ELSEVIER

European Journal of Pharmacology 312 (1996) 385–388

ejp

Short communication

The subtype-selective α_2-adrenoceptor antagonists BRL 44408 and ARC 239 also recognize 5-HT_{1A} receptors in the rat brain

J. Javier Meana [a,*], Luis F. Callado [a], Angel Pazos [b], Bernardo Grijalba [b], Jesús A. García-Sevilla [c]

[a] *Department of Pharmacology, University of the Basque Country, E-48940 Leioa, Bizkaia, Spain*
[b] *Department of Physiology and Pharmacology, Unit of Pharmacology, University of Cantabria, Santander, Spain*
[c] *Laboratory of Neuropharmacology, University of the Balearic Islands, Palma de Mallorca, Spain*

Received 20 May 1996; revised 15 July 1996; accepted 26 July 1996

Abstract

Several α_2-adrenoceptor compounds have been reported to recognize 5-HT_{1A} receptors. The interaction of the $\alpha_{2A/D}$- and $\alpha_{2B/C}$-adrenoceptor antagonists BRL 44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole) methyl]-4,5-dihydroimidazole) and ARC 239 (2-[2-[4-(*o*-methoxyphenyl)piperazin-1-yl] ethyl]-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolinedione) with 5-HT_{1A} receptors was evaluated in rat brain. Competition experiments in cortex with both compounds against the specific binding of the 5-HT_{1A} receptor radioligand [^{3}H]8-OH-DPAT (8-hydroxy-2-(*n*-dipropyl-amine)-tetralin) yielded K_i values in the nanomolar range, fairly close to their previously reported affinities for α_2-adrenoceptors. Similar K_i values were obtained under α_2-adrenoceptor masking conditions by competition assays of these compounds against the α_2-adrenoceptor and 5-HT_{1A} receptor radioligand [^{3}H]RX 821002 (2-methoxy idazoxan) specific binding in hippocampus. The results indicate that BRL 44408 and ARC 239 recognize 5-HT_{1A} receptors in addition to α_2-adrenoceptors. The fact should be considered when using these compounds to study α_2-adrenoceptor subtypes.

Keywords: 5-HT_{1A} receptor; BRL 44408; ARC 239; α_2-Adrenoceptor subtype; Brain, rat

1. Introduction

The properties and functional significance of α_2-adrenoceptors have been subject of particular attention, especially in the central nervous system (Bylund, 1992). The heterogeneity of this type of adrenoceptors is supported by functional, biochemical and, more recently, molecular biology data, and the existence of three different subtypes ($\alpha_{2A/D}$, α_{2B} and α_{2C}) is now widely accepted (for a review see Bylund, 1992). However, the lack of highly selective ligands to identify the different subtypes has hampered the progress in their labeling and pharmacological characterization. In this regard, oxymetazoline and prazosin were earlier proposed as α_{2A}- and α_{2B}-adrenoceptor selective compounds, respectively (Bylund et al., 1988; De Vos et al., 1992). More recently, a number of drugs have been demonstrated to present subtype selectivity for α_2-adrenoceptors. Among them, BRL 44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole) methyl]-4,5-dihydroimidazole) and ARC 239 (2-[2-[4-(*o*-methoxyphenyl)piperazin-1-yl] ethyl]-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolinedione) are now considered as drugs of choice for the specific analysis of $\alpha_{2A/D}$- and $\alpha_{2B/C}$-adrenoceptor populations (Young et al., 1989; Uhlén and Wikberg, 1991; Devedjian et al., 1994; Sastre and García-Sevilla, 1994).

In addition to the differences in their relative selectivity for the three α_2-adrenoceptor subtypes, it has been reported that several α_2-adrenoceptor drugs, some of them used to radiolabel such receptors, also bind with high affinity to 5-HT_{1A} receptors, as it is the case for RX 821002 (2-methoxy idazoxan), yohimbine, oxymetazoline and rauwolscine (Convents et al., 1989; Vauquelin et al., 1990; Schoeffter and Hoyer, 1991; Winter and Rabin, 1992). This fact makes the selective analysis and characterization of the different α_2-adrenoceptor subtypes more difficult, especially in tissues containing both α_2-adrenoceptors and 5-HT_{1A} receptors. However, the selective iden-

* Corresponding author. Fax: +34 4 4648152; e-mail: kfpmemaj@lg.ehu.es

PII S0014-2999(96)00598-5

tification of α_2-adrenoceptor subtypes is crucial when studying their role under certain physiological and pathological conditions. In this regard, we have analyzed in the present work the possible interaction of BRL 44408 and ARC 239 with 5-HT_{1A} receptors in the rat brain.

