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$GABA_B$ Receptor Activation Inhibits Neuronal Excitability and Spatial Learning in the Entorhinal Cortex by Activating TREK-2 K^+ Channels

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SUMMARY

The entorhinal cortex (EC) is regarded as the gateway to the hippocampus and thus is essential for learning and memory. Whereas the EC expresses a high density of $GABA_B$ receptors, the functions of these receptors in this region remain unexplored. Here, we examined the effects of $GABA_B$ receptor activation on neuronal excitability in the EC and spatial learning. Application of baclofen, a specific $GABA_B$ receptor agonist, inhibited significantly neuronal excitability in the EC. $GABA_B$ receptor-mediated inhibition in the EC was mediated via activating TREK-2, a type of two-pore domain K^+ channels, and required the functions of inhibitory G proteins and protein kinase A pathway. Depression of neuronal excitability in the EC underlies $GABA_B$ receptor-mediated inhibition of spatial learning as assessed by Morris water maze. Our study indicates that $GABA_B$ receptors exert a tight control over spatial learning by modulating neuronal excitability in the EC.

INTRODUCTION

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system, where it acts at ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors. Whereas the functions of $GABA_A$ and $GABA_C$ receptors are to mediate fast inhibitory synaptic transmission, stimulation of $GABA_B$ receptors ($GABA_B$Rs) modulates synaptic function via G proteins and intracellular signals (Couve et al., 2000). $GABA_B$Rs consist of heterodimers of $GABA_{B1}$ and $GABA_{B2}$ subunits that are coupled to inhibitory G proteins ($G\alpha_i$ and $G\alpha_o$), which inhibit adenylyl cyclase (AC), resulting in a reduction in cyclic AMP (cAMP) generation and an inhibition of protein kinase A (PKA) (Couve et al., 2000). $GABA_B$Rs play important modulatory roles in cognition, nociception, neuroprotection, depression, addiction, and epilepsy (Couve et al., 2000).

Anatomically, the entorhinal cortex (EC) mediates the majority of the connections between the hippocampus and other cortical areas (Witter et al., 1989, 2000a). Sensory inputs converge onto the superficial layers (layers II–III) of the EC (Burwell, 2000), which give rise to dense projections to the hippocampus; the axons of the stellate neurons in layer II of the EC form the perforant path that innervates the dentate gyrus and CA3 (Steward and Scoville, 1976), whereas those of the pyramidal neurons in layer III form the temporoammonic pathway that synapses onto the distal dendrites of pyramidal neurons in CA1 and the subiculum (Steward and Scoville, 1976; Witter et al., 2000a, 2000b). Moreover, neurons in the deep layers of the EC (layers V–VI) relay a large portion of hippocampal output projections back to the superficial layers of the EC (Dolorfo and Amaral, 1998a, 1998b; Kohler, 1986; van Haeften et al., 2003) and to other cortical areas (Witter et al., 1989). The EC is part of a network that aids in the consolidation and recall of memories (Haist et al., 2001; Squire et al., 2004; Steffenach et al., 2005). Neuronal pathology and atrophy of the EC are potential contributors to Alzheimer's disease (Hyman et al., 1984; Kotzbauer et al., 2001) and schizophrenia (Falkai et al., 1988; Prasad et al., 2004). Furthermore, the EC participates in the induction and maintenance of temporal lobe epilepsy (Avoli et al., 2002; Spencer and Spencer, 1994).

$GABA_B$Rs are densely expressed in the principal cells, especially the stellate neurons, of the EC (Mizukami et al., 2002). However, the functions of $GABA_B$Rs in this brain region remain unexplored. Because $GABA_B$Rs and the EC are closely associated with cognitive function in the brain, we tested the hypothesis that $GABA_B$Rs expressed in the EC modulate neuronal excitability and participate in learning and memory. Our results demonstrate that $GABA_B$R activation drastically inhibited neuronal excitability in the superficial layers of the EC via activation of TREK-2, a type of two-pore domain K^+ (K_{2P}) channels. We also found that the functions of pertussis toxin (PTX)-sensitive G proteins, PKA and A-kinase anchoring proteins (AKAPs) are necessary for $GABA_B$R-mediated inhibition in the EC. Microinjection of the $GABA_B$R agonist, baclofen, and Rp-cAMPS, a specific PKA inhibitor, into the EC of rats prevented spatial learning whereas downregulation of TREK-2 channels using siRNA significantly reduced the effect of baclofen on spatial learning suggesting that $GABA_B$R-mediated inhibition of neuronal excitability contributes significantly to spatial learning.

