CMLS, Cell. Mol. Life Sci. 61 (2004) 857–872
1420-682X/04/080857-16
DOI 10.1007/s00018-003-3269-3

CMLS Cellular and Molecular Life Sciences

Review

Caffeine as a psychomotor stimulant: mechanism of action

G. Fisone*, A. Borgkvist and A. Usiello

Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 171 77 Stockholm (Sweden), Fax: +46 8 320988, e-mail: gilberto.fisone@neuro.ki.se

Received 8 July 2003; received after revision 7 September 2003; accepted 6 October 2003

Abstract. The popularity of caffeine as a psychoactive drug is due to its stimulant properties, which depend on its ability to reduce adenosine transmission in the brain. Adenosine A_1 and A_{2A} receptors are expressed in the basal ganglia, a group of structures involved in various aspects of motor control. Caffeine acts as an antagonist to both types of receptors. Increasing evidence indicates that the psychomotor stimulant effect of caffeine is generated by affecting a particular group of projection neurons located in the striatum, the main receiving area of the basal ganglia. These cells express high levels of adenosine A_{2A} receptors, which are involved in various intracellular processes, including the expression of immediate early genes and regulation of the dopamine- and cyclic AMP-regulated 32-kDa phosphoprotein DARPP-32. The present review focuses on the effects of caffeine on striatal signal transduction and on their involvement in caffeine-mediated motor stimulation.

Key words. Basal ganglia; adenosine; adenosine A_{2A} receptors; immediate early gene; dopamine; dopamine- and cAMP-regulated phosphoprotein of 32 kDa; motor activity; Parkinson's disease.

Introduction

The methylxanthine caffeine is the world's most popular psychoactive drug. The reason for this popularity, which crosses age and cultural boundaries, lies in the psychostimulant properties of caffeine, combined with the absence of substantial or clearly documented negative side effects. Caffeine is contained in coffee, tea, soft drinks and chocolate. In addition, common over-the-counter drugs such as aspirin and appetite suppressants are often combined with caffeine. Upon ingestion, caffeine is efficiently absorbed from the gastrointestinal tract and, because of its hydrophobic properties, rapidly distributed in the organism. Overall, the psychostimulant properties of caffeine are due to its ability to interact with neurotransmission in different regions of the brain, thereby promoting behavioral functions, such as vigilance, attention, mood and arousal. These various responses are often interdependent, and therefore difficult to assess individually, and sometimes even poorly defined. Among the behavioral effects produced by caffeine, the ability to enhance motor activity has received a great deal of attention. Motor activity can be easily measured and is controlled by relatively well characterized cerebral circuits. For these reasons, changes in locomotion often represent the behavioral output of choice utilized in the quantification of the stimulant properties of caffeine, as well as in the study of its mechanism of action. Caffeine-mediated changes in motor activity are attributable to the ability of this drug to affect neurotransmission within the basal ganglia, a group of subcortical nuclei involved in various aspects of motor control. The present review discusses the molecular mechanisms underlying the psychomotor stimulant properties of caffeine, with special reference to the action of this drug in the basal ganglia. The therapeutic significance of caffeine-based therapies in Parkinson's disease, a frequent neurodegenerative illness affecting basal ganglia neurotransmission and motor function, is also discussed.

* Corresponding author.

Molecular targets for the physiological action of caffeine in the brain

Methylxanthines are structurally similar to cyclic nucleotides and have been extensively studied for their ability to interact with cyclic nucleotide phosphodiesterases [1]. Caffeine and theophylline act as competitive inhibitors of cyclic nucleotide phosphodiesterase isozymes in various tissues, including the brain [2]. Their affinity for phosphodiesterases, however, is low, and concentrations in the millimolar range are necessary to attain significant effects [3]. Similarly, millimolar concentrations of caffeine are necessary to mobilize calcium from intracellular stores, an effect mediated via activation of ryanodine-sensitive channels [4, 5]. Studies performed in brain membranes have shown that caffeine inhibits benzodiazepine binding to the γ-aminobutyric acid $(GABA)_A$ receptor [6] with an IC_{50} – 50% inhibition concentration – of 350–500 μM [7, 8]. Although caffeine has been instrumentally important in the study of cyclic nucleotide phosphodiesterases, ryanodine receptors and GABA receptors [8, 9], its physiological effects cannot be accounted for by its ability to regulate these intracellular targets. In fact, a blood concentration of 500 μM caffeine produces lethal intoxication [10], and even after ingestion of three cups of coffee (corresponding to about 300 mg of caffeine), the peak concentration of free caffeine circulating in the plasma does not exceed 30 μM [11].

Caffeine and adenosine transmission

It is now well established that under normal physiological conditions, the effects exerted in the brain by caffeine depend on its ability to act as an antagonist at adenosine receptors [12]. Adenosine is a purine that functions as a general inhibitor of neuronal activity. In spite of its considerable and specific effects produced at the level of the central nervous system [13], adenosine does not fit the criteria normally used to define a neurotransmitter. For instance, adenosine is not accumulated into vesicles, and it is not released from nerve terminals in a calcium-dependent fashion.

Regulation of adenosine synthesis and release

Adenosine is generated extracellularly as a product of the breakdown of adenine nucleotides, such as ATP. A variety of ecto-nucleotidases dephosphorylate ATP to AMP, which is then converted to adenosine [14]. Synthesis of adenosine occurs also intracellularly, by means of a cytoplasmic 5′-nucleotidase [15] or by hydrolysis of *S*-adenosyl-homocysteine [16]. Intracellular adenosine is converted to AMP by adenosine kinase, or to inosine by adenosine deaminase. The K_m of the first reaction (0.2–2 μM) approaches the range of the physiological concentration of adenosine, which in rat brain is between 25 and 250 nM [17, 18]. Thus, adenosine kinase plays a prevalent role in regulating the basal levels of intracellular adenosine [19]. In contrast, the reaction in which adenosine deaminase converts adenosine into inosine has a higher K_m and is especially important in controlling the abnormally elevated levels of adenosine produced during pathophysiological conditions or electrical stimulation (see below; [19, 20]). The extracellular concentration of adenosine is controlled by means of Na^+-dependent equilibrative transporters, which maintain similar intra- and extracellular concentrations of nucleosides [21–23]. Under normal conditions, the activity of intracellular adenosine kinase is sufficiently high to maintain low levels of adenosine, thereby determining an inward transport of adenosine, which is removed from the extracellular space [17]. However, conditions such as ischemia [24], hypoxia [25] or prolonged electrical stimulation [26] augment energy requirements and stimulate ATP hydrolysis. This, in turn, dramatically raises the intracellular levels of adenosine [27], which is then released in the extracellular space by the nucleoside equilibrative transporters.

Adenosine receptors

Adenosine is produced ubiquitously, and its neuroactive properties are determined by the presence of specific receptors in discrete regions of the brain. At present, four heptahelical, G-protein-coupled receptors for adenosine have been identified and named A_1, A_{2A}, A_{2B} and A_3 receptor [28]. Whereas all four receptors are expressed in the brain, the affinity for adenosine of the A_{2B} and A_3 receptors is low, and their basal level of activation is negligible [13, 29, 30]. This implies that under normal physiological conditions, caffeine cannot act via blockade of these receptors. In contrast, adenosine A_1 and A_{2A} receptors bind to caffeine with high affinity and are activated by nanomolar concentrations of adenosine, normally present in the brain [17, 18]. It can therefore be concluded that in resting tissues, the effects of caffeine are mediated via blockade of adenosine A_1 and A_{2A} receptors.

Adenosine A_1 and A_{2A} receptors: transduction mechanisms and distribution

Initial studies showed that adenosine activated two distinct types of receptors, which exerted opposite biochemical effects: the A_1 type of receptor reduced, whereas the A_2 type of receptor increased, the levels of cyclic AMP (cAMP) [31, 32]. Subsequent studies have shown that A_1

receptors are coupled to pertussis toxin-sensitive G_i and G_o proteins, whose stimulation leads to inhibition of adenylyl cyclase, activation of K^+ channels [33] and inhibition of Ca^{2+} channels [34]. Adenosine A_{2A} receptors are instead coupled to G_s and G_{olf} proteins [35], which activate adenylyl cyclase.

The pattern of distribution of adenosine A_1 and A_{2A} receptors in the brain differs strikingly. The A_1 receptor has a widespread distribution, as shown by radioligand binding autoradiography [36] and in situ hybridization [37, 38]. Immunohistochemical analysis demonstrates high levels of A_1 receptors in the hippocampal formation, cerebral cortex, cerebellum and in numerous hypothalamic nuclei [39]. Lower levels of A_1 receptors are found in the basal ganglia, where ~40% of the neurons are labeled in globus pallidus and striatum [39].

At the cellular level, the majority of adenosine A_1 receptors are located on presynaptic nerve terminals, where they mediate the inhibition exerted by adenosine on the release of neurotransmitters [40], including glutamate [41–43], dopamine [44] and acetylcholine [45]. These effects are most likely exerted via cell membrane hyperpolarization caused by activation of G-protein-dependent inwardly rectifying K^+ channels and/or via inhibition of Ca^{2+} channels [33, 34].

The inhibitory control on neurotransmission exerted by adenosine, via A_1 receptors, is thought to account for the positive effect produced by caffeine on arousal, vigilance and attention. Caffeine is likely to stimulate arousal by blocking the A_1 receptor-mediated inhibition of mesopontine cholinergic projection neurons involved in the regulation of cortical activity [46]. The ability of caffeine and methylxanthines to increase cortical [47, 48] and hippocampal [49, 50] activity has been proposed to mediate their facilitatory action on vigilance and information processing cf. [12]. Recently, in vivo microdialysis studies showed that administration of caffeine stimulates acetylcholine release in the rat prefrontal cortex [51], an effect that also occurs during sustained attention tasks [52, 53].

In contrast to the rather ubiquitous distribution of A_1 receptors, the expression of adenosine A_{2A} receptors in the brain is limited to regions heavily innervated by dopamine-containing fibers, such as the striatum and the olfactory tubercle [54–58]. In the striatum, A_{2A} receptors are highly expressed postsynaptically by a large population of medium-sized spiny neurons (cf. below; [58–60]). These cells play a critical role in the functioning of the basal ganglia, a group of nuclei involved in the control of voluntary movements, as well as in motivational, emotional and cognitive aspects of motor behavior. Since one of the major effects of caffeine as a psychostimulant is a prolonged increase in motor activity (see below), the basal ganglia and, particularly, the striatal medium spiny neurons represent an important model to investigate the cellular and molecular mechanism of action of this drug.

General organization of the basal ganglia and control of motor activity

The basal ganglia form a subcortical station where information coming from limbic, prefrontal, oculomotor and motor cortex is collected, integrated, transferred to ventral tier thalamic nuclei and sent back to the cortex. The corticostriatal pathway is organized in parallel, segregated circuits, so that specific cortical areas innervate subregions of the basal ganglia, which feed back on the same cortical areas, resulting in the execution of selected motor programs [61].

The striatum is the main receiving area of the basal ganglia, and ~95% of all striatal neurons consist of GABAergic medium spiny neurons. These cells receive a glutamatergic excitatory input from the cerebral cortex (see above) and a modulatory input from midbrain dopaminergic neurons [62–64] (fig. 1).

In the dorsal striatum, medium spiny neurons give rise to two major outputs responsible for fine motor control: the direct pathway, which contains GABA and substance P, and projects to the substantia nigra pars reticulata/globus pallidus pars interna (Gpi), and the indirect pathway, which contains GABA and enkephalins, and projects to the substantia nigra pars reticulata/Gpi via globus pallidus pars externa (Gpe; entopeduncular nuclei in rodents) and subthalamic nucleus. These two pathways exert opposing effects on movements by controlling the activity of thalamocortical neurons. Activation of the direct striato-nigral/Gpi pathway disinhibits thalamocortical neurons and facilitates motor activity, whereas activation of the indirect striato-Gpe pathway enhances inhibition on thalamocortical neurons and reduces motor activity [65] (fig. 1).

