The Journal of Neuroscience, July 1992, 12(7): 2745–2750

Estrogen Suppresses μ-Opioid- and $GABA_B$-mediated Hyperpolarization of Hypothalamic Arcuate Neurons

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The effects of estrogen on the response of hypothalamic arcuate neurons to μ-opioid and $GABA_B$ agonists were investigated. Intracellular recordings were made from arcuate neurons in slices prepared from ovariectomized guinea pigs that were pretreated with estrogen or vehicle. Estrogen shifted the dose–response curve to the μ-opioid agonist DAMGO (Tyr-D-Ala-Gly-MePhe-Gly-ol) by 3.4-fold; the EC_{50} for DAMGO was 240 ± 25 nM in estrogen-treated females versus 70 ± 12 nM in the controls. The maximal hyperpolarization induced by DAMGO was equivalent in neurons from both groups. The K_e for the naloxone antagonism of the DAMGO response was similar in both groups, which would indicate that the affinity of the μ-receptor was unchanged. To explore where in the receptor/G-protein/K^+ channel cascade estrogen may be acting to attenuate the μ-opioid–mediated hyperpolarization, the response to the $GABA_B$ agonist baclofen was also tested. Estrogen treatment also shifted the dose–response curve for the baclofen-induced hyperpolarization by 3.3-fold without altering the maximum hyperpolarization; the EC_{50} shifted from 11.0 ± 4.0 μM to 36.0 ± 5.0 μM. All of the neurons were identified after linking the intracellular biocytin with streptavidin-FITC, and a subpopulation of cells in both groups were immunoreactive for β-endorphin. We conclude that estrogen decreases the functional coupling of the μ-opioid and $GABA_B$ receptors to the inwardly rectifying K^+ channel possibly through an action on the G-protein.

Morphine and opioid peptides inhibit the reproductive cycle of the mammal at least in part through their direct actions on hypothalamic neurosecretory neurons (Kelly et al., 1990; Loose et al., 1990). These hypothalamic neurons in the arcuate nucleus (ARC) are hyperpolarized by activation of μ-opioid receptors that are linked to an inwardly rectifying potassium conductance (Loose and Kelly, 1990). This mechanism of action of opioids has also been identified in other CNS and PNS neurons (see North et al., 1987).

The efficacy of morphine and the opioid peptides to inhibit reproductive events is dependent on the steroid milieu. The gonadal steroids play a major role in modulating the tonic opioid input onto hypothalamic neurosecretory neurons during the reproductive cycle of the mammal (Ferin et al., 1984; Kalra and Kalra, 1984; Millan and Herz, 1985). There appears to be decreased opioidergic tone at the time of the preovulatory surge of luteinizing hormone (LH; Kalra and Kalra, 1984; Millan and Herz, 1985). We therefore hypothesized that an elevation in serum estrogens plays a role in modifying the opioidergic tone through a modification of the receptor-mediated response in parvocellular neurosecretory neurons (e.g., β-endorphin, dopamine, and gonadotropin-releasing hormone). Since GABA is another potent inhibitory transmitter in the hypothalamus that is present in the ARC (Vincent et al., 1982) and the $GABA_B$ receptor is coupled to the same inwardly rectifying potassium conductance in the rat (Loose et al., 1991), we tested the hypothesis that estrogen suppresses both the μ-opioid and $GABA_B$ responses that are coupled to the inwardly rectifying K^+ channel. The EC_{50} values for the μ-opioid- and the $GABA_B$-mediated hyperpolarization were compared in slices obtained from female guinea pigs exposed to low levels of serum estrogen versus high levels of serum estrogen.

Preliminary results from this work have been presented in abstract form (Kelly et al., 1991a,b).

Materials and Methods

Female guinea pigs (Topeka; 350–600 gm), born and raised in our colony, were maintained on a 14 hr light/10 hr dark lighting schedule (lights on 0630–2030) and were ovariectomized under ketamine (33 mg/kg)/xylazine (6 mg/kg) anesthetic 6–10 d before each experiment. Each animal was given a subcutaneous injection of estradiol benzoate (EB; 25 μg) or oil (100 μl; oil-treated) 24 hr before it was killed by decapitation. EB-treated and oil-treated animals were used alternately throughout the course of these studies. Serum estrogens determined by radioimmunoassay (RIA; Steroid RIA Core, P 30 HD18185) were 100–150 pg/ml in the EB-treated animals and less than 12 pg/ml (sensitivity of the RIA was 2.5 pg/ml) in the oil-treated animals at the time of death. Each animal was decapitated at 0900–0930; the brain was removed, the hypothalamus was dissected, and coronal slices of 450 μm thickness were cut on a vibratome (Loose and Kelly, 1989). A single slice was submerged in an oxygenated (95% O_2, 5% CO_2) salt solution at 35 ± 1 °C; the solution flowed through at 1.5 ml/min and contained (in mM) NaCl, 124; KCl, 5; NaH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; dextrose, 10; and HEPES, 10.

Intracellular recordings were made from arcuate neurons using techniques similar to those previously described (Kelly et al., 1990; Loose and Kelly, 1990; Loose et al., 1990). Microelectrodes were made from borosilicate glass micropipettes (1 mm o.d.; A-M Systems) and were filled with a 3% biocytin solution in 1.75 M KCl and 0.025 M Tris (pH 7.4); resistances varied from 80 to 150 MΩ. Intracellular potentials were amplified and current was passed through the electrode using an Axoclamp 2A (Axon Instruments). Voltage-clamp recordings were made using a 1–3 kHz switching frequency and 30% duty cycle in accordance with the procedures of Finkel and Redman (1985). Current and voltage

Received Nov. 8, 1991; revised Feb. 7, 1992; accepted Feb. 14, 1992.

