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Modulation of adenosine concentration by opioid receptor agonists in rat striatum

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Abstract

There is evidence that adenosine and morphine interact in the striatum. However, little is known about the precise role of the opioid receptor subtypes implicated in the modulation of adenosine tissue concentration and in adenosine receptor expression and function. We sought to evaluate, in the absence of withdrawal symptoms, the effects of the short-term administration of selective μ-, δ- or κ-opioid receptor agonists on adenosine concentration and on adenosine A_{2A} receptor function in rat striatum. Adenosine A_{2A} receptor was chosen because the neuronal sub-population expressing this receptor coexpresses enkephalin, suggesting that adenosine A_{2A} receptor may be regulated by opioid receptor agonists. Oxymorphone hydrochloride (μ-opioid receptor agonist, 6 mg/kg/day), +[-(5α,7α,8β)-(−)-*N*-methyl-*N*(7-(1-pyrrolidinyl)1-oxaspiro (4.5)dec-8-yl) benzenacetamide] (U69593) (κ-opioid receptor agonist, 0.75 mg/kg/day), and (+)-4[(α *R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) (SNC80) (δ-opioid receptor agonist, 9 mm/kg/day), or vehicle, were administered i.p 3 × daily during 5 days to groups of rats ($n = 6$). We also investigated the effects of opioid receptor agonists on adenosine uptake by striatal cell extracts. We found that administration of μ- or δ-opioid receptor agonists significantly decreased adenosine uptake in striatal cell extracts and increased adenosine concentration (mean + 24% and + 45% for μ- and δ-opioid receptor agonist, respectively, relative to controls). None of the receptor agonists tested induced obvious modifications of adenosine A_{2A} receptor function. However, the δ-opioid receptor agonist induced an increase in adenosine A_{2A} mRNA expression (mean 44%). We conclude that μ and δ receptor agonists inhibit adenosine uptake by striatal cell extracts and increase adenosine concentrations in rat striatum.

Keywords: Adenosine; Opioid; Striatum

1. Introduction

There is evidence that endogenous adenosine is implicated in the modulation of opiate action in the central nervous system and that both adenosine A_1 and A_{2A} receptors are involved (Salem and Hope, 1997). Morphine and adenosine inhibit Ca^{2+}-dependent neurotransmitter release (Hedqvist and Fredholm, 1976; Hayashi et al., 1978); this inhibition can be blocked by theophylline, a non selective receptor antagonist of adenosine (Sawynok and Jhamandas, 1976; Sawynok et al., 1991). Conversely, dipyridamole, an inhibitor of adenosine uptake, potentiates the inhibition of transmitter release by both adenosine and morphine (Okwuasaba, 1977; Hayashi et al., 1978). Adenosine kinase inhibitors, which increase adenosine concentration in the extracellular spaces, attenuate opiate withdrawal as a result of adenosine receptor activation (Kaplan and Coyle, 1998). Short-term morphine treatment releases adenosine from capsaicin sensitive afferent nerve terminals (Sweeney et al., 1989) and from spinal synaptosomes (Sweeney et al., 1991) and enhances adenosine release in cerebral cortex (Fredholm and Vernet, 1978; Phillis et al., 1979). Furthermore, chronic opiate treatment raises A_1 receptor concentrations in whole brain homogenates (Ahlijanian and Takemori, 1986) and in cortex (Kaplan et al., 1994). Also, morphine elevates extracellular

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adenosine concentration through an up-regulation of adenosine transporter binding sites that can increase both adenosine efflux and synaptic adenosine levels (Kaplan and Leite-Morris, 1997). Little is known however about the precise role of opioid receptor subtypes implicated in the modultion of adenosine receptor expression and function. The study therefore had two aims: (i) evaluate the effects of the short term administration of selective μ-, δ- and κ-opioid receptor agonists on adenosine concentration in the rat striatum and (ii) evaluate the effects of these receptor agonists on adenosine A_{2A} receptor mRNA expression and on receptor number and function. Rat striatum was chosen because this area is rich in adenosine A_{2A} and opioid receptors (Pert et al., 1976; Alexander and Reddington, 1989; Graybiel, 1990).