Increasing evidence indicates that caffeine exerts its motor stimulant effect by acting on striatal medium spiny neurons. In particular, most of the biochemical and behavioral effects of caffeine have been related to the ability of this drug to reduce the inhibition exerted by endogenous adenosine on striatal dopamine transmission. The following sections deal with the involvement of dopamine in the functioning of the basal ganglia and on the interactions between adenosine and dopamine at the level of striatal projection neurons.

Dopamine and adenosine in the basal ganglia

Dopamine, acting on dopamine D_1 and dopamine D_2 receptors, plays a critical role in the regulation of the activity of striatal medium spiny neurons. Dopamine D_1 receptors are coupled via a G_{olf} protein to stimulation of adenylyl cyclase and increased production of cAMP [35, 66, 67]. In contrast, dopamine D_2 receptors are coupled, via G_i/G_o proteins, to inhibition of adenylyl cyclase and reduction of cAMP [66].

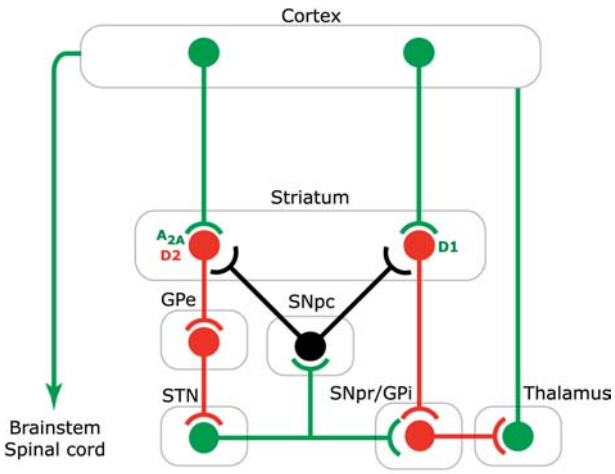

Figure 1. Diagram illustrating the functional organization of the basal ganglia. The striatum receives an excitatory glutamatergic input (green) from cerebral cortex and a modulatory dopaminergic input (black) from the substantia nigra pars compacta (SNpc). GABAergic striatal medium spiny neurons innervate either directly or indirectly [via globus pallidus pars externa (Gpe) and subthalamic nucleus (STN)] the substantia nigra pars reticulata (SNpr)/globus pallidus pars interna (Gpi). Dopamine activates, via D_1 receptors, the direct striato-nigral/Gpi pathway and inhibits, via D_2 receptors, the indirect striato-Gpe pathway. These opposite regulations disinhibit thalamo-cortical glutamatergic neurons and promote motor activity. Adenosine, via A_{2A} receptors, antagonizes the inhibitory effect of dopamine D_2 receptors on the indirect pathway, thereby depressing motor activity. Caffeine produces its psychomotor stimulant effect by blocking adenosine A_{2A} receptors. In addition, caffeine may protect SNpr/Gpi dopaminergic neurons from glutamate-induced neurotoxicity via disinhibition of GABAergic Gpe neurons and inhibition of STN neurons (cf. text). Excitatory (glutamatergic) and inhibitory (GABAergic) inputs are shown in green and red, respectively.

It is generally believed that within the dorsal striatum, activation of dopamine D_1 receptors stimulates the neurons of the direct pathway, whereas activation of dopamine D_2 receptors inhibits the neurons of the indirect pathway [65]. Because of the opposite control exerted by direct and indirect pathway on the activity of thalamocortical neurons (i.e. disinhibition and enhancement of inhibition, respectively; cf. fig. 1), the overall effect of dopamine is motor stimulation. A large proportion of the stimulant effects produced by substances such as cocaine and amphetamine are exerted by interfering with the dopamine transport system, thereby increasing the extracellular concentration of dopamine.

Several studies have shown that adenosine A_{2A} receptors are highly and selectively expressed by the neurons of the indirect, striato-Gpe pathway [58–60]. During the last years it has become clear that most of the psychomotor stimulant effects of caffeine are mediated via regulation of the activity of this particular set of striatal projection neurons.

Adenosine A_{2A} receptor/dopamine D_2 receptor antagonism on striato-Gpe neurons

A large amount of evidence indicates the existence of a complex antagonistic relationship between adenosine A_{2A} and dopamine D_2 receptors, in striatal projection neurons (cf. fig. 2). Studies performed in striatal membrane preparations show that activation of adenosine A_{2A} receptors reduces the affinity of dopamine D_2 receptors for agonists [68, 69]. This intramembrane, receptor-receptor interaction has been proposed to play a critical role in the responses elicited by activation of adenosine A_{2A} receptors [70]. However, the antagonistic relationship between A_{2A} and D_2 receptors is by no means restricted to the level of the plasma membrane. As mentioned above, activation of A_{2A} receptors results in G_{olf}-dependent stimulation of cAMP production [35, 71], whereas activation of dopamine D_2 receptors decreases the production of cAMP [66]. This leads to opposite regulation of the activity of cAMP-dependent protein kinase (PKA), which, in turn, is involved in the control of the state of phosphorylation and activity of numerous phosphoproteins, including the dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), and transcription factors, such as the cAMP-response element binding protein (CREB), which controls the expression of immediate early genes (IEGs) (cf. fig. 2).

The antagonistic interactions described above result in opposite regulation of the activity of striato-Gpe neurons of the indirect pathway, where both A_{2A} and D_2 receptors are highly expressed. This is clearly indicated by studies showing that the increase in enkephalin messenger RNA (mRNA) (a specific marker indicating activation of striato-Gpe neurons [65]) observed in dopamine D_2 receptor knockout mice is counteracted by concomitant genetic inactivation of adenosine A_{2A} receptors [72]. The ability of A_{2A} receptors to enhance the activity of striato-Gpe neurons, thereby opposing the inhibitory action exerted on these cells by dopamine D_2 receptors, is further demonstrated by studies of IEG expression (see below). In addition, neurochemical studies show that the A_{2A} receptor agonist, CGS 21680, prevents the decrease in GABA release produced, in the globus pallidus, by striatal infusion of a dopamine D_2 receptor agonist [73, 74]. In contrast, blockade of striatal A_{2A} receptors with theophylline potentiates the dopamine D_2 receptor-mediated decrease in GABA release [74].

Altogether, the above evidence suggests that caffeine stimulates motor activity by counteracting the inhibitory control exerted by adenosine A_{2A} receptors on striatal dopamine D_2 transmission. This, in turn, would reduce the activity of striato-Gpe neurons and ultimately disinhibit thalamo-cortical projection neurons (figs 1, 2).

It should be noted that the A_{2A} receptor-mediated regulation of striato-Gpe neurons does not depend completely

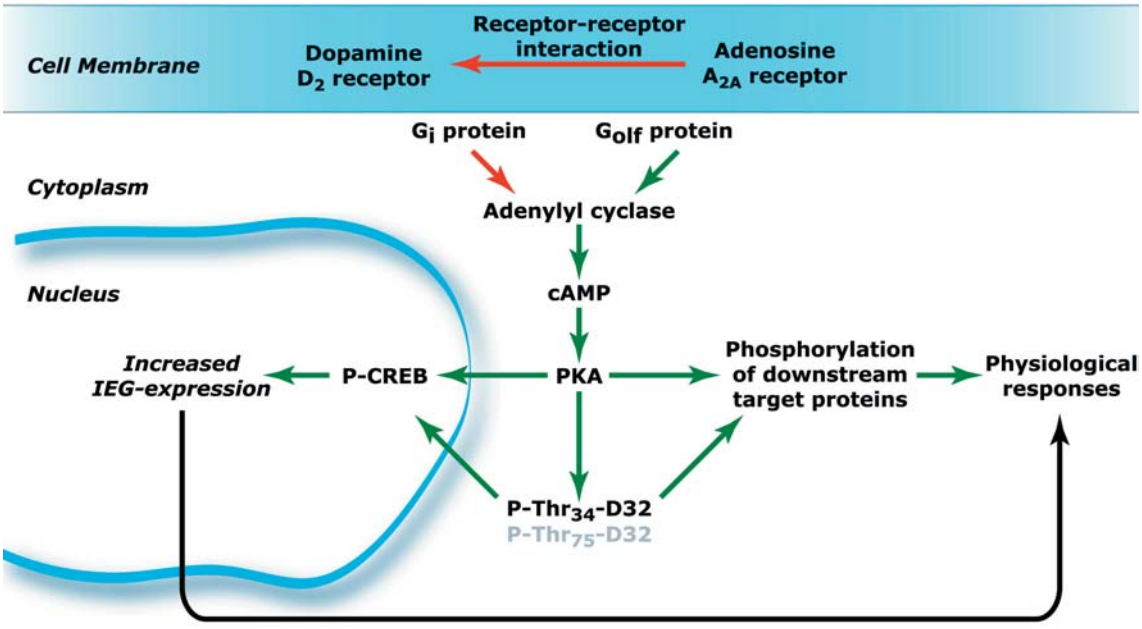

Figure 2. Schematic representation of the antagonistic interactions between adenosine A_{2A} and dopamine D_2 receptors, in striato-Gpe projection neurons. At the plasma membrane level, stimulation of A_{2A} receptors results in decreased affinity of the dopamine D_2 receptor for agonists. At the cytoplasm level, A_{2A} receptors stimulate, whereas D_2 receptors inhibit the production of cAMP. This result in opposite regulation of the state of phosphorylation of DARPP-32 and downstream target proteins involved in the control of the activity of striato-Gpe neurons. In the nucleus, the opposite regulation of the cAMP/PKA pathway results in opposite regulation of CREB phosphorylation and IEG expression. Green and red arrows indicate positive and negative regulations, respectively.

on their antagonistic relationship with D_2 receptors. Thus, the stimulant effect exerted by caffeine [72] or by selective blockade of A_{2A} receptors [75] on motor activity is still present, albeit reduced, in dopamine D_2 receptor-null mice (but see also [76]). The existence of a dopamine-independent component in the action of caffeine and adenosine A_{2A} receptor antagonists is further indicated by the observation that blockade of A_{2A} receptors stimulates motor activity in various experimental models of dopamine-deficient animals (see below and cf. section on caffeine and Parkinson's disease). It therefore appears that endogenous adenosine, via A_{2A} receptors, at least in part promotes striato-Gpe neuron transmission in a D_2 receptor-independent fashion.

Caffeine and motor activity

The ability of caffeine to enhance motor activity in experimental animals is well known [12, 77–79] and has been correlated to its affinity at adenosine receptors [80] and blockade of tonic adenosine transmission [3, 80, 81]. Recently, evidence has been provided indicating that a similar mechanism is involved even in the ability of caffeine to delay fatigue during exercise [82]. Typically, caffeine produces a biphasic stimulation of locomotor activity. In the rat, a peak effect is observed at doses between 15 and 30 mg/kg [83–85], whereas at the dose of 100 mg/kg caffeine is ineffective, or depressant on locomotion [84–86]. A similar biphasic profile, with low doses increasing and high doses decreasing locomotor activity, has been observed in the mouse [87–89].

The locomotor stimulant effect of caffeine has been initially attributed to blockade of adenosine A_1 receptors [80, 90, 91]. These receptors inhibit dopamine release [44], and caffeine has been reported to increase extracellular dopamine in the striatum [92, 93]. However, this effect, which should result in increased locomotion (see above; cf. fig. 1), is elicited by high concentrations (50 µM in the perfusion buffer) [92] or doses (30–75 mg/kg) of caffeine [93], which, as mentioned above, do not produce motor stimulation. In a recent study, Solinas et al. [94] have reported that low, but not high, doses of caffeine increase glutamate and dopamine release in the ventral striatum, and have proposed that this regulation mediate the biphasic motor stimulant response to caffeine. This idea, however, has been challenged in another recent report, which shows that caffeine, administered in a similar range of doses, does not affect dopamine release in the ventral striatum [51]. It should also be noted that whereas the ventral striatum is involved in the psychomotor effects of cocaine and amphetamine, caffeine appears to produce its stimulant action independent of this brain region [95–97].

