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Estimation of apparent pA_2 values for WAY 100635 at 5-HT$_{1A}$ receptors regulating 5-hydroxytryptamine release in anaesthetised rats

Marie-Bernadette Assié *, Wouter Koek

Neurobiology Division II, Centre de Recherche Pierre Fabre, 17 avenue Jean Moulin, 81106 Castres Cedex, France

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Abstract

5-HT$_{1A}$ receptor agonists decrease 5-hydroxytryptamine (5-HT) terminal release by activating somatodendritic 5-HT$_{1A}$ autoreceptors. The selective 5-HT$_{1A}$ receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635) inhibits these effects of 5-HT$_{1A}$ receptor agonists. The present study was aimed at estimating apparent pA_2 values for WAY 100635 to antagonise 5-HT$_{1A}$ receptor agonist-induced decrease in 5-HT release in rat hippocampus. Extracellular concentrations of 5-HT were measured in microdialysis samples after administration of cumulative doses of 5-HT$_{1A}$ receptor agonists with different intrinsic activity, alone or in the presence of increasing doses of WAY 100635. Administration of cumulative doses of (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (0.01–40 mg/kg), 1[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphtyl)piperazine (S 14506) (0.00063–2.5 mg/kg), or buspirone (0.16–40 mg/kg), dose-dependently decreased the extracellular concentrations of 5-HT in the ventral hippocampus. Pre-treatment with WAY 100635 (0.01–0.63 mg/kg) shifted the dose–response curve of each agonist to the right in a dose-dependent manner. WAY 100635 antagonised the effects of all three compounds in a competitive manner, with an estimated apparent in vivo pA_2 value of 7.95 (95% confidence limits: 7.66–8.24). Taken together, the results are evidence that buspirone, S 14506 and 8-OH-DPAT, administered in cumulative doses, decreased 5-HT release by activating similar 5-HT$_{1A}$ receptors, because a common apparent pA_2 value was obtained for WAY 100635. The results also show that orderly microdialysis data can be obtained using cumulative dosing, which enables one to collect dose–response data rapidly, with fewer animals.

Keywords: Microdialysis, in vivo; 5-HT$_{1A}$ receptor; 5-HT (5-hydroxytryptamine, serotonin) release; WAY 100635

1. Introduction

Since the discovery that the 5-HT$_{1A}$ receptor (partial) agonist, buspirone, is effective in the treatment of anxiety and depression (Eison and Temple, 1986), 5-HT$_{1A}$ receptors have been a target for new psychotropic compounds. Drugs acting as agonists at 5-HT$_{1A}$ receptors exhibit anxiolytic- and/or antidepressant-like activity in animal models (see De Vry, 1995). 5-HT$_{1A}$ receptors are localised pre-synaptically on cell bodies in the raphe nuclei (somatodendritic receptors) and post-synaptically in 5-HT forebrain projecting areas. By activating somatodendritic receptors, 5-HT and 5-HT$_{1A}$ receptor agonists decrease the firing of raphe neurons, and subsequently decrease 5-HT terminal release (see Barnes and Sharp, 1999). The selective 5-HT$_{1A}$ receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635), has been shown to antagonise the inhibition of 5-HT release induced by 5-HT$_{1A}$ receptor agonists (Assié and Koek, 1996b; Bosker et al., 1997).

In an effort to characterise this antagonism in more detail, the present study was aimed at estimating apparent pA_2 values for WAY 100635 to antagonise the ability of 5-HT$_{1A}$ receptor agonists to decrease the hippocampal extracellular concentration of 5-HT. The 5-HT$_{1A}$ receptor agonists examined here were 1[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphtyl)piperazine (S 14506), (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), and buspirone, which have different intrinsic activity at 5-HT$_{1A}$ receptors (i.e., high, intermediate and low intrinsic activity, respectively) (e.g., Koek et al., 1998). Changes in extracellular 5-HT concentration in rat hip-

* Corresponding author. Tel.: +33-5-63-71-42-29; fax: +33-5-63-35-66-29.

