ELSEVIER

European Journal of Pharmacology 316 (1996) 43–47

ejp

Short communication

# Raphe 5-$HT_{1A}$ autoreceptors, but not postsynaptic 5-$HT_{1A}$ receptors or β-adrenoceptors, restrain the citalopram-induced increase in extracellular 5-hydroxytryptamine in vivo

Stephan Hjorth *, H. Jörgen Bengtsson, Stéphane Milano

*Department of Pharmacology, University of Göteborg, Göteborg, Sweden*

Received 9 September 1996; accepted 24 September 1996

**Abstract**

In vivo microdialysis in rat ventral hippocampus was used (i) to verify the importance of 5-$HT_{1A}$ autoreceptors in the raphe as targets for drugs that enhance the citalopram-induced elevation of forebrain 5-hydroxytryptamine (5-HT), and (ii) to further examine the specificity of (−)-penbutolol in this regard. The selective 5-$HT_{1A}$ receptor antagonist WAY100635 (s.c., or intra-raphe) or the mixed 5-$HT_{1A/1B}$/β-adrenoceptor antagonist (−)-penbutolol (s.c.), potentiated the citalopram-induced 5-HT rise, whereas local 'reverse' dialysis of WAY100635 into the ventral hippocampus did not. Furthermore, the (−)-penbutolol-induced augmentation proved stereoselective and not mediated by β-adrenoceptors (no effect of s.c. (+)-penbutolol, or $\beta_1$- and $\beta_2$-adrenoceptor blockers (betaxolol, ICI118.551)). These data provide direct evidence that increased stimulation of 5-$HT_{1A}$ autoreceptors in the midbrain raphe impedes the effect of citalopram on forebrain extracellular 5-HT, whereas neither postsynaptic 5-$HT_{1A}$ receptors nor β-adrenoceptors appear to be involved.

*Keywords:* Microdialysis, in vivo; Citalopram; WAY-100635; Penbutolol enantiomer; 5-$HT_{1A}$ autoreceptor, raphe; 5-$HT_{1A}$ receptor, postsynaptic; β-Adrenoceptor; Antidepressant augmentation

## 1. Introduction

Previous in vivo microdialysis studies have indicated that systemic administration of 5-hydroxytryptamine (5-HT) reuptake blockers may elevate extracellular levels of 5-HT more in the midbrain raphe than in corresponding forebrain projections, tentatively due to a concomitant increase in autoreceptor tone (Adell et al., 1991; Bel and Artigas, 1992). Subsequent work suggested that the 5-$HT_{1A}$ autoreceptors, in particular, may be responsible for restraining the elevation of extracellular 5-HT induced by selective serotonin reuptake inhibitors (SSRIs) like citalopram. However, the results of these latter studies are incomplete, since they involve the use of imperfect pharmacological tools (Invernizzi et al., 1992) and/or systemic (Hjorth, 1993) rather than direct intra-raphe drug administration. Moreover, the potential contributions from postsynaptic 5-$HT_{1A}$ receptors and β-adrenoceptors in the SSRI-potentiating effects of selective 5-$HT_{1A}$ and mixed 5-$HT_{1A}$/β-adrenoceptor agents have not been addressed previously. The purpose of the present experiments was two-fold, (i) to complement and verify, by means of subcutaneous (s.c.), intra-raphe or hippocampal 'reverse'-dialysis administration of the new potent and selective 5-$HT_{1A}$ receptor antagonist, WAY100635 (Forster et al., 1995), the importance of 5-$HT_{1A}$ autoreceptors in the raphe (vs. postsynaptic 5-$HT_{1A}$ receptors), as targets for drugs that potentiate the citalopram-induced elevation of extracellular 5-HT, and (ii) to further examine the specificity of the mixed 5-$HT_{1A/1B}$ and β-adrenoceptor blocker (−)-penbutolol – in particular, with respect to stereoselectivity and involvement of β-adrenoceptors – in this regard. Preliminary accounts of some of these findings have been given at the 25th Annual Meeting of the American Society

* Corresponding author. Institute of Physiology and Pharmacology, Department of Pharmacology, University of Göteborg, Medicinareg. 7, S-413 90 Göteborg, Sweden. Tel./fax (direct): +46 (0)31-773 34 28; fax (general): +46 (0)31-82 17 95; e-mail: Stephan.Hjorth@pharm.gu.se


*PII* S0014-2999(96)00779-0

for Neuroscience (Hjorth and Milano, 1995), and the 37th Annual Meeting of the Scandinavian Society for Psychopharmacology (Hjorth et al., 1996).

