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ARTICLE *in* NEUROPHARMACOLOGY · MARCH 1994

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Neuropharmacology Vol. 33, No. 3/4, pp. 501–507, 1994

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Interaction between Enantiomers of Mianserin and ORG3770 at 5-HT_3 Receptors in Cultured Mouse Neuroblastoma Cells

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(Accepted 21 December 1993)

Summary—Stereoselective effects of mianserin and ORG3770 on serotonin 5-HT_3 receptors in mouse neuroblastoma N1E-115 cells have been investigated in radioligand binding and in whole-cell voltage clamp experiments. The specific binding of [^{3}H]GR65630 to 5-HT_3 recognition sites in N1E-115 cell homogenates is reduced by mianserin and ORG3770 and their enantiomers. The pK_i values of the more potent (*R*)enantiomers of mianserin and ORG3770 are 8.44 and 8.62, respectively. The (*R*)enantiomers of mianserin and ORG3770 are 15 and 37 times more potent than their respective (*S*)enantiomers. The racemates are only 1.9 and 3.3 times less potent than the corresponding (*R*)enantiomers. In voltage clamp experiments the (*R*)enantiomers block the 5-hydroxytryptamine(5-HT)-induced ion current with pIC_{50} values of 8.52 for (*R*)mianserin and 8.26 for the (*R*)enantiomer of ORG3770. The (*R*)enantiomers of mianserin and ORG3770 are 24 and 145 times more potent in blocking the 5-HT-induced ion current than their respective (*S*)enantiomers. The racemates are 6 and 13 times less potent than the corresponding (*R*)enantiomers. In addition, the block of 5-HT-induced ion current by the (*R*)enantiomer of ORG3770 is partially reversed by a low concentration of its (*S*)enantiomer. The results indicate that the two enantiomers block the 5-HT_3 receptor-mediated ion current in a mutually dependent manner.

Keywords—N1E-115 cells; 5-hydroxytryptamine; radioligand binding; voltage clamp; ion current; GR65630; mianserin; ORG3770; antidepressant; 5-HT_3 receptor.

Radioligand binding experiments have demonstrated the presence of 5-HT_3 recognition sites in the central nervous system. Specific binding of 5-HT_3 receptor antagonists was described in homogenates of various parts of rat brain and particularly in entorhinal cortex (Kilpatrick *et al.*, 1987; Watling *et al.*, 1988). Receptor autoradiography in mouse and in post mortem human tissues has revealed specific binding of [^{3}H]tropisetron in discrete regions of the brainstem and the spinal cord and, to a lesser extent, in limbic forebrain structures (Waeber *et al.*, 1988, 1989). The detailed investigation of central 5-HT_3 receptors is hampered by the low receptor density (Kilpatrick *et al.*, 1987) and the ensuing difficulties to record 5-HT_3 responses from central neurons (Yakel and Jackson, 1988; Yakel *et al.*, 1988; Ropert and Guy, 1991; Sugita *et al.*, 1992). Conversely, mouse neuroblastoma N1E-115 and neurohybrid NG108-15 (Hoyer and Neijt, 1988; Neijt *et al.*, 1988b) and NCB20 cells (Sharif *et al.*, 1991) express 5-HT_3 receptors at relatively high densities and are readily available to perform ligand binding and electrophysiological experiments. The functional properties of 5-HT_3 receptors in N1E-115 cells have been investigated in detail (Neijt *et al.*, 1986, 1988a, 1989). High correlations between ligand affinities indicate that the 5-HT_3 receptors in neuroblastoma cells are very similar to those in rat cortex (Hoyer *et al.*, 1989; Milburn and Peroutka, 1989; Bolaños *et al.*, 1990; Sharif *et al.*, 1991).

The antidepressants mianserin and its 6-aza-analogue ORG3770 (Smith *et al.*, 1990), are potent histamine H_1 and serotonin 5-HT_2 receptor antagonists in rat brain and enhance the release of noradrenaline and 5-HT from cortex slices and synaptosomes by antagonizing α_2-adrenoceptors. Enantiomers of mianserin and of ORG3770 stereoselectively antagonize 5-HT_1, 5-HT_2 and α_2 receptors, the (*S*)enantiomers being more potent than the (*R*)enantiomers (Alexander and Wood, 1987; De Boer *et al.*, 1988). Binding of racemic mianserin to 5-HT_3 receptors was first demonstrated in N1E-115 cells using [^{3}H]tropisetron as radioligand (Hoyer and Neijt,

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1988). Similar results have been obtained in other neuronal cell lines and in rat brain homogenates (Hoyer *et al.*, 1989; Schmidt and Peroutka, 1989; Bolaños *et al.*, 1990). Preliminary results from our laboratory showed that the enantiomers of ORG3770 stereoselectively block 5-HT_3 receptor-operated ion current in voltage clamped N1E-115 cells (Zwart *et al.*, 1990). Recently, similar stereoselective effects of the enantiomers of mianserin in displacing [^{3}H]granisetron from 5-HT_3 receptors in rat cortex membranes and in blocking the 5-HT-induced depolarizing response in rat vagus nerve have been reported (Wood *et al.*, 1993).