E-mail address: marie.bernadette.assie@pierre-fabre.com (M.-B. Assié).

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pocampus were measured using in vivo microdialysis, following administration of cumulative doses of each agonist, alone or in the presence of increasing doses of WAY 100635. A cumulative dosing procedure was used to minimise the number of animals needed. A preliminary account of this work has been presented at the 8th International Conference on in Vivo Methods (Assié and Koek, 1999).

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats [Ico: OFA SD (I.O.P.S. Caw); Iffa Credo, France], weighing 260–340 g, were group housed (three rats per cage) in the animal-keeping facilities, under controlled conditions (12/12 light/dark cycle: lights on 0700 h; ambient temperature 21 ± 1°C; humidity 55 ± 5%), with rat food (AO4, UAR, France) and filtered (0.2 μm) tap water available ad libitum. At least 5 days were allowed for adaptation before the rats were used in the experiments. The experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH publication 85-23, revised 1985), and were approved by the institutional Protocol Review Committee (protocol 069).

2.2. Microdialysis procedure

Rats were anaesthetised with chloral hydrate (400–500 mg/kg i.p. and supplementary doses to maintain anaesthesia). A microdialysis probe (2 mm length, 0.5 mm diameter, CMA, Microdialysis) was stereotaxically implanted into the ventral hippocampus, stereotaxic coordinates: rostral −4.8 mm, lateral 4.6 mm, ventral −7.5 mm, from bregma and dura surface according to Paxinos and Watson (1986). The probe was continuously perfused (1.1 μl/min) with artificial cerebrospinal fluid containing 1 μM citalopram. Starting approximately 2 h after probe implantation, samples were collected every 20 min. After four baseline samples, saline or WAY 100635 was injected s.c., followed by 5–7 i.p. injections, at 20-min intervals, of increasing doses of the agonist. The increasing doses of the drug treatment were calculated such that the total amount injected before each microdialysis sample was four-fold higher than that of the previous sample. Post-injection levels of 5-HT were expressed as a percentage of the mean value of the four pre-injection control samples. For comparison purposes, in some experiments, the agonist was given i.p., at a single dose, and samples were collected for 140 min after administration of the compound.

At the end of the experiment, the animal was killed by decapitation and the brain was removed, frozen and cut in a cryomicrotome (Jung Frigocut 2800) to verify the placement of the probe.

2.3. Analytical procedure

Analysis of 5-HT was performed by means of an online high performance liquid chromatography (HPLC) system with electrochemical detection equipped with a reverse phase column (Merck, Lichrocart 125-2, Superspher 100 RP-18, length 119 mm, internal diameter 2 mm, granulometry 4 μm). The mobile phase (0.15 M NaH_2PO_4, 0.1 mM EDTA, 0.5 mM 1-octanesulfonic acid sodium salt, 16 % methanol) was pumped through the column at a rate of 0.2 ml/min (HPLC-116 solvent module, Beckman Coulter, Fullerton CA, USA). 5-HT eluted from the column (retention time 7–9 min) was measured with a glassy carbon working electrode maintained at a potential of +0.64 V versus Ag/AgCl reference electrode (DECADE detector, ANTEC Leyden, Leiden, The Netherlands). Data were acquired using a Beckman Gold system. Concentrations of 5-HT were estimated by comparing peak areas from the microdialysis samples with those of external standards of known concentration of 5-HT. The limit of detection (three times baseline noise) was approximately 1–2 fmol/20 μl sample.

2.4. Data analysis

For each agonist, dose–response data were obtained following treatment with saline or various doses of WAY 100635 ($n = 5$/dose), and logistic dose–response curves were simultaneously fitted to the median percentage values by means of the program Allfit (Windows version 2.12, C. and A. De Léan, University of Montreal) assuming a common slope and maximal effect. The ED_{50} estimates derived from these dose–response curves were used to calculate, for each agonist, an apparent pA_2 value for WAY 100635 by means of Schild regression. Schild regressions were analysed simultaneously by partial *F*-tests on the sum of squares between the observed values and those predicted assuming (1) individual slope and pA_2 estimates for each agonist, or (2) a common slope of −1 and a common pA_2 value, as described by Pöch et al. (1992). Differences were considered significant if $P < 0.05$.

