British Journal of Pharmacology (1998) 123, 71–80

Modulation by adenine nucleotides of epileptiform activity in the CA3 region of rat hippocampal slices

F.M. Ross, *M.J. Brodie & [1]T.W. Stone

Institute of Biomedical and Life Sciences, West Medical Building, University of Glasgow, Glasgow, G12 8QQ and *Epilepsy Unit, Department of Medicine & Therapeutics, Western Infirmary, Glasgow, G11 6NT

1 Hippocampal slices (450 μm) generate epileptiform bursts of an interictal nature when perfused with a zero magnesium medium containing 4-aminopyridine (50 μM). The effect of adenine nucleotides on this activity was investigated.

2 ATP and adenosine depressed this epileptiform activity in a concentration-dependent manner, with both purines being equipotent at concentrations above 10 μM.

3 Adenosine deaminase 0.2 u ml^{-1}, a concentration that annuls the effect of adenosine (50 μM), did not significantly alter the depression of activity caused by ATP (50 μM).

4 8-Cyclopentyl-1, 3-dimethylxanthine (CPT), an A_1 receptor antagonist, enhanced the discharge rate significantly and inhibited the depressant effect of both ATP and adenosine such that the net effect of ATP or adenosine plus CPT was excitatory.

5 Several ATP analogues were also tested: α, β-methyleneATP (α, β-meATP), 2-methylthioATP (2-meSATP) and uridine triphosphate (UTP). Only α, β-meATP (10 μM) produced an increase in the frequency of spontaneous activity which suggests a lack of involvement of P2Y or P2U receptors.

6 Suramin and pyridoxalphosphate-6-azophenyl-2′, 4′-disulphonic acid (PPADS), P2 receptor antagonists, failed to inhibit the depression produced by ATP (50 μM). The excitatory effect of α, β-meATP (10 μM) was inhibited by suramin (50 μM) and PPADS (5 μM).

7 ATP therefore depresses epileptiform activity in this model in a manner which is not consistent with the activation of known P1 or P2 receptors, suggesting the involvement of a xanthine-sensitive nucleotide receptor. The results are also indicative of an excitatory P2X receptor existing in the hippocampal CA3 region.

Keywords: ATP; purines; nucleotides; epileptiform bursts; epilepsy; hippocampus

Introduction

There is growing evidence for a physiological role of adenosine 5′-triphosphate (ATP) in the modulation of synaptic transmission in the CNS. ATP release has been demonstrated in response to chemical stimulation in brain synaptosomal preparations (White, 1978) and electrical stimulation in hippocampal slices (Wieraszko *et al*., 1989; Cunha *et al*., 1996) and habenula (Barajas-Lopez *et al*., 1995). This release may be via a classical exocytotic mechanism or through transient electroporation of the cellular membrane (Hamman & Attwell, 1996).

Purine receptors were originally subdivided into two subclasses P1 and P2 with adenosine and adenosine 5′-monophosphate (AMP) acting preferentially on P1 receptors and ATP and adenosine 5′-diphosphate (ADP) being the preferred agonists at P2 receptors (Burnstock, 1978). It is now recognised that there are at least four subtypes of P1 receptor: A_1, A_{2A}, A_{2B} and A_3. A_1 receptors induce hyperpolarization and inhibit the release of several transmitters, and xanthines such as 8-cyclopentyl-1,3-dimethylxanthine (CPT) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) are selective antagonists. The initial subclassification of P2 receptors into P2X and P2Y was based on the selectivity of two ATP analogues, α, β-methyleneATP (α,β-meATP) and 2-methylthioATP (2-meSATP), respectively (Burnstock & Kennedy, 1985). The P2X receptor is also desensitized by α, β-meATP. Three other subtypes also exist P2T, P2Z and P2U which are sensitive to ADP, ATP^{4-} and UTP as agonists, respectively. The P2X receptors are now known to consist of a family of receptors with integral ion channels whereas P2Y receptors are coupled to G proteins (Abbracchio & Burnstock, 1994; Fredholm *et al*., 1997). The receptor from the vas deferens is $P2X_1$ and from PC12 cells $P2X_2$.

Radioligand binding (Michel & Humphrey, 1993) and autoradiography studies (Balcar *et al*., 1995) with [^{3}H]-α, β-meATP have localized areas of high affinity ligand binding to several brain regions. The existence of purinoceptors was further supported by the cloning of P2X receptors from both rat vas deferens (Valera *et al*., 1994) and PC12 cells (Brake *et al*., 1994) and the subsequent detection of these receptors with Northern blotting (Brake *et al*., 1994; Valera *et al*., 1994) and *in situ* hybridization to brain regions (Kidd *et al*., 1995; Séguéla *et al*., 1996). In the hippocampus, the receptor was found in all areas including the CA1, CA2 and CA3 regions and dentate gyrus.

Exogenous ATP applied to dorsal horn neurones produced an inward current, providing the first evidence that ATP could act in an extracellular manner within the CNS (Jahr & Jessel, 1983). ATP has since been shown to exert similar effects in coeliac ganglion cells (Khakh *et al*., 1995), ciliary neurones (Abe *et al*., 1995), cerebellar neurones (Ikeuchi & Nishizaki, 1996), nucleus tractus solitarius neurones (Ueno *et al*., 1992) and hypothalamic neurones (Chen *et al*., 1994). In pontine slices containing the locus coeruleus, ATP analogues enhanced the spontaneous firing rate and produced depolarization of the cells. These effects were displayed by ATP in the presence of DPCPX, an A_1 receptor antagonist (Harms *et al*., 1992; Tschopl *et al*., 1992).

[1] Author for correspondence.

Several studies have examined the role of ATP in synaptically-induced responses which could not be explained by classical neurotransmitter release. Excitatory postsynaptic potentials (e.p.s.p.) and excitatory postsynaptic currents (e.p.s.c.) in cultured coeliac ganglion cells were not inhibited by antagonists acting at receptors for N-methyl-D-aspartate (NMDA), non-NMDA agonists, noradrenaline or 5-HT but were inhibited by the P2 antagonist suramin and desensitized by α, β-meATP suggesting that ATP is the substance released (Evans *et al*., 1992). However this inhibition by suramin and mimicking by ATP was not observed in intact ganglia (Inokuchi & McLachlan, 1995). Evoked and miniature e.p.s.cs in the habenula are also depressed exclusively by suramin and α, β-meATP, suggesting that ATP is a chemical mediator of fast excitatory transmission in the CNS (Edwards *et al*., 1992).

A number of studies regarding ATP and P2 receptors have concentrated on the hippocampus both in slice form and as neuronal cultures, resulting in somewhat conflicting results. In hippocampal slices stable ATP analogues produced no consistent effects on CA1 population spikes or single neurone firing (Stone & Cusack, 1989). The depression of neuronal activity often seen with ATP has been attributed to metabolism to adenosine (Stone & Cusack, 1989). Di Cori & Henry (1984) showed that ATP depressed single unit recordings and concluded that ATP acted post-synaptically to modulate hippocampal activity. ATP may act in a dose-dependent biphasic manner producing sustained potentiation of activity, resembling a form of long-term potentiation (LTP), at nanomolar concentrations and sustained depression at high micromolar concentrations (Wieraszko & Seyfried, 1989). Inward currents mediated by channels carrying calcium (Inoue *et al*., 1992; 1995; Dave & Mogul, 1996) and anions (Balachadran & Bennett, 1996) have been shown to be activated in hippocampal neurones following ATP application.

In view of this evidence for receptor-mediated actions of ATP on single neurones, it was of interest to investigate the effect of ATP and related analogues on the behaviour of a neuronal population. Since CA1 population spikes had proved resistant to ATP analogues (Stone & Cusack, 1989), the present study was designed to examine the modulation of epileptiform bursting which can be triggered from the CA3 region.

Methods

Male Wistar rats (180–250 g) were anaesthetized with urethane (1.3 g kg^{-1}, i.p.) before being killed by cervical dislocation. Transverse hippocampal slices (450 μm) were prepared with a McIlwain Tissue Chopper. The slices were kept within an interface chamber containing artificial cerebrospinal fluid (aCSF) gassed with 95% O_2-5% CO_2 for at least 1 h before use. The composition of the aCSF was as follows (mM): NaCl 115, $NaHCO_3$ 25, KCl 2, KH_2PO_4 2.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2 and glucose 10; saturated with 95% O_2-5% CO_2. After incubation individual slices were transferred to a 1 ml submersion chamber which was continually perfused with aCSF or modified aCSF at a rate of 3.5–4 ml min^{-1}. The temperature of the chamber was maintained at approximately 34°C.

