Early History of Purinergic Signalling

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
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 Meyerhoff 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).
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

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.)
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 chronotropic 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 (Rodríguez 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\textsubscript{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 (Buathal 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 \textit{tibialis anticus}, (Emmelin and Feldberg 1948). The ATP injection led to a ‘complex symptomatology’ 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 synaptosomal fractions 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 [14C]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). Abdulla 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 P2Z receptor was cloned and found to belong to the ATP-gated P2X receptor family and designated P2X7 (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 1972). Later ‘Dale’s Principle’ which, erroneously, came to present the view that one nerve only utilised one transmitter was challenged (Burnstock 1972). 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].

2.6.1 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].
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; Che sher 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 extra-cellular 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 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
taenia coli preparation (Fig. 2.5). Only later was it shown that the response in the top chamber was mimicked by ATP, while the response in the lower chamber was mediated by adenosine (after rapid breakdown by ectonucleotidases of ATP, released from the top preparation).

In 1972, an article in *Pharmacological Reviews* (Burnstock 1972) formulating the purinergic neurotransmission hypothesis was published. Sadly, few believed this hypothesis over the next 25 years and it was often ridiculed at meetings and symposia. Resistance to this concept was perhaps understandable because ATP was well-established as an intracellular energy source involved in the Krebs cycle and other biochemical pathways and it seemed
unlikely that such a ubiquitous molecule would also act as an extracellular messenger. However, it appears that ATP, an ancient biological molecule, evolved both as an intracellular energy source and an extracellular signalling molecule. Nerves utilising ATP as their principal transmitter were subsequently named ‘purinergic’ and a tentative model of storage, release and inactivation of ATP for purinergic nerves was proposed (Burnstock 1971, 1972). Since then a great deal of evidence has followed in support of the purinergic hypothesis (see Burnstock 1975a, b, 1979a; Su 1983; Gordon 1986; White 1988; Olsson and Pearson 1990; Hoyle 1992; Burnstock 1993b; Dubyak and el Moattassim 1993; Zimmermann 1994; North 2002; Burnstock 2007; Abbracchio et al. 2009).

### 2.6.3 ATP as a Cotransmitter

‘Dale’s Principle’, was challenged in 1976 by Burnstock (1976) and the existence of nerves that can synthesise, store and release more than one pharmacologically active substance is now widely accepted [see (Burnstock 1983, 1990, 2004a, 2009; Osborne 1983; Kupfermann 1991)].

By the mid 1950s, it was recognised that ATP was co-stored with catecholamines in adrenal medullary chromaffin cells (Hillarp et al. 1955; Blaschko et al. 1956) and soon after, the corelease of ATP with adrenaline from chromaffin cells was identified (Carlsson et al. 1957; Douglas and Poisner 1966). The molar ratio between NA and ATP in sympathetic nerve terminals was estimated to vary between 7 : 1 and 12 : 1 (NA : ATP) (Schumann 1958; Eulerus et al. 1963; Stjärne and Lishajko 1966; Geffen and Livett 1971; Lagercrantz and Stajarne 1974).

The electrical recordings made during sympathetic neurotransmission in the guinea pig vas deferens in the early 1960s showed excitatory junction potentials (EJPs) in response to single pulses that summed and facilitated until at a critical depolarisation, a spike was generated leading to contraction (Burnstock and Holman 1961). However, what was puzzling was that receptor antagonists to NA as the sole transmitter recognised at that time in sympathetic nerves did not block the EJPs, although bretylium, that prevents release of transmitter from sympathetic nerves, did reduce them. It was not until over 20 years later, that it was shown that α,β-methylene ATP (α,β-meATP), a slowly degradable analogue of ATP that acts as a selective desensitiser of the ATP receptor (Kasakov and Burnstock 1982), abolished the
EJPs and spritzed ATP mimicked the EJP, but NA did not (Sneddon and Burnstock 1984) (Fig. 2.6).

In 1971, Su et al. (1971) demonstrated that stimulation of periarterial sympathetic nerves led to release of tritium from guinea pig taenia coli preincubated in [3H]adenosine (which is taken up and converted largely to [3H]ATP) and that the release of both tritium and NA was blocked by guanethidine. Soon after, Nakanishi and Takeda (1973) showed evidence that ATP might be co-released with NA in synapses from the hypogastric nerve to the seminal vesicle of the guinea pig and Langer and Pinto (1976) suggested that the substantial residual NANC responses of the cat nictitating membrane, following depletion of NA by reserpine, might be due to the release of ATP remaining in sympathetic nerves. It was found that adenosine, following breakdown of released ATP, could limit excessive transmitter release by means of presynaptic inhibition (Hedqvist and Fredholm 1976; Fredholm and Hedqvist 1979, 1980). Finally it was also demonstrated that ATP release is Ca\(^{2+}\) dependent (Fig. 2.4b; Burnstock et al. 1978).

ATP acts as a cotransmitter with ACh in cholinergic nerves in various tissues, including the electric organ of elasmobranch fish (Dowdall et al. 1974; Zimmermann 1978), the phrenic nerve endings in rat diaphragm (Silinsky and Hubbard 1973; Silinsky 1975), and in the excitatory nerves of the guinea pig urinary bladder (Kasakov and Burnstock 1982; McKenzie et al. 1982; Westfall et al. 1983; see also Burnstock 1986b; Hoyle 1996 for reviews).

The most extensive evidence for sympathetic cotransmission, however, came from studies of the vas deferens, initially by Westfall et al. (1978; Fedan et al. 1981). Later studies from

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**Fig. 2.5** This figure shows Loewi-inspired experiments carried out in 1966. The upper guinea pig taenia coli innervated preparation was stimulated at 5 Hz for 40 s every 6 min at 50 V and 2 ms duration, in the presence of atropine and guanethidine to elicit typical non-adrenergic, non-cholinergic responses, fast relaxation followed by rebound contraction. The perfusate passed over the lower taenia coli preparation to produce slow relaxations, but not followed by rebound contractions. In later experiments, we showed that, while the response of the taenia coli in the upper chamber was mimicked by ATP, the response in the lower chamber was mimicked by adenosine, the ATP released from the upper preparation being hydrolysed rapidly by ectonucleotidases to adenosine before reaching the lower preparation. n. (Experiments carried out by Burnstock and Smythe 1966, but not published until 2010 (Burnstock et al. 2010) and reproduced with permission of the Scandinavian Physiological Society)
several laboratories, following on from the initial work of Su (1975), established sympathetic cotransmission in a variety of different blood vessels (see Burnstock 1988).

2.6.4 ATP as an Excitatory Transmitter in the CNS

Although the release of purines from gross brain structures was identified in the mid 1970s (Sulakhe and Phillis 1975), the first indication that ATP may act as a neurotransmitter in central synapses was recognised by Thomas White, who observed the release of ATP from synaptosomes prepared from the whole brain, the cortex and the striatum; the ATP release was triggered by exposure to high extracellular K+ or to veratridine (White 1978; White et al. 1980, 1984). The next important step was electrophysiological recording of ATP-induced depolarisation and ATP-induced currents in both sensory and central neurons (Jahr and Jessell 1983; Krishtal et al. 1983). Almost 10 years later, ATP-mediated synaptic transmission was identified in cultured coeliac ganglion cells (Evans et al. 1992; Silinsky et al. 1992) and in neurons in acute slices from medial habenula (Edwards et al. 1992, 1997).