We have compared the effects of racemic mianserin, ORG3770 and their respective enantiomers on 5-HT_3 receptors in N1E-115 cells in ligand binding and in whole-cell voltage clamp experiments.

METHODS

Cell culture

Mouse neuroblastoma cells of the clone N1E-115 (Amano *et al.*, 1972) were grown under conditions identical to those described previously (Neijt *et al.*, 1989). Subcultures of passage numbers 31–42 were used. For ligand binding studies undifferentiated cells were frozen in external solution at −80°C. For electrophysiology cells were grown in 3.5 cm tissue culture dishes and were initiated to differentiate on day 2 in subculture with culture medium supplemented with 1 mM dibutyryladenosine 3′:5′-cyclic monophosphate and 1 mM 3-isobutyl-1-methylxanthine. This medium was refreshed about every other day. Cells were used for experiments on days 7–12 after differentiation.

Ligand binding

Frozen cells were thawed and diluted in cold incubation buffer to obtain a final concentration of 4×10^5 cells ml^{-1}. Cells were homogenized by a Polytron homogenizer for 20 sec at maximal setting. The tritiated selective 5-HT_3 receptor antagonist [^{3}H]GR65630 (specific activity 79.2 Ci $mmol^{-1}$) was used as radioligand.

For binding assays 400 μl of cell homogenate, 50 μl of radioligand and 50 μl of either control buffer or compound containing buffer were incubated at room temperature (20–24°C). Experiments were started by the addition of cell homogenates to glass incubation vials that contained radioligand and compound. The incubation was stopped by rapid filtration over Whatman GF/B glass fiber filters on a Brandell MR24 cell harvester. Filters were washed three times for 5 sec with ice-cold 5 mM Tris–HCl buffer (pH 7.4). Filter bound radioactivity was counted for 3 min in scintillation vials containing 2.5 ml Ultima Gold MV (Packard) using a Packard instruments 2200CA liquid scintillation spectrophotometer. Non-specific binding was determined in the presence of an excess of the 5-HT_3 receptor antagonist MDL 72222. Displacement was determined from triplicate samples incubated for 15 min with 6 concentrations of compound and 0.3–1.0 nM [^{3}H]GR65630. Exact concentrations of radioligand were calculated from the total radioactivity added to the incubation mixtures.

K_i values were calculated from fitted IC_{50} values according to the equation:

$$K_i = IC_{50}/(1 + [L]/K_D) \quad (1)$$

(Cheng and Prusoff, 1973). A K_D value of 0.68 nM for [^{3}H]GR65630 was determined in separate experiments (A. R. Kooyman, unpublished observation). This is virtually identical to a previously reported value of 0.69 nM (Lummis *et al.*, 1990).

Electrophysiology

Ion currents were recorded using a suction pipette technique for whole-cell voltage clamp (Lee *et al.*, 1978) identical to methods described previously (Neijt *et al.*, 1989). Cells were voltage-clamped at −60 mV. Cells were continuously superfused with external solution or

