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ACCELERATED COMMUNICATION

Inverse Modulation of γ-Aminobutyric Acid- and Glycine-Induced Currents by Progesterone

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SUMMARY

The ability of certain synthetic and endogenous steroids to modulate neuronal responses to γ-aminobutyric acid (GABA) is well documented, but little is known of the effect of steroids on glycine responses. We show here that in voltage-clamped neurons progesterone (10–100 μM) itself enhances GABA-induced chloride currents but, surprisingly, antagonizes those induced by glycine. Some, but not all, progesterone metabolites also display these effects. The effects of progesterone on GABA and glycine responses are dose dependent, with EC_{50} values of 26 and 16 μM and maxima of +156 and −60%, respectively. Progesterone and its reduced metabolite 5α-pregnan-3α-ol-20-one potentiate GABA responses by acting through a common site. The site through which progesterone acts to inhibit glycine responses is distinct from the strychnine and glycine binding sites. These results not only provide an important distinction between chloride-mediated GABA and glycine responses but also suggest that endogenous progesterone or its metabolites may differentially modulate the inhibitory actions of these two neurotransmitters.

GABA and glycine, two major inhibitory neurotransmitters in the vertebrate central nervous system, mediate fast synaptic inhibition via the activation of receptor-linked chloride ionophores (1, 2). Receptors for these two neurotransmitters share many properties (3–6) and are considered members of a superfamily of ligand-gated ion channels (7–9). However, the differences in the pharmacology of these channels indicate that the glycine receptor and the $GABA_A$ receptor are separate molecules (10).

Certain synthetic and endogenous steroids have recently been identified as potent modulators at the $GABA_A$ receptor. The anesthetic alphaxalone and some reduced metabolites of progesterone potentiate GABA responses (11–14), whereas pregnenolone sulfate interacts with the $GABA_A$ receptor in an antagonistic fashion (15–17). In contrast, relatively little is known of the effect of steroids on glycine responses.

In the present study, we examine the effects of steroids on currents induced by GABA and glycine in primary cultures of voltage-clamped chick spinal cord neurons, and we report that progesterone itself is a positive modulator of GABA responses and is also a negative modulator of glycine responses.

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Materials and Methods

Cell cultures. Cultures of spinal cord neurons were prepared from 7-day chick embryos as previously described (18), with minor modifications. Briefly, dissociated cells were plated on collagen-coated 35-mm tissue culture dishes in Eagle's minimum essential medium supplemented with 2.4 mM glutamine, 10% (v/v) heat-inactivated horse serum, 5% (v/v) chick embryo extract, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cultures were maintained at 37° in an atmosphere of 5% CO_2/95% air. Cytosine arabinoside (0.1 μM) was added after 36 hr to control the proliferation of nonneuronal cells. This medium was removed 1 day later and replaced with a similar medium supplemented with 20.5 mM glucose and 18 mM KCl and containing only 2.5% chick embryo extract. Fresh medium was added twice weekly. Cultured neurons were used in experiments 2–4 weeks after plating.

Electrophysiology. Experiments were carried out in 35-mm tissue culture dishes on the stage of an inverted phase-contrast microscope. Whole-cell currents were recorded by the whole-cell variant of the patch-clamp technique (19). Patch electrodes were fabricated from thin-wall borosilicate glass microcapillary pipettes (Fischer) with a double pull on a David Kopf vertical pipette puller (model 700D). Electrode resistance was 5.1 ± 0.08 MΩ (n = 160) when filled with intracellular solution. Unless otherwise indicated, the pipette solution contained (in mM) 140 KCl, 3 NaCl, 1 $MgCl_2$, 11 EGTA, and 10 HEPES (pH adjusted to 7.2 with KOH). The bath solution contained (in mM) 150 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES (pH adjusted to 7.2 with NaOH). All experiments were performed at room temperature (23–25°).

ABBREVIATIONS: GABA, γ-aminobutyric acid; EGTA, ethylene glycolbis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; 3α-OH-DHP, 5α-pregnan-3α-ol-20-one.