We appreciate the helpful comments on the manuscript provided by Drs. John Williams and Susan MacMillan. This work was supported by U.S. Department of Health and Human Services Grants DA 05158 and HD 00718 (Research Career Development Award to M.J.K.). Mrs. Martha Bosch and Mr. Barry Naylor provided valuable technical assistance.

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traces were recorded on a chart recorder (Gould 2200) and periodically stored on an FM tape recorder (Vetter Instruments).

Data are reported from cells that exhibited overshooting action potentials and had membrane potentials of less than −50 mV for at least 20 min. Voltage–current relationships were obtained by applying a series of depolarizing and hyperpolarizing current pulses (>150 msec) and measuring the voltage at the end of each step. The apparent input resistance of the cell (R_{in}) was calculated from the slope of the voltage–current plots in the region between −60 and −80 mV. Voltage–current plots were also done during the drug-induced hyperpolarization in at least half of the cells from both groups of animals to ascertain the reversal potential of the effect (e.g., Fig. 1*A*). The membrane time constant was estimated by measuring the time for a voltage deflection (≈10 mV) to reach 63% of its steady-state level. Numerical data are expressed as mean ± SEM, except as noted. Comparisons between groups were evaluated using the χ^2 statistic, and a p value less than 0.05 was considered significant.

Tetrodotoxin (2 μM; Sigma) was added to the solution prior to application of opioids in the majority of cells. A cumulative dose–response curve was generated to DAMGO (Peninsula Labs), an opioid agonist selective for μ-receptors (Handa et al., 1981; Williams and North, 1984). The membrane hyperpolarization caused by DAMGO (20 nM, 50 nM, 100 nM, 300 nM, 600 nM, 1 μM) was measured. Solutions containing each concentration were applied for 6 min, which was sufficient for the membrane potential to reach a new steady level. The EC_{50} was estimated from a third-order regression function fitted by computer to the experimental points. An unpaired t test was used to compute statistical significance between the EC_{50} values, and a two-way ANOVA was used to compute statistical significance between the curves for the two groups. After washout of 1 μM DAMGO, a second series of DAMGO concentrations (100 nM to 50 μM) was applied in the presence of naloxone (30 nM, 100 nM, or 1 μM). The dissociation constant for naloxone (K_e) was estimated by computing the dose ratio at 50% of the maximal effect (Schild, 1947; Tallarida et al., 1979).

In a second series of experiments, the membrane hyperpolarization to baclofen (1–80 μM), a specific agonist for the $GABA_B$ receptor, was measured. Solutions were applied as described above, and the EC_{50} values were determined. The occlusion of the baclofen response by DAMGO and of the DAMGO response by baclofen was accessed by utilizing supramaximal concentrations of both drugs for both animal groups.

Following a recording, the slices were immersed in 4% paraformaldehyde in 0.03 M Sorensen's phosphate buffer (pH 7.4) for 90–100 min. The slices were then soaked overnight in this phosphate buffer with 30% sucrose. Sections (16 μm) were cut on a cryostat and mounted on slides coated with poly-L-lysine. Sections were washed with a 0.1 M sodium phosphate buffer (pH 7.4) and then processed with streptavidin-FITC as previously described (Ronnekleiv et al., 1990). After localization of the biocytin-filled neurons, the slides containing the appropriate sections were processed with β-endorphin antisera (R13; Weber et al., 1982) at 1:1000 or an affinity-purified tyrosine hydroxylase (TH) antiserum (Pel Freeze) at 1:750 using fluorescence immunohistochemistry (Ronnekleiv et al., 1990).

Results

Fifty-four neurons (N = 16, oil-treated; N = 38, EB-treated) were studied. There were no differences in the resting membrane potential (oil, −57 ± 3 mV; EB, −59 ± 2 mV), R_{in} (oil, 293 ± 32 MΩ; EB, 325 ± 44 MΩ), or τ (oil, 18 ± 3 msec; EB, 15 ± 1 msec) between the two groups of neurons. Neurons from both groups exhibited the conductances that we described previously (Kelly et al., 1990; Loose et al., 1990) and can be summarized as follows: most of the neurons from both groups of animals (oil, 56%; EB, 53%) exhibited a time-dependent inward rectification (I_h), 22% of the cells (oil, 25%; EB, 21%) exhibited a low-threshold spike that is typical of ARC dopamine-containing neurons (Loose et al., 1990), and another 33% of the cells (oil, 38%; EB, 32%) displayed an I_A that is present in some β-endorphin–containing neurons (Kelly et al., 1990) including three out of five of the β-endorphin cells in this study.

A complete dose–response relationship was obtained for 16 neurons from 15 animals (seven cells from six oil-treated, and nine cells from nine EB-treated animals) with 20 nM to 1 μM DAMGO. All neurons that were tested from the oil-treated animals were hyperpolarized by DAMGO; eight of nine neurons in the EB-treated group were hyperpolarized. Typical responses are shown in Figure 1. The maximum membrane hyperpolarization (oil, 11.0 ± 1.3 mV, vs. EB, 12.6 ± 2.1 mV) or the decrease in R_{in} (oil, 48 ± 10%; EB, 46 ± 6%) did not differ between the two groups. However, EB treatment significantly ($p < 0.0001$) shifted the dose–response curve (Fig. 2). The EC_{50} differed significantly ($p < 0.001$) between the two groups (oil, 70 ± 12 nM, vs. EB, 240 ± 25 nM). There was no overlap in the EC_{50} values between any neurons from the two groups (range of oil EC_{50} values, 65–150 nM; EB-treatment EC_{50} values, 160–320 nM). These concentrations of DAMGO are below those at which desensitization has been shown to occur (Harris and Williams, 1991).

