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Opioid Tolerance and the Emergence of New Opioid Receptor-Coupled Signaling

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Abstract

Multiple cellular adaptations are elicited by chronic exposure to opioids. These include diminution of spare opioid receptors, decreased opioid receptor density, and G-protein content and coupling thereof. All imply that opioid tolerance is a manifestation of a loss of opioid function, i.e., desensitization. Recent observations challenge the exclusiveness of this formulation and indicate that opioid tolerance also results from qualitative changes in opioid signaling. In this article, Gintzler and Chakrabarti discuss the evidence that suggests that opioid tolerance results not only from impaired opioid receptor functionality, but also from altered consequences of coupling. Underlying the latter are fundamental changes in the nature of effectors that are coupled to the opioid receptor/G-protein signaling pathway. These molecular changes include the upregulation of adenylyl cyclase isoforms of the type II family as well as a substantial increase in their phosphorylation state. As a result, there is a shift in opioid receptor/G-protein signaling from predominantly $G_{i\alpha}$ inhibitory to $G_{\beta\gamma}$ stimulatory following chronic in vivo morphine exposure. These adaptations to chronic morphine indicate the plasticity of opioid-signal transduction mechanisms and the ability of chronic morphine to augment new signaling strategies.

Index Entries: Opioid; tolerance; adenylyl cyclase; phosphorylation; plasticity; isoforms.

Introduction

A major complicating factor in the medicinal use of narcotics is their diminished pharmacological effectiveness following persistent administration. As a result of tolerance formation, it is not uncommon for the antinociceptive effectiveness of opioids to be reduced by an order of magnitude or more. Thus, responsiveness to opioids includes initial perturbations that are followed by the re-establishment of "normal" physiological equilibrium despite its continued presence. The neurochemical perturbations that underlie opioid tolerance (e.g.,

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alterations in receptors, G proteins, and signaling enzymes) represent potential targets for pharmacotherapies that could be used in combination with narcotics for the treatment of chronic pain.

One predominant focus of research in the opioid field has been the elucidation of the cellular events that underlie this re-establishment of homeostasis following chronic morphine. On a phenomenological level, tolerance to opioids is probably better characterized than any other manifestation of attenuated drug responsiveness. Nevertheless, despite the delineation of many of the physiological, biochemical, and molecular biological sequela of persistent exposure to opioids, elucidation of the cellular events that are causally associated with the re-establishment of homeostasis following chronic morphine remains incomplete. Opioid tolerance is always defined relative to a specific opioid action such as analgesia, sedation, and respiratory depression, which often manifest differential tolerance development. This could suggest that different opioid functions are mediated via different signaling strategies that activate different tolerance-forming mechanisms. Thus, the generality of any model of opioid tolerance should be inferred with appropriate caution.

Acute Opioid Signaling

Inhibitory

In animals not previously exposed to opioids (naive condition), acute neuronal responsiveness to opioids is predominantly inhibitory (Duggan and North, 1984; North, 1989; Keren et al., 1997). Activation of all the major types of opioid receptor (μ, δ, κ) inhibits transmitter release and slows cell firing. These inhibitory effects are attributed to the ability of opioids to hyperpolarize nerve cells via either an increase in specific membrane K^+ conductances (North, 1989; Ikeda et al., 1995) or the inhibition of voltage-gated Ca^{2+} channels) (Gross and Macdonald, 1987; Tallent et al., 1994). Because transmitter release is dependent on intracellular calcium concentrations, which are enhanced by depolarization and decreased by hyperpolarization, these opioid actions are believed to underlie the opioid inhibition of excitatory neurotransmission (e.g., via substance P and glutamate) and thereby produce their well-known antinociceptive effects [see Leslie (1987) for review].

Stimulatory

In addition to the well-documented inhibitory actions of opioids, these substances also stimulate neuronal transmission. Such action includes an increase in the rate of neuronal firing (Lin and Carpenter, 1994), enhancement of Ca^{2++}-dependent responses in visceral primary afferent neurons (Higashi et al., 1982), presynaptic facilitation in sympathetic ganglion (Hirai and Katayama, 1988), calcium channel activation (Lorentz et al., 1988), as well as action potential prolongation (Shen and Crain, 1989). As would be expected based on these electrophysiological findings, opioids have also been shown to enhance the evoked in vivo release of several neurotransmitters. These include dopamine (Devine et al., 1993), acetylcholine (Beani et al., 1982; Neal et al., 1994), substance P (Mauborgne et al., 1987), methionine-enkephalin (Xu et al., 1989; Xu and Gintzler, 1992) and adenosine (Sweeney et al., 1989). Analogous effects have been demonstrated in cells maintained in culture or synaptosomal preparations where opioids have been observed to stimulate the release of substance P, cholecystokinin, and adenosine (Bourgoin et al., 1994; Cahill et al., 1995).

Blockade of inhibitory synaptic action does not necessarily alter opioid stimulatory activity (Lin and Carpenter, 1994; Neal et al., 1994). This suggests the presence of mechanisms in addition to disinhibition that mediate opioid enhancement of transmitter release. In most cases, opioid bimodality exhibits concentration dependence, i.e., usually the concentration of opioid required for excitatory actions (n*M*) is lower than that required for inhibitory effec-

tiveness (Shen and Crain, 1989; Xu et al., 1989; Devine et al., 1993; Bourgoin et al., 1994). However, excitatory actions produced by μ*M* opioid have also been reported (Bourgoin et al., 1994; Cahill et al., 1995). To date, there is no evidence for the existence of separate excitatory and inhibitory opioid receptors. Instead, pharmacological experiments indicate that opioid excitation vs inhibition occurs via the differential coupling of opioid receptors to G_s- and G_i-like G proteins, respectively.

Opioid Receptor-G Protein Coupling

Opioid receptors belong to the superfamily of guanine nucleotide-binding protein (G protein) coupled receptors. Such receptors consist of seven transmembrane-spanning domains, an N-terminus extracellular domain, and a C-terminus intracellular domain. G protein-coupled cell surface receptors do not interact directly with their final effector molecules but communicate indirectly via a variety of G proteins. In several cell lines, opioid receptors are known to couple simultaneously to multiple G proteins (Roerig et al., 1992; Laugwitz and Offermanns, 1993; Prather et al., 1994; Chakrabarti et al., 1995). All physiological actions of opioids are thought to result from their ability to activate G proteins that in turn directly modulate channel activity and/or the formation of second messengers such as cyclic adenosine 5′-monophosphate (cAMP), inositol tris phosphate (IP_3), and so forth. Opioid modulation of second messenger concentration is thought to occur via modulation of the activity of key signaling enzymes such as adenylyl cyclase (AC), phospholipase C, etc (Childers, 1991).