Adenosine A_{2A} receptor was chosen for three reasons. First adenosine A_{2A} receptor gene is expressed mainly by striata and olfactive neurons (Fink et al., 1992; Schiffmann et al., 1991); furthermore, cells expressing adenosine A_{2A} receptor are well identified conversely to cells expressing adenosine A_1 receptors (Schiffmann and Vanderhaegen, 1995). Second, the neuronal sub-population that expresses adenosine A_{2A} receptor is the same as the one that also expresses enkephalines (Schiffmann et al., 1991, 1993; Augood and Emson, 1994). Thus adenosine A_{2A} receptor was the best candidate for a possible regulation by opioid receptor agonists. Finally, there is evidence for a strong interaction between purinergic adenosine A_{2A} and opioid receptors in the striatum (Graybiel, 1990; Schiffmann and Vanderhaegen, 1995).

2. Materials and methods

2.1. Drugs

Oxymorphone hydrochloride (Sigma, St. Quentin Fallavier, France) was dissolved in NaCl 0.9% at the concentration of 2 mg/ml. U69593 = +[-(5α,7α,8β)-(−)-*N*-methyl-*N*(7-(1-pyrrolidinyl)1-oxaspiro (4.5)dec-8-yl) benzenacetamide] was from Sigma (RBI) and was dissolved in 0.1N HCl to a concentration of 10 mg/ml and then diluted in NaCl 0.9% to a final concentration of 0.25 mg/ml. SNC80 = (+)-4[(α *R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) (Tocris, Fisher Scientific, France) was dissolved in 5 mM dimethyl sulfoxide (DMSO, Sigma) to a final concentration of 3 mg/ml.

2.2. Treatment procedure

Studies were done on four groups of six Sprague female rats (250–300 g) bred in our laboratory. Grouping was as follows:

Group 1 was injected with U69593 0.25 mg/kg/injection;
Group 2 was injected with SNC80 3 mg/kg/injection;
Group 3 was injected with oxymorphone hydrochloride 2 mg/kg/injection;
Group 4 was injected with 5 mM DMSO in serum saline.

All injections were done i.p. thrice a day (7 am, 1 and 7 pm) during 5 days. Animals were housed six per cage at a constant temperature (26°C). They had free access to food and water and were subjected to 12-h cycles of light and dark periods.

2.3. Rat striatal dissection

Twenty minutes after the last i.p. injection, rats were anesthetized with sodium pentobarbital (80 mg/kg i.p.). The skull was exposed by making a midline skin incision and the brain was quickly removed and immediately put in nitrogen. Striata were then microdissected under binocular microscope on ice.

2.4. Receptor binding assays

We used the methodology described by Popoli et al. (1998) with some modifications. Binding assays were performed on pooled membranes from rat dorsal striatum. Frozen extracts were rapidly thawed and homogenized in Tris buffer (50 mM Tris, 10 mM $MgCl_2$, pH 7.4; 3 ml/g tissue) and centrifuged at $1200 \times g$ for 10 min at 4°C. The supernatants were then centrifuged at $9000 \times g$ for 60 min at 4°C. The pellets were resuspended in 1.5 ml of Tris buffer containing 2 U/ml of adenosine deaminase (Sigma) and incubated for 30 min at 37°C. The protein contents of preparations were determined in microtitration plates using the Micro BCA protein assay reagent (Interchim, Montluçon, France) with bovine serum albumin as standard. Membrane suspensions were diluted at 2 mg/ml and incubated with increasing concentrations (8–280 nM) of [^{3}H]CGS-21680 = (2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamido-adenosine (New England Nuclear, Life Science Products, Zeventen, Belgium, 42.5 Ci/mmol) for 90 min at 25°C. The reaction was stopped by the addition of cold Tris buffer and the samples were filtered through Whatman GF/C glass fiber filters under vacuum. After drying overnight at 37°C, filters were counted for radioactivity in scintillation fluid (Packard, Les Ullis, France). Nonspecific bindings were determined in a series of parallel experiments with 100 μM of 2-chloroadenosine.

2.5. Tissue preparation for adenosine assay

Adenosine (crystallized, 99% pure), adenosine deaminase (calf intestine, specific activity 200 IU/mg), and dipyridamidole (5 mg/ml) were obtained from Boehringer Mannheim (Meylan, France). α,β-Methylene-adenosine-5′-diphosphate (AOPCP), nitrobenzylthioinosine, and de-

oxycoformycin were from Sigma. 9-Erythro (2-hydroxy-3 nonyl) adenine (EHNA) was from ICN Pharmaceutical (Orsay, France). The reversed phase chromatography column, methanol, and other reagents were from Merck, Darmstadt, Germany).

