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An autoradiographic study of the influence of pindolol upon [^{35}S]GTPγS binding in rat, guinea pig and human brain

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Abstract

The 5-HT$_{1A}$/β-adrenoceptor ligand ($\pm$)pindolol has been used in clinical trials to enhance the anti-depressant effect of selective serotonin (5-HT) reuptake inhibitors (SSRIs). The accelerating effect of ($\pm$)pindolol is thought to derive from its blockade of the SSRI-induced, 5-HT$_{1A}$ autoreceptor-mediated inhibition of serotonergic cell firing and 5-HT release. However, controversial results have been reported in regard to its ability to antagonize the effect of 5-HT at such receptors. In the present study, we have analysed the effect of ($\pm$)pindolol on receptor-mediated G-protein activation by measuring guanylyl 5'-[γ-[^{35}S]thio]-triphosphate ([^{35}S]GTPγS) binding onto tissue sections from the hippocampus and dorsal raphe nucleus from rat, guinea pig and human brain. In these regions, enriched in 5-HT$_{1A}$ receptors, ($\pm$)pindolol antagonized the stimulation of [^{35}S]GTPγS binding induced by 5-HT in a concentration-dependent manner. We found that in both rat and human brain the calculated pEC$_{50}$ values were higher in the dorsal raphe nucleus than in hippocampus. This suggests a higher potency of ($\pm$)pindolol at somato-dendritic 5-HT$_{1A}$ receptors compared to post-synaptic 5-HT$_{1A}$ sites. In the absence of 5-HT, ($\pm$)pindolol (up to 10^{-3} M) did not modify [^{35}S]GTPγS binding, which remained at basal levels, indicating that, in this assay, ($\pm$)pindolol acts as a neutral antagonist rather than a partial agonist as it has been observed in other experimental models. The present data are relevant for the understanding of the neurobiological basis of pindolol acceleration of the action of SSRI antidepressants.

Introduction

Since 1994, the β-adrenoceptor/5-HT$_{1A/1B}$ receptor antagonist pindolol has been used to accelerate the clinical effects of antidepressant drugs acting primar-ily on serotonergic neurons, such as the selective serotonin (5-HT) reuptake inhibitors (SSRIs) (Artigas et al., 1994). Pindolol is thought to act by preventing the acute inhibition of 5-HT release and firing of sero-tonergic cells elicited by antidepressants, due to its ability to antagonize the action of 5-HT at midbrain raphe 5-HT$_{1A}$ autoreceptors (Artigas et al., 1996, 2001). Hence, ($-$)pindolol (the active enantiomer at 5-HT$_{1A}$ receptors) and racemic pindolol antagonize the re-ductions in 5-HT release resulting from the activation of 5-HT$_{1A}$ autoreceptors that follows the adminis-tration of SSRIs and potentiates the elevation of the extracellular concentration of 5-HT induced by these drugs (Hjorth and Auerbach, 1994; Romero et al., 1996). This view has been subsequently strengthened by the observation that ($-$)pindolol antagonizes the inhibitory effects of 5-HT on dorsal raphe 5-HT-cell firing in vitro (Corradetti et al., 1998). More recently, ($-$)pindolol has been shown to antagonize the actions of 5-HT on guanylyl 5'-[γ-[^{35}S]thio]-triphosphate (GTPγS) binding in rat brain and CHO cells trans-fected with human 5-HT$_{1A}$ receptors, while displaying a low intrinsic efficacy (Newman-Tancredi et al., 1998, 2001). On the contrary, other reports suggest that pin-dolol might act as a 5-HT$_{1A}$ receptor agonist because of its ability to suppress 5-HT cell firing in vivo (Clifford et al., 1998; Fornal et al., 1999b; Lejeune and Millan, 2000). Yet with one exception (Clifford et al., 1998), this effect is not associated with a reduction of 5-HT release in the forebrain (Dawson and Nguyen, 2000; Fornal et al., 1999a; Romero et al., 1996).

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Moreover, a substantial discrepancy exists in regard to the ability of pindolol to interact in a differential manner with auto- and hetero-5-HT$_{1A}$ receptors. Previous studies have reported a preferential blockade of the inhibitory action of 5-HT and 5-HT$_{1A}$ agonists at presynaptic somatodendritic autoreceptors (Romero et al., 1996; Tada et al., 1999), whereas others have found a comparable effectiveness at both receptor populations (Corradetti et al., 1998) or an inability to behave as a presynaptic 5-HT$_{1A}$ receptor antagonist (Fornal et al., 1999b).

