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Acute and chronic activation of the μ-opioid receptor with the endogenous ligand endomorphin differentially regulates adenylyl cyclase isozymes

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Abstract

While acute activation of $G_{i/o}$-coupled receptors leads to inhibition of adenylyl cyclase, chronic activation of such receptors produces an increase in cyclic AMP accumulation, particularly evident upon withdrawal of the inhibitory agonist. This phenomenon has been referred to as adenylyl cyclase superactivation and is believed to play an important role in opiate addiction. Nine adenylyl cyclase isozymes have been recently identified and shown by us to be differentially regulated by acute and chronic inhibitory receptor activation. Using COS-7 cells cotransfected with various adenylyl cyclase isozymes, we examined here whether the endomorphins (the most recently discovered of the four classes of endogenous opioid peptides, and which interact selectively with the μ receptor) are able to induce inhibition/superactivation of representatives from the various adenylyl cyclase isozyme classes. Here, we show that adenylyl cyclase types I and V were inhibited by acute endomorphin application and superactivated upon chronic exposure, while adenylyl cyclase type II was stimulated by acute and "superinhibited" by chronic endomorphin exposure. These results show that the endomorphins are capable of regulating adenylyl cyclase activity and that different adenylyl cyclase isozymes respond differently to these endogenous ligands. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Endomorphins; Opioid receptor; Adenylyl cyclase; Isozymes; Inhibition; Superactivation

1. Introduction

Pharmacological studies have defined three types of opioid receptors, termed μ, δ and κ, which differ in their affinity for various opioid ligands, their distribution in the nervous system and their physiological and behavioral profiles (Reisine and Bell, 1993; Uhl et al., 1994). The three opioid receptors are members of the seven-transmembrane domain GTP-binding protein (G protein)-coupled receptor superfamily. Activation of these receptors inhibits the activity of adenylyl cyclase (AC) via the $G_{i/o}$ type of G proteins. Yet, we and others have shown that when these and several other $G_{i/o}$-coupled receptors (e.g. μ-, δ- and κ-opioid, D_2-dopaminergic, m_2- and m_4-muscarinic, α_2-adrenergic, somatostatin) are chronically activated, there is an *increase* in cAMP accumulation, which is particularly apparent upon withdrawal of the inhibitory agonist (Sharma et al. 1975, 1977; Hamprecht, 1977; Parsons and Stiles, 1987; Thomas and Hoffman 1987, 1992; Avidor-Reiss et al., 1995a,b, 1996; McDermott and Sharp, 1995; Nevo et al., 1998). This phenomenon has been referred to as AC superactivation and is believed to represent a possible biochemical substratum for the development of drug tolerance and dependence, commonly observed upon prolonged exposure to opiate drugs (Sharma et al., 1975; Nestler et al., 1993).

In subsequent studies, we and others demonstrated that not all of the nine isozymes of AC which have been identified to date (Mons and Cooper, 1995; Sunahara et al., 1996) behave identically with respect to regulation by $G_{i/o}$-coupled receptor activation. Indeed, using African green monkey kidney (COS-7) cells cotransfected with μ-opioid (Avidor-Reiss et al., 1997), D_2-dopaminergic, m_4-muscarinic (Nevo et al., 1998), or CB1-cannabinoid (Rhee et al., 1998a,b) receptor and various AC isozymes, we observed acute inhibition of

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AC types I, V, VI and VIII, and superactivation of these isozymes upon chronic agonist treatment. Conversely, the activity of AC isozymes II, IV and VII was stimulated by acute agonist application, and reduced upon chronic treatment.

Four classes of structurally related endogenous peptides which interact with the opioid receptors have been discovered. The first three consist of the enkephalins, β–endorphin, and the family of neoendorphin and the dynorphins (Corbett et al., 1993; Hollt, 1993). The latest family to be discovered consists of two tetrapeptides which differ by one amino acid: endomorphin-1 (Tyr–Pro–Trp–Phe–NH_2, endo-1) and endomorphin-2 (Tyr–Pro–Phe–Phe–NH_2, endo-2) (Zadina et al., 1997).

All of the members of the first three classes of endogenous opioid peptides described above have only moderate specificity for the various opioid receptors, although the enkephalins and β–endorphin bind with some preference to the δ- and μ-opioid receptors, while the dynorphins are more selective to the κ-receptors (Corbett et al., 1993). The two endomorphins, however, not only have a high affinity for the μ-opioid receptor (rivaling morphine in both their potency as agonists and their analgesic activity), they are also very selective for the μ receptor and show very low affinity for the δ and κ opioid receptors (Zadina et al., 1997). These characteristics make these endogenous ligands ideal candidates for elucidation of the biochemical mechanisms underlying the physiological effects of the μ receptor. This is especially important, as knock-out experiments have shown that the μ receptor, but not the δ and κ receptors, is the molecular target of morphine in vivo, and that it is a mandatory component of the opioid system for both morphine analgesic effects and physical dependence (Matthes et al., 1996).

It was thus deemed of interest to determine whether the endogenous μ ligands, the endomorphins, are able to induce AC inhibition/superactivation, and to determine the inhibition/activation pattern of the various isozymes upon acute and chronic endomorphin activation of the μ receptor.

