antidepressants when combined (DMI/MIAN). The antidepressants were given for 21–28 days before the behavioral experiments and for 35–42 days before the receptor binding experiments. 8-Hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT, Research Biochemicals Inc., USA) was dissolved in 0.9% NaCl and injected subcutaneously (SC), 0.02 mg/kg, in a volume of 5 ml/kg. 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI, Research Biochemicals Inc.), 0.25 mg/kg in 0.9% NaCl, was administered together with 8-OH-DPAT in some experiments. The doses of 8-OH-DPAT and DOI were based on preliminary experiments, and chosen so as to make possible the observation of both an increase and a decrease in the behavioral response.

Animals

Male Sprague-Dawley rats (Mol: SPRD, Møllegaard, Denmark), weighing 300–380 g at the end of the experiments, were used. The rats were housed at 22–24°C in standard macrolone cages, two to three in each cage, and kept on a 12L : 12D cycle with lights on at 7:00 a.m. Food and tap water were freely available for the control animals, and food and tap water with the antidepressants were freely available for the treatment groups. One week before the behavioral testing started, the animals were put in single cages, and stayed there for the rest of the experiment.

Behavioral Testing

All animals were handled during the period of drug treatment. During this handling, they were exposed once to the increasing temperature hot plate without heating the plate.

The animals were brought to the test room at least 1 h before the behavioral experiments started. Each animal received one injection of 8-OH-DPAT, and one injection of 8-OH-DPAT and DOI SC, with at least 7 days between the two experiments. Control animals were injected with the same volume of the vehicle. Ambient temperature was between 22 and 24°C.

For the scoring of the 5-HT behavioral syndrome, the animal was put back in its home cage after the injection and videotaped for 30 min in a sound-attenuated room, interrupted for the hot-plate test and the measurement of colonic temperature 10 min after the injection. The scoring of the behavioral 5-HT syndrome was modified from that previously described (8). Since a low dose of 8-OH-DPAT was administered, the only behavioral categories observed were flat body posture, hyperlocomotion, and Straub tail (increased tonus or stiffness of the tail). Each category was scored on a scale from 0 to 3 (0: absent, 1: just present, 2: definitely present, 3: intense). For a total score, the scores for all categories were added. The behavior was scored for 1-min sessions, 4–5 min, 9–10 min, 14–15 min, 19–20 min, 24–25 min, and 29–30 min after the injection.

Colonic temperature was measured immediately before each test on the increasing temperature hot plate. A thermocouple probe was inserted 5–6 cm from the anus while the animal was loosely restrained by the tail, allowing some moving around.

The increasing temperature hot-plate test was performed as described previously (39). The temperature of the plate was increased from 42°C at a rate of 3°C per min, and a cut-off temperature of 50°C was set to avoid tissue damage. The second time the animal licked one of the hindpaws was used as the endpoint of the test; the first time was not used to avoid the scoring of nonspecific licking. The response temperature was recorded by means of a computer. The hot-plate test was performed prior to injection of 8 OH-DPAT and 10 and 60 min after the injection.

Binding of [^{3}H]8-OH-DPAT to 5-HT_{1A} Receptors

The rats were killed by decapitation, and the brain and spinal cord were quickly removed. The frontal cortex, the hippocampus, and the spinal cord were dissected on an ice-cold plate, and immediately homogenized in 10 vol. of ice-cold buffer (50 mM Tris HCl, pH 7.8; 4 mM $CaCl_2$; 0.1% ascorbic acid) with a Teflon-glass homogenizer, and then sonificated. The homogenates were centrifuged at 18,000 rpm for 12.5 min, and the supernatant was removed. The pellet was resuspended with the same buffer, sonificated, and centrifuged as the first time. Sucrose (3.0 ml 0.32 M) was added to the pellet and another sonification was done before storing at −35°C until the binding assay. The same buffer was added again,

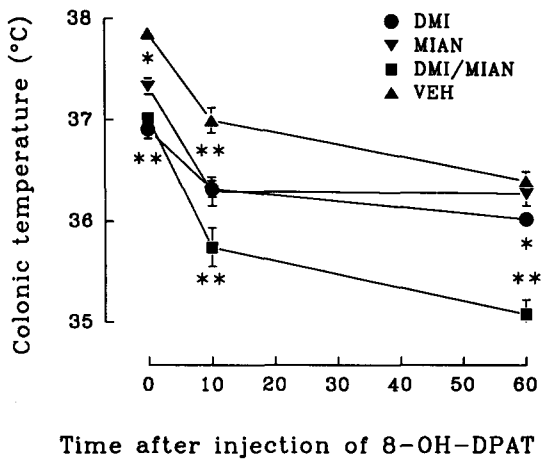

FIG. 1. The colonic temperature (°C) (mean ± SEM; n = 6–10) after the injection of 8-OH-DPAT (0.02 mg/kg SC) to rats pretreated with desipramine (DMI), mianserin (MIAN), the combination of the two (DMI/MIAN), or vehicle (VEH) in the drinking water for 21–28 days. *$p < 0.05$, **$p < 0.005$ (t-test, subsequent to a significant overall ANOVA).

