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The low K_M-phosphodiesterase inhibitor denbufylline enhances neuronal excitability in guinea pig hippocampus in vitro

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Summary. The actions of the phosphodiesterase inhibitor denbufylline on the excitability of hippocampal neurons were investigated by means of extracellular and intracellular recordings. Denbufylline, which has been shown to selectively inhibit a low K_M, Ca^{2+}/calmodulin-independent phosphodiesterase isozyme, concentration-dependently increased the amplitude of the extracellularly recorded CA1 population spike evoked by electrical stimulation of the Schaffer collateral/commissural pathway. Concentration-response-curves yielded an EC_{50} for denbufylline of 0.76 μM. In comparison, the non-selective phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) also produced an increase in the amplitude of the population spike. From the concentration-response-curve, which was steeper than that of denbufylline, an EC_{50} for IBMX of 1.04 μM was obtained. However, despite their similar EC_{50} values, denbufylline was found to be significantly more potent at lower concentrations (≤ 300 nM) than IBMX.

Intracellular recordings from CA1 pyramidal cells revealed postsynaptic actions of denbufylline (300 nM) as indicated by a small drug-induced depolarization (2–5 mV) associated with an increase in membrane input resistance by 10–20%. In addition, denbufylline blocked the accommodation of trains of action potentials evoked by the injection of depolarizing current pulses.

The results suggest i) that accumulation of adenosine-3′,5′-monophosphate (cAMP) in the postsynaptic cell and/or in the presynaptic terminal produced by blockade of phosphodiesterases leads to enhanced synaptic transmission in the CA1 area of the hippocampus and ii) that a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase is an important component involved in the regulation of the intracellular cAMP level at synapses of central nervous system neurons.

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Key words: Hippocampal slice – CA1 cells – Phosphodiesterase – Denbufylline – IBMX – Population spike – Intracellular recordings

Introduction

Phosphodiesterases represent a family of several isozymes which are responsible for the inactivation of the intracellular second messenger adenosine-3′,5′-monophosphate (cAMP) (Beavo 1988). In the cerebral cortex, at least three different isozymes exist. One of them is the so-called low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase (Thompson and Appleman 1971; Strada et al. 1984; Nicholson and Wilke 1987). This isozyme is characterized by a high affinity for cAMP ($K_M = 2$ μM) and a low affinity for cyclic guanosine-3′,5′-monophosphate (cGMP, $K_M = 310$ μM). Furthermore, at low concentrations, cGMP does not inhibit the activity of this phosphodiesterase isozyme ($K_I = 530$ μM, Beavo 1988). The low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase is responsible for about 20–30% of total phosphodiesterase activity in brain tissue (Strada et al. 1984, Nicholson and Wilke 1987).

Many neurotransmitters modulate the activity of central nervous system neurons via changes in the intracellular concentration of the second messenger cAMP (Siggins and Gruol 1986). It might therefore be expected that the selective blockade of a phosphodiesterase isozyme involved in the termination of the action of the second messenger should lead to changes in neuronal excitability.

The alkylxanthine derivative denbufylline (1,3-di-n-butyl-7-(2′-oxopropyl)-xanthine) is a substance which has been shown to enhance oxygen- and glucose-consumption in animal models of cerebrovascular disease (Nicholson et al. 1987). During acute experimental ischemia, this compound increases the pO_2 in the cerebral cortex and prevents or retards the development of brain edema (Nicholson and Angersbach 1986). Furthermore,

denbufylline improves performance of animals during behavioral tests (Nicholson et al. 1988), enhances the pO_2 and function of partially ischemic skeletal muscle (Angersbach and Ochlich 1984), and decreases blood viscosity (Jukna and Nicholson 1987). In clinical studies, denbufylline was effective in the therapy of multi-infarct dementia (O'Connolly et al. 1986).

This broad spectrum of activity of denbufylline is most probably due to a pronounced inhibition of phosphodiesterase activity in brain, skeletal muscle and erythrocytes (Nicholson and Wilke 1986). Biochemical studies on partially purified isozymes derived from rat cerebral cortex revealed that denbufylline selectively blocks a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase, the K_I being 0.7 μM (Nicholson et al. 1989).

In the present study, denbufylline was used to investigate the involvement of a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase in the modulation of synaptic transmission in the CA1 area of guinea pig hippocampus.

