J. Physiol. (1985), **369**, *pp.* 365–377
With 7 text-figures
Printed in Great Britain

ADENOSINE INCREASES SYNAPTIC FACILITATION IN THE *IN VITRO* RAT HIPPOCAMPUS: EVIDENCE FOR A PRESYNAPTIC SITE OF ACTION

BY THOMAS V. DUNWIDDIE*† AND HELMUT L. HAAS‡

*From the *Department of Pharmacology, C236, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262, U.S.A., †the Veterans Administration Medical Research Service, 1055 Clermont Street, Denver, CO 80220, U.S.A. and the ‡Neurophysiological Laboratory, Neurochirurgische Universitätsklinik, CH 8091 Zürich, Switzerland*

(*Received 8 January 1985*)

SUMMARY

1. The effect of adenosine on paired synaptic responses was characterized in the CA1 region of the rat hippocampus *in vitro*. Adenosine increased the degree of synaptic facilitation at a 40 ms conditioning–testing interval under all conditions tested. Even when the stimulation intensity was increased so as to counteract the direct depressant effect of adenosine on synaptic transmission, its effect on facilitation was maintained.

2. The ability of adenosine to increase synaptic facilitation was a complex function of several variables. The effect was enhanced by increasing the calcium concentration of the medium, and was most pronounced at short conditioning–testing intervals and at low response amplitudes.

3. Adenosine was particularly efficacious in blocking the depression of synaptic responses observed in high-calcium medium at short conditioning– testing intervals. Because this depression most probably reflects depletion of the available store of releasable transmitter, one mechanism by which adenosine could reverse this effect would be by blocking the depletion of transmitter.

4. These results suggest that adenosine diminishes transmitter release via an action at the presynaptic terminal. The reduction in the release of neurotransmitter, particularly at excitatory synapses, may be responsible for the depressant effects of adenosine upon the central nervous system.

INTRODUCTION

Purinergic agonists such as adenosine are potent depressants of synaptic transmission in both the central as well as peripheral nervous systems (Ginsborg & Hirst, 1972; Okada & Kuroda, 1975; Scholfield, 1978; Dunwiddie & Hoffer, 1980; Fredholm & Hedqvist, 1980) but the mechanism by which they exert this action remains to be established. At peripheral synapses, where the quantal aspects of synaptic transmission can be determined, adenosine decreases the quantum content without

affecting post-synaptic sensitivity to the neurotransmitter (Ginsborg & Hirst, 1972; Silinsky, 1980, 1984). In the central nervous system, where quantal analysis is by and large not possible, adenosine has been shown to diminish the efflux of various transmitters such as γ-aminobutyric acid, noradrenaline, and acetylcholine from brain slices (Vizi & Knoll, 1976; Hollins & Stone, 1980; Jonzon & Fredholm, 1984), presumably via a presynaptic mechanism of action. High concentrations of adenosine and 2-chloroadenosine inhibit the release of glutamate and [^{3}H]glutamate from hippocampal slices (Dolphin & Archer, 1983; Corradetti, Lo Conte, Moroni, Passani & Pepeu, 1984), and since the former is a likely candidate for the transmitter at these synapses (Nadler, White, Vaca, Redburn & Cotman, 1977; Corradetti, Moneti, Moroni, Pepeu & Wieraszko, 1983), this would provide direct support for a presynaptic mechanism of action. However, in addition to its presynaptic actions, adenosine has post-synaptic effects in the central nervous system (Siggins & Schubert, 1981; Segal, 1982; Proctor & Dunwiddie, 1983; Haas & Greene, 1984). The inhibitory post-synaptic actions of adenosine present a problem, in that they may indirectly lead to a decreased efflux of transmitter from brain slices.

To address this problem we have adopted an indirect approach, which has been to examine the effect of adenosine on electrophysiological responses that are presynaptic in nature. When most excitatory synapses in the hippocampus are stimulated twice in rapid succession there is a facilitation of the second (test) response when compared with amplitude of the initial (conditioning) response (Andersen, 1960*a*,*b*; Lømo, 1971; Alger & Teyler, 1976; Dunwiddie & Lynch, 1978; Buckle & Haas, 1982). At other synapses where this phenomenon is observed, the mechanism underlying such facilitation is an increase in the release of transmitter, and appears to reflect the persistence of calcium in the presynaptic terminal following the initial impulse (Katz & Miledi, 1965, 1968; Rahamimoff, 1968). Superimposed upon this facilitation, particularly under conditions of high transmitter release, is a depression of the test response, which usually results from either a depletion of the store of releasable transmitter or a decrease in the probability of release (Betz, 1970). In the hippocampus either facilitation or depression may predominate, depending upon which synapse is being studied and the conditions under which it is tested.

