Original Paper

Neuroendocrinology 1992;55:1–8

Nicolas Petitti
Anne M. Etgen

Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y., USA

Progesterone Promotes Rapid Desensitization of α_1-Adrenergic Receptor Augmentation of cAMP Formation in Rat Hypothalamic Slices

Key Words
Norepinephrine
Progesterone
cAMP
Hypothalamus
Preoptic area
α_1-Adrenergic receptor, desensitization

Abstract

We previously demonstrated that norepinephrine (NE) induction of cAMP accumulation in slices of the preoptic area (POA) and middle hypothalamus (MH) is reduced by in vivo administration of progesterone to estradiol-primed rats, apparently by eliminating α_1-receptor augmentation of β-receptor-stimulated cAMP formation. The present studies examined whether in vitro exposure to progesterone would also depress NE-stimulated cAMP synthesis. POA and MH slices from estradiol-primed females were incubated with 20 n*M* progesterone for 5–30 min prior to addition of 100 μ*M* NE. Preincubation of slices with progesterone for as little as 5 min significantly suppressed NE-stimulated cAMP formation by greater than 60%. This effect was estrogen-dependent in that progesterone in vitro did not inhibit NE-stimulated cAMP accumulation in slices from ovariectomized rats not pretreated with estradiol. Isoproterenol, a β-adrenergic agonist, elevated cAMP to the same extent in slices from estradiol-primed females incubated with and without progesterone in vitro; however, the α_1-agonist, phenylephrine, was unable to augment cAMP formation in slices incubated in vitro with progesterone for 5 min prior to drug challenge. To determine whether the rapid effects of progesterone may be exerted at the level of the plasma membrane, we employed progesterone conjugated to bovine serum albumin at carbon 3 (P-3-BSA). Slices from estradiol-primed rats incubated with P-3-BSA for 5 min did not exhibit an α_1-receptor augmentation of β-receptor-stimulated cAMP accumulation. These data indicate that progesterone may have rapid, non-genomic effects on α_1-adrenergic receptor coupling to second-messenger systems in the hypothalamus of female rats.

Received:
January 21, 1991
Accepted after revision:
April 24, 1991

Anne M. Etgen
Department of Psychiatry
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461 (USA)

0028–3835/92/0551–0001
$ 2.75/0

Steroid hormones act on the central nervous system to produce a variety of neuroendocrine and behavioral effects. However, the precise molecular and cellular mechanisms by which steroids work in the brain have yet to be clarified. It is generally held that steroid actions are mediated by genomic regulation [1, 2]. However, an additional model of steroid action has been suggested, one involving a direct effect of the steroids on components of cell membranes [3–7].

Progesterone (Pg) is a steroid that may not only act on the genome to regulate gene expression [8] but appears also to possess a relatively rapid, non-genomic, membrane-mediated mechanism of action. This conclusion is based upon data from a number of model systems. For example, Pg has been shown to induce the maturation of *Xenopus laevis* oocytes through interactions with cell membranes [9, 10]; to decrease glutamate and enhance GABA electrophysiological responses of rat cerebellar neurons [11]; to inhibit the binding of muscarinic agonists to hypothalamic and pituitary membranes [12]; to bind to σ-receptors in guinea pig brains [13]; to cause redistribution of oxytocin receptors in the hypothalamus [7], and to stimulate calcium influx in human sperm [14]. In addition, an impermeable analog of Pg has been shown to bind to specific and saturable binding sites on cell membranes [15]; to stimulate in vitro gonadotropin-releasing hormone release from rat hypothalamus [16], and to activate the release of dopamine from striatal neurons [17].

Several years ago, using stimulation of cyclic adenosine 3′5′-monophosphate (cAMP) accumulation in brain slices as an assay, we demonstrated that estradiol and Pg in vivo modulate norepinephrine (NE) signal transduction in brain areas involved in the coordination of female reproductive function. We found that hypothalamic slices from female rats injected with both estradiol and Pg prior to sacrifice demonstrate reduced NE-dependent activation of cAMP accumulation when compared to slices from gonadectomized females [18]. We later demonstrated that this reduction in NE-stimulated cAMP formation is a two-step process. First, estradiol inhibits β-adrenoceptor function as indicated by a reduced ability of the β-agonist, isoproterenol (ISO), to activate adenylyl cyclase. Administration of Pg to estrogen-primed females subsequently eliminates α_1-adrenoceptor augmentation cAMP synthesis as evidenced by a loss of the ability of the α_1-agonist, phenylephrine (PHE), to potentiate ISO- and vasoactive intestinal peptide-stimulated cAMP accumulation [19, 20]. In those studies, Pg suppression of agonist-stimulated cAMP formation was observed 3.5 h after in vivo hormone administration. The relatively short time course of this Pg effect as well as the short time course of Pg facilitation of female reproductive behavior [21, 22] imply that Pg may influence neuroendocrine function by non-genomic as well as genomic mechanisms. Therefore, we examined the possibility that the effect of Pg on NE-stimulated cAMP formation may represent a rapid, non-genomic response to the hormone by determining whether in vitro actions of Pg on NE-stimulated cAMP formation would mimic the in vivo results.

