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Estradiol Elevates Protein Kinase C Catalytic Activity in the Preoptic Area of Female Rats*

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ABSTRACT

Estrogen acts in the brain to regulate female reproductive physiology and behavior, and protein kinase C (PKC) is estrogen-regulated in many estrogen-responsive tissues. We examined whether estrogen regulates PKC in the hypothalamus (HYP) and preoptic area (POA), brain regions which mediate estrogenic control of female reproductive function. PKC activity in tissue from hormone-treated and control female rats was measured, in the presence of phorbol ester and calcium, by quantifying ^{32}P incorporation into a substrate peptide. PKC catalytic activity increased significantly in POA tissue extracts from estradiol-treated, ovariectomized (OVX) female rats but not in HYP or cortical extracts. Phorbol ester potentiation of cAMP accumulation also was examined to determine whether the ability of PKC to potentiate adenylyl cyclase activity was affected by estrogen. PKC stimulation potentiated forskolin-induced cAMP accumulation to a greater degree in POA, but not HYP, slices from estrogen-treated OVX female rats. PKC enzyme levels were examined using phorbol-12,13-dibutyrate binding assays and immunoblots. Estrogen treatment did not change phorbol ester binding affinity or the density of binding sites in the POA or HYP. Immunoblots for the α, β, and γ PKC isoforms combined, or the γ PKC isoform alone, did not detect differences between hormone-treated and control OVX female rats. Therefore, estrogen treatment increased PKC catalytic activity in the POA of OVX female rats but not in the HYP. However, the increased PKC catalytic activity was not correlated with detectable changes in the level of the α, β, or γ PKC isoforms or in the density of phorbol ester binding sites. (*Endocrinology* **139:** 3050–3056, 1998)

ESTROGEN is one of the major ovarian hormones responsible for regulating female reproductive physiology and behavior. Ovariectomized (OVX) female rats require estrogen replacement to reinstate normal gonadotropin release and sexual behavior (1–3). The noradrenergic pathways of the hypothalamus (HYP) and preoptic area (POA) are one site at which estrogen acts to modulate both pituitary secretion of gonadotropins and the expression of female reproductive behavior (2, 3). Previous work demonstrates that estrogen modifies noradrenergic receptors and their linkage to signal transduction pathways in the HYP-POA (2). For example, estrogen treatment of OVX female rats up-regulates α_{1B}-adrenergic receptor binding sites and messenger RNA (mRNA) in the HYP-POA (4, 5). Stimulation of α_{1B}-adrenergic receptors activates phospholipase C (6), which cleaves phosphoinositol 2-phosphate into inositol 1,4,5-triphosphate and diacylglycerol, which in turn, activates protein kinase C (PKC) (7, 8). Presumably, PKC goes on to phosphorylate and activate proteins required for reproductive behavior and gonadotropin release.

Cross-talk has been shown to occur between PKC and estrogen receptors. In mammary, ovarian, and pituitary tissue, estrogen up-regulates PKC protein and mRNA expression (9–15). In these cells, PKC activation augments the ability of estradiol to promote estrogen receptor-mediated increases in gene transcription. In some cases, PKC produces ligand-independent activation of estrogen receptor-induced gene transcription (13, 16, 17). Therefore, present studies examine whether estrogen regulates PKC in the HYP-POA.

We used assays of PKC catalytic activity and measurement of PKC augmentation of cAMP synthesis in brain slices to determine whether estrogen replacement in OVX female rats changes PKC activity in the POA or HYP. We report that estrogen elevates PKC catalytic activity in the POA of OVX female rats. Subsequent phorbol ester binding and immunoblot studies demonstrate that estrogen enhancement of PKC activity does not result from increased density of phorbol ester binding sites or protein levels of classical PKC isoforms.

