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Agonist properties of pindolol at h5-HT_{1A} receptors coupled to mitogen-activated protein kinase

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Abstract

At h5-HT_{1A} receptors, stably transfected into Chinese Hamster Ovary Cells (CHO-h5-HT_{1A}), the selective 5-HT_{1A} receptor agonist, (+)8-hydroxy-dipropyl-amino-tetralin, ((+)8-OH-DPAT), transiently activated mitogen-activated protein kinase (MAPK) with a pEC_{50} of 8.5. The arylalkylamine, (−)-pindolol, also behaved as an agonist with a maximal effect of 57% relative to (+)8-OH-DPAT (100%), and with a pEC_{50} of 7.2. The selective 5-HT_{1A} receptor antagonist, *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane carboxamide (WAY100,635), blocked (+)8-OH-DPAT- and (−)-pindolol-induced MAPK activation with pK_Bs of 9.7 and 9.9, respectively, whereas the selective 5-HT_{1B} receptor antagonist, 1′-Methyl-5-[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-ylcarbonyl]-2,3,6,7-tetrahydro-5*H*-spiro[furo[2,3-f]indole-3,4′-piperidine] (SB224,289) was inactive. Pertussis toxin blocked the actions of (+)8-OH-DPAT and (−)-pindolol demonstrating implication of G_i/G_o proteins. Thus, stimulation of MAPK provides an intracellular marker and signal for expression of the agonist actions of (−)-pindolol at h5-HT_{1A} receptors. © 2001 Published by Elsevier Science B.V.

Keywords: MAPK (Mitogen-activated protein kinase); h5-HT_{1A} receptor; (−)-Pindolol; (+)8-OH-DPAT ((+)8-Hydroxy-dipropyl-amino-tetralin); G protein

1. Introduction

The β-adrenoceptor partial antagonist, (−)-pindolol, potently interacts with 5-HT_{1A} receptors (Meltzer and Maes, 1996; Newman-Tancredi et al., 1998). Correspondingly, its acceleration of the clinical actions of antidepressants has been attributed to blockade of 5-HT_{1A} autoreceptors thereby reinforcing serotonergic and, possibly, frontocortical dopaminergic and adrenergic transmission (Artigas et al., 1996; McAskill et al., 1998; Gobert and Millan, 1999). However, both antagonist and agonist actions of (−)-pindolol have been documented in man (Meltzer and Maes, 1996) and rodents (Lejeune and Millan, 2000). The precise interaction of (−)-pindolol with 5-HT_{1A} receptors remains, thus, uncertain.

To date, several approaches have addressed this issue at the cellular level. First, affinity ratios for displacement of agonist versus antagonist radioligands indicated antagonist and weak agonist properties at cloned (human) and hippocampal (rat) 5-HT_{1A} receptors, respectively (Assié et al., 1999; Watson et al., 2000; Newman-Tancredi et al., 2001b). Second, the inability of GTP to modulate the affinity of (−)-pindolol for rat, hippocampal 5-HT_{1A} sites suggested antagonist properties (Oksenberg and Peroutka, 1988). Third, in studies of [^{35}S]guanosine-5′-*O*-(3-thiotriphosphate ([^{35}S]GTPγS) binding, (−)-pindolol demonstrated weak partial agonist actions at recombinant h5-HT_{1A} receptors transfected into diverse cell lines (Pauwels et al., 1997; Newman-Tancredi et al., 1998) and antagonist actions at cerebral, rat 5-HT_{1A} receptors (Newman-Tancredi et al., 2001a). Fourth, in rat hippocampus, De Vivo and Maayani (1986) observed only weak stimulation of adenylyl cylase activity, and employing this parameter, Oksenberg and Peroutka (1988) reported that (−)-pindolol was a pure antagonist, an observation reproduced by Pauwels et al. (1993).

Although these observations suggest that (−)-pindolol possesses low efficacy, they reflect its influence on the first step of guanine nucleotide-binding protein (G protein) activation. Recently, it was shown that 5-HT_{1A} receptors couple to mitogen-activated protein kinase (MAPK) activation via βγ subunit of G protein (Gβγ) and a multimolecular cascade involving Src-like proteins, phosphatidylinositol 3-kinase, Shc–Grb2–Sos complex (Garnovskaya et al.,

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1996; Mukhin et al., 2000) and a phosphatidylcholine-specific phospholipase C (Cowen et al., 1996). Reflecting signal amplification downstream of G proteins, MAPK is a particularly sensitive parameter for detection of low efficacy agonists (Cussac et al., 1999). Further MAPK may mediate functional actions distinct from the α subunit of G protein (Gα)-mediated adenylyl cyclase axis. Indeed, MAPK can modulate gene expression, synaptic plasticity and neuronal activity (Lopez-Ilasaca, 1998; Orban et al., 1999). Thus, herein, we examined the influence of (−)-pindolol upon 5-HT_{1A} receptor-coupled to MAPK.