Materials and Methods

Animals and Hormone Treatments

Sexually mature female Sprague-Dawley rats obtained from Taconic Farm (Taconic, N.Y.) and weighing 150–175 g were ovariohysterectomized (OVX) bilaterally under Metofane anesthesia 4–7 days prior to use. Estrogen treatment consisted of two subcutaneous injections of 2 μg of estradiol benzoate (EB) given 24 and 48 h before sacrifice. EB was dissolved in peanut oil and injected in a volume of 0.1 ml.

Dissection of Hypothalami

Animals were killed by decapitation, and their brains rapidly removed, dissected over ice and placed into ice-cold artificial cerebrospinal fluid. The entire hypothalamus and preoptic area (POA) were removed, and slices (each 350 μm thick) were cut on a McIlwain tissue chopper beginning approximately 2 mm anterior to the optic chiasm and ending 1 mm anterior to the mammillary bodies. Based on anatomical landmarks observed in comparable slices from fixed tissue, four slices of POA and three of middle hypothalamus (MH) were obtained as described earlier [18, 23]. The MH slices include the arcuate nucleus, the ventromedial nucleus, the dorsomedial nucleus and much of the lateral hypothalamus.

Preparation of Hypothalamic Slices and Stimulation of cAMP Accumulation

Each slice was maintained at 34–35 °C in a shaking water bath (80 oscillations/min) in an individual tissue culture well containing 300 μl of Yamamoto's medium in an O_2/CO_2(95/5)-saturated environment. The incubation conditions were identical to those used in our previous work [18, 19, 23]. Slices were left undisturbed for 75 min to allow nucleotide levels to stabilize [24] and then incubated for another 25–50 min with appropriate drugs or vehicle (see individual experiments).

At the end of the incubation period, the slices were transferred rapidly to 400 μl of ice-cold 5% (w/v) trichloroacetic acid. The slices were disrupted by sonication, and the supernatant (containing cAMP) and pellet (containing tissue protein) separated by centrifugation. The pellet was dissolved in 2.0 *M* NaOH for later determination of protein content [25]. The supernatant was acidified with 1.0 *M* HCl, and trichloroacetic acid was removed with 4 vol of washed ether. The resulting aqueous extracts were concentrated by lyophilization and analyzed for cAMP content using a modified Gilman protein binding assay [26]. Data were converted to picomoles of cAMP per milligram of tissue protein (pmol cAMP/mg

protein). For all experiments, values for the four POA or three MH slices were averaged to give a single value for each brain region for each rat.

Drug Treatment

Drugs were added directly to the incubation wells as concentrated solutions in appropriate vehicle. Distilled water was the vehicle for PHE, ISO, NE and progesterone 3-(O-carboxymethyl)oxime-bovine serum albumin (P-3-BSA). Pg was added at the end of the equilibration period for 5–30 min (see individual experiments) in a volume of 1% ethanol. Control slices received equal volumes of vehicle at the same time. Phosphodiesterase inhibitor D-4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO-20-1724) was added at the beginning of the equilibration period in a volume of 1% ethanol in order to prevent cAMP hydrolysis.

Chemicals

EB and Pg were purchased from Steraloids, Inc. (Wilton, N.H.). Metofane was obtained from Pitman-Moore, Inc. (Atlanta, Ga.). NE, PHE, ISO and P-3-BSA were purchased from Sigma (St. Louis, Mo.). RO-20-1724 was obtained from BioMol Research Labs (Plymouth Meeting, Pa.).

Analysis of Data

Significant differences between means were determined using analysis of variance or t tests. Planned post hoc comparisons were made using a Newman-Keuls multiple range test. Differences were considered statistically significant if $p < 0.05$.

Results

Progesterone and NE-Stimulated cAMP Formation

In our previous studies [18–20] a 3.5-hour exposure to Pg attenuated the cAMP response to NE in hypothalamic and POA slices from EB-primed rats. To determine whether exposure to Pg in vitro would mimic the in vivo result and to see whether this phenomenon is rapid in onset, OVX female rats were injected with oil or with EB prior to sacrifice. Equilibrated slices were then incubated with or without 20 n*M* Pg in vitro for 5–30 min, followed by a 20-min challenge with 100 μ*M* NE.

Pg treatment in vitro has no effect on basal cAMP levels in slices from either hormone group (data not shown). In addition, in slices from OVX animals injected with oil, Pg in vitro has no effect on NE-stimulated cAMP accumulation (fig. 1). That is, a similar cAMP response is observed when slices from OVX rats are incubated with or without 20 n*M* Pg for 5–30 min prior to NE challenge. In contrast, exposure of slices from EB-primed rats to 20 n*M* Pg in vitro significantly depresses NE-stimulated cAMP formation ($p < 0.01$). This effect is rapid in onset since it is observed when slices are incubated with Pg for as little as 5 min prior to NE addition.