Methods

Guinea pigs (200–300 g) were anesthetized with ether and decapitated. The brain was quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF). Following isolation of the hippocampus from each hemisphere, transverse slices (500 μm) were prepared using a vibratome (Vibroslice, Campden Instruments, London, UK) and stored in ACSF at room temperature for about 1 h. The slices were transferred to the recording chamber where they were kept submerged in ACSF consisting of (in mM): NaCl 118, KCl 3, NaH_2PO_4 1.2, $CaCl_2$ 2, $MgCl_2$ 1.3, D-glucose 11, $NaHCO_3$ 25. The solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 in order to obtain a pH value of 7.4 at a recording temperature of 31–32° C.

Extracellular recordings of stimulus-evoked population spikes in stratum pyramidale of area CA1 were made using glass microelectrodes filled with 4 M NaCl (electrode resistance 2–5 MOhms). For stimulation, a concentric bipolar stainless steel electrode (Rhodes Instruments) was positioned into the Schaffer collateral/commissural pathway. Stimulation was performed using pulses of 100–300 μs in duration delivered at a frequency of 0.05 Hz. Stimulus intensities ranged between 50 and 150 μA. Drug effects were investigated on population spikes evoked using half-maximal stimulus strength (half-maximal intensity = $(I_{max}-I_{min})/2+I_{min}$, where I_{min} is the intensity just subthreshold for the elicitation of a response and I_{max} is the intensity producing a maximum amplitude).

The extracellular signals were recorded and amplified by means of an Axoclamp 2 amplifier (Axon Instruments, Burlingame, CA, USA), averaged on-line (4–5 subsequent sweeps), and stored using a laboratory computer system (CED 1401, Cambridge Instruments, UK, in conjunction with a Tandon PCA 40). Hard copies of the digitized data were made using a HP 7470A plotter.

Intracellular recordings from pyramidal cells of area CA1 were obtained by means of glass microelectrodes filled with 3 M KCl (electrode resistance 50–90 MOhms). For amplification of the signals, an npi SEC 1L amplifier (npi, Tamm, FRG) was used. The membrane potential and the input resistance (determined by injection of hyperpolarizing current pulses of 0.2 or 0.3 nA at a frequency of 0.1 or 0.2 Hz) were continuously monitored on a chart recorder (Gould System Instruments, Cleveland, OH, USA). Action potentials and signals obtained during the measurements of current-voltage-relationships were digitized on-line and stored using the laboratory computer system mentioned above.

Denbufylline and 3-isobutyl-1-methylxanthine (IBMX) were applied by addition to the bathing solution. The drugs were dissolved in dimethyl sulfoxide (DMSO) to give stock solutions of 10 or 100 mM. These solutions were diluted with ACSF to reach final concentrations between 10 nM and 10 μM. Depending on the denbufylline or IBMX concentration applied, the final DMSO concentrations ranged between 0.0001% (10 nM denbufylline or IBMX) and 0.1% (10 μM denbufylline or IBMX). In control experiments ($n = 5$), the effects of 0.1% DMSO solutions on the amplitude of the population spike were tested. At this concentration, DMSO produced an insignificant increase in the population spike amplitude by 0.6 mV (control: 2.6 ± 0.4 mV (mean ± SD), 0.1% DMSO: 3.2 ± 0.4 mV, $p = 0.058$).

Denbufylline (BRL 30892) was kindly provided by Beecham-Wülfing (Gronau, FRG). IBMX was purchased from Sigma (Taufkirchen, FRG) and CNQX (6-cyano-7-nitroquinoxaline-2,3-dion) from Tocris Neuramin (UK).

Values are given as mean ± standard deviation (SD). Statistical evaluations were performed using the paired or unpaired, two-tailed Student's *t*-test. The amplitude of the population spike was determined from the negative peak to a tangent drawn between the first and the second maximum positivities.

Results

The actions of denbufylline and IBMX on the population spike recorded in area CA1 of the guinea pig hippocampus were investigated in 74 slices. In every experiment, the stimulus intensity necessary to produce a maximal population spike amplitude was determined by measuring an input-output-curve (Fig. 1 C, control). Only those recordings were included into the data analysis where electrical stimulation using maximal stimulus strength did not evoke a second population spike and where the amplitude of the population spike produced by maximal or half-maximal stimulation intensities was stable for a control period of at least 30 min.

Effects of denbufylline

In 51 out of 54 slices tested, denbufylline led to an enhancement of the amplitude of the orthodromically evoked population spike. Figure 1 A depicts an example of recordings of population spikes from one slice under control conditions and in the presence of increasing concentrations of denbufylline.

