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Short communication

Subtype selectivity of a new α_1-adrenoceptor antagonist, JTH-601: comparison with prazosin

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Abstract

The existence of α_1-adrenoceptors with low affinity for prazosin (α_{1L} group: α_{1L} and α_{1N} subtypes) has been proposed in addition to α_1-adrenoceptor subtypes with high affinity for prazosin (α_{1H} group: α_{1A}, α_{1B} and α_{1D} subtypes). A newly synthesized α_1-adrenoceptor antagonist, JTH-601 (*N*-(3-hydroxy-6-methoxy-2,4,5-trimethylbenzyl)-*N*-methyl-2-(4-hydroxy-2-isopropyl-5-methylphenoxy) ethylamine hemifumarate) showed approximately a 10 times higher affinity for the α_{1L} group, a similar affinity for the α_{1A} subtype, but a more than 10 times lower affinity for the α_{1B} and α_{1D} subtypes when compared with prazosin. These results provide a further pharmacological evidence that α_1-adrenoceptors with low affinity for prazosin exist in addition to those with high affinity for prazosin, suggesting that JTH-601 may be useful for characterising the α_1-adrenoceptor subtypes.

Keywords: α_1-Adrenoceptor subtype; JTH-601; Prazosin; α_{1L}-Adrenoceptor

1. Introduction

Three distinct α_1-adrenoceptor subtypes with a high affinity for prazosin (α_{1H} group: α_{1A}, α_{1B} and α_{1D}) have recently been demonstrated in pharmacological and molecular biological studies (for review, Lomasney et al., 1991; Hieble et al., 1995; Michel et al., 1995). Furthermore, the existence of additional α_1-adrenoceptors showing low affinity for prazosin (α_{1L} group: α_{1L} and α_{1N} subtypes) has been previously suggested based on pharmacological studies (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990, 1995; Ford et al., 1994). However, to date no compounds have been found to show high affinity for the α_{1L} subtype: therefore the characterisation of the α_{1L} subtype has been difficult. We have looked for compounds that show α_{1L} selectivity. Here, we report a new α_1-adrenoceptor antagonist, JTH-601 (Fig. 1), which shows a higher affinity for the putative α_{1L} group than prazosin. The results also strongly support the presence, in addition to the well characterized α_{1H} group, of a class of α_1-adrenoceptors showing low affinity for prazosin.

2. Materials and methods

Functional, native receptor binding and cloned receptor binding experiments were carried out. In the functional experiments, rat thoracic aorta and vas deferens, dog carotid and mesenteric arteries and rabbit thoracic aorta were used. As previously described (Muramatsu et al., 1990, 1995), the isolated preparations were set in organ chambers filled with modified Krebs-Henseleit solution and the isometric tension changes in response to noradrenaline were recorded. Rabbit thoracic aorta was pretreated with 10 μM chloroethylclonidine in order to inactivate the α_{1B} subtype (Oshita et al., 1993). Schild plots were used to determine the affinity constants for the antagonists.

In the native receptor binding experiments, rat liver and submaxillary gland and rabbit thoracic aorta were homogenized and the precipitated fractions after centrifugation ($80000 \times g$) were used as membrane preparations. In these experiments, only rabbit thoracic aorta membranes were preincubated with 10 μM chloroethylclonidine. In the cloned receptor binding experiments, cell membrane preparations were obtained from COS-7 cells transfected with bovine α_{1a}-, hamster α_{1b}- and rat α_{1d}-adrenoceptors (a gift from Dr. R.J. Lefkowitz, Duke University). The membranes were incubated at 30°C for 45 min with 200

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JTH-601

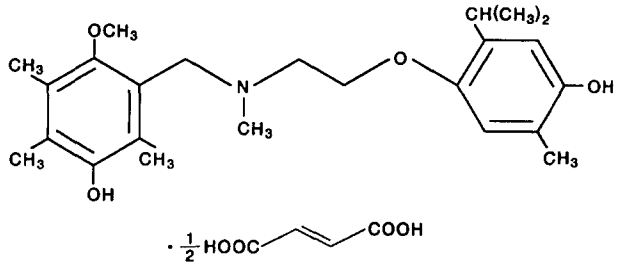

Fig. 1. Chemical structure of JTH-601.

