A Presynaptic Action of the Neurosteroid Pregnenolone Sulfate on GABAergic Synaptic Transmission

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ABSTRACT
The endogenous neurosteroid pregnenolone sulfate (PS) is known to enhance memory and cognitive function at nanomolar concentrations. However, the effect of these low concentrations on synaptic transmission has not been previously studied. The effects of PS on GABA_{A} receptor-mediated inhibitory postsynaptic currents were studied in cultured hippocampal pyramidal neurons. Concentrations of PS similar to those endogenous in the hippocampus (10–30 nM) reduced the frequency of both action potential-dependent (spontaneous inhibitory postsynaptic current) and -independent (miniature inhibitory postsynaptic current; mIPSC) inhibitory postsynaptic currents. This effect of PS was mimicked by the selective σ_{1} receptor agonist [2S-[2α,6α,11R]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-[2-propenyl]-2,6-methano-3-benzazocin-8-ol hydrochloride ([+]SKF 10047)] and blocked the specific σ_{1} receptor antagonists 1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride (BD-1063) and haloperidol and by pertussis toxin. The GABA_{A} antagonist baclofen and the metabotropic glutamate receptor antagonist (R,S)-a-cyclopropyl-4-phosphonophenylglycine had no effect on the PS-mediated inhibition of mIPSC frequency. The postsynaptic effects of PS occurred at micromolar concentrations but not at nanomolar concentrations. A comparison of the pre- and postsynaptic effects of PS demonstrated that it was 100-fold more potent in inhibiting presynaptic GABAergic synaptic mechanisms than GABA_{A} receptors. These studies demonstrate that concentrations of PS, similar to those endogenous in the hippocampus, inhibit GABAergic synaptic transmission by a presynaptic effect. PS causes specific activation of G protein-coupled σ_{1} receptors, resulting in modulation of both action potential-dependent and -independent IPSCs. These findings improve our understanding of the physiological function of PS.

The term neurosteroid refers to a group of compounds that can be synthesized de novo from cholesterol or gonadal and adrenal hormones by glial cells and neurons (Baulieu, 1991). Neurosteroids regulate many physiological processes, including memory, cognitive function, sleep, and nociception and may play a role in the pathogenesis of depression, epilepsy, and stress (Schumacher et al., 1999). Sulfated neurosteroids, such as pregnenolone sulfate (PS), can regulate learning and memory in extremely low concentrations. Early studies demonstrated that femtomolar doses of PS infused into the ventricles of mice could enhance memory (Flood et al., 1992). In aged rats, there is a correlation between cognitive decline and subtle reduction of hippocampal PS levels. Infusion of small doses (5–10 ng) of PS into the hippocampus can reverse cognitive impairment of aged rats (Vallee et al., 1997).

A number of studies demonstrated that micromolar concentrations of PS allosterically inhibited GABA_{A} receptor currents (Majewska et al., 1988; Park-Chung et al., 1999) and enhanced N-methyl-d-aspartate (NMDA) receptor function (Wu et al., 1991). However, it is unclear whether concentrations of PS commonly found in the brain (10–30 nM) (Kimoto et al., 2001) can modulate GABA_{A} and NMDA receptors.

In addition to its effects on ionotropic receptors, PS binds to σ_{1} receptors (Su et al., 1988; Hayashi et al., 1995; Monnet et al., 1995; Debonnel et al., 1996) resulting in the modulation of glutamate release from the presynaptic terminals (Meyer et al., 2002). We tested the possibility that concentrations of PS commonly found in the hippocampus can modulate GABAergic synaptic transmission via σ_{1} receptors. We describe a novel effect of physiologically relevant concentrations of PS on GABAergic synaptic transmission in hippocampal neurons. PS inhibited the vesicular release of

