Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 481 (2003) 141–146
www.elsevier.com/locate/ejphar

Cyclopentyladenosine and some of its low-efficacy derivatives inhibit striatal synaptosomal release of acetylcholine to a similar degree

Tjerk J.H. Bueters[a], Leonie M. van Duivenvoorde[a], Meindert Danhof[b], Ad P. IJzerman[c], Herman P.M. van Helden[a,*]

[a] *Research Group Medical Countermeasures, TNO Prins Maurits Laboratory, Lange Kleiweg 137, P.O. Box 45, 2280 AA Rijswijk, The Netherlands*
[b] *Gorleaus Laboratory, Department of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University, Einsteinweg 55, P.O. Box 9502, 2300 RA Leiden, The Netherlands*
[c] *Gorleaus Laboratory, Department of Medicinal Chemistry, Leiden/Amsterdam Center for Drug Research, Leiden University, Einsteinweg 55, P.O. Box 9502, 2300 RA Leiden, The Netherlands*

Received 24 March 2003; received in revised form 4 September 2003; accepted 10 September 2003

Abstract

The application of adenosine A_1 receptor agonists in regard to cerebral disorders is hampered by serious cardiovascular side effects. This problem might be circumvented by using low-efficacy agonists (partial agonists). The objective of the present study was to characterize the effects of the full agonist N^6-cyclopentyladenosine (CPA) and its low-efficacy derivatives 3′ -deoxy-CPA (3-DCPA), 8-propylamino-CPA (8-PCPA) and 8-butylamino-CPA (8-BCPA) on the 4-aminopyridine (4AP)-evoked release of [^{3}H]-acetylcholine in a rat striatal synaptosomal system. The reason for studying these partial agonists in particular was their established low cardiovascular side effect profile. CPA reached a concentration-dependent maximal inhibition of the evoked acetylcholine release of 38 ± 3%. 3-DCPA and 8-PCPA inhibited the acetylcholine release by 29 ± 5% and 38 ± 3%, respectively. On the other hand, 8-BCPA only diminished the acetylcholine release by 19 ± 3%. This inhibitory effect was reversible upon coadministration of the nonselective adenosine antagonist theophylline, but not by the selective adenosine A_{2A} receptor antagonist 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261). It is concluded that some partial adenosine A_1 receptor agonists behave as full agonists with respect to the inhibition of acetylcholine release, while lacking profound cardiovascular side effects. These preliminary results encourage further investigation of their tissue selectivity and therapeutic potential in vivo.

Keywords: Acetylcholine release; Adenosine A_1 receptor; Low-efficacy agonist; Partial agonist; Neuronal synaptosome; CPA (cyclopentyladenosine); 3D-CPA (3′-deoxy-CPA); 8-PCPA (8-propylamino-CPA); 8-BCPA (8-butylamino-CPA)

1. Introduction

The adenosine A_1 receptor agonists reduce the release of many excitatory neurotransmitters in the brain, such as glutamate (Ambrosio et al., 1997), aspartate (Simonato et al., 1994) and acetylcholine (Kirkpatrick and Richardson, 1993; Materi et al., 2000). Therefore, the adenosine A_1 receptor is considered as an attractive target for therapeutic intervention in several disorders associated with neuro-excitability, such as ischemic stroke (Von Lubitz, 1999), epilepsy (Dunwiddie, 1999) and neuropathic pain (Sollevi et al., 1995). Specifically, the inhibition of central acetylcholine release may be advantageous in the management of organophosphate intoxication in which the inhibition of acetylcholinesterase results in accumulation of acetylcholine and overstimulation of cholinergic receptors (Van Helden and Bueters, 1999).