pM [^{3}H]prazosin except 1000 pM [^{3}H]prazosin in the rabbit thoracic aorta membranes. Non-specific binding was determined with 0.3 μM prazosin. The affinity constants for the antagonists were determined from the [^{3}H]prazosin displacement experiments using the LIGAND program, as described previously (Oshita et al., 1993). The main drugs used were as follows: JTH-601 (*N*-(3-hydroxy-6-methoxy-2,4,5-trimethylbenzyl)-*N*-methyl-2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethylamine hemifumarate, Toyobo, Osaka, Japan, Fig. 1), prazosin hydrochloride (Sigma, St. Louis, MO, USA), [^{3}H]prazosin (specific activity 76.6 Ci/mmol, NEN, Boston, MA, USA), noradrenaline bitartrate (Nacalai Tesque, Kyoto, Japan) and chloroethylclonidine (Funakoshi, Tokyo, Japan).

3. Results

In the functional study, noradrenaline produced concentration-dependent contractions in the rat thoracic aorta and vas deferens, dog carotid and mesenteric arteries and rabbit thoracic aorta (the latter pretreated with 10 μM chloroethylclonidine). The contractions were inhibited by JTH-601 and prazosin, resulting in a parallel shift of the concentration-response curve to the right. The slopes of Schild plot were not significantly different from unity. The resultant pK_B values for JTH-601 in rabbit thoracic aorta, dog mesenteric artery and rat vas deferens were higher than those in rat thoracic aorta and dog carotid artery (Table 1). The affinity for JTH-601 was also approximately 10-fold higher than that for prazosin in rabbit thoracic aorta, dog mesenteric artery and rat vas deferens, while an inverse correlation was seen in rat thoracic aorta and dog carotid artery. In the native receptor binding study, JTH-601 again showed a higher affinity than prazosin in rabbit thoracic aorta membranes (chloroethylclonidine-pretreated), whereas prazosin was higher than JTH-601 in rat liver membranes. In the rat submaxillary gland membranes both antagonists showed similar and high affinity. In the cloned receptor binding study, JTH-601 showed a high and similar affinity to prazosin for the bovine α_{1a} subtype; however, in the case of hamster α_{1b} and rat α_{1d} subtypes the affinity for JTH-601 was lower than prazosin (Table 1).

4. Discussion

As shown in Table 1, different α_1-adrenoceptor subtypes are involved in the functional responses to noradrenaline and [^{3}H]prazosin binding in various tissues. Furthermore, they show different affinities for prazosin consistent with previous findings (Muramatsu et al., 1995;

Table 1
α_1-Adrenoceptor affinities for JTH-601 and prazosin

Source	α_1-Adrenoceptor [a] group (subtype)	pK_B		Ratio
		JTH-601	Prazosin	
Functional study [b]				
Rat thoracic aorta	α_{1H} (α_{1D})	8.0 ± 0.1	10.0 ± 0.1	0.01
Dog carotid artery	α_{1H} (α_{1B})	7.6 ± 0.1	9.2 ± 0.2	0.03
Rabbit thoracic aorta [d]	α_{1L} (α_{1L})	9.1 ± 0.1	8.1 ± 0.2	10.0
Rat vas deferens	α_{1L} (α_{1L})	9.2 ± 0.2	8.2 ± 0.2	10.0
Dog mesenteric artery	α_{1L} (α_{1N})	8.8 ± 0.2	7.9 ± 0.1	7.9
Binding study [c]				
(Native receptor)				
Rat liver	α_{1H} (α_{1B})	8.8 ± 0.2	10.1 ± 0.2	0.05
Rat submaxillary gland	α_{1H} (α_{1A})	9.6 ± 0.1	10.1 ± 0.2	0.32
Rabbit thoracic aorta [d]	α_{1L} (α_{1L})	9.8 ± 0.1	8.6 ± 0.2	15.8
(Recombinant receptor)				
Rat	α_{1H} (α_{1d})	8.9 ± 0.1	10.0 ± 0.2	0.07
Hamster	α_{1H} (α_{1b})	8.9 ± 0.1	10.2 ± 0.1	0.05
Bovine	α_{1H} (α_{1a})	9.4 ± 0.1	9.4 ± 0.3	1.00

Results are mean ± S.E.M. of 3–7 experiments. Ratio = anti-log (pK_B for JTH-601 − pK_B for prazosin). [a] α_1-Adrenoceptor subtypes were classified into two groups (α_{1H} amd α_{1L}) by different affinities for prazosin (see text for further explanation). [b] The subtypes involved predominantly in the contractile response to noradrenaline were listed. [c] Competition of [^{3}H]prazosin binding was analysed with the LIGAND program. [d] Chloroethylclonidine (10 μM)-pretreated preparations.

