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# Physiology, Pharmacology and Development of Epileptogenic Phenomena

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# CONTENTS

# Excitability Changes Induced in Rat Neocortical Neurons by the Selective Blockade of a Low $K_m$, $Ca^{2+}$/Calmodulin - Independent cAMP - Phosphodiesterase

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*Introduction*

By analogy with the effects of neurotransmitters, the action of the intracellular second messenger cyclic adenosine-3',5'-monophosphate (cAMP) has to be terminated by an efficient and effectively regulated inactivation mechanism. Cyclic AMP is degraded by cAMP - phosphodiesterases (cAMP - PDE). Thus, the regulation of the activity of cAMP - PDE may be of crucial importance for normal neuronal excitability as well as for the development of abnormal neuronal behavior, including epilepsies. PDEs are a family of isozymes differing in their biochemical and pharmacological properties (Beavo 1988). The availability of selective inhibitors for a low $K_m$, $Ca^{2+}$/calmodulin-independent isozyme (e.g., denbufylline, see Nicholson et al. 1989) allows one to study the influence of this cAMP - PDE isozyme on the excitability of rat neocortical neurons *in vitro*.

*Methods*

Coronal slices (500 µm) were prepared from the frontal cortex of male Wistar rats (120 - 160 g). In the recording chamber, the slices were kept submerged in artificial CSF consisting of (in mM): 124 NaCl, 3 KCl, 1.25 $NaH_2PO_4$, 2.0.$CaCl_2$, 1.3 $MgCl_2$, 25 $NaHCO_3$, and 10 glucose (continuously gassed with 5% $CO_2$ in $O_2$; pH 7.4 at 32°C). Intracellular recordings were made from superficially located neurons (layer II and III) by means of glass microelectrodes filled with either 4 M potassium acetate (pH 7.2) or 3 M KCl. Postsynaptic potentials were evoked by electrical stimulation using a bipolar silver electrode positioned in cortical layer IV. Stimuli were applied at a frequency of 0.1 Hz. In the present study, the stimulus intensities (30 - 300 µA) are given as multiples of the threshold intensity (T) necessary to evoke an action potential. Denbufylline (DBF) was dissolved in dimethyl sulfoxide (DMSO) at concentrations of $10^{-2}$ M. From this stock solution, appropriate amounts were added to the bathing solution in order to obtain the desired drug concentration. The final solvent concentration never exceeded 0.1%. At this concentration, DMSO did not affect the neuronal electrophysiological properties.

*Results*

The results are based on intracellular recordings from 25 cortical neurons with a mean resting potential of -79.6 ± 3.2 mV (mean ± SD) and a mean input resistance of 26.5 ± 2.3 MΩ. When added to the bathing solution at concentrations between 10 and 100 nM, DBF increased the amplitude of excitatory postsynaptic potentials (EPSPs). Figure 1 depicts an example of DBF-induced changes of EPSPs. At low stimulus intensities (0.4 T, Fig. 1A), this neuron responded with an early EPSP (eEPSP, control trace). Following the application of DBF (100 nM, center trace), the amplitude of the eEPSP increased and a late EPSP (lEPSP) occurred. Upon an increase in stimulus strength to 0.5 T (Fig. 1B), an eEPSP followed by a small lEPSP was observed (see Sutor and Hablitz 1989). Again, DBF (100 nM) produced an enhancement in the amplitude of both EPSPs and, in addition, a decrease in the latency of the lEPSP (center trace). These DBF-induced

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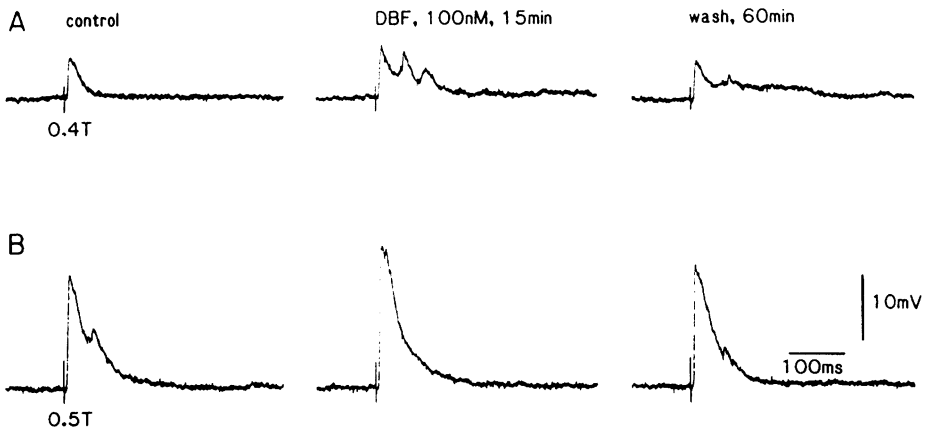


