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Short communication

(−)-Pindolol, but not buspirone, potentiates the citalopram-induced rise in extracellular 5-hydroxytryptamine

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Abstract

Recent open clinical studies suggest that pindolol and buspirone may enhance the efficacy and/or shorten the latency to antidepressant action of selective serotonin reuptake inhibitors (SSRI) in unipolar major depressive disorder. The present investigation addressed the possibility that these agents share the ability to enhance the extracellular 5-hydroxytryptamine (5-HT)-elevating response to the SSRI citalopram. For the purpose, in vivo microdialysis in the rat ventral hippocampus was employed. (−)-Pindolol (8 mg/kg s.c.) augmented the citalopram (5 mg/kg s.c.)-induced rise of extracellular 5-HT levels, whereas buspirone (5 mg/kg s.c.) failed to do so. This effect of (−)-pindolol probably reflects its ability to block 5-HT_{1A} autoreceptors, thereby abating the citalopram-induced indirect activation of these sites (secondary to the inhibition of 5-HT reuptake and elevation of extracellular 5-HT in the midbrain raphe). The lack of effect of buspirone in this model indicates that the clinically observed antidepressant augmentation action of buspirone is not mediated indirectly, via enhanced extracellular levels of 5-HT.

Keywords: 5-HT_{1A} autoreceptor antagonism; (−)-Pindolol; Buspirone; Selective serotonin reuptake inhibitor (SSRI); Citalopram; 5-HT (5-hydroxytryptamine, serotonin), extracellular; Microdialysis, in vivo

1. Introduction

Pindolol and buspirone possess relatively high affinity for brain 5-HT_{1A} receptors (Hoyer, 1988), and may be functionally characterised as antagonist, and partial agonist/antagonist, respectively. Interestingly, however, recent open clinical studies suggest that both of these agents may be used to enhance efficacy and/or shorten the latency to antidepressant action of selective serotonin reuptake inhibitors (SSRI) in unipolar major depressive disorder (Artigas et al., 1994; Blier and Bergeron, 1995; Jacobsen, 1991; Joffe and Schuller, 1993). As regards the underlying mechanism(s), the antidepressant augmentation effect of pindolol may be mediated by 5-HT_{1A} autoreceptor antagonism (Artigas, 1993; Hjorth, 1993; Hjorth and Milano, 1995) whereas that of buspirone is unclear. Previous in vivo microdialysis work in the rat has demonstrated that the pindolol analogue (−)-penbutolol potentiates the 5-hydroxytryptamine (5-HT)-elevating response to citalopram in the rat central nervous system, in all likelihood primarily owing to its 5-HT_{1A} autoreceptor blocking properties (Hjorth, 1993; Hjorth and Milano, 1995). The present experiments were undertaken to address the possibility that (−)-pindolol and buspirone share the ability to enhance the 5-HT-elevating response to the SSRI citalopram, tentatively via interaction with 5-HT_{1A} autoreceptor sites.

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

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Animal Ethics Committee of the University of Göteborg. All efforts were made to minimise animal suffering and to reduce the number of animals used in the experiments.

2.2. Microdialysis procedure

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 (cf. Hjorth and Sharp, 1993). A U-shaped microdialysis probe, continuously perfused with artificial cerebrospinal fluid (aCSF; composition as in 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). 20 min dialysate fractions were collected and immediately analyzed for 5-HT by standard high pressure liquid chromatographic (electrochemical detection) methodology (cf. Hjorth, 1993). Stable baseline dialysate levels of 5-HT were typically obtained 2–3 h after probe implantation.

2.3. Drugs

Citalopram (HBr; H. Lundbeck, Copenhagen-Valby, Denmark) and buspirone (HCl; Bristol-Myers/Squibb, Wallingford, CT, USA) were dissolved in 0.9% saline. (−)-Pindolol (Sandoz, Basel, Switzerland) was dissolved in a minimal quantity of glacial acetic acid, diluted to volume in saline, and neutralized – when necessary – with solid sodium bicarbonate. All compounds were given subcutaneously (s.c.) into the neck region, in a volume of 1 ml/kg rat body weight.