Subsequently, fast ATP/P2X-mediated synaptic transmission was found in various regions of the CNS, including spinal cord (Bardoni et al. 1997), hippocampus (Pankratov et al. 1998; Mori et al. 2001), locus coeruleus (Niebert et al. 1997) and cortex (Pankratov et al. 2002, 2003), see also (North and Verkhratsky 2006). The quantal release of ATP was characterised in

![Fig. 2.6](image-url)
PC12 cells (Fabbro et al. 2004), in peripheral (Silinsky et al. 1999) and in central synapses (Pankratov et al. 2006, 2007). In addition, purinergic transmission is involved in a wide variety of trophic and developmental processes in the nervous system (see Abbracchio et al. 2009; Burnstock and Verkhratsky 2010 for reviews). Purines as signalling molecules are particularly important for transmission in the neuronal-glial circuitry, that forms the brain parenchyma and provides the substrate for CNS function (Verkhratsky 2006; Verkhratsky and Toescu 2006; Verkhratsky 2009). It appears that all types of glial cells, be they of neural (astrocytes and oligodendrocytes) or myeloid (microglia) origin, express an extensive complement of purinoceptors. ATP triggers massive Ca\(^{2+}\) release from the endoplasmic reticulum (ER) (mediated via P2Y/inositol trisphosphate (InsP\(_3\)) pathway) in both astrocytes and oligodendrocytes (Kirischuk et al. 1995a, b; Hamilton et al. 2008) and controls the same pathway in microglial cells (Moller et al. 2000; Farber and Kettenmann 2006). In addition, some types of astrocytes express fast P2X receptors (Lalo et al. 2008), whereas oligodendrocytes and microglial cells possess P2X\(_7\) receptors (Ferrari et al. 1996; Haas et al. 1996; Matute et al. 2007), see also (Verkhratsky et al. 2009) for a comprehensive review. Furthermore, ATP acts as a main gliotransmitter, which provides for both glial-glial and glial-neuronal signalling.

A detailed account of ATP-mediated transmission in the nervous system is presented in Chaps. 6, 7, 8, 9, 10 of this book.

### 2.6.5 Adenosine in the Nervous System

It was shown in the early studies by Kakiuchi et al. (1969) that nerve activity causes release of adenosine in sufficient amounts to activate even...
the low affinity adenosine A<sub>2B</sub> receptors. The adenosine thus released was formed de novo and did not come from pre-existing stores. Furthermore, not even when release was examined from isolated nerve endings could the majority of the adenosine release be accounted for by release of ATP and subsequent extracellular degradation (Fredholm and Vernet 1979; Fredholm and Hedqvist 1980). Inhibition of transmitter release is an important effect of adenosine (see below) and this will cause protection against seizures (Dunwiddie et al. 1981; Dragunow et al. 1985; Fedele et al. 2006; Li et al. 2007). Similarly, there is a very important role of adenosine in limiting the extent of neuronal damage following e.g. ischaemia. This is in keeping with the proposed role as a ‘retaliatory metabolite’ (Newby 1984), the evidence for which is very strong (Rudolphi et al. 1992).

Much focus was initially on the A<sub>1</sub> receptors (see following chapters), but it is becoming clear that A<sub>2A</sub> receptors, that have a highly restricted distribution (Svenningsson et al. 1997b), are particularly important already under physiological conditions as they control the so-called indirect pathway from the basal ganglia and hence contribute to a variety of systems, including sleep-wakefulness (Huang et al. 2005) and locomotion (Svenningsson et al. 1997c; Yacoubi et al. 2000) and mood (Yacoubi et al. 2001).

### 2.6.6 Neuromodulation by Purines

Neuromodulators can influence neurotransmission at two sites, either by acting on presynaptic receptors to reduce or enhance transmitter release, or by acting on postsynaptic receptors to alter the magnitude or time course of the transmitter on the postjunctional cell. The first studies of prejunctional modulation of transmitter release by purines concerned the isolated rat phrenic nerve-diaphragm preparation, where adenosine and adenine nucleotides reduced both the spontaneous and evoked release of ACh from motor nerve terminals (Ginsborg and Hirst 1972; Ribeiro and Walker 1975). These same purine compounds were later shown to cause prejunctional inhibition of NA release from peripheral sympathetic nerves in a wide variety of tissues, including rabbit kidney, canine adipose tissue, guinea pig vas deferens (Hedqvist and Fredholm 1976; Clanachan et al. 1977) and rabbit central ear artery, saphenous vein, portal vein and pulmonary artery (Enero and Saidman 1977; Verhaeghe et al. 1977; Su 1978). Prejunctional modulation of ACh release from peripheral cholinergic nerves by purines was observed in the isolated guinea pig ileum and the myenteric plexus longitudinal muscle preparation (Sawynok and Jhamandas 1976; Moritoki et al. 1978; Moody and Burnstock 1982). Adenosine and related compounds, iontophoretically applied to central synapses, decreased the rate of spontaneous firing of rat cerebral cortical neurons (Phillis et al. 1975, 1979). Similarly, the naturally occurring diadenosine polyphosphates were found to modulate transmitter release from central neurons through presynaptic receptors (Miras-Portugal et al. 1996).

Purine modulation of transmitter release was thought to be mediated largely via presynaptic P1 receptors both in adrenergic systems (Clanachan et al. 1977; Enero and Saidman 1977; Verhaeghe et al. 1977; Hom and Lokhandwala 1981) and cholinergic systems (Ginsborg and Hirst 1972; Sawynok and Jhamandas 1976; Vizi and Knoll 1976; Griffith et al. 1981). Clear evidence for this was presented by De Mey et al. (1979) who showed that the prejunctonal actions of purine nucleotides were mediated by adenosine following the rapid breakdown of ATP, since slowly degradable analogues of ATP were ineffective. Results supporting this hypothesis have been presented for other preparations (Burnstock and Meghji 1981; Moody and Burnstock 1982; Bruns et al. 1983). It has also been suggested that ATP may act per se on P1 purinoceptors in guinea pig atria (Collis and Pettinger 1982) or that both mechanisms operate during the time course of a response to ATP (Moody et al. 1984). Subsequently, evidence has been presented for a prejunctional modulatory action by ATP itself in the iris, rat vas deferens and tail artery via a ‘P3’
receptor (Shinozuka et al. 1990) or a P2Y purinoceptor (Fuder and Muth 1993; von Kugelgen et al. 1994). At the same time, in many of these studies a participation of breakdown products, such as adenosine have not been rigorously excluded, and in at least some preparations presynaptic inhibitory effects of ATP were completely eliminated in mice lacking adenosine A1 receptors (Masino et al. 2002).

Purine nucleotides and nucleosides can also act on postjunctional receptors to modulate cholinergic and adrenergic neurotransmission. Purines increase ACh receptor sensitivity in various preparations, including the rat diaphragm muscle (Ewald 1976), frog skeletal muscle (Akasu et al. 1981) and rabbit iris sphincter (Gustafsson and Wiklund 1986). These interactions are Ca2+-dependent and may involve interaction with the allosteric site of the receptor-ion channel complex. Purine nucleotides and nucleosides have been shown to interact with NA postjunctionally in vitro in guinea pig seminal vesicles (Nakanishi and Takeda 1973), rabbit kidney (Hedqvist and Fredholm 1976), guinea pig and mouse vas deferens (Holck and Marks 1978; Witt et al. 1991), rabbit mesenteric artery (Krishnamurty and Kadowitz 1983) and rat mesenteric bed (Ralevic and Burnstock 1990). All these neuromodulatory actions of purines have been extensively reviewed (Ribeiro 1979; Burnstock and Brown 1981; Stone 1981; Paton 1987; Hoyle 1992; Starke et al. 1996; Dunwiddie and Fredholm 1979; Cunha 2001).

