Pharmacology Biochemistry & Behavior, Vol. 23, pp. 501–504, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Effect of Progesterone Upon Adenylate Cyclase Activity and cAMP Levels on Brain Areas

M. L. COLLADO, G. RODRÍGUEZ-MANZO AND M. L. CRUZ

Sección de Neuroendocrinología, División de Neurociencias, Unidad de Investigación Biomédica, CMN Instituto Mexicano del Seguro Social, Apartado Postal 73-032, México 03020, D. F.

Received 8 November 1984

COLLADO, M. L., G. RODRÍGUEZ-MANZO AND M. L. CRUZ. *Effect of progesterone upon adenylate cyclase activity and cAMP levels on brain areas.* PHARMACOL BIOCHEM BEHAV **23**(4) 501–504, 1985.—Changes induced by progesterone on adenylate cyclase activity and cAMP levels were determined in brain areas involved in the integration of sexual behavior in ovariectomized estradiol benzoate primed rats. Adenylate cyclase and cAMP concentrations were assayed in: preoptic area, ventromedial hypothalamus and cerebral cortex. Our results show a significant increase in both enzyme activity and cAMP levels at 2 and 4 hours after progesterone administration in all brain areas studied. These data suggest that the facilitatory effect of progesterone on sexual behavior involves an activating mechanism of this steroid upon the adenylate cyclase enzyme which results in an intraneuronal cAMP enhancement.

Progesterone Adenylate cyclase cAMP Brain areas Lordosis response

LORDOSIS behavior is induced by the sequential action of estrogen (E) and progesterone (P). The cellular events underlying the E-P interaction on lordosis behavior are not well understood. Estrogen stimulates the synthesis of protein molecules [14] and according to a model recently suggested by Beyer *et al.* [1], the majority of these proteins are in an inactive state and P would facilitate lordosis by stimulating their phosphorylation through a cAMP dependent phosphokinase. The following observations support this hypothesis: (a) administration of cAMP analogues stimulate lordosis in estrogen primed rats [3], (b) treatment with chemicals directly or indirectly activating adenylate cyclase (E.C.4.6.1.1) substitute for P in inducing lordosis (guanine nucleotides, forskolin, cholera toxin) [4, 7, 8, 10], and (c) administration of phosphodiesterase inhibitors potentiate the effect of P in stimulating lordosis [5].

There is no evidence showing that P influences an adenylate cyclase cAMP system in the brain. Therefore, this study was designed to investigate the effects of this steroid on the adenylate cyclase-cAMP activity of estrogen-primed rats.

Changes in adenylate cyclase activity and cAMP levels after P are also correlated with the display of lordosis behavior of ovariectomized estrogen primed rats.

METHOD

Eighty adult female Wistar rats (200–250 g weight), were kept in isolated cages, fed with Purina and water ad lib and maintained in a room at 23°C with a reversed light cycle (14 hr light/10 hr dark). The rats were ovariectomized and twenty days later injected with 4 μg of estradiol benzoate (EB). This dose of EB has been found to be ineffective in stimulation of lordosis behavior in our ovariectomized rats. A single injection of 4 mg of progesterone (P), or oil was given 44 hr after EB. The animals were divided into four groups of 20 rats. Group 1 as control: the rats were injected with oil at 44 hr after EB. Group 2–4: the animals were injected with P at 44 hr after EB.

The rats were tested for lordosis behavior at 1 (group 2), 2 (group 3) and 4 (group 4) hours after P. Tests for lordosis behavior were made in a circular observation cage (Plexiglas) of 53 cm diameter. Sires were vigorous males. Each female received 10 vigorous mounts per test. Receptivity of the females was quantified by the lordosis quotient (LQ = lordosis/10 mounts). Immediately after the conductual test, the rats were sacrificed by decapitation (not by microwave irradiation which inactivates enzymatic activities [31]) and the head immediately frozen in liquid nitrogen, to prevent the increase of nucleotides levels.

