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ADENOSINE INHIBITION OF γ-AMINOBUTYRIC ACID RELEASE FROM SLICES OF RAT CEREBRAL CORTEX

CAROLYN HOLLINS & TREVOR W. STONE

Department of Physiology, St. George's Hospital Medical School, University of London, London SW17 0RE

1 The effect of purine compounds on the potassium-evoked release of ^{14}C-labelled γ-aminobutyric acid (GABA) has been studied in 400 μm slices of rat cerebral cortex *in vitro*.

2 Adenosine and adenosine 5′ monophosphate (AMP) inhibited the release of GABA at 10^{-5} to 10^{-3} M. Adenosine triphosphate (ATP) produced a significant inhibition of release only at 10^{-3} M.

3 Theophylline 10^{-4} or 10^{-3} M reduced the inhibitory effect of adenosine, but did not change basal release of GABA.

4 Dipyridamole 10^{-5} M itself reduced evoked GABA release, but did not prevent the inhibitory effect of adenosine, implying that adenosine was acting at an extracellularly directed receptor.

5 Calcium removal or antagonism by verapamil reduced the evoked release of GABA, but adenosine did not produce any further reduction of the calcium-independent release. This may indicate that the inhibitory effect of adenosine on GABA release results from interference with calcium influx or availability within the terminals.

Introduction

Adenosine and its nucleotides, adenosine 5′ monophosphate (AMP) and triphosphate (ATP) probably have important functions in the central nervous system either as neurotransmitters, or in a more generalised neuromodulatory role (Burnstock, 1972; 1975; McIlwain, 1972; 1977; Stone, 1978). One of the effects of these compounds which has received much attention to date is their ability to inhibit transmitter release from nerve terminals. This action has been studied at both cholinergic (Ginsborg & Hirst, 1972; Ribeiro & Walker, 1975; Sawynok & Jhamandas, 1976; Vizi & Knoll, 1976; Gustafsson, Hedqvist, Fredholm & Lundgren, 1978) and adrenergic (Hedqvist & Fredholm, 1976; Clanachan, Johns & Paton, 1977; Enero & Saidman, 1977; Verhaeghe, Vanhoutte & Shepherd, 1977; Su, 1978; Paton, Bar, Clanachan & Lauzon, 1978) terminals in the peripheral nervous system. It has recently been concluded that adenosine can also reduce acetylcholine release at the synapse between motor axon collaterals and Renshaw cells (Lekić, 1977), and noradrenaline release from terminals in the cerebral cortex *in vivo* (Taylor & Stone, 1980) and *in vitro* (Harms, Wardeh & Mulder, 1978).

What is not clear at present is whether this presynaptic effect is limited to these two classes of neurones, cholinergic and adrenergic, or whether it applies more generally to other transmitter systems in the central nervous system. We have therefore examined the effects of adenosine, AMP and ATP on the release from rat cerebral cortex slices of γ-aminobutyric acid (GABA) which is generally accepted as an important inhibitory transmitter in the central nervous system.

Methods

Male Porton Wistar rats weighing 200 to 350 g were killed by stunning and cervical dislocation. The skull was opened and a section was then made to remove most of the dorsal-lying area of cerebral cortex, which was immediately placed in a solution at 0°C of the following composition (mM): NaCl 124, KCl 5; KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2.2H_2O$ 2.4, $NaHCO_3$ 26, glucose 10 and amino-oxyacetic acid (AOAA), 0.1. When using calcium-free solutions, EGTA was also added at a concentration of 5×10^{-4} M. It has been assumed, in the experiments to be described, that in the presence of AOAA, an inhibitor of GABA transaminase, the released radioactivity was present primarily as GABA. This assumption is consistent with the work of other groups (Bowery & Brown, 1974). The tissue was next transferred to a McIlwain tissue chopper and sagittal slices of cortex were cut at a thickness of 400 μm. Three to six slices weighing approximately 15 mg per slice were placed

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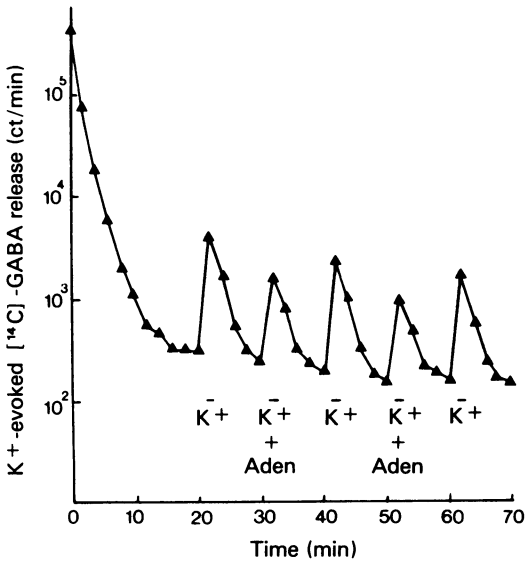