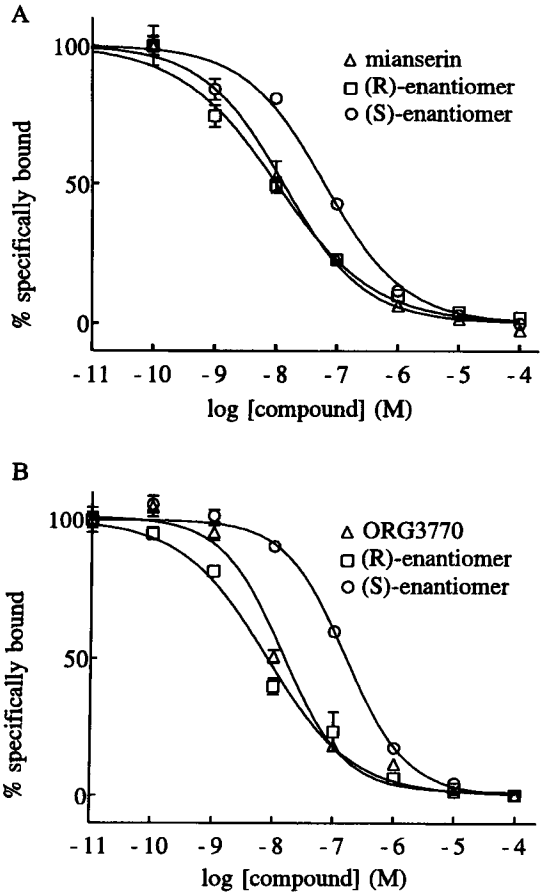

Fig. 1. (A) Displacement of 0.3 nM [^{3}H]GR65630 from 5-HT_3 recognition sites in N1E-115 cell homogenates by (*R,S*)mianserin and its enantiomers. (B) Displacement of 0.3 nM [^{3}H]GR65630 by ORG3770 and its enantiomers. Symbols represent the mean percentage of specifically bound radioligand ± SEM from triplicate measurements in one experiment. The absence of error bars indicates that the SEM is smaller than the size of the symbol. Concentration–effect curves were fitted according to Eqn. (2).

Table 1. Affinity values and slope factors obtained from concentration–effect curves of racemic mianserin, ORG3770 and their respective enantiomers for the displacement of approximately 0.3 nM [^{3}H]GR65630 specifically bound to 5-HT$_3$ recognition sites in N1E-115 cell homogenates. Values represent mean ± SD of 3 independent experiments

	Mianserin			ORG3770		
Enantiomer	IC_{50} (nM)	pK_i	$-n$	IC_{50} (nM)	pK_i	$-n$
$R,S(\pm)$	10.6 ± 2.3	8.15 ± 0.02	0.68 ± 0.04	11.8 ± 2.0	8.10 ± 0.15	0.92 ± 0.07
$S(+)$	73.7 ± 6.3	7.27 ± 0.10	1.07 ± 0.60	150.1 ± 89.6	7.05 ± 0.33	0.73 ± 0.17
$R(-)$	6.2 ± 3.8	8.44 ± 0.30	0.68 ± 0.30	3.9 ± 2.1	8.62 ± 0.17	0.99 ± 0.20

compound-containing external solution. Ion currents were evoked by changing to agonist- or agonist- and compound-containing external solution. Series resistance initially estimated from the instantaneous voltage jump in response to a constant current stimulus was compensated under voltage clamp for about 80–85%. To maintain cells at holding potential in the absence of any stimulation, a steady inward current injection was required that varied between cells in the range of 0.1–0.5 nA. Membrane currents were low-pass filtered (−3 dB at 1 kHz, 12 dB/octave), digitized (8 bits, 1024 points/record) and stored on a magnetic disc for off-line computer analysis. Cells were exposed to the agonist at intervals of at least 100 sec in order to allow complete recovery from desensitization. All experiments were performed at room temperature (20–24°C).

Data are expressed as mean ± SD of n independent experiments. Concentration–effect curves were fitted using a Levenberg–Marquardt non-linear least squares algorithm (Marquardt, 1963) according to the function:

$$i = i_{max}/\{1 + (IC_{50}/[drug])^n\} \quad (2)$$

Solutions

The ionic composition of the pipette solution was (in mM): K-glutamate 100, Na-HEPES 20, sucrose 120. The pH was adjusted to 7.25 with L-glutamic acid. The external solution contained (in mM): NaCl 125, KCl 5.5, HEPES 20, $CaCl_2$, 1.8, $MgCl_2$ 0.8, glucose 24 and sucrose 37, and the pH was adjusted to 7.3 with approx 7 mM NaOH. The incubation buffer used in ligand binding studies was identical to the external solution.

Compounds

[^{3}H]GR65630 ([^{3}H]-3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone) was obtained from DuPont NEN, 's-Hertogenbosch, The Netherlands; MDL 72222 (3-tropanyl-3,5-dichlorobenzoate) from Research Biochemicals Inc., Natick, U.S.A., and 5-HT (5-hydroxytryptamine creatine sulphate) from Sigma, St Louis, U.S.A. (*R,S*)mianserin–HCl (1,2,3,4,10,14b-hexa-hydro-2-methyldibenzo[c,f]pyrazino [1,2-a] monohydro-chloride), ORG3770 (mirtazepine, remeron®) and their respective enantiomers were provided by Organon International B.V., Oss, The Netherlands.

(*R,S*)mianserin and its enantiomers (*R*)mianserin (ORG5859) and (*S*)mianserin (ORG4360) were dissolved in distilled water to obtain 1 and 10 mM stock solutions. ORG3770 and its (*R*)- and (*S*)enantiomers ORG4419 and ORG4420, respectively, were dissolved in dimethylsulfoxide to obtain 1 μM–10 mM stock solutions. A concentrated stock solution of 10 mM 5-HT in distilled water was divided into small quantities, which were frozen at −20°C and thawed prior to the experiments. Test solutions of the compounds were prepared daily by mixing 10–500 μl of stock solutions with 100 ml of external solution.

