Protein Kinase A Maintains Cellular Tolerance to Mu Opioid Receptor Agonists in Hypothalamic Neurosecretory Cells with Chronic Morphine Treatment: Convergence on a Common Pathway with Estrogen in Modulating Mu Opioid Receptor/Effecter Coupling

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ABSTRACT

The present study examined protein kinase A (PKA) and protein kinase C (PKC) involvement in the maintenance of cellular tolerance to mu opioid receptor agonists resulting from chronic opiate exposure in neurosecretory cells of the hypothalamic arcuate nucleus (ARC). The possibility that the diminution of mu opioid receptor/effecter coupling produced by acute 17β-estradiol or chronic opiate exposures is mediated by a common kinase pathway also was investigated. Intracellular recordings were made in hypothalamic slices prepared from ovariectomized female guinea pigs. The mu opioid receptor agonist D-Ala2, N-Me-Phe4, Gly-ol5-enkephalin (DAMGO) produced dose-dependent hyperpolarizations of ARC neurons. Chronic morphine treatment for 4 days reduced DAMGO potency 2.5-fold with no change in the maximal response. This effect was mimicked by a 20-min bath application of the PKA activator cAMP, Sp-isomer, or the PKC activator phorbol-12,13-dibutyrate. A 30-min bath application of the broad-spectrum protein kinase inhibitor staurosporine completely abolished the reduced DAMGO potency seen in morphine-tolerant neurosecretory cells, including those immunopositive for gonadotropin-releasing hormone. The effect of staurosporine was mimicked by the PKA inhibitor camp, Rp-isomer, but not by the PKC inhibitor calphostin C. Finally, a 20-min bath application of 17β-estradiol did not further reduce DAMGO potency in morphine-tolerant ARC neurons. Therefore, increased PKA activity maintains cellular tolerance to mu opioid receptor agonists in ARC neurosecretory cells caused by chronic morphine treatment. Furthermore, acute 17β-estradiol and chronic opiate treatments attenuate mu opioid receptor-mediated responses via a common PKA pathway.

Opioids play an integral role in the regulation of the hypothalamo-pituitary-gonadal axis. For example, opioids inhibit the activity of GnRH and A12 dopamine neurosecretory cells in the hypothalamus (Loose et al., 1990; Lagrange et al., 1995), thereby inhibiting luteinizing hormone secretion and increasing prolactin secretion, respectively, from the anterior pituitary (Ferin et al., 1984; Kapoor and Willoughby, 1990). Opioids inhibit these neurons via a membrane hyperpolarization attributable to the activation of an inwardly rectifying K+ channel (Loose and Kelly, 1990). The inhibitory effect of the mu opioid receptor agonist DAMGO (Goldstein and Naidu, 1989) on neurons from the ARC of the mediobasal hypothalamus is antagonized by naloxone with a Kᵦ fully consistent with a blockade of mu opioid receptors (Lagrange et al., 1997). Thus, the effects of mu opioids, in conjunction with estrogen secreted from the developing ovarian follicle, are largely responsible for the negative feedback control of the reproductive axis (Ferin et al., 1984; Lagrange et al., 1995).

Tolerance to the effects of opioids with chronic morphine administration involves an attenuation or reversal of the inhibitory effects of opioid receptor agonists in both the peripheral and central nervous systems. This phenomenon has been observed in many different paradigms including, but...
not limited to, antinociception (Narita et al., 1994; Bernstein and Welch, 1995; Roerig, 1995), contractility of gastrointestinal smooth muscle (Chavkin and Goldstein, 1984), evoked cAMP formation in the myenteric plexus (Wang et al., 1996) and neuronal excitability in the locus ceruleus (Christie et al., 1987; Guitart and Nestler, 1993). In the mediobasal hypothalamus, tolerance is manifested by a reduction in the potency of the mu opioid receptor agonist DAMGO to hyperpolarize ARC neurons including A12 dopamine neurons, a reduction in DAMGO binding capacity and a down-regulation of mu opioid receptor mRNA (Zhang et al., 1996; Ronneklev et al., 1996; Wagner et al., 1997). The tolerance to mu opioid receptor agonists in A12 dopamine neurons most likely accounts for the tolerance to the proactin-releasing effects of morphine (Deyo et al., 1980).

