Adenosine A₂ Receptors Regulate the Gene Expression of Striatopallidal and Striatonigral Neurons

Serge N. Schiffmann and Jean-Jacques Vanderhaeghen

Laboratory of Neurophysiology, Neuropathology and Neuropeptide Research, Erasme Hospital, Faculty of Medicine, Université Libre de Bruxelles, 1070 Brussels, Belgium

Adenosine acts as a neuromodulator through A₁ and A₂ receptors. The adenosine analogs have been recognized, among other effects, as strong depressors of the locomotor activity by acting on striatal A₂ receptors. Moreover, the A_{2a} receptor subtype is exclusively expressed in the striatum. To elucidate at the cellular level the roles of adenosine in the basal ganglia, the anatomical and functional relationships of the A₂ receptors with the dopamine D₁ and D₂ receptors were studied in the rat striatum.

In situ hybridization histochemistry was used either in combination with retrograde labeling of striatonigral neurons to determine the projection site of A_{2a} receptor expressing neurons, or on consecutive thin sections to address the putative coexpression of the A_{2a} receptor with the D₁ or D₂ receptors in individual neurons. The A_{2a} receptor is mainly expressed by neurons projecting to the globus pallidus and expressing also the dopamine D₂ receptor and enkephalin, but very sparsely by neurons projecting to the substantia nigra that express the dopamine D₁ receptor and substance P.

We have further examined the regulatory effect of the A₂ receptors on striatal gene expression using *in situ* hybridization histochemistry and quantitative autoradiography. Rats unilaterally depleted in dopamine by an unilateral 6-hydroxydopamine-induced lesion of the nigrostriatal pathway used as a model of Parkinson's disease subsequently received chronic injections of saline or the adenosine receptor antagonist caffeine. Intact rats were chronically treated with either saline, caffeine alone, caffeine with N-ethylcarboxamidoadenosine (an equipotent A₁ and A₂ agonist), or caffeine with cyclohexyladenosine (a more selective A₁ agonist). In striatopallidal neurons, caffeine reverses the alterations of enkephalin gene expression induced by the unilateral depletion in striatal dopamine while it has no effect on the alterations of substance P gene expression in striatonigral neurons. Conversely, in the intact striatum, normally innervated by dopaminergic fibers, caffeine acting through

an A₂ receptor induces similar alterations of enkephalin and substance P gene expression compared with the case of dopamine depletion.

This suggests that adenosine and the widely consumed adenosine antagonist caffeine regulate through A₂ receptors the gene expression of striatal neurons both by post- and presynaptic mechanisms.

[Key words: striatum, adenosine A₂ receptor, caffeine, gene expression, enkephalin, substance P, 6-hydroxydopamine]

Any perturbations within the basal ganglia system lead to movement disorders (Albin et al., 1989). The striatum receiving its main input from the cerebral cortex is the first relay of this system. In turn, its medium-sized spiny neurons accounting for more than 90% of the striatal neurons constitute the output pathways. Although all of them utilize GABA as neurotransmitter (Chesselet and Robins, 1989), two subpopulations of these efferent neurons have been identified (for review, see Graybiel, 1990). The striatopallidal neurons express enkephalin whereas the striatonigral neurons coexpress substance P and dynorphin (Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine et al., 1990, 1991), acting finally as an inhibitory and an excitatory pathway to the thalamus, respectively (for review, see Albin et al., 1989). The dopaminergic nigrostriatal pathway influences differentially these two striatal subpopulations. Reduction of dopamine input into the striatum decreases substance P expression in striatonigral neurons whereas it increases enkephalin expression in striatopallidal subpopulation (Young et al., 1986; Normand et al., 1988; Gerfen et al., 1990). Moreover, it has been recently demonstrated that these actions of dopamine are differentially mediated by dopamine D₁ and D₂ receptors (Gerfen et al., 1990), the former being mainly expressed in striatonigral neurons and the latter mainly in striatopallidal neurons (Gerfen et al., 1990; Le Moine et al., 1990, 1991). It has been proposed that hyperkinetic states, such as Huntington's disease or hemiballism, and hypokinetic states, such as parkinsonism, all result from disequilibria between these two striatal subpopulations and/or the specific pathways originating from them (Albin et al., 1989).

