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α_1-Adrenoceptor Augmentation of β-Stimulated cAMP Formation Is Enhanced by Estrogen and Reduced by Progesterone in Rat Hypothalamic Slices

Nicolas Petitti and Anne M. Etgen

Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

These experiments examined the influence of estradiol and progesterone given *in vivo* on norepinephrine (NE) regulation of cAMP synthesis in hypothalamic and preoptic area slices *in vitro*. Administration of progesterone to estrogen-primed female rats attenuated NE-induced slice cAMP accumulation. This hormone-dependent reduction in NE-stimulated cAMP synthesis was observed in slices incubated with TTX and in slices prepared from hypophysectomized rats, suggesting that progesterone effects on NE receptor activation of cAMP-generating systems are not secondary to the release of neurotransmitters that inhibit adenylyl cyclase or to changes in pituitary hormone secretion. Progesterone suppression of NE-induced cAMP formation could be prevented by incubating slices in the presence of a phorbol ester. In additional studies, the activity of β-NE receptors was assessed by measuring isoproterenol (ISO)-stimulated cAMP accumulation in the presence of the phosphodiesterase inhibitor RO-20-1724, and the activity of α_1 receptors was evaluated by measuring phenylephrine (PHE) augmentation of the ISO response. Estradiol reduced the cAMP response to ISO in both hypothalamic and preoptic area slices, and this effect was not reversed by subsequent progesterone treatment. Estradiol also enhanced PHE augmentation of ISO-stimulated cAMP synthesis. Moreover, administration of progesterone subsequent to estradiol eliminated α_1-receptor augmentation of the ISO response. An α_1 enhancement of the ISO response is observed if the progestin receptor antagonist RU 38486 is administered before progesterone. Progesterone also abolished PHE potentiation of vasoactive intestinal polypeptide-stimulated cAMP accumulation. In contrast, neither phorbol ester nor muscarinic (carbachol) potentiation of the cAMP response to ISO was affected by progesterone. The data suggest that ovarian steroids regulate the coupling of both α_1 and β receptors to the membrane effector systems that generate intracellular cAMP.

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Correspondence should be addressed to Nicolas Petitti, Department of Psychiatry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

Extracellular first messengers (e.g., neurotransmitters and water-soluble hormones) alter cell function by binding with membrane receptors that regulate the production of intracellular second messengers. Occupancy of β-adrenergic receptors by agonists results in a conformational change, facilitating the interaction of occupied receptors with the guanyl nucleotide-binding protein Gs (see Freissmuth et al., 1989). Gs then activates the catalytic subunit of adenylyl cyclase, leading to the formation of cAMP. Agonist occupancy of α_1-adrenergic receptors results in the activation of another guanyl nucleotide binding protein, Gp, which activates phospholipase C (Brown et al., 1984; Minneman and Johnson, 1984; Mahan, 1987; Szabadi and Bradshaw, 1987; Okajima et al., 1989), leading to the hydrolysis of membrane inositol phospholipids into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (see reviews in Berridge, 1987; Cockcroft and Stutchfield, 1988; Chuang, 1989).

Intracellular second messengers can play interconnected roles by modulating the generation of other messengers (see reviews in Nishizuka, 1984, 1986; Berridge, 1986, 1987; Enna and Karbon, 1987). A prime example of second-messenger interaction is the norepinephrine (NE) system in the rat brain. In some tissues, the amount of cAMP produced by β-receptor occupancy can be enhanced by concurrent α_1-receptor activation (Perkins and Moore, 1973; Daly et al., 1980; Duman et al., 1985; Sugden et al., 1985; Etgen and Petitti, 1987). There is evidence that activation of protein kinase C (PKC) may mediate α_1 augmentation of cAMP formation. For example, phorbol esters and synthetic diacylglycerols, which directly activate PKC, mimic the synergistic effects of α_1 agonists on cAMP synthesis in some tissues (Sugden et al., 1985; Chik et al., 1988; Ho et al., 1988), including hypothalamic slices (Petitti and Etgen, submitted). We also find that PKC inhibitors (H7 and staurosporine) and a phospholipase C inhibitor (neomycin) attenuate or abolish α_1-receptor enhancement of cAMP synthesis in hypothalamic slices (Petitti and Etgen, submitted). It should be noted that α_2 adrenoceptors may also contribute to NE-stimulated cAMP accumulation in cortical (Pile and Enna, 1986) and hypothalamic slices (Petitti and Etgen, 1989).

Recent evidence suggests that ovarian steroids modulate neurotransmitter-receptor-effector systems involving the second messenger cAMP in the brain (Durant et al., 1983; Collado et al., 1985; Etgen and Petitti, 1986, 1987; Harrelson and McEwen, 1987a, b; Petitti and Etgen, 1989; Zubin and Taleisnik, 1989), as well as other tissues (Riemer et al., 1988; Roberts et al., 1989; Wu et al., 1989). For example, we found that estrogen treatment simultaneously enhances α_1-receptor augmentation and depresses β-receptor stimulation of cAMP formation in female rat

hypothalamic slices (Etgen and Petitti, 1987). We also found that progesterone (P) treatment of estrogen-primed female rats, but not P alone, depresses NE-stimulated cAMP formation (Petitti and Etgen, 1989). Preliminary studies indicated that P may inhibit α_1-receptor augmentation of β-stimulated cAMP synthesis. The purpose of the present study was to determine in greater detail (1) whether the reduced cAMP response to NE in P-treated animals is due to a modification of the α_1 augmentation, (2) what is the influence of ovarian steroids on β-stimulated cAMP synthesis, and (3) whether estrogen and P selectively modulate NE-receptor coupling to cAMP-generating systems in hypothalamic slices.

