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ADENOSINE ANALOGUES

THE TEMPERATURE-DEPENDENCE OF THE ANTICONVULSANT EFFECT AND INHIBITION OF ^{3}H-D-ASPARTATE RELEASE

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Abstract—Following the intraperitoneal administration of the adenosine analogues, 2-chloro-adenosine (1–4 mg/kg) or 5′-*N*-ethylcarboxamidoadenosine (NECA; 0.01–0.5 mg/kg) to audiogenic DBA/2 mice, there is a potent protection against sound-induced seizures and a simultaneous large (2–5°) reduction in body temperature. The anticonvulsant potency of the adenosine analogues is almost completely abolished by (1) pretreatment with methylxanthines or (2) warming the mice to prevent the adenosine-induced temperature decrease.

Adenosine (0.01–1 mM), 2-chloro-adenosine (0.1–1 mM) and NECA (0.1 mM) also significantly inhibit potassium-evoked release of ^{3}H-D-aspartate from rat hippocampal slices. This inhibition is not affected by theophylline (1 mM).

An increased cerebral adenosine level is commonly observed during experimental seizures [1–4]. This is a consequence of the adenine nucleotide catabolism occurring during seizure activity [5]. The vasodilatory properties of the released adenosine [6–7] may contribute to the sustained elevation of cerebral blood flow observed during seizures [5]. Recent evidence indicates that adenosine and adenosine derivatives have anticonvulsant properties, and that the adenosine formed during convulsions may help to diminish the seizure activity [8]. An anticonvulsant action of adenosine was first reported by Mandel and co-workers [9] who used very high doses of adenosine to partially block sound-induced seizures in mice. Subsequent studies using more modest levels of adenosine or adenosine analogues have shown that these compounds inhibit seizures induced by chemical convulsants, such as pentylenetetrazol, 3-mercaptopropionic acid, kainic acid [10, 11] and pilocarpine [12], or amygdaloid-kindled seizures [13, 14].

Properties of adenosine or adenosine analogues that probably relate directly to their anticonvulsant activity include (1) an inhibition of spontaneous or evoked neuronal firing [15–17], and (2) an inhibition of excitatory transmitter release [18–20].

Adenosine and adenosine analogues also affect a wide range of systemic and metabolic parameters, which may indirectly contribute to their anticonvulsant activity: following adenosine administration there is a reduction in spontaneous motor activity, a reduction in body temperature and blood pressure [10, 21], and a multitude of metabolic effects involving the adenylate cyclase system, several neurotransmitter systems, and the benzodiazepine receptor [22–25].

The aim of the present paper is to determine the anticonvulsant activity of two adenosine analogues, one purine-substituted [2-chloroadenosine] and one ribose-substituted [5′-*N*-ethylcarboxamidoadenosine = NECA] against sound-induced seizures in audiogenic DBA/2 mice, and to study the influence of body temperature on the anticonvulsant potency.

Finally, since other anticonvulsant compounds, such as barbiturates, benzodiazepines, phenytoin [26] or the excitatory amino acid antagonists 2-APH and 2-APV [27], all inhibit potassium-evoked release of excitatory amino acids from brain slices, we want to establish if the anticonvulsant effect of adenosine compounds can likewise partially be attributed to a presynaptic effect on excitatory amino acid release.

MATERIALS AND METHODS

Chemicals. Adenosine, 2-chloro-adenosine, 5′-*N*-ethylcarboxamidoadenosine [NECA], theophylline, and aminophylline were purchased from Sigma U.K. (Poole, Dorset).

Animals. DBA/2 mice, male and female, 3–4 weeks old, were purchased from Bantin and Kingman (Hull, U.K.). Male Wistar rats, 180–200 g, were purchased from Charles Rivers (Margate, Kent, U.K.).