sonification was performed, and dilution to the final protein concentration of 0.50–0.66 mg/ml. Pargyline (5 mM) was added to the suspension to a final concentration of 10 μM. [^{3}H]8-OH-DPAT (concentration: 0.2–8.0 nM for the frontal cortex and the hippocampus, and 0.2–6.0 nM for the spinal cord) was used as ligand, and nonspecific binding was determined in the presence of 5-HT (10 μM). The tubes were harvested in a 7025 Skatron cell harvester with auto-wash program using glass fiber filters. The filter discs were placed in scintillation vials, along with 6-ml filter-count scintillation fluid, and radiation was counted in a LKB 1219 RackBeta Spectral Liquid Scintillation Counter, counting efficiency 56–57%. All binding assays were carried out in triplicate, and at least three separate experiments (different groups of animals) were performed with separate homogenates from each treatment group and controls.

Protein concentrations were determined by a standard method, using bovine plasma gamma globulin as the standard.

Statistics

Analysis of variance (ANOVA) and ANOVA with repeated measures were used. Subsequent to a significant overall ANOVA, t-tests were used where appropriate. For the data from the behavioral 5-HT syndrome, nonparametric statistics were performed (Kruskall–Wallis ANOVA by ranks). Statistical significance at the 5% level was accepted.

RESULTS

Body Weight and Drinking Volume

The animals receiving DMI treatment alone or in combination with MIAN drank less than controls, and they gained somewhat less body weight. After 3 weeks of treatment, the mean weight (g ± SEM) of the animals in the different groups were: DMI: 288.3 ± 3.5, MIAN: 325 ± 7.5, DMI/MIAN: 287.3 ± 9.2, and VEH: 321.3 ± 12. The drinking volume (ml/kg body weight/24 h ± SEM) for the same groups after 3 weeks of treatment were: DMI: 54.8 ± 2.3, MIAN: 82.1 ± 3.8, DMI/MIAN: 68.3 ± 1.5, and VEH: 83.9 ± 3.9.

Functional Tests Before the Injection of Receptor Agonists

After pretreatment with DMI (8 mg/kg/24 h), MIAN (12 mg/kg/24 h), or the combined treatment with DMI (5 mg/kg/24 h) and MIAN (5 mg/kg/24 h) (DMI/MIAN), the colonic temperature was lower (Fig. 1) and the hot-plate response temperature higher (Fig. 2) than in controls (VEH) before the administration of 8-OH-DPAT.

Functional Tests After the Injection of 8-OH-DPAT

The low dose of 8-OH-DPAT given (0.02 mg/kg) induced only a weak 5-HT syndrome in the controls (Fig. 3). Pretreat-

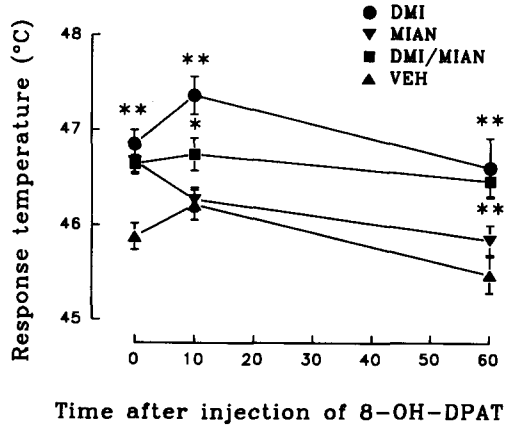

FIG. 2. The response temperature (°C) (mean ± SEM; n = 6–10) in the increasing temperature hot-plate test after injection of 8-OH-DPAT (0.02 mg/kg SC) to groups of rats pretreated with desipramine (DMI), mianserin (MIAN), the combination of the two (DMI/MIAN), or vehicle (VEH), in the drinking water for 21–28days. *$p < 0.05$, **$p < 0.005$ (t-test, subsequent to a significant overall ANOVA).