2. Materials and methods

Male Wistar rats (300–400 g) were decapitated and the brains rapidly removed and placed on ice. Cerebral cortices and hippocampus were promptly dissected and homogenized in 10 volumes of 0.32 M sucrose using a Polytron. This homogenate was centrifuged at $900 \times g$ for 10 min and the supernatant centrifuged at $70000 \times g$ for 15 min. The pellet was resuspended in 10 volumes of 50 mM Tris-HCl buffer (pH 7.5), incubated at 37°C for 15 min and then centrifuged again at $70000 \times g$ for 15 min. The final pellet was resuspended in buffer containing 50 mM Tris-HCl (pH 7.7), 4 mM $CaCl_2$ and 0.1% ascorbic acid and stored at −70°C until used.

[^{3}H]8-OH-DPAT (8-hydroxy-2-(*n*-dipropyl-amine)-tetralin) binding assays were performed in cortical membranes at 37°C as previously described (Hoyer et al., 1986). Radioligand binding was done by incubating, for 30 min, 750 μl of tissue suspension with 100 μl of the radioligand [^{3}H]8-OH-DPAT (New England Nuclear, 132.8 Ci/mmol) and 150 μl of either incubation buffer (the same Tris-HCl buffer containing 10 μM pargyline) or the testing drugs. [^{3}H]RX 821002 ((2-methoxy-1,4-6,7-benzodioxan-2-yl)-2-imidazoline) (Amersham International, 60 Ci/mmol) binding assays were performed in hippocampal membranes at 25°C as previously described (Sastre and García-Sevilla, 1994). These experiments were done by incubating during 30 min 500 μl of membrane preparation with 10 μl of the radioligand and 40 μl of either incubation buffer or the testing drugs. Drug competition studies were carried out with 2 nM [^{3}H]8-OH-DPAT or 10 nM [^{3}H]RX 821002 in the absence or presence of various concentrations of phentolamine (Ciba-Geigy, Barcelona, Spain), (±)8-OH-DPAT HBr (Research Biochemicals International, Natick, MA, USA), BRL 44408 HCl (kindly provided by Dr. M.A. Cawthorne, Smith Kline Beecham, Essex, UK) or ARC 239 HCl (kindly provided by Dr. J.C.A. van Meel, Thomae, Biberach, Germany) (range of concentrations 10^{-10} to 10^{-4} M). [^{3}H]Radioligand bound was measured by rapid filtration under vacuum through Whatman GF/C glass fiber filters. Then, the filters were rinsed twice with 10 ml of ice-cold buffer, air-dried and transferred to plastic counting minivials containing 5 ml of OptiPhase Hisafe II cocktail (LKB, UK). Radioactivity was extracted from the filters by incubation at 60°C for 30 min and subsequent cooling. The radioactivity of the filters was counted by liquid scintillation spectrometry (Beckman, LS 6000). Specific binding was defined as the excess over blank values obtained in the presence of 10 μM 5-HT. Experiments were performed in duplicate and repeated 2 or 3 times.

Competition studies were analyzed by non-linear least-square curve fitting with the computer program LIGAND, allowing fitting to a one- or two-site model, to determine the equilibrium dissociation constants (K_i). Drug affinities are expressed as the best fit ±S.E. of the K_i values as determined by the program.

3. Results

In order to examine the properties of the [^{3}H]8-OH-DPAT binding in rat brain, saturation studies were first carried out. Such experiments demonstrated that the binding of [^{3}H]8-OH-DPAT (0.05–8 nM; 8 concentrations) to cortical membranes was saturable and of high affinity ($K_d = 2.5 \pm 0.8$ nM). The pharmacological profile was compatible with that previously reported for 5-HT_{1A} receptors: 5-HT > buspirone ≫ sumatriptan = mianserin (data not shown).