ABBREVIATIONS: PS, pregnenolone sulfate; NMDA, N-methyl-d-aspartate; IPSC, inhibitory postsynaptic current; PTX, pertussis toxin; DL-AP5, DL-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; BD-1063, 1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride; mIPSC, miniature inhibitory postsynaptic current; sIPSC, spontaneous inhibitory postsynaptic current; (+)-SKF 10047, [2S-[2α,6α,11R]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-[2-propenyl]-2,6-methano-3-benzazocin-8-ol hydrochloride; mGluR, metabotropic glutamate receptor; (R)-CPPG, (R,S)-a-cyclopropyl-4-phosphonophenylglycine; CGP-55845 3-N[(S)-3,4-dichlorophenyl]ethyl]amino-2-(S)-hydroxypropyl-p-benzyl-phosphinic acid.
GABA by diminishing the frequency of inhibitory postsynaptic currents (IPSCs). This effect was mediated by a pertussis toxin (PTX)-sensitive G protein-linked σ1 receptor.

Materials and Methods

Hippocampal Culture. Rat hippocampal cultures were prepared according to the method described by Banker et al. (1988). Briefly, hippocampi were dissected from 18-day-old rat embryos, dissociated by trypsin, and triturated with a Pasteur pipette. The neurons were plated on coverslips coated with poly-L-lysine in minimal essential medium with 10% horse serum at an approximate density of 25,000/cm². Once the neurons had attached to the substrate, they were transferred to a dish containing a glial monolayer and maintained for up to 4 weeks in serum-free minimal essential medium with N2 supplements.

Electrophysiology. Patch electrodes were pulled from borosilicate glass (Sutter Instruments, Novato, CA) on a horizontal Flaming-Brown microelectrode puller (model P-97; Sutter Instruments) using a two-stage pull protocol. Electrode resistances were 4 to 6 MΩ. Electrode tips were filled with an internal recording solution consisting of 153.3 mM CsCl, 1.0 mM MgCl₂, 10 mM HEPES, and 5.0 mM EGTA, pH adjusted to 7.2 with CsOH; osmolality, 285 to 295 mOsm. The internal solution was sterile-filtered before use. For experiments involving GABA_A receptors, CsCl was replaced by an equimolar concentration of KCl. The electrode shank contained an ATP regeneration solution consisting of 3 mM ATP, 0.1 mM GTP, 19 mM phosphocreatine, and 50 units/ml creatinine phosphokinase.

Cover slips with hippocampal neurons were removed from culture medium and placed in a 30-mm × 10-mm polylysine-coated culture dish containing external recording solution consisting of 142 mM NaCl, 1.0 mM CaCl₂, 8.1 mM CsCl, 2.1 mM MgCl₂, 10.0 mM glucose, and 10.0 mM HEPES, pH adjusted to 7.4 with NaOH. The osmolality was adjusted to 305 to 318 mOsm with sucrose. Voltage-clamp recordings were performed at room temperature (22–24°C) with an Axopatch 200A amplifier (Axon Instruments, Union City, CA). The cells, 10 to 21 days in vitro, with the characteristic shape of pyramidal neurons were selected for recording. The cells were voltage-clamped to −60 mV, and synaptic currents were low pass-filtered at 5 kHz with an eight-pole Bessel filter before digitization. The currents were digitized at the rate of 10 kHz by using a Digidata 1200 interface (Axon Instruments) and recorded to a personal computer with Axoscope 8.2 data acquisition software. Five-minute epochs of synaptic activity were recorded. Uncompensated whole cell resistances were digitized at the rate of 10 kHz by using a Digidata 1200 and recorded to a personal computer with Axoscope 8.2 data acquisition software. Five-minute epochs of synaptic current traces. The threshold for detection was set at 5 times the root mean square baseline noise, which was measured for each epoch of recording. Only those events with a 10 to 90% rise time less than 4 ms were included for analysis. The accuracy of detection was confirmed visually. For the analysis of the decay of individual synaptic currents, at least 25 current traces were selected randomly from a subpopulation of events with a 10 to 90% rise time less than 2 ms. For the majority of miniature IPSCs (mIPSCs), the decay time was best fit with a two-exponential decay function, identified visually, and included for the analysis. Data were analyzed using the Prism 3.0 program (GraphPad Software Inc., Mountain View, CA). Fast and slow decay times (τ₁ and τ₂) and amplitudes were compared with an unpaired t test. Frequencies were compared with a Wilcoxon matched pairs test. Data are represented as mean ± S.E.M.