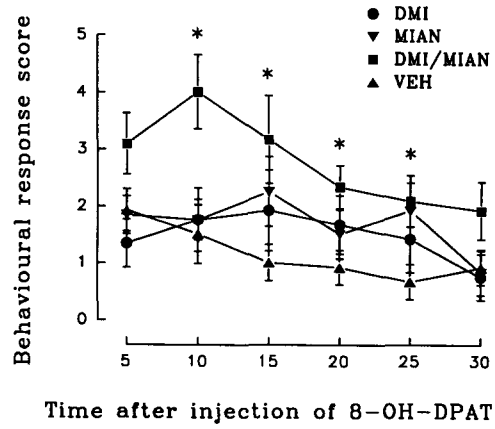

FIG. 3. The behavioral 5-HT syndrome (mean ± SEM; n = 6–10) after the injection of 8-OH-DPAT (0.02 mg/kg SC) to groups of rats pretreated with desipramine (DMI), mianserin (MIAN), the combination of the two (DMI/MIAN), or vehicle (VEH) in the drinking water for 21–28 days. The values given are the sum of the score of three behavioral categories (maximum score 9). *$p < 0.05$, **$p < 0.005$ (Kruskall–Wallis ANOVA by ranks).

ment with DMI or with MIAN tended to increase this behavior, and for the combined treatment (DMI/MIAN), a great and statistically significant increase in the 8-OH-DPAT-induced behavioral syndrome was found after 10, 15, 20, and 25 min (Fig. 3; Kruskall-Wallis ANOVA by ranks, between DMI/MIAN group and controls). Significant differences were also found between the DMI/MIAN group and the DMI group at 5 and 10 min, and between DMI/MIAN group and MIAN group at 10 min (Kruskall-Wallis ANOVA by ranks, data not shown).

The fall in the colonic temperature after injection of 8-OH-DPAT was also greater for the DMI/MIAN-pretreated group than for either pretreatment given alone in higher doses (Fig. 1; ANOVA with repeated measures: $p < 0.001$ for the pretreatment; $p < 0.001$ for the repetition; and $p = 0.004$ for the interaction between the two).

In the increasing temperature hot-plate test, a significant increase in response temperature after 8-OH-DPAT injection was observed in the DMI group, and in the DMI/MIAN group (Fig. 2; ANOVA with repeated measures: treatment variable: $p < 0.001$; repetition factor: $p < 0.001$; and the interaction between the two: $p = 0.007$).

Functional Tests After the Coadministration of 8-OH-DPAT and DOI

When DOI (0.25 mg/kg) was administered together with 8-OH-DPAT (0.02 mg/kg), the fall in colonic temperature and the increase in hot-plate response temperature were greater for all groups except for the DMI/MIAN group, compared to the groups injected with 8-OH-DPAT only (Figs. 4 and 5). In the DMI/MIAN group, the fall in colonic temperature was not significantly changed by the additional administration of DOI (Fig. 4), and neither was the response temperature in the increasing temperature hot plate test (Fig. 5). As for the behavioral 5-HT syndrome, the DMI/MIAN group showed almost no detectable 5-HT syndrome after coadministration of 8-OH-DPAT and DOI (Fig. 6B), in contrast to the group pretreated with DMI only (Fig. 6A) or with VEH (Fig. 6D), where the additional administration of DOI increased the 8-OH-DPAT effect 25 and 30 min after injection. In the MIAN-pretreated group, the additional administration of DOI did not change the behavioral response (Fig. 6C). The administration of this dose of DOI alone to the different treatment groups neither induced any detectable behavioral syndrome, nor any significant effects on colonic temperature or hot-plate response temperature (data not shown).

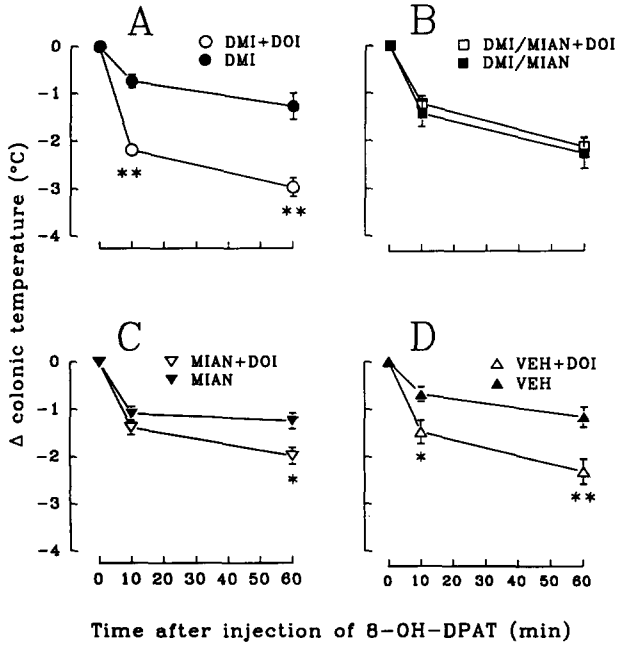

FIG. 4. The difference between test and pretest value for the colonic temperature (°C) after the injection of 8-OH-DPAT (0.02 mg/kg) plus DOI (0.25 mg/kg), or 8-OH-DPAT alone to different groups: (A) pretreatment with DMI, (B) pretreatment with DMI/MIAN, (C) pretreatment with MIAN, (D) VEH. $*p < 0.05$, $**p < 0.005$ (t-test).