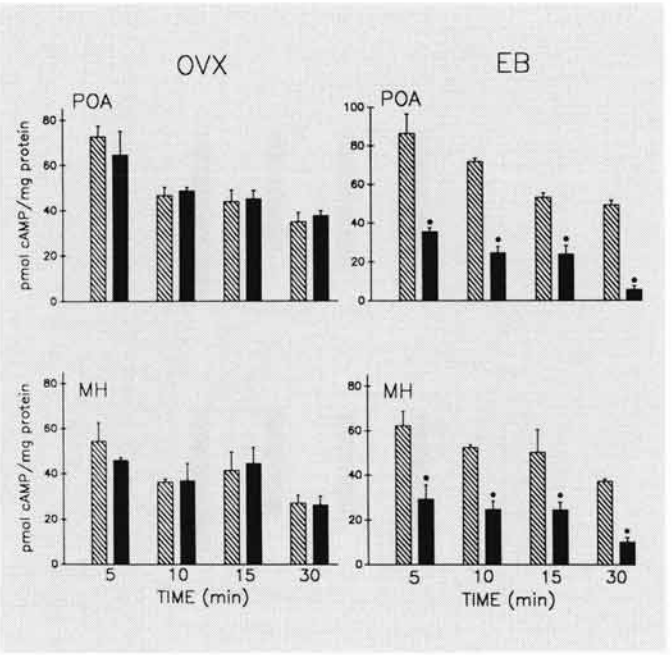

Fig. 1. NE-stimulated cAMP accumulation in slices incubated with vehicle or 20 n*M* Pg in vitro. OVX female rats were injected with oil (OVX) or with 2 μg of EB 24 and 48 h before sacrifice. Equilibrated slices were then incubated an additional 5–30 min with vehicle (hatched bars) or 20 n*M* Pg (solid bars), followed by a 20-min challenge with 100 μ*M* NE. Each value represents the mean (± SEM) of 3–4 independent replications. Basal values ranged from 3 to 8 pmol cAMP/mg protein. * Significantly less than vehicle control: $p < 0.01$.

There is also a significant decrease in NE-stimulated cAMP content of the slices with time ($p < 0.01$). Basal cAMP values also decrease gradually with time (mean: at 5 min, 8 pmol cAMP/mg protein; mean at 30 min, 3 pmol cAMP/mg protein). These decreases may result from hypoxia caused by the experimental manipulations or from the addition of the ethanol vehicle. When the NE data are expressed as the percent of the cAMP response to NE in slices incubated for the same time with vehicle prior to NE challenge (fig. 2), there is no effect of time on the cAMP response to NE. That is, incubation of slices from EB-primed rats with Pg suppresses NE-stimulated cAMP formation by 60–90% at all times ($p < 0.01$).

Effect of Pg in vitro on α_1-Receptor Augmentation of cAMP Formation

Our previous studies indicated that the cAMP response to NE in slices of POA and MH from OVX and EB-treated rats is attributable to a combination of β-receptor activation of cAMP synthesis and α_1-receptor

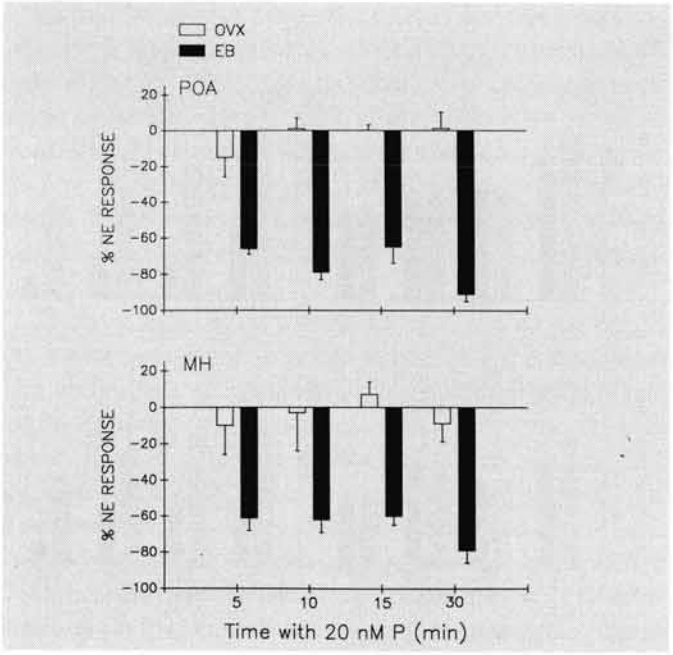

Fig. 2. Inhibition of NE-stimulated cAMP accumulation in slices incubated with 20 n*M* Pg in vitro. OVX female rats were injected with oil (OVX) or with 2 μg of EB 24 and 48 h before sacrifice. Equilibrated slices were then incubated with or without 20 n*M* Pg in vitro for 5–30 min, followed by a 20-min challenge with 100 μ*M* NE. Data from the Pg-exposed slices in figure 1 are expressed as the percent of the cAMP response to NE (% NE response) in slices incubated for the same time with vehicle prior to NE challenge. Each value represents the mean (± SEM) of 3–4 independent replications. Two-way analysis of variance (hormone × time) showed a significant main effect of hormone ($p < 0.00001$) but not of time ($p > 0.10$) in both the POA and MH slices.