Materials and Methods

Tissue slice preparation and stimulation of cAMP accumulation. Sexually mature female Sprague-Dawley rats weighing 150–175 gm were ovariohysterectomized (OVX) bilaterally under Metofane anesthesia 4–7 d prior to use. Hypophysectomized female rats were obtained from Blue-Spruce Farms (Altamont, NY), while all other rats were obtained from Taconic Farm (Taconic, NY). In all cases, estrogen treatment consisted of 2 subcutaneous injections of 2 μg estradiol benzoate (EB) given 24 and 48 hr before sacrifice. The P treatment consisted of a subcutaneous injection of 500 μg P given 3.5 hr before sacrifice. EB and P were dissolved in peanut oil and injected in a volume of 0.1 ml.

Animals were killed by decapitation and their brains rapidly removed, dissected over ice, and placed into ice-cold Yamamoto's medium (Yamamoto, 1972). The entire hypothalamus and preoptic area (POA) were removed, and slices (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, slices of POA and middle hypothalamus (MH) were obtained as described earlier (Etgen and Petitti, 1986, 1987). 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 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 (Etgen and Petitti, 1986, 1987; Petitti and Etgen, 1989). Slices were left undisturbed for 75 min to allow nucleotide levels to stabilize (Fredholm et al., 1984), then incubated another 20 min with the appropriate drug or vehicle.

At the end of the incubation period, the slices were transferred rapidly to 400 μl ice-cold 5% trichloroacetic acid (TCA). The slices were disrupted by sonication, and the supernatant (containing cAMP) and pellet (containing tissue protein) were separated by centrifugation. The TCA pellet was dissolved in 2.0 N NaOH for later determination of protein content (Larson et al., 1986). The supernatant was acidified with 1.0 N HCl, and TCA was removed with 4 vol washed ether. The resulting aqueous extracts were concentrated by lyophilization and analyzed for cAMP content using a modified Gilman protein binding assay (Brostrom and Kon, 1974). Data were converted to pmol cAMP/mg tissue protein. For all experiments, values for the 4 POA or 3 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 the appropriate vehicle: distilled water for adenosine, carbamoyl choline chloride (carbachol), isoproterenol (ISO), phenylephrine (PHE), TTX, and vasoactive intestinal polypeptide (VIP); and dimethyl sulfoxide for phorbol-12,13-dibutyrate (PDB). Control slices received equal volumes of vehicle at the same time. In some experiments, phosphodiesterase inhibitor D-4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO-20-1724) was added at a concentration of 0.7 mM at the beginning of the equilibration period in a volume of 1% ethanol.

Materials. EB and P were purchased from Steraloids, Inc. (Wilton, NH). Metofane was obtained from Pitman-Moore, Inc. (Atlanta, GA). The synthetic antiprogestin 17β-hydroxyl-11β-(4-dimethylaminophenyl)-17α-(1-propyl)estra-4,9-dien-3-one (RU 38486) was a kind gift of Dr. D. Philibert (Roussel-Uclaf, Romaineville, France). VIP was purchased from Bachem (Torrance, CA). Adenosine, carbachol, NE, and TTX 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. Because we wished to evaluate potential effects of hormone and drug treatments on basal cAMP levels, basal cAMP was always included as an independent group in the statistical analysis. Planned post hoc comparisons were made using the Newman-Keuls multiple range test unless noted otherwise. Differences were considered statistically significant if $p < 0.05$.

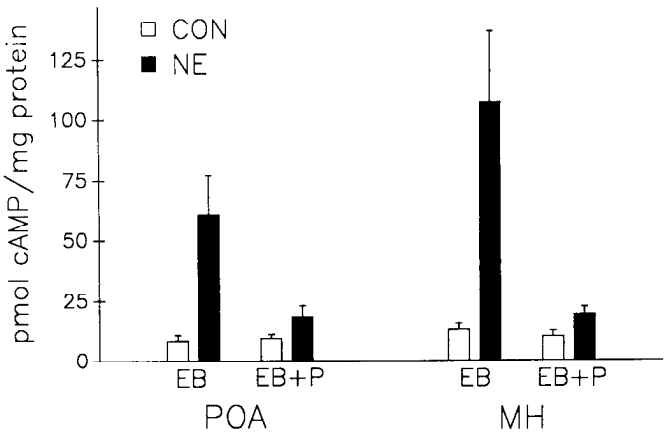

Figure 1. Stimulation of cAMP accumulation in the absence (*CON*) or presence (*NE*) of 10 μM NE in POA and MH slices from hypophysectomized rats. OVX female rats were primed with EB only (2 μg, 24 and 48 hr before sacrifice) or with EB + 500 μg P 3.5 hr before death. Each value represents the mean (±SEM) of 4–5 independent replications. Two-way analysis of variance (hormone × NE treatment) showed significant main effects of hormone ($p < 0.03$), NE treatment ($p < 0.003$), and hormone-treatment interactions ($p < 0.04$) in both the POA and MH slices.

