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The mouse brain adenosine A_1 receptor: functional expression and pharmacology

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Abstract

The adenosinergic system is involved in many important physiological functions. Adenosine exerts its extracellular effects through four types of G-protein-coupled receptors: A_1, A_{2A}, A_{2B} and A_3. Adenosine acts as an important regulator of metabolic processes. In the brain adenosine mediates prominent neuroprotective functions via the adenosine A_1 receptor. Whereas the pharmacological characteristics of the rat and human adenosine A_1 receptor have been intensively studied, the mouse adenosine A_1 receptor has not yet been characterised. Accordingly, we have cloned the mouse brain adenosine A_1 receptor and present here a pharmacological characterisation of the mouse adenosine A_1 receptor using functional studies and radioligand binding assays. The results show that the binding affinities of several ligands for the mouse adenosine A_1 receptor are similar to the affinities for the rat and human adenosine A_1 receptor with some exceptions.

Keywords: Adenosine receptor; Pharmacology; Brain; Mouse; cAMP assay

1. Introduction

The four distinct adenosine receptor subtypes, A_1, A_{2A}, A_{2B} and A_3, belong to the family of G-protein-coupled receptors (Fredholm et al., 1994). Originally adenosine A_1 and A_3 receptors were described to interact mainly with G_i-proteins and induce inhibition of adenylyl cyclase whereas adenosine A_{2A} and A_{2B} receptors are coupled mainly to G_s-proteins, consequently stimulate adenylyl cyclase and increase cAMP levels (Fredholm et al., 2001; Olah and Stiles, 1995). However, since adenosine receptors have also been reported to interact with different G-proteins and signal through various other pathways, independent of adenylyl cyclase, as reviewed recently (Schulte and Fredholm, 2003), the above mentioned classification should be regarded as historical and may not solely reflect the situation in vivo.

Since adenosine was first described to be involved cardiovascular regulation (Drury and Szent-Gyorgyi, 1929), extensive research has been performed and a large number of physiological functions of adenosine has been described (for reviews see: Dunwiddie, 1985; Kaiser and Quinn, 1999; Linden, 2001; Mubagwa and Flameng, 2001; Ribeiro et al., 2002; Stone, 2002; Williams and Jarvis, 2000). After the cloning of the first adenosine receptor (Libert et al., 1989), different adenosine receptor subtypes have been characterised. The adenosine A_1 receptor has been cloned from several species: (Aguilar et al., 1995; Libert et al., 1992; Mahan et al., 1991; Meng et al., 1994; Olah et al., 1992). Fewer reports described the cloning of adenosine A_3 (Murrison et al., 1996; Zhao et al., 1999; Zhou et al., 1992), adenosine A_{2A} (Marquardt et al., 1994) and adenosine A_{2B} (Pierce et al., 1992) receptor subtypes.

Mouse strains with targeted deletions of adenosine A_1, A_{2A} and A_3 receptor subtypes have been generated (Johansson et al., 2001; Ledent et al., 1997; Zhao et al., 2000). These transgenic mice prove to be a useful tool to unravel the function of these adenosine receptor subtypes (Nyce, 1999). Despite the large interest in mouse adenosine A_1 receptors, its pharmacological properties are largely unknown (Fredholm et al., 2001). This is in contrast to the human and rat adenosine receptors, which have been well

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characterised in pharmacological studies. In order to elucidate the pharmacological properties of the mouse adenosine A_1 receptor, we have cloned the adenosine A_1 receptor from mouse brain and expressed it in Chinese hamster ovary (CHO) cells for pharmacological characterisation and functional studies. Additionally, further binding studies were performed on rat and human adenosine A_1 receptor in order to compare the binding affinities for all three species.

2. Materials and methods

2.1. Reagents

Phosphate-buffered saline, Dulbecco's modified Eagle's medium (DMEM), DMEM-F12, fetal calf serum and penicillin/streptomycin were obtained from Gibco. Reverse transcriptase enzyme and buffer were purchased from Promega. Taq polymerase and buffer for polymerase chain reaction (PCR) were obtained from Eppendorf (Boulder, USA). PCR primers were ordered from Genset oligo's. N^6-cyclopentyladenosine (CPA), R-N^6-phenylisopropyladenosine (R-PIA), cyclopentyltheophylline (CPT), N^6-cyclopentyl-9-methyladenine (N-0840) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were obtained from RBI (Natick, MA, USA). *N*-ethylcarboxamidoadenosine (NECA), 2-chloro-N^6-cyclopentyladenosine (CCPA) and forskolin were purchased from Sigma (St. Louis, MO, USA), while adenosine deaminase was from Roche Biochemicals (Mannheim, Germany). 2-[*p*-(2-Carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680) was from Ciba Geigy. 4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241358) was from Zeneca Pharmaceuticals. [^{3}H]DPCPX was purchased from Amersham, whereas [^{3}H]cAMP was from NEN (Du Pont Nemours, 's-Hertogenbosch, NL). GTP was obtained from Aldrich. 2-Chloro-N^6-(3-iodobenzyl)-*N*-methyl-5′-carbamoyladenosine (Cl-IB-MECA) was a kind gift from K.A. Jacobson, NIH, USA.

