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The novel antidyskinetic drug sarizotan elicits different functional responses at human D2-like dopamine receptors

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Abstract

Sarizotan (EMD 128130) is a chromane derivative that exhibits affinity at serotonin and dopamine receptors. Sarizotan effectively suppresses levodopa-induced dyskinesia in primate and rodent models of Parkinson's disease, and tardive dyskinesia in a rodent model. Results from clinical trials suggest that sarizotan significantly alleviates levodopa-induced dyskinesia. The functional effects of sarizotan on individual dopamine receptor subtypes are not known. Here we report the functional effects of sarizotan on human D2-like dopamine receptors (D2S, D2L, D3, D4.2 and D4.4) individually expressed in the AtT-20 neuroendocrine cell line. Using the coupling of D2-like dopamine receptors to G-protein coupled inward rectifier potassium channels we determined that sarizotan is a full agonist at D3 and D4.4 receptors ($EC_{50} = 5.6$ and 5.4 nM, respectively) but a partial agonist at D2S, D2L and D4.2 receptors ($EC_{50} = 29$, 23 and 4.5 nM, respectively). Consistent with its partial agonist property, sarizotan is an antagonist at D2S and D2L receptors ($IC_{50} = 52$ and 121 nM, respectively). Using the coupling of D2-like dopamine receptors to adenylyl cyclase we determined that sarizotan is a full agonist at D2L, D3, D4.2 and D4.4 receptors ($EC_{50} = 0.51$, 0.47, 0.48 and 0.23 nM, respectively) but a partial agonist at D2S receptors ($EC_{50} = 0.6$ nM).

Keywords: Parkinson's disease; Dyskinesia; GIRK channels; Adenylyl cyclase; Sarizotan; Signaling; Functional selectivity; Dopamine receptors; AtT-20 neuroendocrine cells; Partial agonists; Electrophysiology

1. Introduction

The chronic management of Parkinson's disease (PD) using L-3,4-dihydroxyphenylalanine (L-DOPA) or dopamine receptor agonists results in development of debilitating motor problems broadly defined as dyskinesias. Studies suggest that up to 80% of PD patients develop dyskinesia within 5 years of treatment (Rascol et al., 2000; Bezard et al., 2001). The mechanisms underlying development of levodopa-induced dyskinesia are not clear; however, recent studies in non-human primate models have suggested a role for the D3 dopamine receptor (Bezard et al., 2003; Guigoni et al., 2005). The D3 receptor partial agonist, BP897, attenuates levodopa-induced dyskinesia; however, a recent study in squirrel monkeys, showed that BP897 also attenuated the antiparkinsonian effect of L-DOPA, suggesting that BP897 might be less effective for treating levodopa-induced dyskinesia in PD (Hsu et al., 2004). The attenuation of L-DOPA's beneficial effects is consistent with in vitro and in vivo reports that BP897 is an antagonist at D3 dopamine receptors (Wood et al., 2000; Wicke and Garcia-Ladona, 2001; Moreland et al., 2004).

Two recent clinical studies in PD patients have demonstrated that sarizotan (EMD 128130; (*R*)-(−)-2-[5-(4-fluorophenyl)-3-pyridylmethylaminomethyl]-chromane hydrochloride) significantly reduced levodopa-induced dyskinesia (Olanow et al., 2004; Bara-Jimenez et al., 2005). Sarizotan also improves levodopa-induced dyskinesia in rodent and primate models of PD (Bibbiani et al., 2001). More recently, sarizotan was shown

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to effectively reverse antipsychotic-induced tardive dyskinesia in a rat model (Rosengarten et al., 2006). Finally, sarizotan also reduced haloperidol-induced catalepsy in rats, a putative animal model for extrapyramidal symptoms induced by typical neuroleptics (Kleven et al., 2005).

Receptor binding studies suggest that sarizotan has high affinity for 5-HT_{1A} serotonin receptor and the D2-like dopamine receptors (D2S, D3, D4.2 and D4.4) with full agonistic activity at 5-HT_{1A} (Bartoszyk et al., 2004). Among the D2-like dopamine receptors, sarizotan exhibited the highest binding affinity for D4.2, D4.4 and D3 receptor subtypes and approximately 5-fold lower affinities at D2 dopamine receptors (Bartoszyk et al., 2004). In the same study, the in vivo, antagonistic effect of sarizotan on D2-like dopamine receptors was inferred by assessing its effect on striatal DOPA accumulation in normosensitive rats. Interestingly the study also suggested a weak partial agonistic effect of sarizotan on D2-like receptors using hypersensitive (reserpinized) rats (Bartoszyk et al., 2004). However, due to comparable affinity of sarizotan for the different dopamine receptor subtypes this in vivo approach could not be used to determine the effect of sarizotan on individual D2-like dopamine receptor subtypes signaling (Bartoszyk et al., 2004).