Although the mu opioid receptor is negatively coupled to the adenylate cyclase/cAMP/PKA pathway upon acute stimulation (Kluttz et al., 1995), both the PKA and the PKC pathways are up-regulated with chronic morphine treatment (Guitart and Nestler, 1993; Wang et al., 1994; Avidor-Reiss et al., 1995; Tokuyama et al., 1995). This suggests that protein kinases play an adaptive role in attenuating mu opioid receptor-mediated responses. Recent evidence indicates that they are involved in desensitization induced by prolonged exposure (minutes) to high concentrations of agonist (Chen and Yu, 1994; Mestek et al., 1995; Narita et al., 1995), and in the development of antinoceptive tolerance induced by chronic morphine exposure (Narita et al., 1994). This is consistent with the observation that mu opioid receptor phosphorylation decreases receptor/G-protein coupling (Harada et al., 1989, 1990), thereby effectively decreasing receptor/effector coupling. It is unknown, however, whether increased protein kinase activity underlies the cellular tolerance to mu opioid receptor agonists observed in ARC neurons with chronic morphine treatment.

The purpose of the present study was 2-fold: 1) to determine whether protein kinase activation can negatively modulate mu opioid receptor/effector coupling in ARC neurons and 2) to determine to what extent protein kinases are involved in the maintenance of cellular tolerance to mu opioid receptor agonists in ARC neurons with chronic morphine treatment. Because E2 negatively modulates mu opioid receptor/effector coupling in ARC neurons via the activation of the PKA pathway (Lagrange et al., 1995, 1997), we also evaluated the effects of the combined treatments of chronic morphine and acute E2 for potential convergence on a common mechanism. To this end, intracellular recordings were made under current clamp in hypothalamic slices prepared from ovariectomized female guinea pigs treated with placebo or morphine pellets for 4 days. Dose-response relationships for the hyperpolarization to DAMGO were generated to evaluate its potency and its efficacy before and immediately after a 20- to 30-min perfusion of the protein kinase activators Sp-cAMP (Dostmann et al., 1990) or PDBu (Fisone et al., 1995), the protein kinase inhibitors staurosporine (Tamaoki et al., 1986; Nakano et al., 1987; Yanagihara et al., 1991), Rp-cAMP (Dostmann et al., 1990) or calphostin C (Kobayashi et al., 1989) or E2. The results reveal that activation of either PKA or PKC can negatively modulate mu opioid receptor/effector coupling in neurosecretory cells of the ARC. Moreover, increased activity of PKA is responsible for the maintenance of cellular tolerance with chronic morphine treatment, just as it is in the uncoupling of the mu opioid receptor from its effector after acute E2 treatment.

Methods

Animals. Female Topka guinea pigs (440—670 g) were obtained from our institutional breeding facility and maintained under conditions of constant temperature (72.4 ± 0.1°F) and light (lights on between 6:30 A.M. and 8:30 P.M.). Animals were housed individually, with water and food provided ad libitum. The surgical and experimental procedures described in the present study were in accordance with institutional guidelines based on National Institutes of Health standards.