2. Materials and methods

2.1. Drugs

WAY100,635 (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclo-hexanecarboxamide) and SB224,289 (1′-methyl-5-[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-ylcarbonyl]-2,3,6,7-tetrahydro-5*H*-spiro[furo[2,3-f]indole-3,4′-piperidine]) were synthesized by J.-L. Peglion, Servier. (+)8-hydroxy-dipropyl-aminotetralin ((+)8-OH-DPAT), serotonin (5-HT), (−)-pindolol and pertussis toxin were purchased from Sigma (St. Quentin Fallavier, France).

2.2. Cell culture and immunoblotting of MAPK

Chinese Hamster Ovary (CHO) cells, stably expressing h5-HT_{1A} receptors (~2.5 pmol/mg of membrane protein), were grown as described (Newman-Tancredi et al., 1998). MAPK determinations were undertaken essentially as previously (Cussac et al., 1999). Briefly, CHO-h5-HT_{1A} cells were grown until 90% confluent, then starved overnight in serum-free medium. Treatment with pertussis toxin (100 ng/ml) was performed overnight. In antagonist studies, cells were incubated for 10 min with ligands, then stimulated with agonists for 5 min. At the end of incubation, 0.2 ml/well of Laemmi buffer containing 200 mM of dithiotreitol was added and whole cell lysates were boiled for 3 min at 95 °C. "Fully" activated MAPK was revealed using a monoclonal antibody specific for phosphorylated MAPK also known as extracellular signal-regulated kinases, ERK2 and ERK 1, (NanoTools, Teningen, Germany) followed by enhanced chemiluminescence (ECL) detection (Amersham, les Ulis, France). Total MAPK immunoreactivity was determined using an antibody raised against unphosphorylated and phosphorylated forms of ERK1 and ERK2 (Santa Cruz Biotechnology, CA, USA).

2.3. Quantification of MAPK activation and data analysis

Immunoblots shown are from representative experiments repeated on at least three occasions. Autoradiograms were analysed by computerised densitometry using AIS software, (Imaging Research, Ontario, Canada). Only the ERK2 phosphorylated form was quantified. Isotherms were analysed by non-linear regression using 'PRISM' (Graphpad Software, San Diego, CA). K_B values of WAY100,635 for inhibition of (+)8-OH-DPAT and (−)-pindolol-stimulated MAPK phosphorylation were calculated according to the Cheng–Prusoff equation: $K_B = IC_{50}/(1 + (Agonist/EC_{50}))$, where IC_{50} = Inhibitory $Concentration_{50}$ of WAY100,635, Agonist = concentration of (+)8-OH-DPAT (0.1 μM) and (−)-pindolol (1 μM), and EC_{50} = Effective $Concentration_{50}$ of (+)8-OH-DPAT and (−)-pindolol alone.

3. Results

3.1. Coupling of h5-HT_{1A} receptors to MAPK activation

(+)8-OH-DPAT (0.1 μM) elicited a rapid increase in phosphorylation of the ERK2 (~42 kDa) and ERK1 (~44 kDa) forms of MAPK, peaking at 5 min (Fig. 1A), whereas total MAPK was unchanged (not shown). This time was selected for all experiments. In the presence of pertussis toxin, actions of (+)8-OH-DPAT (0.1 μM) and (−)-pindolol (1 μM) were abolished (Fig. 1B). (−)-pindolol (1 μM) increased phosphorylation of MAPK with an E_{MAX} of 57.2 ± 3.9% relative to (+)8-OH-DPAT (0.1 μM, defined as 100%) (Fig. 1B). WAY100,635 (0.1 μM)

