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Sex steroid effects on extrahypothalamic CNS. II. Progesterone, alone and in combination with estrogen, modulates cerebellar responses to amino acid neurotransmitters

Sheryl S. Smith, Barry D. Waterhouse and Donald J. Woodward*

Department of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102-1192 (U.S.A.)

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In a preliminary report we have shown that both intravenous and local application of progesterone (P) are capable of increasing cerebellar Purkinje cell responsiveness to microiontophoretically applied γ-aminobutyric acid (GABA) and decreasing responsiveness to glutamate (GLUT) in the urethane-anesthetized, ovariectomized adult rat. In the present study we have examined the time course of effects of several doses of P and different combinations of both E_2 and P on responses of individual Purkinje cells to GABA and GLUT. Extracellular activity of single Purkinje neurons was recorded using multibarrel glass micropipets. Spontaneous firing rate and responses of neurons to microiontophoretic pulses (10 s pulses every 40 s) of GABA (10–50 nA) and GLUT (3–40 nA) were examined before and after jugular i.v. administration of P or E_2/P combinations to ovariectomized rats. In some cases animals received s.c. injections of E_2 (2 μg) at 24 and 48 h before the day of recording. This injection schedule results in maximal reproductive effects of P. Within 5–15 min after P administration (5,50 or 500 μg) to ovariectomized rats, Purkinje cell responses to GLUT were decreased by 87%, and inhibitory responses to GABA were increased by 50%, with no associated change in spontaneous firing rate. In addition, the magnitude of the change in amino acid response was directly proportional to the dose of P. In most cases, complete recovery was observed 20–45 min after P administration. E_2 pretreatment did not alter these P-induced effects. Combinations of E_2 (300 ng/kg) and P (50 or 500 μg) injected simultaneously resulted in effects on GLUT responsiveness which were similar to those seen with P alone, while effects similar to E_2 alone were observed with administration of E_2 plus P at 5 μg. The administration of a protein synthesis inhibitor, anisomycin (30 mg/kg, i.v.), 20 min before the recording session did not prevent any of the above steroid effects. These results indicate that sex steroids can act to alter neuronal responsiveness to putative neurotransmitters in a CNS region not known to contain steroid receptors and that the particular combination of steroids will determine the neuronal response. These findings further suggest that the observed steroid-induced alterations in Purkinje cell responsiveness do not appear to require genomic mechanisms.

INTRODUCTION

In the preceding paper, we have presented evidence that estrogen augments cerebellar Purkinje cell responsiveness to microiontophoretically applied glutamate (GLUT)[30]. This effect was shown to be specific for the β-isomer of the steroid and not dependent upon classic receptor interactions.

In the present study, another sex steroid, progesterone (P), was tested for its effect on Purkinje cell sensitivity to amino acid neurotransmitters. It is well known that large doses of P (>60 mg/kg) possess anesthetic effects[27]. However, at physiologic levels, P exerts both anticonvulsant[22] and anxiolytic effects in humans[13] and animals[26]. We have previously reported in a preliminary study that both systemic and local application of P, at physiological doses, augment cerebellar Purkinje cell responsiveness to iontophoretically applied γ-aminobutyric acid (GABA) and reduce neuronal responses to GLUT[28]. These al-

* Present address: Dept. of Cell Biology and Anatomy, Univ. of Texas Health Science Center, Dallas, TX 75235, U.S.A.
Correspondence: S.S. Smith, Department of Physiology and Biophysics, Hahnemann University, Broad and Vine, Philadelphia, PA 19102-1192, U.S.A.

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terations in GABA and GLUT function, in a global sense, would be consistent with the functional parameters influenced by the steroid as described above.

Although the hypothalamo-pituitary-gonadal axis is considered a primary target for this sex steroid, moderate uptake of P and its metabolites in the cerebellum subsequent to P administration has been shown both in vivo[14] and in vitro[11]. In addition, P has been demonstrated to increase GABA receptor binding in cerebellar tissue[19].

In the present study, we have evaluated the time course of dose-related P-induced changes in background discharge and the neuronal responses to GABA and GLUT. In addition, the effect of combined E_2/P administration on neurotransmitter responsiveness has been evaluated, because these steroids exert opposite effects on neuronal responsiveness to GLUT[28,30]. Neuromodulatory effects of both steroids have also been tested after administration of a protein synthesis inhibitor.

MATERIALS AND METHODS

Animals

Experiments were performed on adult (200–300 g) Sprague–Dawley female rats, ovariectomized 3–6 weeks. Animals were housed in groups of 5–6 in cages supplied with rat chow and tap water. The light–dark cycle (lights on 05.00–19.00 h) and temperature (23–25 °C) were consistently regulated.

Surgery

On the day of the experiment, animals were surgically prepared as previously described[30]. Briefly, anesthesia was initially achieved with halothane (0.75–2.5% in air) and then with urethane (1.2 mg/kg, i.p.). Jugular cannulations were performed using Silastic tubing[12], followed by stereotaxic surgery to expose the caudal aspect of the anterior lobe and much of the posterior lobe of the cerebellum. Body temperature was monitored by means of a rectal probe and maintained at 36–37 °C with a heating pad.

Electrophysiology

Single unit recordings of Purkinje cells were obtained from the vermis and adjacent paravermal area, lobules IV–VIII. Five-barrel glass micropipets with 5–8 μm tips were used for recording extracellular action potentials and for applying drugs at the recording site by microiontophoresis. The central barrel, filled with 3 M NaCl, was used for recording. Side barrels were filled by diffusion with solutions of γ-aminobutyric acid (GABA; 1.0 M, pH 4.0, Sigma) or sodium L-glutamate (GLUT; 1.0 M, pH 8.0, Sigma). Drug solutions were ejected as cations or anions and retained by application of 15 nA currents of opposite polarity[9]. Automatic current balancing was maintained through a fourth peripheral barrel containing 3 M NaCl. Positive and negative currents passed through this barrel were used to check for possible current artifacts.

