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SHORT COMMUNICATION

Effects of Adenosine A_{2A} Receptor Stimulation *In Vivo* on Dopamine D_3 Receptor Agonist Binding in the Rat Brain

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ABSTRACT. To investigate if adenosine A_{2A} receptor stimulation *in vivo* modulates dopamine D_3 receptor binding, we analyzed the effects of 2-[*p*-(carboxyethyl)-phenylethylamino]-5′-*N*-ethylcarboxyamidoadenosine (CGS 21680) on the binding properties of the selective D_3 receptor agonist [*N*-propyl-2,3,-^{3}H]4a*R*,10b*R*-(+)-*trans*-3,4,4a,10b-tetrahydro-4-*n*-propyl2*H*,5*H*-[1]benzopyrano[4,3-b]1,4-oxazin-9-ol ([^{3}H]PD 128907) in the rat forebrain using quantitative autoradiography. Intraperitoneally administered CGS 21680 (0.1–3 mg/kg) increased the K_d and B_{max} values of [^{3}H]PD 128907 binding in the islands of Calleja and in subregions of the caudate-putamen. These results suggest that stimulation of adenosine A_{2A} receptors *in vivo* causes alterations in the binding characteristics of dopamine D_3 receptors in the basal ganglia, and that this effect may relate to the neuroleptic-like effect of adenosine A_{2A} receptor agonists. BIOCHEM PHARMACOL **58**;12:1961–1964, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. adenosine A_{2A} receptor; basal ganglia; CGS 21680; dopamine D_3 receptor; PD 128907; receptor autoradiography

Adenosine is an extracellular metabolite of ATP that acts on multiple receptor types in the brain. Among these are the G-protein-coupled adenosine A_{2A} receptors [1], which are present in the basal ganglia on medium-sized neurons that contain γ-aminobutyric acid and enkephalins [2, 3]. Agonists at the adenosine A_{2A} receptor show, when injected into the brain, neuroleptic-like activities such as locomotor depression [4] and catalepsy [5], similar to the actions of antagonists for the dopamine D_2 receptor. Interestingly, stimulation of A_{2A} receptors affects the binding properties of ligands acting on the dopamine D_2 receptor, thereby providing a plausible mechanism for the neuroleptic-like effects of adenosine A_{2A} receptor agonists [6]. Specifically, the adenosine A_{2A} receptor agonist CGS 21680§ [7] increases the K_d value of [^{3}H]NPA and reduces the K_H and the K_L values of dopamine on [^{3}H]raclopride binding in rat brain membranes [8] and in transfected cells [9]. The binding properties of [^{3}H]raclopride itself are not affected by CGS 21680 [8], suggesting that only agonist but not antagonist binding is affected by adenosine A_{2A} receptor agonists. *In vivo* administration of CGS 21680 also affects the binding properties of dopamine D_2 receptor ligands but, in contrast to the *in vitro* effects, increases the binding of [^{3}H]NPA and causes an increase in the affinity of dopamine on [^{125}I]iodosulpride binding [10]. However, the ligands used in these studies also bind to dopamine D_3 receptors, which show marked structural homology with the dopamine D_2 receptor [11]. In the rat, dopamine D_3 receptors are mainly present in the ventral basal ganglia, especially the islands of Calleja, as analyzed in studies using autoradiography [12, 13], and *in situ* hybridization [11, 12], although the presence in the caudate-putamen may increase with age [14]. The development of selective dopamine D_3 receptor agonists, such as [^{3}H]PD 128907 [15, 16], has made it possible to investigate the binding properties of dopamine D_3 receptors in the brain. We have therefore in the present study used [^{3}H]PD 128907 to investigate whether adenosine A_{2A} stimulation *in vivo* affects dopamine D_3 receptors, in order to extend and reevaluate earlier studies that used non-selective D_2/D_3 receptor ligands.

MATERIALS AND METHODS

Male specific pathogen-free Sprague–Dawley rats (250 g; about 45 days old; Alab) were used. The rats were injected intraperitoneally (i.p.; 1.25 mL/rat) with CGS 21680 (0.1–3 mg/kg; Research Biochemicals Inc.) or with 0.9% sodium chloride alone one hour before decapitation. All experimental treatments were approved by the Swedish

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§ *Abbreviations:* CGS 21680, 2-[*p*-(carboxyethyl)-phenylethylamino]-5′-*N*-ethylcarboxyamidoadenosine; [^{3}H]NPA, [^{3}H]*N*-propylnoraporphine; [^{3}H]PD 128907, [*N*-propyl-2,3,-^{3}H]4a*R*,10b*R*-(+)-*trans*-3,4,4a,10b-tetrahydro-4-*n*-propyl2*H*,5*H*-[1]benzopyrano[4,3-b]1,4-oxazin-9-ol.