Studies based on the use of selective pharmacological agents and gene targeting have clearly indicated that blockade of A_{2A}, rather than A_1, receptors is involved in the stimulant properties of caffeine. Svenningsson et al. [83] showed that in the rat, administration of SCH 58261, an adenosine receptor antagonist with 100-fold selectivity for A_{2A} over A_1 receptors [98], produced an increase in locomotion comparable to that caused by caffeine. In contrast, administration of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; a specific A_1 receptor antagonist [99]) did not produce significant changes in locomotor activity [83]. Similar results have been obtained in the mouse [89, 100–102]. Demonstration of the specific involvement of A_{2A} receptors in caffeine-mediated motor stimulation came from studies performed in adenosine A_{2A} receptor knockout mice. These animals showed a decrease in locomotion following administration of a dose of caffeine (25 mg/kg) that produced motor stimulation in wild-type mice [103]. The motor depressant effect exerted by caffeine in A_{2A} receptor-null mice has been proposed to occur via blockade of adenosine A_1 receptors [83, 86], an idea supported by the observation that DPCPX reduces locomotor activity in A_{2A} receptor knockout mice [89].

The psychomotor stimulant effect of caffeine appears to be, at least in part, dependent on intact dopaminergic transmission. Administration of reserpine, which depletes endogenous monoamines, or α-methyl-*p*-tyrosine, which blocks the synthesis of catecholamines, prevents the caffeine-induced increase in locomotor activity [79, 104, 105]. Similar results are obtained using dopamine D_1 and D_2 receptor antagonists [106]. The ability of a dopamine D_1 receptor antagonist to counteract the motor stimulant effect of caffeine may seem at first surprising, considering the selective localization of A_{2A} receptors on striato-Gpe neurons, which are mostly devoid of D_1 receptors. However, it should be considered that blockade of dopamine D_1 receptors is sufficient to prevent the increase in locomotion produced by activation of dopamine D_2 receptors [107]. Thus, a dopamine D_1 receptor antagonist should be able to suppress the motor stimulant effect of caffeine, which is for the most part (cf. below) exerted via disinhibition of dopamine D_2 receptors transmission.

The requirement of intact dopaminergic transmission for the psychomotor stimulant action of caffeine is questioned by studies demonstrating the ability of caffeine or a specific adenosine A_{2A} receptor antagonist to prevent akinesia in reserpinized rodents [108]. Moreover, blockade of adenosine A_{2A} receptors stimulates motor activity in dopamine-deficient, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-intoxicated mice [109] and monkeys [110] (cf. section on caffeine and Parkinson's disease).

In summary, the psychomotor stimulant effect of low doses of caffeine, which closely match the amount of drug normally ingested in beverages and food, is produced by antagonism at adenosine A_{2A} receptors. Higher doses of caffeine are ineffective or induce locomotor depression, most likely acting via blockade of adenosine A_1 receptors. The ability of caffeine to stimulate motor activity via A_{2A} receptor blockade appears to involve dopamine-dependent, as well as dopamine-independent mechanisms.

Caffeine effects on striato-Gpe neurons: evidence from immediate early gene expression studies

Changes in the expression of IEG, such as c-*fos*, Δ*fos*B, c-*jun*, *jun*B, *jun*D, *arc*, *zif-268* [or nerve growth factor-inducible (NGFI)-A] and NGFI-B, are generally considered as markers of changes in neuronal activity and synaptic transmission. The rapid increase in IEG expression results in activation of late-response genes involved in plastic and pathological processes, and is generally thought to occur in concomitance with increased neuronal activity [111]. Therefore, changes in IEG expression have been extensively utilized as indicators of the ability of drugs to affect specific neuronal circuits [112]. For example, both neuroleptic drugs (e.g. haloperidol), via blockade of dopamine D_2 receptors [113–115], and psychostimulants (e.g. amphetamine and cocaine), via activation of dopamine D_1 receptors [116, 117], are known to induce c-*fos* expression in the striatum.

It is now well established that the biphasic effect produced by caffeine on motor activity is paralleled by biphasic changes in IEG expression at the level of striatal projection neurons. Administration of 25 mg/kg of caffeine, a dose that induces stimulation of motor activity [84–86], reduces the mRNA levels for *zif-268*, NGFI-B and *jun*B [86]. In contrast, administration of 100 mg/kg of caffeine, a dose that does not affect locomotion, increases the expression of c-*fos*, *zif-268*, NGFI-B, *jun*B, c-*jun* and *arc* [86, 118–120].

Low doses of caffeine decrease IEG expression via blockade of adenosine A_{2A} receptors. Thus, administration of SCH58261 produces a decrease in *zif-268* and NGFI-B similar to that caused by doses of caffeine ranging from 7.5 to 30 mg/kg [83]. Furthermore, the reduction of *zif*-268 produced by low doses of caffeine occurs in striato-Gpe neurons [86], which selectively express A_{2A} receptors [58–60]. In contrast, the stimulation of IEG expression produced by higher, physiologically less relevant, doses of caffeine occurs in both striato-nigral/Gpi and striato-Gpe neurons [86, 118, 119]. This effect, which is mimicked by administration of DPCPX [83, 119], has been attributed to blockade of inhibitory presynaptic adenosine A_1 receptors and increase in the release of dopamine, glutamate and acetylcholine [119], which would affect IEG expression in both subpopulations of striatal projection neurons.

The ability of low doses of caffeine to decrease IEG expression via antagonism at adenosine A_{2A} receptors is most likely mediated via inhibition of the cAMP/protein kinase A (PKA) pathway. Adenosine A_{2A} receptors are positively coupled to adenylyl cyclase (see above), and blockade of their tonic activation by caffeine would reduce cAMP levels. This, in turn, would decrease PKA activity and inhibit the state of phosphorylation and activity of transcription factors, such as CREB [121], which induces IEG expression by interacting with the calcium/cAMP response element [122].

The regulation of IEG expression by caffeine is dependent on the antagonistic interaction between A_{2A} and dopamine D_2 receptors. Blockade of D_2 receptors results in increased c-*fos* expression in striato-Gpe neurons [114, 115]. Moreover, treatment with reserpine causes an increase in striatal Fos-like immunoreactivity, which is prevented by administration of quinpirole, a dopamine D_2 receptor agonist [123]. Using reserpine-treated mice, Pollack and Fink [124] showed that methylxanthines, such as theophylline and the selective A_{2A} receptor antagonist 8-(3-chlorostyryl)caffeine (CSC), potentiate the reduction of c-*fos* expression produced by quinpirole in striato-Gpe neurons. In the same experimental model, CSC inhibited D_2 receptor antagonist-induced Fos-like immunoreactivity [125]. These results indicate that blockade of adenosine A_{2A} receptors, such as that produced by low doses of caffeine, promotes the inhibition exerted by dopamine D_2 receptors on the activity of striato-Gpe neurons.

The behavioral and biochemical evidence presented above indicates that the striato-Gpe neurons of the indirect pathway are a crucial anatomical target involved in the psychomotor stimulant effect exerted by caffeine. Administration of low to moderate doses of caffeine is accompanied by reduced IEG expression in these neurons. Such a reduction is an indicator of decreased activity in the indirect pathway, which, in turn, results in disinhibition of thalamocortical neurons and motor stimulation (cf. fig. 1).

Alterations in IEG expression have been crucial in the identification of the neuroanatomical substrates involved in the stimulant effect of caffeine. However, changes in the levels of Fos and other IEG products occur over a period of h and therefore cannot account for the rapid (min) increase in locomotor activity observed following administration of caffeine. During recent years, evidence has been accumulated indicating that the phosphoprotein DARPP-32 plays a critical role in the acute psychomotor stimulant response to caffeine.

DARPP-32 as an amplification system for cAMP/PKA-mediated responses

DARPP-32 is highly expressed in both striato-Gpe and striato-nigral/Gpi neurons [126], where it acts as a modulator of the cAMP/PKA pathway [127, 128]. Phosphorylation catalyzed by PKA at Thr34 converts DARPP-32 into a selective inhibitor of protein phosphatase-1 (PP-1) [129]. Conversely, phosphorylation catalyzed by cyclin-dependent kinase-5 (Cdk-5) at Thr75 converts DARPP-32 into an inhibitor of PKA [130]. Thus, depending on the site of phosphorylation, DARPP-32 is able to produce opposing biochemical effects (i.e. inhibition of protein phosphatase activity or inhibition of protein kinase activity) (fig. 3).

The state of phosphorylation of Thr34 and Thr75 appears to be reciprocally regulated. An increase in Thr75 phosphorylation results in decreased phosphorylation at Thr34, via inhibition of PKA [130, 131]. Conversely, stimuli that lead to activation of PKA, and increased phosphorylation at Thr34, produce a concomitant decrease in Thr75 phosphorylation [131]. This latter effect is most likely dependent on the ability of PKA to phosphorylate and activate protein phosphatase-2A (PP-2A) [132, 133], which is responsible for dephosphorylation of DARPP-32 at Thr75 [130, 131] (fig. 3).

Responses to stimuli that activate the cAMP/PKA pathway are strongly amplified by concomitant changes in the state of phosphorylation of DARPP-32 at Thr34 and Thr75. Increased phosphorylation at Thr34 amplifies the effects of PKA by reducing dephosphorylation of downstream target proteins, through inhibition of PP-1. In addition, decreased phosphorylation at Thr75 promotes activation of the cAMP/PKA pathway by reducing the inhibition exerted by phospho[Thr75]DARPP-32 on PKA [130] (fig. 3).

DARPP-32 plays a critical role in the functioning of the basal ganglia, as illustrated by its involvement in striatal dopaminergic transmission. Activation of dopamine D_1 receptors results in G_{olf}-mediated stimulation of PKA, which increases phosphorylation of DARPP-32 at Thr34 [134, 135], and decreases phosphorylation at Thr75 [131]. This regulation of DARPP-32 promotes dopamine D_1 receptor-mediated phosphorylation of downstream target proteins critically involved in the control of the state of excitability of striatal projection neurons, including voltage-dependent calcium channels [136], glutamate NMDA [137] and AMPA [138] receptors, and $GABA_A$ receptors [139]. The positive feedback on protein phosphorylation provided by DARPP-32 appears to be critical for eliciting full behavioral responses. Thus, the hyperlocomotor effect of cocaine, a drug which increases Thr34 [140, 141], and decreases Thr75 [140], phosphorylation via stimulation of dopamine D_1 receptors [141], is stronlgy attenuated in DARPP-32-deficient mice [128].

