J Neural Transm [GenSect] (1994) 96: 179–186

— Journal of — Neural Transmission

The 5-HT_{1A} receptor antagonist (S)-UH-301 blocks the (R)-8-OH-DPAT-induced inhibition of serotonergic dorsal raphe cell firing in the rat

L. Arborelius[1], B. Backlund Höök[2], U. Hacksell[2], and T. H. Svensson[1]

[1] Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institutet, Stockholm, and
[2] Department of Organic Pharmaceutical Chemistry, Uppsala University, Uppsala, Sweden

Accepted February 16, 1994

Summary. (S)-UH-301 [(S)-5-fluoro-8-hydroxy-2-(dipropylamino)-tetralin, 0.5–4.0 mg/kg i.v.] did not significantly alter the firing rate of 5-hydroxytryptamine (5-HT) containing neurons in the dorsal raphe nucleus (DRN) as a group, although some individual cells were activated whereas others were depressed. However, (S)-UH-301 (2.0 mg/kg i.v.) consistently reversed the inhibition of DRN-5-HT cells produced by the selective 5-HT_{1A} receptor agonist (R)-8-OH-DPAT (0.5 μg/kg i.v.) and the dose-response curve for this effect of (R)-8-OH-DPAT was markedly shifted to the right by pretreatment with (S)-UH-301 (1.0 mg/kg i.v.). These results support the notion that (S)-UH-301 acts as an antagonist at central 5-HT_{1A} receptors.

Keywords: 5-HT_{1A}, 8-OH-DPAT, (S)-UH-301, 5-HT_{1A} receptor antagonist, electrophysiology, dorsal raphe nucleus

Introduction

Several studies have revealed a high density of both binding sites, protein and mRNA for 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors in the DRN (Pazos and Palacios, 1985; Miguel et al., 1991). Within this nucleus the 5-HT_{1A} receptors are located on the cell bodies and dendrites of 5-HT neurons (Sotelo et al., 1990) and these receptors are involved in the regulation of the activity of DRN-5-HT cells. Thus, electrophysiological studies have shown that systemic administration of selective 5-HT_{1A} agonists such as 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), gepirone and ipsapirone, as well as microiontophoretic application of these compounds directly onto the DRN neurons, potently inhibit the firing of 5-HT cells in this nucleus (Blier et al., 1988; Sprouse and Aghajanian, 1987, 1988; Sinton and Fallon, 1988). A number of putative

5-HT_{1A} antagonists, such as BMY 7378, NAN-190 and recently (−)tertatolol and WAY100,135, have all been found to attenuate the inhibitory effect induced by 8-OH-DPAT on 5-HT cell activity in the DRN (Chaput and de Montigny, 1988; Greul and Glaser, 1992; Jolas et al., 1993; Fletcher et al., 1993; Lejeune et al., 1993). However, the effects of these compounds alone on DRN-5-HT cells suggest that they are either partial agonists at somatodendritic 5-HT_{1A} autoreceptors or possess activity at other central monoaminergic receptors.

(S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin [(S)-UH-301], a drug with relatively high affinity for the 5-HT_{1A} receptor (K_i = 52 nM, Hillver et al., 1990), has been shown to antagonize several effects elicited by the selective and efficacious 5-HT_{1A} receptor agonist (R)-8-OH-DPAT (Cornfield et al., 1991; Björk et al., 1991). For instance, (S)-UH-301, without producing any effect by itself, reverses the (R)-8-OH-DPAT-induced reduction in brain 5-HT synthesis and blocks the 5-HT-produced inhibition of forskolin-stimulated adenylate cyclase activity in hippocampal membranes (Björk et al., 1991). Using the microdialysis technique, we have also shown that (S)-UH-301 does not affect extracellular 5-HT or 5-hydroxyindoloacetic acid (5-HIAA) concentrations in the hippocampus, but still potently blocks the (R)-8-OH-DPAT-induced decrease in 5-HT and 5-HIAA in this area (Nomikos et al., 1992).

In the present study we have used extracellular recording techniques to examine the effects of systemically administered (S)-UH-301 on the activity of 5-HT neurons in the DRN. To further document the antagonistic properties of (S)-UH-301 on central 5-HT_{1A} receptors, the ability of (S)-UH-301 to reverse the (R)-8-OH-DPAT-induced inhibition of the DRN-5-HT cells was investigated and the dose-response curve for the inhibitory effect of (R)-8-OH-DPAT after pretreatment with (S)-UH-301 was established. Preliminary data from these studies have been reported previously (Arborelius and Svensson, 1992).

