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BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors

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Abstract

BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride), a 5-HT_{1A} receptor partial agonist, also binds to α_1-adrenoceptors. Competition assays were performed using (±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone ([^{125}I]HEAT), and membranes prepared from Rat-1 fibroblasts expressing hamster α_{1b}-, bovine α_{1c}-, or rat α_{1d}-adrenoceptor, or their respective human homologues. Results indicate that BMY 7378 is selective for the α_{1D}-adrenoceptor subtype (pK_i: hamster α_{1b}-adrenoceptor 6.2 ± 0.03, human α_{1b}-adrenoceptor 7.2 ± 0.05; bovine α_{1c}-adrenoceptor 6.1 ± 0.02, human α_{1c}-adrenoceptor 6.6 ± 0.20; rat α_{1d}-adrenoceptor 8.2 ± 0.06, human α_{1d}-adrenoceptor 9.4 ± 0.05) and has high affinity (pA_2, 8.9 ± 0.1) for rat aorta α_1-adrenoceptor.

Keywords: α_1-Adrenoceptor; α_{1D}-Adrenoceptor subtype; BMY 7378

The characterization of the α_1-adrenoceptor subtypes (α_{1A} (also called α_{1C}) (Ford et al., 1994), α_{1B}, and α_{1D}) has been difficult due to the lack of α_1-adrenoceptor subtype selective antagonists. 5-Methylurapidil is selective for α_{1A}-adrenoceptor versus α_{1B}- and α_{1D}-adrenoceptors. No compounds have been found to be similarly selective for the α_{1B}- or α_{1D}-adrenoceptor subtypes, though recent studies by Ko et al. (1994), using isolated tissue preparations have suggested that (−)-discretamine is an α_{1D}-adrenoceptor selective antagonist. The purpose of the present study was to determine if BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) is a selective antagonist of the α_{1D}-adrenoceptor subtype. Experiments were designed to compare the binding affinities of BMY 7378 in the hamster α_{1b}-, bovine α_{1c}- and rat α_{1d}-adrenoceptor subtypes as well as in their respective human homologues and rat tissues.

BMY 7378 was synthesized in the department of Medicinal Chemistry, Glaxo Research Institute. Human α_1-adrenoceptors were expressed in Rat-1 fibroblasts. The human α_{1d}-adrenoceptor construct contained the melittin signal sequence at the NH_2-terminus to facilitate stable expression. All other materials, membrane preparations and radioligand binding assays using (±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone ([^{125}I]HEAT) were performed and analyzed as previously reported (Goetz et al., 1994) except as noted. For the human recombinant α_1 binding assays, the buffer was 25 mM piperazine-*N*,*N*′-bis-[2-ethanesulfonic acid (Pipes), 150 mM NaCl, 10 mM $MgCl_2$, 1 mM ethylenediamine-tetraacetic acid (EDTA) pH 7.5, incubated for 90 min at room temperature and washed with 25 mM Tris-HCl. For the rat submaxillary gland and rat brain cortex membrane binding assays, the buffer was 50 mM 3-[*N*-morpholino]propane-sulfonic acid (Mops), 100 mM NaCl, 10 mM $MgCl_2$, 2 mM EDTA pH 7.5, 400 pM [^{3}H]prazosin, incubated for 180 min at room temperature and washed with 25 mM Tris-HCl, pH 7.4. Experiments measuring aortic smooth muscle contraction were conducted as

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previously described (Rimele et al., 1988). Receptor profiles were obtained from Battelle's Receptogram screen (Battelle-Europe Geneva Research Centres). Statistical analyses were performed using JMP 3.0 (SAS Institute, Cary, NC, USA).

