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# The contribution of adenosine to paired-pulse inhibition in the normal and disinhibited hippocampal slice

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**Abstract**

The effects of the adenosine receptor antagonist 1,3-dimethyl-8-cyclopentylxanthine (cyclopentyltheophylline) and the enzyme adenosine deaminase have been examined on paired-pulse inhibition between orthodromic evoked field potentials in the CA1 region of the normal and disinhibited hippocampal slice. In the presence of the $GABA_A$ receptor antagonist (−)-bicuculline methobromide, cyclopentyltheophylline suppressed homosynaptic paired-pulse inhibition between stimuli 300 ms apart. Slices treated with (−)-bicuculline and cyclopentyltheophylline together tended to develop spontaneous burst potentials. In slices in which a surgical cut isolated the CA1 and CA3 areas, thereby preventing the development of bursts in CA1, the effect on paired-pulse inhibition was lessened but was still apparent. Adenosine deaminase, in the presence of (−)-bicuculline showed the same effect as cyclopentyltheophylline, decreasing substantially the amount of paired-pulse inhibition. These results suggest that adenosine may contribute to homosynaptic paired-pulse inhibition in disinhibited slices. For comparison, we also examined the effect of cyclopentyltheophylline in normal ((−)-bicuculline-free) slices. At 100 nM, cyclopentyltheophylline increased reversibly the size of orthodromically evoked synaptic population potentials in the CA1 region of the slices and also reduced reversibly the degree of homosynaptic paired-pulse inhibition between two stimuli delivered only 30 ms apart. This suggests that adenosine may also contribute to shorter latency paired-pulse inhibition in the normal hippocampal slice.

*Keywords:* Adenosine; Purine; (−)-Bicuculline; GABA (γ-aminobutyric acid); Paired-pulse inhibition; Inhibition; Hippocampus; Cyclopentyltheophylline

## 1. Introduction

If field potentials evoked from stratum radiatum are recorded in either the stratum radiatum or the stratum pyramidale of the hippocampus, the delivery of two stimuli in rapid succession results in an inhibition of the second response, known as paired-pulse inhibition. The extent of the inhibition is strongly dependent on the configuration of the inputs and the temporal separation between the two stimuli (Creager et al., 1980; Dunwiddie et al., 1980; Lynch et al., 1981; Sagratella et al., 1991; Steffensen and Hendriksen, 1991), and it is widely accepted that most of the inhibition is due to the activation of local inhibitory interneurones releasing γ-aminobutyric acid (GABA) as transmitter. Since paired-pulse inhibition is observed most markedly and consistently when the interstimulus interval is around 15–100 ms, which corresponds to the time course of the $GABA_A$ mediated fast inhibitory potential recorded intracellularly in pyramidal cells (Davies et al., 1990), it is thought to be largely caused by the activation of $GABA_A$ receptors. It is difficult to test this proposition directly, however, since $GABA_A$ receptor antagonists such as (−)-bicuculline increase the excitability of the pyramidal cells by blocking the effects of spontaneously released GABA. The single population spike normally recorded in the stratum pyramidale in response to a single stimulus in the stratum radiatum or the alveus then becomes a short burst of spikes which may be 40 ms long. Under these conditions of $GABA_A$ inhibition, paired-pulse inhibition between the evoked bursts is actually increased, although the time course is changed in a characteristic fashion such that a marked inhibition is now seen at an interpulse interval of around 300 ms (Higgins and Stone, 1993, 1995). This medium-latency paired-pulse inhibition can be reduced by an antagonist at $GABA_B$ receptors, which are possibly located presynaptically (Higgins and Stone, 1993). However, we have observed that the combination of antag-

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onists at $GABA_A$ and $GABA_B$ receptors often does not abolish paired-pulse inhibition completely.

Adenosine is known to be an effective depressant of neuronal activity, acting both postsynaptically to increase potassium conductances and induce hyperpolarisation (Trussell and Jackson, 1985; Haas and Greene, 1988; Thompson et al., 1992) and presynaptically to suppress the release of neurotransmitters such as glutamate (Corradetti et al., 1984; Fastbom and Fredholm, 1985; Burke and Nadler, 1988), acetylcholine (Spignoli et al., 1984), dopamine (Michaelis et al., 1979; Zetterstrom and Fillenz, 1990) and 5-hydroxytryptamine (Feuerstein et al., 1985). Adenosine is known to be released into the extracellular space following neuronal depolarisation (Stone et al., 1991). Adenosine is at least partly responsible for a prolonged (seconds to minutes) depression of CA1 pyramidal cell epsps caused by a long (25 Hz for 15 s or 5 Hz for 20 s) conditioning tetanus (Sekino and Koyama, 1992; Grover and Teyler, 1993). Recently Mitchell et al. (1993) have shown that field epsps recorded in the stratum radiatum of the CA1 area can be inhibited by a preceding short burst of stimuli to an independent excitatory pathway (heterosynaptic depression) and that this inhibition is mediated by adenosine. This inhibition appears with a latency of 50 ms, is maximal at about 250 ms, increases with the number of stimulations in the conditioning train and is just detectable after a single conditioning stimulus. We were thus interested to see whether adenosine contributed to the medium-latency homosynaptic paired-pulse inhibition we have described in (−)-bicuculline-treated slices or to the well known short-latency homosynaptic paired-pulse inhibition demonstrable in the absence of added agents. The present work was designed to examine these questions by studying the effect of the $A_1$ adenosine receptor antagonist 1,3-dimethyl-8-cyclopentylxanthine (cyclopentyltheophylline) and of adenosine deaminase.

