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Invited Review: Role of Receptor Regulation in Opioid Tolerance Mechanisms

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ABSTRACT The molecular basis of opioid tolerance/dependence has long eluded researchers, but recent advances in receptor regulation have suggested a useful conceptual approach to the problem. In NG108-15 neuroblastoma × glioma hybrid (NG) cells, opioid agonists inhibit adenylate cyclase in a dose-dependent, naloxone-antagonizable fashion. Chronic treatment with opioid agonists results in a series of molecular processes that, in a tolerance-like fashion, counteract this inhibition. These processes include desensitization and down-regulation of receptors and an increase in adenylate cyclase activity.

Opioid inhibition of adenylate cyclase and opioid receptor down-regulation also have been observed in the brain. However, most studies have found that the receptors coupled to adenylate cyclase are not of the mu type, which are thought to be the primary mediators of opioid analgesia. Down-regulation has been observed for both mu and delta opioid receptors in the brain. However, in most cases, the time course of down-regulation is not correlated with that for tolerance development, and chronic morphine treatment does not result in down-regulation. Thus, opioid receptors in the brain, like those in NG cells, are subject to dynamic regulation by agonists, which probably has an important role in their function. However, it remains to be established that opioid receptor regulation is the basis of opioid tolerance and dependence.

INTRODUCTION

Opioid drugs are widely used clinically as analgesics, or painkillers, but a major drawback of their continued use is the development of tolerance and dependence. Tolerance is manifested in decreased potency of the drug, so that progressively larger doses must be administered to achieve a given level of analgesia; sometimes, in fact, tolerance becomes so great that little or no analgesia is possible, even with very high doses (Coombs et al., 1985; Woods and Cohen, 1982). Dependence, which is usually associated with tolerance, is a state in which continued administration of the opioid is necessary to prevent a constellation of painful symptoms known as withdrawal.

Although these phenomena have been appreciated for several thousand years, their basis remains mysterious. It is now widely assumed that chronic opioid administration induces specific, semipermanent chemical changes in the brain, which in some manner counteract the processes underlying the acute actions of these drugs. Intensive efforts in many laboratories, however, have failed repeatedly to demonstrate the changes that can account for tolerance/dependence development.

A major obstacle to progress in this area is the enormous complexity of the brain. Although the major pathways on which opioids act are now fairly well understood (Basbaum and Fields, 1985), it is difficult to isolate these areas for biochemical analyses, particularly when the anticipated changes may involve a very small number of molecular species. Indeed, the molecular and cellular processes underlying even the acute actions of opioids have not been established. Because tolerance to drugs can be defined only in terms of a change in the drug's acute actions, it is unlikely that the chronic effects of opioids will be understood until their short-term actions are understood.

Obviously, it would help greatly if a simplified model system were available for studying opioid tolerance, analogous to those that have been used successfully for genetics studies and, to a lesser extent, learning and memory studies. Such a system is offered by NG108-15 neuroblastoma-glioma hybrid (NG) cells in culture. Sharma et al. (1975a) reported that these cells contain opioid receptors on their plasma membranes and that binding opioid agonists to these receptors inhibits the membrane-bound enzyme adenylate cyclase. This inhibition was dose-dependent and antagonized by naloxone, and the inhibitory potencies of a series of opioid agonists correlated well with their binding affinities to these cells, as well as with their in vivo pharmacologic potencies.

Subsequently, this group found that chronic opioid agonist treatment resulted in a loss of opioid inhibition

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of cyclase, with levels of the enzyme gradually returning to normal (Sharma et al., 1975b). Furthermore, after the withdrawal of agonist or the addition of antagonist, there was an increase in adenylate cyclase above that of control levels. These effects are at least superficially analogous to opioid tolerance and dependence, respectively, suggesting that NG108-15 cells would be a useful model system in which to study these phenomena.

In the past 10 years, many details of the processes underlying the acute and chronic actions of opioids in NG108-15 cells have been elucidated. Whereas these studies make an important contribution to our understanding of fundamental cellular processes, the major question of interest to opioid researchers is whether or not these processes are relevant to opioid tolerance/dependence in whole animals. That is, do opioid tolerance and dependence result from desensitization and/or down-regulation of opioid receptors in the brain?

In this article, we assess the current evidence for this hypothesis. We begin by briefly discussing what is now known about the molecular processes underlying the acute and chronic effects of opioids in NG108-15 and other cell systems. We then consider studies that have attempted to demonstrate that these processes also occur in the brain and are associated with the development of opioid tolerance and dependence.