Competition curves of BRL 44408 and ARC 239 for [^{3}H]8-OH-DPAT binding in rat cortex membranes were monophasic and best explained by a one-site model (Fig. 1A). At relatively high concentrations (10^{-5} M), both compounds inhibited approximately 70% of total [^{3}H]8-OH-DPAT binding. This level of inhibition was similar to

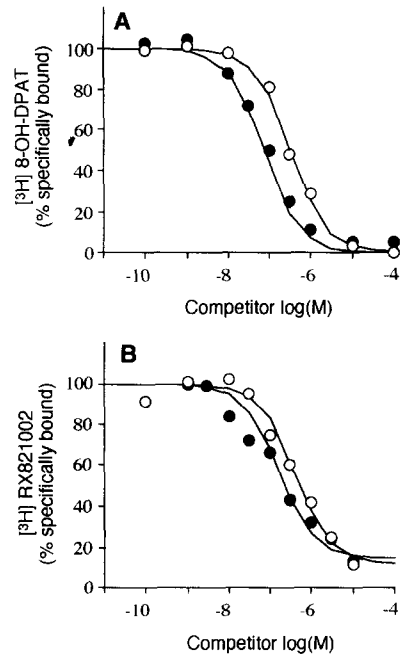

Fig. 1. Representative experiments of competition curves of BRL 44409 (o) and ARC 239 (●) against [^{3}H]8-OH-DPAT specific binding to cortical membranes (A) or [^{3}H]RX 821002 specific binding to hippocampal membranes (B). Phentolamine (50 nM) was included in the [^{3}H]RX 821002-binding assay to mask α_2-adrenoceptors in hippocampus. Abscissae represent the negative logarithm of the concentration of the drugs. Hill slope values (n_H) were not different from unity except for the inhibition of [^{3}H]RX 821002 binding by ARC 239 ($n_H = 0.31 \pm 0.20$).

Table 1
Competition binding parameters in membranes of rat brain cortex and hippocampus

Drug	[^{3}H]8-OH-DPAT K_i (nM)	n	[^{3}H]RX 821002 K_i (nM)	n
8-OH-DPAT	–		15.8 ± 1.4	2
BRL 44408	199 ± 6	3	338 ± 46.3	3
ARC 239	63.1 ± 1.7	3	136 ± 10.9	3

Membranes were incubated with 2 nM [^{3}H]8-OH-DPAT (brain cortex) or 10 nM [^{3}H]RX 821002 (hippocampus) as described in Section 2. Phentolamine (50 nM) was included in [^{3}H]RX 821002 assays to mask α_2-adrenoceptors. Data are the estimated best fit ± S.E. of n independent experiments and were determined by the LIGAND program.

that achieved by 10^{-5} M 5-HT, routinely used to define non-specific binding in these studies. Analysis of binding data revealed K_i values in the nanomolar range (Table 1).

In order to validate the importance of the previous finding, we decided to perform competition experiments with BRL 44408 and ARC 239 against the 5-HT$_{1A}$ component of the [^{3}H]RX 821002 specific binding. The radioligand [^{3}H]RX 821002 is an α_2-adrenoceptor antagonist which also displays high affinity for 5-HT$_{1A}$ receptors (Vauquelin et al., 1990). BRL 44408 and ARC 239 have previously shown potency values in the nanomolar range against the α_2-adrenoceptor component of the [^{3}H]RX 821002 specific binding (Uhlén and Wikberg, 1991; Devedjian et al., 1994; Sastre and García-Sevilla, 1994). To perform these experiments, the hippocampus, a brain area with a high ratio of 5-HT$_{1A}$ receptor/α_2-adrenoceptor density, was chosen. To delineate the proportion of α_2-adrenoceptors and 5-HT$_{1A}$ receptors in hippocampus, preliminary competition experiments with the α_2-adrenoceptor antagonist phentolamine were carried out. Phentolamine was found to displace [^{3}H]RX 821002 binding in a biphasic manner ($K_{iH} = 2.72 \pm 1.43$ nM, $K_{iL} = 162.8 \pm 38.6$ nM; $P < 0.05$), corresponding to 54% and 46% of the binding sites, respectively. Thus, 50 nM phentolamine was included in subsequent [^{3}H]RX 821002 binding experiments to mask α_2-adrenoceptors. Using this approach, the [^{3}H]RX 821002 binding (0.5-64 nM, 8 concentrations) to hippocampal membranes was a saturable process of high affinity ($K_d = 9.1 \pm 0.7$ nM). Competition curves by 8-OH-DPAT against a fixed concentration of [^{3}H]RX 821002 (10 nM) in the presence of phentolamine (50 nM) confirmed that the radioligand labels 5-HT$_{1A}$ receptors, as previously demonstrated (Vauquelin et al., 1990) (Table 1).