Materials and Methods

Tissue slice preparation

Female Sprague-Dawley rats (Taconic Farms, Germantown, NY), weighing 150–175 g, were ovariectomized under Metofane anesthesia (Pitman Moore, Mundelein, IL). Three to 5 days later they were injected sc twice with either 0.1 ml peanut oil or 2 μg of estradiol benzoate (EB; Steraloids Inc., Wilton, NH) dissolved in 0.1 ml peanut oil at 24 and 48 h before decapitation. The animals were rapidly killed, and the brains were placed in artificial cerebral spinal fluid (aCSF). The entire HYP-POA was dissected and sliced (350-μm slices) 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, the first four slices, containing the medial and lateral POA, suprachiasmatic nucleus, and supraoptic nucleus, were taken and were designated POA. The next slice was discarded. The following four slices, containing the anterior, ventromedial, paraventricular, arcuate, and dorsomedial nuclei of the HYP, were kept and were designated HYP. Slices were

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incubated at 35 C in 300 μl aCSF for 75 min in an O_2/CO_2-saturated environment. All animal experimentation was carried out in accordance with guidelines recommended by the NIH Guide for the Care and Use of Laboratory Animals.

Drug treatment

After incubation of the slices, to equilibrate their cAMP level, they were treated with vehicle or drug for 20 min. Each slice received either vehicle, agonist alone, 1 μM phorbol-12,13-dibutyrate (PDB) alone, or agonist and 1 μM PDB dissolved in aCSF. Agonist treatments were either 10 μM forskolin (FOR), 100 μM adenosine (ADE), or 10 μM isoproterenol (ISO). Each experiment used tissue from two control and two EB-treated animals, and each experiment was repeated at least four times.

cAMP determination

After drug treatment, the slices were disrupted by sonication in 5% ice-cold trichloroacetic acid. The supernatant containing cAMP and the pellet containing tissue protein were separated by centrifugation. The supernatant was acidified with 1 N HCl and extracted four times with hydrated ether. The samples were dried by lyophilization and analyzed for cAMP concentration using a modified Gilman cAMP assay (18). The protein concentration was determined by resuspending the pellet in 2 N NaOH and assaying by a modified Lowry assay (19). All cAMP values were expressed as pmoles cAMP per milligram protein.

PKC extraction

OVX female Sprague-Dawley rats (150–175 g) were injected sc twice with either 0.1 ml peanut oil (control) or 2 μg of EB dissolved in 0.1 ml peanut oil at 24 and 48 h before decapitation. Animals were decapitated, and the brains were removed and washed with PBS. The HYP-POA, frontal cortex (CTX), pituitary or HYP, and POA separately were dissected and homogenized in a dounce homogenizer in extraction buffer [20 mM Tris (pH 7.5), 0.5 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, and 25 mg/ml each aprotinin and leupeptin]. The HYP-POA was dissected with an anterior cut approximately 2 mm anterior to the optic chiasm, a posterior cut just caudal to the mammillary bodies, lateral cuts at the hypothalamic fissures, and a final cut 4 mm dorsal to the ventral surface. The HYP and POA were divided by cutting the HYP-POA block just posterior to the optic chiasm. The homogenate was incubated on ice for 30 min, then centrifuged for 2 min at 10,000 × *g*. The supernatant was loaded onto a previously poured diethylaminoethyl-cellulose column. The column was made by suspending 0.5 g Whatman DE 52 in 1 ml washing buffer [20 mM Tris (pH 7.5), 0.5 mM EDTA, 0.5 mM EGTA, and 10 mM β-mercaptoethanol]. Columns were washed with 2 ml washing buffer and stored in a refrigerator until use. The loaded column was washed with 8 ml washing buffer. After washing, PKC was extracted from the column with 5 ml extraction buffer [20 mM Tris (pH 7.5), 0.5 mM EDTA, 0.5 mM EGTA, 10 mM β-mercaptoethanol, and 0.2 M NaCl]. In total, 5 ml extraction buffer was recovered. The protein concentration in the column eluate was determined using a modified Lowry assay.

PKC assay

Protein extracts were assayed for PKC activity using a kit (Life Technologies, Gaithersburg, MD) (20). The assay was linear between 2 and 8 μg protein (data not shown). Samples were assayed for 5 min at 30 C in a mixture containing approximately 5 μg protein extract, 50 μM Ac-MBP(4–14) (an acetylated synthetic peptide corresponding to amino acids 4–14 of myelin basic protein, which is a general PKC substrate), 20 μM ATP, 1 mM $CaCl_2$, 20 mM $MgCl_2$, 4 mM Tris (pH 7.5), 0.1 μCi ^{32}P ATP, 10 μM phorbol 12-myristate 13-acetate, 280 μg/ml phosphatidyl serine, and Triton X-100 mixed micelles. Nonspecific activity was determined in the same manner as total activity, except protein extract was preincubated for 20 min with 20 mM PKC (19–36) (a synthetic peptide corresponding to the pseudosubstrate region of PKC α that specifically inhibits PKC) at room temperature. Four replicates were done of the specific activity and two of the nonspecific activity. After the enzyme activation and incubation, half of the sample was spotted onto phosphocellulose paper and washed in 1% phosphoric acid. Phosphocellulose spotted discs were counted in a scintillation counter. Total PKC activity was determined by subtracting PKC activity in the presence of inhibitor peptide from total phorbol 12-myristate 13-acetate-stimulated activity.