Action potentials of Purkinje cells, identified by their characteristic discharge pattern of single and complex spikes[7], were monitored on an oscilloscope and converted to uniform voltage pulses by a window discriminator. Peristimulus drug histograms summing unit responses during regularly repeated pulses of transmitter substances were used to compute the average agonist response to the neurotransmitter. To quantitate the agonist response, the change in discharge rate during amino acid application was compared with the rate between amino acid pulses and the difference expressed as percentage inhibition or excitation, accordingly. To avoid artifacts due to variation in neuronal activity, all histograms were computed when spontaneous activity was steady. When the control response to an amino acid was determined, progesterone (P; 5, 50 or 500 μg), alone or in combination with 17 β-estradiol (E_2; 300 ng/kg) was infused intravenously (steroid vehicle: 0.01% propylene glycol in saline). Some rats received injections of vehicle only. Cells were generally held for at least 1.5–2 h in order to generate a control and a series of drug effect histograms over time.

Data analysis

The Unit program (written by Dr. J.K. Chapin, Philadelphia) was used to generate a series of histograms with which to compare firing rate during amino acid pulses (evoked responses) and between pulses (spontaneous discharge) before and after steroid administration. Differential changes in putative transmitter-induced and spontaneous activity resulting from hormonal administration were assessed by comparing discharge in identical portions of con-

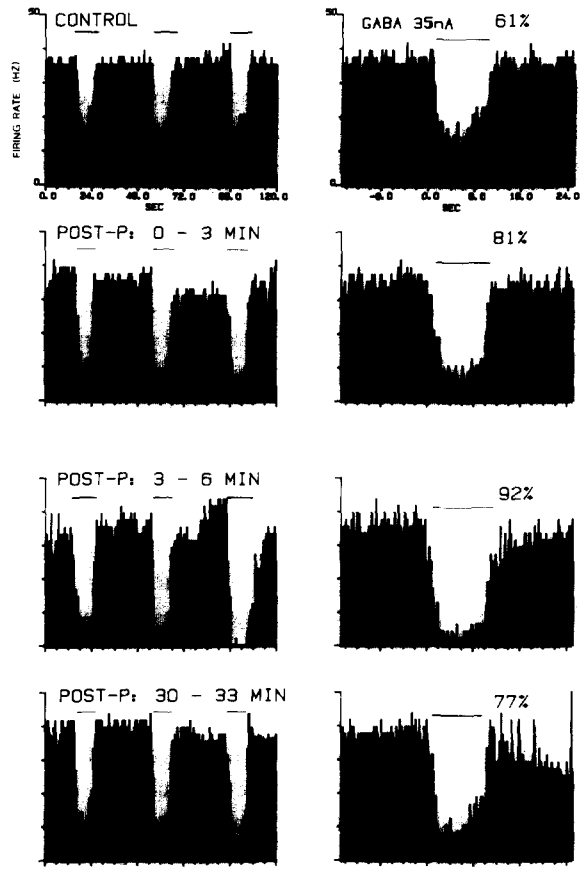

Fig. 1. I.v. administration of progesterone (P) augments Purkinje cell responsiveness to iontophoretically applied GABA. Ratemeter records (left) and corresponding dose–response histograms (right) indicate changes in Purkinje cell (P cell) responsiveness to GABA before and after i.v. administration of progesterone (P) to adult ovariectomized rats (200–300 g). Each histogram sums unit activity from 4 GABA pulses (35 nA, solid bar), of 10 s duration, occurring at 40 s intervals. Control data were collected for a period of 20 min, and the animal injected through a jugular vein cannula with P (in 0.01% propylene glycol–saline) at a dose of 50 μg. P cell responses were monitored continuously until a parital recovery was achieved 30–33 min after steroid administration. Representative excerpts of control, drug effect and recovery data are presented. In this, and subsequent figures, numbers next to bars indicate percent GABA-inhibition or GLUT-excitation relative to background. Potentiation of GABA responsiveness was observed as soon as 3–10 min after P injection. These results are representative of 30 out of 35 cases for all doses of P tested. Injection of vehicle alone resulted in no change in GABA response ($n = 20$).

trol and hormone–drug interaction histograms[8]. The paired-sample t-test was used to assess significant differences between percentage spontaneous activity and response changes induced by sex steroids for each putative transmitter test. The ANOVA and Student-Newman-Keuls statistical procedures were used to compare differences between dose and time-course groups.

Hormone administration

E_2 (300 ng/kg) and P (5, 50 or 500 μg) were administered in combination or alone by intravenous injection over a 3 min period through a previously implanted indwelling jugular cannula. At the lowest dose administered, injection of P would produce blood levels equivalent to those seen on proestrus, when integrated over a 9–10 min period, due to the short half-life of this steroid[25]. In addition, the immediate blood levels produced by 5 μg P would approximate those observed during the last few days of pregnancy (125 ng/ml). The steroids were first dissolved in a small amount of propylene glycol–saline (2:3, v/v) and then diluted to the appropriate concentration with saline. In some cases, rats received prior injections of 2 μg E_2, s.c., 24 and 48 h before the recording session, when they were injected as described above. This injection paradigm, when followed by P administration, has been shown[31] to result in maximal reproductive behavior, and indicates a receptor-mediated mechanism, as E_2-priming upregulates P receptors[3,23].

