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DEPRESSION OF EVOKED POTENTIALS IN BRAIN SLICES BY ADENOSINE COMPOUNDS

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1 A study has been made of the action of adenosine on surface slices of guinea-pig olfactory cortex *in vitro*.

2 With extracellular recordings from the pial surface and stimulation of the presynaptic input, the lateral olfactory tract (LOT) generated a monosynaptic negative wave representing dendritic excitatory potentials. This negative wave was depressed by bath application of 1 μM adenosine with increasing effect up to 1 mM. Adenosine 5'-triphosphate (ATP), adenosine 5'-monophosphate (AMP) and cyclic adenosine 3',5'-monophosphate (cyclic AMP) had similar depressant actions. Adenine and guanosine were very weak depressants.

3 Theophylline concentrations in the range 10 μM to 3 mM progressively antagonized the action of adenosine.

4 Dibutyryl cyclic AMP (100 μM) and agents which increase intracellular cyclic AMP were not depressants, suggesting that the action of adenosine was not cyclic AMP-mediated.

5 Intracellular recordings confirmed the depressant effect of adenosine on excitatory potentials generated by LOT stimulation and also showed that postsynaptic action potentials and the membrane of the soma were unaffected by adenosine.

6 Since presynaptic action potentials were also unaffected by adenosine, these experiments suggest that adenosine reduces excitatory transmission at LOT synapses and fortifies the idea that adenosine has a 'neurohumoral' action.

Introduction

Adenosine 5'-triphosphate (ATP) is released from non-adrenergic nerve terminals of the autonomic nervous system and produces post-synaptic inhibition (see Burnstock, 1972). Pull & McIlwain (1972) have demonstrated that substantial amounts of adenine compounds are released from brain slices on electrical stimulation suggesting that purines might also have some kind of neurotransmitter role in the brain. This view is supported by the experiments of Okada (1975) and those in the present study, where adenine nucleotides are shown to depress synaptic activity in the olfactory cortex *in vitro* by previously described techniques (Yamomoto & McIlwain, 1966; Richards & Sercombe, 1970; Harvey, Scholfield & Brown, 1974). Further experiments were performed to test whether agents which increase intracellular adenosine 5'-monophosphate (AMP) might mimic the action of these nucleotides. A preliminary account of this work has been presented (Scholfield, 1974).

Methods

Guinea-pigs of either sex and weighing 290–500 g were decapitated, the brain removed and a large section cut out to include the lateral olfactory tract (LOT), the olfactory cortex and amygdala. Using a bow cutter and guide of 600 μM thickness, a surface slice was cut (see Figure 1 of Harvey, Scholfield, Graham & Aprison, 1975). These slices were placed in Krebs solution equilibrated with 95% O_2 and 5% CO_2 at room temperature (24°C). In 2 experiments, slices were taken from rat olfactory cortex in a similar manner. After 2–4 h, the slice was placed on a nylon mesh within a 1.5 ml recording bath. Krebs solution flowed over the slice at more than 1 ml/min, the fluid in the chamber being kept level with the nylon mesh. A pair of platinum stimulating electrodes was placed across the LOT about 1 mm away from the severed, rostral end. An Ag/AgCl recording electrode touched the pial surface (uppermost) of the periamygdaloid area of the slice via a balsa wood wick (position 6

in Figure 1 of Harvey *et al.*, 1974). Another similar electrode was placed on the LOT 6 mm away from the stimulating electrodes. An earthed Ag/AgCl electrode was placed in the subjacent bathing solution. These electrodes were connected directly or via a capacitor (time-constant 1 s) to an oscilloscope or chart recorder (frequency response 1 to 75 Hz) through a preamplifier (input resistance 10^{11} ohm). Single, supramaximal stimuli (0.2 ms long and about 10 V) were delivered to the LOT at less than 0.1 hertz. All drugs were prepared in Krebs solution. The tissue was allowed to equilibrate with purines for 5 min and with other drugs for 30 min before experimentation. The drug effects are expressed as a change in the maximum amplitude of the recorded waves from those obtained immediately before drug addition and after recovery therefrom. Dose-response curves to purines (usually adenosine) were constructed before, during and 2 h after removal of theophylline. A limited number of intracellular recordings were made from neurones in the periamygdaloid cortex by the procedure in Scholfield (1978a). Neurones were impaled with single barrelled micro-electrodes filled with 4 M potassium acetate connected to an amplifier containing a current source for injecting current into the cells. Recordings were made with a digital storage oscilloscope (Advance OS 4000) and an X-Y plotter.