## 2. Materials and methods

### 2.1. Animals

The studies were carried out in male Sprague-Dawley rats (280–350 g; B & K Universal, Sollentuna, Sweden). The animals were housed in our animal quarters, 4–5/cage under controlled environmental conditions (temperature 22–25°C; humidity 55–65%; 14/10 h dark/light cycle, lights on 06.00 a.m.; rat chow and tap water available ad libitum) for at least one week after arrival until used in the experiments. The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals, and were approved by the Animal Ethics Committee of the University of Göteborg. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

### 2.2. Drugs

Citalopram (HBr; H. Lundbeck, Copenhagen-Valby, Denmark), betaxolol (Synthelabo, Paris, France), ICI118.551 (erythro-*dl*-1-(7-methylindan-4-yloxy)-3-isopropyl-amino-butan-2-ol HCl; ICI, Macclesfield, UK), (−)- and (+)-penbutolol hemisulphate (Hoechst, Stockholm, Sweden) and WAY100635 (*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(pyridinyl)cyclohexanecarboxamide oxalate; courtesy Dr. C. Sanchez, H. Lundbeck, Copenhagen-Valby, Denmark) were dissolved in 0.9% saline and given subcutaneously (s.c.; either agent). In some experiments, WAY100635 (in saline) was instead administered directly into the dorsal raphe area, or dissolved in artificial cerebrospinal fluid (aCSF) and applied by 'reverse' dialysis into the ventral hippocampus (cf. below).

### 2.3. Microdialysis and raphe injection procedures

The microdialysis experiments were performed in chloral hydrate-anaesthetized rats (initial dose 400 mg/kg, i.p.; suppl. dosing – ≈ 80–100 mg/kg/h – assured adequate surgical anaesthesia for the remainder of the experiment), as described elsewhere (Hjorth and Sharp, 1993; Sharp et al., 1989). A U-shaped microdialysis probe, continuously perfused with aCSF (composition: see e.g. Hjorth, 1993), was implanted in the ventral hippocampus, with the probe tip at AP −4.8, ML +4.6, DV −8.5 vs. bregma and dura surface (Paxinos and Watson, 1986). Twenty-minute dialysates were collected and immediately analysed for 5-HT by standard HPLC-EC methods (Hjorth, 1993). Stable baseline dialysate levels of 5-HT were typically obtained 2–4 h after probe implantation.

For the intra-raphe injection, a 250 μm OD stainless steel hollow guide cannula was stereotaxically implanted at a lateral angle of 32° to the vertical off the midline, with the cannula tip aimed at the border of the dorsa raphe nucleus (AP −7.8, ML +0.2, DV −6.0 vs. bregma and dura surface; Paxinos and Watson, 1986), ipsilaterally to the hippocampal dialysis probe. An injection unit, constructed from fused silica (SGE, UK; 140/75 μm OD/ID) glued to polyethylene tubing and connected to a 5 μl Hamilton syringe, was inserted to the full depth of the guide cannula. The Hamilton syringe was placed in a micro-perfusion syringe pump (MD-1001; BAS, W. Lafayette, IN, USA) which delivered 1 μl of drug (WAY100635) or saline solution over a period of 5 min; after completion of the infusion, the injection unit was left in position for the remainder of the experiment. Immediately following the experiment, 1 μl of Fast Green dye solution was injected in the same manner, to aid in subsequent gross histological verification of the site of the injection. Only data obtained in rats where the dye spots were found within the confines of the dorsa raphe nucleus were included in the calculations.

