

The Striopallidal Neuron: A Main Locus for Adenosine–Dopamine Interactions in the Brain

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Recent pharmacological data suggest that a receptor–receptor interaction between adenosine A₂ and dopamine D₂ receptors in the brain underlies the behavioral effects of adenosine agonists and adenosine antagonists, such as caffeine and theophylline. According to this interaction, stimulation of A₂ receptors inhibits and their blockade potentiates the effects of D₂ receptor stimulation. Furthermore, both A₂ and D₂ receptors are selectively colocalized on GABAergic striopallidal neurons. In this microdialysis investigation the effect of intrastriatal infusion of adenosine and dopamine agonists and antagonists alone or in combination was studied on the release of GABA from the terminals of the striopallidal neuron in awake, freely moving rats. We report that the GABAergic striopallidal neuron, which is a key component of the indirect striatal efferent pathway, is a main locus for A₂–D₂ interactions in the brain and possibly a main target for the central actions of adenosine agonists and antagonists.

[Key words: adenosine A₂ receptor, dopamine D₂ receptor, GABA, striopallidal neuron, receptor–receptor interaction, methylxanthines]

Behavioral and biochemical evidence suggests that a strong and specific interaction between adenosine A₂ and dopamine D₂ receptors exists in the brain. Behavioral data show that stimulation and blockade of the A₂ receptor inhibits and potentiates, respectively, D₂-mediated locomotor activation in mice (Ferré et al., 1991a,b) while stimulation of D₂ receptors counteracts the A₂-mediated cataleptic effect in rats (Ferré et al., 1991c). Biochemical data show that A₂ receptor stimulation decreases both the affinity of D₂ receptors for dopamine agonists (Ferré et al., 1991d) and D₂ transduction (Ferré and Fuxe, 1992; Ferré et al., 1993) in rat striatal membrane preparations. Based on these pharmacological findings we postulated that this A₂–D₂ interaction represents a main mechanism underlying the central effects of adenosine agonists and antagonists (for review, see Ferré et al., 1992).

The biochemical data showing an A₂–D₂ interaction with membrane preparations of rat striatum strongly suggested the existence of a colocalization of A₂ and D₂ receptors on the same

striatal neuron (Ferré et al., 1991d). Both A₂ receptors and A₂ receptor mRNA expression are highly enriched in the striatum, nucleus accumbens, and olfactory tubercle (Jarvis and Williams, 1989; Parkinson and Fredholm, 1990; Martínez-Mir et al., 1991; Schiffmann et al., 1991; Fink et al., 1992), areas also associated with high numbers of D₂ receptors (Boyson et al., 1986). Furthermore, A₂ receptor mRNA is selectively expressed in GABAergic striatal neurons also containing D₂ receptors (Schiffmann et al., 1991; Fink et al., 1992). These GABA- and enkephalin-containing neurons (Gerfen et al., 1990; Le Moine et al., 1990) project to the globus pallidus constituting the “indirect pathway,” one of the two major striatal efferent pathways (Alexander and Crutcher, 1990; Gerfen, 1992). Thus, we have recently postulated that the striopallidal GABAergic neuron is a main locus for A₂–D₂ interactions in the brain and is thus a primary site for the action of adenosine agonists and antagonists (Ferré et al., 1992).

In the present *in vivo* microdialysis study, direct functional evidence is provided that an A₂–D₂ interaction plays a central role in the function of the striopallidal pathway in the awake, freely moving rat. One microdialysis probe was implanted in the striatum, the locus of the striopallidal neuronal bodies and of the hypothetical A₂–D₂ interaction, and a second probe was implanted in the ipsilateral globus pallidus, the locus of the striopallidal nerve terminals, which release the neurotransmitter GABA. By using this experimental preparation in awake, freely moving rats, the effect of the intrastriatal infusion of adenosine and dopamine agonists and antagonists alone or in combination was studied on the release of GABA from the terminals of the striopallidal neuron.