However, the development of adenosine A_1 receptor agonists as centrally active drugs has been impeded by serious side effects due to ubiquitous receptor distribution throughout the body. This problem might be circumvented by using low-efficacy (partial) agonists (IJzerman et al., 1994). Previously it has been shown that some low-efficacy adenosine A_1 receptor agonists exhibit a much improved selectivity of action in vivo. For example, in conscious rats, the low-efficacy 8-alkylamino derivatives of N^6-cyclopentyladenosine (CPA) demonstrated similar anti-lipolytic effects as the full agonist CPA did, but the profound

* Corresponding author. Tel.: +31-15-2843240; fax: +31-15-2843963.
E-mail address: Helden@pml.tno.nl (H.P.M. van Helden).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.09.027

cardiovascular side effects of the latter are lacking (Van der Graaf et al., 1997; Van Schaick et al., 1998).

Referring to the abovementioned attractiveness of the adenosine A_1 receptor in regard to therapeutic interventions in several cerebral disorders, a relevant question is whether this selective action of low-efficacy adenosine A_1 receptor agonists also applies to the effects in the central nervous system, and in particular to the release of neurotransmitters. Differences in adenosine A_1 receptor densities (Spielman et al., 1992) and efficiency in receptor coupling between adipose, cardiac and brain tissues (Dennis et al., 1992) suggest that selective actions in the brain may be obtained. For example, Lorenzen et al. (1997) showed that the low-efficacy agonist 8-aminobutyl-CPA (8-BCPA), which exhibited minimal effects on adenosine A_1 receptor-mediated actions in cortical and cardiovascular tissues, was highly efficacious in inhibiting neurotransmission in hippocampal slices. These results encouraged further research into the selective properties of such low-efficacy compounds for central nervous tissues.

The purpose of the present study was to investigate whether low-efficacy derivatives of CPA, which appeared to lack profound cardiovascular side effects in previous studies, would demonstrate full agonist properties in regard to the reduction of acetylcholine release in the brain. As a first step in this approach, an in vitro neuronal synaptosomal system was used. The synaptosomal preparation is a well-established model to study presynaptic transmitter regulation by a wide range of different modulators, among which adenosine and various compounds selective for the adenosine A_1 receptor (Brown et al., 1990; Kirk and Richardson, 1994; Kirkpatrick and Richardson, 1993; Pedata et al., 1986).

2. Methods

2.1. Animals

Male Wistar rats, supplied by Harlan (Horst, The Netherlands), were housed with two or three animals in macrolon type III cages and allowed to get accustomed to standard conditions for at least 1 week. In the conditioned room, temperature was kept at 19–22 °C, relative humidity at 55–65% and a 12-h light–dark cycle was maintained (lights on at 7 a.m.). Acidified water and standard rodent chow (Teklad Global Diet, Harlan) were freely accessible. The Ethical Committee on Animal Experimentation of TNO approved the experiments described.

2.2. Synaptosomal preparation procedure

The synaptosomal fraction was prepared as described by Breukel et al. (1997). Briefly, after decapitation the brain was removed and placed in ice-cold homogenization buffer containing 0.32 M sucrose, 5 mM HEPES and 0.1 mM ethylenediaminetetraacetic acid, pH 7.5. After the striatal tissue was dissected out and homogenized (900 rpm), the homogenate was centrifuged for 10 min at $1500 \times g$ at 4 °C (Beckman GS-6R, Beckman Instruments, Palo Alto, CA). The supernatant was transferred on a percoll/sucrose gradient and centrifuged for 30 min at $23{,}000 \times g$ in a SW41 rotor at 4 °C (L8-70 Ultracentrifuge, Beckman Instruments), followed by a second centrifugation step to further purify the synaptosomes ($200{,}000 \times g$, 4 °C, 40 min, Ti70 rotor). Finally, the synaptosomes were resuspended in an artificial cerebral spinal fluid (aCSF) buffer which consisted of 2 mM $CaCl_2$, 132 mM NaCl, 3 mM KCl, 2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 10 mM HEPES, 10 mM glucose, pH 7.4, yielding an approximate protein concentration between 4 and 13 $mg \cdot ml^{-1}$, as determined with a Biorad Protein assay.