Data from experiments involving administration of a fixed dose of each agonist were expressed as the mean area under curve (AUC) for the 140-min period after administration of the compound. Logistic dose–response curves were simultaneously fitted to the data by means of the program Allfit assuming a common slope and maximal effect.

2.5. Drugs

(±)-8-OH-DPAT hydrobromide and buspirone hydrochloride were purchased from Sigma-RBI (Saint Quentin

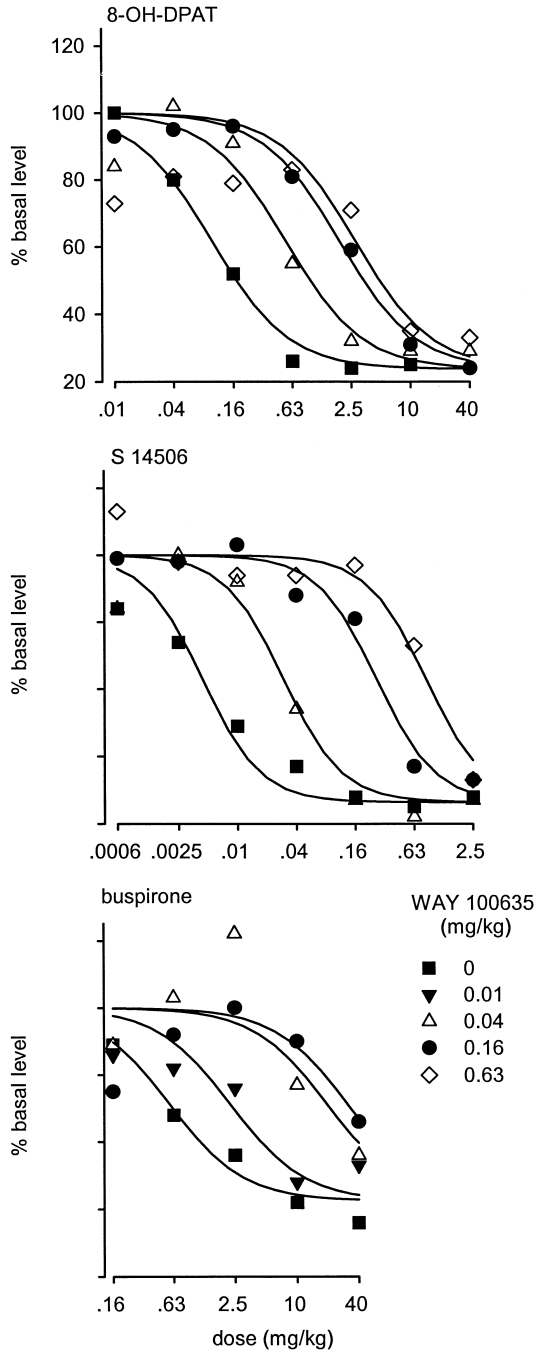

Fig. 1. Effects of WAY 100635 on the ability of cumulative doses of the 5-HT_{1A} receptor agonists, 8-OH-DPAT, S 14506, and buspirone, to decrease extracellular concentration of 5-HT in rat ventral hippocampus. Cumulative doses were injected i.p. at 20-min intervals and WAY 100635 was injected s.c., 20 min before the first dose of the agonist.

Fallavier, France), and chloral hydrate from Acros (Geel, Belgium). Citalopram was kindly donated by Lundbeck (Copenhagen, Denmark). S 14506 and WAY 100635 dihydrochloride were synthesised at the Centre de Recherche Pierre Fabre. The doses of compounds were expressed as the base. WAY 100635 was dissolved in distilled water and the injection volume was 10 ml/kg; all other compounds were suspended in distilled water by adding Tween 80 (2 drops/10 ml) and the injection volumes during cumulative dosing were 0.15–1 ml/rat.

