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# 5-$HT_{1A}$ receptor activation and antidepressant-like effects: F 13714 has high efficacy and marked antidepressant potential

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**Abstract**

To examine further the hypothesis that the magnitude of the intrinsic activity of agonists at 5-$HT_{1A}$ receptors determines the magnitude of their psychotropic activity, we studied the relationship between the maximal receptor activation produced by various 5-$HT_{1A}$ receptor ligands and their antidepressant-like effects (i.e., decreased immobility in the forced swimming test in rats). Using three different in vitro assays suitable to measure differences among high, intermediate, and low efficacy 5-$HT_{1A}$ receptor agonists, ligands were identified with intrinsic activities ranging from low-negative (i.e., the inverse agonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexane-carboxamide (WAY 100635)) to high-positive (i.e., 3-chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methyl-6-methylamino-pyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl-methanone (F 13714)). In addition, novel compounds with intermediate intrinsic activity, like buspirone, but with high selectivity for 5-$HT_{1A}$ receptors, unlike buspirone, were identified. The maximal effects of the 5-$HT_{1A}$ receptor ligands in the forced swimming test correlated positively ($r_S = 0.91$, $P < 0.005$) with the rank order of their intrinsic activity at 5-$HT_{1A}$ receptors. This relationship constitutes evidence that the magnitude of the psychotropic activity of 5-$HT_{1A}$ receptor ligands is a positive function of their intrinsic activity at the receptor, and suggests that F 13714, which had maximal effects in the forced swimming test significantly larger than any of the other compounds examined here, did so because of its higher intrinsic activity at 5-$HT_{1A}$ receptors. 

*Keywords:* 5-$HT_{1A}$ receptor; Intrinsic activity; [$^{35}$S]GTPγS binding; cAMP; Forced swimming test; Antidepressant; (Rat)

## 1. Introduction

The introduction of buspirone for the treatment of anxiety, and also of depression (see reviews by Tunnicliff et al., 1991; Fulton and Brogden, 1997), suggested a novel mechanism of anxiolytic and antidepressant drug action: direct activation of a 5-HT receptor subtype, the 5-$HT_{1A}$ receptor. The clinical effectiveness of buspirone and its analogues, gepirone and ipsapirone, however, does not appear to be an improvement over that of other available treatments (Deakin, 1993). Their limited clinical efficacy has prompted the search for 5-$HT_{1A}$ receptor agonists with enhanced activity in animal models of anxiety and depression.

Recent pre-clinical evidence suggests that the ability of agonists to activate 5-$HT_{1A}$ receptors (i.e., intrinsic activity) may be an important determinant of the magnitude of their psychotropic activity (i.e., clinical effectiveness). In particular, the magnitude of the anxiolytic- and antidepressant-like effects of 5-$HT_{1A}$ receptor agonists appears to be positively related to the magnitude of their intrinsic activity at the receptor (Colpaert et al., 1992; De Vry, 1995, 1996; Koek et al., 1998). This relationship may explain why buspirone and its analogues, gepirone and ipsapirone, have limited clinical effects; these compounds activate the 5-$HT_{1A}$ receptor only weakly and are generally considered to be partial 5-$HT_{1A}$ receptor agonists.

Previously, we reported a significant, positive correlation between the magnitude of the anxiolytic-like effects (i.e., maximal increase of punished responding in a conflict procedure in pigeons) and the intrinsic activity of 5-$HT_{1A}$ receptor agonists (Spearman rank correlation $r_S = 0.78$, $P < 0.005$; Koek et al., 1998). Although a similar positive relation was apparent between intrinsic activity and antidepressant-like effects (i.e., inhibition of immobility in a forced swimming test in rats), the number of compounds studied was too limited to examine this putative relationship in detail.

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Traditionally, the ability of a drug to activate a receptor (i.e., intrinsic activity or efficacy) is thought to be a unique property of the drug, independent of the effector pathway through which its activity is measured. Consistent with this assumption, intrinsic activity is commonly measured in vitro using recombinant receptor systems. Inhibition of forskolin-stimulated cAMP levels in such systems has been shown to provide a measure of 5-$HT_{1A}$ receptor activation that is much more sensitive (e.g., Pauwels et al., 1993) than its measurement in brain membrane preparations (e.g., De Vivo and Maayani, 1986). Therefore, we previously used the maximal inhibition of forskolin-stimulated cAMP levels in HA7 cells as a measure of intrinsic activity at 5-$HT_{1A}$ receptors (Koek et al., 1998). Differences in maximal effects of compounds in such systems, however, are determined not only by their relative efficacies, but also by other factors, such as receptor number (e.g., Kenakin, 1997). Thus, the lack of a detectable difference between the efficacies of compounds under particular conditions does not imply that their efficacies will be identical under all conditions, i.e., the capacity of a system to differentiate maximal effects of compounds may be limited by ceiling or floor effects (Colquhoun, 1998). Indeed, recent findings obtained by measuring GTPγS binding in cells transfected with the human 5-$HT_{1A}$ receptor gene suggest that the ability to differentiate among high-efficacy 5-$HT_{1A}$ receptor agonists can be markedly enhanced (Pauwels et al., 1997), as can the ability to differentiate among low-efficacy 5-$HT_{1A}$ receptor agonists (Cosi and Koek, 2000).

The present study was aimed at examining in detail the relationship between intrinsic activity at 5-$HT_{1A}$ receptors and antidepressant-like effects using a large number of compounds varying over a wide range of intrinsic activities. Included in the study were novel 5-$HT_{1A}$ receptor agonists with buspirone-like intermediate intrinsic activity, but more selective than buspirone with respect to other receptors for which 5-$HT_{1A}$ receptor ligands often have substantial affinity (i.e., dopamine $D_2$ receptors and $\alpha_1$-adrenoceptors; Van Wijngaarden et al., 1990). The results show a strong, positive correlation between intrinsic activity at 5-$HT_{1A}$ receptors and maximal effects in the forced swimming test in rats. Further, they show that F 13714, a recently identified compound belonging to a structurally novel class of selective 5-$HT_{1A}$ receptor agonists (Vacher et al., 1998, 1999), has higher intrinsic activity and larger antidepressant-like effects than all other receptor ligands examined here.