In-vitro autoradiographic studies have revealed similar binding profiles of pindolol at pre- and post-synaptic sites in the rodent, primate and human brain (Raurich et al., 1999) or a slightly higher potency for presynaptic sites in human (but not rodent) brain (Castro et al., 2000). Interestingly, positron emission tomography (PET) studies consistently showed a preferential displacement of [^{11}C]WAY-100635 from pre- vs. post-synaptic 5-HT$_{1A}$ receptors in both rat and human brain (Hirani et al., 2000; Martinez et al., 2001; Rabiner et al., 2000).

The aim of the present study was to further explore the actions of pindolol at 5-HT$_{1A}$ pre- and post-synaptic sites in the rodent and human brain by monitoring its effects on [^{35}S]GTPγS binding as a measure of receptor-mediated G-protein activation.

Method

Adult male Wistar rats (200–250 g) and Dunkin–Hartley Albino guinea pigs (250–300 g) were purchased from Iffa Credo (Lyon, France). Animal care followed the Spanish legislation on 'Protection of animals used in experimental and other scientific purposes' in agreement with the European (EEC) regulations (O.J. of EC L358/1 18/12/1986). The animals were kept in a controlled environment (12-h light–dark cycle at 22 ± 2 °C), with free access to food and water. The animals were sacrificed by decapitation, brains were quickly removed, frozen on dry ice and kept at -20 °C. Frozen human brain tissue samples were obtained from the Neurological Tissue Bank, University of Barcelona, Hospital Clinic (Barcelona, Spain) from four women and two men (mean age 72 yr, range 60–78 yr, mean post-mortem delay 11 h, range 5–23 h) without clinical or histopathological evidence of neurological or psychiatric disease. The procedures followed for using human brain samples were approved by the Ethical Committee of the Hospital Clinic of Barcelona and the Bioethical Committee of the Spanish Research Council (CSIC). Tissue sections, 10 μm thick, were cut using a microtome-cryostat

(HM500M, Microm, Walldorf, Germany), thaw-mounted onto 3-aminopropyltriethoxysilane (APTS)-coated slides and kept at –20 °C until use.

The autoradiographic procedure was essentially as described previously (Dupuis et al., 1998; Sim et al., 1995). The sections were pre-incubated twice in 50 mM Hepes buffer (pH 7.4), containing 10 mM NaCl, 3 mM MgCl$_2$ and 0.2 mM EGTA for 15 min at room temperature, the second time with the addition of 2 mM GDP. Thereafter the sections were incubated for 1 h at room temperature in the same buffer containing 2 mM GDP, 0.2 mM dithiothreitol and 0.04 nM [^{35}S]GTPγS (1033 Ci/mmol; Amersham, Little Chalfont, UK), either in the absence or in the presence of agonist and/or antagonist. Stimulation curves were generated using 5-HT (10^{-10}–10^{-4} M). Pindolol was used at concentrations ranging from 10^{-7} to 10^{-4} M either in the presence or absence of 10 μM 5-HT. Basal [^{35}S]GTPγS binding was defined in the absence of both agonist and antagonist. The incubation was stopped by two washes in ice-cold 50 mM Hepes buffer (pH 7.0). The sections were dried with cold air and exposed to Kodak X-OMAT (Kodak, Rochester, NY, USA) films during 2 wk for rat and guinea pig or 8 wk for human tissue. Quantitative analysis of the autoradiograms was performed with a computerized image analysis system (MCID M4, St Catherine's, Ontario, Canada) using plastic ^{14}C standards (American Radiolabeled Chemicals, St Louis, MO, USA) which had been exposed along with the labelled tissues. Inhibition curves were statistically analysed using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

Serotonin was purchased from Sigma (St Louis, MO, USA) and ($\pm$)pindolol from Research Biochemical International (Natick, MA, USA).