### 2.4. Statistics

The experimental data are expressed as absolute differences (fmol/20 μl sample) from the individual pre-injection 5-HT values (baseline). Statistical analysis of the overall responses in the different treatment groups was carried out by means of a repeated measures analysis of variance (ANOVA) followed by Fisher's protected least significance differences (PLSD) test, using StatView 4.0 for the Macintosh. Probabilities of $\leq 5\%$ were considered statistically significant.

## 3. Results

### 3.1. Basal 5-HT levels, effect of citalopram alone

The average basal 5-HT levels in the dialysates were $6.7 \pm 0.5$ fmol/20 μl sample ($n = 35$). The present data represent results from two separate experimental series: (1) citalopram plus WAY100635 (s.c. injection, intra-dorsa raphe nucleus or hippocampus infusion), and (2) citalopram plus penbutolol isomers or betaxolol/IC118.551. As seen in Figs. 1 and 2, citalopram (5 mg/kg, s.c.) consistently elevated the ventral hippocampal dialysate 5-HT levels throughout, although the absolute magnitude of the increase in the citalopram control groups varied slightly between the two experimental series. By and large, the maximum elevation induced by citalopram corresponded to about a doubling of the initial pre-injection baseline values.

### 3.2. Systemic WAY100635

As shown in Fig. 1A, the citalopram-induced rise in dialysate 5-HT was clearly potentiated by WAY100635 (0.3 mg/kg, s.c.), administered 1 h after the reuptake inhibitor, the resulting levels being approximately 4 times the initial, pre-citalopram, baseline value. This dose of WAY100635 did not significantly change the 5-HT output when given alone (Fig. 1A).

### 3.3. Intra-raphe or intra-hippocampal WAY100635

Also the local bolus injection of WAY100635 (1 μg in 1 μl) into the dorsa raphe nucleus significantly augmented the enhanced extracellular levels of 5-HT as compared to controls injected with citalopram and subsequently infused with saline into the dorsa raphe nucleus (Fig. 1B). In contrast, the local 'reverse'-dialysis perfusion of ventral hippocampus with WAY100635 (10 μM added to the aCSF; commencing 60 min after citalopram injection and continuing for the remainder of the experiment) failed to alter the citalopram-induced elevation of extracellular 5-HT (Fig. 1B).

