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ADENINE NUCLEOTIDES AND SYNAPTIC TRANSMISSION IN THE *in vitro* RAT HIPPOCAMPUS

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1 The effects of adenosine and various derivatives were examined in the *in vitro* hippocampal slice preparation from rat.

2 The amplitudes of extracellularly recorded field potentials from the CA1 region were depressed by adenosine, and this effect could be antagonized by methylxanthines. Because presynaptic field potentials were unaffected by adenosine, while the field e.p.s.p. was depressed, adenosine would appear to act at a synaptic site to depress transmission.

3 Adenosine deaminase, which breaks down adenosine to inosine, increased the amplitude of synaptic responses, while hexobendine, which blocks reuptake of adenosine, had a depressant effect. This strongly suggests that the endogenous release of adenosine from the hippocampal slice preparation is sufficient to exert a tonic inhibitory influence on the amplitude of synaptic responses.

4 Cyclic adenosine 3',5'-monophosphate (cyclic AMP) and its dibutyryl derivative had depressant effects on the amplitude of field responses which were blocked by theophylline, suggesting that they are able to act at the extracellular adenosine receptor. (−)-Isoprenaline (which raises tissue cyclic AMP levels), and the 8-*p*-chlorophenylthio derivative of cyclic AMP both increased the amplitude of population spike responses, and these effects were not blocked by theophylline, suggesting that the physiological effects of adenosine are not mediated via a cyclic AMP-dependent mechanism.

5 Since adenosine is not the transmitter at this CA1 pyramidal cell synapse, but is apparently present in the extracellular compartment in sufficient concentrations to affect the synaptic physiology of this region, this provides strong evidence in favour of the concept of a neuromodulatory role for adenosine in the central nervous system.

Introduction

Recent experiments have focused much attention on adenine nucleotides as possible transmitters or neuromodulators in the mammalian brain. Although much of the work implicating adenosine or related compounds in 'purinergic transmission' has been done with the peripheral nervous system (Burnstock, 1975), the possible relevance to the function of the central nervous system has not been lost on workers in this area. Indeed, adenosine (or related adenine nucleotides) meet many of the criteria for consideration as putative transmitters; these compounds and the enzymes for their synthesis and degradation are ubiquitous in the nervous system, and are released in a calcium-dependent manner by either field stimulation or by activation of discrete synaptic systems (Pull & McIlwain, 1972a & b; Sun, McIlwain & Pull, 1975; Schubert, Lee, West, Deadwyler & Lynch, 1976).

Adenosine and various derivatives have potent depressant actions at many levels of the neuroaxis when administered by microiontophoresis, with the hippocampus, cortex, and caudate being particularly sensitive (Phillis, Kostopoulos & Limacher, 1975; Kostopoulos, Limacher & Phillis, 1975; Kostopoulos & Phillis, 1977; Stone & Taylor, 1978). In experiments using *in vitro* preparations and perfusion with these compounds, a marked reduction in the amplitude of monosynaptic evoked responses has been demonstrated (Kuroda & Kobayashi, 1975; Kuroda Saito & Kobayashi, 1976; Scholfield, 1978).

Nevertheless, a convincing case has yet to be made for adenosine being the sole transmitter at any central synapse. Partly in response to this, and partly because many of the actions of adenosine are seen at synapses where adenosine is clearly *not* the primary transmitter (Ginsborg & Hirst, 1972; Miyamoto & Breckenridge, 1974; Schubert *et al.*, 1976; Hayashi, Mori, Yamada & Kunitomo, 1978), it has come to be regarded more as a putative neuromodulator than as a transmitter *per se* in the central nervous system.

In addition to the powerful electrophysiological actions of adenosine, it is also one of the most potent elevators of cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels in brain tissue (Sattin & Rall,

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1970; Shimizu & Daly, 1970; Schultz, 1975). Because these increases in cyclic AMP can be blocked by a variety of antagonists, primarily methylxanthines (Sattin & Rall, 1970; Huang & Daly, 1972; Kuroda *et al.*, 1976; Mah & Daly, 1976), and because the increases in cyclic AMP can be potentiated by treatments which would be expected to raise *extracellular* adenosine concentrations, such as adenosine uptake blockers (Huang & Daly, 1974), it has been suggested that adenosine may exert its effects via an interaction with an adenylate cyclase-linked receptor located in the cell membrane (Huang & Daly, 1974; Mah & Daly, 1976). In addition to directly increasing cyclic AMP levels, adenosine also potentiates the ability of other amines, most notably noradrenaline (NA), to stimulate cyclic AMP formation.

Thus, adenosine has clearly defined physiological effects, and biochemical evidence suggests an empirically defined 'adenosine receptor' which is coupled to an adenylate cyclase, and which possibly interacts with other amine-sensitive adenylate cyclases.