Figure 1 An example of the potassium-evoked [^{14}C]-GABA release in a typical experiment using slices of rat cerebral cortex. Release is represented on the ordinate on a logarithmic scale. Samples were changed every 2 min, beginning at time 0 on the abscissa scale, after incubation for 20 min with 1 μCi (5 nmol) of [^{14}C]-GABA. Depolarization was achieved by including potassium in the samples indicated to a final concentration of 36 mM. In the samples indicated, Aden, adenosine 10^{-3} M was also included. Note both the consistency of the potassium evoked release, and the reduction of this release by adenosine.

on a nylon mesh in glass incubation chambers of 2.5 ml capacity containing bathing fluid at 37°C, gassed with 95% O_2 and 5% CO_2. The solution in the chambers also contained 1 μCi of [^{14}C]-GABA (Radiochemical Centre, Amersham) specific activity 200 mCi/mmol. After incubation for 20 min the incubating solution was withdrawn and replaced with a further 2.5 ml of gassed medium at 37°C every 2 min. The exchange of medium was effected by hypodermic syringes, via inlet and outlet arms in the base of the incubation chamber.

Release of labelled GABA taken up by the slices was effected by adding 30 mM potassium to every sixth sample (final concentration of potassium 36 mM). Five or six tests were made in each experiment, and two experiments were always conducted in parallel. Adenosine and related compounds were added to alternate tests in early experiments or were bracketed by tests with potassium alone in later experiments. The latter method gave more consistent results, since GABA release evoked by successive potassiuum pulses alone tended to decrease with time. This decrease was not marked, however, and the difference between the first and sixth tests of a control potassium series was rarely more than about 20% (Figure 1).

Samples of the radioactive medium that were withdrawn were mixed with scintillation fluid (60% toluene, 40% ethyleneglycol methylether, containing 8 g butyl-PBD (2-4-*t*-butylphenyl) 5-(4″-biphenyl)-1,3,4-oxadiazole) and 80 g naphthalene) and counted in a liquid scintillation counter. Counting efficiency was estimated at 94% (mean) by comparison with a known sample counted on a fully open window. Results were calculated as the ratio of radioactivity in the high potassium samples to that in the immediately preceding sample and the mean and standard errors of this ratio were then calculated for each experimental condition. The mean ratio for the potassium alone tests was then set at 100 and the mean release under the various test conditions expressed as a percentage of this.

In experiments in which theophylline and dipyridamole were used, these compounds were added to the medium throughout the efflux. Adenosine, AMP and

Table 1 The effects of purines on the potassium-evoked release of [^{14}C]-GABA from slices of cerebral cortex

Purine	*Concentration* (M) 10^{-6}	10^{-5}	10^{-4}	10^{-3}
Adenosine	95.4 ± 2.9 (4)	91.0 ± 2.2* (6)	83.6 ± 3.0* (10)	65.5 ± 1.8* (8)
AMP	96.2 ± 3.6 (4)	91.8 ± 3.2* (6)	86.7 ± 2.1* (6)	68.1 ± 2.7* (5)
ATP	99.1 ± 3.1 (3)	97.3 ± 3.4 (3)	92.0 ± 2.1 (6)	83.9 ± 4.1* (6)

Release is expressed as a percentage of that obtained with potassium alone for which the mean release has been scaled to 100 (±2.8, s.e; $n = 32$).
Results are shown as mean ± s.e. mean (n experiments).
* Significantly different from potassium controls at $P < 0.05$, using one-tailed Student's t test and assuming normal distribution of results.

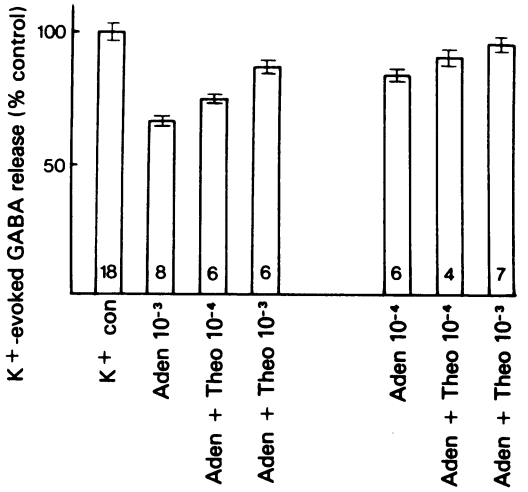

Figure 2 Histogram showing the reduction of potassium evoked [^{14}C]-GABA release, from slices of cerebral cortex, by adenosine 10^{-3} M and 10^{-4} M, and the reduction of that inhibition by theophylline at 10^{-3} and 10^{-4} M. The effective concentration of potassium used was 36 mM and samples were collected over 2 min periods. The mean potassium-evoked release has been set at 100 and release obtained under the various test conditions expressed as a percentage of this. The columns show the mean values and vertical lines show s.e. mean and the numbers within the columns show the number of experimental tests.