### 2.7 Receptors for Purines

#### 2.7.1 Subdivision into P1 and P2 Purinoceptors

Implicit in purinergic transmission is the existence of specific receptors. In 1978 Burnstock, after analysing a wealth of literature dedicated to the effects of purine nucleotides and nucleosides in a wide variety of tissues, proposed the first classification of purinergic receptors (Burnstock 1978). Based on several criteria, subclassification into P1 and P2 purinoceptors was proposed. The P1 (adenosine) purinoceptors are responsive to adenosine and AMP and are selectively and competitively antagonised by methylxanthines, such as theophylline and caffeine. Occupation of P1 purinoceptors leads to changes in adenylate cyclase activity, resulting in alterations in intracellular levels of cAMP. P2 purinoceptors are responsive to ATP and ADP. They are not antagonised by methylxanthines and occupation leads to increase in production of prostaglandins. This was a useful step forward, explaining some of the early confusions in the literature resulting from the rapid extracellular breakdown of ATP to adenosine and extended the concept of purinergic neurotransmission, by identifying postjunctional receptors as P2, while prejunctonal P1 receptors mediated neuromodulatory negative feedback responses or autoregulation of transmitter release.

Perhaps the first indication, contained in a single study, of subtypes of receptors preferring ATP versus adenosine was presented by Gillespie (1934), who described ATP as being more potent than its dephosphorylated relatives in causing relaxation of the guinea pig ileum, and adenosine as being more potent than its phosphorylated derivatives in causing coronary vasodilatation or inducing hypotension in cats and rabbits. Mihich et al. (1954) noted that, although the effects of ATP were similar to those of adenosine in the isolated rabbit intestine, the action of ATP was qualitatively distinct, in that preparations rendered tachyphylactic to adenosine or its 2-substituted derivatives retained their responsiveness to ATP. Adenosine and ATP produced opposite responses in the renal vascular bed; adenosine and AMP caused an increase and ATP a decrease in vascular resistance. Furthermore, theophylline, although antagonising the responses to adenosine and AMP, was unable to reduce the vasodilatation produced by ATP (Haddy and Scott 1968), suggestive of two distinct receptor populations for these compounds in this tissue. Adenosine and ATP appeared to have different actions in guinea pig bladder (Burnstock et al. 1972), rat portal vein (Sjoberg and BA 1975) and chicken rectum
In the guinea pig taenia coli, the log concentration response curves for the inhibitory effects of ATP and ADP were found to be substantially more potent and non-parallel to those for AMP and adenosine (Burnstock et al. 1970; Satchell and Maguire 1975). 2,2'-Pyridylisatogen tosylate was able to block the inhibitory responses of the guinea pig taenia coli to ATP and ADP, but not to adenosine (Spedding and Weetman 1976).

The subclassification of Pl and P2 purinoceptors was supported by numerous pharmacological, biochemical and molecular biological studies performed during the last three decades (Fredholm et al. 1994; Ralevic and Burnstock 1998; Fredholm et al. 2001; Khakh et al. 2001; North 2002; Abbracchio et al. 2006; Surprenant and North 2009).

2.7.2 History of Adenosine (P1) Purinoceptors

The existence of adenosine receptors was postulated already in 1965 by DeGubareff and Sleator (1965) based on studies showing caffeine antagonism of adenosine actions in atrial muscle, and in the early 1970s by several scientists, including Rall and Daly based on their studies showing the ability of adenosine analogues to raise cAMP levels in brain slices from several organs (vide supra). The competitive antagonism between adenosine and methylxanthines were shown (Fig. 2.7) and this suggested that the two structurally similar compounds acted at the same site/receptor to exert their effects. This tentative conclusion was further supported by work examining a series of adenosine analogues and demonstrating the type of dose–response relationships typical of receptors (Cobbin et al. 1974).

Evidence for subclasses of adenosine receptors emerged and a distinction was made between the adenosine receptor-mediated stimulation and inhibition of adenylate cyclase. First, Londos and Woolf (1977) demonstrated that adenosine and its analogs stimulated adenylate cyclase by a mechanism involving an external membrane receptor which required an essentially unmodified ribose moiety (hence called the R site). They also demonstrated a high dose effect directly on adenylate cyclase that required an unchanged purine moiety (and this was called the P-site). Concurrently, Van Calker and colleagues put forward evidence that both...
stimulation and inhibition of adenylate cyclase could be mediated by adenosine at the external R site (van Calker et al. 1978, 1979). Londos and colleagues also demonstrated two different extracellular adenosine receptors, and consistent with his R- vs P- site distinction called the receptor whose activation inhibited adenylate cyclase an R\textsubscript{i} receptor, whereas receptors whose stimulation resulted in the activation of adenylate cyclase were termed R\textsubscript{a} receptors (Londos et al. 1978, 1980). It was soon realised that the two terminologies referred to the same entities and Londos subsequently agreed that the terms A\textsubscript{1} and A\textsubscript{2} would be preferable in the pharmacological literature since activation of the adenosine receptors is not always linked to adenylate cyclise, because it has priority and because it agrees with procedures for naming receptors, and these terms are now firmly established (see Fredholm et al. 1994). The two adenosine receptors were shown to have different agonist profiles (Daly 1982). In general, at the A\textsubscript{1} purinoceptor, N\textsuperscript{6}-substituted adenosine analogues were shown to be more potent than 5\textsuperscript{\prime}-substituted analogues. Numerous specific pharmacological agents acting at P1 receptors have been synthesised since (for reviews see Linden 1994; Olah and Stiles 1995; Fredholm 2001; Baraldi et al. 2006; Gao and Jacobson 2007; Baraldi et al. 2008; Elzein and Zablocki 2008; Borea et al. 2009; Cristalli et al. 2009; Kalla et al. 2009; Kiesman et al. 2009).

Direct characterisation of the adenosine receptor by ligand binding techniques was performed in 1978, when Malbon et al. used $[^3\text{H}]$adenosine to bind to the adenosine receptor in fat cells (Malbon et al. 1978). However, just as with attempts to use labelled adenine nucleotides directly the rapid metabolism and the presence of other sites (e.g. transporters and enzymes) prevented good specific binding to be detected and even when great care was taken both bona-fide receptors and the P-site contributed to binding (Schwabe and Trost 1980); N\textsuperscript{6}-cyclohexyl-adenosine (CHA) and 1,3-diethyl-8-phenylxanthine (DPX) (Fig. 2.8) (Bruns et al. 1980) and 2-chloroadenosine (Williams and Risley 1980). It took much longer to obtain good binding data for A\textsubscript{2} receptors, because the initial attempts have failed. The first entirely satisfactory demonstration used labelled N-ethyl-carboxamido adenosine (NECA) and unlabelled CHA to displace the binding of this non-selective ligand from A\textsubscript{1} receptors (Bruns et al. 1986). The study of A\textsubscript{2} receptors really benefited from the development of a highly specific agonist, CGS 21680 useful in binding studies (Jarvis et al. 1989) and by the later development of really selective antagonists (Poucher et al. 1995; Zocchi et al. 1996).

However, by this time it was clear that there were two types of A\textsubscript{2} receptors. Whereas, the classical cAMP elevating A\textsubscript{2} receptor in cortical brain slices required rather high levels of adenosine analogues for activation and could not be demonstrated in cell free extracts, two groups showed that in the dopamine rich regions of the brain adenylate cyclase in membrane preparations could be stimulated by low concentrations of adenosine analogues (Fredholm 1977; Premont et al. 1977). Subsequent work would clearly demonstrate that the A\textsubscript{2A} subform of the receptor is indeed highly enriched in the basal ganglia (Jarvis and Williams 1989; Parkinson and Fredholm 1990; Svenningsson et al. 1997b). Formal proof for the two (high and low affinity subtypes, A\textsubscript{2A} and A\textsubscript{2B}, respectively) receptors were obtained in Daly’s laboratory on the basis of structure–activity relationships (SAR) and binding studies (Bruns et al. 1980, 1983; Daly et al. 1983). The subclassification into A\textsubscript{1}, A\textsubscript{2A} and A\textsubscript{2B} was further corroborated by molecular cloning (Libert et al. 1989; Maenhaut et al. 1990; Libert et al. 1991; Stehle et al. 1992). The latter cloning studies have revealed a receptor from the rat testis and brain that, when compared to the other G protein-coupled P\textsubscript{1} receptors, was found to correspond to a novel functional adenosine receptor and termed an A\textsubscript{3} receptor (Meyerhof et al. 1991; Zhou et al. 1992). Another potential candidate for the role of A\textsubscript{3}
receptor, identified by Ribeiro and Sebastiao (1986) was, most probably, an A1 receptor coupled to intracellular Ca^{2+} signalling. The physiological role of the A3 receptor is still largely unknown, although it is widely distributed in many peripheral tissues and in the brain (Zhou et al. 1992; Dixon et al. 1996) and in the immune system, where it appears to be involved in the modulation of release of allergic mediators from mast cells and other cells involved in the immediate hypersensitivity reaction (Ramkumar et al. 1993). N^6-BenzylINeCA has been found to be a highly potent and moderately selective agonist at the A3 receptor (van Galen et al. 1994) and BW-A522 has potent antagonist properties, at least at ovine and human A3 receptors (Linden et al. 1993; Salvatore et al. 1993; Fozard and Hannon 1994). For further reading on adenosine receptors, we recommend to the curious reader a number of excellent reviews (Olah and Stiles 2000; Fredholm et al. 2005, 2007; Jacobson and Gao 2006; Dare et al. 2007; Klaasse et al. 2008; Jenner et al. 2009). These reviews also highlight several areas where drugs acting on adenosine receptors are being developed.