2.2. Glial cultures

Mixed astrocyte cell cultures were established as described previously (Biber et al., 1997). In brief, mouse cortex was dissected from newborn mouse pups (< 1 day). Brain tissue was gently dissociated by trituration in phosphate-buffered saline and filtered through a cell strainer (70 mm ∅, Falcon) into Dulbecco's modified Eagle's medium (DMEM). After two washing steps (200 × *g* for 10 min), cells were seeded in culture flasks (75 cm^2,Greiner; 10×10^6 cells/flask). Cultures were maintained up to 4 weeks in DMEM containing 10% fetal calf serum with 0.01% penicillin and 0.01% streptomycin in a humidified atmosphere (5% CO_2) at 37 °C. Culture medium was changed the second day after preparation and every 6 days thereafter.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

Cells were lysed in guanidinium isothiocyanate/mercaptoethanol buffer and total RNA was extracted with one phenol/chloroform step, precipitated according to Chomczynski and Sacchi (1987). Subsequently RNA was treated with RNAse free DNAse (Sigma-Aldrich, Bornhem, Belgium) for 2 h and precipitated. Reverse transcription (RT): 1 μg of total RNA was transcribed into cDNA in a total volume of 25 μl containing 0.5 μl of M-MLV reverse transcriptase, 1 μl of RNase inhibitor, 1 μl of random hexamers (0.2 mM), 5 ml of 5 × buffer, 5 μl of deoxynucleosidetriphosphates (dNTPs) (2.5 mM) and H_2O adjusted to 25 μl. After 60 min at 42 °C, the reaction was stopped by heating at 95° for 5 min. The quality of the cDNA was controlled using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (Biber et al., 2001) and potential contamination by genomic DNA was checked for by running the reactions without reverse transcriptase and using GAPDH primers in subsequent polymerase chain reaction (PCR) amplifications. PCR: 2 μl of the RT reaction was used in subsequent PCR amplification as described previously (Biber et al., 1997). In brief, the following reagents were added: 5 μl 10 × PCR buffer, 2.5 μl $MgCl_2$ (50 mM), 0.5 μl dNTPs (10 mM), 1 μl of each primer, 38 μl H_2O and 0.1 μl Taq polymerase. Primer sequences for mouse full-length adenosine A_1 receptor: forward primer: 5′-CATGCCGCCGTACATCTC-3′; backward primer: 5′-TCTAGTCCTCAGCTTTCTCCTC-3′. Cycle numbers were 35 and annealing temperature was 58 °C. The sequence of the PCR product was verified by TA cloning into pCRII (Invitrogen). In brief, PCR products were stored on ice after the amplification; 20 μl of the resulting PCR product was checked by gel electrophoresis and 2 μl was used for ligation into linearized pCRII (Invitrogen) according to the instructions of the manufacturer. The ligation product was used to transform competent bacteria (TOP-10F, Invitrogen) and resulting bacterial colonies were grown overnight in 5 ml LB medium. Plasmid preparation was done by standard methods and positive plasmids were sequenced by ALF (sequencing facility; University of Groningen). A *Xho*I/*Bam*HI fragment, containing the full-length adenosine A_1 receptor coding region, was excised from the PCR II vector clone and ligated into a *Xho*I/*Bam*HI digested pcDNA3.1(−) expression vector (Invitrogen), incorporating a neomycin resistance gene. Sequence analysis was performed to check the orientation of the adenosine A_1 receptor insert.

2.4. Stable expression in Chinese hamster ovary (CHO) cells

CHO cells were cultured at 37 °C in cell culture flasks (25 cm^2, Corning), containing 5 ml DMEM-F12 medium, supplemented with 10% fetal calf serum and 2 mM L-glutamine

in a humidified atmosphere (5% CO_2). Cells were seeded into a six-well dish and transfected with 1 μg of the pcDNA3.1(−)-mouse adenosine A_1 receptor expression vector, using 3 μl of fuGENE 6 transfection reagent (Roche). After transfection, cells were seeded in cell culture flasks. After 2 weeks of selection with 50 μg/ml G 418, monoclonal CHO cell lines, stably expressing the mouse adenosine A_1 receptor (CHO-mA_1), were generated and adenosine A_1 receptor mRNA expression levels of 16 clones were determined by RT-PCR. The CHO-mA_1 cell line with highest expression levels was used for binding studies and cAMP measurements.

