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Characterisation of ATP-induced facilitation of transmission in rat hippocampus

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Abstract

Superfusion of rat hippocampal slices with ATP induces a form of facilitation that has been poorly characterised. The present study has confirmed that at low concentrations of ATP (10 μM or less), an initial depression of evoked potential size is followed by a rebound facilitation which is not reproduced by αβ-methyleneATP, βγ-methyleneATP, or the dinucleotide P1,P6-diadenosine hexaphosphate. The post-ATP facilitation could be prevented by the adenosine A_1 receptor antagonists 8-phenyltheophylline or 1,3-dipropyl-8-cyclopentyltheophylline (50 nM), or superfusion of adenosine deaminase. The adenosine A_{2A} receptor antagonist 8-(chlorostyryl)-caffeine did not affect the inhibition but prevented the post-ATP facilitation. The NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid prevented the establishment of post-ATP facilitation. The post-ATP facilitation was also blocked by suramin at a concentration (50 μM) that does not block glutamate receptors. Suramin prevented the induction but not the maintenance phase of the post-ATP facilitation. The repeated induction of post-ATP facilitation by bursts of electrical stimulation designed to saturate the normal mechanisms of long-term potentiation prevented the induction of post-ATP facilitation. However, repeated applications of ATP to achieve saturation of its receptor did not prevent the subsequent induction of electrically evoked long-term potentiation. It is concluded that ATP can induce a form of synaptic facilitation which resembles only partially that induced by electrical stimulation and which may require the simultaneous activation of P1 and P2 receptors.

Keywords: Purine; Adenosine; ATP; Nucleotide; Hippocampus; Suramin; Long-term potentiation

1. Introduction

ATP has been shown to produce excitation of neurones in several regions of the central nervous system (Harms et al., 1992; Sun et al., 1992; Tschopl et al., 1992; Frohlich et al., 1996) and to modulate membrane potassium (Nakazawa et al., 1994; Ueno et al., 1992; Ikeuchi and Nishizaki, 1995; Dave and Mogul, 1996; Robertson et al., 1996) or Ca^{2+} conductances (Chen et al., 1994; Koizumi and Inoue, 1997). In addition, it is now recognised that ATP can function as a neurotransmitter in the brain (Edwards et al., 1992; Nieber et al., 1997), and peripheral ganglia (Silinsky et al., 1992; Galligan and Bertrand, 1994) and between cultured neurones (Evans et al., 1992) often with a pharmacology suggestive of a P2 purinoceptor rather than an indirect effect such as ion chelation or metabolic modification.

In the hippocampus, however, the effects of ATP remain unclear. Binding studies have clearly delineated the existence of P2 receptors (Michel and Humphrey, 1993; Bo and Burnstock, 1994; Balcar et al., 1995) and messenger RNAs for several P2 receptor subtypes have been identified in the brain (Kidd et al., 1995; Seguela et al., 1996; Soto et al., 1996; Tanaka et al., 1996; Le et al., 1998). The demonstration of conductance changes on individual cells and coupling to second messenger pathways suggest that the receptors visualised by autoradiography are functionally coupled to channels or other transduction systems.

Despite this volume of evidence, several studies have failed to detect any consistent functional responses to adenine nucleotides on neuronal networks and synaptic transmission in the mammalian hippocampus, which cannot be explained by metabolism to adenosine (Gordon et al., 1986; Harms et al., 1978; Dunwiddie and Hoffer,

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1980; Cunha et al., 1998; Stone and Perkins, 1981; Stone and Cusack., 1989; Lee et al., 1981; Cunha et al., 1994; Piper and Hollingsworth, 1996). Only depressant effects were noted when ATP was applied by microiontophoresis to single neurones, which were spontaneously active or excited by glutamate (Stone and Perkins, 1981; DiCori and Henry, 1984). Furthermore, ATP and derivatives depressed evoked potentials (Dunwiddie and Hoffer, 1980; Stone and Cusack, 1989; Cunha et al., 1998) even when analogues were used which were resistant to hydrolysis and had selective actions on $P2_X$ and $P2_Y$ receptors, respectively (Stone and Cusack, 1989). Cunha et al. (1998) have recently performed a careful analysis suggesting that ATP must first be metabolised by ecto-nucleotidases, and that it was the adenosine generated, acting at P1 purinoceptors, that caused inhibition of hippocampal synaptic transmission (Gordon et al., 1986).