Anisomycin, a protein synthesis inhibitor, (Sigma; 30 mg/kg in saline, pH 7.0) was administered, i.v. 20 min before recording. At a dose of 30 mg/kg, this agent has been shown to inhibit protein synthesis by 80% up to 1 h and by 40% up to 2 h after injection[24], and to prevent classic actions of E_2 and P on reproductive behavior[24].

RESULTS

Effects of systemic administration of progesterone on GABA and glutamate responsiveness of cerebellar Purkinje cells

In this study, iontophoretic application of GABA routinely suppressed spontaneous discharge of cerebellar Purkinje cells by 15–80%. Systemic administration of P (5, 50 or 500 μg) consistently increased the magnitude of this GABA-induced inhibition by an average of 87.5 ± 12.0% ($P < 0.01$). Recovery to control levels of response was observed in a majority of cases. An example of this effect is depicted in Fig. 1. In this case, pulsatile application of GABA decreased firing rate 61%. After intravenous infusion

of P, the same iontophoretic dose of GABA decreased spontaneous discharge by 92%. Thus, P administration potentiated GABA-induced inhibition of spontaneous activity by 50.8%. It is important to note that although background firing rate was reduced 5%, at 3–6 min post-steroid, discharge during the GABA response was decreased 80.1%, yielding a net enhancement of the GABA-mediated inhibition relative to steroid-induced alterations in background discharge. Similar analysis of peri-drug histograms collected at 10 and 20 min post-steroid revealed that this potentiating effect on GABA responsiveness was persistent, although accompanied by a gradual increase in background firing. Recovery toward the control level of spontaneous discharge and GABA response was observed by 30–40 min post-steroid. No change in spike height was observed throughout the experimental period as previously reported[28].

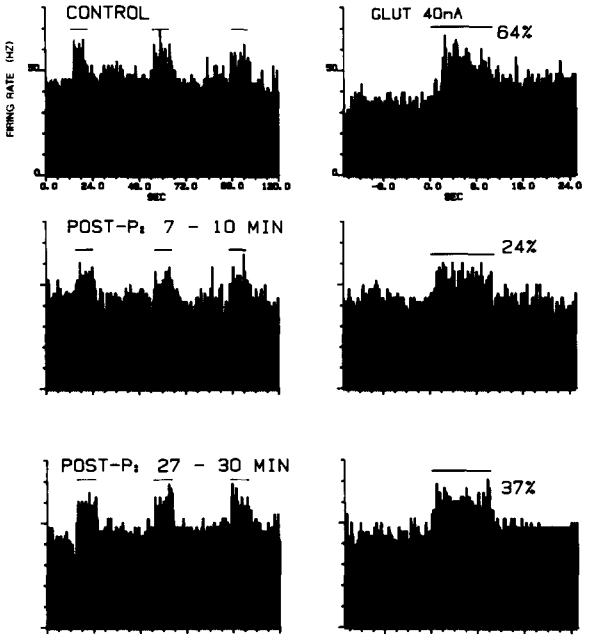

Fig. 2. I.v. administration of progesterone diminishes Purkinje cell responsiveness to iontophoretically applied glutamate. Ratemeter records (left) and corresponding dose–response histograms (right) indicate changes in Purkinje cell (P cell) responsiveness to glutamate (GLUT) before and after i.v. administration of progesterone (50 μg) to adult ovariectomized rats. Each histogram sums unit activity from 4 GLUT pulses (40 nA, solid bar), of 10 s duration, occurring at 40 s intervals. P cell responses to GLUT were monitored continuously for 60 min. Control, recovery and representative pre- and post-steroid excerpts are presented. A marked decrease in GLUT responsiveness is seen after 5 min, unaccompanied by significant alterations in spontaneous discharge. These results are representative of 27 out of 32 cases for all doses of P tested. No alterations in GLUT responsiveness were observed following injection of vehicle alone.

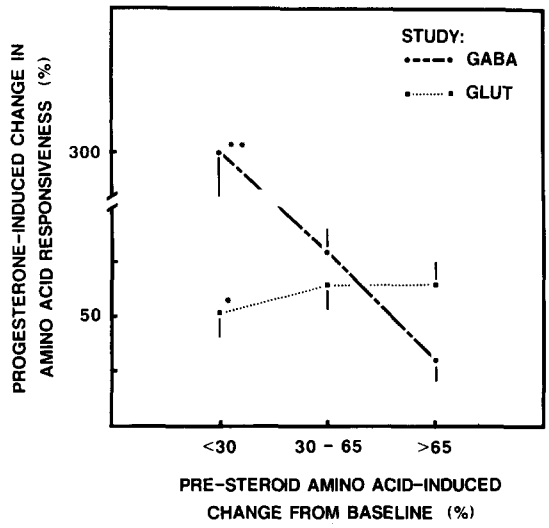

Fig. 3. An amino acid dose–response evaluation of P-induced changes in GABA and GLUT responsiveness of Purkinje cells. Differential P effects on the above parameters (% change from pre-steroid values) are presented for amino acid doses producing an initial (pre-steroid) change from baseline of <30% (n = 5 each), 30–65% (n = 10, GABA; n = 8, GLUT) or >65% (n = 5 each). Bars indicate S.E.M. * $P < 0.05$ vs control (all groups); ** $P < 0.05$ vs control (all groups); $P < 0.05$ for the <30% group vs the 30–65% group and the >65% group.