The composition of the Krebs solution was (mM):— Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 129, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.1 and D-glucose 11.

The drugs used were: adenosine (Sigma); adenosine 5′-triphosphate (ATP) grade II, disodium salt with 2.5 mol of water per mol (Sigma); adenosine 5′-monophosphate from yeast, Na salt, Type II (Sigma); adenine (Sigma); adenosine 3′,5′-cyclic monophosphoric acid, crystalline (Sigma); N^6,O^2-dibutyryl adenosine 3′,5′-cyclic monophosphoric acid (dibutyryl cyclic AMP), monosodium salt (98% purity), contaminant mainly the monobutyryl derivatives (Sigma); guanosine, with 1% ATP as an impurity (Sigma); theophylline anhydrous (Sigma); histamine dichloride (Sigma) and noradrenaline bitartrate (Nutritional Biochemical Corp.).

None of these drugs produced measurable changes in the pH of the solutions.

Results

Extracellular recordings

A single supramaximal stimulus to the lateral olfactory tract (LOT) produced a negative synaptic wave (N-wave) that could be recorded from the pial surface of the slice (Figure 1); this represents the synchronous depolarization (excitatory postsynaptic potential—

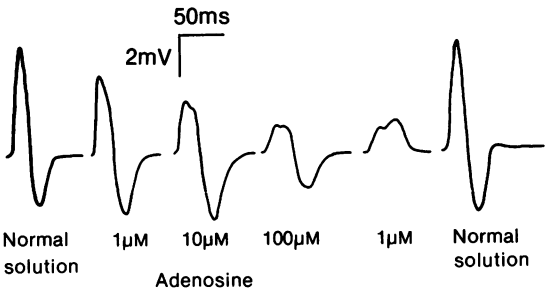

Figure 1 Potentials evoked from the periamygdaloid cortex upon delivering single supramaximal stimuli to the lateral olfactory tract in the presence of different concentrations of adenosine.

e.p.s.p.) in the superficial dendrites (Biedenbach & Stevens, 1969; Richards & Sercombe, 1970; Halliwell, 1976). In the periamygdaloid cortex, the N-wave is followed by a pial-surface positive wave (P-wave) (Figure 1) (Harvey *et al.*, 1974) representing polysynaptic e.p.s.ps generated in the deeper layers of the slice (Halliwell, 1976). The term 'N-wave' is retained to distinguish it from the e.p.s.p. recorded intracellularly from the somas (see below).

Adenosine (1 to 100 μM) produced a dose-related depression of the N-wave; at concentrations above 100 μM little further depression in the N-wave was observed (Figure 1). The depression of the N-wave was rapid in onset and did not show any fade during exposure (Figure 2). The recovery from adenosine action required 1 to 8 min after concentrations of up to 100 μM and 1 to 2 h after 1.0 or 10 mM applications. In contrast, the P-wave was less sensitive than the N-wave to the depressant actions of adenosine (Figure 1).