On the other hand, there are recent reports of ATP contributions to synaptic transmission (Bohmann et al., 1997; Miyahara and Suzuki, 1987; Todorov et al., 1994; Pankratov et al., 1999) as well as reports of long-term potentiation being induced in the hippocampus in response to ATP (Wieraszko and Seyfried, 1989; Fujii et al., 1995; Chen et al., 1996) and we have, therefore, attempted to characterise this phenomenon in greater detail.

2. Methods

Male Wistar rats (150–250 g) were anaesthetised with urethane (1.5 g/kg) i.p. and cooled on ice whilst breathing oxygen enriched air until rectal temperature reached 30°C. This procedure was recommended by Newman et al. (1992) to enhance the viability of slices. The animals were then killed by cervical dislocation, decapitated, and the brain rapidly removed to ice-cold artificial cerebrospinal fluid (aCSF) of composition (mmol l^{-1}) KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5 $MgSO_4$ 1.2, glucose 10 saturated with 95% O_2 and 5% CO_2. The hippocampi were dissected free of surrounding tissue and were cut transversely into slices 450 μm thick using a McIlwain tissue chopper. Slices were maintained in an incubation chamber saturated with 95% O_2/5% CO_2 for at least 1 h before being transferred to a recording chamber in which they were submerged and superfused with pre-gassed aCSF (34–35°C) at a constant flow rate of 2–3 ml/min. Drugs were added to the superfusion fluid.

Test pulses (100 μs, 200–450 μA) were normally delivered at 20-s intervals via a concentric bipolar electrode placed in the stratum radiatum of the CA1 area. The preparation was allowed to stabilise for at least 45 min before recordings of extracellular population excitatory postsynaptic potentials (EPSPs) and population spikes were made from the stratum radiatum and stratum pyramidale, respectively, using glass microelectrodes of tip diameter approximately 2 μm and filled with 0.9% NaCl. Responses were quantified as the amplitude of the population spike (measured as the difference between the peak negativity and the averaged values of the two peak positivities of the population spike), and the rate of rise of the population EPSP (measured as the slope of the negative-going arm of the evoked population EPSP response). Slices were studied when a maximal evoked population spike of 1 mV or greater could be obtained. In such slices, the stimulus strength was then reduced to yield a spike approximately 70% of the maximum in order to ensure that increases or decreases of spike size could be induced. Stimulus intensity was then maintained constant throughout the experiment unless stated otherwise. For input/output studies, five stimuli were delivered at each stimulation intensity and the average computed using a 32-channel signal averager (Cambridge Electronic Design). The data were captured on computer for later off-line analysis.

When appropriate, long-term potentiation was induced electrically by delivering a train of stimuli at 100 Hz for 1 s. The amount of synaptic facilitation induced either by ATP or electrical stimulation was quantified as the increase in the size of the population spike or population postsynaptic potential remaining 30 min after the end of the nucleotide perfusion or of the stimulus train. Unless stated otherwise, potentials were measured at the end of the 10-min period of drug perfusion and, where appropriate, after 30 min of washing. These values were then compared with the size of the potential recorded immediately before drug addition.

2.1. Drugs

Adenosine, ATP, adenosine deaminase, αβ-methyleneATP, βγ-methyleneATP, βγ-imidoATP, ATPγS, 2-amino-5-phosphonopentanoic acid and UTP were obtained from Sigma; 8-phenyltheophylline, suramin, 8-(chlorostyryl)-caffeine, pyridoxalphosphate-6-azo-phenyl-2,4′-disulphuric acid (PPADS) and 8-cyclopentyl-1,3-dipropylxanthine (CPX) were obtained from Research Biochemicals International.

2.2. Data analysis

The maximal changes of evoked potential size were compared using a *t*-test, taking $P < 0.05$ to indicate statistical significance.