In contrast to its potentiation of GABA responses, P suppressed glutamate-induced excitation of Purkinje neurons relative to changes in background firing rate by an average of 45.3 ± 7.5% ($P < 0.02$). For the cell shown in Fig. 2, P decreased glutamate-evoked responses by two thirds from a 64% excitation (pre-steroid) to a 25% excitation at 5 min post-steroid. This net depressant effect on glutamate responsiveness occurred as a result of reductions in glutamate-evoked responses (from 67.2 Hz pre-steroid to 58.1 Hz post-steroid), as well as by a slight increase in spontaneous discharge (from 42.0 Hz pre-steroid to 47.3 Hz post-steroid). By 30 min post-steroid, a partial return to the control level of response was observed. Complete recovery was observed in 60% of the neurons tested. In no case did injection of vehicle alone result in any alteration in Purkinje cell responses to either GABA or GLUT.

Dose-dependent effects of P–GABA interactions

An amino acid dose–response evaluation of P effects on neurotransmitter response revealed that P–

GABA, but not P–GLUT, interactions exhibited significant dose-dependent effects. Fig. 3 presents differential P effects on the magnitude of neurotransmitter-induced alterations in spontaneous discharge, which were initially (pre-steroid): <30%, 30–65% or >65%.

The group with an initial GABA response of <30% exhibited a 300.0 ± 85.4% change after P administration, compared with P-induced changes of 78.2 ± 12.5% and 31.8 ± 9.2% for the 30–65% and >65% groups, respectively. In addition, in all cases, P-induced changes in GABA and GLUT responses were significant with respect to the pre-steroid condition ($P < 0.05$).

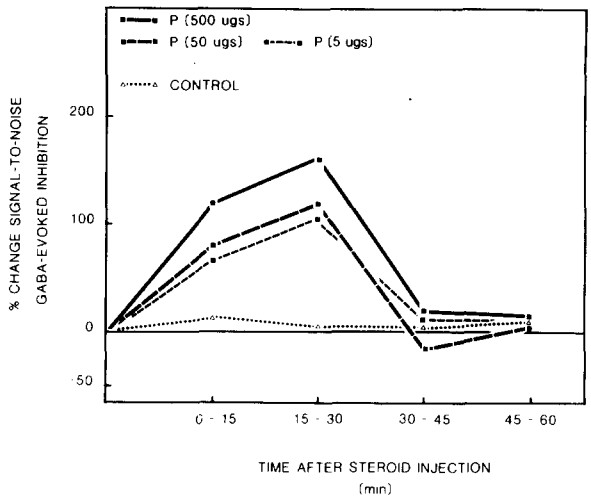

Fig. 4. Dose-dependent effects of P on the signal-to-noise ratio of GABA responses. The percent change in signal-to-noise ratio for GABA-evoked inhibition of P cell firing was calculated by comparing values obtained during the pre-steroid control interval versus the post-steroid time period (0–60 min). Mean values are plotted for each of the groups at 15 min intervals after i.v. injection of the indicated steroid. Administration of all 3 doses of P (5, 50 and 500 μg) to ovariectomized animals resulted in a significant increase in the signal-to-noise ratio for GABA-evoked inhibition ($P < 0.05$; $n = 8$, 20 and 7, respectively).

A dose and time-course evaluation of P-effects on neurotransmitter responses

In this study, a dose–response analysis of the time-course of alterations in the % change in amino acid response ('signal') relative to spontaneous discharge ('noise') was also performed. In Fig. 4, 3 doses of P (5, 50 or 500 μg), administered systemically, are shown to increase the signal-to-noise ratio for GABA-evoked inhibition by 75–155% in a dose-dependent fashion, within 15 min after P administration. Peak responses for all 3 doses were attained by 15–30 min post-steroid. At this time, P increased the signal-to-noise ratio by 105.3 ± 34.2% ($P < 0.05$ vs control), 148.7 ± 29.8% ($P < 0.02$) and 210.8 ± 45.2% ($P < 0.01$) for the 5, 50 and 500 μg doses, respectively. Recovery to control levels of the signal-to-noise ratio was apparent 30–45 min post-steroid at the two lower doses, and by 45–60 min post-P at the highest dose. Systemic administration of vehicle (control) did not produce any significant alteration in the signal-to-noise ratio for the entire 60-min period.

In contrast, P decreased the signal-to-noise ratio for GLUT-evoked excitation by 85–95% by 15 min post-steroid. Unlike the modulatory actions of P on GABA response, P-induced alterations in GLUT response were not dose-dependent, as there was no significant difference in the degree to which the 3 doses depressed the signal-to-noise ratio for GLUT responses. Recovery was attained by 30–45 min post-steroid (data not shown).

Effects of E_2 priming on neuronal responsiveness

This study was conducted to determine whether prior administration of E_2 altered the observed effects of sex steroids on neuronal responsiveness. Animals were injected with E_2 (2 μg, s.c.) at 24 and 48 h before the day of recording. This injection schedule increases P binding capacity in the hypothalamus[3] which permits stimulatory effects of P on LH release and lordosis behavior[31]. Thus, an augmented P effect following E_2 priming would be suggestive of a receptor-mediated event in the classic sense. In addition, this paradigm replicates endogenous hormonal conditions more precisely, as E_2 is elevated for 36 h prior to the increase in P on the PM of proestrus. In this study, E_2 did not alter the P effects on GABA and GLUT responses (see Fig. 5A, B). In the example presented in Fig. 5A, i.v. administration of 50 μg P was followed by a 39% increase in GABA-evoked inhibition relative to spontaneous discharge by 5 min post-steroid. Background firing was decreased by 40%. Within 45 min post-steroid, GABA responsiveness had returned to control levels. In panel B, GLUT responsiveness was decreased by systemic P 15 min post-injection, with no accompanying change in background discharge. Recovery towards the con-