A bipolar stimulation electrode was placed in the stratum radiatum of the hippocampal CA3 region to allow orthodromic stimulation of the mossy fibres. The response was recorded via a glass capillary electrode in the pyramidal cell layer of the CA3. Stimulation (0.2 Hz) was applied briefly to check the viability of the slice and the correct positioning of the recording electrode, after which stimulation was halted and the perfusing medium changed from normal aCSF to magnesium-free aCSF containing 4-aminopyridine (4-AP) at 50 μM. After approximately 5–20 min spontaneous bursts of population spikes occurred which were continuously recorded on a Gould storage oscilloscope and a Grass pen recorder and subsequently plotted as frequency against time. Drugs were perfused for a minimum of 10 min. The control frequency (bursts min^{-1}) was calculated as the mean of the 3 observations immediately preceding the start of drug perfusion. The effect of added agents was taken as the mean of the final 3 observations made during the 10 min period of perfusion. Results are expressed as a percentage of the control rate $\pm$ s.e.mean for n slices. Statistical analysis of control against test rate was carried out with paired Student's t test. Multiple comparisons were made by use of Student's t test (paired or unpaired) or analysis of variance (ANOVA) followed by a Student-Newman-Keul's *post*-test. $P<0.05$ was taken to indicate significance.

Drugs

All drugs except CPT and adenosine deaminase were dissolved in distilled water to form a stock solution before being diluted further in normal or modified aCSF. CPT was dissolved in a 1 : 1 combination of NaOH (0.5 M) and distilled water whereas adenosine deaminase was prepared directly in aCSF.

Adenosine, ATP, α,β-methyleneATP, adenosine deaminase (Type II), 4-aminopyridine, uridine 5′-triphosphate (UTP) and 2-methylthio ATP were purchased from the Sigma Chemical Co. and CPT obtained from Research Biochemicals Incorporated. PPADS (pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid) was from Tocris Cookson. Suramin was a gift of Bayer.

Results

The perfusion of hippocampal slices with medium containing no added magnesium and 4-aminopyridine (4-AP) at 50 μM resulted in the generation of spontaneous activity. Individual bursts had a duration of between 150 and 400 ms with either single or multiple spikes per burst. The frequency of the bursts was in the range of 0.08–0.6 Hz. Burst duration, frequency and amplitude varied between slices but remained stable during rest and washout periods in individual slices.

Adenosine produced a concentration-dependent reduction in the discharge rate (Figure 1). ATP at concentrations greater than 10 μM depressed spontaneous activity to a degree comparable with adenosine. However, at a concentration of 2 μM the concentration-response graphs of adenosine and ATP diverged, with ATP tending to increase the burst frequency, though this did not reach significance. In the case of perfusion with adenosine or ATP, the peak depression of burst frequency occurred within 5 min with some subsequent evidence of recovery towards an equilibrium effect after 10 min (Figures 2, 3 and 4). This may reflect a degree of desensitization.

Adenosine deaminase

ATP is metabolized to adenosine by ecto-nucleotidases. In order to inactivate any adenosine formed during the perfusion of ATP, adenosine deaminase was co-perfused with ATP. The time course of the depression of activity induced by ATP at

50 μM is plotted in Figure 2a. The onset of the ATP effect was almost instantaneous when the perfusion system lag time of 1.5–2 min was taken into consideration.

Adenosine deaminase itself (0.2 u ml^{-1}) tended to increase the rate of activity to a small extent but this did not reach significance. (Figure 2b). Adenosine deaminase (0.2 u ml^{-1}) did not significantly reduce the extent of the ATP (50 μM)-induced depression at equilibrium (10 min). The depression of burst rate by adenosine (50 μM) followed a similar time course to that of ATP, but when perfused in identical experimental conditions the same concentration of adenosine deaminase (0.2 u ml^{-1}) totally abolished any effect of adenosine, as shown in Figure 3.

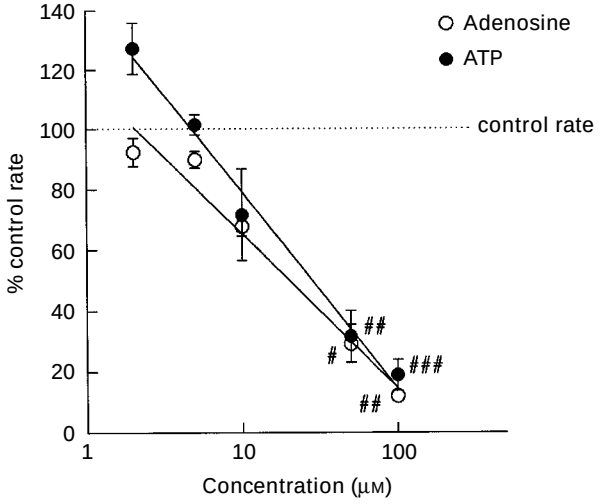

Figure 1 Concentration-response curves for the effect of ATP and adenosine on the frequency of epileptiform activity. Each symbol represents the mean and vertical lines show s.e.mean ($n=4$). #$P<0.05$, ##$P<0.01$, ###$P<0.001$.

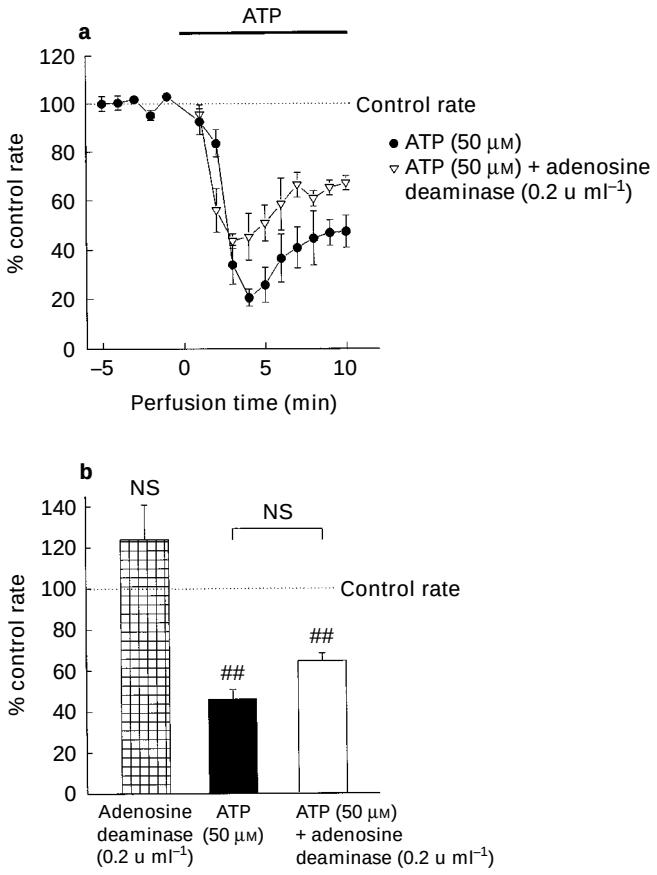

Figure 2 The effect of adenosine deaminase on the depression of epileptiform activity produced by ATP. (a) The time course of ATP effect when perfused alone ($n=6$) and in combination with adenosine deaminase. ($n=4$). (b) Summary of the effects at the end of the perfusion period. Adenosine deaminase did not significantly alter the discharge rate, whereas ATP and ATP + adenosine deaminase did, ##$P<0.01$.

Cyclopentyltheophylline

8-Cyclopentyl-1,3-dimethylxanthine (CPT), an A_1 receptor antagonist, at 100 nM elevated the rate of burst activity by approximately 40% (Figure 4c). When CPT was perfused simultaneously with ATP or adenosine (50 μM) it completely prevented the effect of the purine such that the CPT plus purine curves were almost superimposable upon the effect of CPT alone. The time course of the effects of adenosine and ATP are illustrated in Figure 4a and b, and a summary plot of the changes in burst frequency and their statistics is given in Figure 4c.

Nucleotide analogues

The ATP analogue α, β-meATP is relatively resistant to degradation by ecto-nucleotidases and is considered to activate several subtypes of P2X receptors. At a concentration of 5 μM α, β-meATP had no effect on burst frequency, whereas at 10 μM the frequency was increased significantly (Figure 5). This excitatory effect was not altered when α, β-meATP was

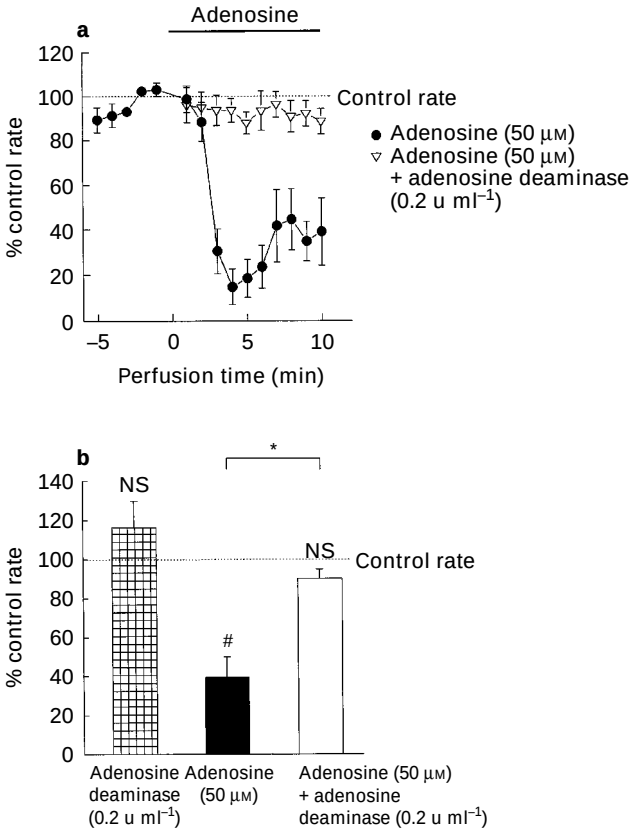

Figure 3 The time course of the decrease in discharge rate by adenosine ($n=5$) and the inhibition of this by adenosine deaminase ($n=4$) is shown in (a). (b) Represents the mean effect at the end of 10 min perfusion. Only adenosine significantly altered the rate from control, #$P<0.05$. The addition of adenosine deaminase reduced the effect of adenosine to a significant degree, *$P<0.05$.