DARPP-32, adenosine transmission and caffeine

Stimulation of striatal slices with the A_{2A} receptor agonist, CGS 21680, results in G_{olf}-dependent activation of

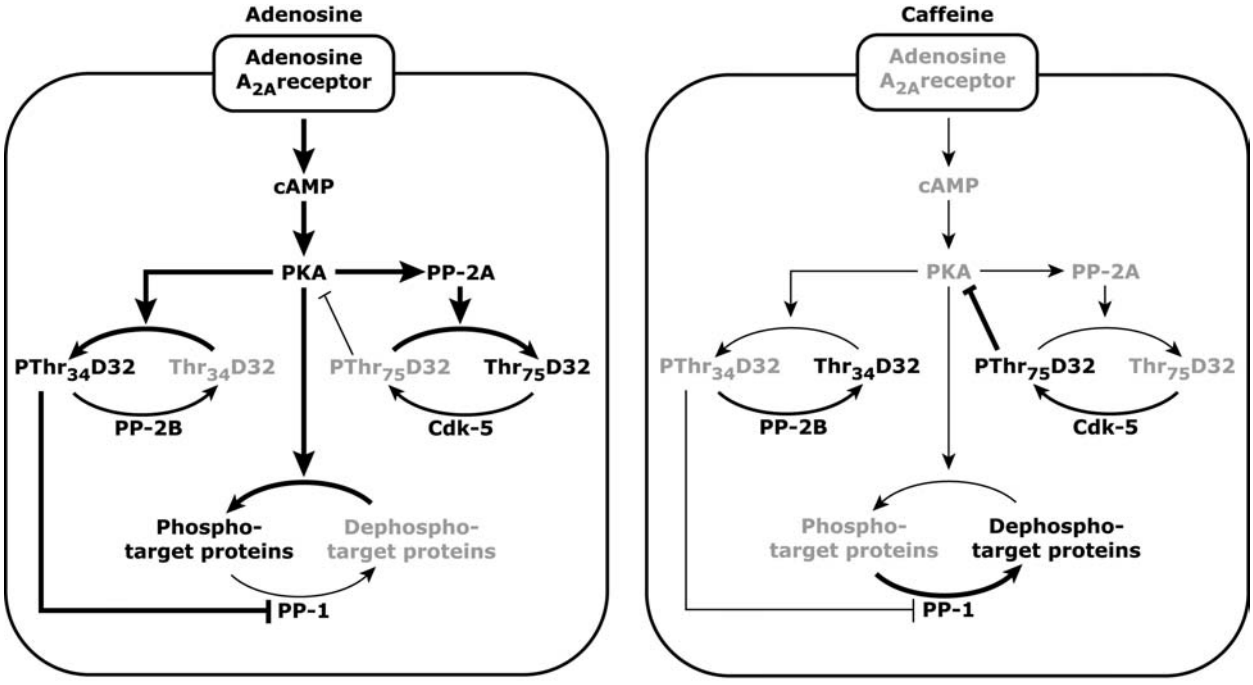

Figure 3. Schematic representation of the regulation of DARPP-32 by adenosine and caffeine. *Left panel*: Adenosine, via A_{2A} receptors, stimulates adenylyl cyclase and increases the production of cAMP. Activation of PKA results in phosphorylation of Thr34 of DARPP-32 (D32), which is converted into an inhibitor of PP-1. PKA also phosphorylates and activates PP-2A, thereby hastening the dephosphorylation of DARPP-32 at Thr75 and reducing the inhibition exerted by phosphoThr75-DARPP-32 on PKA. These effects intensify the phosphorylation of downstream target proteins produced by adenosine via activation of the cAMP/PKA cascade. *Right panel*: Caffeine, via blockade of A_{2A} receptors, reduces the production of cAMP and decreases the activity of PKA. This, in turn, results in diminished phosphorylation of DARPP-32 at Thr34 and increased phosphorylation at Thr75. By further reducing the activity of PKA, phosphoThr75-DARPP-32 provides a positive feedback mechanism able to amplify the inhibition of the cAMP/PKA pathway. Thicker arrows and bars and black color indicate higher activity or levels. PP-2B, protein phosphatase-2B.

adenylyl cyclase [35], increased cAMP levels and PKA-mediated phosphorylation of DARPP-32 at Thr34 [135]. These effects most likely occur in striato-Gpe neurons [135], where CGS 21680 also reduces the phosphorylation of DARPP-32 at Thr75 [102]. The state of phosphorylation of DARPP-32 in striato-Gpe neurons appears to be determined by the combined tonic activation of adenosine A_{2A} and dopamine D_2 receptors, which stimulate and inhibit the production of cAMP, respectively. Thus, blockade of D_2 receptors, achieved with the selective antagonist eticlopride results in increased phosphorylation of DARPP-32 at Thr34, through disinhibition of PKA activity. Furthermore, the effect of eticlopride on DARPP-32 phosphorylation is prevented in adenosine A_{2A} receptor-null mice [141].

The importance of changes in DARPP-32 phosphorylation for adenosine transmission has been demonstrated by studies performed using DARPP-32 knockout mice. In these animals, the motor depressant effect produced by administration of CGS 21680 [142] is significantly attenuated [102].

Recent evidence shows that caffeine produces a prolonged (≥2 h) and dose-dependent increase in the state of phosphorylation of DARPP-32 at Thr75. The peak effect of caffeine is reached at the dose of 7.5 mg/kg, which also produces a sustained increase in motor activity [102]. The ability of caffeine to increase DARPP-32 phosphorylation at Thr75 is most likely mediated via blockade of tonically activated adenosine A_{2A} receptors, since administration of the selective A_{2A} receptor antagonist, SCH 58261, also increases the levels of phospho [Thr75] DARPP-32. Furthermore, caffeine appears to increase DARPP-32 phosphorylation at Thr75 via inhibition of PP-2A activity, rather than via activation of Cdk-5 [102]. The increase in locomotor activity produced in wild-type mice by a low dose (7.5 mg/kg) of caffeine is strongly attenuated in mice lacking DARPP-32. Similar results are obtained following administration of SCH 58261, which also increases motor activity [102]. Thus, the A_{2A} receptor-dependent increase in DARPP-32 phosphorylation at Thr75 produced by caffeine appears to be critically involved in its stimulant action.

Based on these results, a molecular mechanism responsible for the psychomotor stimulant properties of caffeine has been proposed [102]. According to this mechanism, caffeine would increase motor activity by blocking adenosine A_{2A} receptors and reducing tonic activation of the cAMP/PKA pathway in striato-Gpe neurons. Such a

caffeine-mediated inhibition of the cAMP/PKA pathway would reduce phosphorylation of downstream target proteins, thereby affecting the activity of striato-Gpe neurons and ultimately enhancing locomotion. The parallel increase in Thr75 phosphorylation would convert DARPP-32 into an inhibitor of PKA, further reducing phosphorylation of target proteins and amplifying the effect of caffeine (cf. fig. 3).

Studies performed in DARPP-32-null mice show that DARPP-32 prolongs the motor stimulant effect of 7.5 mg/kg of caffeine, but does not affect the response to a higher dose (15 mg/kg) of the drug. In addition, DARPP-32 is not involved in the initial increase in motor activity produced by caffeine, but rather intensifies the late effect of the drug, as its concentration diminishes. These observations indicate that the positive feedback loop provided by DARPP-32 assumes physiological relevance only in association with submaximal inhibitions of the cAMP/PKA pathway, produced by relatively low concentrations of caffeine. When the cAMP/PKA pathway is strongly inhibited by high concentrations of caffeine, the additional reduction of PKA activity provided by phospho [Thr75] DARPP-32 becomes superfluous.

Caffeine and Parkinson's disease

Parkinson's disease is the second most frequent neurodegenerative disorder in people older than 45 years. The cardinal symptoms of Parkinson's disease arise from the degeneration of dopaminergic nigrostriatal neurons of the basal ganglia and consist of a series of motor disturbances ranging from resting, tremor and rigidity, to akinesia, bradykinesia and postural instability. The current therapy for Parkinson's disease relies on substitution treatment with the dopamine precursor, levodopa, which in the initial phases of the disease effectively reduces the motor symptoms. Unfortunately, the therapeutic effects of levodopa wane with time, and prolonged use of this drug is accompanied by the appearance of abnormal involuntary movements, generally referred to as dyskinesia.

The lack of dopaminergic input to the medium spiny neurons occurring in Parkinson's disease is associated with decreased activity of the striato-nigral/Gpi neurons of the direct pathway, as indicated by reduced expression of preprotachykinin in these cells [65]. In contrast, the expression of mRNA for preproenkephalin, a selective marker for striato-Gpe neurons [65], is increased in Parkinsonian patients [143, 144], as well as in experimental animals treated with 6-hydroxydopamine (6-OHDA) [145] or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [146], two toxins that cause a selective disruption of dopaminergic transmission. These changes in the activity of striatal projection pathways are thought to result in the motor dysfunctions typical of Parkinson's disease [65].

Administration of levodopa, or dopamine receptor agonists, such as apomorphine, to rats lesioned unilaterally with 6-OHDA produces a particular form of motor activity consisting in rotations (turning behavior) oriented toward the side contralateral to the lesion [147]. This response, which is attributed to the development of supersensitive dopamine transmission in the lesioned side, is regarded as a measure of the antiparkinsonian properties of a drug [148].

Administraton of caffeine to 6-OHDA-lesioned rats produces contralateral turning behavior and potentiates turning behavior induced by dopaminomimetic drugs, including levodopa and apomorphine [149–154]. The mechanism by which caffeine induces motor activity (i.e. contralateral turning behavior) in the 6-OHDA lesion model of Parkinson's disease differs from the stimulant effect produced on spontaneous locomotion. For instance, whereas both D_1 and D_2 receptor antagonists are able to prevent the locomotor stimulation induced by caffeine in naive rats [84], only dopamine D_2, but not D_1, receptor antagonists block the contralateral turning behavior induced by caffeine in 6-OHDA-lesioned rats [152, 154]. This difference may be due to the functional uncoupling between D_1 and D_2 receptors observed in animal models of Parkinson's disease and in parkinsonian patients cf. [155]. In this pathological situation, dopamine D_1 antagonists lose their ability to prevent the motor stimulant effects produced by D_2 receptor agonists. It is therefore possible that in 6-OHDA-lesioned rats, the inability of dopamine D_1 antagonists to block the stimulant effect of caffeine, which acts by promoting dopamine D_2 receptor-mediated transmission, is a consequence of such a loss of 'cross-antagonism' [155]. In contrast, and in spite of the functional uncoupling, blockade of dopamine D_2 receptors is still able to prevent the motor activation induced by caffeine because of the direct functional interaction between D_2 and A_{2A} receptors, which are highly coexpressed on striato-Gpe neurons.

The ability of caffeine to potentiate levodopa-induced contralateral turning in 6-OHDA-lesioned rats is shared by selective adenosine A_{2A} receptor antagonists [156–158], which are therefore regarded as possible antiparkinsonian drugs [142, 159]. One of these compounds, KW-6002, alleviates parkinsonian symptoms in monkeys treated with MPTP and potentiates the therapeutic efficacy of low-dose levodopa and dopamine receptor agonists [110, 160, 161]. The potential therapeutic efficacy of adenosine A_{2A} receptor antagonists is further demonstrated by studies showing that the motor impairment caused by genetic inactivation of the dopamine D_2 receptor is counteracted by administration of KW-6002 [75]. In addition, caffeine enhances locomotion in mice made dopamine deficient by inactivating the gene coding for tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines [162].

Studies performed in MPTP-treated monkeys show that the anti-parkinsonian effect of KW-6002, administered alone or together with dopaminomimetic drugs, is not accompanied by dyskinesia, even after several days of administration [110, 160, 161]. These results suggest that combined treatment with levodopa and adenosine A_{2A} receptor antagonists improves the symptoms of Parkinson's disease without causing dyskinesia. In support of this idea, it has been reported that in hemiparkinsonian rats, the A_{2A} receptor antagonist CSC prevents levodopa-induced behavioral sensitization, which is considered an indicator of dyskinesia [163]. Lack of behavioral sensitization is also observed following chronic administration of levodopa to A_{2A} knockout mice unilaterally lesioned with 6-OHDA [164]. In addition, coadministration of caffeine reduces the hyperlocomotor effect produced by levodopa in genetically altered, dopamine-deficient mice [162]. The idea that adenosine A_{2A} receptor antagonists possess antidyskinetic properties has been recently challenged by Lundblad et al. [165]. Utilizing a more specific approach to the quantification of levodopa-induced abnormal involuntary movements [166–168], these authors show that coadministration of an adenosine A_{2A} receptor antagonist does not prevent the dyskinetic effect caused by therapeutic doses of levodopa given to severely denervated rats.

In conclusion, blockade of adenosine A_{2A} receptors with caffeine or with selective antagonists improves the symptoms of Parkinson's disease in animal models and potentiates the therapeutic efficacy of dopaminomimetic medications. This latter effect may help to reduce the dosage of levodopa and indirectly diminish the incidence of 'peak-dose' levodopa-induced dyskinesia, which currently represents one of the major problems in the pharmacotherapy of Parkinson's disease. Whereas initial clinical trials did not report any significant improvement following administration of caffeine to parkinsonian patients [169, 170], more recent work indicates the potential therapeutic value of theophylline [171]. It should be noted, however, that methylxanthines, although potentially useful to correct for the psychomotor symptoms of Parkinson's disease, could have negative effects in patients because of their anxiogenic properties and their adverse cardiovascular effects. In this regard, the use of specific adenosine A_{2A} receptor antagonist may be more appropriate, as these drugs do not induce anxiety [172]. More studies will be necessary to establish the efficacy and suitability of adenosine A_{2A} receptor blockade in the treatment of Parkinson's disease.