3. Results

3.1. Post-ATP facilitation

When ATP was superfused over slices at a concentration of 10 μM, the evoked population potentials were reduced in size during the ATP application ($65 \pm 12.8\%$; $n = 5$), but a consistent, reproducible increase of potential size developed on washout (Fig. 1). This increase of

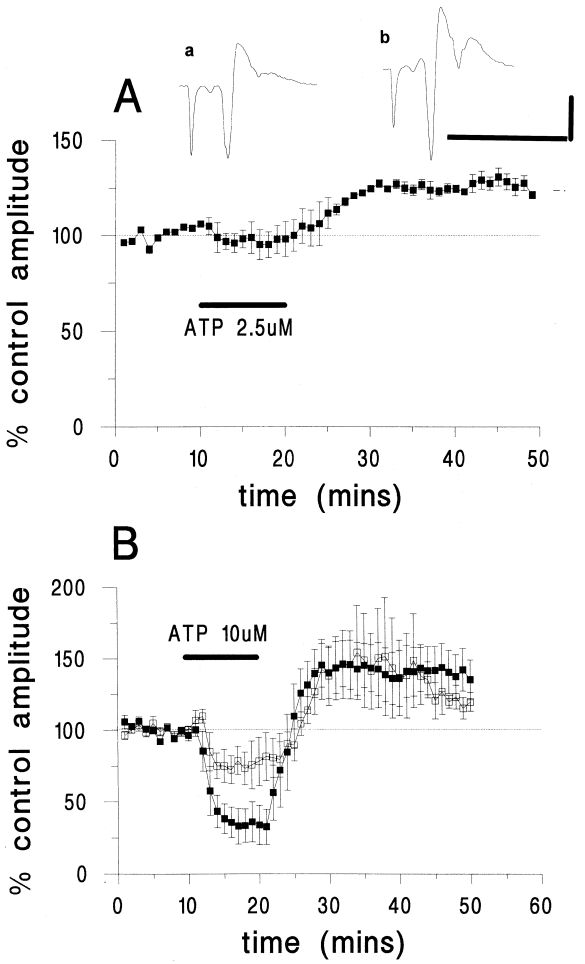

Fig. 1. Plots showing the effect of ATP on the evoked potentials. The plots show the size of the potentials expressed as a percentage of the initial amplitude. In A, ATP produces no effect during its presence, but generates a facilitation of potential size on washout. The insets show sample records obtained (A) immediately before the addition of ATP and (B) 30 min after ending the ATP perfusion. Plot B illustrates the effect of superfusion of ATP 10 μM, which produces an initial depression of population spike (filled squares; $n = 5$) and population EPSP (open squares; $n = 3$), both of which recover to an increased size on washout, though there is a subsequent decline in the EPSP slope towards baseline values. Symbols indicate the mean ± S.E.M. Calibration bars in A: 1 mV and 20 ms.

potential size developed over approximately 10–15 min to reach a plateau level which was maintained for up to at least 30 min after the removal of ATP. At a lower concentration of 2.5 μM ATP no inhibitory response was apparent during the period of application, but the development of long-term enhancement still occurred on washout. The size of the ATP-induced increase of evoked potentials reached a peak of 130.9 ± 4.6% ($n = 3$) at 2.5 μM and 146.4 ± 13.9% ($n = 5$) at 10 μM compared with the control potential size.

Simultaneous measurements of the population spike and population EPSP during the application of ATP at 10 μM revealed that the depression of spike size was greater than the decrease of EPSP size during the application of ATP, but that the subsequent facilitation reached a similar magnitude for both components compared with the control values (Fig. 1). When a higher concentration of 40 μM ATP was used, the inhibitory response was much larger, reaching almost 100% in most slices but this was not followed by any facilitation.

3.2. Occlusion of electrical- and ATP-induced potentiation

In order to assess the relationship between the mechanisms of electrically induced long-term potentiation and the facilitation produced by ATP, experiments were performed in which long-term potentiation was induced by a brief period of high-frequency stimulation (100 Hz for 1 s), or by three such bursts in order to ensure that electrically induced potentiation was fully saturated. The subsequent application of ATP then failed to induce any further increase of potential size (Fig. 2).

Conversely, when synaptic facilitation was first induced by two or three applications of ATP, such that no further increase of potential size was obtained, subsequent electrical stimulation was still able to induce long-term potentiation to the same extent as in control slices, reaching a level of 164.0 ± 55.4% ($n = 3$).