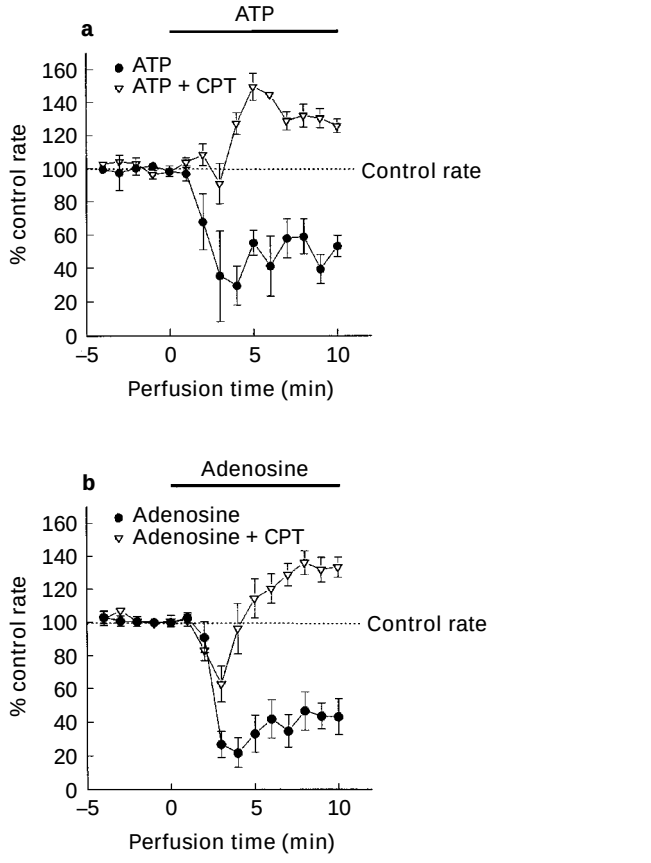

Figure 4 The effect of CPT (100 nM) on the depression of spontaneous activity by ATP (50 μM) and adenosine (50 μM). The time course of the effect of ATP and adenosine are represented in (a) and (b), respectively. The net effect after 10 min perfusion of CPT (100 nM, $n=4$), ATP (50 μM, $n=3$) and adenosine (50 μM, $n=8$), singly or in combination (ATP + CPT, $n=3$; adenosine + CPT, $n=6$) is shown in (c). All combinations altered the rate significantly from control, $\#P<0.05$, $\#\#P<0.01$.

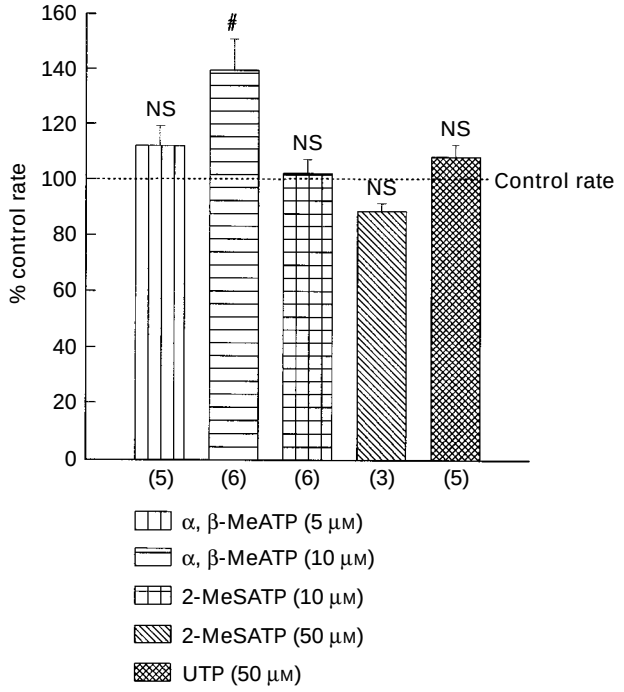

Figure 5 Effect of ATP analogues, α, β-meATP, 2-meSATP and UTP, on epileptiform activity. Results are taken at the end of a 10 min perfusion. Only α, β-meATP (10 μM) significantly changed the rate from control, $\#P<0.05$; $n=$number in parentheses below each column.

co-perfused with adenosine deaminase (data not shown). 2-Methylthio ATP (2-meSATP) is susceptible to a similar degree of metabolic breakdown as ATP and can activate P2 purinoceptors. 2-meSATP failed to alter significantly the frequency of spontaneous activity at concentrations of 10 and 50 μM (Figure 5). However, 2-meSATP is susceptible to metabolism by nucleotidases and the experiments were therefore also performed in the presence of α, β-meADP (50 μM), an inhibitor of 5′-nucleotidase. 2meSATP was still ineffective under these conditions (data not shown). The pyrimidine nucleotide UTP was also not significantly active at 50 μM in this model of epileptiform activity.

ADP modified spontaneous activity to a significant degree at 50 but not 10 μM (Figure 6). Adenosine deaminase (0.2 u ml^{-1}) reduced the effect of ADP (50 μM), although ADP still depressed burst rate to a significant degree in the presence of the enzyme.

Nucleotide antagonists

Suramin and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), P2 receptor antagonists, were perfused independently to determine any effect on discharge frequency.

Preliminary experiments indicated that suramin alone had no effect on burst frequency at concentrations of 10 and 50 μM, whereas at 200 μM there was a significant depression of activity. A concentration of 50 μM was therefore selected for use in experiments with ATP. Similarly PPADS at a concentration of 5 μM did not alter the basal rate of activity. Slices were exposed to suramin (50 μM) and PPADS (5 μM) for periods of 10 and 15 min, respectively, before the addition of ATP or related analogues.

In experiments examining antagonism of the ATP depression, adenosine deaminase was always perfused concurrently with ATP to ensure the elimination of any effect of adenosine. Neither suramin (Figure 7) nor PPADS (Figure 8) inhibited the depressant action of ATP (50 μM). In fact with both compounds there was a marked tendency for the effect of ATP to be enhanced and this enhancement was statistically significant in the case of PPADS (Figure 8c).

Similar conditions were used to investigate the effect of P2 antagonists on the increase of burst frequency induced by α, β-meATP. As in the earlier experiments with α, β meATP alone, this nucleotide again increased bursting rate, an effect which was prevented by suramin (50 μM) (Figure 9). Similarly, in the presence of PPADS (5 μM) α, β-meATP (10 μM) no longer significantly altered the rate from control (Figure 10).

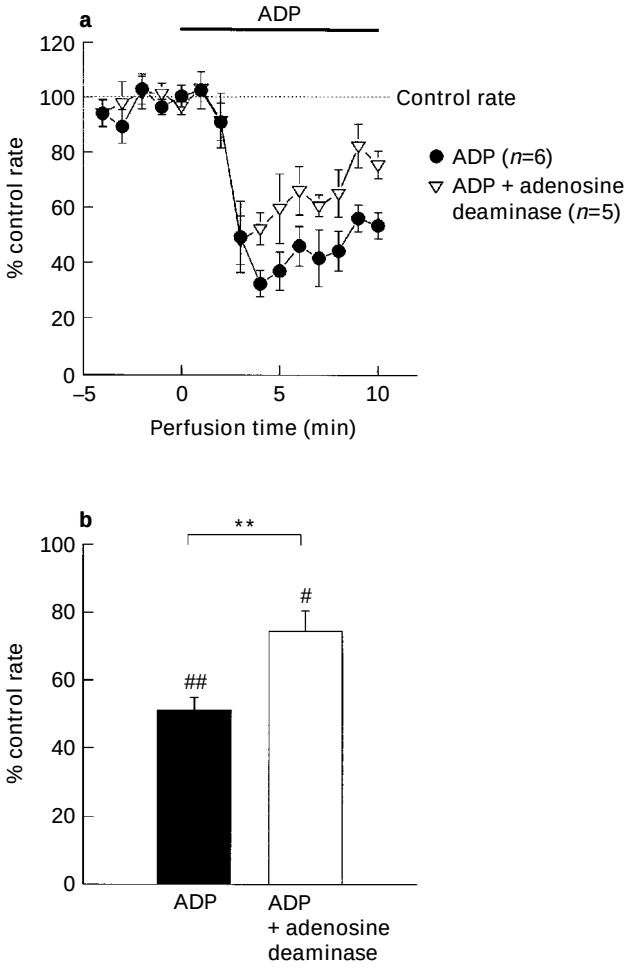

Figure 6 (a) The time course of the effect of adenosine deaminase (0.2 u ml^{-1}) on the depression of discharge rate by ADP (50 μM). (b) A summary of the effect at the end of a 10 min perfusion. ADP (50 μM) significantly decreased the rate when perfused alone or with adenosine deaminase (0.2 u ml^{-1}); $\#P<0.05$, $\#\#P<0.01$. Adenosine deaminase reduced the effect of ADP, $**P<0.01$.