Anticonvulsant evaluation. The DBA/2 mice were divided into groups of 8–10 each and injected intraperitoneally with 0.1 ml of saline, 2-Cl-adenosine [1–4 mg/kg] or NECA [0.01–10 mg/kg]. Some groups were pretreated with aminophylline (50 mg/kg, i.p.) or theophylline (50 mg/kg, i.p.) for 10 min prior to the administration of the adenosine analogues. Following drugs administration the groups of mice were kept in cages at room temperature (Group A, Fig. 1), or warmed by placing a desk lamp (100 W bulb) immediately above the cage (Group B, Fig. 1). Twenty min after the drug administration the rectal temperature was measured, and the mice were placed singly under a perspex dome (diam. 58 cm) fitted with an electric doorbell at the apex. Following

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30 sec of behavioural observation, the mice were exposed to accoustic stimulation (109 db intensity; mixed frequency) for 60 sec or until tonic extension occurred. Sound-induced seizures in DBA/2 mice follow a fixed pattern of sequential phases, and the seizure response for each animal was scored as follows: 0 = no response, 1 = wild running, 2 = clonic seizures, 3 = tonic extension, 4 = respiratory arrest. Statistically significant differences between control groups and drug-treated groups were assessed by Fisher's exact probability test (incidence of seizure phases) and Student's *t*-test (rectal temperature): $^{*}P > 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Aspartate release. Potassium-evoked calcium-dependent release of ^{3}H-D-aspartate from preloaded hippocampal slices was carried out according to a modification of a previously described procedure [28]. Slices were prepared (0.2 mm thickness, cut in two directions) using a McIlwain tissue chopper from freshly dissected hippocampal tissue of male Wistar rats and incubated for 20 min at 30° in an oxygenated solution containing 136 mM NaCl, 2.4 mM KCl, 5 mM KH_2PO_4, 0.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 27.5 mM $NaHCO_3$, 1.66 mM glucose and 0.06 mM ^{3}H-D-aspartate (1.25 μCi/ml). The experiments were performed using a four channel superfusion chamber, two control experiments were performed in parallel with two drug experiments, and carried out in quadruplicate [N = 8]. After a 30 min washout period, 1.0 ml fractions were collected every 2 min at a flow rate of 0.5 ml/min. Release was stimulated by raising the potassium concentration in the superfusion medium from 2.4 mM to 50 mM for two 4 min periods (initiating 6 and 22 min after the start of fraction collection). In the drug experiments, the drug was present only during the second evoked stimulation. Radioactivity in each fraction and that remaining in the tissue at the end of the experiments (more than 95% of the starting amount) was determined by liquid scintillation spectrometry. The release is expressed as fractional release, i.e. amount of radioactivity recovered in the eluent as a percentage of radioactivity remaining in the tissue at the time of collection. The S_2/S_1 ratio expresses the ratio of the mean of the evoked release of the first [S_1] and second [S_2] depolarising stimuli.

Statistically significant differences in the in the evoked release ratios between the control group and experimental groups were evaluated using the Student's *t*-test as above.

RESULTS

Anticonvulsant activity of adenosine analogues

The control group of DBA/2 mice (N = 30) underwent a characteristic sequence of seizure phases when exposed to the sound-stimulus: wild running (100% incidence; latency 3.2 ± 0.1 sec), followed by clonic convulsions (100%; 17.4 ± 2.9 sec latency), tonic extension (83%; 20.8 ± 3.0 sec latency), ending in respiratory arrest (57%; 31.7 ± 2.9 sec latency) or subsequent recovery, with an overall seizure response of 3.4. NECA, when administered i.p. to the mice, provided a dose-dependent protection against the sound-induced seizures with an ED_{50} value for the clonic seizure phase of 0.08 mg/kg [Fig. 1, Group A, top]. At a dose of 0.4 mg/kg NECA, sound-induced seizures were almost completely suppressed, with a 10% incidence of clonic seizures and an overall seizure response of 0.4. Rectal temperature in mice was significantly reduced by the whole range of NECA doses (0.01–0.5 mg/kg) utilized (Fig. 1, Group A, bottom), from a mean of 37.2 ± 0.1° in the overall control group to 32.1 ± 0.5°