2.3. [^{3}H]Acetylcholine release assay

The synaptosomal suspension was incubated with 10 μM crotylsarin at 37 °C for 10 min and subsequently centrifuged at $10{,}000 \times g$ (Eppendorf 5415C, Eppendorf-Netheler-Hinz, Hamburg, Germany) at 4 °C for 15 min. After the nerve endings were resuspended in fresh ice-cold aCSF, they were loaded with 1 μM [^{3}H]choline (2.5 $\mu Ci \cdot ml^{-1}$) for 30 min at 37 °C (Pittel et al., 1990). After labelling, the synaptosomes were washed three times with ice-cold aCSF and centrifuged between the washing steps ($10{,}000 \times g$, 2 min, 4 °C). After the final resuspension, adenosine deaminase was added (4 $U \cdot ml^{-1}$) and a last incubation step of 10 min followed. Finally, 50-μl aliquots (0.2–0.65 mg protein content) were transferred in 37 °C conditioned perfusion chambers, containing Whatman GF/B filters, in which they were perfused at 0.5 $ml \cdot min^{-1}$ with pre-gassed aCSF, to which 10 μM hemicholinium-3 was added. After an equilibration period of 32 min, fractions were collected with a 2-min interval. Transmitter release was evoked by adding 1 mM 4-aminopyridine to the aCSF for 90 s. It is a generally used stimulus in this kind of studies and the effect is tetrodotoxine (TTX)-sensitive like that of spike-induced release, a mechanism more likely to resemble exocitosis triggered by action potentials (Tibbs et al., 1989). This suggests that the calcium (Ca^{2+})-dependent exocytotic release of [^{3}H]acetylcholine can be specifically induced by 4-AP (Santos et al., 1992).

Release was determined by estimating the peak area with subtraction of the basal release.

Modulation of the evoked release of [^{3}H]acetylcholine by adenosine analogues was assessed by changes in the ratio of two consecutive release stimuli S_1 and S_2, which lay 12 min apart. Drugs were only present during the S_2 stimulus, affecting that peak area, which resulted in a changed ratio compared to the control situation. The CPA analogues were added 4 min prior to the S_2 evoked release peak of [^{3}H]acetylcholine.

2.4. Chemicals

Hemicholinium-3, 4-aminopyridine and N^6-cyclopentyladenosine (CPA) were purchased from Research Biochemicals (Zwijndrecht, The Netherlands). Percoll was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden) and [*methyl*-^{3}H]choline chloride from Nycomed Amersham (Buckinghamshire, UK). Dimethylsulfoxide, adenosine deaminase and theophylline were purchased from Sigma (St. Louis, MO). 3′-Deoxy-CPA (3-DCPA), 8-propylamino-CPA (8-PCPA) and 8-butylamino-CPA (8-BCPA) were synthesized at the Department of Medicinal Chemistry of the Leiden/Amsterdam Center for Drug Research. The Chemistry Department of the TNO Prins Maurits Laboratory provided crotylsarin, an irreversible, rapidly aging acetylcholinesterase inhibitor. Dr. A. Monopoli (Schering-Plough, Milan, Italy) kindly donated 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261). The other chemicals used were of standard purity.

All solutions were prepared with water tapped from a Milli-Q system (Millipore, Molsheim, France) except for the stock solutions of CPA, 3-DCPA, 8-PCPA and 8-BCPA, which were made in dimethylsulfoxide, 10^{-2} M, each.

2.5. Data analyses and presentation

The S_2/S_1 ratios were calculated based on the peak areas and expressed relative to the peak ratio with no modulatory drugs present. Statistical analysis was performed with an analysis of variance followed by a Bonferoni posthoc test, whenever appropriate. A P value <0.05 was considered significant. All data are reported as the mean ± S.E.M.