2.7.3 History of P2 Purinoceptors

The functional heterogeneity of ATP responses that hinted at several receptor classes was recognised rather early. Phosphate modified analogues of ATP and ADP showed considerably steeper log dose-response curves for their inhibition of the guinea pig taenia coli than those for ADP and ATP (Maguire and Satchell 1979). Frew and Baer (1979), using these same analogues on the rabbit small intestine, concluded that the x,β-methylene isosteres of ATP and ADP acted at a different receptor site from ATP. The P2 purinoceptor mediating inhibition of the guinea pig taenia coli displayed stereoselectivity and a different relative potency order of agonists, compared to the P2 purinoceptor mediating contraction of the guinea pig bladder and frog heart (Satchell and Maguire 1975; Cusack and Planker 1979; Burnstock et al. 1983). Shuba and Vladimirova (1980) suggested that there might be subclasses of the ATP receptor based on their observations that apamin, a potassium channel blocker, antagonised the inhibitory actions of ATP in guinea pig caecum and stomach (Banks et al. 1979), but not the excitatory actions in the guinea pig bladder and uterus. Su (1981) suggested that postjunctional P2 purinoceptors should be named ‘P2,α-receptors’ and prejunctional P2 purinoceptors named ‘P2,β-receptors’. Fedan et al. (1982) proposed that two P2 purinoceptors may exist in the smooth muscle of the guinea pig vas deferens, based on pharmacological studies using ATP analogues and arylazidoaminopropionyl ATP (ANAPP3), an antagonist at the P2 purinoceptor. In contrast to the P2 purinoceptors on smooth muscle, where ADP and ATP are often equipotent, the receptors on platelets responsible for aggregation are highly specific for ADP, whereas ATP inhibits platelet aggregation (Cusack et al. 1979).

However, it was not until 1985 that Burnstock and Kennedy (1985) proposed the first clear subdivision of P2 purinoceptors into P2X purinoceptors (that mediate vasoconstriction and contraction of visceral smooth muscle, with x,β-meATP as a potent agonist) and P2Y purinoceptors (that mediate vasodilatation as well as relaxation of the smooth muscle of the gut, with 2-methylthio ATP (2-MeSATP) as a particularly potent agonist). Soon after, two further P2 purinoceptors were tentatively proposed (Gordon 1986): an ADP-selective P2T purinoceptor that is present on platelets and thrombocytes, and a P2Z purinoceptor, which appears to be activated by ATP^4− and is prominent in macrophages, lymphocytes and mast cells. Later, a P2U purinoceptor was proposed, where ATP and UTP are equipotent (O’Connor et al. 1991) and a P2D purinoceptor for diadenosine polyphosphates (Miras-Portugal et al. 1996), with some less-accepted subtypes: P2S (Wiklund and Gustafsson 1988a, b), P2R (von Kugelgen and Starke 1990) and the P2u receptor that is synonymous with the P2U receptors (Abbracchio et al. 1993). It was clearly shown that there were ATP-activated ion channel purinoceptors in excitable cells (Bean et al. 1990) and that P2Y purinoceptors involved
G protein activation and were members of the GPCR family (Dubyak 1991). The possibility that some P2Y purinoceptors act via Gi proteins to inhibit adenylate cyclase has been raised (Harden et al. 1995) and the existence of pyrimidine nucleotide-selective G protein-linked receptors has been proposed (Lazarowski and Harden 1994; Chang et al. 1995; Communi et al. 1995a; Nguyen et al. 1995).

The term ‘P3’ has been suggested for an adenine nucleotide receptor claimed to be methylxanthine-sensitive (Shinozuka et al. 1988) in the rat tail artery, on the basis that adenosine and ATP, together with their analogues 2-chloroadenosine and β,γ-meATP, inhibit NA release from sympathetic nerves, an effect that was antagonised by 8-PSPT; this subclass, however, has not been generally accepted.

The first P2 purinoceptors to be cloned were G protein-coupled purinoceptors of the P2Y family: a P2Y1 purinoceptor was isolated from chick brain (Webb et al. 1993) and a P2U purinoceptor (later designated P2Y2) from neuroblastoma cells (Lustig et al. 1993). A year later, two ligand-gated ion channel ATP receptors of the P2X family were also cloned—one from vas deferens (Valera et al. 1994) and another from rat pheochromocytoma PCl2 cells (Brake et al. 1994).

In the paper prepared by the IUPHAR subcommittee concerned with the nomenclature of P2 purinoceptors (Fredholm et al. 1994), it was emphasised that the contemporary purinoceptor subclassification, with so many letters of the alphabet being somewhat randomly added as new receptor subtypes were discovered, was unsatisfactory. The subcommittee supported, in principle, a new system of classification proposed by Abbracchio and Burnstock (1994). In this proposal, it was suggested that P2 purinoceptors should be divided in two major families: a P2X family consisting of ligand-gated cation channels and a P2Y family consisting of G protein-coupled receptors. It was pointed out that this classification brought ATP into line with most other neurotransmitter receptors, such as ACh, γ-amino butyric acid (GABA), glutamate and 5-hydroxytryptamine (5-HT), where ligand-gated and G protein-coupled receptor sub-classifications have already been established (see also Burnstock 1996a). Table 2.1 represents the general overview of tissue distribution and main functions of the main types of purinoceptors, the P1 adenosine (A1, A2A, A2B and A3 receptors), the P2X ionotropic and P2Y metabotropic ATP/nucleoside receptors. A detailed account of purinoceptor structure, properties and molecular pharmacology is presented in Chap. 5 of this book.

Among the more dramatic events in recent years has been the elucidation, by crystallography, of the structures of P2X and adenosine receptors. As expected the adenosine A2A receptor (Jaakola et al. 2008) had a structure that showed considerable similarity to the previously clarified bovine rhodopsin (Palczewski et al. 2000) and the mammalian β-adrenergic receptor (Rasmussen et al. 2007; Warne et al. 2008). It is anticipated that these structures will be of assistance in the targeting of novel drugs (Katrich et al. 2010).

Furthermore, there has been a dramatic expansion of research into purinergic signalling in the last decade. This is partly a consequence of the recognition that purinergic signalling first appeared early in evolution (see Burnstock and Verkhratsky 2009 and see Chap. 6) and is widespread in most non-neuronal as well as neuronal cell types (see Burnstock and Knight 2004) and partly as a consequence of the recognition that there is long-term (trophic) purinergic signalling in cell proliferation, differentiation, motility and death in development and regeneration, as well as short-term purinergic signalling in neurotransmission and secretion (Abbracchio and Burnstock 1998; Neary and Zimmermann 2009; Burnstock and Verkhratsky 2010). There are some exciting
Table 2.1 Identification of purinoceptors in mammalian tissues 

This table is compiled from (Burnstock and Knight 2004, Burnstock 2007, Burnstock and Verkhratsky 2009, Verkhratsky et al. 2009) where readers are advised to find the full list of references; here we present mostly early works, and we apologise in advance for inevitable omissions.