Following a washout of DAMGO and a return to the original resting membrane potential (e.g., Fig. 1), a second cumulative dose–response curve (100 nM to 50 μM) was generated in the presence of the opioid antagonist naloxone (30 nM, 100 nM, or 1 μM) in neurons from each group (Fig. 3). The K_e for naloxone antagonism was not different between the two groups (oil, 8.3 ± 1.8 nM, N = 4, vs. EB, 6.6 ± 1.8 nM, N = 3).

To identify where in the receptor/G-protein/K^+ channel cascade estrogen may be acting to depress the μ-opioid response, we examined the response of an additional group of ARC neurons (N = 8) to baclofen, a $GABA_B$ agonist. Both the μ-opioid receptor and the $GABA_B$ receptor are coupled to the same inwardly rectifying potassium conductance in that the hyperpolarization induced by either agonist was occluded by prior maximal activation of the potassium conductance by the other agonist (e.g., Fig. 4; N = 7). We have previously identified such an occlusion in rat ARC neurons (Loose et al., 1991). Moreover, the dose–response relationship for the $GABA_B$ agonist baclofen exhibited a 3.3-fold rightward shift following estrogen treatment without an alteration in the maximal response (the EC_{50} shifted from 11.0 ± 4.0 μM, N = 4 cells from four oil-treated animals, to 36.0 ± 5.0 μM, N = 3 cells from three EB-treated animals; $p < 0.02$). The maximum membrane hyperpolarization did not differ between the two groups (oil, 15.0 ± 1.4 mV, vs. EB, 12.3 ± 1.4 mV). One cell from an EB-treated female did not respond to baclofen (up to 100 μM).

All of the neurons for which a dose–response relationship was obtained were identified in the ARC with streptavidin-FITC labeling. There were no obvious morphological differences between the two treatment groups. The recorded neurons were small (7 × 11 μm to 11 × 13 μm; 10 cells from oil-treated and 9 cells from EB-treated animals) oval/fusiform cells with one to two processes and larger (12 × 20 μm; three cells from oil-treated and two cells from EB-treated animals) pyramidal cells with three processes. Moreover, two of nine (oil-treated group) and three of eight (EB-treated group) neurons responsive to DAMGO were shown to contain β-endorphin (Fig. 5). Seven cells (five cells from the EB-treated group and two cells from the oil-treated group) were processed for TH immunostaining based on the presence of a low-threshold spike (Loose et al., 1990); none of the cells were immunoreactive for TH. However, the latter cells may have been TH containing, for we do not have complete success in immunostaining all biocytin-filled neurons (Ronnekleiv et al., 1990). We concluded that the recordings were done from the same heterogeneous population of

