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PROGESTERONE ADMINISTRATION ATTENUATES EXCITATORY AMINO ACID RESPONSES OF CEREBELLAR PURKINJE CELLS

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Abstract—We have previously shown that the sex steroid progesterone plays a modulatory role in amino acid physiology by suppressing excitatory responses of cerebellar Purkinje cells to glutamate and augmenting inhibitory responses of these neurons to GABA. In the present study using the rat, progesterone effects on neuronal responses to the specific excitatory amino acid agonists quisqualate, kainate and *N*-methyl-D-aspartate were tested using iontophoretic, extracellular single unit recording techniques. In addition, the effect of systemic administration of progesterone on quisqualate-evoked excitation was evaluated in the presence of the $GABA_A$ blocker bicuculline. Progesterone consistently attenuated excitatory neuronal responses to local application of all three excitatory amino acids by 40–51%, but exerted variable effects on combined administration of quisqualate and *N*-methyl-D-aspartate which were dependent on temporal and dose-related factors.

Progesterone-induced attenuation of the quisqualate response was found to be mediated primarily by a non-*N*-methyl-D-aspartate receptor. In addition, bicuculline application did not block progesterone effects on quisqualate excitation, suggesting that the observed steroidal modulation of excitatory amino acid function is not secondary to progesterone-induced potentiation of GABA inhibition.

Although it is well established that progestins alter GABA receptor function,[9,13,26,27,42,44] ongoing studies in this laboratory have also demonstrated a clear role for the endogenous sex steroid progesterone in modulating excitatory amino acid function in an intact circuit of the extrahypothalamic CNS.[42–44] Both local and systemic application of physiological levels of this steroid markedly attenuate excitatory responses of cerebellar Purkinje cells to iontophoretic application of the excitatory amino acid glutamate, in addition to potentiating inhibitory responses of these neurons to GABA application using the identical paradigm. Both effects displayed a rapid (3–7 min) onset and termination (20–50 min), when recovery to control levels of response was typically observed, and were not dependent upon estrogen priming. The time-course, hormonal milieu and site of action necessary for these observed effects are suggestive of nongenomic actions of the steroid and are not mediated by its identified nuclear receptor unlike classic stimulatory actions of progesterone on reproductive function.[30] In contrast, progesterone modulation of both excitatory and inhibitory amino acid function mimics actions of the benzodiazepines in this system and, globally, may provide a mechanism for the known anxiolytic[10,37,38] and anticonvulsant[20,28] actions attributed to circulating levels of this steroid.

Endogenous glutamate can interact with three distinct receptor subtypes in the cerebellum, which are differentially sensitive to quisqualate/AMPA, kainate or *N*-methyl-D-aspartate (NMDA).[3,32,33,51] Quisqualic acid receptors are localized on both the soma and dendrites of the Purkinje cell.[3,32,41] In contrast, NMDA receptors have been identified on granule and Golgi cells, but are not localized to the Purkinje cell. However, iontophoretic application of all three excitatory amino acids has been shown to result in excitatory or biphasic responses, with quisqualate the most potent excitatory agent in this regard.[36,41] In addition, a recent study from this laboratory has demonstrated that quisqualate and NMDA can interact in a synergistic manner,[45] an action which was dependent on the dose and time-course of administration, and in many cases was a long-term effect. This phenomenon may, at least in part, underlie motor learning or other long-term changes in synaptic efficacy as has been shown for NMDA actions,[2,12,18,21,34] which could result in persistent alterations in cerebellar function. Thus, the purpose of the present study was to explore further the role of progesterone in biasing excitatory amino acid function by testing the actions of this steroid on the observed interactions of quisqualate and NMDA.

An additional aspect of progesterone-induced modulation of excitatory amino acid function, as tested using an intact circuit *in vivo*, is that attenuation of excitatory responses may be an indirect result of increases in the level of GABA inhibition and not a direct action at the level of the excitatory amino acid receptor, as has been shown for other agents.[5,31] A previous report from this laboratory has demonstrated that, in the cerebellum, the modulatory

Abbreviations: APV, 2-amino-5-phosphovalerate; DNQX, 6,7-dinitroquinoxaline-2,3-dione; E_2, estradiol; NMDA, *N*-methyl-D-aspartate.

actions of progesterone on GABA function are exerted at the level of the $GABA_A$ receptor.[47] Therefore, the ability of progesterone to modulate excitatory responses of cerebellar Purkinje cells to quisqualate was also tested in the presence of the $GABA_A$ receptor blocker bicuculline.

EXPERIMENTAL PROCEDURES

Adult, female rats (Long–Evans, Charles River, 200–250 g), tested on the days of proestrus and estrus, were employed for all studies. Animals were housed in groups of five to six and allowed free access to food and tap water. The facilities were maintained at a temperature of 23–25°C and a 14:10 light:dark cycle (lights on: 05:00; lights off: 19:00). Animals were tested between 12:00 and 19:00. The stage of the estrous cycle was determined by daily inspection of the vaginal lavage, which is a routine procedure. Progesterone exerted variable effects on the days of diestrus, and these data will be presented in a separate report. Data from animals in estrus and proestrus were statistically validated as being not significantly different using the intraclass coefficient, r_i (1.2), which established that the degree of variance between the two groups was smaller than the variation within groups. Thus, these data were pooled for the present study; the individual data will be presented in a separate report.

Surgery

Surgical procedures were identical to those previously described.[43,44] Animals were anesthetized with urethane (1.2 g/kg, i.p.) and the jugular vein cannulated with Silastic tubing (0.05 cm i.d., 0.09 cm o.d.) according to the method of Harms and Ojeda.[11] Animals were then placed in a stereotaxic apparatus, and a craniotomy performed to expose parts of the anterior and posterior lobes of the cerebellum. A heating pad was used to maintain body temperature at 36–37°C, as monitored by a rectal probe. Extracellular unit discharge of cerebellar Purkinje cells was obtained from the paravermal area 1–1.5 mm lateral to midline. Electrophysiological recordings were made using five barrel micropipettes, with 4–6-μm tips and a saline-filled central barrel (3 M NaCl). Side barrels, filled by diffusion, contained both excitatory and inhibitory amino acid agonists/antagonists, as described below. Drug solutions to be iontophoresed were ejected as cations or anions with 20-s pulses every 50 s and retained by application of 15 nA currents of opposite polarity. An additional saline-filled side barrel was employed for purposes of current balancing.

