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Sex steroid effects on extrahypothalamic CNS. I. Estrogen augments neuronal responsiveness to iontophoretically applied glutamate in the cerebellum

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The purpose of this study was to test whether 17β-estradiol (E_2) could alter neuronal activity or responsiveness to iontophoretically applied amino acid neurotransmitters in an area not reported to contain classical E_2 receptors. Such a region is the cerebellum, which was selected as a model system for these studies because it has been well characterized electrophysiologically. Extracellular activity of cerebellar Purkinje neurons was recorded from urethane-anesthetized, adult, ovariectomized rats using multibarrel glass micropipets. Spontaneous firing rate and responses of single units to microiontophoretic pulses (10 s pulses every 40 s) of GABA (10–50 nA) or glutamate (GLUT, 3–40 nA) were examined before, during and after iontophoretic (0.25 mM 17β-estradiol hemisuccinate) or jugular i.v. (100, 300 or 1000 ng/kg 17β-estradiol) administration of E_2. Both modes of E_2 administration resulted in a significant increase in Purkinje cell excitatory responses to GLUT, independent of the direction of change in spontaneous firing rate. This effect was seen as early as one minute after iontophoretic application of E_2 and 10–40 min following i.v. E_2. In all cases, recovery to the control level of response was not observed by 2 h following E_2 administration. 17α-E_2 (300 ng/kg) resulted in a less pronounced, transient increase in GLUT response, while a lower dose (100 ng/kg) did not have any effect. Prior administration of the anti-estrogen tamoxifen did not prevent any of the observed E_2 effects. In addition, estrogen-priming did not alter E_2-induced potentiation of GLUT responses. In contrast to the effect of E_2 on GLUT responsiveness, GABA-mediated inhibition of Purkinje cells was either increased, antagonized or unchanged following E_2 application. In summary, this study suggests the hypothesis that circulating levels of E_2 may alter neuronal sensitivity to specific neurotransmitter substances within the cerebellar circuitry.

INTRODUCTION

Although the classic function of estrogen is to act at the level of the hypothalamo-pituitary-gonadal axis and uterus to promote full reproductive function, recent reports by numerous investigators indicate effects of estrogen on a variety of parameters in extrahypothalamic regions of the CNS. Both endogenous and exogenously administered estrogen have also been shown to exert global activational effects on sensorimotor function in the rat[2] and human[14], enhance seizure activity[28] and alter affect[8]. In addition, estrogen has been demonstrated to stimulate dopamine release and neuronal excitability in the striatum[3,5], and alter receptor and synthetic/degradative enzyme systems for amino acid neurotransmitters in extrahypothalamic areas[26]. Local effects of 17β-estradiol (E_2) on membrane permeability have also been demonstrated recently[39], suggesting the possibility that this steroid may act directly at the membrane level.

A pertinent question to be asked at this stage is whether estrogen can alter neuronal physiology in an intact, local circuit of the extrahypothalamic CNS. The Purkinje neuron, the major output cell of the cerebellum, was chosen as a test site because it is well

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characterized, both anatomically and electrophysiologically[10], and contains only low levels of classic cytosol/nuclear E_2 receptors[31]. Thus, it is a good model system with which to test potential non-receptor-mediated extrahypothalamic effects of sex steroids on neurotransmitter function. Specifically, the conceptual idea tested here was whether estrogen could modulate cerebellar Purkinje cell responsiveness to putative neurotransmitters.

In this study, both local and systemically applied E_2 were shown to augment the response of cerebellar Purkinje cells to microiontophoretically applied glutamate, the major excitatory putative neurotransmitter in the cerebellum, independent of the change in spontaneous firing rate. This effect is opposite to that we have reported for progesterone[36]. A preliminary report of these findings has appeared[35].

MATERIALS AND METHODS

Animals

Experiments were carried out on adult (200–300 g) Sprague–Dawley female rats, ovariectomized 3–6 weeks prior to experimentation. Animals were housed in group cages (5–6 rats/cage) with free access to food and tap water and maintained under controlled conditions of light (lights on from 05.00–19.00 h) and temperature (23–25 °C).

Surgery

On the day of the experiment, animals were anesthetized with halothane (0.75–2.5% in air), allowed to breathe spontaneously and the external jugular vein cannulated with Silastic tubing (0.05 cm i.d., 0.09 cm o.d.) according to the method of Harms and Ojeda[17]. Animals were allowed to recover for one hour and injected with 25% urethane (1.2 g/kg in saline). Then, the animals were placed in a stereotaxic apparatus and a craniotomy performed 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), sodium L-glutamate (GLUT; 1.0 M, pH 8.0, Sigma) and, in some cases, 17β-estradiol hemisuccinate (E_2S; 0.25 mM, pH 7.5, Steraloids), 17α-estradiol hemisuccinate (A-E_2S; 0.25 mM, pH 7.5, courtesy of Dr. R.L. Moss, Dallas, TX) or succinate (S; 0.25 mM, pH 7.5, Sigma). Drug solutions were ejected as cations or anions and retained by application of 15 nA currents of opposite polarity using a computer-controlled solid state iontophoresis unit[15]. Steroids were prepared and ejected as described by Kelly et al.[22]. 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[10], were monitored on an oscilloscope and converted to uniform voltage pulses by a window discriminator. The pulses were integrated over 1-s intervals and displayed on a computer terminal (Microeclipse S-20, Data General). The Unit program written by Dr. John K. Chapin (Hahnemann University, Philadelphia) processes incoming spikes into strip chart and/or histogram format, and stores data for eventual histogram construction and data analysis. 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. Once the control response to an amino acid was determined, either 17β-estradiol or 17α-estradiol was infused intravenously at doses of either 100, 300 or 1000 ng/kg or iontophoresed locally. In addition, the vehicle solution (0.01% propylene glycol–saline) was injected

i.v. in a few representative control animals (n = 12). Cells were generally held in isolation for at least 1.5–2 h in order to generate control and drug effect histograms over time.