## 2. Methods

### 2.1. Animals

Male Sprague–Dawley rats (Ico: OFA SD [IOPS Caw], Iffa Credo, l'Arbresle, France), weighing 160–180 g upon arrival, were group-housed (five animals per cage) with food and water freely available in a quarantine room for 4 to 8 days before being used in the experiments. Thereafter, they were housed individually in hanging cages (length × width × height: 28 × 21 × 18 cm) with metal grid floors (RC Iffa Credo), in the room where the experiments were conducted, with unlimited access to filtered (0.22 μm) water and, except when stated otherwise, standard laboratory food (UAR A03; UAR, Epinay/s/Orge, France). Animals were housed in environmentally controlled rooms (21 ± 1°C, relative humidity: 55 ± 5%) under a 12-h light–dark cycle (lights on at 0700 h), both during quarantine and during the experiments. The experimental procedures were in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985), were in compliance with French regulations, and were approved by the institutional Protocol Review Committee.

### 2.2. Radioligand binding

Frozen brains of male Sprague–Dawley rats were purchased from Iffa Credo, and were stored at −70°C before use. Binding affinities for the different receptors were determined by means of radioligand competition assays using the conditions summarized in Table 1. The reactions were stopped by rapid filtration through Whatman GF/B glass fiber filters, and the filters were washed with appropriate buffer. The radioactivity retained on the filters was measured by scintillation spectroscopy in 4-ml scintillation fluid (Emulsifier Safe, Packard). All experiments were performed in triplicate. $K_i$ values were estimated using non-linear regression (EBDA/LIGAND, Biosoft), and the results were expressed as mean $pK_i$ values ± S.E.M. of three independent determinations (two determinations if $pK_i < 5.0$).

### 2.3. Second messenger assays

#### 2.3.1. cAMP formation in HA7 cells

The methods used were identical to those described previously (Koek et al., 1998). The HeLa cell line permanently transfected with the human 5-$HT_{1A}$ receptor gene and permanently expressing approximately 0.5 pmol 5-$HT_{1A}$ receptor/mg protein (HA7) as described by Fargin et al. (1989) was commercially obtained from Duke University (Durham, NC, USA). HA7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco Biocult. Laboratories (Paisley, UK)) supplemented with 10% fetal calf serum, gentamicin (100 μg/ml), geneticin (G418) (400 μg/ml) in 5% $CO_2$ at 37°C in a water-saturated atmosphere. The cells were plated in six-well culture plates and used in the experiments at a confluency of 80–90%. Culturing medium (DMEM, 10% fetal calf

Table 1
Experimental details for 5-$HT_{1A}$ receptor-, dopamine $D_2$ receptor-, and $\alpha_1$-adrenoceptor binding assays

| Binding site | [$^3$H] ligand | | Tissue | | Incubation | | Non-specific binding | | Buffer | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | $K_D$ (nM) | Concentration (nM) | Type | Concentration (mg/ml) | Time (min) | Temperature (°C) | Drug | Concentration (μM) | | |
| 5-$HT_{1A}$ | 8-OH-DPAT (3.1) | 0.2 | rat cortex | 10 | 30 | 23 | 5-HT | 10 | A | Assié and Koek, 1996 |
| $D_2$ | YM-09151-2 (0.036) | 0.05 | rat striatum | 1 | 60 | 23 | (+)-butaclamol | 1 | B | Assié et al., 1993 |
| $\alpha_1$ | prazosin (0.063) | 0.1 | rat cortex | 5 | 30 | 23 | phentolamine | 50 | C | Assié and Koek, 1996 |

Buffers: (A) Tris–HCl 50 mM pH 7.4, pargyline 10 μM, $CaCl_2$ 4 mM, ascorbic acid 0.1%; (B) Tris–HCl 50 mM pH 7.4, NaCl 120 mM, KCl 5 mM; (C) Tris–HCl 50 mM pH 7.4.

serum, gentamicin 100 μg/ml, G418 400 μl/ml) was replaced by DMEM supplemented with 10% fetal calf serum without antibiotics 24 h before experimentation.

Cells were preincubated with DMEM, 10 mM HEPES for 10 min at room temperature. Drugs, at concentrations ranging from 0.1 nM to 100 μM, and appropriate vehicle controls [i.e., water or dimethyl sulfoxide (DMSO)], were then added in DMEM, 10 mM HEPES, 100 μM forskolin, and 100 μM 3-isobutyl-1-methylxanthine (IBMX) to the cells. At the end of the treatment (10 min, room temperature), the reaction was stopped by aspiration of the medium and addition of 0.1 N HCl. Cellular extract was diluted 1:500 or 1:400 in radioimmunoassay buffer, and cyclic AMP (cAMP) content was measured by using a commercially available kit (Dupont NEN: NEK-033). Basal cAMP levels were $10 \pm 0.9$ pmol/well ($n = 8$).

Each concentration–response experiment was performed in triplicate and replicated three to six times. Data were expressed as a percentage of the maximal inhibition by 5-HT of forskolin-stimulated cAMP. $pIC_{50}$ and $E_{max}$ values were estimated from the individual concentration–response data by means of non-linear regression (sigmoidal model with unit slope; Graphpad Prism). $E_{max}$ value differences were analyzed statistically by a one-way analysis of variance followed by paired comparisons by Newman–Keuls tests (Winer, 1971); $P$ values $< 0.05$ were considered statistically significant.

Note that the relative maximal responses of compounds cannot be directly equated to efficacy except by their rank order (i.e., the actual numerical identification of the ratio of maximal responses with efficacy is suspect, but the compound that produces the greater maximal response has a higher efficacy) (Kenakin, 1997, 1999). Thus, possible relations between the maximal effects observed here and other measures were examined by calculating Spearman's rank correlation ($r_S$).