Manipulations that reduce transmitter release (e.g. reduced calcium in the perfusion medium), have previously been shown to increase synaptic facilitation in the hippocampal CA1 region (Creager, Dunwiddie & Lynch, 1980) and to decrease synaptic depression (Creager *et al.* 1980; Harris & Cotman, 1983*a*). If adenosine reduces synaptic efficacy by reducing calcium influx, then corresponding changes in facilitation and depression should be observed. Alternatively, if adenosine reduces synaptic efficacy by another type of mechanism (e.g. by reducing the sensitivity of post-synaptic receptors, or by 'shunting' synaptically generated currents) then presynaptic events should be relatively unaffected. In this case, adenosine would depress the amplitudes of both the conditioning and testing responses, but the ratio between the two would remain unchanged. Thus, in the present experiments, we have examined the effect of adenosine on paired-pulse facilitation and on depression in order to determine whether these effects are consistent with a presynaptic action of adenosine.

METHODS

Male Sprague–Dawley rats weighing 150–250 g obtained from Charles River were decapitated, the hippocampus dissected free of surrounding tissue, and coronal slices prepared as described previously (Dunwiddie & Lynch, 1978; Mueller, Hoffer & Dunwiddie, 1981). Slices were cut at 400 μm on a Sorvall tissue chopper and immediately placed in ice-cold medium consisting of (mM): NaCl, 124; KCl, 3·3; $MgSO_4$, 2·4; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $NaHCO_3$, 25·6; glucose, 10; pre-gassed with 95% O_2/5% CO_2. Slices were transferred within 5 min to a recording chamber maintained at 33–34 °C. In some of the experiments, the calcium concentration of the medium was varied from that in our control medium. Because at the higher concentration used (10 mM) calcium was not soluble in bicarbonate buffered medium, we also used a medium consisting of (mM): NaCl, 150; KCl, 4·5; $MgSO_4$, 2·0; $CaCl_2$, 0·5, 1·0, or 10; Tris HCl, 10 mM; glucose, 10 mM, with the pH adjusted to 7·4 and gassed with 100% O_2. Slices were placed in this medium within 30 min of preparation and were maintained under these conditions throughout the experiment.

Synaptic responses were elicited by stimulation of the Schaffer collateral and commissural afferents in stratum radiatum, and field e.p.s.p. responses were recorded with glass micropipettes in stratum radiatum of the CA1 region, as has been described elsewhere (Haas & Rose, 1982; Dunwiddie, 1984). The field e.p.s.p. response, which reflects currents generated by synapses in the immediate vicinity of the recording electrode, is markedly depressed by perfusion with adenosine (Schubert & Mitzdorf, 1979; Dunwiddie & Hoffer, 1980). In all experiments this pathway to the CA1 neurones was stimulated twice in rapid succession; in control medium in the absence of drug, the second (test) e.p.s.p. was normally facilitated with respect to the first (conditioning) response. In most experiments a 40 ms interpulse interval was used to elicit paired pulse facilitation, although in other experiments conditioning–testing intervals ranging from 15 to 300 ms were employed. The degree of facilitation was expressed as the percentage increase in the peak field e.p.s.p. of the test response in comparison with the conditioning response. The magnitude of the facilitation was determined in some cases by expressing the log percentage facilitation as a function of the interpulse interval, and fitting a line to the experimental data using a least-squares criterion. The intercept with the Y axis provided an estimate of the initial facilitation, and the standard error of the intercept was used to determine whether there were significant differences in facilitation.

Input–output curves were determined by testing the responses every 12–15 s while increasing the stimulation voltage throughout the range of stimulus intensities employed (usually 2–30 V). The stimulation voltage was then reduced to elicit a 1 mV test response, and potentials evoked at 1/min for at least 5 min to acquire a pre-drug base line prior to perfusion with adenosine. Once a stable drug response was achieved, a second input–output series identical to the first was run, and in most cases a third series was run following wash-out of adenosine.

Slices were generally maintained in a 95% O_2/5% CO_2 (or 100% O_2) atmosphere without superfusion of medium until they were to be tested, at which time a constant flow of oxygenated pre-heated medium was initiated at a rate of 2 ml/min. Adenosine was dissolved in distilled water to make a 10 mM stock solution, and this was added directly to the flow of perfusion medium with a calibrated syringe pump.

RESULTS

Effects of adenosine upon facilitation

When excitatory afferents to the CA1 pyramidal neurones are stimulated twice in rapid succession, the response to the second stimulus (test response) is generally facilitated in relation to the initial, or conditioning stimulus (Fig. 1). The degree of facilitation, particularly in low-calcium medium, declines logarithmically as a function of the conditioning–testing interval (Fig. 1*A*; see also Creager *et al.* 1980).

