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Neurosteroids 3β, 20 (R/S)-pregnandiols decrease offset rate of the GABA-site activation at the recombinant $GABA_A$ receptor

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Abstract

Neurosteroids directly modulate ligand gated ion channels such as $GABA_A$ receptors. Two such molecules, 3β-OH A-ring reduced pregnane steroids and pregnenolone sulfate (PS), inhibit recombinant $GABA_A$ receptor. Using a two-electrode voltage-clamp technique, we compared the effect of 5α-pregnan-3β,20(S)-diol (UC1019), 5β-pregnan-3β, 20(R)-diol (UC1020) and PS on the activation onset and offset times of the recombinant $GABA_A$ receptor (*rat* $\alpha_1\beta_2\gamma_{2L}$) in *Xenopus* oocytes. Rapid solution changes allowed the kinetic analysis of GABA-evoked currents. Steroids were co-applied with 30 μM GABA for 10 s, followed by a 80 s washout period. PS (≥0.3 μM) moderately increased the slow onset rate ($k_{on\text{-}S}$) of GABA-response. PS had no significant effects on the fast onset rate ($k_{on\text{-}F}$). UC1019 and UC1020 decreased the $k_{on\text{-}S}$ of the GABA-response in a concentration-dependent manner with no significant effects on the $k_{on\text{-}F}$. Like PS, UC1019 and UC1020 decreased the slow offset rates ($k_{off\text{-}S}$). In addition, PS increased the fast offset rate ($k_{off\text{-}F}$) in a concentration-dependent manner, while UC1019 and UC1020 decreased $k_{off\text{-}F}$. The EC_{50} of PS to increase $k_{off\text{-}F}$ was calculated as 0.47±0.1 μM. The corresponding IC_{50} values of UC1019 and UC1020 to decrease $k_{off\text{-}F}$ were 5.0±0.5 μM and 8.4±0.9 μM, respectively. These results suggest differential actions of PS and 3β, 20(R/S)-pregnandiols on the offset time course of GABA-site activation.

Keywords: $GABA_A$ receptor; GABA; Neurosteroid; Onset/offset rate; Pregnenolone sulfate

1. Introduction

Neurosteroids are synthesized in the central nervous system (CNS) and carry out potent actions on the $GABA_A$ receptor (Covey et al., 2001; Lambert et al., 2003). They can either enhance or inhibit GABAergic neurotransmission (Eisenman et al., 2003; Mennerick et al., 2004; Mennerick et al., 2001; Shen et al., 2000; Stromberg et al., 2006; Wang et al., 2002). Two types of neurosteroids, 3β-hydroxy A-ring reduced pregnane steroids (3β-OH steroids) and pregnenolone sulfate (PS), have been shown to inhibit $GABA_A$ receptor-coupled Cl^- channels (Lundgren et al., 2003; Wang et al., 2002; Wang et al., 2007). Under agonist binding and channel opening conditions, PS and 3β-OH steroids block $GABA_A$ receptors more effectively (Eisenman et al., 2003; Shen et al., 2000; Wang et al., 2002). Interactions between antagonist and agonist steroids are resulted from a use-dependent action at $GABA_A$ receptor level (Wang et al., 2002).

In our previous comparison of 3β-OH steroids and PS on the recombinant *rat* $GABA_A$ receptors in the *Xenopus* oocyte, we found that PS stimulated desensitization rate during prolonged GABA-application in a dose-dependent manner on the *rat* $\alpha_1\beta_2\gamma_{2L}$ receptor. In contrast, 5α-pregnan-3β, 20 (S)-diol (UC 1019) and 5β-pregnan-3β, 20(R)-diol (UC1020) did not (Wang et al., 2007). We concluded that the inhibition by PS, but not by 3β,20(R/S)-pregnandiols, on the recombinant $GABA_A$ receptor was dependent on the receptor desensitization. In order to characterize the underlying difference between PS and 3β-OH steroids on the $GABA_A$ receptor, we compared the onset

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and offset time courses of the GABA-site activation of both types of neurosteroids. This study thus provides further analysis on the $GABA_A$ receptor desensitization and offset rates of the GABA-site activation induced by 3β-OH steroids or pregnenolone sulfate.