[^{3}H] PDB binding

Tissue protein was extracted in the same manner as for the PKC activity assay, from the HYP or POA combined from three animals or from the CTX from a single animal. Binding conditions were derived from a combination of several protocols (21–29). Final concentrations of reagents were 20 mM Tris-HCl (pH 7.5), 500 μg/ml bovine γ-globulin, 0.5 mM $CaCl_2$, 5 mM $MgCl_2$, 10 μg/ml phosphatidylserine, and 40 μg/ml phosphatidylcholine. Before the binding reaction, the components of the binding buffer were vigorously vortexed and sonicated. Binding buffer, protein extract (~15 μg for CTX; > 100 μg for HYP and POA), and [^{3}H] PDB (Amersham Life Sciences, Arlington Heights, IL; 17.2 Ci/mmol), at seven concentrations ranging from 0.5–20 nM, with or without 100 μM cold PDB, were added in triplicate to glass tubes (0.25 ml final vol). The tubes were incubated at room temperature for 1 h. Mixtures were then filtered three times with PBS through a cell harvester onto GF/B filters, which were presoaked for 1 h in 0.3% polyethyleneimine. Filters were placed into vials, filled with scintillation fluid, and counted in a liquid scintillation counter. Data were plotted and analyzed by means of Scatchard analysis using the computer program EBDA.

Immunoblots

Protein was prepared by dounce homogenization of tissue (HYP, POA, or combined HYP-POA) in 1% SDS, 1 mM sodium vanadate, and 10 mM Tris-HCl, pH 7.4. After homogenization, tubes were boiled for 10 min and microwaved for 10–15 sec. Samples were spun for 5 min at 10,000 × *g* to remove insoluble material. Protein concentrations were determined by a modified Lowry assay. Twenty-five micrograms of protein, in duplicate, were applied to 12.5% SDS-polyacrylamide minigels and resolved at 150 volts for 1.5 h. Proteins were transferred electrophorectically to nitrocellulose membranes at 100 A for 1 h. Membranes were then blocked for 0.5 h in 5% nonfat dry milk in Tris-buffered saline (TBS). Membranes were incubated for 1 h at 37 C with primary antibody. Primary antibodies used were αβγ PKC rabbit polyclonal antibody (Life Technologies; 1:500 dilution in 5% milk and 0.1% goat serum), γ PKC mouse monoclonal antibody (Transduction Laboratories, Lexington, KY; 1:250 dilution in 5% milk and 0.1% goat serum) and β-tubulin type I and II mouse monoclonal antibody (Sigma, St. Louis, MO; 1:500 dilution in 5% milk and 0.1% goat serum). After four washes with 0.1% Tween 20 in TBS and two washes in 1× TBS, blots were incubated at room temperature for 1 h with horseradish peroxidase-conjugated antirabbit or antimouse secondary antibodies (Boehringer Mannheim, Indianapolis, IN; 1:1000 dilution in 5% milk and 0.1% goat serum), where appropriate. Blots were then washed in the same manner as for the primary antibody. Peroxidase activity was visualized by means of chemiluminescence (Renaissance, New England Nuclear, Boston, MA).

Blots were exposed to film, at two different times, to obtain signals within the linear range of the film and the densitometer. Standard curves were generated for each antibody, using 5–50 μg whole rat brain protein extract, and these produced linearly increasing signals over the entire range of starting protein. Twenty-five micrograms of protein extract was chosen for semiquantitative analysis, because signal reliably fell within the linear range of the film. Membranes were blotted for β-tubulin after initial blotting for PKC isoforms. β-tubulin blots were used as a control to verify that protein load was comparable among samples. Data were expressed as a ratio of OD of the PKC isoform signal divided by OD of the β-tubulin signal. ODs were obtained by scanning autoradiograms on a Molecular Dynamics densitometer and analyzing the image using Molecular Dynamics ImageQuant v3.03 (Molecular Dynamics, Sunnyvale, CA).

