ELSEVIER

European Journal of Pharmacology 312 (1996) 263–266

ejp

Short communication

Characterization of α_1-adrenoceptor subtypes in rat spinal cord

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Received 25 April 1996; revised 15 July 1996; accepted 19 July 1996

Abstract

The α_1-adrenoceptor subtypes in ventral and dorsal horns of rat lumbar spinal cord were studied. High concentrations of the α_{1D}-adrenoceptor antagonist, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride), displaced bound [^{3}H]prazosin in a single-site manner and the binding affinity was close to those for α_{1A}- and α_{1B}-adrenoceptor binding sites. 5-Methyl-urapidil displaced bound [^{3}H]prazosin in a two-site manner and the high and low affinities were close to those of α_{1A}- and α_{1B}-adrenoceptor binding sites, respectively. The α_1-adrenoceptor subtype populations of ventral and dorsal horns did not differ. The α_{1A}- and α_{1B}-adrenoceptor populations comprised 70% and 30%, respectively, of the total and very few α_{1D}-adrenoceptors were detected.

Keywords: Spinal cord; α_1-Adrenoceptor subtype; BMY 7378; 5-Methyl-urapidil; [^{3}H]Prazosin; (Rat)

1. Introduction

Recently, pharmacologically distinct subtypes of α_1-adrenoceptor were identified. The initial subdivision of the α_1-adrenoceptors in the rat spinal cord into α_{1A} and α_{1B} classes was based on their differential affinities for the competitive antagonist, WB4101, and different sensitivities to the irreversible alkylating agent, chloroethylclonidine, demonstrated by Wilson and Minneman (1989). They showed that α_{1A}- and α_{1B}-adrenoceptors are distributed heterogenously in rat brain and that α_{1A}-adrenoceptors predominate in rat cervical spinal cord. However, α_{1D}-adrenoceptors are also inactivated by chloroethylclonidine (Perez, 1991) and have a relatively high affinity for WB4101 (Minneman and Esbenshade, 1994). Therefore, α_1-adrenoceptor subtypes should be reinvestigated from the viewpoint of the present classification in the spinal cord.

Quite recently, three native and three recombinant α_1-adrenoceptors were identified. The relationships between native and recombinant α_1-adrenoceptor subtypes and a nomenclature to identify them have been proposed (Hieble et al., 1995) and we have followed this nomenclature in the present study.

Descending noradrenergic fibers originating in the brain stem and terminating in the spinal cord play an important role in controlling spinal neuronal activity. These descending noradrenergic fibers facilitate motoneuron activity via α_1-adrenoceptors (Ono and Fukuda, 1995) and, therefore, we feel it is important to characterize the α_1-adrenoceptor subtypes in rat lumbar spinal cord. Although high levels of all α_1-adrenoceptor subtypes are expressed in the brain stem (Alonso-Llamazares et al., 1995), the situation in the spinal cord is not clear. Moreover, it would be of interest to determine whether the distributions of α_1-adrenoceptor subtypes in the subregions of the spinal cord differ.

There are a few reports about α_1-adrenoceptor subtypes in the spinal cord, but, as far as we are aware, none that discuss α_1-adrenoceptor subtypes in the dorsal and ventral horns. We now tried to characterize the α_1-adrenoceptor subtypes in the ventral and dorsal horns of the rat lumbar spinal cord using a radioligand binding assay.

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PII S0014-2999(96)00570-5

2. Materials and methods

2.1. [^{3}H]Prazosin binding assay

The [^{3}H]prazosin binding assay was performed on membranes obtained from the ventral and dorsal horns of the rat lumbar spinal cord. Male Wistar rats (Japan SLC) aged 9–10 weeks were killed with CO_2 and an about 2 cm-long piece of the lumbar enlargement of the spinal cord was isolated quickly and divided into ventral and dorsal horns. Each isolated spinal cord preparation was lysed by sonication in 20 vols. (v/w) of 50 mM Tris-HCl, pH 7.6, the tissue lysates were centrifuged at $1500 \times g$ for 10 min, the pellets was resuspended in 10 vols. of 50 mM Tris-HCl, pH 7.6, and centrifuged at $1500 \times g$ for 10 min. These initial and second supernatants were mixed and centrifuged at $40000 \times g$ for 15 min and binding of [^{3}H]prazosin to membrane preparations (the resulting pellets) of ventral and dorsal horns was assayed in a final reaction volume of 0.25 ml after incubation at 25°C for 30 min. The non-specific binding was determined in the presence of 2 μM phentolamine. The amount of each crude membrane preparation added to the binding reaction mixture was adjusted to 10 mg wet tissue/tube. The reaction was stopped by filtration through 30% polyethylenimine-pretreated GF/B filters using a cell harvester, and the radioactivity due to tritium was determined by liquid scintillation counting. Each assay was performed in duplicate. Saturation curves of [^{3}H]prazosin were obtained using 0.025–3.20 nM [^{3}H]prazosin. Displacement of bound [^{3}H]prazosin by antagonists was determined using a reaction mixture containing 1 nM [^{3}H]prazosin. Under these conditions, the specific/non-specific binding ratio was about 6.

