Brain Research Bulletin, Vol. 20, pp. 151–155. © Pergamon Press plc, 1988. Printed in the U.S.A. 0361-9230/88 $3.00 + .00

Effects of Estradiol on Cerebral Cortical Neurons and Their Responses to Adenosine

JOHN W. PHILLIS AND MICHAEL H. O'REGAN

Department of Physiology, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI 48201

Received 7 August 1987

PHILLIS, J. W. AND M. H. O'REGAN. *Effects of estradiol on cerebral cortical neurons and their responses to adenosine.* BRAIN RES BULL **20**(2) 151–155, 1988.—The effects of iontophoretically applied 17 α- and 17 β-estradiol on the spontaneous firing of, and actions of purines (adenosine, adenosine 5'-N-ethylcarboxamide) on, neurons in the rat cerebral cortex have been determined. Both steroids, applied as acetate, sulphate or hemisuccinate esters, depressed the spontaneous firing of approximately half of the neurons tested. 17 β-Estradiol potentiated the inhibitory actions of adenosine on some neurons (26%) and antagonized the effects of adenosine on others (42%). 17 β-Estradiol antagonized the inhibitory actions of adenosine 5'-N-ethylcarboxamide on 76% of the neurons tested, but did not enhance the actions of this uptake resistant adenosine analog. 17 α-Estradiol potentiated the actions of adenosine and adenosine 5'-N-ethylcarboxamide but failed to antagonize either purine. It is suggested that the potentiating effects of both stereoisomers on adenosine are a result of their ability to block adenosine uptake and that 17 β-estradiol is also able to antagonize the actions of these purines. Antagonism of the effects of endogenously released adenosine may account for the excitant actions of 17 β-estradiol on the central nervous system.

Estradiol Cerebral cortical neurons Adenosine

THE actions of gonadal steroid hormones on neural mechanisms underlying reproductive behavior are well known. Recently, it has been recognized that these hormones may also affect nonreproductive behaviors [2], but these underlying neural mechanisms are poorly understood. Thus, while many of the behavioral effects of estrogen may be a result of its regulation of gonadotropin secretion [16], which can affect behavior indirectly, a second possibility may be that estrogenic hormones have neuropharmacological activities that directly influence behavior.

The influence of ovarian hormones on the excitability of the central nervous system has long been the object of considerable interest amongst neuropharmacologists. Since the pioneering studies of Woolley and Timiras [28,29], it has been known that estradiol can decrease electroshock seizure threshold in rats in a dose-dependent manner and that the threshold for the induction of electroshock convulsions in female rats is lowest during estrus. Furthermore, electroencephalographic seizures can be induced in cats by the topical application of estrogen onto the cerebral cortex [8]. Conversely, progesterone has been shown to increase electroshock seizure thresholds in animals [27]; decrease the frequency of interictal spikes from a penicillin focus in the cat cortex [11]; and have sedative/hypnotic and anticonvulsant actions in humans [1,14].

In earlier reports, it has been suggested that the emotional symptoms associated with the luteal phase of the ovarian cycle could result from an enhancement by progesterone of the inhibitory effects of endogenously released adenosine on neuronal excitability [18,19]. The objective of the present investigation was to explore the possibility that the excitant effects of estrogens on central neurons might result from antagonism of inhibitory purinergic tone.

METHOD

Experiments were performed on eight male Sprague-Dawley rats (350–400 g). The animals were anesthetized with halothane, and after insertion of a tracheal cannula, anesthesia was maintained with a mixture of methoxyflurane, nitrous oxide (70%) and oxygen. The animals were placed in a stereotaxic frame and body temperature was maintained at 37°C via an electric heating pad controlled by a rectal thermal probe. After reflection of the skin overlying the dorsal skull, a small hole was drilled through the parietal bone 2 mm lateral to the sagittal suture and 1.5 mm posterior to the coronal suture line. This hole allowed access to the sensorimotor cortex after a slit had been made in the dura mater. The exposed skin, muscle and bone were covered with a layer of 4% agar in Ringer solution to prevent drying and stabilize the cortical surface.