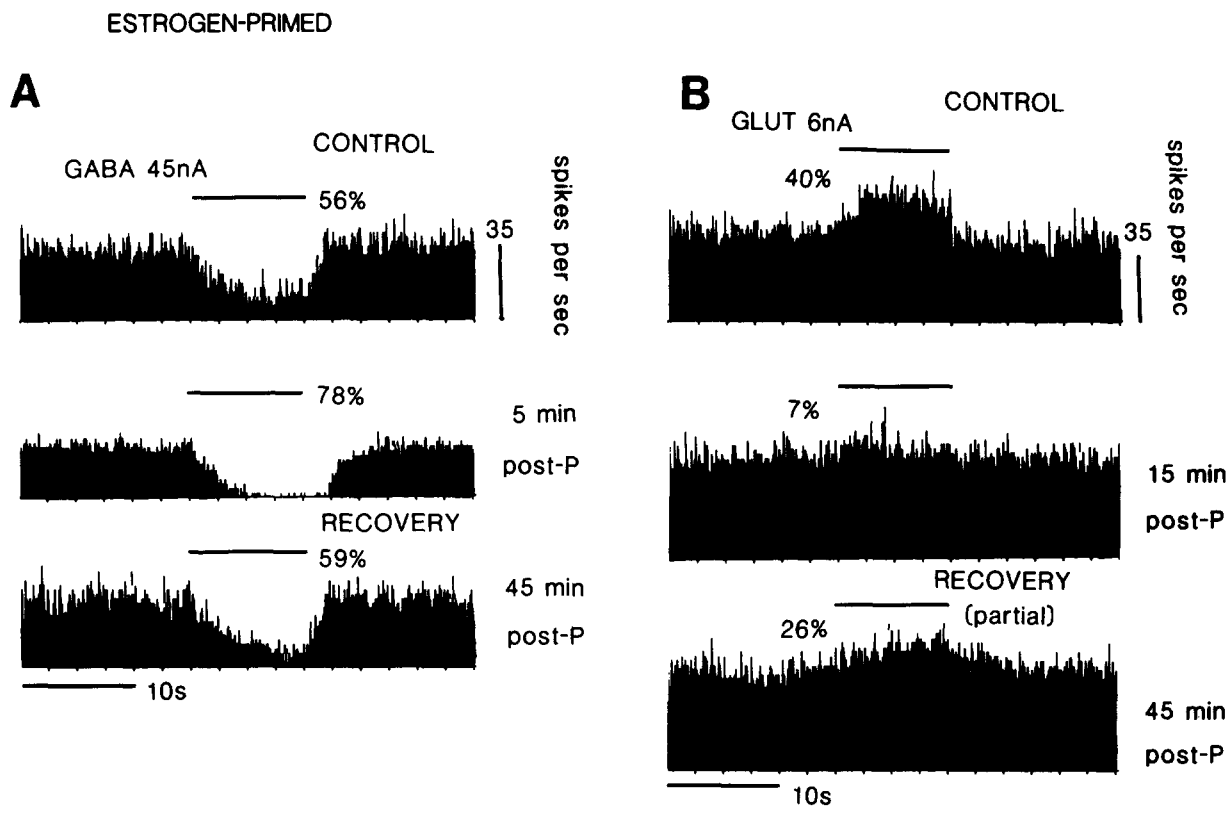

Fig. 5. Estrogen priming does not alter the modulatory effect of progesterone on Purkinje cell responsiveness to GABA and glutamate. Dose–response histograms demonstrate P cell responses to iontophoretically applied GABA (A) and GLUT (B) before and after i.v. P (50 μg) administration to rats pre-treated for two days with E_2 (2 μg, s.c.). This injection schedule has been shown by others to induce reproductive behavior. These histograms sum unit activity from 4–6 pulses of GABA or GLUT. As in the previous study, potentiation of GABA responsiveness (A) and diminution of GLUT responsiveness (B) are observed within 5–15 min after steroid administration and persist for 30–40 min. The histograms displayed are representative of 14 of 14 cases (GABA) and 13 of 14 cases (GLUT).

trol level of GLUT responsiveness was observed 45 min after P administration. Thus, the effects of P in the E_2-primed animal are qualitatively similar to those seen in the non-primed animal.

However, a quantitative assessment was performed in order to further compare results from E_2-primed and non-primed animals. In 26 of 28 neurons tested in non-primed animals, P potentiated GABA-evoked inhibition by an average of 59.5 ± 10.2%, while background activity was altered by a mean 6.5 ± 1.3%. Thus, GABA responses were enhanced by 808.4 ± 45.3% relative to alterations in spontaneous discharge. In contrast, P administration reduced GLUT-induced excitation ($\bar{X}$ = 43.6 ± 12.3%) to a greater degree than spontaneous activity ($\bar{X}$ = 10.7 ± 2.4%), a difference of 306.0 ± 78.8% in 21 of 24 cells.

In estrogen-primed animals, P increased GABA-evoked inhibition by an average of 79.0 ± 23.0%, while spontaneous discharge was altered by 3.6 ± 0.67%. Thus, the P increase in GABA response was 2058.4 ± 568.7% larger than the increase in background. In terms of GLUT response, background firing was increased 4.2 ± 1.2% by systemic P, while GLUT excitation was suppressed by 29.0 ± 2.3%. In this case, the change in evoked discharge was 590.4 ± 123.1% larger than the change in spontaneous discharge. Although intra-group variability and overlap caused mean steroid alterations to not differ significantly, E_2-priming tended to maximize P potentiation of GABA responses.