To overcome this problem, we characterized the functional effects of sarizotan on the individual human D2-like dopamine receptors (D2S, D2L, D3, D4.2, and D4.4) stably expressed in the AtT-20 neuroendocrine cell line. This cell line is an ideal heterologous expression system for studying D2-like dopamine receptors since it natively expresses many of the effector molecules that couple to the different D2-like dopamine receptors and also exhibits neuronal properties (Kuzhikandathil et al., 1998; Kuzhikandathil and Oxford, 1999, 2000, 2002). In particular, AtT-20 cells express endogenous adenylyl cyclase V, the only cyclase isoform that couples robustly to the D3 dopamine receptor subtype (Robinson and Caron, 1997). In addition, AtT-20 cells express endogenous GIRK channels that couple to all D2-like dopamine receptor subtypes (Kuzhikandathil et al., 1998). In this study, we compared the effect of sarizotan on the adenylyl cyclase and GIRK channel signaling pathways as they not only utilize different molecular coupling mechanisms but also affect different cellular function. The D2-like dopamine receptors couple to adenylyl cyclase via the pertussis toxin-sensitive G alpha subunits of the heterotrimeric G-proteins. In contrast, the coupling to GIRK channels is mediated by the $\beta\gamma$ subunits of the heterotrimeric G-proteins. By coupling to adenylyl cyclase, D2-like dopamine receptors regulate the synthesis of neurotransmitters. In contrast, by coupling to GIRK channels, D2-like dopamine receptors regulate membrane potential, firing of action potentials and release of neurotransmitters. Furthermore, recent studies have reported the phenomenon of functional selectivity in which the same agonist can elicit different functional responses on different signaling pathways in the same cell (Gay et al., 2004). We were interested in determining if sarizotan exhibited functional selectivity at D2-like dopamine receptors.

In this paper, we report the characterization of the functional effects of sarizotan on D2S, D2L, D3, D4.2 and D4.4 dopamine receptors individually expressed in AtT-20 cells. The five different D2-like dopamine receptor subtypes were chosen based on the previously reported high receptor binding affinity of sarizotan for these D2-like dopamine receptor subtypes (Bartoszyk et al., 2004). Using two different signaling pathways- the D2-like receptor-mediated inhibition of adenylyl cyclase and the D2-like receptor-mediated activation of G-protein coupled inward rectifier potassium channels (GIRK), we determined that depending on the receptor subtype and signaling pathway, sarizotan is both a partial agonist and a full agonist. Some of the data in this paper were previously presented as posters at the Society for Neuroscience meeting, 2004 (Kuzhikandathil et al., 2004a) and at the International Congress for Parkinson's Disease and Related Disorders, 2005 (Bartoszyk and Kuzhikandathil, 2005).

2. Methods

2.1. Cell culture and transfection

AtT-20 mouse pituitary cells were grown in Ham's F10 medium with 5% fetal bovine serum (FBS), 10% heat-inactivated horse serum, 2 mM glutamine and 50 μg/ml gentamicin (Invitrogen, Carlsbad, CA, USA). AtT-20 cells stabling expressing the human D2S, D2L, D3, D4.2 and D4.4 receptor were maintained in the above F10 culture media supplemented with 500 μg/ml G418 (Invitrogen). For electrophysiological characterization, cells were plated onto glass coverslips coated with 40 μg/ml poly L-lysine (Sigma, St. Louis, MO, USA). The generation and characterization of the AtT-20 cells stably expressing human D2S and D3 dopamine receptors have been previously reported (Kuzhikandathil et al., 1998). The human D2L expression plasmid was obtained from the UMR cDNA Resource Center (www.cdna.org). The human D4.2 and D4.4 expression plasmids (kindly provided by Dr. H.H. Van Tol, University of Toronto, Canada) were recloned into a pcDNA3.0 vector (Invitrogen) to match the human D2S and human D3 expressing plasmids used in previous studies (Kuzhikandathil et al., 1998). The plasmids were sequenced to confirm the identity of the cloned genes. To generate AtT-20 cells stably expressing human D2L, D4.2 and D4.4 receptor genes, cells were transfected with 1.0 μg of plasmid containing the receptor cDNA, using the DMRIE-C reagent (Invitrogen) in a 12-well plate. Following initial selection in 1 mg/ml G418, several clonal lines were obtained and sustained in media containing 500 μg/ml G418. Clonal lines expressing human D2L, D4.2 and D4.4 dopamine receptors were screened for function by both cAMP assay and electrophysiological recording. Three independent AtT-20 clonal lines stably expressing functional D2L, D4.2 and D4.4 dopamine receptors were identified and used in this study.

2.2. Membrane isolation

AtT-20 clonal lines expressing human D2S, D2L, D3, D4.2 and D4.4 dopamine receptors were grown in six 100 mm tissue culture plates to approximately 80% confluence. Membranes were isolated as described previously (Malmberg et al., 1993; Kuzhikandathil et al., 1998). Briefly, cells were scraped in Harvest Buffer (HB: 10 mM Tris–Cl [pH7.4], 5 mM $MgSO_4$) and lysed with 10 strokes of a Dounce A homogenizer. The nuclei were pelleted at 1000 × *g* for 5 min and the supernatant spun at 45,000 × *g* for 20 min to pellet the membrane fraction. The membrane pellet was resuspended in Binding Buffer (BB: 50 mM Tris–Cl [pH7.4], 120 mM NMDG, 5 mM KCl, 4 mM $MgCl_2$, 1 mM EDTA) and stored at −70 °C.

2.3. Radioligand binding assay

Binding assays were performed as described previously (Malmberg and Mohell, 1995). The frozen membrane pellets were thawed on ice and resuspended using three short pulses using a Branson sonifier. Membranes (20–75 μg) were incubated with various concentrations of [^{3}H]quinpirole