Seven barrelled micropipettes, constructed from fiber-fill glass tubes, were used to record the extracellular action potentials of deep (900–1400 μm) spontaneously firing cerebral cortical neurons. The central recording barrel was filled with 2 M NaCl. One side barrel, which was used for current neutralization by the Dagan polarizer, was also filled with 2 M NaCl. The remaining barrels, not all of which were necessarily used, were filled with various combinations of the fol-

TABLE 1

EFFECTS OF VARIOUS ESTRADIOL ESTERS ON CELL FIRING AND ON THE RESPONSES TO ADENOSINE OR NECA

Steroid	Effect On Spontaneous Firing			Responses to Adenosine			Responses to NECA		
	Inc.	Dec.	Nil.	Potentiated	Antagonized	Nil.	Potentiated	Antagonized	Nil.
17-β-estradiol-3-sulphate	0/22*	17/22	5/22	5/7†	0/7	2/7	0/15	12/15	3/15
17-β-estradiol-17-hemisuccinate	4/33	10/33	19/33	2/20	13/20	5/20	0/13	10/13	3/13
17-β-estradiol-17-acetate	2/9	1/9	6/9	1/4	0/4	3/4	0/5	3/5	2/5
	6/64 (9%)	28/64 (44%)	30/64 (47%)	8/31 (26%)	13/31 (42%)	10/31 (32%)	0/33 (0%)	25/33 (76%)	8/33 (24%)
17-α-estradiol-3-acetate	4/28	10/28	14/28	8/12	0/12	4/12	3/16	0/16	13/16
17-α-estradiol-3-sulphate	0/20	14/20	6/20	3/10	0/10	7/10	5/10	0/10	5/10
	4/48 (8%)	24/48 (50%)	20/48 (42%)	11/22 (50%)	0/22 (0%)	11/22 (50%)	8/26 (31%)	0/26 (0%)	18/26 (69%)

*Figures indicate number of neurons showing specific response/total number of neurons tested.

†The seven neurons listed for adenosine were also tested with NECA. NECA was antagonized on all seven neurons.

A Chi-Square analysis for homogeneity indicates that the effects of 17-β-estradiol are significantly different from those with 17-α-estradiol for both adenosine ($p<0.01$) and NECA ($p<0.001$).

lowing compounds: adenosine hemisulphate (0.1 M, pH 4.5); acetylcholine chloride (0.1 M, pH 5.2); adenosine 5'-N-ethylcarboxamide (NECA; 0.002 M in 75 mM NaCl, pH 6.5); 17-β-estradiol-3-sulphate (Sigma, 0.01 M, pH 6.5); 17-β-estradiol-17-hemisuccinate (Sigma, <0.005 M, pH 6.7); 17-β-estradiol-17-acetate (Research Plus Labs., <0.005 M, pH 6.2); 17-α-estradiol-3-acetate (Research Plus Labs., <0.005 M, pH 6.7); 17-α-estradiol-3-sulphate (Research Plus Labs., 0.02 M, pH 6.7). Esters of 17α- and 17 β-estradiol were used because of their greater solubility in aqueous solution than the parent compounds. Even so, with the exception of the 3-sulphate derivatives, the esters were relatively insoluble and saturated solutions were used to fill the electrode barrels. The high resistances of those barrels containing the steroids limited the amount of current that could be passed through individual barrels, and it was frequently necessary to fill 2 barrels with the same steroid and then pass small currents through both barrels simultaneously. All of the substances used were applied with cation ejecting currents. The steroid applications likely resulted from electroosmotic ejection of the solution in which they were dissolved, rather than from iontophoresis. Steroid effects were evaluated by observing changes in the rate of spontaneous firing of cortical neurons or by monitoring their effects on the depressant actions of adenosine and/or NECA. In most instances, adenosine and NECA were not included in the same electrode.

Adenosine (or NECA) was applied repetitively by 12–20 sec pulses of current at interpulse intervals of 90–240 sec. When responses to adenosine had stabilized, a steroid was applied concurrently for periods of up to 7 min. The magnitude and duration of adenosine (or NECA) control responses could then be compared with those evoked during and following estradiol application. For evaluative and statistical purposes, the response of a cell was considered to be either potentiated or antagonized by estradiol when the duration of purine-evoked depression in spontaneous firing was increased or decreased by more than 20% compared to the controls. A Student's *t*-test for paired data was used to determine the significance of changes in response duration. A Chi-Square test for homogeneity [24] was used to analyze the difference between the effects of 17 β- and 17 α-stereoisomers of estradiol on responses to adenosine and NECA.