2.5. Radioligand binding assay

For membrane preparation CHO-mA_1 cells were cultured in cell culture flasks (75 cm^2,Greiner), grown to confluency and harvested by trypsinization for 30 s with 0.25% w/v porcine trypsin at 37 °C. The cell suspension was spun for 10 min at 500 g. After removing the supernatant, the pellet was resuspended in 50 mM Tris/HCl, pH 7.4 (at 25 °C, approximately 10×10^6 cells/ml) and spun for 40 min (4 °C) at 18,000 × *g*.

After discarding the supernatant, the pellet was resuspended in 50 mM Tris/HCl, pH 7.4 (at 4 °C, approximately 20×10^6 cells/ml) and stored at −80 °C until analysis.

Rat cortical membranes were prepared according to the method of Lohse et al. (1984) and incubated with 2 IU/ml adenosine deaminase for 30 min at 37 °C before storage (Pirovano et al., 1989). CHO-hA_1 cell membranes were prepared as described previously (Dalpiaz et al., 1998). For displacement studies, membranes (75 μg) were incubated for 1 h at 25 °C in 50 mM Tris/HCl (pH 7.4) in the presence of 1.6 nM [^{3}H]DPCPX and different concentrations of ligand to determine the K_i. To determine nonspecific binding 100 μM CPA was used. Total volume during incubation was 200 μl.

Incubations were stopped by rapid dilution with 1 ml ice-cold buffer and bound radioligand was subsequently recovered by filtration through Whatman GF/B filters using either Millipore system or Brandel Harvester under reduced pressure. Filters were then washed three times with 2 ml buffer. The retained radioactivity was measured by liquid scintillation counting (LKB Wallac, 1219 Rackbeta).

Saturation experiments were carried out under the similar conditions. Increasing concentrations of [^{3}H]DPCPX (0–4 nM) were used. Filters were washed five times with 2.5 ml buffer to remove excess of radioligand.

2.6. cAMP accumulation assay

CHO-mA_1 cells were seeded in 24-well plate at a density of 2×10^5 cells/well. The next day, growth medium was aspirated and washed twice with DMEM/HEPES (pH 7.4). Cells were then incubated for 30 min at 37 °C

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mouse   MPPYISAFQA  AYIGIEVLIA  LVSVPGNVLV  IWAVKVNQAL  RDATFCFIVS   50
rat     MPPYISAFQA  AYIGIEVLIA  LVSVPGNVLV  IWAVKVNQAL  RDATFCFIVS
human   MPPSISAFQA  AYIGIEVLIA  LVSVPGNVLV  IWAVKVNQAL  RDATFCFIVS

mouse   LAVADVAVGA  LVIPLAILIN  IGPQTYFHTC  LMVACPVLIL  TQSSILALLA  100
rat     LAVADVAVGA  LVIPLAILIN  IGPQTYFHTC  LMVACPVLIL  TQSSILALLA
human   LAVADVAVGA  LVIPLAILIN  IGPQTYFHTC  LMVACPVLIL  TQSSILALLA

mouse   IAVDRYLRVK  IPLRYKTVVT  QRRAAVAIAG  CWILSLVVGL  TPMFGWNNLS  150
rat     IAVDRYLRVK  IPLRYKTVVT  QRRAAVAIAG  CWILSLVVGL  TPMFGWNNLS
human   IAVDRYLRVK  IPLRYKMVVT  PRRAAVAIAG  CWILSFVVGL  TPMFGWNNLS

mouse   EVEQAWIANG  SVGEPVIKCE  FEKVISMEYM  VYFNFFVWVL  PPLLLMVLIY  200
rat     VVEQDWRANG  SVGEPVIKCE  FEKVISMEYM  VYFNFFVWVL  PPLLLMVLIY
human   AVERAWAANG  SMGEPVIKCE  FEKVISMEYM  VYFNFFVWVL  PPLLLMVLIY

mouse   LEVFYLIRKQ  LNKKVSASSG  DPQKYYGKEL  KIAKSLALIL  FLFALSWLPL  250
rat     LEVFYLIRKQ  LNKKVSASSG  DPQKYYGKEL  KIAKSLALIL  FLFALSWLPL
human   LEVFYLIRKQ  LNKKVSASSG  DPQKYYGKEL  KIAKSLALIL  FLFALSWLPL