Results

PS Modulation of IPSCs. We investigated whether PS, in the concentration range found in the hippocampus in vivo (10–35 nm) (Kimoto et al., 2001) could affect inhibitory synaptic transmission. Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded from hippocampal pyramidal neurons, 14 to 21 days in culture, after blocking excitatory transmission with DL-AP5 and CNQX. Addition of 10 μM bicuculline methiodide abolished IPSCs, demonstrating that they were mediated by GABA_A receptors (data not shown). PS was bath applied to a pyramidal neuron by means of a glass micropipette after recording sIPSCs for 5 min in control solution (Fig. 1A). The baseline frequency of sIPSCs was 2.8 Hz. The frequency of sIPSCs recorded from the neuron for 5 min in the presence of 30 nM PS was reduced to 1.73 Hz, corresponding to a 38% reduction of sIPSC frequency. After PS washout and a 40-min interval without drug application, a repeat 5-min recording demonstrated an sIPSC frequency of 2.53 Hz (Fig. 1C). In seven neurons tested, sIPSC frequency was suppressed by PS by 44 ± 8.9% (p = 0.03). In four neurons tested, removal of PS restored sIPSC frequency to control levels (96.3 ± 6.4%; p = 0.58).

The effect of multiple concentrations (300 pM–1 μM) of PS on sIPSC frequency was studied to further characterize PS effects on GABAergic synaptic transmission. PS inhibited the frequency of synaptic events in a concentration-dependent manner; i.e., as the concentration of PS was increased, sIPSC frequency declined. Percentage of reductions in sIPSC frequency as a function of increasing concentrations of PS were fit to the four parameter logistic equation (equation for a sigmoid curve): $F = F_{max}/(1 + 10^{(\log EC_{50} - \log [PS])/\text{Hill slope}})$, where F is the frequency of sIPSCs at a given PS concentration. The IC₅₀ and Hill slope values were derived from the equation that best fit the observed data by the least square fit method and were 26 ± 9.8 nM and −1, respectively (n = 21; Fig. 2).

In the hippocampus, sIPSCs result from action potential-mediated entry of calcium into the presynaptic terminal and vesicular release of GABA. It was unclear from the previous experiments whether the observed reduction of sIPSC frequency was the result of inhibition of action potentials or action potential-independent release of GABA. We separated the events into “small” and “large” amplitude groups, because large events are action potential-mediated and small amplitude events are believed to be action potential-independent (Katz and Miledi, 1967; Edwards et al., 1990). The low-amplitude group consisted of events with amplitudes up to 100 pA, and the high-amplitude group included events 101...
pA and higher. After the application of 30 nM PS (n = 6), the frequency of low-amplitude events was reduced by 42.7 ± 12% and the frequency of high-amplitude events was reduced by 61.2 ± 11%. No difference in inhibition was demonstrated between groups (p = 0.31). These studies suggested that low concentrations of PS affect the release of GABA from presynaptic terminals and that PS modulates both action potential-dependent and -independent GABA release.

To further confirm the finding that PS inhibited action potential-independent release of GABA, 1 μM tetrodotoxin was added to the external solution to record miniature inhibitory postsynaptic currents (mIPSCs). PS (30 nM) reduced the frequency of mIPSCs from 4.04 ± 1.6 to 2.53 ± 1 Hz, corresponding to a decrease in frequency by 37 ± 2.9% (n = 6; p = 0.03; Wilcoxon matched pairs test; Fig. 3, A, B, and D). A lower concentration of PS (10 nM) also reduced the frequency of mIPSCs from 1.96 ± 0.8 Hz to 1.5 ± 0.2 Hz, a 22 ± 1.6% reduction in the frequency of mIPSCs (n = 4; p = 0.04).

