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# Interactions between adenosine $A_{2a}$ and dopamine D2 receptors in the control of [$^3$H]GABA release in the globus pallidus of the rat

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**Abstract**

The interactions between adenosine $A_{2A}$ receptors and dopamine D2 receptors on the modulation of depolarization-evoked [$^3$H]-γ-amino-butyric-acid release (GABA) were examined in slices of the globus pallidus of the rat. The stimulation of release caused by activation of $A_{2A}$ receptors was blocked when dopaminergic influences were eliminated with three independent methods: a) antagonism of D2 receptors with sulpiride; b) alkylation of these receptors with N-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline (EEDQ); c) depletion of dopamine with reserpine. In turn, activation of $A_{2A}$ receptors modified the response to stimulation of D2 receptors: the $EC_{50}$ for quinpirole increased nearly one thousand times when $A_{2A}$ receptors were stimulated. Antagonism of $A_{2A}$ receptors in the absence of added agonists inhibited [$^3$H] GABA release indicating receptor occupancy by endogenous adenosine. The dopamine dependence and the large effects of activating $A_{2A}$ receptors on the potency of dopaminergic agonists clarify some of the therapeutic properties of $A_{2A}$ antagonists in parkinsonian animals and patients.


*Keywords:* Adenosine receptor; Adenosine antagonist; Adenosine/Dopamine interaction; Dopamine receptor; GABA transmission; Striatopallidal transmission

## 1. Introduction

Adenosine $A_{2A}$ receptors in the brain are localized more frequently in structures that have a high density of dopamine D2 receptors such as the basal ganglia (Fredholm et al., 2001). The presence of both types of receptors in the same structure has important physiologic and pharmacologic consequences. Agonists of $A_{2A}$ receptors oppose the effects to activation of D2 receptors on several functions such as motor behavior (Stromberg et al., 2000), neurotransmitter release (Mayfield et al., 1993; Corsi et al., 1999), receptor binding (Ferre et al., 1999; Diaz-Cabiale et al., 2001) and gene expression (Svenningsson et al., 1999).

The literature suggests several explanations for the antagonism between adenosine $A_{2A}$ receptors and dopamine D2 receptors (Ferre et al., 2004). One question on which no agreement has been reached is whether or not concurrent activation of dopamine receptors is required for adenosine action. Even the recent evidence, obtained in studies in D2 receptor knockout mice, leads to conflicting conclusions. The results from one group of studies suggest that the adenosine responses are dependent on the presence of D2 receptors (Zahniser et al., 2000), while other investigators concluded that adenosine effects are not dependent on the presence of D2 receptors (Aoyama et al., 2000). A third group suggests that the effects of adenosine are only partially dopamine-dependent (Chen et al., 2001).

It is possible that these conclusions are contradictory because they are based on comparisons of very different assays ranging from biochemical to behavioral responses. Here we determined whether the response of a single process of physiologic significance, γ-amino-butyric-acid

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release in the globus pallidus, to stimulation of $A_{2A}$ receptors is dependent on the concurrent activation of dopamine D2 receptors. We selected this parameter because in the basal ganglia, adenosine $A_{2A}$ receptors are present in highest density in the GABAergic striatal medium-sized spiny neurons that project to the globus pallidus (Ferre et al., 1997; Fredholm et al., 2001). $A_{2A}$ receptors within the globus pallidus are most likely localized in the terminals of these striatopallidal neurons because the $A_{2A}$ receptor protein is present in the globus pallidus but the $A_{2A}$ receptor mRNA cannot be detected there (Svenningsson et al., 1997, 1999; Schiffmann et al., 1991; Fink et al., 1992; Pollack et al., 1993). Dopamine D2 receptors are also localized at high density in the subpopulation of GABAergic striatal medium-sized spiny neurons that project to the globus pallidus (Gerfen and Young, 1988). Both $A_{2A}$ and D2 receptors are active in the globus pallidus since their stimulation leads to opposing actions on GABA release (Mayfield et al., 1993).

Blockers of adenosine $A_{2A}$ receptors appear to be very effective in the treatment of parkinsonian animals and patients (Chase et al., 2003; Kanda et al., 2000). Therefore, a detailed characterization of the interactions between of $A_{2A}$ and D2 receptors may improve our understanding of these therapeutic effects.