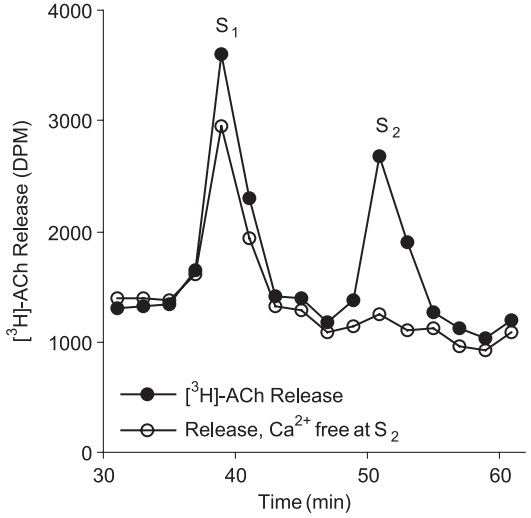

Fig. 1. Typical profile of [^{3}H]-acetylcholine release from striatal synaptosomes. S_1 and S_2 represent the peaks that emerge after a 90-s stimulation period with 1 mM 4-aminopyridine. The ratio between the peak areas S_2 and S_1, corrected for the basal release, was calculated and reflected the modulatory effect of the examined compounds on the evoked release. Absence of calcium in the medium during the second stimulation resulted in a complete abolishment of transmitter release, demonstrating the high calcium-dependency of the observed release.

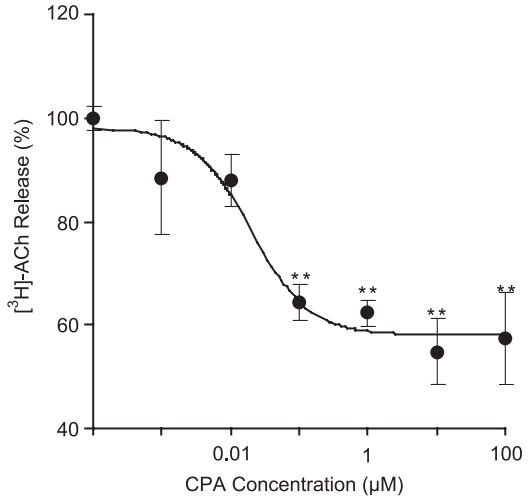

Fig. 2. Concentration-dependent inhibition of [^{3}H]-acetylcholine release from synaptosomes by the selective adenosine A_1 receptor agonist CPA (mean ± S.E.M.; $n=4$-21). The results are expressed as the change in [^{3}H]-acetylcholine release compared to the control incubation with no agonist present. Note that the y-axis starts at 40%. $^{**}P<0.01$, significantly different from basal [^{3}H]-acetylcholine release with no drug present.

3. Results

Fig. 1 illustrates a typical perfusion profile of [^{3}H]acetylcholine release from striatal synaptosomes, in which

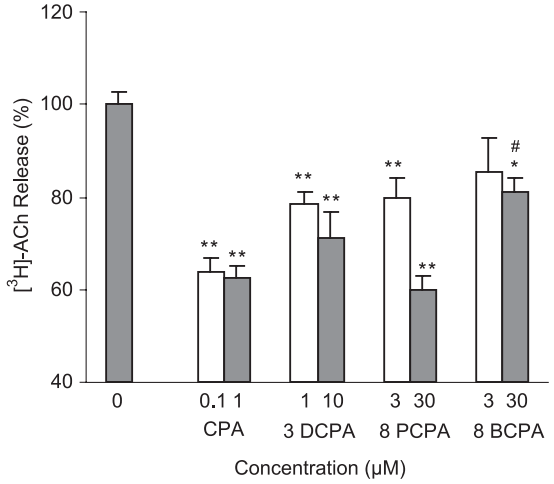

Fig. 3. Adenosine A_1 receptor-mediated inhibition of the 4-aminopyridine evoked [^{3}H]-acetylcholine release from synaptosomes by the selective adenosine A_1 receptor agonists CPA, 3-DCPA, 8-PCPA and 8-BCPA. The results are expressed as the change in [^{3}H]-acetylcholine release compared to the controls with no agonist present (mean ± S.E.M.; $n=3$–16). Note that the y-axis starts at 40%. $^{*}P<0.05$, $^{**}P<0.01$, significantly different from the control value with no adenosine A_1 receptor agonist present; $^{\#}P<0.05$, significantly different from 1 μM CPA and 30 μM 8-PCPA.