The pharmacological properties of this biochemically-defined receptor appear similar to those of the receptor responsible for the electrophysiological effects of adenosine as well; methylxanthines block the actions of exogenously applied adenosine (Phillis & Kostopoulos, 1975; Kuroda *et al.*, 1976; Stone & Taylor, 1977; Scholfield, 1978). The ability of adenine nucleotides to stimulate cyclic AMP formation correlates well with their physiological potency (Kuroda *et al.*, 1976), and the synergism observed between adenosine and NA obtains as well when they are applied via microiontophoresis (Stone & Taylor, 1978).

Nevertheless, gaps remain in our knowledge of the manner in which adenosine is acting in the nervous system, particularly in the way in which this nucleoside might be involved in 'normal' function. To demonstrate that exogenously applied adenosine has physiological and biochemical effects on a tissue does not imply that the usual extracellular concentrations are sufficient to produce similar effects. Moreover, there is no conclusive evidence demonstrating that the physiological effects of adenosine are mediated via the biochemically defined receptor.

In this paper we describe electrophysiological experiments using the *in vivo* hippocampal slice preparation which specifically address these issues. To do so, we have considered a series of related questions concerning the role of adenosine in the hippocampus. First, what is the relationship between electrophysiological responses and extracellular nucleotide (or nucleoside) levels? Second, do the structure-activity relationships of agonists and antagonists, and the drug interactions demonstrated for biochemical studies have electrophysiological correlates? Third, do slices synthesize and release sufficient quantities of adenosine agonists to produce electrophysiological effects? Fourth, do these responses appear to be mediated via cyclic AMP, as would be suggested by biochemical experiments? Finally, since adenosine has been suggested as a neuromodulator, does it appear to play a role in the development of any form of synaptic plasticity observed in the hippocampus?

The *in vitro* hippocampal preparation used for these experiments has a variety of advantages over any *in vivo* preparation; it permits the administration of known concentrations of various drugs, stimulation of specific afferent pathways under visual control, and extracellular recording of both action potential population discharge and e.p.s.p. field potentials from pyramidal neurones. Moreover, the hippocampus is known to be very sensitive to adenine nucleotides *in vivo* (Kostopoulos & Phillis, 1977) and exhibits several forms of synaptic plasticity after repetitive stimulation (Lømo, 1966, 1971; Bliss & Lømo, 1973; Alger & Teyler, 1976).

Methods

The experiments were conducted in the *in vitro* slice preparation of rat hippocampus. Male Sprague–Dawley rats weighing between 200 and 300 g obtained from Charles River were decapitated and the hippocampus dissected free of surrounding tissue. Coronal slices were prepared, as described previously (Spencer, Gribkoff, Cottman & Lynch, 1976; Dunwiddie & Lynch, 1978). Slices were cut at 400 μm on a McIlwain tissue chopper and immediately placed in ice-cold medium consisting of (mM) NaCl 124, KCl 4.9, KH_2PO_4 1.2, $MgSO_4$ 2.4, $CaCl_2$ 2.5, $NaHCO_3$ 25.6 and glucose 10; which was pregassed with 95% O_2 and 5% CO_2. Slices were transferred to a recording chamber maintained at 33°C within 5 min, and the fluid level was maintained at or just below the upper surface of the slice.

Twisted nichrome wire stimulation electrodes were placed under visual guidance in the stratum radiatum near the border of CA1–CA2. Stimuli were monophasic 0.1 ms pulses of 5 to 20 V, delivered via an isolation unit. Stimulating electrodes were lowered into the slice until potentials of maximum amplitude were obtained and the voltage was then set so as to evoke a 1 to 5 mV population spike. Recording was done with 2 to 3 MΩ glass micro-electrodes filled with 2 M NaCl which were also placed under visual guidance. Responses were recorded from the CA1 pyramidal cell layer (population spike response) or from the region of synaptic termination (field e.p.s.p.), averaged, and entered into a NOVA 3/12 computer in digital form for subsequent analysis.

Slices were normally maintained without perfusion until they were to be tested at which time they were

transferred to the test chamber and a constant flow of fresh oxygenated pre-heated medium was initiated at a rate of 2 ml/min. Following an equilibration period, testing of response amplitude was begun; usually 2 responses evoked 5 s apart at 1 min intervals were averaged and displayed throughout the test period. More frequent stimulation was avoided to prevent frequency potentiation in the slice. Drugs were made up in medium at 100 to 1000 × the desired final concentration, then added to the flow of perfusion fluid with a calibrated Sage Model 355 syringe pump.

