Geoffrey Burnstock
Alexei Verkhratsky

Purinergic Signalling and the Nervous System

Springer

Purinergic Signalling and the Nervous System

Geoffrey Burnstock • Alexei Verkhratsky

Purinergic Signalling and the Nervous System

Geoffrey Burnstock
Autonomic Neuroscience Centre
University College Medical School
Rowland Hill Street
London
NW3 2PF
UK

Alexei Verkhratsky
Faculty of Life Sciences
University of Manchester
Oxford Road
Manchester
M13 9PT
UK

ISBN 978-3-642-28862-3 ISBN 978-3-642-28863-0 (eBook)
DOI 10.1007/978-3-642-28863-0
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2012935234

© Springer-Verlag Berlin Heidelberg 2012
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
Product liability: While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

This book is dedicated to our wives and daughters

Preface

Why are we writing this book? It is mostly because, while the field had a slow start from its inception in 1972, it has now exploded in many directions and newcomers will find it difficult to find what they need in the huge purinergic literature—there are over 300,000 reprints in my office. The discovery of non-adrenergic, non-cholinergic neurotransmission in the 1960s in Melbourne was followed by the search for the transmitter involved and to our surprise, as well as that of others, ATP rather than neuropeptides, monoamines or amino acids, turned out to satisfy the criteria needed to establish the identity of a neurotransmitter and soon after a cotransmitter together with classical transmitters. It was a tough time defending this hypothesis during the next 20 years. For example, when I left Australia for London in 1975, at my farewell party, the Professor of Medicine said 'Geoff Burnstock is the inventor of the purimagine hypothesis'! I think that the turning point for the acceptance of purinergic signalling came in the early 1990s when we, and others, cloned and characterised the receptors for ATP. Since then, the field has exploded in many different directions: the recognition that ATP was an ancient signalling molecule utilised early in evolution; the distribution of functional purinoceptors in most cells in the body, the pathophysiological roles of purinergic signalling, and most recently the development of therapeutic strategies for a wide variety of diseases.

In these two volumes we have tried to cover every aspect of this burgeoning field, with a historical approach identifying seminal papers as well as describing the most recent discoveries. The first volume is focussed on the nervous system and special senses, the second on the roles of purines and pyrimidines on non-neuronal cells in respiratory, cardiovascular, endocrine, urinogenital, skeletal, immune and gastrointestinal systems in health and disease. The publishers have fortunately agreed to publish each chapter with references, so that readers can pull out single chapters of special interest to them online. Alex and I very much hope that this ambitious adventure will be helpful to those interested in this exciting field.

Geoff Burnstock

Author's Special Note

We would like to express our special gratitude to Dr. Gilian E. Knight for invaluable help in preparing this book.

Geoff Burnstock
Alexei Verkhratsky

Contents

1 Introduction 1
References 5

2 Early History of Purinergic Signalling 7
2.1 Discovery of Purines and Pyrimidines 7
2.2 Early Studies of the Extracellular Effects of Purines 9
2.3 Early Studies of the Effects of Purines on the Nervous System 11
2.4 Early Studies of Peripheral Effects of Purines 13
2.5 Early Comparative Studies 14
2.6 Discovery of Purinergic Transmission 14
2.6.1 Non-Adrenergic, Non-Cholinergic (NANC) Nerves 14
2.6.2 ATP as a Principal Transmitter 14
2.6.3 ATP as a Cotransmitter 17
2.6.4 ATP as an Excitatory Transmitter in the CNS 19
2.6.5 Adenosine in the Nervous System 20
2.6.6 Neuromodulation by Purines 21
2.7 Receptors for Purines 22
2.7.1 Subdivision into P1 and P2 Purinoceptors 22
2.7.2 History of Adenosine (P1) Purinoceptors . . . 23
2.7.3 History of P2 Purinoceptors 25
2.8 Concluding Remarks: The Expanding Field of Purinergic Signalling in the Past Decade 26
References 37

3 Purinergic Cotransmission 67
3.1 Peripheral Motor Nerves 68
3.2 Sympathetic Nerves 68
3.3 Parasympathetic Nerves 70
3.4 Sensory-Motor Nerves 70
3.5 Intrinsic Neurons in the Gut and Heart 70
3.6 Nerves in the Brain and Spinal Cord 70
3.7 Physiological Significance of Cotransmission 71

3.7.1 Fast and Slow Cotransmitters: Different Firing Patterns 71
3.7.2 Different Cotransmitters Act on Different Postjunctional Cells. 73
3.7.3 Presynaptic Neuromodulation of Cotransmitter Release 73
3.7.4 Synergism 73
3.7.5 Negative Cross-Talk 74
3.7.6 Cotransmitters and Trophic Factors 74
3.7.7 Excitatory and Inhibitory Cotransmitters . . . 74
3.7.8 False Cotransmitters 74
3.7.9 Coexisting Peptide Acting as a Neuromodulator 74
3.8 Cotransmitter Plasticity: Control of Transmitter Expression 74
3.9 Concluding Remarks. 75
References 75

4 Mechanisms of ATP Release and Inactivation 79
4.1 Introduction: General Principles of Cellular ATP Release 79
4.2 Measuring ATP Release 80
4.2.1 Luciferin–Luciferase Technique 80
4.2.2 Local ATP Measurements: Plasmalemma-Tagged Luciferase 81
4.2.3 Biosensors 82
4.2.4 The Amperometric ATP Biosensor Microelectrodes 83
4.2.5 Atomic Force Microscopy 83
4.2.6 Quinacrine-Based Fluorescence Imaging . . . 83
4.2.7 Other Enzymatic Assays 83
4.3 Mechanisms of ATP Release 84
4.3.1 Exocytosis 84
4.3.2 Diffusional ATP Release: The Role of Plasmalemmal Channels 88
4.3.3 ATP Release Following Cell Damage 93
4.3.4 Physiological ATP Release. 93
4.3.5 Release of Uridine Nucleotides and Nucleotide Sugars 96
4.4 ATP Degradation: Roles for Ectoenzymes 96
4.4.1 Nucleoside Triphosphate Diphosphohydrolases 96
4.4.2 Nucleotide Pyrophosphatases/ Diphosphodiesterases. 102
4.4.3 Alkaline and Acid Phosphatases 102
4.4.4 Ecto-5′-Nucleotidase 103

4.4.5 Other Ecto-Enzymes Involved in Metabolism of Purines 103
4.4.6 Pharmacology of Ectonucleotidases 104
4.5 Adenosine Release 104
4.6 Concluding Remarks 105
References 105

5 Receptors for Purines and Pyrimidines 119
5.1 Introduction 119
5.2 Adenosine (P1) Receptors 120
5.2.1 Molecular Cloning 120
5.2.2 Molecular Structure 120
5.2.3 Adenosine Receptor Oligomerisation 123
5.2.4 Adenosine Receptors, G-Proteins and Second-Messenger Systems 126
5.2.5 Distribution of Adenosine Receptors: General Overview 126
5.3 P2X Receptors 128
5.3.1 General Principles of Ionotropic Receptor Architecture 128
5.3.2 Molecular Cloning of P2X Receptors 129
5.3.3 Molecular Structure of P2X Receptors. 130
5.3.4 P2X Receptors: Biophysics and Pharmacology 134
5.3.5 Distribution of P2X Receptors: General Overview 167
5.4 P2Y Receptors 168
5.4.1 Molecular Structure of P2Y Receptors. 168
5.4.2 P2Y Receptor Classification 174
5.4.3 P2Y Receptor Subtypes 176
5.4.4 Molecular Pharmacology: Agonists and Antagonists 190
5.4.5 Molecular Modelling Studies 199
5.4.6 Interactions Between P2Y and Other Receptors 199
5.4.7 P2Y Receptors Distribution: General Overview 204
5.4.8 Gene Activation Regulated by P2Y Receptors 206
5.5 Concluding Remarks 209
References 209

6 Evolution of Purinergic Signalling 245
6.1 Evolution of Chemical Intercellular Transmission 245
6.2 Tree of Life: Remarks on Taxonomy 246
6.3 Viruses 247
6.4 Bacteria 248

6.5 Eucarya 249
6.5.1 Protozoa (Amoebozoa) 249
6.5.2 Algae. 252
6.5.3 Plants 253
6.5.4 Fungi. 255
6.5.5 Sponges 256
6.5.6 Metazoa or Animalia 256
6.6 Evolution of the Purinergic Signalling System: Generalisation 278
6.6.1 Ancient Roots of ATP Release Systems. 278
6.6.2 Purinoceptors 279
6.6.3 Ectonucleotidases 281
References 284