release was evoked by two consecutive 90-s pulses of 1 mM 4-aminopyridine ($n=3$). A complete loss of [^{3}H]acetylcholine release was observed (Fig. 1) upon complexation of calcium with EGTA in the perfusion medium. It is well established in this model that the increased activity during chemical stimulation represents the release of [^{3}H]acetylcholine, whereas the basal release of [^{3}H]label reflects predominantly the release of unchanged choline (Kirk and Richardson, 1994; Pittel et al., 1990; Tapia et al., 1985).

Addition of increasing concentrations of the prototypic adenosine A_1 receptor agonist CPA to the perfusion medium reduced the [^{3}H]acetylcholine release in a concentration-dependent manner (Fig. 2; $n=4-21$). Maximal inhibition of $38 \pm 3\%$ was reached at 1 μM. The acetylcholine-modulatory action of the CPA analogues 3-DCPA, 8-PCPA and 8-BCPA was examined in a similar fashion (Fig. 3; $n=3-4$). The concentrations of the CPA derivatives used were approximately 3- and 30-fold the K_i values of the different agonists in the presence of guanosine 5′- triphosphate (GTP) as determined in radioligand binding studies (Roelen et al., 1996; Van der Wenden et al., 1995). 10 μM 3-DCPA and 30 μM 8-PCPA inhibited the 4-aminopyridine-evoked [^{3}H]acetylcholine release by 29 ± 5 and $38 \pm 3\%$, respectively, which was similar to the effect of CPA. 8-BCPA also attenuated the transmitter outflow, but this effect was less pronounced, $19 \pm 3\%$. Higher concentrations of 8-BCPA were not tested since there was no difference between 3 and 30 μM of this compound with regard to the inhibition of acetylcholine release.

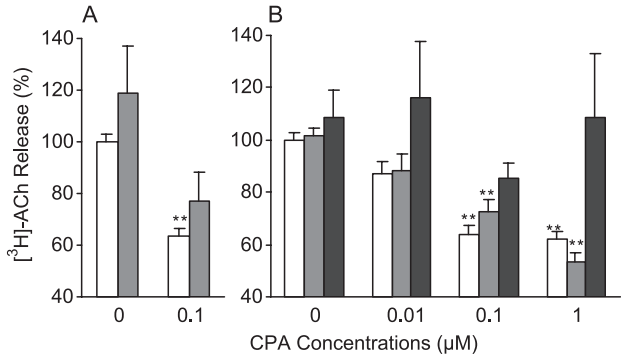

Fig. 4. Inhibition of the [^{3}H]-acetylcholine release from striatal synaptosomes by the selective adenosine A_1 receptor agonist CPA in the presence of (A) the adenosine A_{2A} receptor selective antagonist SCH 58261 or (B) the nonselective antagonist theophylline. Panel A represents the evoked [^{3}H]-acetylcholine release inhibited by CPA (open columns) and CPA with 0.1 μM SCH 58261 (hatched columns) present. Panel B represents the evoked [^{3}H]-acetylcholine release inhibited by CPA (open columns), CPA with 10 μM theophylline (hatched columns) and CPA with 100 μM theophylline (solid columns) present in the perfusion buffer. The results are expressed as the change in [^{3}H]-acetylcholine release compared to the controls with no agonist present (mean ± S.E.M.; $n=3-16$). Note that the y-axis starts at 40%. $^{**}P<0.01$, significantly different from the controls with no CPA present.