The brain was removed and the hypothalamus was separated by a frontal section at the level of optic chiasma, a caudal section through the mammillary bodies and the lateral sections of the hypothalamic fissures. A horizontal section, at 2 mm from the base of the brain, separated the hypothalamus from the rest of the brain. This block of tissue was then divided into two portions by a frontal cut placed at the frontal level of the median eminence; the anterior portion contained the continum preoptic area-anterior hypothalamus (POA) and the posterior portion contained the medial and posterior hypothalamus (HVM). A portion of the motor cortex (Cx) was also dissected and processed.

Tissues were cold homogenized at 30% w/v in a buffer (Tris-HCl, 40 mM, pH = 7.4) containing theophylline 10 mM. The homogenate was divided into three aliquotes: one used for protein determination, one for the enzymatic activity

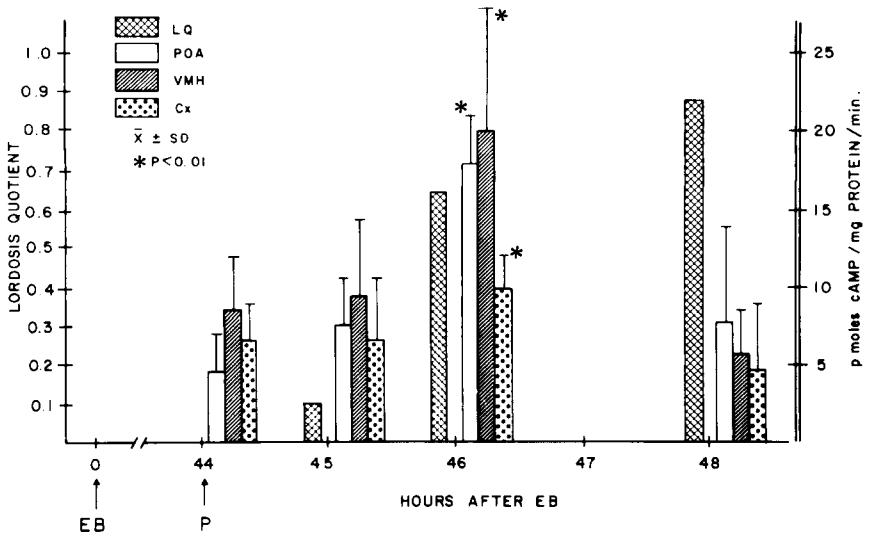

FIG. 1. Brain adenylate-cyclase activity changes induced by sex hormones. The LQ and the adenylate cyclase activity in different brain structures of ovx rats injected with estradiol benzoate (EB) followed 44 hr later by progesterone (P). The animals were tested for lordosis and thereafter sacrificed for biochemical determinations. *Student's *t*-test.

assay, and the last one was stored at −70°C until the determination of cAMP was performed.

Assay of Proteins With the Homogenate Preparation

In all homogenates, proteins were determined by the method of Lowry, [19] and were used as a reference parameter.

Assay of Adenylate Cyclase Activity With Homogenate Preparation

The enzyme activity was determined by the method of Krishna *et al.* [16]. The reaction mixture contained 40 mM Tris-HCl (pH = 7.4), 5 mM $MgSO_4$, 10 mM theophylline, 10 mM NaF, 2 mM ATP (enzyme substrate) and enzyme (homogenate containing 0.06–0.3 mg of proteins) in a total volume of 0.2 ml. After incubation of 5 minutes at 30°C, the reaction was stopped by boiling 3 minutes, and the samples were centrifuged at 3,000 × g for 10 minutes. In one 50 μl aliquote of supernatant, the concentration of cAMP formed was measured in competition with addition of labeled cAMP, and radioassayed by the method of Steiner [32] using the cAMP-Ria Kit of Amersham, England. All values were related per mg of protein per min.

Assay of cAMP Levels With Homogenate Preparation

The homogenate was boiled for 3 minutes, in order to coagulate proteins, and centrifuged at 3,000 × g for 10 min. The cAMP determined in the supernatant by radioimmunoassay following the method of Steiner [32] using the cAMP-Ria Kit of Amersham, England.