2. Materials and methods

2.1. Drug preparation

5α-pregnan-3β, 20(S)-diol and 5β-pregnan-3β, 20(R)-diol were purchased from Steraloids (Newport, RI, USA). GABA and pregnenolone sulfate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pK_a=7.5) was used to buffer the external solution (ND96) to neutral pH. The composition of the standard ND96 solution was (in mM): 96 NaCl, 1 KCl, 1 $MgCl_2$, 2 $CaCl_2$ and 5 HEPES at pH 7.4. Steroids were dissolved in dimethyl sulfoxide (DMSO) except for pregnenolone sulfate which was dissolved in the distilled water and treated with ultrasonic bath (Bransonic 2210) for 10 min before use. All chemicals were then diluted in external solution for experiments. The final concentration of DMSO in experimental solutions was ≤0.1%. We have earlier tested the effect of DMSO in the recombinant *rat* $\alpha_1\beta_2\gamma_{2L}$ receptor and did not find any significant response with DMSO up to 0.2% (Wang et al., 2002).

2.2. In vitro transcription and expression in the Xenopus oocyte

Sexually mature female *Xenopus laevis* (Horst Kähler; Bedarf für Forschung & Lehre, Hamburg, Germany) were kept in optimal condition by authorized animal keepers in the local animal house and fed with standard frog food. Experimental protocols were approved by the Animal Experimentation Ethical Committee in Umeå according to the EU legislation. Stage V–VI oocytes were harvested under 0.1% tricaine (3-aminobenzoic acid ethyl ester) anaesthesia. Oocytes were defolliculated by shaking for 20 min at 37 °C in collagenase (2 mg/ml) dissolved in calcium-free solution containing (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, and 5 HEPES at pH 7.4.

The cDNAs for the *rat* $GABA_A$-receptor subunits were provided by Dr. A. Tobin, University of California, Los Angeles, CA, U.S.A. (α_1), P. Malherbe, Hoffman-La Roche, Switzerland (β_2), C. Fraser, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, U.S.A. (γ_{2L}). Capped mRNAs, encoding *rat* $GABA_A$ receptor subunits were transcribed *in vitro* using the mMESSAGE mMachine™ T7 Kit (Ambion, Austin, TX, U.S.A.) according to manufacturer's instructions from linearized pBluescript vectors containing receptor coding regions. Subunit transcripts were injected in equimolar ratios (20–40 ng total RNA) to construct the ternary receptor (1:1:1) at 24 h following defolliculation. A Nanoject II auto nanoliter injector (Drummond Scientific Company, Broomall, PA, U.S.A.) was used for mRNA microinjection. Oocytes were incubated up to 5 days at 18 °C in the standard ND96 at pH 7.4, supplemented with pyruvate (5 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and gentamycin (50 μg/ml).

2.3. Oocyte electrophysiology

Two-electrode voltage-clamp whole-cell recording was performed with a Warner OC725 amplifier (Warner Instrument Corporation, Hamden, CT, USA) 2–3 days following RNA injection. The extracellular recording solution was ND96 medium with no supplements. Intracellular recording pipettes were filled with 3 M KCl and had open tip resistance of ~1 MΩ. Drugs were bath applied from a common tip via a gravity-driven multibarrel drug-delivery system ValveLink 16 pinch valve perfusion system which was controlled by a Valvelink 16 controller (AutoMate Scientific, Inc, San Francisco, CA, USA). The perfusion system is designed for fast local superfusion and bath perfusion with rapid valve response time at 25 ms. The oocyte perfusion chamber has small working volume (<100 μl) which allows fast solution exchange. In the present study, drugs were always co-applied with GABA in 10 s and were not pre-applied in the absence of GABA for possible comparison between drug effects. A washout period of 80 s was adopted between repeated recordings. Cells were clamped at −70 mV for all experiments. The peak current and the steady-state current following a 10 s drug application were measured for quantification of current amplitudes.

