Current Topics in Neurotoxicity 10

Micaela Morelli
Nicola Simola
Jadwiga Wardas *Editors*

The Adenosinergic System

A Non-Dopaminergic Target in Parkinson's Disease

Springer

Current Topics in Neurotoxicity

Volume 10

Series Editors
Richard Kostrzewa
East Tennessee State University,
Johnson City, USA

Trevor Archer
Gothenburg University, University of Gothenburg,
Gothenburg, Sweden

Current Topics in Neurotoxicity consists of volumes that cover the latest developments in neurotoxicity studies including neurodegeneration, neuroprotection, and neuroregeneration. The series has a translational focus and addresses the causes of neurotoxicity associated with diseases such as Alzheimers, Parkinsons Disease, autism, HIV associated dementia etc.

More information about this series at http://www.springer.com/series/8791

Micaela Morelli • Nicola Simola • Jadwiga Wardas
Editors

The Adenosinergic System

A Non-Dopaminergic Target in Parkinson's Disease

Springer

Editors
Micaela Morelli
Dept. of Biomedical Sciences Section
of Neuropsychopharmacology
University of Cagliari
Cagliari
Italy

Jadwiga Wardas
Dept. of Neuropsychopharmacology
Institute of Pharmacology
Polish Academy of Sciences
Krakow
Poland

Nicola Simola
Dept. of Biomedical Sciences Section
of Neuropsychopharmacology
University of Cagliari
Cagliari
Italy

ISSN 2363-9563 ISSN 2363-9571 (electronic)
Current Topics in Neurotoxicity
ISBN 978-3-319-20272-3 ISBN 978-3-319-20273-0 (eBook)
DOI 10.1007/978-3-319-20273-0

Library of Congress Control Number: 2015947498

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease, and affects more than 5 million people worldwide. Today, the clinical management of Parkinson's disease chiefly relies on the use of the so called "dopamine replacement therapy" in order to re-establish the function of the dopaminergic system, which is affected by the neurodegeneration underlying the disease. While this approach effectively counteracts the motor deficits featuring Parkinson's disease, the chronic use of dopamine replacement therapy eventually leads to the emergence of motor complications (e.g., dyskinesia and motor fluctuations) that greatly limit its therapeutic potential. Moreover, dopamine replacement therapy has no apparent beneficial effects on the progression of dopaminergic degeneration featuring Parkinson's disease. Based on these considerations, there is a need for the development of alternative therapies that could help to overcome these limitations.

In these years, drugs acting as antagonists of the adenosine A_{2A} receptors have emerged as new promising candidates for the therapy of Parkinson's disease. When evaluated in experimental animal models of the disease, these drugs counteract motor deficits and amplify the beneficial effects of dopaminergic drugs without worsening their dyskinetic effect. Moreover, experimental evidence also indicates that adenosine A_{2A} receptor antagonists might slow down or arrest the dopaminergic degeneration that underlies Parkinson's disease. Building on this evidence, the research in this field has recently made significant progress, leading to the approval of the first A_{2A} receptor antagonist for clinical use as adjunct to L-DOPA (istradefylline, marketed under the name of NOURIAST®), and the ongoing clinical evaluation of other promising drugs (e.g., tozadenant).

This book covers basic biological aspects of the adenosine system relevant to Parkinson's disease, and also discusses recent experimental findings at both the preclinical and clinical level. Attention is dedicated to the localization and function of adenosine A_{2A} receptors, to their interaction with dopaminergic and non-dopaminergic receptors in the brain, and to the development of novel molecules that may target A_{2A} receptors. The critical role of the adenosine system in the regulation of neurotrophic factors, neuroinflammation, and neurotoxicity is also covered, and the relevance of these phenomena to the etiology of Parkinson's disease discussed. Moreover, the book thoroughly describes the effects of adenosine A_{2A} receptor

antagonists observed in experimental models of Parkinson's disease on both motor (akinesia, dyskinesia, tremor) and non-motor (cognition, peripheral functions, sleep) symptoms. Finally, attention is dedicated to the clinical relevance of the adenosinergic system, by describing the development of the first ever approved adenosine A_{2A} receptor antagonist (istradefylline), the most advanced clinical trials with these drugs, the use of A_{2A} receptor antagonist in neuroimaging, and the epidemiological evidence that links the adenosine system with the onset and progression of Parkinson's disease.

