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4-AMINOPYRIDINE BLOCKADE OF NEURONAL DEPRESSANT RESPONSES TO ADENOSINE TRIPHOSPHATE

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1 Adenosine, adenosine monophosphate and adenosine triphosphate (ATP) depressed the firing rate of neurones in the rat cerebral cortex when applied by microinontophoresis.

2 4-Aminopyridine, also applied iontophoretically, blocked the depressant effects of the purines, without affecting responses to γ-aminobutyric acid (GABA). This blockade was effected against purine depressions of both spontaneous and glutamate-evoked activity, suggesting that the interaction occurred postsynaptically.

Introduction

Various purine derivatives such as adenosine, adenosine monophosphate (AMP) and triphosphate (ATP) powerfully depress the firing rate of single neurones in the central nervous system when applied by microiontophoresis (Phillis, Kostopoulos & Limacher, 1974; Kostopoulos & Phillis, 1977; Stone & Taylor, 1977, 1978a, b; Taylor & Stone, 1978; 1980). The mechanism of this effect is unclear but in several tissues adenosine or ATP increase cell membrane potassium permeability (de Grubareff & Sleator, 1965; Burnstock, 1972; Tomita & Watanabe, 1973; Burgess, Claret & Jenkinson, 1979; Hartzell, 1979) and a similar effect on central neurones could account for the depression of firing rate.

4-Aminopyridine (4-AP) is a compound widely used experimentally to block membrane potassium channels (Meves & Pichon, 1977; Ulbricht, 1977; Hösli, Andres & Hösli, 1979) and, probably as a result of that action, to increase transmitter release from nerve terminals (Molgo, Lemeignan & Lechat, 1979; Tokunaga, Sandri & Akert, 1979). Although 4-AP would not be expected to block those potassium channels activated by transmitter action (Llinas, Walton & Bohr, 1976; Jankowska, Lundberg, Rudomin & Sykova, 1977; Molgo, Lemeignan & Lechat, 1977; Lundh, 1978; McGeer, Eccles & McGeer, 1978; Hösli *et al.*, 1979) the structural formulae of 4-AP and adenine are sufficiently similar that it was decided to examine the effects of 4-AP on central neurones.

Methods

Male Porton Wistar rats weighing 250 to 350 g were anaesthetized with urethane (1.25 g/kg i.p.). The rat was placed in a stereotaxic frame and the body temperature maintained at 37 to 38°C. An area approximately 4 mm square of cerebral cortex was exposed immediately anterior to the bregma suture, to allow access to the motorsensory cortex. The dura was removed and, after positioning the electrodes, the exposed cortex and muscles were covered with a layer of 5% agar in 0.9% w/v NaCl solution (saline) to prevent cooling and drying, and to reduce respiratory and vascular movements of the brain. The agar layer was changed after each microelectrode penetration, though usually only one or two penetrations were made per experiment.

For the microiontophoretic application of compounds, seven-barrelled micropipettes were used. The barrels were filled immediately before use to approximately 1 cm from the top of the pipette. The central barrel was always left unfilled. The pipettes were broken under microscopic control so the overall tip diameter was 7 to 10 μm. Iontophoretic ejection was effected by a Digitimer Neurophore Unit, incorporating automatic balancing at the electrode tip.

For iontophoresis, the following solutions were used: γ-aminobutyric acid, 0.2 M, pH 3.5; adenosine hemisulphate 0.2 M, pH 4.5; adenosine 5'-monophosphoric acid sodium salt, 0.2 M, pH 5.0; adenosine triphosphate sodium salt, 0.2 M, pH adjusted to 7.0 with NaOH; 4-AP, 0.05 M, pH 9.0; tetraethylammonium bromide (TEA) 0.05 M, pH 4.5; L-glutamate sodium, 0.2 M, pH 7.0. When not being ejected, compounds were subjected to a retaining current of 10 to 15 nA.