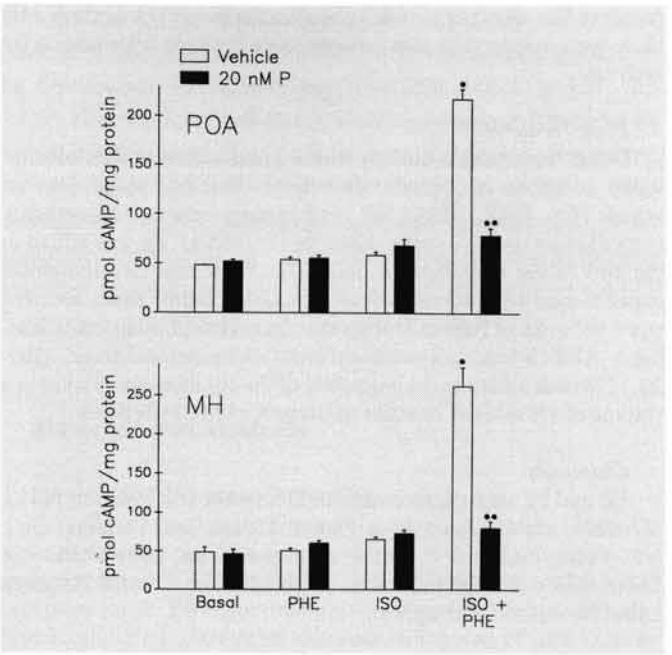

Fig. 3. Inhibition of PHE augmentation of ISO-stimulated cAMP accumulation in POA and MH slices incubated in vitro with 20 n*M* Pg. OVX female rats received the same EB treatments described in figure 1. Slices were equilibrated in the presence of 0.7 m*M* RO-20-1724 throughout the experiment. Slices were then incubated an additional 5 min with Pg or vehicle followed by a 20-min challenge with 1 μ*M* ISO, 10 μ*M* PHE, or a combination of ISO + PHE. Each value represents the mean (± SEM) of 3–4 independent replications. * Significantly greater than ISO or PHE alone ($p < 0.01$). ** Significantly less than ISO + PHE from slices incubated with vehicle ($p < 0.01$).

augmentation of the β response [23]. We also found that Pg administration to EB-primed rats 3–4 h before sacrifice eliminated the α_1-mediated augmenting response [20]. Therefore, we determined whether the inhibition of NE-induced cAMP accumulation in slices incubated with Pg in vitro was also due to modulation of α_1-receptor function. OVX female rats were injected with EB in vivo, and slices were incubated with vehicle or with 20 n*M* Pg in vitro for 5 min prior to the addition of 1 μ*M* ISO (β-agonist), 10 μ*M* PHE (α_1-agonist) or ISO + PHE (fig. 3). When basal and ISO-stimulated cAMP levels were compared across all treatment groups, ISO alone modestly (about 25%) but significantly (t test, $p < 0.01$) elevated cAMP above basal levels. In our laboratory, ISO activation of adenylyl cyclase in slices from EB-primed females is typically decreased by 50–90% when compared to slices from OVX females (20, 23). In the absence of Pg, the α_1-agonist PHE potentiates the cAMP response to the β-agonist ISO (analysis of variance; $p < 0.01$ versus ISO or PHE alone). In contrast, slices exposed to Pg in vitro for 5 min prior to addition of adrenergic agonists show no α_1-receptor-mediated augmentation of cAMP formation.

P-3-BSA Effects on cAMP Accumulation

To determine whether the rapid effects of Pg on α_1-receptor-mediated signal transduction may be exerted at the level of the plasma membrane, we employed Pg conjugated to bovine serum albumin at carbon 3 (P-3-BSA). We chose this conjugated progestin because it is unlikely to diffuse through the plasma membrane into the

cytoplasm, owing to its large size and hydrophilic properties. In addition, P-3-BSA has been shown to bind to specific and saturable binding sites on cell membranes [15] and to stimulate gonadotropin-releasing hormone release from rat hypothalamic tissue in vitro [16]. OVX female rats were injected with EB in vivo, and slices were incubated in vitro with vehicle or a concentration of P-3-BSA (0.045 μg/ml) yielding a final Pg concentration of 20 n*M* for 5 min prior to addition of NE agonists (fig. 4). The α_1-agonist PHE does not modify basal cAMP levels in slices from either hormone group. However, the β-agonist ISO significantly elevates cAMP levels in slices incubated in the presence and absence of P-3-BSA ($p < 0.01$, compared to basal). In the absence of P-3-BSA, the α_1-receptor agonist PHE augments the cAMP response to the β-agonist ISO to produce a greater increase in cAMP than either drug alone ($p < 0.01$). In contrast, in slices exposed to P-3-BSA in vitro for 5 min prior to agonist stimulation, α_1-adrenoceptor activation does not potentiate cAMP formation.

