J. Pharm. Pharmacol. 1997, 49: 802–805
Received January 10, 1997
Accepted April 9, 1997

Chronic Treatment with Prazosin Causes a Subtype-specific Increase in the α_1-Adrenoceptor Density of the Stressed Rat Cerebral Cortex

MANABU TAKITA, TAKANOBU TANIGUCHI, JUN ZHU AND IKUNOBU MURAMATSU

Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan

Abstract

The effects of chronic treatment with prazosin and of immobilization stress on the α_1-adrenoceptor subtypes in rat cerebral cortex have been examined.

Prazosin-treated rats were allowed free access to tap water containing two different concentrations of prazosin (16 or 156 mg L^{-1}) for 5 weeks. The mean plasma concentrations of prazosin were 5 ng mL^{-1} in groups treated with a low dose and 8 or 14 ng mL^{-1} in those treated with a high dose. Immobilization stress (2 h day^{-1}, 2 weeks) or chronic treatment with a low dose of prazosin caused no significant change in the affinity for [^{3}H]prazosin or in the maximum number of α_1-adrenoceptor sites (B_{max}). However, treatment with prazosin (low dose) combined with stress increased the density of α_1-adrenoceptors with low affinity for prazosin. Treatment with a high dose of prazosin increased the density of α_{1L}-adrenoceptors, irrespective of stress loading. The densities of α_{1A}- and α_{1B}-adrenoceptors with high affinity for prazosin were increased only after treatment with a high dose of prazosin in combination with stress.

These results indicate that three distinct α_1-adrenoceptor subtypes, α_{1A}, α_{1B} and α_{1L}, might be affected differently by treatment with prazosin and by stress.

Different subclasses of α_1-adrenoceptor subtypes have been proposed. Two subtypes were first proposed on the basis of binding experiments (Morrow & Creese 1986; Minneman 1988; Oshita et al 1991). The α_{1A}-adrenoceptor subtype has high affinity for 2-(2,6-dimethoxy-phenoxyethyl) aminomethyl-1,4-benzodioxane (WB4101) and 5-methylurapidil, whereas the α_{1B} subtype has extremely low sensitivity. Molecular cloning studies have confirmed the existence of two such subtypes ($\alpha_{1A}=\alpha_{1a}$, $\alpha_{1B}=\alpha_{1b}$; uppercase and lowercase subscripts denote native and cloned receptors, respectively) and further demonstrated the existence of an additional subtype $\alpha_{1D}=\alpha_{1d}$ (Schwinn & Lomasney 1992; Hieble et al 1995). These three subtypes have a high affinity for prazosin. Another subclassification, into α_{1H}, α_{1L} and α_{1N} subtypes, has been proposed on the basis of functional and binding experiments (Flavahan & Vanhoutte 1986; Muramatsu et al 1990, 1995; Ford et al 1994, 1996). The α_{1H} subtype has a high affinity for prazosin, whereas the α_{1L} and α_{1N} subtypes have low affinity for prazosin and are discriminated by means of HV723, a new α_1-antagonist. Subsequent functional studies have suggested the possibility that the previously defined subtypes (α_{1A}, α_{1B} and α_{1D}) are members of the α_{1H} subtype in the α_{1H}, α_{1L}, α_{1N} subclassification (Muramatsu et al 1991, 1995; Ford et al 1994).

Central α_1- and β-adrenoceptors are affected by different treatments. For example, the density of central β-adrenoceptors is reduced by repeated stress and increased by chronic treatment with β-adrenoceptor antagonist (Heal 1990; Takita et al 1993, 1995). In contrast, central α_1-adrenoceptors are not affected by stress (Lynch et al 1983) but are up-regulated by systemic injection of reserpine or intracerebroventricular injection of 6-hydroxydopamine (Bylund & U'Prichard 1983; Szabadi & Bradshaw 1987; Gross et al 1988).

Correspondence: I. Muramatsu, Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan.