Extracellular recordings of unit activity were made with single glass microelectrodes containing 1 M potassium acetate and having d.c. resistances of 2 to 8 MΩ. The tips of these electrodes were bent to an

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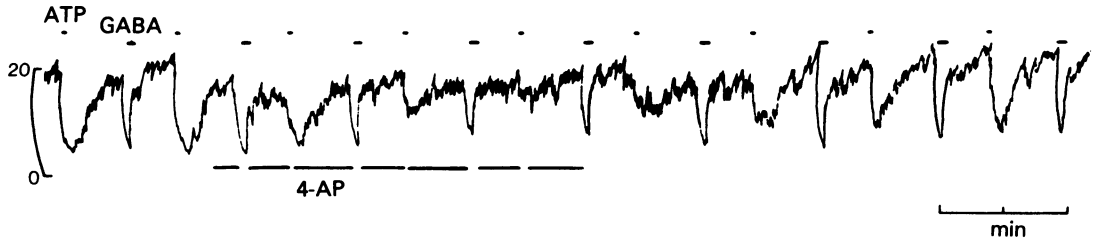

Figure 1 Record of the firing rate of a spontaneously active neurone in the cerebral cortex, depressed by the iontophoretic application of adenosine triphosphate (ATP) with a current of 78 nA, and of γ-aminobutyric acid (GABA) with 60 nA. The application of 4-aminopyridine 30 nA (4-AP) then virtually abolished the purine response, while having no effect on the GABA depression. Responses to both ATP and GABA were submaximal. Compounds were subjected to retaining currents of 5 to 15 nA when not being ejected. Although the net ejecting and retaining currents were balanced to zero throughout all the experiments described here, this record is one of several during which the 4-AP ejection was turned off during the application of the agonists. This was in order to be absolutely certain that no small current imbalance could affect the amount of agonist ejected. Ordinate scale: spikes/second; time bar: 2 min.

angle of 10° to 20° during the pulling process to facilitate fixing alongside the multibarrel pipette. The tips of the single and multibarrel pipettes were initially approximated by eye, and then under microscopic control. During this stage the electrodes were held together by Plasticine, and permanent fixing was then achieved by an epoxy-resin. Tip approximation was always confirmed microscopically before and after each experimental penetration. The recording electrode was arranged to project 10 to 20 μm beyond the multibarrel tip.

Unit activity was amplified by a Grass P511 amplifier, and the spikes were passed through a window discriminator, the output pulses of which were counted and displayed as a record of spikes per s on a Grass polygraph. Spikes were simultaneously displayed on oscilloscopes and were also available for recording on magnetic tape and for on-line generation of post-stimulus time histograms.

Results

Adenosine, AMP and ATP depressed the firing rate of most of the cortical neurones tested. Adenosine was effective on 14 of 24 cells studied, AMP on 15 of 21 and ATP on 33 of 45 (total 62 of 90). All three compounds were effective on sensitive cells when applied with iontophoretic currents in the range 10 to 140 nA for 5 s or less (Figure 1). A cell was classified as insensitive if it failed to respond to five successive applications at 200 nA for 10 s.

4-AP was ejected with positive (outward) currents of 20 to 50 nA. Currents greater than 50 nA tended to cause pipette blockade. Even when applied for several minutes, 4-AP produced no obvious changes of firing rate or of spike shape on 34 cells, while 20 cells showed an increase of firing rate and 2 were depressed.

A regular sequence of reproducible responses to a purine agonist were obtained on 53 neurones to which 4-AP was then applied. The agonist responses were always adjusted so as to be submaximal. On 30 of these cells, the purine responses were reduced by the 4-AP application, although sometimes only after several minutes, while control depressions were unaffected (Figure 1). It was noted that 4-AP antagonism was more easily demonstrated against ATP (21 of 27 cells) than against AMP (5 of 12) or adenosine (7 of 14 cells). Besides being effective against a higher proportion of ATP responses, the degree of blockade by 4-AP was much clearer against ATP than in the case of adenosine or AMP.