7 Peripheral Nervous System 307
7.1 Motor Nerves to Skeletal Muscle 307
7.1.1 Skeletal Neuromuscular Junction. 307
7.1.2 Schwann Cells 311
7.2 Autonomic Nervous System 313
7.2.1 Sympathetic Ganglia 314
7.2.2 Parasympathetic Ganglia 317
7.2.3 Enteric Ganglia. 319
7.2.4 Autonomic Neuroeffector Transmission 329
7.2.5 Smooth Muscle. 334
7.2.6 Heart 363
7.2.7 Other Cell Types 366
7.3 Diseases of Peripheral Nervous System 377
7.3.1 Skeletal Muscle Diseases 377
7.4 Concluding Remarks 388
References 388

8 Purinergic Signalling in the Central Nervous System 433
8.1 Intercellular Communications in the Central Nervous System 434
8.2 Initial Discoveries of the Central Action of ATP 436
8.3 Mechanisms of ATP Release in CNS 437
8.3.1 Vesicular Release of ATP in the CNS 438
8.3.2 Diffusional Release of ATP 443
8.4 Neuronal P2X Receptors: ATP-Mediated Excitatory Synaptic Transmission 445
8.4.1 P2X Receptors in Central Neurons 445
8.4.2 P2X-Mediated Excitatory Postsynaptic Currents (EPSCs) 453
8.4.3 Multiple Functional Roles of Neuronal P2X Receptors 454

8.5 Neuronal P2Y-Mediated Signalling 462
8.5.1 Distribution of P2Y Receptors in the CNS 462
8.5.2 Functional Role of Neuronal P2Y Receptors 462
8.6 Neuronal Adenosine Receptor-Mediated Signalling . . . 466
8.6.1 Mechanisms of Adenosine Release in CNS 466
8.6.2 Neuronal Adenosine Receptors 468
8.7 Purinergic Signalling in Astroglia 470
8.7.1 P2X Receptors 470
8.7.2 Synaptic Activation of Astroglial P2X Receptors 476
8.7.3 Role of P2X Receptors in Astroglial Signalling 479
8.7.4 P2Y Receptors 482
8.7.5 Adenosine (P1) Receptors 488
8.8 Purinergic Signalling in Oligodendroglia 491
8.8.1 P2X Receptors 491
8.8.2 P2Y Receptors 491
8.8.3 Adenosine Receptors 492
8.9 Purinergic Signalling in Ependymal Cells 492
8.10 Purinergic Signalling in Microglia 493
8.10.1 Purinergic Signalling Controls Microglial Function 493
8.10.2 Microglial P2X Receptors 495
8.10.3 $P2X_7$ Receptors and Microglial Function . . . 495
8.10.4 Microglial P2Y Receptors 499
8.10.5 Microglial Adenosine Receptors 501
8.11 Purinergic Transmission in Various Regions of the CNS 501
8.11.1 Cortex 501
8.11.2 Hippocampus 502
8.11.3 Cerebellum. 504
8.11.4 Basal Ganglia 505
8.11.5 Midbrain 506
8.11.6 Thalamus 506
8.11.7 Habenula 506
8.11.8 Spinal Cord 507
8.12 Purinergic Transmission and Higher Brain Functions 508
8.12.1 Learning and Memory 508
8.12.2 Sleep and Arousal 509
8.12.3 Locomotion 510
8.12.4 Feeding 511
8.12.5 Mood and Motivation 511

8.13 Central Control of Autonomic Function 512
8.13.1 Brain Stem and Ventrolateral Medulla: Central Chemoreception 512
8.13.2 Trigeminal Mesencephalic Nucleus 513
8.13.3 Trigeminal Subnucleus Caudalis 513
8.13.4 Area Postrema 514
8.13.5 Locus Coeruleus 514
8.13.6 Nucleus Tractus Solitarius 514
8.13.7 Motor and Sensory Nuclei 516
8.13.8 Hypothalamus 516
8.13.9 Circadian Rhytms 519
8.14 Purinoceptors in Neuropathology 519
8.14.1 Brain Injury and Neuroprotection 519
8.14.2 Neuroinflammatory Disorders 523
8.14.3 Neurodegenerative Diseases 524
8.14.4 Epileptic Seizures 528
8.14.5 Neuropsychiatric Disorders 529
8.14.6 Neuropathic Pain 531
8.14.7 Migraine 534
8.14.8 Cancer 534
8.14.9 Encephalitis 534
8.14.10 Abnormalities in Central Control of Peripheral Function 535
8.15 Concluding Remarks 535
References 535

9 Sensory Nerves 583
9.1 Introduction 583
9.2 Sensory Neurons 584
9.2.1 Dorsal Root Ganglia 586
9.2.2 Nodose Ganglia 588
9.2.3 Trigeminal Ganglia 589
9.2.4 Petrosal Ganglia 591
9.2.5 Retinal Ganglia 591
9.2.6 Intramural Enteric Sensory Neurons 592
9.3 Peripheral Sensory Nerve Terminals 593
9.3.1 Carotid Body 594
9.3.2 Lung 595
9.3.3 Gut 598
9.3.4 Urinary Bladder 598
9.3.5 Skin, Muscle and Joints 598
9.3.6 Heart 599
9.4 Purinergic Mechanosensory Transduction 599
9.4.1 Urinary Bladder 599
9.4.2 Ureter 601

9.4.3 Gut 601
9.4.4 Uterus 601
9.4.5 Tooth Pulp 602
9.4.6 Tongue 602
9.4.7 Skin and Joints 602
9.5 Purinergic Sensory Pathology 602
9.5.1 Pain 602
9.5.2 Bladder Diseases 606
9.5.3 Gut Disorders 607
9.5.4 Arthritis 607
9.5.5 Respiratory Diseases 608
9.6 Development of Purinergic Sensory Signalling 608
9.7 Evolution of Purinergic Sensory Mechanisms 611
9.8 Concluding Remarks 612
References 614

10 Special Senses 627
10.1 Eye 627
10.1.1 Retina 627
10.1.2 Lens 638
10.1.3 Ciliary Body and Choroid 638
10.1.4 Trabecular Meshwork and Schlemme Cells: Regulation of Intra-ocular Pressure 641
10.1.5 Iris 642
10.1.6 Conjunctiva 642
10.1.7 Cornea 643
10.1.8 Vasculature 643
10.1.9 Lacrimal Gland and Tears 644
10.1.10 Development 645
10.1.11 Diseases of the Eye 646
10.2 Ear 650
10.2.1 Cochlea 652
10.2.2 Vestibule 655
10.2.3 Endolymph and Peilymph 656
10.2.4 Development and Ageing 656
10.2.5 Diseases of the Ear 657
10.3 Nasal Organs 658
10.4 Tongue 661
10.5 Concluding Remarks 663
References 663

11 Ontogeny of Purinergic Neurotransmission 681
11.1 Central Nervous System 681
11.2 Peripheral Ganglia 687
11.3 Retina 688
11.4 Skeletal Neuromuscular Junction 690

11.5 Gastrointestinal Tract 693
11.6 Cardiovascular System 694
11.7 Lung 696
11.8 Urinary Bladder 696
11.9 Inner Ear 697
11.10 Vas Deferens and Seminal Vesicles 697
11.11 Other Organs 698
11.12 Embryonic Stem Cells. 698
11.13 Concluding Remarks 701
References 702

Index 709

Introduction 1

Purines and pyrimidines are those fateful molecules which shaped the life on our Earth. These molecules occurred in a prebiotic period (Ponnamperuma et al. 1963; Waldrop 1989) and became essential for the emerging life. Indeed without purines and pyrimidines, construction of RNA and DNA would be impossible and hence the genetic code that sustains life familiar to us would never appear. Furthermore, ATP was selected very early as the main source of biological energy, and thus became an indispensable feature of life. This was a critical evolutionary choice because it shaped enzymatic systems to utilise ATP in energy-dependent reactions and necessitated the appearance of the universal intracellular signalling system based on calcium ions (Case et al. 2007) as keeping cytosolic Ca^{2+} extremely low became vitally important, since otherwise insoluble Ca^{2+}-phopshates would preclude the cell energetics. Thus all living cells on the Earth, beginning from the most primitive ones, had high cytosolic concentrations of ATP (or sometimes GTP) and it is of little surprise that ATP was soon utilised by nature for another fundamental function of sending information from one living cell to another.