Discussion

Several *in vitro* models of epileptiform activity have been used in order to gain greater understanding of the epileptic brain. Abnormal wave activity in an electroencephalogram outwith a seizure is referred to as an interictal spike. The intracellular correlate is a paroxysmal depolarizing shift (PDS) which consists of depolarization of the cellular membrane along with bursts of action potentials (Ayala *et al*., 1970). The removal of magnesium from the perfusing medium or the addition of 4-aminopyridine (4-AP) generally produces epileptiform activity of an interictal-like nature which consists of bursts of population spikes lasting up to several hundred milliseconds (Horne *et al*., 1986; Mody *et al*., 1987; Schneiderman & MacDonald, 1987; Swartzwelder *et al*., 1988; Lewis *et al*, 1989; Tancredi *et al*., 1990). The combination of zero magnesium and 4-AP in the present study produced spontaneous activity of steady frequency and amplitude. Although no intracellular recordings were undertaken in this study, the combination of zero magnesium and 4-AP has been shown to generate paroxysmal depolarizing shifts in neocortical neurones (Siniscalchi *et al*., 1997). Therefore, due to its characteristics, the spontaneous activity generated was considered to represent a form of interictal epileptiform activity. The concentration of 4-AP used was similar to that used by other groups (Voskuyl & Albus, 1985; Avoli *et al*., 1996; Psarropoulou & Avoli, 1996).

Individually the activity produced by 4-AP results from inhibition of the D potassium current (Storm, 1988) and a direct effect on voltage-gated calcium channels (Segal & Barker, 1986), whereas zero magnesium causes increased excitability by reducing membrane charge screening, lessening the antagonism between magnesium and calcium and alleviating the voltage-dependent block of *N*-methyl-D-aspartate (NMDA) receptors (Mody *et al*., 1987). A combination of these factors is proposed to be involved in the generation of epileptiform activity when the two, zero magnesium and 4-AP, are combined. The involvement of both NMDA and non-NMDA receptors was implicated by the depression in discharge rate produced by 2-amino-5-phosphonopentanoic acid, an NMDA receptor antagonist, and kynurenate (data not shown), which is in agreement with the study on coronal slices (Siniscalchi *et al*., 1997).

Over the last few years investigations into the role of extracellular ATP and P2 receptors have established that ATP is an important extracellular compound acting through a variety of P2X and P2Y receptors (Burnstock, 1990; Chen *et al*., 1995). However, there is very little information regarding the effect of ATP on epileptiform activity. The study of ATP is hampered by its degradation to metabolites which are active in their own right, especially adenosine.

ATP and adenosine depressed epileptiform activity in our model to a similar extent, which raised the question of whether ATP was acting directly or after metabolism to adenosine. Adenosine is a potent anticonvulsant in both *in vitro* (Dunwiddie *et al*., 1981; Ault & Wang, 1986) and *in vivo* models (Maitre *et al*., 1974; Dunwiddie & Worth, 1982), an effect which has been attributed to the activation of A_1 receptors (Dunwiddie & Fredholm, 1989). Presynaptic mechanisms involving a reduction in neurotransmitter release (Dunwiddie, 1980; Fredholm & Hedquist, 1980; Scholz & Miller, 1991) and postsynaptic stimulation of potassium currents (Lee *et al*., 1984; Alzheimer & ten Bruggencate, 1991) are involved in producing the inhibitory effect of adenosine. A_1 receptor antagonists make seizures worse (Chu, 1981; Dunwiddie *et al*., 1981; Alzheimer *et al*., 1993), while adenosine uptake inhibitors increase seizure threshold *in vivo* (Dunwiddie & Worth, 1982; Dragunow & Goddard, 1984) and *in vitro* (Ault & Wang, 1986) supporting the notion that adenosine is a mediator of seizure termination.

The action of adenosine *in vivo* is regulated both enzymatically and by uptake. In the present work adenosine deaminase was used at a concentration of 0.2 u ml^{-1}, which was sufficient to abolish fully the effect of added adenosine. At the same concentration adenosine deaminase did not block the inhibitory activity of ATP to a significant extent, suggesting that the depression was not mediated by the formation of adenosine. The use of adenosine deaminase has been criticised due to the large size of the enzyme, which may restrict entry to small compartments where the production of adenosine from ATP is occurring. Hence inactivation of locally formed adenosine would not occur (Rubio *et al*., 1987). However, adenosine deaminase has been used in many tissues with great success to inhibit the production of adenosine from ATP (Côte *et al*., 1993; Barajas-López *et al*., 1995; King *et al*., 1996).

Cyclopentyltheophylline (CPT), an A_1 receptor antagonist with a 140 fold selectivity for A_1 over A_2 receptor subtypes (Bruns *et al*., 1986), increased the rate of burst discharge substantially, a result which is consistent with the view that A_1 receptor antagonists are excitants due to inhibition of basal

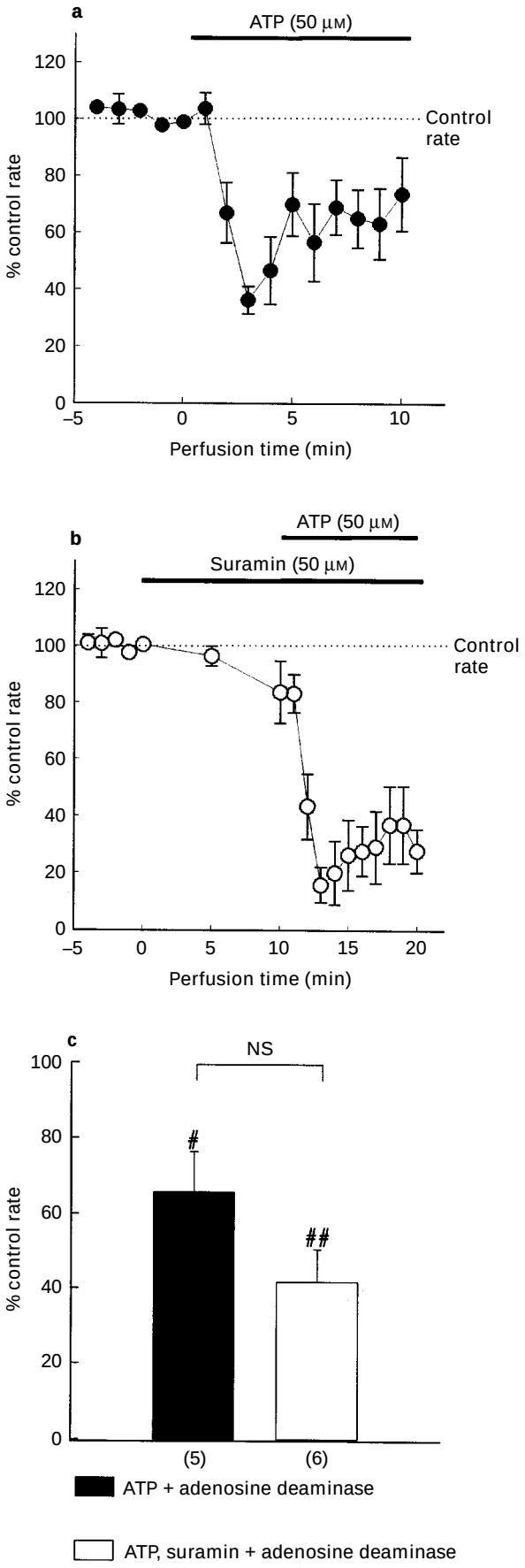

Figure 7 (a) Control responses to ATP (50 μM) in the presence of adenosine deaminase (0.2 u ml^{-1}). Slices were washed with 0 Mg/4-AP medium for a minimum of 15 min between subsequent ATP additions. (b) Effect of suramin, perfused for 10 min before ATP and adenosine deaminase addition, on the response. (c) Statistical analysis of the resultant effects are shown, with #$P<0.05$, ##$P<0.01$; n = number in parentheses below each column.

adenosine activity. As expected, CPT completely prevented the inhibitory effect of adenosine, supporting previous conclusions that adenosine inhibits epileptiform activity through A_1 receptors (Lee *et al*., 1984; Thompson *et al*., 1992; Barbarosie *et al*., 1994).