2. Methods

2.1. Materials

[^{3}H-2]adenine (18.0 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Morphine was obtained from the National Institute of Drug Abuse, Research Technology Branch (Rockville, MD), and naloxone was purchased from Research Biochemical International (Natick, MA). Ionomycin and the phosphodiesterase inhibitors 1-methyl-3-isobutylxanthine (IBMX) and RO–20–1724 were from Calbiochem (La Jolla, CA). Forskolin (FS), cAMP, and thyroid-stimulating hormone (TSH) were from Sigma (St. Louis, MO). Pertussis toxin (PTX) was purchased from List Biological Laboratories (Campbell, CA). Endo-1 and endo-2 were obtained from Tocris Cookson Ltd. (Longford, Bristol, UK) Tissue culture reagents were from Life Technologies (Gaithersburg, MD).

2.2. Plasmids

Plasmids containing AC cDNAs (pXMD1–AC–I, pXMD1–AC–II and pXMD1–AC–V), as well as pXMD1–gal, rat TSH receptor in pSG5, and rat μ-opioid receptor in pCMV-neo have been previously described (Avidor-Reiss et al., 1997).

2.3. Cell transfection

The Chinese hamster ovary (CHO) cell line stably transfected with the rat μ-opioid receptor cDNA (CHO-μ) was previously described (Avidor-Reiss et al., 1995a). The CHO-μ cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8% fetal calf serum, 2 mM glutamine, nonessential amino acids, 0.2 mg/ml G418, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37°C.

Transient transfection of COS–7 cells was performed as previously described (Avidor-Reiss et al. 1996, 1997). In brief, COS–7 cells in 10-cm plates were transfected using the DEAE-dextran chloroquine method with 1 μg/plate of μ-opioid receptor, 2 μg/plate of either one of the AC isozyme cDNAs, or pXMD1–gal (for mock DNA transfection), and, where indicated, with 1 μg/plate of the TSH receptor cDNA. Twenty-four hours later, the cells from each 10-cm plate were trypsinized and recultured in a 24-well plate, and after an additional 24 h, the cells were assayed for cAMP content (as a measure of AC activity) as described below. Transfection efficiencies were normally in the range of 60–80%, as determined by staining for β–galactosidase activity (Lim and Chae, 1989).

2.4. AC assay

The assay was performed in triplicate as described previously (Avidor-Reiss et al. 1995a, 1997; Nevo et al., 1998). In brief, cells cultured in 24-well plates were incubated for 2 h with 0.25 ml/well of fresh growth medium containing 5 μCi/ml of [^{3}H]adenine, and then washed three times with 0.5 ml/well of DMEM containing 20 mM Hepes (pH 7.4) and 0.1 mg/ml bovine serum albumin(BSA). This medium was replaced with 0.5 ml/well of DMEM containing 20 mM Hepes (pH 7.4), 0.1 mg/ml BSA, and the phosphodiesterase inhibitors IBMX (0.5 mM) and RO–20–1724 (0.5 mM). AC activity was stimulated in the presence or absence of the

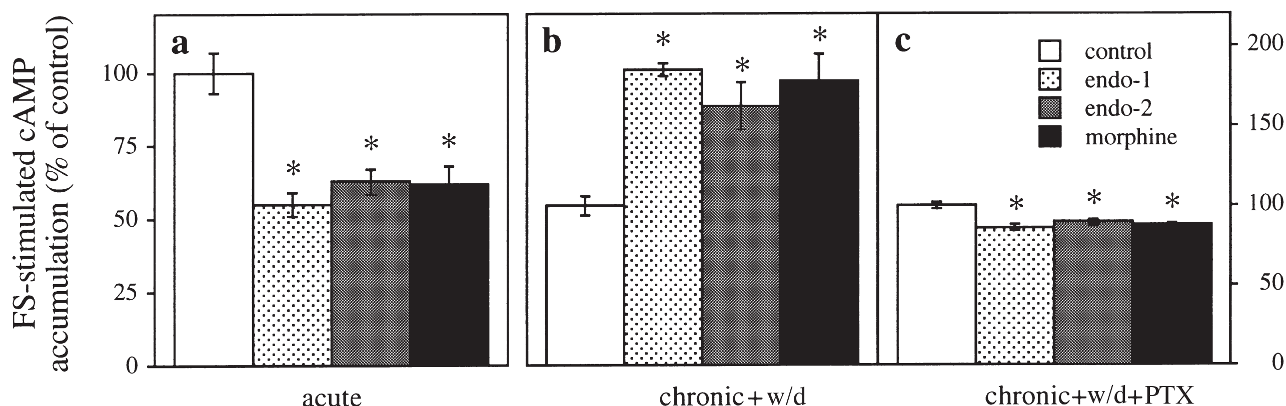

Fig. 1. Effect of acute and chronic treatment with endomorphins or morphine on endogenous AC activity in CHO-μ cells. Endogenous AC activity in CHO-μ cells is estimated as [^{3}H]cAMP formed (a) following acute (10-min) stimulation with 1 μM FS in the presence or absence (control) of 1 μM of the agonists endo-1, endo-2 or morphine, (b) after chronic (18-h) treatment with the agonists followed by withdrawal (by two rapid washes and addition of 1 μM naloxone), or (c) after chronic treatment and withdrawal subsequent to 18-h pretreatment with 100 ng/ml PTX. PTX treatment decreased FS-stimulated AC activity by 30% compared to non-PTX-treated FS-stimulated cells. 100% represents the control AC activity observed in FS-stimulated cells (a, b: 1825±82 cpm of [^{3}H]cAMP; c: 1071±24 cpm of [^{3}H]cAMP). Data represent the means±S.E. of triplicate determinations of a representative experiment. *$P<0.02$ vs control. For a more detailed description of the methods used, refer to the Methods section and references therein.