Recordings were made using a Yale MK V amplifier. After a tight seal was formed (typically 1–10 GΩ), capacitance transients were minimized. The patch of membrane under the pipette tip was then ruptured by gentle suction to obtain the whole-cell configuration. Cells with series resistance greater than 10 MΩ were rejected. Series resistance, which initially measured 7.1 ± 0.09 MΩ (n = 160), was compensated (>60%). Only cells with resting membrane potential greater than −55 mV and input resistance in excess of 150 MΩ were used. All recordings were made with the cell membrane potential clamped at −70 mV. Currents were filtered at 1 kHz using an eight-pole Bessel filter and were digitized (40 msec/point) using an on-line acquisition system (pClamp; Axon Instruments).

Drug solutions were applied to single neurons by pressure ejection (15 psi) from seven-barrel pipettes. Pipettes were pulled from Omega dot tubing to a tip diameter of about 1 μm/barrel and were broken back to about 3–5 μm/barrel after filling. Seven-barrel pressure pipettes were positioned approximately 50 μm from the neuronal soma. Under these conditions, we have established that the drug solution in the pressure pipette rapidly and effectively replaces the solution surrounding the target neuron, with less than 10% dilution (20–22). All drugs used in electrophysiological experiments were obtained from Sigma, with the exception of pregnenolone sodium sulfate, which was purchased from Steraloids. Stock solutions of steroids and strychnine were prepared in dimethyl sulfoxide, the final concentration of which was 0.5% (v/v). To obviate the possible effect of dimethyl sulfoxide on the relevant agonist-induced currents, all other drug solutions, including GABA, glycine, and external buffer (in the pressure pipette), also contained 0.5% dimethyl sulfoxide. In all experiments, neurons received a prepulse of either external buffer solution or drug solution for 10 sec before each agonist application, the duration of which was 10–15 sec. Immediately following each agonist application, neurons were washed with external buffer solution for 10–20 sec. A period of 2 min was allowed between each agonist application.

The degree of modulation of GABA and glycine responses by steroids, the percentage of change, was defined as $(I'/I - 1) \times 100\%$, where I and I' were, respectively, the agonist-induced current responses in the absence and presence of steroids. I was the average of control responses obtained before and after the agonist response in the presence of modulator(s). In all cases complete, or nearly complete, recovery was obtained. Throughout, results are expressed as mean ± standard error; statistical comparison of groups was carried out using Student's t test.

Results and Discussion

At −70 mV, both GABA and glycine produced inward currents, which reversed at or near 0 mV in symmetrical Cl^- solutions, as expected for Cl^--mediated currents. GABA-induced currents were blocked by bicuculline and SR-95531 and potentiated by chlordiazepoxide (not shown), consistent with the activation of $GABA_A$ receptors. Glycine responses were antagonized by nanomolar concentrations of strychnine (see below), indicating that strychnine-sensitive receptors were involved in these responses. In agreement with our previous work (23), current responses to 3 μM GABA (233 ± 16 pA, n = 65) showed little or no desensitization and did not decline with repeated GABA applications. Currents evoked by 50 μM glycine rapidly reached a peak (1363 ± 73 pA, n = 103) and slowly desensitized during the period of glycine application. Unlike 30 μM GABA-elicited responses (23), responses to 50 μM glycine did not show run-down.

The effects of progesterone on currents induced by 3 μM GABA and 50 μM glycine are illustrated in Fig. 1. Pressure application of 100 μM progesterone rapidly and reversibly potentiated the GABA response (Fig. 1A) but reduced the glycine response (Fig. 1B). Progesterone alone produced little or no direct response. These effects of progesterone were also observed when both GABA and glycine were tested in sequence on the same neuron. It should be noted here that, in order to prevent the inhibitory effect from decaying during application of the test glycine pulse, it was necessary for progesterone to be present in the glycine pipette as well as in the modulator pipette. In contrast, it was unnecessary for progesterone to be included in the GABA pipette, because the potentiating effect