## 2. Materials and methods

Male Wistar rats (150–250 g) were anaesthetised with urethane and cooled on ice whilst breathing oxygen enriched air, a procedure shown to improve slice quality by Newman et al. (1992). When the rectal temperature reached 30°C (about 15 min) the animals were killed by cervical dislocation.

Hippocampal slices were prepared 450 μm thick in artificial cerebrospinal fluid of composition (mmol $l^{-1}$) $KH_2PO_4$ 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10, saturated with 95% $O_2$/5% $CO_2$. Slices were maintained in an incubation chamber at room temperature in an atmosphere of 5% $CO_2$ in oxygen saturated with water vapour for at least 1 h prior to recording. For one set of experiments, immediately after slice preparation, the slices were divided by a single cut in the CA2 region to isolate the CA1 from the CA3 region.

### 2.1. Recording

For recording, individual slices were transferred to a perfusion chamber (volume approximately 0.5 ml) and superfused submerged at 30°C and at a flow rate of approximately 3 $ml \cdot min^{-1}$. Drugs were added to the superfusing artificial cerebrospinal fluid. Extracellular population potentials were recorded in the CA1 pyramidal cell layer using single glass microelectrodes filled with artificial cerebrospinal fluid. A concentric bipolar stimulating electrode was placed in the stratum radiatum for orthodromic activation of pyramidal cells (in those slices cut across CA2 stimulating and recording electrode were on the same side of the cut). Stimuli were square wave constant-current pulses of 100 μs duration. Paired stimuli were delivered through the same electrode. Evoked responses were amplified, displayed on digital storage oscilloscopes and plotted onto a chart recorder or recorded and analysed on a Viglen microcomputer (SCAN software, John Dempster, University of Strathclyde). In some experiments electrical activity in the slices was recorded continually on a slow speed chart recorder to monitor the presence or absence of spontaneous burst potentials.

Responses to the first of a pair of stimuli, the conditioning stimulus, have been designated $R_{control}$. The subsequent test response is labelled $R_{test}$. Test and conditioning stimuli were the same strength as each other in all experiments and adjusted so that the size of $R_{control}$ was 70% maximal under control conditions (in artificial cerebrospinal fluid or in (−)-bicuculline alone). Responses in the presence of cyclopentyltheophylline or adenosine deaminase and after subsequent washing were measured (i) with stimulus strength unadjusted from control conditions and (ii) in order to compensate for the direct effect of these agents on single evoked potentials, with stimulus strength adjusted such that $R_{control}$ was returned to its size under control conditions.

The interpulse interval between the conditioning stimulus and the paired test stimulus was 300 ms in experiments which took place with 10 μM (−)-bicuculline in the superfusate and 30 ms in experiments which took place in the absence of this agent. Pairs of stimuli were delivered at intervals of 1 min (0.017 Hz).

Response size was taken to be the size of the population spike measured from the peak positivity to the peak negativity. Bicuculline induces the appearance of a burst of population spikes in response to each stimulus; response size in (−)-bicuculline was taken to be the peak to peak size of the first population spike of the group. Inhibition was expressed as the percentage change in $R_{test}$ compared with $R_{control}$, each value for analysis being the mean of three or four individual responses.

Slices were only accepted for experiment if the evoked maximum orthodromic population spike in artificial cerebrospinal fluid after 10 min in the recording chamber was at least 5 mV and free from secondary spikes.

### 2.2. Data analysis

Statistical analysis was by paired *t*-test. Data were tested for normality using a version of the Shapiro-Wilk test (Minitab Release 8). Any data where normality could be rejected at the $P < 0.05$ level were also tested using the Wilcoxon matched pairs test, the results of which are quoted if they differ importantly from the *t*-test. $P < 0.05$ was taken to indicate statistical significance. Results are presented as mean ± S.E.

Cyclopentyltheophylline and adenosine deaminase were obtained from Sigma, (−)-bicuculline methobromide from Research Biochemicals International.

## 3. Results

### 3.1. Experiments in artificial cerebrospinal fluid

When applied during the delivery of single stimuli to the CA1 region (0.017 Hz), in the absence of other agents, cyclopentyltheophylline at a concentration of 100 nM increased the size of the orthodromic potentials by 14 ± 2.9% ($n = 20$; $P < 0.001$) (Fig. 1A). This increase was reversed on washing for 20 min (Fig. 1A). The mean size of the potentials in the washed slices was 8 ± 2.9% smaller than control ($n = 20$; $P < 0.01$).