Second Messenger Modulation

A predominant consequence of opioid receptor activation is the inhibition of second messenger formation. Many central opioid effects are thought to be consequences of reduced formation of cAMP [*see* Loh and Smith (1990) for review]. However, there is growing evidence for opioid receptor-coupled stimulation of second messenger accumulation.

In guinea pig longitudinal muscle myenteric plexus (LMMP) tissue, nanomolar and micromolar concentrations of μ, δ and κ-opioids stimulate and inhibit, respectively, cAMP formation (Wang and Gintzler, 1994) in rat olfactory bulb, stimulation of cAMP accumulation is the predominant response to μ- and δ-opioids (Olianas and Onali, 1993). In this tissue, activation of opioid receptors enhances basal AC activity and potentiates its stimulation by G_s-coupled neurotransmitter receptors. These excitatory opioid actions are most likely mediated by $G_{\beta\gamma}$ subunits released from G_i/G_o G proteins since they can be greatly attenuated by scavengers of $G_{\beta\gamma}$ (Olianas and Onali, 1999). Analogous findings have been reported in Xenopus oocytes or human embryonic kidney (HEK) cells transfected with κ- or δ-opioid receptors, respectively (Kaneko et al., 1994; Tsu et al., 1995). It should be noted that opioid stimulation of cAMP levels in HEK cells is dependent on the overexpression of AC type II (Tsu et al., 1995). In addition to modulating AC/cAMP signal transduction systems, opioids also transiently stimulate intracellular calcium concentrations (Jin et al., 1992; Eriksson et al., 1993; Jin et al., 1994; Tang et al., 1994; Sarne et al., 1996) and phospholipase C activity (Johnson et al., 1994; Smart et al., 1994, 1995), the latter resulting in increased concentrations of IP_3 (Tsu et al., 1995; Smart and Lambert, 1996). In several systems, stimulatory opioid effects are mediated via a G_s-coupled mechanism(s) (Shen and Crain, 1990; Gintzler and Xu, 1991; Wang and Gintzler, 1997).

Opioid Tolerance

Tolerance to opioid inhibitory signaling is thought to result from multiple cellular adaptations. These include the diminution of spare opioid receptors (Chavkin and Goldstein, 1984), altered opioid receptor density (Chakrabarti et al., 1995), and altered G-protein content (Ammer

and Schulz, 1993). Additionally, studies utilizing GTPγS^{35} binding (that reflect the exchange of GTP for GDP on the heterotrimeric G-protein and thus its activation) have demonstrated decreased opioid receptor G-protein coupling following chronic systemic morphine (Sim et al., 1996). All of these putative mechanisms derive from the formulation that opioid tolerance is a manifestation of a loss of opioid function, i.e., desensitization.

In several cell lines, abrupt removal of morphine from chronic morphine-treated cells reveals a robust upregulation of AC activity. This is often referred to as AC superactivation (Sharma et al., 1975, 1977; Avidor-Reiss et al., 1995). This phenomenon has also been observed in the locus coeruleus, where chronic morphine administration has been associated with an increase in basal as well as forskolin-stimulated AC activity (Duman et al., 1988).

AC superactivation is the immediate increase in AC activity that is manifest upon the acute withdrawal of an opioid after chronic opioid treatment. Its manifestation is often interpreted to indicate the presence of enhanced AC activity in chronic morphine-treated cells prior to agonist withdrawal (although this inference has never been unequivocally demonstrated). Thus, it was postulated that the re-establishment of "normal" levels of cyclase activity in chronic morphine-treated cells results from an upregulation of cyclase activity that counter balances the inhibitory influence of the continued presence of morphine. Importantly, the marked increase in AC activity that occurs following the acute removal of morphine following prolonged exposure indicates that morphine still inhibits AC activity (Sharma et al., 1975). This underscores that opioid receptor desensitization, regardless of the underlying mechanism(s), cannot be the exclusive explanation for opioid tolerance.

Although AC superactivation may contribute to the overall manifestation of opioid tolerance, it is unlikely to be the sole mechanism underlying tolerance. This is indicated by reports that AC superactivation is not uniformly observed in all brain regions. For example, in rats, chronic in vivo administration of morphine increased basal, G-protein, and forskolin-stimulated adenylyl cyclase activity in the locus coeruleus but not in the dorsal raphe, frontal cortex, or neostriatum (Duman et al., 1988). Moreover, even in the locus coeruleus, the chronic morphine-induced increment in basal and stimulated AC activity (~30%) (Duman et al., 1988) is considerably smaller than that manifest in cell lines maintained in culture (~75%) (Sharma et al., 1975). Furthermore, it is now known that there exists a multiplicity of AC isoforms, some of which do not manifest superactivation. Indeed, those of the type II AC family (AC II, IV, VII) manifest *reduced* activity following chronic morphine exposure and its abrupt withdrawal (Avidor-Reiss et al., 1997).

Chronic Morphine Induction of New Signaling Pathways

Recent observations indicate that opioid tolerance is not exclusively the result of quantitative changes in opioid signaling. Qualitative changes in opioid signaling also contribute to its formation. In other words, mounting evidence suggests that opioid tolerance results not only from impaired opioid receptor coupling but also from altered consequences of coupling. Underlying the latter are fundamental changes in the nature of effectors that are coupled to the opioid receptor-G protein-signaling pathway.

Altered, but not lost, opioid receptor-coupled signaling following persistent opioid-receptor activation was inferred from a comparison of the effects of morphine on transmitter release from opioid naive vs tolerant/dependent preparations. In the former, morphine substantially inhibits the electrically evoked release of methionine enkephalin. Paradoxically, however, in tolerant/dependent preparations, morphine is a prerequisite for such release (Gintzler et al., 1987). In other words, following chronic morphine, the loss of

opioid inhibition is accompanied by the manifestation of a facilitative action. This suggested a switch from opioid receptor-coupled inhibitory to excitatory signaling.