Combined administration of E_2 and P

This experiment was performed in order to assess

the combined effect of different doses of E_2 and P on neuronal responsiveness to GLUT, as the steroids exert opposite effects on this parameter. Furthermore, although, for the most part, endogenous P levels peak after the proestrous rise in E_2, elevated levels of both steroids overlap transiently on this day of the cycle as they do during pregnancy. In the present study, E_2 and P injected simultaneously produced variable effects which were dose-dependent. 50 or 500 μg of P injected with 300 ng/kg E_2 produced effects similar to those seen with P alone: in the example presented in Fig. 6A, 10 min after steroid injection, a 68% decrease in GLUT excitation relative to background was observed, accompanied by a 20% increase in spontaneous discharge rate. By 30 min post-steroid, GLUT response had returned to control levels. An additional injection of E_2 alone (2 μg) produced a 10-fold increase in GLUT response over background firing, 20 min post-steroid. These results are representative of those seen in 21 cells, all of which exhibited significant decreases in GLUT excitation ($\bar{X}$ = 85.3 ± 9.2% at 15–30 min post-steroid and $\bar{X}$ = 95.6 ± 13.2% at 45–60 min post-steroid, $P < 0.02$ for both cases). No recovery was observed by 45–60 min post-steroid, however, unlike effects of P alone.

In contrast, a lower dose of P (5 μg) injected simultaneously with 300 ng/kg E_2, doses which resemble those seen in the PM of proestrus when levels of both steroids are relatively high, produced effects similar to those seen after an injection of E_2 alone (see Fig. 6B). By 5 min post-injection, a 281% increase in

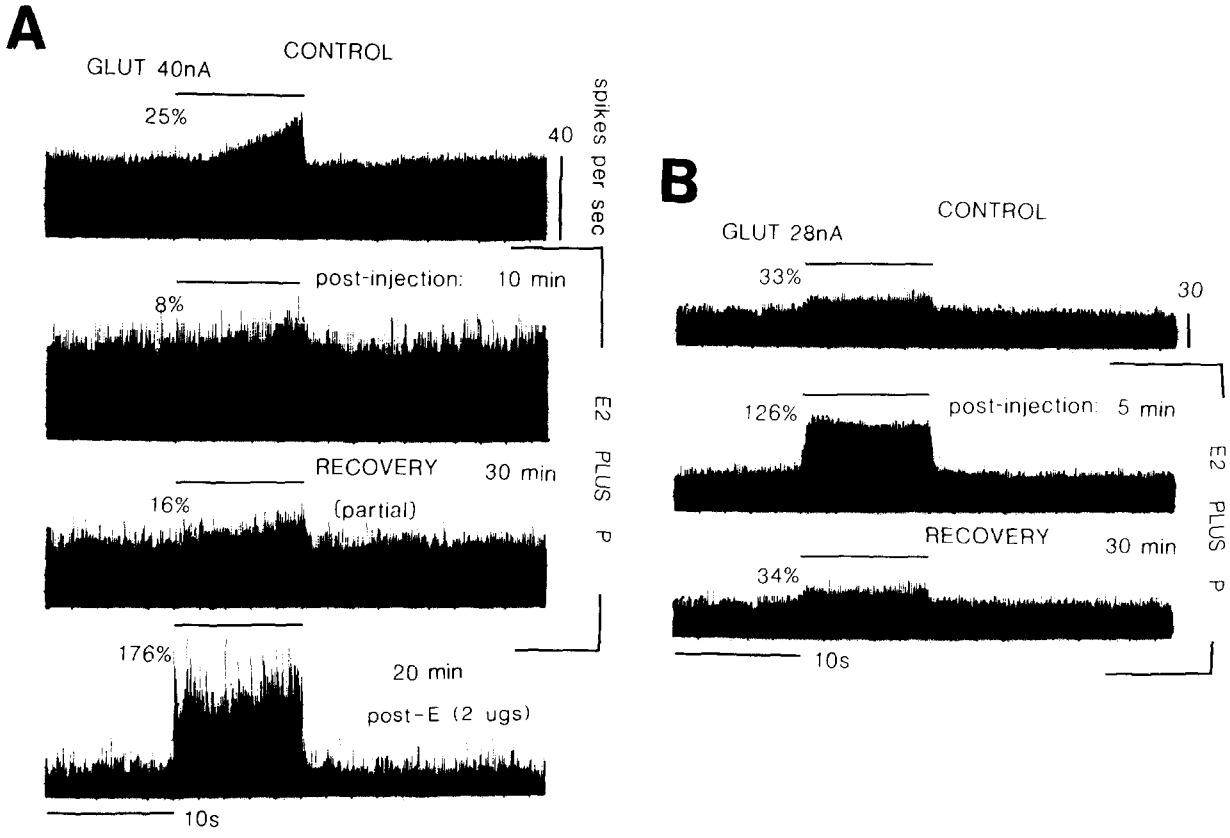

Fig. 6. Simultaneous administration of E_2 and P produce effects on Purkinje cell responses to GLUT which are additive and dose-dependent. Dose–response histograms depict P cell responsiveness to GLUT before and after i.v. injection of a combination of E_2 (300 ng/kg) and P (5, 50 or 500 μg) to E_2-primed rats (2 μg, s.c., for two days prior to the experiment). At the higher dose of P (A), GLUT responsiveness was diminished, a P-like effect. However, only a partial recovery was obtained after 20 min, and administration of E_2 (2 μg) at this time produced a marked potentiation of GLUT responsiveness. Conversely, at the lower dose of P (B), GLUT responsiveness was augmented, an E_2-like effect. In this case, recovery was attained by 30 min after E_2 plus P administration. These results are representative of 10 out of 12 cases (A) and 11 out of 12 cases (B).

