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Neurosteroid paradoxical enhancement of paired-pulse inhibition through paired-pulse facilitation of inhibitory circuits in dentate granule cells

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Abstract

Neurosteroids are produced in the brain independently of peripheral endocrine glands to act locally in the nervous system. They exert potent promnesic effects and play significant roles in mental health-related disorders. In part, neurosteroids act by affecting ligand-gated ion channels and metabotropic receptors through rapid non-genomic processes. We have previously demonstrated that neurosteroids also affect synaptic transmission presynaptically in the CA1 region of the hippocampus. Here we describe the effects of the most abundant neurosteroid in the rodent brain, pregnenolone sulfate (PregS), on signal processing in the dentate subfield of the hippocampus. We show that PregS acts presynaptically at low concentrations (300 nM) to enhance paired-pulse facilitation (PPF) in perforant pathway terminals on dentate granule cells. Similar effects were found with two steroid sulfatase inhibitors demonstrating a potential contribution of endogenous steroids to dentate synaptic plasticity. This enhanced presynaptic facilitation paradoxically increases paired-pulse inhibition (PPI) at short interpulse intervals. Based on these data, a model of dentate gyrus circuit interactions is proposed for the presynaptic action of PregS on the filtering dynamics of the dentate subfield at frequencies similar to those of the endogenous signals from the entorhinal cortex. These modeling studies are consistent with experimental measurements demonstrating positive modulation by PregS at low frequencies and negative modulation at high frequencies. These studies show an important role for the presynaptic action of neurosteroids in modulating input signals to the hippocampus.

Keywords: Dentate gyrus; Hippocampal slice; Recurrent inhibition

1. Introduction

Neurosteroids, produced locally in neurons and glial cells, are important modulators of neuronal function (Baulieu, 1998). Pregnenolone sulfate (PregS), the most common neurosteroid in rodent brain (Corpechot et al., 1983), is produced by a steroid sulfotransferase from pregnenolone and is converted back to pregnenolone by a steroid sulfatase. It is well established that this and other neurosteroids have promnesic and cognitive-enhancing roles within the neocortex (Barrett-Connor and Edelstein, 1994; Flood et al., 1992, 1999; Darnaudery et al., 2000; Akwa et al., 2001; Matthews et al., 2002). Increases in brain levels of neurosteroids have been tied to improvements in memory acquisition and retention including increased performance in the Morris Water Test in rats (Darnaudery et al., 2000; Johnson et al., 2000) and there is a correlation of circulating neurosteroid levels with cognitive performance in humans

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(Barrett-Connor and Edelstein, 1994). These observations strongly suggest that neurosteroids are important endogenous modulators of spatial and/or relational learning and memory processes of the hippocampal formation (Corpechot et al., 1983; Baulieu, 1998).

The mechanism by which neurosteroids produce these effects is not fully understood. PregS is classified as an excitatory neurosteroid that positively modulates the *N*-methyl D-aspartate receptor (NMDAR) and negatively modulates the gamma amino butyric acid-A receptor ($GABA_AR$) (Majewska and Schwartz, 1987; Wu et al., 1991; Majewska, 1992; Bowlby, 1993; Park-Chung et al., 1994, 1999; Eisenman et al., 2003). These postsynaptic actions may, in part, underlie the cognitive-enhancing actions of PregS, although the concentrations required to exert these effects raise the possibility of the involvement of other sites of action. The presynaptic effect of this neurosteroid has been explored in only a few studies and, importantly, these studies have found that PregS is effective presynaptically at equal or lower concentrations than it is postsynaptically. Specifically, PregS has been shown to modulate release of noradrenaline (Monnet et al., 1995), GABA (Teschemacher et al., 1997), acetylcholine (Darnaudery et al., 1998; Darnaudery et al., 2000), dopamine (Barrot et al., 1999), and glutamate (Meyer et al., 2002). We recently reported that PregS increases paired-pulse facilitation without affecting the basal probability of glutamate release in mature neurons (Partridge and Valenzuela, 2001).

To explore further the presynaptic action of neurosteroids, we evaluated the effect of PregS on short-term synaptic plasticity and the consequent effects on the signal-filtering characteristics of the dentate gyrus. We show that by enhancing glutamate release, PregS has both monosynaptic and polysynaptic effects that positively modulate low-frequency transmission and negatively modulate high-frequency transmission between the perforant pathway and the output of the dentate subfield. This regulation of short-term synaptic plasticity in the hippocampus may underlie the neurosteroid enhancement of spatial/relational learning and memory. Because the dentate gyrus is an important input to the associative CA3–CA1 network of the hippocampus that modulates encoded object information transferred from the entorhinal cortex, these neurosteroid-modulated short-term plasticity changes within the dentate subfield will have a substantial impact on the downstream neuronal network.

2. Materials and methods

2.1. Field recordings

All experiments were performed in accordance with the University of New Mexico animal care and use guidelines. Field recordings were obtained from the dentate gyrus of coronal brain slices from 50-day-old male Sprague–Dawley rats deeply anesthetized with 250 mg/kg ketamine (intraperitoneal injection). Slices were cut at 400 μm with a Vibratome (Technical Products, St. Louis, MO) in ice-cold solution consisting of (in mM) 124 NaCl, 5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 10 glucose, followed by incubation at 34 °C in this same solution continuously oxygenated with 95% O_2/5% CO_2. After 45–60 min, slices were transferred to 25 °C artificial cerebrospinal fluid (aCSF) consisting of (in mM) 124 NaCl, 5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 10 glucose continuously oxygenated with 95% O_2/5% CO_2. All recordings were conducted after the slices had been incubated for at least 1 h at 25 °C. For recording, individual slices were transferred to a submerged-type recording chamber (Scientific Systems Design, Mercerville, NJ) and continuously perfused at 34 °C at a flow rate of 2 ml/min with oxygenated aCSF or aCSF to which drugs or vehicle had been added.

Extracellular field potentials were amplified with an Axoclamp 2B amplifier (Axon Instruments, Inc., Foster City, CA). A Digidata 1322A interface and pCLAMP 8.2 software (Axon Instruments) were used for data acquisition and analysis. Potentials were digitized at 67 kHz and filtered at 2 kHz using the low-pass filter of Clampex. Recording electrodes were filled with 3 M NaCl (tip impedance 2–3 MΩ). Pulses (100 μs) were delivered to the perforant pathway with a concentric bipolar electrode (pole separation 25 μm) via an Iso-Flex constant current stimulator (A.M.P.I., Jerusalem, Israel). Stimulation was adjusted to produce ~20% of maximum spike amplitude at a 0.05 Hz stimulation frequency.

The recording electrode was placed in the dense granular layer 2–3 mm from the medial tip of the stratum granulosum. Stimulation was delivered 50–100 μm dorsal and 100–150 μm medial to the most medial portion of the stratum granulosum near the perforant path termination zone (McNaughton, 1980). The position of the stimulation electrode was optimized in each slice to achieve PPI at short intervals and PPF at longer intervals. This placement assured that the majority of fibers stimulated were within the lateral perforant path, thus decreasing the influence of feedforward inhibitory connections (McNaughton, 1980; Sloviter, 1991). After placement of electrodes, field potentials were allowed to stabilize for a minimum of 40 min before recording was begun and recordings with a population spike of less than 1 mV were rejected. Three responses were averaged for each record except during 14 and 50 Hz stimulation where responses were not averaged. Frequency data were obtained with 10 pulses at either 14 or 50 Hz and inter-pulse (IPI) data were obtained at intervals of 12, 20, 30, 40, 50, 60, and