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Evidence for an A_2-like adenosine receptor on cerebral cortical neurons

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The concept of extracellular purine receptors has been generally accepted for both peripheral and central tissues where adenosine and the adenine nucleotides exert dose dependent depressant or excitant effects on activity (Phillis & Wu 1981). Burnstock (1978) has proposed the existence of two types of extracellular receptor in peripheral tissues. The P_1 purinoceptor is more responsive to adenosine and adenosine 5′-monophosphate (AMP) than to adenosine 5′-triphosphate (ATP) and is antagonized by the methylxanthines while the P_2 purinoceptor is activated by ATP and adenosine 5′-diphosphate (ADP), and is antagonized by quinidine and 2,2′-pyridilisatogen tosylate. Studies on the effects of adenosine on adenylate cyclase from various tissues have led to a further classification of the adenosine receptors into a high affinity A_1 type which inhibits, and a lower affinity A_2 type which activates, the enzyme (Van Calker et al 1979; Londos et al 1980). The order of potency of certain adenosine analogues is different for the two receptor subtypes. However both are antagonized by methylxanthines. The purpose of the present study was to determine whether the P_1 receptor on cerebral cortical neurons can be equated with either the A_1 or A_2 subtypes of adenosine receptor associated with adenylate cyclase.

Experiments were performed on 18 male Sprague-Dawley rats (350–500 g). Following induction of anaesthesia with halothane, and tracheal intubation, the animals were placed in a stereotaxic frame and maintained on methoxyflurane in a mixture of nitrous oxide and oxygen (80:20). An electric heating pad controlled by a rectal probe maintained body temperature at 37°C. A small hole was drilled through the parietal bone 2 mm lateral to the sagittal suture and 1·5 mm posterior to the coronal suture line. A small slit was made in the exposed dura to expose the sensorimotor cortex. The exposed subcutaneous areas, muscle and bone were covered with a thin layer of 4% agar in Ringer solution to prevent drying.

Eleven of these rats had a cannula placed in the right femoral vein for intravenous drug injections and an arterial cannula inserted into the right femoral artery for recording arterial blood pressure on a Grass polygraph.

The recording of neuronal activity and iontophoresis of drugs was accomplished with seven barrelled micropipettes with overall tip diameters of 6–10 μm. The central recording barrel contained 2 M NaCl and the remaining barrels were filled by centrifugation with various combinations of the following solutions: adenosine hemisulphate (0·1 M pH 4, Sigma), AMP (0·1 M, pH 6·0, Sigma), 2-chloroadenosine (0·01 M, pH 5·5, Sigma), adenosine 5′-*N*-ethylcarboxamide (NECA, 0·01 M, pH 6·2, Byk Gulden Pharmazeutika), adenosine 5′-*N*-methylcarboxamide (NMCA, 0·01 M, pH 5·8, Abbott), adenosine 5′-*N*-cyclopropylcarboxamide (NCPCA, 0·005 M, Abbott), L-N^6-phenylisopropyladenosine (L-PIA, 0·001 M, pH 5·8 Boehringer Mannheim, GmbH), N^6-cyclohexyladenosine (CHA, 0·001 M, pH 5·5, Calbiochem). Substances were applied from at least three different electrode barrels onto neurons in the sensorimotor cortices of a minimum of two animals. Drugs were tested on deep (800–1200 μm) 'spontaneously firing' cortical neurons. Drug effects were evaluated by observing the alterations in the rate of neuronal firing. The relative depressant potencies of individual compounds were evaluated either on the basis of the magnitude of the currents which had to be passed through the various electrode barrels for a standard time interval to elicit comparable reductions in the firing rate or by observing the magnitude of the depressions elicited when currents of similar magnitude were passed through different barrels.

The adenosine derivatives were also tested as hypotensive agents to ascertain the structure-activity relationships of these compounds in another test system (Phillis & Kostopoulos 1975). The amount of adenosine, administered intravenously, required to produce a discernible fall in blood pressure was determined and then the amounts of the other compounds which produced a comparable fall were ascertained.

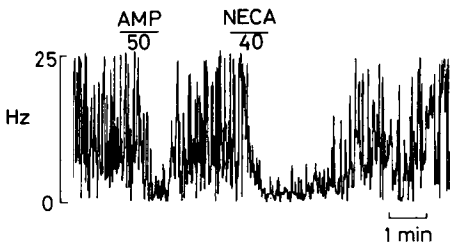

FIG. 1. Firing frequency record from a cerebral cortical neuron. This is a ratemeter record of spontaneous firing with the number of action potentials per second on the ordinate. Horizontal bars above the record indicate periods of drug application. AMP (50 nA) and NECA (40 nA) depressed the firing of the neuron. The NECA effect was more pronounced and of longer duration.