Materials and Methods

Animals. Male Sprague-Dawley rats (Alab, Stockholm) weighing 350–400 gm were used. Animals were maintained on a standard light–dark cycle and allowed free access to food and water.

Surgery. During surgery the animals were mounted into a Kopf stereotaxic frame and body temperature was continuously maintained at 37°C with a temperature controller (CMA 150, Carnegie Medicin, Stockholm, Sweden). The animals were maintained under 1.5% halothane, 98.5% air anesthesia (delivered at 1.4 liters/min). After exposure of the skull and drilling two burr holes, two microdialysis probes (Carnegie Medicin) were stereotaxically implanted: a large probe, with a 4.0 × 0.5 mm membrane, was implanted into the neostriatum (coordinates from bregma: AP +0.7, L +3.0, DV –8.0) and a smaller probe, with a 2.0 × 0.5 mm membrane, was implanted into the ipsilateral globus pallidus (AP –1.1, L +3.1, DV –7.75). The probes were perfused at a rate of 2 μl/min with a modified Ringer solution (1.2 mM CaCl₂, 2.7 mM KCl, 148 mM NaCl, and 0.85 mM MgCl₂) (Drew and Ungerstedt, 1991) throughout the implantation procedure and dialysis experiment. The probes were permanently secured with methacrylic cement and two stainless steel screws that were implanted in the skull.

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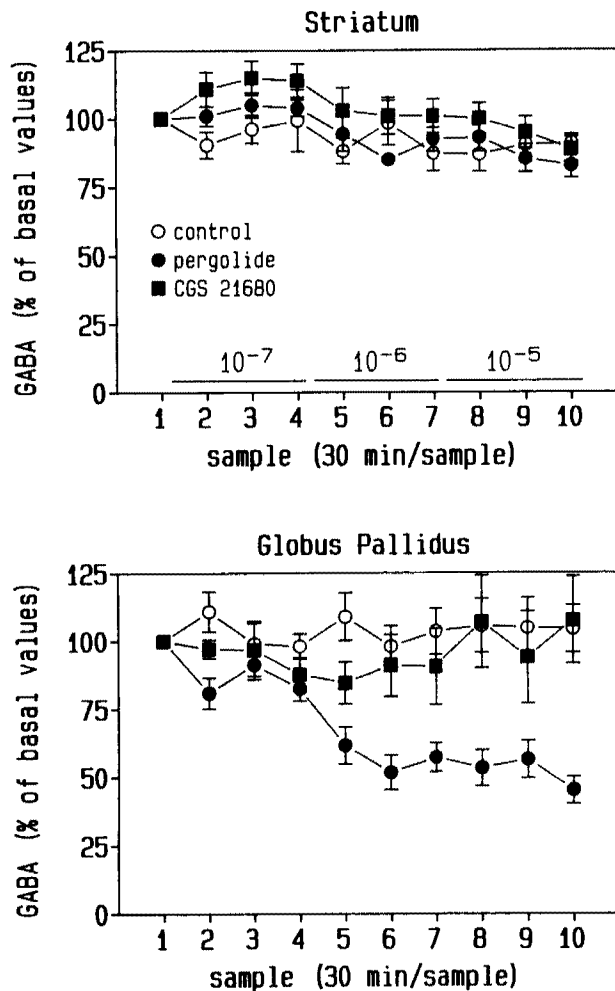


Figure 1. Effect of increasing doses of the intrastrially infused dopamine D_2 agonist pergolide and the adenosine A_2 agonist CGS 21680 on GABA extracellular levels in the striatum and in the ipsilateral globus pallidus of awake, moving rats. The results are expressed as percentage of the three basal values measured prior to drug infusion (means \pm SEM, $n = 5$ or 6/group). Horizontal lines represent the length of time the different drug concentrations were infused. Basal striatal and pallidal GABA levels measured in 30 min perfusate fractions were, respectively, 17.9 ± 1.2 nM ($n = 18$) and 11.8 ± 1.1 nM ($n = 16$).