Drugs and treatments. Pellets containing either placebo or 75 mg of morphine free base were obtained from the Research Technology Branch of the National Institute on Drug Abuse (Research Triangle, NC). TTX (Sigma Chemical Co., St. Louis, MO) was dissolved in Milli-Q H2O and diluted to the appropriate volume with 0.1% acetic acid (final concentration, 1 mM; pH 4–5). DAMGO (Peninsula Laboratories Inc., Belmont, CA) was dissolved in Milli-Q H2O to a stock concentration of 1 mM. Sp-cAMP and Rp-cAMP were dissolved in Milli-Q H2O to a stock concentration of 10 mM. PDBu, 4α-phorbol and staurosporine (9α,10β,11β,13α(+)-2,3,10,11,12,13-hexahydro-10-methoxy-9-methyl-11-(methylamino)-9,13-epoxy-1H,9H-diindolol,1,2,3,4-2',3',4'-lmpyrrole[3,4-j][1,7]benzodiazepin-1-one; Research Biochemicals Inc., Natick, MA) were dissolved in 100% ethanol to a stock concentration of 1 mM. E2 was purchased from Steraloids (Wilton, NH), recrystallized to ensure purity and dissolved in 95% ethanol to a stock concentration of 1 mM. Calphostin C (2-[12-(2-benzoyloxypropyl)-3,10-dihydro-4,9-dihydroxy-2,6,7,11-tetramethoxy-3,10-dioxy-1-perylenyl]-1-methylethyl carbonic acid 4-hydroxymethyl ester) was dissolved in 100% ethanol to a stock concentration of 100 μM. Unless otherwise indicated, all drugs were purchased from Calbiochem (La Jolla, CA). Doses of Sp-cAMP and Rp-cAMP refer to their respective tetrathyrammonium salts. Aliquots of the various stock solutions were stored at −80°C (TTX, DAMGO, staurosporine), −20°C (Sp-cAMP, Rp-cAMP, calphostin C) or 4°C (PDBu, 4α-phorbol, E2) until used for experimentation, and those for calphostin C were protected from light at all times.

The paradigm for chronic morphine treatment is a modification of the procedure described by Chavkin and Goldstein (1984). Animals were ovariectomized while under ketamine/xylazine anesthesia (33 and 6 mg/kg, respectively; i.p.) and were given four pellets containing either morphine or placebo (s.c.). The animals were kept until electrophysiological recording. During experimentation, the animal was decapitated, and its brain rapidly removed from the skull. The brain was rinsed with ice-cold aCSF (in mM: NaCl, 124; KCl, 5; NaH2PO4, 2.6; dextrose, 10; HEPES, 10; MgSO4, 2; CaCl2, 2) and the hypothalamus immediately dissected. Four coronal slices (450 μM) through the rostro-caudal extent of the ARC were cut with a vibrotome. The slices were transferred to a multwell auxiliary chamber containing oxygenated (95% O2, 5% CO2) aCSF, where they were kept until electrophysiological recording. During experiments involving morphine-treated animals, slices were kept in aCSF containing 1 μM morphine until they were transferred to the recording chamber to minimize withdrawal (Zhang et al., 1996; Wagner et al., 1997). The latency between the slice transfer and the start of electrophysiological recording was 105.9 ± 11.5 min.
Electrophysiology. During recording, slices were maintained in a chamber perfused with warmed (35°C), oxygenated aCSF. aCSF and all drugs (diluted with aCSF) were perfused via a peristaltic pump at a rate of 1.5 ml/min. Microelectrodes were assembled from borosilicate glass pipettes (1.2 mm outer diameter; Dagan, Minneapolis, MN) pulled on a P-87 Flaming Brown puller (Sutter Instrument Co., Novato, CA) and filled with either a 3% biocytin solution in 1.75 M KCl and 0.025 M Tris (pH 7.4), or a 3 M KCl/1.5 M KCl citrate solution (20%-50% v/v). Electrode resistances varied from 100 to 300 megohm. The membrane potential (V_m) of hypothalamic neurons was measured in current clamp via intracellular recording from the ARC. Potentials were amplified and current was passed through the electrode by an Axoclamp 2A preamplifier (Axon Instruments, Foster City, CA). Current and V_m traces were stored on a digital oscilloscope (Tektronix 2230, Tektronix, Beaverton, OR) and were recorded on a chart recorder (Gould 2200, Gould Inc., Glen Burnie, MD). They also underwent analog-digital conversion with a CyberAmp 320 signal conditioner (for amplification) connected to a DigiData 1200 A/D converter (sampling frequency: 62 Hz for the gap-free tape mode, 10–50 kHz for the oscilloscope mode) and subsequent storage on a computer containing Axotape or Axoscope software (Axon Instruments).