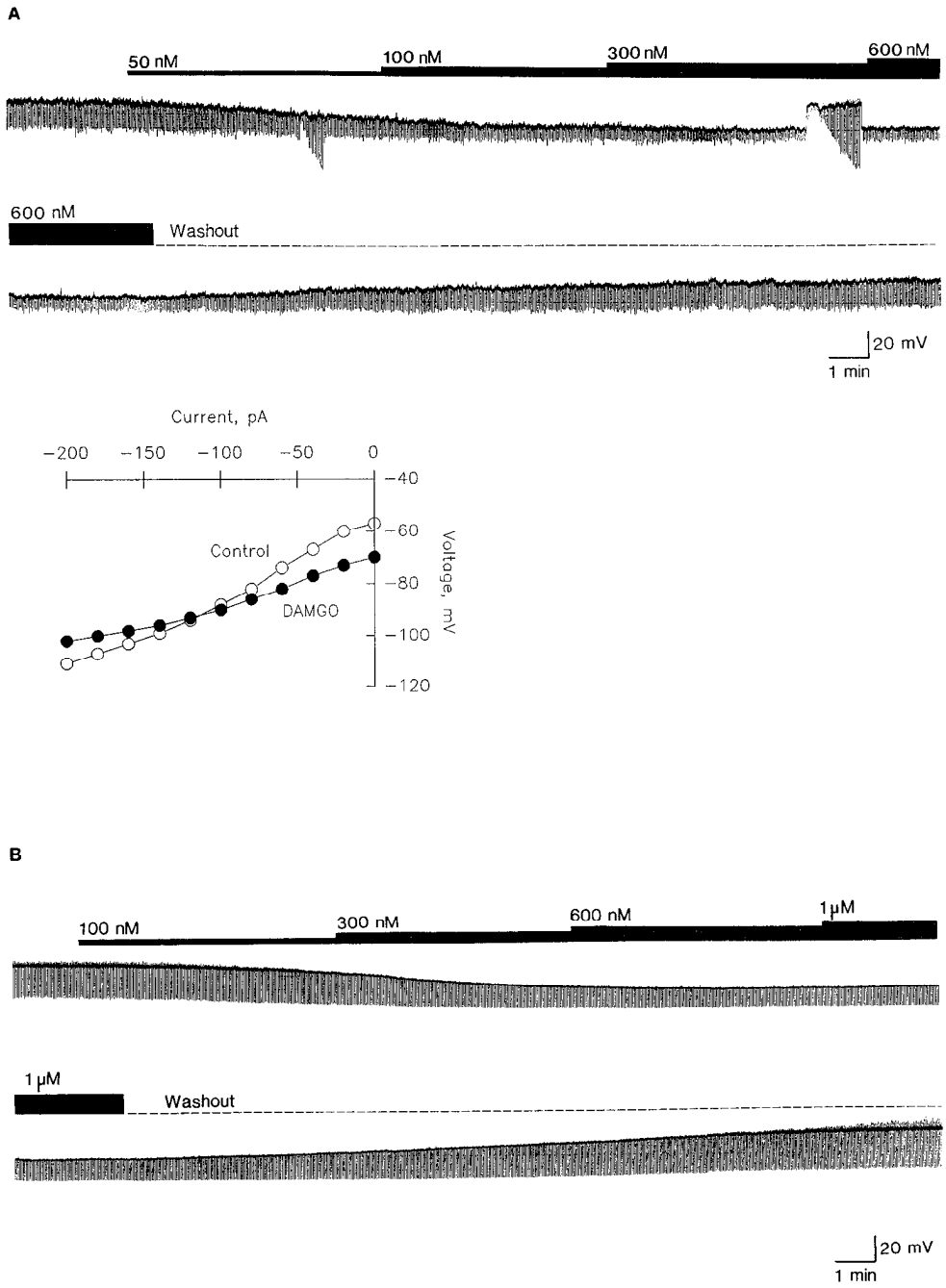

Figure 1. Continuous chart record showing the dose–response to the μ-opioid agonist DAMGO. *A*, Intracellular recording (*top*) is in an ARC neuron from an ovariectomized, oil-treated female guinea pig. Concentrations of 50 nM, 100 nM, and 300 nM hyperpolarized the cell 6, 11, and 16 mV, respectively, and reduced R_{in} 69%, without any further changes at the 600 nM concentration. The EC_{50} was 66 nM. Drug applications were done in the presence of TTX (2 μM), which was not present during the washout (*broken line*). It took ≈24 min to wash out the DAMGO effects. The *downward deflections* represent the membrane voltage responses to constant current hyperpolarizing pulses (1 Hz), which approximated R_{in}. A voltage-matched (+90 pA), current–voltage plot (*bottom*) was done during maximum hyperpolarization, and the reversal for the DAMGO response (*intersection of curves*) was at the calculated E_{K+} (−93 mV). The reversal potential did not differ between the two groups of animals. Resting membrane potential, −50 mV. *B*, Intracellular recording is in an ARC neuron from an ovariectomized, EB-treated female guinea pig. Concentrations of 100 nM, 300 nM, and 600 nM DAMGO hyperpolarized the cell 5, 15, and 23 mV, respectively, and reduced R_{in} 42%, without any further changes at the 1 μM concentration. The EC_{50} was 210 nM. Drug applications were done in the presence of TTX (2 μM), which was not present during the washout (*broken line*). It took ≈24 min to wash out the DAMGO effects. The *downward deflections* represent the membrane voltage responses to constant current hyperpolarizing pulses (1 Hz), which approximated R_{in}. Resting membrane potential, −52 mV (in 2 μM TTX).

ARC neurons within the two treatment groups based on their similar location, morphology, and immunocytochemistry.

Discussion

It has been known for a number of years that morphine inhibits gonadotropin secretion in the mammal (Barraclough and Sawyer, 1955; Kinoshita et al., 1980; Sylvester et al., 1982; Gabriel et al., 1983). Moreover, opioid peptides attenuate the preovulatory increase of LH and prevent ovulation (Ieiri et al., 1980; Kalra, 1981), and naloxone potentiates the duration and magnitude of the ovulatory surge of LH in the rat (Ieiri et al., 1980; Kalra, 1981), elevates portal blood levels of gonadotrophin-releasing hormone in the rhesus monkey (Ferin et al., 1984), and advances ovulation in the human female (Rossmanith et al., 1988). Based on the latter studies, it has been inferred that there is tonic opioid input that is attenuated by the gonadal steroids at the time of the preovulatory surge of LH (Kalra and Kalra, 1984; Millan and Herz, 1985). The present studies suggest a cellular mechanism for this decreased opioidergic tone prior to ovulation. Estrogen at concentrations similar to those observed prior to the LH surge shifted the dose–response curve by 3.4-fold (decreased efficacy) without changing the maximal response. Therefore, opioid input that previously was effective would become less effective as a result of an estrogen-induced decrease in the postsynaptic sensitivity to endogenous opioids. Although estrogen would also disinhibit the β-endorphin neurons that synapse on other neurosecretory neurons, the net effect would be less inhibitory input because of decreased postsynaptic sensitivity.