2.4. Data analysis

Data acquisition and analysis were performed with pClamp software (Axon Instruments, San Francisco, CA, USA). Data plotting and curve fitting were done with Sigma Plot software (SPSS, Chicago, IL, USA). Data are presented as mean±S.E.M. Statistical difference is determined using a two-tailed student *t*-test. $\Delta I_{peak}/\Delta I_{staedy\text{-}state}$ ($\Delta P/S$ ratio) was calculated to determine the desensitization rate of GABA-response. The time courses of composite onset and offset of macroscopic currents were fitted to a biexponential function:

$$A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t),$$

with Sigma Plot using a least-squares criterion, where k_i is the estimated rate and calculated as $1/\tau$. Fitting began after the initial sigmoidal foot when discernible. For ease of comparing effects of several drugs among cells with heterogeneous kinetics, the model-independent 10–90% offset time of GABA-evoked currents was used as primary fitting standard of offset decay time.

In order to reduce the influence of perfusion speed on the onset and offset time courses of the GABA-site activation, data were normalized against the corresponding control GABA-response: $k_i = k_M/k_N$, where k_N (normalizing time rate) and k_M (measured time rate) represent the time course of GABA-site activation in the presence and absence of test substances. Fitting of the dose–response relationships of onset and offset rates (k_{on} and k_{off}) was

performed using the Hill equation with a four-parameter logistic function:

$$k_i = k_{i\min} + \frac{k_{i\max} - k_{i\min}}{1 + 10^{(LogEC_{50} - Log[drug])^n}}$$

where n represents the Hill coefficient.

3. Results

PS, UC1019 and UC1020 decreased the peak and the steady-state currents with 10 s GABA-application (Figs. 1A, 2A and 3A). It was apparent that PS inhibited steady-state GABA-currents more effectively than it did on the peak currents ($\Delta P/S$ ratio > 1; Table 1). UC1019 and UC1020 inhibited steady-state GABA-currents in proportion to their inhibition on the peak currents ($\Delta P/S$ ratio = 1; Table 1). GABA concentration–response curves were obtained before testing antagonist steroids on the $\alpha_1\beta_2\gamma_{2L}$ receptor. EC_{50} values were recorded as 7.4±0.8 μM and 6.5±0.4 μM for peak and steady-state currents, respectively (data not shown). In order to reduce the effect of rapid desensitization on the offset time course of GABA-response, we used EC_{90} GABA-response (30 μM) as our control to investigate steroid-inhibition. IC_{50} values of PS, UC1019 and UC1020 to inhibit the steady-state GABA-currents were recorded as 0.3±0.1 μM (N=7), 1.9±0.3 μM (N=7) and 2.6±0.6 μM (N=7), respectively (data not shown).

The onset and offset rates of the macroscopic current induced by PS, UC1019 or UC1020 were expressed by fitting biexponential functions to current time course. The average parameters of biexponential fitting, together with peak and steady-state current amplitudes, are reported in Table 1. Analysis of the onset time course, superimposed for clarity, revealed little effect with PS ≤ 2 μM on the fast onset rate, $k_{on\text{-}F}$ (Fig. 1B and D). However, a significant increase in $k_{on\text{-}S}$ was observed with PS ≥ 0.3 μM (P<0.05; Fig. 1D). On the contrast, both UC1019 and UC1020 decreased the $k_{on\text{-}S}$ of GABA-current in a dose-dependent manner (Figs. 2D and 3D), although neither changed $k_{on\text{-}F}$ significantly. The corresponding IC_{50} values for UC1019- and UC1020-inhibition on the $k_{on\text{-}S}$ were 3.1±0.7 μM and 3.4±0.5 μM, respectively (Figs. 2D and 3D).