Under α_2-adrenoceptor masking conditions with phentolamine (50 nM), BRL 44408 and ARC 239 displaced [^{3}H]RX 821002 (10 nM) binding to hippocampal membranes with high affinities (Fig. 1B) yielding values in the nanomolar range (Table 1). At 10^{-5} M of both drugs, a portion (9–14%) of the [^{3}H]RX 821002 specific binding remained non-displaceable (Fig. 1B).

4. Discussion

BRL 44408 and ARC 239 have been now considered as very selective compounds for the discrimination of $\alpha_{2A/D}$- and $\alpha_{2B/C}$-adrenoceptors, being currently used for such a purpose in functional and biochemical studies carried out in several tissues (Uhlén and Wikberg, 1991; Smith and Docherty, 1992; Devedjian et al., 1994; Millan et al., 1994; Renouard et al., 1994; Sastre and García-Sevilla, 1994). With regard to ARC 239, previous studies have shown that, in addition to the reported α_1-adrenoceptor antagonist properties, its K_i values for $\alpha_{2B/C}$-adrenoceptors are between 14 nM and 219 nM, depending on the methodological approach used (Uhlén and Wikberg, 1991; Devedjian et al., 1994; Renouard et al., 1994; Sastre and García-Sevilla, 1994). Our results indicate that ARC 239 has a similar affinity for 5-HT$_{1A}$ receptors ($K_i = 63$ nM to 136 nM) (Table 1) to that described for $\alpha_{2B/C}$-adrenoceptors. Therefore, it is evident that the population of sites recognised with high affinity by ARC 239 in a given tissue could in fact include, in addition to α_2-adrenoceptors, 5-HT$_{1A}$ receptors. A relatively similar picture is suggested by our results with BRL 44408. Although its affinity for 5-HT$_{1A}$ receptors ($K_i = 199$ nM to 338 nM) (Table 1) appears to be somewhat lower than that reported for $\alpha_{2A/D}$-adrenoceptors ($K_i = 10$–52 nM) (Uhlén and Wikberg, 1991; Devedjian et al., 1994; Renouard et al., 1994; Sastre and García-Sevilla, 1994), the 5-HT$_{1A}$ component could be relevant when studying the behavior of BRL 44408 in radiometric and functional assays. This serotonergic 'contamination' would be of special relevance when BRL 44408 or ARC 239 are used in competition binding experiments against radiolabeled α_2-adrenoceptor ligands which also bind to 5-HT$_{1A}$ receptors, such as RX 821002, rauwolscine, oxymetazoline or yohimbine (Convents et al., 1989; Vauquelin et al., 1990; Schoeffter and Hoyer, 1991; Winter and Rabin, 1992).

The ability of some α_2-adrenoceptor drugs to bind with a significant affinity to 5-HT$_{1A}$ receptors could be explained by the strong homology in the amino acid sequence between α_2-adrenoceptors and 5-HT$_{1A}$ receptors (Kobilka et al., 1987). This fact suggests that some structural resemblance exists between their binding domains. In this regard, our results contribute to clarify the knowledge of the pharmacological profile of BRL 44408 and ARC 239. In in vivo studies (modulation of 5-HT synthesis in hippocampus), ARC 239 has been shown to be a 5-HT$_{1A}$ receptor agonist (Esteban et al., 1996). Furthermore, the results indicate that caution must be taken when these compounds are used to delineate subtypes of α_2-adrenoceptors. Based on our data, α_2-adrenoceptors should be specifically identified in the brain in the presence of a masking concentration of an unlabeled 5-HT$_{1A}$ drug, especially with most antagonist radioligands, as currently carried out by several authors (Vauquelin et al., 1990; Sastre and García-Sevilla, 1994). This is of special relevance

when such studies are carried out in tissues such as cortex or hippocampus endowed with 5-HT_{1A} receptors.

Acknowledgements

Supported by grants from the Salud 2000 Foundation and the CICYT (SAF 93/0459), FIS (95/1731) and the Basque Government. L.F.C. is a recipient of a predoctoral fellowship from the Basque Government. B.G. is a recipient of a predoctoral fellowship from the University of Cantabria. The authors are indebted to Ms. M. Josefa Castillo for her excellent technical assistance.

References

Bylund, D.B., 1992, Subtypes of α_1- and α_2-adrenergic receptors, FASEB J. 6, 832.