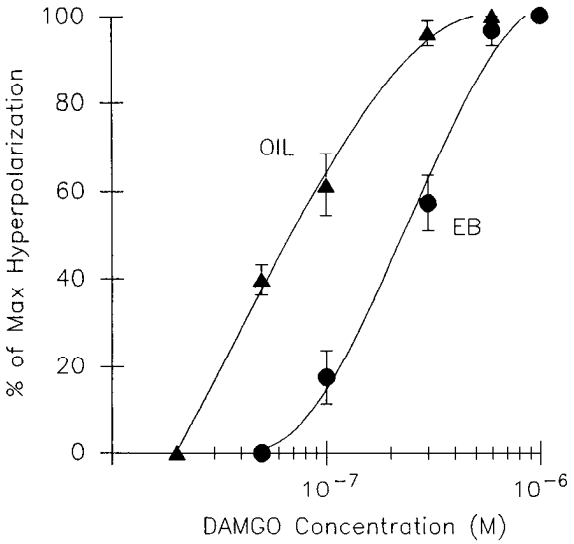

Figure 2. Composite dose–response curves for the μ-opioid–induced hyperpolarization of ARC neurons recorded from ovariectomized, oil-treated females ($N = 7$ cells) and from ovariectomized, EB-treated females ($N = 8$ cells). A third-order regression was used to fit the curve to the points. The EC_{50} for the EB-treated group (240 nM) was significantly ($p < 0.001$) greater than for the oil-treated group (70 nM). Two-way ANOVA for the dose–response curves, $p < 0.0001$ (df = 3).

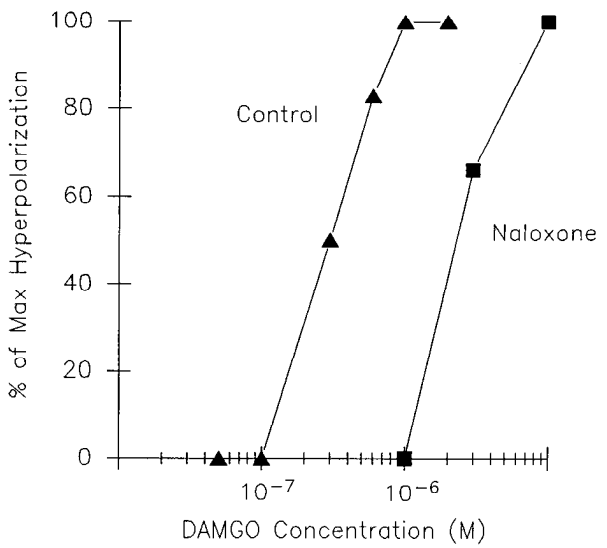

Figure 3. Dose–response curve from an ARC neuron from an ovariectomized, EB-treated female guinea pig illustrating the naloxone inhibition of the μ-opioid response (100 nM naloxone shifted the EC_{50} by about one log unit). The K_e for the naloxone inhibition was 9.0 nM. A similar rightward shift was seen in ARC neurons recorded from ovariectomized, oil-treated females.

The affinity of the μ-opioid receptor for naloxone antagonism of the response (K_e) was the same in both groups of animals. Therefore, there are several possible mechanisms by which estrogen could modify the opioid response in ARC neurons: first, estrogen could decrease the number of μ-opioid receptors; second, estrogen could alter the expression of K^+ channels; or third, estrogen could alter the G-protein coupling of the μ-receptor to the K^+ channel, which may be reflected in a change from the high-affinity, G-protein–coupled state to the low-affinity, G-protein–uncoupled state of the μ-receptor (Ueda et al., 1991). Regarding the first possible mechanism, long-term treatment (5 d) of ovariectomized female rats with estrogen has been shown to increase dopamine D1 and D2 receptors in the striatum (Rajakumar et al., 1987; Hruska and Nowak, 1988) and decrease $GABA_A$ receptors in whole brain (Juptner et al., 1991) without affecting the K_d for the various ligands. However, even long exposure of ovariectomized rats to estrogen (5 d) alters the B_{max} for the various ligands by no more than twofold. For the shift in the dose–response curve seen in the present studies, one would have to hypothesize that estrogen causes an even greater change (decrease) in μ-receptors within a shorter time period (24 hr). Furthermore, since estrogen treatment also shifted the dose–response curve to baclofen, it is more likely that rather than altering the expression of two different populations of membrane receptors, μ-opioid and $GABA_B$ receptors, estrogen could alter a single element (G-protein) that is common to both receptors and couples them to the inwardly rectifying K^+ channel.

Estrogen could also downregulate the expression of K^+ channels. It is known, for example, that estrogen can induce the expression of a unique class of K^+ channels in uterine myometrial cells within 3 hr following a single injection *in vivo* in ovariectomized rats (Pragnell et al., 1990). However, in the present studies the maximum hyperpolarization (11–12 mV) with the accompanying increase in conductance (46–48% decrease in R_{in}) is the same in both groups of animals, suggesting that the number of channels and the conductance state of the channels are unaltered with estrogen treatment.