In order to investigate the concentration-response-relationship of this effect, we first determined the time required to attain the equilibrium level of denbufylline's action. In these experiments, the amplitude of the population spike evoked by half-maximal stimulus strength was monitored before, during, and 50–90 min after the application of denbufylline at a single concentration. As shown in Fig. 1 B, a slowly developing enhancement in the population spike amplitude was observed following addition of denbufylline (300 nM) to the bathing solution. An equilibrium level was reached within 40 min after the start of the denbufylline application. This slow time course was typical for the development of denbufylline's action. In all slices tested using a denbufylline concentration of 300 nM ($n = 5$), the maximum effect was attained within 40 min, independent of the duration of application (20–60 min). Despite prolonged washout of the compound in normal ACSF (up

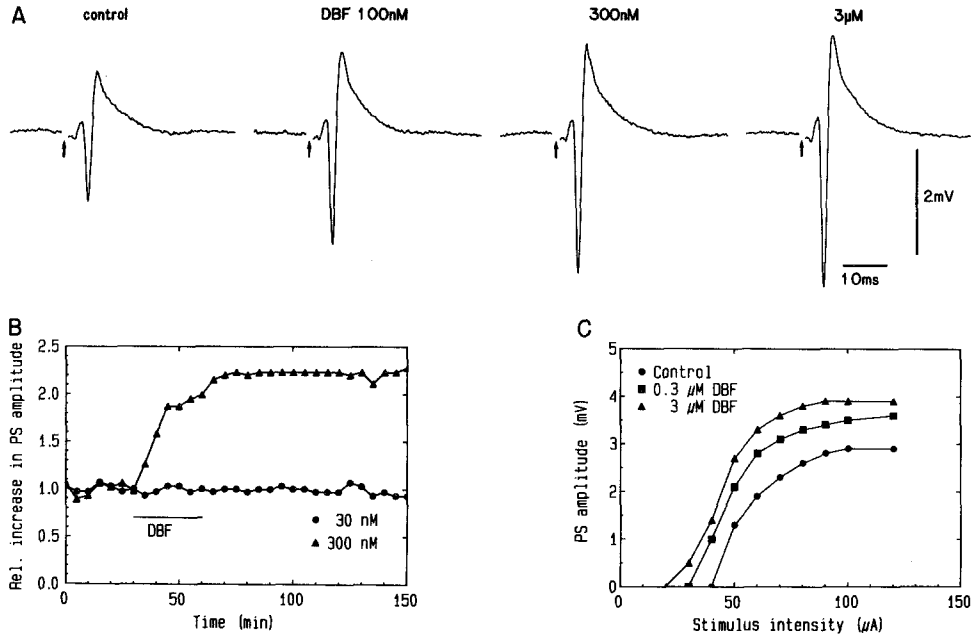

Fig. 1. Action of denbufylline (DBF) on the extracellularly recorded CA1 population spike (PS). **A**: Population spikes were evoked by electrical stimulation of the Schaffer collateral/commissural pathway (time of stimulation indicated by *arrows*). Different concentrations of denbufylline were cumulatively applied. Each trace represents the average of 4 subsequent responses. **B**: Time course of the action of denbufylline. The diagram depicts the measurements made in two different slices. Denbufylline was applied for 30 min (see bar) at the concentrations indicated and measurements of the population spike amplitude were made at 5 min intervals. For the purpose of comparison, the population spike amplitudes were normalized with respect to control. **C**: Effect of increasing concentrations of denbufylline on the input-output-curve. The amplitude of the population spike was measured as a function of the stimulus intensity in the absence (control) and presence of various concentrations of denbufylline. The data presented in this figure were obtained from four different slices

to 90 min), denbufylline's action on the population spike amplitude was found to be apparently irreversible (Fig. 1 B), even in those experiments where denbufylline was applied at low concentrations (30–100 nM). The possibility that this "apparent irreversibility" was due to a denbufylline-independent, progressive increase in the population spike amplitude could be excluded, since in experiments where denbufylline was without effect (3 out of 54 slices), a stable population spike amplitude could be recorded over a time range of 120–150 min (see Fig. 1 B). Furthermore, when the population spike amplitude had reached its maximum increase following the application of denbufylline at concentrations between 100 nM and 1 μM, it remained unchanged for the rest of the recording period (Fig. 1 B).