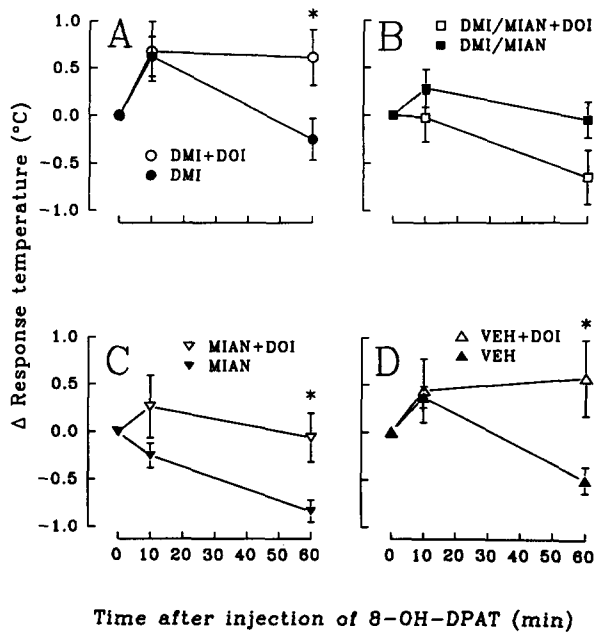

FIG. 5. The difference between test and pretest for the response temperature in the increasing temperature hot-plate test (°C) after the injection of 8-OH-DPAT plus DOI, or 8-OH-DPAT alone to different groups: (A) pretreatment with DMI, (B) pretreatment with DMI/MIAN, (C) pretreatment with MIAN, (D) VEH. $*p < 0.05$ (t-test).

Binding of [^{3}H]8-OH-DPAT

The results of the study of [^{3}H]-8-OH-DPAT binding are shown in Table 1. In the DMI/MIAN group, a statistically significant increase of B_{max} (22.7%) and K_d (46.5%) was found in the spinal cord, and of B_{max} in the hippocampus (11.2%). In the DMI group, a decrease in K_d (10.7%) and B_{max} (12.5%) was found in the frontal cortex, as well as a decrease of K_d in the spinal cord (15.7%). The data for the DMI group have been published previously (24).

Concentration of Desipramine and Mianserin in Serum

The serum concentration (mean ± SEM) of desipramine in the DMI group was 107.8 ± 35.4 ng/ml (356 ± 116.4 nmol/l), and of mianserin in the MIAN group 10.1 ± 0.8 ng/ml (33.4 ± 2.6 nmol/l). In the DMI/MIAN group, the concentration of desipramine was 26.6 ± 5.2 ng/ml (88.5 ± 17.2 nmol/l) and of mianserin 9.6 ± 4.3 ng/ml (32 ± 14.5

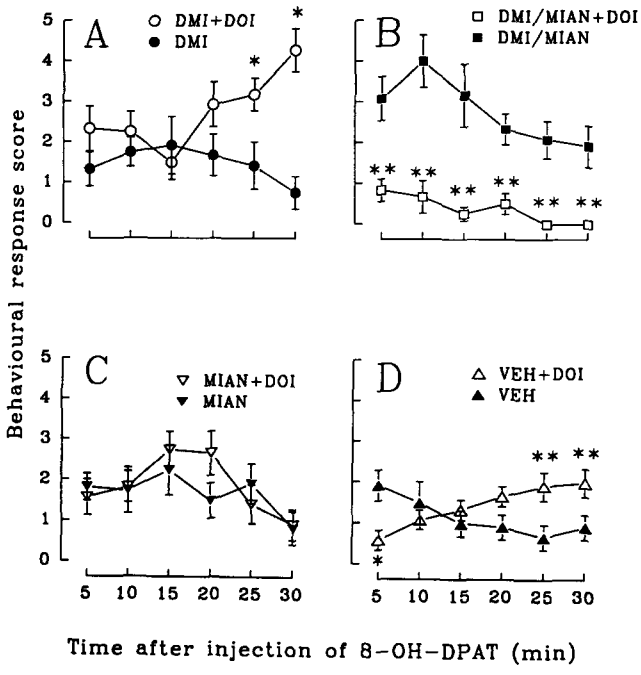

FIG. 6. The behavioral 5-HT syndrome after injection of 8-OH-DPAT (0.02 mg/kg) plus DOI (0.25 mg/kg), or 8-OH-DPAT alone to different groups: (A) pretreatment with DMI, (B) pretreatment with DMI/MIAN, (C) pretreatment with MIAN, (D) VEH. $*p < 0.05$, $**p < 0.005$ (Kruskall-Wallis ANOVA by ranks).

nmol/l). The blood was collected when the animals were sacrificed, after 35–42 days.