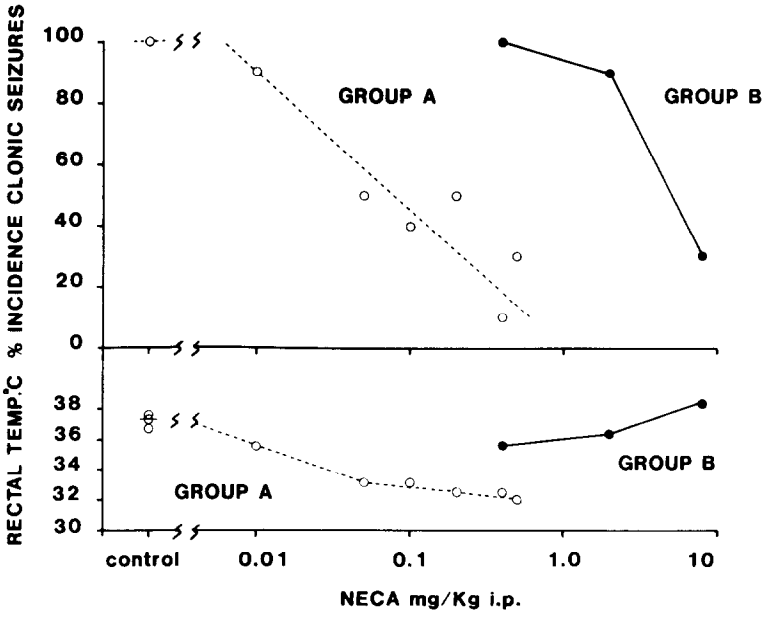

Fig. 1. Effect of NECA administration on sound-induced clonic seizures (top) and body temperature (bottom) in DBA/2 mice. In group A the body temperature was allowed to decrease spontaneously; in group B the temperature decrease was prevented by warming the animals (N = 8–10 per dose of NECA) with a heating lamp.

Table 1. Inhibition of sound-induced seizures in DBA/2 mice by adenosine analogues (i.p. administration). Reversal by pretreatment with methylxanthines, or by the maintenance of body temperature

Group	N	Body temp °C	Clonic seizures % incidence
Control	30	37.2 ± 0.1	100
+NECA, 0.4 mg/kg	10	32.5 ± 0.2***	10***
+NECA, 0.4 mg/kg, warmed	10	35.6 ± 0.3***	100
+NECA, 0.5 mg/kg	10	32.1 ± 0.5***	30***
+NECA, 0.5 mg/kg, + theophylline, 50 mg/kg	10	36.2 ± 0.2***	90
Control	10	37.6 ± 0.2	100
+2-Cl-adenosine, 4 mg/kg	10	34.0 ± 0.3***	0***
+2-Cl-adenosine, 4 mg/kg, warmed	8	37.8 ± 0.4	75*
+2-Cl-adenosine, 4 mg/kg, + aminophylline, 50 mg/kg	10	36.9 ± 0.2	100

Asterisks denote statistically significant differences between experimental and control groups; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

at 0.5 mg/kg NECA. There was a simultaneous severe reduction in spontaneous locomotion activity and some sedation following the NECA administration. When the reduction in body temperature was counteracted by warming with a heating lamp after the administration of NECA, (Fig. 1, Group B, bottom) there was a partial reversal of the behavioural effects of NECA. Spontaneous locomotion was almost completely restored, and much higher doses of NECA (2–8 mg/kg) were required to inhibit sound-induced seizures [Fig. 1, Group B, top]. The ED_{50} value for protection against clonic seizures in mice with a maintained body temperature was 5 mg/kg, an approximate 60-fold decrease in efficacy compared to the group where the temperature fall was not prevented. Pretreatment of the mice with methylxanthines for 10 min prior to the administration of adenosine analogues likewise reversed the drug-induced effects on body temperature, locomotion and seizure-susceptibility. Theophylline (50 mg/kg, i.p.) pretreatment almost completely abolished the protection rendered by 0.5 mg/kg NECA against clonic seizures in DBA/2 mice (Table 1), while at the same time restoring spontaneous locomotion and partially preventing the reduction in body temperature.

Another adenosine analogue, 2-chloro-adenosine, also provided a dose-dependent (1–4 mg/kg; i.p.) protection against sound-induced seizures (Table 1 for highest dose). As with NECA, the anticonvulsant activity was accompanied by a significant reduction in body temperature and spontaneous locomotion.