However, unexpectedly, in view of the results with adenosine deaminase, CPT also reversed the effect of ATP. This combination of results with CPT and adenosine deaminase strongly suggests that the inhibitory effect of ATP on burst frequency may be mediated by the nucleotide acting directly at a P1 receptor, or at a xanthine-sensitive P2 receptor. The frequency of epileptiform activity can vary with time. However, the fact that antagonists can inhibit the effect of ATP, coupled with the reversibility of the depression on wash, suggest that this is an actual effect and not just an artefact. In slices of cortex, ATP and related analogues decrease the evoked overflow of [^{3}H]-noradrenaline in a manner which is not sensitive to adenosine deaminase or suramin but which is inhibited by 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) (von Kügelgen *et al*., 1992). This led the authors to propose that nucleotides could act directly on P1 receptors located presynaptically. A similar suggestion has been made more recently that adenine dinucleotides may act directly on P1 receptors in the heart (Hoyle *et al*., 1996). A third subtype of purine receptor, the P3 purinoceptor, has been proposed (Westfall *et al*., 1990) which is activated by both nucleotides and nucleosides, and which is inhibited by P1 receptor antagonists and α, β-meATP (Shinozuka *et al*., 1990; Todorov *et al*., 1994). A binding protein which could be classified as a putative P3 receptor has been purified from rat brain membranes (Saitoh & Nakata, 1996). In general the P3 purinoceptors are located on the presynaptic membrane.

The initial subclassification of P2 receptors into P2X and P2Y was based on the differential effects of α, β-meATP and 2-meSATP, analogues of ATP (Burnstock & Kennedy, 1985). α, β-meATP is relatively resistant to degradation by ecto-ATPases thus reducing the complicating factor of metabolism to adenosine (Welford *et al*., 1986). Earlier studies have failed to demonstrate a consistent effect of α, β-meATP in hippocampal slices (Stone & Cusack, 1989). The LTP shown to be induced by ATP was not imitated, but rather inhibited by α, β-meATP (Wieraszko & Ehrlich, 1994). In the present study α, β-meATP was found to elevate the level of epileptiform activity. α, β-meATP is considered to activate preferentially P2X receptors (Burnstock & Kennedy, 1985). These receptors involve an intrinsic ion channel which is generally considered to be non-specific for cations (Benham & Tsien, 1987; Bean, 1992) and activation of P2X receptors is usually coupled to situations which involve an increase in cell excitability, for example muscle contraction (Meldrum & Burnstock, 1983; Burnstock & Kennedy, 1985; Khakh *et al*., 1995) and neuronal depolarization (Illes *et al*., 1994).

2-MeSATP is subject to the same metabolism as ATP but can induce inward currents in hippocampal neurones with a greater potency than ATP and α, β-meATP (Balachadran & Bennett, 1996). In this study 2-meSATP failed to alter the rate of epileptiform activity. Due to the potential metabolism of this compound, α, β-meADP, a 5′-nucleotidase inhibitor, was applied together with 2-meSATP but the latter remained ineffective. The lack of effect of 2-meSATP would suggest an absence of P2Y receptors in the hippocampal CA3 region.

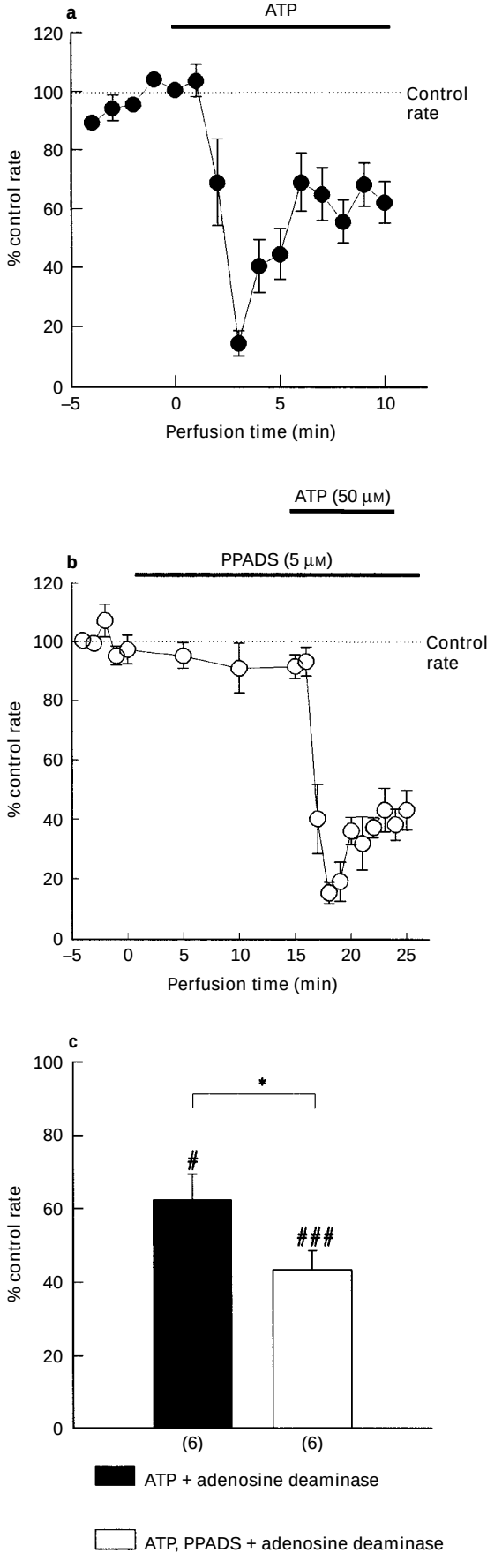

Figure 8 (a) Control responses to ATP (50 μM) and adenosine deaminase (0.2 u ml^{-1}). (b) Effect of PPADS perfused for 15 min before the subsequent addition of ATP and adenosine deaminase. (c) The resultant effects are summarized; $\#P<0.05$, $\#\#\#P<0.001$, $*P<0.05$; n = number in parentheses below each column.

Much evidence is available surrounding the existence of a receptor which is activated by uridine triphosphate (UTP). In many instances it is suggested that ATP and UTP are acting at different receptors since, for example, PPADS antagonizes responses to ATP but not UTP (Connolly, 1994) and there are dissimilarities in the effects produced by the two nucleotides (Haussinger *et al*., 1984; von Kügelgen *et al*., 1987). However, cross-desensitization between ATP and UTP (Brown *et al*., 1991) and their equipotency in a number of cell types has been demonstrated (Lustig *et al*., 1993; Parr *et al*., 1994), suggesting that both compounds may activate the same receptor in some

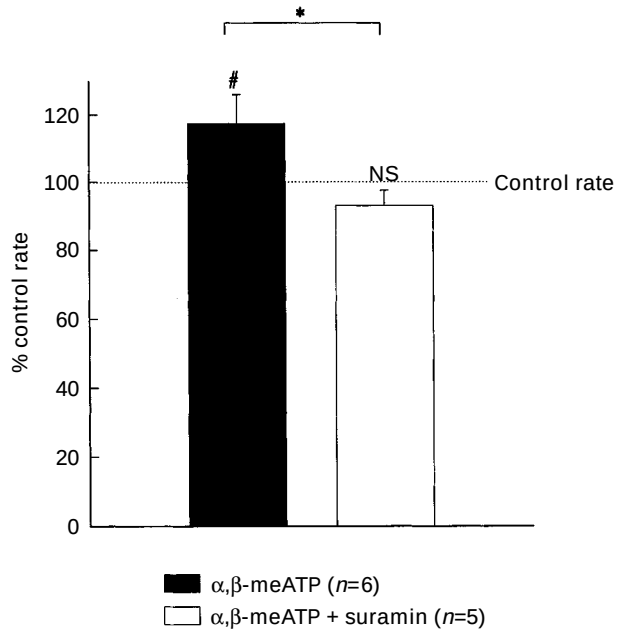

Figure 9 The effect of the P2 antagonist suramin on the excitatory response of α, β-meATP. α, β-meATP (10 μM) increased burst rate to a significant level, $\#P<0.05$. Suramin (50 μM) was perfused for 10 min before α, β-meATP was added. Suramin inhibited this excitation, $*P<0.05$.