Tetraethylammonium ions were applied to 10 cells, but no changes of spontaneous firing rate or of responses to the purine agonists were noted.

Glutamate-evoked activity

A number of neurones were also tested which were quiescent or firing only slowly (5 Hz or less) and which were therefore excited artifically by the iontophoresis of glutamate (Figure 2). All three purines tested reduced the glutamate-evoked firing (17 of 24 cells) and 4-AP reduced this depression on 12 of the 17 neurones (Figure 2). It was of interest that on seven neurones the application of purines reduced spontaneous firing rate but had little effect on glutamate-evoked excitation.

Discussion

The powerful depressant effects of adenosine and ATP on neuronal firing rate have been observed pre-

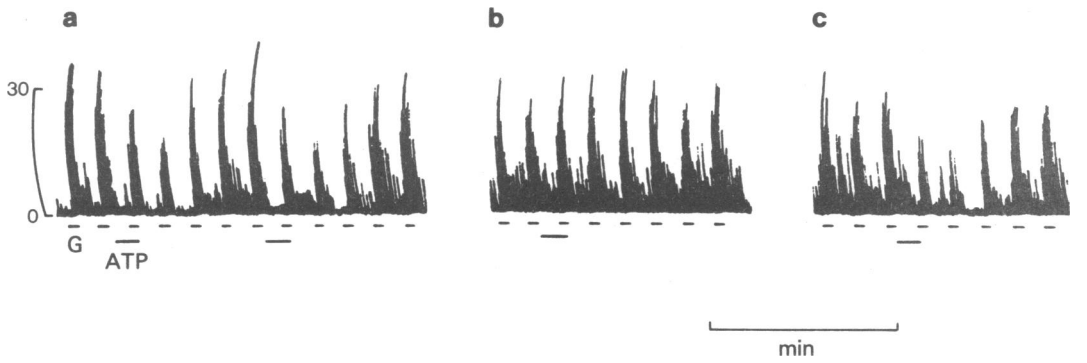

Figure 2 Records of the firing of a cortical neurone evoked by pulses of L-glutamate, 16 nA (G). In (a) the iontophoresis of adenosine triphosphate (ATP) with a current of 74 nA causes a reduction of the glutamate-evoked activity, and this is shown to be reproducible. In (b) the beginning of which is approximately 3 min after starting an ejection of 4-AP with a current of 30 nA, ATP no longer inhibits the glutamate excitation. Record (c) was begun 4 min after ending the 4-AP ejection, at a time when ATP depression has recovered. Ordinate scale: spikes/second; time: 2 min.

viously (Phillis *et al.*, 1974; Kostopoulos & Phillis, 1977; Stone & Taylor, 1977; 1978a, b; Taylor & Stone, 1978; 1980) and support the view that these compounds may act to modulate neuronal activity, whether or not they are released as neurotransmitters (Burnstock, 1972; Stone, 1978).

The particularly interesting finding of the present study is that 4-AP, a compound usually used only as a pharmacological tool to block membrane calcium-dependent and voltage-dependent potassium channels (Meves & Pichon, 1977; Ulbricht, 1977; Hösli *et al.*, 1979) can block the purine-induced depressions. Although this is consistent with the opening of potassium channels by purines and the physiological antagonsim of that action by the channel blocker 4-AP, it contrasts with the general observation that the species of potassium channel opened by transmitter action is not blocked by 4-AP (Llinas *et al.*, 1976; Molgo *et al.*, 1977; Jankowska *et al.*, 1977; Lundh, 1978; McGeer *et al.*, 1978; Hösli *et al.*, 1979).