Statistics

PKC activity data in control and hormone-treated extracts were analyzed using Student's *t* tests for independent samples. PDB binding parameters were also analyzed using Student's *t* tests for independent samples. Data obtained from cAMP experiments were analyzed using

two-way ANOVA, with drug treatment and hormone as the two factors. Significant differences between means were determined for main effects using Tukey's test and least-square means for interactions. Differences were considered significant if $P < 0.05$.

Results

Effects of EB treatment on PKC activity in the brain

PKC catalytic activity was significantly elevated in column-purified protein extract from the combined HYP-POA of EB-treated animals, relative to OVX controls ($P < 0.01$; *t* test), but this was not the case in extracts from the CTX (Fig. 1A). PKC catalytic activity was higher in extracts from CTX than from the combined HYP-POA, and very low in pituitary (data not shown). To test whether estradiol directly modified PKC catalytic activity, 100 nM estradiol or 100 nM EB was added to combined HYP-POA protein extracts from OVX control rats before assaying PKC catalytic activity. *In vitro* addition of estradiol or EB had no effect on PKC catalytic activity in combined HYP-POA extracts (Table 1).

To determine whether estrogen-induced increases in PKC catalytic activity were localized within the combined HYP-POA, the HYP and POA were assayed separately. In POA extracts from EB-treated animals, PKC catalytic activity was elevated, relative to OVX controls ($P < 0.01$; *t* test; Fig. 1B). In HYP extracts, no difference was seen between extracts from control and EB-treated animals (Fig. 1B).

Effects of EB treatment on PKC potentiation of cAMP accumulation

Activation of PKC can increase cAMP synthesis by potentiating both receptor-dependent and receptor-independent activity of adenylyl cyclase (AC). This effect of PKC occurs in the HYP and POA of female rats (6–8). This experiment used the phorbol ester PDB to examine whether brain slices from the HYP or POA of EB-treated animals show greater PKC potentiation of AC than slices from control OVX females. As expected, in EB-treated and control slices, PDB alone had no effect on cAMP accumulation (Fig. 2). The receptor-independent activator of AC, FOR, stimulated cAMP accumulation ($P < 0.001$ *vs.* basal). PDB potentiated FOR-stimulated cAMP accumulation in control and EB-treated slices ($P < 0.01$ *vs.* FOR alone). Finally (as expected, on the basis of PKC catalytic activity assays), EB treatment increased the ability of PKC to potentiate FOR-stimulated cAMP accumulation in POA slices ($P < 0.05$; FOR+PDB in EB *vs.* FOR+PDB in oil; Fig. 2A) but not in HYP slices.

The ability of PKC to potentiate receptor-dependent activation of AC was also examined. The receptor agonists, ISO and ADE, were used for these experiments. PKC potentiation of receptor-dependent activation of AC did not differ between EB-treated and control slices (Table 2).

Effects of EB treatment on PDB binding and PKC enzyme levels in the brain

PDB binding assays were performed on the same protein extracts used to determine PKC catalytic activity separately in the POA and HYP (Fig. 1B). PDB binding affinity and the density of PDB binding sites were the same in POA, HYP, and CTX extracts from OVX control and EB-treated animals (Fig. 3; Table 3). The number of PDB binding sites was highest in CTX, in agreement with published literature (26, 28–30).