The concentration-response-relationship of denbufylline was examined by cumulative application of the drug. Population spikes were evoked using half-maximal stimulation intensities and increasing concentrations of denbufylline were applied. In order to attain a clear equilibrium level, each concentration was administered for 45 min before switching to a solution containing a higher denbufylline concentration. Concentration-response-curves in the concentration range between 30 nM and 10 μM were obtained from 5 slices (specimen recordings see Fig. 1 A). Due to the increasing influence of DMSO at denbufylline concentrations larger than 10 μM, the concentration-response-relationship was examined only up to 10 μM denbufylline. Following normalization of the population spike amplitudes with respect to control, the resulting values of the relative changes in population spike amplitudes were summarized and plotted as a function of the logarithm of the denbufylline concentration (Fig. 2, *circles*). The minimal effective concentration was found to be 30 nM. At this concentration, denbufylline led to an increase in the population spike amplitude by 10–20%. At lower concentrations (10 nM, $n = 3$), no detectable action of denbufylline was observed. In the concentration range tested (up to 10 μM), the concentration-response-curve did not reach a clear maximum. In order to determine an EC_{50} value, a sigmoid curve was fitted to the data points using non-linear least square regression analysis. The resulting curve was obtained on the assumption that denbufylline at a concentration of 1 nM does not produce any effect on the population spike amplitude. From this curve an apparent EC_{50} of 0.76 μM for denbufylline's action was calculated. This apparent EC_{50} is similar to the K_I value of denbufylline for the low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase (Nicholson et al. 1989).

In a next series of experiments, we investigated the influence of denbufylline on the input-output-relationship of the synaptic response in hippocampal slices. For this purpose, electrical stimuli of increasing intensity were applied and the amplitudes of the corresponding population spikes were measured in the absence and presence of various concentrations of denbufylline. The obtained values were plotted as a function of the stimulus intensity

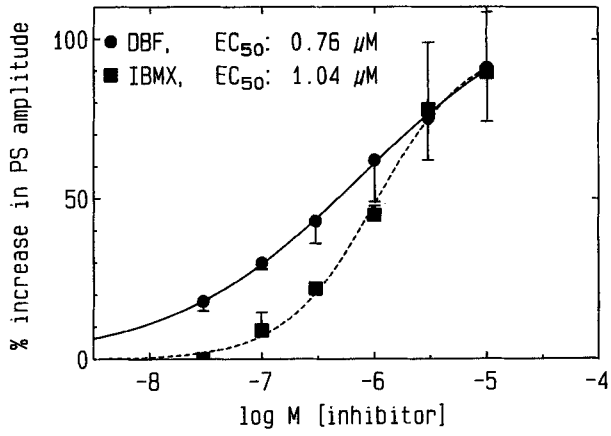

Fig. 2. Concentration-response-curves for denbufylline (DBF) (*solid line, error bars downward*) and IBMX (*dashed line, error bars upward*). For each curve, data were obtained from measurements performed in 5 and 4 different slices, respectively. The phosphodiesterase inhibitors were applied cumulatively at increasing concentrations. The amplitudes of the population spikes (PS) were normalized with respect to control and plotted as a function of the logarithm of the phosphodiesterase inhibitor concentrations (log M [inhibitor]). Data points (mean ± SD) were fitted by a sigmoid function and EC_{50} values were calculated (denbufylline: $r^2 = 1.00$, slope of the curve: 0.52; IBMX: $r^2 = 0.995$, slope of the curve: 1.08). In cases where data points are lacking error bars, the standard deviation is smaller than the size of the symbol