RESULTS

The effects of 17 β-estradiol were studied on 64 neurons (Table 1). The most frequently observed responses were either a decrease in the rate of spontaneous firing (28 neurons, 44%) or a lack of change in the rate of firing (30 neurons, 47%). An increase in the rate of firing was observed with only 6 neurons (9%). With the rather small currents that could be passed through the steroid containing barrels, neither the depressions nor excitations were pronounced and firing returned to control levels within a few minutes of the cessation of steroid application.

Rather similar observations were recorded when 17 α-estradiol was present in the micropipette barrels. The most frequent response was a weak depression of spontaneous firing (24 neurons, 50%), followed by no change in frequency of firing (20 neurons, 42%) and a weak excitation (4 neurons, 8%). As with the 17 β-estradiol the depressant or excitant actions were not pronounced, with recovery occurring within a few minutes of the end of steroid application.

For tests of their interactions with purine-evoked depressions, the steroid application currents were adjusted to have a minimal effect on spontaneous firing rates. Adenosine (and NECA) depresses the spontaneous firing of cerebral cortical

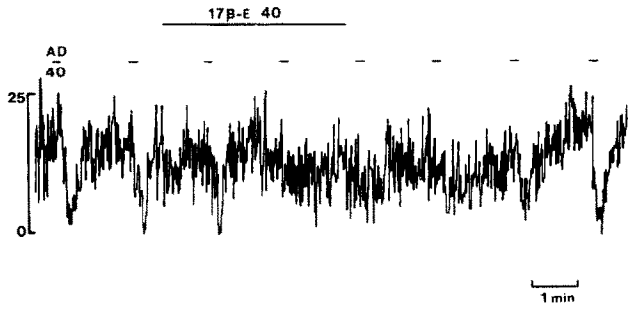

FIG. 1. Firing frequency record of a rat cerebral cortical neuron. This is a rate meter recording with the number of action potentials per second on the ordinate scale. Horizontal bars above the recordings indicate periods of drug application. Adenosine (AD, 40 nA) reduced the rate of firing. During an application of 17 β-estradiol hemisuccinate (17 β-E, 40 nA) the effects of adenosine were reduced. Recovery of the adenosine responses had occurred 5 min after the 17 β-estradiol application.

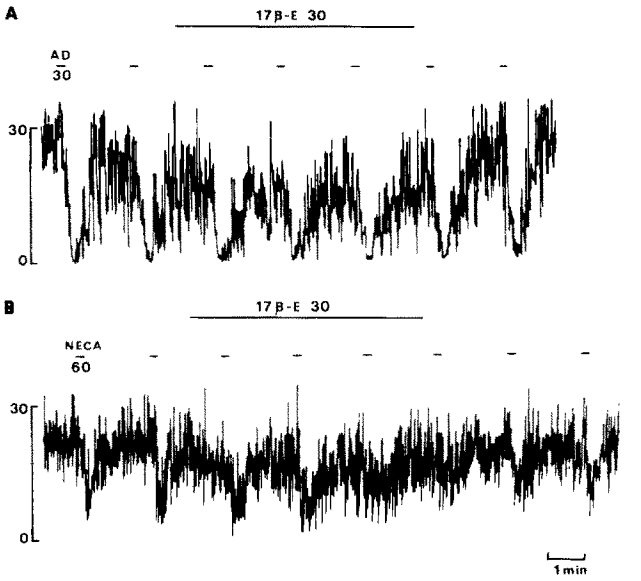

FIG. 2. (A and B) Rate meter recordings from the same rat cerebral cortical neuron. 17 β-Estradiol-3-sulphate (30 nA) had a weak depressant action on firing. Adenosine (30 nA)-evoked depressions of firing were potentiated in magnitude and duration by steroid application (Fig. 2A), whereas NECA (60 nA)-evoked depressions were antagonized (Fig. 2B).

neurons. Once the responses to adenosine had stabilized, β-estradiol was applied concurrently and the changes in magnitude and duration of the purine-evoked inhibition observed.