Fig. 1C shows the effect of PS on offset time course re-plotted from Fig. 1A, by aligning the data at the end of offset time course. The concentration–response inhibitions of PS on the $k_{off\text{-}F}$ and $k_{off\text{-}S}$ are depicted in Fig. 1E. $k_{off\text{-}F}$ of the GABA-response was increased by PS in a concentration-dependent manner (Fig. 1E), indicating that PS shortened the fast offset time course of GABA-response. The EC_{50} of PS to increase $k_{off\text{-}F}$ was estimated to 0.47±0.1 μM (Fig. 1E). In addition, PS decreased $k_{off\text{-}S}$ of the GABA-current in a concentration-dependent manner also (IC_{50} = 0.36±0.1 μM).

The kinetic properties of the offset time course relative to UC1019- and UC1020-induced inhibition were examined.

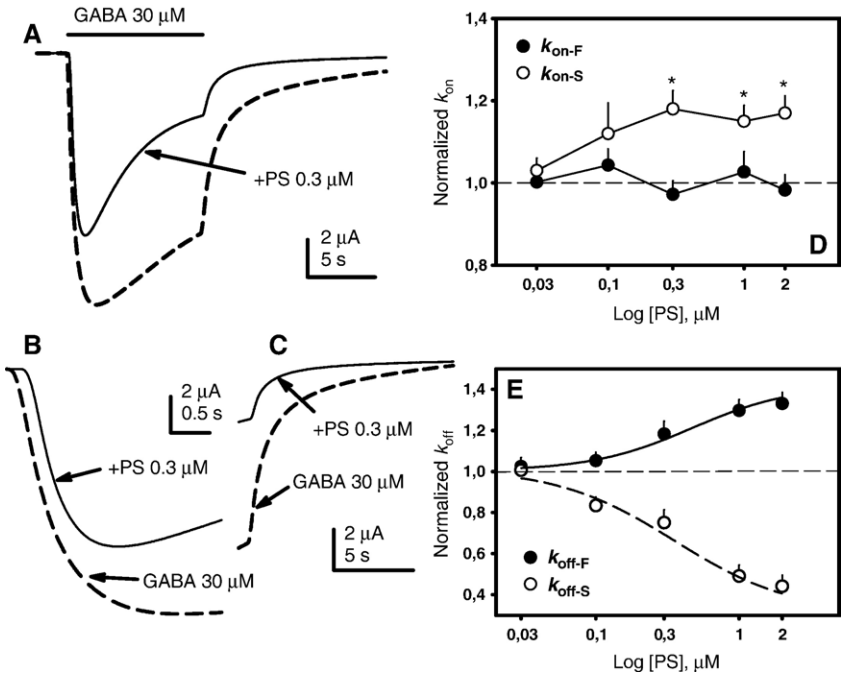

Fig. 1. Pregnenolone sulfate (PS) inhibits the macroscopic GABA-response at the recombinant $\alpha_1\beta_2\gamma_{2L}$ receptor. A, Currents triggered by 30 μM GABA from a single cell in the absence and presence of 0.3 μM PS. Note that PS decrease the steady-state GABA-response more effectively than its inhibition on the peak current. B, Normalized responses shifted in time on an expanded time scale re-plotted from A demonstrating the onset time course of GABA-response. C, Time course of GABA-response offset, aligned at the end of offset phase on an expanded time scale. D, Plot of activation onset rate (k_{on}) of PS action on the onset time course of 30 μM GABA-response (N=7). $k_{on\text{-}F}$ and $k_{on\text{-}S}$ were normalized against the k_N values of control GABA-response (k_N=1). *Significant different from controls (P<0.05). E, Dose–response plot of fast and slow offset rates of GABA-response ($k_{off\text{-}F}$ and $k_{off\text{-}S}$) derived from biexponential fitting of PS action on the offset time course of 30 μM GABA-response. k_N=1, representing the normalizing $k_{off\text{-}F}$ and $k_{off\text{-}S}$ recorded with control GABA-response, respectively. The EC_{50}/Hill coefficient of $k_{off\text{-}F}$ fitting is 0.47±0.1 μM/−1.23 (N=7). The IC_{50}/Hill coefficient of $k_{off\text{-}S}$ fitting is 0.36±0.1 μM/−1.05 (N=7).