We have previously demonstrated that three distinct binding sites for prazosin (presumably α_{1A}, α_{1B} and α_{1L} subtypes) occur in rat cerebral cortex (Oshita et al 1991) and in Mongolian gerbil cerebral cortex (Kimura et al 1993). However, effects of stress and chronic treatment with prazosin on these α_1 subtypes have not yet been determined. The purpose of this study was to examine the effects of chronic treatment with prazosin and of immobilization stress on the α_1-adrenoceptor subtypes in rat cerebral cortex.

Materials and Methods

Materials

[^{3}H] Prazosin (specific activity 79·2 Ci $mmol^{-1}$) was from New England Nuclear (Boston, MA), prazosin hydrochloride was from Sigma (St Louis, MO), and WB4101 hydrochloride (2-(2,6-dimethoxy-phenoxyethyl)aminoethyl-1,4-benzodioxane hydrochloride) and 5-methylurapidil were from Funakoshi (Tokyo, Japan).

Stress and prazosin treatment

The experimental protocol was essentially the same as described previously (Takita et al 1993, 1995). In brief, male Wistar rats, 6 weeks old, 160–180 g, were housed in groups of two or three animals with free access to the usual chow diet and tap-water. One week later, six different experimental groups (6–10 rats) were designated (Table 1). The four groups, prazosin-treated (groups 3 and 5) and stressed prazosin-treated (groups 4 and 6) were provided with tap-water containing prazosin hydrochloride (16 or 156 mg L^{-1}) as prazosin base for 5 weeks, whereas the other two groups, untreated (group 1) and stressed (group 2), received unmodified tap-water. On the basis of a mean water intake of 35 mL day^{-1} for a 250-g rat the daily consumption of prazosin was estimated to be between 2 and 20 mg kg^{-1}. At the end of the study the concentrations

of prazosin in the plasma were measured by the method of Wood et al (1976) with some modification. Rats in the stressed groups were subjected to immobilization stress (2 h day^{-1}) for the last 2 weeks of the 5-week experimental period. Rats were killed by decapitation 2–4 h after completion of the last stress session. Cerebral cortex was rapidly dissected on ice and stored at −80°C until use for binding assays.

α_1-Adrenoceptor binding

α_1-Adrenoceptors from the rat cerebral cortex were measured by assay of binding with [^{3}H]prazosin as described previously (Takita et al 1992, 1995). In brief, the cerebral cortex was homogenized with a Polytron in 80 vols homogenizing buffer (50 mM Tris-HCl, 100 mM NaCl, and 2 mM EDTA; pH 7·4). The homogenate was filtered through gauze and centrifuged at 80 000 *g* for 30 min. The pellets were resuspended in the same volume of assay buffer (50 mM Tris-HCl, 1 mM EDTA; pH 7·4), incubated at 37°C for 15 min, and centrifuged at 80 000 *g* for 30 min. The final pellets were resuspended in the same volume of assay buffer and the membrane fraction was used for binding assays. Except for pre-incubation of the membranes all membrane-preparation procedures were conducted at 4°C, and ice-cold buffers were used. The homogenates were incubated with [^{3}H]prazosin for 45 min at 30°C in a total volume of 1 mL (approximately 100 μg protein). In saturation experiments, 10 concentrations of [^{3}H]prazosin (10, 30, 100, 200, 300, 600, 1000, 2000, 3000, 5000 pM) were used. In competition binding experiments, 12 concentrations of WB4101 and prazosin (1 pM–0·3 μM) or 13 concentrations of 5-methylurapidil (10 pM–10 μM) were tested. Reactions were terminated by rapid filtration through Whatman GF/C filters (Whatman, Clifton, NJ) using a Brander cell-harvester. The filter was washed three times with ice-cold washing buffer (50 mM Tris-HCl, pH 7·4; 4 mL) and dried; the filter-bound radioactivity was then determined by means of a liquid scintillation counter (Aloka, Japan). Non-specific binding was defined as binding in the presence of 0·3 μM prazosin because 10 μM phentolamine was insufficient to inhibit completely the specific binding of high concentrations of [^{3}H]prazosin to α_1-adrenoceptors. The specific binding as a percentage of total binding was 89 ± 2% at ca 200 pM [^{3}H]prazosin. Assays were conducted in duplicate.