Materials

(1) Quisqualic acid: 20 mM, pH 8.0, Tocris Neuramin; (2) kainic acid: 50 mM, pH 8.0, Sigma; (3) NMDA: 100 mM, pH 8.0, Sigma; (4) 2-amino-5-phosphovalerate (APV, NMDA blocker): 50 mM, pH 8.0, Sigma; (5) 6,7-dinitroquinoxaline-2,3-dione (DNQX/FG 9041): 3 mM, pH 8–9, Tocris Neuramin; and (6) bicuculline: 20 mM, pH 4.0, Sigma.

Electrophysiology and data analysis

Action potentials of individual Purkinje cells identified by their characteristic discharge pattern of simple and complex spikes,[8] were monitored on an oscilloscope and converted to uniform voltage pulses by a window discriminator. From these gated pulses perievent histograms were constructed using a microeclipse computer (Data General) and Thoth software (J. K. Chapin, PA). Computer-generated peridrug histograms were used to compute the average agonist response to repeated applications of drug (excitation or inhibition of background discharge). In some cases, the blockers APV, DNQX or bicuculline, or the excitatory amino acid NMDA, were iontophoretically applied in a continuous manner during pulsatile agonist application. Once the control response to an amino acid was determined, progesterone (50 μg in 0.01% propylene glycol–saline, i.v.) or vehicle alone, was infused systemically and neuronal responses determined for an additional 60 min post-steroid. Background discharge (firing rate between amino acid pulses), excitatory amino acid evoked discharge (firing rate during pulses) and the percentage excitation (percentage increase in discharge above background levels produced by the amino acid) were calculated pre- vs poststeroid, as well as during treatment with various blocking agents. Because the data did not follow a normal distribution, Wilcoxon's paired sample t-test or Friedman's analysis of variance procedures were used to statistically assess significant differences between percentage changes in spontaneous activity and transmitter response pre- vs poststeroid or across steroid/amino acid antagonist groups, respectively.

RESULTS

Progesterone suppresses quisqualate excitability of cerebellar Purkinje cells

Systemic administration of progesterone (50 μg, i.v.) consistently reduced excitatory responses of Purkinje cells to iontophoretically applied quisqualate. In the example presented in Fig. 1, progesterone reduced a quisqualate excitation of 106% by 75% within 5 min after its administration. A minimal (5%) increase in background discharge was apparent after injection of the steroid. Although peak levels of maximal quisqualic acid response may not have been attained in the 20-s pulse period, the response of the Purkinje cell to a constant dose of quisqualic acid (i.e. as a function of current and time of application) was significantly decreased by progesterone administration. Recovery to control levels of quisqualic acid excitability was noted by 30 min post-steroid.

When the entire population of cells (52 neurons: 18 rats, 10 estrous, eight proestrous) tested was evaluated with respect to background discharge rate and quisqualate excitation pre- and poststeroid (Table 1), progesterone significantly suppressed ($P < 0.001$) both quisqualate-evoked discharge and the degree of quisqualate excitation by 37.7 ± 7.1 and 39.6 ± 8.1%, respectively, but had no effect on the background discharge level. In the control epoch, quisqualate produced a mean firing rate of 32.5 Hz, which effectively increased the firing rate by 108.3% above background levels. Systemic administration of progesterone decreased quisqualate-evoked discharge to 19.1 Hz and the degree of quisqualate excitation to 73.6%. Only insignificant reductions in the background discharge rate were noted.

Progesterone reduces kainate-evoked excitation

In a similar manner to its effect on quisqualate excitability, progesterone also reduced excitatory effects of kainate on Purkinje cell firing by a mean value of 54.8 ± 10.3%. A representative case is presented in Fig. 2. In this example, systemic administration of progesterone reduced kainate-evoked excitation by 44%, from 50 to 28 Hz by 2–6 min poststeroid. In this case, the background discharge

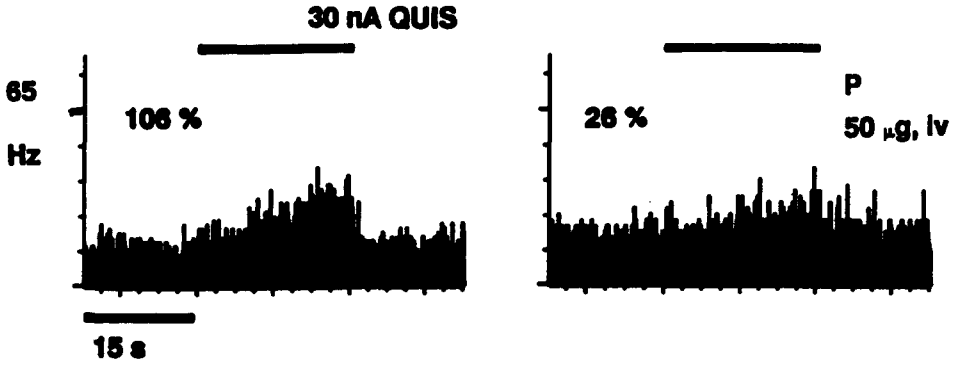

Fig. 1. Progesterone administration decreases quisqualate excitation. The perievent histograms depict the suppressant effect of progesterone (50 μg, i.v.) on quisqualate-induced excitation. Cerebellar Purkinje cell discharge in response to the excitatory amino acid quisqualate, iontophoretically delivered as 30-nA 20-s pulses (solid bars) every 50 s, is presented before and 15 min after administration of the steroid. The degree of quisqualate-evoked excitation (numbers next to bars) was determined as the percentage increase in discharge level above spontaneous. Progesterone significantly reduced the degree of quisqualate-evoked excitation by 3–20 min poststeroid. Recovery to control levels of discharge was observed by 30 min poststeroid. This effect is representative of 48 out of 52 cells.

