within physiological range, can result in increased activity in a biological system[4]. Thus the results of the present experiments are not unexpected. What is surprising is that so many neurobiologists have failed to use this technique to stimulate physiological activity and interactions between anatomically related centers such as the cerebellum and cerebrum. A search of the literature indicates that the hypothalamus is one of the few areas of the brain to be studied in this manner. Nakayama et al.[5] have reported increased activity of single units in the preoptic area with warming to 41 °C and indicate this is directly related to maintenance of body temperature. Additionally, the authors write 'most neurons, even thalamic units, increased their discharge frequency at temperature higher than 41-42 °C'. We have not done single unit studies but the upper traces in figure 1, B and C clearly demonstrate that warming increased cerebellar activity as observed from records obtained from cerebellar white matter deep and adjacent to the warmed cortex. A common finding was an enhancement of activity between 39.5 and 42 °C. The effects of higher temperatures have not been studied, but work in progress indicates that warming to 41 °C enhances activity in the sensori-motor cerebral cortex.

Purkinje cell axons give rise to the major efferent axons of the cortex and the increased activity in figure 1 is a direct result on increased cortical temperature. The enhanced activity (lower trace, figure 1, B and C) in the red nucleus, a known terminus of cerebellar efferent fibres[6], also appears to be related to cerebellar warming since allowing the cerebellar surface temperature to return to 36–37 °C results in decreased activity.

The data shown in figure 2 and the table indicate that increasing the local temperature of the cerebellar anterior lobe reduces the length of experimentally induced paroxysmal discharges. As demonstrated in a number of basic and clinical studies[6–8], electrical stimulation of the cerebellum can suppress and/or stop seizure discharges. The present data show strong compatibility with those obtained by conventional methods for both acute and chronic experiments[7].

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Neuronal responses to extracellularly applied cyclic AMP: Role of the adenosine receptor

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Summary. At low doses, theophylline blocks the neuronal depressant effects of 5′-AMP, but not cyclic AMP. Higher doses (100 mg/kg) block cyclic AMP responses and reduce the effects of noradrenaline and GABA. It is concluded that cyclic AMP does not depress neurones via the adenosine receptor.

Several recent studies have attempted to correlate the response of central neurones to synaptic transmitters and nucleotides by observing changes of firing rate when these substances are applied extracellularly to single neurones by microiontophoresis[4]. Some clear correlations have resulted, particularly for acetylcholine and cyclic GMP, and noradrenaline and cyclic AMP[5–8].

It has now been shown that purines other than cyclic AMP can produce changes of firing rate by acting on an extracellular adenosine receptor[9], raising the possibility that extracellularly applied cyclic AMP might alter neuronal firing via this same receptor. As the adenosine receptor can be blocked by theophylline[9,10], we have used this substance to try to differentiate between neuronal responses to cyclic AMP and related compounds.

Materials and methods. Adult male rats were anaesthetised with urethane, 1.25 g/kg i.p. Recordings were made of the firing rates of neurones in the parietal cerebral cortex, many of which were identified as pyramidal tract cells. Cyclic AMP, adenosine and 5′-AMP were ejected by microiontophoresis from 5- or 7-barrelled micropipettes filled with cyclic AMP sodium salt; adenosine hemisulphate; 5′-AMP sodium salt (all 250 mM); gamma-aminobutyric acid (GABA) 1 M. The net current at the pipette tip was automatically maintained at zero. The centre barrel was filled with 5 M sodium chloride or 1 M sodium acetate for recording unit activity. Aminophylline (theophylline ethylenediamine) was dissolved in saline and injected i.v. at doses of 25–100 mg/kg.

Results and discussion. The 3 nucleotides used in these experiments proved able to depress the firing of many cortical cells. Cyclic AMP was the least effective but it depressed 12 of 26 cells when applied with currents of 40–150 nA[5]. Adenosine and 5′-AMP depressed all but 2 of the cells tested (28 of 30), (figure 1). Gamma-aminobutyric acid (GABA) ejected with currents of 5–80 nA, readily depressed neuronal firing and was used as a control agonist in the examination of the effects of aminophylline.

Aminophylline, 25–100 mg/kg i.v. was injected whilst recording from 16 neurones depressed by both 5′-AMP and cyclic AMP. The drug caused a slowing of firing rate of 10 cells and an acceleration of 4, which lasted for between 30 sec and 4 min, but thereafter firing returned to the pre-injection level. On all the cells, responsiveness to 5′-AMP and adenosine was reduced following the injection, the purine responses being completely blocked after the 50 or 100 mg/kg doses. Responses to cyclic AMP were reduced by no more than 50% on 12 cells following 25 or 50 mg/kg theophylline, (figure 1) although they were substantially reduced in size after doses of 100 mg/kg. Recordings from 8 of the 16 units were sufficiently stable to follow recovery