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<th>Tissue</th>
<th>Purinoceptors</th>
<th>Main functional role</th>
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<tr>
<td></td>
<td>A&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>Stimulation of indirect pathway in striatum; increased transmitter release; neurodegeneration</td>
<td>(Sebastiao and Ribeiro 1992; Cunha et al. 1995; Sebastiao and Ribeiro 1996; Svenningsson et al. 1997a, c, 2000, 1995; Huang et al. 2005)</td>
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<td></td>
<td>A&lt;sub&gt;2B&lt;/sub&gt;; A&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>P2X&lt;sub&gt;1&lt;/sub&gt;; P2X&lt;sub&gt;2&lt;/sub&gt;; P2X&lt;sub&gt;3&lt;/sub&gt;; P2X&lt;sub&gt;4&lt;/sub&gt;; P2X&lt;sub&gt;5&lt;/sub&gt;; P2X&lt;sub&gt;6&lt;/sub&gt;; P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Fast and slow neurotransmission in central synapses; presynaptic modulation; synaptic plasticity</td>
<td>(Jahr and Jessell 1983; Wieraszko and Seyfried 1989; Edwards et al. 1992; Bo and Burnstock 1994; Ergene et al. 1994; Furukawa et al. 1994; Balcar et al. 1995; Li and Perl 1995; Sperlagh et al. 1995; Collo et al. 1996; Soto et al. 1996a; Soto et al. b; Vulchanova et al. 1996; Funk et al. 1997; Nieber et al. 1997; Scislo et al. 1997; Vulchanova et al. 1997; Le et al. 1998; Li et al. 1998; Pankratov et al. 1998; Kanjhan et al. 1999; Jang et al. 2001; Nakatsuka and Gu 2001; Pankratov et al. 2002)</td>
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<td>P2Y&lt;sub&gt;1&lt;/sub&gt;; P2Y&lt;sub&gt;6&lt;/sub&gt;; P2Y&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Regulation of growth and development; metabolic regulation</td>
<td>(Mironov 1994; Salter and Hicks 1994; Ikeuchi et al. 1995; Ikeuchi and Nishizaki 1996; Kirischuk et al. 1996; Chessell et al. 1997a; Lalo et al. 1998; Ralevic et al. 1999; Brown and Dale 2002; Vasiljev et al. 2003)</td>
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<td>CNS</td>
<td>P2X&lt;sub&gt;1&lt;/sub&gt;, P2X&lt;sub&gt;7&lt;/sub&gt; (in pathological conditions?)</td>
<td>Fast neuronal-glial transmission(?) regulation of astrogliosis in pathological conditions</td>
<td>(Magoski and Walz 1992; Walz et al. 1994; Ballerini et al. 1996; Pannicke et al. 2000; Kukley et al. 2001; Panenka et al. 2001; Duan et al. 2003; Lalo et al. 2008)</td>
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<td>P2Y&lt;sub&gt;1&lt;/sub&gt;, P2Y&lt;sub&gt;2&lt;/sub&gt;, P2Y&lt;sub&gt;4&lt;/sub&gt;, P2Y&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; propagating Ca&lt;sup&gt;2+&lt;/sup&gt; waves; release of gliotransmitters; regulation of gap junctions; neuronal-glial and glial-glial signalling</td>
<td>(Pearce et al. 1989; Kastritsis et al. 1992; Bruner and Murphy 1993; Pearce and Langley 1994; Salter and Hicks 1994; Kirischuk et al. 1995a; Salter and Hicks 1995; Chen and Chen 1996; Centemeri et al. 1997; Ishimoto et al. 1997; Bernstein et al. 1998; Troadec et al. 1999; Cotrina et al. 2000; Fam et al. 2000; Jimenez et al. 2000; Wang et al. 2000; Zhu and Kimelberg 2001; Franke et al. 2004; Meme et al. 2004; Fries et al. 2005; Haas et al. 2006)</td>
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<td>Oligodendroglia</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;, A&lt;sub&gt;2A&lt;/sub&gt;, A&lt;sub&gt;2B&lt;/sub&gt;, A&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Control of myelination; may be involved in demyelinating disorders</td>
<td>(Stevens et al. 2002; Turner et al. 2002; Tsutsui et al. 2004)</td>
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<td>P2X&lt;sub&gt;7&lt;/sub&gt;, P2Y&lt;sub&gt;1&lt;/sub&gt;, P2Y&lt;sub&gt;2&lt;/sub&gt;, P2Y&lt;sub&gt;4&lt;/sub&gt;, P2Y&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; axonal-oligodendroglial signalling; development and maturation</td>
<td>(Kirischuk et al. 1995b; Deng et al. 1998; James and Butt 1999, 2001; Moran-Jimenez and Matute 2000; Laitinen et al. 2001; Matute et al. 2007)</td>
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<td>Microglia</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;, A&lt;sub&gt;2A&lt;/sub&gt;, A&lt;sub&gt;2B&lt;/sub&gt;, A&lt;sub&gt;3&lt;/sub&gt;</td>
<td>MAP-kinas signaling; cytokine production; neuronal survival; microglial proliferation</td>
<td>(Gebicke-Haerter et al. 1996; Hammarberg et al. 2003; 2004; Tsutsui et al. 2004; Synowitz et al. 2006; Min et al. 2008; Maggi et al. 2009)</td>
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<td>P2X&lt;sub&gt;4&lt;/sub&gt;, P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; control of microglial activation; regulation of the production and release of inflammatory mediators</td>
<td>(Ferrari et al. 1996; Haas et al. 1996; Illes et al. 1996; Chessell et al. 1997b; Collo et al. 1997; Visentin et al. 1999)</td>
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<td>P2Y&lt;sub&gt;12&lt;/sub&gt;, P2Y&lt;sub&gt;4&lt;/sub&gt;, P2Y&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; control of microglial activation; induction of early genes expression; regulation of K&lt;sup&gt;+&lt;/sup&gt; channels</td>
<td>(Ilschner et al. 1995; Priller et al. 1995; Norenberg et al. 1997; Inoue et al. 1998; Priller et al. 1998; Toescu et al. 1998; McLarnon et al. 1999; Moller et al. 2000)</td>
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<td>PNS</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;, A&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>Antinociception; sensitisation</td>
<td>(Sawynok et al. 1986; DeLander and Wahl 1988; Sosnowski et al. 1989; Karlsten et al. 1992; Sylven 1993; Reeve and Dickenson 1995; Abo-Salem et al. 2004)</td>
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<td>CNS</td>
<td>P2X2; P2X3;</td>
<td>Nociception; thermal sensitivity</td>
<td>(Krishtal et al. 1983; Salt and Hill 1983; Fyffe and Perl 1984; Mori et al. 1985; Bean et al. 1990; Tokimasa and Akasu 1990; Collo et al. 1996; Svichar et al. 1997a, 1997b; Xiang et al. 1998; Souslova et al. 2000; Boldogkoi et al. 2002; Khmyz et al. 2008)</td>