Table 1. Progesterone effects on quisqualate-evoked excitation—summary data

	Background discharge (Hz)	Quisqualate-evoked discharge (Hz)	Quisqualate excitation (%)
Pre-P	12.3 ± 2.1	32.5 ± 3.5	108.3 ± 6.2
Post-P	10.4 ± 2.3	19.1 ± 5.1*	73.6 ± 8.2*

This summary table depicts progesterone effects on quisqualate excitation for the entire population tested (52 neurons tested in 18 rats). Background discharge, discharge during excitatory amino acid application and the degree of excitation above background discharge are presented pre- and post-steroid. Administration of systemic progesterone resulted in a marked reduction in quisqualate-evoked discharge. The degree to which quisqualate produced excitatory responses above background discharge was also significantly reduced after administration of the steroid. *$P < 0.001$ vs control epoch. P, progesterone.

rate was also reduced by 40%. In 50% of the cases, the degree of kainate excitation recovered to control levels by 40 min poststeroid.

Table 2 presents average firing rates for the entire population of cells studied (22 cells, 10 animals: seven estrous, three proestrous). In the control epoch, kainate produced a discharge level of 32.3 Hz, which was reduced to 16.0 Hz after administration of progesterone ($P < 0.05$). The degree of kainate excitation, expressed as a percentage increase in discharge above the background level, was also reduced significantly ($P < 0.05$), from a 222.5% excitation in the control epoch to 106.3% poststeroid. The background discharge level was reduced only insignificantly by steroid administration.

Progesterone reduces N-*methyl-*D*-aspartate-evoked excitatory responses of Purkinje cells*

Systemic progesterone administration suppressed NMDA-evoked discharge by an average of 29.7 ± 6.2% ($P < 0.05$), 1–3 min poststeroid, as exemplified by the cell in Fig. 3. In this case, pulsatile application of 40 nA NMDA produced a discharge rate of 34 Hz, a 108% excitation above background discharge levels. Infusion of 50 μg progesterone four pulses after peak levels of NMDA response were attained reduced NMDA-evoked discharge and degree of excitation to 31 Hz and 82%, respectively, by 1–2 min poststeroid. Throughout the ensuing 12.5-min poststeroid period, both NMDA-evoked discharge and background discharge levels gradually

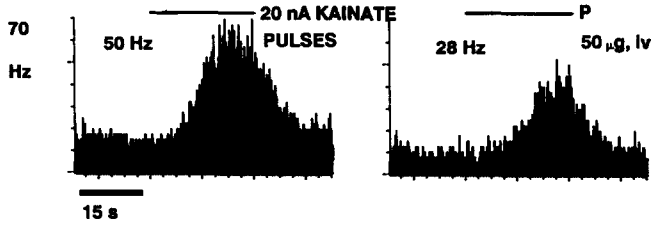

Fig. 2. Progesterone attenuates kainate-induced excitation. Perievent histograms depict the suppressant effect of systemically administered progesterone (50 μg) on excitatory responses of Purkinje cells to iontophoretically applied kainate (20-nA pulses of 20 s duration every 50 s). Pre- vs poststeroid epochs are shown. Progesterone markedly reduced kainate-evoked excitatory responses of Purkinje cells by 4 min poststeroid.

Table 2. Progesterone effects on kainate-evoked excitation—summary data

	Background discharge (Hz)	Kainate-evoked discharge (Hz)	Kainate excitation (%)
Pre-P	9.5 ± 2.7	32.3 ± 3.1	222.5 ± 8.1
Post-P	7.6 ± 4.5	18.3 ± 4.2*	106.3 ± 7.8*

This summary table presents average discharge levels during or between kainate pulses pre-vs post-progesterone. In addition, the degree of kainate excitation is also depicted for both conditions. Progesterone significantly reduced both kainate-evoked discharge as well as the degree of kainate excitation above spontaneous discharge, without significantly affecting the background firing rate. $n = 22$ cells in 10 animals. *$P < 0.05$ vs control epoch. P, progesterone.

increased, the former to control values of 31 Hz. However, background discharge rate nearly doubled subsequent to progesterone administration, yielding a percentage NMDA excitation of 60%. DNQX application at this time resulted in nearly equivalent increases in background and evoked discharge, yielding an NMDA excitation of 58%. Removal of the antagonist DNQX produced no significant change in any parameter. The degree of NMDA excitation remained below control values for the remainder of the recording session. Recovery of NMDA-evoked discharge was observed in 75% of the 18 cells tested (six rats, four estrous, two proestrous).

Effects of progesterone on N-*methyl-*D*-aspartate modulation of quisqualate excitation*

Ongoing studies in the laboratory have shown that NMDA can modulate quisqualate excitation in many cases as a long-term effect.[45] In the present study, progesterone was shown to exert either potentiating or suppressant effects on these NMDA–quisqualate interactions. The data presented in Fig. 4A are representative of the latter effect. In this case NMDA application markedly potentiated quisqualate-evoked discharge to an excitation of 114% above the background level. Subsequent administration of progesterone produced a greater degree of quisqualate excitation (148%) with concomitant reductions in background and evoked activity. A second application of NMDA administered 5 min poststeroid, further decreased quisqualate-evoked discharge to 36 Hz. Recovery to control discharge levels was not observed for the duration of the recording session. Significant reductions (mean = 42.5 ± 10.5%, $P < 0.05$) in post-NMDA levels of quisqualate excitation were observed in 60% of the cells studied.

However, in 40% of the cells studied, progesterone administration produced significant increases ($P < 0.05$) in NMDA-augmented levels of quisqualate excitation. This effect is exemplified in Fig. 4B. In this case, NMDA application indirectly resulted in a potentiation of the percentage quisqualate excitation (from 395 to 498%) secondary to reductions in background discharge. Systemic administration of progesterone (50 μg, i.v.) during local NMDA application markedly increased the percentage quisqualate excitation to a 1479% increase above background discharge levels by 15 min poststeroid. By 30 min poststeroid the degree of quisqualate excitation had recovered to control values (mean increase = 30.5 ± 8.0%); however, absolute levels of background and quisqualate-evoked discharge returned to presteroid post-NMDA values (background discharge: 11 Hz; evoked discharge: 26 Hz).