In general, two types of response measures were taken. The population spike response, which represents the summated firing of the CA1 pyramidal neurones (Andersen, Bliss & Skrede, 1966; Andersen, Holmqvist & Voorhoeve, 1971; Lømo, 1971), was recorded from the cell layer. This response measure is sensitive to at least two different types of influences. A change in this response can represent either a change in the degree of synaptic activation (e.p.s.p. amplitude), or an alteration in the intrinsic excitability of the CA1 pyramids. On the other hand, the potential recorded from the dendritic region appears to reflect the currents generated primarily by the excitatory synaptic events (Andersen *et al.*, 1966; Lømo, 1971; Andersen *et al.*, 1971); hence changes in this response measure represent real alterations in the potency of the synapses being activated. However, since this is really a *summated* e.p.s.p. resulting from the activation of a large number of synapses, it is necessary to establish that the number of presynaptic fibres being activated remains relatively constant. Whenever changes were noted in the field e.p.s.p., controls were run to determine whether parallel changes occurred in the presynaptic afferent fibre potential as well. Thus the actions of various agents could be interpreted on the basis of which response measures were affected. If only the population spike response changed, the drug was affecting the excitability of the CA1 pyramids; if both the e.p.s.p. and population spike changed, it was influencing some aspect of the transmission process, and if the fibre spike fluctuated, it was influencing

Table 1 Drugs used in experiments described in this paper

Drug	*Effects on evoked activity* ↓	↑	↑↓	ϕ
Adenosine (1–100 μM)	30*	0	0	4
5'AMP (5–100 μM)	7	0	0	0
ATP	3	0	0	0
2 Chloroadenosine (0.25–5 μM)	5	0	0	0
(−)-Phenyl isopropyladenosine (0.5–5 μM)	8	0	0	1
(+)-Phenyl isopropyladenosine (1–100 μM)	4	0	0	6
Cyclic AMP (50–1000 μM)	2	0	0	0
db Cyclic AMP (50–1000 μM)	5	0	0	0
8-PCPT cyclic AMP (250–1000 μM) pop spike	1	3	6	2
8-PCPT cyclic AMP (250 μM) + theophylline (500 μM) e.p.s.p.	0	0	0	5
8-PCPT cyclic AMP (250 μM) + theophylline (500 μM) pop spike	0	4	0	0
Theophylline (5–500 μM)	0	18	0	2
IBMX (10–100 μM)	0	5	0	1
Adenosine deaminase (10–30 μg/ml)	0	9	0	4
Hexobendine (10–50 μM)	7	0	0	3
(−)-Isoprenaline (10–50 μM) pop spike	1	15	6	3
(−)-Isoprenaline (10–50 μM) e.p.s.p.	0	0	0	4

Drug interactions with adenosine-evoked depressions

Drug	*Antagonizes*	*Potentiates*	*No effect*
Theophylline (100–500 μM)	4	0	0
IBMX (500 μM)	3	0	0
Adenosine deaminase (25 μg/ml)	3	0	1

The number (*) refers to the number of slices on which a drug had a statistically significant effect ($P < 0.05$) on response amplitude. Field e.p.s.p. and population spike responses were combined in all cases where the drug had similar effects on both measures.

↑ Increased amplitude of evoked activity.

↓ Decreased amplitude of evoked activity. ↑↓ Biphasic effects on evoked activity.

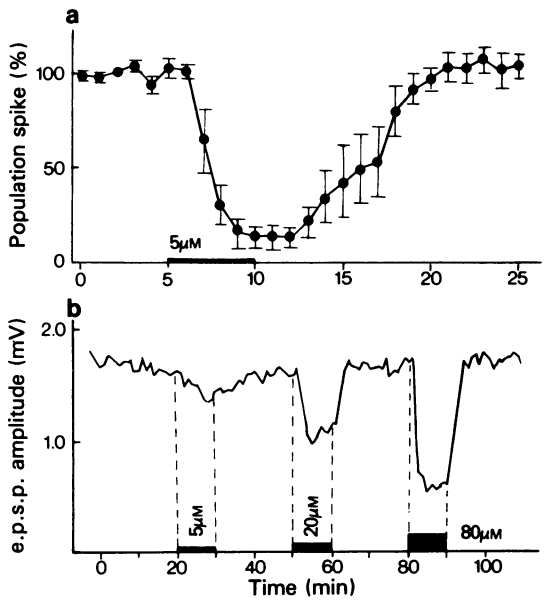

Figure 1 Effects of adenosine on evoked field responses. (a) The effect of perfusion with 5 μM adenosine is shown on the amplitude of the evoked population spike response in the CA1 region. Adenosine produced a rapid and reversible decrease in the amplitude of the population spike (indicated as mean of a group of 5 experiments; vertical lines show s.e. mean). Such decreases were maintained for as long as adenosine was perfused. (b) Perfusion with various concentrations of adenosine demonstrated dose-dependent decreases in the amplitude of the evoked extracellular e.p.s.p. as well. This figure illustrates a continuous record of the peak amplitude of the extracellular e.p.s.p. during successive perfusions with 5 μM, 20 μM and 80 μM adenosine.