Materials and methods

Standard electrophysiological methods were used. Briefly, male SD rats weighing between 250 and 400 g (Alab, Sollentuna, Sweden) were anesthetized with 400 mg/kg chloral hydrate intraperitoneally (i.p.). Body temperature was kept at 37–38 °C by means of an electric heating pad. Extracellular recording electrodes were pulled in a Narishige vertical puller from glass capillaries (Clark Electromedical Instruments) and filled with 2% Pontamine Sky Blue in 2 M NaCl. Coordinates, determined from the atlas of Paxinos and Watson (1986), were 0.8–1.4 mm anterior and 0 ± 0.1 mm lateral to lambda. Presumed 5-HT neurons, with electrophysiological characteristics corresponding to those previously described (Aghajanian et al., 1978), were found 5.0–6.0 mm from brain surface in the DRN. Recordings were made from only one cell in each animal. At the end of the experiment, a 5 μA negative current was passed through the electrode, leaving a blue spot at the recording site. All cells included in this study were verified by standard histological techniques to be located in the DRN.

Extracellular action potentials were amplified, discriminated and monitored on an oscilloscope, an audiomonitor and continuous rate recordings were generated on a pen chart recorder. Discriminated spikes were fed into a IBM PS/2 computer connected to a CED 1401 interface unit (Cambridge Electronic Design, Ltd., Cambridge, UK) and the action potentials were collected and analysed by the CED Spike 2 program.

(S)-UH-301 HCl and (R)-8-OH-DPAT HCl (synthesized at the Department of Organic Pharmaceutical Chemistry, Uppsala University, Sweden) were dissolved in saline and administered i.v.. For the establishment of dose-response curves, drugs were given in exponentially increasing doses at 3 min intervals. Drug effects were assessed by comparison of the mean basal frequency during 1.5 min (baseline values) to the mean frequency during the same time period at maximal drug effect at each dose. For the reversal of the (R)-8-OH-DPAT-induced effect, mean frequences were determined during 1.5 min prior to (R)-8-OH-DPAT and immediately after (S)-UH-301 injections. ED_{50} values were estimated from the respective dose-response graphs.

Results

(S)-UH-301 (0.5–4.0 mg/kg i.v.) variably affected the firing rate of 5-HT neurons in the DRN. Although the overall effect was not significant, individual cells responded with either an increased or a decreased neuronal activity (Figs. 1 A, 1 B, 2 A). The maximal excitatory effect of (S)-UH-301 was 230% of baseline and the maximal inhibition was 45% of baseline.

Administration of a low dose of (R)-8-OH-DPAT (0.5 μg/kg i.v.) completely suppressed the activity of 5-HT neurons in the DRN. This effect was instantly and potently reversed by 2.0 mg/kg i.v. of (S)-UH-301 (Fig. 1 C). In two out of four cells the reversal by (S)-UH-301 actually continued even above baseline firing whereas in the other cells (S)-UH-301 partly or completely reversed the firing to baseline. This differential response is, generally, consonant with the effects seen with (S)-UH-301 alone (see above).

The dose-response curve for the effects of (R)-8-OH-DPAT on DRN 5-HT cell activity was established with an estimated ED_{50} value of 0.4 μg/kg (Figs. 1 D, 2 B). After pretreatment with 1.0 mg/kg (S)-UH-301 the dose-response curve for (R)-8-OH-DPAT was markedly shifted to the right and ED_{50} increased to 8.4 μg/kg (Figs. 1 E, 2 B). This dose of (S)-UH-301, by itself, slightly affected the activity of DRN-5-HT cells although it did not reach statistical significance (paired t-test).

Discussion

The present study provides additional evidence that (S)-UH-301 acts as an antagonist at central $5\text{-}HT_{1A}$ receptors. This antagonism was evidenced by an immediate and consistent reversal by (S)-UH-301 of the (R)-8-OH-DPAT-induced suppression of the activity of DRN-5-HT cells. Generally, (R)-8-OH-DPAT produced a dose-dependent decrease in neuronal activity of serotonergic cells in the DRN, in agreement with previous results (see Introduction). Importantly, the dose-response curve for this inhibitory effect induced by (R)-8-OH-DPAT was potently shifted to the right by pretreatment with (S)-UH-301, thus, providing classical evidence for the $5\text{-}HT_{1A}$ antagonistic action of the compound.