ATP were obtained from Sigma Chemical Co., dipyridamole and verapamil were gifts from Boehringer Ingelheim and Abbott Laboratories, respectively.

Results

As illustrated by the result of a typical experiment shown in Figure 1, adenosine reduced the release of GABA evoked by elevated potassium levels. The inhibition was not significant at 10^{-6} M adenosine (one-tailed Student's *t* test assuming a normal distribution of results), but ranged from 9 percent of control at 10^{-5} M to 34.5% at 10^{-3} M (Table 1). Adenosine 5′ monophosphate (AMP) was almost as effective as adenosine in the same concentration range, while adenosine triphosphate (ATP) was much less effective, producing a significant depression of release only at 10^{-3} M, of 16.1% (Table 1).

None of the compounds used in these experiments, including adenosine, AMP, ATP, theophylline and dipyridamole, affected the basal spontaneous release of GABA.

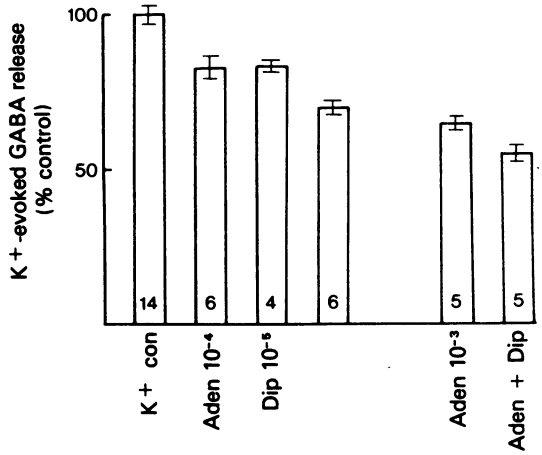

Figure 3 Histogram showing the effect on K^+-evoked [^{14}C]-GABA release of adenosine 10^{-4} M and 10^{-3} M alone and in the presence of dipyridamole 10^{-5} M. Experimental detail as in Methods and Figure 2. Columns show the mean values and vertical lines show s.e. mean; figures within the column are the number of experimental tests. Note the inhibitory effect of dipyridamole alone, and the additive inhibition produced by dipyridamole and adenosine.

Theophylline

The presence of theophylline at 10^{-4} M produced some reduction of the adenosine inhibition, and at 10^{-3} M reduced the inhibition to about one third of its usual value (13%) (Figure 2). Theophylline alone had no effect on the potassium evoked GABA release. The effects of AMP and ATP were also reduced by theophylline.

Dipyridamole

Dipyridamole, 10^{-5} M, present in the bathing fluid throughout the experiment, reduced the potassium evoked GABA release (16.3%). However, the effects of dipyridamole and adenosine at 10^{-4} M or 10^{-3} M, were additive, indicating that the inhibition of cellular uptake of adenosine had not prevented the action of this purine (Figure 3). Responses to AMP and ATP were also additive with dipyridamole.

Calcium removal

The removal of calcium from and inclusion of EGTA in the incubation medium reduced the evoked release of GABA to 34.6% of control. Similarly the presence of the calcium antagonist verapamil in the incubation medium at a concentration of 10^{-4} M reduced the

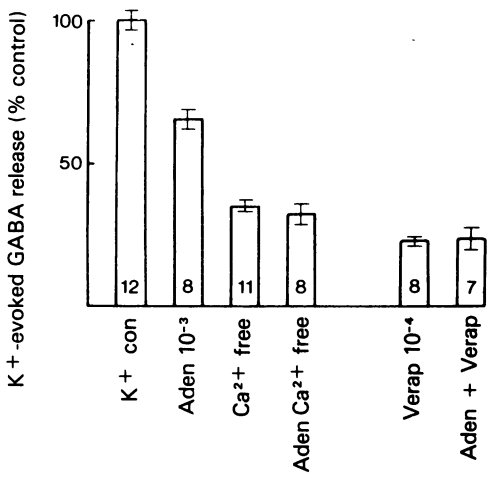

Figure 4 Histograms showing respectively the effects on K^+-evoked [^{14}C]-GABA release of adenosine 10^{-3} M, calcium removal in the presence of EGTA 5×10^{-4} M, adenosine 10^{-3} M in calcium-free medium, verapamil 10^{-4} M and adenosine 10^{-3} M in the presence of verapamil 10^{-4} M. Note that adenosine produces no inhibition of that portion of K^+-evoked GABA release which is not dependent on the presence of calcium. Columns show the mean values; ± vertical lines indicate s.e. mean, and figures within the columns are the number of experimental tests.

release to only 22.5% of control. In neither experimental series however, did adenosine further reduce the calcium-independent release, at either 10^{-4} M or 10^{-3} M (illustrated in Figure 4).