RESULTS

Radioligand binding studies

The 5-HT$_3$ receptor antagonist MDL 72222 displaced the binding of [^{3}H]GR65630 in a concentration-dependent manner. The calculated mean pK_i value as

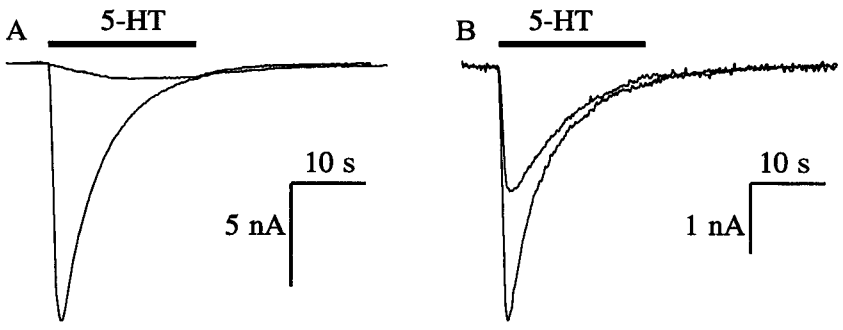

Fig. 2. Block of the 5-HT-induced inward current by (*R*)mianserin. Superimposed ion currents were evoked by superfusion with 5 μM 5-HT (solid bar) before and in the presence of (*R*)mianserin. The concentrations of (*R*)mianserin were 100 nM (A) and 3.3 nM (B). In the presence of the high concentration of (*R*)mianserin the kinetics of the remaining, small inward current are slowed.

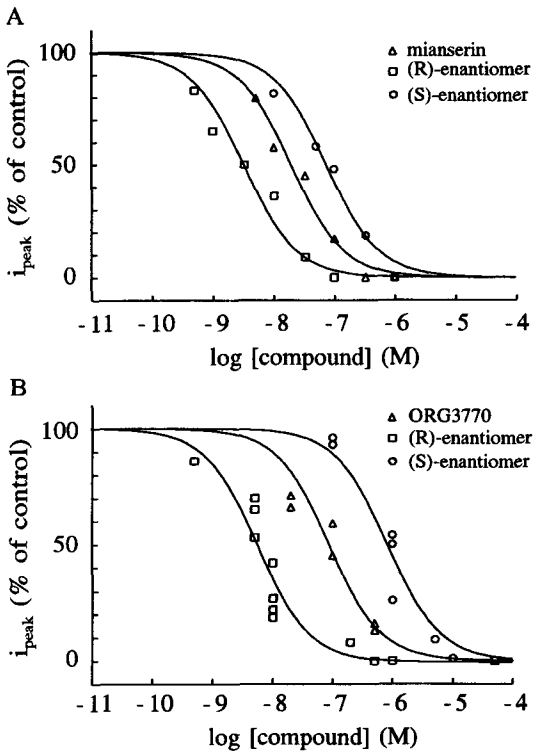

Fig. 3. (A) Concentration–effect curves of the antagonism of 5-HT-induced inward current by (*R,S*)mianserin and its enantiomers (*S*)mianserin and (*R*)mianserin obtained from 6, 6 and 5 cells, respectively. The amplitudes of inward currents evoked with 5 μM 5-HT in the presence of various concentrations of mianserin or its enantiomers were normalized to control inward current amplitudes evoked with the same concentration of 5-HT in the absence of the antagonist. (B) Concentration–effect curve of the antagonism of 5-HT-induced inward current by the racemic ORG3770, its (*R*)enantiomer ORG4419 and its (*S*)enantiomer ORG4420 obtained from 6, 13 and 8 cells, respectively. The amplitudes of inward currents evoked with 5 μM 5-HT in the presence of various concentrations of ORG3770 or its enantiomers were normalized to control inward current amplitudes evoked with the same concentration of 5-HT in the absence of the antagonist. For clarity all curves drawn are computer fits for a one site model.

determined from 4 independent experiments amounts to 7.59 ± 0.07 and the mean slope value of the displacement curves is 0.7. At high concentrations of MDL 72222 a plateau phase was reached. In all subsequent binding experiments the radioactivity remaining in the presence of 100 μM MDL 72222 was subtracted from total binding to calculate specific radioligand binding.

