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Absence of P^2-purinoceptors in hippocampal pathways

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1 Many apparent actions of adenosine 5'-triphosphate (ATP) are mediated by adenosine produced by enzymatic hydrolysis of the nucleotide. Previously described actions of ATP in the CNS have been partly due to this phenomenon. In the present study analogues of ATP, which are not hydrolysed to adenosine, were used to seek responses to activating nucleotide (P_2) receptors in the hippocampus. The analogues used were L-adenosine-5'-(β,γ-methylene)-triphosphonate and 2-methylthioadenosine-5'-(β,γ-difluoromethylene)-triphosphonate.

2 Neither of the stable nucleotides had any effect on orthodromically evoked synaptic potentials in the CA1 region of rat hippocampal slices. Adenosine and ATP had inhibitory actions that could be prevented by the P_1-receptor blocker 8-phenyltheophylline.

3 The stable nucleotides had no consistent effects on the firing rate of single neurones in stratum pyramidale of the CA1 region, although adenosine and ATP produced a xanthine-sensitive inhibition.

4 Adenosine selectively reduced the sensitivity of CA1 neurones to microiontophoretically applied carbachol whereas stable nucleotides did not.

5 It is concluded that there are neither P_{2X}- nor P_{2Y}-receptors for adenine nucleotides on rat hippocampal CA1 pyramidal cells at the Schaffer collateral and commissural terminals in stratum radiatum.

Introduction

In the peripheral nervous system there is now substantial evidence to indicate that adenosine triphosphate (ATP) can be released as a neurotransmitter or cotransmitter together with acetylcholine or noradrenaline (Fedan *et al.*, 1981; Sneddon *et al.*, 1982; Westfall *et al.*, 1983; Sneddon & Burnstock, 1984a,b; Machaly *et al.*, 1988; Stjarne & Astrand, 1985). The nucleotide may then have direct actions on postjunctional sites (Burnstock, 1972; Stone, 1981a,b; Wiklund *et al.*, 1985; Burnstock & Kennedy, 1985; Gordon, 1986), but it is also rapidly metabolised to adenosine 5'-monophosphate (AMP) and adenosine (Cusack *et al.*, 1988), which can have a variety of actions both on the postjunctional surface (Stone, 1981a; Williams, 1987) and on presynaptic terminals (Paton *et al.*, 1978; Ribeiro, 1979; Fredholm & Hedqvist, 1980; Israel *et al.*, 1980; Su, 1983; Stone, 1981a; Schubert *et al.*, 1986). The latter responses have been attributed to activation of xanthine-sensitive nucleoside or P_1-receptors, while the effects of ATP have been ascribed to a distinct population of P_2-sites (Burnstock, 1978).

In the central nervous system most of the actions of nucleotides are sensitive to methylxanthine blockade, implying either the metabolism of the nucleotides to adenosine, or that the nucleotides are able to activate the P_1 population of receptors.

Thus the depressant effects of ATP and relatively stable analogues produced on spontaneous neuronal firing in the cerebral cortex can be prevented by locally applied theophylline (Stone & Perkins, 1981; Phillis & Wu, 1981; Stone, 1982).

However, responses to purine nucleotides which appear to reflect the involvement of P_2-receptors have been described, since they are not reproduced by adenosine and are not sensitive to blockade by methylxanthines. These effects include the excitatory action of ATP on some sympathetic and dorsal root ganglion cells and neurones in the brain stem (Jahr & Jessell, 1983; Salt & Hill, 1983; Salter & Henry 1985). The present experiments were therefore

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carried out to determine whether nucleotide receptors exist in the synaptic pathway from the stratum radiatum (Schaffer collateral and commissural fibres) to pyramidal neurones of the CA1 subfield of the rat hippocampus.

Advantage was taken of the development of two analogues of ATP, 2-methylthio-adenosine-5'-(β,γ-difluoromethylene)-triphosphonate (2-MeS-AMP-PCF_2P) and L-adenosine-5'-(β,γ-methylene)-triphosphonate (L-AMP-PCP), which are not hydrolysed to adenosine (Cusack & Hourani, 1984; Cusack *et al.*, 1987).