We used the procedure described by Delaney and Geiger (1996), with some modifications. Striata were slightly homogenized, using a polytron, in a cold ''stopping'' solution (1 mg protein/ml) containing in 0.9% NaCl: 2 mM dipyridamole; 100 nM nitrobenzylthioinosine; 4.2 mM Na_2 EDTA; 5 mM EHNA; 79 mM AOPCP; 1 μM deoxycoformycin; 0.25 mg/ml of indomethacin. Dipyridamole and nitrobenzylthioinosine were added to stop the uptake and release of adenosine by striatal cells; AOPCP was added to prevent the action of adenosine formation through a 5′ nucleotidase. Deoxycoformycin was used to prevent adenosine deaminase action. Indomethacin was added to prevent adenosine release by cells during centrifugation. Aliquots of 125 μl were centrifuged (0°C, 4 min at 15,000 × *g*) after addition of 200 μl of perchloric acid (10%, v/v) to each supernatant. Samples were vortexed on ice for 5 s and then filtered by centrifugation (15,000 × *g*, 0°C, 5 min) on Millipore ultra free® 0.5 μ filter. A volume of 100 μ l of supernatants was lyophilized. All assays describe above were done in duplicate.

2.6. Tissue preparation for adenosine uptake

After dissection as described above, striata were gently homogenized using a polytron. Samples of 125 μl of striatal cell extracts (six samples each time) were added with 125 μl of a solution containing 0.9% NaCl, 2 μM $CaCl_2$. The samples (250 μl) were continuously mixed by vortex® system and then 2 nmol (in 100 μl) of adenosine was added. The degradation and uptake of adenosine was stopped 5, 30, and 60 s after adenosine addition, using 750 μl of cold stopping solution (the same as in Section 2.5 above). Samples were then centrifuged (0°C, 1,500 × *g*, 10 min), lyophilized, and stored at −80°C before being chromatographed.

2.7. Adenosine assay

2.7.1. Equipment

A Hewlett Packard HP 1100 modular system was used, with a diode array detector (G13135A) and a deuterium lamp (slit 8 nm). The column (150 × 4 mm) was packed with RP8 and the injection loop was 200 μl. The column was equilibrated with 50 mM phosphate buffer, pH 4.

2.7.2. Chromatography

The technical procedure has been described (Guieu et al., 1994, 1996, 1998, 1999). Briefly, 100 μl of sample was mixed with 100 μl of phosphate buffer (NaH_2PO_4/Na_2HPO_4; pH 4) and injected in the column. Samples were eluted with a methanol gradient (3 min with 0%, then 10–25% methanol in 15 min, and return to initial gradient value in 2 min). The intra-test variation coefficient was 0.5% and the limit of detection of adenosine at 254 nm was 1 pmol in 200 μl injected. During chromatography, the spectra of peaks eluted between 12 and 15 min were recorded automatically at the rate of 6 spectra/s, in the 190–400 nm window, at 2 nm intervals.

Identification was done by elution time and by comparing spectra with internal references. Quantitation was done by comparing areas obtained with known quantities of exogenous adenosine.

2.8. RNA extraction

Total cellular RNA was extracted from striatal tissue according to the acid guanidine thiocyanate phenol chloroform method of Chomczynski and Sacchi (1987). The reagent was Trizol LS (Life Technologies, Gaithesburg MD).

2.9. Reverse transcriptase polymerase chain reaction

Total RNA (0.5 μg) was reverse transcribed in the presence of random hexamers by the use of M-MLV reverse transcriptase (Pharmacia Biotech) according to the manufacturer's recommendations. Of the resulting cDNA, 2 μl was amplified by a PCR developed in our laboratory, with primers derived from adenosine A_{2A} receptor cDNA sequences (accession number L08102). Adenosine A_{2A} primers sense 5′ TTGGTGACAGGTGTGAGG 3′ and antisense 5′ GAAGGGGCAGTAACACGAACG 3′, designed to generate 225 bp PCR product. β actin specific primers sense 5′ TTGTAACCAACTGGGACGATATGG 3′ and antisense 5′ GATCTTGATCTTCATGGTGCTTAGG 3′,

Table 1
Adenosine concentrations
Adenosine concentration in striata of rats ($n = 6$ per group) injected with μ (Oxymorphone hydrochloride, 6 mg/kg/day), ∂ (SNC80, 9 mg/kg/day), or k (U69593, 0.75 mg/kg/day) receptor agonist. Controls were injected with 5 mM DMSO in serum saline. All products were injected i.p. in 1 ml solvant, during 5 days.