The differential effect of progesterone on quisqualate–NMDA interactions can be better understood when both initial background discharge and NMDA effects on quisqualate excitability are considered (see Table 3). When the data are analysed as two separate populations, progesterone consistently reduced quisqualate–NMDA responses when the background discharge was less than 15 Hz and NMDA-potentiation of the quisqualate response was clearly observed. In cases of reduced background discharge levels without NMDA-induced potentiation of the quisqualate response, progesterone increased quisqualate excitability post-NMDA ($P < 0.05$). The increased background activity may

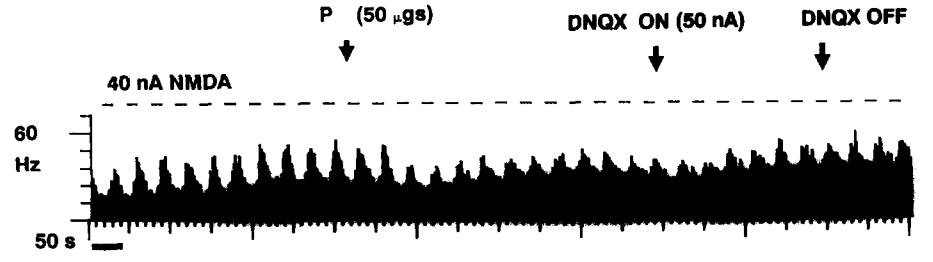

Fig. 3. Progesterone suppresses excitatory responses of Purkinje cells to iontophoresed pulses of NMDA. The NMDA was administered as 40-nA pulses (solid bar), of 20 s duration delivered every 50 s. Excitatory responses were determined as percentage increases in spontaneous discharge before and for 30 min after systemic administration of progesterone (P) (50 μg). Progesterone markedly reduced NMDA-evoked discharge by 2.5 min poststeroid. Concomitant application of DNQX only transiently reduced NMDA-evoked discharge (by 5%). NMDA-evoked discharge recovered to control levels by 15 min poststeroid in conjunction with elevations in the spontaneous discharge rate. These results are representative of 17 out of 18 cells studied in six rats.

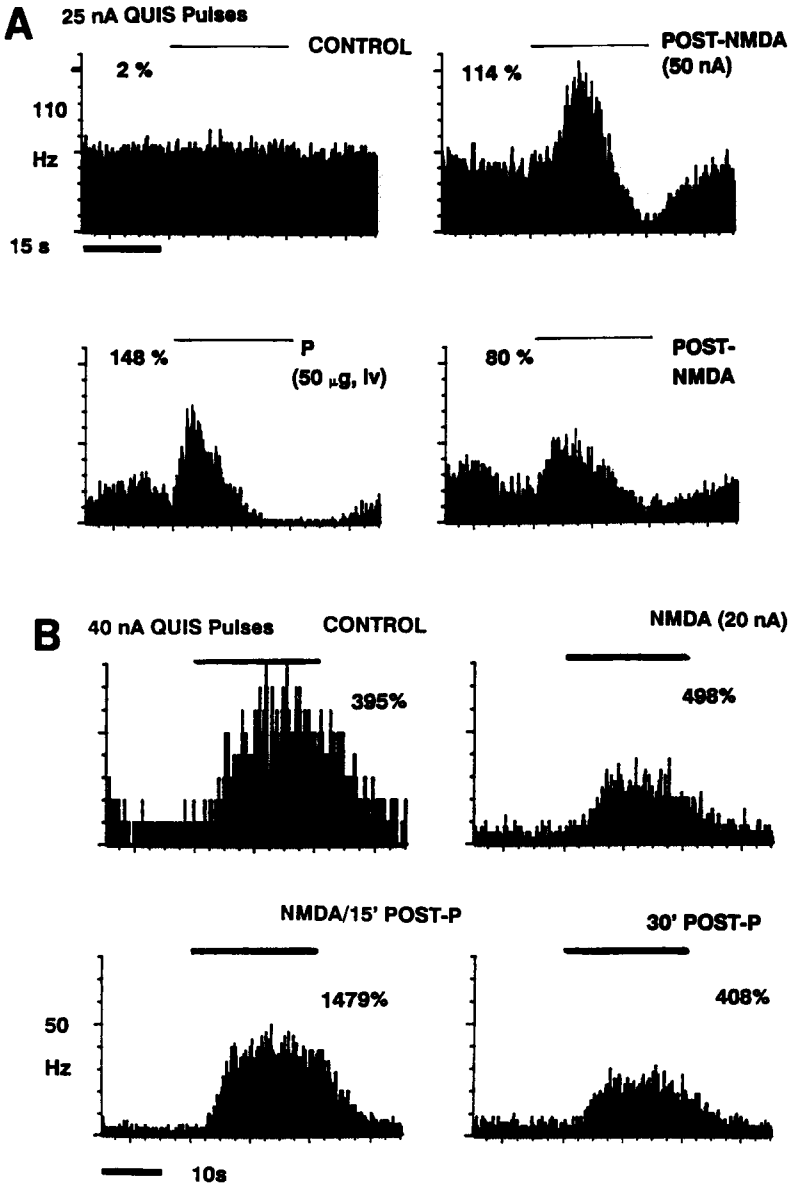

Fig. 4. Progesterone can either suppress or augment the synergistic effects of quisqualate and NMDA on Purkinje cell discharge. Perievent histograms depict Purkinje cell discharge levels in response to pulsatile application of quisqualate (25 or 40 nA for 20 s every 50 s) before, during and after continuous local application of NMDA administered for 5–10 min. The solid bar denotes periods of quisqualate application; numbers next to bars represent the percentage increase in discharge evoked during quisqualate administration relative to spontaneous levels. The sex steroid progesterone (50 μg, i.v.) was administered either during or after the initial dose of NMDA. CONTROL, poststeriod, post-NMDA and recovery histograms are presented for each injection of steroid. (A) NMDA exerts maximal permissive effects on quisqualate excitability at an almost subthreshold dose of quisqualate. This was a long-lasting effect as recovery to control levels of quisqualate response was not noted. Progesterone administration decreased quisqualate-evoked discharge and markedly blunted the facilitatory effect of a second dose of NMDA on quisqualate-evoked discharge. (B) In contrast, progesterone administered during local NMDA application enhanced the ability of NMDA to increase the degree of quisqualate excitation by 15 min poststeroid. A partial recovery to control levels of quisqualate excitation was noted by 30 min after injection of progesterone.