17 β-Estradiol was tested as three different esters and the results for each are summarized in Table 1. When the results with all three compounds were accumulated, the actions of adenosine were potentiated in amplitude and/or duration (duration of control depressions 34.3±1.8 sec, depressions with 17 β-estradiol application 47.0±2.7 sec, $p<0.001$) on 26% of the neurons tested; antagonized on 42% (duration of control depressions 37.3±0.9 sec, depressions with 17 β-estradiol application 22.5±0.7 sec, $p<0.001$) and unaffected on 32%. Potentiation of adenosine depressions was of relatively short duration, with recovery occurring after 5–10 min. Antagonism of adenosine had an even shorter duration after the application of steroid ended, with recovery apparent within 3–5 min. Examples of adenosine antagonism and potentiation by 17 β-estradiol are presented in Figs. 1 and 2A. Adenosine (40 nA) depressed the spontaneous firing of the neuron illustrated in Fig. 1. 17 β-Estradiol hemisuccinate (40 nA) was then applied for a period of 4 min. During this period cell firing frequency was reduced slightly and the responses to adenosine were partially antagonized. Antagonism was still evident during the initial two adenosine applications after the cessation of the 17 β-estradiol application, but then recovery rapidly followed. 17 β-Estradiol-3-sulphate enhanced the depressant effects of adenosine on the neuron presented in Fig. 2A; as it did on the majority of neurons tested with this relatively soluble ester. Potentiation of adenosine-evoked inhibitions is evident both during and following steroid application.

The effects of 17 β-estradiol on NECA-evoked inhibition of cortical cell firing revealed a different pattern of interactions. NECA is a stable analog of adenosine, which is not readily carried across cell membranes by the adenosine transporter [3,30]. 17 β-Estradiol failed to potentiate NECA-evoked inhibitions on any of the 33 neurons tested. However, the steroid antagonized NECA on 76% of these neurons (control duration 47.8±2.5 sec vs. 26.3±2.7 sec, $p<0.001$). An example of this antagonism is presented in Fig. 2B, recorded from the same neuron as Fig. 2A.

17 α-Estradiol (as the -3-acetate and -3-sulphate esters) was tested on 48 neurons. Its effects were similar to those observed with the 17 β-steroid in that the majority of affected neurons underwent a reduction in firing rate. Only a few instances of weak excitation were observed. In contrast to 17 β-estradiol, the 17 α-compound failed to antagonize the depressant actions of either adenosine or NECA on any of the 48 neurons tested. Potentiation of adenosine's depressant action was observed on 50% of the neurons tested (control duration 38.0±1.3 sec vs. 51.2±3.6 sec, $p<0.001$) and a potentiation of NECA-evoked inhibitions was apparent on 8 neurons (control duration 40.9±1.7 sec, vs. 54.8±3.6 sec). An example of adenosine potentiation by 17 α-estradiol-3-acetate is presented in Fig. 3. The magnitude and time course of the enhancement of adenosine's depressant actions by 17 α-estradiol was similar to that observed with the 17 β-steroid.

DISCUSSION

Estrogens have been reported to have both excitant and sedative effects on the central nervous system. Selye [26] attributed weak anesthetic actions to 17 α-estradiol, as well as to the hormonally active estrogens such as estrone and estriol. Subsequently, 17 β-estradiol was recognized as having an excitant action on the CNS, manifested as a reduction in electroshock seizure thresholds following its administration, and during the estrous phase of the reproductive cycle [28,29]. Estrogens have significant epileptogenic activity when applied directly onto the cerebral cortex [8,12] and have been observed to increase spike frequency in epileptic patients [12]. Conversely, 17 β-estradiol has been described as having a hypnogenic action when injected intracerebroventricularly in rabbits [17].

Women characteristically attribute feelings of "wellbeing," "energetic," "active" etc. to the late follicular phase

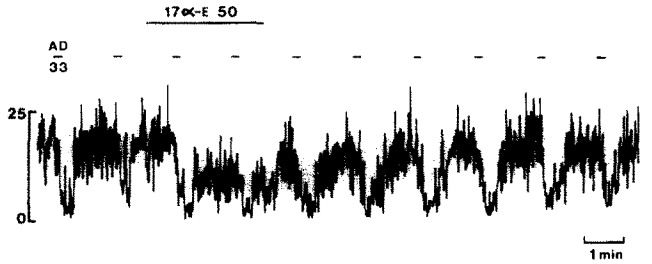

FIG. 3. 17 α-Estradiol-3-acetate (50 nA) reduced the spontaneous firing and potentiated the depressant actions of adenosine (33 nA) on the rat cerebral cortical neuron.