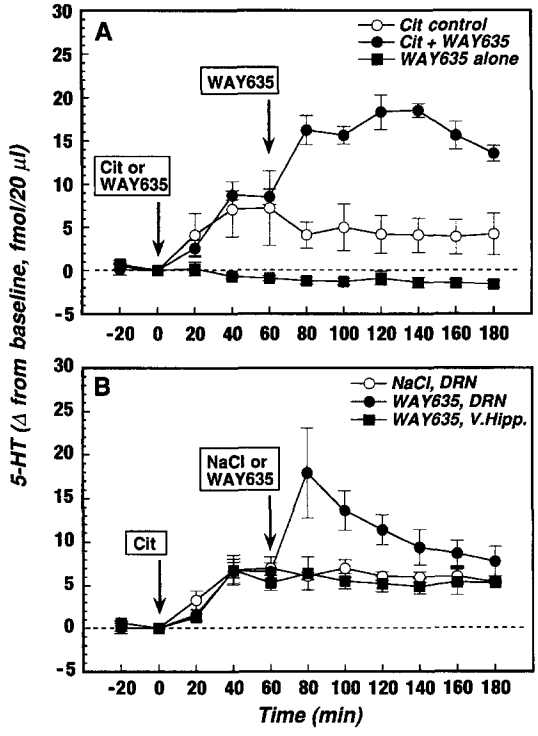


Fig. 1. Effect of systemic, intra-raphe or intra-hippocampal administration of WAY100635 on the citalopram-induced elevation of extracellular 5-HT. (A) Citalopram (5 mg/kg, s.c.) was given at time zero (1st arrow), and WAY100635 (0.3 mg/kg, s.c.) 1 h thereafter (2nd arrow). A separate group received WAY100635 (0.3 mg/kg, s.c.) only, at time zero. (B) Citalopram (5 mg/kg, s.c.) was given at time zero (1st arrow), and, starting 1 h thereafter (2nd arrow), WAY100635 was either injected into the dorsa raphe nucleus (DRN; 1 μg in 1 μl), or applied by 'reverse' dialysis (10 μM via the perfusion medium for 2 h) into the hippocampus. Each point represents the mean ± S.E.M. of 3–6 observations. The dashed line depicts extended zero baseline value. The citalopram response was significantly ($P < 0.01$) potentiated by systemic or intra-raphe WAY100635, but not by the intra-hippocampal 'reverse'-dialysis application of the compound.

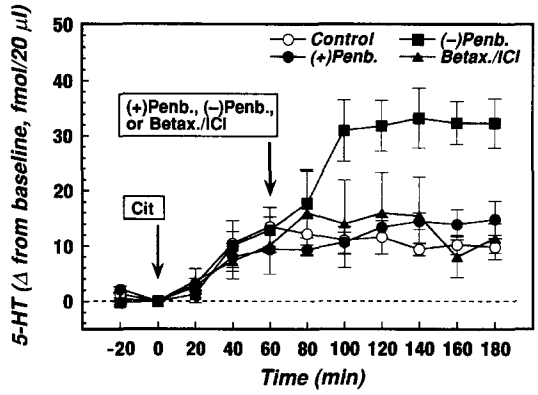


Fig. 2. Effect of penbutolol isomers or betaxolol plus ICI118.551 on the citalopram-induced elevation of extracellular 5-HT. Citalopram (5 mg/kg, s.c.) was given at time zero (1st arrow), and (+)-penbutolol, (−)-penbutolol or betaxolol + ICI118.551 (8 mg/kg, s.c., of either agent) 1 h thereafter (2nd arrow). Each point represents the mean ± S.E.M. of 4–5 observations. Data for (−)-penbutolol were taken from Hjorth (1993). The dashed line depicts extended zero baseline value. The citalopram response was significantly ($P < 0.01$) potentiated by (−)-penbutolol, but not by (+)-penbutolol or by betaxolol + ICI118.551.

### 3.4. (+)-Penbutolol, betaxolol and ICI118.551

In contrast to WAY100635 and (−)-penbutolol (Hjorth and Sharp, 1993), (+)-penbutolol (8 mg/kg, s.c.) did not significantly enhance the citalopram-induced rise in extracellular 5-HT (Fig. 2). Similarly, the combined treatment with betaxolol and ICI118.551 (8 mg/kg, s.c., either agent), $\beta_1$- and $\beta_2$-adrenoceptor blockers, respectively, failed to alter the citalopram response (Fig. 2). Neither the penbutolol enantiomers, nor betaxolol/ICI118.551 affect the output of 5-HT vs. controls under comparable conditions (Hjorth and Sharp, 1993; unpublished data).

## 4. Discussion

The results of this study extend and validate previous observations by our group and others concerning the involvement of 5-HT autoreceptors in the short-term restraint of the 5-HT-promoting action of citalopram. Specifically, the present data provide *direct* evidence that citalopram's effect is impeded by increased stimulation of cell body 5-$HT_{1A}$ autoreceptors (secondary to raised extracellular 5-HT levels) in the midbrain raphe. Thus, the local administration into the dorsa raphe nucleus of WAY100635, a new selective 5-$HT_{1A}$ receptor blocker (Forster et al., 1995), strongly augmented the capacity of systemically given citalopram to increase extracellular levels of 5-HT in the ventral hippocampus. Although we