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<td>P2X2/3; P2Y4;</td>
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<td>Sympathetic neurons</td>
<td>A1; P2X1; P2X2;</td>
<td>Neuronal-effector transmission; presynaptic modulation; regulation of neurotransmitter (noradrenaline) release; Ca2+ signalling</td>
<td>(Hedqvist and Fredholm 1976; Connolly and Harrison 1994; Reekie and Burnstock 1994; Todorov et al. 1994; Boehm et al. 1995; Cloues 1995; Connolly and Harrison 1995; Ishii et al. 1995; Khakh et al. 1995; Haniuda et al. 1997; Simon et al. 1997; Searl et al. 1998; Xiang et al. 1998)</td>
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<td>Parasympathetic neurons</td>
<td>A1; P2X2, P2X3,</td>
<td>Neuronal-effector transmission; pre and postsynaptic modulation; Ca2+ signalling; control of excitability via opening of ion channels</td>
<td>(Ginsborg and Hirst 1972; Hayashi et al. 1978; Horackova et al. 1994; Nishimura and Tokimasa 1996; Sun and Stanley 1996; Zhong et al. 1998; Liu et al. 2000; Zhong et al. 2000; Liu and Adams 2001; Smith et al. 2001; Zhong et al. 2001)</td>
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<td>P2Y2, P2Y6;</td>
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<td>Cardio-vascular system</td>
<td>A1, A3; P2X1; P2X4;</td>
<td>Chronotrophic effects (both negative and positive); preconditioning; regulation of Ca2+ signalling; control of pacemaking activity; regulation of excitability of cardiomyocytes; modulation of Ca2+ channels; activation of Cl-currents; activation of muscarinic K+ channel in atrial cells</td>
<td>(Collis 1983; Liu et al. 1991; Thornton et al. 1992; Auchampach and Gross 1993; Froldi et al. 1994; Kameda et al. 1994; Liu et al. 1994; Parr et al. 1994; Scamps and Vassort 1994; Soto et al. 2003; Lankford et al. 2006; Eckle et al. 2007)</td>
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<td>P2X5; P2Y2; P2Y4;</td>
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<td>Blood vessels/endothelial cells</td>
<td>P2X1, P2X2, P2X3, P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4</td>
<td>Ca²⁺ signalling; platelet aggregation; shape changes; formation of thrombin</td>
<td>(Haslam and Cusack 1981; Paul et al. 1990; Lohse et al. 1991; Cristalli et al. 1994; Gachet et al. 1995; Soslau et al. 1995; MacKenzie et al. 1996; Leon et al. 1997; Savi et al. 1997; Vial et al. 1997)</td>
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<td>Blood</td>
<td>P2X1, P2Y1, P2Y12</td>
<td>Ca²⁺ signalling and [Ca²⁺] oscillations; cytoskeletal remodelling</td>
<td>(Somasundaram and Mahaut-Smith 1994; Uneyama et al. 1994a, b; Kawa 1996)</td>
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<td>Leucocytes</td>
<td>$A_1$; $A_{2A}$; $A_{2B}$; $A_3$; $P2X_1$; $P2X_4$, $P2X_5$; $P2X_7$, $P2Y_2$; $P2Y_4$; $P2Y_6$; $P2Y_{11}$</td>
<td>$Ca^{2+}$ signalling; promotion of adhesion to endothelial cells; stimulation of the oxidative burst; secretion of allergic and pro-inflammatory mediators</td>
<td>(Marone et al. 1985; Roberts et al. 1985; Cronstein et al. 1992; Wollner et al. 1993; Ludowyke and Scrr 1994; O’Flaherty and Cordes 1994; Dawicki et al. 1995; Susztak et al. 1995; Fredholm et al. 1996; Zalavary et al. 1996; Zhang et al. 1996; Gessi et al. 2005; Chen et al. 2006; Inoue et al. 2008)</td>
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<td>Lymphocytes</td>
<td>$A_{2A}$; $A_{2B}$; $P2X_1$; $P2X_2$, $P2X_4$; $P2X_7$; $P2Y_{11}$</td>
<td>Regulation of proliferation, differentiation and cell death; regulation of secretion of IL-2 and IFN-$\gamma$</td>
<td>(Miles et al. 1977; Fredholm et al. 1978; Marone et al. 1978; Schwartz et al. 1978; Sandberg and Fredholm 1981; Wiley et al. 1994; Breitschneider et al. 1995; Baricordi et al. 1996; Chused et al. 1996; Macino et al. 1996; Huang et al. 1997; Markwardt et al. 1997; Varani et al. 1997; Mirabet et al. 1999)</td>
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<td>Monocytes</td>
<td>$A_1$; $A_{2A}$; $A_{2B}$; $A_3$; $P2X_7$; $P2Y_1$; $P2Y_2$; $P2Y_4$; $P2Y_6$</td>
<td>Adenosine control differentiation and cytokine production; ATP acts as a potent chemoattractant</td>
<td>(Najar et al. 1990; Salmon et al. 1993; Akbar et al. 1997; Rassendren et al. 1997; Jin et al. 1998; Mayne et al. 1999; Landells et al. 2000; Link et al. 2000; Broussas et al. 2002; Zhang et al. 2005)</td>
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<td>Mast cells</td>
<td>$A_3$; $A_{2B}$; $P2Y_1$; $P2Y_2$</td>
<td>Adenosine and ATP releases histamine and causes degranulation</td>
<td>(Diamant and Kruger 1967; Ramkumar et al. 1993; Jin et al. 1997; McCluskey et al. 1999; Schulman et al. 1999; Hua et al. 2007)</td>
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<td><strong>Lung</strong></td>
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<td>Airway smooth muscle</td>
<td>$A_1$; $P2Y_2$; $P2Y_6$</td>
<td>$Ca^{2+}$ signalling; regulation of proliferation</td>
<td>(Bjorck et al. 1992; Michoud et al. 1997; Bergner and Sanderson 2002; Brown et al. 2008)</td>
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<td>Tracheal smooth muscle</td>
<td>$P2X_1$; $P2Y_1$; $P2Y_2$</td>
<td>$Ca^{2+}$ signalling</td>
<td>(Michoud et al. 1997; Sawai et al. 1997)</td>
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<td>Tracheal epithelial cells</td>
<td>$A_1$; $P2X_4$; $P2X_7$; $P2Y_1$; $P2Y_2$</td>
<td>$Ca^{2+}$ signalling; regulation of ciliary function; activation of $Ca^{2+}$-dependent Cl- channels</td>
<td>(Aksoy et al. 1995; Satoh et al. 1995; Hwang et al. 1996; Kim et al. 1996; Evans and Sanderson 1999; Gabriel et al. 2000; Nlend et al. 2002; Ma et al. 2006; Brown et al. 2008)</td>
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<td>Tracheal Goblet cells</td>
<td>$P2X_4$; $P2X_7$; $P2Y_1$; $P2Y_2$</td>
<td>Stimulation of mucin secretion</td>
<td>(Marino et al. 1999)</td>
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<td>Acinar cells from submucosal gland</td>
<td>$P2Y$</td>
<td>$Ca^{2+}$ signalling; stimulation of protein secretion</td>
<td>(Shimura et al. 1994)</td>
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Table 2.1 (continued)