When we examined the effect of cyclopentyltheophylline on paired-pulse inhibition at an interpulse interval of 30 ms in artificial cerebrospinal fluid approximately half of the 20 slices tested showed an obvious reduction in paired-pulse inhibition when cyclopentyltheophylline was superfused (Fig. 2). Overall, the amount of inhibition produced was significantly reduced by 47 ± 8.1% ($n = 20$; $P < 0.0001$) (Fig. 1B). The effect of cyclopentyltheophylline was virtually completely reversed after washing.

This result, however, was obtained in a situation in which the size of the first response ($R_{test}$) had been itself increased by cyclopentyltheophylline (Fig. 1A), The experiment was therefore also carried out with an adjustment of stimulus strength to compensate for the increased excitability such that the size of $R_{test}$ was restored to that recorded in the absence of cyclopentyltheophylline. Under these circumstances, cyclopentyltheophylline now decreased the mean paired-pulse inhibition by 34 ± 10.0% ($n = 20$; $P < 0.01$). Again the effect was substantially and significantly ($P < 0.01$) reversed on washing (Fig. 1A and Fig. 2).

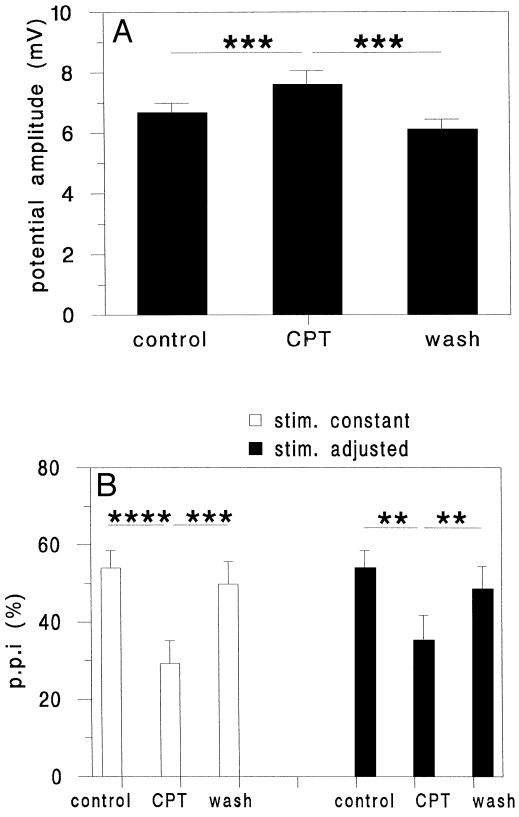


Fig. 1. Effect of cyclopentyltheophylline (CPT) 100 nM (A) on the amplitude of single evoked potentials and (B) on the magnitude of paired-pulse inhibition (p.p.i.) in the same slices. Experiments were carried out in artificial cerebrospinal fluid in the absence of other agents. Stimuli were delivered through a single electrode in the stratum radiatum and responses recorded in the stratum pyramidale of the CA1. Pairs of stimuli were delivered at 1 min intervals with an interpulse interval of 30 ms between each test stimulus and its preceding conditioning stimulus. Test and conditioning stimuli were identical to each other. Stimulus strength was such that the response to the conditioning stimulus ($R_{control}$) was 70% maximal under control conditions in artificial cerebrospinal fluid. Cyclopentyltheophylline (CPT) was superfused for 20 min, with data being collected during the last 5 min of this period, followed by 20 min wash in artificial cerebrospinal fluid. Paired-pulse inhibition is expressed as the percentage difference between $R_{test}$ and $R_{control}$ compared with $R_{control}$. During cyclopentyltheophylline superfusion and subsequent wash paired-pulse inhibition was calculated both using the same stimulus strength as under control conditions (open columns) and with stimulus strength adjusted to counteract the direct effect of cyclopentyltheophylline on response size and return $R_{control}$ to the same size as under control conditions (shaded columns). Slices were initially selected to show obvious paired-pulse inhibition under control conditions. Error bars show S.E. $n = 20$ slices. $^{**}$ $P < 0.01$, $^{***}$ $P < 0.001$, $^{****}$ $P < 0.0001$.

### 3.2. Experiments in the presence of (−)-bicuculline

In (−)-bicuculline 10 μM the evoked orthodromic field potential in the stratum pyramidale consists of a short burst of population spikes up to 50 ms in duration. Under these conditions, cyclopentyltheophylline increased the size of the first spike in each burst in 9 of 12 slices (range 6–24% of control), whereas 3 slices exhibited a large decrease of up to 24% relative to controls. Overall there was no significant change of potential size in these slices (Fig. 3A). In 11 of 12 slices tested, the amplitudes of the evoked potentials remained elevated above control potential size after washing out cyclopentyltheophylline for 20 min. (In one slice the amplitude of the potential decreased in cyclopentyltheophylline and decreased further on washing. When the data from this slice were included in the analy-

sis, the differences in potential size between control, cyclopentyltheophylline and wash were far from normally distributed.) The median amplitude on washing for 20 min was 6% larger than control potentials ($n = 12$; $P < 0.05$; Wilcoxon matched pairs).

