Neurosteroid Modulation of Synaptic and GABA-Evoked Currents in Neurons From the Rat Medial Preoptic Nucleus

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Haage, David and Staffan Johansson. Neurosteroid modulation of synaptic and GABA-evoked currents in neurons from the rat medial preoptic nucleus. J. Neurophysiol. 82: 143–151, 1999. The effects of the neurosteroid 3α-hydroxy-5α-pregnane-20-one (allopregnanolone) on synaptic and GABA-evoked currents in acutely dissociated neurons from the medial preoptic nucleus of rat were investigated by perforated-patch recordings under voltage-clamp conditions. The effect of 2.0 μM allopregnanolone on GABA-evoked currents depended strongly on the GABA concentration: the currents evoked by 100 μM GABA were markedly depressed and the desensitization was faster, but the decay after GABA application was prolonged. In contrast, the currents evoked by 1.0 μM GABA were markedly potentiated, the activation was faster, a prominent desensitization was induced, and the decay after GABA application was prolonged. In the absence of externally applied GABA, 2.0 μM allopregnanolone induced a slow current that could be attributed to Cl⁻. Allopregnanolone did not significantly affect the amplitude of spontaneous tetrodotoxin-insensitive (miniature) synaptic currents (mIPSCs) originating from synaptic terminals releasing GABA onto the dissociated neurons. However, the mIPSC decay phase was dramatically prolonged, with half-maximal effect at ~50 nM allopregnanolone. A qualitatively similar effect of allopregnanolone was seen when KCl was used to evoke synchronous GABA release. The frequency of mIPSCs was also affected, on average increased 3.5-fold, by 2.0 μM allopregnanolone, suggesting a presynaptic steroid action.

INTRODUCTION

A number of steroid hormones or their metabolites have been shown to induce rapid effects on the nervous system. These effects may occur on a second or sub-second time scale and are thought to be mediated by membrane receptors, in contrast to the more classical, slower genomic action of steroids. Some of the steroids can be synthesized de novo from cholesterol in the CNS and have been termed neurosteroids (Baulieu and Schumacher 1996). The most potent neurosteroid, 3α-hydroxy-5α-pregnane-20-one (also termed 3α,5α-tetrahydroprogesterone or, as below, allopregnanolone) has been shown to dramatically potentiate the membrane currents caused by γ-aminobutyric acid (GABA) receptor activation (Majewska et al. 1986; Puia et al. 1990; Wu et al. 1990). However, the effects of allopregnanolone on GABA receptors vary within the nervous system (Gee and Lan 1991), and with GABA receptor subunit composition (Belletti et al. 1996; Hauser et al. 1995, 1996; Lan et al. 1991; Shingai et al. 1991). The effect of the steroid may also vary with GABA concentration. Thus allopregnanolone has been reported to potentiate currents evoked by exogenous application of low (50 μM), but not high (500 μM), concentrations of GABA onto cultured pituitary cells (Poisbeau et al. 1997). Similar results have also been reported for pregnanolone, a 5β-stereoisomer of allopregnanolone (Le Foll et al. 1997).

The dependence on GABA concentration raises the question whether the steroids affect GABA receptors activated by synaptically released GABA, which is likely to reach near-millimolar concentrations (see, e.g., Edwards 1995). Recent results on GABA-mediated synaptic currents have not provided a unified answer. Thus no effect on the peak amplitude, but a prolonged decay (which depended on subunit composition) of synaptic currents was reported by Brussaard et al. (1997) (see also Harrison et al. 1987). Similar effects have been reported for pregnanolone (Reith and Sillar 1997). However, in the study by Poisbeau et al. (1997), although presynaptic effects were suggested, miniature synaptic currents were not affected by allopregnanolone, and thus no evidence was found in favor of a postsynaptic action of allopregnanolone during synaptic transmission.

The present study concerns neurons from the preoptic area of rat. The preoptic area is involved in the regulation of sexually related functions, as well as in thermoregulation, slow-wave sleep, and feeding. The majority of gonadotropin releasing hormone (GnRH)-producing cells are located in the preoptic area (see, e.g., Chappel 1985). The involvement of GABA in the regulation of GnRH release has been clearly shown (Leonhardt et al. 1995), and feedback by gonadal steroid hormones is suggested to affect GABA-mediated transmission in the preoptic area (Grattan et al. 1996; Herbison 1997). However, fast steroid effects on membrane currents of preoptic neurons are, to our knowledge, not known, although it has been demonstrated that medial preoptic neurons can generate currents mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, N-methyl-d-aspartate, GABA and glycine receptors (Karlsson et al. 1997a,b).