Potential problems with pipet drift during the course of the experiment were ruled out by 2 means in the present study: (i) the use of many animals (cumulative n = 50, for all doses) to test the hypothesis that E_2 increases GLUT responsiveness, as consistent steroid effects would not be expected if these phenomena were artifactual, i.e. resulting from changes in the proximity of the pipet to the neuron; and (ii) ensuring that the spike height on the oscilloscope trace remained unchanged throughout the duration of the experimental epoch, ruling out the possibility of local anesthetic effects and indicating no significant alteration in the position of the recording barrel relative to the neuron due to changes in the local microvasculature (shown in Fig. 1 inset). Indeed, others have shown that E_2 does not result in any change in cardiovascular parameters (including blood pressure and hematocrit) or respiratory/metabolic parameters (such as pCO_2, O_2 and pH) for at least 2 h after i.v. infusion of estradiol benzoate (100 ng), which has a longer half-life than unconjugated E_2 (ref. 34).

The problem of volume loading was eliminated by injecting steroids in small volumes of saline (0.2 ml) and with the use of proper controls injected only with vehicle.

Data analysis

Differential changes in putative transmitter-induced and spontaneous activity resulting from hormonal administration were assessed by comparing discharge in identical portions of control and hormone–drug interaction histograms with procedures similar to those employed by Freedman et al.[13]. Epochs of activity during the amino acid response and of spontaneous activity were selected in the control histogram, and the discharge rates in each were calculated by dividing the number of counts (within the interval) by the time (total ms × 100) multiplied by the number of sweeps. The period of transmitter response was selected to begin at the particular time bin where counts deviated significantly from baseline and terminate in the bin where counts reapproached the baseline. Identical epochs of spontaneous and transmitter-induced activity were then compared between control and hormone–drug interaction histograms computed for a cell. To facilitate comparisons between histograms, equal numbers of agonist drug applications were routinely used for each. The paired-sample t-test or one-way analysis of variance and Student-Newman-Keuls tests were used to statistically assess significant differences between percentage changes in spontaneous activity and transmitter response induced by sex steroids.

The following operational definitions were employed to assess the influence of E_2 on the actions of amino acid neurotransmitters. Enhancement of the neurotransmitter response relative to background discharge was declared when the magnitude of the amino acid response (GLUT excitation or GABA inhibition) was increased by at least 15% more by E_2 than was any change in spontaneous discharge in the same direction as the neurotransmitter response. Steroid–amino acid interactions were termed suppressive when the GABA-evoked inhibition or GLUT-evoked excitation was decreased in magnitude by 15% or more than corresponding alterations in spontaneous discharge.

Hormone administration

After 20 min of recording control data, estrogen was administered over a 3 min period at doses of either 100, 300 or 1000 ng/kg, by intravenous injections of solutions of 17β-estradiol (E_2) or its inactive isomer 17α-estradiol (Steraloids, ME). 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 as described by Chiodo and Caggiula[5], such that the final solution contained 0.01% propylene glycol in saline (pH 7.4). In addition, the hemisuccinate derivatives of both estrogen stereoisomers were dissolved in 0.01% propylene glycol–saline (0.25 mM, pH 7.5) and locally administered by iontophoretic administration. Estrogen-primed animals were pretreated with E_2 (2 μg in 0.01% propylene glycol–saline) 24 h prior to the recording session. E_2-priming results in chronic E_2 levels more closely approximating those seen endogenously, as reproductive effects of E_2 only occur 24–36 h after the initial increase in E_2[30].

The anti-estrogen, tamoxifen, blocks the action of estrogen by binding to specific estrogen receptor

sites and preventing the action of estrogen[20]. In this study, ovariectomized rats were injected with 5 mg tamoxifen (in 0.2 ml saline) 2 h before and concomitant with estrogen injection. This injection schedule has been shown to be effective in blocking estrogen actions[16]. The neuronal effects of tamoxifen alone, administered using the same injection schedule, were also determined in terms of GLUT response. Possible estrogenic effects of this drug are minimized by reducing exposure to light[16], a precaution which was taken in the present study.

RESULTS

E_2 effects on GLUT-induced excitation of cerebellar Purkinje cells

Systemic injection of 17β-estradiol (E_2) produced a marked enhancement of the excitatory response of single cerebellar Purkinje cells to iontophoretically applied glutamate (GLUT). This effect was dose-dependent, tested at 100, 300 and 1000 ng/kg, as higher doses of the steroid produced a greater effect on GLUT response relative to spontaneous discharge, than did lower doses (see Table I). Furthermore, only the highest dose tested produced a significant increase in background firing rate. In most cases, E_2 effects on GLUT responsiveness were seen by 15–30 min post-steroid, with no recovery apparent by 2 h after steroid administration. In Fig. 1A, pulsatile application of GLUT (27 nA) increased neuronal discharge by 34%. Within 15 min after intravenous injection of 17β-estradiol (1000 ng/kg), spontaneous discharge was increased by 48%, and GLUT-induced excitation was increased by nearly twice that amount such that it represented a 50% increase in discharge relative to spontaneous firing. By 30–45 min post-steroid, GLUT-evoked excitation relative to background was increased further by 367% over control levels to a 159% excitation over background discharge. The Purkinje cell response to GLUT remained elevated although spontaneous discharge decreased over the last 30 min of recording, yielding significant increases in evoked discharge relative to background (maximum = 1563% by 60–75 min). This case is representative of 10 out of 13 cells tested (Table I). For the second cell depicted in Fig. 1B, a lower dose of 17β-E_2 (300 ng/kg) produced a 49.4% increase in GLUT-evoked excitation from 77 to 115

TABLE I

Average change in glutamate response and spontaneous discharge in estrogen-treated groups