mouse   HILNCITLFC  PTCQKPSILI  YIAIFLTHGN  SAMNPIVYAF  RIHKFRVTFL  300
rat     HILNCITLFC  PTCQKPSILI  YIAIFLTHGN  SAMNPIVYAF  RIHKFRVTFL
human   HILNCITLFC  PSCHKPSILT  YIAIFLTHGN  SAMNPIVYAF  RIQKFRVTFL

mouse   KIWNDHFRCQ  PKPPIEEDLP  EEKAED                            326
rat     KIWNDHFRCQ  PKPPIDEDLP  EEKAED
human   KIWNDHFRCQ  PAPPIDEDLP  EERPDD
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Fig. 1. Alignment of adenosine A_1 receptor amino acid sequences for mouse (AC: AJ555877), rat (AC: M69045) and human (AC: S56143). Differences between the mouse and rat sequence are shown in black. Differences between the human sequence compared to rat and/or mouse are shaded. The mouse protein sequence presented here was identical to the previously described mouse sequence (AC: U05671) except at position 10 (in italics) where a glycine was found at this position instead of the alanine in the rat, human and the mouse sequence presented here.

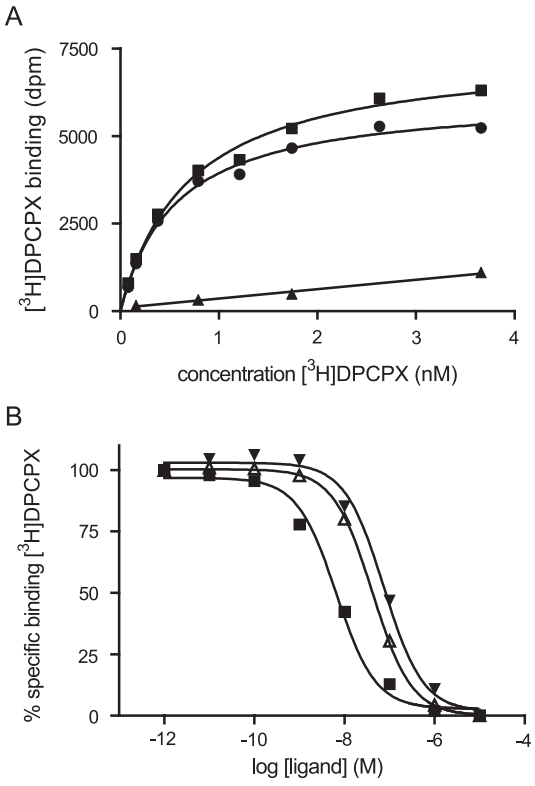

Fig. 2. (A) Saturation analysis of [^{3}H]DPCPX binding to membranes of CHO cells expressing the mouse adenosine A_1 receptor (■: total binding; ▲: nonspecific binding; ●: specific binding). A representative experiment is shown. (B) Displacement studies of specific [^{3}H]DPCPX binding (1.6 nM) from membranes of CHO cells expressing the mouse adenosine A_1 receptor by CPA (■), by CPA + GTP (10^{-4} M) (△) and by R-PIA (▼). A representative experiment is shown.

with DMEM/HEPES supplemented with adenosine deaminase (2 IU/ml), cilostamide (50 μM) and rolipram (50 μM). After incubation different concentrations of CPA, ranging from 1 nM till 100 μM, were added for another 10 min. Subsequently, forskolin (10 μM) was added. After another 15 min, the cAMP generation was stopped by aspirating incubation medium and adding 200 μl ice-cold 0.1 N HCl to the cells.

The amount of cAMP was determined by competition with [^{3}H]cAMP for protein kinase A binding protein (PKA). Briefly, sample or cAMP standard (0–16 pmol), 1.8 nM [^{3}H]cAMP and PKA solution were incubated on ice for at least 2.5 h. The incubation was stopped by dilution with ice-cold Tris/HCl buffer (pH 7.4). Bound radioactivity was recovered by filtration through Whatman GF/C filters using a Brandel harvester.

2.7. Data analysis

All radioligand binding data were analysed using the Software program PRISM 3.0 (GraphPad, San Diego, CA), based on nonlinear curve fitting procedures, yielding K_D and B_{max} values in case of saturation binding experiments and K_i values in case of displacement studies. Data derived from cAMP determinations were also analysed using PRISM (GraphPad), providing EC_{50} values.

3. Results

3.1. Sequence

The sequence that was obtained for the present adenosine A_1 receptor gene cloned from mouse brain (Fig. 1, accession number (AC): AJ555877) showed 99% similarity with the sequence suggested to be the mouse adenosine A_1 receptor previously cloned by Marquardt et al. (1994) (AC: U05671), 95% similarity with the rat adenosine A_1 receptor (AC: M69045) and 89% similarity with the human adenosine A_1 receptor (AC: S56143). See Fig. 1 for an alignment of the protein sequences of the mouse, rat and human adenosine A_1 receptor. The previously cloned mouse adenosine A_1 receptor (Marquardt et al., 1994) differs from the present receptor sequence at position 10. We describe here an alanine, which is also found in the rat and human sequence, whereas the previously described sequence showed a glycine at this position.