The present work was carried out to investigate the effect of the neurosteroid allopregnanolone on GABA receptor–mediated currents in neurons from the rat medial preoptic nucleus (MPN). The aim was to characterize the neurosteroid effects on the currents induced by synaptically released, as well as externally applied, GABA. We used a preparation of acutely dissociated medial preoptic neurons, with adherent functional synaptic terminals co-isolated with the neurons (Haage et al. 1998). We report that allopregnanolone affects the time course of GABA-evoked current in a manner that depends on the GABA concentration and results in either depression or potentiation. We further report that the main effect of allopreg-
nanolone on synaptically evoked currents is to prolong the time course of current decay. In some cases allopregnanolone also causes an increased frequency of spontaneous transmitter release. Some of the results have been reported in a preliminary form (Johansson and Haage 1997).

METHODS

Cell preparation

The cells were prepared from hypothalamic brain slices from young male Sprague-Dawley rats, 50–120 g in weight. Ethical approval was given by the local ethics committee for animal research. The procedures for preparation of slices and for mechanical dissociation of cells have been previously described (Johansson et al. 1995; Karlsson et al. 1997b). In short, after decapitation without use of anesthetics, the brain was removed and placed in preoxygenated ice-cold incubation solution (see Solutions) for 5–10 min. A block of tissue containing the preoptic area and anterior hypothalamus was cut out, and coronal slices, 250–300 μm thick, were cut with a vibroslicer (752 M, Campden Instruments, Pangbourne, UK). The slices were incubated for at least 1 h in incubation solution, at 29–31°C or at room temperature (21–23°C), before isolation of cells. The mechanical vibro-dissociation method by Vorobjev (1991) was used after slight modification (cf. Pasternack et al. 1993), and without enzymes. The mechanical vibration was applied by a glass rod (0.5 mm diam), mounted on a piezo-electric bimorph crystal and directed to the site of the medial preoptic nucleus. The dissociated cells were allowed to settle at the bottom of a Petri dish for 10 min. The cell bodies were 10–15 μm at their longest axis, rounded or elongated in shape, typically with two or more 5- to 30-μm-long dendritic processes, but in some cases with longer neurites or without neurites. In a majority of cells, spontaneous synaptic currents indicated the presence of co-isolated functional synaptic terminals (see RESULTS) (Haage et al. 1998).

Electrophysiological recordings

Whole cell patch-clamp (Marty and Neher 1983) recordings were performed by the use of the amphotericin-B perforated-patch technique (Rae et al. 1991). All currents were recorded under voltage-clamp conditions. Borosilicate glass pipettes (GC150, Clark Electromedical Instruments, Pangbourne, UK), with a resistance of 2–7 MΩ when filled with intracellular solution and immersed in extracellular solution (see Solutions), were used. The liquid-junction potential between pipette solution and extracellular solution was measured as described by Neher (1992), and has been subtracted in all potential values given. The signals were recorded using an Axopatch 200A amplifier, a Digidata 1200 interface, and pClamp software (all from Axon Instruments, Foster City, CA) controlled via a 486-processor based personal computer. Recorded signals were low-pass filtered at 2–10 kHz (–3 dB). Series-resistance compensation was not used, due to its introduction of extra noise. However, to avoid experiments with large changes in series resistance, and to evaluate patch integrity, the time course of capacitative current elicited by a −5-mV voltage step was repeatedly monitored during the experiments. Rare occasions of sudden changes in capacitative current resulting in a faster current decay were followed by a gradual increase of the noise level, decrease in membrane resistance, and usually diminished GABA-mediated currents. This was interpreted as patch rupture followed by amphotericin B–mediated effects on cellular membranes. The latter recordings were therefore discarded.

In a majority of experiments, the currents were recorded at a constant membrane potential of −34 mV. At this potential, the driving force for Cl−-mediated currents was reasonably large, whereas additional noise and instability of membrane properties appearing at more positive potentials were avoided.

All solutions, for continuous perfusion as well as for application of test substances, were applied by a gravity-fed fast perfusion system, controlled by solenoid valves operated from the computer. The solution exchange time, as indicated by the current change measured from a patch pipette in alternating extracellular solution and 140 mM KCl, was in good cases <10 ms. All experiments were performed at room temperature (21–23°C).