PS Modulation of IPSC Frequency Is Mediated by a PTX-Sensitive G Protein-Linked σ1 Receptors. Sulfated neurosteroids such as PS bind to σ1 receptors (Su et al., 1988), which were initially described as a subtype of opiate receptors (Su et al., 1986). If the inhibitory effect of PS is mediated by activation of σ1 receptors, it would be reasonable to expect that other agonists of σ1 receptors would be able to inhibit IPSCs frequency. (+)-SKF 10047 has been shown to bind with high affinity to σ receptors and to bind with low affinity to other receptor types, such as opiate and phencyclidine receptors (Walker et al., 1990). Application of 50 μM (+)-SKF 10047 decreased the frequency of sIPSCs by 55%, from 1.5 ± 0.8 to 0.7 ± 0.3 Hz (p = 0.03; n = 6; Wilcoxon matched pairs test; Fig. 4, A–C) but did not change the mean amplitude of sIPSCs, 49.3 ± 1 pA (n = 332) versus 49.5 ± 1 (n = 119; p = 0.93).

We investigated whether inhibition of σ1 receptors could block the inhibitory effect of PS. Haloperidol and BD-1063 are potent antagonists of σ1 receptors with high binding affinity (Walker et al., 1990; Debonnel et al., 1996).
Fig. 3. PS (30 nM) reduced the frequency of mIPSCs recorded from a cultured hippocampal neuron (A and B). Cumulative probability plot (C) of the mIPSCs frequency in control (solid line) and after application of 30 nM PS (dotted line). The ordinate depicts the cumulative probability of mIPSCs occurrence (density) in a range of 0 to 1, and the abscissa depicts time. Note the decreased frequency of mIPSCs after application of 30 nM PS. Similar to sIPSCs, the distribution of interevent intervals of mIPSCs was significantly different after application of 30 nM PS (Kolmogorov-Smirnov test; \( p = 0.0016 \)).

Fig. 4. Activation of \( \sigma_1 \) receptors by 50 \( \mu \)M (+)-SKF 10047 reduced the frequency of sIPSCs recorded from a cultured hippocampal neuron (A and B). Cumulative probability plot (C) of the sIPSCs frequency in control (solid line) and after application of 50 \( \mu \)M (+)-SKF 10047 (dotted line). The ordinate depicts the cumulative probability of sIPSCs occurrence (density) in a range of 0 to 1, and the abscissa depicts time. The frequency of sIPSCs decreased after application of 50 \( \mu \)M (+)-SKF 10047 (Kolmogorov-Smirnov test).
neurons were incubated with 50 μM haloperidol for 1 h. PS (30 nM) did not reduce the frequency of sIPSCs. The mean frequency of sIPSCs was 1.9 ± 0.5 Hz before and 2.1 ± 0.4 Hz after application of PS (n = 5; p = 0.87). Similarly, the inhibitory effect of 30 nM PS was also abolished by BD-1063. PS (30 nM) was applied to hippocampal neurons in the presence of 300 nM BD-1063 and no change in mIPSC frequency was observed (p = 0.91; two-tailed t test; Fig. 5, A–C). Therefore, blockade of the σ1 receptor by haloperidol and BD-1063 abolished the presynaptic effect of 30 nM PS. Under the conditions of blockade of σ1 receptors by BD-1063, there were no changes in the fast and slow decay time components (τ1 and τ2) or peak amplitude, neither before nor after application of 30 nM PS.

σ1 Receptors are PTX-sensitive G protein-coupled receptors, initially described as a subtype of opiate receptors (Martin et al., 1976). To test whether PS inhibition of IPSCs in hippocampal neurons was mediated by a G protein-linked σ1 receptors the effect of PS was studied after the blockade of the α subunit of G protein by pertussis toxin. The cultures were incubated in the presence of PTX (50 ng/ml) for 12 to 14 h and the effect of 30 nM PS on mIPSC frequency was assessed. PS did not reduce the frequency of mIPSCs in the neurons treated with PTX. The mIPSCs frequency increased by 1.68% after application of 30 nM PS (n = 6; p = 0.97; Wilcoxon matched-pairs test; Fig. 6, A–C). Therefore, PS modulation of GABA release was mediated by a G protein-coupled mechanism.