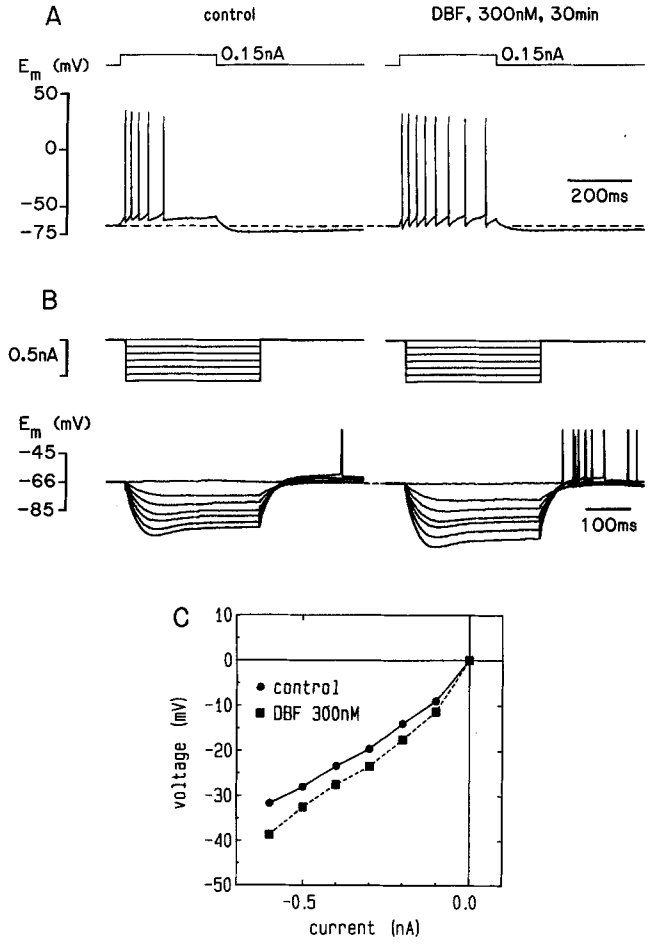

Fig. 3. Actions of denbufylline (DBF) on an intracellulary recorded CA1 pyramidal cell. **A**: Effects of denbufylline on direct evoked discharge of action potentials. A train of action potentials was evoked by the injection of a depolarizing current pulse (300 ms, 0.15 nA, top traces) in the absence (control) and presence of 300 nM denbufylline. *Dashed line* indicates the RMP (−66 mV) under control conditions. **B:** Effects of denbufylline on the current-voltage-relationship in the same cell. The current-voltage-curve was determined as described in the text. The consecutive recordings were superimposed (spikes were truncated by digitization). In this cell, denbufylline (300 nM) produced a depolarization by 3 mV. During the determination of the current-voltage-curve in the presence of denbufylline, the membrane potential was manually voltage-clamped to −66 mV. **C**: current-voltage-curves showing the relationship between injected current and measured voltage deviation in the absence (*circles*) and presence (*squares*) of denbufylline

(Fig. 1 C). The input-output-curves show that the population spike amplitude increased with stimulus strength until a maximum was attained (Fig. 1 C, control). In all slices tested ($n = 20$), denbufylline produced a concentration-dependent shift to left of the input-output-curve and an increase in its maximum (Fig. 1 C).

The maximum of the input-output-curve depends, inter alia, mainly on two factors: 1) on the excitability of afferent fibers, and 2) on the electrophysiological properties of the postsynaptic cells. The excitability of the afferent fibers innervating hippocampal CA1 neurons (Schaffer collateral/commissural pathway) is expressed by the amplitude and duration of the fiber volley preceding the population spike. Under normal conditions, this fiber volley partially overlaps with the synaptic field potential. In order to investigate the effects of denbufylline on the fiber volley in isolation, postsynaptic responses were blocked by the application of the selective quisqualate/kainate-receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dion (CNQX, 5 µM). Under these conditions, the fiber volley was clearly visible (see also Andreasen et al. 1989). The area of the fiber volley was measured and the control values were averaged and normalized to 1. In the presence of CNQX, denbufylline (300 nM) had no effect on the area of the fiber volley. The mean value was found to be 1.01 ± 0.03 ($n = 3$) which was not significantly different from 1. This finding indicates that the denbufylline-induced increase in the amplitude of the population spike and in the maximum of the input-output-curve, respectively, was not due to changes in the excitability of afferent fibers.

The second possibility which might influence the magnitude of synaptic responses, namely changes in electrophysiological properties of the postsynaptic cells, was investigated by means of intracellular recordings from CA1 pyramidal cells. The neurons had resting membrane potentials > −65 mV and input resistances > 60 MOhms. Following application of denbufylline (300 nM for 30 min), a slight depolarization (2 − 5 mV) was observed in all neurons tested ($n = 5$). In the presence of denbufylline, all neurons displayed an enhanced discharge of spontaneous action potentials associated with an increase in the frequency of small spontaneous synaptic potentials. The onset of the denbufylline-induced effects (10 − 20 min following start of application) was similar to that observed during extracellular recordings and