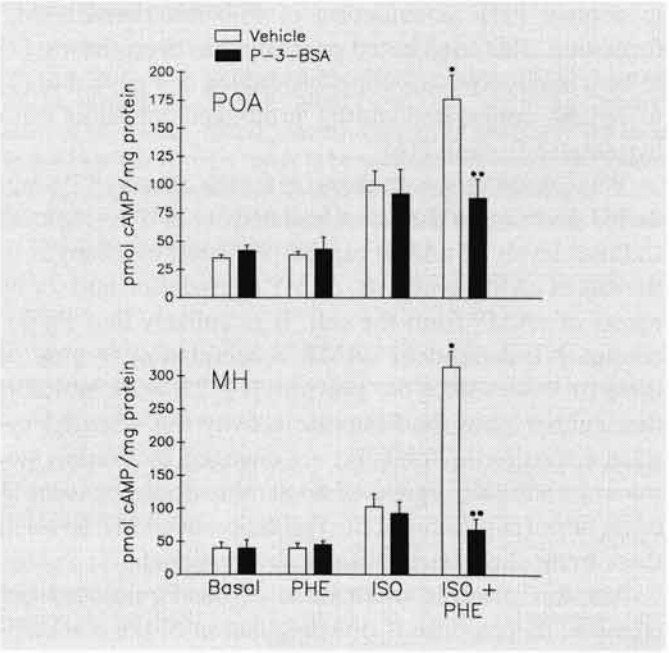

Fig. 4. Inhibition of PHE augmentation of ISO-stimulated cAMP accumulation in POA and MH slices incubated in vitro with 0.045 μg/ml P-3-BSA. OVX female rats received the same EB treatments described in figure 1. Slices were equilibrated in the presence of 0.35 m*M* RO-20-1724 throughout the experiment. Slices were then incubated an additional 5 min with P-3-BSA or vehicle followed by a 20-min challenge with 1 μ*M* ISO, 10 μ*M* PHE, or a combination of ISO + PHE. Each value represents the mean (± SEM) of 4 independent replications. * Significantly greater than ISO or PHE alone ($p < 0.01$). ** Significantly less than ISO + PHE from slices incubated with vehicle ($p < 0.01$).

Discussion

Present studies extend our previous finding that NE-stimulated cAMP formation in hypothalamic slices from estrogen-primed female rats is altered by Pg [19, 20]. Specifically, we demonstrate that in vitro exposure of slices from estrogen-primed females to a physiological concentration of Pg (20 n*M*) prior to addition of NE attenuates NE-stimulated cAMP formation by 60–90%. In our earlier studies, estrogen-primed rats injected with Pg in vivo showed a similarly decreased capacity of NE to stimulate cAMP formation in hypothalamic and POA slices [18, 19]. To date we have not evaluated the effects of Pg on NE-stimulated cAMP formation in brain regions expressing low levels of steroid receptors or in other steroid target tissues.

The Pg-induced reduction in NE-stimulated cAMP accumulation appears to be an estrogen-dependent process since Pg treatment in vivo [19] and in vitro to OVX rats not primed with estradiol does not attenuate NE-stimulated cAMP formation. Although we have not examined the effects of higher Pg concentrations in vitro on slices from OVX rats, in vivo injections of 500 μg of Pg did not affect cAMP synthesis in hypothalamic slices unless the rats had been primed with EB [19]. Hence we believe it unlikely that higher Pg concentrations would affect NE signal transduction in vitro in the absence of estrogen priming. Furthermore, as in slices from EB-primed animals given Pg in vivo [19, 20], slices exposed to Pg for 5 min in vitro lose the ability to show PHE potentiation of the ISO response. This indicates that Pg attenuates the total cAMP response to NE by reducing the augmentation ascribed to α_1-receptors.

This study also supports the hypothesis that the Pg-induced uncoupling of α_1-adrenoceptor augmentation of cAMP formation may represent an interaction of the steroid at the level of the plasma membrane. We draw this conclusion from the following considerations. First, the Pg-dependent decrease in the cAMP response to NE is rapid in onset since it is demonstrable within 5 min of Pg application. Second, incubation of slices from estrogen-primed rats for 5 min in vitro with Pg conjugated covalently to bovine serum albumin (i.e., P-3-BSA) is sufficient

to abolish PHE potentiation of ISO-stimulated cAMP formation. This conjugated progestin has been shown: (1) to be a membrane-impermeable analog of Pg [9], and (2) to remain conjugated during prolonged infusions onto hypothalamic slices [16].