The doses of (S)-UH-301 which were used in this study were in the same range as those previously shown to prevent the (R)-8-OH-DPAT-induced de-

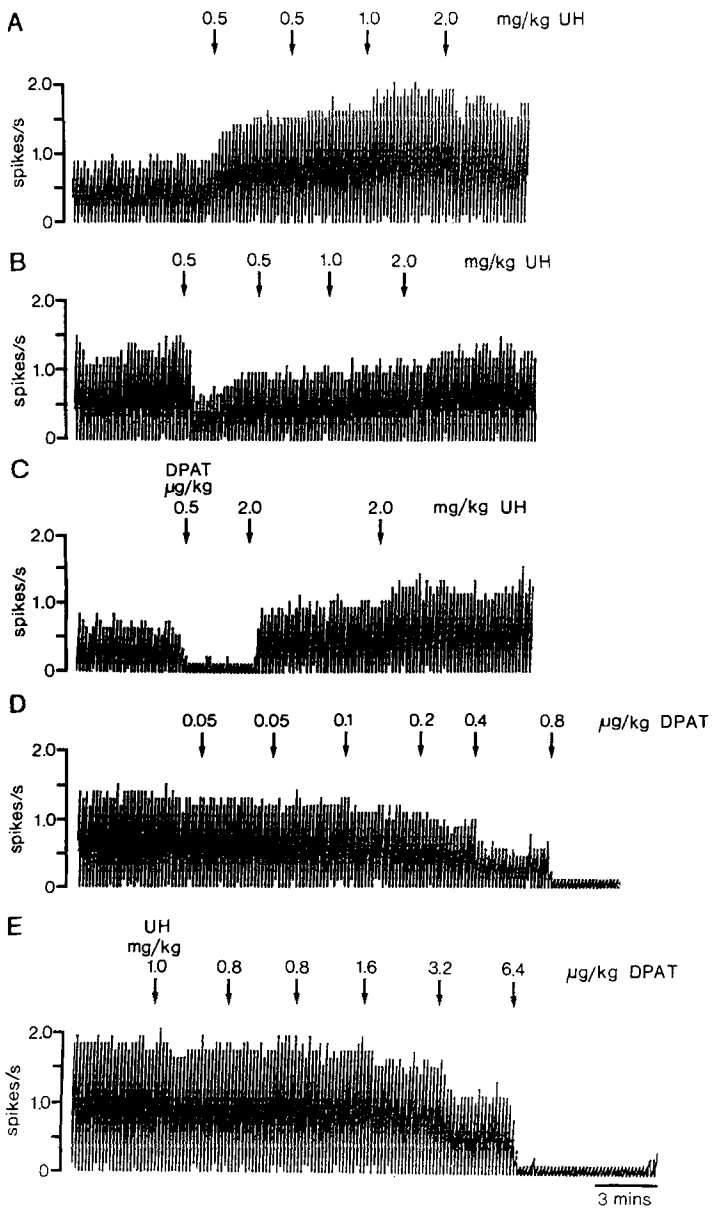

Fig. 1. Integrated firing rate histograms of five single 5-HT neurons in the dorsal raphe nucleus showing the effect of (S)-UH-301 (UH) alone and before and after administration of (R)-8-OH-DPAT (DPAT). **A** A typical 5-HT neuron responding with an increased firing rate after (S)-UH-301 (0.5–4.0 mg/kg i.v.). **B** Showing a 5-HT neuron that responded with a decreased firing rate after (S)-UH-301 (0.5–4.0 mg/kg i.v.). **C** The effects of (R)-8-OH-DPAT (0.5 µg/kg i.v.) and (S)-UH-301 (2.0 + 2.0 mg/kg i.v.) on a 5-HT cell. **D** and **E** Show the effects of cumulative increasing doses of (R)-8-OH-DPAT alone (D: 0.05–1.6 µg/kg i.v.) and after pretreatment with (S)-UH-301 (E: UH; 1.0 mg/kg i.v., DPAT; 0.8–12.8 µg/kg i.v.)