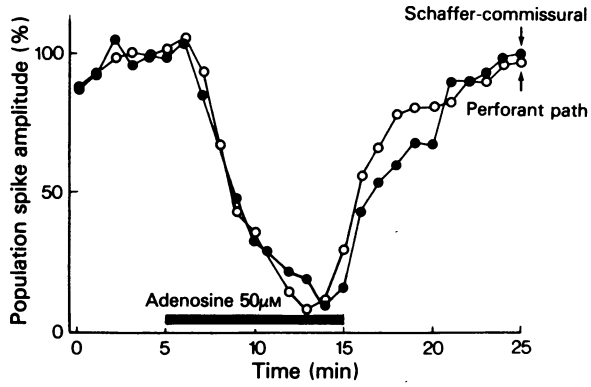

Figure 2 Regional sensitivity to adenosine. Population spike amplitudes were measured from responses evoked in the CA1 region (Schaffer-commissural) and in the perforant path to the dentate gyrus from the same slice. Perfusion with 50 μM adenosine produced similar reductions in spike amplitudes in both regions, and with similar time courses.

presynaptic afferent excitability; however, with the drugs used in these experiments, presynaptic excitability changes were never observed.

Results

Adenosine agonists and antagonists

A summary of the experiments is presented in Table 1. Perfusion of hippocampal slices with adenosine elicited a dose-dependent, reversible decrease in the amplitude of both the population spike (Figures 1, 2) and e.p.s.p. (Figures 1, 3, 4); the EC_{50} for 17 experiments was about 10 μM. At levels which profoundly depressed the extracellular e.p.s.p., no change was seen in the amplitude of the presynaptic afferent fibre volley. Several different adenine nucleotides and other derivatives were also examined; 2-chloroadenosine, a derivative which is also able to elevate brain slice cyclic AMP levels in biochemical studies (Huang, Shimazu & Daly, 1972; Sturgill, Schrier & Gilman, 1975), is at least one order of magnitude more potent than adenosine (Figures 3, 4). Adenosine triphosphate (ATP) and 5'-AMP appeared to be somewhat less potent. Of particular interest were the stereoisomers (−)- and (+)-phenyl isopropyladenosine. In these experiments, the (−)-isomer is about 2 orders of magnitude more potent than the (+)-form in depressing the evoked field e.p.s.p. (Figure 3). The responses to all the adenosine derivatives were qualitatively similar, with the exception of the 2-chloro and 1-phenylisopropyl derivatives. In experiments comparing these derivatives with adenosine, it was noted that an equieffective concentration of adenosine had a more rapid onset as well as a much shorter duration of effect (Figure 4).

The second set of experiments examined the receptor specificity of the adenosine-induced depressions of evoked responses. As was discussed previously, biochemical and physiological evidence indicates that methylxanthines act as antagonists at this receptor. Consequently, we tested the ability of theophylline and isobutylmethylxanthine (IBMX) to block adenosine-induced depressions (Figure 5). Similar receptor interactions were exhibited by all the agonists tested, including 2-chloroadenosine and 1-phenylisopropyl adenosine. In every case, 500 μM theophylline or IBMX was able to reverse the depressant effects of maximal concentrations of any of the agon-

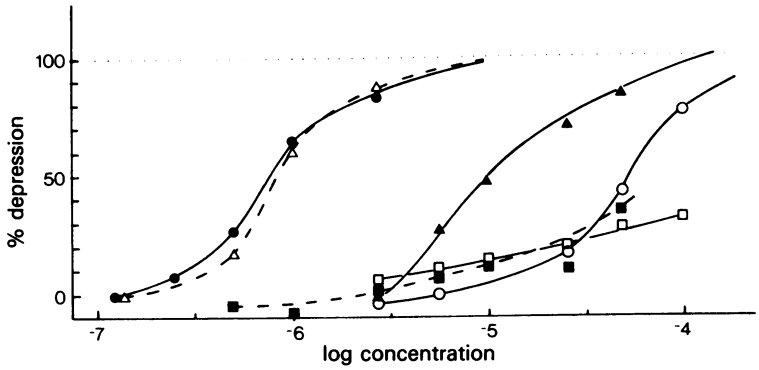

Figure 3 Dose-response curves for adenosine and adenosine analogues. Dose-response curves are shown for adenosine (▲), ATP (○), 5'-AMP (□), 2-chloroadenosine (●), and two stereoisomeric derivatives of adenosine, (−)- and (+)-phenylisopropyl adenosine (△ and ■). The values used in this figure were obtained from 67 individual determinations of the effects of various concentrations of these drugs; individual points represent the results of 1–5 experiments.