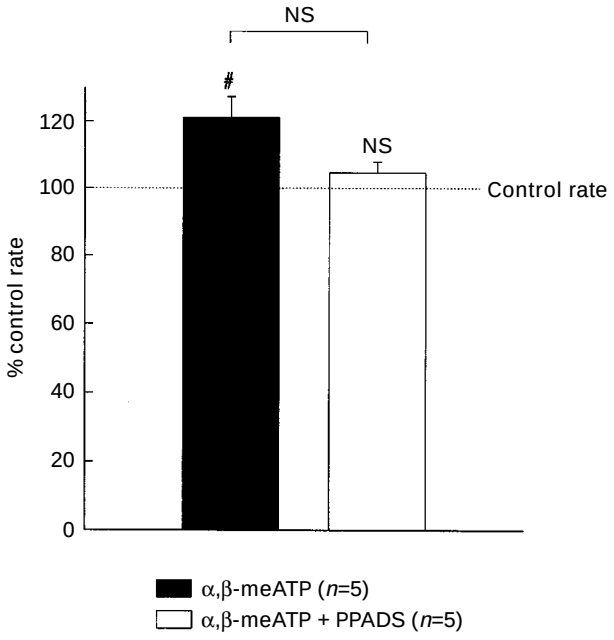

Figure 10 α, β-MeATP (10 μM) enhanced the frequency of epileptiform activity, $\#P<0.05$. The P2 antagonist PPADs (5 μM), with a perfusion period of 15 min, inhibited the increase in rate from control.

systems or some circumstances. A receptor activated by ATP and UTP which depressed potassium currents has been demonstrated in hippocampal neurones (Nakazawa *et al*., 1994). In the present study the inactivity of UTP, at a concentration at which ATP produced a clear effect, suggests that a P2U or similar receptor subtype was not involved in the modulation of CA3 neuronal bursting.

In addition to agonist potency orders, the classification of P2 receptors has been based on antagonist activity. Three main antagonists were used: suramin - a non-selective P2 antagonist (Dunn & Blakeley, 1988), PPADS - an agent with limited selectivity for P2X receptors but which can also inhibit P2Y but not P2U receptors at higher concentrations (Lambrecht *et al*., 1992; Windscheif *et al*., 1994; Brown *et al*., 1995) and reactive blue 2 (RB2) - which has a small concentration window in which it is considered to be selective for P2Y receptors (Burnstock & Warland, 1987).

Suramin and PPADS did not antagonize the inhibitory action of ATP on epileptiform activity. PPADS, and to a small extent suramin, potentiated the effect of ATP, which may be explained if ATP were capable of causing both excitation and inhibition. This possibility is supported by the excitatory effect of α, β-meATP and its inhibition by suramin and PPADS. Overall the results are consistent with the idea that P2X receptors exist in the CA3 region which are capable of increasing neuronal excitability. Seven subtypes of P2X receptor have so far been cloned, all with distinct characteristics with regard to agonist potency, antagonism and desensitization. $P2X_2$ (Kidd *et al*., 1995), $P2X_4$ (Bo *et al*., 1995; Buell *et al*., 1996a, b; Soto *et al*., 1996) and $P2X_6$ (Collo *et al*., 1996) receptors are expressed in regions of the hippocampus and it is possible, therefore, that one of these subtypes mediates the excitatory effect of α, β-meATP in the CA3 region. However, the characteristics of the cloned receptors with expression in the brain do not correlate with those found in this study. It is possible that other subtypes of P2X receptor exist which have yet to be cloned, or that heterologous expression of receptor subtypes produces characteristics dissimilar from those of the cloned receptor.

In summary, the combination of zero magnesium and 4-AP resulted in the generation of epileptiform activity of an interictal nature. ATP depressed this activity in a manner which is not consistent with the characteristics of known P1 or P2 receptors. Hence the involvement of a xanthine-sensitive nucleotide receptor is suggested. The results also indicate the existence of a P2X receptor able to increase the excitability of CA3 neurones.

This work was supported by the Epilepsy Research Association of Scotland.

References

ABBRACCHIO, M.P. & BURNSTOCK, G. (1994). Purinoceptors: are there families of P2X and P2Y purinoceptors. *Pharmacol. Ther*., **64,** 445–475.

ABE, Y., SORIMACHI, M., ITOYAMA, Y., FURUKAWA, K. & AKAIKE, N. (1995). ATP responses in the embryo chick ciliary ganglion cells. *Neuroscience*, **64,** 547–551.

ALZHEIMER, C. & TEN BRUGGENCATE, G. (1991). Postsynaptic inhibition by adenosine in hippocampal CA3 neurons Co^{2+}-sensitive activation of an inwardly rectifying K^+ conductance. *Pflügers Arch*., **419,** 288–295.

ALZHEIMER, C., SUTOR, B. & TEN BRUGGENCATE, G. (1993). Disinhibition of hippocampal CA3 neurons induced by suppression of an adenosine A_1 receptor mediated inhibitory tonus: Pre and postsynaptic components. *Neuroscience*, **57**, 65–75.

AULT, B. & WANG, C.M. (1986). Adenosing inhibits epileptiform activity arising in hippocampal area CA3. *Br. J. Pharmacol*., **87,** 695–703.

AVOLI, M., BARBAROSIE, M., LÜCKE, A., NAGAO, T., LOPANTSEV, V. & KÖHLING, R. (1996). Synchronous GABA-mediated potentials and epileptiform discharges in the rat limbic system *in vitro*. *J. Neurosci*., **16,** 3912–3924.

AYALA, G.F., MATSUMOTO, H. & GRUMNIT, R.J. (1970). Excitability changes and inhibitory mechanism in neocortical neurons during seizures. *J. Neurophysiol*., **33,** 73–85.

BALACHANDRAN, C. & BENNETT, M.R. (1996). ATP-activated cationic and anionic conductances in cultured rat hippocampal neurons. *Neurosci. Lett*., **204,** 73–76.

BALCAR, V.J., LI, Y., KILLINGER, S. & BENNETT, M.R. (1995). Autoradiography of P_{2X} ATP receptors in the rat brain. *Br. J. Pharmacol*., **115,** 302–306.

BARAJAS-LÓPEZ, C., MULLER, M.J., PRIETOGOMEZ, B. & ESPINOSALUNA, R. (1995). ATP inhibits the synaptic release of acetylcholine in submucosal neurons. *J. Pharmacol. Exp. Ther*., **3,** 1238–1245.

BARBAROSIE, M.T., NAGAO, T. & AVOLI, M. (1994). Control of 4-aminopyridine induced synchronous activity by adenosine A1 and μ-opiod receptor agonists in adult rat hippocampus. *Neurosci. Lett*., **182,** 208–212.

BEAN, B. P. (1992). Pharmacology and electrophysiology of ATP-activated ion channels. *Trends Pharmacol. Sci*., **13,** 87–90.

BENHAM, C.D. & TSIEN, R.W. (1987). A novel receptor-operated Ca^{2+} permeable channel activated by ATP in smooth muscle. *Nature*, **328,** 275–278.

BO, X.N., ZHANG, Y., NASSAR, M., BURNSTOCK, G. & SCHOEPFER, R. (1995). A P2X purinoceptor cDNA conferring a novel pharmacological profile. *FEBS Lett*., **375,** 129–133.

BRAKE, A.J., WAGENBACH, M.J. & JULIUS, D. (1994). New structural motif for ligand-gated ion channels defined by an ionotrophic ATP receptor. *Nature*, **371,** 519–523.

BROWN, H.A., LAZAROWSKI, E.R., BOUCHER, R.C. & HARDEN, T.K. (1991). Evidence that ATP and UTP regulate phospholipase C through a common extracellular 5′-nucleotide receptor in human airway epithelial cells. *Mol. Pharmacol*., **40,** 648–655.

BROWN, C., TANNA, B. & BOARDER, M.R. (1995). PPADS: an antagonist at endothelial P_{2Y}-purinoceptors but not P_{2U}-purinoceptors. *Br. J. Pharmacol*., **116,** 2413–2416.

BRUNS, R.F., LU, G.H. & PUGSLEY, T.A. (1986). Characterisation of the A2 adenosine receptor labelled by (3H)NECA in rat striatal membranes. *Molec. Pharmacol*., **29,** 331–346.

BUELL, G., COLLO, G. & RASSENDREN, F. (1996a). P2X receptors: an emerging channel family. *Eur. J. Neurosci*., **8,** 2221–2228.

BUELL, G., LEWIS, C., COLLO, G., NORTH, R.A. & SUPRENANT, A. (1996b). An antagonist insensitive P_{2X} receptor expressed in epithelia and brain. *EMBO J*., **15,** 55–62.

BURNSTOCK, G. (1978). A basis for distinguishing purinergic receptors. In *Cell Membrane Receptors for Drugs and Hormones: a Multidisciplinary Approach*. ed. Bolis, L. & Straub, R.W. pp. 107–118. New York: Raven Press.

BURNSTOCK, G. (1990). Purinergic mechanisms. *Ann. New York Acad. Sci*., **603,** 1–17.