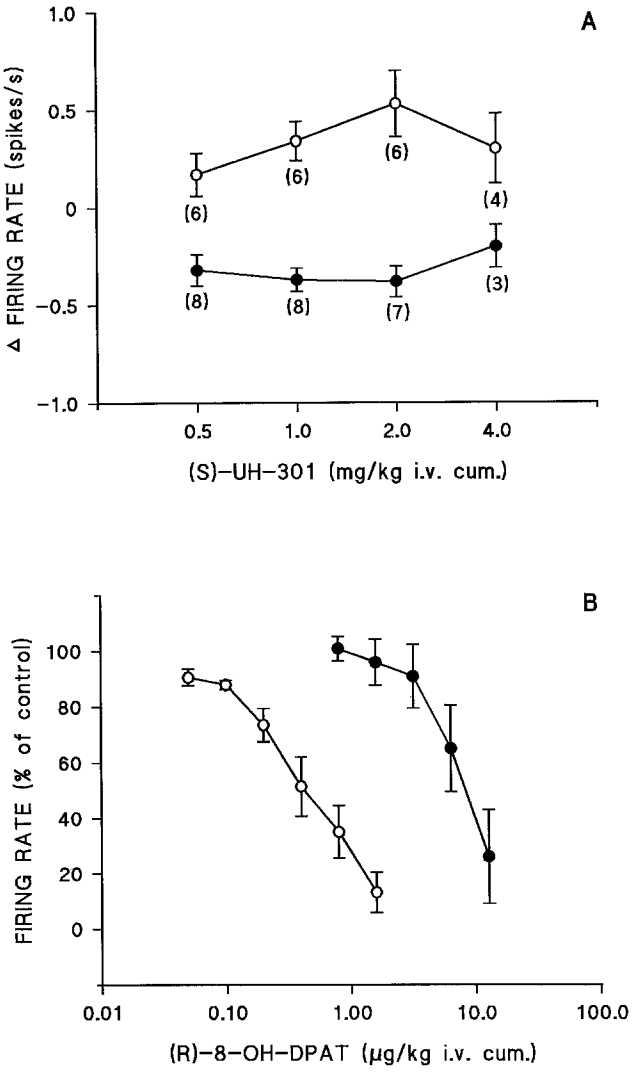

Fig. 2. Effects of cumulative i.v. doses of (S)-UH-301 (0.5–4.0 mg/kg) on the firing rate of DRN-5-HT neurons. Data are presented as the difference in firing rate from baseline values (0 spikes/second). Open circles represent cells responding with increased firing rate (baseline; 1.15 ± 0.28) and solid circles represent cells responding with decreased firing rate (baseline; 1.24 ± 0.28) after (S)-UH-301. Each point is the mean ± S.E.M. of the number of cells in parenthesis. **B** Cumulative log dose-response curves for (R)-8-OH-DPAT on the firing rate of DRN-5-HT neurons expressed as percent of baseline values (mean ± S.E.M.) either without (1.46 ± 0.21 Hz, open circles, n = 6) or with (1.78 ± 0.28 Hz, closed circles, n = 6) 1.0 mg/kg i.v. (S)-UH-301 given 3 min prior to the first dose of (R)-8-OH-DPAT

crease in 5-HT release in the hippocampus and 5-HT synthesis (Björk et al., 1991; Nomikos et al., 1992). The overall effects produced by (R)-8-OH-DPAT, i.e., inhibition of neuronal activity of 5-HT-DRN cells and the concomitant decrease in release and synthesis of 5-HT, are most likely mediated through stimulation of somatodendritic 5-HT_{1A} autoreceptors in the DRN, since local

application of 8-OH-DPAT into the DRN produces similar effects as systemic administration (Sprouse and Aghajanian, 1988; Hjorth and Magnusson, 1988; Hutson et al., 1989). Thus, (S)-UH-301 appears, indeed, to be a potent antagonist at these receptors. However, since (S)-UH-301 also acts as an antagonist at postsynaptic 5-HT_{1A} receptors (Björk et al., 1991) and has some agonistic properties at dopamine$_2$ (D_2) receptors (Hillver et al., 1990; Arborelius et al., 1993), it can not be ruled out that these receptors may play a role in the (S)-UH-301 mediated antagonism of the (R)-8-OH-DPAT-induced suppression of 5-HT cells in the DRN.