Bylund, D.B., C. Ray-Prenger and T.J. Murphy, 1988, Alpha-2A and alpha-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype, J. Pharmacol. Exp. Ther. 245, 600.

Convents, A., J. De Keyser, J.P. De Backer and G. Vauquelin, 1989, [^{3}H]Rauwolscine labels α_2-adrenoceptors and 5-HT_{1A} receptors in human cerebral cortex, Eur. J. Pharmacol. 159, 307.

Devedjian, J.-C., F. Esclapez, C. Denis-Pouxviel and H. Paris, 1994, Further characterization of human α_2-adrenoceptor subtypes: [^{3}H]RX821002 binding and definition of additional selective drugs, Eur. J. Pharmacol. 252, 43.

De Vos, H., G. Vauquelin, J. De Keyser, J.-P. De Backer and I. Van Liefde, 1992, Regional distribution of α_{2A}- and α_{2B}-adrenoceptor subtypes in postmortem human brain, J. Neurochem. 58, 1555.

Esteban, S., J. Lladó and J.A. García-Sevilla, 1996, α_2-Autoreceptors and α_2-heteroreceptors modulating tyrosine and tryptophan hydroxylase activity in the rat brain in vivo: an investigation into the α_2-adrenoceptor subtypes, Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 391.

Hoyer, D., A. Pazos, A. Probst and J.M. Palacios, 1986, Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites, Brain Res. 376, 85.

Kobilka, B.K., H. Matsui, T.S. Kobilka, T.L. Yang-Feng, U. Francke, M.G. Caron, R.J. Lefkowitz and J.W. Regan, 1987, Cloning, sequencing, and expression of the gene coding for the human platelet α_2-adrenergic receptor, Science 238, 650.

Millan, M.J., K. Bervoets, J.-M. Rivet, P. Widdowson, A. Renouard, S. Le Marouille-Girardon and A. Gobert, 1994, Multiple alpha-2 adrenergic receptor subtypes. II. Evidence for a role of rat $R_{Alpha\text{-}2A}$ adrenergic receptors in the control of nociception, motor behavior and hippocampal synthesis of noradrenaline, J. Pharmacol. Exp. Ther. 270, 958.

Renouard, A., P.S. Widdowson and M.J. Millan, 1994, Multiple $Alpha_2$ adrenergic receptor subtypes. I. Comparison of [^{3}H]RX 821002-labeled rat $R_{Alpha\text{-}2A}$ adrenergic receptors in cerebral cortex to human $H_{Alpha2A}$ adrenergic receptor and other populations of alpha-2 adrenergic subtypes, J. Pharmacol. Exp. Ther. 270, 946.

Sastre, M. and J.A. García-Sevilla, 1994, α_2-Adrenoceptor subtypes identified by [^{3}H]RX821002 binding in the human brain: the agonist guanoxabenz does not discriminate different forms of the predominant α_{2A} subtype, J. Neurochem. 63, 1077.

Schoeffter, P. and D. Hoyer, 1991, Interaction of the α-adrenoceptor agonist oxymetazoline with serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors, Eur. J. Pharmacol. 196, 213.

Smith, K. and J.R. Docherty, 1992, Are the prejunctional α_2-adrenoceptors of the rat deferens and submandibular gland of the α_{2A}- or α_{2D}-subtype?, Eur. J. Pharmacol. 219, 203.

Uhlén, S. and J.E.S. Wikberg, 1991, Delineation of rat kidney α_{2A}- and α_{2B}-adrenoceptors with [^{3}H]RX821002 radioligand binding computer modelling reveals that guanfacine is an α_{2A}-selective compound, Eur. J. Pharmacol. 202, 235.

Vauquelin, G., H. De Vos, J.-P. De Backer and G. Ebinger, 1990, Identification of α_2 adrenergic receptors in human frontal cortex membranes by binding of [^{3}H]RX 821002, the 2-methoxy analog of [^{3}H]Idazoxan, Neurochem. Int. 17, 537.

Winter, J.C. and R.A. Rabin, 1992, Yohimbine as a serotonergic agent: evidence from receptor binding and drug discrimination, J. Pharmacol. Exp. Ther. 263, 682.

Young, P., J. Berge, H. Chapman and M.A. Cawthorne, 1989, Novel α_2-adrenoceptor antagonists show selectivity for α_{2A}- and α_{2B}-adrenoceptor subtypes, Eur. J. Pharmacol. 168, 381.