3.2. Radioligand binding

Saturation binding experiments were performed at CHO-mA_1 membranes, using the ligand [^{3}H]DPCPX. A typical experiment is shown in Fig. 2A. Analysis of three experiments resulted in a K_D of 0.53 nM with a 95% confidence limit of 0.44–0.63 nM and a B_{max} of 97 ± 60 (standard deviation) fmol/mg. Competition binding experiments were

Table 1
Binding affinities (K_i values ± S.E.M. in nM or % displacement) of reference ligands for adenosine A_1 receptors from mouse, rat and human

	Mouse[a]	Rat[b]	Human[c]
CCPA	21 ± 2	8.1 ± 0.4	6.4 ± 1.8[d]
CPA	4.2 ± 2.7	6.1 ± 0.8	10 ± 1
R-PIA	14 ± 3	11 ± 5[e]	n.d.
NECA	238 ± 55	98 ± 22	12 (9.6–15)[d]
DPCPX	1.4 ± 0.1	0.73 ± 0.08	1.6 ± 0.1
8-CPT	12 ± 2	11 ± 3	36 ± 2
N0840	303 ± 70	182 ± 53	1081 ± 69
ZM 241385	116 ± 18	n.d.	n.d.
CGS 21680	3–5% (10^{-6})[f]	n.d.	n.d.
	18–20% (10^{-5})[f]	n.d.	n.d.
Cl-IB-MECA	10–12% (10^{-6})[f]	n.d.	n.d.
	45–49% (10^{-5})[f]	n.d.	n.d.

[a] Membranes of CHO cells expressing the mouse adenosine A_1 receptor.
[b] Rat brain cortical membranes.
[c] Membranes of CHO cells expressing the human adenosine A_1 receptor.
[d] K_i value with 95% confidence interval; value taken from Beukers et al. (2003).
[e] Value taken from Van der Wenden et al. (1995).
[f] % displacement of specific binding at the designated molar concentration ($n = 2$).

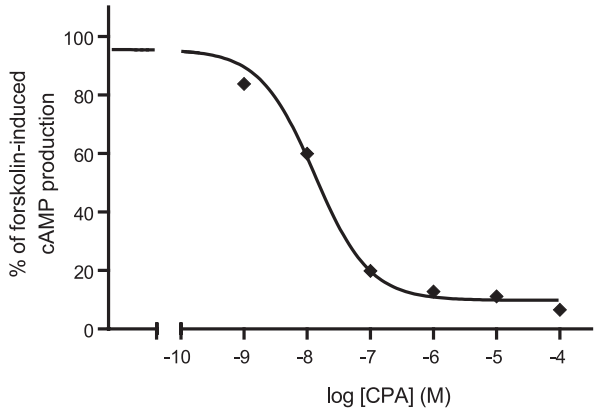

Fig. 3. Inhibition of forskolin-stimulated cAMP production by CPA in CHO cells expressing the mouse adenosine A_1 receptor. A representative experiment is shown.

performed with the following ligands: the selective adenosine A_1 receptor agonists CCPA, CPA and R-PIA, the nonselective adenosine receptor agonist NECA and the selective adenosine A_1 receptor antagonists CPT, DPCPX and N0840. Furthermore, binding affinities towards the mouse adenosine A_1 receptor were determined for ligands selective for other adenosine receptors: the adenosine A_{2A} receptor agonist CGS21680, the adenosine A_{2A} receptor antagonist ZM241385 and the selective adenosine A_3 receptor agonist Cl-IB-MECA. Fig. 2B shows a representative competition experiment of increasing concentrations of selective agonists CPA and R-PIA with the radioligand [^{3}H]DPCPX. Adding GTP (1 mM) shifted the displacement curve of CPA to the right, resulting in a K_i value of 4.2 ± 2.7 nM in the control situation in contrast to a K_i of 17 ± 8 nM in the presence of GTP. This finding suggested agonistic activity of CPA at CHO-mA_1 membranes, which was further corroborated by functional studies (see below). An overview of the affinity values of the binding assays is provided in Table 1.