Caffeine and neuroprotection

Clinical studies have established a positive correlation between dietary caffeine consumption and reduced risk of Parkinson's disease [173, 174]. In agreement with these observations, caffeine has been shown to reduce the neurotoxic effect exerted by MPTP on dopaminergic neurons [175]. The mechanisms underlying the neuroprotective action of caffeine are not completely understood; however, blockade of A_{2A} receptors appears to be involved, since selective A_{2A} receptor antagonists, but not A_1 receptor antagonists, reduce both MPTP [175] and 6-OHDA [176] induced neurodegeneration. In addition, blockade of A_{2A} receptors has been shown to exert neuroprotective action during excitotoxicity and cerebral ischemia [177–180]. It has been proposed that caffeine may protect dopaminergic cells by reducing glutamate excitotoxicity. This action could be exerted via a polysynaptic circuit leading to inhibition of the subthalamic nucleus (cf. fig. 1) [175], a region that sends a glutamatergic input to the substantia nigra pars compacta and that has been proposed as a target for neuroprotective therapies [181].

Concluding remarks and future perspectives

Several lines of evidence indicate that the psychomotor stimulant effect of caffeine is exerted by modulating the state of excitability of striatal medium spiny neurons, via blockade of adenosine A_{2A} receptors. Although caffeine acts, at least in part, by facilitating dopamine D_2 receptor transmission, its mechanism of action appears to be substantially different from that of 'dopaminomimetic' psychostimulants, such as cocaine and amphetamine.

Caffeine acts on the indirect striato-Gpe pathway, whereas cocaine and amphetamine affect the direct, striato-nigral/Gpi pathway (fig. 4). In addition, and in contrast with cocaine and amphetamine, caffeine does not influence dopamine release in the ventral striatum [51], and its psychostimulant effect is independent of this brain region [95–97] (but see [94]). In fact, the stimulant effects of caffeine and cocaine are additive [182, 183].

The motor stimulant effect of caffeine is accompanied by changes in IEG expression and DARPP-32 phosphorylation opposite to those caused by cocaine (and amphetamine) (fig. 4), which also increase motor activity. This apparent discrepancy can be reconciled by considering that the striato-Gpe indirect pathway, which is inhibited by caffeine, and the striato-nigral/Gpi direct pathway, which is activated by cocaine, regulate motor activity in opposite ways (cf. fig. 1). The ability of DARPP-32 to intensify the behavioral effects of cocaine and caffeine indicate that this phosphoprotein functions as a bidirectional modulator, able to amplify responses elicited by activation as well as inhibition of the cAMP/PKA cascade.

One important question to be addressed in future studies is the identification of the downstream target proteins responsible for regulation of the activity of striato-Gpe neurons exerted by caffeine. The involvement of the DARPP-

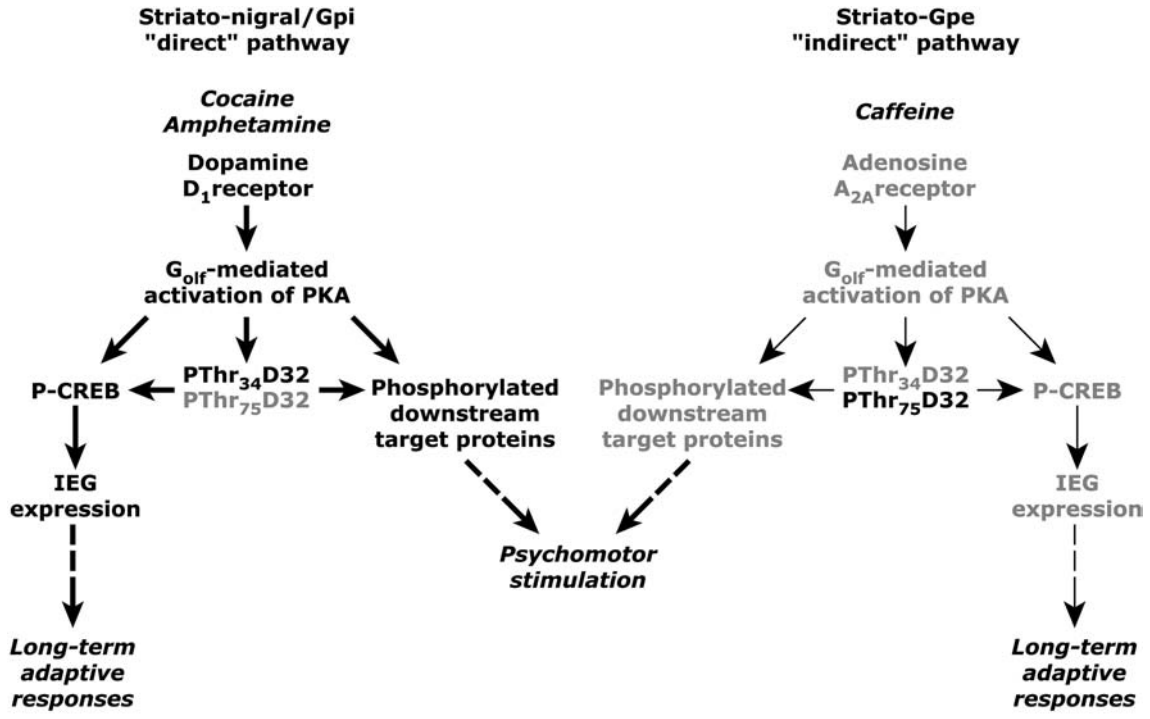

Figure 4. Diagram illustrating the effects of caffeine, and cocaine and amphetamine on striatal projection neurons. Caffeine reduces the activity of the cAMP/PKA pathway in the striato-Gpe indirect pathway, via blockade of adenosine A_{2A} receptors. In contrast, cocaine stimulates the cAMP/PKA pathway in the striato-nigral/Gpi pathway by increasing extracellular dopamine and activating dopamine D_1 receptors. Caffeine and cocaine regulate in an opposite way the state of phosphorylation of DARPP-32 (D32), which in turn amplifies their biochemical and behavioral effects (cf. fig. 3 and text). Thicker arrows and bars and black color indicate higher activity or levels.

32/PKA/PP-1 pathway in dopamine D_1 receptor-mediated regulation of glutamate and GABA receptors has been previously demonstrated [137–139]. These regulations are most likely involved in the activation of striato-nigral/Gpi neurons, since D_1 receptors stimulate IEG expression specifically in these cells [116, 117]. It is possible that caffeine, via stimulation of DARPP-32 phosphorylation at Thr75, regulates a similar set of downstream target proteins in an opposite way, thereby depressing the activity of striato-Gpe neurons.

Caffeine at low doses induces place conditioning [182, 184], and tolerance to its locomotor stimulant effect is observed after repeated administration [12, 87, 152, 185]. However, caffeine has a limited ability to promote self-administration compared with cocaine, amphetamine and other drugs of abuse cf. [186] and is not listed among addictive substances in the *Diagnostic and statistical manual of mental disorders* (4th ed.) [187]. Nevertheless, dependence on caffeine is currently a matter of discussion, and caffeine withdrawal symptoms including headaches, irritability, drowsiness and fatigue have been documented [78, 186, 188]. Caffeine tolerance is accompanied by changes in IEG expression and adenosine receptors [189]. Interestingly, prolonged administration of an adenosine A_{2A} receptor antagonist does not induce tolerance to its motor stimulant effect [190], raising the possibility that caffeine tolerance is dependent on blockade of A_1, rather than A_{2A}, receptors. Future studies will be necessary to examine the possible involvement of DARPP-32 and other intracellular signaling molecules in the adaptive responses produced by long-term exposure caffeine.

Acknowledgements. G. F. is supported by Swedish Research Council grants 13482 and 14518. A. U. is the recipient of a fellowship from the Wenner-Gren Foundations.

1 Butcher R. W. and Sutherland E. W. (1962) Adenosine 3′,5′-monophosphate in biological materials. I. Purification and properties of cyclic 3′,5′-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3′,5′-phosphate in human urine. J. Biol. Chem. **237:** 1244–1250
2 Vernikos-Danellis J. and Harris III C. G. (1968) The effect of in vitro and in vivo caffeine, theophylline and hydrocortisone on the phosphodiesterase activity of the pituitary, median eminence, heart and cerebral cortex. Proc. Soc. Exp. Biol. Med. **128:** 1016–1021
3 Cardinali D. P. (1980) Methylxanthines: possible mechanisms of action in the brain. Trends Pharmacol. Sci. **1:** 405–407
4 McPherson P. S., Kim Y. K., Valdivia H., Knudson C. M., Takekura H., Franzini-Armstrong C. et al. (1991) The brain ryanodine receptor: a caffeine-sensitive calcium release channel. Neuron **7:** 17–25
5 Sitsapesan R., McGarry S. J. and Williams A. J. (1995) Cyclic ADP-ribose, the ryanodine receptor and Ca^{2+} release. Trends Pharmacol. Sci. **16:** 386–391