Previous studies with other putative 5-HT_{1A} antagonists, e.g., BMY 7378, NAN-190, (−)tertatolol and WAY100,135, have shown that these compounds also block the 8-OH-DPAT-induced decrease in firing rate of DRN-5-HT neurons (see Introduction). However, both BMY 7378 and NAN-190 appear to be partial agonists at somatodendritic 5-HT_{1A} autoreceptors (Chaput and de Montigny, 1988; Greul and Glaser, 1992). Also, (−)tertatolol has higher affinity for β-adrenoceptors then 5-HT_{1A} receptors and possesses considerable affinity for 5-HT_{1B} receptors (Jolas et al., 1993). In contrast, WAY100,135 seems to be a more selective 5-HT_{1A} receptor antagonist. However, when given alone it produces a slight but significant suppression of 5-HT cell activity in the DRN, which may be due to a weak, partial agonistic action at 5-HT_{1A} receptors. Alternatively, interaction with other receptors, e.g., α_1 adrenoceptors, can not be ruled out (Fletcher et al., 1993; Lejeune et al., 1993).

In the present study individual DRN-5-HT cells responded with either an increased or a decreased neuronal activity after systemic administration of (S)-UH-301. Tentatively, the excitation produced by (S)-UH-301 in a subset of DRN-5-HT cells may be due to antagonism of a tonically active, inhibitory influence by endogenous 5-HT, mediated through somatodendritic 5-HT_{1A} autoreceptors. However, (S)-UH-301, in addition to its 5-HT_{1A} receptor antagonistic actions, also possesses some agonistic properties at D_2 receptors (see above), which might contribute to its differential effect on the activity of DRN cells. Evidence for a dopaminergic-serotonergic interaction within the DRN has been provided by a number of anatomical studies (Junzo and Shimizu, 1978; Kalén et al., 1988). Moreover, both systemic and local administration of apomorphine, a DA agonist, increases extracellular concentrations of 5-HT in the DRN, an effect probably mediated through D_2 receptors (Ferré and Artigas, 1993; personal communication). Preliminary results from our laboratory have shown that also systemic administration of apomorphine can produce differential effects on the firing rates of 5-HT neurons in the DRN. Thus, the D_2 receptor agonistic properties of (S)-UH-301 may well contribute to the overall response of individual 5-HT cells in the DRN, albeit in a complex way.

In conclusion, the present study provides electrophysiological evidence that (S)-UH-301 acts as an antagonist at central 5-HT_{1A} receptors such as those in the DRN. Furthermore, our data indicate that there may exist at least two subsets of 5-HT neurons in the DRN, the activities of which are differentially

affected by (S)-UH-301. Recently, Jacobs and Azmitia (1992) also identified at least two different subsets of 5-HT neurons in the DRN, based on physiological criteria, i.e., their activation in association with repetitive motor acitivities. Thus, drugs such as (S)-UH-301 might prove interesting to explore as regards their effects on motor control.

Acknowledgements

The present study was supported by the Swedish National Board for Technical Development, the Swedish Medical Research Council (project no. 4747), Torsten och Ragnar Söderbergs Stiftelser, Astra Arcus AB and the Karolinska Institute. We thank Ms. M. Marcus and Ms. A. Malmerfelt for skilful technical assistance.

References

Aghajanian GK, Wang RY, Barban J (1978) Serotonergic and non-serotonergic neurons of the dorsal raphe: reciprocal changes in firing induced by peripheral nerve stimulation. Brain Res 153: 169–175

Arborelius L, Chergui K, Murase S, Nomikos GG, Backlund Höök B, Chouvet G, Hacksell U, Svensson TH (1993) The 5-HT_{1A} receptor selective ligands, (R)-8-OH-DPAT and (S)-UH-301, differentially affect the activity of midbrain dopamine neurons. Naunyn Schmiedebergs Arch Pharmacol 347: 353–362

Arborelius L, Svensson TH (1992) Acute effects of (R)-8-OH-DPAT and a novel 5-HT_{1A} receptor antagonist, UH-301, on the cell firing of dorsal raphe (DRN) 5-HT neurons and DA neurons in the ventral tegmental area (VTA). Behav Pharmacol 3 (S1): 40

Björk L, Cornfield LJ, Nelson DL, Hillver S-E, Andén N-E, Lewander T, Hacksell U (1991) Pharmacology of the novel 5-hydroxytryptamine$_{1A}$ receptor antagonist (S)-5-fluoro-2-(dipropylamino)-tetralin: inhibition of (R)-8-hydroxy-2-(dipropylamino)-tetralin-induced effects. J Pharmacol Exp Ther 258: 58–65

Blier P, Steinberg S, Chaput Y, de Montigny C (1988) Electrophysiological assessment of putative antagonists of 5-hydroxytryptamine receptors: a single-cell study in the rat dorsal raphe nucleus. Can J Physiol Pharmacol 67: 98–105