### *2.3.2. [$^{35}$S]GTPγS binding response in HA7 cells*

HA7 cells were grown as described above, and were plated in 150 $cm^2$ Petri dishes until they reached a 90–100% confluence, after which they were washed with phosphate buffered saline (PBS) and stored at −80°C. The methods used were the same as those described previously (Cosi and Koek, 2000). The membranes were prepared from frozen cells on the day of the experiment, according to Stanton and Beer (1997) with some modifications. Cells were harvested in ice-cold 20 mM HEPES buffer containing 10 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4, room temperature), and were homogenized and centrifuged at $40{,}000 \times g$, at 4°C for 15 min. The pellet was resuspended in ice-cold 20 mM HEPES containing 0.1 mM EDTA (pH 7.4, room temperature) and recentrifuged at $40{,}000 \times g$, at 4°C for 15 min. The final pellet was resuspended in 20 mM HEPES containing 10 mM $MgCl_2$, 10 μM pargyline, and 30 μM GDP, and to which no NaCl was added. The membranes, 100–50 μg/tube, were incu-

bated in the presence of the test compounds for 1 h at 30°C. After cooling the tubes for 15 min with ice, [$^{35}$S]GTPγS (specific activity ≈ 1000 Ci/mmol) (Amersham, Les Ulis, France) was added to a final concentration of 0.1 nM. The membranes were then incubated for an additional 30 min, at 30°C. The reaction was terminated by filtration through Whatman filters using a Brandel harvester and radioactivity was counted by liquid scintillation spectrometry. Each compound was tested at six concentrations and [$^{35}$S]GTPγS binding values were expressed as a percentage of the maximal response obtained with 10 μM 5-HT. Data were analyzed as described above.

*2.3.3. [$^{35}$S]GTPγS binding response in C6 cells*

C6-glial cells stably transfected with a pcDNA3/h5-HT$_{1A}$ plasmid were prepared as a monoclonal cell line expressing approximately 1 pmol 5-HT$_{1A}$ receptor/mg protein (Wurch et al., 1996), cultured as described for C6-glial/h5-HT$_{1B}$ cells (Pauwels et al., 1996), and used for [$^{35}$S]GTPγS binding assays described previously (Pauwels et al., 1997). Transfected C6-glial cells were cultured in DMEM supplemented with 10% heat-inactivated fetal calf serum containing 1.25 mg geneticin/ml.

Cells were collected in phosphate-buffered-saline (pH 7.4) and centrifuged for 20 min at 48,000 × *g*. The pellet was homogenized with a Polytron in 20 mM HEPES containing 10 mM EDTA (pH 7.4) and recentrifuged for 10 min at 48,000 × *g*. The resulting pellet was washed twice in 10 mM HEPES (pH 7.4) containing 0.1 mM EDTA. The pellet was stored at −80°C in fractions of 600 to 750 μg-protein. The pellet was thawed, diluted 20 times in 20 mM HEPES (pH 7.4) supplemented with the 30 μM GDP, 100 mM NaCl, 3 mM $MgCl_2$ and 0.2 mM ascorbic acid. Incubation mixtures were prepared in glass tubes and consisted of 0.4 ml of membrane preparation (16 to 38 μg of protein) and 0.05 ml of compound. After an incubation period of 30 min at 25°C, 0.05 ml [$^{35}$S]GTPγS (500 pM) was added for an additional period of 30 min. The reactions were stopped by adding 3 ml of ice-cold 20 mM HEPES (pH 7.4) containing 3 mM $MgCl_2$ and rapid filtration over Whatman GF/B glass fiber filters using a Brandel harvester. The filters were rinsed three additional times with 3 ml HEPES buffer, placed in scintillation vials and the radioactivity was extracted in 4 ml of Emulsifier-Safe (Packard, Warrenville, PA, USA). Nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. Maximal stimulation of [$^{35}$S]GTPγS binding was defined in the presence of 10 μM 5-HT. Membrane protein levels were estimated with the dye-binding assay using the Bio-Rad kit (Bradford, 1976). Bovine serum albumin was used as a standard. Each compound was tested at six concentrations and [$^{35}$S]GTPγS binding values were expressed as a percentage of the maximal response obtained with 10 μM 5-HT. Data were analyzed as described above.

*2.4. Forced swimming test*

Adapted from the procedure described in detail by Porsolt et al. (1978), rats were placed in a Plexiglas cylinder (height 45 cm, diameter 20 cm) containing 17 cm water (at 25°C) for 15 min on the first day of the experiment (training session), and 24 h later placed again in the cylinder for 5 min (test session). From 24 h before the training session until the test session, the animals were housed in individual cages (described above), with water and food freely available. The duration of immobility during the 5-min test session was measured by an observer who was unaware of the treatment conditions.

Each animal was treated p.o. or i.p. with vehicle immediately after the first session, and with a particular dose of a drug ($n = 10$ per dose), or its vehicle control, p.o. 1 h or i.p. 15 min before the second session. All experiments were conducted between 0900 and 1700 h. During each successive block of experiments, each of the various drug conditions was tested in two to three animals.

Drug effects on the median immobility time were analyzed statistically by a Kruskall–Wallis nonparametric analysis of variance, followed, where appropriate, by individual comparisons using the Mann–Whitney *U*-test (Siegel and Castellan, 1988).

Dose–response functions were determined from the percentage of rats with immobility times less than 134 s (a criterion based on the immobility times obtained in controls; see Results), and $ED_{50}$ values and their associated confidence limits were calculated with the Litchfield–Wilcoxon procedure using the PHARM/PCS program by Tallarida and Murray (1987); when less than two intermediate percentages were observed, 0% and/or 100% effects were transformed by Berkson's adjustment (Hubert, 1984) to enable the use of the Litchfield–Wilcoxon procedure.