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 significant difference (PLSD) test, using StatView 4.0 for the Macintosh. Probabilities of ≤ 5% were considered statistically significant.

3. Results

The basal levels of 5-HT in the hippocampal dialysates were 6.1 ± 1.0 (4), 6.0 ± 0.4 (4) and 6.6 ± 0.8 (5) fmol/20 μl sample, for the citalopram only, citalopram/(−)-pindolol and citalopram/buspirone combination groups, respectively.

As seen in Fig. 1, and consistent with previous findings (Hjorth, 1993), citalopram (5 mg/kg, s.c.) elevated the ventral hippocampal dialysate 5-HT levels, by a maximum of 5–7 fmol above pre-injection baseline values. The citalopram-induced increase in dialysate 5-HT was significantly ($P < 0.025$) potentiated by (−)-pindolol (8 mg/kg, s.c.; 60 min after citalopram), the resulting levels being approximately three times the initial, pre-citalopram, baseline value (Fig. 1A). In contrast, buspirone (5 mg/kg, s.c.; 60 min after citalopram) failed to appreciably alter the citalopram response (Fig. 1B). Given alone, buspirone reduces 5-HT output (e.g., Routledge et al., 1993), whereas (−)-pindolol has little effect (e.g., Hjorth and Sharp, 1993).

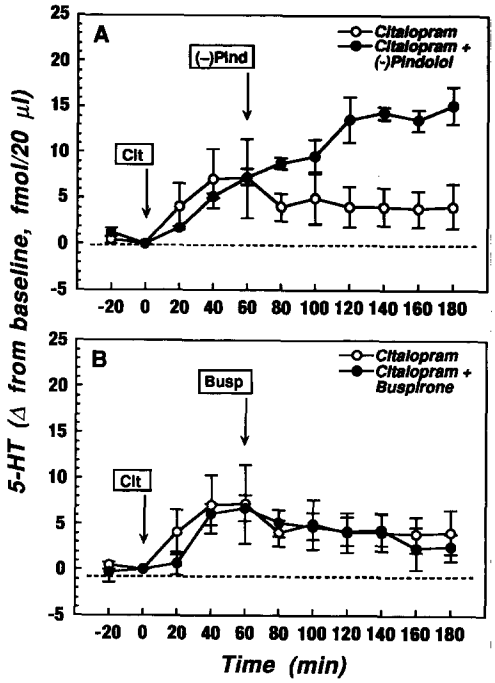

Fig. 1. Effect of (−)-pindolol and buspirone on the citalopram-induced elevation of extracellular 5-HT in the rat hippocampus. A: Rats were given citalopram (5 mg/kg, s.c.) at time zero, and (−)-pindolol (8 mg/kg, s.c.) 1 h thereafter (●). Controls received citalopram only (○). B: Rats were given citalopram (5 mg/kg, s.c.) at time zero, and buspirone (5 mg/kg, s.c.) 1 h thereafter (●). Citalopram control (○) data as in A. Shown are the absolute changes in dialysate 5-HT from the pre-citalopram baseline value, means ± S.E.M. of 4–5 observations in each time point. The dashed line depicts extended zero baseline value. The citalopram response was significantly ($P < 0.025$) potentiated by (−)-pindolol, but not by buspirone.

4. Discussion

The results of this study show that (−)-pindolol significantly augments the citalopram-induced rise of extracellular 5-HT levels in the ventral hippocampus, whereas buspirone does not. This effect of (−)-pindolol tentatively reflects its ability to block 5-HT_{1A} autoreceptors, and thereby to abate the citalopram-induced indirect activation of these sites, which occurs secondary to the inhibition of

5-HT reuptake and elevation of extracellular 5-HT in the midbrain raphe.

(−)-Pindolol possesses, in addition to its 5-HT_{1A} receptor blocking actions, strong nonselective β-adrenoceptor antagonist properties. β-Adrenoceptor blockade does not, however, appear to underlie the citalopram-promoting action of (−)-pindolol. Thus, a combination of the lipophilic, selective β_1- and β_2-adrenoceptor blocking agents betaxolol and ICI 118,551, respectively, which lack affinity for the 5-HT_{1A} receptors (Middlemiss et al., 1985), fails to significantly augment the citalopram-induced elevation of 5-HT (Hjorth and Milano, 1995).