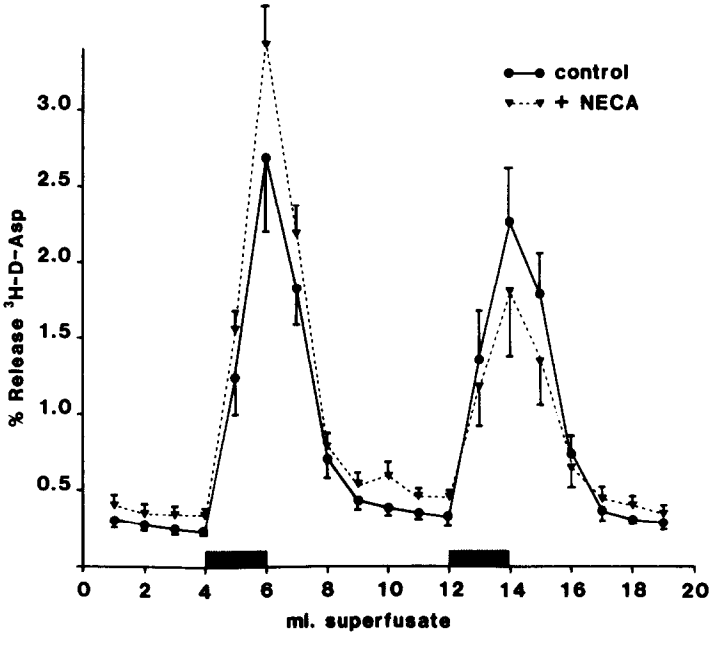

Fig. 2. Inhibitory effect of NECA on potassium-evoked release of ^{3}H-D-aspartate from preloaded rat hippocampal slices. The bars along the abscissa indicate the two potassium (50 mM) pulses. The solid line represents control fractional release, and the dotted line represents release in the presence of 100 μM NECA during the second pulse. The values are expressed as mean ± S.E.M. (indicated by error-bars); N = 8.

Table 2

Adenosine compound	N	Evoked release ratio (S_2/S_1) Control	N	+adenosine compound
Adenosine, 0.01 mM	7	0.79 ± 0.039	8	0.66 ± 0.033*
Adenosine, 1 mM	8	0.91 ± 0.057	8	0.72 ± 0.073*
2-Cl-adenosine, 0.1 mM	7	0.78 ± 0.051	7	0.49 ± 0.058***
2-Cl-adenosine, 1 mM	8	0.80 ± 0.049	8	0.54 ± 0.027***
NECA, 0.1 mM	8	0.94 ± 0.047	8	0.65 ± 0.101*
Theophylline, 1 mM	9	0.86 ± 0.027	8	0.81 ± 0.037
Theophylline, 1 mM, + adenosine, 0.01 mM	7	0.78 ± 0.039	7	0.63 ± 0.037**

Asterisks denote statistically significant differences between experimental groups and matched control groups as in Table 1.

Warming the animals to prevent the temperature reduction, or pretreating the animals with aminophylline (50 mg/kg; i.p.) reversed the 2-chloroadenosine inhibition of sound-induced seizures and restored locomotion and body temperature (Table 1).

Inhibition of D-aspartate release by adenosine and adenosine-analogues

When ^{3}H-D-aspartate release from hippocampal slices was evoked by elevation of the potassium concentration in the perfusion medium to 50 mM in two sequential pulses, the second evoked release was, as has been routinely observed in previous studies, smaller than the first (Fig. 2). Expressed as fractional release (amount of ^{3}H-D-aspartate released as a percentage of radioactivity remaining in the tissue) the ratio of the evoked release peaks [S_2/S_1] in control hippocampal tissue was 0.84 ± 0.018 (Table 2). Adenosine or adenosine analogues present in the perfusion medium during the second evoked release significantly inhibited the release of D-aspartate from preloaded hippocampal slices when compared to matched drug-free controls (Table 2). Adenosine at a concentration of 10 μM reduced the S_2/S_1 ratio to 0.66 ± 0.033. No further reduction of D-aspartate release was observed when the adenosine concentration was increased to 1 mM. NECA (100 μM; Fig. 2) or 2-chloroadenosine (100 μM or 1 mM) likewise significantly reduced the S_2/S_1 evoked release ratio. Theophylline alone (1 mM) had no effect on the hippocampal D-aspartate release, nor did it reverse the adenosine (10 μM) induced inhibition of the aspartate release (Table 2). Cortical D-aspartate release was similarly inhibited by 2-chloro-adenosine (100 μM; data not shown).

DISCUSSION

Adenosine is metabolically unstable, and following its administration it is rapidly removed from plasma by adenosine kinase-catalysed uptake systems and by deamination to inosine [1, 21, 23]. A massive dose (130 mg/kg) of adenosine is therefore required to produce an anticonvulsant effect against audiogenic seizures in mice [9]. Under these conditions the vasodilatory properties of adenosine and the metabolite inosine result in a substantial [40–50%] decrease in the blood pressure [9, 21] and a prolonged sedative effect [9]. When adenosine is derivatized in the 2′ (as in 2-chloro-adenosine) or 6′ position of the purine moiety it becomes unavailable for deamination to inosine. When derivatized in the 5′ position of the ribose moiety (as in NECA) adenosine is no longer a substrate for adenosine kinase. These metabolically stable adenosine derivatives are therefore much more potent in producing the physiological effect analogous to adenosine.