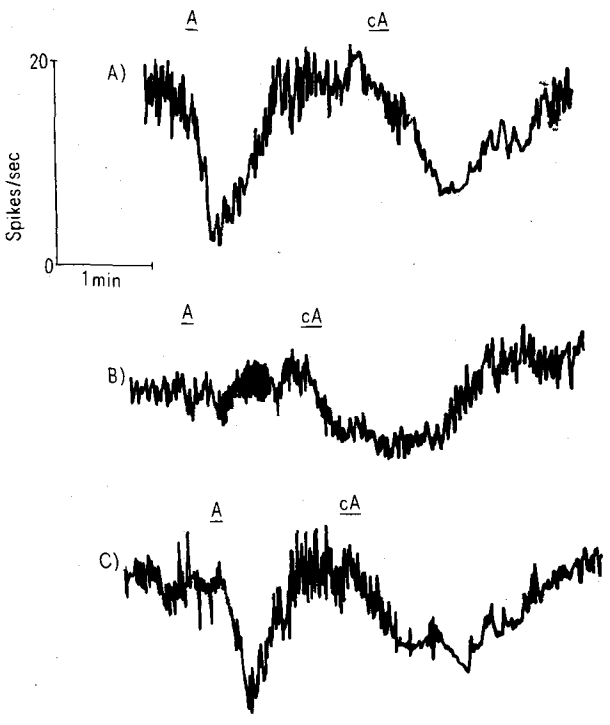

Fig. 1. Records of the firing rate of a cortical neurone showing the depression of firing by the iontophoresis of adenosine, 25 nA (A) and cyclic AMP, 80 nA (cA). *A* was recorded before the i.v. injection of aminophylline, 50 mg/kg, *B* 5 min after the injection, and *C* 150 min after the injection. The effect of aminophylline was to block completely the response to adenosine, while leaving cyclic AMP responses virtually unchanged.

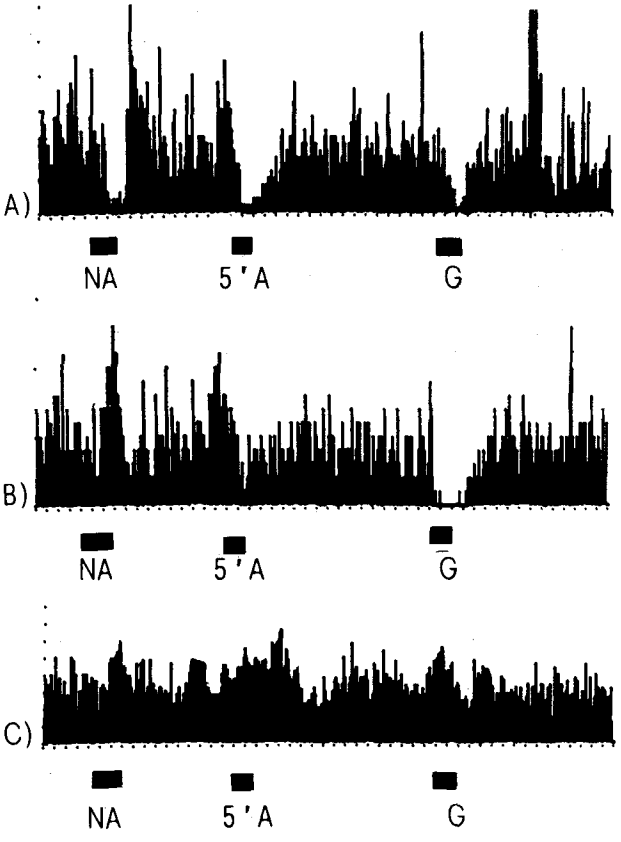

Fig. 2. Computer-generated on-line post-stimulus time histograms, showing neuronal firing rate (ordinate) responses to the iontophoresis of noradrenaline, 40 nA (NA); 5′-AMP. 15 nA (5′A) and GABA, 70 nA (G). Each trace has a duration of 128 sec and is composed of 3 superimposed sweeps.
A control record. *B* 10 min after the i.v. injection of 50 mg/kg of aminophylline. The noradrenaline and 5′-AMP responses are substantially reduced, though GABA is at this time potentiated. *C* 10 min after an additional dose of 50 mg/kg (total 100 mg/kg) given 20 min after the 1st dose. The responses to all 3 agonists have been abolished.

of the adenosine or 5′-AMP responses, which reached pre-injection size after 90–250 min (figure 1).

The depressant responses to GABA were unaffected or even increased by 25–50 mg/kg of aminophylline, but were substantially reduced by higher doses of the drug. Figure 2 shows poststimulus time histograms of the firing rate of a neurone depressed by 5′-AMP, noradrenaline and GABA. After 100 mg/kg of aminophylline responses to all 3 substances were abolished.

These results confirm reports that adenosine and 5′-AMP are potent depressants of cortical neurones[11]. The ability of low doses of aminophylline to abolish these responses supports the biochemical evidence that their action is mediated through a theophylline-sensitive adenylate cyclase[10,12].

Since responses to cyclic AMP were sometimes also reduced by the higher doses of aminophylline it might be concluded that the effects of extracellularly applied cyclic AMP may be exerted partly via the adenosine sensitive cyclase. Mah and Daly[13] have shown that cyclic AMP can activate this system in vitro. However, since cyclic AMP responses were not totally abolished at a time when 5′-AMP or adenosine responses were completely blocked, such a mechanism could not wholly explain the depressant effects of cyclic AMP.

As responses to noradrenaline and GABA were reduced by the high doses of aminophylline needed to reduce cyclic AMP responses, it seems likely that such doses of aminophylline were producing a nonspecific reduction of neuronal sensitivity to depressant compounds. We conclude that the activation of the extracellular adenosine receptor plays little part in mediating neuronal depressant responses to cyclic AMP, indirectly supporting the idea that recently reported effects of cyclic AMP on central neurones may be related to the effects of endogenous cyclic AMP produced by activation of membranal adenylate cyclase[5,14].

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