indicated concentrations of the examined ligands (endo-1, endo-2 or morphine) by the addition of either 1 μM FS, 1 μM ionomycin or 0.1 μM TSH (in the latter case, the cells were transfected with the TSH receptor). After 10 min incubation at room temperature, the medium was removed and the reaction terminated by addition of perchloric acid containing 0.1 mM unlabeled cAMP, followed by neutralization with KOH, and the amount of [^{3}H]cAMP was determined by a two-step column separation procedure as previously described (Salomon, 1991; Avidor-Reiss et al., 1996). Unless otherwise indicated, chronic treatment was achieved by incubating the cells for 18 h with the indicated concentrations of the agonists, followed by agonist withdrawal (achieved by quick removal of medium and the addition of new medium containing the 1 μM of the opioid antagonist naloxone) and the addition of the appropriate AC stimulator (see above) to assay cAMP accumulation. The incubation with [^{3}H]adenine took place during the last two hours of the chronic exposure. Uptake of [^{3}H]adenine into the cells was not affected by the chronic agonist treatments.

3. Results and discussion

3.1. Effect of acute and chronic endomorphins on AC activity in CHO-μ cells

Acute (10 min) application of 1 μM endo-1, endo-2 or morphine to CHO-μ cells resulted in a significant reduction (of 40–50%) in FS-stimulated cAMP accumulation in these cells (Fig. 1a). In contrast, chronic treatment (18 h) of these cells with these agonists followed by their rapid withdrawal led to an increase (of 60–85%) in FS-stimulated cAMP accumulation (Fig. 1b), in agreement with the phenomenon of chronic agonist-induced AC superactivation (Sharma et al., 1975; Thomas and Hoffman, 1987; Avidor-Reiss et al., 1995a,b, 1996). The similarity between morphine and the endomorphins is interesting, since it has recently been shown that chronic treatment with endo-1 leads to μ-opioid receptor internalization, while chronic morphine does not (Burford et al., 1998). This demonstrates that the superactivation of AC following chronic agonist exposure is not dependent on receptor internalization.

PTX is known for its ability to catalyze the ADP-ribosylation of inhibitory G proteins ($G_{i/o}$) at the cysteine residue at the C′-terminus of the Gα subunit, thus preventing the activation of these G proteins (Birnbaumer et al., 1990). Chronic treatment with PTX alone was found to have an inhibitory effect on the level of cAMP accumulation (by ca. 35%; see legend to Fig. 1). Upon chronic application of the opioid ligands to PTX-pretreated cells, not only was no superactivation observed, there was actually a very small, albeit significant, reduction in cAMP accumulation as compared to control PTX-pretreated cells (Fig. 1c). At this stage, we cannot rule out that this very small reduction in cAMP formation could be due to an alteration of cell physiology by the chronic agonist treatment in the PTX-pretreated cells.

Taken together, these results indicate that like morphine, endo-1 and endo-2 activate the μ-opioid receptor in this cell line, leading to inhibition of the endogenous AC upon acute activation and to AC superactivation upon chronic treatment. Furthermore, the absence of AC superactivation when the cells are pre-treated with PTX suggests that the superactivation of the endogenous AC found in CHO-μ cells by chronic endomorphin or morphine is mediated via the $G_{i/o}$ family of G proteins.

Dose-response curves for the effects of acute and chronic agonist exposures in CHO-μ cells are depicted in Fig. 2. Significant inhibition of FS-stimulated cAMP accumulation was observed in cells acutely treated with endo-1 or endo-2, with IC_{50} concentrations of ca. 100 pM and 3 nM, respectively (Fig. 2a). Moreover, a dose-dependent superactivation was observed for both of these ligands following chronic exposure and agonist withdrawal (Fig. 2b). As the chronic dose-response curves did not attain saturation at the highest concentration employed (1 μM), it was not possible to reliably calculate the ED_{50} values for AC superactivation upon chronic endo-1 or endo-2 treatment. It can, however, be concluded that the ED_{50} concentrations for superactivation of these opioid ligands are at least several orders of magnitude higher than the IC_{50} concentrations for inhibition. This shift in the ED_{50} suggests that the chronic endomorphin activation may possibly lead to some desensitization of the μ-opioid receptor. This is consistent with results obtained previously which showed that the enkephalin analog[D–Ala^2, *N*–methyl–Phe^4, Gly–ol^5]enkephalin (DAMGE), which induces μ-opioid receptor desensitization (Carter and Medzihradsky, 1993), demonstrated a much larger shift in ED_{50} between acute inhibition and chronic agonist-induced superactivation than did morphine (Avidor-Reiss et al., 1995a), which does not cause significant densensitization or internalization (Blake et al., 1997; Burford et al., 1998).