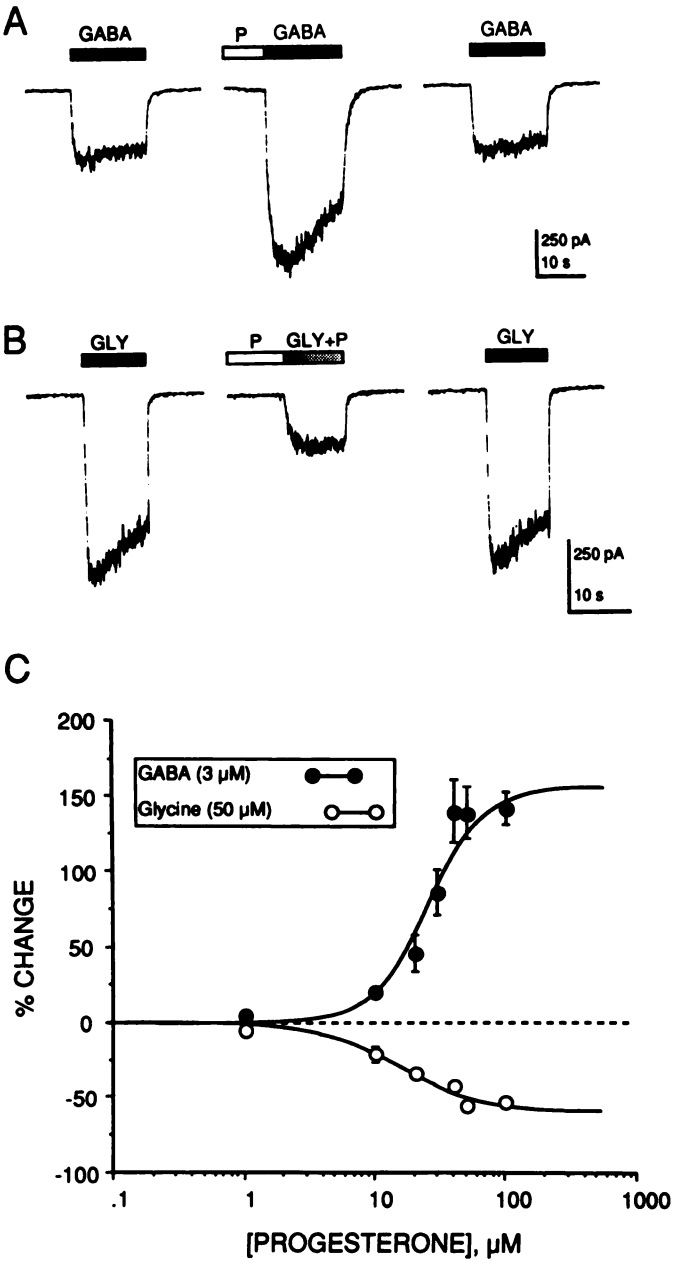


Fig. 1. Progesterone produces opposite effects on whole cell current responses induced by GABA and glycine. Holding potential, −70 mV. A, The potentiating effect of progesterone (*P*) on the current induced by GABA. Progesterone (100 μM) reversibly enhanced the GABA (3 μM) response. B, In a different cell, the current induced by 50 μM glycine (*GLY*) was reversibly antagonized by 100 μM progesterone. *Horizontal bar* above each trace, period of drug application. C, Dose-response curves for progesterone. *Data points*, percentage of change in peak current, mean of 4–18 experiments. *Error bars*, standard errors. Each set of data points is fitted by nonlinear regression to the logistic equation (31). For GABA, EC_{50} = 25.5 μM, n_H = 2.07, and the maximum potentiation = 155.8%. For glycine, EC_{50} = 15.6 μM, n_H = 1.42, and the maximum inhibition = 59.6%.

did not decline during application of the test GABA pulse. The effects of progesterone on GABA and glycine responses were evaluated over the concentration range 1–100 μM and were found to be dose dependent, with EC_{50} values of 26 and 16 μM, maxima of +156 and −60%, and Hill coefficients of 2.1 and 1.4, respectively (Fig. 1C). The threshold concentration for an effect of progesterone on GABA- and glycine-induced currents was 10 μM in both cases. Maximal effects on GABA and glycine responses were achieved at approximately 50 μM progesterone.