Results

NE-stimulated cAMP synthesis in hypophysectomized animals and TTX-exposed tissue

In our previous study (Petitti and Etgen, 1989), P treatment of EB-primed rats attenuated the cAMP response to NE in MH and POA slices. To rule out the possibility that pituitary secretions mediate the depressant effect of P on NE-stimulated cAMP, the cAMP response to NE was compared in EB- and EB + P-treated, hypophysectomized rats. Figure 1 shows that, in MH and POA slices from EB-treated animals, 10 μM NE elicits 7- to 9-fold increases in cAMP. In contrast, in slices from EB + P-treated animals, 10 μM NE produces only a 2-fold increase in cAMP. These patterns replicate our earlier observations in slices of POA and MH from nonhypophysectomized rats (Etgen and Petitti, 1986; Petitti and Etgen, 1989).

Figure 2 shows that P inhibition of NE-stimulated cAMP formation in EB-treated rats is apparent when slices are incubated with 2 μM TTX, a sodium channel blocker, to prevent neuronal firing. POA and MH slices from EB- and EB + P-treated animals show similar patterns of NE-stimulated cAMP in the presence (Fig. 2) or absence of TTX (data not shown).

Effect of steroids on β-stimulated cAMP formation and its augmentation by α_1 receptors

We demonstrated in an earlier study (Etgen and Petitti, 1987) that slices from OVX and EB-treated animals exhibit an α_1-receptor augmentation of β-stimulated cAMP accumulation. A later study (Petitti and Etgen, 1989) suggested that P treatment of EB-primed rats reduces the α_1 facilitation of β-stimulated cAMP synthesis. The present experiment determined more systematically whether ovarian steroid treatment of OVX rats influences α_1 enhancement of β-stimulated cAMP formation. Figure 3 shows the cAMP response to increasing ISO doses and

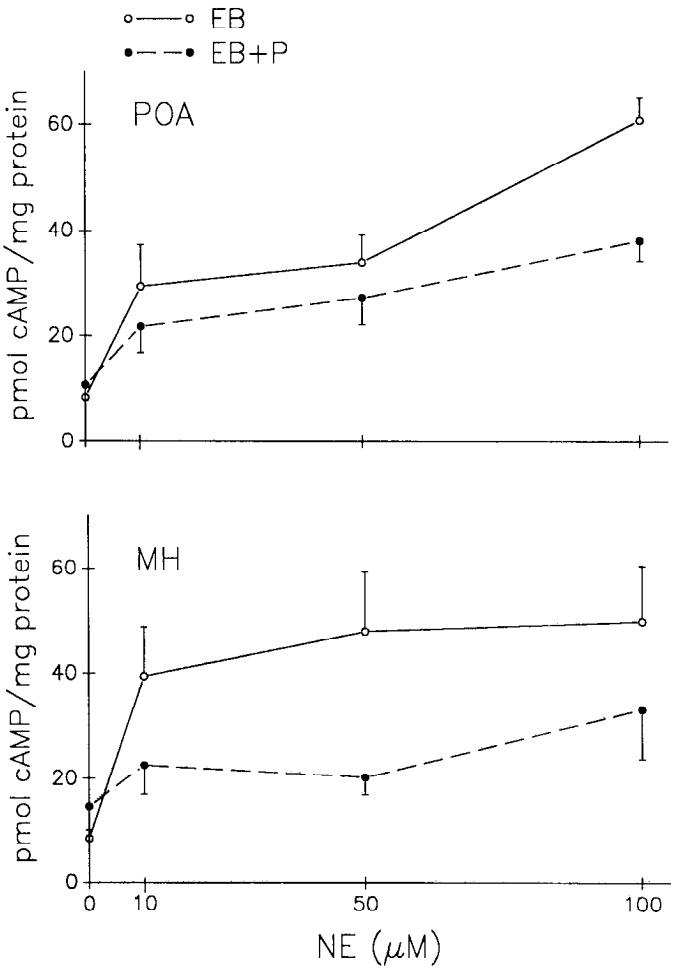

Figure 2. Concentration-dependent elevation of cAMP content by NE in slices incubated with TTX (2 μM). OVX female rats received the same EB and EB + P treatments described in Figure 1. Each value represents the mean (±SEM) of 4 independent replications. Two-way analysis of variance (hormone × NE concentration) showed significant main effects of hormone ($p < 0.05$) and NE concentration ($p < 0.01$) in both the POA and MH slices.

the ability of the α_1 agonist PHE (10 μM) to augment the ISO response in POA and MH slices from OVX and EB- and EB + P-treated rats. PHE potentiation of the ISO response was calculated after subtracting basal cAMP levels. PHE alone did not change basal cAMP levels in any hormone treatment group. Although the total cAMP response to ISO + PHE (pmol/mg) was similar in OVX and EB-exposed rats, slices from EB-treated animals demonstrated a relatively greater α_1-receptor potentiation of the β response than did slices from OVX animals. For example, PHE potentiated the cAMP response to 1 μM ISO by 30% in OVX animals, but by 300% in slices from EB-treated animals. In contrast to the clear α_1-receptor augmentation of β-stimulated cAMP synthesis observed in OVX and EB conditions, PHE did not potentiate the cAMP response to ISO in POA or MH slices from EB + P-treated animals. In agreement with our previous study (Etgen and Petitti, 1987), Figure 3 also shows that ISO-stimulated cAMP accumulation is significantly decreased in POA and MH slices from EB-treated animals when compared to OVX animals (1 and 10 μM ISO; $p < 0.05$). This effect of EB was not reversed by P in that the response to ISO in EB + P-treated animals was not significantly different from that seen in EB-treated animals.