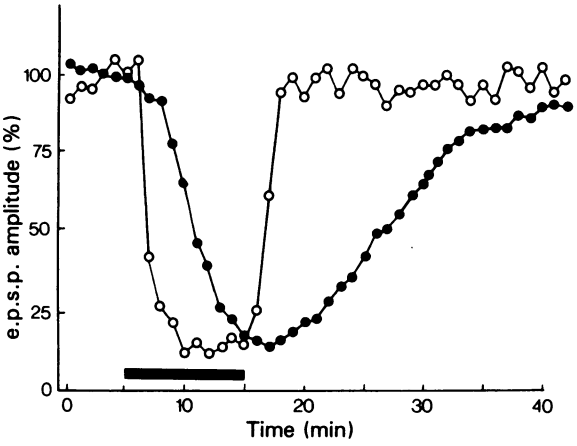

Figure 4 Effects of adenosine and 2-chloroadenosine on extracellular e.p.s.ps. Slices were perfused with 80 μM adenosine (○) or 2.5 μM 2-chloroadenosine (●) during the period marked along the horizontal axis. While both drugs produced nearly identical maximal depressions when used in these concentrations, the time course of the effect produced by the 2-chloro derivative was slower, both in onset and in decline. ($n = 2$ for each condition).

ists. In some experiments, it was demonstrated that this antagonism was surmountable by using increased concentrations of agonist (Figure 5).

Endogenous release of adenosine

In addition to blocking the effects of adenosine, methylxanthines directly affected the amplitudes of both population spike and e.p.s.p. responses; they produced increases in both response measures which were rapid, long-lasting, and were reversed upon washing out of the drug (Figure 6). In an effort to determine whether these effects were related to the actions of the methylxanthines on adenosine receptors, we studied other agents known to influence extracellular adenosine levels. As a first step, we used adenosine deaminase, the enzyme which converts adenosine to inosine (inosine is ineffective in raising cyclic AMP levels; Sattin & Rall, 1970). Superfusion of slices with adenosine deaminase totally blocks the depressant effect of adenosine when both agents are administered together (Figure 7), but more importantly, the population spike amplitude is also significantly elevated by adenosine deaminase alone (Figure 7). Clearly, this would suggest that there is sufficient adenosine in the extracellular compartment to depress tonically synaptically mediated responses. In order to test this hypothesis further, we perfused slices with hexobendine, which blocks adenosine reuptake and can produce small increases in cyclic AMP by itself (Huang & Daly, 1974). Hexobendine was found consistently to produce a depression of synaptic responses with relatively long onset (Figure 7), which would also support the hypothesis of an endogenous release of adenosine from the slice.

Role of cyclic nucleotides

Much controversy surrounds the electrophysiological effects of cyclic AMP in the nervous system. While most investigators now obtain responses to extracellular administration of cyclic AMP, the mechanism of this response is alternatively postulated as due to

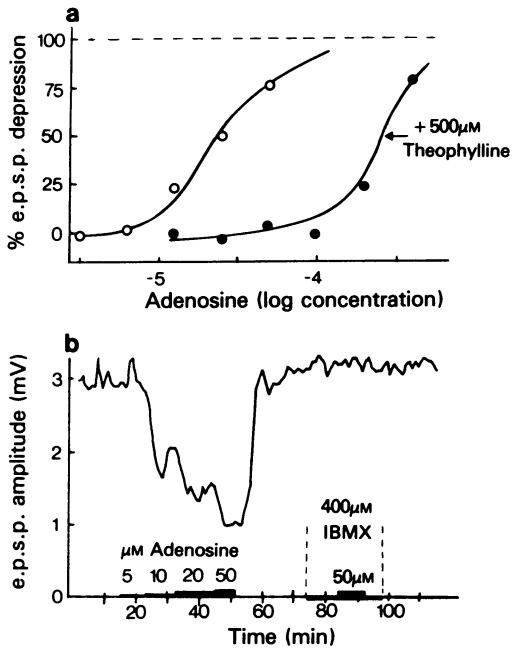

Figure 5 Methylxanthine antagonism of adenosine effects. (a) The effect of 500 μM theophylline (●) in this experiment is seen to be a shift of roughly one order of magnitude in the dose-response curve for adenosine-mediated depression (○) of the e.p.s.p. All the points in this figure were derived from a single slice with the indicated concentrations of adenosine. (b) Isobutylmethylxanthine (IBMX) also antagonized adenosine-mediated depressions. In this experiment, adenosine produced a dose-dependent decrease in the amplitude of the extracellular e.p.s.p. When the slice was pretreated with IBMX, the effect of the highest concentration of adenosine used in the first part (50 μM) was completely abolished.