Adenosine concentration (pg/mg protein)				
	Controls	oxymorphone	SNC80	U69593
Mean	92	121	166	82.5
SD	22	27	34	23

p=0.046
p=0.0032
p=0.23

Table 2
A_{2A} receptor number and function
Effects of opioid receptor agonists on A_{2A} adenosine receptor binding. Saturation experiments were done using increasing concentrations of [^{3}H]CGS-21680 as A_{2A} receptor agonist. The estimates of equilibrium dissociation constants, K_D, and density of binding sites, B_{max}, were deduced from non-linear regression curves using a computer program (Mc Ligand, adapted for McIntosh by Williams, UCLA].

	K_D (nM)	B_{max} (fmol/mg of protein)
Oxymorphone hydrochloride	35	380
SNC80	18	274
U69593	28	350
Controls	46	338

designed to generate a 838 bp PCR product, served as internal standards. PCR was performed on a Perkin-Elmer® thermocycler 9600 with 3 cycles at 94°C for 15 s, 58° for 30 s, and 72° for 30 s, and then a simple extension step of 72°C for 3 min. We followed the recommendations of Kwok and Higuchi (1989) to avoid contamination. All reverse transcriptase polymerase chain reaction (RT-PCR) reactions were performed on the same preparation for the analysis of the different mRNA receptors. After PCR, a 10 μl aliquot of each sample was electrophoresed on a 2% agarose gel, and the PCR product was visualized by ethidium bromide staining and UV illuminator. PCR products were verified by sequence analysis.

2.10. Relative RT-PCR quantitation of adenosine A_{2A} receptor mRNA concentration

To compensate for variations in RNA isolation and tube to tube variations in RT and PCR reactions, multiplex RT-PCR can be performed by relative RT-PCR using β-actin as an internal standard. Relative RT-PCR allows direct comparisons between multiple samples. To increase reproducibility, the level of PCR product is adjusted, on the basis of the signal of co-amplified internal standard. The PCR products were analyzed while the PCR was still in linear amplification for the target and the β-actin. Quantitation was performed on the digital photography by a NIH image 6.1 software.

2.11. Statistical analysis

We used the Mann–Whitney test to compare adenosine concentrations and adenosine A_{2A} mRNA receptor concentrations in striatal cell extracts. A variance analysis (ANOVA, one way analysis) was used to evaluate adenosine uptakes. A *p*-value less than 0.05 was considered significant.