3. Results

The mean basal extracellular concentration of 5-HT in the rat ventral hippocampus was 36.9 ± 1.3 fmol/20 μl ($n = 60$) in the presence of 1 μM of the 5-HT uptake inhibitor, citalopram.

Administration of cumulative doses of 8-OH-DPAT (0.01–40 mg/kg), S 14506 (0.00063–2.5 mg/kg), or buspirone (0.16–40 mg/kg), dose-dependently decreased the extracellular 5-HT concentration in the ventral hippocampus (Fig. 1); the estimated ED_{50} values were 0.097, 0.0043, and 0.57 mg/kg, respectively. In comparison, in experiments involving administration of a fixed dose of the compounds, the ED_{50} values were 0.13 mg/kg for 8-OH-DPAT (data from Assié and Koek, 1996a), 0.0033 mg/kg for S 14506 and 1.47 mg/kg for buspirone (data not shown).

Pre-treatment with WAY 100635 (0.01–0.63 mg/kg) dose-dependently shifted the dose–response curves of the agonists to the right. For each agonist, the dose–response curves obtained after pre-treatment with different doses of WAY 100635 could be fitted by means of a logistic equation using a common maximum of 100% and a common minimum and slope for each agonist (minimum: 24%, 25% and 37%, slope: 1.1, 0.8 and 0.9 for 8-OH-DPAT, S 14506 and buspirone, respectively). The quality of the fit did not improve significantly when slopes were unconstrained; thus the curves could be assumed to be parallel. For each agonist, the logistic curve-fitting procedure yielded estimates of ED_{50} values after different doses of WAY 100635 (Table 1), which were used to construct a Schild regression plot (Fig. 2) and to estimate apparent pA_2 values. For each of the agonists, the estimated slope of the Schild regression was not significantly different from -1, consequently a pA_2 value was calculated also with the slope of the regression constrained to -1. The apparent pA_2 values were 7.55 (95% confidence limits: 6.94–8.15) for 8-OH-DPAT, 8.03 (7.49–8.57) for S 14506

Table 1
ED_{50} values (mg/kg) for each 5-HT_{1A} receptor agonist alone (given in cumulative doses) or in the presence of different doses of WAY 100635

WAY 100635 (mg/kg)	8-OH-DPAT	S 14506	Buspirone
0	0.097 (0.038)	0.0043 (0.0010)	0.57 (0.36)
0.01	–	–	2.24 (1.42)
0.04	0.54 (0.21)	0.029 (0.006)	19.6 (12.3)
0.16	1.89 (0.73)	0.26 (0.06)	31.3 (20.3)
0.63	2.75 (1.06)	0.83 (0.19)	–

Numbers between parentheses are approximate standard errors calculated by the non linear regression program Allfit.

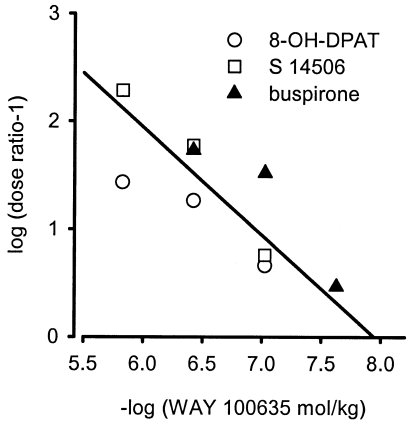

Fig. 2. Schild regression plot for the ability of WAY 100635 to antagonise 5-HT_{1A} receptor agonist-induced decrease in extracellular 5-HT concentration in rat ventral hippocampus. Dose ratios are the ED_{50} values of each agonist in the presence of WAY 100635 (0.01–0.63 mg/kg) divided by the ED_{50} value of the agonist in the presence of saline. ED_{50} values were estimated from the logistic curves shown in Fig. 1. A single regression line with a slope of -1 provided a satisfactory, simultaneous fit to the data obtained with all four drugs, and yielded a common apparent pA_2 value of 7.95 (95% confidence limits: 7.66–8.24).

and 8.26 (7.65–8.88) for buspirone. Constraining the Schild regressions for each of the agonists to have a common slope of -1 and a common pA_2 value did not significantly degraded the goodness of fit as compared with a model with individual slope and pA_2 estimates ($F(5,3) = 2.95$, $P = 0.2$). Thus, WAY 100635 antagonised the effects of each of the agonists with the same apparent pA_2 value of 7.95 (95% confidence limits: 7.66–8.24).