By gathering updated and high-quality chapters written by world-leading experts in the field, this book provides essential information to preclinical and clinical researchers interested in the development of new therapies against Parkinson's disease and related neurodegenerative disorders.

Cagliari, Italy Micaela Morelli
Cagliari, Italy Nicola Simola
Krakow, Poland Jadwiga Wardas

Contents

Contributors

Aderbal Silva Aguiar Laboratório Experimental de Doenças Neurodegenerativas (LEXDON), Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil

Laboratório de Neurobiologia do Exercício Físico, Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil

Rachit Bakshi Molecular Neurobiology Laboratory, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Boston, MA, USA

Olivier Barret Molecular NeuroImaging, LLC, New Haven, CT, USA

Jordi Bonaventura Integrative Neurobiology Section, National Institute on Drug Abuse, IRP, Intramural Research Program, National Institutes of Health, Baltimore, MD, USA

Daniel Caldeira Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Vicent Casadó Centro de Investigacion Biomedica en Red sobre Enfermedades Neurodegenerativas, Department of Biochemisry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain

Jiang-Fan Chen Department of Neurology, Boston University School of Medicine, Boston, MA, USA

Mercè Correa Behavioral Neuroscience Division, Department of Psychology, University of Connecticut, Storrs, CT, USA

Area de Psicobiologia, Universitat Jaume I, Castello, Spain

Giulia Costa Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy

João Costa Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Clinical Pharmacology Unit, Instituto de Medicina Molecular, Lisbon, Portugal

Evidence Based Medicine Centre, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Portuguese Collaborating Centre of the Cochrane Iberoamerican Network, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Rodrigo A. Cunha CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

FMUC-Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Maria J. Diógenes Institute of Pharmacology and Neurosciences, Faculty of Medicine and Unit of Neurosciences, Instituto de Medicina Molecular, Universidade de Lisboa

Sergi Ferré Integrative Neurobiology Section, National Institute on Drug Abuse, IRP, Intramural Research Program, National Institutes of Health, Baltimore, MD, USA

Joaquim J. Ferreira Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Jimmy George CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Catarina Gomes CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

FMUC-Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Robert A. Hauser USF Byrd Parkinson's Disease and Movement Disorders Center, Byrd Institute, University of South Florida, Tampa, USA

Peter Jenner Neurodegenerative Diseases Research Group, Institute of Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College, London, UK

Michael Lazarus International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Tsukuba, Ibaraki, Japan

Peter LeWitt Departments of Neurology, Henry Ford Hospital and Wayne State University School of Medicine, West Bloomfield, Michigan, USA

Carme Lluís Centro de Investigacion Biomedica en Red sobre Enfermedades Neurodegenerativas, Department of Biochemisry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain

Robert Logan Molecular Neurobiology Laboratory, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Lauren L. Long Behavioral Neuroscience Division, Department of Psychology, University of Connecticut, Storrs, CT, USA

Filipe Carvalho Matheus Laboratório Experimental de Doenças Neurodegenerativas (LEXDON), Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil

Micaela Morelli Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy

Estefanía Moreno Centro de Investigacion Biomedica en Red sobre Enfermedades Neurodegenerativas, Department of Biochemisry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain

Morgana Moretti Laboratório Experimental de Doenças Neurodegenerativas (LEXDON), Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil

Akihisa Mori Medical Affairs Department, Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan

Christa E. Müller PharmaCenter Bonn, Pharmaceutical Chemistry I, Pharmaceutical Institute, Universität Bonn, Bonn, Germany

Gemma Navarro Centro de Investigacion Biomedica en Red sobre Enfermedades Neurodegenerativas, Department of Biochemisry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain

Eric J. Nunes Behavioral Neuroscience Division, Department of Psychology, University of Connecticut, Storrs, CT, USA

Paulo Alexandre de Oliveira Laboratório Experimental de Doenças Neurodegenerativas (LEXDON), Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil

Annalisa Pinna Institute of Neuroscience, National Research Council of Italy (CNR), Cagliari, Italy

Samantha J. Podurgiel Behavioral Neuroscience Division, Department of Psychology, University of Connecticut, Storrs, CT, USA

Emmanuelle Pourcher Faculty of Medicine, Clinique Sainte Anne Mémoire et Mouvement, Laval University, Quebec, QC, Canada

Rui Daniel Prediger Laboratório Experimental de Doenças Neurodegenerativas (LEXDON), Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil

Daniel Rial Laboratório Experimental de Doenças Neurodegenerativas (LEXDON), Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil
CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Joaquim A. Ribeiro Institute of Pharmacology and Neurosciences, Faculty of Medicine and Unit of Neurosciences, Instituto de Medicina Molecular, Universidade de Lisboa

Filipe B. Rodrigues Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

John D. Salamone Behavioral Neuroscience Division, Department of Psychology, University of Connecticut, Storrs, CT, USA

Michael A. Schwarzschild Molecular Neurobiology Laboratory, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Ana M. Sebastião Institute of Pharmacology and Neurosciences, Faculty of Medicine and Unit of Neurosciences, Instituto de Medicina Molecular, Universidade de Lisboa

John P. Seibyl Molecular NeuroImaging, LLC, New Haven, CT, USA

Nicola Simola Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy

Ana Cristina Guerra de Souza Laboratório Experimental de Doenças Neurodegenerativas (LEXDON), Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil

Gilles D. Tamagnan Molecular NeuroImaging, LLC, New Haven, CT, USA

Adriana Alexandre S. Tavares Molecular NeuroImaging, LLC, New Haven, CT, USA

Yoshihiro Urade International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Tsukuba, Ibaraki, Japan

Nora D. Volkow National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA

Jadwiga Wardas Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Chapter 1
Adenosine A_{2A} Receptors: Localization and Function

Nicola Simola and Jadwiga Wardas

Abstract Adenosine is an endogenous purine nucleoside present in all mammalian tissues, that originates from the breakdown of ATP. By binding to its four receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3), adenosine regulates several important physiological functions at both the central and peripheral levels. Therefore, ligands for the different adenosine receptors are attracting increasing attention as new potential drugs to be used in the treatment of several diseases.

This chapter is aimed at providing an overview of adenosine metabolism, adenosine receptors localization and their signal transduction pathways. Particular attention will be paid to the biochemistry and pharmacology of A_{2A} receptors, since antagonists of these receptors have emerged as promising new drugs for the treatment of Parkinson's disease. The interactions of A_{2A} receptors with other non-adenosinergic receptors, and the effects of the pharmacological manipulation of A_{2A} receptors on different body organs will be discussed, together with the usefulness of A_{2A} receptor antagonists for the treatment of Parkinson's disease and the potential adverse effects of these drugs.

Keywords Adenylate cyclase · Basal ganglia · Dopamine · G protein-coupled receptors · Heteromeric complexes · Nucleoside · Purine · Striatonigral · Striatopallidal

N. Simola (✉)
Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy
e-mail: nicola.simola@unica.it

J. Wardas
Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna St., 31-343 Kraków, Poland
e-mail: wardas@if-pan.krakow.pl

M. Morelli et al. (eds.), *The Adenosinergic System,* Current Topics in Neurotoxicity 10,
DOI 10.1007/978-3-319-20273-0_1

Introduction

The concept of purinergic neurotransmission was first introduced by Burnstock in 1972 and subsequently adenosine 5' triphosphate (ATP) was shown to act either as a transmitter or a co-transmitter in most nerves in both the peripheral and central nervous system (CNS) (Abbracchio and Burnstock 1998; Abbracchio et al. 2008; Burnstock 1972, 2013). At present, it is known that ATP acts as a fast excitatory neurotransmitter or neuromodulator, and has a potent long-term trophic role in cell proliferation, growth and development as well as in disease and cytotoxicity (Abbracchio and Burnstock 1998; Abbracchio et al. 2008; Burnstock 2013).