Racemic (*R,S*)mianserin and the pure (*R*)- and (*S*)enantiomers displaced [^{3}H]GR65630 from 5-HT$_3$ recognition sites in homogenates of N1E-115 cells in a concentration-dependent manner. The maximum effect obtained at high concentrations of the drugs was the same as that of 100 μM MDL 72222. The displacement curves of the radioligand by (*R,S*)mianserin and its enantiomers [Fig. 1(A)] shows that the racemic mixture is only slightly less potent than the (*R*)enantiomer and that both the racemate and the (*R*)enantiomer are more potent than the (*S*)enantiomer. Similar stereoselective displacement of [^{3}H]GR65630 bound to 5-HT$_3$ recognition sites was obtained with ORG3770, and with its enantiomers [Fig. 1(B)]. The mean pK_i values and slope factors obtained from three independent experiments are given in Table 1. The mean values of the slope factors were close to unity. Based on the mean pK_i values the (*S*)enantiomers are less potent than the corresponding (*R*)enantiomers, i.e. by a factor of 15 for mianserin and by a factor of 37 for ORG3770. Compared with their racemates (*R*)mianserin and the (*R*)enantiomer of ORG3770 were 1.9 and 3.3 times more potent, respectively.

Electrophysiology

In whole-cell voltage clamped N1E-115 cells mianserin, ORG3770 and their respective enantiomers block the ion current evoked by superfusion with 5 μM 5-HT. Figure 2 shows effects of two concentrations of (*R*)mianserin on 5-HT$_3$ receptor-operated ion current. Ion current block is induced rapidly and in most cases steady effects were obtained within 2 min of superfusion with the drug. At the higher concentrations of the drugs [Fig. 2(A)] the kinetics of the remaining 5-HT-induced inward current are slowed. Effects of low concentrations of the drugs were completely reversed on washing with control external solution, but the reversal of the effects of high concentrations of the drugs was generally incomplete. In control experiments the superfusion of cells with external solution containing 500 nM of the (*R*)enantiomer of ORG3770 for 20 sec did not evoke any detectable membrane current, indicating the absence of agonistic activity. In all other experiments in which mianserin and ORG3770 were superfused agonist effects

Table 2. Potencies of racemic mianserin, ORG3770 and their respective enantiomers for the block of 5 μM 5-HT-induced inward current in voltage clamped N1E-115 cells

	Mianserin			ORG3770		
Enantiomer	IC_{50} (nM)	pIC_{50}	$-n$	IC_{50} (nM)	pIC_{50}	$-n$
R,S(±)	19 ± 3	7.72	0.9 ± 0.1	76 ± 18	7.12	0.7 ± 0.2
S(+)	72 ± 12	7.14	0.9 ± 0.2	870 ± 110	6.06	1.4 ± 0.4
R(−)	3 ± 0.6	8.52	0.8 ± 0.1	6 ± 1	8.26	2.0 ± 0.5

Values represent mean ± estimated SD by fitting concentration–effect curves to data obtained from 5–13 cells. The IC_{50} value estimates were not significantly altered when a one site model with a fixed slope factor of −1 was fitted.

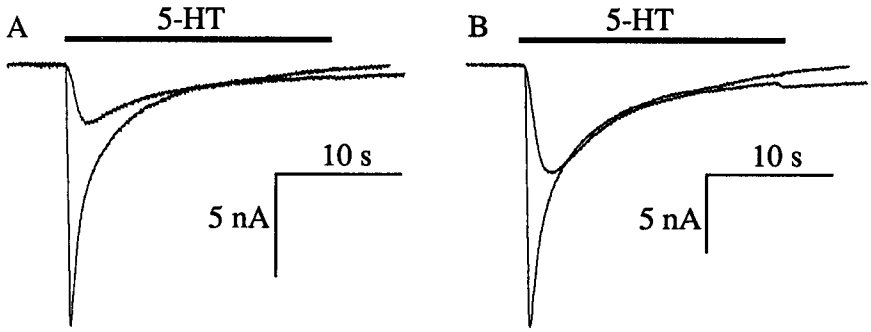

Fig. 4. Functional interaction between (*R*)- and (*S*)enantiomers of ORG3770. (A) Block of the 5-HT-induced ion current by 10 nM of the (*R*)enantiomer. Control response evoked by superfusion with 5 μM 5-HT (solid bar) before and the response evoked by the same concentration of 5-HT in the presence of 10 nM of the (*R*)enantiomer are superimposed. The peak amplitude is reduced to 23% of the control value. (B) Block of the 5-HT-induced ion current in the presence of both (*R*)- and (*S*)enantiomers of ORG3770. Control response and the response in the presence of 10 nM of each enantiomer are superimposed. The peak amplitude is reduced to 41% of the control value of the mixture of enantiomers, which is less than the block observed with the (*R*)enantiomer alone (A). The responses in (B) were recorded first and after washout of the effect the responses in (A) were recorded from the same cell. A reversed experimental protocol in two other cells yielded identical results.

were never observed (results not shown). These results indicate that the drugs reversibly antagonize the 5-HT$_3$ receptor-mediated response and lack agonist activity.