THE NG108-15 NEUROBLASTOMA-GLIOMA HYBRID CELL MODEL OF OPIOID ADDICTION

Demonstrating that opioids inhibit adenylate cyclase in NG108-15 cells, and that this inhibitory effect is eliminated or reduced following chronic opioid treatment, is not unique to the opioid class of drugs. Several other ligand families, including beta-adrenergic and cholinergic agonists and glucagon, have analogous effects in certain homogeneous cell preparations. Although some of these ligands stimulate rather than inhibit adenylate cyclase, chronic administration of ligand, in a fashion analogous with opioids, results in a progressive reduction of this stimulatory effect.

In all of these examples, the acute effect of ligand on adenylate cyclase has been found to be mediated by a guanosine triphosphate (GTP)-binding protein that couples the ligand's receptor to the catalytic subunit of adenylate cyclase (Rodbell, 1980). Agonists that stimulate adenylate cyclase couple with a stimulatory subunit (G_s), whereas inhibitory agonists couple with an inhibitory subunit, (G_i). In both cases, the association of receptor with G-protein is accompanied by an exchange of bound guanosine diphosphate (GDP) for GTP on the latter. In its GTP-binding form, the G-protein then stimulates or inhibits adenylate cyclase.

The chronic effect, on the other hand, appears to involve at least two steps (Su et al., 1980; Lefkowitz et al., 1980). In the first step, called desensitization, the receptor becomes uncoupled from the GTP-binding subunit, with an accompanying reduction of affinity for agonist binding. Subsequently, the receptors are removed from the cell surface, passing into the cell interior, where they may be degraded or later recycled to the surface. This step, called down-regulation, results in a loss of receptor binding sites, detectable as a reduction in B_{max}.

Both our and others' studies of opioid action in NG108-15 cells have indicated that these ligands act through generally similar mechanisms. Thus, opioid agonist inhibition of adenylate cyclase is mediated through G_i, as demonstrated by the observation that opioids do not inhibit cyclase if GTP is replaced by its nonhydrolyzable analog Gpp(NH)p and by the correlation of potencies of various opioids to inhibit cyclase with their stimulation of GTP hydrolysis (Koski and Klee, 1981; Koski et al., 1982). In addition, the acute effects of opioids on adenylate cyclase in NG cells can be blocked by pertussis toxin, which prevents coupling with G_i by ADP-ribosylating the latter (Katada et al., 1984).

The chronic effects of opioids in NG cells likewise appear to result from a multistep process, featuring both desensitization and down-regulation. Desensitization, as in other systems, is associated with an uncoupling of opioid receptors from G_i (Law et al., 1984b). This process is homologous (Law et al., 1984b); that is, it does not affect the cell's response to other classes of ligands, indicating that the changes occur in the receptors themselves, not in G_i or adenylate cyclase. Down-regulation involves an energy-dependent accumulation of receptors inside the cell, where they are subsequently degraded (Blanchard et al., 1983; Law et al., 1984a). G_i appears not to be involved in this process either, as pertussis toxin treatment does not prevent down-regulation, and G_i labelled with 32p by this treatment is not found in the cytosol (Law et al., 1985).

In addition to desensitization and down-regulation, there is evidence for a third process, distinct from either of these, which accounts for the increase in adenylate cyclase that accompanies withdrawal from opioid in these cells (Law et al., 1984b). This increase seems to reflect an increase in the intrinsic activity of adenylate cyclase molecules rather than in their number (Sharma et al., 1977) and probably results from the synthesis or mobilization of some activating factor (Griffin et al., 1983; Law et al., 1984b; Wilkening and Nirenberg, 1980). At any rate, this increase appears to be completely independent of any changes in opioid receptors, for under some conditions it can be induced without any alteration in receptor number (Law et al., 1983).

To summarize, opioids administered acutely to NG108–15 neuroblastoma-glioma hybrid cells inhibit adenylate cyclase, whereas chronic treatment results in tolerance- and dependence-like effects. Tolerance in this system, manifested as the requirement for increasingly higher doses of drug to achieve a given level of adenylate cyclase inhibition, seems to involve changes only in opioid receptors (desensitization and down-regulation), and not in processes that occur subsequent to opioid binding. Dependence, on the other hand, which can be defined as the requirement for continued opioid administration to prevent an increase in adenylate cyclase above control levels, involves a distinct process that is not related to changes in opioid receptors. Thus, the site of dependence in this system is presumably in processes subsequent to opioid receptor activation. It is of interest to note that a similar conclusion was reached in studies of opioid dependence in a somewhat more complex in vitro system, the guinea pig ileum (Collier and Tucker, 1984).