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Committee for Ethical Experiments on Laboratory Animals. Some of the rats had also been used in a previous study [10]. After decapitation with a guillotine, the brains were rapidly dissected out and frozen. Twenty consecutive 20-μm thick coronal forebrain sections were cut at bregma 1.00–1.40 mm [14]. The cryostat sections were incubated for 1 hr at room temperature with 3 nM [^{3}H]PD 128907 (108 Ci/mmol; Amersham) in 50 mM Tris–HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 5.7 mM L(+)-ascorbic acid. Twenty concentrations (i.e. 18 + two controls) of PD 128907 (Research Biochemicals Inc.) were added to consecutive sections, randomly arranged either in the rostro-caudal or caudo-rostral direction. Incubations were terminated by washing the sections three times for 5 min in ice-cold Tris buffer (as specified above) and rinsed briefly in ice-cold distilled water before drying under a stream of cold air. The dried sections were exposed to a ^{3}H-sensitive film (^{3}H-Hyperfilm, Amersham) for 4 months. The receptor autoradiograms were measured and analyzed as previously described [14]. Competition curves were analyzed according to a one-binding-site model yielding B_0, IC_{50}, and non-specific binding values using the EasyBound software for non-linear, least-squares iterative fitting [17]. Data points having absolute standard residual values >2 were excluded from the analysis. Eighteen out of 228 curves were excluded on the basis of having standard error of IC_{50} values of individual curves greater than 100%. K_d values were calculated according to equation $K_d = IC_{50} - L$, where L represents the radioligand concentration. B_{max} values were calculated as $B_{max} = (B_0 \times IC_{50}) / L$. Dose dependency was tested using the Jonckheere–Terpstra test for ordered alternatives.

RESULTS AND DISCUSSION

[^{3}H]PD 128907 binding was localized to the islands of Calleja, islands of Calleja major, accumbens nucleus (core and shell), and caudate-putamen (Fig. 1). The binding of [^{3}H]PD 128907 in the cerebral cortex was too low to be analyzed with regard to IC_{50} values, B_0 values of the control group being 2.6 ± 0.7 fmol/mg protein, N = 5. The binding in the caudate-putamen was measured in two subregions as defined by the marked differences in binding levels. The caudate-putament subregion 1 may correspond to the dopamine nerve terminal network region termed the medial part of the caudate-putamen, characterized by relatively few neurons and a lack of white matter [14]. This distribution is similar to that observed in previous studies using D_3-selective compounds [12, 13, 18], but different from the regional distribution of non-selective D_2/D_3 receptor ligands such as [^{3}H]NPA and [^{125}I]iodosulpride [10], indicating that [^{3}H]PD 128907, at 3 nM, selectively binds to dopamine D_3 receptors.

Unlabeled PD 128907 displaced [^{3}H]PD 128907 binding with IC_{50} (and thus K_d values of 10–30 nM in all regions of the control group (Table 1). These values lie between the K_d values of 1–2 and 400 nM obtained for cell-transfected

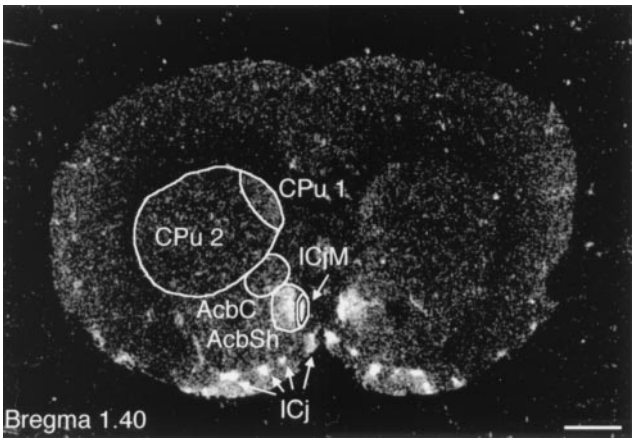

FIG. 1. Representative autoradiograms of total [^{3}H]PD 128907 binding (2 nM) in coronal sections of the rat forebrain. Brain region abbreviations used: caudate-putamen subregion 1, CPu 1; caudate-putamen subregion 2, CPu 2; accumbens nucleus core, AcbC; accumbens nucleus shell, AcbSh; islands of Calleja, ICj; and islands of Calleja major, ICjM. Bar = 1 mm.