Methods

Four hundred μm thick transverse hippocampal slices were prepared from adult male rats which had been killed by stunning and cervical dislocation. Slices were cut using a McIlwain tissue chopper and stored in artificial CSF at room temperature under an atmosphere of water-saturated $95\%O_2/5\%CO_2$. After a minimum recovery period of one hour, individual slices were transferred to a perfusion chamber and superfused with warmed (30°C) artificial CSF containing (mM): NaCl 115, KCl 3.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and D-glucose 10, gassed with 95% O_2/5% CO_2. The slices were completely submerged in the medium which flowed continuously from a gravity feed at a rate of approximately 3 ml min^{-1}.

The recorded signals were amplified and displayed on oscilloscopes and were processed on line via a Grafitek signal processing interface linked into a BBC microcomputer. The software package used (SAP, Harvard Instruments) allowed the positioning of cursors onto the graphical display of the recorded waveform in order to measure the amplitude and rate of rise of the population spike (Brooks & Stone, 1988). These amplitude values were printed on-line and subsequently plotted in histogram form. Potentials were evoked by a concentric bipolar electrode by use of stimuli of 0.1 ms duration and 5–200 μA amplitude delivered at 0.1 or 0.2 Hz.

Carbachol and NMDA were applied iontophoretically (5–100 nA, 5–25 s) at appropriate, constant time intervals to ensure recovery between applications. Purines and purine analogues were applied via the perfusate at known concentrations.

Responses of single cells were elicited by microiontophoretically administered compounds and recorded as described above using combined multibarrel pipettes and single recording electrodes. The multibarrel pipettes were constructed from 7-barrelled blanks which contained a glass fibre (Clarke Electromedical) and were filled immediately before use with a selection of the following solutions: carbachol, 10 mM, pH 6; kainic acid, 10 mM, pH 5; quisqualic acid 10 mM, pH 7; NMDA, 20 mM, pH 7.

Unit activity was recorded in the pyramidal cell layer of the CA1 region. Action potentials were amplified and monitored on oscilloscopes, gated and counted, and are presented in histogram form as records of cell firing rate in spikes per second. All calculations of response size are based upon a measure of total spike count during a drug response, expressed as a percentage of the total count obtained in the same period before drug application, determined using a computer programme written specifically for the purpose. Results are presented as means $\pm$ s.e. for n cells or slices. Statistical significance of differences was assessed by use of a two-tailed t test.

Drugs

Adenosine, ATP, adenosine 5'-(α,β-methylene)-triphosphonate (AMPCPP), adenosine 5'-(β,γ-methylene)-triphosphonate (AMP-PCP), 8-phenyltheophylline, carbamyl choline chloride (carbachol) and kainic acid were purchased from the Sigma Chemical Co, and quisqualic acid and N-methyl-D-aspartic acid (NMDA) obtained from Cambridge Research Biochemicals. L-Adenosine 5'-(β,γ-methylene)-triphosphonate (L-AMP-PCP) was synthesised as described by Cusack & Hourani (1984), 2-methylthioadenosine 5'-triphosphate (2-Mes-ATP) as described by Gough *et al.* (1973), and 2-methylthioadenosine 5'-(β,γ-difluoromethylene)-triphosphonate (2-MeS-AMP-PCF_2P) as described by Cusack *et al.* (1987).

Results

Adenosine itself inhibited orthodromic activation of CA1 pyramidal cells, as reflected by a depression of the evoked field postsynaptic potential (p.s.p.) and the population spike (Figure 1). A 50% reduction in the size of this potential was produced by

Figure 1 Records of the orthodromic population spike evoked in the CA1 region of a hippocampal slice. The records were taken before (a), during (b) and following (c) superfusion of the slice with adenosine (10 μM). Calibrations 2 mV and 5 ms.

22.4 ± 3.5 μM adenosine ($n = 12$). The effect of ATP was qualitatively similar though the concentration producing 50% inhibition was significantly greater (35.6 ± 3.2 μM, $n = 6$). Responses to both purines were totally abolished by superfusion with 8-phenyltheophylline (1 μM). Neither adenosine nor ATP had any effect on the antidromic population spike at concentrations up to 100 μM.

Superfusion with AMP-PCP produced a small depression of synaptic potentials, but even at 100 μM this was substantially less (21 ± 6%, $n = 3$) than that seen with ATP.

The other stable analogues of ATP tested, AMPCPP, 2-MeS-ATP and 2-MeS-AMP-PCF_2P, had no effect on the orthodromic or antidromic evoked potentials in the CA1 region when tested at concentrations up to 10 μM (Figure 2).

In separate experiments the various purines were tested on the single cell firing which occurred either spontaneously or was induced by NMDA or carbachol.