In an attempt to increase the difference between full and partial agonist responses, which was only 42% inhibition, the selective adenosine A_{2A} receptor antagonist SCH 58261 (Zocchi et al., 1996) and the nonselective adenosine receptor antagonist theophylline were applied. Administration of 0.1 μM SCH 58261 ($n=3$) alone resulted in an insignificant increase of the [^{3}H]acetylcholine release, but the inhibition in combination with 0.1 μM CPA (Fig. 4A; $n=3$) remained 38%. Addition of 10 mM or 100 mM theophylline did not have an effect on the acetylcholine release itself (Fig. 4B; $n=3-12$). In combination with CPA, however, only attenuation of transmitter release was observed. Apparently, 40% inhibition is the maximal response that could be reached with adenosine A_1 receptor agonists.

4. Discussion

In the introductory paragraph of this paper, it was emphasized that the application of adenosine A_1 receptor agonists in regard to cerebral disorders is hampered by serious cardiovascular side effects and that this problem might be circumvented by using low-efficacy agonists (partial agonists). The most interesting but preliminary observation of this study is that the CPA-derived low-efficacy derivatives 3-DCPA and 8-PCPA decreased the 4-aminopyridine-evoked [^{3}H]acetylcholine release with maximal inhibitory responses similar to that of CPA, suggesting a highly efficacious stimulus-response system in the striatal tissue on the presynaptic modulation of the acetylcholine release. In a previous study, it had been demonstrated that the latter CPA analogues had only moderate hemodynamic effects in vivo (Van der Graaf et al., 1997; Van Schaick et al., 1998).

Comparable concentrations of 8-BCPA led only to a partial reduction of the synaptosomal acetylcholine release. This contrasts with the results of 8-BCPA found in hippocampal slices models (Lorenzen et al., 1997; Harrison et al., 2003), where full agonist properties were demonstrated. Additional research is required to resolve this discrepancy.

It was attempted in this study to better differentiate between full and partial agonists at the central adenosine A_1 receptor. For that purpose the selective adenosine A_{2A} receptor antagonist SCH 58261 or the nonselective antagonist theophylline was added to the perfusion medium in order to increase the inhibitory range in which partial and full agonism could be assessed more accurately. Addition of SCH 58261 prevents interfering modulatory actions via the adenosine A_{2A} receptor. Kirkpatrick and Richardson (1993) found an extra 30% reduction of the acetylcholine release with R-PIA (R-N^6-(2-phenylisopropyl)adenosine) in the presence of the poorly selective (13-fold) adenosine A_{2A} receptor antagonist 4-amino-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxaline (CP 66,713; Sarges et al., 1990). However, in our case, addition of SCH 58261 did not result in the desired effect, i.e., the maximal CPA-mediated [^{3}H]acetyl-

choline inhibition remained 40%. The nonselective antagonist theophylline was added to the medium to block the adenosine A_1 and A_{2A} receptors, thereby excluding any presynaptic influence of endogenous adenosine that might be present. Subsequent addition of CPA was expected to result in a rightward shift of the inhibitory curve together with a larger effective window, due to the absence of modulation by endogenous adenosine. However, only adenosine A_1 receptor antagonism was observed with 100 μM theophylline. Although theophylline is also a well-known inhibitor of phosphodiesterase activity in the millimolar range, the concentrations used in the present study were within the micromolar range and thus the absence of inhibition of the acetylcholine release has to be attributed only to competitive adenosine A_1 receptor antagonism. Apparently, neither putative binding of CPA to the adenosine A_{2A} receptor nor the presence of endogenous adenosine had an appreciable influence on the reduction of acetylcholine release in our synaptosomal preparation.

It has been mentioned that the inhibition of central acetylcholine release may be advantageous in the management of organophosphate intoxication in which the inhibition of acetylcholinesterase results in accumulation of acetylcholine and overstimulation of cholinergic receptors causing electroencephalogram (EEG)-seizures and epileptic activity (Van Helden and Bueters, 1999). In a recent study, it has been shown that CPA, 2-DCPA and 8-BCPA terminated epileptiform activity induced by the organophosphate sarin in a guinea-pig hippocampal slice system in a concentration-related manner (Harrison et al., 2003).