## 2. Experimental procedures

### 2.1. Slice preparation

The slices were obtained from male Wistar rats weighing 180–220 g, maintained and handled according to the guidelines of the CINVESTAV-IPN Animal Care Committee, taking all efforts to minimize suffering and the number of animals used. After rapid decapitation, the brain was removed and submerged in ice-cold artificial cerebrospinal fluid (aCSF), composed as follows (mM): NaCl, 134; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 1; $KH_2PO_4$, 1.25; $NaHCO_3$, 25; glucose 10; pH, 7.4. The brain was glued with cyanoacrylate to a metal cube mounted on a Petri dish filled with ice-cold aCSF, and brain slices (300 μm thick) containing the globus pallidus were obtained with a vibroslicer (Campden Inc., Cambridge UK). The slices were transferred to cold slides and under a stereoscopic microscope the globus pallidus was micro-dissected as previously described (Aceves and Cuello, 1981).

### 2.2. Pretreatment with EEDQ or reserpine

N-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline (EEDQ) is an N-carboxydihydroquinoline that binds and inactivates both D1 and D2 receptors (Meller et al., 1985; Hemsley and Crocker, 1998; Yeghiayan et al., 1998; Undie et al., 2000) and other receptors such as adrenergic (Ribas et al., 1998), and 5-HT receptors (Kettle et al., 1999). Injection of specific receptor antagonists before the administration of EEDQ selectively protects the involved receptor from inactivation (Meller et al., 1985; Kettle et al., 1999). Thus it is possible to test whether the effects of EEDQ treatment are caused by selective inactivation of a given receptor by comparing its effects in preparations with and without protection of the receptor with its antagonist.

Our EEDQ treatment is based on the protocol of Hemsley and Crocker (1998). All animals were injected intraperitoneally with 60 μmol/kg of EEDQ 2 h before sacrifice. When required (see Results) dopaminergic receptors were protected by injecting either R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390) (1.7 μmol/kg i.p.) for D1 receptors, or sulpiride (234 μmol/kg s.c.) for D2 receptors, 1 h before EEDQ administration.

To deplete endogenous dopamine, reserpine (10 mg/kg s.c.) was injected subcutaneously 16 h before sacrificing the animals. This treatment has previously been shown to reduce striatal dopamine levels by 95% (Garcia et al., 1997).

### 2.3. [$^3$H]GABA release

GABA release was determined using methods similar to those currently used by us (Floran et al., 2002) and others (Katona et al., 2000; Schoffelmeer et al., 2000). $K^+$ depolarization was used because the release patterns found with continuous and repetitive stimulation are quite comparable, thus permitting reliable determination of dose–response curves (Frankhuyzen and Mulder, 1982). Slices from 10 rats were pooled and allowed to equilibrate for 30 min in aCSF maintained at 37 °C and gassed continuously with $O_2/CO_2$ (95 : 5, v/v). The slices were then incubated for 30 min with 80 nM [$^3$H]GABA in 2 ml aCSF containing 10 μM aminooxiacetic acid (to prevent degradation of the label). At the end of this period, excess radiolabel was removed by washing twice with aCSF containing 10 μM aminooxiacetic and 10 μM nipecotic acids (to block the GABA transporter). Both compounds remained in the superfusion solution for the rest of the experiment. Thin layer chromatography determinations showed that more than 90% of the $^3$H label of our samples is in GABA (Garcia et al., 1997).

The slices were then apportioned randomly between the chambers (80 μl volume) of a superfusion apparatus (20 superfusion chambers in parallel) and superfused with the medium at a rate of 0.5 ml/min. Each chamber contained 4–5 slices. The design of the superfusion chambers was essentially described by Aceves and Cuello (1981), except that the electrodes for electrical stimulation were omitted. A multichannel peristaltic pump (Brandel Inc. Gaithersburg, MD, USA) was used to superfuse the slices. The aCSF for superfusion was contained in reservoirs placed in a constant temperature bath maintained at 38 °C. When the aCSF reached the chambers, its temperature was 36–37 °C. To further wash out the [$^3$H]GABA trapped in the interstitial space, the slices were superfused with normal aCSF for 1 h before collecting the fractions for counting radioactivity. Fractions of the superfusate were collected in a fraction