dopamine D_3 receptors in the high- and low-affinity states, respectively [16]. However, they are higher than the K_d values of 0.7 nM obtained in membrane preparations from the rat ventral striatum [13], most likely because the latter experiments were performed using incubation media that did not include Na^+, K^+, Mg^{2+}, or Ca^{2+}. The possibility of unwanted binding to dopamine D_2 receptors when increasing the concentration of PD 128907 has to be considered in view of the K_d values of 20–40 nM determined for D_{2L} receptors in the high-affinity state in transfected cells [15, 16], and the fact that PD 128907 stimulates dopamine D_2-receptor-mediated [^{3}H]thymidine incorporation in cell lines with IC_{50} values of 34 nM [19]. However, the regional distribution of [^{3}H]PD 128907 did not change as unlabeled PD 128907 was added (data not shown), indicating that D_3-selectively is also retained at 30 nM of PD 128907 and above. Therefore, the K_d and B_{max} values, as calculated using the IC_{50} values, seem to specifically refer to the dopamine D_3 receptor, and not to the D_2 receptor.

CGS 21680 increased the K_d values of [^{3}H]PD 128907 in caudate-putamen subregions 1 and 2 and in the islands of Calleja (Table 1). Representative curves from the islands of Calleja showing the effect of CGS 21680 (3 mg/kg) are shown in Fig. 2. There seemed to be little or no effect at 1 nM CGS 21680 in all regions, possibly because the putative presence of two distinct effects that for some reason cancel each other out at this concentration. There were no effects of CGS 21680 on the B_0 values of [^{3}H]PD 128907 in any region (Table 1), in spite of the decreased affinities implied by the increased K_d values. Hence, the calculated B_{max} values of [^{3}H]PD 128907 were significantly increased in the same subregions as those with affected K_d values, i.e. caudate-putamen subregion 1 and in the islands of Calleja (Table 1). The magnitude of the B_{max} increases must be interpreted with caution in view of the low proportion of specific binding in many regions. The non-specific values

TABLE 1. Effects of CGS 21680 on binding parameters of [^{3}H]PD 128907 in coronal cryostat sections from rat forebrain

Area	CGS 21680 (mg/kg)	K_d (nM)	B_0 (fmol/mg protein)	B_{max} (fmol/mg protein)
CPu 1				
	0	22 (17–29)	11 ± 1.9	131 ± 39
	0.1	67 (33–133)	7.1 ± 2.5	339 ± 149
	0.3	56 (28–115)	10 ± 2.7	1370 ± 1150
	1	27 (13–56)	13 ± 2.5	422 ± 312
	3	199 (149–264)	8.1 ± 0.7	843 ± 149
		$P < 0.05$		$P < 0.05$
CPu 2				
	0	33 (18–60)	8.6 ± 2.1	185 ± 57
	0.1	125 (97–161)	5.3 ± 2.1	304 ± 140
	0.3	449 (303–665)	10 ± 3.3	2520 ± 1070
	1	48 (20–118)	11 ± 2.9	662 ± 521
	3	338 (267–428)	6.6 ± 1.2	1350 ± 414
		$P < 0.05$		
AcbC				
	0	23 (13–41)	15 ± 2.2	216 ± 75
	0.1	17 (9–33)	9.1 ± 1.0	163 ± 106
	0.3	47 (26–86)	9.6 ± 1.5	435 ± 247
	1	24 (13–45)	13 ± 3.6	241 ± 86
	3	86 (46–161)	10 ± 2.0	545 ± 197
AcbSh				
	0	26 (22–30)	25 ± 1.9	320 ± 60
	0.1	36 (27–49)	21 ± 2.3	374 ± 71
	0.3	28 (16–49)	24 ± 3.4	564 ± 234
	2	16 (10–26)	40 ± 7.9	342 ± 80
	3	41 (21–78)	22 ± 3.6	660 ± 309
ICj				
	0	9 (6–12)	51 ± 4.0	272 ± 65
	0.1	16 (12–22)	47 ± 2.2	475 ± 133
	0.3	21 (15–28)	59 ± 2.4	759 ± 244
	1	11 (7–16)	77 ± 11	444 ± 85
	3	32 (26–39)	47 ± 4.0	799 ± 67
		$P < 0.05$		$P < 0.01$
ICjM				
	0	32 (25–42)	50 ± 2.5	826 ± 266
	0.1	60 (50–74)	43 ± 5.2	1257 ± 231
	0.3	24 (15–38)	58 ± 7.2	970 ± 308
	1	12 (7–20)	56 ± 7.7	586 ± 359
	3	38 (27–52)	47 ± 3.5	911 ± 165