OPIOID RECEPTOR REGULATION OF ADENYLATE CYCLASE IN THE BRAIN

In assessing the relevance of findings in NG cells to understanding the processes underlying opioid tolerance/dependence in whole animals, the first question

one might ask is whether or not opioids regulate adenylate cyclase in mammalian systems. This is not a necessary requirement, as receptor regulation has been observed in systems not coupled to adenylate cyclase. However, if the acute effects of opioids in whole animals could be shown to involve adenylate cyclase inhibition, this would increase greatly the likelihood that the specific processes set in motion by chronic opioid treatment of NG cells also occur in the brain. This demonstration would also, of course, constitute a major step in our understanding of opioid analgesia itself.

Considerable circumstantial evidence suggests that adenylate cyclase is associated with the acute and chronic effects of opioids in whole organisms. For example, cyclic adenosine monophosphate (cAMP) has been reported to antagonize opioid analgesia and to accelerate opioid tolerance development (Yano et al., 1980; Levy et al., 1981, 1983). Moreover, opioid agonists can inhibit adenylate cyclase in brain membranes in vitro (Law et al., 1981; Cooper et al., 1982). As in the homogeneous cell systems, this inhibition is dose-dependent and antagonized by naloxone, and it correlates well with the affinity of ligands for opioid receptors.

However, the receptors that mediate adenylate cyclase inhibition do not seem to be the same as those responsible for opioid analgesia. Initial studies reported maximum inhibition in the striatum, which is not involved in the analgetic effects of these drugs; opioid inhibition of adenylate cyclase was much less detectable or nondetectable in other brain regions. Furthermore, in the striatum, analysis indicated that only delta (enkephalin-selective) receptors were involved. These are the same type of opioid receptors present in NG cells (Chang et al., 1979), but most evidence indicates that they do not mediate the analgetic effects of opioids in the brain (Chaillet et al., 1984; Feng et al., 1986; Ward and Takemori, 1983).

Although there is no convincing evidence that opioid analgesia is mediated by adenylate cyclase, this possibility should not be ruled out yet. Opioid binding to mu receptors, like that to delta receptors, is regulated by GTP (Blume, 1978; Childers and Snyder, 1980), and this class of receptors has been reported to regulate adenylate cyclase in one homogeneous cell system (Frey and Kebabian, 1984). Moreover, some preliminary studies in our laboratory indicate that the mu receptors in the periaqueductal area gray (PAG) area of the brain, which is known to be involved in opioid analgesia, also can regulate adenylate cyclase (Fedynyshyn and Lee, unpublished data).

In fact, the demonstration of opioid inhibition of adenylate cyclase in the striatum required certain conditions, such as the presence of Na^+ and GTP, the absence of which apparently accounted for many earlier failures to demonstrate this inhibition in brain. Thus, it is conceivable that opioid inhibition of adenylate cyclase does occur in other areas of the brain, but only under as yet undefined conditions. Some support for this idea is provided by Childers and LaRiviere (1984), who have reported that opioid inhibition of adenylate cyclase in striatal membranes is enhanced by preincubation of the membranes at low pH. These authors suggest that certain membrane components removed by the acid pH treatment might regulate opioid receptor-G-protein coupling.

EVIDENCE FOR OPIOID RECEPTOR DOWN-REGULATION IN THE BRAIN

Even before the discovery of opioid receptor desensitization and down-regulation in NG108–15 cells, many investigators had attempted to demonstrate changes in the affinity or in the number of opioid receptors in tolerant-dependent animals. The results of these studies, however, were disappointing. In almost all cases, either no changes were observed, or changes occurred that did not correlate well, in either magnitude or time course, with tolerance development (Creese and Sibley, 1981; Harris and Kazmierowski, 1975; Hitzemann et al., 1974; Hollt et al., 1975; Klee and Streaty, 1974; Pert and Snyder, 1976).