3.3. cAMP measurements

In recombinant CHO cells stably expressing the mouse adenosine A_1 receptor baseline cAMP levels were too low to allow analysis of A_1 receptor-induced inhibition of cAMP production. Therefore, cAMP levels were raised by adding forskolin at a concentration of 10 μM. Co-administration of 1 nM up to 100 μM of the selective adenosine A_1 receptor agonist CPA concentration-dependently inhibited the forskolin-induced cAMP accumulation in CHO-mA_1 cells (Fig. 3). An EC_{50} value of 16.7 ± 2.2 (S.E.M.) nM was calculated from three independent experiments.

4. Discussion

The adenosinergic system has been studied intensively for several decades. A large number of physiological and metabolic functions of adenosine have been described. Thus adenosine is essentially involved in the coupling of metabolic requirement to energy supply. In order to control metabolic rate, adenosine, for example, retains neuronal firing, increases heart rate and increases blood flow by inducing vasodilatation of smooth muscle cells (Cunha, 2001; Dirnagl et al., 1994; Haas and Selbach, 2000; Mubagwa and Flameng, 2001; Tabrizchi and Bedi, 2001). Furthermore, adenosine is also involved in other functions such as inducing sleep and causing analgesic effects and anti-inflammatory effects (Dunwiddie, 1985; Fredholm et al., 1999; Linden, 2001).

Especially in the brain adenosine shows its importance as a metabolic control factor. Adenosine inhibits the release of excitatory neurotransmitters and causes a hyperpolarization of the membrane potential in neurons. Adenosine exerts these neuroprotective effects mainly by stimulation of adenosine A_1 receptors present throughout the brain (Dunwiddie, 1985; Rudolphi and Schubert, 1996). Although adenosine is obviously involved in essential physiological functions, studies show that adenosine A_1 receptor deficient mice function relatively normal compared to wild type mice (Gimenez-Llort et al., 2002; Johansson et al., 2001). However, exposed to pathophysiological conditions like hypoxia, mice lacking adenosine A_1 receptors show more neuronal damage and have a lower survival rate. It is therefore concluded that adenosine A_1 receptors are primarily important in mediating effects of adenosine during pathophysiological conditions (Gimenez-Llort et al., 2002; Johansson et al., 2001).

Despite the fact that human and rat adenosine receptors have been extensively characterised, the pharmacological properties of the mouse adenosine receptors have not yet been investigated in detail compared to other species (Fredholm et al., 2001). The two previous studies that investigated binding affinities of adenosine A_1 receptor ligands in membrane fractions from fresh material, used only a limited set of adenosine receptor ligands (Maemoto et al., 1997; Tatsis-Kotsidis and Erlanger, 1999). Therefore, we have cloned the adenosine A_1 receptor from mouse brain, expressed it in CHO cells, a cell line that has been successfully used in a detailed study on rat and human adenosine receptors (Klotz et al., 1998) and studied its characteristics in radioligand binding assays and second messenger studies.

The sequence of the present adenosine A_1 receptor gene cloned from mouse brain was almost identical to the mouse adenosine A_1 receptor gene that was previously described by Marquardt and colleagues (Marquardt et al., 1994) and the sequence was similar to the rat and human adenosine A_1 receptor sequence. The radioligand binding assays revealed that the binding characteristics of the mouse receptor in this study are similar to the characteristics of the rat and human adenosine A_1 receptor (Table 1), although the nonselective ligand NECA showed a slightly lower affinity for the mouse receptor than for the rat receptor. Moreover, inhibition of cAMP production was observed after receptor stimulation

with CPA with an EC_{50} value of 16.7 ± 2.2 nM. The results of the radioligand binding and cAMP assays show that ligands commonly used to study the rat and human adenosine A_1 receptor have similar binding affinities to the mouse adenosine A_1 receptor. It is thus concluded that these ligands can be used to study pharmacological and physiological effects of the mouse adenosine A_1 receptor.

In summary, we have cloned the mouse adenosine A_1 receptor, which showed to be functional after expression in CHO cells. Our results provide a specification of mouse adenosine A_1 receptor pharmacology. This information will be of use for the characterisation of physiological effects of the adenosine A_1 receptor in mouse models.

References

Aguilar, J.S., Tan, F., Durand, I., Green, R.D., 1995. Isolation and characterization of an avian A_1 adenosine receptor gene and a related cDNA clone. Biochem. J. 307 (Pt. 3), 729–734.

Beukers, M.W., Wanner, M.J., von Frijtag Drabbe Kunzel, J.K., Klaasse, E.C., IJzerman, A.P., Koomen, G.J., 2003. N^6-cyclopentyl-2-(3-phenylaminocarbonyltriazene-1-yl)adenosine (TCPA), a very selective agonist with high affinity for the human adenosine A_1 receptor. J. Med. Chem. 46, 1492–1503.