Results

In the presence of 2 mM GDP, 5-HT (10 μM) stimulated [^{35}S]GTPγS binding in sections from rat, guinea pig and human hippocampus and dorsal raphe (Figure 1). The maximal effect of 5-HT in terms of per cent increment of [^{35}S]GTPγS binding relative to basal values varied among regions, being most marked in the CA1 hippocampal field (CA1), specifically in the layers oriens and radiatum in rat and guinea pig and in layers oriens and pyramidale in humans. The response to 5-HT was less marked in the dentate gyrus (molecular layer) and entorhinal cortex and still weaker in the dorsal raphe (2- to 4-fold lower than in CA1). The apparent potencies (pEC$_{50}$) displayed by 5-HT were 7.24 ± 0.17 and 6.88 ± 0.25 in the rat CA1 and dorsal
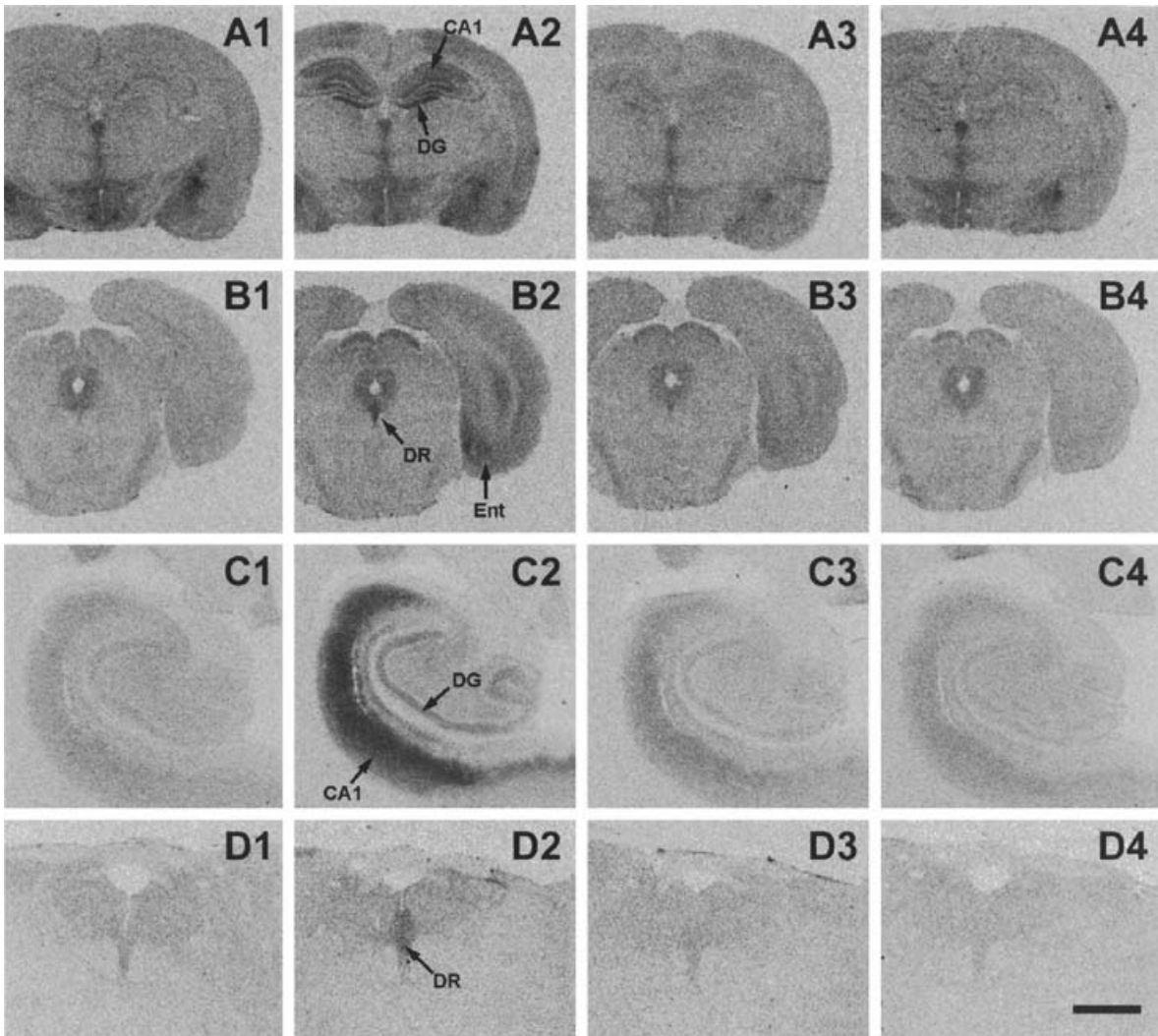
Figure 1. Autoradiographic images from rat (A, B) and human (C, D) brain sections showing the distribution of [^{35}S]GTPγS labelling in the absence of agonist (basal binding, A1–D1), or in the presence of 10 μM 5-HT alone (A2–D2), 10 μM 5-HT and 100 μM ($\pm$)pindolol (A3–D3), or 100 μM ($\pm$)pindolol alone (A4–D4). Note that 5-HT-stimulated [^{35}S]GTPγS binding is antagonized by the addition of ($\pm$)pindolol, whereas ($\pm$)pindolol on its own is without effect. CA1, CA1 hippocampal field; DG, dentate gyrus; DR, dorsal raphe; Ent, entorhinal cortex. (Bar, 3 mm.)