Concentration–effect curves were obtained for the racemic mixtures and their enantiomers. To avoid problems of cumulative effects and reversibility cells were exposed to a single concentration of antagonist only. Effects were standardized by normalizing the peak amplitude of the (partially) blocked response in the steady presence of the drug to the peak amplitude of a control response obtained from the same cell before application of the drug. Fitting the data obtained in this way from 5–13 cells for each compound yielded estimates of the IC_{50} values and of their standard deviations. The fitted curves in Fig. 3 and the estimated IC_{50} values presented in Table 2 demonstrate that the (*R*)enantiomers are the more potent functional antagonists with half maximum blocking concentrations of 3 nM for (*R*)mianserin and of 6 nM for the (*R*)enantiomer of ORG3770. From the IC_{50} values obtained in the functional assay the (*R*)enantiomers of mianserin and ORG3770 appear 24 and 145 times more potent than the corresponding (*S*)enantiomers and the (*R*)enantiomers are 6 and 13 times more potent than the respective racemates (see Table 2). The relative potencies of the racemates obtained in the voltage clamp experiments are less than might be expected from the radioligand binding experiments, in which the (*R*)enantiomers are only 2–4 times more potent than the racemates.

In order to investigate this apparent difference between the relative potencies of the racemates in radioligand binding and voltage clamp experiments the effect of 10 nM of the (*R*)enantiomer of ORG3770 on 5-HT$_3$ receptor-operated ion current was measured in the absence and in the presence of 10 nM of the (*S*)enantiomer. At this low concentration both the ability to displace [^{3}H]GR65630 and the ion current blocking effect of the (*S*)enantiomer on itself were very small (see Figs 1 and 3). In three cells 10 nM of the (*R*)enantiomer reduced the peak amplitude of 5-HT-induced inward current to 22.5 $\pm$ 4.3% of control. In the same cells the superfusion of 10 nM of the (*R*)enantiomer and 10 nM of the (*S*)enantiomer, simultaneously, did not cause enhanced block. Instead, the fraction of the 5-HT-induced inward current remaining in the presence of both (*R*)- and (*S*)enantiomers was 2.0 $\pm$ 0.5 times larger than in the presence of the (*R*)enantiomer alone (Fig. 4).

DISCUSSION

The results show that the antidepressants mianserin and ORG3770 show considerable affinity for the 5-HT$_3$ recognition sites and that these drugs are functional antagonists of the 5-HT$_3$ receptor-operated ion current in cultured mouse neuroblastoma cells. Mianserin and ORG3770 enantiomers appear devoid of any agonist activity.

Within experimental error racemic mianserin and ORG3770 as well as the respective enantiomers are equipotent in displacing [^{3}H]GR65630 binding to 5-HT$_3$ receptors in N1E-115 cell homogenates (Table 1). The recently reported pK_i values for the displacement of [^{3}H]granisetron in rat cortical membranes by (*R*)- and (*S*)mianserin of 8.46 and 6.95 (Wood *et al.*, 1993) are very similar to the presently calculated values of 8.44 and 7.27. The comparison suggests that the 5-HT$_3$ receptors in the two preparations are very similar with respect to the stereoselective interaction of the enantiomers of mianserin. In addition, the results further corroborate that the stereoselectivity of mianserin and ORG3770 enantiomers for 5-HT$_3$ receptors (Zwart *et al.*, 1990;

Wood *et al.*, 1993) is reversed with respect to the stereoselectivity for other subtypes of 5-HT receptors, H_1 receptors and for α_2 receptors (Alexander and Wood, 1987; De Boer *et al.*, 1988).

Using the radioligand [^{3}H]tropisetron pK_i values of racemic mianserin were 7.2 for N1E-115 and 7.0 for NG108-15 cells (Hoyer and Neijt, 1988; Neijt *et al.*, 1988b) and in rat cortex membranes racemic mianserin inhibits [^{3}H]quipazine binding with a pIC_{50} of 7.2 (Schmidt and Peroutka, 1989) and [^{3}H]zacopride binding with a pK_i of 6.7 (Bolaños *et al.*, 1990). These values are well below the presently obtained pK_i of 8.15 and pIC_{50} of 7.72 for racemic mianserin. It has previously been suggested that the use of distinct radioligands is a potential source of variability in the apparent affinity of antidepressants for 5-HT_3 receptors (Hoyer *et al.*, 1989; Wood *et al.*, 1993). This notion is supported by the recent finding that [^{3}H]granisetron and [^{3}H](*S*)zacopride may label different sites on the 5-HT_3 receptor in NG108-15 cells (Barnes and Barnes, 1993).