**Fig. 1A,B.** Actions of DBF (100 nM) on EPSPs in rat neocortical neurons. The resting potential was -85 mV. EPSPs were evoked by electrical stimulation of cortical layer IV using different stimulus strength (**A** and **B**). The time point of stimulation is indicated by the stimulus artifact. Stimulus intensities are given as multiples of 1 T

changes of EPSPs were reversible upon washout of the drug (Fig. 1A and B, right traces). At these low concentrations (10 - 100 nM) no significant DBF-induced effects on inhibitory postsynaptic potentials (IPSPs) were observed. However, at higher concentrations (1 - 10μM), DBF produced a marked prolongation of GABAergic, $Cl^-$-dependent IPSPs (Fig. 2). At a stimulus strength of 0.5 T (Fig. 2A), an eEPSP was observed (control trace), the amplitude of which was enhanced following the application of DBF (10 μM). Stimulation with an intensity of 0.9 T (Fig. 2B) evoked a sequence of postsynaptic potentials consisting of an EPSP followed by a depolarizing, $Cl^-$-dependent IPSP and a small hyperpolarizing, $K^+$-dependent IPSP (control trace, see Howe et al. 1987). In the presence of DBF (10 μM), both the amplitude of the EPSP and the duration of the depolarizing IPSP increased (center trace; see also superimposed traces). At a stimulus intensity of 1 T (Fig. 2C), the DBF-induced effects were similar to those observed at 0.9 T. The action of DBF on the depolarizing IPSP was reversible upon washout of the drug.

DBF affected neither the membrane potential nor the input resistance (measured with hyperpolarizing current pulses). In the depolarizing direction, a slight enhancement of the steady state inward rectification (see Sutor and Zieglgänsberger 1987) was observed. The latter effect is probably responsible for the DBF-induced increase in direct excitability.

*Discussion*

These experiments demonstrate a concentration-dependent effects of the selective cAMP - PDE inhibitor, DBF, on synaptic potentials in rat neocortical neurons. At low concentrations, DBF facilitates excitatory synaptic transmission without affecting inhibitory transmission. At higher concentrations, DBF predominantly prolongs the duration of GABAergic, $Cl^-$-dependent IPSPs. In this context, two other observations are of interest: (1) In cytochemical studies, we were able to show that the cAMP - PDE activity is predominantly located in the postsynaptic density of axospineous or axodendritic synapses of cortical neurons (see also Florendo et al. 1971) and (2) by measuring the effects of DBF on the total PDE activity in rat frontal cortex we found that at concentrations which effectively facilitate synaptic transmission (up to 10 μM), only a maximum of 10% of the total PDE activity was blocked. These results suggest that the low $K_m$, $Ca^{2+}$/calmodulin-independent cAMP - PDE might be of enormous importance for synaptic transmission in the neocortex. The ultrastructural localization of most of the PDE activity and the

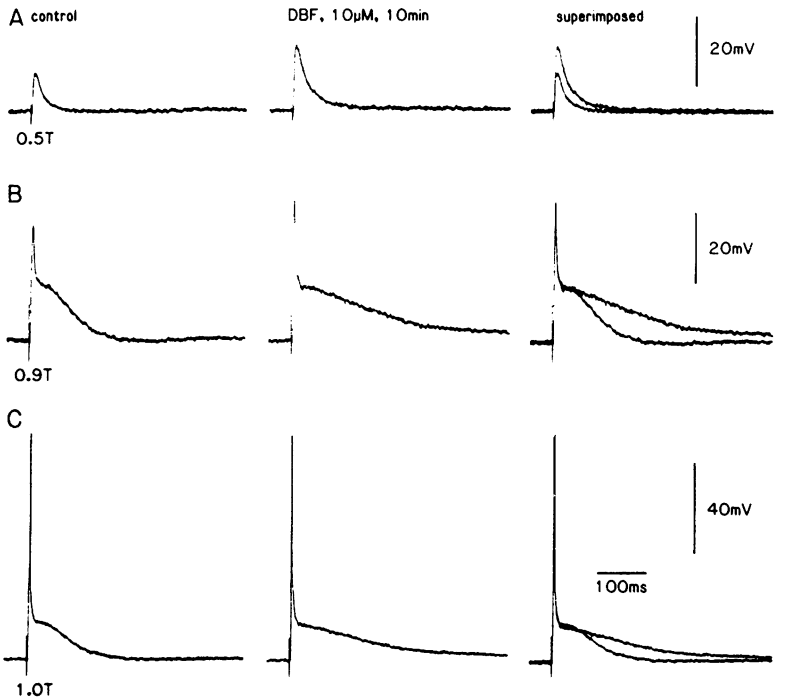


**Fig. 2A-C.** Actions of DBF (10 μM) on IPSPs in rat neocortical neurons. The resting potential was -81 mV. **A** At an intensity of 0.5 T, the stimulus produced an EPSP (control) the amplitude of which increased upon application of DBF. In this and the following two panels, the single recordings were superimposed and are shown on the right-hand side of the figure. **B** At a just subthreshold stimulus intensity (0.9 T), the stimulus evoked an EPSP/IPSP sequence. GABA-mediated, $Cl^-$-dependent IPSPs display a depolarizing time course in rat neocortical neurons in vitro. **C** At 1.0 T, the stimulus induced a slowly decaying depolarizing IPSP following the synaptically evoked action potential. Note the enhancing effect of DBF on the IPSP duration

observation that the inhibition of only a small fraction of the total PDE activity leads to marked facilitation of synaptic transmission suggest that the low $K_m$ cAMP - PDE is located at a "strategically" significant site and that this isozyme might be the determining factor in the regulation of intracellular cAMP concentration in the synaptic region.

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