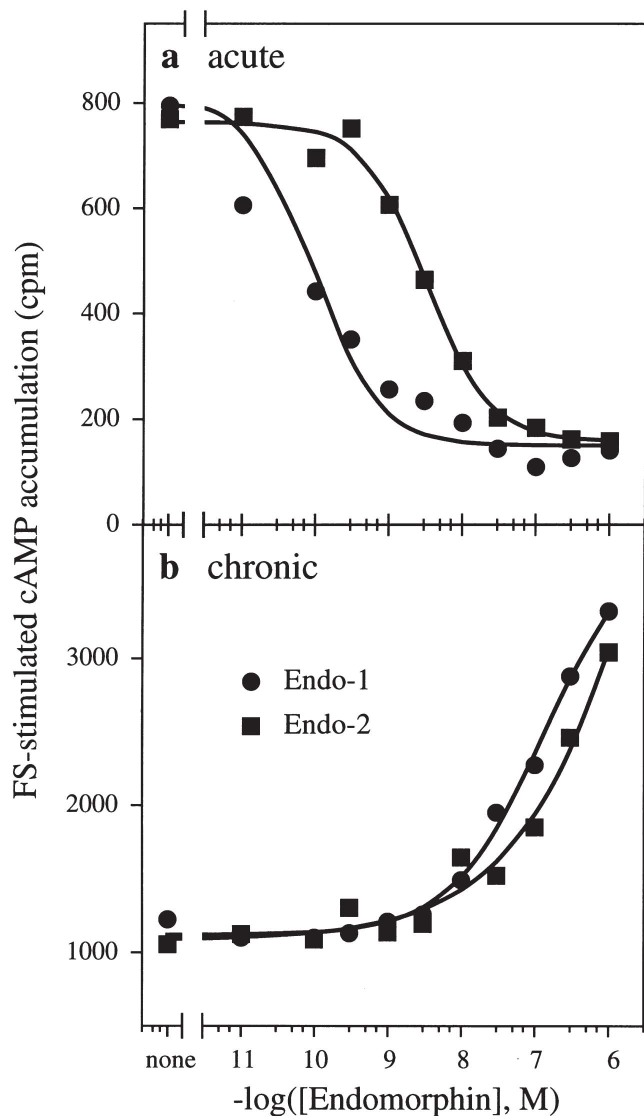

Fig. 2. Dose-response of acute and chronic endomorphins on endogenous AC activity in CHO-μ cells. (a) Inhibition of 1 μM FS-stimulated endogenous AC activity by various concentrations of endo-1 or endo-2 applied acutely during the assay. (b) FS-stimulated endogenous AC activity following chronic treatment (18 h) with the indicated concentrations of endo-1 or endo-2 and subsequent withdrawal of the agonist (by two rapid washes and addition of 1 μM naloxone). AC inhibition/superactivation curves were drawn using the equation: $y=(a-d)/[1+(x/c)^b]+d$, where a is the asymptotic maximum, b is the value of the slope, c is the inflexion point (which is equivalent to the EC_{50} value), and d is the asymptotic minimum. Data are expressed in cpm of [^{3}H]cAMP formed and represent the means±S.E. of triplicate determinations of a representative experiment out of three which produced similar results.

The development of superactivation upon withdrawal following chronic endo-1 or endo-2 treatment in CHO-μ cells is time dependent. Fig. 3 depicts the kinetics of this process as a function of time of treatment with 300 nM of either of these ligands. For both ligands, a maximal level of superactivation was attained following 2–4 h of chronic treatment. Re-addition of agonist (at the same concentration) following chronic treatment (Fig. 3; *chronic treatment+agonist*) prevented the appearance of superactivation and nearly completely inhibited cAMP accumulation. This result demonstrates that the μ-receptor did not undergo complete desensitization and remained partially functional even after the chronic exposure to the endomorphins.

3.2. Differential regulation of AC isozymes by endomorphin activation of the μ-opioid receptor

In order to examine the differential effect of the endomorphins on the various AC isozymes, we employed the COS–7 cell system, which readily allows transient transfection of the μ-opioid receptor together with the desired AC isozymes.

Three types of AC isozymes, representing the three major classes of AC isozymes according to their properties and sequence homologies, were studied: AC–I, which, like AC–VIII, is stimulated by Ca^{2+}/calmodulin; AC–V, which, like AC–VI, is inhibited by Ca^{2+} ions, stimulated by FS and inhibited by $G_{\beta\gamma}$; and AC–II, which is similar to AC–IV and AC–VII, and is stimulated by $G_{\beta\gamma}$ subunits (Choi et al., 1993; Mons and Cooper, 1995; Sunahara et al., 1996; Bayewitch et al., 1998b). The choice of these particular isozymes was also based on the fact that these are the only three AC isozymes whose