This formulation is supported by the results of experiments that directly assessed the effect of chronic in vivo morphine on opioid regulation of cAMP formation. The μ-selective opioid receptor agonist, sufentanil, can either increase or decrease the electrically stimulated formation of cAMP in the myenteric plexus. The direction of the opioid modulation of this second messenger depends on the concentration of opioid employed. Enhancement and inhibition of the magnitude of cAMP that is formed in response to electrical stimulation is observed with low (n*M*) and high (μ*M*) concentrations of opioid (Wang and Gintzler, 1994). As would be expected, following chronic in vivo morphine exposure, high-dose opioid inhibition of electrically stimulated cAMP formation manifests tolerance. This is evidenced by the fact that the magnitude of electrically stimulated cAMP formation in opioid naive preparations is indistinguishable from that which occurs in chronic morphine-treated preparations maintained in the presence of inhibitory (μ*M*) concentration of sufentanil (Wang and Gintzler, 1995).

This manifestation of tolerance, however, does not result from the loss of opioid-signaling capability since in chronic morphine-treated LMMP tissue, there is not only a (paradoxical) increase in the content of $G_{i\alpha}$ (Lang and Schulz, 1989) but in its coupling to opioid receptors as well (Wang and Gintzler, 1997). Furthermore, in LMMP tissue that had been treated in vivo chronically with morphine and abruptly withdrawn, the electrically stimulated formation of cAMP was significantly compromised (Wang and Gintzler, 1995). Analogous findings were subsequently demonstrated in COS-7 cells transfected with AC type II, IV, and VII (Avidor-Reiss et al., 1997). Thus, the "normal" response observed in tolerant/dependent LMMP tissue, while in the presence of sufentanil, must result from its ability to restore stimulatory responsiveness of AC. In other words, in the LMMP tissue, chronic exposure to morphine results in the acquisition (manifestation) of a qualitatively opposite response to opioids. One adaptive consequence of this qualitative shift in opioid responsiveness is that the ability to stimulate cAMP formation would be maintained despite the compromised functionality of AC (isoforms II, IV, VII) that results from persistent opioid receptor stimulation. Such adaptations could underlie the permissive effect of morphine on transmitter (methionine-enkephalin) release in chronic morphine-treated tissue (Gintzler et al., 1987).

Predominance of Facilitative Opioid Effects Does Not Result From a Shift from $G_{i\alpha}$ to $G_{s\alpha}$ Signaling

Plasticity of G protein-coupled receptor signaling following persistent agonist exposure is not unique to the opioid receptor. For example, sustained activation of the β_2-adrenergic receptor shifts signaling of this receptor from the $G_{s\alpha}$-protein kinase A pathway to the $G_{\beta\gamma}$-(G_i-derived) mitogen-activated protein kinase pathway (Daaka et al., 1997). In contrast, although chronic morphine exposure reveals an analogous plasticity of opioid receptor-coupled signaling, it dose not result from a shift in the G protein through which opioid receptor-coupled signaling occurs (G_i–G_s) but rather from the altered consequences of that coupling.

As has been reported for the α_2-adrenergic receptor (Eason et al., 1992), in opioid naive myenteric plexus, G_s- and G_i-like G proteins mediate positive and negative sufentanil modulation of stimulated transmitter release and cAMP formation, respectively (Gintzler and Xu, 1991; Wang and Gintzler, 1997). However, the chronic morphine-induced shift from opioid inhibition to facilitation results neither from the loss of inhibitory, G_i-mediated, responsiveness (such as that which occurs fol-

lowing treatment with pertussis toxin), nor from an augmentation of the opioid-facilitative G_s pathway (Wang and Gintzler, 1997). Instead, molecular changes that underlie the shift from opioid receptor-coupled inhibitory to stimulatory signaling reflect the plasticity of opioid signal-transduction mechanisms and the ability of chronic morphine to augment new signaling strategies.

Plasticity of the consequences of opioid-receptor G_i coupling was initially inferred based on two demonstrations. Although the G_s-mediated, low-dose μ-opioid facilitative pathway is augmented in tolerant/dependent preparations, it is not related to tolerant-associated reversal of high-dose sufentanil inhibition to enhancement, because the former is abolished by cholera toxin, whereas the latter is not (Wang and Gintzler, 1997). Moreover, in chronic morphine-treated LMMP tissue, despite the shift from inhibitory to excitatory opioid modulation, there appears to be a paradoxical augmentation of μ-opioid receptor coupling to the inhibitory (G_i mediated) opioid pathway. This finding is consistent with (a) the subsequent demonstration (Ingram, 1998) of enhanced efficacy of coupling of μ-opioid receptors and inhibition of GABAergic evoked inhibitory postsynaptic potentials in periaquiductal gray neurons following in vivo chronic morphine; and (b) the seemingly contradictory observation (Lang and Schulz, 1989) that the LMMP content of G_α is elevated following chronic in vivo morphine exposure, despite the loss of opioid receptor-coupled inhibitory signaling. Thus, despite the numerous demonstrations of an opioid stimulatory G_s-signaling pathway, it seems unlikely that its augmentation along with the diminution of the inhibitory (G_i) pathway contributes to the tolerant-associated predominance of sufentanil facilitative effects. In other words, in tolerant/dependent tissue, the predominance of opioid excitatory responses is, most likely, not mediated via an alteration of the balance between facilitative and inhibitory mechanisms that are operative in opioid-naive tissue.

Chronic Morphine Alters Consequences of Opioid Receptor G_i-AC Coupling

The earlier observations point to the chronic morphine-induced emergence of a μ-opioid receptor-coupled facilitative pathway. This pathway is either not expressed or not fully manifest in opioid-naive LMMP tissue. Recent reports indicate the relevance of opioid receptor-coupled $G_{\beta\gamma}$ stimulatory AC signaling to opioid tolerant/dependent mechanisms.

It is now well-established that the relative preponderance of specific AC isoforms are critical determinants of excitatory vs inhibitory AC responsiveness (Federman et al., 1992; Tang and Gilman, 1991, 1992). Specifically, the relative abundance of specific AC isoforms can influence the directionality of opioid regulation thereof (Yoshimura et al., 1996; Avidor-Reiss et al., 1997). Moreover, chronic morphine can influence the content of AC in an apparently isoform-specific fashion.