What could be the mechanism for the observed Pg-mediated decrease in the functional activity of α_1-receptors? Cellular levels of cAMP can be regulated by changes in the rate of cAMP synthesis, cAMP degradation and/or by egress of cAMP from the cell. It is unlikely that Pg decreases NE-dependent cAMP accumulation by any of these processes since our previous [19, 23] work indicates that neither phosphodiesterase activity nor adenylyl cyclase activation by forskolin are changed by ovarian steroids. In addition, egress of nucleotide does not seem to be an important factor in the regulation of cAMP levels in these brain slices [unpublished observations].

Another possible mechanism of the Pg-induced decrease in α_1-response is downregulation of the α_1-receptor. This explanation seems unlikely since downregulation of receptors is usually a relatively slow process [27, 28]. In addition, ligand-binding studies reveal that α_1-receptor number and antagonist binding affinity are similar in POA and MH membranes from animals injected in vivo with the EB and Pg doses used in our previous slice studies [29; unpublished observations]. Thus, Pg does not appear to downregulate α_1-receptors in these brain regions.

NE α_1-receptors appear to augment cAMP synthesis through a cascade involving a variety of membrane proteins. Agonist occupancy of α_1-receptors results in the activation of a guanyl nucleotide-binding protein (Gp) which activates phospholipase C [30–32] leading to the hydrolysis of membrane inositol phospholipids into diacylglycerol and inositol 1,4,5-*tris*-phosphate [33–35]. These products in turn activate protein kinase C (PKC) and induce calcium influx, respectively. Because α_1-receptors appear to augment cAMP formation by a cascade involving Gp-phospholipase C-PKC activation [36–38], Pg could modify one or more of these molecular components. Pg may affect the coupling between α_1-adrenergic receptors and Gp or between Gp and the effector enzyme (phospholipase C). It is unlikely that Pg acts on Gp, because the steroid in vivo does not modify the ability of carbachol, an agonist of muscarinic receptors coupled to Gp, to augment ISO-stimulated cAMP formation [20]. It is also unlikely that Pg acts on PKC since slices incubated with phorbol ester, a direct activator of PKC, do not exhibit Pg suppression of NE-induced cAMP formation [20].

Another potential mechanism for decreasing receptor function, which is commonly observed in many biological systems, is receptor phosphorylation and desensitization. For example, α_1-adrenergic receptors can be covalently modified by phosphorylation [39–41]. Phosphorylation often leads to desensitization of receptor activity by causing a reversible uncoupling of the receptor from its G protein. However, phosphorylation is usually promoted by agonist interactions with receptors [28, 40, 41]. Nonetheless, steroid regulation of protein phosphorylation/dephosphorylation has been observed in *X. laevis* oocytes, where it decreases protein phosphatase activity as early as 5 min [42]. Thus there is some precedent for proposing such a mechanism of Pg action in brain.

The rapid time course of Pg action strongly suggests that Pg modulates α_1-receptor signaling by acting directly on the plasma membrane. A mechanism by which Pg may cause rapid uncoupling of α_1-receptors that does not require metabolic energy is the modification of membrane lipid fluidity. Pg could intercalate into the lipid bilayer of the cell membrane [3, 4] or alter membrane phospholipid methylation [43, 44], leading to changes in membrane microviscosity. Because receptors, G proteins and effector enzymes are able to undergo independent free lateral diffusion within the plasma membrane, Pg could influence cAMP generation by modifying the microviscosity of the lipid bilayer. Furthermore, Pg need not work on the entire fluid state of the membrane, but may operate on specific subdomains.

Another explanation for the rapid effects of Pg on hypothalamic α_1-receptor function is a conformational change of active α_1-receptors to an inactive state. Such conformational changes could be mediated by Pg binding to specific sites on the membrane or by altered protein-lipid interactions. Such a mechanism is similar to the one proposed above, except that it does not necessitate alterations in diffusion rates of membrane components, but rather a change in the three-dimensional structure of the α_1-receptor as a consequence of a modified lipid environment. Different, interconvertible affinity states have been proposed for G protein-linked receptors [45, 46], an active state with high affinity for NE and its agonists, and an inactive, low affinity state. In contrast to agonists, antagonists bind to both conformational states with similar affinity [45–47]. Thus binding experiments performed to date [29] would not distinguish changes in α_1-receptors from a high to low affinity agonist-binding form.

In summary, this study demonstrates that, in estrogen-primed rats, Pg in vitro rapidly depresses NE-stimulated cAMP formation by eliminating the α_1-receptor-medi-

ated augmentation of β-receptor-stimulated cAMP synthesis. These results indicate that Pg influences α_1-adrenergic receptor signal transduction in hypothalamic and POA slices of estrogen-primed rats, probably via a nongenomic process. Thus both rapid membrane mechanisms as well as genomic mechanisms are likely to mediate steroid effects in brain regions that regulate reproductive function.

Acknowledgments

This work was supported by DHHS grants MH41414 and RSDA MH00636 to A.M.E., BRSG RR05397, and by the Department of Psychiatry, Albert Einstein College of Medicine. The data in this paper are from a thesis to be submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Sue Golding Division of Medical Sciences, Albert Einstein College of Medicine, Yeshiva University.