Binding data were analysed by the weighted least-squares iterative curve-fitting program LIGAND (Munson & Rodbard 1980). The data were first fitted to one- and then two-site models, and if the residual sums of squares, as determined by an *F*-test comparison, were statistically less for a two-site fit of the data than for a one-site fit then the two-site model was accepted. *P* values <0·05 were considered as indicative of significance. Proteins were assayed according to the method of Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

All values were expressed as mean ± s.e.m. Results were analysed by analysis of variance or Student's *t*-test and a probability of less than 0·05 was considered significant.

Results

The plasma prazosin concentrations measured at the end of the study were 5 ± 1, 5 ± 2, 14 ± 2 and 8 ± 1 ng mL^{-1} for groups 3, 4, 5 and 6, respectively (n = 5–7).

Saturation experiments with [^{3}H]prazosin in rat cerebral cortex

[^{3}H]prazosin at concentrations ranging from 10 to 5000 pM was used to label the α_1-adrenoceptors of the rat cerebral cortex. In non-stressed rats, a Scatchard plot of [^{3}H]prazosin binding to cerebral cortex was curvilinear, indicative of more than a single type of binding site. LIGAND analysis fitted the data to a two-site model. The pK_D values (negative logarithm of the equilibrium dissociation constants) at high- and low-affinity sites were 9·99 ± 0·05 and 8·45 ± 0·08, respectively, and the B_{max} values (maximum number of α_1-adrenoceptor sites) for both types of site were 242 ± 12 and 97 ± 19 fmol mg^{-1} protein, respectively (Table 1).

Immobilization stress (2 h day^{-1}, 2 weeks) or chronic treatment with a low dose of prazosin (5 weeks) caused no significant change in the affinity and B_{max} of the α_1-adrenoceptors in rat cerebral cortex. However, treatment with a low dose of prazosin in combination with immobilization stress increased the number of sites with low affinity for prazosin. In the rats treated with a higher dose of prazosin the number of sites with low affinity for prazosin increased irrespective of stress loading. The number of sites with high affinity for prazosin also increased in rats treated with a high dose of prazosin in combination with immobilization stress, but no significant change in the high-affinity sites was seen under the other conditions used.

Table 1. Effects of stress and prazosin on [^{3}H]prazosin binding to rat cerebral cortex.

Group	Treatment	[^{3}H]Prazosin binding			
		Negative log of equilibrium dissociation constant		Maximum number of low affinity sites (fmol mg^{-1} protein)	Maximum number of high affinity sites (fmol mg^{-1} protein)
		Low affinity sites	High affinity sites		
1	Control	9·99 ± 0·04	8·30 ± 0·06	242 ± 12	97 ± 19
2	Stress	10·22 ± 0·03	8·85 ± 0·14	210 ± 27	128 ± 12
3	Prazosin (low dose)	10·12 ± 0·04	8·43 ± 0·14	218 ± 3	122 ± 35
4	Prazosin (low dose) + stress	10·03 ± 0·04	8·66 ± 0·19	228 ± 27	161 ± 9*
5	Prazosin (high dose)	9·96 ± 0·08	8·55 ± 0·30	245 ± 16	163 ± 14†
6	Prazosin (high dose) + stress	9·88 ± 0·03	8·18 ± 0·06	316 ± 10*	190 ± 19*

Prazosin-treated rats had free access to tap water containing a low or high dose (6 or 156 mg L^{-1}) of prazosin. Scatchard curves were individually analysed by means of the LIGAND program. Data shown are means ± s.e.m. of results from three experiments. *$P < 0·05$, significantly different from group 2; †$P < 0·05$, significantly different from group 1.