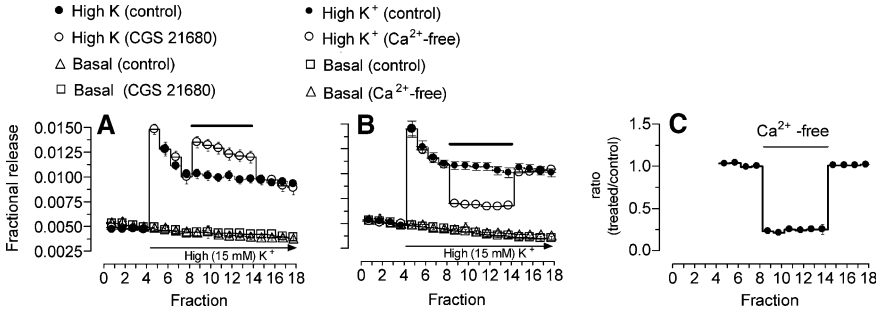


Fig. 1. Depolarization-dependence of the effects of $A_{2A}$ receptor agonist CGS 21680 and $Ca^{2+}$-free solutions on [$^3$H] GABA release. (A) illustrates the effect of the $A_{2A}$ agonist CGS 21680 (0.1 μM) on depolarized and non-depolarized slices. Depolarization was produced by increasing [$K^+$] from 6.25 to 15 mM. The unlabeled horizontal bar indicates the presence of the agonist in both depolarized and non-depolarized slices. The increase in release in depolarized slices is significant ($P<0.001$). (B) shows the effects of $Ca^{2+}$-free solutions on release in depolarized and non-depolarized slices. $Ca^{2+}$ was substituted equimolarly with $Mg^{2+}$. Ordinates in (A) and (B) are fractional release. (C) shows the depolarization-induced [$^3$H] GABA release from B expressed as the ratio of the fractional release of the slices exposed to $Ca^{2+}$-free solutions over that of control slices. The unlabeled horizontal bar indicates the period in which $Ca^{2+}$ was omitted from the medium. The effects of $Ca^{2+}$-free medium were significant ($P<0.001$). Points are mean ± S.E.M of 3 independent experiments (six replicates for experiment).

collector every 4 min. The superfusion medium contained 0.1 μM SCH 23390 to prevent activation of D1 receptors (Floran et al., 1997).

Four fractions were first collected to determine basal release then [$K^+$] in the aCSF was increased from 6.25 to 15 mM for the rest of the experiment (Frankhuyzen and Mulder, 1982; Floran et al., 2002). After collecting 4 fractions in the high $K^+$ medium, the drugs were added, remaining in the medium for 24 min, during which 6 fractions were collected. After removing the drugs, 4 more fractions were collected to test for reversibility of drug-effects. The sequence of a typical experiment is illustrated in Fig. 1. The radioactivity released into the superfusion medium in each fraction was measured by liquid scintillation counting. The radioactivity remaining in the tissues at the end of the experiment was assessed after extracting the radioactivity with 0.1 N HCl.

When we increased [$K^+$] we kept the [$K^+$][$Cl^-$] product constant, thus avoiding a possible swelling of the tissues caused by Donnan effects. The composition of the high $K^+$-depolarizing solution was (mM): NaCl, 55.58; $Na_2SO_4$, 39.21; $K_2SO_4$, 6.87; $KH_2PO_4$, 1.25; $CaCl_2$, 2; $MgSO_4$, 1; $NaHCO_3$, 25; glucose, 10; pH, 7.4 (Floran et al., 1988).

All drugs were obtained from Sigma-RBI (St. Louis Missouri. USA), except for ZM 241385 that was purchased from Tocris Cookson Inc. (Ballwin, MO, USA).

### 2.4. Data analysis

In all experiments we first calculated the fractional release for each sample i.e., the radioactivity present in the fraction divided by the total radioactivity present in the tissue at the moment of collecting the fraction (data expressed as fractional release are shown in Fig. 1A and B). The total radioactivity present in the tissue at the moment of collection was calculated by adding back all the radioactivity collected between the end of the experiment and the time of sampling to the radioactivity remaining in the tissue at the end of the experiment.