In conclusion, the results demonstrate that the full adenosine A_1 receptor agonist CPA, and some of its low-efficacy derivatives lacking profound cardiovascular side effects, inhibit the release of acetylcholine concentration-dependently to a similar degree in a striatal synaptosome preparation. These preliminary results encourage further investigation of the tissue selectivity and therapeutic potential of these low-efficacy agonists in vivo.

Acknowledgements

We thank Prof. W.E.J.M. Ghijsen and Mrs. E. Besselsen from the University of Amsterdam, The Netherlands for their assistance concerning the synaptosomal preparation, and Dr. A. Monopoli from Schering-Plough, Milan, Italy for the generous gift of SCH 58261.

References

Ambrosio, A.F., Malva, J.O., Carvalho, A.P., Carvalho, C.M., 1997. Inhibition of N-, P/Q- and other types of Ca^{2+} channels in rat hippocampal nerve terminals by the adenosine A_1 receptor. Eur. J. Pharmacol. 340, 301–310.

Breukel, A.I., Besselsen, E., Ghijsen, W.E., 1997. Synaptosomes: a model system to study release of multiple classes of neurotransmitters. Methods Mol. Biol. 72, 33–47.

Brown, S.J., James, S., Reddington, M., Richardson, P.J., 1990. Both A_1 and A_{2a} purine receptors regulate striatal acetylcholine release. J. Neurochem. 55, 31–38.

Dennis, D., Jacobson, K., Belardinelli, L., 1992. Evidence of spare A_1-adenosine receptors in guinea pig atrioventricular node. Am. J. Physiol. 262, H661–H671.

Dunwiddie, T.V., 1999. Adenosine and suppression of seizures. Adv. Neurol. 79, 1001–1010.

Harrison, P.K., Bueters, T.J.H., IJzerman, A.P., Van Helden, H.P.M., Tattersall, J.E.H., 2003. Partial adenosine A_1 receptor agonists inhibit sarin-induced epileptiform activity in the hippocampal slice. Eur. J. Pharmacol. 471, 97–104.

IJzerman, A.P., Van der Wenden, E.M., Von Frijtag Drabbe Kunzel, J.K., Mathot, R.A., Danhof, M., Borea, P.A., Varani, K., 1994. Partial agonism of theophylline-7-riboside on adenosine receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 638–645.

Kirk, I.P., Richardson, P.J., 1994. Adenosine A_{2a} receptor-mediated modulation of striatal [^{3}H]GABA and [^{3}H]acetylcholine release. J. Neurochem. 62, 960–966.

Kirkpatrick, K.A., Richardson, P.J., 1993. Adenosine receptor-mediated modulation of acetylcholine release from rat striatal synaptosomes. Br. J. Pharmacol. 110, 949–954.

Lorenzen, A., Sebastiao, A.M., Sellink, A., Vogt, H., Schwabe, U., Ribeiro, J.A., IJzerman, A.P., 1997. Biological activities of N^6,C^8-disubstituted adenosine derivatives as partial agonists at rat brain adenosine A_1 receptors. Eur. J. Pharmacol. 334, 299–307.

Materi, L.M., Rasmusson, D.D., Semba, K., 2000. Inhibition of synaptically evoked cortical acetylcholine release by adenosine: an in vivo microdialysis study in the rat. Neuroscience 97, 219–226.

Pedata, F., Giovannelli, L., De Sarno, P., Pepeu, G., 1986. Effect of adenosine, adenosine derivatives, and caffeine on acetylcholine release from brain synaptosomes: interaction with muscarinic autoregulatory mechanisms. J. Neurochem. 46, 1593–1598.

Pittel, Z., Heldman, E., Rubinstein, R., Cohen, S., 1990. Distinct muscarinic receptor subtypes differentially modulate acetylcholine release from corticocerebral synaptosomes. J. Neurochem. 55, 665–672.