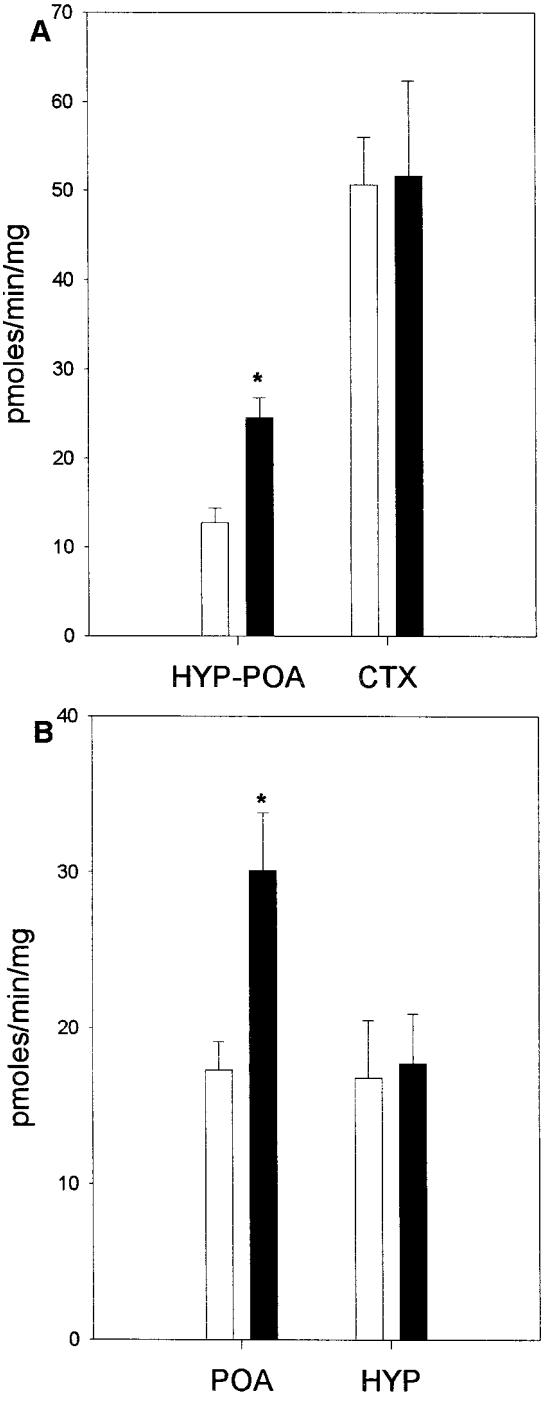

FIG. 1. Effects of hormonal status on PKC activity. Female Sprague-Dawley rats, ovariectomized 3–5 days before, were injected twice with either 0.1 ml peanut oil (*open bars*) or 2 μg EB (*filled bars*) at both 24 and 48 h before decapitation. All measurements of pmoles ^{32}P incorporated into a substrate were done in quadruplicate. A, Data are means ± SEM of 7 independent replications for HYP-POA and 6 independent replications for CTX (*, significantly greater than oil, $P < 0.05$); B, data are means ± SEM of 11 independent replications for POA and 10 independent replications for HYP (*, significantly greater than oil, $P < 0.01$).

EB effects on PKC enzyme levels were further examined

TABLE 1. Estradiol *in vitro* has no effect on PKC catalytic activity (pmol ^{32}P/min·mg protein extract)

	Control	Treated
100 nM EB	13.6 ± 4.5	15.3 ± 4.1
100 nM Estradiol	18.0 ± 8.5	19.2 ± 7.9

Treatments were 100 nM EB or estradiol added to HYP-POA tissue extracts from oil-treated, OVX rats 5 min before addition of ATP and peptide substrate. Measurements of ^{32}P incorporation were done in quadruplicate. Data represent the mean ± SEM of four independent measurements.

by immunoblotting. Immunoblots, with a polyclonal PKC antibody recognizing the α, β, and γ isoforms, were done on combined HYP-POA tissue. EB treatment for 48 h had no effect on PKC immunoreactivity for the combined α, β, and γ isoforms (Fig. 4A). Immunoblots with a monoclonal PKC antibody recognizing only the γ isoform were done on separate HYP and POA extracts. The γ isoform is highly expressed in brain, so we wished to verify that a hormone-dependent change in PKC immunoreactivity was not missed in the initial blots because they were done on combined HYP-POA using a PKC antibody that recognizes multiple isoforms. EB treatment for 48 h did not affect γ PKC immunoreactivity in the HYP (data not shown) or the POA (Fig. 4B). Immunoblots with a monoclonal PKC antibody specific for the ϵ isoform were done on POA extracts. PKC ϵ immunoreactivity in the POA did not increase after EB treatment (data not shown).

Discussion

Estrogen treatment, sufficient to reinstate gonadotropin surges and female reproductive behavior, elevated PKC catalytic activity in the POA of OVX female rats. An increase in PKC catalytic activity was detectable in the combined HYP-POA of estrogen-treated animals. Further dissection localized the increase to the POA. The cAMP studies verified that this estrogen-induced increase in PKC catalytic activity has functional consequences. PKC potentiation of receptor-independent activation of AC was elevated in the POA slices from estrogen-treated animals. Interestingly, PKC potentiation of receptor-dependent activation of AC was unaffected by hormonal condition.