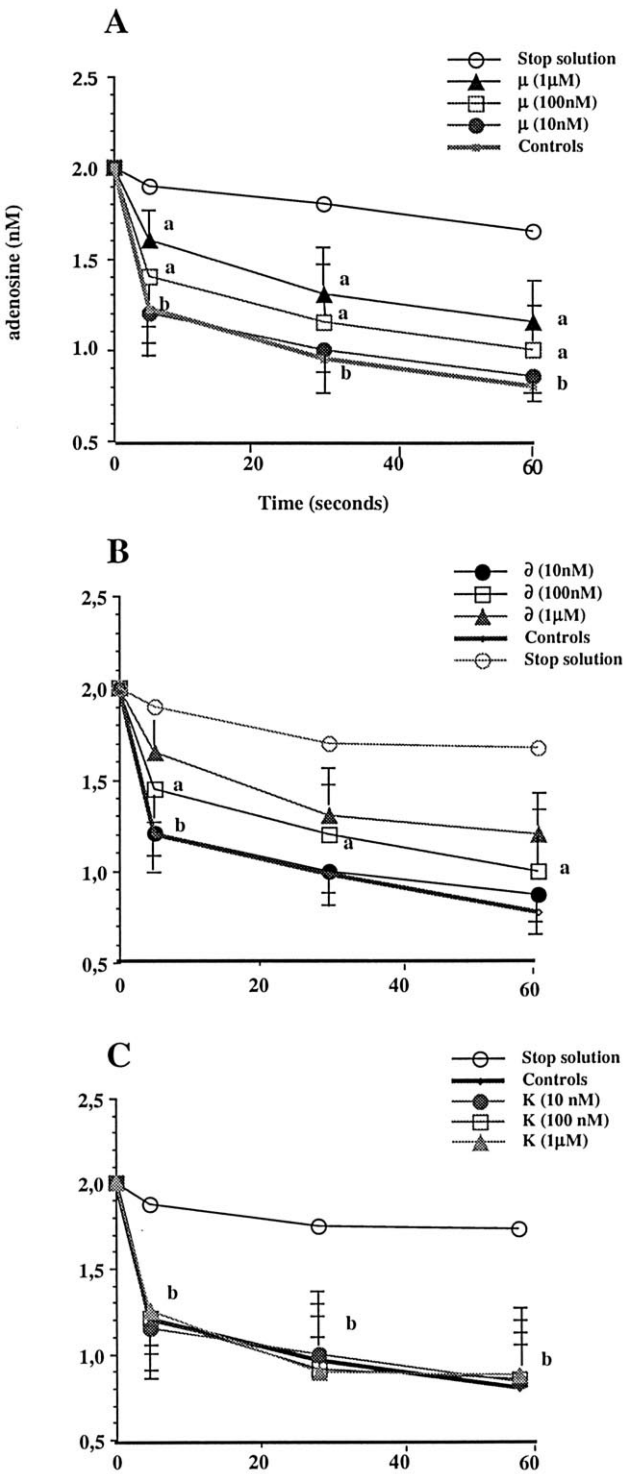

Fig. 1. Adenosine uptake. Means and standard deviations of adenosine concentrations as a function of time in the supernatant of striatal cell extracts, in the presence of oxymorphone hydrochloride (A), SNC80 (B), or U69593 (C) at three different concentrations. Striatal cell extracts of rats were vortexed continuously. Then at time 0, 2 nmol of adenosine in 100 μl of phopshate buffer was added. Then the uptake of adenosine by striatal cells was stopped 3, 15, 30, and 60 s after adenosine was added, using 1 ml of a cold stopping solution containing 1 μMof deoxycoformycin, 2 mM dipyridamole, and 100 nM nitrobenzylthioinosine. The supernatants were pipetted off and lyophilized, and the adenosine concentrations were evaluated as indicated in Section 2. (a) ANOVA $p < 0.05$; (b) $p > 0.05$ compared with controls.

3. Results

3.1. Adenosine concentration in striata

Adenosine concentration in striata was significantly higher in rats treated with oxymorphone or SNC80 than in controls (mean + 24% and +45% respectively relative to controls; conversely U69593 did not significantly modify adenosine concentration in striata (see Table 1).

3.2. Effects of opioid receptor agonists on adenosine receptor binding

Saturation experiments were done using increasing concentrations of [^{3}H]CGS-21680 as an adenosine A_{2A} receptor agonist. The estimates of equilibrium dissociation constants, K_D, and density of binding sites, B_{max}, were deduced from non-linear regression curves by a computer program (Mc Ligand, adapted for McIntosh by R.E. Williams, UCLA]. The K_{Ds} of CGS-21680 for adenosine A_{2A} receptor were 35, 18, 28, and 46 nM in striata of rats injected with oxymorphone, SNC80, U69593 and vehicle, respectively. B_{max} values were 380, 274, 350, and 338 fmol/mg of proteins, respectively (Table 2).

3.3. Adenosine uptake

Oxymorphone hydrochloride and SNC80 increased the adenosine concentration in the supernatant of striatal cell extracts 30 and 60 s after adenosine addition in a dose dependent manner (Fig. 1A and B). Conversely, U69593 did not modify adenosine uptake by striatal slices (Fig. 1C).

Table 3
mRNA expression
A_{2A} mRNA adenosine receptor concentration in striata of rats ($n = 6$ per group) injected in the same condition as in Table 1. mRNA was evaluated by relative RT-PCR. Levels of adenosine A_{2A} mRNA were expressed as the A_{2A} receptor/β-actin ratio of RT-PCR products.

	Controls	μ	δ	κ
Mean	0.55	0.48	1.1	0.55
SD	0.1	0.07	0.2	0.2

p=0.09
p=0.001
p=0.36

3.4. Adenosine A_{2A} mRNA concentrations

Levels of adenosine A_{2A} receptor mRNAs were expressed as the adenosine A_{2A} receptor/β-actin ratio of RT-PCR products (Table 3). Adenosine A_{2A} mRNA concentration was significantly higher in striata of rats treated with SNC80 than in controls (mean + 44%; $p = 0.01$), and see Table 3). Conversely, oxymorphone or U69593 did not significantly modify adenosine A_{2A} mRNA expression ($p = 0.09$ and $p = 0.36$, respectively).