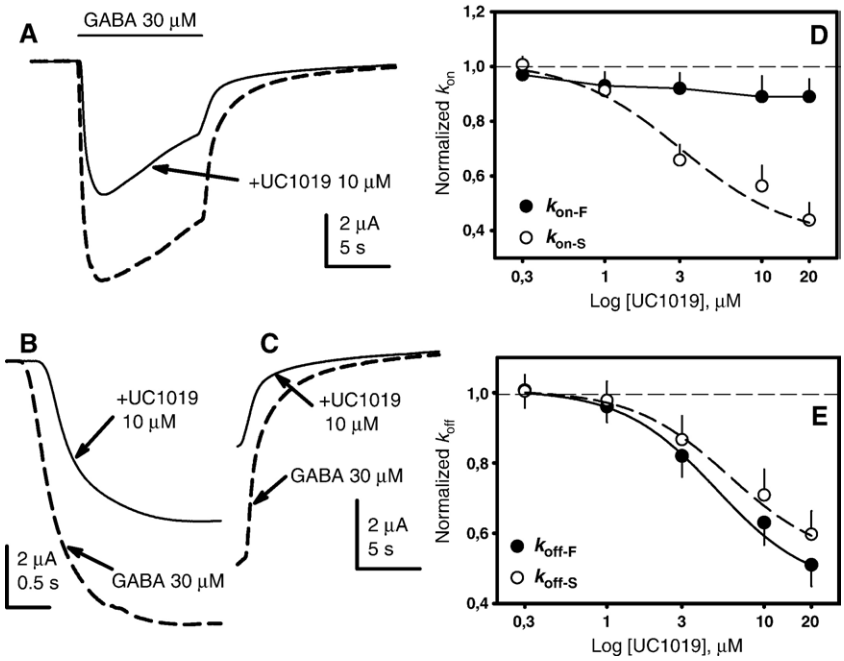

Fig. 2. 5α-pregnan-3β, 20(S)-diol (UC1019) inhibits the macroscopic GABA-response. A, Currents triggered by 30 μM GABA from a single cell in the absence and presence of 10 μM UC1019. Note that the inhibition on steady-state GABA-response was proportional to the inhibition on the peak current. B, Normalized responses shifted in time on an expanded time scale re-plotted from A demonstrating the onset time course of GABA-response. C, Time course of GABA-response offset, aligned at the end of offset phase on an expanded time scale. D, Plot of activation onset rate (k_{on}) of UC1019 action on the onset time course of 30 μM GABA-response (N=8). UC1019 decreased $k_{on\text{-}S}$ in a dose-dependent manner with the IC_{50}/Hill coefficient recorded as 3.1±0.7 μM/−1.48 (N=8). UC1019 did not change $k_{on\text{-}S}$ significantly compared to controls. E, Dose–response plot of fast and slow activation offset rates ($k_{off\text{-}F}$ and $k_{off\text{-}S}$) derived from biexponential fitting of UC1019 action on the offset time course of 30 μM GABA-response. The IC_{50}/Hill coefficient of $k_{off\text{-}F}$ fitting is 5.0±0.5 μM/−1.46 (N=8). The IC_{50}/Hill coefficient of $k_{off\text{-}S}$ fitting is 6.0±1.1 μM/−1.41 (N=8).