Chronic Morphine Alters the Relative Abundance of AC Isoforms

Following chronic systemic morphine, mRNA encoding AC IV (Rivera and Gintzler, 1998) and AC VII (Wang, Chakrabarti, and Gintzler, unpublished observations) are significantly elevated in LMMP tissue. Additionally, as expected, AC protein is also significantly augmented (56%) in these preparations (Chakrabarti et al., 1998a). Given the isoform specificity of the monoclonal AC antibody employed in the Western-blot analysis (AC II, IV, VII, and I), in combination with changes or lack thereof in isoform-specific mRNA, this increment is, most likely, comprised of AC isoforms IV and/or VII. Both isoforms are stimulated by $G_{\beta\gamma}$ (Gao and Gilman, 1991; Watson et al., 1994; Yoshimura et al., 1996) (*See* Table 1 for summary of of chronic morphine-induced AC isoform changes and $G_{\beta\gamma}$ stimulatory responsiveness).

Table 1
Effect of Chronic Systemic Morphine on the Content of mRNA Encoding AC Isoforms[a]

AC isoforms	AC mRNA/total RNA (pg/μg ± SEM)		$G_{\beta\gamma}$ Regulation	Effect of PKC on $G_{s\alpha}/G_{\beta\gamma}$ stimulation
	Opioid naive	Chronic morphine		
AC I	0.64 ± 0.14 (4)	0.71 ± 0.13 (4)	Inhibitory	None
AC II	0.93 ± 0.16 (4)	1.0 ± 0.1 (4)	Stimulatory	Facilitation
AC IV	0.49 ± 0.04 (4)	0.67 ± 0.04*(4)	Stimulatory	Inhibition
AC VII	0.52 ± 0.06 (4)	0.66 ± .07* (4)	Stimulatory	Facilitation

[a] The content of AC isoform-specific mRNA in opioid naive and tolerant/dependent tissue was quantified as previously described (Rivera and Gintzler, 1998) using quantitative solution hybridization. Chronic treatment with morphine was accomplished via the subcutaneous implantation of 5 morphine pellets for 6 d. Chronic systemic morphine increases the myenteric content of mRNA encoding AC IV and VII. This suggests that these isoforms comprise the ~56% increase in AC protein that has been observed in these preparations. *See* Sunahara et al., (1995) for review of AC isoform-specific regulation by $G_{\beta\gamma}$ and PKC.

*$p > 0.05$.

Chronic Morphine Augments AC Phosphorylation

Persistent activation of opioid receptors also has profound effects on the phosphorylation state of AC. A dramatic increase in phosphorylation of the type II family of AC was observed in LMMP preparations obtained from chronic morphine-treated guinea pigs (Chakrabarti et al., 1998b). Moreover, the magnitude of the chronic morphine-induced augmented AC phosphorylation is substantially attenuated by chelerythrine, a protein kinase C (PKC)-selective inhibitor, pretreatment with which also blocks the chronic morphine-induced reversal of opioid receptor-coupled inhibition to stimulation and reinstates the former (Wang et al., 1996). This underscores the potential relevance of increased (PKC-mediated) phosphorylation of AC to opioid tolerant/dependent mechanisms. This would be consistent with the ability of chronic systemic morphine to increase the content of the α and β isoforms of PKC in guinea pig LMMP tissue (Wang et al., 1996) as well as the membrane-bound (translocated) content of the γ isoform of PKC in rat spinal cord (Mao et al., 1995; Mayer et al., 1995).

Augmented phosphorylation of certain AC isoforms has substantial functional implications. For example, phosphorylated AC II and VII manifest increased stimulatory responsiveness to $G_{s\alpha}$ (Jacobowitz and Iyengar, 1994; Watson et al., 1994; Zimmerman and Taussig, 1996). Moreover, $G_{s\alpha}$-independent $G_{\beta\gamma}$ stimulation of AC II is augmented following its phosphorylation (Isu and Wong, 1996; Zimmerman and Taussig, 1996).

Functional Consequences of AC Isoform-Specific Synthesis and Phosphorylation

Upregulation of AC isoforms IV and VII would have physiologic consequences complimentary to those produced by augmented phosphorylation of AC II and VII. Both, in combination, would be expected to result in a shift in receptor/G protein signaling from predominantly $G_{i\alpha}$ inhibitory to $G_{\beta\gamma}$ stimulatory. Indeed, it has recently been demonstrated that (activated) recombinant $G_{s\alpha}$ dose dependently stimulates AC activity in LMMP membranes obtained from opioid-naive as well as tolerant LMMP tissue. However, the magnitude of effect in the latter is significantly greater than that observed in the former (Chakrabarti et al., 1998a). Moreover,

Table 2
Stimulatory Responsiveness of Membranes Obtained from Opioid Naive and Tolerant/Dependent Myenteric Plexus Tissue to Recombinant $G_{s\alpha}$ and its Alteration Following $G_{\beta\gamma}$ Innactivation[a]

	Stimulation of AC activity	
Modulatory agents	Opioid naive	Morphine tolerance
rGsα[b]	+++	+++++
rGsα + QEHA	+	+
rGsα + SKEE	+++	+++++
QEHA	NS	NS
SKEE	NS	NS

[a] Chronic treatment with morphine was accomplished as described for Table 1. $G_{\beta\gamma}$ innactivation was accomplished by pretreatment with the $G_{\beta\gamma}$ blocking peptide QEHA (20 μ*M*). This peptide has the sequence corresponding to residues 956–982 of AC II (Chen et al., 1995). SKEE is the inactive peptide whose sequence corresponds to the same residues of AC III, an AC isoform not stimulated by $G_{\beta\gamma}$ AC activity was determined by measuring the synthesis of [^{32}P]cAMP from [α-^{32}P]ATP (Chakrabarti et al., 1998a).

[b] $rG_{s\alpha}$ is recombinant $G_{s\alpha}$ activated using GTPγS. NS, no significant change ($p < 0.5$) in AC activity. The magnitude by which $rG_{s\alpha}$ stimulates AC activity is much greater (+++++) in chronic morphine-treated myenteric membranes than in opioid-naive preparations (+++). QEHA reduced the stimulatory activity in both (+) and eliminated the differential stimulatory responsiveness. This indicates that augmented AC stimulation by $rG_{s\alpha}$ following chronic morphine results from increased $G_{s\alpha}$-dependent $G_{\beta\gamma}$ AC stimulation.

sub-optimum concentrations of the $G_{\beta\gamma}$-blocking peptide QEHA (Chen et al., 1995) abolishes the incremental AC stimulation by recombinant $G_{s\alpha}$ in tolerant LMMP membranes, indicating augmented $G_{\beta\gamma}$ stimulatory AC responsiveness in these preparations (*see* Table 2 and Fig. 1). These biochemical changes could underlie the previously demonstrated shift from high-dose opioid inhibition to stimulation of AC activity (Wang and Gintzler, 1995, 1997) and transmitter release (Gintzler et al., 1987). Chronic morphine-induced (PKC-mediated) phosphorylation of AC II and/or AC VII and the attendant increased stimulatory AC responsiveness to $G_{s\alpha}$ could also underlie the previously reported ability of chronic morphine treatment to markedly enhance low-dose sufentanil facilitation of stimulatory AC responsiveness (Wang and Gintzler, 1997).