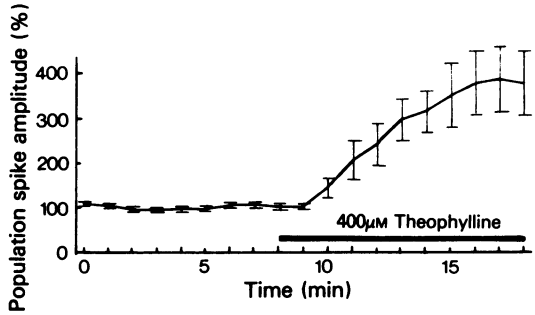

Figure 6 Effects of theophylline on evoked population spike responses. Theophylline produced a rapid increase in the amplitude of the field population spike response. These increases were reversible upon perfusion with control medium, but could also be maintained for long periods of time if theophylline was continuously perfused. The threshold for effects of theophylline was in the range of 10 to 25 μM.

extracellular activation of the adenosine receptor, or to intracellular activation of cyclic nucleotide-dependent protein kinases. In the hippocampal slice, superfusion of cyclic AMP or its dibutyryl derivative had a potent depressant effect on synaptic responses (Figure 8). However, this response was completely blocked by superfusion of theophylline, suggesting mediation by an extracellular adenosine receptor. The effect of 8-parachlorophenylthio cyclic AMP (8-PCPT-cyclic AMP), a derivative which is very resistant to phosphodiesterase and a potent activator of bovine brain cyclic AMP-dependent protein kinase (Miller, Boswell, Muneyama, Simon, Robins & Shuman, 1973), was more complex. As shown in Figure 8, administration of 8-PCPT-cyclic AMP in control slices produced a biphasic augmentation and depression of population spike amplitude. After blockade of adenosine receptors with theophylline, a much larger and more consistent increase in population spike amplitude was seen, without subsequent depression. However, 8-PCPT-cyclic AMP had no effect on field e.p.s.p. amplitudes.

If an increase in intracellular cyclic AMP elicits an increase in population spike amplitude, this would suggest that other drugs which induce similar increases via receptor coupled adenylate cyclases might produce similar increases in the population spike. Since the hippocampus is known to possess β-receptors (Alexander, Davis & Lefkowitz, 1975; Minneman, Hegstrand & Molinoff, 1979) stimulation of which elevates cyclic AMP in many brain regions (Kakiuchi & Rall, 1968; Forn & Krishna, 1971), superfusion of the β-adrenoceptor agonist, isoprenaline, was studied. As with 8-PCPT-cyclic AMP, isoprenaline in concentrations of 10 to 50 μM was found to produce significant elevations in population spike amplitude without having any effect on the field e.p.s.p.

Adenosine and synaptic plasticity

Since adenosine has frequently been assigned a role as a putative neuromodulator rather than neurotransmitter, we considered the possibility that besides its immediate effects on synaptic strength, adenosine might trigger some persistent change as well. Several forms of synaptic plasticity, manifested primarily as changes in synaptic efficacy following repetitive stimulation, have been described for the hippocampus (Lømo, 1968; 1971; Bliss & Lømo, 1973; Alger & Teyler, 1976). We studied a long-lasting form of

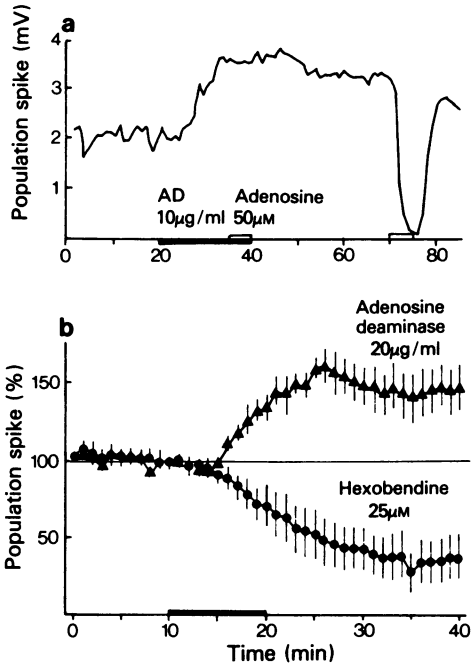

Figure 7 Effects of adenosine deaminase and hexobendine. (a) Perfusion with 10 μg/ml adenosine deaminase (AD) produced direct increases in the amplitude of the population spike response by itself. In addition, adenosine deaminase antagonized the response to exogenously applied 50 μM adenosine as well. However, the response to adenosine recovered upon washout of the adenosine deaminase. (b) The results of 7 experiments with adenosine deaminase (20 μg/ml, ▲), and 6 experiments with hexobendine (25 μM, ●) are illustrated; vertical lines show s.e. mean. Adenosine deaminase produced statistically significant ($P < 0.05$) increases in the amplitude of the population spike, indicating the presence of endogenous adenosine sufficient to inhibit the response. Hexobendine produced decreases in response amplitude, which would be consistent with its action as a blocker of adenosine uptake. Both types of effects were seen in terms of the extracellular e.p.s.p. as well.