Then we calculated treated/control ratios following the procedures of Frankhuyzen and Mulder (1982) and Floran et al. (2002). Only the effect of the drugs on the release of [$^3$H]GABA evoked by high (15 mM) $K^+$ (that is from fraction 4 to 18) is illustrated (see Figs. 1C–5). For comparison, we illustrate the same data expressed as fractional release (Fig. 1B) and treated/control ratios (Fig. 1C).

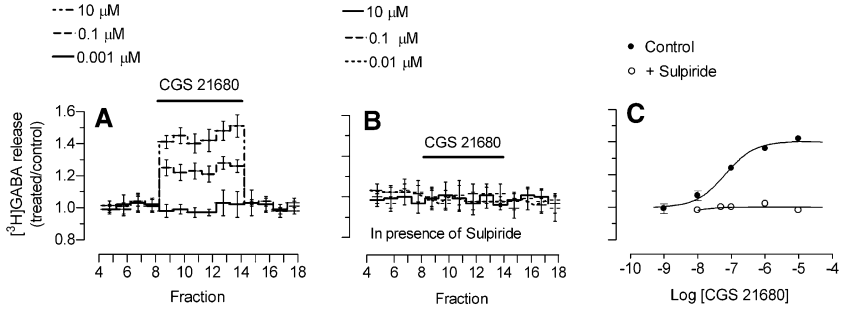


Fig. 2. Effects of the blockade of dopamine D2 receptors with sulpiride on the stimulatory effects of CGS 21680 on depolarization-induced [$^3$H]GABA release. (A) shows the effects of 3 doses of CGS 21680 on [$^3$H]GABA release. (B) shows the effects of CGS 21680 in slices treated with sulpiride (10 μM). (C) shows the concentration–response curve obtained in the absence or presence of sulpiride. The $EC_{50}$ for CGS 21680 was 65 nM. Points are mean ± S.E.M. of 3 independent experiments (three replicates per experiment). The increases in GABA release produced by CGS 21680 concentrations of 0.01 μM or higher were significant at least at $P<0.05$.

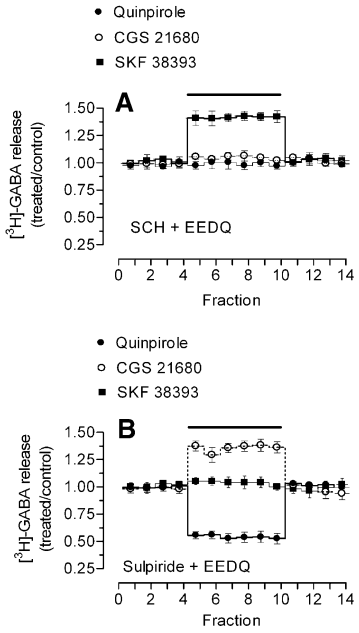


Fig. 3. (A). Alkylation of D2 receptors with EEDQ abolishes the stimulatory effect of activation of $A_{2A}$ receptors with CGS 21680. (A) illustrates the effect of quinpirole (1 μM), CGS 21680 (0.1 μM) and SKF 38393 (10 μM) in slices protected by pretreatment with the D1 antagonist SCH 23390 (1.7 μmol/kg). The increase produced by SKF is significant ($P<0.001$). (B) shows the effects of the same compounds in slices protected by systemic pretreatment with the D2 antagonist sulpiride (234 μmol/kg). The inhibition produced by quinpirole as well as the stimulation produced by CGS is significant ($P<0.001$). Experimental points are the mean±S.E.M. of 3 independent experiments (4–6 replicates per experiment). Horizontal bars indicate the presence of the drugs in the superfusion medium. In each experiment, conditions were run in parallel.

### 2.5. Statistics

Treated/control ratios were used to determine the significance of the effect of $Ca^{2+}$-free media or drugs in release experiments. They were analyzed using Wilcoxon *t*-test comparing with a theoretical value of 1, before, during and after drug-treatment. Differences in effects between three or more groups were assessed by Kruskal–Wallis test for unpaired data followed by Dunn's test using version 4.0 of Prism (Graph-Pad Software, San Diego, CA). Dose–response curves were analyzed by nonlinear regression which provided estimations of $EC_{50}$ and $IC_{50}$ and the confidence intervals (CI). These data were analyzed with the same software.