In voltage clamp experiments mianserin and ORG3770 blocked the 5-HT-induced ion current with an enantioselectivity qualitatively similar to that found in the binding assays. However, the potencies of the racemates relative to those of the enantiomers appeared to be different. In the radioligand binding experiments the racemates were only slightly less potent than the active enantiomers, which is to be expected from the reduced amount of the (*R*)enantiomers in the racemates and from the observed low potency of the (*S*)enantiomers alone (Fig. 1). In the electrophysiological experiments the concentration–effect curves of the racemates are at approximately the same logarithmic distance from those of the (*R*)- and (*S*)enantiomers (Fig. 3). This indicates that the two enantiomers interact either directly or at the receptor level. A direct chemical interaction between the enantiomers can be excluded from the finding that the racemates and the (*R*)enantiomers displace the radiolabeled antagonist [^{3}H]GR65630 with only slightly different potencies (Fig. 1). Moreover, a functional interaction between the two enantiomers of ORG3770 is directly demonstrated by the result in Fig. 4, which shows that the blocking effect of the (*R*)enantiomer on 5-HT-induced ion current is partially reversed in the presence of a low concentration of the (*S*)enantiomer. The clear evidence for a functional interaction between the enantiomers and the apparent absence of such interaction in the binding assay suggest that the effects of mianserin and ORG3770 on 'agonist' and 'antagonist' sites of the 5-HT_3 receptor are different. This could be explained by assuming different, partial overlaps between the binding sites for agonists, selective antagonists and the antidepressants. A possible approach to test this hypothesis would be to perform additional binding experiments with radiolabeled enantiomers of the antidepressants. Further, it would be interesting to establish whether similar interactions occur between the enantiomers of selective 5-HT_3 receptor antagonists, e.g. SDZ 210-204 and SDZ 210-205 with a 7-fold difference in potency in N1E-115 cells (Hoyer and Neijt, 1988) and zacopride with a 10-fold difference in enantiomer potency in rat cortex and in NG108-15 cells (Sharif *et al.*, 1991).

Acknowledgements—The authors wish to thank Ms Paula Martens for cell culture and Ing. Aart de Groot for technical assistance. Drs Gé Ruigt and Thijs de Boer of Organon International B.V., Oss, The Netherlands are acknowledged for suggesting experiments and for providing facilities to perform radioligand binding experiments. This investigation has been supported by the NWO foundation for Medical and Health Research (grant no. 900–553–021).

REFERENCES

Alexander B. S. and Wood M. D. (1987) Stereoselective blockade of central [^{3}H]5-hydroxytryptamine binding to multiple sites (5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}) by mianserin and propranolol. *J. Pharm. Pharmac.* **39:** 664–666.

Amano T., Richelson E. and Nirenberg P. G. (1972) Neurotransmitter synthesis by neuroblastoma clones. *Proc. Natn. Acad. Sci. U.S.A.* **69:** 258–263.

Barnes J. M. and Barnes N. M. (1993) Differential binding characteristics of agonists at 5-HT_3 receptor recognition sites in NG108-15 neuroblastoma-glioma cells labelled by [^{3}H](*S*)-zacopride and [^{3}H]granisetron. *Biochem. Pharmac.* **45:** 2155–2158.

Bolaños F. J., Schechter L. E., Miquel M. C., Emerit M. B., Rumigny J. F., Hamon M. and Gozlan H. (1990) Common pharmacological and physicochemical properties of 5-HT_3 binding sites in the rat cerebral cortex and NG108-15 clonal cells. *Biochem. Pharmac.* **40:** 1541–1550.

Cheng Y. C. and Prusoff W. H. (1973) Relationship between inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmac.* **22:** 3099–3108.

De Boer T. H., Maura G., Raiteri M., De Vos C. J., Wieringa J. and Pinder R. M. (1988) Neurochemical and autonomic pharmacological profiles of the 6-aza-analogue of mianserin, ORG3770 and its enantiomers. *Neuropharmacology* **27:** 399–408.

Hoyer D. and Neijt H. C. (1988) Identification of serotonin 5-HT_3 recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding. *Molec. Pharmac.* **33:** 303–309.

Hoyer D., Gozlan H., Bolaños F. J., Schechter L. E. and Hamon M. (1989) Interaction of psychotropic drugs with central 5-HT_3 recognition sites: fact or artifact? *Eur. J. Pharmac.* **171:** 137–139.

Kilpatrick G. J., Jones B. J. and Tyers M. B. (1987) Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. *Nature* **330:** 746–748.