The ability of adenosine to depress synaptic responses and to increase synaptic facilitation is illustrated in Fig. 2. Adenosine not only depressed the magnitude of the conditioning response (Fig. 2*A*), but *increased* the facilitation of the test response relative to the conditioning response. This increase in facilitation could be observed

regardless of the conditioning–testing interval employed. However, the degree of facilitation is related to the amplitude of the conditioning response (see pre-adenosine and wash-out lines, Fig. 3*A*), and it was important to establish that the increase in facilitation was not secondary to a diminished conditioning response amplitude. Therefore, slices were tested with a range of stimulation voltages before, during and

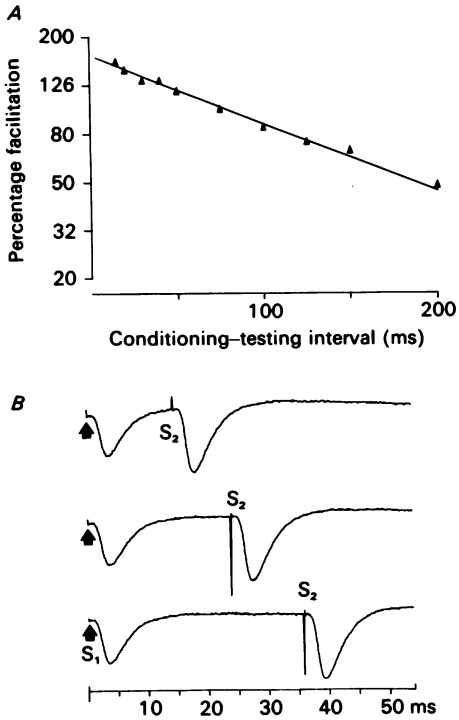

Fig. 1. Paired-pulse facilitation in the rat hippocampal CA1 region *in vitro*. In *A*, the log percentage facilitation of a synaptic response is illustrated as a function of the conditioning–testing interval. The decline in facilitation conforms quite closely to a simple log linear function. In low-calcium medium (illustrated), the test response is facilitated at all time points. In high-calcium medium, there is a substantial deviation from linearity, in that less facilitation is observed than would be expected at short (< 50 ms) conditioning–testing intervals (see Fig. 6*A*), but the time constant of decay is unaffected (see Creager *et al.* 1980). Actual responses to paired stimuli delivered to the Schaffer collateral and commissural fibres of the rat hippocampus are illustrated in *B*. The first stimulus (S_1; conditioning response) results in a facilitation of the second response (S_2; test response), shown here with conditioning–testing intervals of 15–35 ms. Each conditioning response in this series was set to 1 mV.

after adenosine perfusion at a fixed conditioning–testing interval (40 ms). As seen in Fig. 3*A*, adenosine consistently increased the amount of facilitation, and this effect was reversed upon washing with control medium. Highly significant increases in facilitation were observed at response amplitudes between 0·5 and 2 mV (Fig. 3*B*).

Interactions between calcium and adenosine

In a second series of experiments, the effect of adenosine was examined on slices maintained in medium containing different concentrations of calcium. The results of one such experiment are illustrated in Fig. 4. When conditioning responses of the same amplitude were tested adenosine actually reduced facilitation slightly in low-calcium

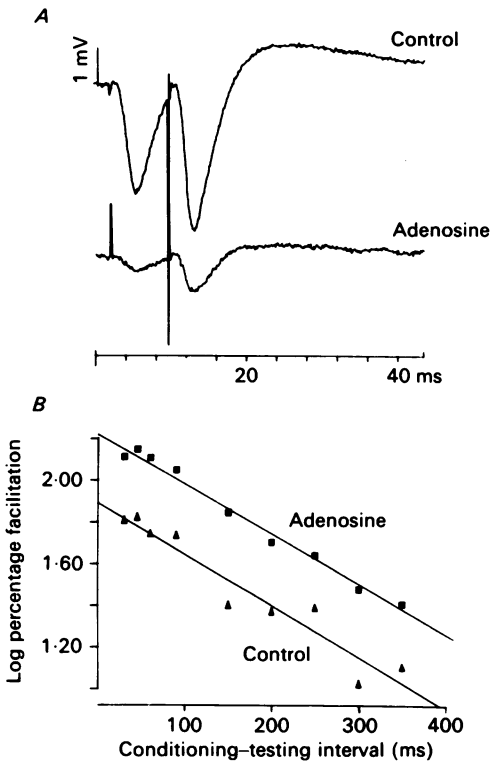

Fig. 2. Effect of adenosine upon synaptic responses and facilitation. *A*, facilitation of a test response with a 10 ms conditioning–testing interval is illustrated before (control) and during (adenosine) perfusion of a single slice with 50 μM-adenosine. Whereas the amplitude of the initial response (conditioning response) was reduced 85% by this concentration of adenosine, the degree of facilitation was increased from 55 to 150%. *B*, the log percentage facilitation is expressed as a function of the conditioning–testing interval, as in Fig. 1*A*. Adenosine clearly increased the degree of facilitation at all time points without significantly affecting the rate at which facilitation decayed. This particular slice was maintained in medium containing 2·5 mM-calcium and 10 mM-magnesium; the effect of this medium was qualitatively similar to low-calcium medium containing 1 mM-calcium and 2·4 mM-magnesium.

medium (Fig. 4*A*), whereas in high-calcium medium adenosine almost doubled the facilitation of the test response (Fig. 4*B*).