4. Discussion

The most important finding of the present study is evidence that buspirone, S 14506 and 8-OH-DPAT, administered in cumulative doses, decreased 5-HT release by activating similar 5-HT_{1A} receptors, because a common apparent pA_2 value was obtained for WAY 100635, which antagonised their effects in a competitive manner.

The 5-HT_{1A} receptor agonists 8-OH-DPAT, S 14506 and buspirone, when administered in cumulative doses, inhibited 5-HT release. The dose–response curves obtained by this method were not markedly different from those obtained when the compounds were administered at a single dose. The ED_{50} values obtained by the two methods were similar (i.e., 0.097 and 0.13 mg/kg for 8-OH-DPAT, 0.0043 and 0.0033 mg/kg for S 14506, and 0.57 and 1.47 mg/kg for buspirone, using cumulative and single dose administration, respectively). This suggests that, using the present experimental conditions, administration of cumulative doses can be used reliably to estimate the effects of 5-HT_{1A} receptor agonists. Note that the use of cumulative doses afforded a considerable reduction (by a factor 5–7) of the number of animals needed to establish complete dose–response curves for each agonist after increasing doses of the 5-HT_{1A} receptor antagonist, WAY 100635.

The agonists used here have different efficacy (intrinsic activity) at 5-HT_{1A} receptors in vitro; maximal inhibition of forskolin-stimulated cAMP production in transfected cells was 0.95 for S 14506, 0.81 for 8-OH-DPAT, and 0.49 for buspirone expressed relative to the maximal effects produced by 5-HT (Koek et al., 1998). In microdialysis experiments, such differences in efficacy do not appear to cause differences in the maximal effect produced by 5-HT_{1A} receptor agonists, likely because of the high receptor reserve in the raphe (Meller et al., 1990). Accordingly, in the present experiment, the maximal decrease in 5-HT concentration was not markedly different for the three compounds tested (24%, 25%, and 37%, for 8-OH-DPAT, S 14506, and buspirone, respectively).

Parallel shifts in the dose–response curves of the 5-HT_{1A} receptor agonists were observed after pre-treatment with increasing doses of WAY 100635, and the slopes of the Schild plots did not differ significantly from the theoretical value of -1. This suggests that the antagonism by WAY 100635 was of a competitive nature. In addition, antagonism by WAY 100635 could be characterised by the same apparent pA_2 value, irrespective of the agonist used. The apparent pA_2 value of 7.95, obtained here for WAY 100635 in anaesthetised animals, is similar to previously reported in vivo pA_2 values in rats. Using a cumulative dosing protocol, WAY 100635 antagonised 5-HT_{1A} receptor agonist-induced lower lip retraction, which is thought to be mediated by pre-synaptic 5-HT_{1A} receptors (Berendsen et al., 1994), with an apparent pA_2 value of 7.8 (Koek et al., 2000). Also, Kleven and Koek (1998) reported a pA_2 value of 7.8 for WAY 100635 to antagonise the discriminative stimulus effects of 8-OH-DPAT, which is mediated predominantly by post-synaptic 5-HT_{1A} receptors (Schreiber et al., 1995). Thus, in rats, the apparent in vivo pA_2 of WAY 100635 appears to be similar for 5-HT_{1A} receptors mediating pre- and post-synaptic responses. Previously, an apparent in vitro pA_2 value of 9.71 has been reported for WAY 100635 (Forster et al., 1995). In vivo and in vitro pA_2 values, however, cannot be directly compared, because they are expressed in a different manner (in mol/kg and in mol/l, respectively).