Microdialysis procedure. The animals were allowed to recover for 48 hr after probe implantation. To prevent induction of adaptive mechanisms, the experiments were performed in a random order on either the second or third day after surgery. On the day of the experiment the rat was placed in a modified activity bowl. The inlet tubing of the probe was connected to a liquid swivel and perfused with the modified Ringer solution at a flow rate of $2 \mu\text{l}/\text{min}$. The striatal probe was used both to infuse dopamine and adenosine agonists and antagonists and to measure the extracellular concentrations of dopamine and GABA. The pallidal probe was used to measure GABA extracellular levels. Dialysates were collected every 30 min during the experiment. At the end of each experiment the animal was disconnected from the swivel, the inlet and outlet tubings were cut and sealed, and the animal was returned to its home cage. At the end of the study the rats were killed with an overdose of Mebumal ($120 \text{ mg}/\text{kg}$ i.p.; Nord Vacc, Stockholm, Sweden). The brain was removed from the skull and the position of the microdialysis probes was verified by sectioning in a cryostat and microscopic examination.

Dopamine and GABA analysis. Three and 10 microliters of each dialysate sample ($60 \mu\text{l}$) were assayed for dopamine and GABA, respectively. Reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection was used to assay dopamine. The dopamine system consisted of a Sepstick microbore column (in-

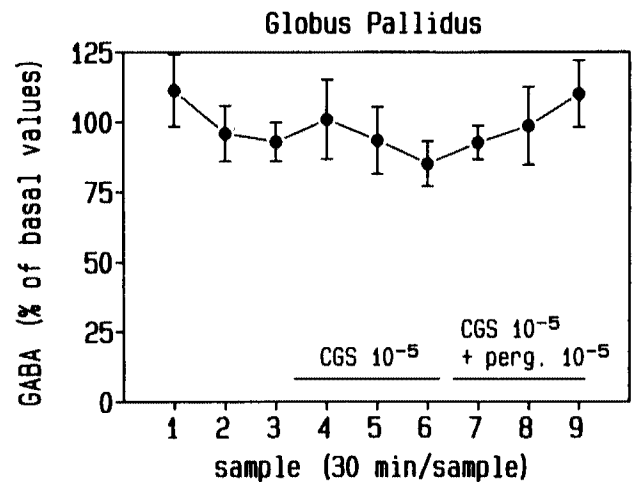


Figure 2. Counteraction of the effect of the intrastrially infused dopamine D_2 agonist pergolide (10^{-5} M) (see also Fig. 4) on GABA extracellular levels in the ipsilateral globus pallidus when it was coinfused in the presence of the adenosine A_2 agonist CGS 21680 (10^{-5} M). CGS 21680 alone was infused 90 min prior to coinfusion with pergolide. The results are expressed as percentage of the three basal values measured prior to drug infusion (means \pm SEM, $n = 4$ or 5/group). Horizontal lines represent the length of time the different drug combinations were infused. Basal pallidal GABA levels measured in 30 min perfusate fractions were 10.4 ± 1.8 nM ($n = 4$).

ternal diameter = 1 mm; length = 10 cm; BAS, West Lafayette, IN) containing $3 \mu\text{m}$ ODS packing material, a Spectra Physics (SP) 8810 precision isocratic pump, an on-line CMA 260 degasser (Carnegie Medicin), an SP 4270 integrator, and a BAS LC 4B detector. The composition of the mobile phase for the dopamine system was 0.1 M NaH_2PO_4 , 0.3 mM EGTA, 1.35 mM sodium octane sulfonate acid, 4% acetonitrile, 0.5% tetrahydrophurane, and 0.1 M acetic acid, pH 4.0. The flow rate of this mobile phase was $70 \mu\text{l}/\text{min}$ and was maintained under isocratic conditions. The limit of sensitivity for dopamine was 2 fmol/sample. The GABA assay employed in this study has been previously described in detail (Kehr and Ungerstedt, 1988). Briefly, the assay was based on precolumn derivatization with *o*-phthalaldehyde/*t*-butyl thiol reagent and separation by reverse-phase HPLC on a Nucleosil 3, C18 column with electrochemical detection under isocratic conditions. The mobile phase for the GABA system was 0.15 M Na acetate, 1 mM EDTA, and 50% acetonitrile, pH 5.4. The flow rate of this mobile phase was 0.8 ml/min. The limit of sensitivity for GABA was 20 fmol/sample.