Besides dopamine, which is considered as a major regulator of striatal function, adenosine also seems to play a crucial role, far less understood though, in the basal ganglia physiology. Adenosine modulates neuronal functions through receptors that have been classified as A₁ and A₂ receptors (Van Calcar et al., 1979). The recent cloning and characterization of the cDNAs encoding adenosine A₁ and A₂ receptor subtypes (Libert et al., 1989, 1991; Maenhaut et al., 1990) have permitted the brain localization of cells expressing their respective mRNAs by *in situ* hybridization

Received June 1, 1992; revised Aug. 28, 1992; accepted Sept. 17, 1992.

We are thankful to P. Halleux and R. Menu, and to J.-L. Conreur for technical assistance and photography, respectively. Thanks are due also to Dr. R. Karmili, who scrutinized the English. This work was supported by grants from the Belgian Fund for Medical Scientific Research (FRSM 3.4523-86), the Queen Elizabeth Medical Foundation (Belgium) (Neurobiology 89-92), and Ministère de la Politique Scientifique (Pole d'Attraction Interuniversitaire 90-95). S.N.S. is a Senior Research Assistant of the Belgian National Fund for Scientific Research (FNRS 1990-1992).

Correspondence should be addressed to S. N. Schiffmann, Laboratory of Neurophysiology, Neuropathology and Neuropeptides Research, Université Libre de Bruxelles, Campus Erasme, CP 601, 808 Route de Leuven, 1070 Brussels, Belgium.

Copyright © 1993 Society for Neuroscience 0270-6474/93/131080-08\$05.00/0

Figure 1. Combination of retrograde labeling of striatonigral neurons with fluorogold and detection of enkephalin (*A*), adenosine A_{2a} receptor (*B*), dopamine D_2 receptor (*C*), substance P (*D*), and dopamine D_1 receptor (*E*) mRNAs by *in situ* hybridization. Striatonigral neurons express substance P and D_1 receptor mRNAs (arrowheads in *D*, *E*), while fluorogold-negative neurons, presumably striatopallidal, express enkephalin, D_2 receptor, and adenosine A_{2a} receptor mRNAs (arrows in *A–C*). Scale bar, 20 μ m.

histochemistry (Schiffmann et al., 1990, 1991a,b; Schiffmann and Vanderhaeghen, 1992). The A_{2a} receptor is exclusively expressed by medium-sized neurons of the striatum (Schiffmann et al., 1990, 1991b) and mainly by the enkephalinergic striatal subpopulation (Schiffmann et al., 1991a). The adenosine analogs have been recognized, among other effects (for reviews, see Dunwiddie, 1985; Snyder, 1985), as strong depressors of the locomotor activity. More recent data strongly suggest that this action is mediated by the striatal A_2 receptor (Durcan and Morgan, 1989; Ferre et al., 1991a) and could be antagonized by methylxanthines such as caffeine, one of the most widely used psychoactive drugs (Ferre et al., 1991b). Moreover, intramembrane A_{2a}/D_2 receptor interactions have been observed in the rat striatum (Ferre et al., 1991c).

To understand the influence of adenosine through the A_2 receptor in the striatum, we studied the anatomical and functional relationships of the A_2 receptors with the D_1 and D_2 receptors.

Materials and Methods

Animals. For the colocalization study, intact adult Wistar rats were killed by decapitation and their brains were frozen and processed for *in situ* hybridization as follows.

One microliter of a 2.5% solution of fluorogold, a fluorescent retrograde axonal tracer (Gerfen and Young, 1988; Gerfen et al., 1990), was injected into the substantia nigra of adult Wistar rats anesthetized with chloral hydrate (3 gm/100 gm body weight). Stereotaxic injections were placed 5.6 mm posterior to the bregma, 2 mm lateral to the midline, and 8 mm ventral to the surface of the skull, according to the atlas of Paxinos and Watson (1986). After 10 d, the rats were killed by decapitation and their brains removed, fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered solution (pH 7.4), and thereafter processed for *in situ* hybridization as follows.

Adult Wistar rats were anesthetized with chloral hydrate (3 gm/100 gm body weight) and 8 μ g of 6-hydroxydopamine (6-OHDA) in 4 μ l of 0.1% ascorbate and 0.9% saline was injected in the right medial forebrain bundle. Stereotaxic injections were placed 4 mm posterior to the bregma, 1.6 mm lateral to the midline, and 8.2 mm ventral to the surface of the skull, according to the atlas of Paxinos and Watson (1986). After 18 d, only rats displaying significant apomorphine-induced contralateral rotation behavior (>7 turns/min) were selected. They subsequently received daily intraperitoneal injections of saline or caffeine (75 mg/kg/d) for 15 d. The lesion of the nigrostriatal pathway was confirmed by a loss of more than 90% nigral dopamine neurons as assessed by *in situ* hybridization with tyrosine hydroxylase probe (not shown).