Most slices examined in the presence of (−)-bicuculline demonstrated marked paired-pulse inhibition at an interpulse interval of 300 ms, as reported previously (Higgins and Stone, 1993, 1995). In the presence of (−)-bicuculline, cyclopentyltheophylline decreased paired-pulse inhibition by $62 \pm 4.9\%$ ($n = 12$; $P < 0.0001$) when stimuli were not adjusted throughout the experiment, and by $55 \pm 7.5\%$ ($n = 12$; $P = 0.0001$) when stimuli were adjusted to restore the size of the first potential in cyclopentyltheophylline to the same size as the control potential (Fig. 3 and Fig. 4B). The cyclopentyltheophylline-induced decrease in paired-pulse inhibition was significantly ($P < 0.01$) reversed upon washing for 20 min whether or not stimulus strength was adjusted to account for the effect

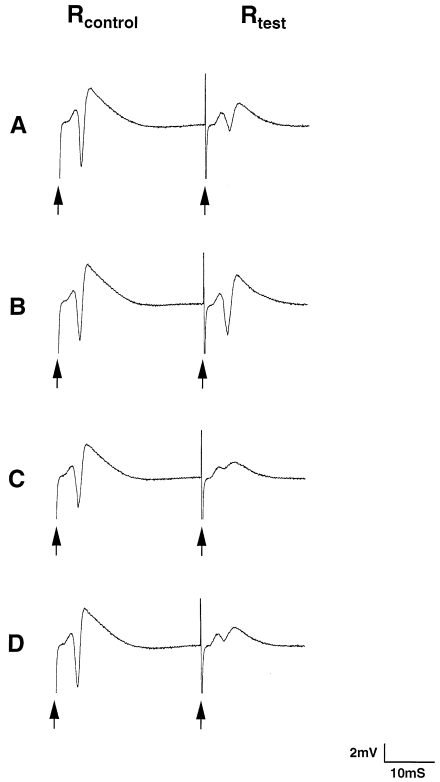


Fig. 2. Typical records of pairs of evoked conditioning ($R_{control}$) and test ($R_{test}$) potentials showing the effect of cyclopentyltheophylline 100 nM on paired-pulse inhibition at an interpulse interval of 30 ms in the absence of other agents. See legend to Fig. 1 for experimental details. (A) Artificial cerebrospinal fluid control. (B) Cyclopentyltheophylline 100 nM. (C) Wash in artificial cerebrospinal fluid. (D) Wash with stimulus readjusted to return $R_{control}$ to its control size in artificial cerebrospinal fluid. Stimulus strength is identical for records A, B and C. $R_{control}$ in this slice showed no change in size on perfusion with cyclopentyltheophylline and a slight decrease on washing. Arrows mark stimulus artifacts. Records were captured on computer (SCAN software, John Dempster, University of Strathclyde) and plotted on a Hewlett Packard plotter.

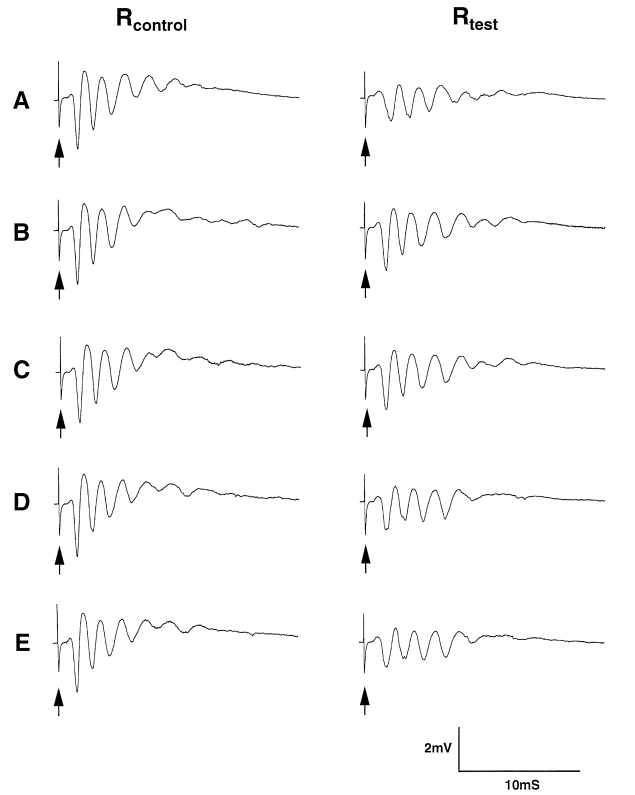


Fig. 3. Typical records of pairs of evoked conditioning ($R_{control}$) and test ($R_{test}$) potentials showing the effect of cyclopentyltheophylline 100 nM on paired-pulse inhibition at an interpulse interval of 300 ms in the presence of 10 μM bicuculline. Experimental details were as in Fig. 4. (A) Control. (B) Cyclopentyltheophylline 100 nM. (C) Cyclopentyltheophylline 100 nM with stimulus readjusted to return $R_{control}$ to control size. (D) Wash. (E) Wash with stimulus readjusted to return $R_{control}$ to its control size in bicuculline alone. Stimulus strength was identical for records A, B and D. Bicuculline 10 μM was present throughout. Arrows mark stimulus artifacts. Records were captured on computer (SCAN software, John Dempster, University of Strathclyde) and plotted on a Hewlett Packard plotter.

of cyclopentyltheophylline on individual evoked potentials. Reversal was greater when the stimulus strength had been adjusted. When stimulus strength was adjusted the recovering slices showed paired-pulse inhibition which was only $19 \pm 7.9\%$ ($n = 12$; $P < 0.05$) less than controls after 20 min wash (Fig. 4B), but when stimulus strengths were not adjusted, potentials in washed slices showed paired-pulse inhibition which was still $41 \pm 6.2\%$ ($n = 12$; $P < 0.0001$) less than controls.