*2.5. Drugs*

The compounds used were: [$^3$H](±)-8-hydroxy-2-(di-*n*-propylamino)tetralin ([$^3$H]8-OH-DPAT) (160–240 Ci/mmol) and [$^3$H]prazosin (65–85 Ci/mmol) (Amersham); [$^3$H](±)-*cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide ([$^3$H]YM-09151-2) (70–87 Ci/mmol) (New England Nuclear); 5-HT creatinine sulphate, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) hydrobromide, buspirone hydrochloride, and prazosin hydrochloride (Sigma–RBI); forskolin (Prolabo); (*s*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide hydrochloride [(*s*)-WAY 100135], *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide dihydrochloride (WAY 100635), 3-chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methyl-6-methylamino-pyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl-methanone fumaric acid salt (F 13714) (MW = 539), 4-methyl-2-[4-(4-pyrimidin-2-yl-piperazin-1-yl)-butyl]-2H[1,2,4] tri-

azin-3,5-dione maleic acid salt (eptapirone; maleic acid salt of F 11440) (MW = 461), 2-[4-[4-(7-methoxy-1-naphtyl) piperazino]butyl]-4-methyl-2*H*,4*H*-1,2,4-triazin-3,5-dione, maleic acid salt (F 11461) (MW = 540), and 2-[4-[4-(7-benzofuranyl)piperazino]butyl]-4-methyl-2*H*,4*H*-1,2,4-triazin-3,5-dione (F 12826) (MW = 383) (J.-L. Maurel, Centre de Recherche Pierre Fabre).

For receptor binding studies, buspirone was dissolved in ethanol (10–20%), and all other compounds were dissolved in distilled water. In the second messenger experiments, compounds were dissolved in DMSO, except 5-HT and 8-OH-DPAT, which were dissolved in water.

For in vivo studies, F 11461 and F 12826 were suspended in distilled water by adding Tween 80 (2 drops/10 ml). All other drugs were dissolved in distilled water. An injection volume of 1 ml/100 g was used throughout. Doses are expressed as the weight of the free base.

## 3. Results

### 3.1. Binding affinity for and selectivity at 5-HT$_{1A}$ receptors

All the 5-HT$_{1A}$ receptor ligands examined here had moderate to high affinity for 5-HT$_{1A}$ binding sites (Table 2), but their selectivity for these sites differed. F 13714 was more selective for 5-HT$_{1A}$ sites than any of the other compounds tested: its 5-HT$_{1A}$ affinity was more than 1000-fold higher than its affinity for dopamine D$_2$ receptors and $\alpha_1$-adrenoceptors. All the compounds listed in Table 2 were more selective for 5-HT$_{1A}$ sites than the prototypical 5-HT$_{1A}$ partial agonist, buspirone. The affinity of buspirone for dopamine D$_2$ receptors was only 2-fold higher than its 5-HT$_{1A}$ affinity, and its affinity for $\alpha_1$-adrenoceptors was less than 30-fold higher.

### 3.2. Activation of 5-HT$_{1A}$ receptors

All 5-HT$_{1A}$ receptor ligands, with the exception of (*s*)-WAY 100135 and WAY 100635, had agonist effects in HA7 cells stably expressing 5-HT$_{1A}$ receptors, as evidenced by their ability to decrease forskolin-stimulated cAMP levels in a concentration-dependent manner (Fig. 1, open circles; Table 2). Their maximal effects, however, were different. The maximal effect of F 13714 was significantly higher than that of all other compounds, except eptapirone. In addition, the effects, if any, of (*s*)-WAY 100135 and WAY 100635 were significantly different from those of all other compounds, but not from each other.

Using maximal inhibition of forskolin-stimulated cAMP levels in HA7 cells as a measure of intrinsic activity, eptapirone had intrinsic activity higher than that of the partial agonists 8-OH-DPAT, F 11461, buspirone, and F 12826; its intrinsic activity was similar to that of 5-HT, and slightly lower than that of F 13714. Maximal stimulation of [$^{35}$S]GTPγS binding in C6 cells (Fig. 1, filled circles; Table 2) as a measure of intrinsic activity showed, however, that eptapirone did not behave as a full agonist, and that the intrinsic activity of F 13714 was significantly higher than that of eptapirone. Thus, F 13714 had intrinsic activity at 5-HT$_{1A}$ receptors higher than that of all other compounds examined here.

Table 2
F 13714 and other 5-HT$_{1A}$ receptor ligands: affinity for 5-HT$_{1A}$ receptors, dopamine D$_2$ receptors, and $\alpha_1$-adrenoceptors, and ability to activate 5-HT$_{1A}$ receptors in vitro

All compounds were examined for their ability to inhibit forskolin-stimulated cAMP production in HA7 cells expressing 5-HT$_{1A}$ receptors. In addition, F 13714 and eptapirone were examined for their ability to stimulate [$^{35}$S]GTPγS binding in membranes of C6 cells expressing 5-HT$_{1A}$ receptors, and (*s*)-WAY 100135 and WAY 100635 were examined for their ability to stimulate or inhibit [$^{35}$S]GTPγS binding in membranes of HA7 cells.

The cAMP values of 8-OH-DPAT and buspirone in HA7 cells are recalculated from Koek et al. (1998), the [$^{35}$S]GTPγS values of (*s*)-WAY 100135 and WAY 100635 in HA7 cells are recalculated from Cosi and Koek (2000), and the p$K_i$ values of these compounds are from Kleven et al. (1997).

| Compound | Affinity for 5-HT$_{1A}$ and other receptors | | | Activation of 5-HT$_{1A}$ receptors | | | |
|---|---|---|---|---|---|---|---|
| | 5-HT$_{1A}$ | D$_2$ | $\alpha_1$ | Cell type | Measure | Potency | Maximum effect |
| | p$K_i$ | p$K_i$ | p$K_i$ | | | pEC$_{50}$ (S.E.M.) | % 5-HT (S.E.M.) |
| F 13714 | 10.23 | 5.89 (22,000) | 7.17 (1100) | C6 | GTPγS | 8.31 (0.18) | 60.6 (4.6) |
| | | | | HA7 | cAMP | 8.70 (0.04) | 109.7 (2.8) |
| Eptapirone | 8.48 | < 5.0 (> 3020) | 6.31 (150) | C6 | GTPγS | 7.01 (0.14) | 37.8 (6.3) |
| | | | | HA7 | cAMP | 7.11 (0.31) | 100.1 (1.3) |
| 8-OH-DPAT | 8.85 | 6.26 (390) | 5.88 (930) | HA7 | cAMP | 7.65 (0.25) | 81.5 (5.2) |
| F 11461 | 10.49 | 8.71 (60) | 7.77 (520) | HA7 | cAMP | 8.92 (0.15) | 66.8 (7.1) |
| Buspirone | 7.65 | 7.49 (2) | 6.19 (29) | HA7 | cAMP | 6.56 (0.23) | 46.5 (5.1) |
| F 12826 | 10.39 | 7.41 (960) | 8.03 (230) | HA7 | cAMP | 9.29 (0.12) | 31.2 (13.7) |
| (*s*)-WAY 100135 | 8.35 | 6.54 (65) | 6.64 (51) | HA7 | cAMP | – | −6.4 (9.3) |
| | | | | | GTPγS | 8.58 (0.20) | 13.5 (4.0) |
| WAY 100635 | 9.02 | 6.55 (300) | 7.24 (60) | HA7 | cAMP | – | −14.2 (3.3) |
| | | | | | GTPγS | 9.59 (0.18) | −66.3 (10) |