References

1 Pfaff DW, McEwen BS: Action of estrogens and progestins on nerve cells. Science 1982; 219:808–814.

2 Pfaff DW: Molecular approaches to steroid hormone effects on hypothalamic nerve cells. Biomedical Res 1989;10:87–94.

3 Alfsen A: Biophysical aspects of the mechanism of action of steroid hormones. Prog Biophys Mol Biol 1983;42:79–93.

4 Duval D, Durant S, Homo-Delarche F: Nongenomic effects of steroids: Interaction of steroid molecules with membrane structures and functions. Biochim Biophys Acta 1983;737: 409–442.

5 Erulkar SD, Wetzel DM: Steroid effects on excitable membranes; in Straus JF, Pfaff DW (eds): Molecular Neurobiology: Endocrine Approaches. Current Topics in Membranes and Transport. New York, Academic Press, 1987, vol 31, pp 141–190.

6 Garcia-Segura LM, Olmos G, Tranque P, Naftolin F: Rapid effects of gonadal steroids upon hypothalamic neuronal membrane ultrastructure. J Steroid Biochem 1987;27: 615–623.

7 Schumacher M, Coirini H, Frankfurt M, McEwen BS: The neuronal membrane: A target for steroid hormone action; in Balthazart J (ed): Hormones, Brain and Behavior in Vertebrates. 2. Behavioral Activation in Males and Females: Social Interaction and Reproductive Endocrinology. Comp Physiology. Basel, Karger, 1990, vol 9, pp 91–115.

8 Savouret JF, Misrahi M, Milgrom E: Molecular action of progesterone. Int J Biochem 1990;6:579–594.

9 Baulieu E-E, Godeau JF, Schorderet M, Schorderet-Slatkine S: Steroid-induced meiotic division in *Xenopus laevis* oocytes: Surface and calcium. Nature 1978;275:593–598.

10 Baulieu E-E: Steroid-membrane-adenylate cyclase interactions during *Xenopus laevis* oocyte meiosis reinitiation: A new mechanism of steroid hormone action. Exp Clin Endocrinol 1983;81:3–16.

11 Smith SS, Waterhouse BD, Chapin JK, Woodward DJ: Progesterone alters GABA and glutamate responsiveness: A possible mechanism for its anxiolytic action. Brain Res 1987;400:353–359.

12 Klangkalya B, Chan A: Inhibition of hypothalamus and pituitary muscarinic receptor binding by progesterone. Neuroendocrinology 1988;47:294–302.

13 Su TP, London E, Jaffe JH: Steroid binding at σ receptors suggests a link between endocrine, nervous and immune systems. Science 1988; 240:219–221.

14 Blackmore PF, Beebe SJ, Danforth DR, Alexander N: Progesterone and 17α-hydroxyprogesterone: Novel stimulators of calcium influx in human sperm. J Biol Chem 1990;265: 1376–1380.

15 Ke F-C, Ramirez VD: Binding of progesterone to nerve cell membranes of rat brain using progesterone conjugated to ^{125}I-bovine serum albumin as a ligand. J Neurochem 1990;54: 467–472.

16 Ke F-C, Ramirez VD: Membrane mechanism mediates progesterone stimulatory effect on LHRH release from superfused rat hypothalami in vitro. Neuroendocrinology 1987;45: 514–517.

17 Dluzen DE, Ramirez VD: Progesterone effects upon dopamine release from the corpus striatum of female rats. II. Evidence for a membrane site of action and the role of albumin. Brain Res 1989;476:338–344.

18 Etgen AM, Petitti N: Norepinephrine-stimulated cyclic AMP accumulation in rat hypothalamic slices: effects of estrous cycle and ovarian steroids. Brain Res 1986;375:385–390.

19 Petitti N, Etgen AM: Progesterone depression of norepinephrine-stimulated cAMP accumulation in hypothalamic slices. Mol Brain Res 1989;5:109–119.

20 Petitti N, Etgen AM: α_1-Adrenoceptor augmentation of β-stimulated cAMP formation is enhanced by estrogen and reduced by progesterone in rat hypothalamic slices. J Neurosci 1990;10:2842–2849.

21 Lisk RD: A comparison of the effectiveness of intravenous as opposed to subcutaneous injection of progesterone for the rat. Can J Biochem Physiol 1960;38:1381–1383.

22 Kubli-Garfias C, Whalen RE: Induction of lordosis behavior in female rats by intravenous administration of progestins. Horm Behav 1977;9:380–386.

23 Etgen AM, Petitti N: Mediation of norepinephrine-stimulated cyclic AMP accumulation by adrenergic receptors in hypothalamic and preoptic area slices: Effects of estradiol. J Neurochem 1987;49:1732–1739.

24 Fredholm BB, Dunwiddie TV, Bergman B, Lindstrom K: Levels of adenosine and adenine nucleotides in slices of rat hippocampus. Brain Res 1984;295:127–136.