Roelen, H., Veldman, N., Spek, A.L., Von Vrijtag Drabbe, K.J., Mathôt, R.A., IJzerman, A.P., 1996. N^6,C^8-distributed adenosine derivatives as partial agonists for adenosine A_1 receptors. J. Med. Chem. 39, 1463–1471.

Santos, M.S., Rodriguez, R., Carvalho, A.P., 1992. Effect of depolarizing agents on the Ca(2+)-independent and Ca(2+)-dependent release of [^{3}H]GABA from sheep brain synaptosomes. Biochem. Pharmacol. 44 (2), 301–308.

Sarges, R., Howard, H.R., Browne, R.G., Lebel, L.A., Seymour, P.A., Koe, B.K., 1990. 4-Amino[1,2,4]triazolo[4,3-a]quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants. J. Med. Chem. 33, 2240–2254.

Simonato, M., Varani, K., Muzzolini, A., Bianchi, C., Beani, L., Borea, P.A., 1994. Adenosine A_1 receptors in the rat brain in the kindling model of epilepsy. Eur. J. Pharmacol. 265, 121–124.

Sollevi, A., Belfrage, M., Lundeberg, T., Segerdahl, M., Hansson, P., 1995. Systemic adenosine infusion: a new treatment modality to alleviate neuropathic pain. Pain 61, 155–158.

Spielman, W.S., Klotz, K.N., Arend, L.J., Olson, B.A., LeVier, D.G., Schwabe, U., 1992. Characterization of adenosine A_1 receptor in a cell line (28A) derived from rabbit collecting tubule. Am. J. Physiol. 263, C502–C508.

Tapia, R., Sitger, M., Morales, E., 1985. Mechanism of the calcium-dependent stimulation of transmitter release by 4-aminopyridine in synaptosomes. Brain Res. 361, 373–382.

Tibbs, G.R., Barrie, A.P., Van Mieghem, F.J., McMahon, H.T, Nicholl, D.G., 1989. Repetitive action potential in isolated nerve terminals in the presence of 4-aminopyridine: effects on cytosolic free Ca2+ and glutamate release. J. Neurochem. 53 (6), 1693–1699.

Van der Graaf, P.H., van Schaick, E.A., Mathot, R.A., IJzerman, A.P., Danhof, M., 1997. Mechanism-based pharmacokinetic-pharmacodynamic modeling of the effects of N^6-cyclopentyladenosine analogs on heart rate in rat: estimation of in vivo operational affinity and efficacy at adenosine A_1 receptors. J. Pharmacol. Exp. Ther. 283, 809–816.

Van der Wenden, E.M., Von Vrijtag Drabbe Kunzel, K.J., Mathôt, R.A., Danhof, M., IJzerman, A.P., Soudijn, W., 1995. Ribose-modified adenosine analogues as potential partial agonists for the adenosine receptor. J. Med. Chem. 38, 4000–4006.

Van Helden, H.P.M., Bueters, T.J., 1999. Protective activity of adenosine receptor agonists in the treatment of organophosphate poisoning. Trends Pharmacol. Sci. 20, 438–441.

Van Schaick, E.A., Tukker, H.E., Roelen, H.C., IJzerman, A.P., Danhof, M., 1998. Selectivity of action of 8-alkylamino analogues of N^6-cyclopentyladenosine in vivo: haemodynamic versus anti-lipolytic responses in rats. Br. J. Pharmacol. 124, 607–618.

Von Lubitz, D.K., 1999. Adenosine and cerebral ischemia: therapeutic future or death of a brave concept? Eur. J. Pharmacol. 371, 85–102.

Zocchi, C., Ongini, E., Conti, A., Monopoli, A., Negretti, A., Baraldi, P.G., Dionisotti, S., 1996. The non-xanthine heterocyclic compound SCH58261 is a new potent and selective A_{2a} adenosine antagonist. J. Pharmacol. Exp. Ther. 276, 398–404.