ATP and other nucleotides are stored in secretory and synaptic vesicles, and exocytotic vesicular release of ATP from neurons and astrocytes is well established (Abbracchio et al. 2008; Bowser and Khakh 2007; Burnstock 2013; Pankratov et al. 2006, 2007). There are also evidences indicating additional mechanisms of the release of this nucleotide, including ATP-cassette transporters, connexin or pannexin hemichannels, plasmalemmal voltage-dependent anion channels and the ATP-sensitive P2X7 receptors (Abbracchio et al. 2008; Burnstock 2013). After release, ATP and other nucleotides undergo rapid enzymatic degradation to adenosine by ectonucleotidases (Bonan 2012; Kovacs et al. 2013; Yegutkin 2008; Zimmermann 2006).

Adenosine Metabolism

Adenosine, an endogenous purine ribonucleoside present in all mammalian tissues, modulates a variety of important synaptic processes and signaling pathways, and regulates the functions of several neurotransmitters in the CNS. Adenosine is considered to be a neuromodulator rather than a neurotransmitter, since it is not stored in synaptic vesicles, and is not released from nerve terminals by exocytosis. Adenosine affects neural activity through multiple mechanisms; presynaptically by controlling neurotransmitter release, postsynaptically by hyperpolaryzing or depolarizing neurons, and non-synaptically mainly via regulatory effects on glial cells (Boison et al. 2010; Dare et al. 2007; Fredholm et al. 2005). Although adenosine is generally known to be produced by the ectoenzymatic breakdown of ATP, there might be a subpopulation of neurons and/or astrocytes that release adenosine directly in an activity-dependent manner (Wall and Dale 2007).

It is well established that adenosine may be formed in the CNS either intracellularly, after degradation of ATP to cyclic-adenosine monophosphate (cAMP) and 5'-AMP, and then transported by nucleotide transporters to the synapse, or extracellularly from nucleotides released into the synaptic cleft (Fig. 1.1). Thus, the formation of adenosine is dependent on the availability of oxygen and energetic compounds as well as on the rate of synthesis and degradation of ATP, released from both neuronal and glial cells. However, it is the release of ATP from astrocytes,