	Group:	*17$_\beta$-E_2*			*E_2-primed + 17$_\beta$-E_2*	*17α-E_2*		*Tamoxifen + 17β-E_2*
	Dose E_2: (ng/kg, i.v.)	*100*	*300*	*100*	*300*	*100*	*300*	*300*
	n:	*(12)*	*(30)*	*(13)*	*(12)*	*(6)*	*(12)*	*(12)*
Background discharge								
% increase		12.1	15.3	58.0*	34.6	7.2	10.1	11.2
S.E.		12.3	11.1	14.6	19.6	3.8	8.2	27.6
Latency to significant response (min)		15	15	15	15	15	15	30–45
Glutamate response								
% increase		21.4	66.7*	150.3*	91.5*	6.8	30.0	55.2*
S.E.		9.2	20.6	49.1	38.5	5.2	22.3	19.3
Latency to significant response (min)		15	15	15	15	15	15	30–45
Relative change in evoked response over background								
% increase		67.8*	95.7*	141.4*	68.6*	−5.3	58.3*	59.1*
S.E.		23.1	27.1	49.6	12.3	10.2	32.8	27.3
Latency to significant response (min)		15	15	30	15	15	15	30–45

*$P < 0.05$ compared with pre-steroid values.

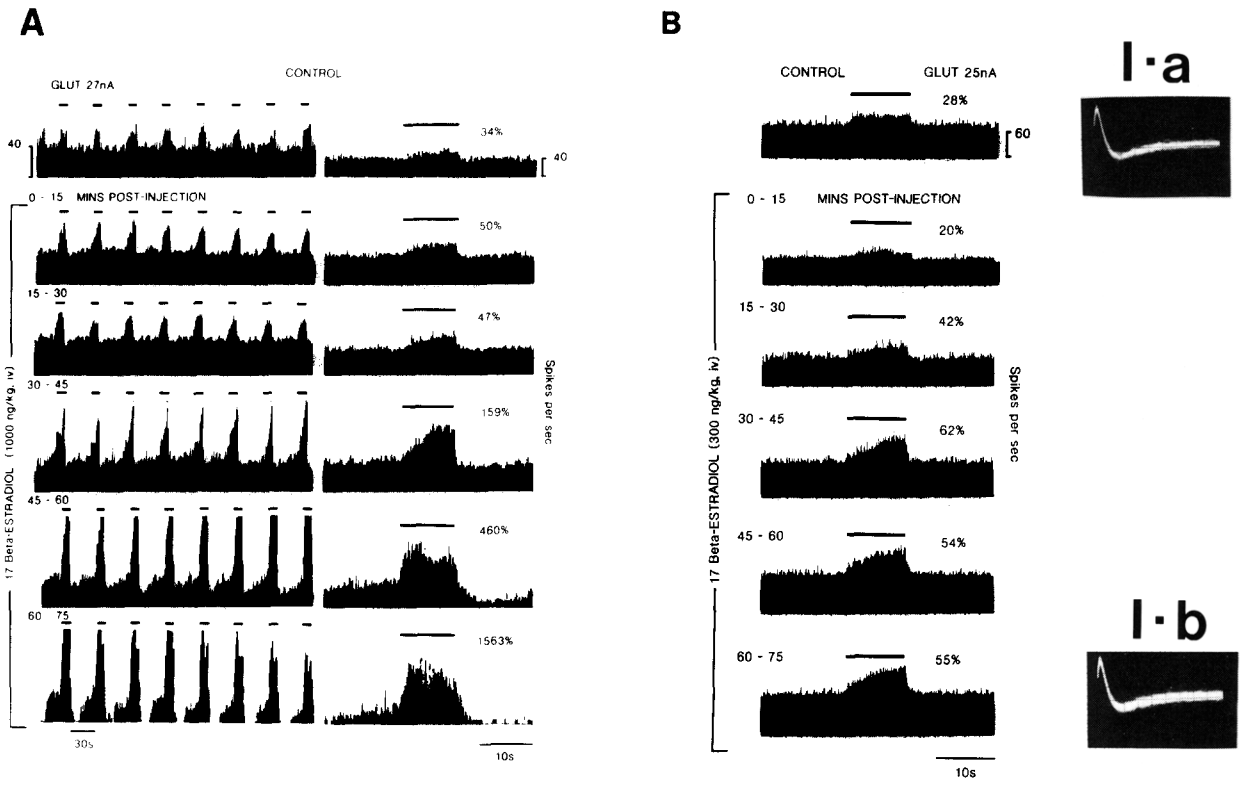

Fig. 1. E_2 augments Purkinje cell responsiveness to iontophoretically applied GLUT. Ratemeter records (left) and corresponding dose–response histograms (right) indicate changes in Purkinje cell (P cell) responsiveness to glutamate (GLUT) subsequent to i.v. administration of 17β-estradiol (E_2) to adult ovariectomized rats. Each histogram sums unit activity from 8 GLUT pulses (solid bar), of 10 s duration, occurring at 40 s intervals. GLUT-induced excitation is indicated as a percent change in firing rate relative to spontaneous discharge (numbers next to bars). Control records were collected for a period of 20 min, and the animal injected through a jugular vein cannula with E_2 (in 0.01% propylene glycol–saline) at a dose of either 1000 ng/kg (panel A) or 300 ng/kg (panel B). P cell responses to GLUT were monitored continuously for 75 min. Representative excerpts taken at 15 min intervals are presented. Injection of vehicle alone produced no alteration in Purkinje cell response to GLUT (n = 12). Marked increases in GLUT responsiveness are seen in both cases after 30 min; spontaneous discharge is either increased (A) or unchanged (B). These results are representative of 10 out of 13 cases (1000 ng/kg) and 26 of 30 cases (300 ng/kg). Inset: oscilloscope trace of a unit before (I-a) and 60–75 min after (I-b) systemic E_2 administration. There was no change in spike height despite an increase in spontaneous firing rate.

spikes/s unaccompanied by changes in baseline firing, 15–30 min post-steroid. Although spontaneous discharge increased slightly to 70 spikes/s, GLUT-evoked excitation more than doubled compared to control values, such that the relative increase in GLUT-evoked discharge above spontaneous firing rate was increased by 121.4%. Augmentation of GLUT responsiveness persisted for the entire recording session, although spontaneous discharge increased further to 80 spikes/s by 75 min post-steroid, with no recovery. The cell in Fig. 1B is representative of 26 of 30 cases (Table I). An oscilloscope trace (Fig. 1B inset) of the spike waveform before (Inset I-a) and 60–75 min after (Inset I-b) systemic E_2 administration reveals no change in spike height subsequent to the systemic administration of the steroid. This observation would argue against the possibility that local anesthetic effects of E_2 or pipet displacement resulting from possible steroid-induced changes in local microvasculature could account for the observed changes.

