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Transient and selective blockade of adenosine A₁-receptors by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) causes sustained epileptiform activity in hippocampal CA3 neurons of guinea pigs

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The effects of endogenously released adenosine on the excitability of hippocampal neurons were studied using the novel and highly selective adenosine A₁-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). Extra- and intracellular recordings performed in area CA1 and CA3 of the guinea pig hippocampal slice preparation revealed that a transient suppression of an inhibitory purinergic tonus by DPCPX leads to sustained interictal-like epileptiform activity arising in area CA3. Once induced, the spontaneous burst discharges were apparently irreversible within the observation period, even after prolonged washout (2–3 h) in normal solution. In contrast, the hyperpolarizing action of exogenous adenosine, which was substantially reduced by DPCPX, recovered within 30–60 min of drug washout, indicating that DPCPX was not irreversibly bound to the A₁-receptor.

Several lines of evidence suggest the existence of an inhibitory purinergic tonus on CNS neurons generated by the release of endogenous adenosine [4–7]. The action of adenosine is mediated by specific receptors which have been classified into A₁- and A₂-subtypes [6]. However, electrophysiological investigations of the effects of adenosine on CNS neurons have been hampered by the lack of selective antagonists able to distinguish selectively between adenosine A₁- and A₂-receptors [4, 6]. Therefore, the recent development of the highly selective, competitive A₁-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), which has a 700-fold A₁ selectivity [9], should provide a useful tool to separate the various actions of adenosine in terms of receptor subtype activation.

Slices (500 μm) were prepared from the guinea pig hippocampus and kept submerged in bathing solution (30–31°C) containing (in mM): NaCl 121, KCl 3,

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NaHCO₃ 22, NaH₂PO₄ 1.2, MgCl₂ 1, CaCl₂ 1.5, glucose 11, gassed with a mixture of 95% O₂ and 5% CO₂. Electrical stimulation of the Schaffer collateral/commissural pathway and the mossy fiber input to CA3 was performed using concentric steel electrodes. Field potentials were recorded in the CA1 and CA3 region by means of glass microelectrodes (2–4 MΩ) filled with 3 M NaCl. Intracellular signals obtained from CA3 neurons were recorded using electrodes (40–80 MΩ) filled with 3 M KCl. The membrane potential and the input resistance (R_N) were continuously monitored on a chart writer and stored on tape for off-line analysis. DPCPX was taken from a stock solution (10 mM, dissolved in dimethylsulfoxide, DMSO) and added to the medium to reach final concentrations between 10 and 100 nM. Maximum final solvent concentration was 0.001%. At this concentration, DMSO neither affected the extracellularly recorded field potentials nor the neuronal properties determined by intracellular recordings. Adenosine (10–50 μM) and bicuculline methiodide (10 μM) were dissolved directly in bathing solution.

Extracellular recordings of orthodromically evoked population spikes and field-EPSPs were performed in the CA1 and CA3 subfields of hippocampal slice preparations ($n=27$). DPCPX (30–100 nM) produced a dose-dependent increase (20–60%) in the amplitude of the population spike recorded in stratum pyramidale and a comparable increase in the amplitude of the field-EPSP recorded in stratum radiatum of area CA3 in all slices tested ($n=9$). In 6 out of 9 slices, DPCPX (30–100 nM) caused a dramatic change in the response to stimulation of the mossy fiber input. Thus, sub-maximal stimulation intensities, which evoked single field-EPSPs under control conditions (Fig. 1A, left), elicited multiple afterdischarges superimposed on a prolonged negative DC-shift following superfusion with DPCPX (Fig. 1A, right). Similar afterdischarges were observed in stratum pyramidale of area CA3. In addition, spontaneous field-EPSPs and population spikes (Fig. 1B, left inset) and interictal-like events at a frequency of 1–6/min (Fig. 1B, right inset) developed within 10–15 min upon application of DPCPX (Fig. 1B). Under control conditions, these slices did not display spontaneous field activity or afterdischarges. The minimum concentration of DPCPX necessary to induce spontaneous epileptiform activity was 30 nM. Upon washout of the drug, both the spontaneous bursts and the afterdischarges persisted for up to one hour, before gradually declining (Fig. 1C). However, within the observation period (3–5 h), recovery to control conditions was never achieved. When tested in area CA1, 30–100 nM DPCPX produced similar patterns of epileptiform activity in 4 out of 10 slices. In contrast to area CA3, the compound (100 nM) consistently failed to induce epileptiform activity in the CA1 region of 'minislices', in which area CA3 was surgically removed ($n=8$). However, the amplitude of the population spike was found to be increased by 40%. This finding indicated that area CA3 represented the focus of hyperexcitability in the intact slice.