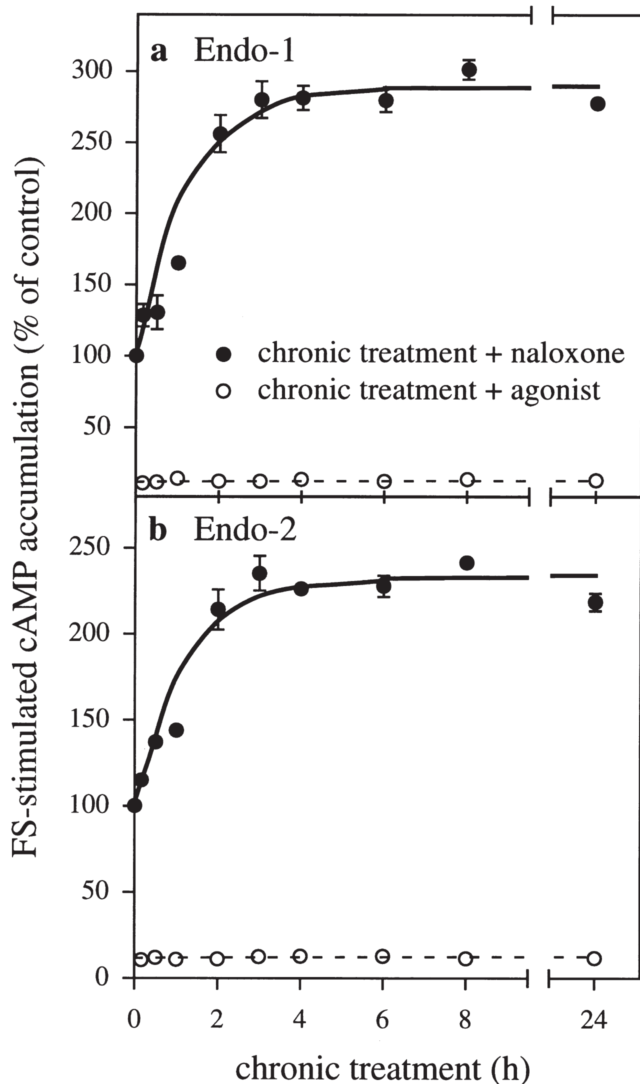

Fig. 3. Time course of the development of AC superactivation in CHO-μ cells upon chronic endo-1 or endo-2 treatment. The figure shows the rate of development of superactivation of endogenous AC (stimulated with 1 μM FS) following chronic treatment for the indicated times with 300 nM endo-1 (a) or endo-2 (b), and withdrawal of the agonist (two rapid washes and addition of 1 μM naloxone) immediately prior to the AC assay (●). The figure also presents a curve depicting the absence of the development of superactivation when the same concentrations of the agonists were re-added following the chronic treatment and withdrawal by two rapid washes (○). Data represent the means±S.E. of triplicate determinations of a representative experiment. 100% represents FS-stimulated AC activity in the absence of agonist.

mRNAs are expressed at high levels in the rat brain (Mons et al., 1995). All three of these isozymes are stimulatable by FS (Sunahara et al., 1996; Avidor-Reiss et al., 1997). However, FS also activates endogenous AC in COS, making for a higher background activity. While this was not a problem with AC–V, which exhibited a high AC activity upon transfection (Avidor-Reiss et al., 1996), it was important to reduce this background activity with AC–I and II. AC–I (but none of the other isozymes examined in this study, including the endogenous AC in COS) is stimulated by Ca^{2+}, and therefore ionomycin stimulation activates this isozyme efficiently with very low background activity for non-transfected cells (Avidor-Reiss et al., 1997; Nevo et al., 1998). As for AC–II, by co-transfecting it with the TSH receptor, the same population of cells will express both the receptor and AC–II, and thus TSH activation stimulates AC–II activity without increasing the background of the cells which did not undergo transfection. Thus, the Ca^{2+} ionophore ionomycin was used to activate AC–I, FS to activate AC–V, and α_s activation (i.e. via activation of the TSH receptor cotransfected into the COS cells) was used to activate AC–II.

In cells co-transfected with the μ-opioid receptor and AC–I or AC–V, stimulation with 1 μM ionomycin (AC–I) or 1 μM FS (AC–V) resulted in a large increase in cAMP accumulation as compared to the unstimulated basal level (Avidor-Reiss et al., 1997). The finding that AC–I-transfected cells show strong activation by ionomycin is in agreement with previous reports, as described above, demonstrating that Ca^{2+}/calmodulin has a strong stimulatory effect on this isozyme (Mons and Cooper, 1995; Sunahara et al., 1996), and indicates that the transfected AC–I is expressed and functionally active. Acute activation of the μ-opioid receptor by either endo-1 or endo-2 led to inhibition of the stimulated activity of both AC–I and AC–V, while chronic activation of the μ-opioid receptor with endo-1 or endo-2 followed by agonist withdrawal (by two rapid washes) led to superactivation of both of these isozymes (Fig. 4a and b). Re-addition of the endomorphins to the cells following chronic treatment and withdrawal prevented the superactivation and produced levels of inhibition similar to those observed upon acute application, indicating that the chronic treatment did not completely desensitize the functional coupling of the μ-opioid receptor to the G proteins or thereby eliminate the capacity of the endomorphins to inhibit AC activity.

The above results demonstrate that AC–I and V both exhibit inhibition and superactivation by acute and chronic exposure, respectively, of the μ-opioid receptor to endomorphins, and that these two functions are not dependent on the agents used to stimulate the particular AC activity, i.e. FS (used for AC–V) or Ca^{2+} (ionomycin, used for AC–I).