6 Marangos P. J., Paul S. M., Parma A. M., Goodwin F. K., Syapin P. and Skolnick P. (1979) Purinergic inhibition of diazepam binding to rat brain (in vitro). Life Sci. **24:** 851–858
7 Davies L. P., Chow S. C. and Johnson G. A. R. (1984) Interaction of purines and related compounds with photoaffinity-labelled benzodiazepine binding sites. Eur. J. Pharmacol. **97:** 325–329
8 Shi D., Padgett W. L. and Daly J. W. (2003) Caffeine analogs: effects on ryanodine-sensitive calcium-release channels and $GABA_A$ receptors. Cell. Mol. Neurobiol. **23:** 331–347
9 Daly J. W. (2000) Alkylxanthines as research tools. J. Auton. Nerv. Syst. **81:** 44–52
10 Dews P. B. (1982) Caffeine. Annu. Rev. Nutr. **2:** 323–341
11 Bonati M., Latini R., Galletti F., Young J. F., Tognoni G. and Garattini S. (1982) Caffeine disposition after oral doses. Clin. Pharmacol. Ther. **32:** 98–106
12 Fredholm B. B., Bättig K., Holmén J., Nehlig A. and Zvvartau E. E. (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. **51:** 83–133
13 Dunwiddie T. V. and Masino S. A. (2001) The role and regulation of adenosine in the central nervous system. Annu. Rev. Neurosci. **24:** 31–55
14 Zimmerman H. and Braun N. (1999) Ecto-nucleotidases – molecular structures, catalytic properties and functional roles in the nervous system. Prog. Brain Res. **120:** 371–385
15 Schubert P., Komp W. and Kreutzberg G. W. (1979) Correlation of 5′-nucleotidase activity and selective transneuronal transfer of adenosine in the hippocampus. Brain Res. **168:** 419–424
16 Broch O. J. and Ueland P. M. (1980) Regional and subcellular distribution of S-adenosylhomocysteine hydrolase in the adult rat brain. J. Neurochem. **35:** 484–488
17 Dunwiddie T. V. and Diao L. H. (1994) Extracellular adenosine in hippocampal brain slices and the tonic inhibitory modulation of evoked excitatory responses. J. Pharmacol. Exp. Ther. **268:** 537–545
18 Ballarin M., Fredholm B. B., Ambrosio S. and Mahy N. (1991) Extracellular levels of adenosine and its metabolites in the striatum of awake rats: inhibition of uptake. Acta Physiol. Scand. **142:** 97–103
19 Lloyd H. G. and Fredholm B.B. (1995) Involvement of adenosine deaminase and adenosine kinase in regulating extracellular adenosine concentration in hippocampal slices. J. Neurochem. **26:** 387–395
20 Arch J. R. and Newsholme E. A. (1978) The control of the metabolism and the hormonal role of adenosine. Essays Biochem. **14:** 82–123
21 Cass C. E., Young J. D. and Baldwin S. A. (1998) Recent advances in the molecular biology of nucleoside transporters of mammalian cells. Biochem. Cell Biol. **76:** 761–770
22 Williams T. C. and Jarvis S. M. (1991) Multiple sodium-dependent nucleoside transport systems in bovine renal brush-border membrane vesicles. Biochem. J. **274:** 27–33
23 Anderson C. M., Xiong W., Young J. D., Cass C. E. and Parkinson F. E. (1996) Demonstration of the existence of mRNA encoding N1/cif and N2/cit sodium/nucleoside cotransporters in the brain. Brain Res. Mol. Brain Res. **42:** 358–361
24 Berne R. M., Rubio R. and Curnish R. R. (1974) Release of adenosine from ischemic brain. Effect on cerebral vascular resistance and incorporation into cerebral adenine nucleotide. Circ. Res. **35:** 262–271
25 Zetterström T., Vernet L., Ungerstedt U., Tossman U. and Jonzon B. (1982) Purine levels in the intact brain. Studies with an implanted perfused hollow fibre. Neurosci. Lett. **29:** 111–115
26 Pull I. and McIlwain H. (1972) Adenine derivatives as neurohumoral agents in the brain. The quantities liberated on excitation of superfused cerebral tissues. Biochem. J. **130:** 975–981
27 Lloyd H. G., Linström K. and Fredholm B. B. (1993) Intracellular formation and release of adenosine from rat hippocampal slices evoked by electrical stimulation or energy depletion. Neurochem. Int. **23:** 173–185
28 Fredholm B. B., Abbracchio M. P., Burnstock G., Daly J. W., Harden T. K., Jacobson K. A. et al. (1994) Nomenclatuure and classification of purinoceptors. Pharmacol. Rev. **46:** 143–156
29 Jacobson K. A. (1998) Adenosine A_3 receptors: novel ligands and paradoxical effects. Trends Pharmacol. Sci. **19:** 184–191
30 Feoktistov I. and Biaggioni I. (1997) Adenosine A_{2B} receptors. Pharmacol. Rev. **49:** 381–402
31 Londos C., Cooper D. M. F. and Wolff J. (1980) Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. USA **77:** 2551–2554
32 van Calker D., Mueller M. and Hamprecht B. (1979) Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J. Neurochem. **33:** 999–1005
33 Trussel L. O. and Jackson M. B. (1985) Adenosine-activated potassium conductance in cultured striatal neurons. Proc. Natl. Acad. Sci. USA **82:** 4857–4861
34 Macdonald R. L., Skerrit J. H. and Werz M. A. (1986) Adenosine agonists reduce voltage-dependent calcium conductance of mouse sensory neurones in cell culture. J. Physiol. **370:** 75–90
35 Hervé D., Le Moine C., Corvol J.-C., Belluscio L., Ledent C., Fienberg A. A. et al. (2001) $G\alpha_{olf}$ levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. J. Neurosci. **21:** 4390–4399
36 Goodman R. R. and Snyder S. H. (1982) Autoradiographic localization of adenosine receptors in rat brain using [^{3}H]cyclohexyladenosine. J. Neurosci. **2:** 1230–1241
37 Reppert S. M., Weaver D. R., Stehle J. H. and Rivkees S. A. (1991) Molecular cloning and characterization of a rat A_1-adenosine receptor that is widely expressed in brain and spinal cord. Mol. Endocrinol. **5:** 1037–1048
38 Mahan L. C., McVittie L. D., Smyk-Randall L. D., Nakata H., Monsma Jr. F. J., Gerfen C. R. et al. (1991) Cloning and expression of an A_1 adenosine receptor from rat brain. Mol. Pharmacol. **40:** 1–7
39 Rivkees S. A., Price S. L. and Zhou F. C. (1995) Immunohistochemical detection of A1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum and basal ganglia. Brain Res. **677:** 193–203
40 Fredholm B. B. and Dunwiddie T. V. (1988) How does adenosine inhibit transmitter release? Trends Pharmacol. Sci. **9:** 130–134
41 Marchi M., Raiteri L., Risso F., Vallarino A., Bonfanti A., Monopoli A. et al. (2002) Effects of adenosine A_1 and adenosine A_{2A} receptor activation on the evoked release of glutamate from cerebrocortical synaptosomes. Br. J. Pharmacol. **136:** 434–440
42 Flagmeyer I., Haas H. L. and Stevens D. R. (1997) Adenosine A1 receptor-mediated depression of corticostriatal and thalamostriatal glutamatergic synaptic potentials in vitro. Brain Res. **778:** 178–185
43 Dunwiddie T. V. and Hoffer B. J. (1980) Adenine nucleotides and synaptic transmission in the in vitro rat hippocampus. Br. J. Pharmacol. **69:** 59–68
44 Okada M., Mizuno K. and Kaneko S. (1996) Adenosine A1 and A2 receptors modulate extracellular dopamine levels in rat striatum. Neurosci. Lett. **212:** 53–56
45 Brown S. J., James S., Reddington M. and Richardson P. J. (1990) Both A1 and A2a purine receptors regulate striatal acetylcholine release. J. Neurochem. **55:** 31–38
46 Rainnie D. G., Grunze H. C., McCarley R. W. and Greene R. W. (1994) Adenosine inhibition of mesopontine cholinergic neurons: implications for EEG arousal. Science **263:** 689–692

47 Arushanian E. B. and Belozertsev Y. B. (1978) The effect of amphetamine and caffeine on neuronal activity in the neocortex of the cat. Neuropharmacology **17:** 1–6

48 Phillis J. W. and Kostopoulos G. K. (1975) Adenosine as a putative transmitter in the cerebral cortex: studies with potentiators and antagonists. Life Sci. **17:** 1085–1094

49 Dunwiddie T. V., Hoffer B. J. and Fredholm B. B. (1981) Alkylxanthines elevate hippocampal excitability: evidence for a role of endogenous adenosine. Naunyn-Schmiedeberg's Arch. Pharmacol. **316:** 326–330

50 Popoli P., Sagratella S. and Scotti de Carolis A. (1987) An EEG and behavioural study on the excitatory properties of caffeine in rabbits. Arch. Int. Pharmacodyn. Ther. **290:** 5–15

51 Acquas E., Tanda G. and Di Chiara G. (2002) Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naive and caffeine pretreated rats. Neuropsychopharmacology **27:** 182–193

52 Himmelheber A. M., Sarter M. and Bruno J. P. (2000) Increases in cortical acetylcholine release during sustained attention performance in rats. Cogn. Brain Res. **9:** 313–325

53 Passetti F., Dalley J. W., O'Connell M. T., Everitt B. J. and Robbins T. W. (2000) Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. Eur. J. Neurosci. **12:** 3051–3058

54 Jarvis M. F. and Williams M. (1989) Direct autoradiographic localization of adenosine A_2 receptors in the rat brain using the A_2-selective agonist [^{3}H]CGS 21680. Eur. J. Pharmacol. **168:** 243–246

55 Parkinson F. E. and Fredholm B. B. (1990) Autoradiographic evidence for G-protein coupled A_2-receptor in rat neostriatum using [^{3}H]-CGS 21680 as a ligand. Naunyn-Schmiedeberg's Arch. Pharmac. **342:** 85–89

56 Wan W., Sutherland G. R. and Geiger J. D. (1990) Binding of the adenosine A_2 receptor ligand [^{3}H]CGS 21680 to human and rat brain: evidence for multiple affinity sites. J. Neurochem. **55:** 1763–1771

57 Martinez-Mir M. I., Probst A. and Palacios J. M. (1991) Adenosine A_2 receptors: selective localization in the human basal ganglia and alterations with disease. Neuroscience **42:** 697–706

58 Schiffman S. N., Jacobs O. and Vanderhaegen J. J. (1991) 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

59 Fink J. S., Weaver D. R., Rivkees S. A., Peterfreund R. A., Pollack A. E., Adler E. M. et al. (1992) Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptor in rat striatum. Mol. Brain Res. **14:** 186–195

60 Schiffman S. N. and Vanderhaeghen J.-J. (1993) Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J. Neurosci. **13:** 1080–1087

61 Alexander G. E., DeLong M. R. and Strick P. L. (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu. Rev. Neurosci. **9:** 357–381

62 Ungerstedt U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. **367:** 1–48

63 Dahlström A. and Fuxe K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in cell bodies of brain stem neurons. Acta Physiol. Scand. **62:** 1–55

64 Andén N.-E., Carlsson A., Dahlström A., Fuxe K., Hillarp N.-Å. and Larsson K. (1964) Demonstration and mapping out of nigro-neostriatal dopamine neurons. Life Sci. **3:** 523–530

65 Gerfen C. R. (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Ann. Rev. Neurosci. **15:** 285–320

66 Stoof J. C. and Kebabian J. W. (1981) Opposing roles for D-1 and D-2 dopamine rceptors in efflux of cyclic AMP from rat neostriatum. Nature **294:** 366–368

67 Zhuang X., Belluscio L. and Hen R. (2000) G_{OLFa} mediates dopamine D_1 receptor signaling. J. Neurosci. **20:** RC91 (1–5)

68 Ferré S., Snaprud P. and Fuxe K. (1993) Opposing actions of an adenosine A_2 agonist and a GTP analogue on the regulation of dopamine D_2 receptors in rat neostriatal membranes. Eur. J. Pharmacol. **244:** 311–315

69 Ferré S., von Euler G., Johansson B., Fredholm B. B. and Fuxe K. (1991) Stimulation of high-affinity adenosine A_2 receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes. Proc. Natl. Acad. Sci. USA **88:** 7238–7241

70 Ferré S., Fredholm B. B., Morelli M., Popoli P. and Fuxe K. (1997) Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci. **20:** 482–487

71 Fredholm B. B. (1977) Activation of adenylate cyclase from rat striatum and tuberculum olfactorium by adenosine. Med. Biol. **55:** 262–267

72 Chen J.-F., Moratalla R., Impagnatiello F., Grandy D. K., Cuellar B., Rubinstein M. et al. (2001) The role of the D_2 dopamine receptor (D_2R) in A_{2A} adenosine receptor (A_{2A} R)-mediated behavioral and cellular responses as revealed by A_{2A} and D_2 receptor knockout mice. Proc. Natl. Acad. Sci. USA **98:** 1970–1975

73 Mayfield R., Larson G., Orona R. A. and Zahniser N. R. (1996) Opposing actions of adenosine A_{2a} and dopamine D_2 receptor activation on GABA release in the basal ganglia: evidence for an A_{2a}/D_2 receptor interaction in globus pallidus. Synapse **22:** 132–138

74 Ferré S., O'Connor W. T., Fuxe K. and Ungerstedt U. (1993) The striopallidal neuron: a main locus for adenosine-dopamine interactions in the brain. J. Neurosci. **13:** 5402–5406

75 Aoyama S., Kase H. and Borrelli (2000) Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A2A receptor antagonist. J. Neurosci. **20:** 5848–5852

76 Zahniser N. R., Simosky J. K., Mayfield R. D., Negri C. A., Hanania T., Larson G. A. et al. (2000) Functional uncoupling of adenosine A_{2A} receptors and reduced response to caffeine in mice lacking dopamine D_2 receptors. J. Neurosci. **20:** 5949–5957

77 Nehlig A., Daval J. L. and Debry G. (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res. Rev. **17:** 139–170

78 Daly J. W. and Fredholm B. B. (1998) Caffeine – an atypical drug of dependence. Drug Alcohol Dep. **51:** 199–206

79 Waldeck B. (1975) Effect of caffeine on locomotor activity and central catecholamine mechanisms: a study with special reference to drug interaction. Acta Pharmacol. Toxicol. **36 (Suppl. IV):** 1–23