Chemical transmission, which utilises small molecules for cell-to-cell information transfer, was an essential evolutionary step, which allowed continuous progression of life-forms. Our knowledge of the initial appearance and early forms of chemical transmission is virtually non-existent (Trams 1981), and yet some generalisation can be drawn from observations of phylogenetic development and from evidence of distribution of different signalling systems in the higher life-forms. Our conjectures of the modus operandi and habits of the first life-forms lie entirely in the realm of speculation, and yet we may assume that some of these nascent living creatures were born and existed in the ocean, and thus the intercellular communication called for a diffusible messenger. Choices for these diffusible messengers were only a few; they can be ions or small diffusible molecules. Ions (possibly with the exception of protons) can be excluded from extracellular communication pathways because of their high background concentrations in the primordial seas, and thus only the relatively small soluble molecules existing in abundance within the cells can be employed. These could be some amino acids, or some forms of gaseous transmitters (for example nitric oxide (NO)), or protons, which may accumulate in cells following metabolism or indeed purines, and especially purines endowed with pyrophosphate bonds.

As usual during evolution, several possibilities were explored. Indeed the most ancient receptors discovered in prokaryotes are those for glutamate (in a form of a potassium-selective glutamate receptor identified in *Synechocystis* (Chen et al. 1999)) and further development resulted in the appearance of glutamate receptors in early eukaryotes (Chiu et al. 1999, 2002) and for protons, cloned from the cyanobacterium *Gloeobacter violaceus* (Bocquet et al. 2007). The early evolution of gaseous extracellular

G. Burnstock and A. Verkhratsky, *Purinergic Signalling and the Nervous System*,
DOI: 10.1007/978-3-642-28863-0_1, © Springer-Verlag Berlin Heidelberg 2012

signalling molecules remains quite obscure; we know that NO was present already in primitive nervous systems (Garthwaite 2008). However, there is little evidence to date of gaseous transmitters in primitive unicellular life-forms.

Arguably, the first function of ATP as the extracellular signaller was to report danger. Indeed, cell disintegration inevitably would cause ATP leakage and a gradient of ATP would appear in the surrounding water. This may have been the initial form of chemical transmission, which, in fact, remained throughout evolution, as in most living tissues massive release of ATP acts as an indicator of damage. Most amazingly, this role of ATP is preserved even in the very complex defensive and behavioural reactions: ATP is a mediator of pain; ATP receptors control activation of immune cells, while in the brain ATP initiates astrogliosis and activation of microglia. It is possibly difficult to realise that virtually every cell type or single-celled organism does display some form of sensitivity to ATP and its derivatives.

The early deployment of ATP as an intercellular signalling molecule led to a rapid evolution of the purinergic signalling system, which includes, apart from the signalling molecule, the systems for regulated release of this molecule from undamaged cells, the receptive molecules and the system for termination of the signalling action of the transmitter.

The mechanisms of ATP release (as we shall reiterate many times in this book) are many. Fundamentally, they include a diffusion route through plasmalemmal channels (and this route is important for ATP because of an exceptionally high transmembrane concentration gradient—cytosolic ATP is kept at a millimolar range, whereas ambient extracellular ATP concentration does not exceed several nanomolars resulting in $\sim$1,00,000 times difference) and regulated exocytosis. The diffusional route was possibly the first to operate: the ATP can be released through volume/mechanical stress regulated anion channels that already appeared in *Escherichia coli* (which possess both mechano-sensitive and anion channels (Booth et al. 2003, 2007)). Eukaryotes acquired a more sophisticated way for controlled release of signalling molecules—the Ca^{2+}-regulated exocytosis of neurotransmitter-containing vesicles. An important step in vesicular development was associated with the appearance of specific transporters that enrich the vesicles with specific transmitters. These transporters appear within the family of hugely diversified families of solute carriers (which in the human genome are represented by 384 genes and constitute 48 different classes). Out of this huge variety, the SLC17 family contains vesicular transporters for amino acids, glutamate and nucleotides, the latter known as VNUT. Interestingly, the SLC17A9 member, which is a VNUT, has the longest evolutionary history and was already present in *Caenorhabditis elegans* (Sreedharan et al. 2011), indicating that vesicular release of ATP has been operational at the very dawn of the formation of neuronal networks.

The ancestral receptors to ATP appeared in the very early eukaryotes, such as social amoeba, tardigrades and schistosoma (Burnstock and Verkhratsky 2009; for a detailed account of the purinergic system evolution see Chap. 6 of this book). These were typical ionotropic receptors, which already at that stage were represented by several subtypes and which are biophysically similar to the more advanced forms. These ancestral forms gave rise to a family of P2X receptors present in most of the life-forms (with surprising disappearance in *C. elegans* and *Drosophila*). Somewhat later the metabotropic purinoceptors of P2Y and P1 (adenosine) varieties became ubiquitous in various cell types of more advanced organisms. Finally, the termination of purinergic signalling events is achieved by an enzymatic activity of an extended family of ectonucleotidases, the ancestral forms of which are present already in bacteria (Vivian et al. 2010).

The purinergic system was probably one of the very first to be constructed and it is little wonder that this early phylogenetic root stipulated an omnipresence of this system within different tissues and cell types and its almost ubiquitous involvement in the regulation of a wide variety of physiological processes.

Several extracellular signalling systems are present in the human body, these systems being divided into classic transmitters (which mediate signal transmission in neural networks and in neuronal-endocrine and neuromuscular junctions), paracrine and autocrine transmission, that act through the extracellular space and hormonal transmission, which exerts its action through blood flow. As a rule, transmitter systems are anatomically and functionally segregated. For example: glutamate acts as an excitatory neurotransmitter in the central nervous system (CNS); cholinergic transmission is prominent at somatic and autonomic neuroeffector junctions and in some brain areas; γ-aminobutyric acid acts largely as a transmitter of inhibitory responses in the brain; noradrenaline (NA) is a major transmitter in the sympathetic nervous system and some parts of the brain; glycine is localised as an inhibitory transmitter largely in the spinal cord; and 5-hydroxytryptamine, while diffusely distributed, is limited in its transmission activities. Even stricter segregation applies to other neurotransmitter systems, such as domapinergic or peptidergic. Some of these transmitters are also released from non-neuronal cells.

The purinergic signalling system, however, is unique, as it has virtually no anatomical segregation. Indeed, in the nervous system ATP acts as a cotransmitter in nerves in both CNS and peripheral nervous system (PNS), whereas adenosine appears as the universal inhibitory neuromodulator. In the PNS ATP is released as the only transmitter from sympathetic nerves supplying submucosal arterioles in the intestine, while NA released from these nerves acts only as a prejunctional neuromodulator. ATP also acts as a major gliotransmitter, and all types of glia studied so far express various subtypes of purinoceptors. However, the role of ATP as a signalling molecule is not limited to the nervous system, as indeed ATP sensitivity and ATP-mediated signalling has been identified in virtually all tissues and cell types as we shall discuss in detail in the chapters of this book. ATP and its derivatives truly appear to be most widespread and omnipresent of all known extracellular signalling molecules.

While early studies were largely focused on short-term signalling in events such as neurotransmission, neuromodulation, secretion, chemoattraction and acute inflammation, there has been increasing interest in long-term (trophic) signalling involving cell proliferation, differentiation, motility and death in development, regeneration, wound healing, restenosis, epithelial cell turnover, cancer and ageing (see Abbracchio and Burnstock 1998; Burnstock and Verkhratsky 2010). For example, in blood vessels, there is dual short-term control of vascular tone by ATP released as an excitatory cotransmitter from perivascular sympathetic nerves to act on P2X receptors on smooth muscle, while ATP released from endothelial cells during changes in blood flow (producing shear stress) and hypoxia acts on P2X and P2Y receptors on endothelial cells leading to production of nitric oxide and relaxation (Burnstock 2002). In addition, there is long-term control of cell proliferation and differentiation, migration and death involved neovascularisation, restenosis following angioplasty and atherosclerosis (Erlinge and Burnstock 2008). Involvement of purinergic signalling in development, ageing and regeneration has been described (see Burnstock 2007).

For many years, the source of ATP acting on receptors was considered to be damaged or dying cells, except for exocytotic vesicular release from nerves. However, it is now known that many cell types release ATP physiologically in response to gentle mechanical distortion, hypoxia or to some agents (Bodin and Burnstock 2001). The mechanism of ATP transport is currently being debated and includes in addition to vesicular release, ABC transporters, connexin or pannexin hemichannels, maxi-ion channels and even $P2X_7$ receptors (Burnstock 2007).

There is now much known about the extracellular breakdown of released ATP by various types of ectonucleotidases, including: E-NTPDases, E-NPPS, alkaline phosphatase and ecto-5′-nucleotidose (Zimmermann et al. 2007).

It is well known that the autonomic nervous system shows high plasticity compared to CNS.

