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Research report

Interaction between allopregnanolone and pregnenolone sulfate in modulating GABA-mediated synaptic currents in neurons from the rat medial preoptic nucleus

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Abstract

The two neurosteroids 3α-hydroxy-5α-pregnane-20-one (allopregnanolone; AlloP) and pregnenolone sulfate (PregS) affect neuronal $GABA_A$ receptors differently. While AlloP mainly potentiates the currents through $GABA_A$ receptors, PregS reduces such currents. The present study aimed at clarifying the interaction of AlloP and PregS at $GABA_A$ receptors in neurons from the medial preoptic nucleus of male rat. AlloP has previously been shown to dramatically prolong GABA-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) in these neurons. Here, by recording sIPSCs under voltage-clamp conditions with the perforated-patch technique, it was shown that PregS by itself did not significantly affect the amplitude or time course of such currents. However, PregS, in a concentration-dependent manner, reduced the AlloP-evoked prolongation of sIPSC decay when the two neurosteroids were applied together. In contrast to sIPSC amplitude and time course, sIPSC frequency was significantly reduced by 10 μM PregS alone. Further, although 1.0 μM AlloP alone induced a clear increase in sIPSC frequency, the frequency was not significantly different from control when 1.0 μM AlloP was applied in combination with 10 μM PregS. In addition to the effects on sIPSC parameters, PregS reduced the baseline current evoked by 1.0 μM AlloP in the absence of GABA application or synaptic activity. PregS by itself did not significantly affect the baseline current. The main effects of AlloP and PregS on the sIPSC time course were mimicked by a simplified model with AlloP assumed to reduce the rate of GABA unbinding from the receptor and PregS assumed to increase the rate of desensitization.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: GABA receptors

Keywords: Neurosteroid; $GABA_A$ receptor; Patch clamp; Inhibitory postsynaptic current; Allopregnanolone; Pregnenolone sulfate

1. Introduction

A number of steroid hormones and their metabolites affect nervous function. Such neuroactive steroids may be involved not only in various physiological functions such as the regulation of sexual behavior [10], responses to stress [41], emotional behavior and mood [45], but also in cognitive functions such as memory formation [8,19,33,52,53]. In addition, they may play critical roles in disorders like epilepsy [2,27], premenstrual syndrome [3,55], depression [51], and anxiety [43]. Among the most potent neuroactive steroids are 3α-hydroxy-5α-pregnane-20-one (allopregnanolone, subsequently called AlloP) and pregnenolone sulfate (PregS). These steroids are present [4,23] and may be synthesized within the nervous system in males as well as in females [41,48,50]. Further, the neuronal synthesis may be regulated in an activity-dependent manner [48]. Thus, it seems likely that AlloP and PregS play sophisticated roles in modulating neuronal communication.

At the cellular level, AlloP as well as PregS has been shown to affect $GABA_A$ receptor function [25,29–31,44]. In

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addition, PregS may affect other ligand-gated channels, such as NMDA receptors [5,32]. Whereas AlloP potentiates currents through $GABA_A$ receptors [30], PregS reduces such currents [29,31,37,46]. Therefore, it is of interest to learn how these endogenous substances, with opposing effects on the $GABA_A$ receptor, function in concert. It has been shown that sulfated steroids other than PregS may noncompetitively antagonize the potentiating effect of AlloP and some other steroids on synaptic currents in cultured hippocampal neurons [57], but direct information on the interaction of AlloP and PregS in modulating synaptic function is lacking.

In the present study, we investigate the effect of PregS alone and in combination with AlloP on spontaneous $GABA_A$-receptor-mediated postsynaptic currents in neurons from the medial preoptic nucleus (MPN) of male rat. Among other functions, the MPN is an integrating node for sensory and hormonal stimulation involved in controlling male sexual behavior [6]. In these neurons, we have previously shown that AlloP modulates $GABA_A$-receptor-mediated miniature postsynaptic currents primarily by prolonging the time course of current decay [13]. At micromolar concentrations, AlloP also directly induces a current in the absence of GABA, and in addition a presynaptic action at $GABA_A$ receptors is likely to explain an increased frequency of spontaneous GABA release [14]. The effects of PregS at these synapses are unknown.