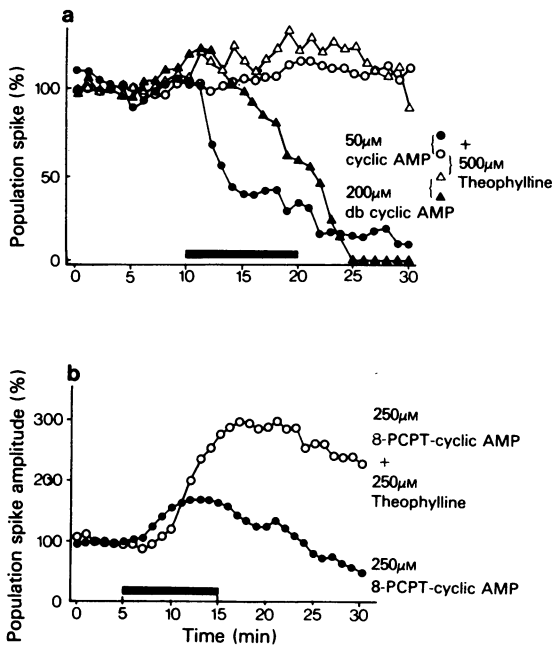

Figure 8 Effects of cyclic AMP analogues. (a) Both cyclic AMP itself (●) and its dibutyryl derivative (▲) produced potent depressant effects on the amplitude of population spike responses. However, when slices were pretreated with theophylline (500 μM) (○ and △), the depressant effects of both drugs were abolished, suggesting that the effects were due to an interaction at an extracellular site with the adenosine receptor. In each case, the original response to the drug and the subsequent test with theophylline pretreatment were conducted on the same slice. (b) Effects of 250 μM 8-*p*-chlorophenylthio cyclic AMP (8-PCPT-cyclic AMP), with and without pretreatment with theophylline are illustrated on the amplitude of the field population spike. In both cases, increases rather than decreases were initially observed; in this case, theophylline potentiated rather than antagonized the effect. The indicated values represent the means from 9 experiments; both increases were significantly different ($P < 0.05$) from control values.

synaptic plasticity which is characteristic of many hippocampal synapses, long-term potentiation (LTP). This phenomenon consists of marked increases in both population spike and e.p.s.p. amplitude, which often persist for hours without decrement following a high frequency tetanization of the slice. Since a significant release of adenosine has been described in brain slices after tetanic stimulation (Heller & McIlwain, 1973; Pull & McIlwain, 1972a & b; Sun *et al.*, 1975) the possible role of adenosine release in synaptic plasticity was explored. In these experiments slices were perfused either with control medium, or with medium containing 500 μM theophylline or 20 μM adenosine, until responses had stabilized. They were then tetanized with a 400 s^{-1}, 0.5 s stimulation train. In all 18 cases, statistically significant increases in population spike amplitude of 400 to 700% were observed ($P < 0.05$). However, neither adenosine nor theophylline significantly altered LTP magnitude.

Discussion

Several lines of evidence suggest that the inhibitory effect of adenosine on synaptic transmission is mediated via an interaction with a membrane-bound 'adenosine receptor' which has many properties in common with the biochemically defined receptor. The rough parallels between the electrophysiological potency of various adenosine derivatives, and their ability to increase cyclic AMP levels would suggest such a conclusion. These parallels in physiological and biochemical potency extended to the (+)- and (−)-stereoisomers of phenyl isopropyl adenosine, as well. Smellie, Daly, Dunwiddie & Hoffer (1978) showed that the (−)-isomer is more potent in elevating cyclic AMP levels than is the (+)-isomer. Studies regarding the potency of the other adenosine derivatives as activators of adenylate cyclase in rat hippocampus are currently being conducted, since any definite conclusions as to the identity of the receptors mediating both of these types of effects must rest upon experiments utilizing the same preparation.

The results of the experiments with 2-chloroadenosine, which unlike many of the other adenosine derivatives cannot be broken down to adenosine, demonstrates that all these compounds do not necessarily act by conversion to adenosine, which then binds to the receptor, or by conversion to cyclic AMP.