Most of these studies, however, examined opioid binding in whole brain, and because opioid receptor heterogeneity was not appreciated at that time, binding to specific receptor types was not analyzed. Thus, tolerance-related changes in opioid binding restricted to specific brain regions, or to specific receptor types, might well have been missed. In support of this, Dingledine et al. (1983) found that chronic opioid treatment of hippocampal slices in vitro could result in a selective decrease of opioid receptors, depending on the agonist used for chronic exposure. Incubation of slices for 4 hours with D-ala^2-D-leu^5-enkephalin (DADLE) resulted in a decrease of delta receptors, but not in mu receptors. Incubation with the mu agonists morphine or morphiceptin had no effect on either mu or delta receptors, although physiologic tolerance to morphiceptin was observed. Dingledine et al. (1983) concluded that down-regulation could account for tolerance to delta agonists in this preparation, but not tolerance to mu agonists.

More recently, our laboratory observed region and receptor type-specific down-regulation of opioid receptors in vivo (Tao et al., 1987). A critical factor in this work was the chronic administration of etorphine, rather than morphine. In our earlier studies of NG108–15 cells, we showed that chronic morphine treatment did not induce down-regulation of opioid receptors, apparently because it was only a partial agonist in this delta receptor system. Down-regulation in this system was induced only by delta-selective ligands, such as enkephalin, and by alkaloids with significant affinity for delta receptors, such as etorphine and ethylketocyclazocine.

We reasoned that a similar situation might exist in certain parts of the brain, particularly in the striatum, where, as discussed earlier, opioids regulate adenylate cyclase in a manner similar to that demonstrated in NG cells (Law et al., 1981). Accordingly, rats were chronically infused with etorphine by means of implanted osmotic minipumps. Under these conditions, we observed a time-dependent decrease in ^{3}H-diprenorphine binding in all three brain regions examined: cortex, midbrain and striatum. This decrease was due to a decrease in receptor number, rather than affinity, and was accompanied by a twofold increase in the IC_{50} for etorphine inhibition of striatal adenylate cyclase. Analysis of specific receptor types revealed that mu receptor binding was decreased in all three brain regions examined, whereas delta binding was decreased significantly only in the midbrain. In agreement with earlier studies, however, no changes in receptor number or affinity accompanied chronic morphine treatment.

In a subsequent study (Tao et al., 1988), we obtained somewhat similar results following chronic administration of DADLE. A decrease in B_{max} of ^{3}H-diprenorphine binding was found in the cortex, midbrain and striatum. When the binding to mu and delta opioid sites was distinguished by assaying ^{3}H-diprenorphine binding in the presence of morphiceptin, the decrease was found to be specific to delta receptors, except in the striatum.

These results establish that the levels of both mu and delta opioid receptors in the brain can be regulated by certain agonists. However, in most cases, there was no correlation between the time course of this down-regulation and that for tolerance development. Except for the effects of chronic DADLE in the striatum, the receptor number reached minimum levels within 1–3 days of chronic treatment, whereas tolerance continued to develop for at least 7 days. This observation, together with the fact that no changes were observed to accompany the chronic administration of morphine, call into question the relevance of these findings to morphine tolerance.

However, these results do demonstrate that both mu and delta opioid receptors in the brain are under dynamic regulation by agonist. Moreover, it is still an open question whether or not desensitization may be better correlated with tolerance development. Because of the existence of multiple opioid receptors in the brain, each of which probably has sites of different affinities, it is not feasible at this time to test whether desensitization of mu or other receptors occurs during tolerance.

Another potentially complicating factor needs to be considered. When brain tissue is removed from a tolerant animal, in preparation for the opioid binding assay, any changes in receptors that might have developed in vivo conceivably could be reversed. This reversal could result from changes during homogenization, or simply from withdrawal, due to the washing out of the chronic opioid. A few studies support this conclusion. Davis et al. (1979) reported that when opioid binding was assayed in unwashed brain stem slices of chronically treated animals before homogenization, a reduction occurred in tolerant animals. The presence of opioid, of course, interferes with the binding assay; thus, very careful controls must be carried out to demonstrate that a real change in opioid receptors has in fact occurred.

More recently, Rogers and Fakahany (1986) found that chronic morphine treatment resulted in a reduction of ^{3}H-naloxone binding in brain when the binding assays were carried out on dissociated whole cells; however, when the assays were carried out on homogenates, there was no change, or even an increase, in binding, depending on the buffer used. Although these results are not readily explainable by any current model, they provide further evidence that homogenization, as well other conditions of the binding assay, may alter observed opioid receptor levels.