Specificity of PS Action. Activation of presynaptic GABA<sub>A</sub> receptors is mediated by a G protein-linked σ1 receptors the effect of PS was studied after the blockade of the α subunit of G protein by pertussis toxin. The cultures were incubated in the presence of PTX (50 ng/ml) for 12 to 14 h and the effect of 30 nM PS on mIPSC frequency was assessed. PS did not reduce the frequency of mIPSCs in the neurons treated with PTX. The mIPSCs frequency increased by 1.68% after application of 30 nM PS (n = 6; p = 0.97; Wilcoxon matched-pairs test; Fig. 6, A–C). Therefore, PS modulation of GABA release was mediated by a G protein-coupled mechanism.

Metabotropic glutamate receptors (mGluRs) are a family of G protein-coupled receptors that are widely distributed throughout the brain. In the hippocampus, activation of mGluR can inhibit (Desai and Conn, 1991), enhance (Sciancalepore et al., 1995), or have both effects on GABA<sub>A</sub> receptor-mediated transmission (Poncer et al., 1995). We studied whether activation of mGluR receptors could mediate the PS-induced reduction in mIPSCs frequency. PS (30 nM) decreased the frequency of mIPSCs by 36%, from 0.3 ± 0.01 to 0.2 ± 0.05 Hz (n = 7; p = 0.03; Wilcoxon matched pairs test). Therefore, PS decreased the release of GABA independent of presynaptic GABA<sub>A</sub> receptors.

A Comparison of Pre- and Postsynaptic Effects of PS. It is well established that micromolar concentrations of PS inhibit GABA<sub>A</sub> receptors (Majewska et al., 1990). However, previous studies did not examine whether 10 to 30 nM PS could modulate GABA<sub>A</sub> receptor function. PS (10 or 30 nM) did not have any effect on peak amplitude or fast and slow decay time components (τ1 and τ2) or peak amplitude, neither before nor after application of PS.
decay time constants ($\tau_1$ and $\tau_2$) of mIPSCs. Fifty individual mIPSCs were selected randomly and analyzed. In control recordings, the mean peak amplitude of mIPSCs was $45 \pm 2.3$ pA, and after application of 30 nM PS it was $51 \pm 3.0$ pA ($p = 0.16; t$ test). The fast and slow decay time constants of mIPSCs ($\tau_1$ and $\tau_2$) with a rise time of $<2$ ms were $24 \pm 2.7$ and $116 \pm 14$ ms in control solution, respectively, and $23 \pm 2.5$ and $122 \pm 15.5$ ms in the presence of 30 nM PS ($p = 0.34; t$ test). Because the number and properties of GABA$_A$ receptors strongly influence the amplitude and decay of mIPSCs, these results suggested that ambient concentrations of PS in the brain (30 nM) did not alter GABA$_A$ receptor function.

This was further confirmed by recording whole cell GABA$_A$ receptor currents from cultured hippocampal neurons. The currents were elicited by application of 30 $\mu$M GABA with increasing concentrations of PS (100 nM–300 $\mu$M). Low concentrations of PS (300 nM and 1 $\mu$M) did not modulate whole cell GABA$_A$ receptor currents. Higher concentrations of PS (3 $\mu$M) decreased peak amplitude of the currents by $8.2 \pm 1.8\%$.

**Fig. 6.** Blockade of the $\alpha$ subunit of the G protein abolished the inhibitory effect of 30 nM PS. The mIPSCs were recorded in the presence of 50 ng/ml PTX (A) and application of 30 nM PS did not reduce the frequency of mIPSCs (B). Cumulative probability plot (C) shows the frequency distribution of mIPSCs in the same neuron before and after application of 30 nM PS.