After successful impalement, action potentials were collected for subsequent determination of the frequency, height (measured at ½ height) and the HAP. Slices then were perfused with 2 μM TTX for at least 6 min to block spontaneous firing, and supplemented with 1 μM TTX in all subsequent drug solutions. Cumulative dose-response relationships were generated as described previously (Wagner et al., 1997). Doses of DAMGO were perfused until a new steady-state V_m had been obtained (2–7 min), at which time incrementally larger doses of the drugs were given, until finally the maximum steady-state hyperpolarization (∆V_max) had been reached. Individual estimates of agonist EC_{50} were obtained from single neurons via the logistic equation:

\[
\Delta V_{\text{max}} = 100 \cdot \left( \frac{[\text{DAMGO}]^n}{([\text{DAMGO}]^n + \text{EC}_{50}^n)} \right)
\]

fitted by computer (SigmaPlot) from the experimental data points. Before starting a DAMGO dose response, a predrug voltage-current (V/I) relationship was established by giving hyperpolarizing and depolarizing current pulses (0.2 Hz, 1-s duration) of varying magnitudes, and monitoring the resultant voltage deflections. The membrane time constant (τ) was calculated as the time necessary for a voltage deflection (∼10 mV) to reach 63% of its maximum. Immediately after the completion of the DAMGO dose response, or during the application of the maximal dose, the V_m was returned to its original resting state and a second, postdrug V/I relationship was established. The conductance was measured by taking the inverse of the original resting state and a second, postdrug V/I relationship was established. The conductance was measured by taking the inverse of the original resting state and a second, postdrug V/I relationship was established. The conductance was measured by taking the inverse of the original resting state and a second, postdrug V/I relationship was established. The conductance was measured by taking the inverse of the original resting state and a second, postdrug V/I relationship was established.

After recording with biocytin-filled electrodes, slices were cut on a cryostat and were mounted on slides coated with poly-l-lysine.

**Results**

The present study included a total of 92 cells obtained from 66 animals. In all, 38 of 43 cells from placebo-treated animals and 47 of 49 cells from morphine-treated animals responded to the mu opioid receptor agonist DAMGO with a dose-dependent, membrane hyperpolarization. Chronic morphine treatment produced a rightward shift of the DAMGO dose-response curve (fig. 1), increasing the DAMGO EC_{50} 2.5-fold, but did not affect the ∆V_{max}. Although opiate-tolerant myenteric S neurons show a depolarized resting V_m compared with placebo-treated controls (Meng et al., 1997), chronic morphine treatment did not affect the resting V_m of the ARC neurosecretory cells in the present study (placebo, −51.0 ± 1.3 mV; morphine, −49.8 ± 1.1 mV). In addition, it did not affect any of the following parameters: input resistance, τ, firing rate, action potential height, action potential width, HAP, the DAMGO-induced ∆g or the reversal potential for the DAMGO response (data not shown). This lack of effect on membrane properties is consistent with other studies involving opiate-tolerant central nervous system neurons (Christie et al., 1987; Zhang et al., 1996; Wagner et al., 1997).

![Fig. 1. Composite cumulative dose-response curves for DAMGO with placebo or morphine treatment. Cells from placebo-treated (open circles) or morphine-treated (solid circles) animals were perfused with successively higher concentrations of DAMGO (20, 50, 100, 200, 300, 500, 600 and 1000 nM; 4–7 min/dose; n = 38 for placebo; n = 40 for morphine). Curves were produced from logistic equations fitted by computer to the data points representing a particular treatment. The mean DAMGO EC_{50} values were 43.6 ± 3.1 nM for placebo and 108.2 ± 10.4 nM for morphine (P < .05; Mann-Whitney test), and the mean DAMGO ∆V_{max} values were 11.1 ± 0.8 mV for placebo and 10.9 ± 0.7 mV for morphine.](https://jpet.aspetjournals.org/article/285/8/1268/1)
We then ascertained whether protein kinase activation could attenuate the DAMGO response as did chronic opiate, and if so, whether protein kinase activation is capable of further diminishing the response attenuated by chronic morphine treatment. Cumulative dose-response relationships obtained in an ARC neuron from a placebo-treated animal before and immediately after a 20-min perfusion of the selective PKA activator Sp-cAMP (Dostmann et al., 1990) are