raphe respectively, and 7.37 ± 0.18 and 7.06 ± 0.37 in the human counterparts.

The stimulation induced by 5-HT in the aforementioned structures was completely antagonized by ($\pm$)pindolol in a concentration-dependent monophasic manner (see Figures 1 and 2). As summarized in Table 1, the pEC$_{50}$ values displayed by ($\pm$)pindolol against 10 μM 5-HT varied depending on the brain region studied, the highest potency corresponding to the dorsal raphe nucleus in the three species. Statistical analysis (two-way ANOVA) performed on the pEC$_{50}$ values obtained for ($\pm$)pindolol showed a significant effect of region factor ($F_{3,26}=8.649$; $p=0.000378$).

Post-hoc Tukey t test indicated significant differences between dorsal raphe nucleus and the other regions. Two-way ANOVA analysis (region, concentration) of the inhibition curves taken as individual values revealed a significant effect of region in human brain ($F_{3,54}=7.477$; $p=0.0003$) as well as region ($F_{3,54}=5.952$; $p=0.0014$), and region $\times$ concentration interaction ($F_{15,54}=2.051$; $p=0.0279$) in the rat brain. Post-hoc Tukey t test indicated a statistically significant difference between dorsal raphe nucleus and hippocampal structures in human and rat.

In the absence of 5-HT, ($\pm$)pindolol on its own did not modify [^{35}S]GTPγS binding in the regions
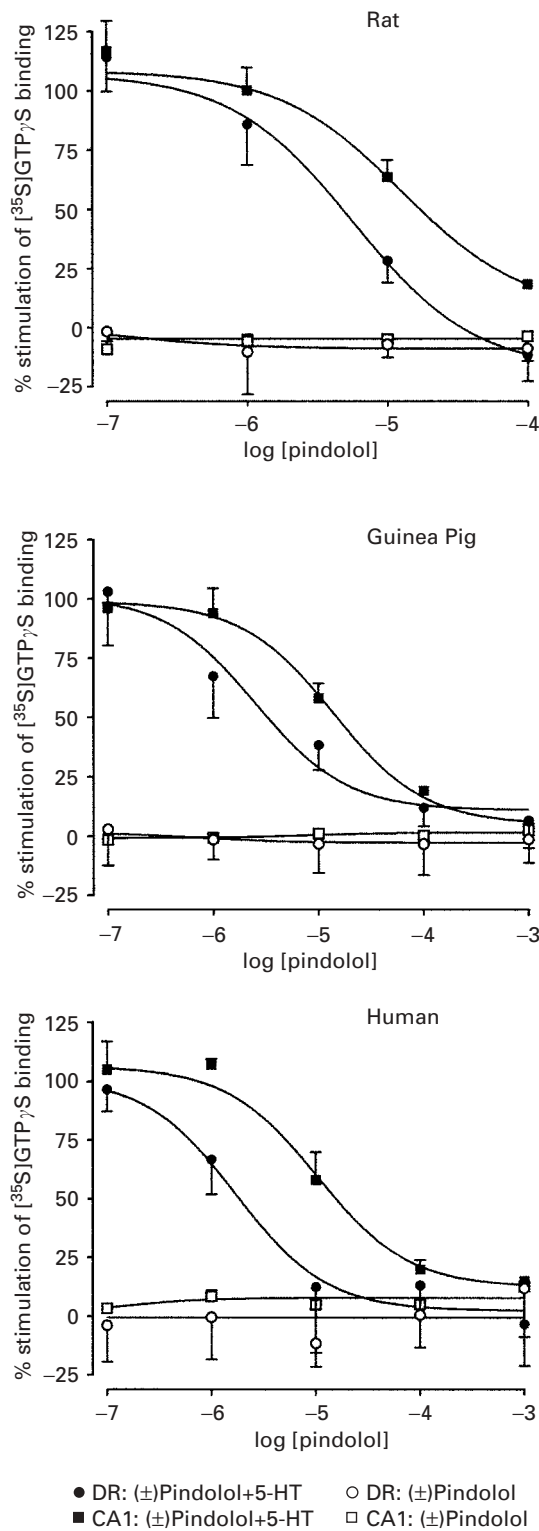
Table 1. pIC$_{50}$ values of ($\pm$)pindolol for 5-HT-stimulated [^{35}S]GTPγS binding to tissue sections