of the menstrual cycle, in contrast to the "fatigue" and "depression" associated with the luteal phase of the cycle [15,25]. It is therefore apparent, that the presence of physiological levels of 17 β-estradiol is associated with behavioral and neurophysiological manifestations of elevated brain excitability, whilst at higher concentrations the estrogenic hormones can elicit a sedative type of activity, a property which they share with 17 α-estradiol. The latter has been described as a weak or inactive estrogen since it cannot promote true uterine growth when compared to 17 β-estradiol and has a relative uterotropic potency less than 17 β-estradiol [7,10]. The 17 α-isomer is often used as a negative control in experiments to demonstrate estrogen-specificity of steroid elicited actions.

A generally accepted concept has been that the hormonal actions of 17 β-estradiol are mediated via RNA-dependent protein synthesis. This mechanism of action has been established for the uterus and was adopted for steroid action on hypothalamic neurons [13]. Therefore, long term modulation of genome activity was assumed to be the primary mechanism of gonadal feedback control of the hypothalamic-pituitary axis. However, microiontophoretic studies of the effects of estrogen on hypothalamic unit activity revealed excitatory and inhibitory effects of the steroid on medial preoptic-septal rat neurons, which occurred within seconds of the onset of application [9]. Comparable, short latency, excitatory or inhibitory effects were observed when 17 β-estradiol was pressure ejected from micropipettes onto septopreoptic neurons [23]. This rapid effect of iontophoretically applied estrogen on spontaneous activity suggested that estrogenic influences on nerve cells might occur through membrane-mediated actions, in addition to modification of genomic activity [16].

Estrogenic effects on neuronal activity are not limited to hypothalamic cells. Evidence has been cited above for an epileptogenic effect of topically applied estrogens on the in situ cerebral cortex and 17 β-estradiol has demonstrable excitant actions on pyramidal cells in a hippocampal slice preparation, increasing the amplitude of synaptically evoked field potentials [5]. The peak excitant effect of the addition of 17 β-estradiol occurred at a concentration of 100 pM, and at higher concentrations the steroid depressed neuronal activity. Once again, the effects were rapid in onset, involving actions at the cell membrane. At a concentration of 100 pM, 17 α-estradiol failed to enhance field potentials in the hippocampal slice [4].

The insolubility of 17 β-estradiol in the aqueous solutions used for iontophoretic experiments has led investigators to use steroid esters in their experiments [9]. Esterases are present in the extracellular space [9] but it is not clear if the changes in responses observed in the present experiments are due to the free (17 β-estradiol) steroid and/or to its ester. To eliminate any possibility that the acid used in the esterification process contributed to the effects of the steroid, we have used three different esters of 17 β-estradiol, and two of 17 α-estradiol. The specific composition of the ester does not appear to have contributed to the overall tenor of the results.

17 β-Estradiol depressed the spontaneous firing of 44% of the neurons tested and enhanced the firing of 9%. Similar results were observed with 17 α-estradiol which depressed the spontaneous firing of 50% of the neurons tested; exciting 8%. Neither depressant (nor excitant) actions were pronounced with the small application currents which could be passed through the high-resistance steroid-containing electrodes.

In confirmation of our earlier findings [19], 17 β-estradiol was observed to potentiate the depressant effects of adenosine on cortical neurons. This effect was observed with all three esters and was most pronounced with the -3-sulphate compound. 17 β-Estradiol is a potent inhibitor of adenosine uptake into rat cerebral cortical synaptosomes [19,22] and a reduction in the rate of sequestration of exogenously applied adenosine into brain cells would account for this potentiation of its action. This conclusion is supported by our observation that the depressant action of NECA, an uptake resistant analog of adenosine [3,30] was not enhanced by the 17 β-estradiol esters. Adenosine evoked inhibitions were antagonized by 17 β-estradiol hemisuccinate, although this was not apparent with the other two esters. All three esters, however, effectively antagonized the depressant effects of NECA on the majority of neurons tested. It is likely that the apparent lack of antagonism of adenosine on many neurons was a result of conflicting potentiative and antagonistic interactions.