Spontaneous activity of CA1 pyramidal cells was inhibited by adenosine or ATP superfused at concentrations above 10 μM and 15 μM, respectively. These actions could be prevented by 8-phenyltheophylline at a concentration of 1 μM. In contrast AMPCPP (10 μM) had no effect on any of the 11 neurones tested (in different slices), although 2-MeS-ATP caused an increase of firing rate of 2 of 14 cells and 2-MeS-AMP-PCF_2P caused excitation of one and inhibition of one of the 12 cells (Figure 3). None of these effects could be repeated by subsequent applications of L-AMP-PCP or 2-MeS-ATP.

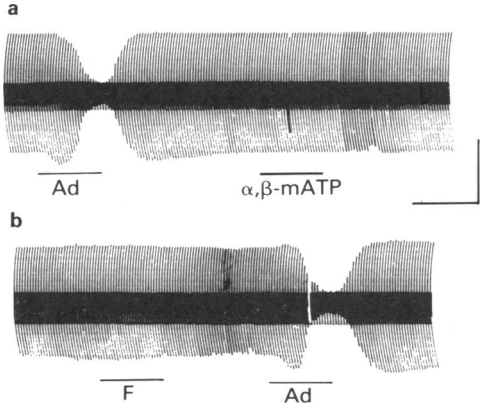

Figure 2 Chart records of the CA1 population spike potential plotted from a digital storage oscilloscope. The potential was almost abolished by adenosine (50 μM) (Ad), but unchanged by α,β-methylene ATP (10 μM) (α,β-mATP) or by 2-methylthioadenosine 5′-(β,γ-difluoromethylene)-triphosphonate (10 μM) (F). The two sections of recording are not consecutive but were made on the same slice. Calibrations 2 mV and 4 min.

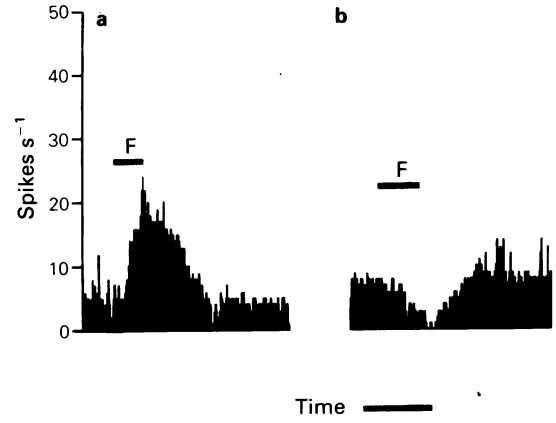

Figure 3 Records of the firing rate of 2 neurones in different slices of rat hippocampus. Neurone (a) is excited and cell (b) inhibited by superfusion with 2-methylthioadenosine 5′-(β,γ-difluoromethylene)-triphosphonate (10 μM) (F). Time bar 1 min.

Superfusion of slices with adenosine (20 μM) prevented excitation of neurones by carbachol applied by microiontophoresis, while sensitivity to the excitant amino acid NMDA was not significantly modified (5 cells) (Figure 4). None of the stable analogues, AMPCPP (4 cells), L-AMP-PCP (4 cells) and 2-MeS-AMP-PCF_2P (2 cells) changed neuronal responses to carbachol or NMDA when tested at a concentration of 10 μM.

Discussion

Of the nucleotide analogues tested here AMPCPP is a relatively stable compound in which the introduction of the α,β-methylene bridge into the phosphate side chain confers resistance to enzymic hydrolysis (Cusack *et al.*, 1988).

AMP-PCP is more susceptible to metabolism to adenosine and this probably explains the xanthine-sensitive effects of this compound on evoked potentials. Its enantiomer L-AMP-PCP which was synthesised and examined by Cusack & Hourani (1984), is also resistant to hydrolysis, but in any case would yield L-adenosine as a product. Unlike the naturally occurring (D) form, L-adenosine is unable to act on P_1-receptors (Cusack & Planker, 1979) and any effects of L-AMP-PCP can therefore be attributed to activation of P_2 sites. The failure of L-AMP-PCP, 2-MeS-ATP and 2-MeS-AMP-PCF_2P to affect evoked p.s.ps or population spikes, therefore, strongly implies the absence of P_2 nucleotide receptors capable of modulating either presynaptic transmitter release or pyramidal cell excitability.