For example, substantial changes in cotransmitter and receptor expression occur during development and ageing, in the nerves that remain following trauma or surgery and in disease situations (Burnstock 2006). For example, a P2Y-like receptor was identified in *Xenopus* that was transiently expressed in the neural plate and again later in secondary neuralation in the tail bud, suggesting involvement of purinergic signalling in the development of the nervous system (Bogdanov et al. 1997). There is transient expression of $P2X_5$ and $P2X_6$ receptors during development of myotubules and of $P2X_2$ receptors during development of the neuromuscular junction (Ryten et al. 2001). In the rat brain, $P2X_3$ receptors are expressed first at embryonic (E)11, $P2X_2$ and $P2X_7$ receptors appear at E14, $P2X_4$, $P2X_5$ and $P2X_6$ receptors at P1 and $P2X_1$ receptors at P16 (Cheung et al. 2005).

Primitive sprouting of central neurons was shown in experiments in which the enteric nervous system was transplanted into the striatum of the brain (Tew et al. 1992). It was later shown that a growth factor released from enteric glial cell acting synergistically with ATP (and its breakdown product, adenosine) and NO were involved (Höpker et al. 1996). It is suggested that similar synergistic activity of purines and growth factors might be involved in stem cell activity (Burnstock and Ulrich 2011).

It was established early that ATP was a major cotransmitter with acetylcholine in parasympathetic nerves mediating contraction of the urinary bladder of rodents (Burnstock et al. 1978). In healthy human bladder, the role of ATP as a cotransmitter is minor. However, in pathological conditions, such as interstitial cystitis, outflow obstruction and most types of neurogenic bladder, the purinergic component is increased to about 40% (Burnstock 2001, 2006). Similarly, in spontaneously hypertensive rats, there is a significantly greater cotransmitter role for ATP in sympathetic nerves (Vidal et al. 1986). $P2X_3$ receptors were cloned in 1995 and shown to be largely located in small nociceptive sensory nerves that label with isolectin B4 (Chen et al. 1995; Bradbury et al. 1998).

Central projections are located in inner lamina 2 of the dorsal horn of the spinal cord and peripheral extension in skin, tongue and visceral organs. A unifying purinergic hypothesis for the initiation of pain was published (Burnstock 1996) and a hypothesis describing purinergic mechanosensory transduction in visceral organs in 1999, where ATP, released from lining epithelial cells during distension, acts on $P2X_3$ and $P2X_{2/3}$ receptors on subepithelial sensory nerve endings to send nociceptive messengers via sensory ganglia to the pain centres in the brain (Burnstock 1999). Supporting evidence including epithelial release of ATP, immuno-localization of $P2X_3$ receptors on subepithelial nerves and activity recorded in sensory nerves during distension that is mimicked by ATP and reduced by $P2X_3$ receptor antagonists has been reported in the bladder (Vlaskovska et al. 2001), ureter (Rong and Burnstock 2004) and gut (Wynn and Burnstock 2006). Purinergic mechanosensory transduction is also involved in urine voiding as evidenced in $P2X_3$ knockout mice (Cockayne et al. 2000). For neuropathic and inflammatory pain $P2X_4$, $P2X_7$ and $P2Y_{12}$ receptors on microglia have been implicated and antagonists to these receptors are very effective in abolishing allodynia (Inoue 2007; Burnstock 2009). There is much current interest in neuron-glial cell interactions in the CNS (Fields and Burnstock 2006) and there is also strong interest in the potential roles of purinergic signalling in trauma and ischaemia, neurodegenerative conditions including Alzheimer's, Parkinson's and Huntington's diseases, multiple sclerosis and amyotrophic lateral sclerosis. There are also studies in progress on purinergic signalling in epilepsy, neuropsychiatric diseases and mood disorders (see Burnstock 2008).

This book, which is intended to be published in two volumes represents our long-lasting effort to produce a comprehensive coverage of the purinergic signalling. In the first volume of the book we shall overview the general features of the purinergic signalling system and concentrate on purinergic transmission in the brain. In the second volume we will cover purinergic signalling in all non-neuronal tissues.

References

Abbracchio MP, Burnstock G (1998) Purinergic signalling: pathophysiological roles. Jpn J Pharmacol 78:113–145

Bocquet N, Prado de Carvalho L, Cartaud J, Neyton J, Le Poupon C, Taly A, Grutter T, Changeux JP, Corringer PJ (2007) A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. Nature 445:116–119

Bodin P, Burnstock G (2001) Purinergic signalling: ATP release. Neurochem Res 26:959–969

Bogdanov YD, Dale L, King BF, Whittock N, Burnstock G (1997) Early expression of a novel nucleotide receptor in the neural plate of *Xenopus* embryos. J Biol Chem 272:12583–12590

Booth IR, Edwards MD, Miller S (2003) Bacterial ion channels. Biochemistry 42:10045–10053

Booth IR, Edwards MD, Black S, Schumann U, Miller S (2007) Mechanosensitive channels in bacteria: signs of closure? Nat Rev Microbiol 5:431–440

Bradbury EJ, Burnstock G, McMahon SB (1998) The expression of $P2X_3$ purinoceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. Mol Cell Neurosci 12:256–268

Burnstock G, Ulrich H (2011) Purinergic signalling in embryonic and stem cell development. Cell Mol Life Sci 68:1369–1394

Burnstock G, Verkhratsky A (2009) Evolutionary origins of the purinergic signalling system. Acta Physiologica 195:415–447

Burnstock G, Verkhratsky A (2010) Long-term (trophic) purinergic signalling: purinoceptors control cell proliferation, differentiation and death. Cell Death Dis 1:e9

Burnstock G (1996) A unifying purinergic hypothesis for the initiation of pain. Lancet 347:1604–1605

Burnstock G (1999) Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. J Anat 194:335–342

Burnstock G (2002) Purinergic signalling and vascular cell proliferation and death. Arterioscler Thromb Vasc Biol 22:364–373

Burnstock G (2006) Pathophysiology and therapeutic potential of purinergic signaling. Pharmacol Rev 58:58–86

Burnstock G (2007) Physiology and pathophysiology of purinergic neurotransmission. Physiol Rev 87:659–797

Burnstock G (2008) Purinergic signalling and disorders of the central nervous system. Nat Rev Drug Discov 7:575–590

Burnstock G (2009) Purinergic receptors and pain. Curr Pharm Des 15:1717–1735

Burnstock G, Cocks T, Kasakov L, Wong HK (1978) Direct evidence for ATP release from non-adrenergic, non-cholinergic ("purinergic") nerves in the guinea-pig taenia coli and bladder. Eur J Pharmacol 49:145–149

Burnstock G (2001) Purinergic signalling in lower urinary tract. In: Abbracchio MP, Williams M (eds) Handbook of experimental pharmacology, vol 151/I. Purinergic and pyrimidinergic signalling I—molecular nervous and urinogenitary system function. Springer, Berlin, pp 423–515

Case RM, Eisner D, Gurney A, Jones O, Muallem S, Verkhratsky A (2007) Evolution of calcium homeostasis: from birth of the first cell to an omnipresent signalling system. Cell Calcium 42:345–350

Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN (1995) A P2X purinoceptor expressed by a subset of sensory neurons. Nature 377:428–431

Chen GQ, Cui C, Mayer ML, Gouaux E (1999) Functional characterization of a potassium-selective prokaryotic glutamate receptor. Nature 402:817–821

Cheung K–K, Chan WY, Burnstock G (2005) Expression of P2X receptors during rat brain development and their inhibitory role on motor axon outgrowth in neural tube explant cultures. Neuroscience 133: 937–945

Chiu JC, Brenner ED, DeSalle R, Nitabach MN, Holmes TC, Coruzzi GM (2002) Phylogenetic and expression analysis of the glutamate-receptor-like gene family in Arabidopsis thaliana. Mol Biol Evol 19:1066–1082

Chiu J, DeSalle R, Lam HM, Meisel L, Coruzzi G (1999) Molecular evolution of glutamate receptors: a primitive signaling mechanism that existed before plants and animals diverged. Mol Biol Evol 16:826–838

Cockayne DA, Hamilton SG, Zhu Q-M, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford APDW (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in $P2X_3$-deficient mice. Nature 407:1011–1015

Erlinge D, Burnstock G (2008) P2 receptors in cardiovascular physiology and disease. Purinergic Signal 4:1–20

Fields D, Burnstock G (2006) Purinergic signalling in neuron-glial interactions. Nature Rev Neurosci 7:423–436

Garthwaite J (2008) Concepts of neural nitric oxide-mediated transmission. Eur J Neurosci 27:2783–2802