Effect of ovarian steroids on PKC augmentation of cAMP formation

There is evidence in some tissues that α_1-receptor augmentation of cAMP formation is mediated by PKC. Activators of PKC can mimic the potentiating effect of PHE on cAMP synthesis, and PKC inhibitors remove this potentiation (Chik et al., 1988; Ho et al., 1988; Petitti and Etgen, submitted). As part of the evaluation of the hormonal modification of cAMP formation in hypothalamic slices (Fig. 3), we also examined the ability of PDB (a phorbol ester that activates PKC) to augment ISO-stimulated cAMP generation in OVX and EB- and EB + P-treated animals. Comparison between Figures 3 and 4 reveals that the direct activation of PKC by phorbol ester markedly enhanced the cAMP response to ISO in OVX and EB- and EB + P-treated slices ($p < 0.01$ vs. ISO alone; Fig. 3). At 0.1 μM ISO + PDB, total cAMP content was significantly higher in POA slices from OVX than from EB- and EB + P-treated animals ($p < 0.01$). This difference can probably be ascribed to the 2-fold higher basal level of cAMP observed in PDB-treated slices from OVX animals than in slices from EB- or EB + P-treated rats; the percent increase in cAMP level from 0 to 0.1 μM ISO was similar in all hormone treatment groups (OVX, 120%; EB, 100%; EB + P, 110%).

Further studies determined whether activation of PKC by PDB would prevent P suppression of NE-stimulated cAMP formation. As observed previously (Petitti and Etgen, 1989), P treatment of EB-primed rats attenuates NE-stimulated cAMP accumulation in POA and MH slices by about 50%. In contrast, slices from EB- and EB + P-treated rats incubated in the presence of PDB showed comparable cAMP responses to 10, 50, and 100 μM NE; i.e., the depressive effect of P was not observed when PKC was directly activated (data not shown). Interestingly, incubation of slices with phorbol-12-myristate-13-acetate (PMA) did not influence cAMP formation. This may reflect the existence in the brain of several PKC isozymes with different potencies of activation by phorbol esters or diacylglycerol (Huang, 1989). For example, recent data indicate that PDB and PMA differentially activate one PKC isoform (Mauduit et al., 1989).

Effect of the antiprogestin RU 38486

To determine if P elimination of the α_1-augmenting response is mediated by neuronal progestin receptors, the cAMP response to ISO in the presence or absence of PHE was evaluated in slices from EB + P-treated female rats given the progestin receptor antagonist RU 38486 (Philibert, 1984; Etgen and Barfield, 1986). Animals received the same EB and P treatments described previously, but they were also subcutaneously injected with 1 mg RU 38486 1 hr prior to the P injection. In slices from rats given RU 38486, α_1-receptor augmentation of β-stimulated cAMP formation is clearly present (Fig. 5).

Effect of steroids on α_1 augmentation of other Gs-coupled receptors

These experiments assessed whether ovarian steroids modulate α_1-receptor augmentation of the cAMP response induced by the activation of other Gs-coupled receptors. Adenosine and VIP were chosen for this examination because previous studies have shown that the cAMP response to both agents can be augmented by α_1-receptor activation (Szabadi and Bradshaw, 1987; Chik