If the interaction between purines and 4-AP is indeed at the level of the potassium channel, then it follows that the purine-activated channel may be fundamentally different from the transmitter-activated channel, and even seems more closely related to the calcium-dependent and voltage-dependent channels which are blocked by 4-AP than to the conventional transmitter-activated channels. In this regard it is of some interest that quinine and related drugs, which block the effects of ATP in many tissues (Burnstock, 1972) also specifically uncouple increases of intracellular calcium levels from the activation of the (calcium-dependent) potassium channels (Armando-Hardy, Ellory, Ferreira, Fleminger & Lew, 1975; Hanani & Shaw, 1977). It is therefore feasible that the release or local mobilization of a purine derivative such as ATP could form the link between intracellular calcium levels and potassium channels.

Another suggestion that arises out of the present study is that the increase of transmitter release produced by 4-AP could result from the blockade by 4-AP of purines released endogenously in the region of the nerve terminal. Adenosine in particular is a potent inhibitor of transmitter release (Ginsborg & Hirst, 1972; Hedqvist & Fredholm, 1976; Vizi & Knoll, 1976; Clanachan, Johns & Paton, 1977; Enero & Saidman, 1977; Hollins & Stone, 1979) and such an effect could explain this action of 4-AP whether or not the interaction occurs at the level of the potassium channel.

It might be argued that the failure of TEA to block purine depressions opposes the idea of interaction at potassium channels. However, in some tissues TEA blocks potassium channels only when applied intracellularly (Ulbricht, 1977). Unlike 4-AP, therefore, which crosses cell membranes to block potassium channels whether applied intracellularly or extracellularly (Meves & Pichon, 1977), TEA may not have gained adequate access to blocking sites.

Finally, it should be emphasised that, as an alternative to an interaction at the potassium channels, 4-AP could possibly act as a blocking agent at the purine receptor. Such a possibility will require investigation in a simpler tissue than brain.

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References

ARMANDO-HARDY, M., ELLORY, J.C., FERREIRA, H.G., FLEMINGER, S. & LEW, V.L. (1975). Inhibition of the calcium-induced increase in the potassium permeability of human red blood cells by quinine. *J. Physiol.*, **250**, 32–33P.

BURGESS, G.M., CLARET, M. & JENKINSON, D.H. (1979). Effects of catecholamines, ATP and ionophore A 23187 on potassium and calcium movements in isolated hepatocytes. *Nature*, **279**, 544–546.

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24**, 509–581.

CLANACHAN, A.S., JOHNS, A. & PATON, D.M. (1977). Presynaptic inhibitory actions of adenine nucleotides and adenosine on neurotransmission in the rat vas deferens. *Neuroscience*, **2**, 597–602.

DE GRUBAREFF, T. & SLEATOR, W. (1965). Effects of caffeine on mammalian atrial muscle and its interaction with adenosine and calcium. *J. Pharmac. exp. Ther.*, **148**, 202–214.

ENERO, M.A. & SAIDMAN, B.Q. (1977). Possible feedback inhibition of noradrenaline release by purine compounds. *Naunyn Schmiedebergs Archs Pharmac.*, **297**, 39–46.

GINSBORG, B.L. & HIRST, G.D.S. (1972). The effects of adenosine on the release of the transmitter from the phrenic nerve of the rat. *J. Physiol.*, **224**, 629–645.

HANANI, M. & SHAW, C. (1977). A potassium contribution to the response of the barnacle photoreceptor. *J. Physiol.*, **270**, 151–163.

HARTZELL, H.C. (1979). Adenosine receptors in frog sinus venous: slow inhibitory potentials produced by adenine compounds and acetylcholine. *J. Physiol.*, **293**, 23–49.

HEDQVIST, P. & FREDHOLM, B.B. (1976). Effects of adenosine on adrenergic neurotransmission: prejunctional inhibition and postjunctional enhancement. *Naunyn Schmiedebergs Archs Pharmac.*, **293**, 217–224.

HOLLINS, C. & STONE, T.W. (1980). Adenosine inhibition of γ-aminobutyric acid release from slices of rat cerebral cortex. *Br. J. Pharmac.* (in press).