Solutions

The incubation solution used during the preparation and for storage of slices contained (in mM) 150 NaCl, 5.0 KCl, 2.0 CaCl2, 10 HEPES, 10 glucose, and 4.93 Tris-base. This solution was used supplemented with a gas mixture containing 95% O2-5% CO2. The extracellular solution used during recording of currents contained (in mM) 137 NaCl, 5.0 KCl, 1.0 CaCl2, 10 HEPES, and 10 glucose. Glycine (3 μM) and tetrodotoxin (2 μM; Sigma, St. Louis, MO, or Alomone Labs, Jerusalem, Israel) were routinely added, and pH adjusted to 7.4 with NaOH. When used (see RESULTS), GABA, allopregnanolone (3α-hydroxy-5α-pregnan-20-one), or bicuculline methiodide (all from Sigma) was added to the extracellular solution. The steroid was dissolved in ethanol (max ethanol concentration 0.01%). Control experiments without steroid revealed that ethanol did not significantly affect the recorded parameters. In some experiments, bovine serum albumin (0.01%, wt/vol) was also added, without causing any significant difference in recorded parameters. The intracellular solution, used for filling the pipette, contained (in mM) 140 K-gluconate, 3.0 NaCl, 1.2 MgCl2, 1.0 EGTA, and 10 HEPES; pH was adjusted to 7.2 with KOH, Amphotericin B (Sigma), prepared from a stock solution (6 mg amphotericin B dissolved in 100 μl dimethylsulphoxide), was added to a final concentration of 120 μg amphotericin B per ml intracellular solution.

Analysis

The analysis was performed by the use of pClamp software (see Electrophysiological recordings) and Origin software (version 4.0–4.1, Microcal Software, Northampton, MA). Miniature synaptic currents were detected by visual inspection, and the amplitude and time course of each event were measured semi-manually by using cursors and the curve fitting routines provided by the software. The lower limit for detection was 5 pA. For estimating the number of exponential components, the standard deviation of the difference between recorded data and the theoretical curve obtained by a simplex fitting algorithm was compared. When this standard deviation was reduced <2.0% by an additional component, the latter was not accepted. The same criterion was used for evaluating the time course of current evoked by external GABA application. In all cases, for miniature inhibitory postsynaptic current (mIPSC) decay, GABA-evoked desensitization and deactivation, a majority of events were best described by a single exponential function. The steady leak current has been subtracted in the figures. The continuous line (dose-response curve) in Fig. 4B, relating mIPSC decay time constant (τ) to the allopregnanolone concentration (C), is described by the equation

$$\tau = (\tau_0 - \tau_{max})[1 + (C/K)^p] + \tau_{max}$$

(1)

where $\tau_0$ is the time constant in the absence of allopregnanolone, $\tau_{max}$ is the maximum time constant, $K$ is the half-saturating concentration, and $P$ is an exponent (usually termed Hill coefficient). During the fitting procedure, $\tau_0$ was fixed to the mean value obtained from a large number of events in control solution. The data are presented as means ± SE, unless stated otherwise.

RESULTS

The results presented below were obtained from 93 medial preoptic neurons. As previously described, all neurons tested
respond to application of 100 μM to 1 mM GABA with currents that can be attributed to the activation of GABA<sub>A</sub> receptors (Karlsson et al. 1997a). The currents evoked by GABA show desensitizing components that recover only slowly (time constant 5–10 s) after wash out of GABA. In the present study, therefore, GABA was applied (for 0.32–2.56 s) at intervals of 20–40 s. This resulted in similar responses at repeated applications.

**Effects of perfusion with allopregnanolone on responses to 100 μM GABA**

The current responses to application of 100 μM GABA were recorded at a holding membrane potential of −34 mV. We used 100 μM GABA because this is probably near the concentration reached in central synapses (see Edwards 1995, for review). When GABA was applied, an outward current that reached a peak of 209 ± 34 pA (mean ± SE; 10–90% rise time 111 ± 11 ms; n = 21) was generated (Fig. 1A). The subsequent desensitization in the presence of GABA was examined in 13 cells to which GABA was applied for 2.56 s. The desensitization was best described by a single exponential function (see METHODS), with a time constant (τ<sub>desens</sub>) of 1.7 ± 0.1 s in 10 of the 13 cells. In the remaining three cells, the time constant was too long to be estimated from the 2.56 s of GABA application. The current amplitude after 2.56 s was 52 ± 4% (n = 13) of the peak amplitude. After the end of the GABA application, the current decay (deactivation) to baseline was also best described by a single exponential function, with a time constant (τ<sub>off</sub>) of 694 ± 70 ms (n = 21).