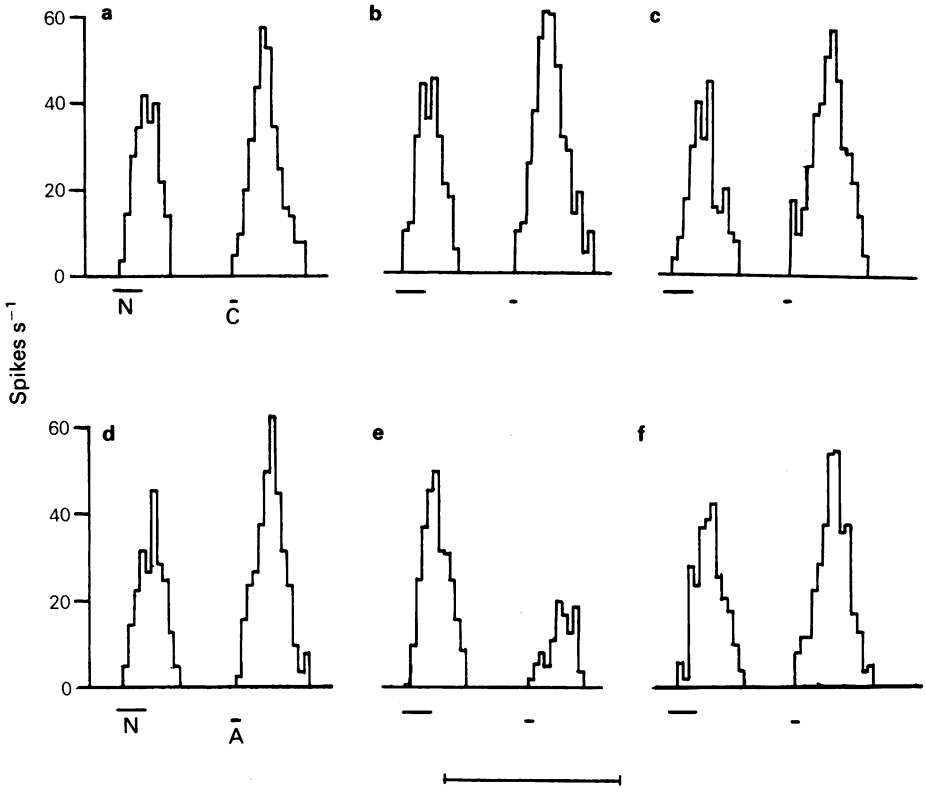

Figure 4 Records of the firing rate of a CA1 pyramidal neurone excited by the iontophoretic application of carbachol, 20 nA (C) and N-methyl-D-aspartate, 32 nA (N). (a) and (d) show control responses, and (c) and (f) recovery following superfusion with L-adenosine 5'-(β,γ-methylene)-triphosphonate, 10 μM (b) or adenosine, 20 μM (e). Adenosine selectively inhibits the excitation by carbachol whereas the nucleotide does not modify sensitivity to either agonist. Time bar: 1 min.

The ability of adenosine to suppress selectively pyramidal cell sensitivity to carbachol appears to be associated with an atypical adenosine receptor since, although the effect is xanthine-sensitive, it has characteristics which differentiate it from the usual A_1 or A_2 subtypes of the P_1 receptor (Brooks & Stone, 1988). Even here the nucleotide analogues had no apparent effect.

The changes of single cell firing rate are difficult to interpret. The restricted quantities of the analogues available precluded testing a larger population of neurones and the significance of unreproducible effects on 4 of 26 neurones must be questioned.

The P_2 population of nucleotide receptors has recently been reviewed by Burnstock & Kennedy (1985) and the proposal made that two subtypes may exist, the P_{2X} and P_{2Y} sites. The former may mediate contraction of a variety of smooth muscle tissues, including the vas deferens and urinary bladder of rats and guinea-pigs and the rat femoral artery. The P_{2Y} site on the other hand may mediate relaxation of the guinea-pig taenia coli and the rabbit portal vein. Of the compounds tested in the present study, L-AMP-PCP has been proposed as a specific agonist at the P_{2X}-receptor (Hourani *et al.*, 1985; Cusack *et al.*, 1987) and both 2-MeS-ATP and 2-MeS-AMP-PCF_2P are very potent at the P_{2Y} site. The present results therefore suggest the absence of either P_{2X}- or P_{2Y}-receptors in the CA1 subfield of rat hippocampus.

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