2.2. Data analysis

All the results are presented as means ± S.E.M. of the numbers of experiments as given. The [^{3}H]prazosin binding saturation data were analyzed using Scatchard plots, and the antagonist displacement data were analyzed using a non-linear regression program (PRISM; GraphPAD software, San Diego, CA). The data were fitted to one- and two-site models and the latter was accepted if it fitted significantly better than the one-site model. The K_i values were calculated by the method of Cheng and Prusoff (1973).

2.3. Drugs

The drugs used were BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride), 5-methyl-urapidil (Research Biochemicals International), phentolamine mesylate (Regitin; Ciba-Geigy, Japan), 30% polyethylenimine P-70 solution (Wako Pure Chemical Industries, Osaka, Japan), prazosin HCl (Sigma), HCl and Tris (Junsei Chemical Co., Tokyo, Japan).

[^{3}H]Prazosin (specific activity, 2819.4 Bq/mmol) was purchased from Du Pont/NEN Research Products.

3. Results

Scatchard transformations of the equilibrium saturation data for [^{3}H]prazosin binding to membranes prepared from ventral and dorsal horns of rat lumbar spinal cord yielded linear plots (Fig. 1 inset). Therefore, [^{3}H]prazosin labeled a homogenous binding site. The K_d values for binding to

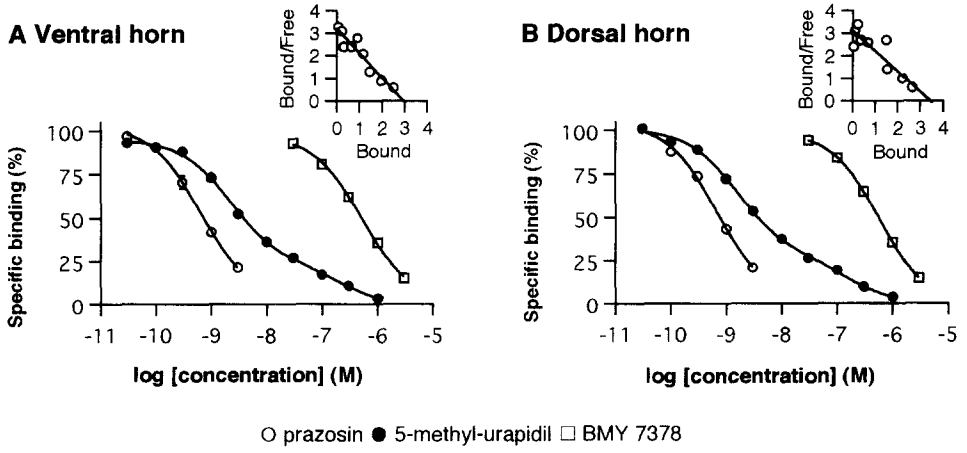

Fig. 1. Competition by prazosin, 5-methylurapidil and BMY 7378 for [^{3}H]prazosin binding to α_1-adrenoceptors in the ventral (A) and dorsal (B) horns of the rat spinal cord. Data are means ± S.E.M. of four experiments, but almost all the symbols mask the error bars. The quantitative analytical results for these data are shown in Table 1. The insets show the Scatchard plots for specific [^{3}H]prazosin binding and each point represents data for a single experiment representative of two experiments (unit of abscissae: fmol/mg tissue; unit of ordinates: fmol/mg tissue/nM). Each assay was performed in duplicate.

Table 1
Inhibition of [^{3}H]prazosin binding to α_1-adrenoceptors of rat spinal cord

Antagonists	Slope factor	pK_i	pK_i high	pK_i low	Percentage high
Ventral horn					
Prazosin	1.17 ± 0.12	9.54 ± 0.06	–	–	–
BMY 7378	0.80 ± 0.14	6.62 ± 0.06	–	–	–
5-Methyl-urapidil	0.64 ± 0.02	–	9.00 ± 0.03	6.98 ± 0.09	72.53 ± 0.01
Dorsal horn					
Prazosin	0.83 ± 0.17	9.50 ± 0.06	–	–	–
BMY 7378	0.99 ± 0.07	6.56 ± 0.03	–	–	–
5-Methyl-urapidil	0.53 ± 0.03	–	9.13 ± 0.06	7.15 ± 0.07	70.75 ± 0.01

The pK_i high and pK_i low values are negative logs of the equilibrium dissociation constants and refer to high- and low-affinity sites, respectively. Percentage high is the percentage of the high-affinity site. Data are means ± S.E.M. of four experiments.

ventral and dorsal horns were similar, as were the B_{max} values: the respective K_d values were 0.84 and 0.92 nM and the B_{max} values were 2.9 and 3.3 fmol/mg tissue, respectively.