Höpker VH, Saffrey MJ, Burnstock G (1996) Neurite outgrowth of striatal neurons in vitro: involvement of purines in the growth promoting effect of myenteric plexus explants. Int J Dev Neurosci 14:439–451

Inoue K (2007) P2 receptors and chronic pain. Purinergic Signal 3:135–144

Ponnamperuma C, Sagan C, Mariner R (1963) Synthesis of adenosine triphosphate under possible primitive Earth conditions. Nature 199:222–226

Rong W, Burnstock G (2004) Activation of ureter nociceptors by exogenous and endogenous ATP in guinea pig. Neuropharmacology 47:1093–1101

Ryten M, Hoebertz A, Burnstock G (2001) Sequential expression of three receptor subtypes for extracellular ATP in developing rat skeletal muscle. Dev Dyn 221:331–341

Sreedharan S, Shaik JH, Olszewski PK, Levine AS, Schioth HB, Fredriksson R (2011) Glutamate, aspartate and nucleotide transporters in the SLC17 family form four main phylogenetic clusters: evolution and tissue expression. BMC Genomics 11:17

Tew EMM, Anderson PN, Burnstock G (1992) Implantation of the myenteric plexus into the corpus striatum of adult rats: survival of the neurones and glia and interactions with host brain. Restor Neurol Neurosci 4:311–321

Trams EG (1981) On the evolution of neurochemical transmission. Differentiation 19:125–133

Vidal M, Hicks PE, Langer SZ (1986) Differential effects of α, β-methylene ATP on responses to nerve stimulation in SHR and WKY tail arteries. Naunyn Schmiedebergs Arch Pharmacol 332:384–390

Vivian JP, Riedmaier P, Ge H, Le Nours J, Sansom FM, Wilce MC, Byres E, Dias M, Schmidberger JW, Cowan PJ, d'Apice AJ, Hartland EL, Rossjohn J, Beddoe T (2010) Crystal structure of a Legionella pneumophila ecto-triphosphate diphosphohydrolase, a structural and functional homolog of the eukaryotic NTPDases. Structure 18:228–238

Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford APDW, Burnstock G (2001) $P2X_3$ knockout mice reveal a major sensory role for urothelially released ATP. J Neurosci 21:5670–5677

Waldrop MM (1989) Did life really start out in an RNA world? Science 246:1248–1249

Wynn G, Burnstock G (2006) Adenosine 5′-triphosphate and it's relationship with other mediators that activate pelvic afferent neurons in the rat colorectum. Purinergic Signal 2:517–526

Zimmermann H, Mishra SK, Shukla V, Langer D, Gampe K, Grimm I, Delic J, Braun N (2007) Ecto-nucleotidases, molecular properties and functional impact. An R Acad Nac Farm 73:537–566

Early History of Purinergic Signalling

2

Contents

2.1 Discovery of Purines and Pyrimidines 7

2.2 Early Studies of the Extracellular Effects of Purines 9

2.3 Early Studies of the Effects of Purines on the Nervous System 11

2.4 Early Studies of Peripheral Effects of Purines 13

2.5 Early Comparative Studies 14

2.6 Discovery of Purinergic Transmission 14
2.6.1 Non-Adrenergic, Non-Cholinergic (NANC) Nerves 14
2.6.2 ATP as a Principal Transmitter 14
2.6.3 ATP as a Cotransmitter 17
2.6.4 ATP as an Excitatory Transmitter in the CNS 19
2.6.5 Adenosine in the Nervous System 20
2.6.6 Neuromodulation by Purines 21

2.7 Receptors for Purines 22
2.7.1 Subdivision into P1 and P2 Purinoceptors 22
2.7.2 History of Adenosine (P1) Purinoceptors 23
2.7.3 History of P2 Purinoceptors 25

2.8 Concluding Remarks: The Expanding Field of Purinergic Signalling in the Past Decade 26

References 37

2.1 Discovery of Purines and Pyrimidines

The history of purines and pyrimidines began in 1776 when the Swedish pharmacist Carl Wilhelm Scheele isolated uric acid from bladder stones (Scheele 1776). Almost seven decades later, in 1844, guanine was isolated by Unger from the faeces of Peruvian guano sea birds (Unger 1846). At the end of the nineteenth century, several principal purines (adenine, xanthine and hypoxantine) and pyrimidines (thymine, cytosine and uracil) were discovered by Ludwig Karl Martin Leonhard Albrecht Kossel (1853–1927; see Jones 1953; Bendich 1955; Persson 2012; the original Kossel report appeared in *Chem. Ber.*, 1885, 18, 79). Interestingly, already at that stage it was believed that these substances constitute the main part of cell nuclei; Kossel followed experimental protocols of Friedrich Miescher (1844–1895), who was the first to isolate the nuclear material rich in phosphorus that was called 'nuclein' (Miescher 1874; Hoppe-Seyler 1871). In the same period the great Emil Fischer started to investigate the structure of caffeine and related compounds (Fischer 1881). He solved the structures and confirmed them by synthesis. It was also Emil Fischer who, based on his structural studies, introduced the term 'purines' (*purum uricum*) (Fischer 1907); this was one of the reasons for his Nobel Prize in 1902. The term 'pyrimidines' was introduced by (Pinner 1885). An arduous

G. Burnstock and A. Verkhratsky, *Purinergic Signalling and the Nervous System*,
DOI: 10.1007/978-3-642-28863-0_2, © Springer-Verlag Berlin Heidelberg 2012

Fig. 2.1 Discoverers of purinergic signalling

task of determining the sugar part of nucleosides (and nucleotides) followed and was finally solved by Phoebus Aaron Levene (Levene and Jacobs 1908; Levene and Tipson 1931).

In 1927, Gustav Embden and Margarete Zimmermann described adenosine monophosphate in skeletal muscle (Embden and Zimmermann 1927). Adenosine 5′-triphosphate (ATP) was discovered in 1929, independently by Karl Lohmann in Germany and by Cyrus Hartwell Fiske and Yellagaprada SubbaRow in the USA (Fiske and SubbaRow 1929; Lohmann 1929). Lohman (1898–1978) was in those days working as the assistant of Otto Meyrhoff in Berlin; Fiske (1890–1978) was an associate professor in Harvard Medical School in Boston, and SubbaRow (1896–1948) was Fiske's PhD student (Fig. 2.1). Lohman's publication appeared several months earlier (in August 1929) than the paper by Fiske and SubbaRow (which was published in October 1929), and yet the latter had obtained the first evidence for ATP probably as early as 1926. It all came to a climax in August 1929, during the thirteenth Physiological Congress in Boston when Lohman and Fiske discussed the priority matters. Whether Fiske briefed Otto Meyerhof, who was Lohmann's director, about his discovery (and then Meyerhof pushed Lohman's publication) or not, remains a matter of doubt (the dramatic history of ATP discovery is described in detail in Maruyama 1991). In the following decade, the role of ATP in cell energetics was firmly established and the concept of the 'high-energy phosphate bond' was introduced by Fritz Lipman (Lipman 1941).

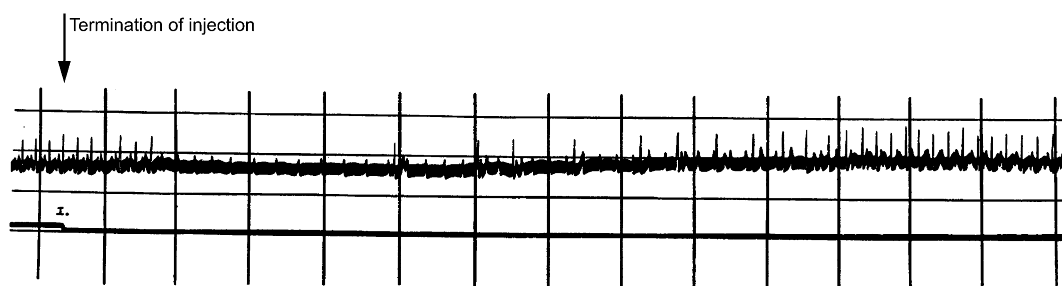

Fig. 2.2 The first experimental recording of the action of purines-enriched tissue extract on heartbeat. The electrocardiogram shows the influence of intravenous injection of 1 c.c. of extract from heart muscle. Injection commenced 3 s. before and terminated at point 'I' (also marked by an *arrow*). Time marker = 1 s. (Figure is reproduced from Drury and Szent-Györgyi 1929, with permission from Wiley.)