Four groups of adult Wistar intact rats received daily intraperitoneal injections of saline, caffeine (75 mg/kg/d) alone, or caffeine with *N*-ethylcarboxamidoadenosine (NECA) (0.25 mg/kg/d) or with cyclohexyladenosine (CHA) (0.5 mg/kg/d) for 15 d.

Figure 2. Comparison of the neuronal localization of adenosine A_{2a} receptor mRNA (*A*, *C*) with that of dopamine D₂ (*B*) and D₁ (*D*) receptor mRNAs, respectively, in the same fields of thin adjacent sections of striatum. Neurons recognized in adjacent sections are numbered. All neurons positive for D₂ receptor mRNA also expressed A_{2a}r mRNA, while coexpression of D₁ receptor and A_{2a}r mRNAs was not observed. Scale bar, 20 μ m.

Treated rats were killed by decapitation 3 hr after the last injection, and their brains were frozen and processed for *in situ* hybridization as follows.

In situ hybridization histochemistry. Serially cut 15- μ m-thick sections, or 4–5- μ m-thick sections for the colocalization analysis, were thaw-mounted onto slides coated with poly-L-lysine and stored at -20°C until use.

With the exception of sections from already fixed fluorogold-injected brains, the sections were immersed in a 4% paraformaldehyde solution for 30 min and rinsed. All sections were dehydrated and dipped for 5 min in chloroform. After air drying, the sections were incubated overnight at 37°C or 42°C with 0.8×10^6 cpm per section of ^{35}S -labeled probes ($4\text{--}8 \times 10^8$ cpm/ μg) diluted in hybridization buffer, which consisted of 50% formamide (Fluka), $4 \times \text{SSC}$ ($1 \times \text{SSC}$: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.4), $1 \times$ Denhardt's solution (0.02% each of polyvinylpyrrolidone, bovine serum albumin, Ficoll), 1% sarcosyl, 0.02 M sodium phosphate at pH 7.4, 10% dextran sulfate, yeast tRNA (Sigma) at 500 $\mu\text{g}/\text{ml}$, salmon sperm DNA (Sigma) at 100 $\mu\text{g}/\text{ml}$, and 60 mM dithiothreitol (Sigma). After hybridization, the sections were rinsed for 4×15 min in $1 \times \text{SSC}$ at 45°C or 55°C , covered with Hyperfilm β -max film (Amersham) for 5–12 d, and thereafter dipped in Kodak NTB3 emulsion for 4–7 weeks.

The specificity of *in situ* hybridization was assessed by the comparison

of localizations of the different mRNAs in adjacent sections using these oligonucleotide probes of the same size and similar guanosine-cytosine content. This demonstrated different distributions throughout the brain that agreed with the previous reports. In addition, an extinction of the signal was observed after RNase (Sigma) pretreatment or after incubation with labeled probe in the presence of excess cold probe.

Oligonucleotide probes. Forty-four-mer oligonucleotides were synthesized on an Applied Biosystems 381A DNA synthesizer. The adenosine A_{2a} receptor (A_{2a}r) probe was complementary to nucleotides 916–959, the enkephalin probe to nucleotides 105–148; the substance P probe to nucleotides 124–167; the D₂ probe to nucleotides 539–582; and the D₁ probe to nucleotides 688–731; of the dog adenosine A_{2a} receptor (RDC8 in Libert et al., 1989), rat preproenkephalin A (Howells et al., 1984), substance P (Krause et al., 1987), D₂ dopamine receptor (Bunzow et al., 1988), and D₁ dopamine receptor (Sunahara et al., 1990) mRNAs, respectively.

Oligonucleotides were labeled with α - ^{35}S dATP (New England Nuclear) at the 3' end by terminal deoxynucleotidyltransferase (GIBCO–Bethesda Research Labs).

Quantitative autoradiography. Average optical densities (ODs) over the striatum were measured on x-ray film autoradiographs using the IBAS image analyzer (Zeiss Kontron, Munich, Germany). An MTI video camera with fixed black level and gain was used to generate the