HÖSLI, L., ANDRES, P.E. & HÖSLI, E. (1979). Effects of 4-aminopyridine and tetraethylammonium on the depolarisation by GABA of cultured satellite glial cells. *Neurosci. Lett.*, **11**, 193–196.

JANKOWSKA, E., LUNDBERG, A., RUDOMIN, P. & SYKOVA, E. (1977). Effects of 4-aminopyridine on transmission in excitatory and inhibitory synapses in the spinal cord. *Brain Res.*, **136**, 387–392.

KOSTOPOULOS, G.K. & PHILLIS, J.W. (1977). Purinergic depression of neurones in different areas of rat brain. *Expl Neurol.* **55**, 719–724.

LLINAS, R., WALTON, K. & BOHR, V. (1976). Synaptic transmission in squid giant synapses after K^+ conductance blockade with external 3- and 4-aminopyridine. *Biophysiol. J.*, **16**, 83–86.

LUNDH, H. (1978). Effects of 4-aminopyridine on neuromuscular transmission. *Brain Res.*, **153**, 307–318.

MCGEER, P.L., ECCLES, J.C. & MCGEER, E.G. (1978). *Molecular Neurobiology of the Mammalian Brain.* p. 98. New York: Plenum Press.

MEVES, H. & PICHON, Y. (1977). The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axons. *J. Physiol.*, **268**, 511–532.

MOLGO, J. LEMEIGNAN, M. & LECHAT, P. (1977). Effects of 4-aminopyridine at the frog neuromuscular junction. *J. Pharmac. exp. Ther.*, **203**, 653–663.

MOLGO, J., LEMEIGNAN, M. & LECHAT, P. (1979). Analysis of the action of 4-aminopyridine during repetitive stimulation at the neuromuscular junction. *Eur. J. Pharmac.*, **53**, 307–312.

PHILLIS, J.W., KOSTOPOULOS, G.W. & LIMACHER, J.J. (1974). Depression of corticospinal cells by various purines and pyrimidines. *Canad. J. Physiol. Pharmac.*, **52**, 1226–1229.

STONE, T.W. (1978). Possible roles for purine compounds in neuronal adaptation. *Biochem. Soc. Trans.*, **6**, 858–862.

STONE, T.W. & TAYLOR, D.A. (1977). Microiontophoretic studies of the effects of cyclic nucleotides on excitability of neurones in the rat cerbral cortex. *J. Physiol.*, **226**, 523–543.

STONE, T.W. & TAYLOR, D.A. (1978a). An electrophysiological demonstration of a synergistic interaction between adenosine and norepinephrine in the cerebral cortex. *Brain Res.*, **147**, 396–400.

STONE, T.W. & TAYLOR, D.A. (1978b). Blockade by clonidine of neuronal depressant responses to adenosine, AMP and ATP. *Br. J. Pharmac.*, **64**, 369–374.

TAYLOR, D.A. & STONE, T.W. (1978). Neuronal responses to extracellularly applied cyclic AMP: role of the adenosine receptor. *Experientia*, **34**, 481–482.

TAYLOR, D.A. & STONE, T.W. (1980). The action of adenosine on noradrenergic neuronal inhibition induced by stimulation of locus coeruleus. *Brain Res.*, **183**, 367–376.

TOKUNAGA, A., SANDRI, C. & AKERT, K. (1979). Ultrastructural effects of 4-aminopyridine on the presynaptic membrane in the rat spinal cord. *Brain Res.*, **163**, 1–8.

TOMITA, T. & WATANABE, H. (1973). A comparison of the effects of ATP with noradrenaline and with the inhibitory potential of the guinea-pig taenia coli. *J. Physiol.*, **231**, 167–178.

ULBRICHT, W. (1977). Ionic channels and gating currents in excitable membranes. *A. Rev. Biophys. Bioeng.*, **6**, 7–31.

VIZI, E.S. & KNOLL, J. (1976). The inhibitory effect of adenosine and related nucleotides on the release of acetylcholine. *Neuroscience*, **1**, 391–398.

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