As described above, AC–II belongs to a family of isozymes (together with AC–IV and VII) which, in contrast to isozymes of the AC–I/VIII or AC–V/VI families, is activatable by free $G_{\beta\gamma}$ subunits. In addition, AC–II can be stimulated by α_s-activating receptors or by a constitutively active α_s mutant, but is less sensitive to FS activation (Mons and Cooper, 1995; Sunahara et al., 1996; Avidor-Reiss et al., 1997). Stimulation of AC–II, co-transfected together with μ-opioid and TSH receptors, by 0.1 μM TSH, led to a large increase in cAMP

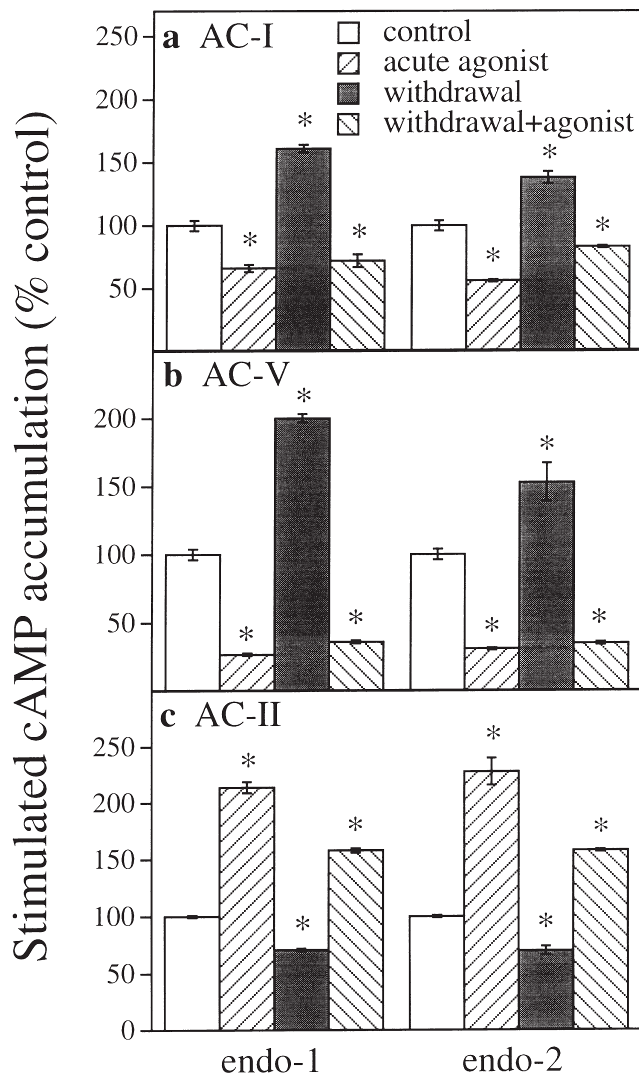

Fig. 4. Acute and chronic μ-opioid receptor activation by endo-1 or endo-2 differentially regulates the various AC isozymes transfected into COS–7 cells. COS–7 cells were transfected with the cDNAs of the indicated AC isozymes together with the cDNAs of the μ-opioid receptor and, in the case of AC–II, also of the TSH receptor. (a) AC–I was stimulated with 1 μM ionomycin, (b) AC–V was stimulated with 1 μM FS, and (c) AC–II was stimulated with 0.1 μM TSH. These stimulations increased AC activity by 2.7, 6.1 and 2.5 fold, respectively. AC activity observed under these stimulating conditions (in the absence of endomorphins) was defined as 100% (control; ▭). The figure also depicts AC activity following stimulation in the presence of acute 1 μM endo-1 or endo-2 (▨); following withdrawal (by two rapid washes) after chronic endo-1 or endo-2 (18 h, 1 μM) treatment (■); and after re-addition of either endo-1 or endo-2 (1 μM) following chronic treatment and withdrawal by two rapid washes (▧). Data represent the means±S.E. of triplicate determinations of a representative experiment. *$P<0.02$ vs control.

accumulation as compared to the unstimulated basal level (Nevo et al., 1998). In contrast to the results observed with AC–I or V, acute exposure of cells transfected with AC–II to either endo-1 or endo-2 induced a significant *increase* in AC–II activity (Fig. 4c), a result similar to the observations made earlier with several other $G_{i/o}$-coupled receptor agonists (Federman et al., 1992; Tsu et al., 1995; Avidor-Reiss et al., 1997; Nevo et al., 1998). Moreover, following withdrawal from chronic agonist treatment (by two rapid washes), not only was no superactivation of this isozyme apparent, but a reduction in the activity of AC–II was actually observed. Re-addition of the endomorphins to the cells following chronic treatment and withdrawal resulted in an elevated level of cAMP accumulation as compared to control (no chronic treatment) cells, albeit to a lesser degree than upon acute application of the ligands. It is very likely that this inhibition of AC–II upon chronic endomorphin treatment is far more complex than a simple downregulation of this isozyme's activity, but further experiments must be performed in order to attempt to understand the molecular basis for this phenomenon.

It is important to note that the phenomenon of inhibition of AC–I and V, in contrast to the acute stimulation of AC–II by acute $G_{i/o}$-coupled receptor activation, has now been observed in several cell lines and with several $G_{i/o}$-coupled receptors (Federman et al., 1992; Tsu et al., 1995; Avidor-Reiss et al. 1996, 1997; Thomas and Hoffman, 1996; Nevo et al., 1998). Moreover, the superactivation of AC–V/VI by chronic μ-opiate or D_2-dopaminergic receptor activation has been observed in both HEK-293 and COS–7 cells (Thomas and Hoffman, 1996; Avidor-Reiss et al., 1997; Nevo et al., 1998). It thus seems that this pattern of regulation is dictated by the AC isozymes involved. However, the repertoire of "free" $G_{\beta\gamma}$ and/or $G_{\alpha i/o}$ subunits present in a given cell can have an effect on the modulation of AC isozymes (see Federman et al., 1992). Indeed, we have previously shown that the $G_{\beta 1}$ subunit differs from $G_{\beta 5}$ in its capacity to regulate the activity of AC–II, as well as of AC–V and VI (Bayewitch et al., 1998a,b).