The interactions between adenosine and calcium on paired-pulse facilitation are clarified in Fig. 5. Slices maintained in low-calcium medium typically showed a high degree of facilitation that was increased (although not significantly) by adenosine. Facilitation in medium containing 10 mM-calcium was initially much less, but was

markedly enhanced by adenosine. The effect of adenosine in control medium (cf. Fig. 3*B*) was intermediate. The difference between high- and low-calcium medium was apparent not only from the magnitude of the changes in facilitation (Fig. 5), but also in terms of the percentage of individual slices that exhibited statistically significant

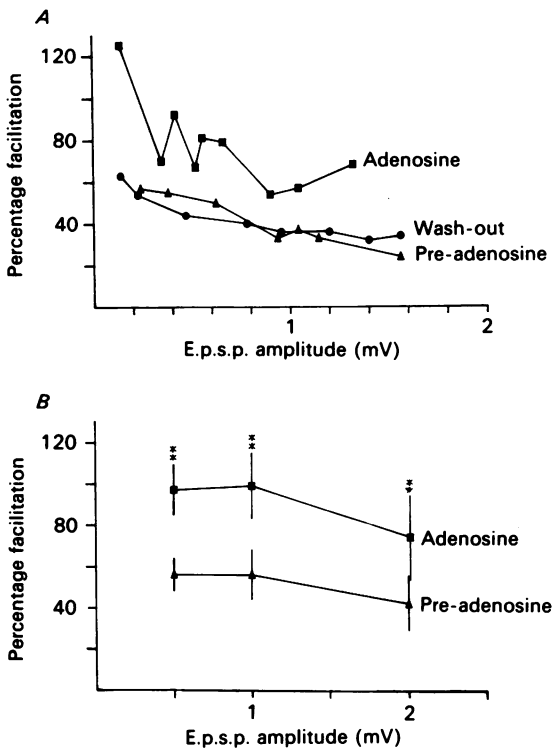

Fig. 3. Interaction between adenosine and response amplitude. *A*, facilitation of a test response with a 40 ms conditioning–testing interval was examined before, during and after perfusion of a single slice with 50 μM-adenosine. Because the facilitation is affected by the response amplitude, responses were obtained with a range of stimulation intensities; the degree of facilitation is shown here as a function of the conditioning response amplitude, so that facilitation can be compared across equivalent conditions. Facilitation was increased at every point during adenosine perfusion, and had recovered to control values 15 min following perfusion with control medium (wash-out). *B*, the mean $\pm$ S.E. of mean facilitation before and during adenosine perfusion is shown for a group of twelve slices tested as in *A*. For each individual slice, the percentage facilitation was taken from the conditioning response closest in amplitude to the indicated points on the abscissa. Actual response amplitudes did not vary more than 10% in range from the indicated values. Changes in facilitation were tested using a paired *t* test for repeated measures. At every response amplitude there was a highly significant increase ($P < 0{\cdot}01$) in the degree of facilitation in the presence of 50 μM-adenosine.

increases in facilitation. Using this criterion, the ability of adenosine to enhance facilitation was significantly less in low-calcium medium (26% of the slices) than in medium containing higher concentrations of calcium (65 and 70% for control and high-calcium medium respectively).

Effects of adenosine at different conditioning–testing intervals

The previous results indicate that adenosine can increase paired-pulse facilitation at a fixed conditioning–testing interval of 40 ms. However, in high-calcium medium, where transmitter release is relatively large, facilitation does not decline logarithmically but deviates substantially from this model at short time intervals (see Creager