### *3.3. Separation of CA1 and CA3 regions in (−)-bicuculline*

Ten slices were prepared in which the CA1 and CA3 regions were isolated by means of a surgical cut in the CA2 region. In these slices, cyclopentyltheophylline increased the single evoked potentials by $13 \pm 2.0\%$ ($n = 10$; $P < 0.0001$) (Fig. 5A). This effect reversed upon washing for 20 min.

The inclusion of cyclopentyltheophylline at 100 nM decreased paired-pulse inhibition by $42 \pm 4.1\%$ ($n = 10$; $P < 0.0001$) when stimuli were not adjusted. This effect was partly but significantly reversible ($n = 10$; $P < 0.05$) (Fig. 5B); inhibition remained decreased by $28 \pm 6\%$ compared with controls after 20 min washing ($n = 10$; $P < 0.01$).

When stimulus strength was adjusted so as to restore the size of the first potential in cyclopentyltheophylline to the same size as the control potential, the xanthine still decreased paired-pulse inhibition significantly, though to a lesser extent ($16 \pm 3.7\%$; $n = 10$; $P < 0.01$). In this case there was no significant reversal of the effect on washing for 20 min (Fig. 5B).

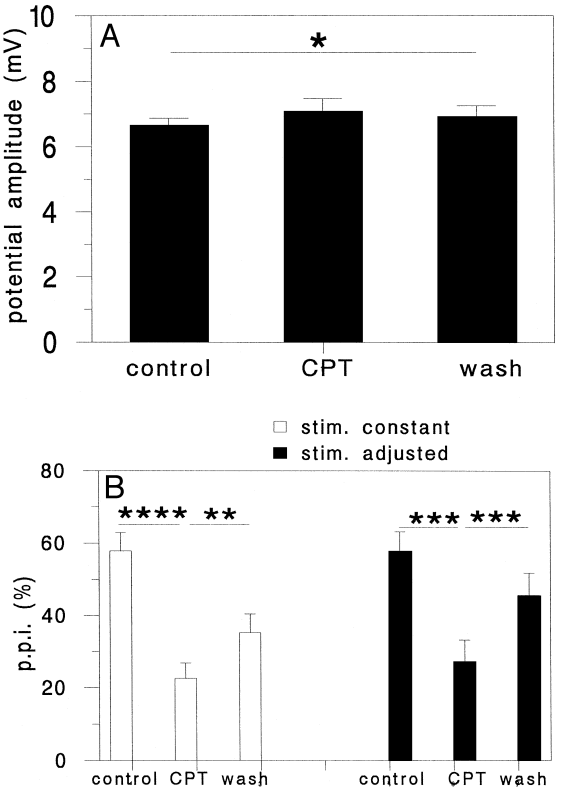


Fig. 4. Effect of cyclopentyltheophylline (CPT) 100 nM (A) on the amplitude of single evoked potentials and (B) on the magnitude of paired-pulse inhibition (p.p.i.) in the same slices in the presence of bicuculline. (−)-Bicuculline methobromide 10 μM was present throughout each experiment and paired-pulse inhibition was measured at an interpulse interval of 300 ms, otherwise experimental details are as for Fig. 1. Stimulus strength was initially set such that the response to the conditioning stimulus ($R_{control}$) was 70% maximal under control conditions in bicuculline. In the presence of cyclopentyltheophylline and after washing paired-pulse inhibition was calculated both using the same stimulus strength as under control conditions (open columns) and with stimulus strength adjusted to counteract the direct effect of cyclopentyltheophylline on response size and return $R_{control}$ to the same size as under control conditions (shaded columns). Error bars show S.E. $n = 12$ slices. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