cannot entirely exclude that WAY100365 also diffused to areas outside the dorsa raphe nucleus in our studies, it should be noted that the local application of WAY100635 by 'reverse' dialysis in the hippocampus was ineffective, arguing against the involvement of postsynaptic 5-$HT_{1A}$ receptors in this area. These data thus extend previous reports, where less selective drugs or systemic rather than intra-raphe drug administration (Hjorth, 1993; Invernizzi et al., 1992) were used to potentiate the 5-HT-elevating action of citalopram. Also consistent with this are recent findings that the reduction in frontal cortical 5-HT release elicited by intra-raphe infusion of citalopram is antagonized by the systemic administration of WAY100635 (Romero and Artigas, personal communication), and that this antagonist offsets the inhibitory action of paroxetine (both drugs given systemically) on 5-HT neuronal firing in the dorsa raphe nucleus, and, at the same time, promotes the elevation of extracellular 5-HT in the rat frontal cortex (Gartside et al., 1995). (It should be noted that the ability of 5-$HT_{1A}$ receptor blocking drugs to enhance the SSRI-induced elevation of 5-HT output is not dependent on whether or not anaesthetized animals are used (cf., e.g., Dreshfield et al., 1996).)

The present data additionally show that the ability of the 5-$HT_{1A \pm 1B}$ receptor antagonist (−)-penbutolol to promote the 5-HT response to citalopram (Hjorth, 1993) (cf. Fig. 2) is stereoselective, and is not due to its β-adrenoceptor blocking properties. Thus, whereas (−)-penbutolol causes a marked accentuation of the rise in extracellular 5-HT following citalopram, an identical dose of the inactive isomer – (+)-penbutolol – failed to do so. Further, a combination of the potent lipophilic $\beta_1$- and $\beta_2$-adrenoceptor blocking agents betaxolol and ICI118.551, respectively, which lack 5-$HT_{1A}$ receptor affinity (Middlemiss et al., 1985), did not significantly alter the citalopram response.

It is well established that drugs which enhance 5-HT neurotransmission are effective in the treatment of depression. Current theory assumes that a net rise in 5-HT availability is important for clinical efficacy. In particular, the mechanism of action of drugs like citalopram has been proposed to involve a sustained increase in agonistic tone at, and thus a gradual down-regulation of, raphe 5-$HT_{1A}$ autoreceptors during prolonged 5-HT reuptake blockade – corresponding to the 2–3 week delay typically required for a therapeutic response to occur (Blier et al., 1987; Chaput et al., 1991). Logically, the direct drug-induced blockade of 5-$HT_{1A}$ autoreceptors should be functionally comparable to a desensitization of these sites. If the above is correct, it can thus be expected that co-treatment with 5-$HT_{1A}$ autoreceptor blocking drugs would avoid the therapeutic time lag of 5-HT reuptake-blocking antidepressants, and possibly also enhance their effectiveness in refractory patients. Interestingly, the results of recently reported open clinical studies are consistent with this idea. In patients with unipolar major depression, the addition of the 5-$HT_{1A}$ receptor (/β-adrenoceptor) antagonist pindolol to antidepressant medication with 5-HT reuptake blocking agents resulted in an accelerated onset of action, and remission of depression also in previously treatment-refractory patients (Artigas et al., 1994; Blier and Bergeron, 1995; Hjorth et al., in preparation).

In summary, our data corroborate the conclusion that indirect stimulation of 5-$HT_{1A}$ autoreceptors – secondary to increased extracellular 5-HT in the raphe cell body areas – limits the overall 5-HT transmission-promoting action of SSRI; postsynaptic 5-$HT_{1A}$ receptors and β-adrenoceptors do not appear involved. The data are thus also relevant to the aforementioned clinical strategy to use pindolol to augment the efficacy and accelerate the onset of action of 5-HT reuptake blockers in the treatment of depression. Based on current hypotheses, selective 5-$HT_{1A}$ autoreceptor antagonists, or novel drugs carrying both 5-HT reuptake and 5-$HT_{1A}$ autoreceptor blocking properties, may be anticipated to become of clinical value as augmentation treatment or mono-therapy, respectively, in the management of conditions with presumed 5-HT deficiency, such as depression. However, as yet no such agents are available for clinical use. Meanwhile, it might therefore be suggested that not only pindolol, but also penbutolol, and possibly other similar-acting agents, could be tried as augmentation therapy in combination with 5-HT reuptake-blocking drugs.