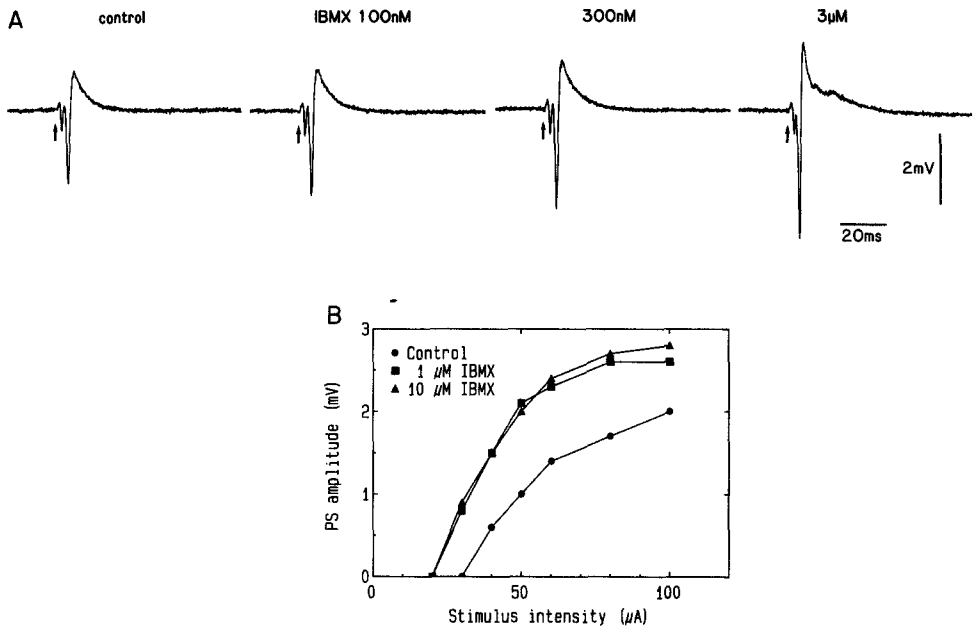

Fig. 4. Effects of IBMX on the CA1 population spike amplitude. **A**: population spikes were evoked at half-maximal stimulation intensities and IBMX was applied cumulatively at increasing concentrations. Application time of each concentration was 30 min. Each trace is the average of 4 subsequent events. *Arrows* indicate the time of stimulation. **B**: Determination of input-output-curves in the absence (*circles*) and presence of IBMX (*squares*: 1 μM; *triangles*: 10 μM). Recordings in **A** and results in **B** were obtained from two different slices

these effects were apparently irreversible within the observation period (up to 3 h). In Fig. 3A, the actions of denbufylline on the direct evoked action potential discharge is shown. Under control conditions (Fig. 3A, left-hand side), a depolarizing current pulse (0.15 nA, 300 ms) induced a train of action potentials only within the first 150 ms of the pulse (accommodation). At a concentration of 300 nM (Fig. 3A, right-hand side), denbufylline blocked this accommodation and a spike train was observed throughout the pulse. The effect of denbufylline on the neurons' current-voltage-relationship was investigated by injecting a series of hyperpolarizing current pulses of various amplitudes and measuring the corresponding voltage deviations in the absence and presence of denbufylline (Fig. 3B). The measured values were then plotted as a function of the current strength injected (Fig. 3C). For the purpose of comparison, denbufylline-induced depolarizations were compensated for by injection of hyperpolarizing direct currents. In the presence of denbufylline, an increase in the slope of the current-voltage-curve was observed in all neurons tested ($n = 3$, Fig. 3C). However, denbufylline did not influence the rectifying behaviour of the membrane. A rectification ratio was calculated by dividing the value of the input resistance determined using an 0.1 nA current pulse by the value obtained following the injection of a 0.6 nA pulse. Under control conditions, the mean value of the cell shown in Fig. 3 was found to be 2.07, indicating inward rectification. Denbufylline had no effect on this rectification ratio (2.03). Similar results were obtained from another two cells. In all neurons tested, an overall increase in input resistance by 10–20% was observed.

Effects of IBMX

The actions of the non-selective phosphodiesterase inhibitor IBMX were studied in 6 experiments. Qualitatively, the effects of IBMX on neuronal excitability were similar to those of denbufylline: upon application of IBMX (100 nM – 10 μM), the amplitude of the population spike concentration-dependently increased (Fig. 4A), the input-output-curve shifted to the left and an increase in the maximum of the input-output-curve was observed in all slices tested (Fig. 4B). Furthermore, IBMX did not influence the amplitude and duration of the fiber volley. Comparable to denbufylline, the IBMX-induced effects developed over a period of 15–25 min and were apparently irreversible following washout of the compound. However, there were two differences which distinguished the action of IBMX from that of denbufylline. First, at higher concentrations (> 1 μM), IBMX led to the occurrence of a second population spike (Fig. 4A, 3 μM) and sometimes (in 2 out of 6 slices) to epileptiform afterdischarges. Second, lower concentrations of IBMX (< 1 μM) were significantly less potent than those of denbufylline. In 4 slices, the concentration-response-relationship for IBMX was determined using a similar paradigm as with denbufylline (Fig. 2, *squares*). The minimal effective IBMX concentration was found to be 100 nM. On the assumption that 10 nM IBMX did not produce any effect on the population spike amplitude, a sigmoid curve was fitted to the data points. From this curve, an EC_{50} of 1.04 μM for IBMX was calculated. The comparison of the concentration-response-curves in Fig. 2 reveals that 300 nM IBMX enhanced the popu-