Results

The intrastriatal infusion of the dopamine D_2 agonist pergolide (Arnt and Hyttel, 1984; Arnt, 1985) did not change local striatal GABA extracellular levels but caused a significant decrease in GABA extracellular levels in the ipsilateral globus pallidus compared to controls (repeated-measures ANOVA: drug effect, $p < 0.001$; drug effect \times dose effect, $p < 0.01$). Post hoc comparisons (repeated-measures ANOVA with Newman-Keuls test) showed significant differences between the pergolide-treated group and the control group during the infusion of pergolide at 10^{-6} M ($p < 0.01$) and pergolide at 10^{-5} M ($p < 0.01$) (Fig. 1).

The intrastriatal infusion of the adenosine A_2 agonist CGS 21680 (Jarvis et al., 1989; Lupica et al., 1990) did not produce any significant change in striatal or pallidal GABA extracellular levels compared to controls (repeated-measures ANOVA with Newman-Keuls test) (Fig. 1). However, CGS 21680 (10^{-5} M) completely counteracted the effect of pergolide (10^{-5} M) on pallidal GABA extracellular levels when they were coinfused in the striatum. Basal pallidal GABA extracellular levels were not statistically different from the GABA levels obtained following the

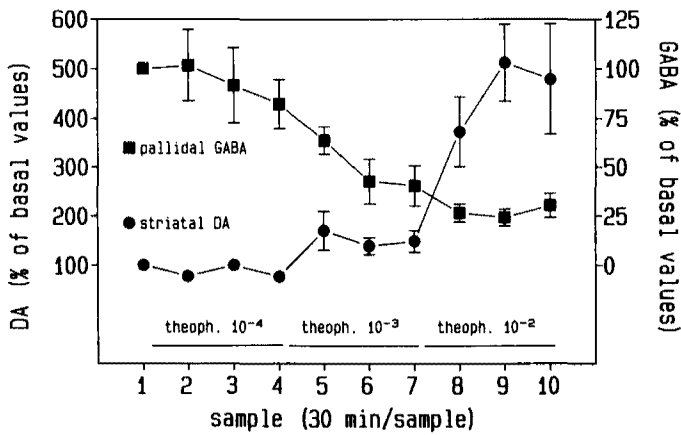


Figure 3. Effect of increasing doses of the intrastratially infused adenosine antagonist theophylline on dopamine extracellular levels in the striatum and on GABA extracellular levels in the ipsilateral globus pallidus of awake, moving rats. The results are expressed as percentage of the three basal values measured prior to drug infusion (means \pm SEM, $n = 4$ or 5/group). Horizontal lines represent the length of time the different drug concentrations were infused. Basal striatal dopamine and pallidal GABA levels measured in 30 min perfusate fractions were 3.0 ± 0.5 nM ($n = 5$) and 14.4 ± 4.6 nM ($n = 4$), respectively.

infusion of CGS 21680 or the infusion of CGS 21680 plus pergolide (repeated-measures ANOVA, no significant treatment effect) (Fig. 2).

The intrastratial infusion of the adenosine A_1/A_2 antagonist theophylline (Jarvis et al., 1989) was associated with a dose-dependent increase in striatal dopamine extracellular levels (repeated-measures ANOVA: dose effect, $p < 0.01$). Post hoc comparisons (Newman-Keuls) showed that the effect of theophylline at 10^{-3} M was significantly greater than the effect of theophylline at 10^{-4} M ($p < 0.05$) and that the effect of theophylline at 10^{-2} M was significantly greater than the effect of theophylline at 10^{-3} M ($p < 0.01$) (Fig. 3). Furthermore, intrastratial theophylline infusion dose dependently decreased pallidal GABA extracellular levels (repeated-measures ANOVA: dose effect, $p < 0.01$). Post hoc comparisons (Newman-Keuls) also showed that the effect of theophylline at 10^{-3} M and 10^{-2} M was significantly greater than the effect of theophylline at 10^{-4} M ($p < 0.05$ in both cases) (Fig. 3).