Biber, K., Klotz, K.N., Berger, M., Gebicke-Harter, P.J., van Calker, D., 1997. Adenosine A_1 receptor-mediated activation of phospholipase C in cultured astrocytes depends on the level of receptor expression. J. Neurosci. 17, 4956–4964.

Biber, K., Sauter, A., Brouwer, N., Copray, S.C., Boddeke, H.W., 2001. Ischemia-induced neuronal expression of the microglia attracting chemokine Secondary Lymphoid-tissue Chemokine (SLC). Glia 34, 121–133.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Cunha, R.A., 2001. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. Neurochem. Int. 38, 107–125.

Dalpiaz, A., Townsend-Nicholson, A., Beukers, M.W., Schofield, P.R., IJzerman, A.P., 1998. Thermodynamics of full agonist, partial agonist, and antagonist binding to wild-type and mutant adenosine A_1 receptors. Biochem. Pharmacol. 56, 1437–1445.

Dirnagl, U., Niwa, K., Lindauer, U., Villringer, A., 1994. Coupling of cerebral blood flow to neuronal activation: role of adenosine and nitric oxide. Am. J. Physiol. 267, 296–301.

Drury, A.N., Szent-Gyorgyi, A., 1929. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. J. Physiol. (Lond.) 68, 213–237.

Dunwiddie, T.V., 1985. The physiological role of adenosine in the central nervous system. Int. Rev. Neurobiol. 27, 63–139.

Fredholm, B.B., Abbracchio, M.P., Burnstock, G., Daly, J.W., Harden, T.K., Jacobson, K.A., Leff, P., Williams, M., 1994. Nomenclature and classification of purinoceptors. Pharmacol. Rev. 46, 143–156.

Fredholm, B.B., Battig, K., Holmen, J., Nehlig, A., Zvartau, 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.

Fredholm, B.B., AP, I.J., Jacobson, K.A., Klotz, K.N., Linden, J., 2001. International Union of Pharmacology: XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–552.

Gimenez-Llort, L., Fernandez-Teruel, A., Escorihuela, R.M., Fredholm, B.B., Tobena, A., Pekny, M., Johansson, B., 2002. Mice lacking the adenosine A_1 receptor are anxious and aggressive, but are normal learners with reduced muscle strength and survival rate. Eur. J. Neurosci. 16, 547–550.

Haas, H.L., Selbach, O., 2000. Functions of neuronal adenosine receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 362, 375–381.

Johansson, B., Halldner, L., Dunwiddie, T.V., Masino, S.A., Poelchen, W., Gimenez-Llort, L., Escorihuela, R.M., Fernandez-Teruel, A., Wiesenfeld-Hallin, Z., Xu, X.J., Hardemark, A., Betsholtz, C., Herlenius, E., Fredholm, B.B., 2001. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 9407–9412.

Kaiser, S.M., Quinn, R.J., 1999. Adenosine receptors as potential therapeutic targets. Drug Discov. Today 4, 542–551.

Klotz, K.N., Hessling, J., Hegler, J., Owman, C., Kull, B., Fredholm, B.B., Lohse, M.J., 1998. Comparative pharmacology of human adenosine receptor subtypes—characterization of stably transfected receptors in CHO cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 1–9.

Ledent, C., Vaugeois, J.M., Schiffmann, S.N., Pedrazzini, T., El Yacoubi, M., Vanderhaeghen, J.J., Costentin, J., Heath, J.K., Vassart, G., Parmentier, M., 1997. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A_{2a} receptor. Nature 388, 674–678.

Libert, F., Parmentier, M., Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M.J., Dumont, J.E., Vassart, G., 1989. Selective amplification and cloning of four new members of the G protein-coupled receptor family. Science 244, 569–572.

Libert, F., Van Sande, J., Lefort, A., Czernilofsky, A., Dumont, J.E., Vassart, G., Ensinger, H.A., Mendla, K.D., 1992. Cloning and functional characterization of a human A_1 adenosine receptor. Biochem. Biophys. Res. Commun. 187, 919–926.

Linden, J., 2001. Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. Annu. Rev. Pharmacol. Toxicol. 41, 775–787.

Lohse, M.J., Lenschow, V., Schwabe, U., 1984. Interaction of barbiturates with adenosine receptors in rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 326, 69–74.

Maemoto, T., Finlayson, K., Olverman, H.J., Akahane, A., Horton, R.W., Butcher, S.P., 1997. Species differences in brain adenosine A_1 receptor pharmacology revealed by use of xanthine and pyrazolopyridine based antagonists. Br. J. Pharmacol. 122, 1202–1208.

Mahan, L.C., McVittie, L.D., Smyk-Randall, E.M., Nakata, H., Monsma Jr., F.J., Gerfen, C.R., Sibley, D.R., 1991. Cloning and expression of an A_1 adenosine receptor from rat brain. Mol. Pharmacol. 40, 1–7.