BURNSTOCK, G. & KENNEDY, C. (1985), Is there a basis for distinguishing two types of P_2-purinoceptor. *Gen. Pharmacol*., **16,** 433–440.

BURNSTOCK, G. & WARLAND, J.J.I. (1987). P_2-purinoceptors of two subtypes in the rabbit mesenteric artery: reactive blue 2 selectively inhibits responses mediated by the P_{2Y} but not the P_{2X}-purinoceptor. *Br. J. Pharmacol*., **90,** 383–391.

CHEN, Z.P., LEVY, A. & LIGHTMAN, S.L. (1994). Activation of specific ATP receptors induces a rapid increase in intracellular calcium ions in rat hypothalamic neurons. *Brain Res*., **641,** 249–256.

CHEN, Z.-P., LEVY, A. & LIGHTMAN, S.L. (1995). Nucleotides as extracellular signalling molecules. *J. Neuroendocrinol*., **7,** 83–96.

CHU, N.S. (1981). Caffeine and aminophylline-induced seizures. *Epilepsia*, **22,** 85–94.

COLLO, G., NORTH, R.A., KAWASHIMA, E., MERLO-PICH, E., NEIDHART, S., SUPRENANT, A. & BUELL, G. (1996). Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP -gated ion channels. *J. Neurosci.*, **16,** 2495 – 2507.

CONOLLY, G.P. (1994). Evidence from desensitisation studies for distinct receptors for ATP and UTP on the rat superior cervical ganglion. *Br. J. Pharmacol.*, **112,** 357 – 359.

CÔTE, S., VAN-SANDE, J. & BOEYNAEMS, J.M. (1993). Enhancement of endothelial cAMP accumulation by adenine nucleotides: Role of methylxanthine-sensitive sites. *Am. J. Physiol.—Heart Circulatory Physiol.*, **264,** H1498 – H1503.

CUNHA, R.A., VIZI, E.S., RIBERIO, J.A. & SEBATIÃO, A.M. (1996). Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. *J. Neurochem.*, **67,** 2180 – 2187.

DAVE, S. & MOGUL, D.J. (1996). ATP receptor activation potentiates a voltage-dependent Ca channel in hippocampal neurons. *Brain Res.*, **715,** 208 – 216.

DI CORI, S. & HENRY, J.L. (1984). Effects of ATP and AMP on hippocampal neurones of the rat *in vitro*. *Brain Res. Bull.*, **13,** 199 – 201.

DRAGUNOW, M. & GODDARD, G.V. (1984). Adenosine modulation of amydgala kindling. *Exp. Neurol.*, **84,** 654 – 665.

DUNN, P.M. & BLAKELEY, A.G.H. (1988). Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol.*, **93,** 243 – 245.

DUNWIDDIE, T.V. (1980). Endogenously released adenosine regulates excitability in the *in vitro* hippocampus. *Epilepsia*, **21,** 541 – 548.

DUNWIDDIE, T.V. & FREDHOLM, B.B. (1989). Adenosine A_1 receptors inhibit adenylate cyclase activity and neurotransmitter release and hyperpolarise neurons in rat hippocampus. *J. Pharmacol. Exp. Ther.*, **249,** 31 – 37.

DUNWIDDIE, T.V., HOFFER, B.J. & FREDHOLM, B.B. (1981). Alkylxanthines elevate hippocampal excitability. Evidence for a role of endogenous adenosine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **316,** 326 – 330.

DUNWIDDIE, T.V. & WORTH, T. (1982). Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. *J. Pharmacol. Exp. Ther.*, **220,** 70 – 76.

EDWARDS, F.A., GIBB, A.J. & COLQUHOUN, D. (1992). ATP receptor mediated synaptic currents in the central nervous system. *Nature*, **359,** 144 – 147.

EVANS, R.J., DERKACK, V. & SUPRENANT, A. (1992). ATP mediates fast synaptic transmission in mammalian neurons. *Nature*, **357,** 503 – 505.

FREDHOLM, B.B. & HEDQUIST, P. (1980). Modulation of neurotransmission by purine nucleotides and nucleosides. *Biochem. Pharmacol.*, **29,** 1635 – 1643.

FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DUBYAK, G.R., HARDEN, T.K., JACOBSON, K.A., SCHWABE, U. & WILLIAMS, M. (1997). Towards a revised nomenclature for P1 and P2 receptors. *Trends Pharmacol. Sci.*, **18,** 79 – 82.

HAMANN, M. & ATTWELL, D. (1996). Non-synaptic release of ATP by electrical stimulation in slices of rat hippocampus, cerebellum and habenula. *Eur. J. Neurosci.*, **8,** 1510 – 1515.

HARMS, L., FINTA, E.P., TSCHÖPL, M. & ILLES, P. (1992). Depolarisation of rat locus coeruleus neurons by adenosine 5′-triphosphate. *Neuroscience*, **48,** 941 – 952.

HÄUSINGER, D., STEHLE, T. & GEROK, W. (1987). Actions of extracellular UTP and ATP in perfused rat liver. A comparative study. *Eur. J. Biochem.*, **167,** 65 – 71.

HORNE, A.L., HARRISON, N.L., JONATHON, P., TURNER, P. & SIMMONDS, M.A. (1986). Spontaneous paroxysmal activity induced by zero magnesium and bicucculline: suppression by NMDA antagonists and GABA mimetics. *Eur. J. Pharmacol.*, **122,** 231 – 238.

HOYLE, C.H.V., ZIGANSHIN, A.U., PINTOR, J. & BURNSTOCK, G. (1996). The activation of P1- and P2-purinoceptors in the guinea-pig left atrium by diadenosine polyphosphates. *Br. J. Pharmacol.*, **118,** 1294 – 1300.

IKEUCHI, Y. & NISHIZAKI, T. (1996). P_2 purinoceptor-operated potassium channel in rat cerebellar neurons. *Biochem. Biophys. Res. Commun.*, **218,** 67 – 71.

ILLES, P., SEVEIK, J., FINTA, E.P., FROHLICH, R., NIEBER, K. & NORENBERG, W. (1994). Modulation of locus coeruleus neurons by extra- and intracellular adenosine 5′-triphosphate. *Brain Res. Bull.*, **35,** 513 – 519.

INOKUCHI, H. & MACLACHAN, E.M. (1995). Lack of evidence for P_{2X}-purinoceptors involvement in fast synaptic responses in intact sympathetic ganglia isolated from guinea-pigs. *Neuroscience*, **69,** 651 – 659.

INOUE, K., NAKAZAWA, K., FUJIMORI, K., WATANO, T. & TAKANAKA, A. (1992). Extracellular adenosine 5′-triphosphate-evoked glutamate release in cultured hippocampal neurons. *Neurosci. Lett.*, **134,** 215 – 218.

INOUE, K., KOIZUMI, S. & NAKAZAWA, K. (1995). Glutamate-evoked release of adenosine 5′ triphosphate causing an increase in intracellular calcium in hippocampal neurons. *NeuroReport*, **6,** 437 – 440.

JAHR, C.E. & JESSEL, T.M. (1983). ATP excites a subpopulation of rat dorsal horn neurons. *Nature*, **304,** 730 – 733.

KHAKH, B.S., HUMPHREY, P.P.A. & SUPRENANT, A. (1995). Electrophysiological properties of P_{2X}-purinoceptors in rat superior cervical, nodose and guinea-pig coeliac ganglion neurones. *J. Physiol.*, **484,** 385 – 395.

KIDD, E.J., GRAHAMES, B.A., SIMON, J., MICHEL, A.D., BARNARD, E.A. & HUMPHREY, P.P.A. (1995). Localisation of P_{2X} purinoceptor transcripts in the rat nervous system. *Mol. Pharmacol.*, **48,** 569 – 573.

KING, B.F., PINTOR, J., WANG, S., ZIGANSHIN, A.U., ZIGANSHINA, L.E. & BURNSTOCK, G. (1996). A novel P1 purinoceptor activates an outward $K+$ current in follicular oocytes of *Xenopus laevis*. *J. Pharmacol. Exp. Ther.*, **276,** 93 – 100.

LAMBRECHT, G., FRIEBE, T., GRIMM, U., WINDSCHIEF, U., BUNGARDT, E., HILDERBRANDT, C., BÄUMERT, H.G., SPATZ-KÜMBEL, G. & MUTSCHLER, E. (1992). PPADS, a novel functionally selective antagonist of P_2 purinoceptor mediated responses. *Eur. J. Pharmacol.*, **217,** 217 – 219.

LEE, K.S., SCHUBERT, P. & HERNEMANN, U. (1984). The anticonvulsive action of adenosine: a postsynaptic dendritic action by a possible endogenous anticonvulsant. *Brain Res.*, **321,** 160 – 164.

LEWIS, D.V., JONES, L.S. & SWARTZWELDER, H.S. (1989). The effects of baclofen and pertussis toxin on epileptiform activity induced in the hippocampal slice by magnesium depletion. *Epilepsy Res.*, **4,** 109 – 118.