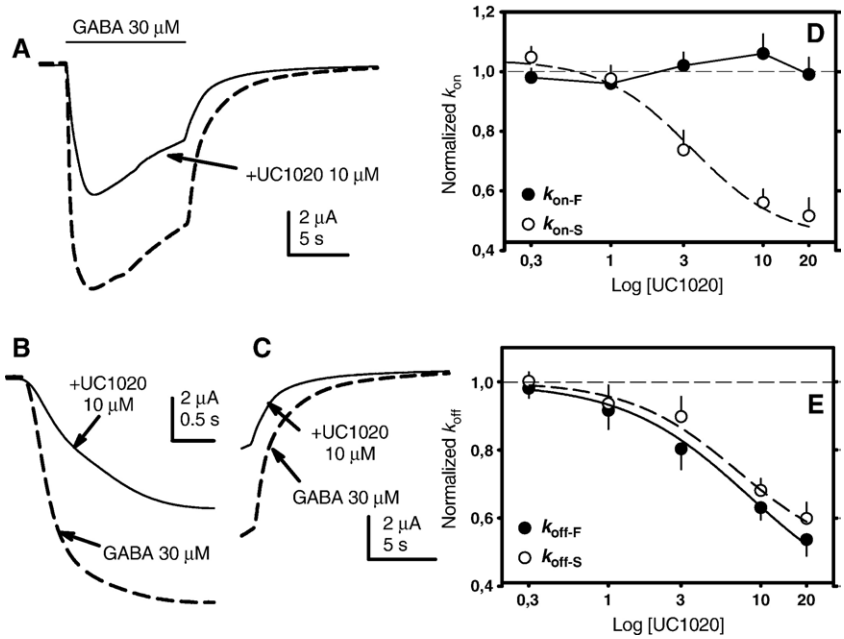

Fig. 3. 5β-pregnan-3β, 20(R)-diol (UC1020) inhibits the macroscopic GABA-response. A, Currents triggered by 30 μM GABA from a single cell in the absence and presence of 10 μM UC1020. Note that the inhibition on steady-state GABA-response was proportional to the inhibition on the peak current. B, Normalized responses shifted in time on an expanded time scale re-plotted from A demonstrating the onset time course of GABA-response. C, Time course of GABA-response offset, aligned at the end of offset phase on an expanded time scale. D, Plot of fast activation onset rate (k_{on}) of UC1020 action on the onset time course of 30 μM GABA-response. UC1020 decreased $k_{on\text{-}S}$ in a dose-dependent manner with the IC_{50}/Hill coefficient recorded as 3.4±0.5 μM/−1.48 (N=7). UC1020 did not change $k_{on\text{-}F}$ significantly. E, Dose–response plot of fast and slow rates of activation offset ($k_{off\text{-}F}$ and $k_{off\text{-}S}$) derived from biexponential fitting of UC1020 action on the offset time course of 30 μM GABA-response. The IC_{50}/Hill coefficient of $k_{off\text{-}F}$ fitting is 8.4±0.9 μM/−1.05 (N=7). The IC_{50}/Hill coefficient of $k_{off\text{-}S}$ fitting is 7.1±0.8 μM/−1.22 (N=7).

Table 1
Average fitting parameters and current amplitudes of 30 μM GABA-response co-applied with 0.3 μM pregnenolone sulfate (PS), 10 μM 5α-pregnan-3β, 20(S)-diol (UC1019) and 10 μM 5β-pregnan-3β, 20(R)-diol (UC1020), respectively

Drugs (*N*)	Onset				I_{steady} (μA)	I_{steady} (μA)	Offset			
	A_1 (μA)	A_2 (μA)	τ_1 (ms)	τ_2 (ms)			A_1 (μA)	A_2 (μA)	τ_1 (ms)	τ_2 (ms)
GABA 30 μM (7)	−5.6	20.6	134±8	336±14	−17.0	−9.3	−4.7	−2.9	914±65	4087±299
+PS 0.3 μM (7)	−6.7	19.8	143±11	260±11	−14.1	−4.3	−2.2	−1.4	779±57	6819±487
GABA 30 μM (8)	−1.8	11.6	159±13	520±14	−10.2	−7.2	−2.5	−4.3	897±53	4538±325
+UC1019 10 μM (8)	−3.4	12.9	161±11	708±22	−7.3	−4.2	−1.9	−2.1	1208±77	6278±443
GABA 30 μM (7)	−1.1	7.0	146±11	422±12	−9.6	−6.3	−2.2	−3.8	972±49	4063±212
+UC1020 10 μM (7)	−2.3	12.4	142±9	565±14	−6.9	−3.5	−1.3	−2.1	1290±71	5736±403

τ_1 and τ_2 denote that fast and slow time constant of the onset or offset time course.