Figure 3. Histograms representing, for enkephalin (*A*, *C*, *E*) and substance P (*B*, *D*, *F*), the ratio ($\pm$ SEM) of labeling as measured in arbitrary OD units in the lesioned versus the intact striatum in the 6-OHDA-lesioned animals (*A*, *B*), the OD values ($\pm$ SEM) in the lesioned and the intact striatum in the 6-OHDA-lesioned animals (*C*, *D*), and the OD values ($\pm$ SEM) in the striatum of intact animals (*E*, *F*). Statistical analysis using two-tailed Student's *t* test demonstrates significant changes. *A*: *, $p < 0.001$. *B*: *, $p < 0.002$. *C*: #, $p < 0.001$ 6-OHDA + saline versus intact + saline; ##, $p < 0.005$ 6-OHDA + caffeine versus intact + caffeine; *, $p < 0.05$ intact + caffeine versus intact + saline; **, $p < 0.02$ 6-OHDA + caffeine versus 6-OHDA + saline. *D*: #, $p < 0.001$ 6-OHDA + saline versus intact + saline; ##, $p < 0.005$ 6-OHDA + caffeine versus intact + caffeine; *, $p < 0.02$ intact + caffeine versus intact + saline. *E*: *, $p < 0.001$ versus saline; #, $p < 0.005$ caffeine + NECA versus caffeine and caffeine + CHA. *F*: *, $p < 0.01$ versus saline; **, $p < 0.001$ versus saline; ***, $p < 0.01$ versus caffeine; #, $p < 0.03$ versus caffeine; ##, $p < 0.001$ versus caffeine + CHA.

digitized images of 512×512 pixels with 256 gray levels. The borders of the measuring fields were interactively defined, and the average ODs were calculated. On each section, the average OD over a white matter area taken as a background level was subtracted from that of the left and right dorsal striatum to obtain a corrected OD value. In unilaterally dopamine-depleted rats, the ratio between corrected ODs in the lesioned and intact striatum (L:I ratio) was calculated in each section. For the

intact rats receiving chronic treatments, the mean of the corrected ODs in the right and left striatum was used as the final OD value in each section. These results expressed in arbitrary OD units (see Fig. 3*C-F*) or in L:I ratio (see Fig. 3*A,B*) were plotted in the Figure 3. They were statistically analyzed using the two-tailed Student's *t* test. In Figures 4 and 5, representative autoradiograms were taken to illustrate these quantitative data.

Figure 4. Autoradiograms generated by hybridization in the striatum with an enkephalin probe (*A, B*) (5 d exposure) or a substance P probe (*C, D*) (12 d exposure) in right side 6-OHDA-lesioned animals subsequently treated with saline (*A, C*) or caffeine (*B, D*). The increase in enkephalin mRNA induced by a 6-OHDA lesion (*A*) was partly reversed by caffeine (*B*), which did not affect the decrease in substance P mRNA (*C, D*). Caffeine increased enkephalin mRNA (*B*) and decreased substance P mRNA (*D*) in the intact contralateral striatum.

Results

Phenotypic characterization of striatal neurons expressing the A_{2a} receptor

The expression of A_{2a} receptor, substance P, enkephalin, D₁, and D₂ receptors was assessed in the striatopallidal and striatonigral neurons using *in situ* hybridization combined with retrograde fluorogold labeling of striatonigral neurons. A large proportion of fluorogold-labeled striatonigral neurons express substance P (147 of 181) and the D₁ receptor (149 of 183), while a small proportion of these neurons express enkephalin (13 of 207), the D₂ receptor (11 of 171), and the A_{2a} receptor (3 of 163) (Fig. 1*A–E*). The majority of neurons that express enkephalin (157 of 170), the D₂ (166 of 177), and the A_{2a} (149 of 152) receptors are fluorogold negative (Fig. 1*A–C*) and are assumed to be striatopallidal neurons (Gerfen et al., 1990). To address more directly the putative coexpression of A_{2a} with D₁ or D₂ receptors in individual striatal neurons, hybridization was performed on thin (5-μm-thick) adjacent sections alternatively with the A_{2a} probe and the D₁ or the D₂ probes. Although significant results were previously obtained using the same approach (Burgunder and Young, 1989; Weiner et al., 1990; Schiffmann et al., 1991a), the inability to recognize all neurons in these thin adjacent sections results in some limitations since only portions of the total neuronal population were taken into account. The neurons expressing the A_{2a} receptor often contain D₂ receptor transcripts (120 of 126) (Fig. 2*A, B*), while they seldom express D₁ receptor (3 of 87) (Fig. 2*C, D*). On the other hand, 120 out of 125 D₂ receptor-expressing neurons and 3 out of 111 D₁ receptor-expressing neurons also contain A_{2a} receptor transcripts. The large cholinergic neurons that never express A_{2a} receptor (Schiffmann et al., 1991a) were excluded.