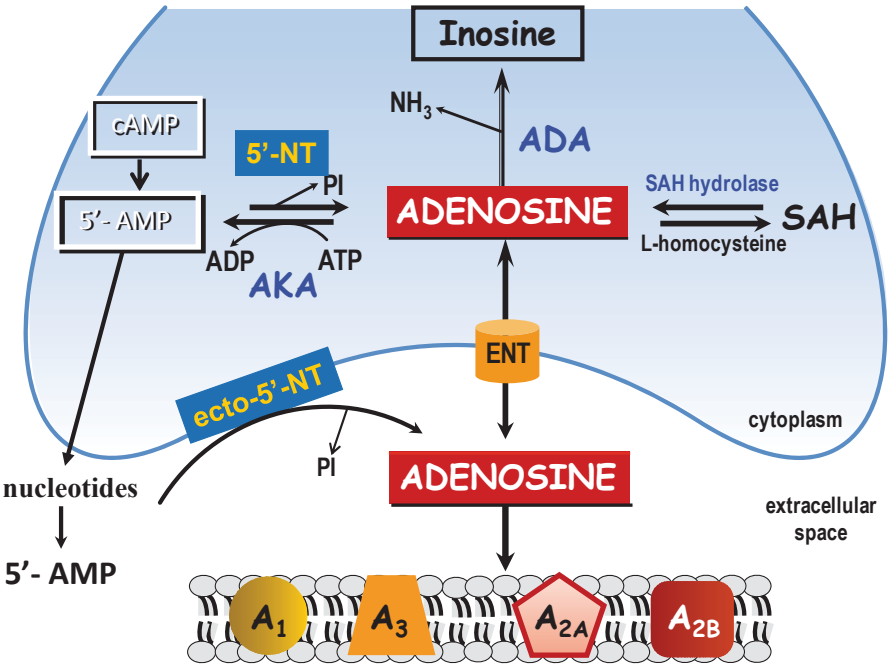

Fig. 1.1 Adenosine synthesis and metabolic pathways. Adenosine is formed both *intracelullarly* from 5'-AMP by the cytosolic 5'-NT, and *extracelullarly* in the metabolism of nucleotides (*ATP*, *ADP*, *AMP*) released from the cell, through the action of ecto-5'-nucleotidase. Another intracellular source of adenosine may be the hydrolysis of SAH by SAH hydrolase. Hence, adenosine formation depends on ATP breakdown and synthesis. Extracellular adenosine is primarily inactivated by uptake through the transporters (*ENT*), which are mainly bidirectional, followed by either phosphorylaton to AMP by AKA (under physiological conditions), or, to a lesser degree, deamination to inosine by ADA. Another possible catabolic pathway of adenosine, though of minor significance, is a reversible reaction catalysed by SAH hydrolase, leading to formation of SAH from adenosine and L-homocysteine (*for more details see the text, and* Abbracchio et al. 2008; Burnstock 2013; Latini and Pedata 2001; Sperlagh and Vizi 2011). *ADA* adenosine deaminase, *AKA* adenosine kinase, *ADP* adenosine diphosphate, *AMP* adenosine monophosphate, *ATP* adenosine 5'-triphosphate, A_1, A_{2A}, A_{2B} *and* A_3—adenosine receptors, *ecto-5'-NT* ecto-5'-nucleotidase, *ENT* nucleoside transporter, *5'-NT* 5'-nucleotidase, *SAH* S-adenosylhomocysteine

either vesicular (Pascual et al. 2005) or via secretion through hemichannels, that is the major source of synaptic adenosine (Kang et al. 2008; Kawamura et al. 2010). Moreover, adenosine can be directly released by nucleoside transporters from astrocytes when its intracellular level is augmented in response to a variety of physiological and pathological stimuli (e.g. increased cellular activity, hypoxia/hypoglycemia, ischemia). Then, adenosine may function as a nonsynaptic signalling molecule that diffuses far away from the site of origin and tonically influences neurotransmission, inflammation, and immune responses, as described below (Bours et al. 2006; Dare et al. 2007; Geiger and Fyda 1991; Sperlagh and Vizi 2011).

Intracellular Formation of Adenosine

In the cell, adenosine may be formed in the process of adenosine monophosphate (AMP) hydrolysis catalyzed by 5'-nucleotidase, which belongs to the family of enzymes called ectonucleotidases (Fig. 1.1, Kovacs et al. 2013; Yegutkin 2008). Seven types of 5'-nucleotidases have been cloned, characterized and demonstrated in various tissues, including brain tissue (Hunsucker et al. 2005; Kovacs et al. 2013). This pathway of adenosine formation via the cytosolic ATP catabolism seems to represent a very sensitive signal of increased metabolic rate or metabolic stress (Latini and Pedata 2001).

Another intracellular source of adenosine may be the hydrolysis of S-adenosylhomocysteine (SAH) by SAH hydrolase (Fig. 1.1), an enzyme present in brain areas, such as the neocortex, hippocampus, and cerebellum (Latini and Pedata 2001). However, this pathway is not strictly dependent upon the energetic state of the cells, and it does not significantly contribute to adenosine production in the brain under either physiological or ischemic conditions (Latini and Pedata 2001).

Extracellular Formation of Adenosine

The extracellular nucleotide and nucleoside levels in the synaptic cleft are controlled by a cascade of enzymes, belonging to the family of ectonucleotidases. There are four major families of ectonucleotidases, namely ectonucleoside triphosphate diphosphohydrolases (E-NTPDases), ectonucleotide pyrophosphatase/phosphodiesterases (E-NPPs), alkaline phosphatases, and ecto-5'-nucleotidase (ecto-5'-NT) (Bonan 2012; Kovacs et al. 2013; Yegutkin 2008; Zimmerman 2006).