reflect increased input from the parallel fibers, and thus increased granule cell activity. If progesterone was acting at the level of the granule cell, one would expect to see decreases in background discharge post-steroid resulting from progesterone-induced suppression of excitatory input from this afferent system. Thus, in Fig. 4A, progesterone may be acting to decrease granule cell input, in addition to its action at the level of the excitatory amino acid receptor population on the Purkinje cell.

Table 3. Progesterone effects on NMDA modulation of quisqualate responses

	Background discharge (Hz)	Quisqualate-evoked discharge (Hz)	Background discharge	Quisqualate-evoked discharge
Control	20.3 ± 3.8	30.85 ± 1.2	9.6 ± 1.4	26.26 ± 7.9
Post-NMDA	13.94 ± 3.1	36.84 ± 2.3*	4.0 ± 0.8*	15.0 ± 2.9*
Post-P	10.09 ± 2.3*	20.68 ± 2.3*	5.63 ± 2.4*	31.30 ± 3.4*
Recovery	15.01 ± 3.5	27.01 ± 4.2	6.84 ± 2.3	18.76 ± 4.6

Average values (S.E.M.) for quisqualate-evoked discharge (Hz) and background discharge (Hz) are presented for two populations of data categorized on the basis of background discharge rate and NMDA effects on quisqualate response: left, background discharge above 15 Hz with demonstrable potentiating effects of NMDA on quisqualate responses ($n = 6$). Right, background discharge below 15 Hz with either no effect or attenuation of the quisqualate response post-NMDA ($n = 6$). Control, post-NMDA, post-P (progesterone) and recovery values are presented. Progesterone significantly reduced both background and quisqualate-evoked discharge when background discharge was above 15 Hz and NMDA increased quisqualate-evoked discharge. Conversely, progesterone augmented quisqualate-evoked discharge when background discharge was below 15 Hz and NMDA failed to potentiate quisqualate-evoked discharge. *$P < 0.05$ vs control.

*Progesterone acts through a non-*N*-methyl-*D*-aspartate receptor to alter* N*-methyl-*D*-aspartate modulation of quisqualate excitation*

The selective non-NMDA blocker DNQX was administered after systemic injection of progesterone in order to determine the role of specific excitatory amino acid receptor subtypes in mediating the observed effects of progesterone on excitatory responses of cerebellar Purkinje cells to quisqualate application. A summary of the population data is presented in Table 4: average values for background and quisqualate-evoked discharge are presented across the various drug treatment groups (10 cells from five rats). Progesterone administration reduced both values below control ($P < 0.05$) levels to 6.1 and 9.3 Hz, respectively, without abolishing the background-quisqualate differential. However, subsequent post-progesterone administration of DNQX reduced both parameters to nearly baseline ($P < 0.05$) and flattened the background-quisqualate differential, thereby abolishing the quisqualate response. Concomitant NMDA application was unable to increase quisqualate-evoked discharge significantly. Removal of the quisqualate blockade by DNQX restored both parameters to poststeroid, pre-DNQX values. Thus a non-NMDA receptor, probably quisqualate-specific, is necessary for progesterone modulation of NMDA–quisqualate interactions. These data summarize results obtained from 10 cells. The accompanying table (Table 5) illustrates the degree of specificity of selective antagonists DNQX and APV for progesterone effects on quisqualate and NMDA responses, respectively.

Progesterone-induced attenuation of quisqualate excitation is not mediated through increases in GABA inhibition

Progesterone consistently depressed excitatory quisqualate responses by an average of 37.6 ± 7.5% ($P < 0.01$) in the presence of bicuculline. Data from this laboratory have shown that progesterone-induced potentiation of the GABA response is exerted primarily at the $GABA_A$ receptor and can be blocked by concomitant application of bicuculline.[47] In the present study, the ability of progesterone to alter quisqualate-evoked discharge was tested during local application of bicuculline used at a dose which completely suppresses responses to $GABA_A$ agonists

Table 4. Specificity of progesterone effects on quisqualate responses

	Control	P	P/DNQX	P/DNQX + NMDA	P alone
Background discharge (Hz)	18.3 ± 3.1*	6.1 ± 0.8	2.0 ± 0.5**	2.8 ± 0.6**	6.1 ± 1.5
Quisqualate-evoked discharge (Hz)	25.6 ± 3.0*	9.3 ± 1.1	2.7 ± 1.0	4.2 ± 0.7	12.3 ± 1.3

These summary data illustrate the effects of the non-NMDA receptor blocker DNQX on progesterone-induced modulation of NMDA-induced enhancement of quisqualate excitation. Both background and quisqualate-evoked discharge are indicated across different drug epochs. As previously described, quisqualate was delivered with a pulsatile administration paradigm, while NMDA and DNQX were locally applied in a continuous manner concomitant with quisqualate either before or after systemic administration of progesterone (50 μg). The first column depicts control levels of discharge. Progesterone decreased quisqualate excitation as previously shown. Application of DNQX at this time decreased both quisqualate excitation as well as background discharge in the presence or absence of NMDA. After application of DNQX and NMDA was discontinued, all parameters increased to poststeroid values but below initial control values. This table averages results from 10 cells (five rats). *$P < 0.05$ vs all groups (except DNQX treated): background discharge. *$P < 0.05$ vs all groups (except NMDA treated): quisqualate-evoked discharge **$P < 0.01$ P, progesterone.