Observations of similar offset time courses were made from comparable experiments between UC1019 and UC1020 (Figs. 2C and 3C). Both steroids decreased $k_{off\text{-}F}$ and $k_{off\text{-}S}$ in a dose-dependent manner (Figs. 2E and 3E). It is noted that UC1019 and UC1020 affected $k_{off\text{-}F}$ in an opposite direction compared to PS. The IC_{50} values for UC1019 and UC1020 to decrease $k_{off\text{-}F}$ were calculated as 5.0±0.5 μM and 8.4±0.9 μM, respectively (Figs. 2E and 3E). The IC_{50} values for UC1019 and UC1020 to decrease $k_{off\text{-}S}$ were 6.0±1.1 μM and 7.1±0.8 μM, respectively (Figs. 2E and 3E).

4. Discussion

Both 3β-OH and 3β-sulfate steroids block $GABA_A$ receptors effectively under conditions that promote channel opening. This suggests that direct antagonism of GABA-site activation may be exemplified by ligand-dependent or state-dependent blockade (Eisenman et al., 2003; Haage et al., 2005; Wang et al., 2007; Shen et al., 2000; Wang et al., 2002). We reported previously that PS-induced inhibition after current peak enhanced the desensitization rate of the GABA-site activation, a property that was not shared by 3β,20(R/S)-pregnandiols (Wang et al., 2007). This disparity prompted us to examine the onset and offset time courses of GABA-site activation as an effort to clarify the mechanism underlying the inhibition of $GABA_A$ receptor by 3β-OH steroids. It is well recognized that the time course of GABA-mediated IPSCs is influenced strongly by the kinetics of the $GABA_A$ receptor desensitization (Celentano and Wong, 1994; Jones and Westbrook, 1995). It is thought that receptors in bound conformations are desensitized to facilitate channel re-opening. During the fast phase of desensitization, probability of the channel opening is limited and its peak synaptic currents are affected. These in turn contribute to the fast component of IPSC decay. The slow phase of IPSC decay is a result of re-opening of GABA channels after they are desensitized (Jones and Westbrook, 1995). Recombinant $GABA_A$ receptors with defined subunits generate also currents with complex decay kinetics (Haas and Macdonald, 1999; Lavoie et al., 1997). Previously we used a GABA subunit with a homologous *serine* mutation at 2′position closest to the cytoplasmic end of the M_2 helix on both α_1 and β_2 subunits (α_{1V256S} and β_{2A252S}). This mutant showed reduced desensitization rate of GABA-site activation at the saturating dose (Wang et al., 2007). The PS-induced inhibition in this mutant combination was also greatly reduced (Wang et al., 2007). Meanwhile, PS has been shown to increase the deactivation rate of the GABA-evoked sIPSCs in a freshly dissociated neuronal model from brain slices recorded on patch clamp (Haage et al., 2005).

In the current study, we confirm the findings that PS increased the fast offset rate of GABA-site activation. The slow component of the offset time course was decreased by PS in a dose-dependent manner. To our knowledge, this is a novel discovery concerning the inhibitory properties of PS on the activation kinetics of $GABA_A$ receptor, thereby giving further insights to the molecular mechanism underlying the blocking effect of sulfated steroids on this receptor. UC1019 and UC1020 have significantly lower potencies on the offset time course of GABA-site activation than PS, in agreement with earlier findings that PS was a significantly stronger inhibitor to both peak and steady-state GABA-currents compared with 3β-OH steroids (Wang et al., 2002; Wang et al., 2007). PS-induced inhibition could be easily seen at low dose of GABA-response ($\leq EC_{20}$) (Eisenman et al., 2003), whereas the inhibition by 3β-OH steroids was only demonstrable at high end ($>EC_{50}$) of the GABA dose–response curve (Wang et al., 2002). Findings that UC1019 and UC1020 prolonged the fast offset time course of GABA-response suggest that the inherent association between 3β,20(R/S)-pregnandiols and receptor is of high affinity nature. However, one cannot fully exclude a multivalent interaction model between hydrophobic steroids and the receptor. Furthermore, based on the fact that EC_{50} of $k_{off\text{-}F}$ for PS did not differ significantly from the IC_{50} of $k_{off\text{-}S}$ for PS, and the IC_{50} values of $k_{off\text{-}F}$ for UC1019 and UC1020 did not differ significantly from that of $k_{off\text{-}S}$, we concluded that the fast and slow phases of offset rates are correlated. It is possible that the offset rate of antagonist steroids on the $GABA_A$ receptor might be determined by the same binding component regulating the rapid state transition.