As noted in Table I, a dose of 100 ng/kg E_2, which results in circulating levels of E_2 within the physiologic range by 2 min after injection, produced results similar to those seen after injection of higher doses of E_2. The relative increase in GLUT excitation over background was half that seen after a dose of 1000 ng/kg E_2. In contrast, injection of vehicle alone

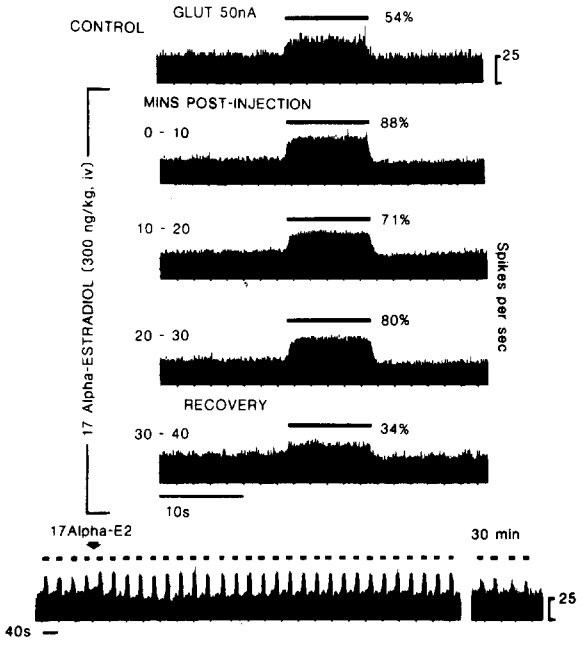

Fig. 2. 17α-E_2, at an intermediate dose, can potentiate GLUT responsiveness of P cells. Strip chart records and peri-event histograms depict one effect of i.v. 17α-E_2 (300 ng/kg in 0.01% propylene glycol–saline) on GLUT response of a P cell. Solid bar indicates period of iontophoretic GLUT ejection (10 s every 40 s). After a stable control response was obtained (upper record), 17α-E_2 was administered systemically through an indwelling jugular cannula and GLUT response monitored continuously for a period of 60 min. Representative post-steroid records indicate GLUT response calculated for 10-min intervals. A significant increase in GLUT response was seen by 10 min post-steroid. However, unlike results obtained with the β-isomer, recovery was apparent by 30–40 min post-steroid. This result is representative of 5 out of 12 cells tested.

(0.01% propylene glycol–saline) did not produce any alteration in Purkinje cell responses to GLUT (n = 12).

17α-Estradiol effects on GLUT responsiveness

The aim of this experiment was to determine the specificity of the observed E_2 effect on P cell function by comparing effects of the less active 17α-isomer with those of the 17β-isomer on neuronal responsiveness to GLUT. 17α-Estradiol was injected i.v. at doses of 100 or 300 ng/kg (Table I). Only the higher dose was able to increase GLUT-evoked excitation to a significant degree, but this increase was of a transient nature as noted in Fig. 2. In this case, a 50% increase in GLUT responsiveness was observed by 10 min after injection, with no change in spontaneous discharge. Recovery of GLUT responsiveness to control levels was observed after 30 min post-steroid. This result was found in 40% of the cells tested. An additional 20% of the cells demonstrated a persistent decrease in GLUT responsiveness ($\bar{X}$ = 50%) by 15–20 min after injection of 17α-estradiol. The remaining 40% of cells failed to exhibit any change in GLUT responsiveness after injection of 17α-E_2. At a lower dose of 17α-E_2 (100 ng/kg), no alteration in GLUT responsiveness was observed by 2 h post-injection.

Direct application of E_2-hemisuccinate and Purkinje cell responses to GLUT

Direct iontophoretic administration of estrogen, which is possible with the use of 17β-estradiol hemisuccinate (E_2S, 20 nA), yielded results similar to those seen after i.v. 17β-E_2: responses of P cells to GLUT were increased by 100–200%, 7–10 min after the onset of E_2S application (see Fig. 3). In this case, GLUT responsiveness was increased by 173%, in the face of a 50% reduction in background discharge. Similar effects were observed in 17 of 20 cells tested. No recovery was achieved by 35 min after termination of hormone applicaton. Iontophoretic application of succinate alone (20 nA) also increased P cell responsiveness to GLUT by 100% (see Fig. 4), but onset and termination of this effect were concomitant with onset and termination of iontophoretic application of the succinate (7 of 12 cells). In 5 out of 12 cells, succinate had no effect. In addition, local application of 17α-E_2 hemisuccinate (17ALPHA-E_2S) also increased GLUT responsiveness by 50% immediately after onset of iontophoretic application in 4 of 10 cells tested (see Fig. 5). Thus, both systemic and local application of E_2 result in potentiation of GLUT excitation in the cerebellar Purkinje cell. Administration of succinate produced similar results as E_2S, but with parameters which were markedly different; i.e. the effects were transient and of lesser magnitude (see Fig. 6).