A more likely site of action of estrogen would be at the G-pro-

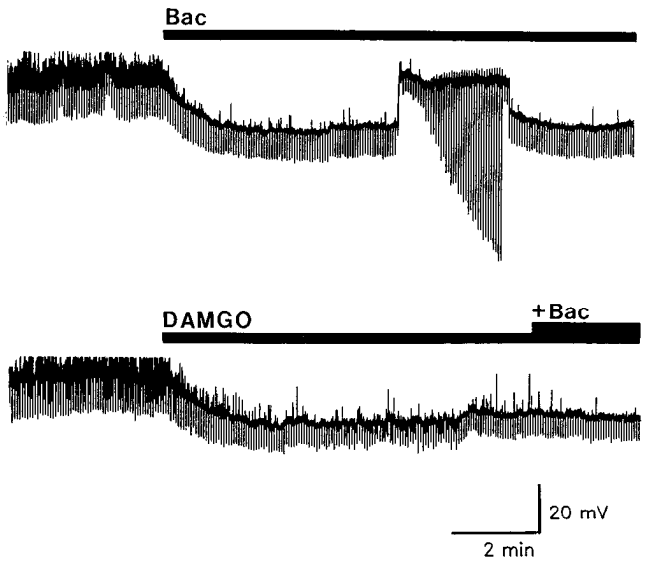

Figure 4. Occlusion of baclofen-induced hyperpolarization by DAMGO. *A*, Intracellular recording in an ARC neuron from an ovariectomized, oil-treated guinea pig. Baclofen at 10 μM (*Bac*) hyperpolarized the membrane 19 mV and decreased R_{in} by 45%. PSPs were also suppressed. A voltage-matched (+60 pA), current–voltage relationship was done during maximum hyperpolarization and compared to control conditions. The reversal for the baclofen-induced hyperpolarization was at −93 mV (E_{K+}). Resting membrane potential, −55 mV. *B*, Recording is from same cell as in *A* after baclofen was washed out and membrane returned to resting membrane potential (≈23 min). DAMGO at 300 nM hyperpolarized the cell 20 mV, with a 45% reduction in R_{in}. PSPs were also suppressed. With the addition of 10 μM baclofen there was no further hyperpolarization of the membrane.

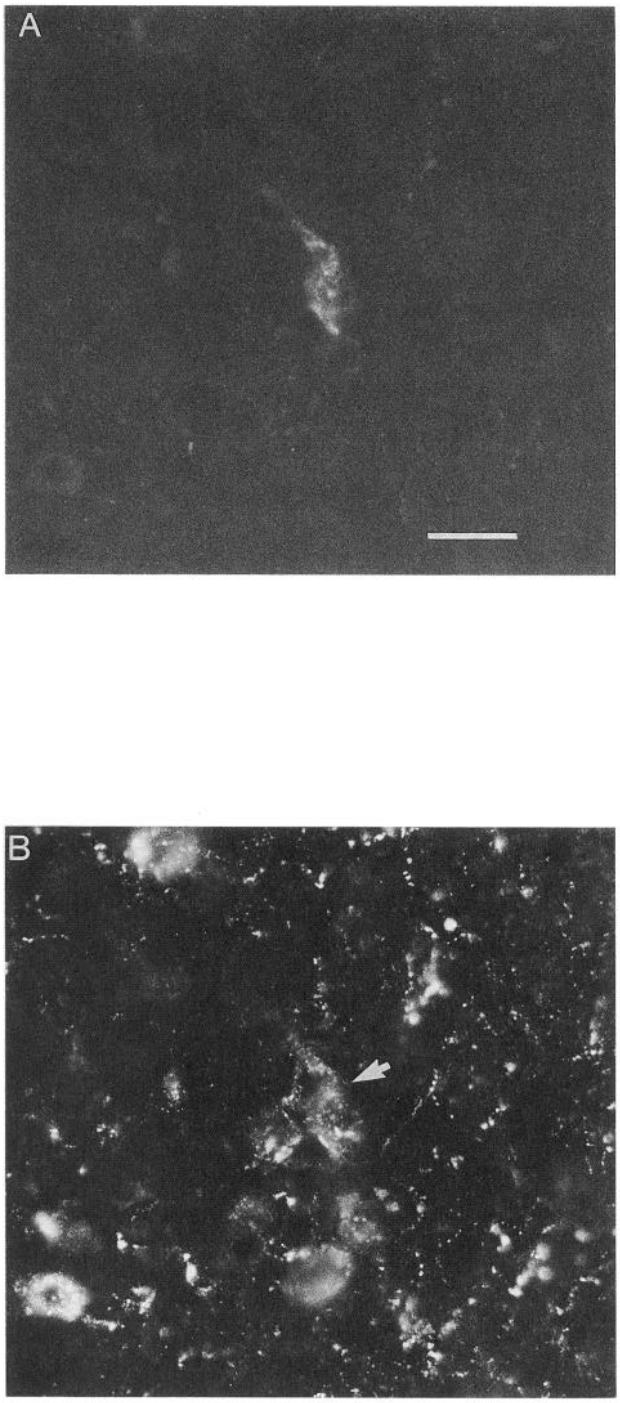

Figure 5. An example of a double-labeled β-endorphin neuron that was hyperpolarized by DAMGO. Three β-endorphin neurons were identified in the EB-treated group and two in the oil-treated group. *A*, Photomicrograph of biocytin-streptavidin-FITC labeling of intracellularly recorded ARC neuron. The small fusiform soma was present in two sections. *B*, Photomicrograph of Texas red staining of β-endorphin in soma and proximal fiber of cell in *A* (*arrow*). Part of the soma in the adjacent section was also double labeled (not shown). Scale bar, 10 μm (for *A* and *B*).

tein. Recent studies on the D1- and D2-dopamine–sensitive adenylate cyclase activity in the striatum indicate that estrogen enhances the D1-stimulated activity and suppresses the D2-inhibited activity (Maus et al., 1989) through its actions on $G_{\alpha o,i}$ protein subunits (Maus et al., 1989, 1990). Estrogen amplifies the pertussis toxin–catalyzed ADP-ribosylation of Gα subunits without affecting levels of Gα subunits in the striatum (Maus et al., 1990). Maus and colleagues conclude that these actions of the gonadal steroid are exerted through a stabilization of the $G_{\alpha_{o,i}\beta,\gamma}$ complex since pertussis toxin preferentially ADP-ribosylates this heterotrimer (Maus et al., 1990). These effects of estrogen are manifested within 24 hr following *in vivo* administration. The μ-opioid receptor is coupled to a K^+ channel via a pertussis-sensitive G-protein (Miyake et al., 1989). Therefore, in the present studies, estrogen could inhibit the expression of G_{α_i} or alter the $G_{\alpha_i\beta,\gamma}$ complex (via phosphorylation?) such that it would be less likely to dissociate with binding of agonist, thereby decreasing the efficacy of the coupling of the receptor to the channel and subsequently decreasing the hyperpolarizing response.