Adenosine A₂ receptor-regulated gene expression of striatonigral and striatopallidal neurons

Since the A_{2a} and D₂ receptors have opposite effects on adenylyl cyclase (Van Calker et al., 1979; Stoof and Kebabian, 1981) and dopamine/adenosine interactions have been described (Ferre et al., 1991a,b), we examined the regulatory effect of A₂ receptor on striatal gene expression.

Rats unilaterally depleted in dopamine by an unilateral 6-OHDA-induced lesion of the right nigrostriatal pathway subsequently received chronic saline or caffeine treatments. In the saline-treated animals, the mean ratio between OD in the 6-OHDA-lesioned striatum and the intact striatum (L:I ratio) was 2.33 for enkephalin mRNA (Figs. 3*A, 4A*) and 0.54 for substance P mRNA (Figs. 3*B, 4C*). In the animals subsequently treated with caffeine, this L:I ratio of enkephalin mRNA was dramatically reduced to 1.45 (Fig. 3*A*) and the L:I ratio of substance P mRNA was slightly increased to 0.78 (Fig. 3*B*). However, when the absolute values of OD were considered, it appeared that the dramatic reduction of the L:I ratio for enkephalin mRNA was due not only to a significant 19% decrease in the lesioned side but also to a significant 32% increase in the intact side (Figs. 3*C, 4B*). In contrast, the caffeine-induced increase of the L:I ratio for substance P mRNA was only due to a significant 24% decrease in the intact side without any significant changes in the lesioned side (Figs. 3*D, 4D*).

Modifications of striatal enkephalin and substance P expression were similarly analyzed in four groups of intact rats treated with either saline, caffeine alone, caffeine with NECA (an equipotent A₁ and A₂ agonist), or caffeine with CHA (a more selective A₁ agonist) (Bruns et al., 1986). Caffeine, as compared to saline, significantly increased OD values for enkephalin mRNA by 67% (Figs. 3*E, 5C*) and decreased OD values for substance P mRNA

Figure 5. Autoradiograms generated by hybridization in the striatum with an enkephalin probe (*A, C, E, G*) (5 d exposure) or a substance P probe (*B, D, F, H*) (12 d exposure) in intact animals chronically treated with saline (*A, B*) or with caffeine alone (*C, D*) or in combination with NECA (*E, F*) or CHA (*G, H*). The caffeine-induced increase in enkephalin mRNA (*C*) and decrease in substance P mRNA (*D*) were reversed by NECA (*E, F*) and not by CHA (*G, H*).

by 26% (Figs. 3*F, 5D*). Moreover, these caffeine-induced modifications were significantly reversed by a concomitant treatment with NECA but not by a concomitant treatment with CHA (Figs. 3*E, F, 5E-H*).

Discussion

The main findings of this study are the nearly exclusive coexpression of A_{2a} receptors and D_2 receptors by the striatopallidal neurons and the two opposite ways by which adenosine

and its antagonist, caffeine, regulate through A_2 receptors, the neuropeptide gene expression in the striatum.

As has been previously demonstrated (Gerfen and Young, 1988; Burgunder and Young, 1989; Weiner et al., 1990; Schiffmann et al., 1991a), significant results were obtained in the first part of this study both for colocalization using different radiolabeled probes on thin adjacent sections and for the characterization of the projections of labeled neurons by combination of retrograde labeling with *in situ* hybridization. These results un-

doubtedly demonstrate a main coexpression of A_{2a} and D₂ receptors in striatopallidal neurons but not of A_{2a} and D₁ receptors in striatonigral neurons as already suggested by the main coexpression of enkephalin and A_{2a} receptor mRNAs (Schiffmann et al., 1991a).

The experiments of gene expression regulation demonstrate two sites of action of A₂ receptor in the striatum. First, as expected from the A_{2a}-D₂ receptor coexpression in the enkephalinergic striatopallidal neurons and from their stimulatory and inhibitory actions on the adenylyl cyclase, respectively, they have opposite effects in striatopallidal neurons: A₂ blockade, like D₂ stimulation (Gerfen et al., 1990), decreases specifically enkephalin gene expression. Behavioral studies in rodents have suggested that stimulation of striatal A₂ receptor causes an inhibition of responses elicited by D₂ activation (Ferre et al., 1991a). The mechanism described above could be interpreted as the molecular and cellular events leading to these observations.