2.2 Early Studies of the Extracellular Effects of Purines

Adenine was identified in blood in 1914, most probably in the form of the adenosine 5′-monophosphate, AMP (Bass 1914), and slightly later it was suggested that it has inhibitory effects on cardiovascular system (Freund 1920). At about the same time, Thannhauser and Bommes (1914) claimed that, unlike adenosine, adenine injected subcutaneously in man was not toxic. In 1926, IG Farben in Germany started to isolate potential cardio-stimulant substances from the heart and developed an extract that contained mostly AMP.

The role of purines as extracellular signalling molecules was experimentally discovered by Alan Drury and Albert Szent-Györgyi von Nagyra-Polt (Drury and Szent-Györgyi 1929) when they found that crude extracts from several different tissues (heart muscle, brain, kidney and spleen) from bullock and sheep, when injected intravenously, exerted profound pharmacological effects, including a negative chronotropic effect (up to a complete cardiac arrest—Fig. 2.2) on the guinea pig, rabbit, cat and dog heart: it further produced dilatation of coronary blood vessels that resulted in profound hypotensive actions, and inhibited spontaneous activity of intestinal smooth muscle. The active constituent in their extracts was identified as adenylic acid (adenosine-5′-monophosphate, 5′-AMP). Further, they showed that intravenous injection of both adenosine and adenylic acid fully mimicked effects of heart extracts causing sinus bradycardia and heart block, and that they were approximately equiactive. In addition, Drury and Szent-Györgyi also found that the purines could normalise supraventricular tachyarrhythmia.

This seminal discovery prompted further work on the IG Farben preparation, called Lacarnol, which was already available. Many studies followed, confirming that purine nucleosides and nucleotides acted as potent vasodilators of coronary (Bennett and Drury 1931; Lindner and Rigler 1931; Wedd 1931; Wedd and Drury 1934; Winbury et al. 1953; Wolf and Berne 1956); renal (Houck et al. 1948) and pulmonary vessels (Gaddum and Holtz 1933), and produce blood pressure changes if administered systemically (Gillespie 1934; Emmelin and Feldberg 1948; Folkow 1949; Davies et al. 1951; Duff et al. 1954). There were also early reports using the IG Farben extract of physiological effects in humans. The first was largely positive and suggested therapeutic usefulness (Rothman 1930), but later reports in man found little therapeutic benefit, perhaps because the patients treated had chronic atrial fibrillation which is not amenable to normalisation by adenosine (Honey et al. 1930). At the same time, the depressing effects of purines on heart muscle were demonstrated on perfused frog heart (Lindner and Rigler 1931; Ostern and Parnas 1932; Loewi 1949). When studying the guinea pig heart, Drury (1936) noted that ATP was more effective than adenosine at producing heart

block. During the war there was much interest in traumatic shock, and one hypothesis is particularly relevant, namely that crushed tissues, especially muscle, would release ATP and other adenylates and then they would contribute to vasodilatation (Green 1943; Bielschowsky and Green 1944). Harry Norman Green and Harry Berrington Stoner, who during World War II were employed for studying the role of ATP in wound shock, published a book on the *Biological Actions of Adenine Nucleotides* in 1950 (Green and Stoner 1950), in which they correlated activity of the nucleotides with the length of the phosphate chain, and came to the conclusion that adenosine was the least active and ATP the most active of the purine compounds. The hypothesis that circulating adenine compounds were responsible for the rapid decrease in blood pressure was refuted by cross-transfusion experiments (Green and Stoner 1950) and, particularly, by the careful measurements of adenine levels made by Herman Kalckar using his new enzymatic detection methods; it appeared that adenine levels were many times too low to mediate vasodilatation (Kalckar 1947a; Kalckar and Lowry 1947). These results also demonstrated the very rapid degradation of adenine compounds in blood.

Extracellular effects of purines were also identified in non-cardiovascular preparations, including adenosine- and ATP-induced contraction of the uterus (Deuticke 1932; Watts 1953) and intestine (Gillespie 1934; Ewing et al. 1949; Mihich et al. 1954). From the very early studies it had already became apparent that the presence of additional phosphates conferred differences in activity, although these differences were not to be resolved until purinoceptors were discovered more than half a century later. In retrospect, a major problem in the interpretation of the early data was the impurity of the compounds available (Gillespie 1934) as well as the extremely rapid metabolism of extracellular adenine nucleotides and nucleosides (Kalckar and Lowry 1947).

Studies of the actions of purine nucleosides and nucleotides were continued in the 1960s on a variety of tissues. In the guinea pig taenia coli, exogenously applied adenylate compounds were shown to suppress spontaneous electrical activity and hyperpolarise the membrane (Axelsson et al. 1965; Axelsson and Holmberg 1969). In these experiments adenosine 5′-diphosphate (ADP), AMP and adenosine were found to be much less effective than ATP (Axelsson and Holmberg 1969). Purines were shown to alter systemic blood pressure (Flesher et al. 1960; Gordon and Hesse 1961; Rowe et al. 1962; Haddy and Scott 1968) and change the tone of isolated arteries from the mesentery, kidney and skeletal muscle (Hashimoto and Kumukura 1965; Scott et al. 1965; Walter and Bassenge 1968). Further experiments confirmed the effects of purines on heart rhythm; in particular it was demonstrated that ATP, ADP, AMP and adenosine all have strong negative chronotorpic effects when acting on the whole heart or directly on the sinoatrial node (Angelakos and Glassman 1965; James 1965; Stafford 1966). At the same time, ATP-induced stimulation of insulin secretion was also demonstrated (Rodriguez Candela and Garcia-Fernandez 1963).

The effects of administration of purines in humans was widely explored in the 1930 and 1940s, especially in geriatric patients with cardiovascular disorders. In 1934, (Richards 1934) found that, in striking contrast to animals, injection of adenosine and AMP invariably induced tachycardia and did not affect blood pressure. During this time, clinical studies were initiated for the use of adenosine to treat cardiac arrhythmias (Honey et al. 1930). However, large boluses of adenosine triggered heart arrest and the short half-life of adenosine further confounded attempts to utilise this nucleoside as an antihypertensive agent (Honey 1930; Jezer et al. 1933). In other studies, the effect of ATP on the heart was found to be dose-dependent; although small doses of ATP produced transient tachycardia, its usual effect was to slow the heart and to produce AV block, probably following breakdown to adenosine (Stoner and Green 1945; Wayne et al. 1949; Johnson and McKinnon 1956; Hollander and Webb 1957). An extensive review was published by Boettge et al. (1957), describing the physiological

significance, pharmacological action and therapeutic use of adenylyl compounds in man.

An important and influential hypothesis was developed by Berne (1963) and Gerlach et al. (1963), who elaborated on the earlier proposal by Lindner and Rigler (1931). This hypothesis postulated that adenosine was the physiological mediator of the coronary vasodilatation associated with myocardial hypoxia; intracellular ATP in myocardial cells was suggested to be degraded to adenosine that then left the cells and induced vasodilatation of the coronary resistance vessels acting through adenosine receptors. This suggestion was based largely on the observation that adenosine and its degradation products were found in the effluent from isolated perfused cat hearts and in the coronary sinus blood of dog hearts, following severe coronary hypoxia, and on the correspondence between the levels of measured adenosine (Olsson and Pearson 1990). This hypothesis was later questioned for the following reasons: (i) ATP is more potent than adenosine in inducing coronary vasodilatation (Winbury et al. 1953; Wolf and Berne 1956; Walter and Bassenge 1968; Moir and Downs 1972); (ii) methylxanthines block adenosine-induced coronary vasodilatation, but have very little effect on that produced by ischaemia or ATP (Eikens and Wilcken 1973; Olsson et al. 1978); and (iii) an increased level of ATP in the effluent from perfused hypoxic hearts was detected by a sensitive and specific assay system (Paddle and Burnstock 1974). An alternative hypothesis has been put forward [see (Burnstock 1982, 1993a)], namely that hypoxia and shear stress induced the release of ATP from endothelial cells that regulate coronary vascular resistance by acting on endothelial ATP receptors, resulting in the release of nitric oxide (NO) and subsequent vasodilatation, whereas adenosine controls the longer-lasting component of reactive hyperaemia. This is not the appropriate place to critically assess the current data on coronary vasodilatation, but a comparative study shows that several factors, including adenosine receptors, NO and K_{ATP} channels contribute, and may act synergistically (Tune et al. 2004).