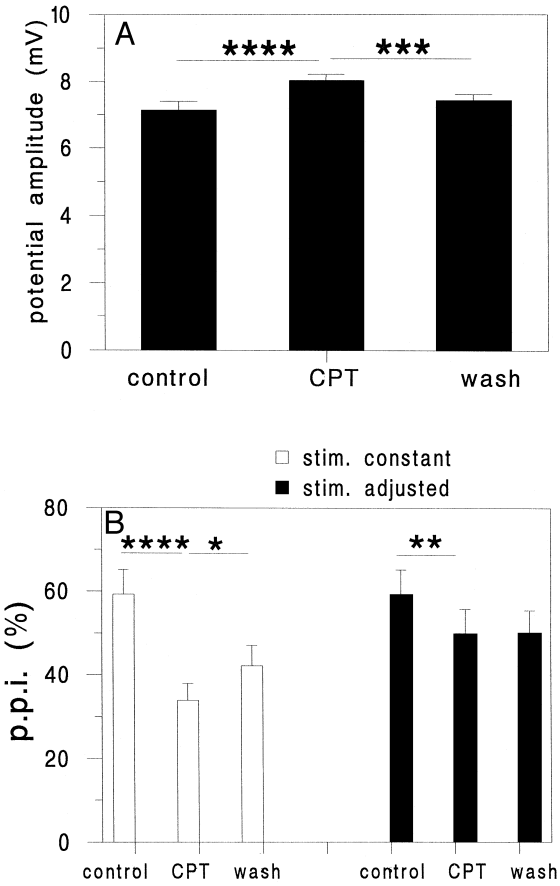


Fig. 5. Effect of cyclopentyltheophylline (CPT) 100 nM (A) on the amplitude of single evoked potentials and (B) on the magnitude of paired-pulse inhibition in the same slices in the presence of bicuculline. All slices were cut to divide the CA1 from the CA3 area. Experimental details are otherwise as for Fig. 4. In the presence of cyclopentyltheophylline and after washing paired-pulse inhibition was calculated both using the same stimulus strength as under control conditions (open columns) and with stimulus strength adjusted to counteract the direct effect of cyclopentyltheophylline on response size and return $R_{control}$ to the same size as under control conditions (shaded columns). Error bars show S.E. $n = 12$ slices. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

### 3.4. Adenosine deaminase

At an activity of 1 $U \cdot ml^{-1}$ adenosine deaminase had no significant effect on the mean amplitude of single (0.017 Hz) orthodromic potentials in 10 slices (Fig. 6A). Individual potentials showed a range of effects, with 5 slices exhibiting a decrease (range 6–48%) in potential size and 4 slices an increase (range 1–13%). Adenosine deaminase decreased the extent of paired-pulse inhibition by $43 \pm 6.2\%$ ($n = 10$; $P < 0.001$) when stimulus strength was not adjusted to compensate for the effect of the enzyme on individual single evoked potential size (Fig. 6B). An even greater effect was observed when stimulus strength was adjusted, paired-pulse inhibition now being reduced by $50 \pm 6.2\%$ ($n = 10$; $P < 0.001$). The decrease

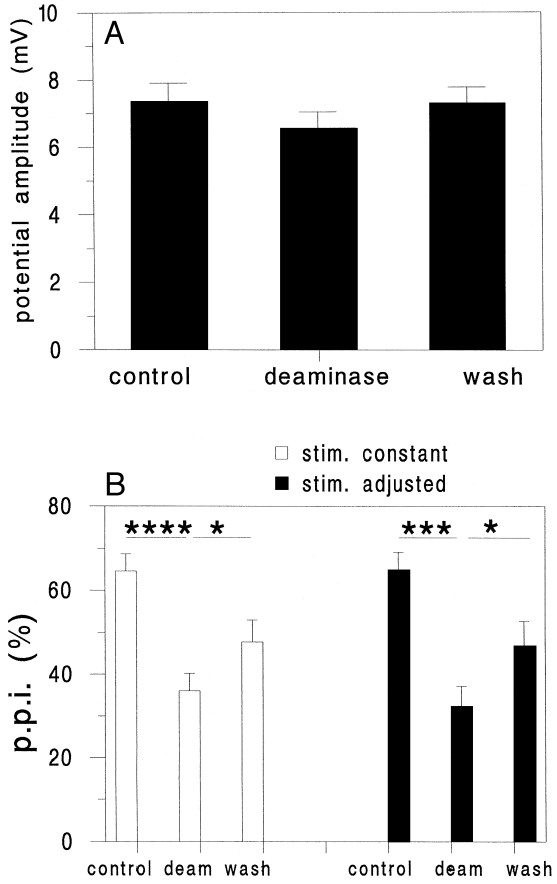


Fig. 6. Effect of adenosine deaminase (deam) 1 U·ml$^{-1}$ (A) on the amplitude of single evoked potentials and (B) on the magnitude of paired-pulse inhibition in the same slices in the presence of bicuculline. Experimental details are as for Fig. 4 except that the slices were perfused with adenosine deaminase in the place of cyclopentyltheophylline. In the presence of adenosine deaminase and after washing paired-pulse inhibition was calculated both using the same stimulus strength as under control conditions (open columns) and with stimulus strength adjusted to counteract any direct effect of adenosine deaminase on individual response sizes and return $R_{control}$ to the same size as under control conditions (shaded columns). Error bars show S.E. $n = 12$ slices. $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$, $^{***}$ $P < 0.001$, $^{****}$ $P < 0.0001$.

in inhibition was partly but significantly reversed after 20 min washing in experiments with or without stimulus adjustments (Fig. 6B).

When stimulus strength was adjusted, the recovering slices showed paired-pulse inhibition which was only 28 ± 7.6% ($n = 10$; $P < 0.01$) less than controls after 20 min wash (Fig. 6B), and when stimulus strengths were not adjusted, potentials in washed slices showed paired-pulse inhibition which was 27 ± 5.7% ($n = 10$; $P < 0.01$) less than controls.