Competition experiments with 200 pM [^{3}H]prazosin in rat cerebral cortex

As sites with high affinity for prazosin in the cerebral cortex have been reported to comprise two subtypes (α_{1A} and α_{1B}), the effects of immobilization stress and chronic treatment with prazosin on α_{1A} and α_{1A} subtypes were examined in competition-binding experiments with WB4101, 5-methylurapidil and prazosin. Prazosin displaced the 200 pM [^{3}H]prazosin binding in a monophasic manner. The pK_i value for prazosin was high ($9{\cdot}66 \pm 0{\cdot}05$). In contrast with prazosin, WB4101 and 5-methylurapidil displaced 200 pM [^{3}H]prazosin binding in a biphasic manner (Table 2). The proportion of sites with high affinity for WB4101 and 5-methylurapidil was approximately 50%. Immobilization stress, prazosin (low or high dose) or the combined treatment either did not affect, or only slightly changed, the proportions of binding sites with high and low affinity for WB4101 and 5-methylurapidil.

Discussion

In this study we have confirmed our previous observations that the α_1-adrenoceptors of the rat cerebral cortex are separated into two distinct types by different affinities for prazosin (Oshita et al 1991). The sites with high affinity for prazosin were further subdivided into two subclasses, which also showed different affinities for WB4101 and 5-methylurapidil. These results, together with those of previous reports, indicate that the α_1-adrenoceptors of rat cerebral cortex comprise two subtypes with high affinity for prazosin (α_{1A} and α_{1B}) (Morrow & Creese 1986; Minneman 1988; Hanft & Gross 1989) and a subtype with low affinity for prazosin (α_{1L}) (Oshita et al 1991).

Lynch et al (1983) have previously reported that immobilization stress (2 h day^{-1}, 7 days) caused no change in [^{3}H]prazosin binding sites in rat cerebral cortex. In the current study also, three distinct α_1-adrenoceptor subtypes were not affected by immobilization stress (2 h day^{-1}, 14 days). Unlike α_1-adrenoceptors, however, such chronic immobilization stress has been reported to cause down-regulation in α_2- and β-adrenoceptors (Stone & Platt 1982; Lynch et al 1983; Takita et al 1993, 1995). As stress can activate the adrenergic and other nerves in the central nervous system (Heal 1990), the current results imply either that regulation of central α_1-adrenoceptors might be relatively insensitive to stress as compared with the other adrenoceptors or that the down-regulation of α_1-adrenoceptors might already be maximum under stress-unloaded conditions.

In contrast, up-regulation of the α_1-adrenoceptors of the rat cerebral cortex has been observed after treatment such as systemic injection of reserpine or intracerebroventricular injection of 6-hydroxydopamine (Bylund & U'Prichard 1983; Szabadi & Bradshaw 1987; Gross et al 1988). In this study also, chronic treatment with a high dose of prazosin, but not with a low dose, significantly increased α_1-adrenoceptor density, especially α_{1L} subtype. Such up-regulation of the central α_1-adrenoceptor has also been reported to be caused by once-daily injection of prazosin (1 mg kg^{-1}) for 4 days or 3 weeks (Swann et al 1981). Thus, it is likely that central α_1-adrenoceptors can be up-regulated in the same way as reported for β-adrenoceptors (Lynch et al 1983; Takita et al 1993, 1995), in turn suggesting that cerebral α_1-adrenoceptors might be down-regulated under normal (stress-unloaded) conditions. Furthermore, these results suggest the possibility that treatment with high doses of prazosin can result in entry of the drug into the brain, although penetration has been reported to be poor (Wood et al 1976; Taylor et al 1977).

In contrast with unstressed rats, prazosin even in low doses increased the number of α_{1L}-adrenoceptors in the stressed rats.

Table 2. Inhibition of 200 pM [^{3}H]prazosin binding to α_1-adrenoceptors of rat cerebral cortex by WB4101, 5-methylurapidil and prazosin.