Marquardt, D.L., Walker, L.L., Heinemann, S., 1994. Cloning of two adenosine receptor subtypes from mouse bone marrow-derived mast cells. J. Immunol. 152, 4508–4515.

Meng, F., Xie, G.X., Chalmers, D., Morgan, C., Watson Jr., S.J., Akil, H., 1994. Cloning and characterization of a pharmacologically distinct A_1 adenosine receptor from guinea pig brain. Brain Res. Mol. Brain Res. 26, 143–155.

Mubagwa, K., Flameng, W., 2001. Adenosine, adenosine receptors and myocardial protection: an updated overview. Cardiovasc. Res. 52, 25–39.

Murrison, E.M., Goodson, S.J., Edbrooke, M.R., Harris, C.A., 1996. Cloning and characterisation of the human adenosine A_3 receptor gene. FEBS Lett. 384, 243–246.

Nyce, J.W., 1999. Insight into adenosine receptor function using antisense and gene-knockout approaches. Trends Pharmacol. Sci. 20, 79–83.

Olah, M.E., Stiles, G.L., 1995. Adenosine receptor subtypes: characterization and therapeutic regulation. Annu. Rev. Pharmacol. Toxicol. 35, 581–606.

Olah, M.E., Ren, H., Ostrowski, J., Jacobson, K.A., Stiles, G.L., 1992. Cloning, expression, and characterization of the unique bovine A_1 adenosine receptor. Studies on the ligand binding site by site-directed mutagenesis. J. Biol. Chem. 267, 10764–10770.

Pierce, K.D., Furlong, T.J., Selbie, L.A., Shine, J., 1992. Molecular cloning and expression of an adenosine A_{2b} receptor from human brain. Biochem. Biophys. Res. Commun. 187, 86–93.

Pirovano, I.M., IJzerman, A.P., Van Galen, P.J., Soudijn, W., 1989. Influ-

ence of the molecular structure of N^6-(omega-aminoalkyl)adenosines on adenosine receptor affinity and intrinsic activity. Eur. J. Pharmacol. 172, 185–193.

Ribeiro, J.A., Sebastiao, A.M., de Mendonca, A., 2002. Adenosine receptors in the nervous system: pathophysiological implications. Prog. Neurobiol. 68, 377–392.

Rudolphi, K.A., Schubert, P., 1996. Purinergic interventions in traumatic and ischemic injury. In: Peterson, P.L., Willis, P.J. (Eds.), Novel Thearapies for CNS Injuries. CRC Press, Boca Raton, pp. 327–342.

Schulte, G., Fredholm, B.B., 2003. Signalling from adenosine receptors to mitogen-activated protein kinases. Cell. Signal. 15, 813–827.

Stone, T.W., 2002. Purines and neuroprotection. Adv. Exp. Med. Biol. 513, 249–280.

Tabrizchi, R., Bedi, S., 2001. Pharmacology of adenosine receptors in the vasculature. Pharmacol. Ther. 91, 133–147.

Tatsis-Kotsidis, I., Erlanger, B.F., 1999. A1 adenosine receptor of human and mouse adipose tissues: cloning, expression, and characterization. Biochem. Pharmacol. 58, 1269–1277.

Van der Wenden, E.M., Hartog-Witte, H.R., Roelen, H.C., von Frijtag Drabbe Kunzel, J.K., Pirovano, I.M., Mathot, R.A., Danhof, M., Van Aerschot, A., Lidaks, M.J., IJzerman, A.P., 1995. 8-Substituted adenosine and theophylline-7-riboside analogues as potential partial agonists for the adenosine A_1 receptor. Eur. J. Pharmacol. 290, 189–199.

Williams, M., Jarvis, M.F., 2000. Purinergic and pyrimidinergic receptors as potential drug targets. Biochem. Pharmacol. 59, 1173–1185.

Zhao, Z., Francis, C., Ravid, K., 1999. Characterization of the mouse A_3 adenosine receptor gene: exon/intron organization and promoter activity. Genomics 57, 152–155.

Zhao, Z., Makaritsis, K., Francis, C.E., Gavras, H., Ravid, K., 2000. A role for the A_3 adenosine receptor in determining tissue levels of cAMP and blood pressure: studies in knock-out mice. Biochim. Biophys. Acta 1500, 280–290.

Zhou, Q.Y., Li, C., Olah, M.E., Johnson, R.A., Stiles, G.L., Civelli, O., 1992. Molecular cloning and characterization of an adenosine receptor: the A_3 adenosine receptor. Proc. Natl. Acad. Sci. U. S. A. 89, 7432–7436.