Data obtained for LQ were analyzed by the Mann-Whitney U test, and values obtained for AC activity and cAMP levels were analyzed by the Student's *t*-test.

RESULTS

Figure 1 summarizes the results obtained in the experiment in which adenylate cyclase activity was measured following either EB or P. Values of adenylate cyclase activity varied significantly between areas, highest values being observed in the POA and lowest in the VMH. Progesterone administration resulted, in the three brain areas studied, in a significant increase in adenylate cyclase activity that reached its peak 2 hr after the administration of the steroid. Four hours after P, levels of enzyme activity were not statistically different from control values (0 hr). Figure 2 presents the cAMP intraneuronal values obtained after P administration in ovariectomized estrogen primed rats. It can be seen that cAMP levels increased significantly 2 hr after P in all brain regions studied, i.e., POA, VMH and Cx, but two hours later (4 hr after P) only the motor cerebral cortex still showed levels significantly higher than control values. At the dose level employed, P (4 mg) elicited lordosis behavior in all treated estrogen primed rats.

Figures 1 and 2 show the temporal development of lordosis behavior following P, together with values of adenylate cyclase (Fig. 1) and cAMP (Fig. 2) at the various test intervals. It can be seen that initiation of overt lordosis behavior coincides with the significant rise of both adenylate cyclase activity and cAMP levels occurring at 2 hr after P, but that the activity of the adenylate cyclase-cAMP system returned to normal values when lordosis behavior was at its peak level (4 hr after P).

DISCUSSION

Several studies have demonstrated the important role played by the adenylate cyclase enzyme in brain as well as its participation in sexual behavior [2].

Several adenylate cyclase activation mechanisms upon each of its three different constitutive units have been proved [11]: (a) The receptor unit contains specific receptors for several substances which, when administered, increase AC activity and facilitate lordosis behavior, for example: neu-

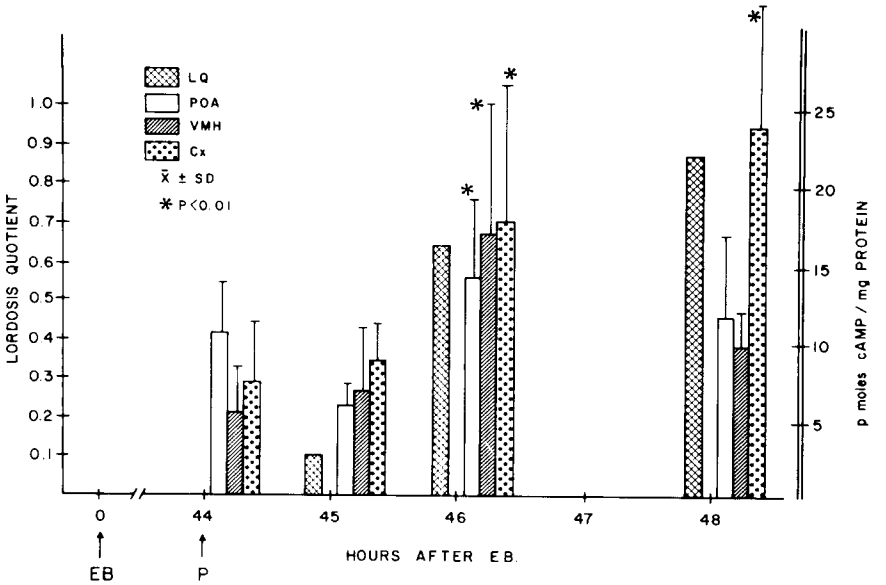

FIG. 2. cAMP levels in brain areas after treatment with sex hormones. The LQ and the intraneuronal cAMP levels in different brain structures of ovx rats injected with estradiol benzoate (EB) followed 44 hr later by progesterone (P). The animals were tested for lordosis and thereafter sacrificed for biochemical determinations. *Student's *t*-test.