Intracellular recordings

Neurones of the periamygdaloid cortex had similar properties to neurones in the prepyriform cortex (Scholfield, 1978a & b). Whilst recording intracellularly from a neurone in the periamygdaloid cortex, stimulation of the LOT elicited the following potentials: an e.p.s.p. of about 25 mV (which usually generated an action potential) followed by a prolonged, low amplitude depolarization which had the properties of an inhibitory post-synaptic potential (i.p.s.p.) (Figure 3). It is difficult to estimate drug effects on supra-threshold e.p.s.ps since their true height is unknown. This is because the peak of the e.p.s.p. passes into the low resistance part of the membrane current-voltage curve (Scholfield, 1978a). To study the action of adenosine, 5 neurones were selected that generated orthodromic spikes of long latency or no spike, leaving some initial e.p.s.ps free of spike. This avoided

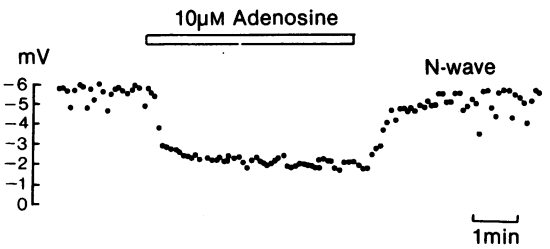

Figure 2 Time courses of adenosine action on the peak amplitude of the N-wave. During the period marked by the bar, adenosine (10 μM) was added to the superfusate. The dead space in the system caused a 10 s delay.

the use of lower LOT stimulus voltages where possible changes in axonal excitability might affect the response.

Figure 3 shows an orthodromic response where the e.p.s.p. amplitude was not quite sufficient to evoke a spike. With the lower concentrations of adenosine (10 to 100 μM), the early part of the e.p.s.p. was depressed (rather than the latency of the e.p.s.p. being increased). The depression of the e.p.s.p. by 1 μM adenosine was very small; in Figure 3, it is negligible, cf. the effect on the N-wave (Figure 1). At the higher adenosine concentrations all of the e.p.s.p. was depressed and also the i.p.s.p. (Figure 3). Adenosine (1 μM to 10 mM) had no effect on the resting input resistance and membrane potential.

Because of the difficulty in measuring the intracellular e.p.s.p., further experimentation on the action of adenosine and its analogues was performed with extracellular recording methods.

Action of other purines

AMP, ATP and cyclic AMP (1 μM to 1 mM) in the bathing solution depressed the extracellularly recorded synaptic potentials in a manner indistinguishable from the depression produced by adenosine (Figure 4), whereas adenine (the base of adenosine) was virtually ineffective. The depressant action of guanosine at lower concentrations was much less than that of the adenine nucleotides (limited solubility dictated the highest concentrations) (Figure 4). The depressant action of cyclic AMP is probably mimicking adenosine action, rather than penetrating the cells and acting on cell metabolism (i.e., an extracellularly directed action).

Effect of agents influencing intracellular cyclic AMP

The concentrations of purines used in the above experiments are capable of increasing intracellular cyclic AMP in cortical brain slices (Sattin & Rall, 1970).

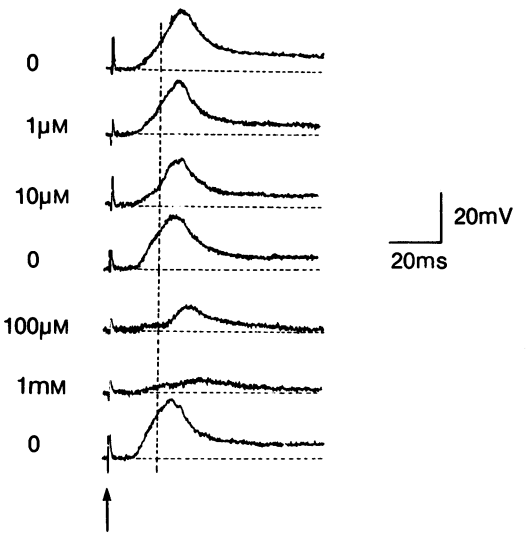

Figure 3 Intracellular recordings from a neurone in the periamygdaloid cortex showing the depression of the e.p.s.p. by adenosine. In this cell, the e.p.s.p. was not quite sufficient to evoke a spike. Adenosine was added to the superfusate at 1, 10, 100 and 1000 μM. The point of lateral olfactory tract stimulation is marked by the arrow and the horizontal dashed lines are the extrapolations of the resting membrane potential. The vertical dashed line is added as a guide to the most sensitive part of the e.p.s.p. to adenosine.