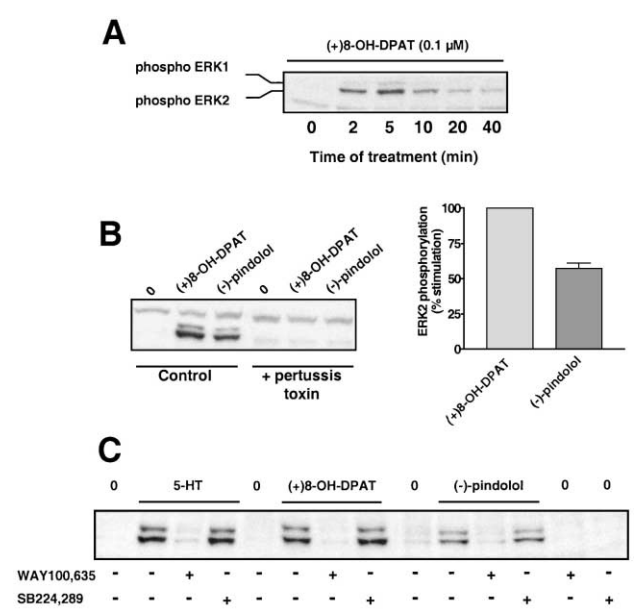

Fig. 1. MAPK activation in CHO-h5-HT_{1A} cells. (A) Time-course of (+)8-OH-DPAT (0.1 μM)-induced MAPK activation. (B) Effect of pertussis toxin upon (+)8-OH-DPAT (0.1 μM)- and (−)-pindolol (1 μM)-induced MAPK activation. (C) Effect of WAY100,635 (0.1 μM) and SB224,289 (0.1 μM) upon 5-HT (0.1μM)-, (+)8-OH-DPAT (0.1 μM)- and (−)-pindolol (1 μM)-induced MAPK activation. Histogram in B expresses actions of (−)-pindolol (1 μM) as a percentage of (+)8-OH-DPAT (0.1 μM, 100%).

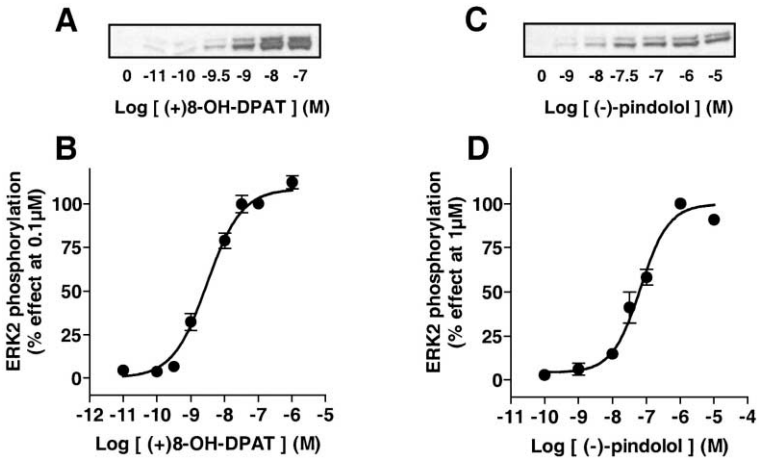

Fig. 2. Concentration-dependence of (+)8-OH-DPAT- and (−)-pindolol-induced MAPK activation. (A, C) Immunoblots of representative experiments. (B, D) Quantification: 100% represents the effects of (+)8-OH-DPAT and (−)-pindolol at 0.1 and 1 μM, respectively.

blocked stimulation induced by 5-HT (0.1 μM), (+)8-OH-DPAT (0.1 μM) and (−)-pindolol (1 μM), whereas SB224,289 (0.1 μM) was ineffective (Fig. 1C). Both antagonists (0.1 μM) did not induce MAPK activation when tested alone. WAY100,635 (0.1 μM) also abolished the agonist actions of racemic (±)pindolol (1 μM) (not shown).

3.2. Concentration-dependent effects of (+)8-OH-DPAT, (−)-pindolol and WAY100,635

Activation of MAPK by (+)8-OH-DPAT was concentration-dependent (pEC_{50}, 8.52 ± 0.08) and maximal at about 0.1 μM (Fig. 2A,B). (−)-pindolol (pEC_{50}, 7.17 ± 0.07) was 20-fold less potent than (+)8-OH-DPAT, attaining a maximal effect at 1 μM (Fig. 2C,D). WAY100,635 concentration-dependently blocked stimulation of MAPK by (+)8-OH-DPAT (0.1 μM) and (−)-pindolol (1 μM) with pIC_{50}s of 8.17 ± 0.09 and 8.72 ± 0.12, respectively, yielding pK_Bs of 9.70 and 9.93 (Fig. 3).

4. Discussion

Several arguments indicate that (−)-pindolol and (+)8-OH-DPAT activated MAPK via 5-HT_{1A} receptors coupled to G_i/G_o proteins. First, in corroboration of previous work (Cowen et al., 1996; Garnovskaya et al., 1996), activation of MAPK by (+)8-OH-DPAT and (−)-pindolol (present study) was abolished by pertussis toxin. Second, the CHO cell line employed possesses ~2.5 pmol/mg of h5-HT_{1A} receptors, and the MAPK response to (+)8-OH-DPAT corresponded well in terms of potency (pEC_{50}, ~8.5) to other quantitative studies employing comparable levels of 5-HT_{1A} receptor (Cowen et al., 1996; Mendez et al., 1999). Third, the highly selective 5-HT_{1A} receptor antago-