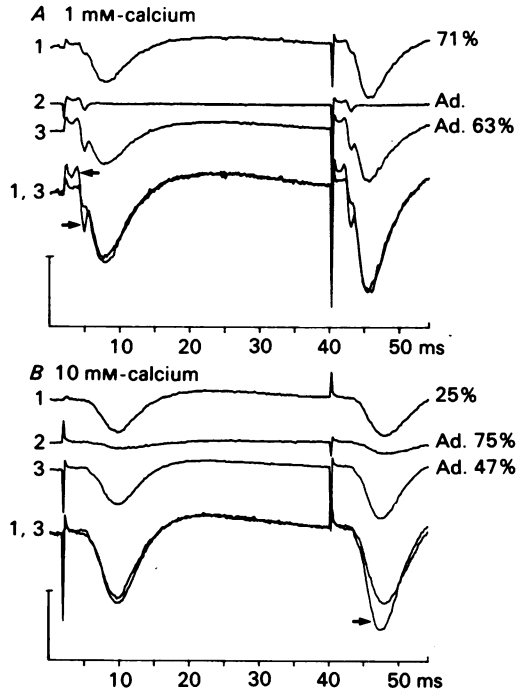

Fig. 4. Effects of adenosine on facilitation in high- and low-calcium medium. The degree of facilitation of a 1 mV conditioning response is shown in medium containing 1 mM-calcium (*A*), and 10 mM-calcium (*B*); the values to the right of each record indicate the percentage facilitation of the test response, and Ad. indicates responses recorded during perfusion with adenosine. Responses are shown prior to adenosine perfusion (1), during perfusion with 50 μM-adenosine (2), and during adenosine perfusion with the stimulation voltage increased so as to elicit the standard 1 mV test response (3). The last trace of each group (1, 3) is the superposition of the first and third responses at increased gain. There was a slight decrease in the amount of facilitation observed in low-calcium medium during perfusion with adenosine, but almost a doubling in high-calcium medium. The single arrow in *B* denotes the facilitated response recorded during adenosine perfusion. The presynaptic fibre spike (indicated by arrows in *A*) associated with a 1 mV field e.p.s.p. response in adenosine is much larger than under control conditions, indicating that many more synapses must be activated to achieve the same post-synaptic response, but the degree of facilitation observed is virtually identical.

et al. 1980, fig. 3), most probably due to a transient depletion of neurotransmitter immediately following the first impulse. Thus, in high-calcium medium, changes in 'facilitation' at a 40 ms conditioning–testing interval could reflect alterations not only in facilitation but also in the degree of post-stimulus depression. Therefore, in

a separate series of experiments we characterized the manner in which adenosine affected paired-pulse facilitation at different intervals. These experiments were carried out in high-calcium medium, which maximizes the post-stimulus depression, so that the effects of adenosine could be characterized on both depression and facilitation.

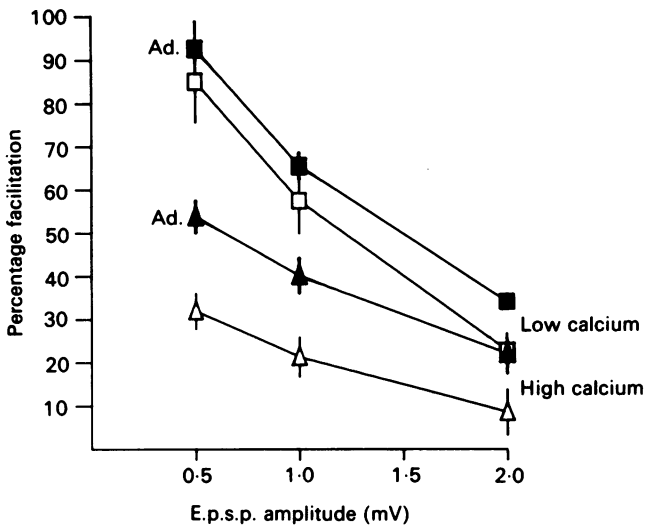

Fig. 5. Interactions between calcium and adenosine on facilitation. Groups of slices maintained in low (1 mM) and high-calcium (10 mM) medium were tested before and during perfusion with 50 μM-adenosine (Ad.) as in Fig. 4. The mean $\pm$ S.E. of mean facilitation observed with a 40 ms conditioning–testing interval is shown for nine slices in low-calcium and eleven slices in high-calcium medium for three different conditioning response amplitudes. No error bar is indicated for the adenosine–low calcium condition because the 2 mV field e.p.s.p. response could only be elicited in two slices under these conditions.

When slices maintained in high-calcium medium were tested with different conditioning–testing intervals, it was found that the major effect of adenosine was at short intervals, with little effect on facilitation at longer intervals. In Fig. 6 results are shown from a single slice in which the test response was depressed at conditioning–testing intervals less than 50 ms. Although there was a marked effect of adenosine on the ratio of testing/conditioning responses at intervals less than 90 ms, there was no significant enhancement of facilitation at longer intervals. This would suggest that the primary effect of adenosine, at least in high-calcium medium, is to reverse the depression of test responses at short conditioning–testing intervals.

This conclusion is supported by the results illustrated in Fig. 7, in which the most striking effects of adenosine are seen at conditioning–testing intervals less than 100 ms. At longer intervals however (Fig. 7*B*), there was a significant enhancement in facilitation as well. The degree of facilitation extrapolated from the lines in Fig. 7*B* was statistically significant (72% for control, *vs.* 144% for adenosine; $P < 0{\cdot}01$), suggesting that the effect of adenosine was not confined solely to the shorter time points.