rotransmitters [6], LH-RH [20], PG E_2 [30]; (b) The regulatory unit has a protease activity which is decreased "in vitro" by protease inhibitors such as Nα-tosyl-L-arginine-methyl-ester (TAME), Nα-tosyl-L-lysine chloro-methyl-ketone (TLCK), etc. [25,28], and activated by GTP and its analogs by a covalent mechanism involving the enzyme phosphorylation [8] and by cholera toxin [7]. Recent reports have shown the activation of brain adenylate cyclase by vanadate via guanine nucleotides, regulatory proteins [18]. (c) The catalytic unit has been activated by forskolin in the brain [9] and in E_2 primed rats in order to facilitate lordosis behavior [10]. This unit can be normally activated by NaF in the presence of Mg ions [26]. At the same time it is responsible for neuronal cAMP production which may mediate synaptic transmission [12].

In some physiological events P increases cAMP levels [15], however, this effect has not been shown in the central nervous system (CNS).

Progesterone can be substituted in its behavioral effect by cAMP and cAMP analogs [3]; by GTP and GTP analogs [11]; by forskolin [9]; and its effect is strongly synergized with phosphodiesterase inhibitors [5]. According to Sutherland [33], these are the basic characteristics to catalog a drug, in this case progesterone, as an adenylate cyclase activating drug.

Recent works reported that P facilitates sexual response with a very short latency when intravenously administered [17], and its facilitatory effect is not blocked by protein synthesis inhibitors [27].

Hitherto, data suggest indirectly that P could have a direct effect upon adenylate cyclase activity related to the induction of lordosis behavior in EB primed rats.

Our present results show that P administration to ovariectomized estrogen primed rats both activates adenylate cyclase activity and raises cAMP levels (this dose also induced a clear facilitation of lordosis behavior in the same rats). Suprisingly, the activation of the adenylate cyclase cAMP system occurred with a rather long latency (1 hr) since no significant elevations in either adenylate cyclase activity or cAMP levels were observed until two hours after P, when peak values were observed in the hypothalamus and the POA. A similar, long latency to raise hypothalamic cAMP levels has been reported for prostaglandine E_2 in the rat, a phenomenon presumably due to the fact that this effect is mediated by catecholamine release [22]. An indirect effect of P on cAMP must also be considered since there is evidence that this steroid stimulates the release of noradrenaline (NA) in anterior hypothalamic tissue [21]. This idea is not directly supported by our finding that propanolol, a β adrenergic antagonist, and phentolamine, an α-adrenergic antagonist, interferes with the facilitatory effect of P on lordosis behavior in estrogen primed rats [29]. The possibility, however, that P can interact with membrane receptors linked to adenylate cyclase cannot be excluded, since membrane receptors for P have been recently found in brain synoptosome system [34].

The dose of P effective to activate the adenylate cyclase cAMP system also induced a clear facilitation of lordosis in the same rats. However, a close temporal relationship between cAMP levels and lordosis behavior was not observed. Peak values of cAMP were observed in the VMH and the POA two hours after P when significant levels of lordosis behavior were already apparent. However, highest LQ values were observed at 4 hr when cAMP levels have clearly diminished in these structures. This lack of temporal correlation between cAMP values and the display of lordosis suggests that a raise in cAMP level is involved in the triggering of cellular events essential for the initiation, but not for the maintenance, of lordosis.

In addition to the "classic" mechanism of steroid action, compelling data suggest that P, at high concentrations, acts directly on the outer cellular membrane [1] to alter calcium uptake [15] which could ultimately influence cyclic nucleotide activity by a variety of mechanisms [22].

The finding that the cerebral cortex, a structure intended to serve as a control responsive tissue, also responded to P administration was surprising. However, there is evidence for the existence of cytosolic progestin receptors in the cerebral cortex and hypothalamus induced by estrogen, and several studies suggest that this structure mediates receptivity in the female rat [24].