The effective inhibitory doses of UC1019 and UC1020 on saturated GABA-response were in the low micromolar range, and non-specific effects on the membrane stiffness cannot be excluded at the doses used in the current study. It is possible that certain portions of 3β,20(R/S)-pregnandiols dissociated rapidly, enabling it accessible to membrane portion, whereas the remainder still bound to the receptor. Despite that they are partially attached to the receptor (Shu et al., 2004), the steroids are rendered inactive as a result of their interaction with the

membrane. To explain PS action on the offset time courses of GABA-site activation, we favor a simple model of complete ligand dissociation because the PS molecule is water soluble.

Different mechanisms have been proposed for the decrease in the current deactivation rate at the $GABA_A$ receptor (Eisenman et al., 2003; Haage et al., 2005; Shen et al., 2000). One was based on the idea that the modulator changes the dissociation rate of GABA thereby prolonging the deactivation state (Haage et al., 2005; Jones and Westbrook, 1995). The other was postulated according to the fact that when the receptors are activated by GABA they are buffered to a slow desensitized state that will subsequently lead to a prolongation of the deactivation rate (Zhu and Vicini, 1997). In our study, PS and 3β,20(R/S)-pregnandiols did not significantly influence the fast onset rate of GABA-response. However, their actions on the slow onset rate of GABA-response were different. PS ($\geq$0.3 μM) increased $k_{on\text{-}S}$ significantly, whereas 3β,20(R/S)-pregnandiols dose-dependently decreased $k_{off\text{-}S}$. It remains unclear what functional significance does such differences mean and this is obviously a subject for our follow-up studies. In conclusion, our analysis of kinetic properties of GABA-site activation reveals different patterns of inhibition between sulfated steroids and 3β-OH steroids. We have shown that PS promotes the desensitization rate and increases the fast offset rate of the GABA-site activation. 3β,20(R/S)-pregnandiols decrease the fast offset rate of GABA-response but their effect on desensitization rate differs from that of PS.

Neurosteroids with 3α-OH configuration such as allopregnanolone (3α-OH-5α-pregnan-20-one) and 5α-pregnan-3α,21-diol-20-one (3α5αTHDOC) are potent and effective modulators of $GABA_A$ receptor and have been demonstrated to possess potent anxiolytic activity in several animal anxiety models (Crawley et al., 1986; Reddy and Kulkarni, 2000; Wieland et al., 1995). Allopregnanolone can induce mild cognitive impairment and modulate anxiety and stress levels (Reddy, 2003). A number of recent reports indicate that allopregnanolone induces aggression and anxiety at low concentrations (Fish et al., 2001; Gulinello et al., 2001; Miczek et al., 2003). Therefore, it has been suggested that allopregnanolone may have a biphasic effect (Miczek et al., 2003). At low doses it has an adverse anxiogenic effect while at higher doses it is an anxiolytic agent (Beauchamp et al., 2000; Fish et al., 2001). $GABA_A$ receptor antagonist molecules such as 3β-OH steroids may have potential therapeutic values in treating cognitive impairment from allopregnanolone induced brain disease (Reddy, 2002; Zorumski et al., 2000).

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