However, this A₂ receptor effect was revealed only when the nigrostriatal dopaminergic pathway was disrupted. This would suggest, therefore, the involvement of adenosine receptors in the regulation of striatal gene expression through a second mechanism that depends on the integrity of the dopaminergic fibers. Caffeine induced an increase in enkephalin mRNA and a decrease in substance P mRNA in the intact rats striatum and in the intact striatum of 6-OHDA-lesioned rats through blockade of an A₂ receptor. This could be inferred from the effects of NECA, an equipotent A₁ and A₂ agonist, and CHA, a selective A₁ agonist on the caffeine-induced modifications. Indeed, NECA completely reversed these caffeine-induced modifications while CHA did not exhibit any effect. Considering that the equipotent A₁ and A₂ agonist NECA possesses a 10-fold lower potency at the A₁ receptor than the A₁ agonist CHA (Bruns et al., 1986), it is doubtful that an A₁ receptor was involved in the effect of NECA. Therefore, in this condition, the equipotent A₁ and A₂ agonist NECA most probably acted on an A₂ receptor. Like the chronic caffeine treatment, a 6-OHDA lesion of the nigrostriatal pathway, resulting in a striatal dopamine depletion, also induced an increase of enkephalin mRNA associated with a decrease of substance P mRNA (see Results; see also Young et al., 1986; Normand et al., 1988; Gerfen et al., 1990). A chronic caffeine treatment could result in a relative dopamine depletion by the blockade of presynaptic A₂ receptors located on nigrostriatal terminals. Indeed, it has been reported that these receptors stimulate synaptic tyrosine hydroxylase activity (Onali et al., 1988) and striatal dopamine release (Zetterström and Fillenz, 1990). While it remains to be confirmed, these presynaptic receptors could be of the A_{2b} subtype (Bruns et al., 1986). This is suggested by the failure to detect A_{2a} receptor mRNA in the substantia nigra (Schiffmann et al., 1990) and by the fact that CGS21680, a selective A_{2a} receptor agonist (Lupica et al., 1990), failed to stimulate striatal dopamine release (Lupica et al., 1990), converse to other A₂ agonists (Onali et al., 1988; Zetterstrom and Fillenz, 1990).

Although more doubtful, other mechanisms leading to the effects of caffeine in the intact striatum could also be proposed. Indeed, the gene expression of substance P and enkephalin in striatal medium-sized neurons is also regulated by glutamatergic and cholinergic fibers (Uhl et al., 1988; Somers and Beckstead, 1990; Pollack and Wooten, 1992). Like the dopaminergic terminals, these fibers exhibit presynaptic adenosine receptors. The releases of glutamate and ACh are modulated by A₁ receptors (Fredholm and Dunwiddie, 1988) and both A₁ and A₂ receptors

(Fredholm and Dunwiddie, 1988; Brown et al., 1990), respectively. However, converse to dopamine, glutamate and ACh regulate substance P and enkephalin gene expression in the same direction (Somers and Beckstead, 1990; Pollack and Wooten, 1992). Blockade of the enzyme glutamine synthetase or cortical cerebral aspiration lesions resulted in a decrease of both substance P and enkephalin mRNAs (Somers and Beckstead, 1990). Scopolamine, a muscarinic antagonist, reduced the 6-OHDA-induced increase in enkephalin mRNA and decreased the substance P mRNA level in the intact striatum (Pollack and Wooten, 1992). It would therefore be expected that, by acting on adenosine receptors located on either glutamatergic or cholinergic terminals, caffeine modifies substance P and enkephalin mRNA levels in the same direction. Conversely, in the present study, caffeine increased enkephalin mRNA and decreased substance P mRNA. Finally, other complex but not fully understood mechanisms could be also involved in the caffeine-induced changes in gene expression in the intact striatum, such as the existence of unidentified adenosine receptor subtypes.

Adenosine through the A₂ receptor is involved in at least two different regulatory mechanisms in the basal ganglia physiology. First, it stimulates the dopamine release, reinforcing the opposite effect of dopamine on striatonigral and striatopallidal neurons mediated by D₁ and D₂ receptors, respectively (Gerfen et al., 1990). On the other hand, its stimulatory effect directed specifically on striatopallidal neurons counteracts the inhibitory action of dopamine through D₂ receptor. This effect is particularly revealed in the case of striatal dopamine depletion as encountered in Parkinson's disease characterized by reduced voluntary movements (Hornykiewicz, 1963). To counteract the hyperactivity of the striatopallidal pathway resulting from the dopamine depletion and therefore from the loss of the inhibitory action of dopamine through D₂ receptor, our data suggest that, in addition to dopamine agonist treatments, selective A_{2a} antagonists should be useful. They should widen the therapeutical arsenal against Parkinson's disease and other syndromes characterized by parkinsonism.