Fig. 2. Cumulative dose-response relationships for DAMGO in an ARC neuron from a placebo-treated animal obtained before and immediately after the bath application of the selective PKA activator Sp-cAMP. The resting $V_m$ of this cell was −52 mV. Successively increasing doses of DAMGO (20, 50, 100 and 200 nM), perfused until a new steady-state $V_m$ had been reached (4–7 min), hyperpolarized the cell 3.5, 6.5, 8.5 and 10 mV, respectively. The break in the recording during the 200 nM DAMGO application represents the time necessary to perform a second V/I relationship (∼4 min). The estimated EC$_{50}$ before Sp-cAMP was 40.7 nM. The break in the abscissa represents the subsequent drug washout, the 20-min perfusion of Sp-cAMP (100 μM) and the generation of a second pre-DAMGO V/I relationship. Immediately after the perfusion of Sp-cAMP, successively increasing doses of DAMGO (50, 100, 300 and 500 nM) were again perfused until a new steady-state $V_m$ had been reached (4–7 min), which hyperpolarized the cell 1.5, 4, 8 and 9 mV, respectively. The estimated EC$_{50}$ after Sp-cAMP was 122.1 nM.

Fig. 3. (A) The effect of bath application of Sp-cAMP on the DAMGO EC$_{50}$ in ARC neurons from placebo- and morphine-treated animals. Cells ($n = 4$ for both placebo and morphine groups) were perfused with successively higher concentrations of DAMGO (20, 50, 100, 200, 300 and 500 nM; 4–7 min/dose) before (open columns) and immediately after a 20-min perfusion of Sp-cAMP (100 μM; solid columns). *EC$_{50}$ values after Sp-cAMP application which are significantly different (Friedman’s/Mann-Whitney test; $P < .05$) from those before Sp-cAMP application. #EC$_{50}$ values from the morphine-treated groups which are significantly different (Friedman’s/Mann-Whitney test; $P < .05$) from placebo-treated controls. (B) The effect of bath application of the PKC activator PDBu on the DAMGO EC$_{50}$ in ARC neurons from placebo- and morphine-treated animals. Cells ($n = 4$ for both placebo and morphine groups) were perfused with successively higher concentrations of DAMGO (20, 50, 100, 200, 300, 500, 600 and 1000 nM; 4–7 min/dose) before (open columns) and immediately after a 20-min perfusion of PDBu (1 μM; solid columns). *EC$_{50}$ values after PDBu application which are significantly different (two-way ANOVA/LSD; $P < .05$) from those before PDBu application. #EC$_{50}$ values from the morphine-treated groups which are significantly different (two-way ANOVA/LSD; $P < .05$) from placebo-treated controls. (C) Lack of effect of the inactive 4α-phorbol on the DAMGO EC$_{50}$ in ARC neurons from placebo-treated controls. Cells ($n = 3$) were perfused with successively higher concentrations of DAMGO (20, 50, 100, 200, 300 and 500 nM; 4–7 min/dose) before (open columns) and immediately after a 20-min perfusion of 4α-phorbol. (1 μM; solid columns).
shown in figure 2. Sp-cAMP (100 μM) increased the DAMGO EC₅₀ in ARC neurons from placebo- but not from morphine-treated animals (fig. 3A). On the other hand, a 1 μM concentration of the PKC activator PDBu (Fisone et al., 1995) increased the DAMGO EC₅₀ in ARC neurons from both placebo- and morphine-treated animals (fig. 3B). This effect was not mimicked by the inactive 4α-phorbol (1 μM; fig. 3C). Neither Sp-cAMP, PDBu nor 4α-phorbol had any effect on the resting Vₘ, the ΔVₘₐₓ, the DAMGO-induced Δg or the reversal potential for the DAMGO response (data not shown).