The reduced metabolites of progesterone, 5β-pregnan-3α-ol-20-one and 3α-OH-DHP, have recently been shown to be potent positive modulators of GABA-induced currents (12, 14). Our results (Fig. 1C) indicate that progesterone exerts similar potentiating actions on the $GABA_A$ receptor, but with less potency and efficacy. In order to examine the possibility that progesterone and 3α-OH-DHP act through a common regulatory site on the $GABA_A$ receptor, the effect of their combined action on GABA-induced currents was determined. In this experiment, we used maximal concentrations of progesterone and 3α-OH-DHP, which were 100 and 1 μM, respectively. The enhancing effects of progesterone and 3α-OH-DHP on current responses evoked by 3 μM GABA were not additive (Fig. 2A). In 7 cells, the average potentiation produced by progesterone and 3α-OH-DHP was 142 ± 22% and 1373 ± 225%, respectively. In the presence of progesterone, potentiation by 3α-OH-DHP was significantly reduced to 470 ± 66%, presumably due to the displacement of 3α-OH-DHP by the less efficacious progesterone (Fig. 2B). These results are not easily explained by a nonspecific mechanism of action, such as perturbation of the lipid bilayer (24), but argue instead that progesterone and 3α-OH-DHP potentiate GABA responses by acting through a common, specific, saturable site at which progesterone is a partial agonist.

Responses induced by glycine have been demonstrated to be preferentially blocked by the convulsive alkaloid strychnine (25). Our data (Fig. 1C) reveal that progesterone also antagonizes glycine-evoked currents, but with less potency and efficacy. To investigate the possibility that progesterone and strychnine may act through a common site on the glycine receptor to inhibit glycine responses, we determined the effect of coapplication of these two compounds. As expected, 50 nM strychnine readily and reversibly blocked currents elicited by 50 μM glycine (Fig. 3A). The average inhibition produced by strychnine was 68 ± 3.3% ($n = 10$). A maximal concentration of progesterone (100 μM) reversibly antagonized glycine-induced currents, and subsequent addition of 50 nM strychnine further blocked glycine responses (Fig. 3B). The inhibitory effects of progesterone and strychnine on glycine-evoked currents were additive. In 9 cells, the average inhibition produced by progesterone was 55 ± 3.8%. In the presence of strychnine, inhibition produced by progesterone was increased to 87 ± 1.1%, which was significantly greater than that produced by strychnine alone ($p < 0.001$, unpaired t test). These results indicate that progesterone blocks glycine-activated currents by acting through a site distinct from the strychnine binding site.