The first step of ATP inactivation is mediated by the family of E-NTPDases, which are able to hydrolyse ATP and adenosine diphosphate (ADP) to AMP (Zimmermann 2006). Moreover, ATP can be dephosphorylated by E-NPPs and alkaline phosphatases which, like E-NTPDases, have widespread distribution in the CNS (Wang and Guidotti 1998; Zimmermann 2006). The next step of extracellular ATP inactivation involves the hydrolysis of AMP to adenosine and phosphate by the ecto-5'-NT, also known as CD73 (Fig. 1.1), which is attached via a GPI anchor to the outer surface of the plasma membrane. Ecto-5'-NT, which is the rate-limiting step in the formation of adenosine (Sperlagh 1996; Sperlagh and Vizi 2007), is also widely expressed in the brain (e.g. in hippocampal and striatal nerve terminals), and it is predominantly associated with glial cells (Cunha et al. 1992; Hunsucker et al. 2005; James and Richardson 1993; Kovacs et al. 2013; Schoen et al. 1987).

Another pathway of extracellular adenosine formation may originate from the cAMP or 5'-AMP released into the synapse. Both these nucleotides are responsible for the slow change in the adenosine concentration. The cAMP can be released through non-specific energy-dependent transporters and then, when in the synapse, it can first be converted to 5'-AMP by ecto-phosphodiesterases and then to adenosine by ecto-5'-NT. Another possibility also exists that the cAMP can be converted to 5'-AMP inside the cell and then 5'-AMP can be released into the synaptic

cleft, becoming a source of adenosine (e.g. after the NMDA (N-methyl-D-aspartate) stimulation in cortical sections) (Latini and Pedata 2001; Sperlagh and Vizi 2011).

The process of extracellular adenosine formation is very fast, and occurs within seconds (Dunwiddie et al. 1997). Adenosine is normally present in a concentration between 30–300 nM, but under hypoxic or ischemic conditions adenosine concentrations in the hippocampus can reach 20–30 μM (Dunwiddie et al. 1997; Latini et al. 1999). It seems that *in vivo* a large part of adenosine present in the synapse under basal conditions comes from the extracellular metabolism of nucleotides (Latini and Pedata 2001; Sperlagh and Vizi 2011). In contrast, numerous studies have suggested that in conditions of hypoxia or ischemia adenosine is mainly formed intracellularly and released to the synaptic space by transportes (Latini and Pedata 2001; Sperlagh and Vizi 2011).

Nucleoside Transporters

The level of extracellular adenosine is regulated by the process of bidirectional transport of nucleosides, which allows for rapid exchange between extra and intracellular adenosine. In contrast to conventional neurotransmitters, the reuptake of adenosine does not depend on energy-driven transporter-mediated systems. This transport is driven by chemical gradients and by unidirectional concentrative processes, regulated by sodium electrochemical gradient (Dos Santos-Rodrigues et al. 2014; Parkinson et al. 2011). There are two functionally distinct types of nucleoside transporters:

1. equilibrative nucleoside transporters (ENT), which predominate in the CNS, and carry both purine and pyrimidine nucleosides in both directions across cell membranes, depending on their concentration gradient. Four types of ENT transporters have been characterized: ENT1-2-3-4; type 1 and 2 appear to be present in all cell types, including neurons and glia (Baldwin et al. 2004; Dos Santos-Rodrigues et al. 2014; King et al. 2006; Parkinson et al. 2011).
2. concentrative nucleoside transporters (CNT, sodium-dependent) which mediate the influx of nucleosides under the force of transmembrane sodium gradient (Dos Santos-Rodrigues et al. 2014; Latini and Pedata 2001; Parkinson et al. 2011). Five subtypes of these transporters have been identified, and two types of CNT were cloned and detected in the rat brain, mainly in the posterior hypothalamus, superior colliculus, brainstem, striatum, hippocampus, cerebellum and cortex (Anderson et al. 1996; Dos Santos-Rodrigues et al. 2014; Latini and Pedata 2001; Parkinson et al. 2011).

Since the ENT transporters, which seem to dominate in the CNS, are bi-directional, they can not only increase the flow of adenosine into the cell when its extracellular level exceeds its intracellular one, but they may mediate the efflux of adenosine from the cell, when its intracellular level increases. On the other hand, when the Na^+ gradient is reversed, also the concentrative nucleoside transporters can release adenosine from the cell (Dos Santos-Rodrigues et al. 2014; Latini and Pedata 2001; Parkinson et al. 2011).