## 3. Results

### 3.1. $K^+$ and $Ca^{2+}$ dependence of [$^3$H]GABA release

To define the components of [$^3$H]GABA release modulated by adenosine receptor ligands we determined their effects in slices superfused with either normal or elevated $K^+$. Fig. 1A shows that the selective adenosine $A_{2A}$ receptor agonist CGS21680 stimulated [$^3$H]GABA release from slices superfused with high $K^+$ but did not affect the release of slices bathed with a medium containing normal (6.25 mM) $K^+$.

Fig. 1B and C show that equimolecular substitution of all $Ca^{2+}$ with $Mg^{2+}$ in the superfusion solution produced a drastic reduction (83±5%) of $K^+$-evoked [$^3$H]GABA release but did not modify (Fig. 1B) the release from slices superfused with normal $K^+$.

### 3.2. Sulpiride

The increase in $K^+$-evoked GABA release caused by CGS 21680 was dose-dependent (Fig. 2A and C); the $EC_{50}$ for this effect was of 65 nM (CI, 16–261). The blockade of D2 receptors with sulpiride blocked totally the CGS 21680-induced increase in GABA release (Fig. 2B and C).

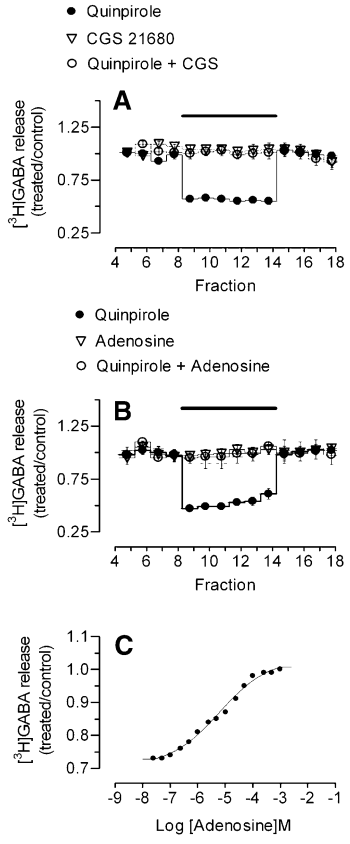


Fig. 4. Reserpinization abolishes the effect of activation of $A_{2A}$ receptors on depolarization-induced [$^3$H]GABA release. (A) shows the effect of CGS 21680 ( 0.1 μM), quinpirole (1 μM) and both compounds together. The quinpirole inhibition is significant ($P<0.001$). (B) shows the effect of adenosine (400 μM), quinpirole (1 μM) and both compounds together. CGS 21680 or adenosine was added to the medium concurrently with quinpirole. Horizontal bar indicates the presence of the compound in the medium. Points are the mean±S.E.M. of 3 experiments (six replicates per experiment). In each experiment conditions were run in parallel. (C) illustrates the concentration–response curve of the inhibition of the effects of 1 μM quinpirole by adenosine. For clarity the ordinate axis was expanded. The $IC_{50}$ was 6.0 μM.