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<td>Lung Goblet cells</td>
<td>P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Increase of mucin secretion</td>
<td>(Conway et al. 2003)</td>
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<td>Alveolar type II cells</td>
<td>P2X&lt;sub&gt;4&lt;/sub&gt;; P2Y&lt;sub&gt;2&lt;/sub&gt;; P2Y&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Facilitation of mucociliary clearance; activation of Cl&lt;sup&gt;−&lt;/sup&gt; currents</td>
<td>(Gobran et al. 1994; Rice et al. 1995; Buell et al. 1996)</td>
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<td>Ciliated epithelium</td>
<td>P2X&lt;sub&gt;4&lt;/sub&gt;; P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Potentiation of surfactant release; increase in ciliary beat frequency</td>
<td>(Stutts et al. 1994; Ma et al. 1999)</td>
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<td>Nonciliated epithelum</td>
<td>P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Stimulation of Cl&lt;sup&gt;−&lt;/sup&gt; and HCO&lt;sub&gt;3&lt;/sub&gt;− secretion</td>
<td>(Van Scott et al. 1995; Kishore et al. 2000)</td>
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<td>(Clara cells)</td>
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<td>Neuroepithelial bodies</td>
<td>P2X&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Mechanosensory transduction and O&lt;sub&gt;2&lt;/sub&gt; sensing</td>
<td>(Brouns et al. 2000)</td>
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<td>Gastrointestinal tract</td>
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<td>Stomach</td>
<td>P2X&lt;sub&gt;1&lt;/sub&gt;; P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Stimulation of contraction/relaxation; Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; prostaglandin production</td>
<td>(Soediono and Burnstock 1994; Baccari et al. 1996; Blottiere et al. 1996; Mashimo et al. 1996; Otsuguro et al. 1996; Curro and Preziosi 1998)</td>
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<td>Duodenum</td>
<td>P2X(?); P2Y&lt;sub&gt;1&lt;/sub&gt;; P2Y&lt;sub&gt;2&lt;/sub&gt;; P2Y(?);</td>
<td>ATP (acting through P2Y(?)) induces relaxation, whereas UTP (acting through P2Y&lt;sub&gt;2&lt;/sub&gt;)–stimulates contraction</td>
<td>(Irie et al. 1994; Johnson and Hourani 1994; Zagorodnyuk et al. 1995)</td>
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<td>Ileum</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;; P2X&lt;sub&gt;1&lt;/sub&gt;; P2X&lt;sub&gt;7&lt;/sub&gt;; P2Y&lt;sub&gt;1&lt;/sub&gt;; P2Y&lt;sub&gt;2&lt;/sub&gt;; P2Y(?);</td>
<td>Regulation of ACh release and muscle relaxation process</td>
<td>(Gustafsson et al. 1978; Hayashi et al. 1978; Kennedy and Humphrey 1994; Nitahara et al. 1995; Longhurst et al. 1996; Smits and Lefebvre 1996)</td>
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<td>Colon</td>
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<td>ATP induces contraction of circular muscle and relaxation of longitudinal muscle; Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; modulation of Cl&lt;sup&gt;−&lt;/sup&gt; currents</td>
<td>(Venkova et al. 1994; Zagorodnyuk and Maggi 1994; Briejer et al. 1995; Qian and Jones 1995; Maggi and Giuliani 1996)</td>
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<td>A&lt;sub&gt;1&lt;/sub&gt;; A&lt;sub&gt;2A&lt;/sub&gt;; A&lt;sub&gt;2B&lt;/sub&gt;; P2X(?); P2Y&lt;sub&gt;1&lt;/sub&gt;; P2Y&lt;sub&gt;2&lt;/sub&gt;; P2Y&lt;sub&gt;13&lt;/sub&gt;</td>
<td>Regulation of gluconeogenesis, stimulation of glycogen breakdown and inhibition of glycolysis and fatty acid synthesis; Ca&lt;sup&gt;2+&lt;/sup&gt; signalling</td>
<td>(Cooper and Londos 1979; Carmichael et al. 1988; Ohigashi et al. 1993; Nagy 1994; Guzman et al. 1996; Capiod 1998; Dixon et al. 2000, 2003; Che et al. 2007)</td>
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<td>Kidney/ glomerulus</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Urine production; renal constriction; tubuloglomerular feedback; Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; stimulation of mitogenesis; induction of apoptosis and necrosis via P2X&lt;sub&gt;7&lt;/sub&gt; receptors in development</td>
<td>(Hedqvist et al. 1978; Murray and Churchill 1984; Schnermann 1988; Schnermann et al. 1990; Briner and Kern 1994; Ishikawa et al. 1994; Takeda et al. 1996; Huwiler et al. 1997; Schulze-Lohoff et al. 1998; Brown et al. 2001)</td>
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<td>Kidney/Loop of Henle</td>
<td>P2Y&lt;sub&gt;1&lt;/sub&gt;; P2Y&lt;sub&gt;2&lt;/sub&gt;; P2Y&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signalling</td>
<td>(Paulais et al. 1995; Bailey et al. 2000)</td>
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<td>Bladder/smooth muscle</td>
<td>P2X&lt;sub&gt;1&lt;/sub&gt;; P2X&lt;sub&gt;2&lt;/sub&gt;; P2X&lt;sub&gt;4&lt;/sub&gt;; P2X&lt;sub&gt;5&lt;/sub&gt;; P2X&lt;sub&gt;6&lt;/sub&gt;; P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>ATP is a main parasympathetic cotransmitter; ATP induces contraction via P2X receptors and relaxation via P2Y; triggers micturition reflex</td>
<td>(Bo et al. 1994, 1995; Bolego et al. 1995; Evans et al. 1995; Michel et al. 1996; Zhao et al. 1996)</td>
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<td>Bladder/urothelium</td>
<td>P2X&lt;sub&gt;3&lt;/sub&gt;; P2X&lt;sub&gt;5&lt;/sub&gt;; P2X&lt;sub&gt;6&lt;/sub&gt;; P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>?</td>
<td>(Lee et al. 2000a)</td>
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<td>Urethra</td>
<td>P2X; P2Y</td>
<td>ATP acts as a cotransmitter; induces relaxation via P2Y receptors</td>
<td>(Pinna et al. 1996; Ohnishi et al. 1997)</td>
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<td><strong>Genital system</strong></td>
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<td>P2X&lt;sub&gt;1&lt;/sub&gt;; P2X&lt;sub&gt;2&lt;/sub&gt;; P2Y&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Relaxation of corpus cavernosum via NO-dependent (humans) and NO-independent pathways; role in prapism</td>
<td>(Broderick et al. 1994; Levin et al. 1995; Ragazzi et al. 1996; Kaya et al. 1998; Shalev et al. 1999)</td>
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<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signalling; regulation of secretion of fluids, estradiol and testosterone</td>
<td>(Nakata 1990; Zhou et al. 1992; Filippini et al. 1994; Foresta et al. 1995; Rudge et al. 1995; Foresta et al. 1996b; Meroni et al. 1998)</td>
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<td>Sperm</td>
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<td>Modulation of spermatogenesis and stimulation of acrosomal exocytosis; capacitation</td>
<td>(Tomiyama et al. 1995; Foresta et al. 1996a; Loir 1999; Glass et al. 2001; Minelli et al. 2004)</td>
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<td>Vas deference</td>
<td>P2X&lt;sub&gt;1&lt;/sub&gt;; P2X&lt;sub&gt;2&lt;/sub&gt;; P2X&lt;sub&gt;4&lt;/sub&gt;; P2X&lt;sub&gt;7&lt;/sub&gt;; P2Y&lt;sub&gt;1&lt;/sub&gt;; P2Y&lt;sub&gt;2&lt;/sub&gt;; P2Y (?)</td>
<td>ATP is a main sympathetic cotransmitter (with NA); triggers contraction and regulates muscle tone</td>
<td>(Bultmann and Starke 1994; Michel and Humphrey 1994; Bo et al. 1995; Westfall et al. 1997; Mulryan et al. 2000)</td>