Table 1. Depressant effects of adenosine analogues on cerebral cortical neurons.

Compound	Depressant action on cerebral cortical neuronal firing: Number of cells tested	Relative potency	Duration of effect
Adenosine or adenosine 5'-monophosphate		----	+
2-Chloroadenosine	24	-----	+++
5'-*N*-Methylcarboxamide adenosine	36	------	++
5'-*N*-Ethylcarboxamide adenosine	61	------	++
5'-*N*-Cyclopropyl-carboxamide adenosine	36	-----	++
L-N^6-Phenylisopropyl adenosine	62	---(-)*	++++
N^6-Cyclohexyladenosine	42	---(-)*	++++

* When applied for brief (20–30 s) periods these compounds were less effective than adenosine. With longer applications the depressions became more pronounced.

Results and discussion

Adenosine, AMP, 2-chloroadenosine, NECA, NMCA, NCPCA, L-PIA and CHA all caused dose-dependent reductions in the rate of firing of cerebral cortical neurons (Table 1). NMCA and NECA were the most potent of these compounds, eliciting equivalent depressions of neuronal firing with application currents that were about one half of those passed through the adenosine or AMP barrels. The duration of the depression was often greater than that observed with adenosine or AMP (Fig. 1). NCPCA was slightly less effective as a depressant, being comparable to 2-chloroadenosine. Both compounds were however more potent than adenosine and AMP. 2-Chloroadenosine had a longer depressant action, with neuronal firing remaining depressed for 2–3 min after its application had been terminated. L-PIA and CHA required larger application currents than did adenosine or AMP to elicit comparable levels of depression when applications were of a standard duration. The effects of both compounds were, however, quite prolonged, with recovery often taking several mintues (Fig. 2).

A remarkably similar structure-activity relationship was observed when these compounds were tested on the arterial blood pressure by intravenous administration. Adenosine caused a brief fall in arterial blood pressure and a slowing of the heart. The threshold dose of adenosine for this effect was between 5–10 μg kg^{-1}. NMCA and NECA were the most potent hypotensive analogues with the threshold dose for both compounds being in the 5–20 ng kg^{-1} range. NCPCA was slightly less potent (threshold dose 10–50 ng kg^{-1}). 2-Chloroadenosine, L-PIA and CHA were less potent (threshold doses, 0·5–2 μg kg^{-1}) than the carboxamides, but more potent than adenosine. L-PIA and CHA had

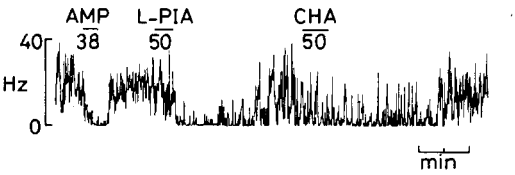

FIG. 2. Depression of the firing of a cerebral cortical neuron by AMP (38 nA), L-PIA (50 nA) and CHA (50 nA). The L-PIA and CHA applications were of longer duration than for AMP.

long-lasting hypotensive actions and the recovery phase after larger doses (40–100 μg kg^{-1}) frequently lasted for up to 2 h.

The results of our experiments with these adenosine analogues demonstrate that in both experimental paradigms (depression of neuronal firing and hypotensive actions) the adenosine 5'-carboxamides are more potent than L-PIA and CHA. The similarity of the structure-activity relationships is encouraging in that it suggests that the results obtained by iontophoretic application represent valid approximations of agonist relative potencies which have not been unduly distorted by differing efficacies of drug ejection from micropipette tips.

The potency of the carboxamides as depressants of neuronal firing indicates that the receptor involved is similar in its properties to the A_2 type of adenosine receptor associated with adenylate cyclase. NECA and NCPCA are potent antagonists at the A_2 receptor, being about 10 fold more active than L-PIA and 2-chloroadenosine (Londos et al 1980; Daly et al 1981), whereas at the A_1 receptor the order of potency is reversed. The question of whether the adenosine receptor responsible for the depression of neuronal firing (the P_1 receptor) is identical with the A_2 receptor remains to be answered.

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