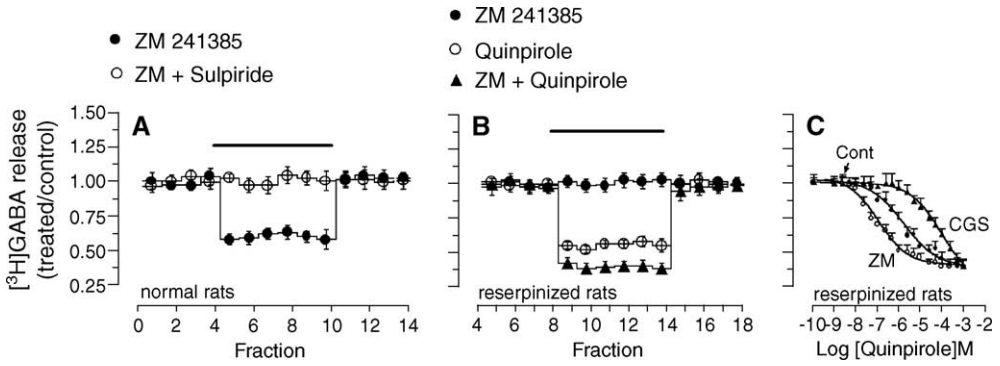


Fig. 5. Effect of endogenous adenosine on depolarization-induced [$^3$H]GABA release. (A) shows the effect of the selective $A_{2A}$ antagonist ZM 241385 (0.01 μM) either alone or in the presence of sulpiride (1 μM). The response to ZM 241385 in presence of sulpiride was calculated using controls that were superfused with medium containing sulpiride only. The inhibition of ZM 241385 (0.01 μM) is significant ($P<0.001$). (B) shows the effect ZM 241385 in the presence or absence of quinpirole (1 μM). The increase of the effect of quinpirole by ZM 241385 is significant as compared to quinpirole alone ($P<0.05$). Horizontal bars indicate the presence of the drugs in the medium. Points are the mean±S.E.M. of 3 independent experiments (4–6 replicates per experiment). (C) shows the effects of ZM 24385 (0.01 μM) and CGS 21680 (0.1 μM) on the concentration-dependence of the inhibitory effect of quinpirole. In the presence of ZM 24385 the $EC_{50}$ for quinpirole was reduced from 1.4 to 0.09 μM. CGS 21680 shifted the $EC_{50}$ to 85.8 μM. The experimental points are the mean±S.E.M. of 3 independent experiments (3–5 replicates per experiment).

### 3.3. EEDQ

The inactivation of D2 receptors by their alkylation with EEDQ prevented the stimulation of GABA release normally caused by CGS 21680 (Fig. 3A). It also prevented the inhibition of [$^3$H]GABA release that quinpirole normally causes in untreated tissues (for an illustration of the effect in untreated tissues see Floran et al., 1997). In this group of experiments EEDQ was administered in animals previously treated with the D1 antagonist SCH 23390 to prevent their alkylation. When the effect of the activation of D1 receptors was tested. (Fig. 3A) SKF 38393 showed its characteristic stimulatory effect (Floran et al., 1990).

We next used the protection protocol to test whether the loss of CGS 21680-stimulated GABA release could have been caused by the alkylation of $A_{2A}$ receptors by EEDQ rather than by the inactivation of D2 receptors. As shown in Fig. 3B when D2 receptors were protected by sulpiride pretreatment the effect of quinpirole and CGS 21680 were preserved. However in this condition the D1 agonist SKF 38393 did not stimulate GABA release.

### 3.4. Reserpine

We further tested the dopamine dependence of the effect of activation of $A_{2A}$ in slices depleted of dopamine. These slices were obtained from reserpine-treated animals (see Experimental procedures). The depletion of endogenous dopamine in the globus pallidus of these animals is of about 90% (Floran et al., 1997). In this condition, the stimulation of GABA release produced by CGS 21680 was lost (Fig. 4A). In this condition the effect of even a high dose (400 μM) of adenosine was not seen. (Fig. 4B).

To determine whether the loss of the effect was caused by a deficiency in $A_{2A}$ receptors in reserpinized tissues we measured whether activation of these receptors modifies the effects of the selective D2 agonist quinpirole. Fig. 4A and B shown that both CGS 21680 and adenosine behaved in a similar fashion: they antagonized the inhibitory effect of quinpirole.

Fig. 4C illustrates the dose-dependency of the antagonism of the quinpirole inhibition by adenosine. The $IC_{50}$ for this effect was of 6.0 μM (CI, 2.8–13.1).

### 3.5. Endogenous adenosine

To determine whether endogenous adenosine acting on $A_{2A}$ receptors in the globus pallidus modulates $K^+$-evoked GABA release, we investigated the effects of the selective adenosine $A_{2A}$ receptor antagonist ZM 241385 in slices obtained from rats without prior reserpine treatment. In this condition, ZM 241385 reversibly reduced depolarization-induced [$^3$H]GABA release (Fig. 5A). When the D2 antagonist sulpiride was also included to the superfusion solution the inhibitory effects of the blockade of $A_{2A}$ receptors with ZM 241385 were prevented (Fig. 5A).The inhibitory effects of ZM 241385 on GABA release were also absent in slices obtained from reserpine-treated animals, therefore depleted of dopamine (Fig. 5B).