Numbers between parentheses after p$K_i$ values: ratios of the affinities for D$_2$ and $\alpha_1$ receptors and the affinity for 5-HT$_{1A}$ receptors.

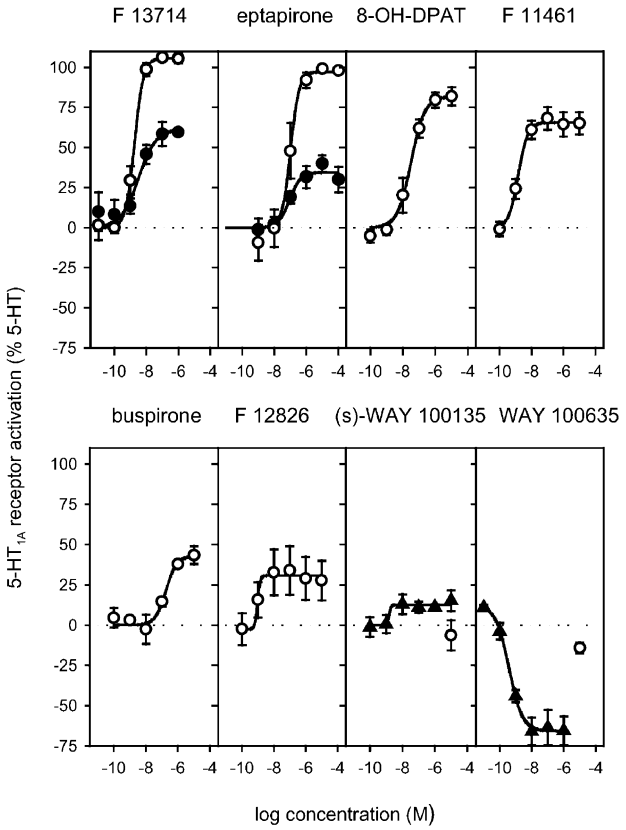


Fig. 1. Ability of F 13714 to activate 5-$HT_{1A}$ receptors in vitro, in comparison with effects of other 5-$HT_{1A}$ receptor ligands. Open circles: inhibition of forskolin-stimulated cAMP production in HA7 cells stably expressing 5-$HT_{1A}$ receptors. Filled circles: stimulation of [$^{35}$S]GTPγS binding in membranes from C6-glial cells stably expression 5-$HT_{1A}$ receptors. Filled triangles: stimulation or inhibition of [$^{35}$S]GTPγS binding, in the absence of added NaCl, in HA7 cells stably expressing 5-$HT_{1A}$ receptors. Values, expressed as a percentage of the maximal effect of 5-HT, are means ± S.E.M. of three to six independent experiments each performed in triplicate.

Although (*s*)-WAY 100135 and WAY 100635 did not differ markedly in their ability to affect forskolin-stimulated cAMP levels, their intrinsic activity as measured by [$^{35}$S]GTPγS binding in HA7 cells was significantly different (Fig. 1, filled triangles; Table 2). In the latter assay, (*s*)-WAY 100135 behaved as a weak partial agonist at 5-$HT_{1A}$ receptors, whereas WAY 100635 behaved as a strong inverse agonist.

### 3.3. Inhibition of immobility in the forced swimming test

The results obtained with F 13714 and the other 5-$HT_{1A}$ receptor ligands are summarized in Fig. 2 and Table 3, and are expressed as percentage inhibition of the median immobility time observed in control animals using the following formula: ((median immobility time in controls − median immobility time in experimental animals)/median immobility time in controls) × 100. Control medians ranged from 182 to 197 s. To calculate $ED_{50}$ values, the results were expressed also as the percentage of animals showing significant inhibition of immobility (i.e., an immobility time shorter than 134 s, because immobility times meeting this criterion occurred in less than 5% of all vehicle-treated controls).

All compounds, except buspirone, (*s*)-WAY 100135, and WAY 100635, significantly inhibited immobility, and the following potency order was obtained: F 11461 < F 13714 < F 12826 < 8-OH-DPAT < eptapirone (Table 3). The relative potencies of these compounds to inhibit immobility appeared to be positively related to their relative potencies to activate 5-$HT_{1A}$ receptors in vitro ($pEC_{50}$ values to decrease forskolin-stimulated cAMP levels in HA7 cells; Table 2). The Pearson product-moment correlation ($r$) between $pEC_{50}$ values and log $ED_{50}$ values (in mol/kg) was 0.87 ($P = 0.052$), which fell short of statistical significance, but the power of the test, which involved only five compounds, was low (i.e., 0.56).