The action of DPCPX was further investigated by means of intracellular recordings from CA3 neurons. The cells ($n=11$) had a resting membrane potential (RMP) of -64.2 ± 1.2 mV (mean \pm S.E.M.), an input resistance (R_N) of 90.5 ± 3.5 MΩ and an action potential (AP) overshoot of 20–30 mV. Nine out of 11 neurons displayed spontaneous activity consisting of either single spikes or bursts of 2–4 APs riding on

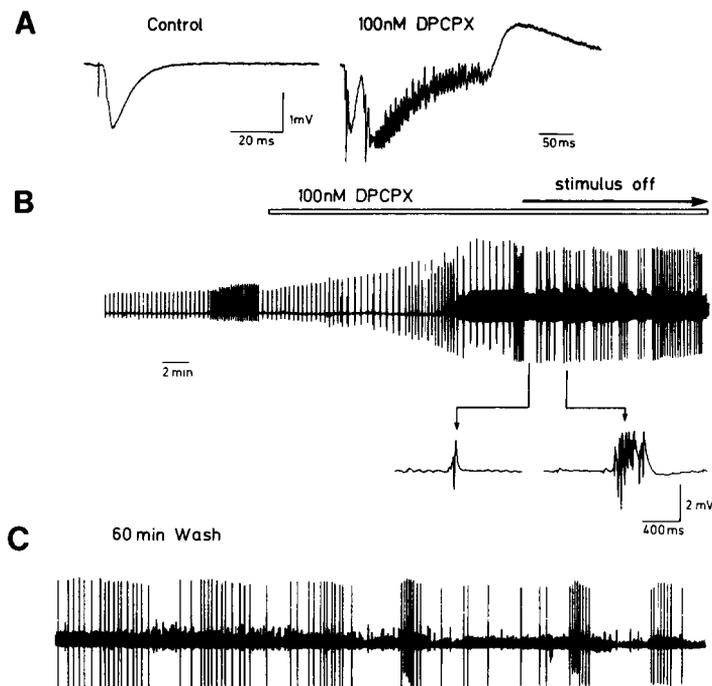


Fig. 1. Actions of DPCPX on field potentials recorded in area CA3. A: dendritic field responses evoked by orthodromic stimulation of the mossy fiber input using a single stimulus (100 μ s, submaximal intensity, time of stimulation indicated by the stimulus artefact) in the absence (control) and presence of 100 nM DPCPX. Note the different time scales on the left- and right-hand side. B: continuous chart records of field activity in CA3 stratum pyramidale before and during application of DPCPX (100 nM). The mossy fiber input was stimulated at a frequency of 0.05 Hz. To demonstrate the sustained spontaneous discharges, the stimulus was switched off for the rest of the experiment. Examples of both spontaneous population spikes (left inset) and interictal-like events (right inset) are depicted below the chart record. C: spontaneous activity recorded after 60 min of washing in normal bathing solution. Time calibration = 2 min (see B).