The effects of estrogen on the response of ARC neurons to the $GABA_B$ agonist baclofen would support the hypothesis that the steroid is altering the coupling of the receptor(s) to the potassium channel. The $GABA_B$ and the μ-opioid receptors have been shown to be coupled to the same potassium conductance in parabrachial neurons (Christie and North, 1988) and rat ARC neurons (Loose et al., 1991). Presently, we have shown that the DAMGO response occludes the baclofen response in guinea pig ARC neurons (Fig. 4), and that estrogen treatment causes an equivalent shift in the dose–response to baclofen without affecting the maximal response. Therefore, rather than altering the expression of two different populations of membrane receptors, μ-opioid and $GABA_B$ receptors, estrogen could alter a single element (G-protein) common to both receptors and bring about an equivalent suppression of the hyperpolarizing response.

In morphine-tolerant animals, a similar but smaller rightward shift in the dose–response relationship for DAMGO is observed in locus coeruleus neurons (Christie et al., 1987). It appears that the number of functional μ-receptors is reduced due to an uncoupling of the receptor to the G-protein (Werling et al., 1989). There also appears to be a reduction in the expression of the $G\alpha_i$ subunit (Attali and Vogel, 1989). However, estrogen causes a suppression of the membrane hyperpolarization mediated by two heterologous receptor populations, whereas morphine tolerance causes a suppression of the μ-opioid response without affecting the α_2 response, which is coupled to the same potassium conductance in locus coeruleus neurons (Christie et al., 1987). Moreover, a recent report has shown that corticosterone treatment suppresses the $5\text{-}HT_{1A}$–mediated hyperpolarization of hippocampal CA1 neurons without affecting the $GABA_B$ response, which is coupled to the same potassium conductance (Joëls et al., 1991). Therefore, the actions of estrogen on ARC neurons may be unique and would argue for an action downstream from the binding of agonists with their receptors. Since the activation of the inwardly rectifying potassium conductance is one of the most powerful inhibitory mechamisms identified in hypothalamic neurosecreotry neurons (Kelly et al., 1990; Loose and Kelly, 1990; Loose et al., 1990), these actions of estrogen would serve to modulate this input during the reproductive cycle of the mammal. Although the G-protein coupling is a likely site for estrogen's actions, it is unclear at this time as to how rapidly and what other intracellular pathways (kinase, etc.) are involved. Regardless of the mechanism of action, the attenuation of the μ-opioid and $GABA_B$ responses by estrogen is further indication that the steroid has widespread effects on many cellular processes.

References

Attali B, Vogel Z (1989) Long-term opiate exposure leads to reduction of the αi-1 subunit of GTP-binding proteins. J Neurochem 53:1636–1639.

Barraclough CA, Sawyer CH (1955) Inhibition of the release of pituitary ovulatory hormone in the rat by morphine. Endocrinology 57: 329.

Christie MJ, North RA (1988) Agonists at μ-opioid, M2-muscarinic and $GABA_B$-receptors increase the same potassium conductance in rat lateral parabrachial neurones. Br J Phamarcol 95:896–902.

Christie MJ, Williams JT, North RA (1987) Cellular mechanisms of opioid tolerance: studies in single brain neurons. Mol Pharmacol 32: 633–638.

Ferin M, Van Vugt D, Wardlaw S (1984) The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. Recent Prog Horm Res 40:441–485.

Finkel AS, Redman SJ (1985) Optimal voltage clamping with single microelectrode. In: Voltage and patch clamping with microelectrodes (Smith TG Jr, Lecar H, Redman SJ, Gage PW, eds), pp 95–120. Bethesda, MD: American Physiological Society.

Gabriel SM, Simpkins JW, Kalra SP (1983) Modulation of endogenous opioid influence on luteinizing hormone secretion by progesterone and estrogen. Endocrinology 113:1806–1811.

Handa BK, Lane AC, Lord AH, Morgan BA, Rance MJ, Smith CFC (1981) Analogues β-LPH^{61-64} possessing selective agonist activity at μ-opiate receptors. Eur J Pharmacol 70:531–540.

Harris GC, Williams JT (1991) Transient homologous μ-opioid receptor desensitization in rat locus coeruleus neurons. J Neurosci 11: 2574–2581.

Hruska RE, Nowak MW (1988) Estrogen treatment increases the density of D1 dopamine receptors in the rat striatum. Brain Res 442: 349–350.

Ieiri T, Chen HT, Campbell GA, Meites J (1980) Effects of naloxone and morphine on the proestrous surge of prolactin and gonadotropins in the rat. Endocrinology 106:1568–1570.

Joëls M, Hesen W, de Kloet ER (1991) Mineralocorticoid hormones suppress serotonin-induced hyperpolarization of rat hippocampal CA1 neurons. J Neurosci 11:2288–2294.

Juptner M, Jussofie A, Hiemke C (1991) Effects of ovariectomy and steroid replacement on $GABA_A$ receptor binding in female rat brain. J Steroid Biochem Mol Biol 38:141–147.