Thus, the depressant action of adenosine might be mediated through increased intracellular cyclic AMP. If so, then increasing intracellular cyclic AMP by other methods might produce a similar depression. Histamine (0.1 mM) alone or with noradrenaline (0.1 mM) can produce substantial increases in intracellular cyclic AMP in neocortical slices (Kakeuchi & Rall, 1968; Schultz, 1974). However, in the present experiments, application of 0.1 mM histamine alone or with 0.1 mM noradrenaline failed to inhibit any of the potentials with exposures of up to 60 minutes. The depressant action of adenosine (0.1 to 100 μM) was unchanged in the presence of these drugs.

Action of dibutyryl cyclic AMP

Extracellularly applied dibutyryl cyclic AMP (about 100 μM) penetrates cells and mimics the action of intracellular cyclic AMP (Robison, Butcher & Sutherland, 1971). The application of 0.1 to 100 μM dibutyryl cyclic AMP failed to produce any depression of the synaptic potentials (Figure 4). At 100 μM dibutyryl cyclic AMP there was a modest *increase* in the amplitude of the N-wave instead of a depression (Figure

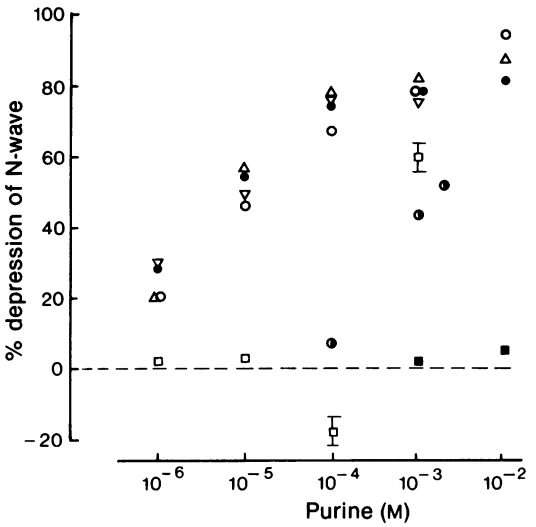

Figure 4 Dose-depression curves for the N-wave with the various purines, adenosine (●), ATP (○), AMP (△), cyclic AMP (▽), dibutyryl cyclic AMP (□), guanosine (◑) and adenine (■). Ordinate scale: the depression of the N-wave amplitude as a % of the amplitude in normal solution. Abscissa scale: concentrations of purine, molar (M). The points represent means of experiments on 2–19 slices except for adenine and guanosine which are single points. Where given, the bars are the standard errors. Standard errors varied between ±0.3% (for 1 μM ATP) to ±5.5% (for 10 mM adenosine).

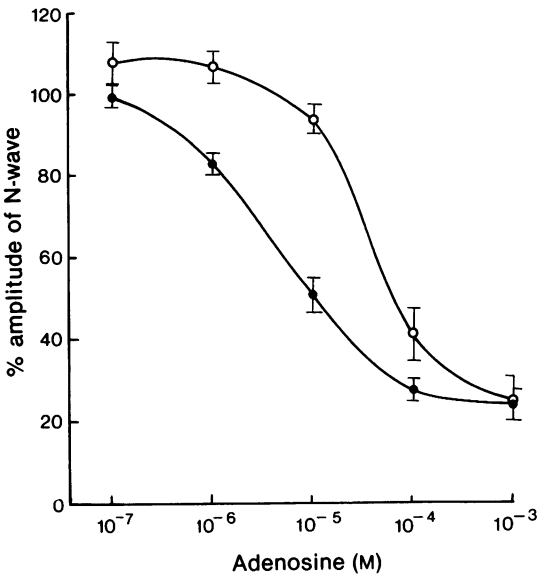

Figure 5 The effect of various concentrations of adenosine in the bathing solution (abscissa scale) alone (●) and in the presence of theophylline 0.3 mM (○). The ordinate scale is the maximum height of the N-wave in the presence of adenosine, expressed as a percentage of the potential's height in normal Krebs solution immediately before and after the action of adenosine. Bars are s.e. means of 10 slices.