lation spike amplitude by $21.9 \pm 2.1\%$ ($n = 4$), whereas, at the same concentration, denbufylline caused an increase by $43.8 \pm 6.3\%$ ($n = 5$, Fig. 2). The difference between these mean values is significant at a level of $p < 0.005$. In the higher concentration range (> 1 µM), both compounds seem to be equally potent (Fig. 2).

Discussion

Our results demonstrate that the alkylxanthine derivative denbufylline produces a considerable increase in excitability of hippocampal neurons in vitro. Denbufylline has been shown to be a potent blocker of cAMP-phosphodiesterase activity in brain (Nicholson et al. 1989). Therefore, the most important question is, whether the action of denbufylline on neuronal excitability can be ascribed exclusively to an increase in the intracellular cAMP concentration induced by depression of phosphodiesterase activity. There are several experimental observations providing indirect evidence that denbufylline acted via an increase in the intracellular cAMP concentration: 1) Nicholson et al. (1989) demonstrated that denbufylline selectively blocks a phosphodiesterase isozyme which was isolated from rat cerebral cortex. This isozyme was characterized by a high affinity for cAMP (K_M: 2 µM) and a low affinity for cGMP (K_M: 50 µM). The activity of this isozyme was not inhibited by cGMP and was independent of the presence of Ca^{2+} and calmodulin. According to Beavo (1988), this isozyme can be classified as a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase. 2) Using radiolabelled rolipram, which is another selective blocker of the low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase (Davis 1984), Kaulen et al. (1989) revealed a high density of rolipram binding sites in the CA1 subfield of the rodent hippocampus. These binding sites were most probably identical to low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterases. Thus, in the hippocampus, the presence of this isozyme has been indirectly demonstrated. 3) Challiss and Nicholson (1988) showed that denbufylline enhances both the basal and isoproterenol-stimulated intracellular cAMP concentration in rat neocortical slices.

From this evidence, it is legitimate to assume that, in our experiments, denbufylline led to an increase in intracellular cAMP concentration in hippocampal slices by blockade of a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase. However, similar to all other alkylxanthine derivatives, denbufylline in addition binds to adenosine receptors and adenosine re-uptake sites (Nicholson et al. 1989). Therefore, it might be possible that denbufylline produces an increase in the population spike amplitude by antagonizing a purinergic inhibitory tonus present in hippocampal slices (Haas and Greene 1988). This possibility can be excluded on the basis of the following reasons: 1) when compared to its inhibition constant for the low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase, the K_I values of denbufylline at adenosine A_1 and A_2 receptors, respectively, as well as the adenosine re-uptake site have been shown to be significantly higher (K_I for the phosphodiesterase: 0.7 µM; K_I for A_1 receptors: 20 µM; K_I for A_2 receptors: 46 µM; K_I for the adenosine re-uptake site: 200 µM, Nicholson et al. 1989). In our study, the EC_{50} of the action of denbufylline on the population spike amplitude was almost similar to the K_I of denbufylline for the phosphodiesterase isozyme (i.e. 25 times smaller than the K_I of denbufylline at the adenosine A_1 receptor) and the minimal effective denbufylline concentration was found to be 30 nM (i.e. 670 times smaller than the K_I of denbufylline at the adenosine A_1 receptor); 2) the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX) often evokes epileptiform discharges in the CA1 subfield of the hippocampus (see Alzheimer et al. 1989), whereas denbufylline never produced such discharges, even when applied at high concentrations (10 µM). Taken together, the significant difference between the effective denbufylline concentrations observed in our experiments and the K_I values of denbufylline at adenosine receptors on the one hand, and the lack of "DPCPX-like" actions of denbufylline on the other hand strongly indicates that denbufylline did not produce its effects by antagonizing an adenosine A_1 receptor-mediated inhibitory tonus. Therefore, we conclude that denbufylline increased the efficacy of synaptic transmission in hippocampal slices by an intracellular accumulation of cAMP produced by a selective blockade of a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase.