The effect of the steroid allopregnanolone (2.0 μM) was tested after a minimum of 30-s perfusion with allopregnanolone-containing extracellular solution and subsequent application of a test solution that contained 100 μM GABA as well as 2.0 μM allopregnanolone. The evoked current now was markedly depressed (to 54 ± 5% at peak, n = 21) compared with the control situation described above (Fig. 1A). Further, the desensitization was faster in allopregnanolone (time constant, τ<sub>desens</sub>, 1.1 ± 0.1 s; n = 10), although the fraction of the peak amplitude reached after 2.56 s of GABA application was not significantly affected. The decay to baseline current after the end of the test application was, in 20 of 21 cells, prolonged. The time constant (τ<sub>off</sub>) was 1,541 ± 205 ms in the 21 cells; in 10 of the 21 cells the time constant was more than twice as long as in control solution. The relative effects of allopregnanolone on different current parameters are summarized in Fig. 1B. The effects were reversible within 1 min of wash with control solution.

From above, it is thus clear that in MPN neurons allopregnanolone reduces the current during application of 100 μM GABA but prolongs the decay phase after GABA application.

**FIG. 1.** Effects of allopregnanolone on currents evoked by externally applied GABA. A: currents evoked by 100 μM GABA in the absence and presence of 2.0 μM allopregnanolone. Note the reduction in peak amplitude, and the prolongation of current decay (τ<sub>off</sub>) after the GABA application, in the presence of allopregnanolone. B: summary of the relative change induced by 2.0 μM allopregnanolone in current parameters at application of 100 μM GABA as in A. Mean values obtained from the number of neurons indicated within parentheses. Error bars denote SE; “Off/max amplitude” denotes the fraction of the peak amplitude reached after 2.56 s of GABA application; τ<sub>desens</sub> and τ<sub>off</sub> are time constants of desensitization and decay after GABA application, respectively. C: currents evoked by 1.0 μM GABA in the absence and presence of 2.0 μM allopregnanolone. Note the increase in amplitude, the faster rise time, the prominent desensitization, and the prolongation of τ<sub>off</sub>, in the presence of allopregnanolone. D: summary of the relative change induced by 2.0 μM allopregnanolone in current parameters at application of 1.0 μM GABA as in C. Symbols as in B. Holding potential −34 mV in A–D; duration of GABA application marked by bars in A and C.
Effects of perfusion with allopregnanolone on responses to 1 μM GABA

In several previous studies reporting steroid-induced potentiation of GABA responses, lower concentrations of GABA have been used (Hauser et al. 1995; Kokate et al. 1994; Shingai et al. 1991; Wu et al. 1990; Zhang and Jackson 1994). Although the GABA concentration used above in the present study was chosen to be in the near-physiological range, we considered the differences in concentration as a possible reason for the discrepancy between the results above and earlier reports (see also Introduction). We therefore investigated the responses to application of 1.0 μM GABA. When 1.0 μM GABA was applied in control solution (standard extracellular solution, see Methods) at −34 mV, a slowly rising outward current was generated (10–90% rise time, 1,162 ± 150 ms; n = 9), reaching a relatively steady level of 32 ± 8 pA (n = 9). No or a very weak desensitization was seen during a standard application of 2.56 s duration, nor (as tested in 1 cell) during longer applications of nearly 20 s duration. The return to baseline after the end of the GABA application was mono-exponential with a time constant of 398 ± 63 ms (n = 9).

The effect of allopregnanolone was studied as above, after a minimum of 30 s perfusion with allopregnanolone-containing solution. When 1.0 μM GABA was applied in the presence of 2.0 μM allopregnanolone, the results differed dramatically from those described above. The GABA-evoked current was markedly potentiated (201 ± 28% at peak, n = 9) compared with control. The onset of current was more rapid with a distinct peak reached after a rise time (10–90%) of 291 ± 30 ms (n = 9) (Fig. 1C). Thus a prominent desensitization was seen, to 66 ± 6.7% of peak amplitude after 2.56 s of GABA application (n = 9). The time constant of return to baseline after the GABA application, τoff, was prolonged to 707 ± 59 ms (n = 9). The relative effects of allopregnanolone are summarized in Fig. 1D. It was thus clear that the effect of allopregnanolone depended dramatically on the GABA concentration, with a reduction of peak current at 100 μM GABA and a potentiation at 1.0 μM GABA.