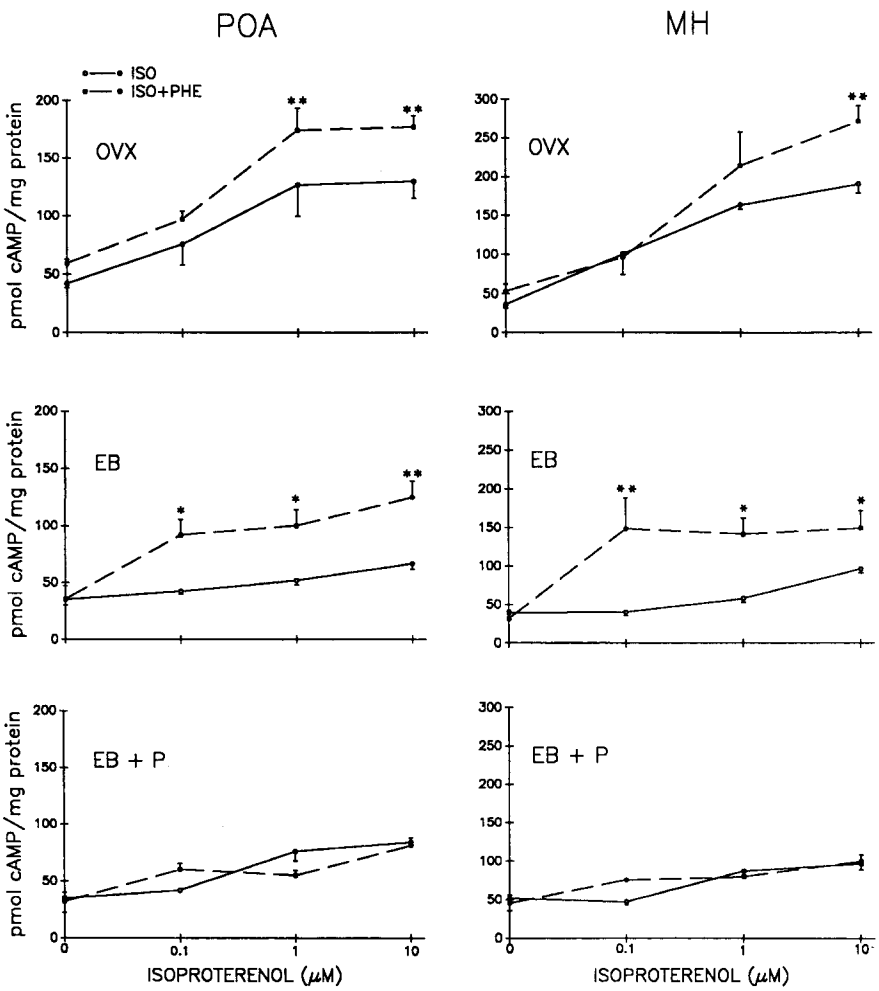

Figure 3. Effects of α_1- and β-adrenergic-receptor agonists on cAMP content of POA and MH slices from OVX and EB- and EB + P-treated rats. OVX female rats received the same EB and EB + P treatments described in Figure 1. Slices were equilibrated in the presence of 0.7 mM RO-20-1724 throughout the experiment, then incubated an additional 20 min with ISO (*solid line*) alone or in combination with 10 μM PHE (*dashed line*). Each value represents the mean (±SEM) of 3–4 independent replications. *, significantly different from ISO alone ($p < 0.005$, t test); **, significantly different from ISO alone ($p < 0.05$).

et al., 1988). It should be noted that PHE alone does not modify basal cAMP in any hormone treatment group (e.g., Figs. 3, 5; Etgen and Petitti, 1987; Petitti and Etgen, 1989).

Figure 6 shows adenosine-stimulated cAMP formation and the influence of PHE on this response. In OVX animals, PHE significantly augmented adenosine-stimulated cAMP by 65% in POA ($p < 0.05$ vs. adenosine alone) but not in MH slices ($p > 0.05$). In contrast to EB effects on PHE potentiation of the ISO response, estrogen induced no modification in the percent of α_1 augmentation of the adenosine response in POA slices. However, EB did promote a significant α_1 potentiation of adenosine-stimulated cAMP formation in the MH ($p < 0.05$ vs. adenosine alone). P treatment of EB-primed animals did not eliminate PHE augmentation of adenosine-stimulated cAMP synthesis in POA or MH slices. Interestingly, EB potentiated the cAMP response to adenosine alone in both POA and MH slices ($p < 0.01$ vs. OVX).

Figure 7 shows the effect of PHE on VIP-stimulated cAMP synthesis. There was little evidence of PHE potentiation of the cAMP response to VIP in POA slices. In MH slices, PHE augments VIP-stimulated cAMP accumulation in OVX ($p < 0.01$) and EB-treated animals ($p < 0.05$). The addition of P to EB-primed rats eliminated PHE potentiation of the VIP response in MH slices.

Effect of steroids on carbachol augmentation of ISO-stimulated cAMP synthesis

Because α_1 receptors are coupled to phospholipase C through Gp, we determined if P selectively inhibits the α_1-receptor augmenting response or if the augmentation produced by other receptors coupled through Gp is also affected by P. Figure 8 depicts the effect of carbachol, an agonist for muscarinic receptors that activate phospholipase C via Gp (Martinson et al., 1989), on ISO-stimulated cAMP formation. In POA slices, carbachol did not significantly potentiate the cAMP response to ISO. Because not all muscarinic receptor subtypes are coupled to Gp (Baumgold and Drobnick, 1989), the POA may well contain predominantly muscarinic receptors coupled to other membrane effector systems. However, the cAMP response in MH slices was augmented by carbachol at both doses of ISO employed ($p < 0.01$). The addition of P to EB-treated rats did not attenuate carbachol augmentation of β-stimulated cAMP synthesis.

Discussion

Present studies confirm and extend our previous finding (Petitti and Etgen, 1989) that P depresses NE-stimulated cAMP formation in estrogen-primed female rats. First, P inhibition of the