Effects of estrogen priming on neuronal responsiveness to GLUT

This study was conducted to determine whether prior administration of E_2 altered the previously observed effects of the sex steroid on neuronal responsiveness. E_2 administration to an E_2-primed rat re-

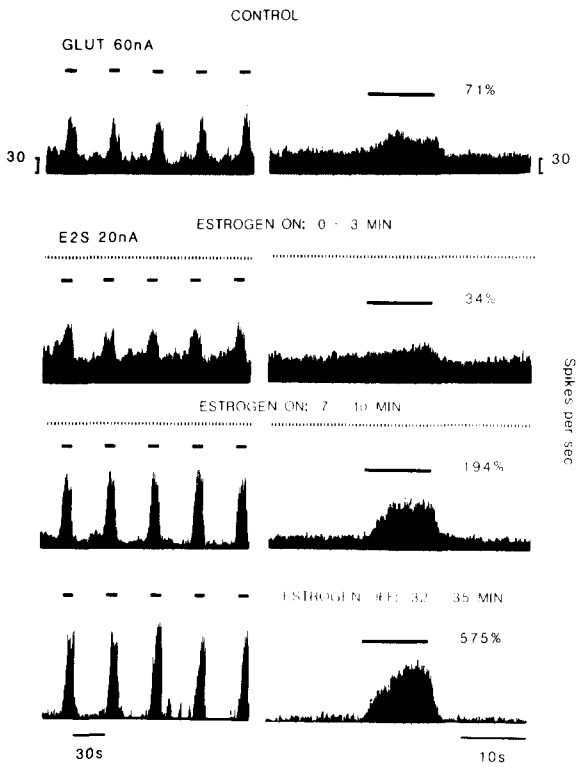

Fig. 3. Iontophoretic application of 17β-estradiol hemisuccinate increases P cell responsiveness to GLUT. In order to test whether local application of estrogen (17β-estradiol hemisuccinate, E_2S) to cerebellar P cells could also effect a change in sensitivity to GLUT. E_2S was continuously ejected iontophoretically (20 nA current) for a period of 20 min concomitant with pulsed iontophoretic application of GLUT (60 nA, 10 s duration at 40 s intervals). GLUT responses during the control period, at 0–3 and 7–10 min after onset of E_2S application, and at 32–35 min after the termination of iontophoretic application of the steroid are presented. GLUT-evoked excitation was markedly increased (by at least 100%) after 5 min of continuous E_2S application (17/20 cells), and this increase was sustained throughout the experimental period. No recovery was achieved by 35 min after drug termination.

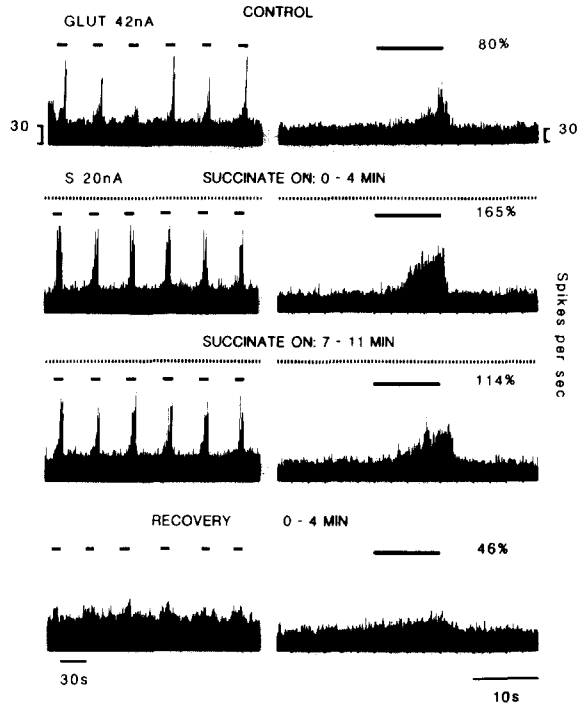

Fig. 4. Succinate can potentiate P cell responsiveness to GLUT. Local iontophoretical application of succinate (20 nA current) resulted in an immediate increase (0–4 min) in GLUT-evoked excitation, in contrast to iontophoresed E_2S which required 5 min of continuous application for a similar effect to occur. (In 7/12 cells succinate demonstrated this effect; in 5/12 cells succinate had no effect.) P cell responsiveness to GLUT returned to levels below the baseline immediately upon termination of drug application (recovery period 0–4 min), again in contrast to E_2S which did not exhibit a recovery.

sulted in a marked increase in GLUT responsiveness in a manner similar to that seen after E_2 administration to an ovariectomized rat. GLUT responses were increased by an average of 91.5% unaccompanied by significant alterations in background firing in 10 of 12 cells tested (see Table I). This E_2-induced augmentation in GLUT responsiveness was persistent, with no recovery by 2 h post-steroid.

Tamoxifen and E_2-induced effects on GLUT responsiveness

The effect of an estrogen receptor blocker was tested in this study to explore the possibility that receptor-mediated actions might be involved in the observed E_2 effects on GLUT responsiveness. The administration of an antiestrogen, tamoxifen, delayed by 30–45 min but did not prevent the E_2-induced increase in GLUT responsiveness normally occurring 30 min after E_2 administration (Fig. 7). Tamoxifen (5 mg in saline) was administered in 2 doses 2 h prior to and concomitant with an i.v. injection of E_2 (300 ng/kg). In this case, a gradual decrease in GLUT responsiveness is observed, which by 30–45 min after injection of E_2 was reduced by 38%, concomitant with a 30% reduction in background discharge. By 60 min post-steroid, however, a 138% increase in GLUT-induced excitation was seen, accompanied by a 50% increase in background discharge. This cell is representative of 10 out of 12 cases; in 2 of 12, the potentiating effect of E_2 on GLUT was not observed. These results show that tamoxifen does not prevent