Furthermore, the percentage of inhibition of glycine-induced

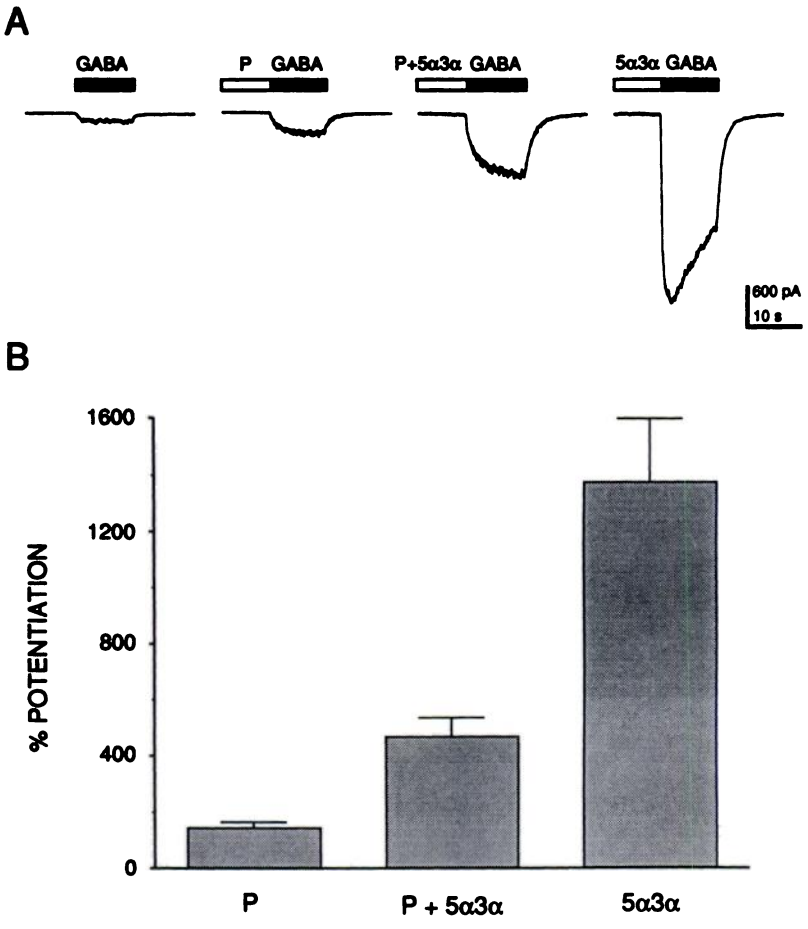


Fig. 2. Progesterone and 3α-OH-DHP act through the same site to potentiate current responses evoked by GABA. Holding potential, −70 mV. A, Current responses from a single cell. Application of GABA (3 μM) was repeated following 10-sec prepulses of 100 μM progesterone (*P*), 100 μM progesterone and 1 μM 3α-OH-DHP in combination (*P+5α3α*), or 1 μM 3α-OH-DHP (*5α3α*). The potentiating effects of progesterone and 3α-OH-DHP on peak current responses evoked by GABA were not additive. *Horizontal bar* above each trace, period of drug application. B, Pooled results from seven cells. *Error bars*, standard errors. Progesterone significantly reduced the potentiation produced by 3α-OH-DHP ($p < 0.005$, paired t test).

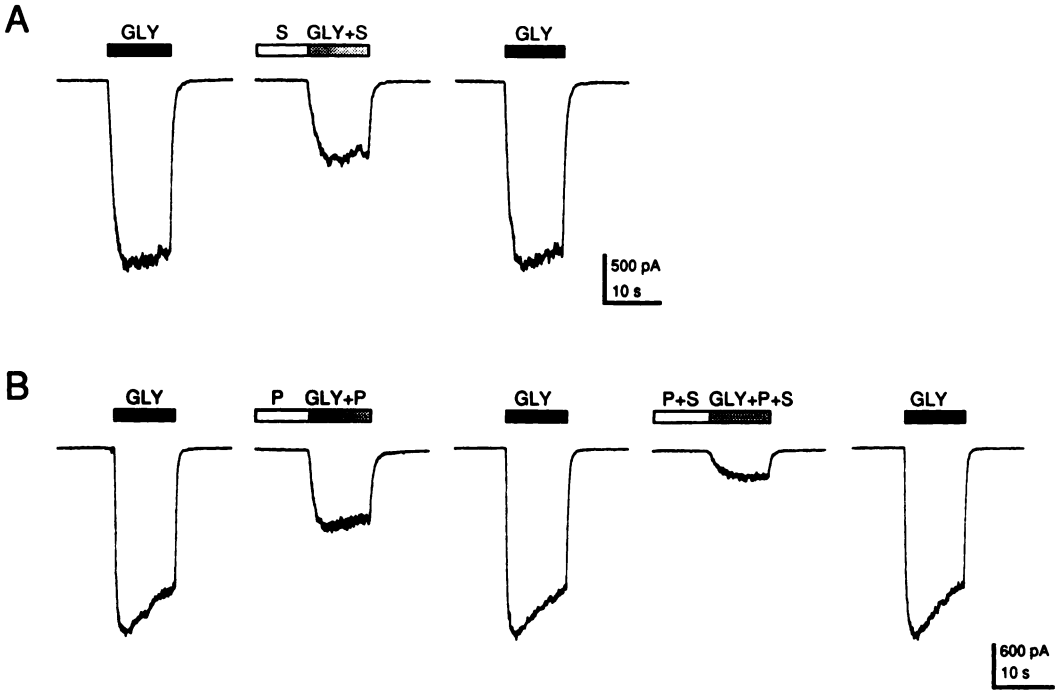


Fig. 3. Progesterone and strychnine inhibit glycine-activated currents by acting through different sites. Holding potential, −70 mV. A, The peak current response activated by 50 μM glycine (*GLY*) was reversibly blocked by 50 nM strychnine (*S*). B, In a different cell, a maximum concentration (100 μM) of progesterone (*P*) reversibly reduced the current activated by 50 μM glycine and subsequent addition of 50 nM strychnine (*P+S*) further reduced the glycine response. *Horizontal bar* above each trace, period of drug application.