Although it appears probable that 5-HT_{1A} autoreceptor antagonism is the main factor in the citalopram-potentiating action of (−)-pindolol, a possible contribution from nerve terminal 5-HT_{1B} autoreceptor blockade cannot be excluded. However, the blockade of 5-HT_{1B} autoreceptors *only*, is less likely to account for the effect of (−)-pindolol. Thus, local 'reverse'-dialysis infusion of the pindolol analogue, (−)-penbutolol, to separately block 5-HT_{1B} autoreceptors in the hippocampus, did not significantly alter the citalopram response, but produced a somewhat stronger potentiation when combined with systemic administration of the 5-HT_{1A} receptor antagonist, (*S*)UH-301 ((*S*)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin), than did this latter treatment alone (Hjorth, 1993). The primary role of the 5-HT_{1A} autoreceptors in restraining the acute 5-HT-elevating effect of compounds like citalopram is also indicated by several other studies (Hjorth, 1993; Hjorth and Milano, 1995; Invernizzi et al., 1992). Indeed, we have recently obtained evidence that local intra-raphe administration of WAY100635 (*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(pyridinyl)cyclohexanecarboxamide) to selectively block somatodendritic 5-HT_{1A} autoreceptors, results in a clearcut potentiation of the 5-HT rise caused by citalopram; 'reverse'-dialysis infusion of WAY100635 into the hippocampal 5-HT terminal region was ineffective in this regard (Hjorth et al., 1996).

In contrast to pindolol, buspirone did not significantly affect the 5-HT response to citalopram. Classical theory on the behaviour of partial agonists predicts that the 5-HT_{1A} autoreceptor *agonistic* action of buspirone is likely to be expressed under conditions involving low endogenous tone, whereas the compound may instead display an *antagonistic* profile when the occupancy of these sites is increased – e.g., as a consequence of raised extracellular levels of 5-HT in the raphe following inhibition of the reuptake process. Thus, consistent with the view that 5-HT_{1A} autoreceptors receive relatively little basal tone (Hjorth, 1993; Hjorth and Sharp, 1993), buspirone given alone decreases the release of 5-HT in the forebrain (Routledge et al., 1993) – similar to other agents known to stimulate the 5-HT_{1A} autoreceptors. However, the inability of the compound to enhance the citalopram-induced increase in hippocampal 5-HT suggests that its antagonistic properties are not recognized by the 5-HT_{1A} autoreceptors, even under these conditions of elevated endogenous tone. In turn, this probably at least partly relates to the high agonist responsiveness (transduction efficiency/receptor reserve) known to exist for this system (Bohmaker et al., 1993; Cox et al., 1993). The lack of significant *suppression* of 5-HT by buspirone (present data) or the full 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin, 8-OH-DPAT (Hjorth and Milano, 1995) following citalopram treatment, may tentatively suggest that the 5-HT_{1A} autoreceptors are already thoroughly occupied by the endogenous ligand. Although buspirone and 8-OH-DPAT are both efficient competitors for the 5-HT_{1A} autoreceptor sites, their agonist character may not be sufficiently distinct from that of the natural agonist, 5-HT, to have appreciable functional consequences in the present model.

In conclusion, whereas the ability of pindolol to increase the SSRI-induced elevation of forebrain extracellular 5-HT levels may provide a possible rationalization of the antidepressant augmentation obtained clinically with the compound, the mechanism by which buspirone can enhance the therapeutical efficacy of SSRI compounds remains to be clarified. The present findings clearly indicate that the action of buspirone is not mediated indirectly, via elevated extracellular levels of 5-HT in the forebrain. It should be recalled in this context that buspirone may also interact with dopamine receptors and, through its metabolite 1-PP (1-phenyl-piperazine), with α-adrenoceptors. More studies are warranted to further define the neurobiological substrates and modes of action of pindolol and buspirone in relation to their clinical use as adjunctive therapy in depression.

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