DISCUSSION

The combined pretreatment with DMI and MIAN for 21–28 days induced a significant increase in sensitivity of the 5-HT_{1A} receptor to 8-OH-DPAT stimulation, as measured functionally by both the 5-HT syndrome and the fall in colonic temperature. After pretreatment with either DMI or MIAN alone, only moderate changes in the 5-HT syndrome and the colonic temperature were found. In the increasing temperature hot-plate test, however, pretreatment with DMI alone induced the greatest increase in response temperature after injection of 8-OH-DPAT. Thus, various behavioral tests reflect differently the upregulation of 5-HT_{1A} receptor sensitivity after pretreatment with various antidepressants. The 5-HT syndrome and the increasing temperature hot-plate test both seem to reflect the function of postsynaptic receptors after stimulation with 8-OH-DPAT, while for the fall in colonic temperature it is somewhat unsettled whether pre- or postsynaptic involvement is of major importance in rats [see references in (24)].

In animals pretreated with the combination of DMI and MIAN, the additional administration of the 5-HT_2 agonist, DOI, almost completely inhibited the 5-HT syndrome induced by injection of 8-OH-DPAT. There was no significant change in the colonic temperature or the hot-plate response temperature when compared to injection of 8-OH-DPAT alone. In the VEH- and the DMI-pretreated rats, however, there was an increase in the effect in all the behavioral tests used after the coadministration of 8-OH-DPAT and DOI. In MIAN-pretreated rats, the additional administration of DOI induced either a weak enhancement of the 8-OH-DPAT effect (colonic

TABLE 1

BINDING OF [^{3}H]8-OH-DPAT

Pretreatment	Hippocampus		Frontal Cortex		Spinal Cord	
	K_d	Diff. (%)	K_d	Diff. (%)	K_d	Diff. (%)
DMI	1.88 ± 0.09	−2.2 (3)	2.03 ± 0.07	−10.7* (3)	1.68 ± 0.10	−15.7* (4)
VEH	1.92 ± 0.09		2.27 ± 0.11		1.99 ± 0.14	
MIAN	1.47 ± 0.13	−0.8 (3)	1.59 ± 0.16	−1.0 (3)	2.19 ± 0.33	−0.5 (4)
VEH	1.48 ± 0.13		1.60 ± 0.14		2.20 ± 0.40	
DMI/MIAN	1.67 ± 0.13	+12.6 (3)	1.71 ± 0.17	+6.7 (3)	3.23 ± 0.48	+46.5† (4)
VEH	1.48 ± 0.13		1.60 ± 0.14		2.20 ± 0.40	
	B_{max}		B_{max}		B_{max}	
DMI	616.9 ± 21.8	+1.1 (3)	242.4 ± 5.1	−12.5† (3)	29.8 ± 2.8	−2.9 (4)
VEH	610.5 ± 21.6		276.9 ± 9.8		30.7 ± 2.8	
MIAN	446.3 ± 22.3	+1.7 (3)	198.3 ± 11.9	+6.8 (3)	21.4 ± 2.6	−5.3 (4)
VEH	438.9 ± 21.9		185.7 ± 9.3		22.6 ± 2.9	
DMI/MIAN	488.1 ± 24.4	+11.7* (3)	201.5 ± 12.1	+8.5 (3)	27.8 ± 3.3	+22.7* (4)
VEH	438.9 ± 21.9		185.7 ± 9.3		22.6 ± 2.9	

K_d (nM) and B_{max} (fmol/mg protein), and the difference (%) compared to controls (VEH). Rats were treated with desipramine (DMI), mianserin (MIAN), or DMI/MIAN for 35–42 days, and [^{3}H]8-OH-DPAT binding was studied in membrane preparations from hippocampus, frontal cortex, and spinal cord. Number of separate experiments is indicated in parentheses.

*$p < 0.05$.

†$p < 0.01$.

temperature and hot plate response temperature) or no additional effect (5-HT syndrome).