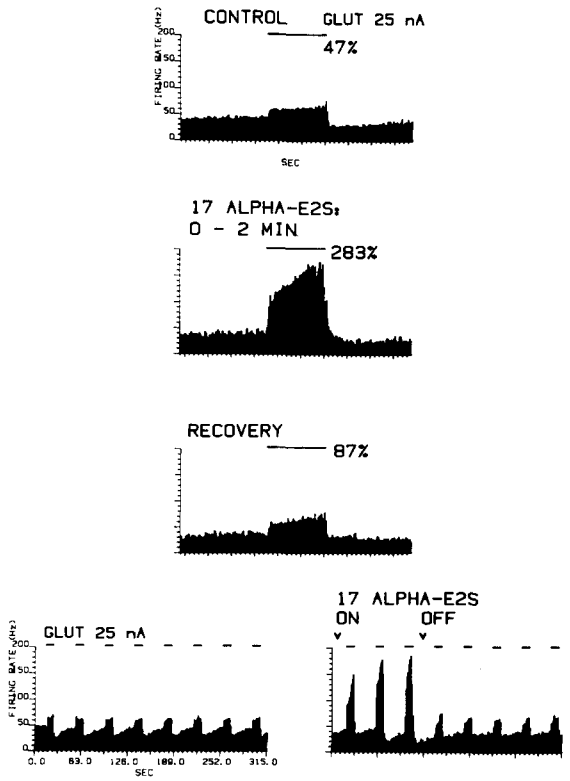

Fig. 5. 17α-Estradiol hemisuccinate increases P cell responsiveness to GLUT in a similar manner to succinate. Drug response histograms and ratemeter records (bottom panel) illustrate one case of GLUT potentiation by iontophoretic application of 17α-E_2S (17α-E_2S, 0.25 mM, pH 7.4). Histograms indicate the response of a P cell to pulsatile GLUT application (25 nA, 10 s pulses at 40 s intervals) during pre-steroid, continuous steroid application (0–2 min, 20 nA) and recovery periods. Direct application of 17α-E_2S resulted in an immediate increase in GLUT responsiveness from 47% to a 283% excitation. A partial recovery was observed within 1 min after termination of iontophoresis. In 4 of 10 cells examined 17α-E_2S resulted in this effect, in 1 cell it increased GLUT responsiveness with a longer latency, in 3 the steroid decreased GLUT responsiveness and in 2 there was no effect.

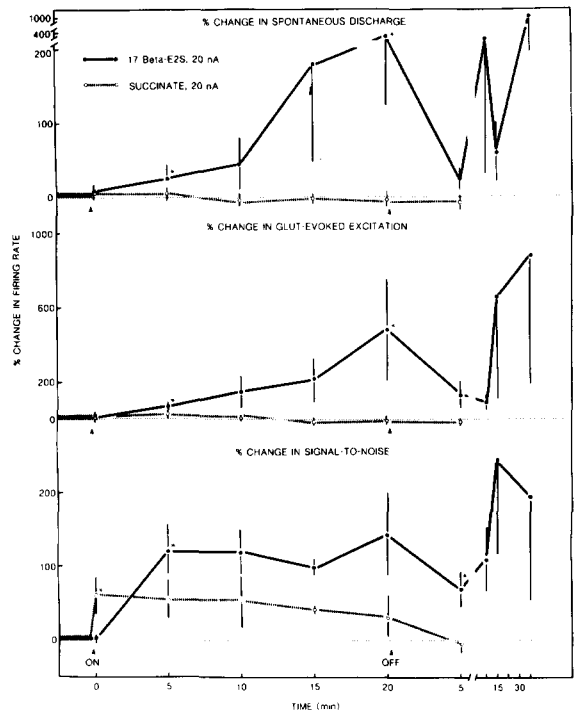

Fig. 6. Iontophoretic application of 17β-estradiol hemisuccinate results in increases in spontaneous, evoked and signal-to-noise activity that are of greater magnitude and slower time course than those elicited by succinate alone. This figure illustrates differences in percent change in spontaneous discharge (upper panel), percent change in GLUT-evoked excitation (middle panel) and percent change in the signal-to-noise ratio (evoked activity vs background) for P cells after local application of E_2S or succinate alone. Bars indicate S.E.M.; arrows indicate drug on (ON) and drug off (OFF). E_2S application resulted in a large percent change in spontaneous discharge (upper panel) by 5 min after the onset of drug application (significantly greater than succinate at 5 min and 20 min). In addition, E_2S application resulted in a proportionately greater increase in percent change of GLUT-evoked excitation (middle panel), again significantly larger than succinate at 5 and 20 min after drug onset. The most significant result, however, is found in the lower panel (percent change in signal-to-noise). In this case, succinate resulted in a percent change in signal-to-noise ratio 50 times greater than that produced by E_2S ($P < 0.001$) immediately (0–1 min) after drug onset. Within 5 min after drug application the percent change in signal-to-noise produced by E_2S application increased by more than 100% such that it was now significantly greater than that produced by succinate throughout the test period. In addition, although the succinate group achieved complete recovery by 5 min after drug termination, cells treated with E_2S had not achieved control levels of signal-to-noise by 35 min after drug termination. E_2S, $n = 20$ cells; succinate, $n = 12$ cells. $^*P < 0.05$.

the effect of E_2, although it altered the latency to response. Administration of tamoxifen, alone, using the same injection schedule, did not produce any alteration in spontaneous firing rate or GLUT responses of Purkinje cells (data not shown).