Direct effects of allopregnanolone

The results above describe the effects of allopregnanolone on GABA-evoked currents. However, the steroid by itself induced a membrane current, in the absence of applied GABA. Thus, when 2.0 μM allopregnanolone was applied at −34 mV, an outward current was generated (Fig. 2, A and B). The current showed a transient component, with a peak of 32 ± 6 pA (n = 10) reached within 0.2–4.3 s, and a subsequent decay to a steady component of 0–27 pA. (Half-peak amplitude was reached after 10–35 s in 8 cells, but was not reached within 35 s in 2 cells.) The relation between peak current and membrane potential (Fig. 2C, ■) showed some outward rectification, and a reversal potential near −74 mV (2 cells), in similarity with the currents evoked by GABA (Karlsson et al. 1997a). When a high Cl− concentration was used in the pipette solution (140 mM KCl substituted for K-gluconate), the reversal potential was 0 ± 2 mV (n = 3), as expected from a Cl− current (Fig. 2C, ●). The current evoked by allopregnanolone was completely, but reversibly, blocked when the GABA_A receptor blocker bicuculline methiodide (100 μM) was added to the extracellular solution (n = 3; not shown).

The direct effect of allopregnanolone described here may be of importance for interpreting the reduction of GABA-evoked currents described above. It seems possible that part of the allopregnanolone-induced reduction of current in response to 100 μM GABA is due to a number of receptors already being activated or desensitized by allopregnanolone itself (i.e., before GABA application), thus reducing the channel population available for contributing to the response on GABA activation. However, the peak current directly evoked by 2.0 μM allopregnanolone (32 ± 6 pA; n = 10) was considerably smaller than the reduction (96 ± 22 pA; n = 21) of the peak response to 100 μM GABA caused by allopregnanolone. Similarly, when compared in the same cell, the current evoked by

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**FIG. 2.** Direct effect of allopregnanolone. A and B: current, recorded from a neuron at −34 mV, for clarity shown at different time scales in A and B. Allopregnanolone (2.0 μM) was added at the time marked by an arrow, and present for the remainder of the traces shown. C: relation between peak current evoked by 2.0 μM allopregnanolone and membrane potential, with control pipette solution ([(Cl−)] = 5.4 mM; ■) and with a “high Cl−” pipette solution ([(Cl−)] = 145.5 mM; ●).
allopregnanolone showed a peak amplitude that constituted only 29 ± 7% (n = 4) of the reduction of current evoked by 100 μM GABA. Thus the number of channels contributing to the peak response caused by allopregnanolone in the absence of GABA is not sufficient to account for the reduction of current activated by GABA.

Effects of allopregnanolone on spontaneous synaptic currents

From above, it is clear that the effect of allopregnanolone depends on the GABA concentration. It is thus important, for evaluating the physiological role of steroids in the nervous system, to investigate the effects on synaptically released GABA in physiological concentrations. For this purpose, we took advantage of the synaptic boutons that are easily co-isolated with the medial preoptic neurons. These synaptic boutons spontaneously release GABA and thereby give rise to tetrodotoxin-insensitive mIPSCs (Haage et al. 1998). When such mIPSCs were recorded in control solution, at a holding potential of −34 mV (59 cells), they showed a rapid (usually <2 ms) rise phase followed by a monoexponential or doubly exponential decay. The amplitude distribution deviated from a Gaussian, being skewed to the right (Fig. 3A). The peak occurred at 17 pA, and the mean amplitude was 26 pA (±1 pA, n = 1,470; range of mean 9–46 pA for 34 individual cells). In the presence of 2.0 μM allopregnanolone, the mIPSC amplitude distribution (mean amplitude 27 ± 1 pA; n = 1,298) was not significantly different from control (Fig. 3B).