3.3. Pharmacology of ATP-induced synaptic facilitation

3.3.1. αβ-MethyleneATP

The ATP analogues αβ-methyleneATP, βγ-methyleneATP, and the pyrimidine nucleotide UTP were examined at concentrations of 5, 10 and 50 μM. αβ-methyleneATP had no significant effect at 5 and 10 μM (potential size 81.6 ± 10.2% at 10 μM, not significant) but reduced population spike size to 64.5 ± 16.8% ($n = 4$, $P < 0.05$) at 50 μM. These depressions were not followed by any

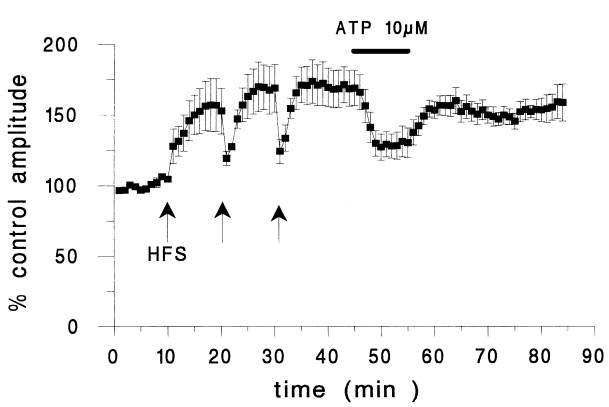

Fig. 2. Plot showing the effect of electrically induced long-term potentiation and of ATP on the evoked potentials. The ordinate indicates the size of population spike potentials expressed as percentage of the initial amplitude. At the arrows, bursts of stimuli at 100 Hz for 1s were applied to induce long-term potentiation. After three such bursts, when LTP appears to be maximal, the superfusion of ATP 10 μM results in a depression of potential size with no subsequent enhancement ($n = 3$). Symbols indicate the mean ± S.E.M.

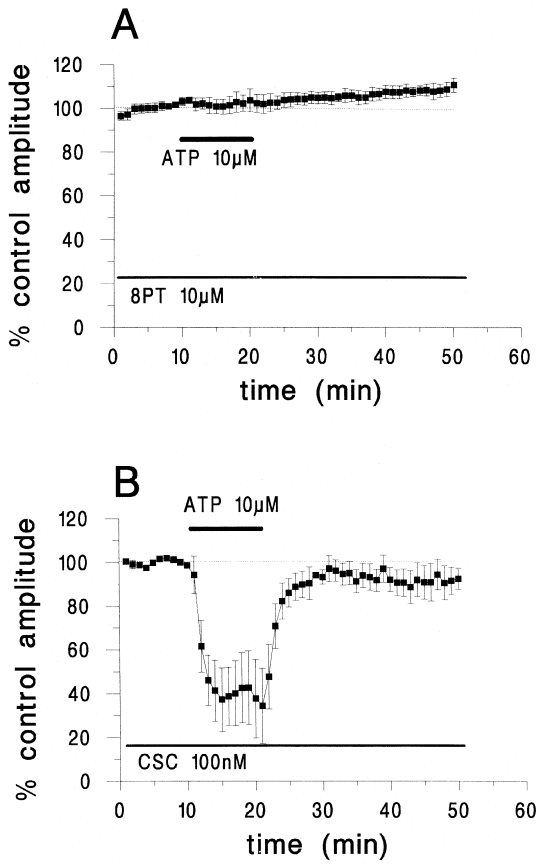

Fig. 3. Plot showing the effect of ATP on the evoked potentials in the presence of (A) 8-phenyltheophylline (8PT, 10 μM) and (B) 8-chlorostyrylcaffeine (CSC, 100 nM). The size of population spike potentials is expressed as a percentage of the initial amplitude. Superfusion with 8-chlorostyrylcaffeine (CSC, 100 nM) does not affect the depressant effect of ATP but prevents the subsequent potentiation ($n = 3$). Symbols indicate the mean ± S.E.M.

potentiation of spike size. βγ-methyleneATP and βγ-imido ATP similarly reduced the spike size (to 4.5 ± 5.6%, $n = 3$ and 36.4 ± 12.2%, $n = 4$, respectively at 50 μM). Although some of these depressant effects were followed by a transient increase of potential size over control size, these enhancements were not maintained and there was no development of facilitation. Neither ATPγS nor UTP had any effect on population spike size.