## Acknowledgements

Gerd Leonsson provided skilful technical assistance in these studies. The generous gifts of citalopram, WAY100635 (H. Lundbeck A/S), penbutolol enantiomers (Hoechst), betaxolol (Synthelabo) and ICI118.551 (ICI) are gratefully acknowledged. This study was financially supported in part by Krapperup and Lundbeck Foundations, Astra Arcus, and the Sw. MRC (#07864 to S.H.). S.M. was the recipient of a fellowship from the Sw. Institute, and H.J.B. held a Göteborg University Summer Student fellowship during the course of this work.

## References

Adell, A., A. Carceller and F. Artigas, 1991, Regional distribution of extracellular 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in the brain of freely moving rats, J. Neurochem. 56, 709.

Artigas, F., V. Perez and E. Alvarez, 1994, Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors, Arch. Gen. Psychiatry 51, 248.

Bel, N. and F. Artigas, 1992, Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei – an in vivo microdialysis study, Eur. J. Pharmacol. 229, 101.

Blier, P. and R. Bergeron, 1995, Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression, J. Clin. Psychopharmacol. 15, 217.

Blier, P., C. De Montigny and Y. Chaput, 1987, Modifications of the serotonin system by antidepressant treatments: implications for the

therapeutic response in major depression, J. Clin. Psychopharmacol. 7, 24S.

Chaput, Y., C. De Montigny and P. Blier, 1991, Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments – an in vivo electrophysiologic study in the rat, Neuropsychopharmacology 5, 219.

Dreshfield, L.J., D.T. Wong, K.W. Perry and E.A. Engleman, 1996, Enhancement of fluoxetine-dependent increase of extracellular serotonin (5-HT) levels by (−)-pindolol, an antagonist at 5-$HT_{1A}$ receptors, Neurochem. Res. 21, 557.

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

Gartside, S.E., V. Umbers, M. Hajos and T. Sharp, 1995, Interaction between a selective 5-$HT_{1A}$ receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT, Br. J. Pharmacol. 115, 1064.

Hjorth, S., 1993, Serotonin 5-$HT_{1A}$ autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo – a microdialysis study, J. Neurochem. 60, 776.

Hjorth, S. and S. Milano, 1995, Further studies on the role of 5-$HT_{1A}$ autoreceptors in the effect of 5-HT reuptake blockade on extracellular 5-HT levels in the rat brain, Soc. Neurosci. Abstr. 21, 1368.

Hjorth, S. and T. Sharp, 1993, In vivo microdialysis evidence for central serotonin$_{1A}$ and serotonin$_{1B}$ autoreceptor blocking properties of the beta-adrenoceptor antagonist (−)penbutolol, J. Pharmacol. Exp. Ther. 265, 707.

Hjorth, S., H.J. Bengtsson and S. Milano, 1996, The 5-$HT_{1A}$ antagonists, WAY100635 and (−)pindolol, augment the 5-HT-elevating effect of the SSRI citalopram in vivo, whereas buspirone does not; systemic and intra-raphe drug injection studies, Nordic J. Psychiat. 50, 96.

Invernizzi, R., S. Belli and R. Samanin, 1992, Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex, Brain Res. 584, 322.

Middlemiss, D.N., J. Neill and M.D. Tricklebank, 1985, Subtypes of the 5-HT receptor involved in hypothermia and forepaw treading induced by 8-OH-DPAT, Br. J. Pharmacol. 85, 251P.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates, 2nd edn. (Academic Press, San Diego).

Sharp, T., S.R. Bramwell, D. Clark and D.G. Grahame-Smith, 1989, In vivo measurements of brain extracellular 5-hydroxytryptamine using microdialysis: changes in relation to 5-hydroxytryptaminergic neuronal activity, J. Neurochem. 53, 234.