	Rat (pIC$_{50}\pm$ s.e.m.)	Guinea pig (pIC$_{50}\pm$ s.e.m.)	Human (pIC$_{50}\pm$ s.e.m.)
CA1	5.11 ± 0.13	4.86 ± 0.10	4.98 ± 0.15
DG	4.89 ± 0.15	4.74 ± 0.20	5.36 ± 0.22
EntCx	4.56 ± 0.22	4.92 ± 0.23	5.02 ± 0.21
DR	5.41 ± 0.28**	5.60 ± 0.33	5.76 ± 0.31**

CA1, CA1 hippocampal field; DG, dentate gyrus; EntCx, entorhinal cortex; DR, dorsal raphe.

($\pm$)Pindolol was used at concentrations ranging from 10^{-7} to 10^{-4} M. The concentration of 5-HT was 10 μM. [^{35}S]GTPγS was used at 0.04 nM. Data are mean$\pm$s.e.m., $n=3$ (rat and guinea pig) and $n=6$ (human) independent determinations. Two-way ANOVA of pIC$_{50}$ values obtained for ($\pm$)pindolol showed a significant effect of region factor ($F_{3,26}=8.649$; $p=0.000378$). Post-hoc Tukey t test indicated significant differences between DR nucleus and the other regions (** $p<0.01$).

analysed, which remained at basal levels (Figures 1 and 2).

Discussion

The present data show that ($\pm$)pindolol inhibits 5-HT-stimulated [^{35}S]GTPγS binding in tissue sections from rat, guinea pig and human brain in a concentration-dependent manner. In both rat and human brain, ($\pm$)pindolol displays a slightly greater potency in the dorsal raphe nucleus than in the hippocampus and entorhinal cortex. On the other hand, pindolol behaves as a neutral antagonist and does not stimulate [^{35}S]GTPγS binding in any of the species examined.

It is probable that the effects of 5-HT upon [^{35}S]GTPγS binding, as well as the ($\pm$)pindolol-mediated inhibition reported here, are primarily mediated through 5-HT$_{1A}$ receptors. This contention is based on several previous observations. First, 5-HT$_{1A}$ receptors are particularly enriched in the brain regions showing high densities of 5-HT-stimulated [^{35}S]GTPγS binding, e.g. the dorsal raphe nucleus, the entorhinal cortex or the hippocampus (Köhler et al., 1986; Pazos et al., 1987; Pazos and Palacios, 1985; Waeber et al.,

Figure 2. Effect of ($\pm$)pindolol on basal (open symbols) and 5-HT-stimulated (solid symbols) [^{35}S]GTPγS binding to hippocampus (stratum radiatum of the CA1 field in rat and guinea pig or stratum pyramidale in human) (squares) and dorsal raphe nucleus (DR) (circles) in rat, guinea pig and human brain sections. Values are means$\pm$s.e.m. obtained from three (rat and guinea pig) or six (human) specimens, and are expressed as percentage of the maximal binding obtained with 10 μM 5-HT.

1989a). It is of note that in the hippocampus the anatomical pattern of 5-HT effects was identical to the regional and laminar distribution of 5-HT$_{1A}$ receptors and clearly differed from that of 5-HT$_{1B/1D}$ receptors (Bruinvels et al., 1993, 1994). Secondly, in these regions, [^{35}S]GTPγS binding stimulated by 5-HT or 5-HT$_{1A}$ selective and non-selective agonists is inhibited by 5-HT$_{1A}$ antagonists such as WAY-100635, S15535 or NAN-190 (Dupuis et al., 1998; Newman-Tancredi et al., 1999, 2001; Sim et al., 1997; Waeber and Moskowitz, 1997; Wang et al., 1997). Thirdly, (±)pindolol is known to have high affinity (∼10 nM) for this receptor subtype (Hoyer and Schoeffter, 1991). Nonetheless, since 5-HT can also stimulate [^{35}S]GTPγS binding through 5-HT$_{1B}$ receptors, and pindolol shows a similar affinity for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in the rat brain (Hoyer and Schoeffter, 1991), the involvement of the 5-HT$_{1B}$ subtype cannot be completely ruled out in this species. However, given the negligible affinity of pindolol for human and guinea pig 5-HT$_{1B}$ receptors (Hoyer et al., 1986; Metcalf et al., 1992; Parker et al., 1993; Waeber et al., 1989b), these are unlikely to play a significant role in the reversal of the 5-HT-stimulated [^{35}S]GTPγS binding by pindolol.