In essence, the demonstrated shift from opioid receptor-coupled $G_{i\alpha}$ inhibitory to $G_{\beta\gamma}$ stimulatory AC signaling represents the mirror image of the superactivation formulation that heretofore has pervaded models of opioid tolerance. As was demonstrated by Tsu et al. (1995), ACs of the type II family (AC II) are stimulated by an opioid receptor G_i-coupled mechanism, as are ACs in the olfactory bulb (Olinas and Onali, 1999). Thus, one could envision that persistent exposure to opioids could result in sustained activation of AC isoforms that comprise the type II family (as opposed to inhibition of other isoforms). Adaptation to this would result in its attenuated activity in order to maintain normative levels of cAMP. Thus, just as the abrupt removal of opioid following chronic treatment results in the superactivation (overshoot) of AC isoforms I, V, VI, and VIII (Avidor-Reiss et

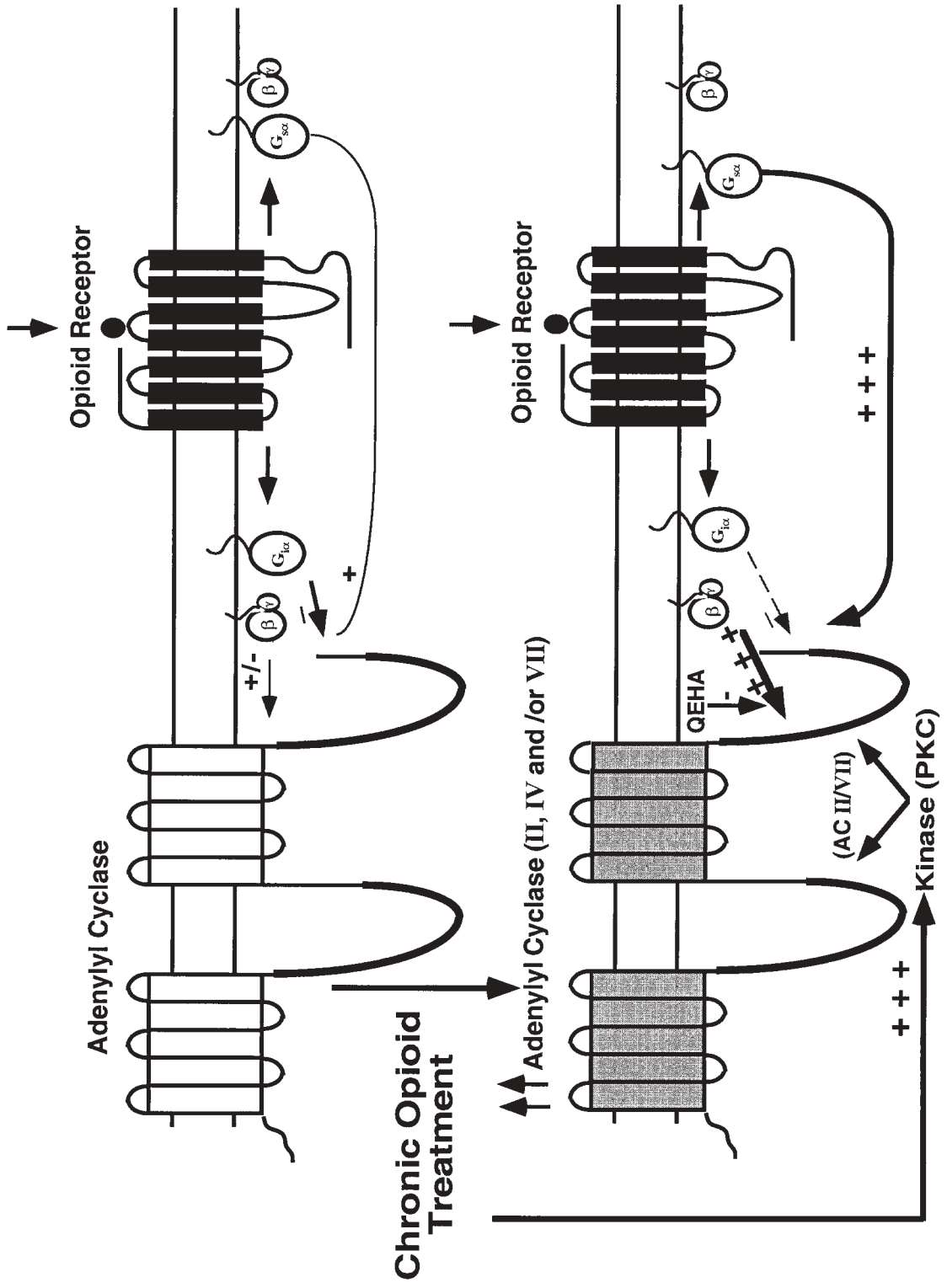

Fig. 1. Altered Opiod Receptor Signaling after Chronic Opioid treatment.

al., 1997), it would be expected to result in the decreased activity of AC II, IV, and VII. Indeed, AC activity has been shown to decrease in the LMMP tissue following chronic morphine treatment and its abrupt withdrawal (Wang and Gintzler, 1995), which was also recently demonstrated in cos-7 cells transiently transfected with ACs of the type II family (Avidor-Reiss et al., 1997). AC isoform-specific stimulation (or inhibition) by opioids would thus restore AC activity to opioid-naive levels, resulting in the appearance of opioid tolerance. Alternatively, or additionally, the persistent opioid inhibition of AC I, V, VI, and VIII via the generation of $G_{i\alpha}$ would be mitigated by increasing the concentration and phosphorylation state of AC isoforms of the type II family, whose activity would be stimulated by persistent opioid receptor activation of G_i/G_o (via the generation of $G_{\beta\gamma}$). The differential adaptation of AC isoforms to chronic morphine could explain why AC superactivation is either absent from or only modestly manifest in most brain regions.