There is now abundant evidence for the participation of phosphorylation reactions in the regulation of neuron excitability. Thus, phosphorylation reaction participates in the activation of several proteins involved in synaptic transmission, and in the affinity of membrane receptors to neurotransmitters [13]. It has been shown that the phosphorylation of several proteins can be modified by various steroids, including androgen, and corticoids, but the effect of P on the phosphorylation rate of these proteins has not been tested. The fact that clear activation of the adenylate cyclase cAMP system was achieved with P in the present study suggests that this steroid exerts some of its action on brain function through changes in protein phosphorylation. More detailed studies, however, will be required to establish a casual relationship, if any, between the raise in cAMP levels induced by P and the facilitation of lordosis by this steroid in estrogen primed rodents.

REFERENCES

1. Baxter, J. D. and J. W. Funder. Hormone receptor. *N Eng J Med* **301:** 1149-1161, 1979.
2. Beyer, C., K. Larsson and M. L. Cruz. Neuronal mechanisms probably related to the effect of sex steroids on sexual behavior. In: *Endocrine Control of Sexual Behavior*, edited by C. Beyer. New York: Raven Press, 1979, pp. 365-387.
3. Beyer, C., E. Canchola and K. Larsson. Facilitation of lordosis behavior in the ovariectomized estrogen primed rat by dibutyryl cAMP. *Physiol Behav* **26:** 249-251, 1981.
4. Beyer, C., A. Fernández-Guasti and G. Rodríguez-Manzo. Induction of female sexual behavior by GTP in ovariectomized estrogen primed rats. *Physiol Behav* **28:** 1073-1076, 1982.
5. Beyer, C. and E. Canchola. Facilitation of progesterone induced lordosis behavior by phosphodiesterase inhibitors in estrogen primed rats. *Physiol Behav* **27:** 731-733, 1981.
6. Carter, C. B., and J. M. Davis. Biogenic amines reproductive hormones and female sexual behavior: A Review. *Biobehav Rev* **1:** 213-224, 1977.
7. Cassel, D. and Z. Selinger. Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolisis at the regulatory site. *Proc Natl Acad Sci USA* **74:** 3307-3311, 1977.
8. Cuatrecasas, P., S. Jacobs and V. Bennett. Activation of adenylate cyclase by phosphoramidate and phosphonate analogs of GTP: Possible role of covalent enzyme-substrate intermediates in the mechanism of hormone activation. *Proc Natl Acad Sci USA* **72:** 1739-1743, 1975.
9. Daly, J. W., W. L. Padgett and K. B. Seamon. Forskolin: A unique triterpene activator of cyclic AMP generating system. *J Cyclic Nucleotide Protein Phosphor Res* **7:** 201-224, 1981.
10. Fernández-Guasti, A., G. Rodríguez-Manzo and C. Beyer. Synergistic effect of progesterone and forskolin on lordosis behavior. Presented in the Eastern Conference on Reproductive Behavior, Boston, MA, 1983, p. 36.
11. Fernández-Guasti, A., G. Rodríguez-Manzo and C. Beyer. Effect of guanine derivatives on lordosis behavior of estrogen primed rats. *Physiol Behav* **31:** 589-592, 1983.
12. Greengard, P. In: *Cyclic Nucleotides, Phosphorylated Proteins and Neuronal Function*. New York: Raven Press, 1978.
13. Greengard, P. Cyclic nucleotides, phosphorylated proteins and the nervous system. *Fed Proc* **38:** 2208-2218, 1979.
14. Jensen, E. V. On the mechanism of estrogen action. *Perspect Biol Med* **6:** 47-59, 1962.
