The Increase in Cl⁻ Permeation Across the Deiters’ Neuron Membrane by GABA on Its Cytoplasmic Side Is Abolished by Protein Kinase C (PKC) Activators

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SUMMARY

1. Cl⁻ ion outward permeation across microdissected Deiters’ neuron plasma membranes is augmented by GABA on the membrane cytoplasmic side. When these neurons are preincubated with a PKC activator, phorbol-12,13-dibutyrate (PdBu), there is a complex pattern of effects on basal and GABA-activated ³⁶Cl⁻ in→out permeation. A distinct fact is an increase in basal Cl⁻ passage and a disappearance of the 10⁻⁶ M GABA effect at [PdBu] = 0.1 µM.

2. Likewise, 0.1 µM oleylacylglycerol (OAG) treatment erases the effect completely, further supporting a role for PKC in modulating GABA-stimulated Cl⁻ in → out permeation.

3. The inactive ester, phorbol-12,13-didecanoate (Pdd), at 0.1 µM, does not affect GABA stimulation of Cl⁻ passage.

4. High concentrations (15–20 µM) of OAG and PdBu block the “intracellular” GABA effect. However, the 20 µM PdBu effect is reversed by 30 µM H7.

5. These results indicate a role of endogenous PKC in Cl⁻ extrusion by GABA_A receptors on the cytoplasmic side of the Deiters’ neuron membrane.

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INTRODUCTION

A micromethod allowing study of the permeation of Cl⁻ ions across single neuronal membranes has been applied by our group to the investigation of GABA receptors on the Deiters' vestibular neurons in the rabbit. As implied by classical neurophysiological experiments in the cat (Obata et al., 1967, 1970), we found GABA A receptors on the extracellular side of the Deiters' neuron membrane (Hydén et al., 1986).

However, further experiments have yielded the unexpected result that GABA could stimulate Cl⁻ in→out permeation when it was present on the membrane cytoplasmic side. Moreover, we could show that the cytoplasmic side GABA effect was reversed by the classical GABA A antagonists, bicuculline and picrotoxin. These data led us to the conclusion that structures which behave like GABA A receptors are present on the Deiters' neuron membrane cytoplasmic side (Hydén et al., 1987).

These indications were confirmed in many further studies (Rapallino et al., 1988a,b, 1989a,b, 1990). On the basis of the specific characteristics of the mechanism of Cl⁻ permeation increase by GABA, we proposed that such GABA A receptors intervene in an intracellular GABA-operated process for Cl⁻ extrusion from the Deiters' neurons (Cupello et al., 1991). This process would create an electrochemical gradient for Cl⁻ influx under the action of synaptically released GABA. The existence of such a gradient is implied by the hyperpolarizing nature of the GABA-mediated intracellular postsynaptic potentials (IPSPs) in these neurons in the cat (Ito et al., 1966; Obata et al., 1967). Most importantly for our proposal, hyperpolarizing IPSPs have been demonstrated in Deiters' nucleus neurons in the rabbit as well (Akaike et al., 1973).

Within the proposal for the role of cytoplasmic side GABA A receptors, we hypothesized a phosphorylation step which ultimately would provide the biochemical energy for Cl⁻ extrusion against an electrochemical gradient.

In particular, we suggested that phosphorylation intervenes in a desensitization step for cytoplasmic side GABA A receptors. In this process extra Cl⁻ ions would be trapped in the desensitized closed Cl⁻ channel and allowed only to exit toward the cell's outside (Cupello et al., 1991; Hydén et al., 1991).

We report herein evidence that activation of a protein kinase (PKC) results in the block of Cl⁻ channels activated by GABA on the Deiters' membrane cytoplasmic side.

METHODS

In these experiments, adult male rabbits weighing about 2.5 kg were used. From the animals, single Deiters' nerve membranes were prepared by the microdissection procedure described in detail previously (Hydén et al., 1986; Hydén and Cupello, 1987). The membranes were freed from synaptic knobs on their external surface due to previous exposure of the isolated cells to isotonic sucrose as already demonstrated (Figs. 1 and 2) (Hydén and Cupello, 1987).