When compared to denbufylline, the actions of the non-selective phosphodiesterase inhibitor IBMX were qualitatively similar (increase in the population spike amplitude with a similar time course). The main difference between both phosphodiesterase inhibitors was a significantly greater potency of denbufylline in the lower concentration range (≤ 300 nM). At higher concentrations (≥ 1 µM), both compounds were found to be equipotent (see Fig. 2). However, in contrast to denbufylline, we have to assume that the actions of IBMX on neuronal excitability consisted of both, a blockade of phosphodiesterase activity and a disinhibitory action via blockade of adenosine A_1 receptors. The EC_{50} value determined in our experiments (1.04 µM) is very similar to the K_I value of IBMX at adenosine A_1 receptors (1.8 µM, Nicholson et al. 1989) and significantly smaller than the K_I values of IBMX at different phosphodiesterase isozymes (8–26 µM, Davis 1984). Furthermore, at higher concentrations (≥ 1 µM), IBMX tended to produce epileptiform activity, an effect which might be explained by an antagonistic action of IBMX at adenosine A_1 receptors (Okada and Ozawa 1980). Thus, the concentration-response-curve for IBMX shown in Fig. 2 probably reflects a superimposition of two different effects of IBMX. Consequently, it is not possible to compare the inhibitory efficacy of denbufylline and IBMX on phosphodiesterase activity on the basis of the concentration-response-curves determined in our experiments.

The time course of the development of the denbufylline-induced effects agreed well with results from biochemical studies on cortical slices (Challiss and Nicholson 1988), which demonstrated that maximum cAMP levels are achieved within 10–20 min following

the application of phosphodiesterase inhibitors (denbufylline and IBMX, respectively). However, in contrast to these investigations, the electrophysiologically determined actions of denbufylline were found to be irreversible within the observation period. A possible explanation for this discrepancy is that the initial rise in the intracellular cAMP concentration produced by denbufylline triggered a cascade of processes leading to sustained changes in neuronal excitability. An analogous phenomenon was observed in hippocampal CA3 pyramidal cells following application of DPCPX (Alzheimer et al. 1989). DPCPX induced sustained epileptiform discharges, which might be triggered by a transient increase in the intracellular cAMP concentration due to blockade of adenosine A_1 receptors.

From our results, it is not possible to decide, whether denbufylline changes synaptic transmission in the hippocampus by a presynaptic or postsynaptic mechanism. Intracellular recordings revealed that denbufylline changed the electrophysiological properties of CA1 pyramidal cells in a way similar to noradrenaline acting via β_1-adrenergic receptors (Madison and Nicoll 1986a). The block of accommodation associated with a small depolarization and an increase in input resistance, suggests an inhibitory effect of denbufylline on a calcium-activated potassium conductance, which would tend to amplify the amplitude of synaptic responses. In fact, Madison and Nicoll (1986b) demonstrated that the selective inhibitor of a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase, Ro 20-1724 (Beavo 1988) depresses a calcium-activated potassium conductance resulting in a blockade of spike train accommodation. However, although we could show that denbufylline does not influence the excitability of afferent fibers, an action of denbufylline on the release of excitatory transmitters cannot be excluded. An increase in the intracellular cAMP concentration in presynaptic terminals might facilitate transmitter release by changing the degree of phosphorylation of phosphoproteins involved in this process (e.g. synapsin I, see Sihra et al. 1989). Those changes are not reflected in the amplitude of the fiber volley.

Based on the selectivity of denbufylline for a low K_M, Ca^{2+}/calmodulin-independent cAMP-phosphodiesterase, our results suggest that this isozyme might play an important role in the modulation of synaptic transmission in the hippocampus. Similar findings were obtained from rat neocortical slices, where denbufylline facilitates synaptic transmission (Sutor et al. 1989). The importance of phosphodiesterases in synaptic transmission is further substantiated by two other observations: 1) using cytochemical techniques, Florendo et al. (1971) demonstrated that phosphodiesterase activity is predominantly located in the postsynaptic density of rat cortical neurons; 2) following selective destruction of the inferior olive, a marked reduction in calmodulin-dependent phosphodiesterase immunoreactivity was observed in rat cerebellar Purkinje cells, suggesting a transsynaptic regulation of phosphodiesterase activity (Balaban et al. 1989).

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