15. Kostellow, A. B., D. Ziegler and G. A. Morrill. Regulation of Ca^{2+} and cyclic AMP during the first meiotic division in amphibian oocytes by progesterone. *J Cyclic Nucleotide Protein Phosphor Res* **6:** 347-358, 1980.
16. Krishna, G., B. Weiss and B. B. Brodie. A simple sensitive method for assay of adenyl cyclase. *J Pharmacol Exp Ther* **163:** 379-385, 1968.
17. Kubli-Garfias, C. and R. E. Whalen. Induction of lordosis behavior in female rats by intravenous administration of progestins. *Horm Behav* **9:** 380-386, 1977.
18. Kurivan, K. J. Vanadate and brain adenylate cyclase. Effect of spreading depression. *Neuroscience* **10:** 545-552, 1983.
19. Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem* **193:** 165-175, 1951.
20. Moss, R. L. and S. M. McCann. Action of luteinizing hormone releasing factor (LRF) in the initiation of lordosis behavior in the estrone-primed ovariectomized female rat. *Neuroendocrinology* **17:** 309-318, 1975.
21. Munaro, N. I. The effect of ovarian steroids on hypothalamic norepinephrine neuronal activity. *Acta Endocrinol (Copenh)* **86:** 235-242, 1977.
22. Nathanson, J. A. Cyclic nucleotides and nervous system function. *Physiol Rev* **57:** 157-256, 1977.
23. Palmer, G. C. and S. J. Palmer. Adenylate cyclase sensitivity to catecholamines and forskolin in rat pia-arachnoid and cerebral microvessels. *Neuropharmacology* **22:** 213-219, 1983.
24. Parsons, B., T. C. Rainbow, D. W. Pfaff and B. S. McEwen. Oestradiol, sexual receptivity and cytosol progestin receptors in rat hypothalamus. *Nature* **292:** 58-59, 1981.
25. Partington, C. R. and J. W. Daly. Effect of proteases and protease inhibitors on adenylate cyclase activity in rat cerebral cortical membranes. *Arch Biochem Biophys* **198:** 255-262, 1979.
26. Perkins, J. P. and M. M. Morre. Adenylate cyclase of rat cerebral cortex activation by sodium floride and detergents. *J Biol Chem* **246:** 62-68, 1971.
27. Quadagno, D. M., J. Shryne and R. A. Gorski. The inhibition of steroid induced sexual behavior by hypothalamic actinomicin-D. *Horm Behav* **2:** 1-10, 1971.
28. Richert, N. D. and R. J. Ryan. Protease inhibitors block hormonal activation of adenylate cyclase. *Biochem Biophys Res Comm* **78:** 799-805, 1977.
29. Rodríguez-Manzo, G., M. L. Collado and M. L. Cruz. Inhibition of estrogen-progesterone and estrogen isoproterenol induced by proporanolol. 16th Conference on Reproductive Behavior, 1984.
30. Rodríguez-Sierra, J. F. and B. R. Komisaruk. Effect of prostaglandin E_2 and indomethacin on sexual behavior in the female rat. *Horm Behav* **9:** 281-289, 1977.
31. Schmidt, M. J., D. E. Schmidt and S. A. Robinson. Cyclic adenosine monophosphate in brain areas: microwave irradiation as a means of tissue fixation. *Science* **173:** 1142-1143, 1971.
32. Steiner, A., L. A. B. Pagliara, L. R. Chase and D. M. Kiphis. Radioimmunoassay for cyclic nucleotides II. Adenosine 3′, 5′ monophosphate and guanosine 3′ 5′ monophosphate in mammalian tissues and body fluids. *J Biol Chem* **247:** 1114-1120, 1972.
33. Sutherland, E. W. and G. A. Robinson. The role of cyclic 3′ 5′ AMP in responses to cathecholamines and other hormones. *Pharmacol Rev* **18:** 145-161, 1966.
34. Towle, A. C. and P. Y. Sze. Steroid binding to synaptic plasma membrane: Differential binding of clucocorticoids and gonadal steroids. *J Steroid Biochem* **18:** 135-143, 1983.
35. Young, W. C. The hormones and mating behavior. In: *Sex and Internal Secretions. Vol II*, edited by W. C. Young. Baltimore: Williams and Wilkins, 1961, pp. 1173-1239.