Various concentrations of CGS 21680 or NaC1 (0.9%) only were administered i.p. one hour before decapitation. K_d values are shown as geometric means ± SE limits of the geometric mean. Other data show means ± SEM values. Significances refer to the Jonckheere–Terpstra test for ordered alternatives. Abbreviations: caudate-putamen subregion 1, CPu 1; caudate-putamen subregion 2, CPu ; accumbens nucleus core, AcbC; accumbens nucleus shell, AcbSh; islands of Calleja, ICj; and islands of Calleja major, ICjM. N = 3–6 in each group.

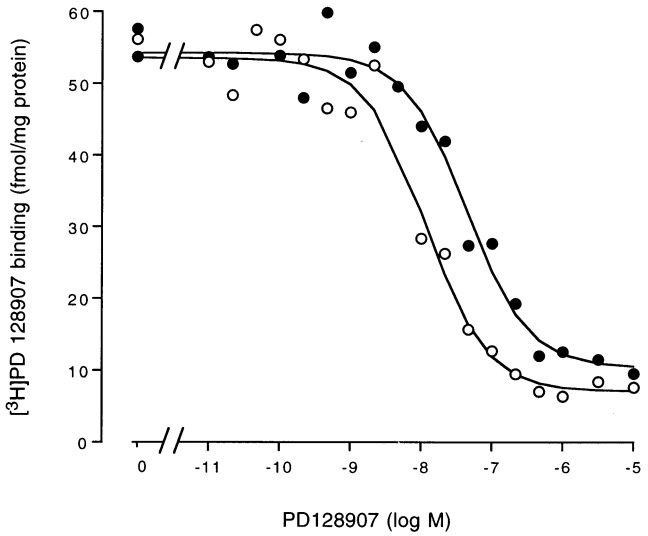

FIG. 2. Representative competition curves of PD 128907 on [^{3}H]PD 128907 binding showing the effects of CGS 21680 (3 mg/kg;●) against control (0.9% NaC1 only;○) in the islands of Calleja. The calculated K_d and B_{max} values were 9.4 nM and 235 fmol/mg protein for the control curve and 42 nM and 834 fmol/mg protein for the CGS 21680 curve, respectively.

were about 10 fmol/mg protein in all regions (data not shown). Taken together, these results suggest that stimulation of adenosine A_{2A} receptors causes alterations of D_3 receptor function. Hence, the effects of CGS 21680 on [^{3}H]NPA and dopamine-displaced [^{3}H]raclopride binding observed *in vitro* in membrane preparations from rat basal ganglia [8] may, at least in part, be due to an effect on the D_3 receptor.

CGS 21680 administered *in vivo* has been shown to increase the binding of [^{3}H]NPA and decrease the K_L values of dopamine on [^{125}I]iodosulpride binding, without affecting the K_H values or the proportion of high-affinity binding sites [10]. The increase in [^{3}H]NPA binding may correspond to the increased B_{max} values of [^{3}H]PD 128907 seen in the present study, and thus reflect an increase in D_3 receptor agonist binding. Thus, it may be speculated that CGS 21680 *in vivo* selectively increases the proportion of high-affinity binding sites of the D_3 receptor. This could possibly be mediated by an adenosine A_{2A}-induced decoupling of G-proteins associated with the dopamine D_3 receptor. The lack of effect of CGS 21680 *in vivo* on the K_H value of dopamine on [^{125}I]iodosulpride binding may possibly reflect a lack of effect on high-affinity D_2 receptor agonist binding sites. The decrease in the K_L value of dopamine on [^{125}I]iodosulpride binding by CGS 21680 *in vivo* may possibly reflect increases in the affinity of low-affinity D_2 and D_3 receptor agonist binding sites, which are not labeled either by [^{3}H]NPA or [^{3}H]PD 128907.

In conclusion, the present study indicates that A_{2A} agonists modulate D_3 receptor agonist binding *in vivo*. This D_3 receptor modulation may partly underlie the neuroleptic effects of A_{2A} agonists.

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