Thus, the combination of DMI and MIAN results in an increased function of the 5-HT_{1A} receptor, compared to either DMI or MIAN alone. In the combined treatment group, additional stimulation of the 5-HT_2 receptors either antagonizes 5-HT_{1A} stimulation (5-HT behavior) or is without effect (colonic temperature and hot-plate response temperature), in contrast to the findings in the groups pretreated with either DMI or MIAN alone, where stimulation of the two 5-HT receptor subtypes has synergistic effects.

The increased effect of 5-HT_{1A} receptor stimulation after pretreatment with the combination of DMI and MIAN could not be explained by changes in serum concentration after this combined treatment. The enhanced function was neither clearly reflected in the receptor binding experiment, except for the increase in B_{max} in the spinal cord. However, the affinity of the receptor in the spinal cord was decreased (increased K_d). The functional consequences of these partly opposing or small changes of the receptor are difficult to predict. It seems possible that the functional changes may have its main substrate on the postreceptor signal transducing system (32).

In order to keep the number of animals at a minimum, the same animals were used in the behavioral experiments and in the receptor binding part of the study, performed at least 1 week after the behavioral experiment. Because of this, the treatment periods before the behavioral testing and the receptor binding experiments were different. This difference is not likely to affect our conclusions, since previous experiments have shown that treatment with these antidepressants in rats induces the same behavioral changes both after 3 and 6 weeks of treatment (unpublished data).

We have not used any washout period for the antidepressants before the experiments were conducted in order to obtain information about the functional and in vitro status during antidepressant therapy, and not about a withdrawal phenomenon some days after discontinuation of treatment. With this design, the antidepressants investigated may of course influence the investigated receptors. For mianserin, the pk_i is approximately 6 for the 5-HT_{1A} receptor and approximately 8 for the 5-HT_2 receptor. Nevertheless, the mianserin group has a greater fall in colonic temperature and a higher response temperature in the increasing temperature hot-plate test after the combined injection of 8-OH-DPAT and DOI than after the injection of 8-OH-DPAT alone. This indicates that DOI has an effect at the 5-HT_2 receptor also during mianserin treatment.

8-OH-DPAT, which is used both as an agonist in the functional studies and as a ligand in the in vitro receptor binding part of the study, has been shown to bind selectively to the 5-HT_{1A} receptor (17), and has become a reference compound in both binding studies and in behavioral studies of 5-HT_{1A} receptor function. A small dose of 8-OH-DPAT, based on previous experiments in our group, was used to make possible the observation of increases in receptor sensitivity. DOI has an affinity for both 5-HT_2 and 5-HT_{1C} receptors, but the affinity for the 5-HT_2 receptor is fortyfold higher (38).

The serum concentrations of desipramine and mianserin measured are comparable to those reported to be therapeutic in humans (12,19,29), and it seems unlikely that any important pharmacokinetic interaction between the drugs has taken place. With most antidepressants, a plasma concentration of 25 nM or higher is required to obtain a clinical effect, although 1–5 nM is sufficient to occupy the binding sites for these antidepressants on the serotonin transporter (25). It has also been claimed that serum concentrations above 160 ng/ml in humans may reduce the therapeutic response (12).

The study indicates an interaction between 5-HT_{1A} and 5-HT_2 receptor subtypes, as previously reported (1). In the DMI/MIAN group, no synergistic interaction between the 5-HT_{1A} agonist and the 5-HT_2 agonist was found. This lack of enhancement may indicate that the 5-HT_{1A} receptors are either maximally upregulated, as measured functionally after the combined treatment, or that the 5-HT_2 receptors are almost inactive functionally after the combined treatment, at least as regards the influence on the 5-HT_{1A} receptors, and that no additional effect is possible to gain from the interaction between these two receptor subtypes.

In agreement with many clinical reports, this study supports the hypothesis of an increased sensitivity of the 5-HT_{1A} receptors after chronic antidepressant treatments. The combination of desipramine and mianserin further enhances this increase. The functional response to costimulation of the 5-HT_{1A} and 5-HT_2 receptor subtypes is altered after the combined treatment, indicating that a further elucidation of the interaction between them may be of importance for our understanding of the action of antidepressant treatments.

ACKNOWLEDGEMENTS

The authors wish to thank Arve Madsen, Christian Helsengren, Anita Kloster, and Torhild Sunde for excellent technical assistance. The study was supported by the Norwegian Research Council for Science and the Humanities and by the Norwegian Cancer Society.