2.3 Early Studies of the Effects of Purines on the Nervous System

In 1947 Buchtal, Engback, Sten-Knudsen and Thomasen reported to the Physiological Society (Buhthal et al. 1947) that arterial injection of ATP to the cervical segments of the spinal cord of cats resulted in tetanus-like contractions of muscles of the upper extremities. The authors attributed this action to the direct excitation of anterior horn cells of the spinal cord. This initial finding of central effects of ATP was soon to be corroborated by ‘an incidental observation made in decerebrated cats when adenosine triphosphate (ATP) was injected into the artery supplying a leg muscle, the *tibialis anticus*, (Emmelin and Feldberg 1948). The ATP injection led to a ‘complex symptomathology’ which involved bradycardia, obstruction of the pulmonary circulation, peristalsis, micturition, vomiting, defaecation and generalised muscular contraction. This broad response, was, at least in part, mediated by nervous centres. Subsequently, several reports appeared which demonstrated that injections of ATP into the ventricles or into the brain resulted in ataxia, sleepiness and motor weakness, and triggered electrophysiological or biochemical responses (Babskii and Malkiman 1950; Feldberg and Sherwod 1954; Galindo et al. 1967; Shneour and Hansen 1971).

There was early recognition for a physiological role for ATP at the neuromuscular junction. Buchthal and Folkow (1948) found that acetylcholine (ACh)-evoked contraction of skeletal muscle fibres was potentiated by exposure to ATP. The first indication that ATP might act as a neurotransmitter in the peripheral nervous system arose when Holton and Holton (1954) proposed that ATP released from sensory nerves during antidromic nerve stimulation of the great auricular nerve caused vasodilatation in the rabbit ear artery. Some years later Pamela Holton, using the firefly luminescence method for ATP detection (Strehler and Totter 1952, 1954), found that electrical stimulation of great

auricular nerves of rabbits resulted in transient elevation of extracellular ATP (see the original trace in Chap. 4). She then concluded that 'when noradrenaline is liberated from sympathetic nerve endings ATP may also be liberated into the tissue spaces' (Holton 1959), thus providing the first hint for the concept of purinergic co-transmission (Burnstock 1976).

Subsequently, the presynaptic modulation of ACh release from the neuromuscular junction by purines was reported by Ginsborg and Hirst (1972) and Ribeiro and Walker (1975). ATP was found in vesicular fractions of synaptosomes of neuromuscular junctions (Dowdall et al. 1974) and ATP release following electrical stimulation of the presynaptic nerve was identified (Zimmermann 1978). It was also demonstrated that ATP increased ACh sensitivity of both rat diaphragm and the frog skeletal muscle endplate (Ewald 1976; Akasu et al. 1981).

ATP effects on physiological activity in the autonomic ganglia was initially reported in 1948 when Feldberg and Hebb (1948) demonstrated that intra-arterial ATP injection excited neurons in the cat superior cervical ganglia (SCG). Subsequent experiments performed in de Groat's laboratory demonstrated that in rat SCG and in the cat vesical parasympathetic ganglia, purines suppressed synaptic transmission through adenosine receptors; at the same time high concentrations of ATP excited the postganglionic neurons (Theobald and De Groat 1977). The earliest intracellular recordings of the action of ATP on neurons were obtained in frog sympathetic ganglia where ATP produced a depolarisation through a reduction in K^+ conductance (Siggins et al. 1977; Akasu et al. 1983).

The initial discoveries of peripheral purinergic transmission (Burnstock 1972) stimulated an increase in the interest in purinergic mechanisms in the central nervous systems (CNS). In the early 1970s, Pull and McIlwain (1972a, b, 1973) described the release of adenine nucleotides and their derivatives from superfused guinea pig neocortex that had been electrically stimulated in vitro. Subsequently, Heller and McIlwain (1973) showed release of labelled nucleotides from isolated superior colliculus and lateral geniculate body incubated in [^{14}C]adenine and stimulated through an incoming optic tract, but not from preparations of piriform cortex stimulated through the lateral olfactory tract. McIlwain and his colleagues discussed their results in terms of a neurohumoral role for adenine derivatives in the brain.

Another major stimulus to the interest in purines in the CNS was the finding from Ted Rall's group that the accumulation of cyclic AMP (cAMP) was not increased by theophylline, despite its being an inhibitor of phosphodiesterase inhibitor and therefore able to reduce cAMP breakdown. The finding was resolved when it became apparent that theophylline antagonised the effects of endogenous (and exogenous) adenosine, which provided a major stimulus for cAMP production in brain slices (Sattin and Rall 1970). These results were soon confirmed and extended in a series of papers from John Daly′s laboratory, which also provided an explanation for an earlier finding that electrical field stimulation caused an increase in cAMP in the stimulated slice (Kakiuchi et al. 1969).

These in vitro experiments were soon extended to the intact cerebral cortex (Sulakhe and Phillis 1975). It was shown that iontophoretic application of adenosine and several adenine nucleotides depressed the excitability of cerebral cortical neurons, including identified Betz cells; cAMP, adenine and inosine were less effective, whereas ATP caused an initial excitation followed by a depression (Phillis et al. 1974; 1975). Adenosine and ATP also depressed firing in cerebellar Purkinje cells (Kostopoulos et al. 1975). ATP was shown to activate units of the emetic chemoreceptor trigger zone of the area postrema of cat brain (Borison et al. 1975). Premature arousal of squirrels from periods of hibernation was evoked by adenosine nucleotides, but not by other purine nucleotides, and it was suggested that this effect was due to their direct action on central neurons (Twente et al. 1970). The infusion of cAMP into the hypothalamus of fowl induced behavioural and electrophysiological sleep, whereas dibutyryl cAMP produced arousal (Marley and Nistico 1972). Local or systemic administration of adenosine in

normal animals produced EEG and behavioural alterations of the hypnogenic type (Haulica et al. 1973).

Two groups demonstrated that low concentrations of adenosine caused a rise in the levels of cAMP in slices of guinea pig cerebral cortex (Shimizu et al. 1969; Sattin and Rall 1970; Shimizu and Daly 1970) and that this rise was antagonised by the methylxanthines, theophylline and caffeine (Sattin and Rall 1970). Other investigators showed that adenosine and 2-chloroadenosine stimulated cAMP production in membrane fractions of human platelets (Mills and Smith 1971) and that this action was antagonised by aminophylline (Haslam and Lynham 1972). Subsequently, adenosine was shown to stimulate adenylate cyclase in a variety of membrane preparations, including those from adipocytes (Fain et al. 1972), turkey erythrocytes (Sevilla et al. 1977), liver (Londos and Wolff 1977) and a glioma cell line (Clark and Seney 1976).

At the same time Cornford and Oldendorf (1975) described two independent transport systems across the rat blood–brain barrier, one for adenine and the other for adenosine, guanosine, inosine and uridine, thus showing that purine homeostasis in the brain parenchyma is tightly controlled. High levels of 5′-nucleotidase were demonstrated histochemically in the substantia gelatinosa of mouse spinal cord (Suran 1974).

Observations of mentally ill patients suggested that purines may play a role in the cognitive and emotional functions of the human brain. Thus, adenine nucleotides have been implicated in depressive illness (Abdulla and McFarlane 1972; Hansen 1972). Abdullah and McFarlane (1972) suggested the indirect effects of adenine nucleotides on prostaglandin biosynthesis that mediated development of depression. Blood levels of ATP and/or adenosine and urinary cAMP excretion were found to be significantly elevated in patients diagnosed with schizophrenia or in psychotic and neurotic depression (Abdulla and Hamadah 1970; Paul et al. 1970; Brown et al. 1972; Hansen and Dimitrakoudi 1974), however these results were not reproduced in the study of Jenner et al. (1975). Inherited disorders of purine metabolism in the brain have been related to psychomotor retardation, athetosis and self-mutilation (Lesch-Nyhan syndrome) (Lesch and Nyhan 1964; Rosenbloom et al. 1967; Seegmiller et al. 1967; Berman et al. 1969). Adenine therapy has been used for Lesch-Nyhan syndrome (Schulman et al. 1971) and therapeutic effects of ATP in the treatment of nerve deafness were also claimed (Ohsawa et al. 1961).

2.4 Early Studies of Peripheral Effects of Purines

The first experiments demonstrating that ADP causes aggregation of blood platelets were performed almost 50 years ago. Initially, it was found that a small molecule derived from red blood cells stimulated platelet adhesion (Hellem 1960). Subsequently, the same compound was found to induce platelet aggregation (Ollgaard 1961) and was finally identified as ADP (Gaarder et al. 1961; Born 1962). Later, adenosine was found to inhibit ADP-induced platelet aggregation (Born and Cross 1963); a similar inhibitory potency was found for ATP (Macfarlane and Mills 1975); adenosine tetraphosphate (Harrison and Brossmer 1976) and β,γ-methylene ATP (β,γ-meATP) (Born and Foulks 1977). For full reviews of developments in this field, see e.g. (Haslam and Cusack 1981; Gachet and Cazenave 1991; Hourani and Cusack 1991).