Having established that both PKA and PKC activators mimic the effects of chronic opiate exposure in attenuating the DAMGO response, we then sought to determine whether increased protein kinase activity is responsible for the attenuation elicited by chronic morphine treatment. Cumulative dose-response relationships obtained in a GnRH-positive ARC neurosecretory cell (fig. 4, A and B) from a morphine-tolerant animal before and immediately after a 30-min perfusion of the broad-spectrum protein kinase inhibitor staurosporine (Tamaoki et al., 1986; Nakano et al., 1987; Yanagihara et al., 1991) are shown in figure 5. Staurosporine (100 nM) completely abolished the increase in the DAMGO EC₅₀ caused by chronic morphine treatment but was without effect in neurosecretory cells from placebo-treated controls (fig. 6A), an example of which is shown in figure 7, A and B. As shown in figure 6, B and C, this effect of staurosporine in neurosecretory cells from morphine-tolerant animals was mimicked by 100 μM of the selective PKA inhibitor Rp-cAMP (Dostmann et al., 1990) but not by 100 nM of the selective PKC inhibitor calphostin C (Kobayashi et al., 1989). Neither staurosporine, Rp-cAMP nor calphostin C had any effect on the resting Vₘ, the ΔVₘₐₓ, the DAMGO-induced Δg or the reversal potential for the DAMGO response (data not shown).

We have shown previously that a 20-min perfusion of E₂ rapidly attenuates the DAMGO response in ARC neurons via activation of a PKA pathway (Lagrange et al., 1995, 1997). To determine whether E₂ can further attenuate the DAMGO response in morphine-tolerant ARC neurons, a 20-min application of E₂ (100 nM) was given during the DAMGO washout after a cumulative dose response, followed by a second cumulative dose response. As shown in figure 8, E₂ was without effect on the DAMGO EC₅₀ in morphine-tolerant ARC neurons. Furthermore, there was no change in the ΔVₘₐₓ of the DAMGO response (not shown). Taken together, the results indicate that activation of PKA or PKC mimics the effect of chronic opiate in ARC neurosecretory cells in selectively reducing DAMGO potency, that activation of PKC can further reduce DAMGO potency in cells from morphine-tolerant animals and that increased PKA activity mediates the reduction in DAMGO potency caused by chronic morphine treatment. Furthermore, the reduction in DAMGO potency produced by either chronic opiate or acute E₂ exposure arises from the activation of a common PKA pathway.

**Discussion**

The results of the present study demonstrate that in ARC neurosecretory cells, including GnRH and A₁₂ dopamine cells, increased PKA activity is responsible for the maintenance of cellular tolerance to mu opioid receptor activation caused by chronic morphine treatment. This conclusion is based on the observations that PKA activation mimics the mu opioid receptor/effector uncoupling elicited by chronic morphine exposure as manifested by a reduction in mu opioid receptor agonist potency, and that PKA inhibition abolishes this mu opioid receptor/effector uncoupling. These results also indicate that chronic morphine and acute E₂ treatments converge on a common PKA pathway to uncouple the mu opioid receptor from its effector. This observation is based on the observed occlusion of the E₂-mediated uncoupling by chronic morphine exposure.

In the present study chronic morphine treatment produced a 2.5-fold reduction in mu opioid receptor agonist potency. This reduction is similar to what we showed previously in ARC A₁₂ dopamine neurosecretory cells (Wagner et al., 1997) and to what was observed in the locus ceruleus (Christie et al., 1987). In the locus ceruleus, morphine tolerance and dependence is associated with the elevated adenylate cyclase and PKA activities, as well as increased levels of pertussis toxin-sensitive G-proteins (Guitart and Nestler, 1993). In *vitro* studies have revealed a similar supersensitivity in adenylate cyclase activity with chronic morphine treatment in Chinese hamster ovary cells transfected with mu opioid receptor (Avidor-Reiss et al., 1995). Furthermore, PKA-mediated phosphorylation of the purified mu opioid receptor preparation prevents the functional coupling of the receptor when reconstituted with purified G₂-protein as measured by agonist-stimulated, low-Kₘ GTPase activity (Harada et al., 1990). Collectively, these studies suggest an adaptive role for increased PKA-mediated phosphorylation in attenuating mu opioid receptor-mediated responses caused by continuous ex-