Estrogen effects on G-protein-coupled receptors may explain the discrepancy between the results on receptor-independent and receptor-dependent activation of AC. Previously, we showed that β-adrenergic receptors were uncoupled from G-proteins in the HYP and POA of estrogen-treated animals (31). Therefore, activation of AC by β-adrenergic receptors was suppressed in estrogen-treated animals. The present data confirmed that activation of β-adrenergic receptors with ISO increased cAMP accumulation in control but not in EB-treated slices (Table 2). That there was no difference between cAMP levels of estrogen-exposed and control slices after treatment with ISO and PDB, despite the absence of an ISO response in hormone-treated slices, suggests that PKC potentiation of receptor-dependent AC activity may have increased in slices from estrogen-treated animals. Estrogen treatment did not suppress ADE receptor activation of AC or modify PKC potentiation of the ADE response. This may suggest that estrogen does not regulate PKC in cells expressing ADE receptors.

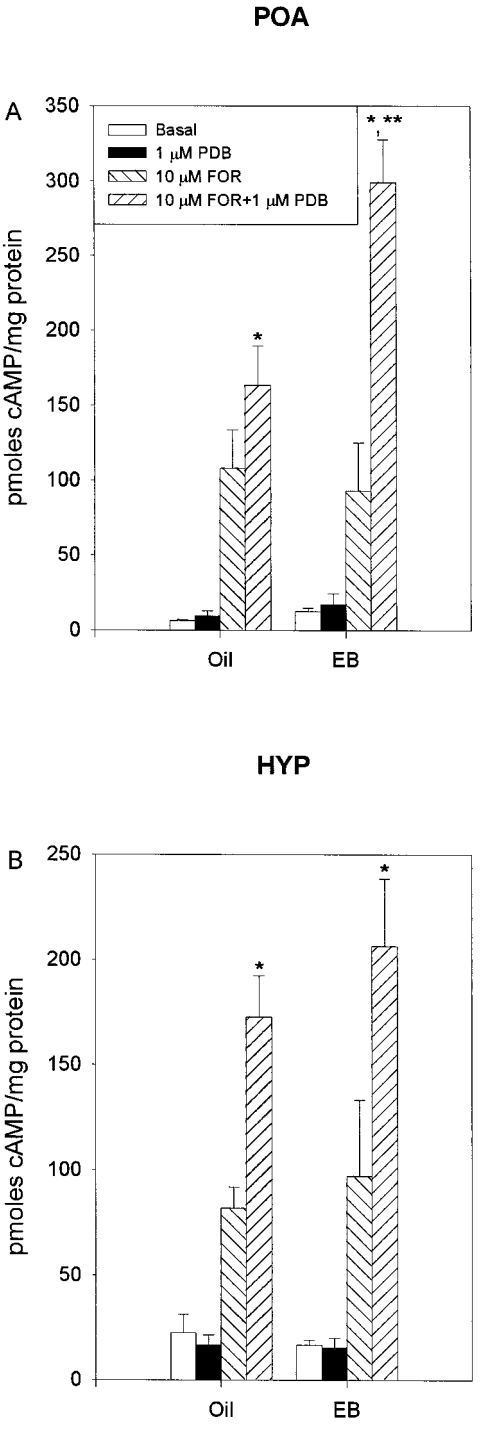

FIG. 2. Effects of hormonal status on PKC potentiation of receptor-independent stimulation of cAMP accumulation. Slices were incubated, with vehicle (basal) or the indicated drug concentrations, for 20 min. Data are means ± SEM of four to eight independent replications. *, Significantly greater than FOR alone; **, significantly greater than oil control slice treated with PDB and FOR.