80 Snyder S. H., Katims J. J., Annau A., Bruns R. F. and Daly J. W. (1981) Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. Life Sci. **28:** 2083–2097

81 Fredholm B. B. (1980) Are methyllxanthine effects due to antagonism of endogenous adenosine? Trends Pharmacol. Sci. **1:** 129–132

82 Davis J. M., Zhao Z., Stock H. S., Mehl K. A., Buggy J. and Hand G. A. (2003) Central nervous system effects of caffeine and adenosine on fatigue. Am. J. Physiol. Regul. Integr. Comp. Physiol. **284:** R399–R404

83 Svenningsson P., Nomikos G. G., Ongini E. and Fredholm B. B. (1997) Antagonism of adenosine A_{2A} receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A and

NGFI-B in caudate-putamen and nucleus accumbens. Neuroscience **79:** 753–764
84 Garrett B. and Holtzman S. G. (1993) D1 and D2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. Pharmacol. Biochem. Behav. **47:** 89–94
85 Finn I. B. and Holtzman S. G. (1986) Tolerance to caffeine-induced stimulation of locomotor activity in rats. J. Pharmacol. Exp. Ther. **238:** 542–546
86 Svenningsson P., Nomikos G. G. and Fredholm B. B. (1995) Biphasic changes in locomotor behavior and in expresssion of mRNA for NGFI-A and NGFI-B in rat striatum following acute caffeine administration. J. Neurosci. **15:** 7612–7624
87 Nikodijeviç O. and Jacobson K. A. (1993) Locomotor activity in mice during chronic treatment with caffeine and withdrawal. Pharmacol. Biochem. Behav. **44:** 199–216
88 Logan L., Seale T. W. and Carney J. M. (1986) Inherent differences in sensitivity to methylxanthines among inbred mice. Pharmacol. Biochem. Behav. **24:** 1281–1286
89 El Yacoubi M., Ledent C., Menard J. F., Parmentier M., Costentin J. and Vaugeois J. M. (2000) The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A_{2A} receptors. Br. J. Pharmacol. **129:** 1465–1473
90 Nikodijeviç O., Sarges R., Daly J. W. and Jacobson K. A. (1991) Behavioral effects of A_1- and A_2-selective adenosine agonists: evidence for synergism and antagonism. J. Pharmacol. Exp. Ther. **259:** 286–294
91 Katims J. J., Annau Z. and Snyder S. H. (1983) Interactions in the behavioral effects of methylxanthines and adenosine derivatives. J. Pharmacol. Exp. Ther. **227:** 167–173
92 Okada M., Kiryu K., Kawata Y., Mizuno K., Wada K., Tasaki H. et al. (1997) Determination of the effects of caffeine and carbamazepine on striatal dopamine release by in vivo microdialysis. Eur. J. Pharmacol. **321:** 181–188
93 Morgan M. E. and Vestal R. E. (1989) Methylxanthine effects on caudate dopamine release as measured by in vivo electrochemistry. Life Sci. **45:** 2025–2039
94 Solinas M., Ferré S., You Z.-B., Karcz-Kubicha M., Popoli P. and Goldberg S. R. (2002) Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. J. Neurosci. **22:** 6321–6324
95 Swerdlow N. R. and Koob G. F. (1985) Separate neural substrates of the locomotor-activating properties of amphetamine, heroin, caffeine and corticotropin releasing factor (CRF) in the rat. Pharmacol. Biochem. Behav. **23:** 303–307
96 Pulvirenti L., Swerdlow N. R. and Koob G. F. (1991) Nucleus accumbens NMDA antagonist decreases locomotor activity produced by cocaine, heroin or accumbens dopamine, but not caffeine. Pharmacol. Biochem. Behav. **40:** 841–845
97 Joyce E. M. and Koob G. F. (1981) Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Psychopharmacology (Berl.) **73:** 311–313
98 Zocchi C., Ongini E., Conti A., Monopoli A., Negretti A., Baraldi P. G. et al. (1996) The non-xanthine hetericyclic compound, SCH 58261, is a new potent and selective A_{2A} adenosine receptor antagonist. J. Pharmacol. Exp. Ther. **276:** 398–404
99 Bruns R. F., Fergus J. H., Badger E. W., Bristol J. A., Santay L. A., Hartman J. D. et al. (1987) Binding of the A_1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. Naunyn-Schmiedeberg's Arch. Pharmac. **335:** 59–63
100 Griebel G., Misslin R. and Vogel E. (1991) Behavioural effects of selective A2 adenosine receptor antagonists, CGS 21197 and CGS 22706, in mice. Neuroreport **2:** 139–140
101 Griebel G., Saffroy-Spittler M., Misslin R., Remmy D., Vogel E. and Bourguignon J.-J. (1991) Comparison of the behavioural effects of an adenosine A1/A2-receptor antagonist, CGS 15943A and an A1-selective antagonist, DPCPX. Psychopharmacology (Berl.) **103:** 541–544
102 Lindskog M., Svenningsson P., Pozzi L., Kim Y., Fienberg A. A., Bibb J. A. et al. (2002) Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine. Nature **418:** 774–778
103 Ledent C., Vaugeois J. M., Schiffman S. N., Pedrazzini T., El Yacoubi M., Vanderhaegen J. J. et al. (1997) Aggressiveness, hypoalgesia and increased blood pressure in mice deficient for the adenosine A2a receptor. Nature **388:** 674–678
104 Ferré S., Herrera-Marschitz M., Grabowska-Andén M., Casas M., Ungerstedt U. and Andén N.-E. (1991) Postsynaptic dopamine/adenosine interaction: II. Postsynaptic dopamine agonism and adenosine antagonism of methylxanthines in short-term reserpinized mice. Eur. J. Pharmacol. **192:** 31–37
105 White B. C., Simpson C. C., Adams J. E. and Harkins D. J. (1978) Monoamine synthesis and caffeine-induced locomotor activity. Neuropharmacology **17:** 511–513
106 Garrett B. E. and Holtzman S. G. (1994) Caffeine cross-tolerance to selecctive dopamine D_1 and D_2 receptor agonists but not to their synergistic interaction. Eur. J. Pharmacol. **262:** 65–75
107 Braun R. L., Barone P. and Chase T. N. (1986) Interaction of D1 and D2 dopamine receptors in the expression of dopamine agonist-induced behavior. Adv. Med. Biol. **204:** 151–166
108 Popoli P., Caporali M. G. and Scotti de Carolis A. (1991) Akinesia due to catecholamine depletion in mice is prevented by caffeine. Further evidence for an involvement of adenosinergic system in the control of motility. J. Pharm. Pharmacol. **43:** 280–281
109 Shiozaki S., Ichikawa S., Nakamura J., Kitamura S., Yamada K. and Kuwana Y. (1999) Actions of adenosine A2A receptor antagonist KW-6002 on drug-induced catalepsy and hypokinesia caused by reserpine or MPTP. Psychopharmacology (Berl.) **147:** 90–95
110 Kanda T., Jackson M. J., Smith L. A., Pearce R. K., Nakamura J., Kase H. et al. (1998) Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann. Neurol. **43:** 507–513
111 Dragunow M. and Faull R. (1989) The use of c-fos as a metabolic marker in neuronal pathways tracing. J. Neurosci. Methods **29:** 261–265
112 Hughes P. and Dragunow M. (1995) Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. Pharmacol. Rev. **47:** 133–178
113 Miller J. C. (1990) Induction of c-fos mRNA expression in rat striatum by neuroleptic drugs. J. Neurochem. **54:** 1453–1455
114 Robertson G. S. and Fibiger H. C. (1992) Neuroleptics increase c-fos expression in the forebrain: contrasting effects of haloperidol and clozapine. Neuroscience **46:** 315–328
115 Dragunow M., Robertson G. S., Faull R. L. M., Robertson H. A. and Jansen K. (1990) D2 dopamine receptor antagonists induce c-fos and related proteins in rat striatal neurons. Neuroscience **37:** 287–294
116 Young S. T., Porrino L. J. and Iadarola M. J. (1991) Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. Proc. Natl. Acad. Sci. USA **88:** 1291–1295
117 Graybiel A. M., Moratalla R. and Robertson H. A. (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc. Natl. Acad. Sci. USA **87:** 6912–6916
118 Svenningsson P., Ström A., Johansson B. and Fredholm B. B. (1995) Increased expression of c-jun, junB, AP-1 and preproenkephalin mRNA in rat striatum following a single injection of caffeine. J. Neurosci. **15:** 3583–3593

119 Dassesse D., Vanderwinden J.-M., Goldberg I., Vanderhaegen J.-J. and Schiffmann (1999) Caffeine-mediated induction of c-fos, zif-268 and arc expression through A_1 receptors in the striatum: different interactions with the dopaminergic system. Eur. J. Neurosci. **11:** 3101–3114

120 Nakajima T., Daval J. L., Morgan P. F., Post R. M. and Marangos P. J. (1989) Adenosinergic modulation of caffeine-induced c-*fos* mRNA expression in mouse brain. Brain Res. **501:** 307–314

121 Gonzales G. A. and Montminy M. R. (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell **59:** 675–680

122 Sheng M. and Greenberg M. E. (1990) The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron **4:** 477–485

123 Cole D. G. and DiFiglia M. (1994) Reserpine increases fos activity in the rat basal ganglia via a quinpirole-sensitive mechanism. Neuroscience **60:** 115–123

124 Pollack A. E. and Fink J. S. (1995) Adenosine antagonists potentiate D_2 dopamne-dependent activation of Fos in the striatopallidal pathway. Neuroscience **68:** 721–728

125 Boegman R. J. and Vincent S. R. (1996) Involvement of adenosine and glutamate receptors in the induction of c-fos in the striatum by haloperidol. Synapse **22:** 70–77

126 Ouimet C. C., Langley-Guillion K.-C. and Greengard P. (1998) Quantitative immunochemistry of DARPP-32-expressing neurons in the rat caudatoputamen. Brain Res. **808:** 8–12

127 Greengard P., Allen P. B. and Nairn A. (1999) Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron **23:** 435–447

128 Fienberg A. A., Hiroi N., Mermelsten P., Song W.-J., Snyder G. L., Nishi A. et al. (1998) DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science **281:** 838–842

129 Hemmings J., H.C., Greengard P., Tung H. Y. L. and Cohen P. (1984) DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. Nature **310:** 503–505

130 Bibb J., Snyder G. L., Nishi A., Yan Z., Meijer L., Fienberg A. A. et al. (1999) Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. Nature **402:** 669–671

131 Nishi A., Bibb J. A., Snyder G. L., Higashi H., Nairn A. C. and Greengard P. (2000) Amplification of dopaminergic signaling by a positive feedback loop. Proc. Natl. Acad. Sci. USA **97:** 12840–12845

132 Usui H., Imazu M., Maeta K., Tsukamoto H., Azuma K. and Takeda M. (1988) Three distinct forms of type 2A protein phosphatase in human erythrocyte cytosol. J. Biol. Chem. **263:** 3752–3761

133 Usui H., Inoue R., Tanabe O., Nishito Y., Shimizu M., Hayashi H. et al. (1998) Activation of protein phosphatase 2A by cAMP-dependent protein kinase-catalyzed phosphorylation of the 74-kDa B″ (delta) regulatory subunit in vitro and identification of the phosphorylation sites. FEBS Lett. **430:** 312–316

134 Nishi A., Snyder G. L. and Greengard P. (1997) Bidirectional regulation of DARPP-32 phosphorylation by dopamine. J. Neurosci. **17:** 8147–8155

135 Svenningsson P., Lindskog M., Rognoni F., Fredholm B. B., Greengard P. and Fisone G. (1998) Activation of adenosine A_{2A} and dopamine D_1 receptors stimulates cyclic AMP-dependent phosphorylation of DARPP-32 in distinct populations of striatal projection neurons. Neuroscience **84:** 223–228

136 Surmeier D. J., Bargas J., Hemmings H. C., Nairn A. C. and Greengard P. (1995) Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. Neuron **14:** 385–397