REFERENCES

1. Arnt, J.; Hyttel, J. Facilitation of 8-OHDPAT-induced forepaw treading of rats by the 5-HT_2 agonist DOI. Eur. J. Pharmacol. 161:45–51; 1989.
2. Avtrutsky, G. Treatment of resistant endogenous depression. Biol. Psychiatry 29:624S; 1991.
3. Baldessarini, R. J. Drugs and the treatment of psychiatric disorders. In: Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. The pharmacological basis of therapeutics. New York: Pergamon Press; 1990:383–435.
4. Blier, P.; De Montigny, C.; Chaput, Y. A role for the serotonin system in the mechanism of action of antidepressant treatments: Preclinical evidence. J. Clin. Psychiatry 51:14–20; 1990.
5. Cowen, P. J. Prolactin response to tryptophan during mianserin treatment. Am. J. Psychiatry 45:193–194; 1988.
6. Cowen, P. J.; McCance, S. L.; Cohen, P. R.; Julier, D. L. Lithium increases 5-HT-mediated neuroendocrine responses in tricyclic resistant depression. Psychopharmacology (Berlin) 99:230–232; 1989.
7. Cowen, P. J. Antidepressant: Current concepts of mode of action. Int. Clin. Psychol. Pharmacol. 5:45–55; 1990.
8. Deakin, J. F. W.; Green, A. R. The effects of putative 5-hydroxytryptamine antagonists on the behaviour produced by administration of tranylcypromine and L-tryptophan ortranylcypromine and L-DOPA to rats. Br. J. Pharmacol. 64:201–209; 1978.
9. Deakin, J. F. W. 5-HT receptor subtypes in depression, In: Bevan, P.; Cools, A. R.; Archer, T., eds. Behavioural pharmacology of 5-HT. Hillsdale, NJ: Lawrence Erlbaum Associates; 1989: 179–204.
10. Deakin, J. F. W.; Pennell, I.; Upadhyaya, A. J.; Lofthouse, R.