**Fig. 7.** Nanomolar concentrations of PS decrease the frequency of sIPSCs, but do not affect GABA-elicited whole cell currents. A, control current was elicited by application of 30 $\mu$M GABA. PS (300 nM), when coapplied with 30 $\mu$M GABA, did not alter the current. PS (3 $\mu$M) reduced the peak amplitude of GABA-evoked current. Horizontal bars show duration of the drug application. B, micromolar concentrations of PS decreased the peak amplitude of 30 $\mu$M GABA-elicited currents in a concentration-dependent manner. The ordinate depicts the percentage of the peak current amplitude as a fraction of that evoked by the control GABA application. The abscissa shows the concentration of PS (300 nM–1 mM). The circles represent mean peak amplitude $\pm$ S.E.M. The solid line represents the best fit to the sigmoidal function. To illustrate the striking difference between effects of low and high concentrations of PS, the sigmoidal graph from Fig. 2 has been replicated in Fig. 6B, left curve. Note that an approximately 150-fold concentration of PS was required for inhibition of postsynaptic GABA$_A$ receptor currents than for inhibition of sIPSC frequency.
Increasing concentrations of PS decreased peak amplitude of GABA-evoked currents in a concentration-dependent manner. Percentage of decrease of peak amplitude of GABA-evoked currents as a function of increasing concentrations of PS was fitted to the equation for a sigmoidal function as defined above. The IC$_{50}$ and Hill slope values were derived from the equation that best fit the observed data by the least-square fit method and were 4 μM and $-0.68$, respectively ($n=8$; Fig. 7B, curve on right). A comparison of the two concentration response curves demonstrated more than a 100-fold difference in the potency of pre- and postsynaptic effects of PS (Fig. 7B). PS was far more potent in inhibiting presynaptic GABAergic synaptic mechanisms.

**Discussion**

In summary, we found that: 1) The concentrations of PS commonly found in the hippocampus reduced the frequency of sIPSCs and mIPSCs recorded from these neurons. 2) The presynaptic effect of PS was mediated by pertussis toxin-sensitive σ₁ receptors. 3) The effect of PS was specific to the σ₁ receptor and not mediated by activation of GABA$_A$ or metabotropic glutamatergic receptors. 4) The presynaptic effect of PS was far more potent than its postsynaptic effect. These findings may help explain the physiological actions of PS in the hippocampus.

The ability of PS to decrease the frequency of sIPSCs was described in cultured hippocampal neurons (Moss and Smart, 2001). However, the lowest concentration of PS used in that study was 1 μM. Effects of PS at this concentration, which exceeds endogenous concentrations of PS in hippocampus severalfold, may have been distinct from effects of nanomolar PS reported in this article. The presynaptic effect of PS described in the current study is similar in some respects to its effect on glutamate release described recently (Meyer et al., 2002). In cultured hippocampal neurons, PS increased the frequency of miniature excitatory postsynaptic currents mediated by amino-3-hydroxy-5-methylisoxazole-4-propionate receptors, but did not alter their amplitude. Similar to our findings, the PS effect was mediated by pertussis toxin-sensitive σ₁ receptors. However, an important difference lies between the effects of PS on IPSC frequency and excitatory postsynaptic current frequency. PS modulated glutamate release at concentrations of 10 μM, which might be a higher concentration than expected for a physiologically relevant action, whereas its effects on GABA release probably occur at far lower, physiologically relevant concentrations.

PS is synthesized and circulates in brain in nanomolar concentrations (Kimoto et al., 2001). Studies have reported hippocampal PS levels in the 5-ng/g range. It was suggested that brain homogenate concentrations of PS might not accurately represent synaptic concentrations of this neurosteroid (Meyer et al., 2002). PS is synthesized by glia and there is no evidence suggesting that it might be synthesized in presynaptic terminals, or a buildup of its local concentrations may occur because of specific transporters. Thus, the synaptic concentration of PS is unlikely to be 1,000-fold higher than that in homogenates or cerebrospinal fluid.

In the hippocampus, nanomolar concentrations of PS modulate learning and memory. Intracerebroventricular administration of PS in mice immediately after training causes improved memory retention in footshock active avoidance training. The dose-response curves show that PS has significant effects at doses as low as 3.5 fmol/mouse (Flood et al., 1992). In follow-up studies, the hippocampus was demonstrated as a potential site of action of PS (Flood et al., 1995) because intrahippocampal injection of PS resulted in enhancement of memory at a lower dose than when infused into the septum or mammillary bodies. The plasma and brain concentrations of sulfated neurosteroids decrease with age, and their low levels correlate with poor learning and memory performance. Concentrations of PS in the hippocampus of 24-month-old rats were found to be decreased compared with that of 3-month-old rats. Systemic administration of PS restored retention deficit in aged rats, and this effect lasted for 10 days (Vallee et al., 2001). In the same study, intrahippocampal infusion of PS immediately after training restored memory retention in aged rats. The present study demonstrated that a physiological concentration of PS diminished the release of GABA from presynaptic terminals. This PS-mediated disinhibition may allow long-term potentiation to occur and thus facilitate learning and memory. For example, endogenous cannabinoids, which exert a disinhibitory effect by suppressing release of GABA, facilitate long-term potentiation in CA1 pyramidal neurons (Carlson et al., 2002).