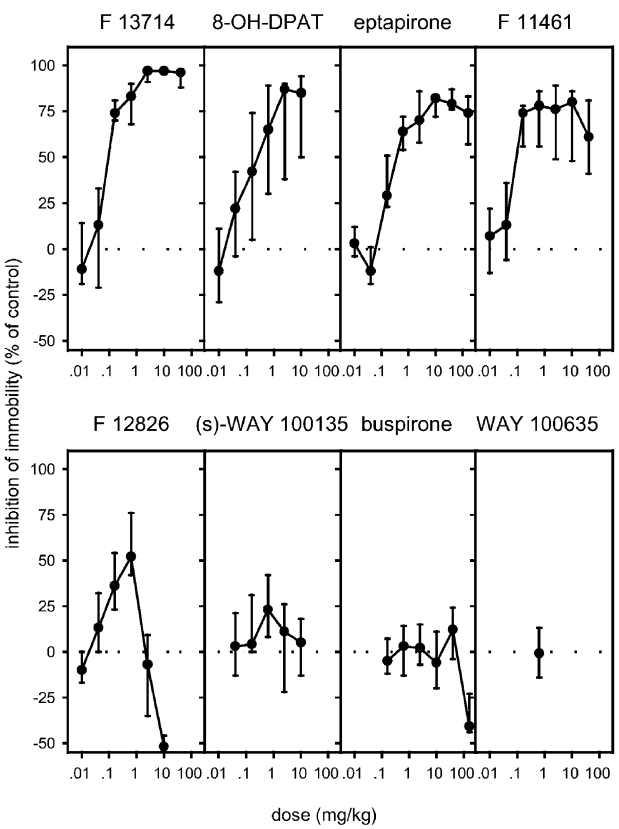


Fig. 2. Ability of F 13714 to produce antidepressant-like effects (inhibition of immobility in the forced swimming test in rats), in comparison with the effects of other 5-$HT_{1A}$ receptor ligands. All F compounds, eptapirone, and buspirone were administered p.o. 60 min before testing; 8-OH-DPAT, (*s*)-WAY 100135, and WAY 100635 were administered i.p. 15 min before testing. Values, expressed as percentage of vehicle control, are medians and interquartile ranges. Each dose was tested in 10 rats. Values obtained in individual rats at maximally effective doses are shown in Fig. 3.

Table 3
Ability of F 13714 and other 5-$HT_{1A}$ receptor ligands to produce antidepressant-like effects (inhibition of immobility in the forced swimming test) in rats. All compounds were administered p.o. 60 min before test, except 8-OH-DPAT, (*s*)-WAY 100135, and WAY 100635, which were administered i.p. 15 min before test

| Compound | Inhibition of immobility in the forced swimming test | | | | |
|---|---|---|---|---|---|
| | Potency | | | Maximum effect | |
| | $ED_{50}$ (mg/kg) | 95% C.L. | MSD (mg/kg) | % Animals | % Control median (I.Q.R.) |
| F 13714 | 0.065 | 0.034–0.12 | 0.16 | 100 | 97 (95–98) |
| Eptapirone | 0.19 | 0.068–0.52 | 0.16 | 100 | 82 (72–84) |
| 8-OH-DPAT | 0.10 | 0.035–0.31 | 0.04 | 100 | 87 (38–90) |
| F 11461 | 0.049 | 0.021–0.11 | 0.16 | 100 | 80 (48–86) |
| Buspirone | – | – | 160 | 10 | 12 ((−4)–24) |
| F 12826 | 0.08 | 0.03–0.21 | 0.16 | 90 | 52 (42–76) |
| (*s*)-WAY 100135 | – | – | – | 50 | 23 (8–42) |
| WAY 100635 | – | – | – | 10 | −1 ((−14)–13) |

MSD: minimum significant dose; I.Q.R.: interquartile range.

The maximal effect of F 13714 (i.e., a median of 97% inhibition; individual values are shown in Fig. 3) was significantly higher than that of all other compounds. The maximal effects of 8-OH-DPAT, eptapirone and F 11461 did not differ significantly from each other, and the maximal effects of eptapirone and F 11461 were significantly higher than those of the compounds shown in the lower panel of Fig. 2. The maximal effect of F 12826 was significantly higher than that of (*s*)-WAY 100135, buspirone, and WAY 100635, and the maximal effect of (*s*)-WAY 100135 was significantly higher than that of WAY 100635. The magnitude of the inhibition of immobility produced by the 5-$HT_{1A}$ receptor ligands examined here correlated positively (Spearman's rank correlation $r_S = 0.91$, $P < 0.005$, $n = 8$) with their intrinsic activity at 5-$HT_{1A}$ receptors (Fig. 4).

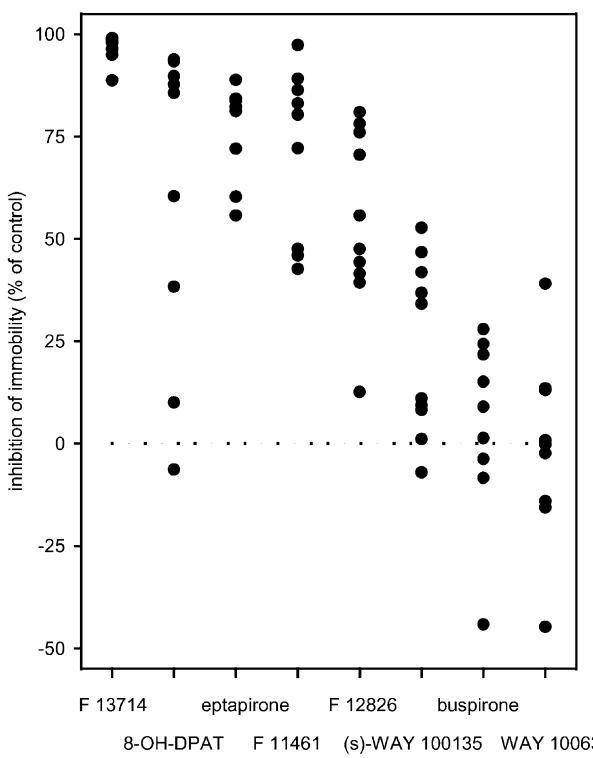


Fig. 3. Inhibition of immobility values (expressed as percentage of vehicle control) obtained in the forced swimming test in individual rats tested with maximally effective doses of F 13714 and other 5-$HT_{1A}$ receptor ligands (i.e., F 13714, eptapirone, F 11461: 10 mg/kg; 8-OH-DPAT: 2.5 mg/kg; buspirone: 40 mg/kg; F 12826, (*s*)-WAY 100135, WAY 100635: 0.63 mg/kg). Each dose was tested in 10 rats. Medians and interquartile ranges are shown in Fig. 2.