4. Discussion

The results show (i) that short term administration of μ- or δ- but not κ-opioid receptor agonist (see Dhawan et al., 1996), in the absence of withdrawal symptoms, decreases adenosine uptake in striatal cell extracts, in a dose dependent manner and (ii) that it increases adenosine concentration in rat striata. Also (iii) the δ-opioid receptor agonist, SNC80, induces an increase in mRNA receptor expression relative to controls. This increase, however, is not associated with obvious modifications in adenosine A_{2A} receptor number.

To our knowledge, there is no data on the influence of selective opioid receptor agonists on the adenosine receptor modulation and on the uptake of adenosine by striatal cells.

It is known that even if brains are quickly removed and put immediately in nitrogen, the levels of adenosine may increases rapidly. However, we have compared the adenosine concentration in four groups of rats in the same conditions of brain removal. And intra group comparisons are of more interest than the absolute value of adenosine concentration in rat striatum. Finally, the adenosine concentrations we found are in the range of those in the previous study which used microwave fixation (Delaney and Geiger, 1996).

We found also that δ-opioid receptor agonist increases the mRNA expression by more than 40% in a relative quantitative RT-PCR evaluation. We hypothesize that this ''up regulation'' does not result from acute adenosine concentration variations during brain removal, but may be due to δ-opioid receptor agonist administration for 5 days, since we found that the δ-opioid receptor agonist inhibited adenosine uptake by rat striatal cell extracts, and then increased adenosine concentrations. Even if relative RT-PCR does not permit any absolute quantitation of mRNAs, in the case of our study, intergroup comparisons are of more interest than absolute mRNA concentrations. However, this increase in mRNA expression did not correlate with any change in receptor number and function.

Nucleoside transport across mammalian cell membranes is complex and involves several distinct nucleoside trans-

porters (Plagemann et al., 1998; Cass et al., 1985). The most common transport system is the equilibrative, facilitated diffusion system found in all mammalian cells (Plagemann et al., 1998; Gati et al., 1989), including the central nervous system (Sweeney, 1996). Extracellular adenosine concentrations are regulated by this bidirectional nucleoside transport system and by cellular release of ATP followed by its metabolism to adenosine (Cunha et al., 1996). Nucleoside transporters regulate both adenosine re-uptake and release and can therefore increase or decrease synaptic adenosine concentrations (Gu et al., 1995; Sweeney, 1996). Our results strongly suggest that, in the absence of withdrawal symptoms, short-term administration of μ- or δ-opiod receptor agonist inhibits the adenosine uptake by the facilitated diffusion system. This inhibition increases the extracellular adenosine concentration. However, uptake inhibition is probably not the only mechanism involved in the adenosine concentration increase, induced in extra-cellular spaces by opioid receptor agonists. Indeed, it was shown that μ (but not δ) selective opioid receptor agonists release adenosine from spinal cord synaptosomes (Cahill et al., 1993), and that μ- and δ-opioid receptors interact to enhance the release of adenosine (Cahill et al., 1996), and that this release occurs via the activation of N-type voltage sensitive Ca^{2+} channel (Cahill et al., 1993).

Also, we found that short term administration of SNC80, a δ-opioid receptor agonist, but not the other opioid receptor agonists, induces an increase in adenosine A_{2A} receptor mRNA expression in rat striatum. This increase is not accompanied by obvious modifications of adenosine A_{2A} receptor number or function since B_{max} and K_D values were in the same range in all groups of rats, including controls.

Various physiological and behavioral roles have been reported for adenosine in the mammalian central nervous, including in locomotor activity (Durcan and Morgan, 1989). The actions of adenosine are mediated via P1 receptors, which are further divided into four subtypes A_1, A_{2A}, A_{2B}, and A_3 on the basis of their distinct molecular structures, area distributions, and pharmacological profiles. All couple to G proteins (for review see Ralevic and Burnstock, 1998).

Striatum is modulate by dopaminergic nigrostriatal pathway and by pallido-sub-thalamo pallidal loop. GABA is the principal neurotransmitter in the striatum and in striatopallidal and striato nigral pathway (for review see Schiffman and Vanderhaegen, 1995).