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<td>Prostate gland</td>
<td>P2X₁</td>
<td>ATP is a main sympathetic cotransmitter (with NA); regulation of excitability of epithelial cells</td>
<td>(Janssens et al. 1996; Longhurst et al. 1996; Lee et al. 2000b)</td>
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<td>Vagina and cervix</td>
<td>P2X₂; P2X₄;</td>
<td>Regulation of relaxation; stimulation of mucus and Cl⁻ secretion; regulation of transepithelial electrical conductance; regulation of cell turnover</td>
<td>(Gorodeski and Hopfer 1995; Gorodeski et al. 1996; Gorodeski and Goldfarb 1997; Groschel-Stewart et al. 1999; Bardini et al. 2000; Min et al. 2003)</td>
</tr>
<tr>
<td>Uterus</td>
<td>A₁; A₂A; A₂B; P2X₁; P2X₅; P2X₇; P2X₁₀; P2X₁₁; P2Y₁; P2Y₂; P2Y₆; P2Y₁₀; P2Y₁₁</td>
<td>Ca²⁺ signalling; stimulation of contraction; regulation of Na⁺ transport in endometrial endothelial cells</td>
<td>(Schiemann and Buxton 1991; Schiemann et al. 1991; Haynes and Pennefather 1993; Piper and Hollingsworth 1996; Gillman and Pennefather 1998; Blackburn et al. 1999; Bardini et al. 2000; Tassell et al. 2000; Aitken et al. 2001; Shmigol et al. 2001)</td>
</tr>
<tr>
<td>Ovary</td>
<td>P2X₁; P2X₂; P2Y₂</td>
<td>Ca²⁺ signalling; increase in ciliary beat frequency; regulation of fluid formation</td>
<td>(Cox and Leese 1995; Dickens et al. 1996; Bardini et al. 2000)</td>
</tr>
<tr>
<td>Placenta</td>
<td>P2X₁; P2X₂; P2X₄; P2X₇; P2Y₁; P2Y₂; P2Y₆; P2Y₁₀; P2Y₁₁</td>
<td>Stimulation of PLC/InsP₃ production; Ca²⁺ signalling</td>
<td>(Petit and Belisle 1995; Karl et al. 1997; Somers et al. 1999)</td>
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<tr>
<td>Exocrine glands</td>
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<tr>
<td>Salivary glands</td>
<td>P2X₄; P2X₇; P2Y₂</td>
<td>Ca²⁺ signalling; regulation of ion balance; regulation of Zn²⁺ uptake; ATP acts as a cotransmitter in excitation-secretion coupling</td>
<td>(Hurley et al. 1994; Dehaye 1995; Jorgensen et al. 1995; Amsallem et al. 1996; Lachish et al. 1996; Fukushi et al. 1997; Park et al. 1997; Park et al. 1997; Zeng et al. 1997; Mizuno-Kamiya et al. 1998; Tenneti et al. 1998)</td>
</tr>
<tr>
<td>Exocrine pancreas</td>
<td>A₂A; P2X₁; P2X₄; P2X₇; P2Y₁; P2Y₂; P2Y₄</td>
<td>Ca²⁺ signalling; stimulation of mucin and anions secretion</td>
<td>(Chan et al. 1996; Christoffersen et al. 1998; Nguyen et al. 1998; Dubyak 1999; Hede et al. 1999; Luo et al. 1999; Iwatsuki 2000)</td>
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<tr>
<td>Endocrine glands</td>
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<tr>
<td>Tissue</td>
<td>Purinoceptors</td>
<td>Main functional role</td>
<td>References</td>
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<tr>
<td>Adrenal gland</td>
<td>P2X1, P2X2, P2X3, P2X5, P2X6, P2X7, P2X8, P2Y1, P2Y2, P2Y4</td>
<td>Ca²⁺ signalling; modulation of secretion of catecholoamines; modulation of aldosterone production and secretion; modulation of exocytosis; control of excitability</td>
<td>(Castro et al. 1995; Lin et al. 1995; Reichsman et al. 1995; Lim et al. 1997; Szalay et al. 1998; Afework and Burnstock 1999; Xu and Enyeart 1999)</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>P2X2; P2X3, P2X4, P2X5, P2Y2</td>
<td>Ca²⁺ signalling; regulation of the release of vasopressin and prolactin</td>
<td>(Carew et al. 1994; Chen et al. 1994; 1995; Tomic et al. 1996; Nunez et al. 1997; Troadec et al. 1998; Sperlagh et al. 1999)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>A1; A2A, P2X3, P2X4, P2X5, P2Y1, P2Y2</td>
<td>Ca²⁺ signalling; Increase and decrease in cAMP; inhibition of Na⁺ absorption</td>
<td>(Fradkin et al. 1982; Frauman and Moses 1989; Okajima et al. 1989; Schofl et al. 1995; Bourke et al. 1999; Harii et al. 1999)</td>
</tr>
<tr>
<td>Endocrine pancreas</td>
<td>A1; A2A, P2X1, P2X3, P2Y1, P2Y4</td>
<td>Inhibition or stimulation of insulin and glucagon release; regulation of pulsatility</td>
<td>(Hillaire-Buys et al. 1987; Squires et al. 1994; Petit et al. 1998; Coutinho-Silva et al. 2001; Johansson et al. 2007; Salehi et al. 2009)</td>
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<tr>
<td>Muskulo-skeletal system</td>
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<tr>
<td>Bone: Osteoblasts</td>
<td>P2X2; P2X3, P2X5, P2X7, P2Y1, P2Y2</td>
<td>Ca²⁺ signalling; stimulation of osteoclasts formation; regulation of proliferation; control of cell death</td>
<td>(Dixon et al. 1997; Jones et al. 1997; Bowler et al. 1999; Hoebertz et al. 2000, 2001, 2002)</td>
</tr>
<tr>
<td>Bone: Osteoclasts</td>
<td>A1, P2X2; P2X3, P2X7, P2Y1, P2Y2</td>
<td>Stimulation of osteoclasts activity; stimulation of resorption pit formation; regulation of acid transport; regulation of intercellular communications via Ca²⁺ waves; inhibition of bone resorption (P2X7?)</td>
<td>(Modderman et al. 1994; Yu and Ferrier 1995; Weidema et al. 1997; Morrison et al. 1998; Hoebertz et al. 2000, 2001; Kara et al.)</td>
</tr>
<tr>
<td>Cartilage</td>
<td>P2X2; P2X3, P2Y1, P2Y2, P2Y4</td>
<td>Ca²⁺ signalling; stimulation of cartilage resorption and production of prostaglandins</td>
<td>(Leong et al. 1994; Bulman et al. 1995; Kaplan et al. 1996; Hung et al. 1997; Koolpe and Benton 1997)</td>
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<tr>
<td>Tissue</td>
<td>Purinoceptors</td>
<td>Main functional role</td>
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<tr>
<td><strong>CNS</strong></td>
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<tr>
<td>Skeletal muscle</td>
<td>A1, P2X1; P2X2, P2X3; P2X5; P2X7; P2Y1, P2Y(?), P2X7; P2X(?), P2Y1; P2Y2; P2Y(?)</td>
<td>Modulation of neuromuscular junction transmission; Ca²⁺ signalling; inhibition of proliferation and stimulation of differentiation; glucose metabolism</td>
<td>(Ayyanathan et al. 1996; Henning 1997; Urano et al. 1997; Cheng et al. 2000)</td>
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<tr>
<td>Connective tissue</td>
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<tr>
<td>Fibroblasts</td>
<td>P2X7; P2X(?), P2Y1; P2Y2; P2Y(?)</td>
<td>Ca²⁺ signalling; regulation of proliferation; activation of volume-sensitive Cl⁻ channels</td>
<td>(Grierson and Meldolesi 1995; Arav and Friedberg 1996; Hofer et al. 1996; Tepel et al. 1996; Zheng et al. 1998)</td>
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</table>
studies of purinergic signalling in the special senses area (Housley et al. 2009). There is increasing interest in the roles of purines and pyrimidines in pathophysiology and the therapeutic potential of the signalling system (Burnstock 2006b) for the treatment of diseases, including thrombosis and stroke (employing clopidogrel, an antagonist to the P2Y12 receptor, that mediates platelet aggregation and made $8.6 billion in 2007), osteoporosis, kidney failure, bladder incontinence, cystic fibrosis, dry eye and cancer. There is also considerable interest in the involvement of P2X receptor antagonists for the treatment of acute and neuropathic pain (Inoue 2007) and diseases of the CNS (Burnstock 2008).

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Purinergic Signalling and the Nervous System
Burnstock, G.; Verkhratsky, A.
2012, XVIII, 715 p. 135 illus., 46 illus. in color.,
Hardcover
ISBN: 978-3-642-28862-3