A neuroendocrine study of 5HT function in depression: Evidence for biological mechanisms of endogenous and psychosocial causation. Psychopharmacology (Berlin) 101:85–92; 1990.
11. Dijcks, F. A.; Ruigt, G. S.; De Graaf, J. S. Antidepressants affect amine modulation of neurotransmission in the rat hippocampal slice-I. Delayed effects. Neuropharmacology 30:1141–1150; 1991.
12. Friedel, R. O.; Veith, R. C.; Bloom, V.; Bielski, R. J. Desipramine plasma levels and clinical response in depressed outpatients. Commmun. Psychopharmacol. 3:81–87; 1979.
13. Glennon, R. A. Serotonin receptors: Clinical implications. Neurosci. Biobehav. Rev. 14:35–47; 1990.
14. Godbout, R.; Chaput, Y.; Blier, P.; de Montigny, C. Tandospirone and its metabolite, 1-(2-pyrimidinyl)-piperazine-l. Effects of acute and long-term administration of tandospirone on serotonin neurotransmission. Neuropharmacology 30:679–690; 1991.
15. Goodwin, G. M. The effects of antidepressant treatments and lithium upon 5-HT_{1A} receptor function. Prog. Neuropsychopharmacol. Biol. Psychiatry 13:445–451; 1989.
16. Grahame-Smith, D. G. Serotonin function in affective disorders. Acta Psychiatr. Scand. 80:7–12; 1989.
17. Hamon, M.; Emerit, M. B.; El Mestikawy, S.; Vergé, D.; Daval, G.; Marquet, A.; Gozlan, H. Pharmacological, biochemical and functional properties of 5-HT_{1A} receptor binding sites labelled by [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin in the rat brain. In: Dourish, C. T.; Ahlenius, S.; Hutson, P. H., eds. Brain 5-HT_{1A} receptors. Chichester: Ellis Horwood Ltd.; 1987:34–51.
18. Hamon, M.; Collin, E.; Chantrel, D.; Daval, G.; Verge, D.; Bourgoin, S.; Cesselin, F. Serotonin receptors and the modulation of pain. In: Besson, J.-M., ed. Serotonin and pain. Amsterdam: Excerpta Medica; 1990:53–72.
19. Hollister, L. E. Overview: Measuring plasma concentrations of psychotherapeutic drugs. In: Gram, L. F.; Usdin, E.; Dahl, S. G.; Kragh-Sørensen, P.; Sjøqvist, F.; Morselli, P. L., eds. Clinical pharmacology in psychiatry. London: Macmillan Press; 1983: 129–135.
20. Hoyer, D. Functional correlates of serotonin 5-HT_1 recognition sites. J. Recept. Res. 8:59–81; 1988.
21. Lesch, K. P.; Mayer, S.; Disselkamp-Tietze, J.; Hoh, A.; Wiesmann, M.; Osterheider, M.; Schulte, H. M. 5-HT_{1a} receptor responsivity in unipolar depression. Evaluation of ipsapirone-induced ACTH and cortisol secretion in patients and controls. Biol. Psychiatry 28:620–628; 1990.
22. Lesch, K. P.; Mayer, S.; Disselkamp-Tietze, J.; Hoh, A.; Schoellnhammer, G.; Schulte, H. M. Subsensitivity of the 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor-mediated hypothermic response to ipsapirone in unipolar depression. Life Sci. 46:1271–1277; 1990.
23. Lund, A.; Mjellem Joly, N.; Hole, K. Chronic administration of desipramine and zimelidine changes the behavioural response in the formalin test in rats. Neuropharmacology 30:481–487; 1991.
24. Lund, A.; Mjellem-Joly, N.; Hole, K. Desipramine administered chronically influences the 5-hydroxytryptamine$_{1A}$ receptors as measured by behavioural tests and receptor binding in rats. Neuropharmacology 31:25–32; 1992.
25. Lunde, A.; Mellerup, E. T.; Plenge, P. Receptor changes in rat brain after treatment with combinations of antidepressants. Biol. Psychiatry 29:302S; 1991.
26. Mann, J. J.; Arango, V.; Marzuk, P. M.; Theccanat, S.; Reis, D. J. Evidence for the 5-HT hypothesis of suicide: A review of postmortem studies. Br. J. Psychiatry 155:7–14; 1989.
27. Medhus, A.; Heskestad, S.; Thue, J. F. A combination effect of mianserin (Tolvon) and tricyclic antidepressive agents. Improved antidepressive therapy. J. Norwegian Med. Assoc. 110:3527–3528; 1990.
28. Morris, J. B.; Beck, A. T. The efficacy of antidepressant drugs. A review of research 1958 to 1972. Arch. Gen. Psychiatry 30: 667–674; 1974.
29. Nelson, J. C.; Jatlow, P.; Quinlan, D. M.; Bowers, M. B. Desipramine plasma concentration and antidepressant response. Arch. Gen. Psychiatry 39:1419–1422; 1982.
30. Peroutka, S. J. 5-Hydroxytryptamine receptor subtypes. Annu. Rev. Neurosci. 11:45–60; 1988.
31. Pinder, R. M.; van Delft, A. M. L. Pharmacological aspects of mianserin. Acta Psychiatr. Scand. Suppl. 302:59–71; 1983.
32. Racagni, G.; Tinelli, D.; Bianchi, E.; Brunello, N.; Perez, J. CAMP-dependent proteins and endogenous phosphorylation after antidepressant treatment. In: Sandler, M.; Coppen, A.; Harnett, S., eds. 5-Hydroxytryptamine in psychiatry. Oxford: Oxford University Press; 1991:116–123.
33. Rausch, J. L.; Ruegg, R.; Moeller, F. G. Gepirone as a 5-HT_{1A} agonist in the treatment of major depression. Psychopharmacol. Bull. 26:169–171; 1990.
34. Sleight, A. J.; Pierce, P. A.; Schmidt, A. W.; Hekmatpanah, C. R.; Peroutka, S. J. The clinical utility of serotonin receptor active agents in neuropsychiatric disease. In: Peroutka, S. J., ed. Serotonin receptor subtypes: Basic and clinical aspects. New York: Wiley-Liss; 1991:211–227.
35. Smith, R. L.; Barrett, R. J.; Sanders-Bush, E. Adaptation of brain 5HT_2 receptors after mianserin treatment: Receptor sensitivity, not receptor binding, more accurately correlates with behaviour. J. Pharmacol. Exp. Ther. 254:484–488; 1990.
36. Sulser, F. Serotonin-norepinephrine receptor interactions in the brain: Implications for the pharmacology and pathophysiology of affective disorders. J. Clin. Psychiatry 48:12–18; 1987.
37. Syha, K.; Schraven, E. Effects of a chronic administration of the new antidepressant pirlindole on biogenic amine receptors in rat prefrontal cortex and hippocampus. Arzneimittelforschung 40: 843–847; 1990.
38. Titeler, M.; Lyon, R. A.; Glennon, R. A. Radioligand binding evidence implicates the brain 5-HT_2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology (Berlin) 94:213–216; 1988.
39. Tjølsen, A.; Rosland, J. H.; Berge, O.-G.; Hole, K. The increasing temperature hot plate test: An improved test of nociception in mice and rats. J. Pharmacol. Methods 25:241–250; 1991.
40. Van Praag, H. M.; Kahn, R.; Asnis, G. M.; Lemus, C. Z.; Brown, S. L. Therapeutic indications for serotonin-potentiating compounds: A hypothesis. Biol. Psychiatry 22:205–212; 1987.
41. Willner, P. Antidepressants and serotonergic neurotransmission, an integrative review. Psychopharmacology (Berlin) 85:387–404; 1985.