currents by progesterone was not reduced by increasing glycine to 1 mM. Because 1 mM is a saturating concentration of glycine (26), this suggests that the antagonism of glycine responses by progesterone is noncompetitive in nature and that progesterone inhibits glycine responses by acting through a site different from the glycine recognition site. In this experiment, we used a low Cl^- (20 mM) pipette solution to reduce the amplitude of the current induced by 1 mM glycine when the cell was held at −70 mV. This solution contained (in mM) 130 potassium gluconate, 20 KCl, 3 sodium gluconate, 11 EGTA, and 10 HEPES (pH adjusted to 7.2 with KOH). Under these conditions, 100 μM progesterone blocked 1 mM glycine-induced currents by 80 ± 3.4% (n = 6). The average inhibition of 300 μM glycine-induced currents produced by 100 μM progesterone was 75 ± 1.9% (n = 3).

We also examined the effects of a variety of other chemically related steroids, including pregnenolone sulfate and some metabolites of progesterone (Table 1). Deoxycorticosterone, with a hydroxyl at C-21, had activity similar to that of progesterone, whereas 17α-hydroxylprogesterone, with a hydroxyl at C-17α, produced lesser effects. Corticosterone, which differs from deoxycorticosterone by the presence of a hydroxyl group at C-11, had weaker activity. Hydrocortisone, with three hydroxyl groups at C-11, C-17α, and C-21, was totally inactive. Thus, addition of a C-21 hydroxyl to the progesterone structure does not change its effects on GABA and glycine responses, whereas addition of hydroxyl group(s) at C-11 or C-17α results in reduction or complete loss of activity. Consistent with previous findings (12, 14), 3α-OH-DHP (1 μM) dramatically potentiated GABA-induced currents but produced little effect on glycine responses. Its stereoisomer, 5α-pregnan-3β-ol-20-one (20 μM), was without effect on both GABA and glycine responses, confirming that the interaction of steroids with the $GABA_A$ receptor is stereospecific (12, 14). These results also suggest that structure-activity requirements for steroid interaction with the $GABA_A$ receptor and the glycine receptor are different. The endogenous neurosteroid pregnenolone sulfate has recently been shown to antagonize $GABA_A$ receptor-mediated responses, presumably by acting through the picrotoxin site (15–17). We found that pregnenolone sulfate (100 μM) blocked both GABA- and glycine-induced currents to the same extent. Although progesterone and pregnenolone sulfate produced opposite effects on GABA responses, both compounds exerted inhibitory effects on glycine responses, the latter being more efficacious.

These experiments demonstrate that progesterone and deoxycorticosterone potentiate GABA responses at the 3α-OH-DHP recognition site and noncompetitively inhibit glycine responses by operating through a site distinct from the strychnine binding site. In view of the fact that GABA- and glycine-activated chloride channels share many properties (3–6) and exhibit close structural and amino acid sequence homology (7–9), the opposite effects of these steroids are intriguing. In particular, the inverse modulation of two such functionally similar receptor systems by a single endogenous compound suggests the possible existence of a hitherto unrecognized form of neuromodulation.

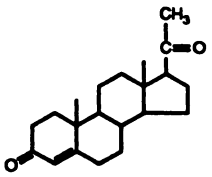
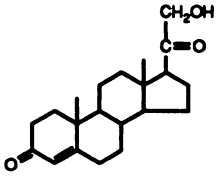
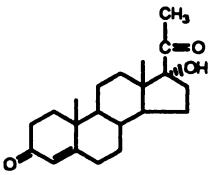
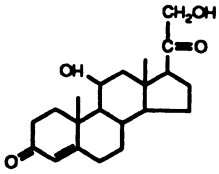
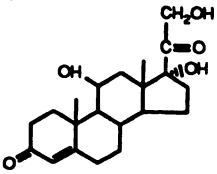
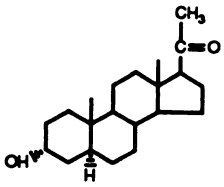
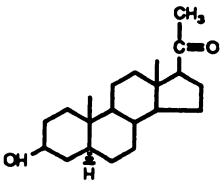
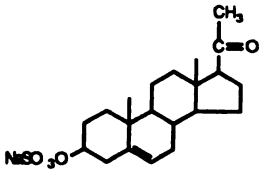
The precise physiological significance of progesterone and deoxycorticosterone as modulators of inhibitory neurotransmission is unknown. However, the ability of steroid hormones to profoundly influence brain excitability is well documented (27, 28). Peripheral administration of progesterone or deoxycorticosterone produces sedative and anesthetic effects in mammals (29), thought to be due to the potentiating effect of their reduced metabolites on GABA responses (12, 14). The observation that progesterone and deoxycorticosterone at higher concentrations are able to potentiate GABA responses implies that these steroids may also contribute directly to central depression.