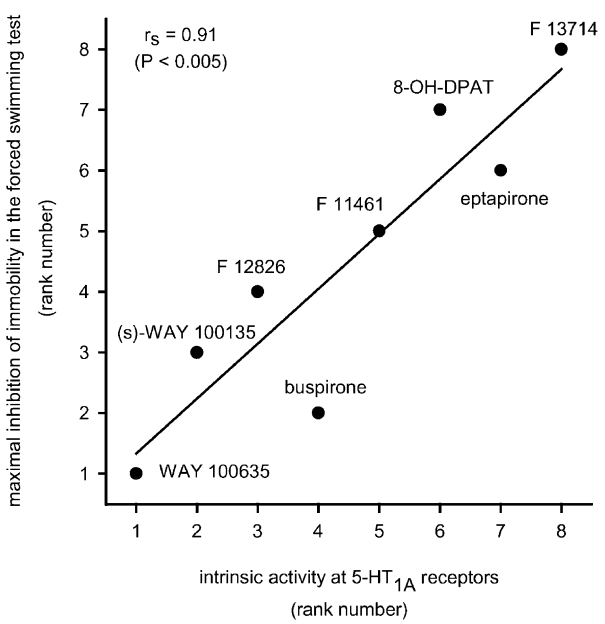


Fig. 4. Relationship between the ability of F 13714 and other 5-$HT_{1A}$ receptor ligands to activate 5-$HT_{1A}$ receptors in vitro (higher rank numbers represent higher intrinsic activity, and are based on the maximal effects of the curves shown in Fig. 1 and described in Table 2) and to produce antidepressant-like effects (higher rank numbers represent higher maximal inhibition of immobility in the forced swimming test in rats, and are based on the maximal effects of the curves shown in Fig. 2 and described in Table 3). $r_S$ represents Spearman's rank correlation coefficient.

## 4. Discussion

The main finding of the present study is that the magnitude of the antidepressant-like effects of 5-$HT_{1A}$ receptor ligands in the forced swimming test in rats correlated positively with their intrinsic activity at 5-$HT_{1A}$ receptors. This finding, together with a previously reported positive relationship between intrinsic activity and anxiolytic-like effects (Koek et al., 1998), offers further support for the hypothesis that high intrinsic activity is necessary for 5-$HT_{1A}$ receptor agonists to produce large anxiolytic and antidepressant effects (Colpaert et al., 1992; De Vry, 1996). A recent outcome of a research effort guided by this hypothesis is F 13714 (Vacher et al., 1998, 1999), a structurally novel and selective 5-$HT_{1A}$ receptor agonist with higher intrinsic activity and larger antidepressant-like effects than all other 5-$HT_{1A}$ receptor agonists examined here.

A combination of three different in vitro measures appeared to be necessary to identify 5-$HT_{1A}$ receptor ligands with intrinsic activities ranging from low-negative (i.e., the inverse agonist, WAY 100635) to high-positive (i.e., the highly efficacious agonist, F 13714). Using maximal inhibition of forskolin-stimulated cAMP levels in HA7 cells as a measure, the intrinsic activity of buspirone was about 50% of that of 5-HT and the intrinsic activity of 8-OH-DPAT was about 80%, i.e., higher than that of buspirone, but lower than that of 5-HT, in agreement with previous findings (e.g., Pauwels et al., 1997; Newman-Tancredi et al., 1998). Using the same measure, eptapirone was found to have intrinsic activity similar to that of 5-HT, confirming previous findings (Koek et al., 1998). The ability of this measure to differentiate among compounds with high efficacy, however, was limited, as was its ability to differentiate among compounds with low efficacy. Using maximal stimulation of [$^{35}$S]GTP$\gamma$S binding in the presence of 30 $\mu$M GDP in C6 cells (Pauwels et al., 1997), F 13714 was found to have higher intrinsic activity than eptapirone, but lower than that of 5-HT, whereas all three compounds inhibited forskolin-stimulated cAMP levels in HA7 cells to a similar extent. Using [$^{35}$S]GTP$\gamma$S binding under low sodium conditions in HA7 cells (Cosi and Koek, 2000), we found WAY 100635 to act as an inverse agonist and (*s*)-WAY 100135 as a weak agonist, whereas both compounds had similar effects on forskolin-stimulated cAMP levels in HA7 cells. Thus, the combined use of the three in vitro procedures enabled the various compounds to be ranked based on their intrinsic activity, allowed a detailed examination of the relationship of this ranking with their antidepressant-like effects, and may be useful to examine the relationship between the efficacy of 5-$HT_{1A}$ receptor agonists and other elements of their psychotropic activity profile.

The forced swimming test (Porsolt et al., 1978) is considered to be a predictively valid and reproducible procedure to examine the antidepressant potential of compounds (e.g., Borsini and Meli, 1988; Lucki et al., 1994). The finding that eptapirone inhibited immobility by about 80% of control confirms and extends results obtained previously with F 11440 (Koek et al., 1998). Consistent with previous studies (see review by Lucki et al., 1994), 8-OH-DPAT inhibited immobility, and did so by more than 80%, which is higher than found in some studies (e.g., Moser and Sanger, 1999), but similar to that shown in others (e.g., Wieland and Lucki, 1990). In contrast to eptapirone and 8-OH-DPAT, buspirone increased immobility, both in the present and in the previous (Koek et al., 1998) study, compatible with reports that buspirone, although decreasing immobility after repeated administration (e.g., Wieland and Lucki, 1990), failed to do so after a single injection (Cervo et al., 1988; Przegalinski et al., 1990). The maximal effects of the 5-$HT_{1A}$ receptor ligands in the forced swimming test correlated positively with their intrinsic activity, compatible with other data (Schreiber and De Vry, 1993; Singh and Lucki, 1993; De Vry, 1996; Koek et al., 1998). The correlation was high, but the magnitude of the antidepressant-like effects of buspirone was less than expected from its intrinsic activity; possibly, the antidepressant-like effects were attenuated by its metabolite, 1-(2-pyrimidinyl)-piperazine (1-PP) (Cervo et al., 1988; Przegalinski et al., 1990). The positive, statistically significant correlation between intrinsic activity and forced swimming test performance supports the hypothesis that high intrinsic activity is necessary for 5-$HT_{1A}$ receptor agonists to produce large antidepressant-like effects. Indeed, it is likely because of its high intrinsic activity that F 13714 produced antidepressant-like effects more important than any of the other compounds examined here, thereby showing marked antidepressant potential.