3.3.2. Adenosine antagonists

A concentration of 10 μM ATP was selected with which to examine in more detail the pharmacology of the potentiation. The adenosine P1 receptor blocker 8-phenyltheophylline (10 μM) and the selective adenosine A_1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (CPX) (50 nM) both abolished the inhibitory component of the response to ATP (from control values of 72 ± 14.5% and 66 ± 13.2%, respectively) and also completely prevented the establishment of post-ATP facilitation ($n = 3$ and 5, respectively).

The adenosine A_{2A} receptor antagonist 8-(chlorostyryl)-caffeine was tested at 100 nM. It did not change the depressant effect of ATP applied at 10 μM, which reduced population spike size to 34.3 ± 17.2% ($n = 3$) of the control amplitude, but it completely prevented the subsequent enhancement of potential size (Fig. 3).

None of these adenosine receptor blockers had any consistent or significant effects themselves on potential size.

3.3.3. Adenosine deaminase

Superfusion with adenosine deaminase at a concentration of 0.2 U ml^{-1} abolished the inhibitory component of the response to ATP. Following removal of the nucleotide, the potentials increased in size to a value greater than the control level, reaching 117.1 ± 4.1% ($n = 4$; $P < 0.01$) but this was not maintained, declining to control values within approximately 20 min (Fig. 4A).

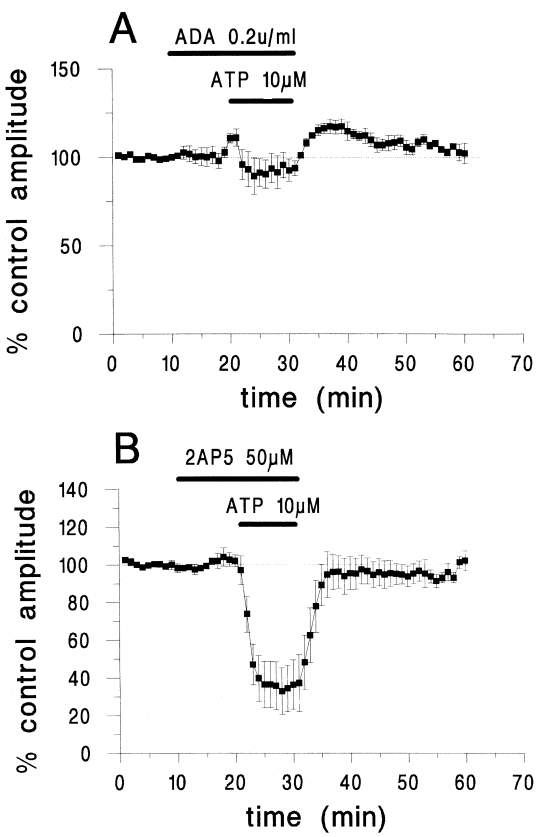

Fig. 4. Plot showing the effect of ATP on the evoked potentials in the presence of (A) adenosine deaminase (0.2 U/ml) or (B) 2-amino-5-phosphonopentanoic acid (50 μM). The size of population spike potentials is expressed as a percentage of the initial amplitude. In A, superfusion of adenosine deaminase abolishes the inhibitory response to ATP 10 μM, and prevents the subsequent increase of potential size ($n = 4$). In B, superfusion with 2-amino-5-phosphonopentanoic acid 10 μM does not change the inhibition produced by ATP but prevents the later development of an increased in potential size upon washout ($n = 3$). Symbols indicate the mean ± S.E.M.

3.3.4. Antagonist of N-methyl-D-aspartate (NMDA)

Inclusion of the NMDA antagonist 2-amino-5-phosphonopentanoic acid (50 μM) for 10 min before and during the application of ATP did not change the inhibitory effect of ATP (to 33.0 ± 12.3%, $n = 5$) but prevented completely the development of the post-ATP facilitation (Fig. 4B).