It is important to note that this formulation of opioid tolerance does not necessarily negate the relevance of previously proposed mechanisms underlying tolerance, e.g., receptor uncoupling (Sim et al., 1996), desensitization of μ-opioid receptor coupled potassium channels (Kovoor et al., 1995), but is complementary to them. Although $GTP_{\gamma}S^{35}$ binding in response to opioid agonist challenge does significantly decrease in tolerant tissue, this decrement is not manifest in all brain regions (Sim et al., 1996). Furthermore, the effector systems associated with this uncoupling have not been unequivocally identified and undoubtedly there will be variability in the extent to which the activity of specific transduction mechanisms is diminished as a consequence of chronic morphine. For example, morphine dependence is associated with increased coupling of μ-opioid receptors to presynaptic inhibition in GABAergic nerve terminals, but not glutamatergic nerve terminals, via AC- and protein kinase A-dependent processes in the periaqueductal gray (Ingram et al., 1998).

Given the plethora of G protein (G_i) coupled receptors that signal via AC, upregulation of $G_{\beta\gamma}$-stimulated AC isoforms in combination with covalent modifications thereof (e.g. phosphorylation) that increase $G_{\beta\gamma}$-stimulated responsiveness would be expected to have very wide-ranging physiological consequences, beyond those directly under the influence of opioids. Thus, one consequence of chronic morphine would be the upregulation of divergent receptor-coupled stimulatory AC signaling. The chronic morphine-induced shift from predominantly $G_{i\alpha}$ AC inhibitory to $G_{\beta\gamma}$ AC stimulatory signaling provides a mechanism for the well-documented heterologous consequences of chronic morphine exposure.

References

Ammer H. and Schulz R. (1993) Alterations of the expression of G proteins and regulation of adenylate cyclase in human neuroblastoma SH-SY5Y cells chronically exposed to low-efficacy mu-opioids. *Biochem. J.* **295,** 263–271.

Avidor-Reiss T., Bayewitch M., Levy R., Matus-Leibovitch N., Nevo I., and Vogel Z. (1995) Adenylyl cyclase supersensitization in mu-opioid receptor-transfected Chinese hamster ovary cells following chronic opioid treatment. *J. Biol. Chem.* **270,** 29,732–29,738.

Avidor-Reiss T., Nevo I., Saya D., Bayewitch M., and Vogel Z. (1997) Opiate-induced adenylyl cyclase superactivation is isozyme-specific. *J. Biol. Chem.* **272(8),** 5040–5047.

Beani L., Bianchi C., and Siniscalchi A. (1982) The effect of naloxone on opioid-induced inhibition and facilitation of acetylcholine release in brain slices. *Br. J. Pharmacol.* **76,** 393–401.

Bourgoin S., Benoliel J. J., Collin E., Mauborgne A., Pohl M., et al. (1994) Opioidergic control of the spinal release of neuropeptides. Possible significance for the analgesic effects of opioids. *Fundam. Clin. Pharmacol.* **8,** 307–321.

Cahill C. M., White T. D., and Sawynok J. (1995) Spinal opioid receptors and adenosine release: neurochemical and behavioral characterization of opioid subtypes. *J. Pharmacol. Exp. Ther.* **275,** 84–93.

Chakrabarti S., Prather P. L., Yu L., Law P.-Y., and Loh H. H. (1995a) Expression of the μ-opioid

receptor in CHO cells: ability of μ-opioid ligands to promote α-azidoanilido[^{32}P]GTP labeling of multiple G protein α subunits. *J. Neurochem.* **64,** 2534–2543.

Chakrabarti S., Law P.-Y., and Loh H. H. (1995b) Neuroblastoma neuro$_{2A}$ cells stably expressing a cloned μ-opioid receptor: a specific cellular model to study acute and chronic effects of morphine. *Mol. Brain Res.* **30,** 269–278.

Chakrabarti S., Rivera M., Yan S.-Z., Tang W.-J., and Gintzler A. R. (1998a) Chronic morphine augments $G_{\beta\gamma}/G_{S\alpha}$ stimulation of adenylyl cyclase: relevance to opioid tolerance. *Mol. Pharmacol.* **54,** 655–662.

Chakrabarti S., Wang L., Tang W.-J., and Gintzler A. R. (1998b) Chronic morphine augments adenylyl cyclase phosphorylation: relevance to altered signaling during tolerance/dependence. *Mol. Pharmacol.* **54,** 949–953.

Chavkin C. and Goldstein A. (1984) Opioid receptor reserve in normal and morphine-tolerant guinea pig ileum myenteric plexus. *Proc. Natl. Acad. Sci. USA* **81(22),** 7253–7257.

Chen J., DeVivo M., Dingus J., Harry A., Li J et al. (1995) A region of adenylyl cyclase 2 critical for regulation by G protein βγ subunits. *Science* **268,** 1166–1169.

Childers S. R. (1991) Opioid receptor-coupled second messenger systems. *Life Sci.* **48,** 1991–2003.

Daaka Y., Luttrell L. M., and Lefkowitz R. J. (1997) Switching of the coupling of the β_2-adrenergic receptor to different G proteins by protein kinase A. *Nature* **390,** 88–91.

Devine D. P., Leone P., Pocock D., and Wise R. A. (1993) Differential involvement of ventral tegmental mu, delta and kappa opioid receptors in modulation of basal mesolimbic dopamine release: in vivo microdialysis studies. *J. Pharmacol. Exp. Ther.* **266,** 1236–1246.

Duggan A. W. and North R. A. (1984) Electrophysiology of opioids. *Pharmacol. Rev.* **35,** 219–281.

Duman R. S., Tallman J. F., and Nestler E. J. (1988) Acute and chronic opiate-receptor regulation of adenylate cyclase in brain: specific effects in locus coerulus. *J. Pharmacol. Exp. Ther.* **246(3),** 1033–1039.

Eason M. G., Kuroses H., Holt B. D., Raymond J. R., and Liggett S. B. (1992) Simultaneous coupling of α_2-adrenergic receptors to two G-proteins with opposing effects. *J. Biol. Chem.* **267(22),** 15,795–15,801.

Eriksson P. S., Nilsson M., Wagberg M., Hansson E., and Ronnback L. (1993) Kappa-opioid receptors on astrocytes stimulate L-type Ca^{2+} channels. *Neuroscience* **54,** 401–407.

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

Gao B. and Gilman A. G. (1991) Cloning and expression of a widely distributed (type IV) adenylyl cyclase. *Proc. Natl. Acad. Sci. USA* **88,** 10,178–10,182.