Discussion

It is clear that adenosine, AMP and ATP can inhibit the release of GABA from neurones of the cerebral cortex *in vitro*. This is consistent with the evidence for an inhibition of transmitter release from both peripheral and central cholinergic and adrenergic neurones (Ginsborg & Hirst, 1972; Ribeiro & Walker, 1975; Sawynok & Jhamandas, 1976; Hedqvist & Fredholm, 1976; Vizi & Knoll, 1976; Clanachan *et al.*, 1977; Enero & Saidman, 1977; Gustafsson *et al.*, 1978; Paton *et al.*, 1978).

The ability of theophylline to prevent this effect also suggests that a receptor is involved similar to that which has been found to mediate effects of adenosine on transmitter release in other systems (Ginsborg & Hirst, 1972; Sawynok & Jhamandas, 1976; Verhaeghe *et al.*, 1977; Su, 1978) as well as the effects of adenosine on adenylate cyclase (Sattin & Rall, 1970; Blume, Dalton & Sheppard, 1973; Sattin, Rall & Zanella, 1975) depression of neuronal firing (Phillis & Kostopoulos, 1975; Stone & Taylor, 1977; Taylor & Stone, 1978) and vascular tone (Wahl & Kuschinsky, 1976). Further the antagonism by theophylline and the greater potency of adenosine compared with ATP suggests that the receptor involved is that recently classified as PI by Burnstock (1978). Dipyridamole inhibits membrane transport processes for adenosine, and thus reduces the uptake of adenosine into cells (Subbarao, Rucinski, Rausch, Schmid & Niewiarowski, 1977) and the finding that dipyridamole does not prevent the depression of GABA release by adenosine therefore suggests that the receptor is extracellularly directed, as in other systems (Kuroda, Saito & Kobayashi, 1976; Olsson, Davis, Khouri & Patterson, 1976; Schrader, Nees & Gerlach, 1977; Moritoki, Kaube, Maruoka, Ohara & Ishida, 1978).

The reduction of GABA release produced by dipyridamole alone may be mediated by endogenous adenosine released spontaneously and which is normally removed by dipyridamole-sensitive uptake mechanisms. However, the effective concentration of any such released adenosine is presumably maintained at a subthreshold level as theophylline alone had little effect on GABA release. This explanation is supported by the observation that in two experiments in which theophylline (10^{-3} M) was present, dipyridamole alone did not reduce GABA release.

The failure of calcium removal or verapamil to eliminate completely the potassium-evoked release of GABA has been noted previously (Srinivasan, Neal & Mitchell, 1969) and has recently been discussed in detail by Haycock, Levy, Denner & Cotman (1978). The latter group concluded that the calcium-independent release was a reflection of membrane transport processes for GABA not directly related to excitation-secretion coupling. As adenosine no longer produced any reduction of the calcium-independent GABA release, the implication may be that the effect of adenosine is mediated by an interference with calcium influx or availability within the synaptic terminal, a suggestion which has been made by previous groups (Schrader, Rubio & Berne, 1975; Kazic & Milosavljević, 1976; Su, 1978).

Having discussed the ability of adenosine to inhibit potassium-evoked GABA release, it must be emphasized that this action seems to be much weaker than the inhibition of noradrenaline release from the same preparation reported by Harms *et al.* (1978). Thus, while the latter group observed decreases of noradrenaline release ranging from 20% at 10^{-7} M adenosine to 34% at 10^{-4} M adenosine, the statistically significant depression of GABA release in the present work seems to vary from 9% at 10^{-5} M to 35% at 10^{-3} M.

This difference may partly explain recently observed electrophysiological differences in the effects of adenosine which can depress transmitter release from noradrenergic axons ascending from locus coer-

uleus (Taylor & Stone, 1980) more readily than it appears to affect local inhibition in the cerebral cortex (Stone & Taylor, unpublished observations) a phenomenon which is probably mediated by GABA (Dreifuss, Kelly & Krnjević, 1969). The inhibition of transmitter release by adenosine, which would seem to be of some general importance for central neurones irrespective of their transmitter, may therefore involve a degree of selectivity which may permit a differential modulation of different neuronal pathways.

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