The intrastratial infusion of pergolide at 10^{-5} M and that of pergolide at 10^{-7} M plus theophylline at 10^{-4} M caused a significant decrease in striatal dopamine extracellular levels (repeated-measures ANOVA: treatment effect, $p < 0.0001$; treatment \times dose effect, $p < 0.0001$). Post hoc comparisons (Newman-Keuls) showed that the effect of pergolide 10^{-5} M was significantly greater than the effect of pergolide at 10^{-7} M plus theophylline at 10^{-4} M ($p < 0.05$), and that the effect of pergolide at 10^{-7} M plus theophylline at 10^{-4} M was significantly different from the control group ($p < 0.01$) (Fig. 4). Furthermore, the intrastratial infusion of pergolide and pergolide plus theophylline caused a significant decrease in pallidal GABA extracellular levels (repeated-measures ANOVA: drug effect, $p < 0.01$; drug effect \times dose effect, $p < 0.01$). Post hoc comparisons (Newman-Keuls) showed that the effect of pergolide at 10^{-5} M was not significantly different than the effect of pergolide at 10^{-7} M plus theophylline at 10^{-4} M, and that both effects were significantly different from the control group ($p < 0.05$ in both cases) (Fig. 4).

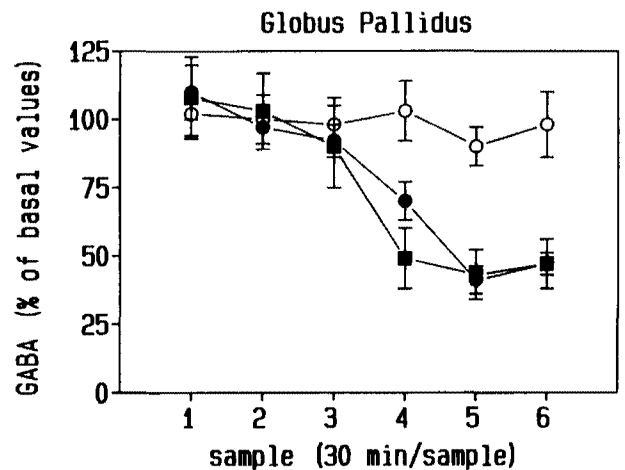
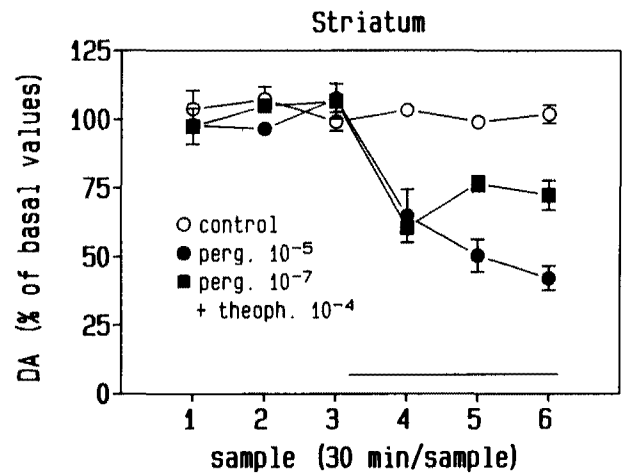


Figure 4. Effect of the intrastratial coinfusion of the dopamine D_2 agonist pergolide (10^{-7} M) with the adenosine antagonist theophylline (10^{-4} M) on dopamine extracellular levels in the striatum and on GABA extracellular levels in the ipsilateral globus pallidus of awake, moving rats. The effect of the intrastratial administration of a maximal dose of pergolide (10^{-5} M) and a control group were used for comparisons. The results are expressed as percentage of the three basal values prior to drug infusion (means \pm SEM, $n = 4$ –6/group). Horizontal lines represent the length of time the different drug combinations were infused. Basal striatal dopamine and pallidal GABA levels measured in 30 min perfusate fractions were 3.0 ± 0.5 nM ($n = 14$) and 12.3 ± 2.4 nM ($n = 13$), respectively.