Two esters of 17 α-estradiol were utilized. Both potentiated the inhibitory actions of adenosine, and to a lesser extent NECA. No antagonism of the effects of either adenosine or NECA was apparent. 17 α-Estradiol also inhibits the transport of adenosine by rat cerebral cortical synaptosomes although less potently than the 17 β-stereoisomer [19], and this action could account for its potentiation of adenosine-evoked inhibitions. The enhancement of NECA-elicited depressions is less readily explained, but may have resulted from a summation of NECA's action with those of accumulated endogenously released adenosine.

The important observation arising from the present study is that whilst both 17 α- and 17 β-stereoisomers of estradiol can depress the firing of rat cerebral cortical neurons and potentiate the depressant actions of adenosine, only the 17 β-isomer possesses the ability to antagonize the actions of adenosine and, especially, that of its uptake resistant analog NECA. The stereoselectivity of NECA-antagonism offers an explanation for the excitant actions of 17 β-estradiol on pyramidal neurons in the hippocampal slice [4]. Adenosine antagonists are known to have excitant actions on the hippocampal slice preparation, which have been attributed to the antagonism of endogenously released adenosine [6]. The excitant actions of 17 β-estradiol may therefore have a similar origin. Higher concentrations of 17 β-estradiol, by inhibiting the re-uptake of adenosine, would have a depressant action on cell excitability [4].

The actions of 17 β-estradiol described in this report bear some similarities to those of certain benzodiazepine receptor ligands, which appear to share the ability to both antagonize and potentiate adenosine. Ro 5-4864 and Ro 15-1788 possess

both anxiolytic-sedative and anxiogenic-proconvulsant activities, which are manifested in the appropriate dosage and species. Both compounds are able to antagonize and potentiate adenosine, [20,21] and, as with 17 β-estradiol, it was necessary to use the uptake resistant analog of adenosine, NECA, to clearly demonstrate the antagonism. Thus, it appears possible that, with various substances, recognition by the transporter site may go hand-in-hand with recognition at the appropriate adenosine receptor site. The pharmacological actions of such compounds on central neurons would ultimately depend on their affinity for, and efficacy at, the two sites and, for endogenously occurring compounds such as 17 β-estradiol, their concentrations in the interstitial fluid. Thus, whereas 17 β-estradiol concentrations in the extracellular fluid of the brain are unlikely to reach levels at which the activity of the adenosine transporter is compromised, they may be sufficient to antagonize the action of endogenously released adenosine, exerting a stimulatory action on central nervous function. Such an action could account for the emotional feelings attributed to the follicular phase of the menstrual cycle.

ACKNOWLEDGEMENT

Supported by NIH grant RR 08167-08.