The lack of an intrinsic action of pindolol on [^{35}S]GTPγS binding and its full reversal of the 5-HT stimulatory effects in all regions examined suggest that, in the experimental conditions used, pindolol behaves as a neutral antagonist. Following the clinical observations on the acceleration of antidepressant effects of SSRIs by pindolol (see Introduction), its action at 5-HT$_{1A}$ receptors has been scrutinized in the search for possible novel mechanisms to improve the clinical antidepressant action. Given the lack of selective 5-HT$_{1A}$ receptor antagonists available for human use, pindolol was used in clinical trials for its ability to antagonize several responses induced by 5-HT$_{1A}$ receptor agonists in rats and humans (Aulakh et al., 1988; De Vivo and Maayani, 1990; Gehlbach and Vandermaelen, 1987; Lesch et al., 1990; Tricklebank et al., 1987). Interestingly, the agonist/antagonist actions of pindolol depend to a large extent on the experimental models used (see Artigas et al., 2001 for review). Thus, pindolol has been shown to act as a partial agonist at recombinant 5-HT$_{1A}$ receptors where it elicits a modest activation of [^{35}S]GTPγS binding (Kobayashi et al., 1987; Pauwels et al., 1997). Consistently, pindolol exhibits a low intrinsic efficacy to inhibit adenylate cyclase in rat brain in vitro (De Vivo and Maayani, 1990). However, and in agreement with the present data, in a study using [^{35}S]GTPγS autoradiography Newman-Tancredi et al. (2001) reported a neutral antagonist action of pindolol in the rat brain.

These discrepancies may perhaps be explained by differences in receptor density and/or coupling to $G_{i/o}$ proteins between transfected cells and tissue sections, since the agonist character increases with receptor density in stable cell lines (Hoyer and Boddeke, 1993).

Similarly, the agonist/antagonist character of pindolol at 5-HT$_{1A}$ receptors in vivo is largely controversial (Artigas et al., 2001). When administered alone, pindolol reduced 5-HT synthesis (Hjorth and Carlsson, 1985) and 5-HT cell firing in anaesthetized rats (Sprouse et al., 2000) but failed to reduce 5-HT release in the rat forebrain (Romero et al., 1996). Interestingly, pindolol did not hyperpolarize 5-HT cells in the dorsal raphe nucleus or CA1 hippocampal cells in the slice preparation – an effect mediated by 5-HT$_{1A}$ receptors – and, in common with WAY-100635, blocked 5-HT-induced hyperpolarizations (Corradetti et al., 1998). Similarly, pindolol blocked the hyperpolarization of dorsal raphe 5-HT cells induced by the 5-HT$_{1A}$ agonist gepirone (Gehlbach and Vandermaelen, 1987). These electrophysiological observations fully agree with the data from the present study and give further support to the notion that pindolol behaves as a neutral antagonist at 5-HT$_{1A}$ receptors. The discrepancy between these results and the suppression of 5-HT cell firing observed in vivo may lie in a putative action at 5-HT$_{1A}$ receptors outside the dorsal raphe nucleus. Although this possibility has not been experimentally tested so far, it is supported by several observations. First, more than one third of the dorsal raphe 5-HT neurons are not inhibited by systemic pindolol administration (Clifford et al., 1998), despite 5-HT neurons expressing 5-HT$_{1A}$ autoreceptors (the sensitivity to 8-OH-DPAT or selective 5-HT$_{1A}$ receptor agonists is a basic criterion to identify a dorsal raphe neuron as serotonergic). Secondly, a low dose of pindolol antagonizes the inhibition of 5-HT cell firing produced by lysergic acid diethylamide (LSD), which is thought to act preferentially on 5-HT$_{1A}$ autoreceptors, but not that produced by 8-OH-DPAT agonist at both pre- and post-synaptic 5-HT$_{1A}$ receptors (Haddjeri et al., 1999). Finally, pre- and post-synaptic 5-HT$_{1A}$ receptors contribute to the suppression of 5-HT cell firing and 5-HT release elicited by 5-HT$_{1A}$ agonists (Casanovas et al., 1999; Ceci et al., 1994; Celada et al., 2001; Hajos et al., 1999; Romero et al., 1994). Thus, it cannot be dismissed that post-synaptic 5-HT$_{1A}$ receptors feeding back onto dorsal raphe 5-HT neurons via long loops may mediate the inhibitory action of systemic pindolol.