4). Dibutyryl cyclic AMP 1 mM produced about 60% depression of the N-wave (Figure 4).

Theophylline action

Theophylline increases intracellular cyclic AMP by antagonizing phosphodiesterase (Robison *et al.*, 1971). It also blocks the effects of adenosine on cyclic AMP stimulation (Sattin & Rall, 1970) presumably by an extracellularly directed action. Theophylline 'antagonized' the depression of the N-wave by adenosine (Figure 5). However, the dose-response curve for adenosine was shifted to the right in a non-parallel fashion and the responses to 0.1 and 1.0 μM adenosine were reversed from a depression to an apparent facilitation of the N-wave. Theophylline produced this 'antagonism' of the depressant effects of adenosine over the concentration range of 0.01 to 3 mM (Figure 6).

Lack of effect of adenosine on the action potential

Action potentials generated by injecting depolarizing current through the intracellular recording microelectrode were unaffected by adenosine up to 10 mM (Figure 7a). This figure also demonstrates that 10 mM adenosine had no effect on membrane resistance (as judged by the rising phase of the current-induced depolarization) and membrane potential (as judged by the amplitude of the action potential).

To assess the effect of adenosine on the presynaptic action potential, two approaches were used:— (1) In extracellular surface recordings from the LOT, the N-wave is preceded by a positive–negative transient due to the synchonous discharge of presynaptic LOT action potentials. It is difficult to measure the LOT action potential because it merges with the following N-wave. So the effect of adenosine was studied in thin slices (310 μm thick) where the N-wave is small (Scholfield & Harvey, 1975). Adenosine (1 μM to 10 mM) had no effect on this compound action potential. (2) The LOT action potential is generated mostly from activity of non-synapsing tract axons. Synapsing axons pass over the surface of the periamygdaloid cortex. In 2 slices of rat olfactory cortex a small but distinct compound action potential could be recorded from these fibres, (but not in the guinea-pig olfactory cortex) (Figure 7b). Adenosine (10 μM to 10 mM) had no effect on this action potential (Figure 7b).

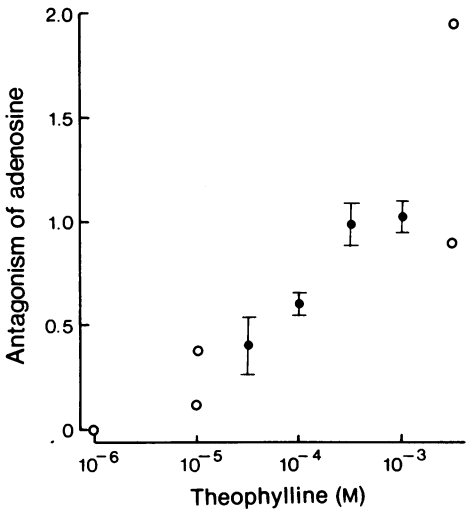

Figure 6 The antagonism by various concentrations of theophylline of the depression of the N-wave induced by adenosine. The dose of adenosine causing a 50% depression in the N-wave (ID_{50}) was measured in experiments similar to that shown in Figure 4. Ordinate scale: the logarithm of the ratio of the ID_{50} for adenosine with theophylline, to the ID_{50} for adenosine alone. Open circles represent individual measurements and bars are s.e. means of experiments from 3–11 slices.