Table 5. Progesterone effects on excitatory amino acid responses in the presence of specific antagonists

	Control	P	DNQX/P	APV/P	P alone	Recovery
Quisqualate						
EVOK	26 ± 4.2	12 ± 1.3*	5 ± 0.8*	14 ± 5.7*	14 ± 6.6*	27 ± 5.4
BKG	18 ± 2.3	10 ± 1.6*	4 ± 0.5*	9 ± 4.4*	11 ± 4.5	17 ± 5.5
NMDA						
EVOK	25 ± 6.3	17 ± 5.3*	18 ± 6.1	5 ± 0.3*	17 ± 6.1*	23 ± 7.5
BKG	11 ± 4.8	6 ± 2.3	4 ± 0.4*	4 ± 0.9*	8 ± 3.2	9 ± 4.6

Both excitatory amino acid-evoked discharge (average firing rate expressed as spikes/s during eight to 10 quisqualate or NMDA pulses) and background discharge (average firing rate between excitatory amino acid pulses) of cerebellar Purkinje cells before and after i.v. injection of progesterone (50 μg) are presented. The specific excitatory amino acid antagonists DNQX and APV were administered by local continuous iontophoretic application in a sequential manner during pulsatile application of quisqualate or NMDA 10 min post-P and both evoked and background discharge levels monitored. Final effects of progesterone alone and eventual recovery to control values of response are presented. In all cases, progesterone significantly reduced excitatory amino acid responses. The excitatory amino acid antagonists DNQX and APV selectively blocked excitatory responses to either quisqualate or NMDA, respectively. Each value is the mean of 20 cells recorded in 10 animals. *$P < 0.05$ vs control. EVOK, evoked discharge (spikes/s); BKG, background discharge.

such as muscimol.[47] In all cases studied previously[47] and currently, concomitant alterations in background discharge rate subsequent to application of this blocker have not been observed. Thus, in the anesthetized rat, tonic GABAergic input may not control the spontaneous Purkinje cell discharge rate. A representative case is presented in Fig. 5. Bicuculline (60 nA) modestly reduced a quisqualate excitation of 500 to 450% accompanied by a 30% reduction in the background discharge rate. Systemic administration of progesterone during bicuculline application further decreased the degree of quisqualate excitation by 56% to a level of 199% concomitant with recovery of the background discharge rate to control levels. Removal of the $GABA_A$ blockade, resulted in a modest 24% reduction in the degree of quisqualate excitation,

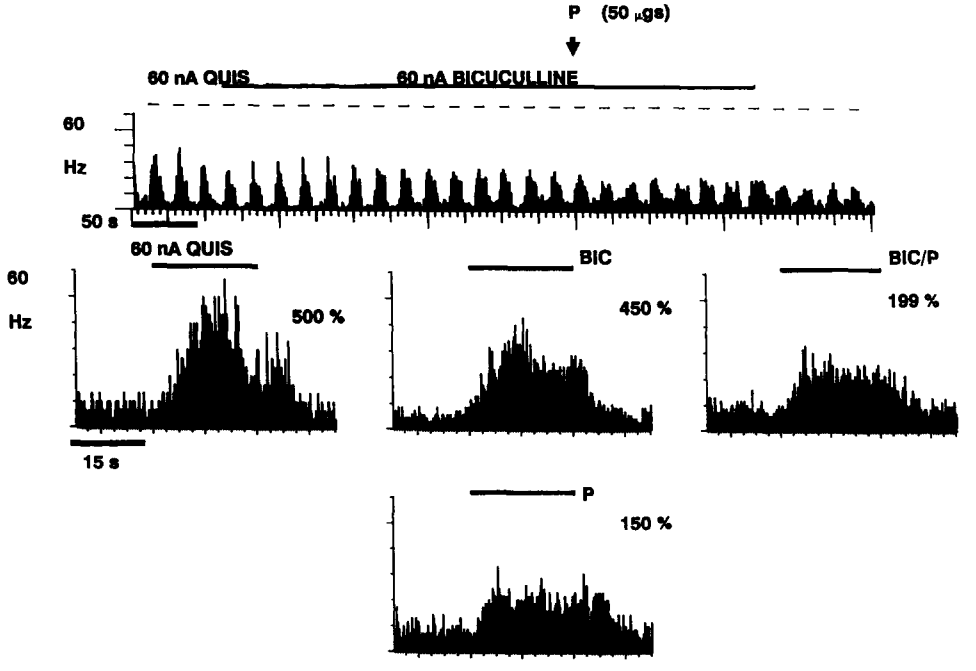

Fig. 5. Progesterone-induced attenuation of quisqualate excitation is not mediated through increases in GABA inhibition. Strip chart records (upper panel) and corresponding perievent histograms (lower panels) depict progesterone effects on quisqualate excitation in the presence of the $GABA_A$ blocker bicuculline. Continuous application of bicuculline (60 nA) slightly reduced excitatory responses to iontophoretic pulses of quisqualate (60 nA). Subsequent administration of progesterone (50 μg, i.v.) significantly reduced quisqualate excitation by 50 s poststeroid in the presence of the blocker. Further reductions in the degree of quisqualate excitation were insignificant after removal of the GABA blocker. This level of quisqualate response did not change over the next 20-min period. *$P < 0.05$ vs control and bicuculline-treated groups (% quisqualate excitation).

Table 6. Bicuculline effects on progesterone–quisqualate interactions—summary data

	Control	Bicuculline	P/bicuculline	P
Background discharge (Hz)	13.5 ± 4.1	11.7 ± 5.2	10.8 ± 6.2	14.3 ± 5.1
Quisqualate excitation (%)	90.0 ± 7.1	82.3 ± 8.0	43.2 ± 7.8*	1.0 ± 7.6*

This summary table depicts average levels of background discharge and quisqualate excitation seen subsequent to progesterone administration in the presence and absence of the $GABA_A$ receptor blocker bicuculline. Administration of bicuculline by continuous iontophoretic application did not significantly alter either parameter. However, subsequent administration of progesterone in the presence of bicuculline produced significant reductions in the degree of quisqualate excitation which were not significantly altered post-progesterone after removal of the GABA blockade. $n = 20$ cells; 11 animals. *$P < 0.05$ vs control and bicucculline-treated groups (percentage quisqualate excitation).

with no further change in the background discharge rate. Recovery to control levels of quisqualate response occurred by 45 min poststeroid.