In an attempt to identify which PKC isoforms are regulated by estrogen, we carried out PDB binding and immunoblots for specific PKC isoforms. Neither PDB binding af-

TABLE 2. PKC potentiation of receptor-dependent stimulation of cAMP accumulation (pmoles cAMP/mg protein)

		Basal	PDB	Agonist	Agonist + PDB
Isoproterenol					
POA	Oil	4.3 ± 1.3	6.5 ± 1.7	9.1 ± 2.3	19.2 ± 3.1[a]
	EB	6.4 ± 2.1	5.8 ± 1.5	7.0 ± 0.7	17.8 ± 3.7[a]
HYP	Oil	5.5 ± 0.8	9.1 ± 2.8	12.2 ± 2.8	18.3 ± 4.2[a]
	EB	9.5 ± 1.6	7.5 ± 2.5	8.0 ± 1.0	24.0 ± 0.6[a]
Adenosine					
POA	Oil	12 ± 5.3	7.5 ± 0.7	54 ± 28	132.2 ± 46[a]
	EB	6.8 ± 4.6	8.7 ± 0.6	31 ± 4.9	88.3 ± 58[a]
HYP	Oil	6.4 ± 1.6	9.2 ± 0.8	28 ± 7.3	213.7 ± 26[a]
	EB	5.9 ± 2.7	16.8 ± 1.1	29 ± 4.9	158.2 ± 22[a]

Slices were incubated with vehicle (basal), 1 μM PDB, 10 μM isoproterenol, 100 μM adenosine, 1 μM PDB + 10 μM isoproterenol, or 1 μM PDB + 100 μM adenosine for 20 min. Each value represents the mean ± SEM of four independent experiments.

[a] Significantly greater than agonist alone, $P < 0.05$.

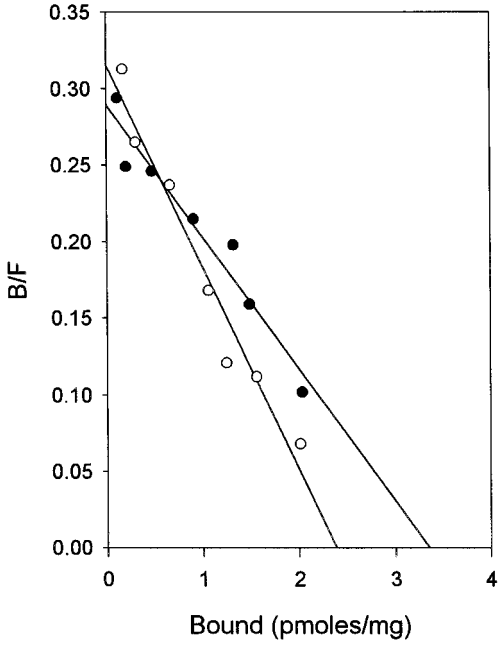

FIG. 3. Representative Scatchard plots of PDB binding in POA tissue extracts from oil (*closed circles*) and EB-treated (*open circles*) female rats. B/F, Bound/free.

TABLE 3. ^{3}H-PDB binding in protein extracts

		K_d (nM)	B_{max} (pmol/mg)	n
POA	Oil	7.6 ± 1.7	3.1 ± 0.5	5
	EB	5.3 ± 0.5	3.5 ± 0.6	5
HYP	Oil	4.8 ± 0.6	2.9 ± 0.6	5
	EB	6.6 ± 0.7	4.6 ± 1.3	5
CTX	Oil	3.3 ± 0.8	12.9 ± 1.9	2
	EB	3.6 ± 0.2	11.8 ± 1.2	2

^{3}H-PDB binding was done on DEAE cellulose column-purified protein from three pooled animals for POA and HYP and individual animals for CTX. Values were obtained by Scatchard analysis of binding data. K_d, Dissociation constant; B_{max}, Binding site density.

finity nor the density of PDB binding sites differed in any brain region as a function of estrogen treatment. Semiquantitative immunoblots, using antibodies recognizing either the classical (α, β, and γ) PKC isoforms or the γ PKC isoform specifically, detected no differences in POA extracts from animals with and without estrogen treatment. These results suggest that either the protein levels of the phorbol ester-sensitive PKC isoforms are not elevated by estrogen or that PDB binding and PKC immunoblots are not sensitive enough methods to detect small differences in protein expression.