The data for the inhibition of specific [^{3}H]prazosin binding to the ventral and dorsal horn preparations by the competitive antagonist, BMY 7378, 5-methyl-urapidil and prazosin are shown in Fig. 1. In the prazosin and BMY 7378 experiments, the high concentration ranges were not used. High concentrations of prazosin seem to bind additional receptors beyond α_{1A}-, α_{1B}-adrenoceptor and α_{1D}-adrenoceptors (Hieble et al., 1995) and a higher concentration of BMY 7378 was not thought to be needed because BMY 7378 inhibition of [^{3}H]prazosin binding was slight at the concentration which appeared to be selective for α_{1D}-adrenoceptors. Therefore, competition data were analyzed on the assumption that high concentration of prazosin and BMY 7378 would inhibit [^{3}H]prazosin binding completely. The results for both horns were virtually identical and did not differ significantly (Table 1). The α_{1D}-adrenoceptor antagonist, BMY 7378, displaced bound [^{3}H]prazosin in a single-site manner and the pK_i value of BMY 7378 was higher than those usually found in the literature for α_{1D}-adrenoceptors. The slope factor for 5-methyl-urapidil was much less than 1, suggesting binding site heterogeneity. The slope factors for BMY 7378 and prazosin seemed to show some differences, but these differences were not significant. The parameters for a two-site model, determined by non-linear regression analysis, are presented in Table 1 and the 5-methyl-urapidil inhibition curves fit a two-site model significantly better than a one-site model. The proportions of the two sites from which 5-methyl-urapidil displaced [^{3}H]prazosin in the ventral and dorsal horns were the same. The average pK_i values for the high- and low-affinity sites were 9 and 7, respectively, and the high-affinity sites accounted for about 70% of the total binding sites.

4. Discussion

BMY 7378 is a selective α_{1D}-adrenoceptor antagonist (pK_i: α_{1b}-adrenoceptor 6.2 ± 0.03; bovine α_{1c} (α_{1a})-adrenoceptor 6.1 ± 0.02; rat α_{1d}-adrenoceptor 8.2 ± 0.06) (Goetz et al., 1995). (Note that cloned α_1-adrenoceptor subtypes are denoted by lower case letters.) Our study of the inhibition of [^{3}H]prazosin binding to membranes prepared from the ventral and dorsal horns of rat lumbar spinal cord yielded pK_i values for BMY 7378 of 6.6 ± 0.06 and 6.6 ± 0.03, respectively, which were close to its pK_i values for α_{1A}- and α_{1B}-adrenoceptors. Therefore, few α_{1D}-adrenoceptors were present in the rat lumbar spinal cord. It is interesting that the expression level of α_{1D}-adrenoceptor mRNA in the rat brain is high (Price et al., 1994), but we detected almost no α_{1D}-adrenoceptors in the rat lumbar spinal cord in this study. Taken together, these findings show that α_{1D}-adrenoceptors are distributed very heterogeneously in the central nervous system.

5-Methyl-urapidil is known to be a selective α_{1A}-adrenoceptor antagonist in comparison with its affinities against α_{1B}- and α_{1D}-adrenoceptors and its averaged pK_i values for α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors, taken from published studies, are 8.63 ± 0.32, 6.97 ± 0.50 and 7.31 ± 0.66, respectively (Michel et al., 1995). In our study, the pK_i values of 5-methyl-urapidil for the high and low affinity sites were about 9 and 7, respectively, which match those for α_{1A}- and α_{1B}-adrenoceptors, respectively. As few α_{1D}-adrenoceptors were present and the ratio of high to low affinity sites for 5-methyl-urapidil was about 7 : 3, the α_{1A}-adrenoceptor, rather than the α_{1B}-adrenoceptor, appears to be the predominant subtype in rat lumbar spinal cord.

Only a few reports deal with α_1-adrenoceptors in the spinal cord. The in vitro experiments of Wilson and Minneman (1989) showed that 42% of the α_1-adrenoceptors of the cervical spinal cord was inactivated by chloroethylclonidine. In our experiment, α_{1B}-adrenoceptors comprised about 30% of the lumbar spinal cord α_1-adrenoceptors. Therefore, the distributions of α_{1B}-adrenoceptors in the cervical and lumbar cords may differ and/or chloroethylclonidine may inactivate not only α_{1B}-adrenoceptors but also other adrenoceptors. The inactivation by chloroethylclonidine has also been suggested by other investigators (Michel et al., 1993). The in vivo experiment of Bervoets and Millan (1994) suggested that spinal α_{1A}-adrenoceptors

mediate the spontaneous tail flicks induced by 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin). Based on the viewpoint that α_{1A}-adrenoceptors predominate, our results are similar to theirs, although their experiment was done in vivo and they did not divide the spinal cord into ventral and dorsal horns.

In summary, our results suggest that α_{1D}-adrenoceptors, expression levels of which are known to be high in brain areas, are sparsely distributed in the rat lumbar spinal cord, in which the α_{1A}-adrenoceptor, not the α_{1B}-adrenoceptor, is the main subtype. Moreover, the α_1-adrenoceptor subtype distributions in the ventral and dorsal horns do not differ.

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