LUSTIG, K.D., SHIAU, A.K., BRAKE, A.J. & JULIUS, D. (1993). Expression cloning of an ATP receptor from mouse neuroblastoma-cells. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 5113 – 5117.

MAITRE, M., CIESIELSKI, L., LEHMAN, A., KEMPF, E. & MANDEL, P. (1974). Protective effect of adenosing and nicotinamide against audiogenic seizures. *Biochem. Pharmacol.*, **23,** 2807 – 2816.

MELDRUM, L.A. & BURNSTOCK G. (1983). Evidence that ATP acts as a cotransmitter with noradrenaline in sympathetic nerves supplying the guinea-pig vas deferens. *Eur. J. Pharmacol.*, **92,** 161 – 163.

MICHEL, A.D. & HUMPHREY, P.P.A. (1993). Distribution and characterisation of [^{3}H] α, β-methylene ATP binding sites in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **348,** 608 – 617.

MODY, I., LAMBERT, J.D.C. & HEINEMANN, U. (1987). Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. *J. Neurophysiol.*, **57,** 869 – 888.

NAKAZAWA, K., INOUE, K. & INOUE, K. (1994). ATP reduces voltage activated K^+ current in cultured rat hippocampal neurons. *Pflügers Archiv.-Eur. J. Pharmacol.*, **429,** 143 – 145.

PARR, C.E., SULLIVAN, D.M., LAZAROWSKI, E.R., BURCH, L.H., OLSEN, J.C., ERB, L., WEISMAN, G.A., BOUCHER, R.C. & TURNER, J.T. (1994). Cloning and expression of a human-P(2U) nucleotide receptor, a target for cystic-fibrosis pharmacology. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 3275 – 3279.

PSARROPOULOU, C. & AVOLI, M. (1996). CPP, an NMDA-receptor antagonist, blocks 4 aminopyridine-induced spreading depression episodes but not epileptiform activity in immature rat hippocampal slices. *Neurosci. Lett.*, **135,** 139 – 143.

RUBIO, R., KNABB, R.M., ELY, S.W. & BERNE, R.M. (1987). A critique on the use of adenosine deaminase to test the adenosine hypothesis: Disregarded implicit assumptions. In *Topics and Perspectives in Adenosine Research*. ed Gerlach, E. & Becker, B.F. Berlin, Heidelberg: Springer-Verlag.

SAITOH, Y. & NAKATA, H. (1996). Photoaffinity labelling of a P_3 purinoceptor-like protein purified from rat brain membrane. *Biochem. Biophy. Res. Commun.*, **219,** 469 – 474.

SCHOLZ, K.P. & MILLER, R.J. (1991). Analysis of adenosine actions on Ca^{2+} currents and synaptic transmission in cultured rat hippocampal pyramidal neurones. *J. Physiol.*, **435,** 373 – 393.

SCHNEIDERMAN, J.H. & MACDONALD, J.F. (1987). Effects of reduced magnesium on hippocampal synchrony. *Brain Res.*, **410,** 174–178.

SEGAL, M. & BARKER, J.L. (1986). Rat hippocampal neurones in culture: Ca^{2+} and Ca^{2+}-dependent K^+ conductance. *J. Neurophysiol.*, **55,** 751–770.

SÉGUÉLA, P., HAGHIGHI, A., SOGHOMONIAN, J.-J. & COOPER, E. (1996). A novel neuronal P_{2X} ATP receptor ion channel with widespread distribution in the brain. *J. Neurosci.*, **16,** 448–455.

SHINOZUKA, K., BJUR, R.A. & WESTFALL, D.P. (1990). Effects of α, β-methylene ATP on the prejunctional purinoceptors of the sympathetic nerves of the rat caudal artery. *J. Pharmacol. Exp. Ther.*, **254,** 900–904.

SINISCALCHI, A., CALABRESI, P., MERCURI, B. & BERNARD, G. (1997). Epileptiform discharge induced by 4-aminopyridine in a magnesium-free medium in neocortical neurons: physiological and pharmacological characterisation. *Neuroscience*, **81,** 189–197.

SOTO, F., GARCIA-GUZMAN, M., GOMEZ-HERNANDEZ, J.M., HOLLMANN, M., KARSCHIN, C. & STÜHMER, W. (1996). $P2X_4$: An ATP-activated inotrophic receptor cloned from rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **93,** 3684–3688.

STONE, T.W. & CUSACK, N.J. (1989). Absence of P_2-purinoceptors in hippocampal pathways. *Br. J. Pharmacol.*, **97,** 631–635.

STORM, J.F. (1988). Temporal integration by a slowly inactivating K^+ current in hippocampal neurons. *Nature*, **336,** 379–381.

SWARTZWELDER, H.S., ANDERSON, W.W. & WILSON, W.A. (1988). Mechanism of electrographic seizure generation in hippocampal slice in Mg^{2+}-free medium: the role of $GABA_A$ inhibition. *Epilepsy Res.*, **2,** 239–245.

TANCREDI, V., HWA, G.G.C., ZONA, C., BRANCATI, A. & AVOLL, M. (1990). Low magnesium epileptogenesis in the rat hippocampal slice: electrophysiological and pharmacological features. *Brain Res.*, **511,** 280–290.

THOMPSON, S.M., HAAS, H.L. & GÄHWILER, B.H. (1992). Comparison of the actions of adenosine at pre- and post-synaptic receptors in the rat hippocampus *in vitro*. *J. Physiol.*, **451,** 347–363.

TODOROV, L.D., BJUR, R.A. & WESTFALL, D.P. (1994). Inhibitory and facilitory effects of purines on transmitter release from sympathetic nerves. *J. Pharmacol. Exp. Ther.*, **268,** 985–989.

TSCHOPL, M., HARMS, L., NORENBERG, W. & ILLES, P. (1992). Excitatory effects of adenosine 5′-triphosphate on rat locus coeruleus neurones. *Eur. J. Pharmacol.*, **213,** 71–77.

UENO, S., HARATA, N., INOUE, K. & AKAIKE, N. (1992). ATP-gated current in dissociated rat nucleus solitarii neurons. *J. Neurophysiol.*, **68,** 778–785.

VALERA, S., HUSSY, N., EVANS, R.J., ADAMI, N., NORTH, R.A., SUPRENANT, A. & BUEL, G. (1994). New class of ligand-gated ion-channel defined by P_{2X} receptor for extracellular ATP. *Nature*, **371,** 516–519.

VON KÜGELEGEN, I., HÄUSSINGER, D. & STARKE, K. (1987). Evidence for a vasoconstriction-mediating receptor for UTP, distinct from the P_2 purinoceptor, in rabbit ear artery. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **336,** 556–560.

VON KÜGELGEN, I., SPÄTH, L. & STARKE, K. (1992). Stable adenine nucleotides inhibit [^{3}H]-noradrenaline release in rabbit brain cortex slices by direct action at presynaptic adenosine A_1-receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **346,** 187–196.

VOSKUYL, R.A. & ALBUS, H. (1985). Spontaneous epileptiform discharges in hippocampal slices by 4-aminopyridine. *Brain Res.*, **342,** 54–66.

WELFORD, L.A., CUSACK, N.J. & HOURANI, S.M.O. (1986). ATP analogues and the guinea-pig taenia coli: a comparison of the structure-activity relationships of ectonucleotidases with those of the P_2-purinoceptor. *Eur. J. Pharmacol.*, **129,** 217–224.

WESTFALL, D.P., SHINOZUKA, K., FORSYTH, K.M. & BJUR, R.A. (1990). Presynaptic purine receptors. *Ann. New York Acad. Sci.*, **604,** 130–135.

WHITE, T.D. (1978). Direct detection of depolarisation-induced release of ATP from synaptosomal preparations. *Nature*, **267,** 329–336.

WIERASZKO, A. & EHRLICH, Y.H. (1994). On the role of extracellular ATP in the induction of long-term potentiation in the hippocampus. *J. Neurochem.*, **63,** 1731–1738.

WIERASZKO, A., GOLDSMITH, G. & SEYFRIED, T.N. (1989). Stimulation-dependent release of adenosine triphosphate from hippocampal slices. *Brain Slices*, **485,** 244–250.

WIERASZKO, A. & SEYFRIED, T.N. (1989). ATP-induced synaptic potentiation in hippocampal slices. *Brain Res.*, **491,** 356–359.

WINDSCHEIF, U., RALEVIC, V., BÄUMERT, H.G., MUTSCHLER, E., GÜNTER, L. & BURNSTOCK, G. (1994). Vasoconstrictor and vasodilator responses to various agonists in the rat perfusion mesenteric arterial bed: selective inhibition by PPADS of contractions mediated by P_{2X}-purinoceptors. *Br. J. Pharmacol.*, **113,** 1015–1021.

(Received June 16, 1997
Revised September 22, 1997
Accepted October 6, 1997)