REFERENCES

1. Bäckström, T., B. Zetterlund, S. Blom and M. Ramano. Effects of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. *Acta Neurol Scand* **69:** 240–248, 1984.
2. Beatty, W. W. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. *Horm Behav* **12:** 112–163, 1979.
3. Ehinger, B. and M. T. R. Perez. Autoradiography of nucleoside uptake into the retina. *Neurochem Int* **6:** 369–381, 1984.
4. Foy, M. R. and T. J. Teyler. 17-α-Estradiol and 17-β-estradiol in hippocampus. *Brain Res Bull* **10:** 735–739, 1983.
5. Foy, M. R., T. J. Teyler and R. M. Vardaris. Δ^9-THC and 17-β-estradiol in hippocampus. *Brain Res Bull* **8:** 341–345, 1982.
6. Fredholm, B. B., T. V. Dunwiddie, B. Bergman and K. Lindstrom. Levels of adenosine and adenine nucleotides in slices of rat hippocampus. *Brain Res* **295:** 127–136, 1984.
7. Huggins, C., E. V. Jensen and A. S. Cleveland. Chemical structure of steroids in relation to promotion of growth of the vagina and uterus of the hypophysectomized rat. *J Exp Med* **100:** 225–240, 1954.
8. Julian, R. M., G. W. Fowler and M. G. Danielson. The effects of antiepileptic drugs on estrogen-induced electrographic spike-wave discharge. *J Pharmacol Exp Ther* **193:** 647–656, 1975.
9. Kelly, M. J., R. L. Moss, C. A. Dudley and C. P. Fawcett. The specificity of the response of preoptic-septal area neurons to estrogen: 17 α-estradiol versus 17 β-estradiol and the response of extrahypothalamic neurons. *Exp Brain Res* **30:** 43–52, 1977.
10. Korenman, S. G. Comparative binding affinity of estrogens and its relation for estrogenic potency. *Steroids* **13:** 163–177, 1969.
11. Landgren, S., T. Backstrom and G. Kalistratov. The effect of progesterone on the spontaneous interictal spike evoked by the application of penicillin to the cat's cerebral cortex. *J Neurol Sci* **36:** 119–133, 1978.
12. Logothetis, J., R. Harner, F. Morrell and F. Torres. The role of estrogens in catamenial exacerbation of epilepsy. *Neurology* **9:** 352–360, 1959.
13. McEwen, B. S., R. E. Zigmond and J. L. Gerlach. Sites of steroid binding and action in the brain. In: *The Structure and Function of Nervous Tissue,* edited by G. Bourne. New York: Academic Press, 1972, pp. 205–291.
14. Merryman, W., R. Boiman, L. Barnes and I. Rothchild. Progesterone "anesthesia" in human subjects. *J Clin Endocrinol Metab* **14:** 1567–1569, 1954.
15. Moos, R. H., B. S. Kopell, F. T. Melges, I. D. Yalom, D. T. Lunde, R. B. Clayton and D. A. Hamburg. Fluctuations in symptoms and moods during the menstrual cycle. *J Psychosom Res* **13:** 37–44, 1969.
16. Moss, R. L. and C. A. Dudley. Molecular aspects of the interaction between estrogen and the membrane excitability of hypothalamic cells. *Prog Brain Res* **61:** 3–21, 1984.
17. Paisley, A. C. and A. J. S. Summerlee. Relationships between behavioral states and activity of the cerebral cortex. *Prog Neurobiol* **22:** 155–184, 1984.
18. Phillis, J. W. Potentiation of the depression by adenosine of rat cerebral cortical neurones by progestational agents. *Br J Pharmacol* **89:** 693–702, 1986.
19. Phillis, J. W., A. S. Bender and W. Marszalec. Estradiol and progesterone potentiate adenosine's depressant action on rat cerebral cortical neurones. *Gen Pharmacol* **16:** 609–612, 1985.
20. Phillis, J. W., M. W. O'Regan and R. E. Stair. Adenosine potentiation and antagonism may account for the diverse behavioral actions of Ro 5-4864. *Brain Res* **416:** 171–174, 1987.
21. Phillis, J. W. and R. E. Stair. Ro 15-1788 both antagonizes and potentiates adenosine-evoked depression of cerebral cortical neurons. *Eur J Pharmacol* **136:** 151–156, 1987.
22. Phillis, J. W. and P. H. Wu. The role of adenosine and its nucleotides in central synaptic transmission. *Prog Neurobiol* **16:** 187–239, 1982.
23. Poulain, P. and B. Carette. Pressure ejection of drugs on single neurons *in vivo:* Technical considerations and application to the study of estradiol effects. *Brain Res Bull* **7:** 33–40, 1981.
24. Roscoe, J. T. *Fundamental Research Statistics for the Behavioral Sciences.* New York: Holt, Rinehart and Winston, 1975.
25. Sanders, D., P. Warner, T. Backstrom and J. Bancroft. Mood, sexuality, hormones and the menstrual cycle. I. Changes in mood and physical state: description of subjects and method. *Psychosom Med* **45:** 487–501, 1983.
26. Selye, H. Studies concerning the correlation between anesthetic potency, hormonal activity and chemical structure among steroid compounds. *Anesth Analg* **21:** 41–47, 1942.
27. Spiegel, E. and H. Wycis. Anticonvulsant effects of steroids. *J Lab Clin Med* **30:** 947–953, 1945.
28. Woolley, D. E. and P. S. Timiras. The gonad-brain relationship: effects of female sex hormones on electroshock convulsions in the rat. *Endocrinology* **70:** 196–209, 1962.
29. Woolley, D. E. and P. Timiras. Estrous and circadian periodicity and electroshock convulsions in rats. *Am J Physiol* **202:** 379–382, 1962.
30. Wu, P. H., R. A. Barraco and J. W. Phillis. Further studies on the inhibition of adenosine uptake into rat brain synaptosomes by adenosine derivatives and methylxanthines. *Gen Pharmacol* **15:** 251–254, 1984.