GLUT-evoked excitation was seen compared with control values. Unlike E_2 alone, this combination of steroids produced transient effects, with recovery apparent 30 min post-injection. Mean values of potentiation of GLUT response were significant: 209.5 ± 70.2% (n = 12, $P < 0.01$). Recovery to pre-steroid levels of GLUT response was noted by 30–45 min post-steroid, unlike GLUT-enhancing effects of E_2 alone, which do not recover by 120 min post-steroid.

Anisomycin and steroid effects on neuronal responsiveness

An important consideration was whether the observed sex steroid effects on P cell responsiveness require protein synthesis. The administration of a protein synthesis inhibitor, anisomycin (30 mg/kg, i.v.), 20 min before the recording session did not block any of the steroid effects described above, although it delayed the response to P by 30 min in the E_2-primed rat. These results are summarized in Fig. 7. Administration of E_2 alone resulted in a 180% increase in GLUT excitation. After prior administration of anisomycin, E_2 induced a 110% increase in this parameter, an effect which was not significantly different due to the large degree of variability. P decreased GLUT responses by 90% and 80–83%, respectively, in ovariectomized and estrogen-primed animals. Anisomycin treatment did not alter these responses. In addition, inhibition of protein synthesis did not significantly alter P-induced augmentation of GABA inhibition, although in both ovariectomized and E_2-primed animals, GABA inhibition was increased from 120–140% to 210%. These results indicate that the observed steroid-induced alterations in Purkinje cell responsiveness are most likely due to non-genomic actions.

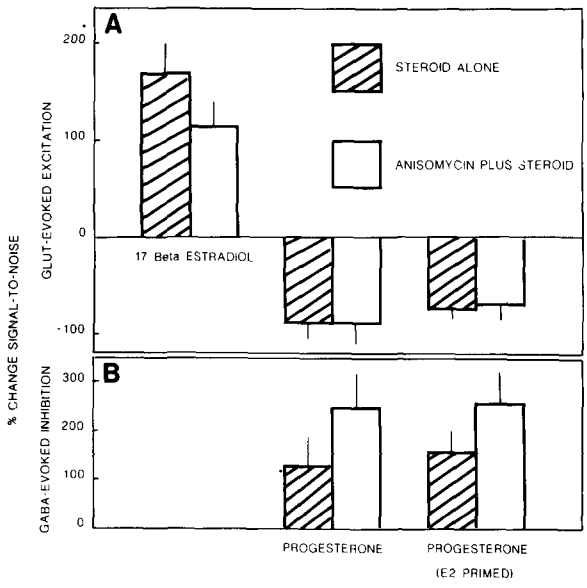

Fig. 7. Inhibition of protein synthesis does not significantly alter steroid-induced changes in the signal-to-noise ratio (%SN) for GABA- and GLUT-evoked responses. The percent change in signal-to-noise ratio for GLUT-evoked excitation (A) and GABA-evoked inhibition (B) were determined after i.v. administration of E_2 (300 ng/kg) or P (50 μg) to ovariectomized or E_2-primed animals. Mean values of the greatest response for each steroid-dose treatment are represented by the individual bar graphs for steroid alone and steroid plus anisomycin (30 mg/kg, i.v., 20 min prior to the experiment). (Bars indicate SEM.) Anisomycin treatment dit not significantly alter the observed steroid effects on the signal-to-noise ratio for P cell responses to iontophoretically applied neurotransmitter application, indicating that these effects are not dependent upon protein synthesis. E_2: control, n = 14; aniso, n = 10; P: control, n = 20; aniso, n = 12; P (E_2-primed): control, n = 14; aniso, n = 8.

DISCUSSION

The results from this study confirm an earlier report[28] that physiologic levels of P can alter the sensitivity of a model neuronal system, by increasing GABA responses and decreasing GLUT responses of cerebellar Purkinje cells. Specifically, P altered both GABA-evoked inhibition and GLUT-evoked excitation in a dose-dependent fashion to a much greater degree than alterations in spontaneous discharge, indicating a modulatory action on amino acid responses, rather than a generalized suppressive effect of the steroid. These results demonstrate an effect opposite to that previously shown for estrogen[30], and unlike the action of estrogen, P effects exhibit recovery to control levels. In addition, the P-induced potentiation of GABA responses observed in the present study is in contrast to the 'fading' of the GABA response normally observed during its continuous iontophoretic application to cortical and hippocampal neurons in vivo[17,32].

This study also demonstrates that E_2 and P injected simultaneously produce effects which are additive and dose-dependent. These results taken together with earlier findings demonstrating independent effects of E_2 on synaptic physiology, suggest that physiological combinations of the sex steroids are ca-

pable of altering extrahypothalamic CNS function and that cyclic endogenously fluctuating sex steroid levels may serve to exert a modulatory tone or bias over neuronal circuits in the intact rat.

Functional implications

The results obtained with P are consistent with the reported anxiolytic actions of this steroid. A recent study demonstrates that P administration increases the frequency of responding during a behavioral conflict paradigm[26], an established animal model of anxiety. P was also seen to potentiate the action of chlordiazepoxide, an anxiolytic benzodiazepine, using this paradigm. The anxiety sometimes associated with premenstrual syndrome, a common disorder, has been correlated with low levels of P relative to other ovarian steroids[1], and can be attenuated by administration of P[6]. In addition, this steroid also exerts anticonvulsant actions[22]. It is of particular interest, that P, as seen in the present study, and anxiolytic, anticonvulsant benzodiazepines, both potentiate GABA responses, while concomitantly also decreasing GLUT responses of cerebellar Purkinje neurons[5,10].