The actions of pindolol at pre- and post-synaptic receptors have been compared in a number of studies,

also yielding controversial results (Castro et al., 2000; Wieland and Chen, 1999). We have previously reported that (−)pindolol exhibits comparable affinities for 5-HT$_{1A}$ receptors in the dorsal raphe and those located in projection areas in different species including humans (Raurich et al., 1999). In contrast, a similar study reported a slightly higher affinity of (±)pindolol for 5-HT$_{1A}$ receptors of the dorsal raphe than for 5-HT$_{1A}$ sites in the hippocampus in the human brain, but not in the rat (Castro et al., 2000). In the present study, the potency of pindolol to antagonize the effect of 5-HT in the dorsal raphe was higher than in structures receiving serotonergic innervation. Since the vast majority of 5-HT$_{1A}$ receptors in the dorsal raphe are located on 5-HT neurons (Sotelo et al., 1990) and only a minor population appears to be expressed in non-serotonergic cells (Kirby et al., 2003), the divergence we observed is probably due to differences between pre- and post-synaptic receptors. Thus, the differential antagonism by pindolol may result from a higher affinity for presynaptic 5-HT$_{1A}$ autoreceptors, but also could be a consequence of diversities in receptor–G-protein coupling efficacy and receptor reserve between 5-HT$_{1A}$ auto- and hetero-receptors. Pindolol has also been reported to exert a selective antagonist action at presynaptic 5-HT$_{1A}$ receptors (Romero et al., 1996; Tada et al., 1999). Similarly, in PET scan studies, pindolol administration results in a preferential occupancy of pre- vs. post-synaptic 5-HT$_{1A}$ receptors both in rat and human brain (Hirani et al., 2000; Martinez et al., 2000). For instance, Rabiner et al. (2000) have shown that, in humans, a single dose of pindolol (10 mg) occupies approx. 37% of 5-HT$_{1A}$ receptors in the dorsal raphe, whereas in other brain structures the occupancy is only 13%. Similar results have been reported by Martinez et al. (2000, 2001) using repeated doses of pindolol (7.5 mg/d) during 1 wk. Therefore, pindolol at the dose used in most clinical trials (3 × 2.5 mg/d) (Artigas et al., 2001) seems to bind predominantly to presynaptic 5-HT$_{1A}$ autoreceptors, although reaching relatively low levels of occupancy.

Our results appear to indicate that 5-HT is slightly more potent in the hippocampus than the dorsal raphe, although regional differences in pEC$_{50}$ did not reach statistical significance in our study. Such variations, although minor, could contribute to the preferential occupancy of dorsal raphe 5-HT$_{1A}$ receptors observed in the aforementioned PET studies and might also explain the differences in the apparent potency of pindolol among regions.

An involvement of 5-HT receptor subtypes other than 5-HT$_{1A}$ sites could also account for the differences in the potency of pindolol between the dorsal raphe and hippocampus found in the present study. In particular, 5-HT$_{1B}$ receptors are known to be present in the dorsal raphe, and in the rat (but not in guinea pig and humans), these are sensitive to pindolol. However, as discussed above, the contribution of other 5-HT receptor subtypes seems to be minor.

In summary, the present study indicates that pindolol behaves as a neutral antagonist of 5-HT$_{1A}$ receptors in vitro, as assessed with [^{35}S]GTPγS autoradiography and inhibits the binding induced by 5-HT. The different potency of pindolol between pre- and post-synaptic sites is in keeping with some previous in-vivo and in-vitro data and may be related to the preferential occupancy of midbrain vs. post-synaptic 5-HT$_{1A}$ receptors observed in brain-imaging studies. Overall, these results contribute to a better understanding of the mechanisms involved in the potentiation of antidepressant drug action by pindolol.

Acknowledgements

Statement of Interest

None.

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