![Image](https://example.com/image.png)
Exposure to opiates. They are fully consistent with the complete abolition by PKA inhibition of the chronic opioid-induced reduction in agonist potency observed in the present study. Whereas the present study does not address a role for PKA in the induction of morphine tolerance, such a role has been described for PKA in the development of antinociceptive tolerance (Narita et al., 1994). However, the present study clearly demonstrates a predominant role for high-turnover, PKA-mediated phosphorylation in maintaining cellular tolerance to chronic opiate exposure in ARC neurosecretory cells.

E2 rapidly attenuates the response in ARC neurons to mu opioid agonists, which is manifested by the selective reduction in agonist potency via the activation of a PKA pathway (Lagrange et al., 1995, 1997). E2 was unable, however, to uncouple further the mu opioid receptor from its effector in morphine-tolerant, neurosecretory cells. This is identical with what we observed with exogenous, PKA activator application. These latter two findings indicate that receptor/effector uncoupling caused by acute E2 or chronic morphine treatment is via a common PKA pathway and that the PKA-mediated receptor/effector uncoupling observed with chronic opiate exposure is maximal.

Activation of PKC also was effective in uncoupling the mu opioid receptor from its effector in ARC neurons. This is consistent with numerous studies implicating PKC in attenuating G-protein-coupled receptor-mediated responses with either acute or prolonged agonist exposure. For example,
PKC activation potentiates the desensitization caused by repeated agonist exposure in *Xenopus* oocytes coexpressing the mu opioid receptor and a G-protein-activated, inwardly rectifying K⁺ channel (Chen and Yu, 1994; Mestek *et al.*, 1995). Furthermore, PKC inhibition blocks the development of acute, antinociceptive tolerance to mu opioid receptor agonists (Narita *et al.*, 1995), and abolishes the reversal of opioid-induced inhibition of evoked cAMP formation to enhancement observed in the opiate-tolerant myenteric plexus (Wang *et al.*, 1996). Finally, PKC mediates the 5-hydroxytryptamine2c receptor-mediated attenuation of the inwardly rectifying K⁺ current in *Xenopus* oocytes (Di-Magno *et al.*, 1996).

In the present study, however, PKC activation elicited receptor/effecter uncoupling in neurosecretory cells from both placebo-treated and morphine-tolerant animals. Moreover, the 100 nM concentration of calphostin C used in the present study was without effect on the uncoupling induced by chronic opiate exposure. This concentration is twice the IC₅₀ for its inhibition of PKC (Kobayashi *et al.*, 1989) and is effective in blocking the negative modulatory effects of E₂ on mu opioid receptor agonist potency in ARC neurons (Lagrange AH, Rønnekleiv OK and Kelly MJ, unpublished observation). The latter finding suggests that the attenuation of the mu opioid response by E₂ may involve an upstream PKC component in series with the PKA component of this modulatory pathway. Conversely, activation of PKA reduces the mu opioid receptor/effecter coupling in cells from placebo- but not morphine-treated animals. Inhibition of PKA restores mu opioid receptor agonist potency to levels observed in cells from placebo-treated controls. Thus, activation of PKC and PKA apparently uncouples the mu opioid receptor from its effecter in ARC neurosecretory cells through serial and parallel pathways. However, increased PKC activity is not implicated in the decreased mu opioid agonist response caused by chronic opiate exposure. Future studies will examine the interaction between PKA and PKC in regulating mu opioid receptor/effecter coupling in ARC neurons.

In conclusion, the results presented in this study reveal that increased PKA but not PKC activity is responsible for the maintenance of cellular tolerance in ARC neurosecretory cells caused by chronic morphine treatment. They also reveal that acute E₂ and chronic opiate exposures negatively modulate mu opioid receptor coupling via a common PKA pathway.

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**References**


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