The decay of the mIPSCs was best described by a single exponential function in a majority of cases (64% of 56 events from 29 different cells used for an initial evaluation). Although some mIPSCs were better described by two exponential components according to the criterion used (see METHODS), the improvement was not marked as judged by visual inspection. Thus, to simplify the comparison with mIPSCs recorded in the presence of the steroid (when also ~64% of mIPSCs showed a monoexponential decay), the decay is for all events described in terms of best fitted single exponential function. In control solution, the time constant of the mIPSC decay was 19.1 ± 0.4 ms (599 events pooled from 31 cells; range of mean 8–31 ms for individual cells). In the presence of 2.0 μM allopregnanolone, the time course of decay was considerably prolonged (Fig. 4, A–D); mean time constant was 133 ± 4 ms (n = 165; range of mean 90–181 ms for 15 individual cells).

We investigated the relation between allopregnanolone concentration and the effect on the mIPSC decay. A slight prolongation of the mIPSC decay was observed already at concentrations of 6.3–20 nM, and a near-maximal effect at >200 nM allopregnanolone. Although the effect was less at 2.0 μM than at 200 nM allopregnanolone, thus suggesting a more complex interaction, the data are shown together with a curve described by Eq. 1 (see METHODS) for a comparison. The concentration for half-maximal effect was ~50 nM (Fig. 4B).

Effects of allopregnanolone on mIPSC frequency

In many cases, perfusion with allopregnanolone (200 nM to 2.0 μM) resulted in an increased frequency of mIPSCs (Fig. 4A), thus suggesting a presynaptic steroid action. The effect was evaluated by comparing the mIPSC frequency during 30-s intervals immediately before and immediately after the onset of allopregnanolone perfusion. Although there was a prominent effect in some cases (Figs. 4A and 5A), there was a large variability between repeated allopregnanolone applications in individual cells and also a large variability between cells. The overall effect of 2.0 μM allopregnanolone was to increase the mIPSC frequency by a factor of 3.5 ± 0.8 (n = 36: number of cells). Although not evaluated quantitatively, the effect was often more prominent when there was a low mIPSC frequency in control solution (Fig. 5A), whereas with high control frequency, allopregnanolone sometimes reduced the mIPSC frequency (Fig. 5B).

Effects of allopregnanolone on KCl-evoked synaptic currents

Because the effects of allopregnanolone depended on GABA concentration and included changes in current kinetics, it seemed possible that the effects on evoked transmitter release might differ from the effects on the spontaneous mIPSCs. The effect on mIPSC frequency also suggested that the release probability was affected, which could possibly be reflected in the transmitter release evoked by depolarization. We therefore studied the effects of GABA release evoked by KCl-induced depolarization of the synaptic boutons attached to the medial
preoptic neurons. When the cells with synaptic boutons are transiently perfused with 140 mM KCl, postsynaptic GABA-mediated currents can be recorded under voltage-clamp conditions. At a steady voltage, in a range of 234 mV to 16 mV, these bicuculline-sensitive currents show a major transient outward component that is most likely due to the synchronized exocytosis of GABA from several release sites (Haage et al. 1998). In control experiments without steroid application, the current evoked by KCl usually reached a peak within 30–130 ms and showed a subsequent roughly exponential decay with a time constant of 60 ± 6 ms (n = 58). With allopregnanolone present >30 s before the KCl application, the time course of decay was prolonged [Fig. 6; time constant 362 ± 85 ms (n = 5) in 2.0 μM allopregnanolone; 195 ± 9 ms (n = 3) in 200 nM allopregnanolone]. The amplitude of the KCl-evoked current was not significantly affected, neither by 200 nM nor by 2.0 μM allopregnanolone. The allopregnanolone effect was thus qualitatively similar to the effect on spontaneous mIPSCs, described above.

DISCUSSION

In the present work, the neurosteroid allopregnanolone was shown to dramatically affect the currents evoked by GABA in acutely dissociated neurons of the medial preoptic nucleus. Although these cells are believed to be an important target for steroid actions (see, e.g., Herbison 1997), effects of steroids on membrane currents in preoptic neurons have, to our knowledge, previously not been investigated.

Effect of allopregnanolone depends on GABA concentration

Allopregnanolone was here shown to reduce the currents evoked by 100 μM GABA, but to potentiate those evoked by 1.0 μM GABA. The dependence on GABA concentration suggests that the strong potentiation reported in earlier studies may partly be due to the use of a relatively low GABA concentration. However, at present we cannot exclude that differences in GABA<sub>α</sub> receptor subunit composition partly contributes to the differences in effect of allopregnanolone among the studies reported, since α-, γ-, and ε-subunits have all been reported to influence the effect of allopregnanolone (Belelli et al. 1996; Brussaard et al. 1997; Davies et al. 1997; Hauser et al. 1996; Shingai et al. 1991; see also Smith et al. 1998).