Taken together, the results indicate that the inhibition of 5-HT release by the 5-HT_{1A} receptor agonists, buspirone, S 14506 and 8-OH-DPAT is mediated by similar 5-HT_{1A} receptors, which are blocked by WAY 100635. The results also show that orderly microdialysis data can be obtained using cumulative dosing, which enables one to collect dose–response data rapidly, with fewer animals.

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References

Assié, M.-B., Koek, W., 1996a. (−)-Pindolol and (±)-tertatolol affect rat hippocampal 5-HT levels through mechanisms involving not only 5-HT_{1A}, but also 5-HT_{1B} receptors. Neuropharmacology 35, 213–222.

Assié, M.-B., Koek, W., 1996b. Effects of 5-HT_{1A} receptor antagonists on hippocampal 5-hydroxytryptamine levels: (*S*)-WAY100135, but not WAY100635, has partial agonist properties. Eur. J. Pharmacol. 304, 15–21.

Assié, M.-B., Koek, W., 1999. Antagonism of the ability of 5-HT_{1A} agonists to inhibit 5-HT release in vivo: estimation of apparent pA_2 values for WAY 100635 at presynaptic 5-HT_{1A} receptors. In: Rollema, H., Abercrombie, E.D., Sulzer, D., Zackheim, J. (Eds.), Monitoring Molecules in Neuroscience: Proceedings of the 8th Conference on in vivo Methods. pp. 259–260.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Berendsen, H.H.G., Bourgondien, F.G.M., Broekkamp, C.L.E., 1994. Role of dorsal and median raphe nuclei in lower lip retraction in rats. Eur. J. Pharmacol. 263, 315–318.

Bosker, F.J., Vrinten, D., Klompmakers, A., Westenberg, H., 1997. The effects of a 5-HT_{1A} receptor agonist and antagonist on the 5-hydroxytryptamine release in the central nucleus of the amygdala: a microdialysis study with flesinoxan and WAY 100635. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 347–353.

De Vry, J., 1995. 5-HT_{1A} receptor agonists: recent developments and controversial issues. Psychopharmacology 121, 1–26.

Eison, A.S., Temple, D.L. Jr., 1986. Buspirone: review of its pharmacology and current perspectives on its mechanism of action. Am. J. Psychiatry 80, 1–9.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635. Eur. J. Pharmacol. 281, 81–88.

Kleven, M.S., Koek, W., 1998. Discriminative stimulus effects of 8-hydroxy-2-(di-*n*-propylamino)tetralin in pigeons and rats: species similarities and differences. J. Pharmacol. Exp. Ther. 284, 238–249.

Koek, W., Patoiseau, J.F., Assié, M.-B., Cosi, C., Kleven, M.S., Dupont-Passelaigue, E., Carilla-Durand, E., Palmier, C., Valentin, J.P., John, G.W., Pauwels, P.J., Tarayre, J.P., Colpaert, F.C., 1998. F 11440, a potent, selective, high efficacy 5-HT_{1A} receptor agonist with marked anxiolytic and antidepressant potential. J. Pharmacol. Exp. Ther. 287, 266–283.

Koek, W., Assié, M.-B., Zernig, G., France, C.P., 2000. In vivo estimates of efficacy at 5-HT_{1A} receptors: effects of EEDQ on the ability of agonists to produce lower-lip retraction in rats. Psychopharmacology 149, 377–387.

Meller, E., Goldstein, M., Bohmaker, K., 1990. Receptor reserve for 5-hydroxytryptamine$_{1A}$-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine$_{1A}$ agonists. Mol. Pharmacol. 37, 231–237.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pöch, G., Brunner, F., Kuhberger, E., 1992. Construction of antagonist dose–response curves for estimation of p*A*2-values by Schild-plot analysis and detection of allosteric interactions. Br. J. Pharmacol. 106, 710–716.

Schreiber, R., Brocco, M., De Ladonchamps, B.L., Monneyron, S., Millan, M.J., 1995. A drug discrimination analysis of the actions of novel serotonin$_{1A}$ receptor ligands in the rat using the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin. J. Pharmacol. Exp. Ther. 275, 822–831.