top of a small depolarizing wave. Upon application of DPCPX (30–100 nM), the membrane depolarized by 4–8 mV, associated with an increase in R_N (Fig. 2A). After 10–15 min of superfusion with DPCPX, the neurons gradually increased their spiking frequency (Fig. 2A) and, within another 3–5 min, 8 out of 11 neurons started to generate rhythmic burst discharges (frequency: 3–9/min, Fig. 2A), sometimes resembling paroxysmal depolarizing shifts (see Fig. 3B). The remaining 3 neurons displayed a strongly enhanced spiking frequency and sporadic bursts (see Fig. 3A). The bursts consisted of high frequency spike discharges elicited by a depolarizing envelope of 150–1000 ms in duration followed by prolonged afterhyperpolarizations (AHP) of up to 20 mV in amplitude (Figs. 2A and 3B). Even after long washout periods (2–3 h), the bursting activity persisted (Fig. 2B). To test whether this sustained epileptiform activity was due to a reduction in GABAergic inhibition caused by an increase in the intracellular calcium concentration [2, 10], the influence of the

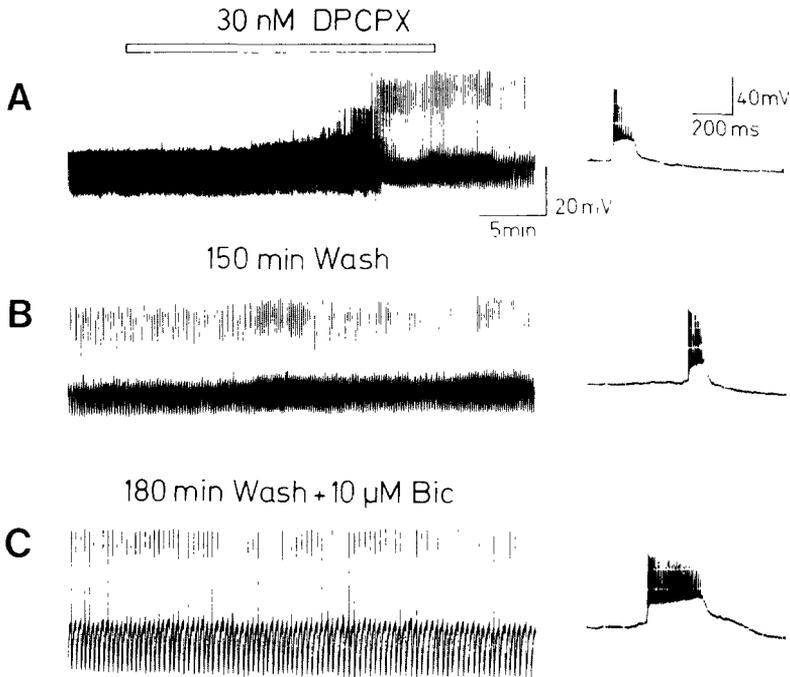


Fig. 2. Actions of DPCPX on a CA3 neuron. Chart records of the membrane potential (left-hand side) and photographs of single bursts (right-hand side) are shown. A: in normal medium the neuron (RMP: -65 mV) did not display spontaneous activity. The downward deflections on the chart record represent the voltage deviations produced by hyperpolarizing current pulses in order to monitor R_N (control value: 80 M Ω). At the onset of bursting activity, the inward current pulse was switched off. On the right-hand side an example of a single burst is depicted on an expanded time scale. B: the epileptiform activity persisted for up to 150 min of washout of the drug. C: following 180 min of washing in normal bathing solution, addition of 10 μ M bicuculline to the ACSF resulted in an increase in burst duration and in a change in the sequence of afterpotentials (see single burst shown on the right-hand side).

GABA_A antagonist, bicuculline, on the bursting behavior was investigated. Superfusion with bicuculline (10 μ M) led to a two-fold increase in the burst duration associated with an increase in the number of spikes per burst (Fig. 2C). In addition, both the duration and the amplitude of the burst-AHP increased thereby leading to a decrease in bursting frequency (Fig. 2C). These findings indicated that the GABA_A-mediated inhibition was not substantially reduced.