25 Larson E, Howlett B, Jagendorf A: Artificial reductant enhancement of the Lowry method for protein determination. Anal Biochem 1986;155:243–248.

26 Brostrom CO, Kon C: An improved protein binding assay for cyclic AMP. Anal Biochem 1974;58:459–468.

27 Doss RC, Perkins JP, Harden TK: Recovery of β-adrenergic receptors following long term exposure of astrocytoma cells to catecholamine: Role of protein synthesis. J Biol Chem 1981;256:12281–12286.

28 Harden TK: Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase. Pharmacol Rev 1983;35:5–32.

29 Etgen AM, Karkanias GB: Estradiol regulates the number of α_1 but not β or α_2 noradrenergic receptors in hypothalamus of female rats. Neurochem Int 1990;16:1–19.

30 Brown E, Kendall DA, Nahorski SR: Inositol phospholipid hydrolysis in rat cerebral cortical slices. 1. Receptor characterization. J Neurosci 1984;1:49–59.

31 Minneman KP, Johnson RD: Characterization of α_1-adrenergic receptors linked to ^{3}H inositol metabolism in rat cerebral cortex. J Pharmacol Exp Ther 1984;30:317–323.

32 Okajima F, Sato K, Sho K, Kondo Y: Stimulation of adenosine receptor enhances α_1-adrenergic receptor-mediated activation of phospholipase C and Ca^{2+} mobilization in a pertussis toxin-sensitive manner in FRTL-5 thyroid cells. FEBS Lett 1989;248:145–149.

33 Berridge MJ: Inositol trisphosphate and diacylglycerol: two interacting second messengers. Annu Rev Biochem 1987;56:159–193.

34 Cockcroft S, Stutchfield J: G-proteins, the inositol signalling pathway, and secretion. Phil Trans R Soc Lond 1988;320:247–265.

35 Chuang D-M: Neurotransmitter receptors and phosphoinositide turnover. Annu Rev Pharmacol Toxicol 1989;29:71–110.
36 Sugden D, Namboodiri MAA, Klein DC, Pierce JE, Grady R Jr, Mefford IN: Ovine pineal α_1-adrenoceptors: Characterization and evidence for a functional role in the regulation of serum melatonin. Endocrinology 1985; 116:1960–1967.
37 Chik CL, Ho AK, Klein DC: α_1-Adrenergic potentiation of vasoactive intestinal peptide stimulation of rat pinealocyte adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate: Evidence for a role of calcium and protein kinase-C. Endocrinology 1988;122:702–708.
38 Petitti N, Etgen AM: Protein kinase C and phospholipase C mediate α_1- and β-adrenoceptor intercommunication in rat hypothalamic slices. J Neurochem 1991;56:628–635.
39 Leeb-Lundberg, LMF, Cotecchia S, Lomasney JW, De Bernardis JF, Lefkowitz RJ, Caron MG: Phorbol esters promote α_1-adrenergic receptor phosphorylation and receptor uncoupling from inositol phospholipid metabolism. Proc Natl Acad Sci USA 1985; 82:5651–5655.
40 Leeb-Lundberg, LMF, Cotecchia S, DeBlasi A, Caron MG, Lefkowitz RJ: Regulation of adrenergic receptor function by phosphorylation. I. Agonist-promoted desensitization and phosphorylation of α_1-adrenergic receptors coupled to inositol phospholipid metabolism in DDT_1 MF-2 smooth muscle cells. J Biol Chem 1987;262:3098–3105.
41 Bouvier M, Leeb-Lundberg LMF, Benovic JL, Caron MG, Lefkowitz RJ: Regulation of adrenergic receptor function by phosphorylation. II. Effects of agonist occupancy on phosphorylation of α_1- and β_2-adrenergic receptors by protein kinase C and the cyclic AMP-dependent protein kinase. J Biol Chem 1987; 262:3106–3113.
42 Cormier P, Mulner-Lorillon O, Belle R: In vivo progesterone regulation of protein phosphatase activity in *Xenopus* oocytes. Dev Biol 1990;139:427–431.
43 Chien EJ, Kostellow AB, Morrill GA: Progesterone induction of phospholipid methylation and arachidonic acid turnover during the first meiotic division in amphibian oocytes. Life Sci 1986;39:1501–1508.
44 Morrill GA, Doi K, Kostellow AB: Progesterone induces transient changes in plasma membrane fluidity of amphibian oocytes during the first meiotic division. Arch Biochem Biophys 1989;269:690–694.
45 Rodbell M: The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 1980;284:17–22.
46 Casey PJ, Gilman AG: G protein involvement in receptor-effector coupling. J Biol Chem 1988;263:2577–2580.
47 Maguire ME, Van Arsdale PM, Gilman AG: An agonist-specific effect of guanine nucleotides on binding to the beta adrenergic receptor. Mol Pharmacol 1976;12:335–339.