137 Snyder G. L., Fienberg A. A., Huganir R. L. and Greengard P. (1998) A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. J. Neurosci. **18:** 10297–10303

138 Snyder G. L., Allen P. B., Fienberg A. A., Valle C. G., Huganir R. L., Nairn A. C. et al. (2000) Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. J. Neurosci. **20:** 4480–4488

139 Flores-Hernandez J., Hernandez S., Snyder G. L., Yan Z., Fienberg A. A., Moss S. J. et al. (2000) D_1 dopamine receptor activation reduces $GABA_A$ receptor currents in neostriatal neurons through a PKA/DARPP-32/PP-1 signaling cascade. J. Neurophysiol. **83:** 2996–3004

140 Bibb J. A., Chen J., Taylor J. R., Svenningsson P., Nishi A., Snyder G. L. et al. (2001) Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature **410:** 376–380

141 Svenningsson P., Lindskog M., Ledent C., Parmentier M., Greengard P., Fredholm B. B. et al. (2000) Regulation of the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa in vivo by dopamine D_1, dopamine D_2 and adenosine A_{2A} receptors. Proc. Natl. Acad. Sci. USA **97:** 1856–1860

142 Svenningsson P., Le Moine C., Fisone G. and Fredholm B. B. (1999) Distribution, biochemistry and function of striatal adenosine A_{2A} receptors. Prog. Neurobiol. **59:** 355–396

143 Grafe M. R., Forno L. S. and Eng L. F. (1985) Immunocytochemical studies of substance P and met-enkephalin in the basal ganglia and substantia nigra in Huntington's, Parkinson's and Alzheimer's disease. J. Neuropathol. Exp. Neurol. **44:** 47–59

144 Nisbet A. P., Foster O. J., Kingsbury A., Eve D. J., Daniel S. E., Marsden C. D. et al. (1995) Preproenkephalin and preprotachykinin messenger RNA expression in normal human basal ganglia and in Parkinson's disease. Neuroscience **66:** 361–376

145 Gerfen C. R., Engber T. M., Mahan L. C., Susel Z., Chase T. N., Monsma J. F. J. et al. (1990) D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science **250:** 1429–1432

146 Augood S. J., Emson P. C., Mitchell I. J., Boyce S., Clarke C. E. and Crossman A. R. (1989) Cellular localisation of enkephalin gene expression in MPTP-treated cynomolgus monkeys. Mol. Brain Res. **6:** 85–92

147 Ungerstedt U. and Arbuthnott G. W. (1970) Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. Brain Res. **24:** 485–493

148 Ungerstedt U. (1971) Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. **367:** 69–93

149 Casas M., Ferré S., Cobos A., Grau J. N. and Jané F. (1989) Relationship between rotational behaviour induced by apomorphine and caffeine in rats with unilateral lesions of the nigrostriatal pathway. Neuropsychopharmacology **28:** 407–409

150 Fredholm B. B., Herrera-Marschitz M., Jonzon B., Lindström K. and Ungerstedt U. (1983) On the mechanism by which methylxanthines enhance apomorphine-induced rotation behaviour in the rat. Pharmac. Biochem. Behav. **19:** 535–541

151 Fuxe K. and Ungerstedt U. (1974) Action of caffeine and theophyllamine on supersensitive dopamine receptors: considerable enhancement of receptor response to treatment with DOPA and dopamine receptor agonists. Med. Biol. **52:** 48–54

152 Garrett B. E. and Holtzman S. G. (1995) Does adenosine receptor blockade mediate caffeine-induced rotational behavior? J. Pharmacol. Exp. Ther. **274:** 207–214

153 Herrera-Marschitz M., Casas M. and Ungerstedt U. (1988) Caffeine produces contralateral rotation in rats with unilateral dopamine denervation: comparisons with apomorphine-induced responses. Psychopharmacology (Berl.) **94:** 38–45

154 Jiang H., Jackson-Lewis V., Muthane U., Dollison A., Ferreira M., Espinosa A. et al. (1993) Adenosine receptor antagonists potentiate dopamine receptor agonist-induced rotational behavior in 6-hydroxydopamine-lesioned rats. Brain Res. **613:** 347–351
155 Wooten G. F. (2001) Anatomy and function of dopamine receptors: understanding the pathophysiology of fluctuations in Parkinson's disease. Park. Rel. Dis. **8:** 79–83
156 Pinna A., Fenu S. and Morelli M. (2001) Motor stimulant effects of the adenosine A(2A) receptor antagonist SCH 58261 do not develop tolerance after repeated treatments in 6-hydroxydopamine-lesioned rats. Synapse **39:** 233–238
157 Fenu S., Pinna A., Ongini E. and Morelli M. (1997) Adenosine A_{2A} receptor antagonism potentiates L-DOPA-induced turning behaviour and *c-fos* expression in 6-hydroxydopamine-lesioned rats. Eur. J. Pharmacol. **321:** 143–147
158 Koga K., Kurokawa M., Ochi M., Nakamura J. and Kuwana Y. (2000) Adenosine A(2A) receptor antagonists KF17837 and KW-6002 potentiate rotation induced by dopaminergic drugs in hemi-Parkinsonian rats. Eur. J. Pharmacol. **408:** 249–255
159 Richardson P. J., Kase H. and Jenner P. (1997) Adenosine A_{2A} receptor antagonists as new agents for the treatment of Parkinson's disease. Trends Neurosci. **18:** 338–344
160 Grondin R., Bedard P. J., Hadj Tahar A., Gregoire L., Mori A. and Kase H. (1999) Antiparkinsonian effect of a new selective adenosine A2A receptor antagonist in MPTP-treated monkeys. Neurology **52:** 1673–1677
161 Kanda T., Jackson M. J., Smith L. A., Pearce R. K., Nakamura J., Kase H. et al. (2000) Combined use of the adenosine A(2A) antagonist KW-6002 with L-DOPA or with selective D1 or D2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. Exp. Neurol. **162:** 321–327
162 Kim D. S. and Palmiter R. D. (2003) Adenosine receptor blockade reverses hypophagia and enhances locomotor activity of dopamine-deficient mice. Proc. Natl. Acad. Sci. USA **100:** 1346–1351
163 Bové J., Marin C., Bonastre M. and Tolosa E. (2002) Adenosine A_{2A} antagonism reverses levodopa-induced motor alterations in hemiparkinsonian rats. Synapse **46:** 251–257
164 Fredduzzi S., Moratalla R., Monopoli A., Cuellar B., Xu K., Ongini E. et al. (2002) Persistent behavioral sensitization to chronic L-DOPA requires A_{2A} adenosine receptors. J. Neurosci. **22:** 1054–1062
165 Lundblad M., Vaudano E. and Cenci M. A. (2003) Cellular and behavioural effects of the adenosine A_{2a} receptor antagonist KW-6002 in a rat model of L-DOPA-induced dyskinesia. J. Neurochem. **84:** 1398–1410
166 Winkler C., Kirik D., Björklund A. and Cenci A. M. (2002) L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. Neurobiol. Dis. **10:** 165–186
167 Lundblad M., Andersson M., Winkler C., Kirik D., Wierup N. and Cenci A. M. (2002) Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. Eur. J. Neurosci. **15:** 120–132
168 Cenci A. M., Whishaw I. Q. and Schallert T. (2002) Animal models of neurological deficits: how relevant is the rat? Nat. Rev. Neuroscience **3:** 574–579
169 Kartzinel R., Shoulson I. and Calne D. B. (1976) Studies with bromocriptine: III. Concomitant administration of caffeine to patients with idiopathic parkinsonism. Neurology **26:** 741–743
170 Shoulson I. and Chase T. N. (1975) Caffeine and the antiparkinsonian response to levodopa or piribedil. Neurology **25:** 722–724
171 Mally J. and Stone T. W. (1994) The effect of theophylline on parkinsonian symptoms. J. Pharm. Pharmacol. **46:** 515–517
172 El Yacoubi M., Ledent C., Parmentier M., Costentin J. and Vaugeois J. M. (2000) The anxiogenic-like effect of caffeine in two experimental procedures measuring anxiety in the mouse is not shared by selective A_{2A} adenosine receptor antagonists. Psychopharmacology (Berl.) **148:** 153–163
173 Ross G. W., Abbott R. D., Petrovich H., Morens D. M., Grandinetti A., Tung K.-H. et al. (2000) Association of coffee and caffeine intake with the risk of Parkinson disease. JAMA **283:** 2674–2679
174 Ascherio A., Zhang S. M., Hernán M. A., Kawachi I., Colditz G. A., Speizer F. E. et al. (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann. Neurol. **50:** 56–63
175 Chen J.-F., Xu K., Petzer J. P., Staal R., Xu Y.-H., Beilstein M. A. et al. (2001) Neuroprotection by caffeine and A2A adenosine receptor inactivation in a model of Parkinson's disease. J. Neurosci. **21:** RC143 (1–6)
176 Ikeda K., Kurokawa M., Aoyama S. and Kuwana Y. (2002) Neuroprotection by adenosine A_{2A} receptor blockade in experimental models of Parkinson's disease. J. Neurochem. **80:** 262–270
177 Bona E., Ådén U., Gilland E., Fredholm B. B. and Hagberg H. (1997) Neonatal cerebral hypoxia-ischemia: the effect of adenosine receptor antagonists. Neuropharmacology **36:** 1327–1338
178 Jones P. A., Smith R. A. and Stone T. W. (1998) Protection against hippocampal kainate excitotoxicity by intracerebral administration of an adenosine A_{2A} receptor antagonist. Brain Res. **800:** 328–335
179 Monopoli A., Lozza G., Forlani A., Mattavelli A. and Ongini E. (1998) Blockade of adenosine A2A receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. Neuroreport **9:** 3955–3959
180 Chen J. F., Huang Z., Ma J., Zhu J., Moratalla R., Standaert D. et al. (1999) A_{2A} adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J. Neurosci. **19:** 9192–9200
181 Rodriguez M. C., Obeso J. A. and Olanow C. W. (1998) Subthalamic nucleus-mediated excitotoxicity in Parkinson's disease: a target for neuroprotection. Ann. Neurol. **44:** S175–S188
182 Bedingfield J. B., King D. A. and Holloway F. A. (1998) Cocaine and caffeine: conditioned place preference, locomotor activity and additivity. Pharmacol. Biochem. Behav. **61:** 291–296
183 Misra A. L., Vadlamani N. L. and Pontani R. B. (1986) Effect of caffeine on cocaine locomotor stimulant activity in rats. Pharmacol. Biochem. Behav. **24:** 761–764
184 Brockwell N. T., Eikelboom R. and Benninger R. J. (1991) Caffeine- induced place and taste conditioning: production of dose-dependent preference and aversion. Pharmacol. Biochem. Behav. **38:** 513–517
185 Holtzman S. G., Mante S. and Minneman K. P. (1991) Role of adenosine receptors in caffeine tolerance. J. Pharmacol. Exp. Ther. **256:** 62–68
186 Nehlig A. (1999) Are we dependent upon coffee and caffeine? A review on human and animal data. Neurosci. Biobehav. Rev. **23:** 563–576
187 American Psychiatric Association (APA) (1992) Diagnostic and Statististical Manual of Mental Disorders, 4th edn, American Psychiatric Association, Washington, DC
188 Evans S. M. and Griffiths R. R. (1999) Caffeine withdrawal: a parametric analysis of caffeine dosing conditions. J. Pharmacol. Exp. Ther. **289:** 285–294
189 Svenningsson P., Nomikos G. G. and Fredholm B. B. (1999) The stimulatory action and the development of tolerance to caffeine is associated with alterations in gene expression in specific brain regions. J. Neurosci. **19:** 4011–4022
190 Halldner L., Lozza G., Lindstrom K. and Fredholm B. B. (2000) Lack of tolerance to motor stimulant effects of a selective adenosine A(2A) receptor antagonist. Eur. J. Pharmacol. **406:** 345–354