Concentrations of steroid hormones in plasma may be sufficient to cause significant effects on GABA and glycine re-

TABLE 1

Effects of steroids on currents evoked by GABA and glycine

Holding potential was −70 mV. Concentrations of all steroids were 100 μM, with the exception of 3α-OH-DHP and 5α-pregnan-3β-ol-20-one, which were 1 and 20 μM, respectively. Values are means ± standard errors. Number of cells is indicated in parentheses.

Steroid	Structure	Change of response	
		3 μM GABA	50 μM Glycine
		%	
Progesterone		+142 ± 10.6 (18)	−53 ± 2.5 (18)
Deoxycorticosterone		+131 ± 22.0 (6)	−51 ± 3.0 (5)
17α-OH-progesterone		+67 ± 5.5 (6)	−32 ± 3.2 (6)
Corticosterone		+60 ± 12.0 (7)	−23 ± 2.8 (6)
Hydrocortisone		0 ± 2.8 (5)	+1 ± 4.2 (5)
3α-OH-DHP		+1373 ± 225 (7)	+2 ± 2.4 (5)
5α-Pregnan-3β-ol-20-one		+4 ± 3.1 (5)	0 ± 1.5 (6)
Pregnenolone sodium sulfate		−91 ± 1.8 (6)	−91 ± 1.9 (6)

sponses. For example, progesterone concentrations in rat plasma range from 6 μM during late proestrus of the estrous cycle to about 20 μM in late pregnancy (30), the latter being close to our EC_{50} values for progesterone effects on GABA and glycine responses. Because of the high lipophilicity of progesterone and its possible localized accumulation in the central nervous system, it is possible that progesterone under normal physiological conditions may interact with both $GABA_A$ and glycine receptors in the mammalian central nervous system.