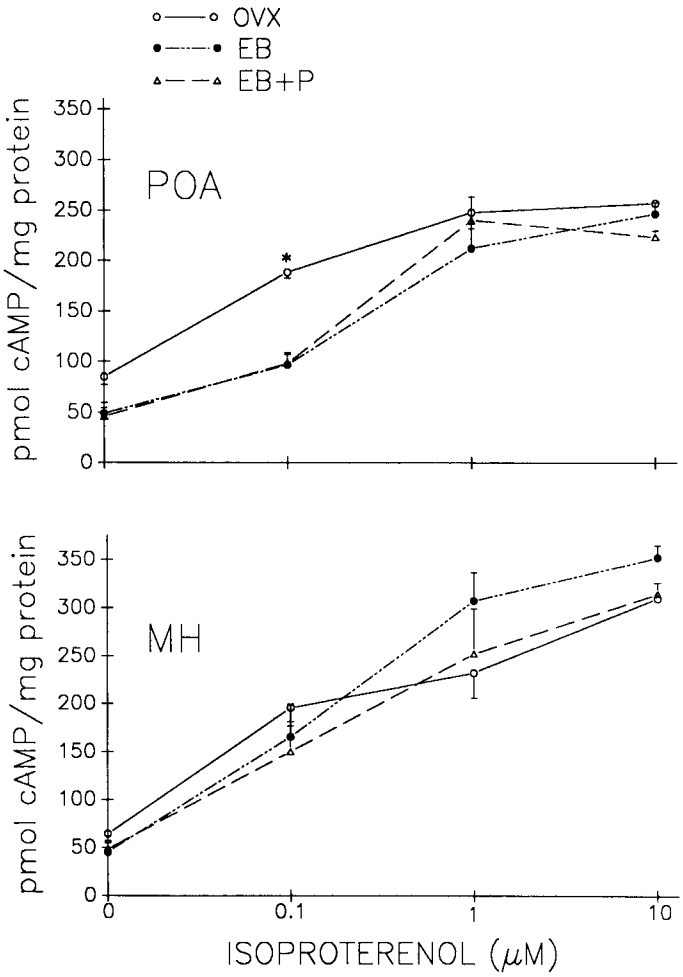

Figure 4. Concentration-dependent elevation of cAMP content by ISO in the presence of 1 μM PDB in POA and MH slices from OVX (*open circles*) or EB- (*solid circles*) or EB + P-treated (*open triangles*) rats. OVX female rats received the same EB and EB + P treatments described in Figure 1. Each value represents the mean (±SEM) of 3–4 independent replications. Data from slices incubated concurrently with ISO alone are shown in Figure 3. *, significantly different from EB and EB + P ($p < 0.01$).

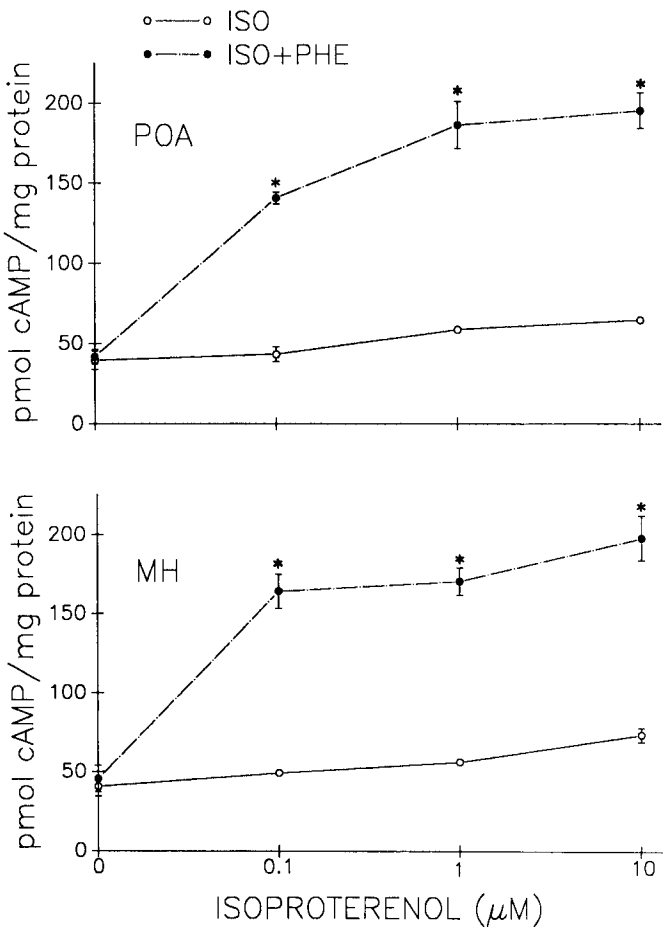

Figure 5. cAMP response to ISO alone (*open circles*) or in combination with 10 μM PHE (*solid circles*) in slices from EB + P-treated female rats injected with the antiprogestin RU 38486. OVX female rats received the same EB and EB + P treatments described in Figure 1 but also received 1 mg RU 38486 1 hr prior to the P injection. Each value represents the mean (±SEM) of 4 independent replications. *, significantly different from ISO alone ($p < 0.01$).

cAMP response is not mediated by pituitary secretions because it was replicated in slices from hypophysectomized animals. Second, it is unlikely that P decreases NE-dependent cAMP accumulation by releasing neurotransmitters that inhibit adenylyl cyclase. When slices were incubated with TTX to prevent neuronal firing, NE-stimulated cAMP formation in EB + P-treated tissue was significantly less than in EB-treated tissue. These observations, plus our previous (Etgen and Petitti, 1987; Petitti and Etgen, 1989) demonstration that neither phosphodiesterase activity nor adenylyl cyclase activation by forskolin are changed by ovarian hormones, indicate that steroids modify NE-receptor regulation of the hypothalamic cAMP-generating system.

This study also demonstrates that steroid-induced modifications in the total cAMP response involve changes in the functional activity of specific NE-receptor subtypes. Slices from OVX and EB-treated animals exhibited an α_1-receptor augmentation of β-stimulated cAMP formation. However, the magnitude of α_1 potentiation of the cAMP response to β-adrenergic and adenosine receptor stimulation was greater in hypothalamic slices

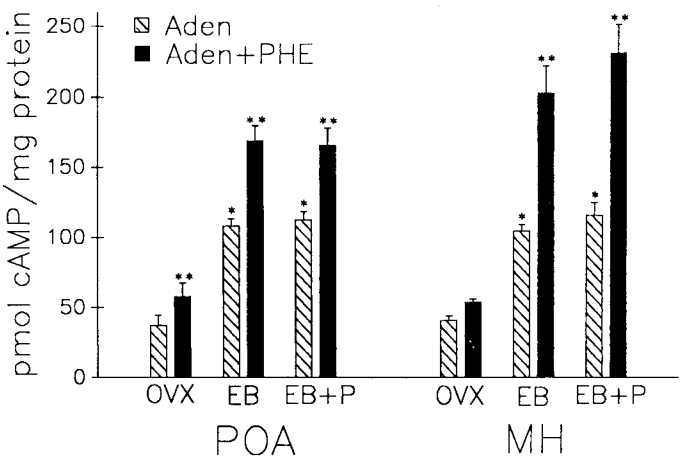

Figure 6. Effects of OVX and EB or EB + P treatments on the stimulation of cAMP formation by 100 μM adenosine (*Aden*) alone (*hatched bars*) or in combination with 10 μM PHE (*solid bars*). OVX female rats received the same EB and EB + P treatments described in Figure 1. Each value represents the mean (±SEM) of 4 independent replications. *, significantly different from OVX ($p < 0.01$); **, significantly different from adenosine alone ($p < 0.01$).