### 3.5. Spontaneous bursts

Many of the slices which were exposed to (−)-bicuculline developed spontaneous bursts when they were additionally perfused with cyclopentyltheophylline or adenosine deaminase. The spontaneous bursts had comparable amplitudes to the evoked potentials, generally occurred with a frequency considerably less than 1 Hz, were not present before the addition of adenosine deaminase or cyclopentyltheophylline and decreased in frequency or disappeared when slices were washed in (−)-bicuculline alone. Such spontaneous bursts did not occur in any of the slices in which a surgical cut in CA2 divided the CA1 region from the CA3.

## 4. Discussion

Several previous groups (e.g. Dunwiddie and Haas, 1985) have reported seeing paired-pulse facilitation with homosynaptic stimulation paradigms, but paired-pulse inhibition was observed routinely in the present study. This is probably the result of employing slices in which excitability was increased and GABA-mediated inhibition suppressed by the inclusion of (−)-bicuculline.

Cyclopentyltheophylline was used throughout this work at a concentration of 100 nM which pilot experiments showed to be sufficient to reduce by 70% the effect of 20 μM adenosine on single evoked potentials. At 20 μM, adenosine itself in our hands produced an approximately 60% decrease in the size of single evoked potentials in artificial cerebrospinal fluid. The ability of cyclopentyltheophylline to increase significantly the size of single orthodromic evoked potentials is consistent with several previous studies and probably reflects the presence of sufficient endogenous adenosine in the hippocampal slice preparation to exert a tonic restraint on transmitter release (Dunwiddie, 1980). It is curious, however, that this effect is no longer clearly observed in the presence of (−)-bicuculline. It might be anticipated that the increased neuronal excitability engendered by the block of GABA receptors would increase the ambient level of adenosine and thus invite a greater relative increase of potential size by cyclopentyltheophylline than in drug-naive slices. However, no overall effect on potential size was noted with cyclopentyltheophylline or adenosine deaminase in the presence of (−)-bicuculline. This may be related to the production of spontaneous bursts of action potentials in (−)-bicuculline-treated slices since the surgical isolation of CA1 and CA3 regions eliminated the large amplitude spontaneous bursts and restored the ability of cyclopentyltheophylline to increase evoked potential size. Wong and Traub (1983) showed spontaneous bursts in the disinhibited hippocampus to be initiated in the CA3 region and transmitted along the stratum radiatum to the CA1.

Both cyclopentyltheophylline and adenosine deaminase proved to have a marked effect on paired-pulse inhibition at an interpulse interval of 300 ms in the presence of (−)-bicuculline, producing a highly significant depression. This effect was seen in most of the individual slices tested and occurred irrespective of whether the stimulus strength

was adjusted to compensate for the effect of cyclopentyltheophylline on control potential size. This is important since paired-pulse inhibition between the responses to pairs of equal stimuli tends to decrease as stimulus strength and therefore response size is increased (Higgins and Stone, 1995). Adenosine deaminase, the enzyme responsible for metabolising adenosine to inosine was used in order to exclude possible non-specific effects of cyclopentyltheophylline.

Taken at face value these results might suggest that in the presence of (−)-bicuculline adenosine is released as a result of the conditioning stimulus and the resulting short synchronised burst of pyramidal cell firing and that this adenosine contributes directly to the suppression of the subsequent test response. This interpretation is consistent with the results of Mitchell et al. (1993) (see Introduction). However, a common effect of both adenosine deaminase and cyclopentyltheophylline was to induce spontaneous bursts in the (−)-bicuculline-treated slices. Spontaneous bursts were not seen in the slices which had been cut to separate the CA1 from the CA3 areas. Cyclopentyltheophylline had a smaller, but still significant effect on paired-pulse inhibition in these cut slices suggesting that the presence of spontaneous bursts per se decreased paired-pulse inhibition and that some but not all of the effect of cyclopentyltheophylline and adenosine deaminase to reduce paired-pulse inhibition in the uncut slices was related to the induction of spontaneous bursts. This effect of spontaneous bursting on paired-pulse inhibition is itself an interesting observation. The mechanism is by no means clear but may be related to the fact that GABA-mediated inhibition in the normal hippocampus is known to fatigue at frequencies greater than 0.1 Hz because of the activation of inhibitory $GABA_B$ autoreceptors on inhibitory interneurones (Davies et al., 1990; Nathan and Lambert, 1991). Baclofen, a $GABA_B$ receptor agonist, decreases (−)-bicuculline-resistant paired-pulse inhibition (Higgins and Stone, 1993).

Cyclopentyltheophylline also reduced paired-pulse inhibition at an interpulse interval of 30 ms in drug naive slices. The effect was not seen in all slices but 11 out of the 20 slices tested showed an obvious marked decrease in paired-pulse inhibition with perfusion of cyclopentyltheophylline (Fig. 1) and the effect was highly significant over the group of 20 slices. In each of the 11 slices the effect of cyclopentyltheophylline on paired-pulse inhibition was substantially reversible on washing in artificial cerebrospinal fluid for 20 min. This marked effect of cyclopentyltheophylline on paired-pulse inhibition in drug-naive slices was an unexpected finding, particularly in view of the short-latency (30 ms) between conditioning and test response. This interval seems too short for the generation of a response mediated by a G-protein-coupled receptor.