The anticonvulsant action of adenosine-analogues against sound-induced seizures in DBA/2 mice (group A with a spontaneous temperature fall) observed in the present study is in agreement with previously published *in vivo* and *in vitro* evidence for the anticonvulsant activity of these compounds. Adenosine and adenosine analogues inhibit spontaneous firing of cortical neurons following their iontophoretic application [15] and this inhibition is potentiated by the anticonvulsant drug, diphenylhydantoin [29]. Adenosine also inhibits penicillin-induced interictal spiking in hippocampal slices [8, 30] and the evoked response from CA_1 region of hippocampal slices [31]. These effects which are attributed to action at the A_1 receptor [17, 32] are blocked by the adenosine antagonist, theophylline or by other methylxanthines [8, 30, 33]. Methylxanthines also reverse the anticonvulsant action of adenosine analogues against pentylenetetrazole-induced or kindled seizures [11, 13]. The protection against sound-induced seizures in DBA/2 mice by adenosine analogues is likewise reversed by methylxanthines in the present study. The incidence of sound-induced clonic seizures is already maximal (100%) under control conditions, so it is not possible to demonstrate if the methylxanthines alone have a proconvulsant effect, as has previously been reported [10, 12, 14].

However, the most striking observation is a significant reversal of the anticonvulsant potency of 2-chloro-adenosine or NECA against sound-induced seizures merely by warming the mice to prevent the drug-induced temperature decrease. The anticonvulsant potency of NECA was reduced 60-fold under these conditions. The hypothermia induced by anticonvulsant doses of adenosine analogues has previously been described (but not reversed) [10]. It is also known that a decrease in body temperature to below approximately 32–33° reduces the seizure

susceptibility in audiogenic mice [34], which is comparable to the NECA-induced temperature decrease observed in the present study. Maintaining the body temperature also partially reverses the sedation and the inhibitory effects of adenosine analogues on locomotor activity. It therefore seems probable that the anticonvulsant action of adenosine analogues is partially a secondary consequence of the systemic effects of these compounds on body temperature and circulation.

Adenosine and adenosine analogues can modulate transmitter action by inhibiting release of acetylcholine and noradrenaline [23, 35]. Excitatory amino acid transmitter release is also inhibited by adenosine analogues: potassium-evoked glutamate release is inhibited by 5 μM 2-chloro-adenosine in rat dentate gyrus slices [18], and 300 μM adenosine inhibits stimulation-induced hippocampal release of aspartate and glutamate [19]. Stimulation-induced release of labelled glutamate from hippocampal slices is also inhibited by 1–10 μM 1-phenylisopropyl-adenosine [20].

In the present study we observe an inhibition of potassium-evoked D-aspartate release from rap hippocampal (or cortical) slices by adenosine (10 μM–1 mM) or by 100 μM concentration of 2-chloroadenosine or NECA. In contrast to previous reports [18, 20] theophylline neither reverses the inhibition of the adenosine analogues, nor potentiates the amino acid release on its own. The reason for this discrepancy remains to be established.

It is difficult to assess the physiological significance of the anticonvulsant action of adenosine. The brain level of adenosine under control conditions is approximately 1 nmoles/g cortex or 1 μM [21]. During different types of experimental seizures transient 3–30-fold increases in adenosine levels have been reported [5] resulting in cortical adenosine concentrations during seizures in the range of 3–30 μM. This rise would be sufficient to affect D-aspartate release according to the present data or affect neuronal firing according to previous studies, where it has been suggested that modulation of endogenous adenosine levels may regulate hippocampal firing [8, 16]. However, convulsions are known to be sustained despite the observed rise in brain adenosine levels [4]. Seizure activity is normally associated with a rise in body temperature, therefore the anticonvulsant action of endogenous adenosine would be further diminished (the ED_{50} value for the NECA inhibition of sound-induced clonic seizures at 37° is approximately 5 mg/kg or 17 μM), since the present results demonstrate a strong temperature-dependence of the anticonvulsant effect of adenosine or adenosine analogues.

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