Dose–response studies

In the present study, the magnitude of the neuromodulatory effect of P was dependent upon the initial degree of GABA inhibition. This relationship was in the direction that would be predicted, i.e. indicating a ceiling effect: an initial GABA inhibition of >65% could not be enhanced to as great an extent as an initial GABA inhibition of less than 30%. However, the degree to which this parameter exhibited dose-dependent differences, especially in view of the failure of GLUT-evoked responses to demonstrate a similar dose-dependent effect, suggests that other factors may be operant. It may be that at the higher dose of GABA, considerable desensitization has already occurred during the 20-min control period, as described by Thalmann and Hershkowitz[32] prior to the onset of P administration. Thus, P would not be capable of creating as potent an augmentation of the GABA response as with the lower dose of the amino acid.

The variable recovery rate for P effects on neurotransmitter responses may result from a number of factors. It may be that P is sequestered within the membrane for varying lengths of time dependent on the relative distribution of membrane constituents such as cholesterol. It has been shown that the particular cholesterol content of the membrane is critical in regulating P permeability and membrane actions[4]. Thus, the duration of the observed neuromodulatory effect of P could vary considerably as a result of alterations in this parameter. Alternatively, P might be capable of interacting with other hormones, such as adrenal steroids, to produce effects of varying duration on neurotransmitter responses. Circulating levels of these hormones fluctuate endogenously (i.e. as a result of stress level), and several of the adrenal steroids have been shown to alter GABA responses[21]. In addition, levels of synthetic/degradative enzymes or receptors for the neurotransmitters themselves may vary from rat to rat, and these factors may influence the duration of the P effect.

Possible mechanisms of action

A likely mechanism for the observed neuromodulatory effects of this lipophilic steroid is by a direct membrane interaction, as we have previously shown that locally applied P exerts effects on GABA and GLUT responses similar to those seen after systemic injection of the steroid[28]. P has been shown to alter the phospholipid domain[4] and permeability of several membrane systems[2,15]. Recently, membrane actions of P were also shown to mediate release of LHRH from hypothalamic tissue[16]. In addition, a metabolite of P localized in the cerebellum, 3α-OH-DHP, has been shown to increase the membrane chloride ion current both independent of and in conjunction with GABA action in cultured hippocampal and spinal cord embryonic neurons[21]. This metabolite also increases GABA binding in whole brain homogenates[21]. It is possible that a metabolite of P may act in such a fashion to mediate the observed P-induced potentiation of GABA inhibition reported in the present study, as results from our lab demonstrate that this steroid, applied locally, increases GABA responses in a manner similar to P[29].

A less likely possibility is that P may be acting through classic receptor mechanisms. Only low levels of P receptors (6–7 fmol/mg protein) have been localized to cerebellum[18]. In addition, estrogen-priming, which has been shown to upregulate P receptors[3], did not alter either the direction or duration of the observed effect of P on neuronal responsiveness. If the observed actions of P were receptor-mediated in the

classic sense, then E_2-priming should produce more pronounced alterations in P-induced changes in neurotransmitter response than were actually seen. Enhancement of P-induced GABA potentiation subsequent to E_2-priming was only a trend, but not statistically significant. Furthermore, P produced a less pronounced suppression of GLUT excitation in the estrogen-primed animal than in the ovariectomized animal. The latter phenomenon may be due to the prolonged potentiating action of E_2 on this parameter. In contrast, estrogen-priming using a similar paradigm enables P to elicit lordosis behavior and LHRH release, two receptor-mediated events[23,31].

In addition, unlike classic actions of sex steroids on reproductive function[24], the observed effects of sex steroids on neuronal responsiveness do not appear to require protein synthesis, as anisomycin did not block any of the observed neuromodulatory actions of either E_2 or P. In view of these findings, it seems reasonable to suspect that the observed actions of P in the cerebellum are not primarily dependent upon conventional steroid receptor mechanisms, although multiple modes of action are a possibility, including interactions with membrane steroid binding sites[33].

Combined E_2–P administration

The present results also indicate that E_2 and P, injected simultaneously, exert effects on GLUT responsiveness which are dose-dependent; i.e. at higher P:E_2 ratios, P-like effects are seen, while at higher E_2:P ratios, E_2-like effects are seen. However, the duration of these effects was altered by combined steroid treatment: P-like effects were prolonged, while E_2-like effects were more transient compared with effects seen after the injection of either steroid alone. The present electrophysiological findings are consistent with reports demonstrating that E_2 or P alone exert opposite effects on various parameters of GABA and GLUT systems[20], as well as on seizure activity[22]. Thus, it is not surprising that complex interactions occur after injection of the steroids in combination.

Summary

These results demonstrate an effect of P on neuronal function in an extrahypothalamic area of the brain. The increased GABA responsiveness and decreased GLUT responsiveness observed after P administration, are opposite to changes observed after E_2 administration. These results suggest that one function of circulating levels of P may be to enhance the inhibitory tone of neuronal circuits in regions of the CNS which are not concerned with reproductive function. In addition, these P-induced phenomena indicate subtle alterations of the steroid on neuronal function which would tend to decrease neuronal excitability, an effect which is consistent with the reported anxiolytic and anti-convulsant effects of P reported by others[13,22]. Furthermore, the effects of E_2 and P are additive, indicating that the particular ratio of E_2:P would determine the dominant steroid effect on neurotransmitter function during the estrous cycle.

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