DISCUSSION

As discussed in the Introduction, the mechanism by which adenosine inhibits synaptic transmission in the central nervous system is still somewhat obscure. However, several aspects of the present results would indicate that adenosine has significant actions at presynaptic as well as post-synaptic sites. First, the depressant effect of adenosine was greater on the first response of a conditioning–testing pair, rather than affecting both responses equally, so that the degree of synaptic

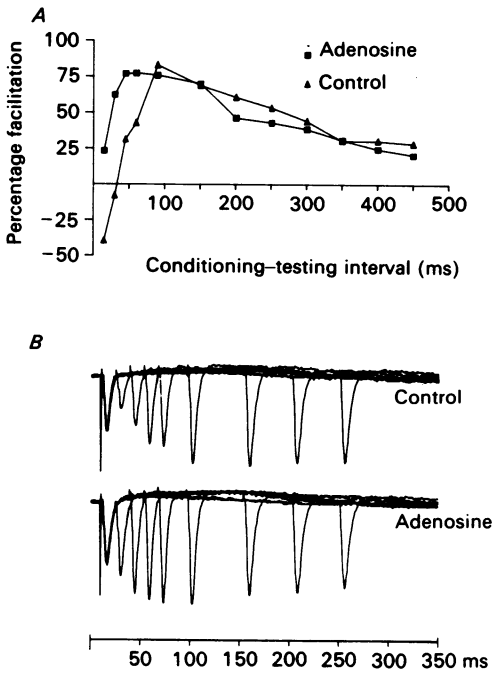

Fig. 6. Effect of adenosine on facilitation or depression in high-calcium medium. *A*, the percentage facilitation is shown as a function of the conditioning–testing interval for a single slice tested in high-calcium medium to enhance the depression observed at short conditioning–testing intervals. The same slice was tested under control conditions, and during perfusion with 50 μM-adenosine. In *B*, the actual field e.p.s.p. responses are illustrated prior to and during perfusion with adenosine. The most striking difference between the two conditions was clearly the loss of the depression of the test response at short conditioning–testing intervals; differences at longer time points were negligible.

facilitation was consistently enhanced by adenosine. In this context, the recent results of Harris & Cotman (1983*a*, *b*) appear to be quite consistent with our findings. In the perforant path to the dentate gyrus of the hippocampus, baclofen, adenosine, and decreases in calcium, all decreased synaptic responses but caused an increase in paired-pulse facilitation, whereas kynurenic acid, which is hypothesized to be a post-synaptic receptor antagonist, depressed conditioning and testing responses equally. On this basis it was suggested that agents that reduce transmitter release increase synaptic facilitation, whereas agents which act post-synaptically do not alter

facilitation. The effects reported for adenosine and calcium are consistent with the findings of the present study in the CA1 region, and suggest that the mechanism of action of adenosine in these two systems is quite similar.

A second salient aspect of the present results was the finding that the effect of adenosine was dependent upon the conditioning–testing interval, in that the most

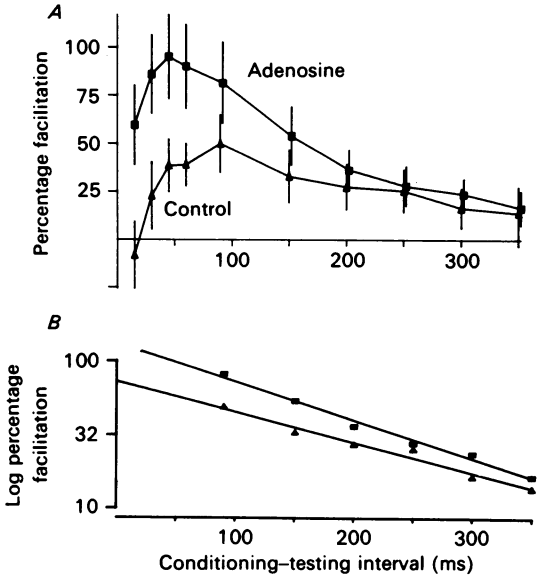

Fig. 7. Effect of adenosine on facilitation or depression. Responses from ten slices tested as in Fig. 6 are shown as the mean ± S.E. of mean facilitation before and during perfusion with 50 μM-adenosine. As with the single slice in Fig. 6, the primary effect of adenosine was to partially reverse the depression of responses at short conditioning–testing intervals. However, the amount of facilitation still was not a simple log–linear function such as is typically seen in low-calcium medium (e.g. Fig. 1). Analysis of the facilitation during the later log–linear portion of the curve (*B*) indicated that adenosine increased facilitation at longer conditioning–testing intervals in addition to its readily apparent effects on the short-term depression (see text).

pronounced effects were observed at short time intervals when a second process, synaptic depression, appeared to be superimposed upon the facilitation. At least two hypotheses might be proposed to account for this apparently selective effect of adenosine at shorter conditioning–testing intervals. Adenosine might reduce transmitter release, minimize the consequent depletion of releasable transmitter, and thus lead to a larger second response. Alternatively, adenosine could antagonize a post-synaptic conductance (e.g. a feed-forward or recurrent GABAergic inhibition that occurs in the dendrites and 'shunts' e.p.s.p. responses: Alger & Nicoll, 1982) that is activated by the conditioning response. In line with this latter hypothesis, intracellularly recorded i.p.s.p.s have been reported to be antagonized by adenosine (Siggins & Schubert, 1981), although this is most probably an indirect effect resulting from reduced transmission at the excitatory pyramidal neurone/inhibitory neurone synapse. The hypothesis that inhibition may be indirectly involved is further supported by the fact that facilitation is reduced at high stimulation intensities

(Fig. 3), and in high-calcium medium (Fig. 5); the relative importance of polysynaptic inhibitory circuits would be expected to be greater under either of these conditions.