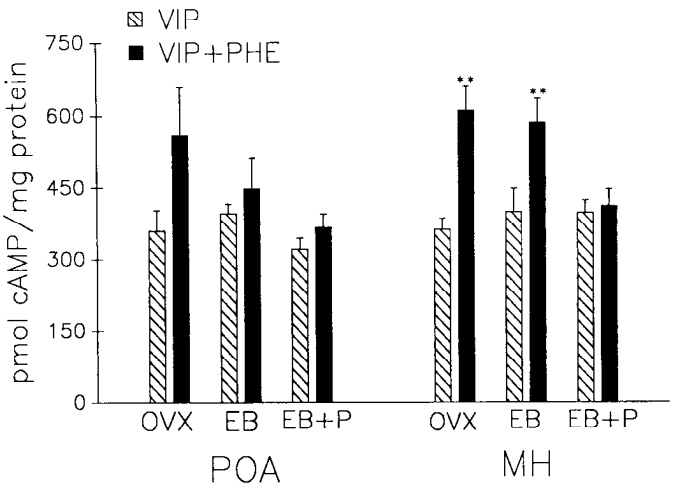

Figure 7. Effects of OVX and EB or EB + P treatments on the stimulation of cAMP formation by 1 μM VIP alone (*hatched bars*) or in combination with 10 μM PHE (*filled bars*). OVX female rats received the same EB and EB + P treatments described in Figure 1. Each value represents the mean (±SEM) of 4 independent replications. **, significantly different from VIP alone ($p < 0.05$).

from EB-treated than from OVX animals. This confirms our earlier observations utilizing prazosin, an α_1-receptor antagonist, which blocked NE-stimulated cAMP formation in slices from EB-treated rats but not from OVX animals (Etgen and Petitti, 1987). Binding studies with ^{3}H-prazosin suggest that the increase in α_1 responsiveness is associated with a 20–30% increase in α_1-receptor number (Etgen and Karkanias, 1990).

Enhancement of α_1-receptor function may be a general mechanism of estrogen action. Estradiol increases the responsiveness of α_1 receptors in rabbit myometrium (Riemer et al., 1988) and the rabbit urinary bladder (Levin et al., 1980). Estradiol also enhances the sensitivity of vascular smooth muscle to α stimulation (Colucci et al., 1982), apparently through an increase in α_1-receptor affinity. Recently, Condon et al. (1989), using electrophysiological methods, reported that estrogen increases α_1-receptor responsiveness in hypothalamic neurons.

Subsequent to the estrogen-induced increase in α_1-receptor response, P abolishes α_1-receptor augmentation of β-stimulated cAMP formation in MH and POA slices. PHE augmentation of VIP-stimulated cAMP formation in hypothalamic slices is also attenuated by P treatment. Despite this P-induced loss of the augmenting response, ligand binding studies reveal that α_1-receptor number and antagonist binding affinity are similar in POA and MH membranes from EB- and EB + P-treated animals (Etgen and Karkanias, 1990). Thus, P does not simply downregulate α_1 receptors. It is unlikely that P is acting on Gp, given that the steroid does not modify the ability of carbachol, an agonist of muscarinic receptors coupled to Gp, to augment ISO-stimulated cAMP formation. The finding that P does not reduce PHE enhancement of adenosine-stimulated cAMP formation was unexpected. Dissimilarities in the anatomical codistribution of adenosine and VIP receptors with steroid-regulated α_1 receptors, either in different cells or through compartmentalization within the same cell, could account for this finding. Adenosine could be distributed to cells or compartments affected by estradiol, while VIP receptors could be distributed to cells or compartments influenced by P.