Hieble et al., 1995). It had previously been proposed that the α_1-adrenoceptors could be classified into two main groups based on different affinities for prazosin (α_{1H} group: α_{1A}, α_{1B} and α_{1D} subtypes, and α_{1L} group: α_{1L} and α_{1N} subtypes) (Muramatsu et al., 1990, 1995; Ford et al., 1994). Cloned α_{1a}, α_{1b} and α_{1d} subtypes correspond to native α_{1A}, α_{1B} and α_{1D} subtypes, respectively (Hieble et al., 1995; Michel et al., 1995). In the present study, however, the grouping based on the different affinities for prazosin was completely reversed: JTH-601 exhibits high affinity for the α_{1L} group and low affinity for the α_{1H} group, except the α_{1A} (or α_{1a}) subtype which has the same affinity for JTH-601 and prazosin.

JTH-601 at 0.1 μM had no significant affinity for α_2-adrenoceptors ([^{3}H]rauwolscine-binding sites in rat cerebral cortex membranes and prejunctional receptors of sympathetic purinergic nerves in rat vas deferens), β-adrenoceptors (receptors involving positive inotropic response to isoproterenol in rat atria and [^{3}H]dihydroalprenolol-binding sites in rat cerebral cortex membranes), muscarinic receptor ([^{3}H]quinuclidinylbenzilate-binding sites in rat cerebral cortex membranes) and 5-HT_{1A} receptor (binding sites for 8-hydroxy-[^{3}H]DPAT (8-hydroxydipropylaminoline) in rat cerebral cortex membranes) (Ohmura and Muramatsu, unpublished observations). These results provide the first indication that JTH-601 is a novel α_1-adrenoceptor antagonist showing a higher affinity for the α_{1L} group (α_{1L} and α_{1N} subtypes) when compared with prazosin. JTH-601 may be useful for characterising α_1-adrenoceptors with different affinities for prazosin.

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References

Flavahan, N.A. and P.M. Vanhoutte, 1986, α_1-Adrenoceptor subclassification in vascular smooth muscle, Trends Pharmacol. Sci. 7, 347.

Ford, A.P.D.W., T.J. Williams, D.R. Blue and D.E. Clarke, 1994, α_1-Adrenoceptor classification: sharpening Occam's razor, Trends Pharmacol. Sci. 15, 167.

Hieble, J.P., D.B. Bylund, D.E. Clarke, D.C. Eikenburg, S.Z. Langer, R.J. Lefkowitz, K.P. Minneman and R.R. Ruffolo Jr., 1995, International Union of Pharmacology. 10. Recommendation for nomenclature of α_1-adrenoceptors: consensus update, Pharmacol. Rev. 47, 267.

Lomasney, J.W., S. Cotecchia, R.J. Lefkowitz and M.G. Caron, 1991, Molecular biology of α-adrenergic receptors: implications for receptor classification and for structure-function relationships, Biochim. Biophys. Acta 1095, 127.

Michel, M.C., B. Kenny and D.A. Schwinn, 1995, Classification of α_1-adrenoceptor subtypes, Naunyn-Schmied. Arch. Pharmacol. 352, 1.

Muramatsu, I., T. Ohmura, S. Kigoshi, S. Hashimoto and M. Oshita, 1990, Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle, Br. J. Pharmacol. 99, 197.

Muramatsu, I., T. Ohmura, S. Hashimoto and M. Oshita, 1995, Functional subclassification of vascular α_1-adrenoceptors, Pharmacol. Commun. 6, 23.

Oshita, M., S. Kigoshi and I. Muramatsu, 1993, Pharmacological characterization of two distinct α_1-adrenoceptor subtypes in rabbit thoracic aorta, Br. J. Pharmacol. 108, 1071.