Group	Treatment	Drug	Negative log of equilibrium dissociation constant (−log M)		Proportion binding at high affinity sites
			High affinity site	Low affinity site	
1	Control	WB4101	$9{\cdot}55 \pm 0{\cdot}07$	$8{\cdot}25 \pm 0{\cdot}06$	49 ± 2
		5-Methylurapidil	$8{\cdot}96 \pm 0{\cdot}06$	$7{\cdot}05 \pm 0{\cdot}03$	46 ± 1
		Prazosin	$9{\cdot}66 \pm 0{\cdot}05$		100
2	Stress	WB4101	$9{\cdot}35 \pm 0{\cdot}07$	$8{\cdot}02 \pm 0{\cdot}05$	50 ± 5
		5-Methylurapidil	$9{\cdot}12 \pm 0{\cdot}06$	$7{\cdot}12 \pm 0{\cdot}02$	49 ± 1
		Prazosin	$9{\cdot}66 \pm 0{\cdot}05$		100
3	Prazosin (low dose)	WB4101	$9{\cdot}45 \pm 0{\cdot}14$	$8{\cdot}06 \pm 0{\cdot}10$	47 ± 6
		5-Methylurapidil	$8{\cdot}52 \pm 0{\cdot}14$	$6{\cdot}79 \pm 0{\cdot}05$	34 ± 4*
		Prazosin	$10{\cdot}19 \pm 0{\cdot}09$		100
4	Prazosin (low dose) +stress	WB4101	$9{\cdot}57 \pm 0{\cdot}08$	$8{\cdot}17 \pm 0{\cdot}10$	37 ± 7
		5-Methylurapidil	$8{\cdot}66 \pm 0{\cdot}07$	$6{\cdot}79 \pm 0{\cdot}17$	48 ± 3
		Prazosin	$10{\cdot}10 \pm 0{\cdot}06$		100
5	Prazosin (high dose)	WB4101	$9{\cdot}37 \pm 0{\cdot}20$	$8{\cdot}14 \pm 0{\cdot}13$	43 ± 8
		5-Methylurapidil	$8{\cdot}67 \pm 0{\cdot}13$	$6{\cdot}85 \pm 0{\cdot}06$	41 ± 3
		Prazosin	$10{\cdot}20 \pm 0{\cdot}10$		100
6	Prazosin (high dose) +stress	WB4101	$9{\cdot}48 \pm 0{\cdot}12$	$8{\cdot}30 \pm 0{\cdot}14$	37 ± 3†
		5-Methylurapidil	$8{\cdot}61 \pm 0{\cdot}11$	$6{\cdot}81 \pm 0{\cdot}08$	40 ± 3†
		Prazosin	$10{\cdot}09 \pm 0{\cdot}01$		100

Displacement curves were individually analysed by means of the LIGAND program. *$P < 0{\cdot}05$, significantly different from group 1; † $P < 0{\cdot}05$, significantly different from group 2.

This effect is not because of a change in prazosin plasma concentrations, because the plasma concentrations were the same for non-stressed and stressed rats (5 ± 1 and 5 ± 2 ng mL^{-1}, respectively). The permeability of the blood-brain barrier has been reported to increase under conditions of stress (Rapaport et al 1971; Anagnostakis et al 1992). The blood-brain barrier might be disrupted by stress (Sharma et al 1991). It was recently demonstrated that pyridostigmine, a peripherally acting acetylcholinesterase inhibitor, could, when administered under stress, reach the brain and affect centrally controlled functions (Friedman et al 1996). Therefore, although we have no direct evidence, it might be considered that prazosin can enter into the brain easily in stressed rats and then block α_1-adrenoceptor-mediated transmission, giving rise to the increase in α_1-adrenoceptor density (up-regulation). In contrast, the total numbers of α_{1A} and α_{1B} subtypes showing high affinity for prazosin, and the proportions of both subtypes, were little affected by stress or prazosin alone; a small change was observed after treatment with a high dose of prazosin in combination with stress. These results might reflect the different sensitivities of regulatory mechanisms against stress and prazosin in three distinct α_1-adrenoceptor subtypes.

In conclusion, this study indicates that the chronic treatment with prazosin increases the density of α_1-adrenoceptors with low affinity for prazosin, especially the α_{1L} subtype, in rat cerebral cortex. The predominant increase in α_{1L} subtype suggests that three distinct α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1L}) coexist and can be differently regulated.

Acknowledgements

We thank N. Aoki for secretarial assistance and H. Tanaka for technical assistance. This work was supported in part by a grant from the Smoking Research Foundation of Japan and from the Ministry of Education and Culture, Japan.

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