However, there are aspects of this hypothesis that are inconsistent with the present results. If adenosine enhances the test response by antagonizing an underlying inhibitory conductance activated by the conditioning stimulus, then the effects of adenosine should be maximal when this inhibitory response is maximal, viz. at high stimulation intensities. In fact, the opposite result was obtained: the effect of adenosine on facilitation was greatest at the lowest stimulation intensity (e.g. Fig. 3). In a group of slices tested as in Fig. 3, the maximal effect of adenosine was observed at the minimum response amplitude in eight out of thirteen slices (data not shown). Thus, it would appear unlikely that adenosine is increasing facilitation selectively at short intervals solely by antagonizing a short-lived conductance that shunts the response to the test stimulus.

The alternative hypothesis is that adenosine decreases the initial release of transmitter, and hence reduces the depletion that leads to depression of the second response at short intervals. Thus, in high-calcium medium where the release of transmitter is maximal, depletion would be greatest and the effect of adenosine on facilitation or depletion would be maximal as well. This was in fact observed to be the case. However, this hypothesis by itself cannot explain why adenosine increased facilitation to the greatest extent at the smallest response amplitudes. Presumably the amount of transmitter released by an individual terminal is largely independent of the number of activated terminals, so the extent of depletion would be constant. This more complex interaction between the action of adenosine on facilitation and response amplitude probably reflects the effects of non-linear summation: as the response amplitude increases, the degree of non-linear summation increases and the degree of facilitation is correspondingly reduced (see Dunwiddie, 1984, for a discussion of this phenomenon; see also McNaughton, Barnes & Andersen, 1981). Reducing the conditioning response, either by reducing the stimulation intensity or by diminishing release (adenosine), would have the same effect, viz. an increase in the degree of facilitation that is observed.

Thus, although other hypotheses are consistent with the experimental results, the simplest explanation for the present findings is that adenosine acts presynaptically to reduce transmitter release. Other actions of adenosine on the post-synaptic neurone and upon interneuronal inhibitory pathways certainly contribute to the electrophysiological effects of this neuromodulator in the hippocampus, but may not contribute in any major way to its depressant effect upon synaptic transmission.

This research was supported by research grants DA 02702 to T.V.D. and by the Veterans Administration Medical Research Service.

REFERENCES

ALGER, B. E. & NICOLL, R. A. (1982). Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied *in vitro*. *Journal of Physiology* **328**, 105–123.

ALGER, B. E. & TEYLER, T. J. (1976). Long-term and short-term plasticity in the CA1, CA3 and dentate regions of the rat hippocampal slice. *Brain Research* **110**, 463–480.

ANDERSEN, P. (1960*a*). Interhippocampal impulses. II. Apical dendritic activation of CA1 neurons. *Acta physiologica scandinavica* **48**, 178–208.

ANDERSEN, P. (1960*b*). Interhippocampal impulses. III. Basal dendritic activation of CA3 neurons. *Acta physiologica scandinavica* **48**, 209–230.

BETZ, W. J. (1970). Depression of transmitter release at the neuromuscular junction of the frog. *Journal of Physiology* **206**, 629–644.

BUCKLE, P. J. & HAAS, H. L. (1982). Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat. *Journal of Physiology* **326**, 109–122.

CORRADETTI, R., LO CONTE, G., MORONI, F., PASSANI, M. B. & PEPEU, G. (1984). Adenosine decreases aspartate and glutamate release from rat hippocampal slices. *European Journal of Pharmacology* **104**, 19–26.

CORRADETTI, R., MONETI, G., MORONI, F., PEPEU, G. & WIERASZKO, A. (1983). Electrical stimulation of the stratum radiatum increases the release and neosynthesis of aspartate, glutamate, and gamma-aminobutyric acid in rat hippocampal slices. *Journal of Neurochemistry* **41**, 1518–1525.

CREAGER, R., DUNWIDDIE, T. & LYNCH, G. (1980). Paired-pulse and frequency facilitation in the CA1 region of the *in vitro* rat hippocampus. *Journal of Physiology* **299**, 409–424.

DOLPHIN, A. C. & ARCHER, E. R. (1983). An adenosine agonist inhibits and a cyclic AMP analogue enhances the release of glutamate but not GABA from slices of rat dentate gyrus. *Neuroscience Letters* **43**, 49–54.

DUNWIDDIE, T. V. (1984). Interactions between the effects of adenosine and calcium on synaptic responses in rat hippocampus *in vitro*. *Journal of Physiology* **350**, 545–559.

DUNWIDDIE, T. V. & HOFFER, B. J. (1980). Adenine nucleotides and synaptic transmission in the *in vitro* rat hippocampus. *British Journal of Pharmacology* **69**, 59–68.

DUNWIDDIE, T. V. & LYNCH, G. S. (1978). Long-term potentiation and depression of synaptic responses in the rat hippocampus: localization and frequency dependency. *Journal of Physiology* **276**, 353–367.