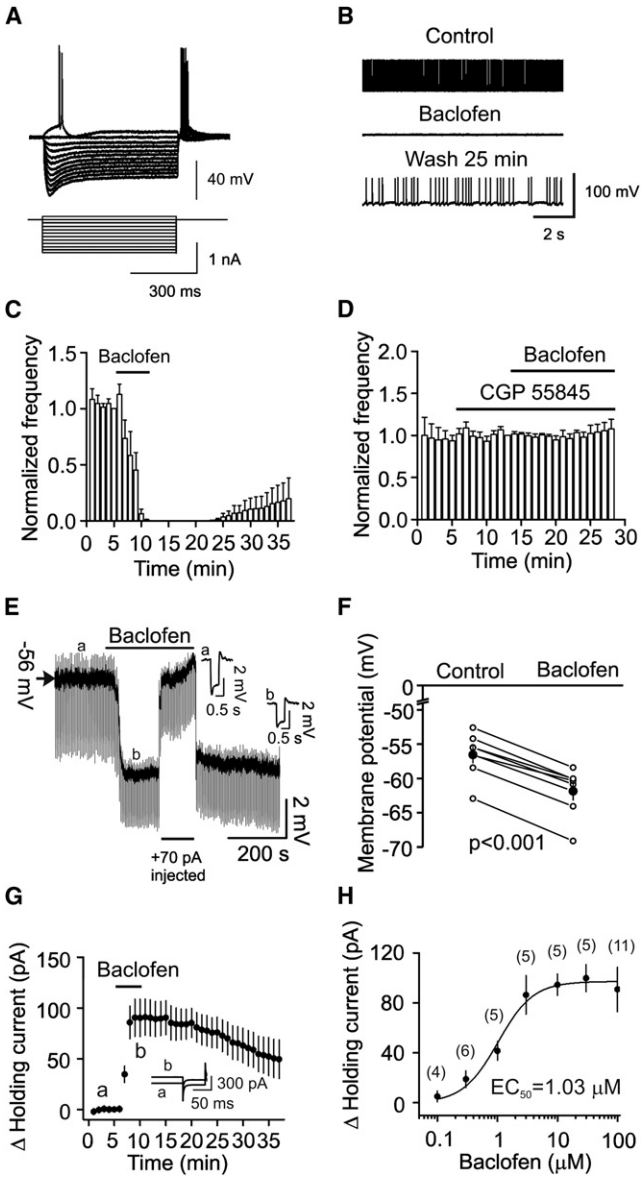

Figure 1. Baclofen Reduces the Excitability of Stellate Neurons by Generating Membrane Hyperpolarization

(A) Voltage responses (upper panel) generated by current injection from +0.1 nA to −1 nA at an interval of −0.1 nA (lower panel) recorded from a stellate neuron in layer II. Note the depolarizing voltage sags in response to hyperpolarizing current pulses.

(B) AP firing recorded prior to, during, and after application of baclofen from the stellate neuron in A.

(C) Pooled time course of AP firing frequency before, during, and after application of baclofen.

(D) Baclofen-mediated inhibition of AP firing was blocked in the presence of the GABA$_B$R blocker, CGP 55845.

(E) Baclofen generated membrane hyperpolarization and reduced input resistance. A negative current (−50 pA for 500 ms) was injected every 5 s to assess the changes of input resistance. Insets are the voltage traces taken before (a) and during (b) the application of baclofen. Note that baclofen induced membrane hyperpolarization and reduced the voltage responses induced by the negative current injections, suggesting a reduction in input resistance. To exclude the influence of baclofen-induced membrane hyperpolarization on input resistance, a constant positive current (+70 pA indicated by the horizontal bar) was injected briefly to elevate the membrane potential to the initial level. Under these conditions, the voltage responses induced by the negative current injections (−50 pA) were still smaller compared with control, suggesting that baclofen-induced reduction in input resistance is not secondary to its effect on membrane hyperpolarization.

(F) Summarized data for baclofen-induced changes in RMPs. Filled circles denote the averaged values.

(G) Baclofen induced an outward HC. HCs were averaged per min and zeroed to the level just prior to the application of baclofen. Inset shows the averaged HCs recorded at the time points denoted in the figure. A −5 mV hyperpolarizing voltage step was used at the end of each trace to monitor potential changes of series resistance during recordings.

(H) Concentration-response curve for baclofen-induced changes in HCs. Numbers in the parenthesis were number of cells recorded. Data represent means ± SEM.

RESULTS

GABA$_B$R Activation Inhibits Action Potential Firing in the EC

GABA$_B$Rs are densely expressed in the EC, especially in the stellate neurons of the superficial layers (Mizukami et al., 2002). Accordingly, we examined first the effects of GABA$_B$R activation on the excitability of stellate neurons. The extracellular solution contained DNQX (10 μM), dl-APV (50 μM), and bicuculline (10 μM) to block synaptic transmission. Stellate neurons were differentiated by their location, morphology and electrophysiology properties (Deng et al., 2007). Figure 1A shows the current-voltage responses from a stellate neuron in layer II of the EC. This neuron showed profound depolarizing voltage sags in response to hyperpolarizing current pulses (Deng et al., 2007). Application of baclofen (100 μM), a specific GABA$_B$R agonist, completely blocked action potential (AP) firing within 5–6 min of application ($n = 6$, $p < 0.001$, Figures 1B and 1C). AP firing frequency recovered partially after wash for ~25 min (Figure 1C). The effect of baclofen was mediated by GABA$_B$Rs because application of CGP 55845 (2 μM), a specific GABA$_B$R inhibitor, completely blocked baclofen-induced inhibition of AP firing (101% ± 2% of control, $n = 7$, $p = 0.76$, Figure 1D). These results indicate that GABA$_B$R activation drastically inhibits neuronal excitability in the EC.

GABA$_B$R Activation Generates Hyperpolarization

Inhibition of AP firing could be attributable to GABA$_B$R-induced membrane hyperpolarization. We therefore recorded the resting membrane potentials (RMPs) in current-clamp in the presence of TTX (0.5 μM) to block potential indirect effects from synaptic transmission. A negative current (−50 pA for 500 ms) was injected every 5 s to assess the changes of input resistance. Application of baclofen (100 μM) generated membrane hyperpolarization (control: −56.5 ± 1.3 mV, baclofen: −61.8 ± 1.4 mV, $n = 7$, $p < 0.001$, Figures 1E and 1F) and reduced the input resistance (control: 98.3 ± 13.1 MΩ, baclfen: 75.0 ± 7.7 MΩ, $n = 7$, $p < 0.006$, Figure 1E), suggesting that GABA$_B$R activation increases membrane conductance. We then recorded the holding currents (HCs) in voltage-clamp at −60 mV, a potential close to the RMPs. Under these conditions, application of baclofen (100 μM) generated an outward HC. The maximal effect usually occurred

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