On the other hand, whatever might be the mechanisms involved, this study reveals that caffeine's capacity to modify gene expression in a major structure of the basal ganglia circuitry could account, in animal models, for the effects of caffeine on motor activity. They could be partly related to the well-recognized behavioral effects of caffeine consumption in humans.

Note added in proofs: In the course of submission of the present article, the rat adenosine A_{2a} (Fink et al., 1992) and A_{2b} (Stehle et al., 1992) receptors have been cloned. The coexpression of the A_{2a} and D₂ receptors has been demonstrated in striatal neurons (Fink et al., 1992). Although Northern blot analysis demonstrated an A_{2b} receptor expression in the brain, no signal could be detected using *in situ* hybridization (except in the hypophyseal pars tuberalis) (Stehle et al., 1992). Therefore, further studies will be necessary to clarify the A_{2b} receptor distribution.

References

- Albin RL, Young AB, Penney B (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* 12:366-375.
- Brown SJ, James S, Reddington M, Richardson PJ (1990) Both A₁ and A_{2a} purine receptors regulate striatal acetylcholine release. *J Neurochem* 55:31-38.
- Bruns RF, Lu GH, Pugsley TA (1986) Characterization of the A₂ adenosine receptor labeled by [³H]NECA in rat striatal neurons. *Mol Pharmacol* 29:331-346.
- Bunzow JR, Van Tol HHM, Grandy DK, Albert P, Salon J, McDonald