Discussion

Adenosine exerts a strong depressant action on synaptic transmission in the olfactory cortex. These results are in accord with the depressant action of adenosine compounds in the cerebral cortex (Phillis, Kostopoulos & Limacher, 1975). Pull & McIlwain (1972) calculated that the extracellular concentrations of adenosine would be about 100 μM during electrical stimulation of brain slices. If this concentration of endogenous adenosine produced a comparable effect to that of bath-applied adenosine, then adenosine or an analogue could have a neurohumoral role in brain.

Since adenosine did not appear to change the presynaptic spike or postsynaptic membrane resistance of the soma, adenosine apparently acts somewhere in the vicinity of the LOT synapses onto dendrites. Adenosine could reduce the release of excitatory transmitter as is the case at the neuromuscular junction (Ribeiro & Walker, 1975) and in the ileum (Mori, Yamada & Hayashi, 1975). However, the mechanism of adenosine action is not clear. A cyclic AMP-mediated effect seems unlikely since conditions which increase, or mimic intracellular cyclic AMP did not act like adenosine.

There were two conditions where the N-wave was apparently facilitated: (i) with 100 μM dibutyryl cyclic AMP and (ii) with the adenosine-theophylline com-

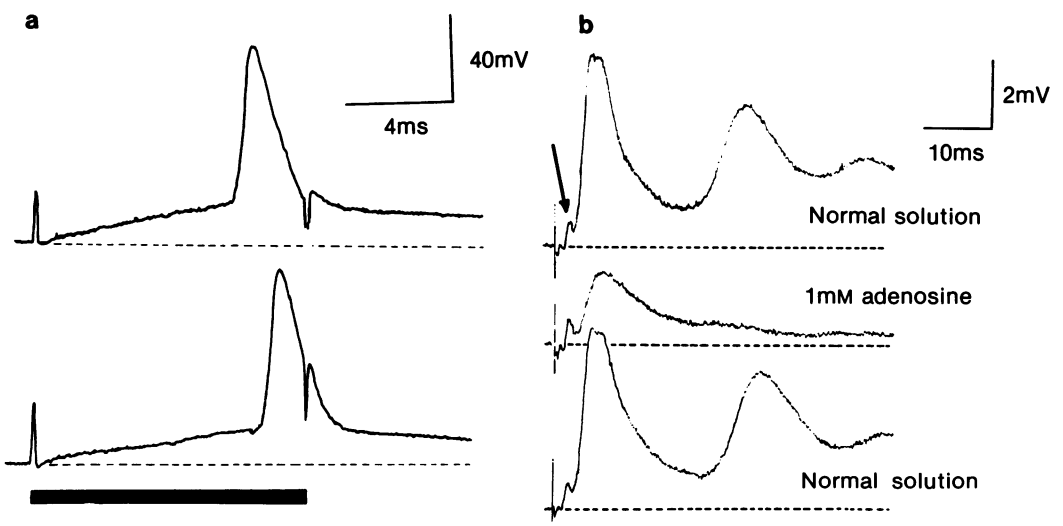

Figure 7 (a) Intracellular recording of a depolarization and action potential generated by passing +0.6 nA of current through the recording electrode during the period marked by the filled bar. The upper trace is in normal solution and the lower trace is in adenosine 10 mM. The depolarizing and hyperpolarizing transients at the beginning and end of the current pulses are current switching artefacts. (b) This is an extracellular recording from the pial surface of a rat olfactory cortex where a small action potential was observed on the periamygdaloid cortex away from the lateral olfactory tract (marked by arrow). The slice was superfused sequentially with (a) normal Krebs solution, (b) adenosine 1 mM for 5 min and (c) normal Krebs solution for 60 minutes. Voltage calibration, 2 mV; time scale, 10 milliseconds.

bination. Both of these effects might involve the intracellular cyclic AMP system, with dibutyryl cyclic AMP mimicking cyclic AMP and with theophylline inhibiting phosphodiesterase, thus reducing the hydrolysis of cyclic AMP stimulated by adenosine. This facilitation of transmission with increased intracellular cyclic AMP is similar to the effect of cyclic AMP stimulation in the neuromuscular junction (Miyamoto & Beckenridge, 1974).

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