In the present study, allopregnanolone at a concentration of 2.0 μM also induced membrane currents in the complete absence of externally applied GABA. The shift in reversal
strongly affect the time course of GABAA receptor deactivation, and transitions to and from a desensitized state may be seen as an increased duration of closed intervals or as a reduced duration of open intervals in the presence of agonist, which is in disagreement with reported effects of steroids (Twyman and Macdonald 1992; Zhu and Vicini 1997). It is thus clear that different or more complex models are required to account for even the most obvious effects of steroid interaction with the GABA<sub>A</sub> receptor.

Desensitization as mediator of steroid effects?

Not only the peak current amplitude, but also the current kinetics was affected by allopregnanolone. The prolongation of the deactivation, τ<sub>off</sub>, was the most prominent effect seen when 100 μM as well as when 1.0 μM GABA was used. It has been shown that transitions to and from a desensitized state may strongly affect the time course of GABA<sub>A</sub> receptor deactivation (Jones and Westbrook 1995). In accordance with this idea, it was recently suggested that another neurosteroid, 3α,21dihydroxy-5α-pregn-20-one, prolonged GABA<sub>A</sub> receptor deactivation in cerebellar granule cells mainly by reducing the rate of recovery from desensitization (Zhu and Vicini 1997).

Interestingly, in the latter study the steroid also reduced the peak current evoked by (1 mM) GABA. Also this effect was suggested as due to desensitization, of part of the available receptor population as a consequence of preapplication of the steroid (Zhu and Vicini 1997). However, although an increased rate of desensitization was reported in the present study, a similar mechanism could not account for all our results. Thus desensitization of part of the available receptor population would not be expected to cause the increased rate of activation and increased peak current at 1.0 μM GABA as reported here. Neither can effects on desensitization explain the currents evoked by allopregnanolone in the absence of GABA. Further, at the single-channel level, an increased desensitization should be seen as an increased duration of closed intervals or as a reduced duration of open intervals in the presence of agonist, which is in disagreement with reported effects of steroids (Twyman and Macdonald 1992; Zhu and Vicini 1997). It is thus clear that different or more complex models are required to account for even the most obvious effects of steroid interaction with the GABA<sub>A</sub> receptor.

It should further be clear that not only transitions to and from desensitized states are of importance in shaping the "macroscopic" deactivation. Thus, for instance, alterations in rate constants involved in ligand binding alone affect deactivation as well as activation and desensitization in a detailed model of GABA<sub>A</sub> receptor function (Gingrich et al. 1995). There should thus be room for alternative models of neurosteroid interaction with GABA<sub>A</sub> receptors.

Effects of allopregnanolone on synaptic GABA-mediated currents

The results reported above imply that the effect of allopregnanolone on GABA-mediated transmission in the CNS do not only depend on the cell type and receptor composition studied, but also on the concentration of GABA in the synapse. Thus, if the GABA concentration varies in different synapses, as has been suggested for synapses of different cleft volume (Nusser et al. 1997), it seems likely that the allopregnanolone effect will vary even if the GABA receptors are identical. Further, because, as shown above, the current kinetics is dramatically affected by allopregnanolone, differences in time course of

potential with Cl<sup>−</sup> concentration implies that these currents can be attributed to Cl<sup>−</sup> ions, and the effect of bicuculline further suggests that they were due to GABA<sub>A</sub> receptor activation. This is consistent with several earlier studies reporting that allopregnanolone in micromolar concentration may directly activate the GABA<sub>A</sub> receptor (Majewska et al. 1986; Puia et al. 1990; Shingai et al. 1991).

FIG. 5. Effects of allopregnanolone on mIPSC frequency. A and B: frequency of mIPSCs recorded from 2 different postsynaptic neurons. The frequency was measured during 10 consecutive, 30-s long episodes (as indicated) with alternating perfusion with control solution (odd-number episodes; □) and a solution with 2.0 μM allopregnanolone added (even-number episodes; ■). Wash out of allopregnanolone was maintained for at least 1 min before each control episode. Note that there was a systematic increase in mIPSC frequency during allopregnanolone perfusion, for the cell in A, which showed a low frequency in control solution, but a lack of effect or opposite effect on the cell in B, which showed a relatively high frequency in control solution.