Our data are consistent with the hypothesis that P elimination

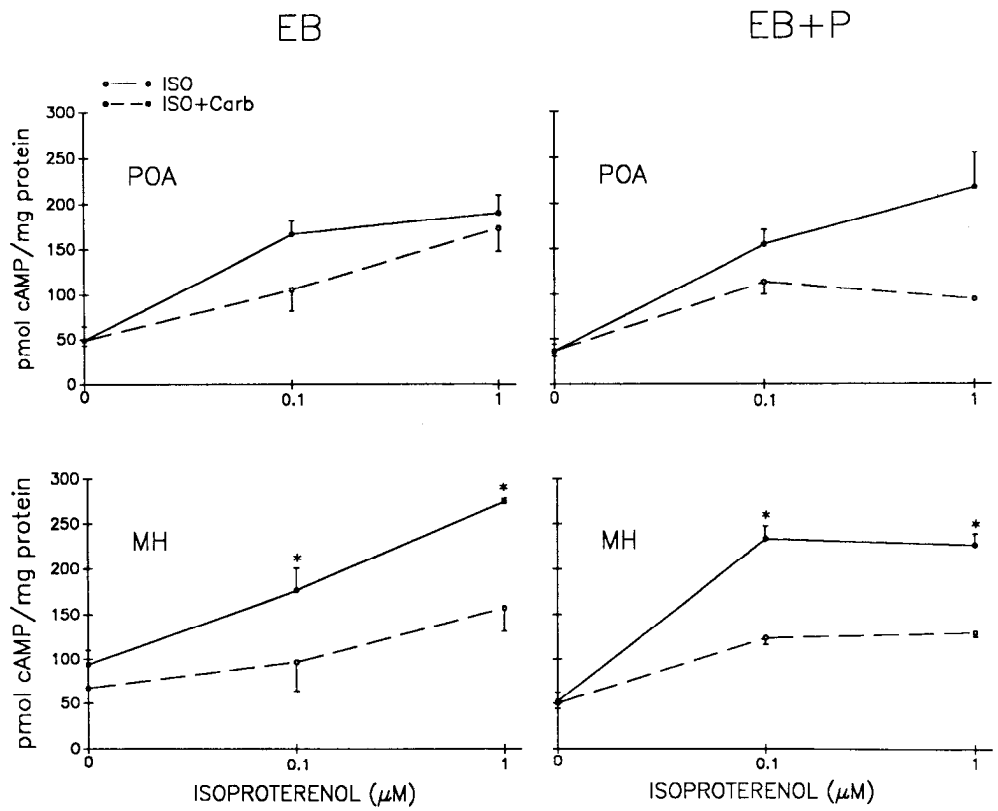

Figure 8. Effects of 1 mM carbachol (*Carb*), an agonist for muscarinic receptors, on ISO-stimulated cAMP formation in POA and MH slices from EB- or EB + P-treated animals. OVX female rats received the same EB and EB + P treatments described in Figure 1. Each value represents the mean (±SEM) of 3–4 independent replications. *, significantly different from ISO alone ($p < 0.01$).

of the α_1-receptor augmenting response may be mediated by neuronal progestin receptors. *In vivo* treatment with the progestin-receptor antagonist RU 38486 prevented the loss of α_1-receptor augmentation of β-stimulated cAMP formation normally seen following P administration. Possible membrane actions of P cannot be eliminated by our RU 38486 results, however, because RU 38486 interferes with membrane actions of P (Sadler et al., 1985), in addition to competing for intracellular progestin receptors (Philibert, 1984; Etgen and Barfield, 1986).

In agreement with our earlier work (Etgen and Petitti, 1987), and now extended over a concentration range of ISO (Fig. 3), we found that estradiol reduces β-stimulated cAMP formation in POA and MH slices. The EB-dependent reduction in ISO-stimulated cAMP synthesis is not reversed by P treatment of EB-primed animals. Moreover, the estrogen-induced blunting of β-stimulated cAMP synthesis does not correlate with changes in β-receptor binding (Etgen and Karkanias, 1990). Our studies also indicate that the EB-induced decrease in β-stimulated cAMP formation in hypothalamic slices is a relatively specific phenomenon. Estrogen does not reduce activation of adenylyl cyclase by VIP or adenosine; in fact, adenosine-stimulated cAMP accumulation increases significantly.

It is possible that estrogens have widespread regulatory effects on β-adrenoceptor function. Riemer et al. (1988) have demonstrated that EB reduces β-mediated cAMP production in rabbit myometrium with relatively little change in β-receptor number. This effect was correlated with a reduction in Gs levels. Estradiol also decreases β-stimulated cAMP formation in the rat cerebral cortex (Wagner et al., 1979; Wagner and Davis, 1980) and hippocampus (Harrelson and McEwen, 1987a). Regulation of β-receptor-stimulated cAMP synthesis may be a general mechanism of steroid action because β responsiveness in adipocytes is also decreased after adrenalectomy (De Mazancourt et al., 1989).

Estrogen-induced changes in α_1- and β-receptor function may modulate neuronal activity in hypothalamic areas that regulate reproductive behavior. Estradiol-initiated increases in neuronal excitability may well be a mechanism by which estrogen and NE interact to facilitate estrous responses. Electrophysiological evidence for an excitatory role of α_1 receptors has been shown in the brain, spinal cord, and parasympathetic autonomic ganglia (see Szabadi and Bradshaw, 1987). Studies in slices of hypothalamus and POA also suggest that α_1 agonists excite and β agonists inhibit neuronal activity (Kow and Pfaff, 1988; Condon et al., 1989; Kim et al., 1989). Therefore, it is interesting to speculate that estrogen may enhance the excitability of hypothalamic neurons by potentiating the excitatory effect of α_1 receptors and reducing the inhibitory effect of β receptors.

In summary, this study demonstrates that estradiol and P act relatively selectively on NE signal transduction processes in the hypothalamus and POA. Specifically, estradiol enhances α_1-receptor augmentation and reduces β-receptor activation of cAMP synthesis. Furthermore, in estrogen-primed rats, P eliminates the α_1 augmentation of β-stimulated cAMP synthesis without affecting the blunted β response. It is postulated that (1) estrogen-induced increases in α_1 responsiveness are due to an increase in α_1-receptor number, (2) the removal of the α_1 component of cAMP synthesis is a relatively selective effect of P on the coupling of α_1 receptors to phospholipase C, and (3) the estrogen-dependent decrease in β responsiveness involves uncoupling of β receptors from the stimulation of adenylyl cyclase.

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