We were concerned that slices obtained from reserpine treated animals may not respond to blockade of $A_{2A}$ receptors because they might contain insufficient endogenous adenosine to modulate the activation of D2 receptors. To test this point we determined the effect of ZM 241385 on the response to exogenous activation of D2 receptors with quinpirole in slices obtained from reserpinized animals. ZM 241385 enhanced the effects of quinpirole on GABA release in these slices (Fig. 5B).

To quantify the effect of $A_{2A}$ receptors on the D2 receptor-mediated control of GABA release, we measured the effect of either activation or blockade of the $A_{2A}$ receptors on the dose–response curve of quinpirole (Fig.

5C). Blockade with ZM 241385 (0.1 μM) decreased the $EC_{50}$ from 1.4 (CI, 0.98–2.23) to 0.09 (CI, 0.06–0.15) μM while stimulation with CGS 21680 (0.1 μM) increased it to 85.8 (CI, 50.0–147.3) μM (see Fig. 5C). Neither of the compounds affected the maximum inhibitory effect of quinpirole. These experiments were done in pallidal slices from reserpinized animals to eliminate effects of endogenous dopamine.

## 4. Discussion

In our experiments, the stimulation of GABA release produced in the globus pallidus by activating $A_{2A}$ receptors was blocked by eliminating dopaminergic influences mediated by D2 receptors. The effect was demonstrated with three independent methods of interfering with dopamine transmission at D2 receptors: antagonism with the specific blocker sulpiride, alkylation of D2 receptors with EEDQ and depletion of dopamine with reserpine.

We also showed that activation of $A_{2A}$ receptors produces a marked decrease in the potency of D2 agonists; the value of $EC_{50}$ for quinpirole was nearly one thousand times larger in tissues incubated with an $A_{2A}$ agonist than when $A_{2A}$ receptors were blocked.

### 4.1. Source of GABA release

Potassium-depolarization can stimulate GABA release from both nerve terminals and glial cells (Bernath, 1992). GABA release from glial cells is stimulated only when concentrations of $K^+$ higher than 50 to 60 mM are used and is not $Ca^{2+}$-dependent (Bernath, 1992). In our experiments GABA release was stimulated with 15 mM $K^+$ and was $Ca^{2+}$-dependent (Fig. 1). These results also indicate that the depolarization-induced increase in [$^3$H]GABA release is not mediated by reversed activity of the GABA transporter because release through the transporter is not $Ca^{2+}$ dependent (Bernath, 1992).

There are two major sources of GABA release in the rat globus pallidus: the projections from striatal medium-sized spiny neurons and the intranuclear collaterals of pallidal GABAergic neurons (see Gerfen and Young, 1988; Parent and Hazrati, 1995; Stanford and Cooper, 1999). We suggest that modulation of GABA release by $A_{2A}$ receptors occurs at the projections from striatal medium-sized spiny neurons because this cell population appears to be the only one that expresses $A_2$ receptors and provides a high density of GABAergic terminals within the globus pallidum.

The effects of both adenosine (see Fig. 1) and dopamine agonists were observed only when the tissues were depolarized. We did not detect significant changes in basal release of GABA during activation of $A_{2A}$ receptors by CGS21680. In contrast, Shindou et al. (2001) detected a 35% increase in the frequency of miniature inhibitory postsynaptic currents. Although we cannot explain the discrepancy, two differences in methods may be significant. Our determinations include GABA released by both striatopallidal and pallidopallidal GABAergic terminals. Since the latter don't have $A_{2A}$ receptors, a relatively modest effect on the basal release by striatopallidal projections may be diluted by unmodified release from pallidopallidal terminals. The other difference is that Shindou et al. (2001) used newborn rats while we used adult rats. Adenosine modulation of basal GABA release is age dependent; the effects of adenosine on basal release disappear as animals become older. (Corsi et al., 1999).