The in vivo regulation of AC activity and the resulting changes in cAMP concentration by released endomorphins are not yet known but could be quite complex, as different areas in the brain differ in their distribution of AC isozymes (Glatt and Snyder, 1993; Mons and Cooper, 1995), as well as in the concentrations of endomorphins (Martin-Schild et al., 1997; Schreff et al., 1998). However, such regulation could be important for the physiological behavior of the animal under pain, stress, and possibly other physiological conditions. Indeed, a recent report showed that chronic administration of morphine, which targets the same receptors as the endogenous endomorphins, increases levels of AC–I and VIII, as well as of cAMP-dependent protein kinase (PKA) activity in the locus coeruleus, a brain area known to be involved in drug reward pathways (Lane-Ladd et al., 1997). Other recent reports suggest that endomorphin, as a natural ligand for the μ-opioid receptor, is likely to be involved in the modulation of nociceptive transmission and reward-seeking behavior (Martin-Schild et al., 1997; Schreff et al., 1998),

Taken together, the results presented here indicate that the endogenous ligands for the μ-opioid receptor, the endomorphins, selectively regulate the various AC isozymes, with AC–I and V undergoing acute inhibition and chronic ligand-induced superactivation, while AC–II is stimulated by acute and inhibited upon chronic endomorphin treatment.

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References

Avidor-Reiss, T., Bayewitch, M., Levy, R., Matus-Leibovitch, N., Nevo, I., Vogel, Z., 1995a. Adenylylcyclase supersensitization in μ-opioid receptor-transfected Chinese hamster ovary cells following chronic opioid treatment. Journal of Biological Chemistry 270, 29732–29738.

Avidor-Reiss, T., Zippel, R., Levy, R., Saya, D., Ezra, V., Barg, J., Matus-Leibovitch, N., Vogel, Z., 1995b. κ-Opioid receptor-transfected cell lines: modulation of adenylyl cyclase activity following acute and chronic opioid treatments. FEBS Letters 361, 70–74.

Avidor-Reiss, T., Nevo, I., Levy, R., Pfeuffer, T., Vogel, Z., 1996. Chronic opioid treatment induces adenylyl cyclase V superactivation: involvement of $G_{\beta\gamma}$. Journal of Biological Chemistry 271, 21309–21315.

Avidor-Reiss, T., Nevo, I., Saya, D., Bayewitch, M., Vogel, Z., 1997. Opiate-induced adenylyl cyclase superactivation is isozyme-specific. Journal of Biological Chemistry 272, 5040–5047.

Bayewitch, M.L., Avidor-Reiss, T., Levy, R., Pfeuffer, T., Nevo, I., Simonds, W.F., Vogel, Z., 1998a. Differential modulation of adenylyl cyclases I and II by various Gβ subunits. Journal of Biological Chemistry 273, 2273–2276.

Bayewitch, M.L., Avidor-Reiss, T., Levy, R., Pfeuffer, T., Nevo, I., Simonds, W.F., Vogel, Z., 1998b. Inhibition of adenylyl cyclase isoforms V and VI by various $G_{\beta\gamma}$ subunits. FASEB Journal 12, 1019–1025.

Birnbaumer, L., Abramowitz, J., Brown, A.M., 1990. Receptor-effector coupling by G proteins. Biochemistry Biophysiology Acta 1031, 163–224.

Blake, A.D., Bot, G., Freeman, J.C., Reisine, T., 1997. Differential opioid agonist regulation of the mouse μ opioid receptor. Journal of Biological Chemistry 272, 782–790.

Burford, N.T., Tolbert, L.M., Sadee, W., 1998. Specific G protein activation and μ-opioid receptor internalization caused by morphine DAMGO and endomorphin I. European Journal of Pharmacology 342, 123–126.

Carter, B.D., Medzihradsky, F., 1993. Receptor mechanisms of opioid tolerance in SH-SY5Y human neural cells. Molecular Pharmacology 43, 465–473.

Choi, E.-J., Xia, Z., Villacres, E.C., Storm, D.R., 1993. The regulatory diversity of the mammalian adenylyl cyclases. Current Opinions in Cell Biology 5, 269–273.

Corbett, A.D., Paterson, S.J., Kosterlitz, H.W., 1993. Selectivity of ligands for opioid receptors. In: Herz, A. (Ed.), Opioids, Vol. 1. Springer-Verlag Press, Berlin, pp. 645–680.

Federman, A.D., Conklin, B.R., Schrader, K.A., Reed, R.R., Bourne, H.R., 1992. Hormonal stimulation of adenylyl cyclase through G_i-protein βγ subunits. Nature 356, 159–161.

Glatt, C.E., Snyder, S.H., 1993. Cloning and expression of an adenylyl cyclase localized to the corpus striatum. Nature 361, 536–538.

Hamprecht, B., 1977. Structural, electrophysiological, biochemical, and pharmacological properties of neuroblastoma-glioma cell hybrids in cell culture. In: Hamprecht, B. (Ed.), International Review of Cytology, Vol. 49. Academic Press, New York, pp. 99–170.