The results obtained here show that PregS by itself only slightly affects the $GABA_A$-receptor-mediated postsynaptic currents, but that it substantially reduces the effects of AlloP. We present a simplified kinetic model that is compatible with the main properties of synaptic currents as well as with the main effects of AlloP and PregS on current time course. The model suggests that the two steroids may act on different state transitions and explains how the effect of PregS could be more pronounced in the presence of AlloP.

2. Materials and methods

The methods used for electrophysiological recordings and cell preparation have been described elsewhere [18,22]. A short account of the procedures is given below. Ethical approval of the procedures used was given by the local ethics committee for animal research.

2.1. Cell preparation

Young (50–120 g) male Sprague–Dawley rats were killed by decapitation without the use of anesthetics. The brain was removed and a block of tissue containing the preoptic area was cut out. Coronal slices, 250–300 μm thick, were cut and thereafter incubated for at least 1 h in "incubation solution" (see below) at room temperature (21–23 °C). Subsequently, single cells with adhering synaptic nerve terminals were isolated by vibrodissociation (as modified after Vorobjev [54]) without addition of enzymes. The mechanical vibration was applied at the medial preoptic nucleus by a thin (~0.5 mm diameter) glass rod. The cell bodies of obtained neurons were round or oval, 10–15 μm at their longest axis, often with two or more dendritic processes extending up to ~100 μm from the cell body. Previous studies have demonstrated that the isolated cells as well as the attached presynaptic terminals are in excellent conditions with electrophysiological properties similar to those in slice preparations (see e.g., Refs. [12–14,18,22]). The preparation used provides many advantages for studies of synaptic transmission, as reviewed by Akaike and Moorhouse [1].

2.2. Electrophysiological recordings

Amphotericin-B-perforated patch techniques [42] were used to record whole-cell currents from postsynaptic neurons under voltage-clamp conditions. The perforated-patch technique prevents wash-out of internal cellular components while still allowing for control of internal Na^+, K^+, and Cl^- concentrations within a physiological range (cf. Ref. [17]). Details of the techniques used are described by Haage and Johansson [13]. Patch pipettes were filled with "intracellular solution" and the cells were bathed in an "extracellular solution" (see below). In all experiments, after compensation for the liquid-junction potential (for details see Ref. [14]), a steady holding potential of −17 mV was used. Extracellular solutions, with or without test substances, were applied by a gravity-fed fast perfusion system controlled by solenoid valves. All experiments were performed at room temperature (21–23 °C) to improve resolution of current kinetics, to improve giga-seal formation, and to slow down cellular deterioration. Since previous studies demonstrated that steroid binding to $GABA_A$ receptors is independent of temperature changes [39,58], steroid effects similar to those recorded are also expected at higher temperatures.

2.3. Solutions

The incubation solution used during the preparation and for storage of slices contained (in mM) 150 NaCl, 5 KCl, 2.0 $CaCl_2$, 10 HEPES, 10 glucose, and 4.94 Tris-base. The solution was supplemented with O_2 gas. The extracellular solution used during recording of currents contained (in mM) 137 NaCl, 5.0 KCl, 1.0 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, and 10 glucose. Glycine (3.0 μM) was routinely added and pH was adjusted with NaOH to 7.4. AlloP (3α-hydroxy-5α-pregnane-20-one) was dissolved by sonification in this extracellular solution without organic solvents. The intracellular solution used for filling the patch pipettes contained (in mM) 140 Cs-gluconate, 3.0 NaCl, 1.2 $MgCl_2$, and 10 HEPES. pH was adjusted to 7.2 with CsOH. Amphotericin B was dissolved in dimethylsulphoxide and added to a final concentration of 120 μg amphotericin B per ml intracellular solution.