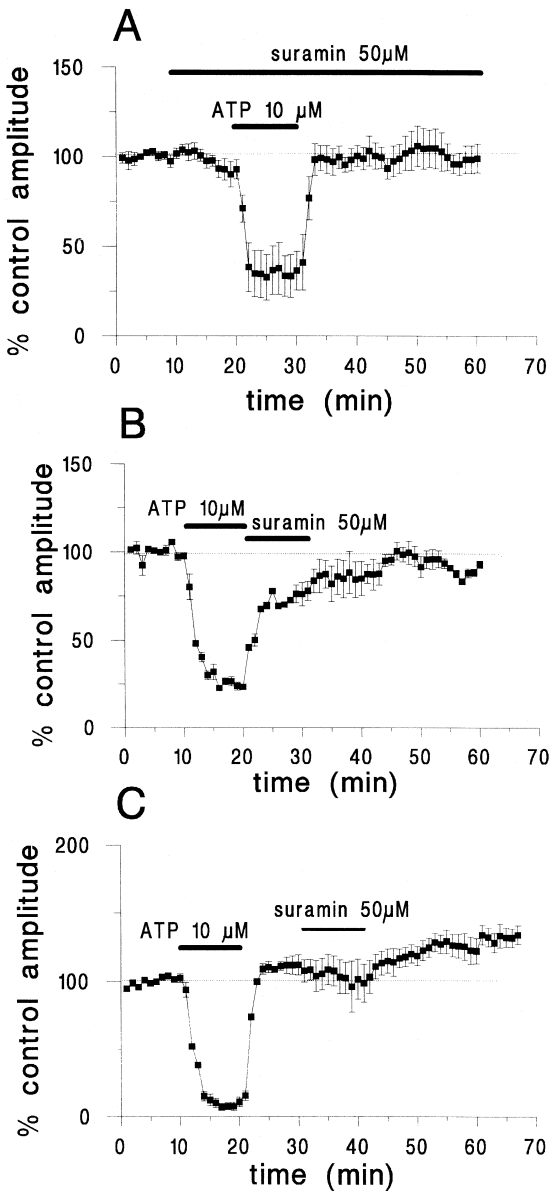

Fig. 5. Plot showing the effect of ATP on the evoked potentials in the presence of suramin. The size of population spike potentials is expressed as a percentage of the initial amplitude. In A, superfusion of suramin 50 μM does not change the inhibition produced by ATP, but prevents the subsequent LTP ($n = 4$). In B, the application of suramin for 10 min immediately following ATP slows the recovery of the potential to control levels, but still prevents the development of LTP ($n = 3$). In C, suramin applied after a washout time of 10 min produces only a transient decrease of potential size but does not prevent the development of LTP ($n = 3$). Symbols indicate the mean ± S.E.M.

3.3.5. ATP antagonists

In order to explore the possible contribution of ATP (P2) receptors to these ATP responses, two antagonists were examined, suramin and pyridoxalphosphate-6-azophenyl-2,4′-disulphuric acid (PPADS).

3.3.5.1. Suramin. Suramin (50 μM) was perfused for 10 min before the addition of ATP to the slices and left in contact with the slices during the washout phase. Suramin did not modify the inhibitory effect of ATP, which still depressed spike potentials to 36.4 ± 10.8% ($n = 4$; n.s.) of the controls but prevented completely the development of the facilitation during ATP washout (Fig. 5A). In order to determine whether suramin was affecting the induction or the maintenance phases of the facilitation, this experiment was repeated with suramin being applied for 10 min beginning immediately after the application of ATP. Under these conditions, suramin delayed the return of potential size to control values, and no post-ATP facilitation of the population spike was recorded (Fig. 5B). However, if suramin were applied for the same period of 10 min beginning 10 min after the ending of the ATP perfusion, at a time when facilitation had already been initiated, the effect was only of transient depression during the period of application. The post-ATP facilitation continued to develop thereafter (Fig. 5C).

3.3.5.2. Pyridoxalphosphate-6-azo-phenyl-2,4′-disulphuric acid (PPADS). PPADS (50 μM) was perfused for 10 min before the addition of ATP to the slices and left in contact with the slices during the washout phase. This compound did not modify the inhibitory effect of ATP, which depressed spike potentials to 44.3 ± 12.0% of the controls ($n = 4$; n.s. from ATP alone) but prevented completely the development of facilitation during ATP washout.

3.3.6. Dinucleotides

P1,P6-diadenosine hexaphosphate (AP6A) was applied at concentrations of 1 and 5 μM. At both concentrations, a depression of both the population spike and population EPSP were observed. At the higher concentration, population spikes were decreased to 30.7 ± 10.2% of control size ($n = 5$) and population EPSPS declined to 62.6 ± 3.15% ($n = 5$). However, at neither concentration was there any evidence for a subsequent increase of potential amplitude.