FREDHOLM, B. B. & HEDQVIST, P. (1980). Modulation of neurotransmission by purine nucleosides and nucleotides. *Biochemical Pharmacology* **25**, 1583–1588.

GINSBORG, B. L. & HIRST, G. D. S. (1972). The effect of adenosine on the release of the transmitter from the phrenic nerve of the rat. *Journal of Physiology* **224**, 629–645.

HAAS, H. & GREENE, R. W. (1984). Adenosine enhances afterhyperpolarization and accommodation in hippocampal pyramidal cells. *Pflügers Archiv* **402**, 244–247.

HAAS, H. L. & ROSE, G. (1982). Long-term potentiation of excitatory synaptic transmission in the rat hippocampus: the role of inhibitory processes. *Journal of Physiology* **329**, 541–552.

HARRIS, E. W. & COTMAN, C. W. (1983*a*). Antagonism of medial perforant path responses by baclofen, adenosine and kynurenic acid: differentiation of pre- and postsynaptic antagonism. *Neuroscience Abstracts* **9**, 1141.

HARRIS, E. W. & COTMAN, C. W. (1983*b*). Effects of acidic amino acid antagonists on paired-pulse potentiation at the lateral perforant path. *Experimental Brain Research* **52**, 455–460.

HOLLINS, C. & STONE, T. W. (1980). Adenosine inhibition of gamma-aminobutyric acid release from slices of rat cerebral cortex. *British Journal of Pharmacology* **69**, 107–112.

JONZON, B. & FREDHOLM, B. B. (1984). Adenosine receptor mediated inhibition of noradrenaline release from slices of rat hippocampus. *Life Sciences* **35**, 1971–1979.

KATZ, B. & MILEDI, R. (1965). The effect of calcium on the release of acetylcholine from motor nerve terminals. *Proceedings of the Royal Society* B **161**, 496–503.

KATZ, B. & MILEDI, R. (1968). The role of calcium in neuromuscular facilitation. *Journal of Physiology* **195**, 481–492.

LØMO, T. (1971). Potentiation of monosynaptic e.p.s.p.s in the perforant path–dentate granule cell synapse. *Experimental Brain Research* **12**, 46–63.

MCNAUGHTON, B. L., BARNES, C. A. & ANDERSEN, P. (1981). Synaptic efficacy and e.p.s.p. summation in granule cells of rat fascia dentata studied *in vitro*. *Journal of Neurophysiology* **46**, 952–966.

MUELLER, A. L., HOFFER, B. J. & DUNWIDDIE, T. V. (1981). Noradrenergic responses in rat hippocampus: evidence for mediation by alpha and beta receptors in the *in vitro* slice. *Brain Research* **214**, 113–126.

NADLER, J. V., WHITE, W. F., VACA, K. W., REDBURN, D. A. & COTMAN, C. W. (1977). Characterization of putative amino acid transmitter release from slices of rat dentate gyrus. *Journal of Neurochemistry* **29**, 279–290.

OKADA, Y. & KURODA, Y. (1975). Inhibitory action of adenosine and adenine nucleotides on the postsynaptic potential of olfactory cortex slices of the guinea pig. *Proceedings of the Japan Academy* **51**, 491–494.

PROCTOR, W. R. & DUNWIDDIE, T. V. (1983). Adenosine inhibits calcium spikes in hippocampal pyramidal neurons *in vitro*. *Neuroscience Letters* **35**, 197–201.

RAHAMIMOFF, R. (1968). A dual action of calcium ions on neuromuscular facilitation. *Journal of Physiology* **195**, 471–480.

SCHOLFIELD, C. N. (1978). Depression of evoked potentials in brain slices by adenosine compounds. *British Journal of Pharmacology* **63**, 239–244.

SCHUBERT, P. & MITZDORF, U. (1979). Analysis and quantitative evaluation of the depressant effect of adenosine on evoked potentials in hippocampal slices. *Brain Research* **172**, 186–190.

SEGAL, M. (1982). Intracellular analysis of a postsynaptic action of adenosine in the rat hippocampus. *European Journal of Pharmacology* **79**, 193–199.

SIGGINS, G. R. & SCHUBERT, P. (1981). Adenosine depression of hippocampal neurons *in vitro*: an intracellular study of dose-dependent actions on synaptic and membrane potentials. *Neuroscience Letters* **23**, 55–60.

SILINSKY, E. M. (1980). Evidence for specific adenosine receptors at cholinergic nerve endings. *British Journal of Pharmacology* **71**, 191–194.

SILINSKY, E. M. (1984). On the mechanism by which adenosine receptor activation inhibits the release of acetylcholine from motor nerve endings. *Journal of Physiology* **346**, 243–256.

VIZI, E. S. & KNOLL, J. (1976). The inhibitory effect of adenosine and related nucleotides on the release of acetylcholine. *Neuroscience* **1**, 391–398.