There is evidence for an interaction between adenosine receptors, dopamine receptors, and opioids receptors in the striatum and for their co-implication in the locomotion control. Adenosine A_{2A} and dopamine D_2 receptors are colocalized in the GABAergic striopallidal neurons (Fink et al., 1992), whereas adenosine A_1 and dopamine D_1 receptors are colocalized in the GABAergic strionigral neurons (Ferré et al., 1996). On the other hand, μ-opioid receptor is coexpressed with dopamine D_1 receptor in striatal neurons, whereas δ-opioid receptor is expressed by striato pallidal neurons and by striatal efferent neurons (Noble and Cox, 1995); furthermore, adenosine A_{2A} receptors are present on the neurons that also express enkephalin and dopamine D_2 receptors (Fink et al., 1992; Schiffmann et al., 1991).

Adenosine receptor activation has been shown to inhibit dopaminergic transmission in striatal region (Jin et al., 1993). Indeed, adenosine A_1 and A_{2A} receptors antagonistically modulate the binding and function of dopamine D_1 and D_2 receptors, respectively (Ferré et al., 1991, 1992, 1993). These modulations occur via direct interaction between A_1/D_1 and A_2/D_2 receptors (see Fuxe et al., 1998). At the behavioral level, adenosine A_1 and A_{2A} receptor agonists counteract the motor activating effects induced by dopamine D_1 and D_2 receptor agonists, respectively (Ferré et al., 1994). Furthermore, in transgenic mice lacking adenosine A_{2A} receptor, the locomotor stimulating effect of caffeine, a non-specific antagonist of adenosine receptors, is reversed into a depressant effect (Ledent et al., 1997). In summary, adenosine A_{2A} receptor produce behavioral effects that are comparable with those obtained with D_2 receptor antagonists (Hauler and Munkle, 1997).

On the other hand, the affinity between adenosine and opiates effects is further supported by the fact that the blockade of adenosine receptors, as obtained by caffeine administration, produces in rats and mice symptoms similar to those observed during the opiate abstinence syndrome (Butt et al., 1979). Furthermore, A_2 receptor function is largely decreased in morphine dependent rats (De Montis et al., 1992) and more recently, it was shown that adenosine receptor antagonists inhibits the development of morphine sensitization in mice (Weisberg and Kaplan, 1999). Furthermore, an absence of opiate rewarding effects in mice lacking D_2 dopamine receptors was reported (Maldonado et al., 1997). During morphine dependence, dopamine and morphine exert opposite effects on striatonigral neurons; by another way, withdrawal is associated with a down regulation of the postsynaptic D_1 and D_2 receptors (Georges et al., 1999).

Concerning locomotion, the predominant effects of μ-opioid agonists are behavioral depression whereas κ-opioid agonists induce hyperactivity (Jackson and Kitchen, 1989). However, opposite effects have been reported depending on the site of administration in the CNS (Matsumoto et al., 1988).

Finally, some δ_1-opioid receptor agonist is able to reduce adenosine A_{2A} receptor activity but not the D_1 dopamine receptor-stimulated adenylcyclase activity whereas δ_2-opioid agonist inhibits both D_1 and adenosine A_{2A} stimulated adenylcyclase activity (Noble and Cox, 1995).

Our findings suggest that μ and δ but not κ activation, which induce an increase in striata adenosine concentration, contribute to the inhibition of dopaminergic function.

We found that δ-opioid receptor agonist was more effective than the μ-opioid receptor agonist in adenosine uptake inhibition in striatal cells. Because enkephalins are endogenous ligands for δ-opioid receptors (Dhawan et al., 1996), this agrees with Augood et al. who suggested that adenosine plays a role in modulating the activity of enkephalin striato-pallidal neurons though the interaction with adenosine A_{2A} receptors (Augood and Emson, 1994).

In a previous work, we found that β-endorphin (an endogenous non-selective μ-opioid receptor agonist) inhibits adenosine uptake by whole brain cells (unpublished data). Furthermore, Kaplan et al. have demonstrated that exogenous morphine elevates extracellular adenosine concentration in rat striatum (Kaplan and Leite-Morris, 1997). Thus endogenous opioids may also be implicated in the inhibition of adenosine uptake.

In conclusion, μ- and δ-opioid receptor agonists inhibit adenosine uptake by striatal cells and increase adenosine concentration in striatal tissues. δ-opioid receptor agonists increase the mRNA expression of adenosine A_{2A} receptor, however, this increase is not accompanied by any modification in adenosine A_{2A} receptor number and function in the rat striatum.

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