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References

1. Coombs, J. S., J. C. Eccles, and P. Fatt. The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol. (Lond.)* **130**:326–373 (1955).
2. Krnjevic, K. Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.* **54**:418–540 (1974).
3. Barker, J. L., and R. N. McBurney. GABA and glycine may share the same conductance channel on cultured mammalian neurons. *Nature (Lond.)* **277**:234–236 (1979).
4. Faber, D. S., and H. Korn. Single-shot channel activation accounts for duration of inhibitory postsynaptic potentials in a central neuron. *Science (Wash. D. C.)* **208**:612–615 (1980).
5. Graham, D., F. Pfeiffer, and H. Betz. Avermectin B1a inhibits the binding of strychnine to the glycine receptor of rat spinal cord. *Neurosci. Lett.* **29**:173–176 (1982).
6. Bormann, J., O. P. Hamill, and B. Sakmann. Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurones. *J. Physiol. (Lond.)* **385**:243–286 (1987).
7. Grenningloh, G., A. Rienitz, B. Schmitt, C. Methfessel, M. Zensen, K. Beyreuther, E. D. Gundelfinger, and H. Betz. The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature (Lond.)* **328**:215–220 (1987).
8. Schofield, P. R., M. G. Darlison, N. Fujita, D. R. Burt, F. A. Stephenson, H. Rodriguez, L. M. Rhee, J. Ramachandran, V. Reale, T. A. Glencorse, P. H. Seeburg, and E. A. Barnard. Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. *Nature (Lond.)* **328**:221–227 (1987).
9. Barnard, E. A., M. G. Darlison, and P. Seeberg. Molecular biology of the $GABA_A$ receptor: the receptor/channel superfamily. *Trends Neurosci.* **10**:502–509 (1987).
10. Betz, H. Biology and structure of the mammalian glycine receptor. *Trends Neurosci.* **10**:113–117 (1987).
11. Harrison, N. L., and M. A. Simmonds. Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res.* **323**:287–292 (1984).
12. Majewska, M. D., N. L. Harrison, R. D. Schwartz, J. L. Barker, and S. M. Paul. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science (Wash. D. C.)* **232**:1004–1007 (1986).
13. Barker, J. L., N. L. Harrison, G. D. Lange, and D. G. Owen. Potentiation of γ-aminobutyric acid-activated chloride conductance by a steroid anesthetic in cultured rat spinal neurons. *J. Physiol. (Lond.)* **386**:485–501 (1987).
14. Harrison, N. L., M. D. Majewska, J. W. Harrington, and J. L. Barker. Structure-activity relationships for steroid interaction with the γ-aminobutyric $acid_A$ receptor complex. *J. Pharmacol. Exp. Ther.* **241**:346–353 (1987).
15. Majewska, M. D., and R. D. Schwartz. Pregnenolone-sulfate: an endogenous antagonist of the γ-aminobutyric acid receptor complex in brain? *Brain Res.* **404**:355–360 (1987).
16. Majewska, M. D., J.-M. Mienville, and S. Vicini. Neurosteroid pregnenolone sulfate antagonizes electrophysiological responses to GABA in neurons. *Neurosci. Lett.* **90**:279–284 (1988).
17. Mienville, J.-M., and S. Vicini. Pregnenolone sulfate antagonizes $GABA_A$ receptor-mediated currents via a reduction of channel opening frequency. *Brain Res.* **489**:190–194 (1989).
18. Farb, D. H., D. K. Berg, and G. D. Fischbach. Uptake and release of [^{3}H]-*gamma*-aminobutyric acid by embryonic spinal cord neurons in dissociated cell culture. *J. Cell Biol.* **80**:651–661 (1979).
19. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* **391**:85–100 (1981).
20. Choi, D. W., D. H. Farb, and G. D. Fischbach. Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures. *Nature (Lond.)* **269**:342–344 (1977).
21. Chan, C. Y., T. T. Gibbs, L. A. Borden, and D. H. Farb. Multiple embryonic benzodiazepine binding sites: evidence for functionality. *Life Sci.* **33**:2061–2069 (1983).
22. Chan, C. Y., and D. H. Farb. Modulation of neurotransmitter action: control of the γ-aminobutyric acid response through the benzodiazepine receptor. *J. Neurosci.* **9**:2365–2373 (1985).
23. Gyenes, M., M. Farrant, and D. H. Farb. "Run-down" of γ-aminobutyric $acid_A$ receptor function during whole-cell recording: a possible role for phosphorylation. *Mol. Pharmacol.* **34**:719–723 (1988).
24. Fesik, S. W., and A. Makriyannis. Geometric requirements for membrane perturbation and anesthetic activity: conformational analysis of alphaxalone and Δ^{16}-alphaxalone and ^{2}H-NMR studies on their interactions with model membranes. *Mol. Pharmacol.* **27**:624–629 (1985).
25. Curtis, D. R., L. Hosli, and G. A. R. Johnston. A pharmacological study of the depression of spinal neurones by glycine and related amino acids. *Exp. Brain Res.* **6**:1–18 (1968).
26. Tokutomi, N., M. Kaneda, and N. Akaike. What confers specificity on glycine for its receptor site? *Br. J. Pharmacol.* **97**:353–360 (1989).
27. Pfaff, D. W., and B. S. McEwen. Actions of estrogens and progestins on nerve cells. *Science (Wash. D. C.)* **219**:808–814 (1983).
28. Majewska, M. D. Steroids and brain activity: essential dialogue between body and mind. *Biochem. Pharmacol.* **36**:3781–3788 (1987).
29. Selye, H. Anesthetic effects of steroid hormones. *Proc. Soc. Exp. Biol. Med.* **46**:116–121 (1941).
30. Ichikawa, S., T. Sawada, Y. Nakamura, and H. Morioka. Ovarian secretion of pregnane compounds during the estrous cycle and pregnancy in rats. *Endocrinology* **94**:1615–1620 (1974).
31. Hooke, R., and T. A. Jeeves. "Direct search" solution of numerical and statistical problems. *J. Ass. Comput. Mach.* **8**:212–229 (1961).

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