The population data (20 cells, 11 animals) from this series of experiments are summarized in Table 6 ($n = 15$). Average values of background discharge levels as well as the percentage quisqualate excitation are presented across serial treatment groups. Administration of bicuculline decreased the level of quisqualate-evoked discharge from 28.5 to 24.2 Hz, and decreased the degree of quisqualate excitation by 14% to a level of 82.3%. Systemic administration of progesterone during local application of bicuculline reduced both quisqualate-evoked discharge and the degree of quisqualate excitation by 33.5 ± 2.9 and 37.6 ± 7.5%, respectively ($P < 0.05$), below presteroid levels obtained in the presence of bicuculline. Removal of the bicuculline blockade resulted in a non-significant increase in quisqualate-evoked discharge to 21.1 Hz, but because of a concomitant 22% increase in the level of background discharge, secondarily decreased the degree of quisqualate excitation to a level of 41% ($P < 0.05$).

DISCUSSION

In addition to its well-characterized effect on $GABA_A$ receptor function,[9,13,27,47] progesterone was demonstrated in the present study to exert significant effects on excitatory amino acid function. Consistent steroid-induced decreases in the magnitude of excitatory responses were demonstrated using agonists specific for all three excitatory amino acid receptor subtypes, quisqualate, kainate and NMDA. As NMDA receptors are not present on Purkinje cells,[3] the observed suppression of Purkinje cell activity subsequent to application of NMDA is most probably due to indirect presynaptic mediation by the granule cell population. In all cases, the time-course for onset (2–5 min) and termination (20–35 min) of these effects was similar to those observed for progesterone modulation of glutamate excitation.[42,44] Thus, results from the present study suggest that systemic administration of progesterone is followed by significant changes in glutamate physiology at multiple excitatory amino acid receptor subtypes.

A combined effect of the steroid in both decreasing excitatory amino acid function and increasing GABA inhibition such as reported in the present study could have far-reaching effects in terms of the physiological outcome alterations in circulating progesterone may have on CNS function. Specifically, this two-pronged effect of the steroid is similar to that reported for anxiolytic drugs, such as the benzodiazepines, using the same system, and may underlie the reported anxiolytic effects of the steroid.[10,37] Although the time-course for progesterone effects on both GABA and glutamate responses is more rapid than the reported effect of the steroid on response rate in animal models of anxiety, it is possible that alterations in receptor function which lead to complex behavioral phenomena such as anxiety reduction may require an entire sequence of events at the level of multiple CNS circuits in order to result in a particular behavioral endpoint, as has been reported for antidepressant drugs. In the human, the reported psychotropic properties of the steroid may relate to premenstrual anxiety, which has been linked to rapidly falling levels of endogenous progesterone and can be ameliorated by progesterone administration.[6] Additionally, progesterone modulation of GABA/glutamate may relate to the known anticonvulsant properties of the steroid;[20,28] especially relevant in this context is the observed reduction of NMDA excitability subsequent to progesterone administration, as NMDA has been implicated in a seizurogenic role.[48] The fact that progesterone reduces seizure activity during the luteal phase of the cycle when both steroids are elevated and exerts anxiolytic actions following estradiol (E_2) administration[28] is especially relevant to the present findings as progesterone effects on excitatory amino acids can be observed on the days of proestrus and estrus following increases in circulating endogenous E_2. The role of progesterone-induced alterations in excitatory amino acid function in mediating the behavioral sequelae of the steroid described above is further suggested by the fact that agents which are potent GABA agonists are not necessarily anxiolytic or anticonvulsant.

The present study utilized systemic injection as the most physiological means of administration since endogenous progesterone is released into the general circulation, crosses the blood–brain barrier and would come into contact with the cerebellar Purkinje cell through its dense vascular network. However,

although this is a physiological paradigm, mechanistic issues are harder to assess. Previous reports from this laboratory have demonstrated that locally applied progesterone decreases glutamatergic responses of cerebellar Purkinje cells.[42,43] Thus, progesterone appears capable of altering excitatory amino acid function at the level of the cerebellar Purkinje cell. Effects of systemic progesterone on other cerebellar cell types are also possible, however. Progesterone effects on Purkinje cell activity following local application of NMDA would reflect a circuit effect on granule and/or Golgi cell populations as no NMDA receptors are present on Purkinje cells. In addition, that progesterone reduced background discharge levels in cases with high control levels of this parameter also suggests that other cell types, such as the granule cell population may be influenced by circulating progesterone levels. Although indirect effects on other neurotransmitter systems, such as serotoninergic inputs to the cerebellum, could provide a possible mechanism for the present results, these projections are of limited density, and further studies must be completed to clarify this issue.

The present study also established that progesterone exerts specific effects on quisqualate receptor function, as the non-NMDA receptor blocker DNQX abolished all response to the amino acid. Because the content of quisqualate receptors is higher than that for kainate, it is very probable that DNQX application in the cerebellum is acting at the quisqualate receptor almost exclusively. Besides producing decreases in the magnitude of the quisqualate response, progesterone administration produced a delay in the onset of the quisqualate response by a mean 3.2 s in 30% of the cases studied.