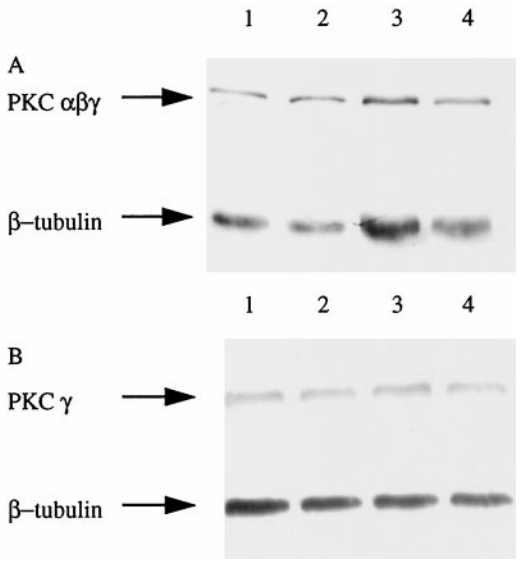

FIG. 4. Immunoblots of PKC α, β, and γ immunoreactivity in tissue extracts from OVX control and EB-treated rats. Whole-cell protein extracts from OVX rats injected with oil (lanes 1–2) or EB (lanes 3–4), both 24 and 48 h before death. A, Immunoblot with an antibody reactive to PKC α, β, and γ on 25 μg whole-cell protein extract from the HYP-POA; B, immunoblot with an antibody reactive to PKC γ on 25 μg whole-cell protein extract from the POA.

Some information about the identity of PKC isoforms affected by estrogen can be deduced from the methods used to evaluate PKC catalytic activity. The PKC activity assay used a substrate that is preferentially phosphorylated by the classical (α, β, and γ) and novel (δ, ε, η, μ, and θ) PKC isoforms (21, 28). The assay also measured phorbol ester-stimulated kinase activity, which would predominantly measure the classical and novel PKC isoforms. Moreover, we used PDB, a phorbol ester, to activate PKC in the cAMP studies. Therefore, estrogen regulation of PKC catalytic activity in the POA is likely to involve phorbol ester-sensitive PKC isoforms. Estrogen could modulate PKC catalytic activity by influencing PKC phosphorylation state or PKC helper proteins, such as receptor for activated C-kinase (32–35), rather than by increasing protein expression.

Estrogen elevation of PKC catalytic activity in the POA

may have implications for noradrenergic actions that are vital for gonadotropin release and reproductive behavior (1–3). PKC is a downstream mediator of α_{1B}-adrenergic receptor signaling in the POA and elsewhere (2, 6–8, 36). We have already shown that α_{1B}-adrenergic receptor binding and mRNA are elevated in the HYP-POA of estrogen-treated female rats (4, 5). Estrogen treatment of OVX female rats thus elevates signaling through the α_{1B}-adrenergic pathway in the HYP-POA. Another lab has shown increased phospholipase C-α mRNA in the rat uterus and ventromedial HYP after estrogen treatment (37). Because PKC is a mediator of α_{1B}-adrenergic signaling, increased PKC catalytic activity would further potentiate any response caused by activation of α_{1B}-adrenergic receptors.

Another consequence of elevated PKC activity may be desensitization of other G-protein-coupled receptors. PKC can promote heterologous desensitization, which is the phosphorylation and subsequent uncoupling of a G-protein-coupled receptor from its associated G-protein (38–39). PKC phosphorylation can also elevate G-protein-coupled receptor kinase activity, which is responsible for homologous desensitization (38). Our laboratory showed that estrogen treatment of OVX female rats uncouples both α_2- and β-adrenergic receptors from G-proteins in the HYP-POA (31, 40). Thus, increased PKC catalytic activity, by promoting either heterologous or homologous desensitization, could be involved in these processes.

Elevation of PKC catalytic activity could also have a wide range of consequences on gene transcription in cells of the POA. In mammary and ovarian cells, PKC potentiates the ability of liganded estrogen receptor to augment gene transcription by phosphorylation of the estrogen receptor (41–45). Both PKC and estrogen receptor can also augment transcription by increasing the phosphorylation or protein level of transcription factors such as c-fos and c-jun. (42, 43, 46–48). Therefore, PKC may contribute to estrogen facilitation of reproductive function by amplifying estrogen actions on target genes (49, 50).

In summary, estrogen elevated PKC catalytic activity in the POA, an estrogen-responsive brain region, but not in the HYP. This elevation of PKC catalytic activity seemed to have functional relevance, in that the ability of PKC to potentiate AC activity is also increased by estrogen. Finally, immunoblots and PDB binding were unable to detect a change in PKC protein expression in the POA of estrogen-treated rats. Therefore, future studies will need to examine whether the increased PKC catalytic activity in estrogen-treated rats is caused by increased expression of one or more PKC isoforms or an alteration in the activity of the PKC isoforms already present.

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