- C, Machida CA, Neve KA, Civelli O (1988) Cloning and expression of a rat D₂ dopamine receptor cDNA. *Nature* 336:783-787.
- Burgunder JM, Young WS III (1989) Distribution, projection and dopaminergic regulation of the neurokinin B mRNA containing neurons of the rat caudate-putamen. *Neuroscience* 32:323-335.
- Chesselet M-F, Robbins E (1989) Characterization of striatal neurons expressing high levels of glutamic acid decarboxylase messenger RNA. *Brain Res* 492:237-244.
- Dunwiddie TV (1985) The physiological role of adenosine in the central nervous system. *Int Rev Neurobiol* 27:63-139.
- Durcan MJ, Morgan PF (1989) Evidence for adenosine A₂ receptor involvement in the hypomotility effects of adenosine analogues in mice. *Eur J Pharmacol* 168:285-290.
- Ferre S, Herrera-Marschitz M, Grabowska-Anden M, Ungerstedt U, Casas M, Anden N-E (1991a) Postsynaptic dopamine/adenosine interaction. I. Adenosine analogues inhibit dopamine D₂-mediated behaviour in short-term reserpinized mice. *Eur J Pharmacol* 192:25-30.
- Ferre S, Herrera-Marschitz M, Grabowska-Anden M, Casas M, Ungerstedt U, Anden N-E (1991b) Postsynaptic dopamine/adenosine interaction. II. Post-synaptic dopamine agonism and adenosine antagonism of methylxanthines in short-term reserpinized mice. *Eur J Pharmacol* 192:31-37.
- Ferre S, Von Euler G, Johansson B, Fredholm BB, Fuxe K (1991c) Stimulation of adenosine A₂ receptors decreases the affinity of dopamine D₂ receptors in rat striatal membranes. *Proc Natl Acad Sci USA* 88:7238-7241.
- Fredholm BB, Dunwiddie TV (1988) How does adenosine inhibit transmitter release? *Trends Pharmacol Sci* 9:130-134.
- Gerfen CR, Young WS III (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an *in situ* hybridization histochemistry and fluorescent retrograde tracing study. *Brain Res* 460:161-167.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250:1429-1432.
- Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci* 13:244-254.
- Hornykiewicz O (1963) Die topische Lokalisation und das Verhalten von Noradrenalin und Dopamin (3-Hydroxytyramin) in der Substantia Nigra des normalen und Parkinsonkranken Menschen. *Klin Wochenschr* 75:309-312.
- Howells RD, Kilpatrick DL, Bhatt R, Monahan JJ, Poonian M, Undenfriend S (1984) Molecular cloning and sequence analysis determination of rat preproenkephalin cDNA: sensitive probe for studying transcriptional changes in rat tissue. *Proc Natl Acad Sci USA* 81:7651-7655.
- Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD (1987) Three rat preprotachykinin mRNAs encode for the neuropeptides substance P and neurokinin A. *Proc Natl Acad Sci USA* 84:881-885.
- Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B (1990) Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proc Natl Acad Sci USA* 87:230-234.
- Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D₁ dopamine receptor gene. *Proc Natl Acad Sci USA* 88:4205-4209.
- Libert F, Parmentier M, Lefort A, Dinsart C, Van Sande J, Maenhaut C, Simons M-J, Dumont JE, Vassart G (1989) Selective amplification and cloning of four new members of the G protein-coupled receptor family. *Science* 244:569-572.
- Libert F, Schiffmann SN, Lefort A, Parmentier M, Gerard C, Dumont JE, Vanderhaeghen J-J, Vassart G (1991) The orphan receptor cDNA RDC7 encodes an A1 adenosine receptor. *EMBO J* 10:1677-1682.
- Lupica CR, Cass WA, Zahniser NR, Dunwiddie TV (1990) Effects of the selective adenosine A2 receptor agonist CGS 21680 on *in vitro* electrophysiology, cAMP formation and dopamine release in rat hippocampus and striatum. *J Pharmacol Exp Ther* 252:1134-1142.
- Maenhaut C, Van Sande J, Libert F, Abramovicz M, Parmentier M, Vanderhaeghen J-J, Dumont JE, Vassart G, Schiffmann SN (1990) RDC8 codes for an A2 receptor with physiological constitutive activity. *Biochem Biophys Res Commun* 173:1169-1178.
- Normand E, Popovici T, Onteniente B, Fellmann D, Piatier-Tonneau D, Auffray C, Bloch B (1988) Dopaminergic neurons of the substantia nigra modulate preproenkephalin A gene expression in rat striatal neurons. *Brain Res* 439:39-46.
- Onali P, Olanas MC, Bunse B (1988) Evidence that adenosine A₂ and dopamine autoreceptors antagonistically regulate tyrosine hydroxylase activity in rat striatal synaptosomes. *Brain Res* 456:302-309.
- Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2d ed. New York: Academic.
- Pollack AE, Wooten GF (1992) D2 dopaminergic regulation of striatal preproenkephalin mRNA levels is mediated at least in part through cholinergic interneurons. *Mol Brain Res* 13:35-41.
- Schiffmann SN, Vanderhaeghen J-J (1992) Ontogeny of gene expression of adenosine A2 receptor in the striatum: early localization in the patch compartment. *J Comp Neurol* 317:117-128.
- Schiffmann SN, Libert F, Vassart G, Dumont JE, Vanderhaeghen J-J (1990) A cloned G protein-coupled protein with a distribution restricted to striatal medium-sized neurons. Possible relationship with D1 dopamine receptor. *Brain Res* 519:333-337.
- Schiffmann SN, Jacobs OP, Vanderhaeghen J-J (1991a) Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an *in situ* hybridization histochemistry study. *J Neurochem* 57:1062-1067.
- Schiffmann SN, Libert F, Vassart G, Vanderhaeghen J-J (1991b) Distribution of adenosine A2 receptor mRNA in the human brain. *Neurosci Lett* 130:177-181.
- Snyder SH (1985) Adenosine as a neuromodulator. *Annu Rev Neurosci* 8:103-124.
- Somers DL, Beckstead RM (1990) Striatal preprotachykinin and preproenkephalin mRNA levels and the levels of nigral substance P and pallidal met-enkephalin depend on corticostriatal axons that use the excitatory amino acid neurotransmitters aspartate and glutamate: quantitative radioimmunocytochemical and *in situ* hybridization evidence. *Mol Brain Res* 8:143-158.
- Stoof JC, Keabian JW (1981) Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature* 294:366-368.
- Sunahara RK, Niznik HB, Weiner DM, Stormann TM, Brann MR, Kennedy JL, Gelernter JE, Rozmahel R, Yang Y, Israel Y, Seeman P, O'Dowd BF (1990) Human dopamine D₁ receptor encoded by an intronless gene on chromosome 5. *Nature* 347:80-83.
- Uhl GR, Navia B, Douglas J (1988) Differential expression of preproenkephalin and preprodynorphin mRNAs in striatal neurons: high levels of preproenkephalin expression depend on cerebral cortical afferents. *J Neurosci* 8:4755-4764.
- Van Calcar D, Muller M, Hamprecht B (1979) Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J Neurochem* 33:99-105.
- Weiner DM, Levey AI, Brann MB (1990) Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc Natl Acad Sci USA* 87:7050-7054.
- Young WS III, Bonner TI, Brann MB (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptides mRNAs in the rat forebrain. *Proc Natl Acad Sci USA* 83:9827-9831.
- Zetterström T, Fillenz M (1990) Adenosine agonists can both inhibit and enhance *in vivo* striatal dopamine release. *Eur J Pharmacol* 180:137-143.