Further evidence of a direct effect of the steroid on quisqualate function is provided by the fact that concomitant application of bicuculline did not prevent progesterone modulation of quisqualate excitation, and removal of the GABA blockade did not significantly alter the magnitude of the steroid's effects on quisqualate activity. As progesterone appears to act selectively at the $GABA_A$ receptor subtype in the cerebellum,[47] it would appear that progesterone-induced decreases in quisqualate excitability are not an indirect consequence of GABA potentiation. This is in contrast to the glutamate suppressant effects of a number of other agents, which have been demonstrated to be indirectly due to potentiation of GABA inhibition,[5] but similar to drugs such as pentobarbital which appear to act at the level of both GABA and excitatory amino acid receptors.[31,40] In addition, this observed action of progesterone is different from the steroid anesthetic alphaxalone which decreases Ca^{2+} conductance, a postexcitatory amino acid receptor mechanism, only indirectly through its potentiating action at the level of the $GABA_A$ receptor.[31] Other anesthetics, however, such as ketamine, act exclusively at the NMDA but not the GABA receptor.[1] The significance of this finding relates to the possibility that multiple mechanisms are responsible for the observed effects of progesterone on amino acid function. A considerable amount of evidence has accumulated suggesting that 3αOH-dihydroprogesterone, a metabolite of progesterone found in several CNS areas including the cerebellum,[43] allosterically alters Cl^- channel function[13] either by binding directly to the $GABA_A$ receptor or altering the membrane/receptor milieu. However, the results of the present study suggest that progesterone or a metabolite may also act at the level of the quisqualate receptor or its associated membrane domain. A second possibility is that progesterone may be acting at a postreceptor level to alter second messengers common to both excitatory amino acids and steroids as well as related to long-term changes in synaptic efficacy, such as Ca^{2+} influx,[7,23,25,49] as both traditional and novel Ca^{2+} channels exist in the cerebellar cortex.[22]

Mechanistically, it is also possible that separate and distinct metabolites of progesterone act directly to alter either GABA or quisqualate function, as suggested by a previous study from this laboratory.[43] Local application of progesterone produced alterations in both GABA and glutamate physiology with a latency of 3 min. However, 5α-dihydroprogesterone exerted immediate and more potent effects on glutamate excitation, while 3α-hydroxy-dihydroprogesterone exerted faster and greater actions on GABA inhibition.[43] The latter metabolite does not appear to alter excitatory amino acid-induced currents assessed *in vitro* (J. Lambert, personal communication). Thus, the specific configuration of the steroid appears to determine the temporal aspects and potency of inhibitory vs excitatory amino acid modulation, a fact which further suggests that specific binding sites may exist either on the receptor or receptor/membrane interface. Although classic cytosolic/nuclear progesterone receptors do not exist in the cerebellum,[25] a recent study has demonstrated the existence of specific membrane binding sites for progesterone in a variety of CNS areas,[50] including the cerebellum.[19]

As rats in estrus and proestrus, but not diestrus 1 or 2 (unpublished results) exhibit similar results, the background steroid milieu also plays a role in modulating progesterone effects on the excitatory amino acid system. However, unlike conventional progesterone effects on classic reproductive function which depend on a 24–36 h period following estrogen priming,[29,30] the role of circulating E_2 levels in the present study are related less to E_2 priming than to the relative E_2/progesterone ratio, as administration of high levels of E_2 acutely reverses the suppressant effect of progesterone to a facilitatory effect (unpublished results), and E_2 alone exerts effects opposite to progesterone on excitatory amino acid responses.[46]

The presently observed progesterone modulation of quisqualate–NMDA function may result from a mechanism different from that underlying progesterone–quisqualate interactions. Previous studies from

this laboratory have shown that NMDA acting at the level of the granule or Golgi cell, can potentiate quisqualate-evoked excitatory responses of Purkinje cells, in many cases, as a long-term effect.[45] This phenomenon may be physiologically relevant as increases in quisqualate sensitivity which can be blocked by APV are seen after induction of long-term potentiation,[4] an established model of learning. Further, the sex steroid 17β estradiol was shown to augment this synergistic action of the two amino acids.[46] In the present study, progesterone actions on this phenomenon were variable. However, in most cases progesterone reduced post-NMDA levels of quisqualate excitation that were greater than control levels and increased this parameter when it represented a decrease from control values. Furthermore, prior application of the specific NMDA blocker APV prevented progesterone actions on this phenomenon. These results suggest that progesterone may be acting at the level of the NMDA receptor to alter quisqualate excitability when both excitatory amino acids are interacted. Alternatively, progesterone may be acting at the parallel fiber synapse to reduce increased activity resulting from NMDA-induced increases in granule cell discharge. The differential effect of NMDA on quisqualate excitation may also be related to stimulatory[2] vs neurotoxic[39] effects of NMDA. The presence of progesterone would then either block the neurotoxic effect and result in an increased quisqualate excitation or block the stimulatory effect and result in a decreased quisqualate excitation. Alternatively, the background discharge level may have determined the direction of the progesterone effect as lower background discharge rates were associated with progesterone-induced increases in post-NMDA quisqualate excitability, while higher background discharge rates were associated with a postprogesterone decrease in quisqualate–NMDA interactions. Thus, a voltage-dependent mechanism either at the level of the excitatory amino acid receptor or second messenger system may be involved in this phenomenon. Because of the complexity of this interaction, however, elucidation of the mechanism awaits further study.

The significance of progesterone modulation of these quisqualate–NMDA interactions at the level of the parallel fiber–Purkinje cell circuit may relate to long-term changes in synaptic efficacy, as both NMDA[2,12,18,21,34] and quisqualate[4,17] have been suggested as mediators of changes in learning and plasticity. Quisqualate has also been implicated in the process of long-term depression,[16] the only demonstrated model of cerebellar plasticity reported to date. In addition, there is evidence that progesterone, along with E_2, exerts effects on sprouting in the hippocampus.[35] Further, potentiating actions of progesterone on this parameter may help explain some of the synergistic effects of progesterone and E_2 on cyclic changes in CNS excitability which lead to initiation of lordosis behavior and positive feedback effects of E_2 which culminate in the preovulatory LH surge.[29,30]

CONCLUSION

The present results suggest that the sex steroid progesterone markedly suppresses neuronal responsiveness to excitatory amino acids in addition to its well-known potentiating effect on GABA inhibition. These effects were ultimately demonstrated at multiple receptor subtypes and not secondary to GABA modulation. The observed modulatory effects of progesterone on excitatory amino acid physiology were seen with low, systemic levels of the steroid and may subserve its reported anxiolytic and anticonvulsant effects.

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