The disinhibitory effect of PS was mediated via σ₁ receptors, which are nonopioid metabotropic, G protein-coupled receptors. PS also acted as a σ₁ receptor agonist in vivo in a number of behavioral studies, and in vitro (Hayashi et al., 1995; Monnet et al., 1995; Debonnel et al., 1996). σ₁ receptor agonists possess potent antiamaesic properties, similar to those of PS (Urani et al., 1998; Maurice et al., 2001). Several studies demonstrate that the σ₁ receptors are expressed in hippocampus. Autoradiographic localization of σ₁ receptors binding sites suggested a high concentration in hippocampal pyramidal neurons (Gundlach et al., 1986). More recently, immunocytochemical studies suggested a high level of expression of the σ₁ receptor in the hippocampus. Intense staining was described in the granule cell layer and moderate staining in pyramidal neurons (Alonso et al., 2000). Ultrastructural immunostaining studies revealed σ₁ receptors at the synapses, typically at the postsynaptic membrane.

It is possible that PS diminished mIPSC frequency via action of σ₁ receptors on Ca$_{2+}$ channels and homeostasis. Ligand activation of σ₁ receptors elicited a dose-dependent reduction in intrasynaptosomal free calcium levels (Brent et al., 1996). Presynaptic internal Ca$_{2+}$ stores are known to modulate mIPSC frequency and GABA release (Bardo et al., 2002); thus, it is possible that the presynaptic effect of PS occurred by mobilizing internal Ca$_{2+}$ stores. The modulation of IPSCs by PS could also have occurred by modulating calcium entry into the presynaptic terminal. PS and other neurosteroids inhibited voltage-gated calcium channel currents in acutely isolated CA1 pyramidal neurons (french-Mullen et al., 1994). This inhibition of calcium currents was diminished by pretreatment with PTX, suggesting involvement of σ₁ receptors. A recent study suggested that σ₁ receptors inhibited high voltage-activated calcium channels in rat autonomic ganglion neurons (Zhang and Cuevas, 2002), although this effect was probably mediated via s$_e$ receptors.

This study demonstrated that PS inhibition of GABA$_A$ receptors occurred at far higher concentrations than what is necessary to inhibit GABA release, because the IC$_{50}$ value for inhibition of GABA$_A$ receptors was more than 100-fold higher.
than that for inhibition of frequency of sIPSCs. Inhibition of GABA_A receptors is a commonly studied mechanism of PS. PS negatively modulates GABA_A receptor currents in neonatal rat cortical neurons (Majewska et al., 1988), recombinant α1β2γ2S GABA_A receptor currents (Park-Chung et al., 1999) and inhibits synaptic GABA currents in hypothalamic neurons in micromolar concentrations (Poisbeau et al., 1997). PS enhances desensitization of GABA_A receptor currents in hippocampal membrane patches (Shen et al., 1999) and reduces single channel cluster opening duration independently of GABA concentration, thus acting as a noncompetitive antagonist in a voltage-independent manner (Akk et al., 2001).

In conclusion, nanomolar concentrations of PS inhibited the frequency of GABA_A receptor mediated mIPSCs and sIPSCs in cultured hippocampal neurons, whereas decay times of postsynaptic currents were not affected. This effect was independent from postsynaptic inhibition of GABA_A receptors and was achieved by activation of G protein-coupled metabotropic _G_ receptors. In conclusion, nanomolar concentrations of PS inhibited the frequency of GABA_A receptor mediated mIPSCs and sIPSCs in cultured hippocampal neurons, whereas decay times of postsynaptic currents were not affected. This effect was independent from postsynaptic inhibition of GABA_A receptors and was achieved by activation of G protein-coupled metabotropic _G_ receptors.

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