Discussion

The infusion of the dopamine D_2 agonist pergolide (Arnt and Hyttel, 1984; Arnt, 1985) in the striatum caused a strong decrease (up to 50%) in the GABA extracellular levels of the ipsilateral globus pallidus without changing striatal GABA levels. With higher levels of calcium into the perfusion medium, a pergolide-induced decrease in striatal GABA levels can also be found (Drew and Ungerstedt, 1991; Fuxe et al., 1992). The striatal infusion of the A_2 agonist CGS 21680 (Jarvis et al., 1989; Lupica et al., 1990) did not alter either striatal or pallidal GABA levels. However, when the A_2 agonist was coinfused with the D_2 agonist pergolide it completely counteracted the effect of the D_2 agonist on pallidal GABA extracellular levels.

Intrastratial infusion of the A_1/A_2 antagonist theophylline (Jarvis et al., 1989) was associated with a stronger inhibition (75%) of pallidal GABA levels compared with that for pergolide

(50%). However, the effects of striatal infusion of pergolide and theophylline on striatal dopamine levels were qualitatively different: pergolide caused a decrease (up to 60%) and theophylline caused a strong increase (up to 400%) of striatal dopamine extracellular levels. These results could be explained by a presynaptic effect of both drugs on dopamine terminals. D₂ and A₁ receptors have been shown to modulate, by different mechanisms, striatal dopamine release, the stimulation of either receptor causing inhibition and their blockade causing stimulation of dopamine release (Morgan and Vestal, 1989; Drew et al., 1990; Cass and Zahniser, 1991).

Consequently, the decrease of pallidal GABA levels after the striatal infusion of theophylline could be explained by the striatal theophylline-induced dopamine release, which would stimulate D₂ receptors on the striopallidal neuron. In fact, theophylline caused a similar but opposite stepwise dose effect in striatal dopamine and pallidal GABA extracellular levels. Nevertheless, a dose of theophylline (100 μ M), which did not produce any change in striatal dopamine or pallidal GABA levels, caused a strong decrease (of about 50%) of pallidal GABA levels when coinfused with a dose of pergolide (100 nM), which was without effect on pallidal GABA levels. Furthermore, this drug combination was associated with a decrease in striatal dopamine levels, strongly indicating that the mechanism involved was an enhancement of postsynaptic D₂ receptor transduction due to blockade of A₂ receptors. Although the presynaptic effect of methylxanthines on the striatal dopamine terminals, that is, an increase in dopamine release, may contribute to the behavioral effects of these drugs, our data suggest that this mechanism of action is only in operation at higher doses. Systemic administration of an optimal dose of theophylline (20 mg/kg), which causes locomotor activation in the rat, is associated with an extracellular striatal concentration of between 50 μ M and 90 μ M (Fredholm et al., 1983; Stähle et al., 1990). This is in the concentration range most probably reached in the vicinity of the microdialysis probe following intra-striatal infusion of theophylline at 100 μ M (Stähle et al., 1990). Thus, the locomotor activation associated with theophylline can be explained on the basis of the presently observed A₂-D₂ interaction on the striopallidal neuron.

These results strongly suggest that, through a postsynaptic A₂ receptor-D₂ receptor interaction, the striopallidal neuron is a main locus for the interaction between the neurotransmitter dopamine and the neuromodulator adenosine in the brain and, thus, a main target for adenosine agonists and antagonists. Furthermore, these results suggest that new therapeutic strategies, incorporating specific A₂ agonists and antagonists, could be useful in some basal ganglia disorders, like Parkinson's disease and Huntington's chorea, as there is considerable evidence showing that the impairment in the functioning of the striopallidal neuron plays a key role in mediating the symptoms of these disorders (Albin et al., 1989; DeLong, 1990).

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