Lee K. S., Akaike N. and Brown A. M. (1978) Properties of internally perfused, voltage clamped, isolated nerve cell bodies. *J. Gen. Physiol.* **71:** 489–507.

Lummis S. C. R., Kilpatrick G. J. and Martin I. L. (1990) Characterization of 5-HT_3 receptors in intact N1E-115 neuroblastoma cells. *Eur. J. Pharmac.* **189:** 223–227.

Marquardt D. W. (1963) An algorithm for least-squares estimation of nonlinear parameters. *J. Soc. Ind. Appl. Math.* **11,** 431–441.

Milburn C. M. and Peroutka S. J. (1989) Characterization of [^{3}H]quipazine binding to 5-hydroxytryptamine$_3$ receptors in rat brain membranes. *J. Neurochem.* **52:** 1787–1792.

Neijt H. C., Vijverberg H. P. M. and van den Bercken J. (1986) The dopamine response in mouse neuroblastoma cells is mediated by serotonin 5-HT_3 receptors. *Eur. J. Pharmac.* **127:** 271–274.

Neijt H. C., te Duits I. J. and Vijverberg H. P. M. (1988a) Pharmacological characterization of serotonin 5-HT_3 receptor-mediated electrical response in cultured mouse neuroblastoma cells. *Neuropharmacology* **27:** 301–307.

Neijt H. C., Karpf A., Schoeffter P., Engel G. and Hoyer D. (1988b) Characterization of 5-HT_3 recognition sites in membranes of NG108-15 neuroblastoma-glioma cells with [^{3}H]ICS 205-930. *Naunyn-Schmiedeberg's Archs Pharmac.* **337:** 493–499.

Neijt H. C., Plomp J. J. and Vijverberg H. P. M. (1989) Kinetics of the membrane current mediated by serotonin 5-HT_3 receptors in cultured mouse neuroblastoma cells. *J. Physiol., Lond.* **411:** 257–269.

Ropert N. and Guy N. (1991) Serotonin facilitates GABAergic transmission in the CA1 region of rat hippocampus *in vitro*. *J. Physiol., Lond.* **441,** 121–136.

Schmidt A. W. and Peroutka S. J. (1989) Antidepressant interactions with 5-hydroxytryptamine$_3$ receptor binding sites. *Eur. J. Pharmac.* **163:** 397–398.

Sharif N. A., Wong E. H. F., Loury D. N., Stefanich E., Michel A. D., Eglen R. M. and Whiting R. J. (1991) Characteristics of 5-HT_3 binding sites in NG108-15, NCB-20 neuroblastoma cells and rat cerebral cortex using [^{3}H]quipazine and [^{3}H]GR65630 binding. *Br. J. Pharmac.* **102:** 919–925.

Smith W. T., Glaudin V., Panagides J. and Gilvary E. (1990) Mirtazapine vs amitriptyline vs placebo in the treatment of major depressive disorder. *Psychopharmac. Bull.* **26:** 191–196.

Sugita S., Shen K. Z. and North R. A. (1992) 5-Hydroxytryptamine is a fast excitatory transmitter at 5-HT_3, receptors in rat amygdala. *Neuron* **8:** 199–203.

Waeber C., Dixon K., Hoyer D. and Palacios J. M. (1988) Localization by autoradiography of neuronal 5-HT_3 receptors in the mouse CNS. *Eur. J. Pharmac.* **151:** 351–352.

Waeber C., Hoyer D. and Palacios J. M. (1989) 5-Hydroxytryptamine$_3$ receptors in the human brain: autoradiographic visualization using [^{3}H]ICS 205-930. *Neuroscience* **31:** 393–400.

Watling K. J., Aspley S., Swain C. J. and Saunders J. (1988) [^{3}H]Quaternised ICS 205-930 labels 5-HT_3 receptor binding sites in rat brain. *Eur. J. Pharmac.* **149:** 397–398.

Wood M. D., Thomas R. D., Watkins C. J. and Newberry N. R. (1993) Stereoselective interaction of mianserin with 5-HT_3 receptors. *J. Pharm. Pharmac.* **45:** 711–714.

Yakel J. L. and Jackson M. B. (1988) 5-HT_3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. *Neuron* **1:** 615–621.

Yakel J. L., Trussell L. O. and Jackson M. B. (1988) Three serotonin responses in cultured mouse hippocampal and striatal neurons. *J. Neurosci.* **8:** 1273–1285.

Zwart R., Kooyman A. R. and Vijverberg H. P. M. (1990) Antagonist effect of antidepressants on 5-HT_3 receptor-mediated inward current in cultured mouse neuroblastoma cells. IUPHAR Satellite Symposium on Serotonin, Basle: P46.