A number of other drugs which have been reported to affect adenosine receptors and/or adenosine levels biochemically were also studied. Methylxanthines, which block adenosine receptors (Sattin & Rall, 1970; Huang & Daly, 1972), antagonized the electrophysiological changes elicited by adenine nucleotides. While it is tempting to speculate that these data suggest a tonic inhibitory role for endogenously released adenosine, methylxanthines have many other effects which must be considered. These drugs inhibit phosphodiesterase activity (Butcher & Sutherland, 1962; Beavo, Rogers, Crofford, Hardman, Sutherland & Newman, 1970), alter Ca^{2+} distribution (Weber & Herz, 1968; Johnson & Inesi, 1969; Chapman & Miller, 1974) and change presynaptic release of transmitter (Goldberg & Singer, 1969; Ginsborg & Hirst, 1972; Wilson, 1974).

While the effects of methylxanthines alone do not provide very convincing evidence for this hypothesis, the fact that adenosine deaminase, a rather specific enzyme for the breakdown of adenosine, also increases the size of the evoked field responses would make it appear that synaptic conduction at this site may be tonically inhibited by endogenous adenosine release. This conjecture was further strengthened by the studies with hexobendine. This drug, which blocks adenosine reuptake and appears to elevate extracellular adenosine levels (Huang & Daly, 1974), depressed the amplitude of both the e.p.s.p. and the population spike, as does adenosine itself. Thus, the presence of adenosine in the extracellular space is apparent; however, in the absence of any definitive histochemical data the source of this postulated adenosine release, whether from neurones, glial cells, or specific purinergic nerve fibres, is unclear.

Data from this paper have several implications for the mechanisms of electrophysiological responses to cyclic AMP. While most investigators now find responses in many brain regions to iontophoretic administration, it is not clear if they are due to stimulation of an external adenosine receptor (Phillis & Kostopoulos, 1975), or to increases in intracellular levels of the nucleotide and subsequent activation of protein kinase (Stone & Taylor, 1977; Taylor & Stone, 1978). In our hands, superfusion of cyclic AMP or dibutyryl cyclic AMP elicited a strong depression of the population spike. Since effects of these nucleotides were blocked by theophylline, it is probable that they were mediated by external adenosine receptors. On the other hand, after theophylline administration, 8-PCPT-cyclic AMP induced a large increase in population spike amplitude without corresponding increases in e.p.s.p. amplitude. Since this particular cyclic nucleotide derivative is a potent activator of protein kinase and quite resistant to phosphodiesterase (Miller *et al.*, 1973), perhaps this latter response is the direct result of elevating intracellular cyclic AMP levels. The physiological response to isoprenaline, a β-adrenoceptor agonist also thought to act via cyclic AMP-dependent mechanism, supports this conjecture. Administration of 10 to 50 μM isoprenaline increased population spike amplitudes (but not field e.p.s.p.) just as did 8-PCPT-cyclic AMP. However, this hypothesis may not be universally applicable to other brain regions. Thus, while Kuroda *et al.* (1976) and Schofield (1978) have described depression in cortical slice evoked potentials by adenosine, neither dibutyryl cyclic AMP nor adrenoceptor agonists had significant effects.

Where might adenosine act in the hippocampal slice preparation to produce depression? In view of the parallel changes in population spike and e.p.s.p. amplitude, a general decrease in postsynaptic excitability is unlikely. The data suggest either a decrease in excitatory transmitter release, as has been reported in the ileum (Hayashi *et al.*, 1978) and neuromuscular junction (Ribeiro & Walker, 1975) or a reduction in sensitivity of the postsynaptic excitatory receptor. Intracellular recording during adenosine administration in rat cortical neurones (Phillis & Edstrom, 1976) and cortical slices (Scholfield, 1978) suggests a presynaptic site of action. However, since the excitatory transmitter at the commissural/Shaffer collateral synapse to pyramidal neurones has not been conclusively identified, direct studies of the effect

of adenosine on transmitter release would be premature.

In view of the depressant effects of adenosine, and the increased responses to 8-PCPT-cyclic AMP and isoprenaline, it is difficult to argue that adenosine effects are mediated by elevation of intracellular cyclic AMP levels. Our data would seem to suggest that increasing cyclic AMP levels elicits an augmentation of the population spike. However, it should be emphasized that compartmentalization is a prominent feature in the central nervous system, and that adenosine could increase intracellular cyclic AMP in a different pre-(or post-)synaptic compartment, and as a consequence, the physiological effect might be far different from that induced by perfusion with 8-PCPT-cyclic AMP, which would presumably reach many more sites of possible cyclic AMP action. Immunocytochemical localization of cyclic AMP (Wedner, Hoffer, Battenberg, Steiner, Parker & Bloom, 1972) in the hippocampal slice after adenosine or isoprenaline administration may be critical in resolving this problem. If electrophysiological experiments with some of the more generalized blockers of adenylate cyclases (which block all hormone-stimulated increases in cyclic AMP) show no antagonism of adenosine-mediated depressions, this would further eliminate cyclic AMP as a 'second messenger' in terms of the physiological effects of this nucleotide.

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