Hollt, V., 1993. Regulation of opioid peptide gene expression. In: Herz, A. (Ed.), Opioids, Vol. 1. Springer-Verlag Press, Berlin, pp. 307–346.

Lane-Ladd, S.B., Pineda, J., Boundy, V.A., Pfeuffer, T., Krupinski, J., Aghajanian, G.K., Nestler, E.J., 1997. CREB (cAMP response element-binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. Journal of Neuroscience 17, 7890–7901.

Lim, K., Chae, C.B., 1989. A simple assay for DNA transfection by incubation of the cells in culture dishes with substrates for beta-galactosidase. Biotechniques 7, 576–579.

Martin-Schild, S., Zadina, J.E., Gerall, A.A., Vigh, S., Kastin, A.J., 1997. Localization of endomorphin-2-like immunoreactivity in the rat medulla and spinal cord. Peptides 18, 1641–1649.

Matthes, H.W., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., LeMeur, M., Dolle, P., Tzavara, E., Hanoune, J., Roques, B.P., Kieffer, B.L., 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid receptor gene. Nature 383, 819–823.

McDermott, A.M., Sharp, G.W.G., 1995. Noradrenaline induces supersensitivity of adenylyl cyclase in NG108-15 and HT29-18 cells but not in the β-cell RINm5F. Cell Signal 7, 277–285.

Mons, N., Cooper, D.M.F., 1995. Adenylate cyclases: critical foci in neuronal signaling. Trends in Neuroscience 18, 536–542.

Mons, N., Harry, A., Dubourg, P., Premont, R.T., Iyengar, R., Cooper, D.M.F., 1995. Immunohistochemical localization of adenylyl-cyclase in rat-brain indicates a highly selective concentration at synapses. Proceedings of the National Acadamy of Sciences USA 92, 8473–8477.

Nestler, E.J., Hope, B.T., Widnell, K.L., 1993. Drug addiction: a model for the molecular basis of neural plasticity. Neuron 11, 995–1006.

Nevo, I., Avidor-Reiss, T., Levy, R., Bayewitch, M., Heldman, E., Vogel, Z., 1998. Regulation of adenylyl cyclase isozymes upon acute and chronic activation of inhibitory receptors. Molecular Pharmacology 54, 419–426.

Parsons, W.J., Stiles, G.L., 1987. Heterologous desensitization of the inhibitory A_1 adenosine receptor-adenylate cyclase system in rat adipocytes. Journal of Biological Chemistry 262, 841–847.

Reisine, T., Bell, G.I., 1993. Molecular biology of opioid receptors. Trends in Neuroscience 16, 506–510.

Rhee, M.-H., Avidor-Reiss, T., Vogel, Z., 1998a. Adenylyl cyclase regulation by acute and chronic activation of the CB1 cannabinoid receptor. Neuroscience Letters S51, S34.

Rhee, M.H., Bayewitch, M., Avidor-Reiss, T., Levy, R., Vogel, Z., 1998b. Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. Journal of Neurochemistry 71, 1525–1534.

Salomon, Y., 1991. Cellular responsiveness to hormones and neuro-

transmitters: conversion of [^{3}H]adenine to [^{3}H]cAMP in cell monolayers, cell suspensions and tissue slices. Methods in Enzymology 195, 22–28.
Schreff, M., Schulz, S., Wiborny, D., Hollt, V., 1998. Immunofluorescent identification of endomorphin-2-containing nerve fibers and terminals in the rat brain and spinal cord. NeuroReport 9, 1031–1034.
Sharma, S.K., Klee, W.A., Nirenberg, M., 1975. Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. Proceedings in the National Acadamy of Science USA 72, 3092–3096.
Sharma, S.K., Klee, W.A., Nirenberg, M., 1977. Opiate-dependent modulation of adenylate cyclase. Proceedings of the National Academy of Sciences USA 74, 3365–3369.
Sunahara, R.K., Dessauer, C.W., Gilman, A.G., 1996. Complexity and diversity of mammalian adenylyl cyclases. Annual Review of Pharmacological Toxicology 36, 461–480.
Thomas, J.M., Hoffman, B.B., 1987. Adenylate cyclase supersensitivity: a general means of cellular adaptation to inhibitory agonists? Trends in Pharmacological Science 8, 308–311.
Thomas, J.M., Hoffman, B.B., 1992. Adaptive increase in adenylyl cyclase activity in NG108-15 and S49 cells induced by chronic treatment with inhibitory drugs is not due to a decrease in cyclic AMP concentrations. Cell Signal 4, 417–428.
Thomas, J.M., Hoffman, B.B., 1996. Isoform-specific sensitization of adenylyl cyclase activity by prior activation of inhibitory receptors: role of βγ subunits in transducing enhanced activity of the type VI isoform. Molecular Pharmacology 49, 907–914.
Tsu, R.C., Chan, J.S.C., Wong, Y.H., 1995. Regulation of multiple effectors by the cloned δ-opioid receptor: stimulation of phospholipase C and type II adenylyl cyclase. Journal of Neurochemistry 64, 2700–2707.
Uhl, G.R., Childers, S., Pasternak, G., 1994. An opiate-receptor gene family reunion. Trends in Neuroscience 17, 89–93.
Zadina, J.E., Hackler, L., Ge, L.J., Kastin, A.J., 1997. A potent and selective endogenous agonist for the μ-opiate receptor. Nature 386, 499–502.