Chaput Y, de Montigny C (1988) Effects of the 5-hydroxytryptamine$_1$ receptor antagonist, BMY 7378, on 5-hydroxytryptamine neurotransmission: electrophysiological studies in the rat central nervous system. J Pharmacol Exp Ther 246: 359–370

Cornfield LJ, Lambert G, Arvidsson L-E, Mellin C, Vallgårda J, Hacksell U, Nelson DL (1991) Intrinsic activity of enantiomers of 8-hydroxy-2-(di-n-propylamino)tetralin and its analogs at 5-hydroxytryptamine$_{1A}$ receptors that are negatively coupled to adenylate cyclase. Mol Pharmacol 39: 780–787

Ferré S, Artigas F (1993) Dopamine D_2 receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. J Neurochem 61: 772–775

Fletcher A, Bill DJ, Bill SJ, Cliffe IA, Dover GM, Forster EA, Haskins JT, Jones D, Mansell HL, Reilly Y (1993) WAY 100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors. Eur J Pharmacol 237: 283–291

Greuel JM, Glaser T (1992) The putative 5-HT_{1A} receptor antagonists NAN-190 and BMY 7378 are partial agonists in the rat dorsal raphe nucleus in vitro. Eur J Pharmacol 211: 211–219

Hillver S-E, Björk L, Li Y-L, Svensson B, Ross S, Andén N-E, Hacksell U (1990) (S)-5-fluoro-8-hydroxy-2-(dipropylamino)-tetralin: a putative 5-HT_{1A}-receptor antagonist. J Med Chem 33: 1541–1544

Hjorth S, Magnusson T (1988) The 5-HT_{1A} receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. Naunyn Schmiedebergs Arch Pharmacol 338: 463–471

Hutson PH, Sarna GS, O'Connell MT, Curzon G (1989) Hippocampal 5-HT synthesis and release in vivo is decreased by infusion of 8-OHDPAT into the nucleus raphe dorsalis. Neurosci Lett 100: 276–280

Jacobs BL, Azmitia EC (1992) Structure and function of brain serotonin system. Physiol Rev 72: 165–229

Jolas T, Haj-Dahmane S, Lanfumey L, Fattaccini CM, Kidd EJ, Adrien J, Gozlan H, Guardiola-Lemaitre B, Hamon M (1993) (−)Tertatolol is a potent antagonist at pre- and postsynaptic serotonin 5-HT_{1A} receptors in the rat brain. Naunyn Schmiedebergs Arch Pharmacol 347: 453–463

Junzo O, Shimizu K (1978) Occurrence of dopamine-containing neurons in the midbrain raphe nuclei of the rat. Neurosci Lett 8: 317–320

Kalén P, Skagerberg G, Lindvall O (1988) Projections from the ventral tegmental area and mesencephalic raphe to the dorsal raphe nucleus in the rat. Exp Brain Res 73: 69–77

Lejeune F, Rivet J-M, Gobert A, Canton H, Millan MJ (1993) WAY100,135 and (−)-tertatolol act as antagonists at both 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors in vivo. Eur J Pharmacol 240: 307–310

Miquel M-C, Doucet E, Boni C, El Mestikawy S, Matthiessen L, Daval G, Vergé D, Hamon M (1991) Central serotonin$_{1A}$ receptors: respective distributions of encoding mRNA, receptor protein and binding sites by in situ hybridisation histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain. Neurochem Int 19: 453–465

Nomikos GG, Arborelius L, Svensson TH (1992) The novel 5-HT_{1A} receptor antagonist (S)-UH-301 prevents (R)-8-OH-DPAT-induced decrease in interstitial concentrations of serotonin in the rat hippocampus. Eur J Pharmacol 216: 373–378

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, Sydney

Pazos A, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res 346: 205–230

Sinton CM, Fallon SL (1988) Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT_1 receptor. Eur J Pharmacol 157: 173–181

Sotelo C, Cholley B, El Mestikawy S, Gozlan H, Hamon M (1990) Direct immunohistochemical evidence of the existence of 5-HT_{1A} autoreceptors on serotonergic neurons in midbrain raphe nuclei. Eur J Neurosci 2: 1144–1154

Sprouse JS, Aghajanian GK (1987) Electrophysiological responses of sertonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonist. Synapse 1: 3–9

Sprouse JS, Aghajanian GK (1988) Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons. Neuropharmacology 27: 707–715

Authors' address: Dr. T. H. Svensson, Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institutet, S-17177 Stockholm, Sweden

Received December 14, 1993