Effect of systemic estrogen administration on Purkinje cell responses to GABA application

In contrast to its effect on GLUT responsiveness E_2 produced variable effects on GABA responsiveness (Table II). Out of 14 cells tested, approximately 21.4% exhibited decreases in GABA responses relative to background ($\bar{X}$ = 58.4%) by 20–30 min after systemic administration of 17β-estradiol. 21.4% of the cells demonstrated no change in Purkinje cell responsiveness to GABA after injection of E_2, while

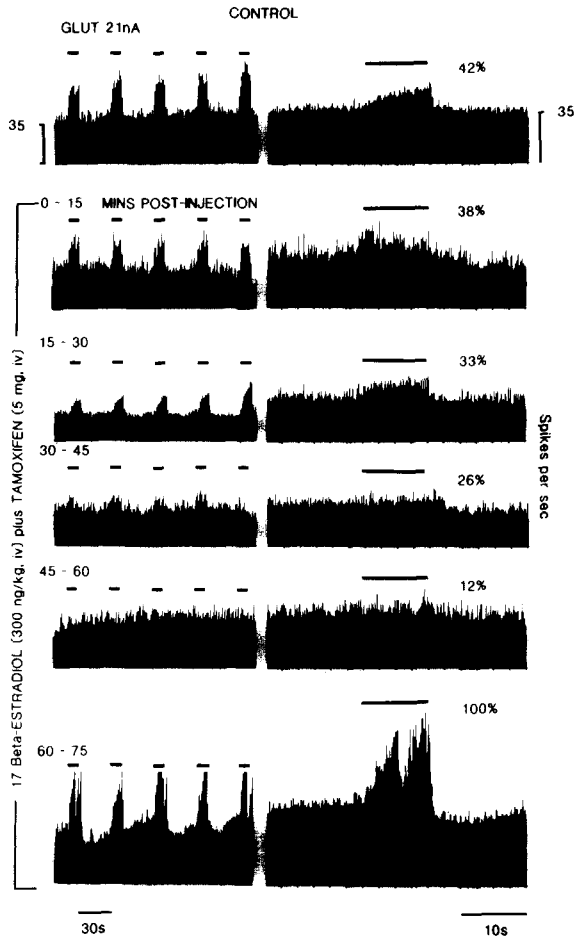

Fig. 7. I.v. tamoxifen does not prevent the effect of E_2-induced potentiation of GLUT responsiveness. As in the other figures, ratemeter records are displayed in the left column and corresponding dose–response histograms on the right. These records indicate that administration of an anti-estrogen, tamoxifen, does not prevent but delays until 60 min post-steroid the E_2-induced increase in GLUT responsiveness normally occurring 30 min after E_2 administration. Tamoxifen (5 mg, in saline) was administered in 2 doses 2 h prior to and concomitant with an i.v. injection of E_2 (300 ng/kg). This delay was apparent in 10 of 12 of the cases; in 2 of 12, the potentiating effect of E_2 on GLUT responsiveness was not observed.

57.1% exhibited an increase in GABA responsiveness ($\bar{X}$ = 69.2%), 10–15 min subsequent to E_2 administration (see Smith et al.[36] for examples of GABA potentiation in Purkinje cells).

Inconsistent effects were also noted after local application of E_2S (Table II). 26.3% of the cells tested responded to E_2S with an increase, 68.4% with a decrease and 5.3% with no change in background discharge. GABA response was increased in 42.1%, decreased in 52.6% and unchanged in 5.3% of the cells.

TABLE II

E_2-induced alteration in GABA responsiveness

Change relative to pre-steroid values (%)

Parameter	*Increase*		*Decrease*		*No change*	
	n	$\bar{X}$	*n*	$\bar{X}$	*n*	$\bar{X}$
Background discharge						
E_2, i.v.	5	12.3	5	20.5	4	1.8
E_2S, ionto.	5	41.2	13	12.2	1	1.1
GABA response						
E_2, i.v.	5	63.5	5	10.6	4	2.5
E_2S, ionto.	8	120.1	10	42.4	1	4.2
Evoked response: background						
E_2, i.v.	8	69.2	3	58.4	3	1.5
E_2S, ionto.	5	30.7	8	26.3	6	3.2

When the change in GABA-evoked inhibition relative to background firing rate was examined, 26.3% of the cells demonstrated an increase ($\bar{X}$ = 30.7%), 42.1% a decrease ($\bar{X}$ = 26.3%) and 31.6% no change.

DISCUSSION

The results from this study demonstrate that E_2, both systemically (at physiological doses) and locally administered, increases cerebellar Purkinje cell responsiveness to glutamate with a latency of 10–35 min. The present results also demonstrate that E_2 selectively alters neuronal responsiveness to a greater degree than background firing, suggesting that this hormone exerts modulatory actions at the synaptic level. A simple excitatory or inhibitory effect of the steroid would be expected to influence mean activity and evoked discharge to the same degree. At the highest dose, however, background discharge was also increased. In addition, E_2-induced potentiation of GLUT responsiveness appeared to be a relatively specific effect as the inactive 17α-isomer of estradiol only produced transient effects on Purkinje cell function at high doses. The observed E_2-induced neuromodulatory effect was also shown to be specific for the amino acid GLUT, as no consistent E_2–GABA interactions were observed.

Neuronal effects of systemic and local E_2

Besides the present study, a number of reports

have noted widespread activational effects of systemically administered E_2 on neuronal excitability, a phenomenon which has been demonstrated primarily in the hypothalamus[21] and, more recently, striatum[42]. In the hypothalamic slice preparation, pretreatment with E_2 has been shown to potentiate excitatory neuronal responses to afferent stimulation and selected neurotransmitter agents[23]. Local effects of E_2 on extrahypothalamic[12,27] neuronal excitability have also been noted. Although these studies clearly suggest a role for circulating and local E_2 in mediating increases in neuronal excitability, hormonal interactions with specific excitatory neurotransmitters have not been thoroughly established in the extrahypothalamic CNS.

In the present study, the potentiation of GLUT response observed after administration of 17β-estradiol hemisuccinate, iontophoresed onto the Purkinje cell, suggests that E_2 can alter neuronal responsiveness locally. Although, in the present study, succinate alone resulted in increases in glutamate responsiveness, these were of shorter latency and duration, as well as of lesser magnitude, than those obtained with E_2S which suggests that E_2S is exerting effects on the neuronal membrane independent of any succinate effect. This possibility is strengthened by recent preliminary results from our laboratory which demonstrate similar augmentation of GLUT responsiveness after local application of 17β-E_2 by pressure ejection[37]. In addition, only 50% of cells treated locally with 17α-E_2S demonstrated an increase in GLUT response, an effect which was transient and resembled the effect of succinate on this parameter rather than that of the 17β-isomer. This finding suggests that like the effect of i.v. E_2, the local effect of the steroid also appears to be relatively specific for the 17β-isomer.