4. Discussion

The results support the view that ATP can induce a form of long-term synaptic facilitation in the hippocampal slice. The absence of any change in the ratio of population spike size to population EPSP size during the early phase of post-ATP enhancement indicates that there was no immediate change of EPSP-spike coupling and, therefore,

of action potential threshold, in the postsynaptic neurones. However, the later decline of EPSP slope suggests that a change of EPSP-spike coupling may occur subsequent to the establishment of potentiation of the population spike.

The inhibitory component of the response to ATP was blocked by the P1 receptor antagonist 8-phenyltheophylline and the A_1 receptor selective compound CPX. This implies either that ATP is being hydrolysed to adenosine, which is responsible for the inhibitory activity, or that it is acting directly on the P1 receptor. The former is more likely since perfusion of ATP in the presence of adenosine deaminase resulted in only a transient period of potentiation. However, both the P1 receptor antagonists reduced the size of the ATP-induced potentiation, and adenosine deaminase prevented its maintenance, leaving only a short-lasting potentiation of potential size. In addition, the stable analogues of ATP had no excitatory effects on the slices. Taken together, these results suggest that the depressant action of ATP is due to its conversion to adenosine, but that the subsequent enhancement of potential size requires the activation of both P1- and P2-receptors. Finding the precise balance between the activation of these sites to produce long-term potentiation experimentally and confirm this idea may be difficult, but preliminary data show that a combination of adenosine 50 μM with αβ-methyleneATP 10 μM can produce a long-lasting but non-significant trend to increased potential size (O'Kane, unpublished results).

4.1. ATP-induced long-term potentiation

In the absence of selective P2 receptor blocking agents, it is difficult to establish the role of P2 receptors in any biological response. Nevertheless, it is likely that the ability of suramin to block the induction of the facilitation by ATP represents blockade of a P2 receptor. The concentration of suramin used, 50 μM, was one which does not block glutamate receptors, as we have found in previous studies (Ross et al., 1998). The nature of the P2 receptor involved cannot be determined from the present study. Binding and molecular biology data suggest the presence of $P2_{X3}$, $P2_{X4}$ and $P2_{X6}$ receptor subunits in the hippocampus (Michel and Humphrey, 1993; Bo and Burnstock, 1994; Balcar et al., 1995; Kidd et al., 1995; Seguela et al.,1996; Soto et al., 1996; Tanaka et al., 1996; Le et al, 1998). Homomeric assemblies of $P2_{X4}$ subunits respond poorly to αβ-methyleneATP and are relatively insensitive to suramin. Combinations of $P2_{X4}$ and $P2_{X6}$ subunits, however, have been shown recently to be sensitive to the agonist effects of αβ-methyleneATP and blockade by suramin (Le et al., 1998). The profile of the putative P2 receptor activated by ATP in this study is not consistent with any of these, and may indicate the involvement of a different heteromeric subunit combination.

The ability of ATP to generate facilitation at a concentration of 10 μM but not 40 μM, is probably a simple reflection of the fact that a certain critical level of neuronal excitability is required for the induction of the phenomenon. At the higher concentrations, the neurone pool and transmission is sufficiently depressed such that this threshold level cannot be attained.

It should be noted that the present experiments were performed in the absence of any blockade of inhibitory neurone activity, so that the receptors responsible for the effects described here could be present on main or interneurones. Indeed, we have preliminary evidence that the latter is the case, with single neurone recordings indicating a subpopulation of interneurones sensitive to ATP and its analogues (Stone, 1999).

4.2. Relationship to electrically induced long-term potentiation

The blockade of ATP-induced facilitation by 2-amino-5-phosphonopentanoic acid suggests similarities in the processes of induction with long-term potentiation induced by electrical stimulation, since the latter is at least partly dependent upon the activation of NMDA receptors and can therefore be prevented by 2-amino-5-phosphonopentanoic acid (Collingridge et al., 1983). Indeed, ATP has been shown to elicit the release of glutamate (Inoue et al., 1992) and such an effect could account for the production of the long-lasting facilitation. The involvement of a common mechanism is supported by the finding that the saturation of electrically induced long-term potentiation prevents the establishment of ATP-induced long-term facilitation. This in turn raises the question of whether electrically induced long-term potentiation might involve the activation of ATP receptors. Complicating this issue, however, the prior application of ATP did not prevent electrically induced long-term potentiation, implying a significant difference at some point along the signalling pathways employed by the two procedures. The existence of differences is supported by our finding that E–S dissociation is not associated with ATP-induced long-term facilitation (Fig. 1B), whereas an enhancement of E–S coupling always accompanies electrically evoked long-term potentiation (Bernard and Wheal, 1995).