ATP has been known to induce the release of histamine from mast cells for some time (Diamant and Kruger 1967; Sugiyama 1971). Since close apposition of autonomic and sensory nerve varicosities with mast cells has been described (Heine and Forster 1975; Wiesner-Menzel et al. 1981; Newson et al. 1983; Bienenstock et al. 1991), it seems likely that ATP released as a neural cotransmitter is involved in the physiological control of histamine release from mast cells. Adenosine has been shown to modulate ADP-induced release of histamine (Marquardt et al. 1978; Lohse et al. 1987). The receptor for ATP on mast cells was studied in depth by Cockcroft and Gomperts (1980) and was designated a P_{2Z}-purinoceptor by Gordon (1986).

About 15 years later, this P_{2Z} receptor was cloned and found to belong to the ATP-gated P2X receptor family and designated $P2X_7$ (Surprenant et al. 1996).

2.5 Early Comparative Studies

Comparative studies of the actions of purines in invertebrates and lower vertebrates were scanty before 1972. Exceptions include: the depolarising actions of ATP on amoeba (Nachmias 1968), the ATP-mediated increase of ciliary beat and locomotion in paramecium (Organ et al. 1968), adenosine actions on the oyster heart (Aikawa and Ishida 1966) and the initiation of feeding behaviour in blood sucking insects by ATP (Galun 1966, 1967). Reviews of the developments concerned with the comparative physiology and evolution of purinergic actions in the animal kingdom are available (Burnstock 1975a, 1979b, 1996b; Burnstock and Verkhratsky 2009; Fountain and Burnstock 2009).

2.6 Discovery of Purinergic Transmission

The brilliant pioneers of chemical neurotransmission, including Langley, Elliot, Loewi, von Euler and Dale, focused on ACh and noradrenaline (NA), and it was not until 1970 that non-adrenergic, non-cholinergic (NANC) neurotransmission was recognised and ATP proposed as a neurotransmitter (see Burnstock 1972). Later 'Dale's Principle' which, erroneously, came to present the view that one nerve only utilised one transmitter was challenged (Burnstock 1976) and it is now clear that ATP is a cotransmitter in most, if not all, nerves in the peripheral nervous system (PNS) and CNS (Burnstock 2004a, 2007, 2009).

2.6.1 Non-Adrenergic, Non-Cholinergic (NANC) Nerves

The ATP tale begun on one day in the early 1960s, when one of the authors of this book (GB), together with his students Max Bennett and Graham Campbell, decided to stimulate the nerves supplying the smooth muscle of the guinea pig taenia coli in the presence of atropine and bretylium to block cholinergic and adrenergic neurotransmission and expected to see depolarisation and contraction in response to direct stimulation of the muscle. However, to their surprise the responses to single stimuli were rapid hyperpolarisations and relaxation (Burnstock et al. 1963). This was a moment of excitement (Burnstock 2004b) for them because they felt that they were on to something important. Interpretation of their results was discussed internationally for a while and that tetrodotoxin (from the puffer fish) had just been shown to block nerve conduction, but not smooth muscle activity. Tetrodotoxin abolished the hyperpolarisations, so they were identified as inhibitory junction potentials in response to NANC neurotransmission (Fig. 2.3; Burnstock et al. 1964). Later it was shown that they were present in intrinsic enteric neurons controlled by vagal or sacral parasympathetic nerves (Burnstock et al. 1966). A comparable demonstration of NANC mechanical responses was made by Martinson and colleagues in the stomach upon stimulation of the vagus nerve (Martinson and Muren 1963; Martinson 1965). By the end of the 1960s, evidence had accumulated for NANC nerves in the respiratory, cardiovascular and urinogenital systems as well as in the gastrointestinal tract (Burnstock 1969). Hughes and Vane (1967, 1970) also demonstrated the presence of a NANC inhibitory innervation of the rabbit portal vein. The existence of NANC neurotransmission is now firmly established in a wide range of peripheral and central nerves and fuller accounts of the development of this concept and the people involved are available [see (Burnstock 1981, 2006a, c) for comprehensive reviews].

2.6.2 ATP as a Principal Transmitter

The next step was to try to identify the transmitter released during NANC inhibitory transmission in the gut and by NANC excitatory transmission in the urinary bladder. From the

work of Jack Eccles and others, several criteria were shown to be needed to be satisfied to establish a neurotransmitter: synthesis and storage in nerve terminals; release by a Ca^{2+}-dependent mechanism; mimicry of the nerve-mediated responses by the exogenously applied transmitter; inactivation by ectoenzymes and/or neuronal uptake and parallel block or potentiation of responses to stimulation by nerves and exogenously applied transmitter. Many different substances were considered in the late 1960s, including amino acids, monoamines, neuropeptides, but none satisfied the criteria. There was, in fact, even an early recognition of atropine-resistant responses of the gastrointestinal tract to parasympathetic nerve stimulation (Langley 1898; McSwiney and Robson 1929; Ambache 1951; Paton and Vane 1963). As for the gastrointestinal tract, at the end of the nineteenth century, it was demonstrated that the excitatory response of the mammalian urinary bladder to parasympathetic nerve stimulation was only partially antagonised by antimuscarinic agents (Langley and Anderson 1895). It was postulated that the atropine-resistant response was due to the release of a non-cholinergic excitatory transmitter (Henderson and Roepke 1934; Chesher and James 1966; Ambache and Zar 1970). However, it was also postulated that atropine was unable to block the subjunctional receptors at which the endogenous ACh acts (Dale and Gaddum 1930) or that it was displaced from these receptors by the high local concentration of ACh released upon parasympathetic stimulation (Hukovic et al. 1965).

However, hints in the literature, including the above-mentioned seminal paper by Drury and Szent-Györgyi (1929) showing powerful extracellular actions of purines on heart and blood vessels, papers by Feldberg showing extracellular actions of ATP on autonomic ganglia (Feldberg and Hebb 1948) and a paper by Pamela Holton in 1959, which showed release of ATP during antidromic stimulation of sensory nerves supplying the rabbit ear artery (Holton 1959) led Burnstock and his colleagues to try ATP and to their surprise it beautifully satisfied all the criteria needed to establish it as a

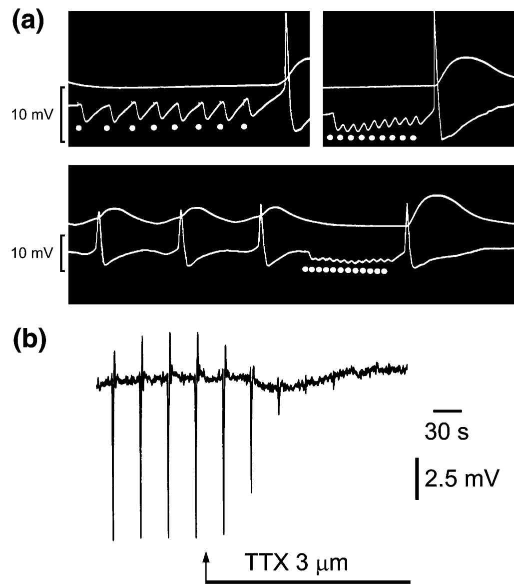

Fig. 2.3 Non-adrenergic, non-cholinergic (NANC) neurotransmission. **a** Sucrose gap records from smooth muscle of guinea pig taenia coli showing hyperpolarisations in response to different stimulation frequencies (1, 3 and 5 Hz) of intrinsic nerves in the presence of atropine and guanethidine. **b** Sucrose gap recording of membrane potential changes in smooth muscle of guinea pig taenia coli in the presence of atropine (0.3 μM) and guanethidine (4 μM). Transmural field stimulation (0.5 ms, 0.033 Hz, 8 V) evoked transient hyperpolarisations, which were followed by rebound depolarisations. Tetrodotoxin (TTX, 3 μM) added to the superfusing Kreb's solution (applied at *arrow*) rapidly abolished the response to transmural field stimulation establishing these as inhibitory junction potentials in response to NANC neurotransmission. (Figure is reproduced with permission from Burnstock 1986a)

transmitter involved in NANC neurotransmission (Fig. 2.4; Burnstock et al. 1970; 1978). An early study, before ATP was identified as the principal transmitter mediated by NANC nerves, was inspired by Loewi's experiments establishing ACh as a neurotransmitter (Loewi 1921). In this study, Burnstock (unpublished experiments carried out by Burnstock and Smythe in 1966) showed that stimulation of NANC nerves to the taenia coli in a top chamber produced the typical nerve-mediated response (fast relaxation, followed by rebound contraction), while the perfusate produced a slower relaxation (without rebound contraction) when reaching a lower