The apparent irreversibility of the DPCPX-induced epileptiform activity might be ascribed to either an irreversible binding of the drug to the A₁-receptor or to an unspecific accumulation of the substance in the slice tissue. To investigate these possibilities, the hyperpolarizing action of adenosine was tested before, during and various times after application of DPCPX in the same neuron. In all cells tested ($n=5$), adenosine (50 μ M) produced a reversible hyperpolarization of the membrane by 10 – 12 mV, associated with a decrease in R_N by about 50% (Fig. 3A,B, control). In the presence of DPCPX (100 nM), the effectiveness of adenosine was substantially reduced

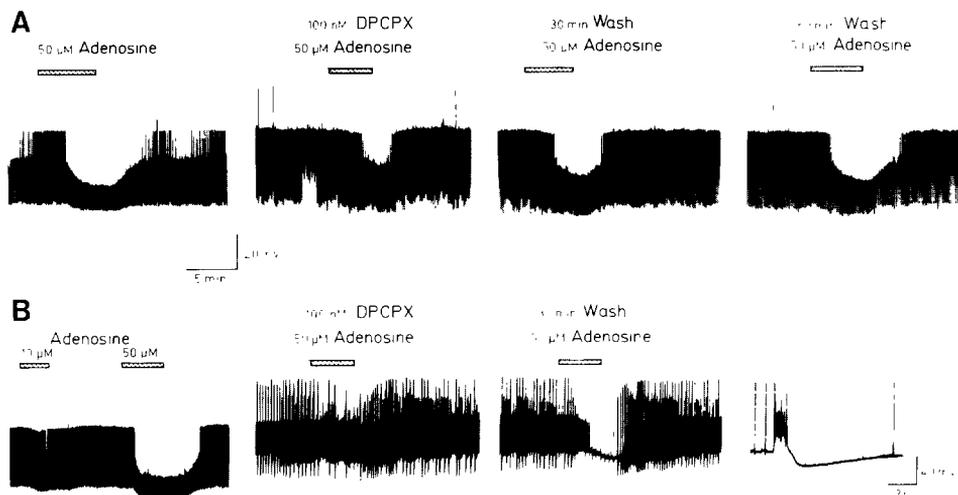


Fig. 3. Reversible antagonism of the adenosine-induced hyperpolarization and induction of sustained hyperexcitability in the same neuron by DPCPX. Chart records of two neurons are shown (A, RMP: -63 mV, R_N : 97 M Ω , B, RMP: -64 mV, R_N : 90 M Ω). Adenosine (50 μ M) was applied before, during and at intervals of 30 min following washout of 100 nM DPCPX. On the right-hand side in B a single PDS is displayed on an expanded time scale. In both neurons, the DPCPX-induced epileptiform activity persisted within the observation period (90 – 120 min washout of DPCPX).

(Fig. 3) or, in one neuron, even abolished. Upon washing in normal bathing solution for 30 – 60 min, a complete or at least partial recovery of adenosine's hyperpolarizing action was observed (Fig. 3), indicating that DPCPX was neither irreversibly bound to the receptor nor did it accumulate in the tissue. As shown in Fig. 3B, adenosine (50 μ M) failed to suppress bursting activity in the presence of DPCPX, but did so following 30 min of DPCPX washout. The striking observation of these experiments was the persistence of the DPCPX-induced epileptiform activity occurring simultaneously with a recovery of the adenosine-induced hyperpolarization (Fig. 3B).

The present results confirm the existence of a powerful purinergic inhibitory tonus in area CA3 of the hippocampus [1] which appears to be exclusively mediated via A_1 -receptors. The selective blockade of A_1 -receptors induced epileptiform discharges in area CA3 resembling those observed following suppression of GABAergic inhibition [11] or elevation of the extracellular potassium concentration [3]. In contrast to the reversibility of both bicuculline's blockade of GABAergic inhibition [8] and high-potassium-induced epileptiform activity [3], the epileptogenic effects of DPCPX were found to be apparently irreversible. Since the hyperpolarizing action of adenosine recovered during washout of DPCPX, the irreversibility of the DPCPX-induced bursting activity was not due to the enduring presence of the antagonist in the tissue. This suggests that a transient and selective blockade of A_1 -receptors initiates, probably by modulation of a second-messenger system, a self-sustaining process leading to persistent epileptiform activity in area CA3.

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