Gintzler A. R., Chan W. C., and Glass J. (1987) Evoked release of methionine-enkephalin from tolerant/dependent enteric ganglia: paradoxical dependence on morphine. *Proc. Natl. Acad. Sci. USA* **84,** 2537–2539.

Gintzler A. R. and Xu H. (1991) Different G proteins mediate the opioid inhibition or enhancement of evoked [5-methionine]enkephalin release. *Proc. Natl. Acad. Sci. USA* **88,** 4741–4745.

Gross R. A. and Macdonald R. L. (1987) Dynorphin A selectively reduces a large transient (N-type) calcium current of mouse dorsal root ganglion neurons in cell culture. *Proc. Natl. Acad. Sci. USA* **84,** 5469–5473.

Higashi H., Shinnick-Gallagher P., and Gallagher J. P. (1982) Morphine enhances and depresses Ca^{2+}-dependent responses in visceral primary afferent neurons. *Brain Res.* **251,** 186–191.

Hirai K. and Katayama Y. (1988) Methionine-enkephalin presynaptically facilites and inhibits bullforg sympathetic ganglionic transmission. *Brain Res.* **448,** 299–307.

Ikeda K., Kobayashi T., Ichikawa T., Usui H., and Kumanishi T. (1995) Functional couplings of the delta- and the kappa-opioid receptors with the G-protein-activated K^+ channel. *Biochem. Biophys. Res. Commun.* **208,** 302–308.

Ingram S. L., Vaughan C. W., Bagley E. E., Connor M., and Christe M. J. (1998) Enhanced opioid efficacy in opioid dependence is caused by an altered signal transduction pathway. *J. Neurosci.* **18,** 10,269–10,276.

Jacobowitz O. and lyengar R. (1994) Phorbol ester-induced stimulation and phosphorylation of adenylyl cyclase 2. *Proc. Natl. Acad. Sci. USA* **91,** 10,630–10,634.

Jin W., Lee N. M., Loh H. H., and Thayer S. A. (1992) Dual excitatory and inhibitory effects of opioids on intracellular calcium in neuroblastoma x gldioma hybrid NG108-15 cells. *Mol. Pharm.* **42,** 1083–1089.

Jin W., Lee N. M., Loh H. H., and Thayer A. (1994) Opioids mobilize calcium from inositol 1,4,5-

trisphosphate-sensitive stores in NG 108-15 cells. *J. Neurosci.* **14,** 1920–1929.

Johnson P. S., Wang J. B., Wang W. F., and UhI G. R. (1994) Expressed mu opiate receptor couples to adenylate cyclase and phosphatidyl inositol turnover. *Neuroreport* **5(4),** 507–509.

Kaneko S., Nakamura S., Adachi K., Akaike A., and Satoh M. (1994) Mobilization of intracellular Ca^{2+} and stimulation of cyclic AMP production by kappa opioid receptors expressed in Xenopus oocytes. *Mol. Brain Res.* **27,** 258–264.

Keren O., Gafni M., and Sarne Y. (1997) Opioids potentiate transmitter release from SK-N-SH human neuroblastoma cells by modulating N-type calcium channels. *Brain Res.* **764,** 277–282.

Kovoor A., Henry D. J., and Chavkin C. (1995) Agonist-induced desensitization of the Mu opioid receptor-coupled potssium channel (GIRK1). *J. Biol. Chem.* **270,** 589–595.

Lang J. and Schulz R. (1989) Chronic opiate receptor activation in vivo alters the level of G-protein subunits in guinea pig myenteric plexus. *Neuroscience* **32(2),** 503–510.

Laugwitz K. L. and Offermanns S. (1993) μ and δ opioid receptors differentially couple to G protein subtypes in membranes of human neuroblastoma SH-SY5Y cells. *Neuron* **10,** 233–242.

Leslie F. M. (1987) Methods used for the study of opioid receptors. *Pharmacol. Rev.* **39,** 197–249.

Lin Y. and Carpenter D. O. (1994) Direct excitatory opiate effects mediated by nonsynaptic actions on rat medial vestibular neurons. *Eur. J. Pharmacol.* **262,** 99–106.

Loh H. H. and Smith A. P. (1990) Molecular characterization of opioid receptors. *Ann. Rev. Pharmacol. Toxicol.* **30,** 123–147.

Lorentz M., Hedlund B., and Arhem P. (1988) Morphine ativates calcium channels in cloned mouse neuroblastoma cell lines. *Brain Res.* **445,** 157–159.

Mao J., Price D. D., Phillips L. L., Lu J., and Mayer D. J. (1995) Increases in protein kinase C gamma immunoreactivity in the spinal cord of rats associated with tolerance to the analgesic effects of morphine. *Brain Res.* **677,** 257–267.

Mauborgne A., Lutz O., Legrand J.-C., Hamon M., and Cesslin F. (1987) Opposite effects of delta and mu opioid receptor agonists on the in vitro release of substance P-like material from the rat spinal cord. *J. Neurochem.* **48,** 529–537.

Mayer D. J., Mao J., and Price D. D. (1995) The development of tolerance and dependence is associated with translocation of protein kinase C. *Pain* **61,** 365–374.

Neal M. J., Paterson S. J., and Cunningham J. R. (1994) Enhancement of retinal acetylcholine release by DAMGO: possibly a direct opioid receptor-mediated excitatory effect. *Br. J. Pharmacol.* **113,** 789–794.

North R. A. (1989) Drug receptors and the inhibition of nerve cells. *Br. J. Pharmacol.* **98,** 13–28.

Olianas M. C. and Onali P. (1993) Characterization of opioid receptors mediating stimulation of adenylate cyclase activity in rat olfactory bulb. *Mol. Pharmacol.* **42,** 109–115.

Olianas M. C. and Onali P. (1999) Mediation by G protein βγ subunits of the opioid stimulation of adenylyl cyclase activity in rat olfactory bulb. *Biochem. J. Pharmacol.* **57,** 649–652.

Prather P. L., Loh H. H., and Law P. Y. (1994) Interaction of δ-opioid receptors with multiple G proteins: a non-relationship between agonist potency to inhibit adenylyl cyclase and to activate G proteins. *Mol. Pharmacol.* **45,** 997–1003.