BMY 7378 is 126-fold selective for α_{1d}- (pK_i 8.2 ± 0.11, $n = 10$) versus α_{1c}-adrenoceptor (pK_i 6.1 ± 0.03, $n = 7$) and 100-fold selective for α_{1d}- versus α_{1b}-adrenoceptor (pK_i 6.2 ± 0.01, $n = 6$) when assayed in nonhuman recombinant α_1-adrenoceptor. Comparable results were obtained for the human recombinant α_1-adrenoceptor where BMY 7378 was found to be 630-fold selective for α_{1d}- (pK_i 9.4 ± 0.05, $n = 4$) versus α_{1c}-adrenoceptor (pK_i 6.6 ± 0.17, $n = 4$) and 158-fold selective for α_{1d}- versus α_{1b}-adrenoceptor (pK_i 7.2 ± 0.05, $n = 4$). Analysis of variance using Tukey-Kramer honestly significant difference post-hoc analysis demonstrated that BMY 7378 had significantly ($P < 0.05$) greater affinity for the α_{1d}-adrenoceptor subtype among both human and nonhuman receptors. Binding to membranes prepared from rat submaxillary gland (α_{1A}-adrenoceptor) and rat brain (α_{1A}- and α_{1B}-adrenoceptor) yielded pK_i values of 6.7 ± 0.04 ($n = 4$) and 6.8 ± 0.01 ($n = 3$) respectively. BMY 7378 showed no agonist activity in rat aortic smooth muscle, while it inhibited phenylephrine induced contraction with a pA_2 value of 8.9 ± 0.1 ($n = 4$). Table 1 shows the receptor binding profile for BMY 7378 against a wide range of receptors.

Table 1
Receptor profile for BMY 7378

Receptor	pIC_{50}	Receptor	pIC_{50}
Adenosine A_1	< 5.0	CCK_A	< 5.0
Adenosine A_2	< 5.0	CCK_B [a]	< 5.0
α_2-Adrenoceptor	5.1	Muscarinic M_1	< 5.0
β_1-Adrenoceptor	5.1	Muscarinic M_2	< 5.0
β_2-Adrenoceptor	< 5.0	Muscarinic M_3	< 5.0
Dopamine D_1	5.0	Nicotinic	4.9
Dopamine D_2	7.4	Histamine H_1	5.7
$GABA_A$	< 5.0	Histamine H_2 [a]	5.3
$GABA_B$	< 5.0	Histamine H_3	< 5.0
5-HT_{1A}	8.3	NMDA	< 5.0
5-HT_{1C} [c]	6.4	Angiotensin AT_1	< 5.0
5-HT_{1D} [b]	5.9	Angiotensin AT_2	< 5.0
5-HT_2	5.5	Bradykinin [a]	< 5.0
5-HT_3	< 5.0	CGRP	< 5.0
Neurokinin NK_1	< 5.0	Endothelin	< 5.0
Neurokinin NK_2	< 5.0	μ-Opiate	< 5.0
Neurokinin NK_3	< 5.0	δ-Opiate	< 5.0
Somatostatin	< 5.0	κ-Opiate [a]	< 5.0

Radioligand binding assays were performed using membrane preparations from rat tissues except [a] guinea pig, [b] cow, and [c] pig. Data are expressed as pIC_{50} from a single experiment.

BMY 7378 is the first α_{1D}-adrenoceptor subtype selective ligand that has high affinity and provides a clear pharmacological distinction between the α_{1B}- and α_{1D}-adrenoceptor subtypes. This ligand has high affinity and is more selective than (−)-discretamine which Ko et al. (1994) described as a selective α_{1D}-adrenoceptor antagonist (15- to 25-fold). Our experiments compare the binding affinities in the three recombinant subtypes expressed in fibroblast cells rather than nonhomogenous subtypes in tissues. Aortic tissues have been shown to contain various α_1-adrenoceptor subtypes in binding and functional studies (Piascik et al., 1991; Han et al., 1990; Aboud et al., 1993). In addition, Piascik et al. (1994) has found mRNA for α_{1B}-, α_{1C}- and α_{1D}-adrenoceptor in aortic tissue. The high affinity of BMY 7378 in rat aortic contractions confirms Ko's suggestions that the α_{1D}-adrenoceptor is the predominant α_1-adrenoceptor subtype involved in rat aorta contractions. BMY 7378 will be a useful tool in discriminating α_1-adrenoceptor subtypes when used with due caution as BMY 7378 has high affinity for dopamine and serotonin receptors. The use of BMY 7378 should help to delineate the distribution of the α_1-adrenoceptor subtypes.

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