Possible mechanisms of action

Classic actions of sex steroids are mediated through specific receptor binding sites and genomic mechanisms[30]. Although the rat cerebellum is not known to contain significant levels of cytosol/nuclear E_2 receptors[31], specific binding sites for E_2 have been localized in mouse cerebellum[11]. However, the present results indicate that the observed E_2-effects on neuronal responsiveness are of a different nature than classic E_2 effects on reproductive function, implying that a different mechanism of action for these effects may exist. First, doses of the 17α-isomer which have no effect on reproductive function produce modest, transient increases in GLUT responsiveness. The cytosol/nuclear E_2 receptor has only a very low affinity for the 17α-isomer, and administration of this isomer should not produce any effect if classic steroid receptor mechanisms were involved. Secondly, the anti-estrogen tamoxifen, which acts by binding to specific cytosol/nuclear E_2 receptors[20] and has been shown to prevent classic E_2 effects (i.e. the LH surge and lordosis) did not prevent E_2 effects on neuronal responsiveness. Finally, E_2 priming did not alter the magnitude or the direction of its effect on GLUT responses. In terms of neuroendocrine function, E_2-priming typically results in increased E_2 sensitivity[4], which may be due to the effect of positive cooperativity of E_2 binding to traditional cytosol/nuclear receptors. Thus, the observed E_2 effects do not appear to be mediated through classic steroid receptors.

In terms of specific mechanisms of action, it is our hypothesis that E_2 may be acting directly on the membrane to alter neurotransmitter function. E_2-induced alterations in local membrane permeability have been reported in liposomes[39]. In addition, a specific membrane receptor binding site for E_2 has been identified[41], and rapid, non-genomic effects of E_2 have been demonstrated on uterine microvilli[40] and hypothalamic neurons[22]. However, none of the existing reports directly addresses the possibility that E_2 could alter GLUT-mediated changes in ionic conductance. A second possibility is that the steroid is altering the degradation rate of GLUT, as previous studies have shown that E_2 can alter amino acid enzyme systems[26]. Therefore, a specific mechanism for the observed potentiation of GLUT responsiveness is not yet resolved.

Although not as likely, E_2–receptor interactions with afferent systems should not be overlooked. Several reports have shown that E_2 is localized autoradiographically to the locus coeruleus[18], the source nucleus for an extensive noradrenergic projection to the cerebellum. This could be a relevant interaction because NE potentiates GLUT responses of Purkinje neurons[33], and E_2 can alter noradrenergic turnover[6]. However, E_2 neuromodulatory effects are of greater magnitude than those of NE, suggesting that if

noradrenergic input is found to be a factor, then a combination of interactions may be involved.

Technical aspects of systemic steroid administration

Systemic injection, as utilized in the present study, is the most physiologic means of administration of sex steroids, as endogenously circulating E_2 would come into contact with cerebellar tissue in a similar manner through its dense vascular network. The doses used in this study, injected manually over a 2–3 min interval, were in the physiologic range when integrated over the experimental time period, as evidence indicates that exogenously administered steroids clear very rapidly from the blood and are reduced to barely detectable levels 5 min after i.v. injection due to uptake by target organs, fat stores and CNS areas[24]. Average blood levels of E_2, at a dose of 300 ng/kg, calculated over the entire 15–35 min latency period would be in the physiologic range of a proestrous animal (40–50 pg/ml). The lowest dose shown here to have a significant effect on GLUT response (100 ng/kg) would result in immediate blood values double those seen endogenously, physiologic levels after 2 min, and the average level integrated over a 5 min period would be of proestrous magnitude. Therefore, the doses tested produce physiologic blood levels of E_2 within the time frame necessary for the observed neuronal effect of the steroid.

The time course for the observed E_2 effect on neuronal responsiveness was shorter than observed for most classic reproductive effects of the steroid, but this effect occurred after both the peak and eventual decline in blood levels of the steroid. Although most of the classic actions of the steroid occur with latencies ranging from hours to days, much more rapid effects have been demonstrated in other systems. Genomic actions of sex steroids can be seen as early as 2 min after administration[32]. Non-genomic actions of E_2 have been reported msecs to secs after systemic injection[21,40], and the negative feedback effect of systemic E_2 on LHRH release can be observed within 15 min[43]. Certainly, E_2 would come into contact with cerebellar Purkinje cells very rapidly after systemic injection, as one complete pass through the circulatory system of the rat is accomplished in 6 s[7], the lipophilic sex steroid would cross the blood–brain barrier[1] and be taken up by cerebellar tissue within minutes as has been shown both in vivo[9] and in vitro[19] using radiolabelled E_2. Therefore, the observed latency of 10–35 min is reasonable for a steroid effect.

Summary

In summary, systemic, as well as locally applied, E_2 appears to exert long-lasting modulatory effects on neuronal function in an extrahypothalamic area of the CNS important for sensorimotor coordination and learning[25,29]. The observed E_2-induced increase in Purkinje cell responsiveness to glutamate suggests that endogenously fluctuating levels of this hormone may tend to increase the overall excitatory tone of neuronal circuits in the cerebellum. This effect would be consistent with the reported activating effects of the steroid on seizure activity[28] and sensorimotor function[2] (i.e. balance beam accuracy[38]). Although a direct causal relationship between these behaviors and alterations in GLUT response cannot at this time be concluded, the widespread nature of these steroid effects on sensorimotor behavior are consistent with a generalized action of the steroid in extrahypothalamic circuits.

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