### 4.2. Endogenous adenosine

The inhibition of release by the $A_{2A}$ receptor antagonist ZM 241385 (Fig. 5A) in the absence of added agonists indicates receptor occupancy by the endogenous ligand i.e., interstitial concentrations of adenosine in the $K^+$-depolarized slice are sufficient to modulate GABA release. In agreement with this view is the $EC_{50}$ of 6.0 μM for adenosine (Fig. 4C); this value is close to the concentrations measured in the interstitial fluid of the brain (Ballarin et al., 1987, 1991; Park et al., 1988; Dunwiddie and Masino, 2001). Moreover, interstitial levels of adenosine increase when brain activity and oxygenation are modified (Park et al., 1988).

### 4.3. Mechanisms of D2 and $A_{2A}$ receptor interactions

In the globus pallidus; increased cytoplasmic cAMP stimulates GABA release (Shindou et al., 2001). Because in medium-sized spiny neurons stimulation of adenosine $A_{2A}$ receptors leads to activation of adenylate cyclase via $G_{s/olf}$-proteins while activation of dopamine D2 receptors inhibits adenylate cyclase via $G\alpha_{i/o}$-proteins (Sibley and Monsma, 1992), one would expect that the rate of adenylate cyclase activity would simply be the sum of the two opposing influences. Our results appear to exclude this possibility. Thus, the stimulatory effects of activating $A_{2A}$ receptors ought to be largest when dopaminergic influences are absent. In contrast, the effects of adenosine were lost in the absence of dopaminergic influences (Figs. 2–4). Also the effects of stimulating D2 receptors would be greatly diminished when adenosine $A_{2A}$ receptors are blocked. In contrast, dopamine potency increased when adenosine receptors were blocked (Fig. 5).

Other properties of dopamine D2 receptor suggest some alternative explanations; dopamine D2 receptors can change the coupling of other receptors to G proteins. For example, D2 receptor activation shifts $CB_1$ receptor coupling from an inhibitory effect on the formation of cAMP to a stimulatory response (Glass and Felder, 1997; Watts, 2002).

Another possible mechanism could involve the heterodimerization of $A_{2A}$ and dopamine D2 receptors demonstrated in isolated membranes (Fuxe et al., 1998; Gines et al., 2000; Hillion et al., 2002; Diaz-Cabiale et al., 2001).

Experiments in isolated enkephalin-expressing medium-sized spiny neurons suggest another possibility. In these cells stimulation of D2 receptors inactivates L-type voltage-dependent $Ca^{2+}$ channel currents in the absence of inactivation of adenylate cyclase (Hernandez-Lopez et al., 2000). Instead, D2 receptor activation increases calcineurin activity, leading to channel dephosphorylation and current inactivation (Hernandez-Lopez et al., 2000). Thus, in the absence of dopamine action, calcineurin activity could be so depressed that the channels are nearly fully phosphorylated and little or no additional phosphorylation would be produced when adenosine $A_{2A}$ receptors are activated.

The different signaling pathways may be behind the differences in dopamine-dependence of adenosine action (Zahniser et al., 2000; Aoyama et al., 2000; Chen et al., 2001); a given response will depend on the specific signaling pathways present in a particular cell domain.

### *4.4. Potentiation of dopamine action by adenosine blockade and the therapeutic effects of adenosine antagonists*

The antagonism of dopaminergic effects by adenosine is extremely powerful: the value of $EC_{50}$ for quinpirole was nearly one thousand times smaller in tissues incubated with an $A_{2A}$ antagonist than in the presence of an $A_{2A}$ agonist (Fig. 5C).

In parkinsonian patients and animals the $A_{2A}$ antagonist KW6002 potentiates the anti-parkinsonian effects of low doses of L-dopa; however, it has no effect when administered in conjunction with optimal doses of L-dopa (Chase et al., 2003; Kanda et al., 2000). Furthermore, KW6002 given alone does not affect the severity of parkinsonian symptoms (Chase et al., 2003; Kanda et al., 2000).

These therapeutic effects of KW6002 are consistent with our experiments. The results of Fig. 5 suggest that the therapeutic effects of $A_{2A}$ antagonists should be largest in the presence of low or modest levels of dopamine and diminished or absent when dopaminergic receptors are saturated. While the results of Figs. 2–4, suggest that the anti-parkinsonian effects of adenosine $A_{2A}$ antagonists should be absent when dopaminergic transmission is eliminated.

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