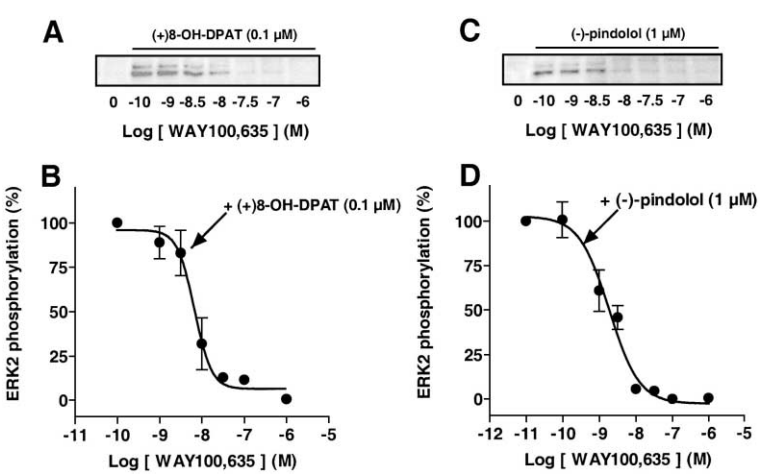

Fig. 3. Antagonism by WAY100,635 of (+)8-OH-DPAT- and (−)-pindolol-induced MAPK activation. (A, C) Immunoblots of representative experiments. (B, D) Quantification: 100% represents the effects of (+)8-OH-DPAT and (−)-pindolol at 0.1 and 1 μM, respectively.

nist, WAY100,635, blocked (−)-pindolol- and (+)8-OH-DPAT-induced stimulation of MAPK with similar potencies (pK_{Bs}, 9.9 and 9.7, respectively), close to its affinity at h5-HT_{1A} sites (pK_i, 9.9; Newman-Tancredi et al., 2001b). Fourth, although CHO cells endogenously express 5-HT_{1B} receptors which couple to MAPK with high efficacy (Mendez et al., 1999), the selective 5-HT_{1B} antagonist, SB224,289, failed to block MAPK activation by 5-HT, (+)8-OH-DPAT and (−)-pindolol.

Although (−)-pindolol was less efficacious than (+)8-OH-DPAT in activating MAPK (E_{MAX}, ~60%), its efficacy was more marked than for 5-HT_{1A}-receptor-induced [^{35}S]GTPγS binding (~20%) in these cells (Newman-Tancredi et al., 1998). Pronounced signal amplification downstream of Gβγ may explain this greater efficacy. An alternative explanation would be that stimulation of MAPK reflects engagement of G proteins different to those activating adenylyl cyclase, in line with the concept of agonist-directed trafficking (Kenakin, 1995). On the other hand, 5-HT_{1A} receptors couple to different G proteins, such as Gi_2 and Gi_3 (Garnovskaya et al., 1997). Thus, Gβγ derived from multiple G proteins might stimulate MAPK with greater efficacy than a single population of G proteins-coupling to adenylyl cyclase.

In fact, a consensus has emerged that (−)-pindolol acts as an agonist in reducing electrical activity of raphe-localized serotonergic perikarya in vivo (Romero et al., 1996; Lejeune and Millan, 2000). This observation differs to cellular models in which (−)-pindolol persistently displays low efficacy at 5-HT_{1A} receptors (see Introduction), including [^{35}S]GTPγS binding studies of raphe nucleus (Corradetti et al., 1998; Serrats et al., 2000; Newman-Tancredi et al., 2001a). Interestingly, adenylyl cyclase, which is weakly responsive to (−)-pindolol, is not involved in the influence of 5-HT_{1A} receptor agonists upon serotonergic cell bodies (Clarke et al., 1996). It is conceivable, thus, that MAPK participates in transduction mechanisms triggered by activation of 5-HT_{1A} autoreceptors. Notably, they are expressed at a high density on raphe nuclei, favouring engagement of MAPK (Mendez et al., 1999). More generally, high versus low efficacy actions of (−)-pindolol at 5-HT_{1A} receptors may principally reflect signalling via the Gβγ-MAPK versus Gα-adenylyl cyclase cascades, respectively.

In conclusion, the present observations identify MAPK as a cellular marker and signal for agonist properties of (−)-pindolol at 5-HT_{1A} receptors. Inasmuch as MAPK participates in trophic, anti-apoptopic and neuroprotective actions of 5-HT_{1A} receptor agonists (Azmitia et al., 1995; Adayev et al., 1999), the influence of (−)-pindolol upon such processes would be of interest to examine. Finally, in view of the functional importance of MAPK in short- and long-term modulation of neuronal activity (Orban et al., 1999; Elorza et al., 2000), these data are of importance to clinical and experimental studies of the influence of (−)-pindolol upon monoaminergic transmission and mood.

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