As noted above, the suppression of paired-pulse inhibition by cyclopentyltheophylline was more pronounced in slices which exhibited spontaneous bursting. This is also apparent from a comparison of the hatched columns in Fig. 3B and Fig. 5B. The greater effect of cyclopentyltheophylline in slices showing bursts may be related to the finding that activation of receptors for *N*-methyl-D-aspartate, which would be induced by the repeated activity which constitutes a burst, can induce a significant release of adenosine (Chen et al., 1992; Manzoni et al., 1994).

Mitchell et al. (1993) demonstrated an adenosine-mediated depression of field epsps in the stratum radiatum of the CA1 after single and multiple conditioning stimuli to an independent excitatory pathway (heterosynaptic depression), but the effect was relatively small when only a single conditioning stimulus was used (about 6% inhibition) and occurred with a latency of greater than 50 ms, with a maximal effect at about 250 ms. In the present work it is unlikely that the effect of cyclopentyltheophylline on paired-pulse inhibition was a direct consequence of its facilitatory effect on single evoked potentials since it could be demonstrated both before and after compensatory adjustment of stimulus strength in cyclopentyltheophylline. Furthermore, there was no significant correlation between the effect of cyclopentyltheophylline on evoked potential size and on paired-pulse inhibition ($r = 0.297$, $n = 20$, n.s.) with the non-significant trend in the wrong direction to favour this explanation. This does not remove the possibility that the cyclopentyltheophylline-mediated reduction in background adenosine tone may have other effects, not directly reflected by changes in PS size, which may account for the decrease in paired-pulse inhibition.

Adenosine itself decreases paired-pulse inhibition, possibly by increasing simultaneous paired-pulse facilitation, both at short latency in the absence of other drugs (Dunwiddie and Haas, 1985) and at 300 ms in the presence of (−)-bicuculline (Higgins and Stone, 1993, 1995). The inhibitory effect of cyclopentyltheophylline on paired-pulse inhibition was thus the reverse of that which would be expected from decreasing the activity of basal levels of endogenous adenosine. Since we have used only a single adenosine antagonist agent we cannot exclude a non-purine-related effect of cyclopentyltheophylline, although at the concentration used (100 nM) cyclopentyltheophylline does not interact with any other known receptor population to any significant extent, nor does it affect purine metabolic enzymes. The similar depressant action of adenosine and cyclopentyltheophylline is therefore intriguing but remains consistent with the possibility that release of adenosine or another adenosine receptor agonist may have contributed directly to short-latency (30 ms) paired-pulse inhibition in more than half of the hippocampal slices examined. The similarity between the effects of adenosine and cyclopentyltheophylline presumably reflects a greater activity of exogenous adenosine in enhancing paired-pulse facilitation. Alternatively it is conceivable that, since adenosine is able to activate $A_1$ and $A_2$ receptors, the similarity of action reflects a differing contribu-

tion of these receptors to the regulation of synaptic transmission. Further examination of selective ligands will be necessary to explore this possibility.

The effects of cyclopentyltheophylline on single evoked potentials and paired-pulse inhibition in drug-naive slices in artificial cerebrospinal fluid were readily reversible. In contrast, in the presence of (−)-bicuculline, the suppression of paired-pulse inhibition by cyclopentyltheophylline was only partly, though still significantly, reversible. It is unlikely that this represents a problem of washout of the xanthine, which is relatively hydrophilic, in view of the ready reversibility on normal slices. The effect of adenosine deaminase on paired-pulse inhibition was also only partly reversible. It is possible this observation may reflect a role for adenosine in the long-term control of synaptic transmission such that in the absence of GABA-mediated inhibition, a blockade of adenosine receptors yields a long-term depression of neuronal inhibition. This may be a general effect due to the increased transmitter release obtained by blocking adenosine receptors.

It is also possible that the observation reflects a specific interaction between GABA and adenosine receptors. Akhondzadeh and Stone (1994) have reported a mutual potentiation between these agonists on pyramidal neurones. It is conceivable that functional $GABA_A$ receptors are required to maintain adenosine receptors in a functional (agonist-sensitive) state. In the absence of $GABA_A$ receptors (in the presence of (−)-bicuculline), a transient block of adenosine receptors is then sufficient to switch the receptors to an antagonist affinity state which cannot be reversed even on removing the adenosine antagonist. Conceptually this is equivalent to a long-term potentiation of the inhibited synapses. Indeed, the observation that cyclopentyltheophylline can induce long-term decreases of paired-pulse inhibition may help to account for the long-lasting development of spontaneous epileptiform bursting reported by Alzheimer et al. (1990) following their use of the less hydrophilic adenosine receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine.

In summary, the present results demonstrate that an adenosine receptor antagonist, cyclopentyltheophylline, and the adenosine- metabolising enzyme adenosine deaminase can reduce paired-pulse inhibition in slices in which GABA-mediated inhibition has been blocked by (−)-bicuculline. Cyclopentyltheophylline also reduced paired-pulse inhibition in normal hippocampal slices. The partial recovery from adenosine blockade in the (−)-bicuculline-treated slices may indicate an involvement of adenosine in long-term changes of neuronal excitability.

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