FIG. 6. Effects of allopregnanolone on KCl-evoked synaptic currents. Synaptic currents evoked by KCl-induced depolarization of synaptic terminals. KCl (140 mM) was applied at the time indicated by a bar. Current in control solution (bottom trace) and in the presence of 200 nM allopregnanolone (top trace). Note the prolonged current decay during KCl application in the presence of allopregnanolone. (The GABA-mediated currents, with peaks after the onset and end of KCl application, are added to a negative leak current caused by the raised K<sup>+</sup> concentration during KCl application.) Each trace shown is an average of 6 individual raw data traces. Holding potential −34 mV.
GABA concentration at the receptors may be important. It should thus be clear that it is essential, for understanding the modulation caused by allopregnanolone, to study the effects on synaptically released GABA, that is, in a physiological concentration and time course within the synapse.

In contrast to the majority of previous studies of allopregnanolone effects, carried out on cultured cells or on heterologous expression systems, we used an acute preparation with functional synapses and thus had the possibility to study synaptic currents (mIPSCs). The results presented above showed that, in GABAergic synapses on MPN neurons, allopregnanolone did not significantly affect the mIPSC amplitude, but the decay time course was dramatically prolonged. A lack of potentiation of mIPSC amplitude is consistent with the current reduction reported here for exogenous application of 100 μM GABA and recent reports that GABA may reach near millimolar concentrations during synaptic transmission (see Edwards 1995, for review). The prolongation of synaptic currents implies that allopregnanolone will affect the temporal characteristics of synaptic integration. Thus, although a single inhibitory synaptic current will not be larger in amplitude, the prolonged duration implies that temporal summation of inhibitory signals will be favored.

The above analysis of the allopregnanolone-induced prolongation of mIPSC decay demonstrated that allopregnanolone is effective already in concentrations as low as 6.3–20 nM (half-maximal effect at ~50 nM). It has earlier been demonstrated that allopregnanolone concentrations as high as 20 nM may be rapidly (~5 min) reached in the male rat brain after exposure to ambient temperature swim stress (Purdy et al. 1991). It thus seems likely that allopregnanolone effects on neurotransmission similar to those demonstrated in the present work may occur under physiological conditions.

Presynaptic steroid effects

In the present study, allopregnanolone affected not only the time course of mIPSCs but also the frequency of mIPSCs. The mIPSC amplitude distribution, however, was not affected (Fig. 3), implying that the observed increase in frequency was of similar magnitude for mIPSCs of all amplitudes. Thus the effect on mIPSC frequency could not be explained as merely apparent due to increased mIPSC amplitudes, but was interpreted as an effect on the frequency of GABA release due to a presynaptic steroid action. Although the effect was not prominent in all cells, it was dramatic in some cases (Fig. 4A). Previously, not much attention has been paid to the possible presynaptic effects of allopregnanolone. However, recently Poisbeau et al. (1997) reported an allopregnanolone-induced increase in mIPSC frequency in cultured pituitary cells, and a similar effect of pregnanolone in Xenopus embryos was reported by Reith and Sillar (1997). Thus our findings suggest that allopregnanolone may also modulate transmitter release from terminals on MPN neurons in an acute mammalian preparation.

Although our results imply an increased probability of spontaneous transmitter release, we found no significant effect of allopregnanolone on the amplitude of KCl-evoked synaptic current. Thus there is no evidence for steroid-mediated potentiation of the probability of transmitter release that is triggered by depolarization. The functional consequence of the presynaptic steroid action is an increase in “basal” (spontaneous) inhibition that adds to the postsynaptic steroid effect discussed above.

Conclusions

In conclusion, the results presented above demonstrate that the neurosteroid allopregnanolone modulates GABA_A receptors–mediated currents in medial preoptic neurons in a manner that depends critically on GABA concentration and involves kinetic changes of activation/deactivation as well as of desensitization. In functional GABAergic synapses on MPN neurons, allopregnanolone modulates transmission via presynaptic as well as postsynaptic mechanisms. The modulatory effect is not a simple amplification of the synaptic signal, but implies altered temporal characteristics in terms of a prolonged time course of postsynaptic currents and an increased frequency of spontaneous synaptic signals. A full understanding of the precise role of allopregnanolone-induced synaptic modulation for the main functions ascribed to preoptic neurons, regulation of sexual behavior, thermoregulation, and slow-wave sleep, will require further investigation.

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