Kalra SP (1981) Neural loci involved in naloxone-induced luteinizing hormone release: effects of a norepinephrine synthesis inhibitor. Endocrinology 109:1805–1810.

Kalra SP, Kalra PS (1984) Opioid-adrenergic-steroid connection in regulation of luteinizing hormone secretion in the rat. Neuroendocrinology 38:418–426.

Kelly MJ, Loose MD, Ronnekleiv OK (1990) Opioids hyperpolarize β-endorphin neurons via μ-receptor activation of a potassium conductance. Neuroendocrinology 52:268–275.

Kelly MJ, Loose MD, Ronnekleiv OK (1991a) Estrogen attenuates the responsiveness of hypothalamic arcuate neurons to μ-opioid inhibition. In: IBRO World Congress of Neuroscience abstracts, p 386.

Kelly MJ, Loose MD, Ronnekleiv OK (1991b) Convergence of μ-opioid and $GABA_B$ receptors at an inwardly rectifying K^+ conductance in hypothalamic neurons: modulation by estrogen. Soc Neurosci Abstr 17:1040.

Kinoshita F, Nakai Y, Katakami H, Kato Y, Yajima H, Imura H (1980) Effect of β-endorphin on pulsatile luteinizing hormone release in conscious castrated rats. Life Sci 27:843–846.

Loose MD, Kelly MJ (1989) Opioid inhibition of spontaneously active neurons of the rat arcuate nucleus *in vitro*. Brain Res Bull 22:819–823.

Loose MD, Kelly MJ (1990) Opioids act at μ-receptors to hyperpolarize arcuate neurons via an inwardly rectifying potassium conductance. Brain Res 513:15–23.

Loose MD, Ronnekleiv OK, Kelly MJ (1990) Membrane properties and response to opioids of identified dopamine neurons in the guinea pig hypothalamus. J Neurosci 10:3627–3634.

Loose MD, Ronnekleiv OK, Kelly MJ (1991) Neurons in the rat arcuate nucleus are hyperpolarized by $GABA_B$ and μ-opioid receptor agonists: evidence for convergence at a ligand-gated potassium conductance. Neuroendocrinology 54:537–544.

Maus M, Bertrand P, Drouva S, Rasolonjanahary R, Kordan C, Glowinski J, Prémont J, Enjalbert A (1989) J Neurochem 52:410–418.

Maus M, Homburger V, Bockaert J, Glowinski J, Prémont J (1990) Pretreatment of mouse striatal neurons in primary culture with 17β-estradiol enhances the pertussis toxin-catalized ADP-ribosylation of Gαo,i protein subunits. J Neurochem 55:1244–1251.

Millan MJ, Herz A (1985) The endocrinology of the opioids. Int Rev Neurobiol 26:1–83.

Miyake M, Christie MJ, North RA (1989) Single potassium channels opened by opioids in rat locus ceruleus neurons. Proc Natl Acad Sci USA 86:3419–3422.

North RA, Williams JT, Surprenant A, Christie MJ (1987) μ and δ receptors belong to a family of receptors that are coupled to potassium channels. Proc Natl Acad Sci USA 84:5487–5491.

Pragnell M, Snay KJ, Trimmer JS, MacLusky NJ, Naftolin F, Kaczmarek LK, Boyle MB (1990) Estrogen induction of a small, putative K^+ channel mRNA in rat uterus. Neuron 4:807–812.

Rajakumar G, Chiu P, Johnson RL, Mishra RK (1987) 17β estradiol-induced increase in brain dopamine D-2 receptor: antagonism by MIF-1. Peptides 8:997–1002.

Ronnekleiv OK, Loose MD, Erickson KR, Kelly MJ (1990) A method for immunocytochemical identification of biocytin-labeled neurons following intracellular recording. Biotechniques 9:432–438.

Rossmanith WG, Mortola JF, Yen SSC (1988) Role of endogenous opioid peptides in the initiation of the midcycle luteinizing hormone surge in normal cycling women. J Clin Endocrinol Metab 67:695–700.

Schild HO (1947) pA, a new scale for the measurement of drug antagonism. Br J Pharmacol 2:189–206.

Sylvester PW, Van Vugt DA, Aylsworth CA, Hanson EA, Meites J (1982) Effects of morphine and naloxone on inhibition by ovarian hormones of pulsatile release of LH in ovariectomized rats. Neuroendocrinology 34:269–273.

Tallarida RJ, Cowan A, Adler MW (1979) pA2 and receptor differentiation: a statistical analysis of competitive antagonism. Life Sci 25:637–654.

Ueda H, Nozaki M, Satoh M (1991) Multiple opioid receptors and GTP-binding proteins. Comp Biochem Physiol 98:157–169.

Vincent SR, Hökfelt T, Wu J-Y (1982) GABA neuron system in hypothalamus and pituitary gland: immunohistochemical demonstration using antibodies against glutamate decarboxylase. Neuroendocrinology 34:117–125.

Weber E, Evans CJ, Chang JK, Barchas JD (1982) Antibodies specific for α-*N*-acetyl-β-endorphins: radioimmunoassays and detection of acetylated β-endorphins in pituitary extracts. J Neurochem 38:436–447.

Werling LL, McMahon PN, Cox BM (1989) Selective changes in μ opioid receptor properties induced by chronic morphine exposure. Proc Natl Acad Sci USA 86:6393–6397.

Williams JT, North RA (1984) Opiate-receptor interactions on single locus coeruleus neurones. Mol Pharmacol 26:489–497.