Rivera M and Gintzler A. R. (1998) Differential effect of chronic morphine on mRNA encoding adenylyl cyclase isoforms: relevance to physiological sequela of tolerance/dependence. *Mol. Brain Res.* **54,** 165–169.

Roerig S. C., Loh H. H., and Law P. Y. (1992) Identification of three separate guanine nucleotide-binding proteins that interact with the delta-opioid receptor in NG108-15 neuroblastoma X glioma hybrid cells. *Mol. Pharmacol.* **41(5),** 822–831.

Sarne Y., Gafni M., Fields A., and Keren O. (1996) Stimulatory effects of opioids on transmitter release and possible cellular mechanisms: overview and original results. (review). *Neurochem. Res.* **21,** 1353–1361.

Sharma S. K., Nirenberg M., and Klee W. A. (1975a) Morphine receptors as regulators of adenylate cyclase activity. *Proc. Natl. Acad. Sci. USA* **72,** 590–594.

Sharma S. K., Klee W. A., and Nirenberg M. (1975b) Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. *Proc. Natl. Acad. Sci. USA* **72,** 3092–3096.

Sharma S. K., Klee W. A., and Nirenberg M. (1977) Opiate-dependent modulation of adenylate cyclase. *Proc. Natl. Acad. Sci. USA* **74(8),** 3365–3369.

Shen K.-F. and Crain S. M. (1989) Dual opioid modulation of the action potential duration of mouse dorsal root ganglion neurons in culture. *Brain Res.* **491,** 227–242.

Shen K.-F. and Crain S. M. (1990) Cholera toxin A blockade of opioid excitatory effects on sensory neuron action potentials indicates mediation by G_s-linked opioid receptors. *Brain Res.* **525,** 225–231.

Sim L. J., Selley D. E., Dworkin S. I., and Childers S. R. (1996) Effects of chronic morphine administration on mu opioid receptor-stimulated [^{35}S]GTPgammaS autoradiography in rat brain. *J. Neurosci.* **16(8),** 2684–2492.

Smart D., Smith G., and Lambert D. G. (1994) mu-Opioid receptor stimulation of inositol (1,4,5)trisphosphate formation via a pertussis toxin-sensitive G protein. *J. Neurochem.* **62,** 1009–1014.

Smart D., Smith G., and Lambert D. G. (1995) Mu-opioids activate phospholipase C in SH-SY5Y human neuroblastoma cells via calcium-channel opening. *Biochem. J.* **305,** 577–581.

Smart D. and Lambert D. G. (1996) delta-Opioids stimulate inositol 1,4,5-trisphosphate formation, and so mobilize Ca^{2+} from intracellular stores, in undifferentiated NG108-15 cells. *J. Neurochem.* **66,** 1462–1467.

Sunahara R. K., Dessauer C. W., and Gilman A. G. (1995) Complexity and diversity of mammalian adenylyl cyclases. *Ann. Rev. Pharmacol. Toxicol.* **36,** 461–480.

Sweeny M. I., White T. D., and Sawynok J. (1989) Morphine, capsaicin and K^+ release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord. *J. Pharmacol. Exp. Ther.* **248,** 447–454.

Tallent M., Dichter M. A., Bell G. I., and Resine T. (1994) The cloned kappa opioid receptor couples to an N-type calcium current in undifferentiated PC-12 cells. *Neuroscience* **63,** 1033–1040.

Tang W.-J. and Gilman A. G. (1991) Type-specific regulation of adenylyl cyclase by G protein βγ subunits. *Science* **254,** 1500–1503.

Tang W.-J. and Gilman A. G. (1992) Adenylyl cyclases. *Cell* **70,** 869–872.

Tang T., Kiang J. G., and Cox B. M. (1994) Opioids acting through delta receptors elicit a transient increase in the intracellular free calcium concentration in dorsal root ganglion-neuroblastoma hybrid ND8-47 cells. *J. Pharmacol. Exp. Ther.* **270,** 40–46.

Tsu R. C., Chan J. S. C., and Wong Y. H. (1995) Regulation of multiple effectors by the cloned delta-opioid receptor: stimulation of phospholipase C and type II adenylyl cyclase. *J. Neurochem.* **64,** 2700–2707.

Tsu R. C. and Wong Y. H. (1996) G_i-mediated stimulation of type II adenylyl cyclase is augmented by G_q-coupled receptor activation and phorbol ester treatment. *J. Neurosci.* **16(4),** 1317–1323.

Wang L. and Gintzler A. R. (1994) Bimodal opioid regulation of cAMP formation: implications for positive and negative coupling of opiate receptors to adenylyl cyclase. *J. Neurochem.* **63,** 1726–1730.

Wang L. and Gintzler A. R. (1995) Morphine tolerance and physical dependence: reversal of opioid inhibition to enhancement of cAMP formation. *J. Neurochem.* **64(3),** 1102–1106.

Wang L., Medina V. M., Rivera M., and Gintzler A. R. (1996) Relevance of phosphorylation state to opioid responsiveness in opiate naive and tolerant/dependent tissue. *Brain Res.* **723,** 61–69.

Wang L. and Gintzler A. R. (1997) Altered μ-opiate receptor-G-protein signal transduction following chronic morphine exposure. *J. Neurochem.* **68(1),** 248–254.

Watson P. A., Krupinski J., Kempinski A. M., and Frankenfiedl C. D. (1994) Molecular cloning and characterization of the type VII isoform of mammalian adenylyl cyclase expressed widely in mouse tissues and in S49 mouse lymphoma cells. *J. Biol. Chem.* **269(46),** 28,893–28,898.

Xu H., Smolens I., and Gintzler A. R. (1989) Opioids can enhance and inhibit the electrically evoked release of methionine-enkephalin. *Brain Res.* **504,** 36–42.

Xu H. and Gintzler A. R. (1992) Opioid enhancement of evoked [Met^5]enkephalin release requires activation of cholinergic receptors: possible involvement of intracellular calcium. *Proc. Natl. Acad. Sci. USA* **89,** 1978–1982.

Yoshimura M., Ikeda H., and Tabakoff B. (1996) μ-Opioid receptors inhibit dopamine-stimulated activity of type V adenylyl cyclase but enhance dopamine-stimulated activity of type VII adenylyl cyclase. *Mol. Pharmacol.* **50,** 43–51.

Zimmermann G. and Taussig R. (1996) Protein kinase C alters the responsiveness of adenylyl cyclase to G protein α and βγ subunits. *J. Biol. Chem.* **271(43),** 27,161–27,166.