It has been suggested that ATP-induced long-term facilitation is due to ecto-protein kinase enzymes using ATP as a substrate, on the basis that, as confirmed here, stable analogues such as βγ-methyleneATP, αβ-methyleneATP and βγ-imidoATP do not mimic the facilitation induced by ATP (Wieraszko and Ehrlich, 1994) and that an inhibitor of ecto-protein kinase, K-252b, prevents the establishment of long-term facilitation by ATP (Fujii et al., 1995). Overall, therefore, the accumulated evidence suggests that ATP itself is needed to activate long-term facilitation by a mechanism that may involve protein phosphorylation, but that P2 receptors are also implicated. The latter statement is supported by our use of both suramin and PPADS as receptor blockers. Suramin is known to

exhibit a range of other actions, most particularly that of inhibiting ecto-nucleotidases (Ziganshin et al., 1996; Zimmerman, 1996; Schwarzbaum et al., 1998), an action which could prevent the use of ATP for phosphorylation and thus be consistent with a metabolic, rather than a receptor-mediated involvement in long-term potentiation. Of course, there is no reason why both these mechanisms should be exclusive, and a situation could be envisaged in which the activation of P2 receptors and at the same time, or within a defined temporal window around, the use of ATP in a phosphorylation process, is required. Interestingly, the involvement of ecto-protein kinase in electrically induced long-term potentiation has been proposed by Chen et al. (1996) with the demonstration that long-term potentiation can be prevented by a monoclonal antibody to the catalytic domain of protein kinase C, increasing the possibility that ATP may contribute to electrically induced long-term potentiation.

The requirement for a simultaneous activation of adenosine A_1 and A_2 receptors, which are suggested by the ability of CPX and 8-(chlorostyryl)-caffeine to prevent the post-ATP facilitation, might be associated with either of these two nucleotide actions. The use of 8-(chlorostyryl)-caffeine was based on the premise that adenosine A_{2A} receptors have been shown to increase presynaptic Ca^{2+} fluxes and increase the release of glutamate (Goncalves et al., 1997). Conceivably, therefore, the activation of adenosine A_{2A} receptors by adenosine formed from the metabolism of ATP could increase glutamate release and contribute to the establishment of long-term facilitation. This view would be consistent with evidence that, in the hippocampus, adenosine formed from nucleotides has a preferential action on excitatory receptors, which could contribute to long-term potentiation or increase glutamate release (Cunha et al. 1996). A role of adenosine A_{2A} receptors in long-term potentiation is suggested by findings that the A2 antagonist 4-amino-8-chloro-1-phenyl(1,2,4)-triazolo(4,3a)quinoxaline (CP66,713) can inhibit the induction of, and facilitate the reversal (depotentiation) of, long-term potentiation of evoked EPSPS (though not population spikes) (Sekino et al., 1991). The effect of 8-(chlorostyryl)-caffeine in the present work, preventing ATP-induced long-term facilitation but not the inhibitory effects, would be consistent with a role of adenosine A_{2A} receptors.

4.3. Adenine dinucleotides

The adenine dinucleotides have been thought to act on ATP (P2) receptors since the original discovery of their actions on smooth muscle (Stone, 1981; Stone and Paton, 1988). In the present study, the most potent of these, AP6A, produced only inhibition of the slices, suggesting that the receptors responsible for long-term facilitation induction by ATP are not sensitive to the dinucleotides (Klishin et al., 1994).

In summary, the present study has examined a form of long-term potentiation that can be induced in rat hippocampal slices by superfusion with ATP. The phenomenon shows several similarities to electrically induced long-term potentiation, and may involve the simultaneous activation of P1 and P2 purinoceptors and the direct activation of P2 purinoceptors, in addition to the previously proposed involvement of protein kinases.

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