Compounds with high intrinsic activity at 5-$HT_{1A}$ receptors can produce elements of the 5-HT syndrome in rats, such as forepaw treading (Tricklebank, 1985). Consistent with their high intrinsic activity at 5-$HT_{1A}$ receptors, F 13714 and eptapirone produced forepaw treading, like 8-OH-DPAT. They were, however, at least 35-fold less potent to produce forepaw treading than to inhibit immobility, and had significantly different maximal effects on immobility (Figs. 2 and 3) at doses that produced forepaw treading (unpublished observations). Further evidence that the 5-HT syndrome induced by 5-$HT_{1A}$ receptor agonists does not underlie their effects on immobility are findings that their ability to induce the 5-HT syndrome shows tolerance, but their ability to produce antidepressant-like effects does not (e.g., De Vry, 1995). Like the 5-HT syndrome, the ability of 5-$HT_{1A}$ agonists to produce hypothermia shows tolerance (e.g., De Vry, 1995). Hypothermia is an effect that can be produced not only by 5-$HT_{1A}$ agonists with high intrinsic activity, but also by compounds with lower intrinsic activity similar to that of buspirone (e.g., Colpaert et al., 1992; Millan et al., 1993), and thus cannot account for the differences between the effects of intermediate and high intrinsic activity com-

pounds in the forced swimming test. F 13714 produced effects in the forced swimming test larger than any of the other compounds examined here. It is unlikely that these effects were the result of general changes in motor activity, because F 13714, at a dose of 10 mg/kg that had maximal effects in the forced swimming test, had no effect on locomotion (unpublished observations). Taken together, the results suggest that the marked effects of F 13714 in the forced swimming test do not involve its ability to induce the 5-HT syndrome, to produce hypothermia, or to affect locomotion, but are likely related to its high intrinsic activity at 5-$HT_{1A}$ receptors.

Previously, intrinsic activity at 5-$HT_{1A}$ receptors was found to be positively related to the magnitude of antidepressant-like effects (i.e., maximal inhibition of immobility in the forced swimming test in rats) and anxiolytic-like effects (i.e., maximal increase of punished responding in a conflict procedure in pigeons) of 5-$HT_{1A}$ receptor agonists, and among the compounds that were examined, F 11440 produced the largest antidepressant- and anxiolytic-like effects (Koek et al., 1998). The present study shows F 13714 to have intrinsic activity even higher than that of F 11440 maleate (eptapirone) and to produce more marked antidepressant-like effects, thereby extending the previously reported positive relation between intrinsic activity and antidepressant-like effects to very high levels of intrinsic activity. Interestingly, however, the anxiolytic-like effects of F 13714 appear to be less substantial than those of eptapirone (unpublished observations), suggesting that the previously found positive relationship between intrinsic activity and anxiolytic-like effects may cease to exist at very high levels of intrinsic activity. The availability of a series of selective 5-$HT_{1A}$ receptor ligands with very high, but different, intrinsic activities will help to further explore this possibility.

5-$HT_{1A}$ receptors are located presynaptically on 5-HT cell bodies in the dorsal and medial raphe nuclei and postsynaptically in various brain regions. Many findings suggest that the antidepressant-like effects of 5-$HT_{1A}$ receptor agonists are mediated by postsynaptic 5-$HT_{1A}$ receptors, but the involvement of presynaptic 5-$HT_{1A}$ receptors cannot be excluded (e.g., Lucki et al., 1994; De Vry, 1995). Presynaptic 5-$HT_{1A}$ receptors are involved in the ability of 5-$HT_{1A}$ agonists to decrease the extracellular level of 5-HT in the hippocampus (e.g., Sharp and Hjorth, 1992), and 5-$HT_{1A}$ agonist-induced increases of plasma corticosterone levels in rats result from activation of central, postsynaptic 5-$HT_{1A}$ receptors (e.g., Przegalinski et al., 1990). Eptapirone had higher intrinsic activity than buspirone and produced larger effects in the forced swimming test, in agreement with previous findings with F 11440 (Koek et al., 1998). In contrast, buspirone decreased hippocampal 5-HT levels and increased plasma corticosterone levels to the same extent as F 11440 (Koek et al., 1998). Together, these findings suggest that 5-$HT_{1A}$ receptors mediating the antidepressant-like effects of 5-$HT_{1A}$ agonists require a higher level of intrinsic activity for activation than presynaptic receptors mediating effects on hippocampal 5-HT levels and postsynaptic receptors mediating effects on plasma corticosterone levels. The positive correlation between intrinsic activity assessed in vitro and antidepressant-like activity suggests that the in vitro procedure used here is predictive of the ability of 5-$HT_{1A}$ receptor agonists to activate in vivo the 5-$HT_{1A}$ receptors involved in their antidepressant-like effects. The brain regions were these receptors are located, and the effector systems to which they are coupled, remain to be further explored.

In summary, the present finding that the intrinsic activity of 5-$HT_{1A}$ receptor ligands correlates positively with the magnitude of their antidepressant-like effects, and the positive relationship between intrinsic activity at 5-$HT_{1A}$ receptors and magnitude of anxiolytic-like effects found previously (Koek et al., 1998), together support the hypothesis that high intrinsic activity is necessary for 5-$HT_{1A}$ receptor ligands to produce marked psychotropic effects. Consistent with this hypothesis, F 13714, which had the highest intrinsic activity than all the other receptor ligands examined here, produced larger antidepressant-like effects than any of the other ligands.

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