Finally, Zaitsev et al. (1986) reported a selective effect of chronic morphine on brain delta receptors. The parameter they measured, however, was not receptor number, which they found to vary significantly among individual animals, but rather the ratio of receptor number [Q] to the ligand-receptor dissociation constant (K). They found that [Q]/K values were quite constant from one animal to the next, and this value was decreased by more than 50% for delta receptors in morphine-tolerant animals. Corresponding values for mu and kappa receptors were unchanged.

UP-REGULATION OF OPIOID RECEPTORS IN BRAIN

Although down-regulation of opioid receptors has been difficult to detect in the brain, opioid receptor up-regulation in this tissue is well established (Hitzemann et al., 1974; Pert and Snyder, 1976; Schulz et al., 1979; Tang and Collins, 1978; Zukin et al., 1982, 1984). In theory, agonist-induced up-regulation of opioid receptors could account for tolerance, but such a model has major difficulties explaining certain data (Smith et al., 1988), and probably could not involve regulation of adenylate cyclase. Nevertheless, such studies are important and worth discussing, as they provide further evidence of the dynamic state of opioid receptor number.

As with the up-regulation reported in other receptor systems (Creese et al., 1977), this appears to be an adaptive response to treatments that block opioid agonist activity, as it is induced by both lesions of certain brain pathways (Gardner et al., 1980; Simantov and Amir, 1983; Young et al., 1982) and, most commonly, by chronic administration of antagonist (Zukin et al., 1982). In a detailed characterization of the phenomenon, Zukin et al. (1984) found an increase of nearly 100% in ^{3}H-etorphine binding in rats chronically treated with the opiate antagonist naltrexone, the maximum being reached after 8 days. The increase was due entirely to a change in receptor number, with no change in affinity, paralleled by an increase in sensitivity of the animals to morphine, suggesting functional significance of the up-regulation. These investigators also reported an increased sensitivity of opiate binding to guanine nucleotides, suggesting an increase in receptor coupling to adenylate cyclase or to some other functional molecule. Autoradiographic studies indicated an uneven distribution of up-regulation, with ventromedial hypothalamus, ventral tegmental area, substantia nigra compacta, and amygdala showing the most increase in etorphine binding; there were also increases in met-enkephalin in some areas. Most significantly, the up-regulation was observed with both mu and delta receptors, but not with kappa receptors (Tempel et al., 1985).

Zukin and colleagues (1984) have also demonstrated up-regulation in the isolated spinal cord-dorsal root ganglion preparation, where the underlying processes can be examined under more controlled conditions (Tempel et al., 1983). The time course of this phenomenon in the presence of 10 μM naloxone (50% up-regulation in 5 days) was similar to that observed in the spinal cord in vivo. The up-regulation was not blocked by cycloheximide, leading these investigators to conclude that up-regulation results from unmasking previously inactive receptors, rather than from the synthesis of new ones. Very recently, Holaday et al. (1985) have reported up-regulation of opioid receptors in the brain following chronic agonist (morphine) treatment. The up-regulation was region and delta-receptor specific and also could be induced by repeated electroconvulsive shock treatments, which the authors found produced many of the effects of opioids. Furthermore, there was cross-tolerance between the effects of the two treatments.

In further studies of this effect, Rothman et al. (1986) found that the up-regulation was restricted not only to

delta receptors, but to a specific subclass of them. Earlier work by this group (Rothman et al., 1982) led to the conclusion that two kinds of delta receptors exist in the brain: 1) a low affinity site that is allosterically coupled to mu receptors and that inhibits binding to the latter noncompetitively and 2) a high affinity site, binding to which is competitive with binding to mu sites. Chronic morphine treatment resulted in almost 50% up-regulation of the low affinity, mu noncompetitive sites, while not affecting the number of high affinity, mu competitive sites.

Because opioid agonists are present in the brain, treatment with antagonists presumably displaces agonists from opioid receptors. If this results in up-regulation of the receptors, the presence of endogenous agonist can be seen as a down-regulating factor. These studies thus support those of Dingledine et al. (1983) and Tao et al. (1987, 1988), suggesting that although opioid receptor down-regulation may not be involved in opioid tolerance, it nevertheless has an important role in brain function.

CONCLUSIONS

Opioid regulation of adenylate cyclase in NG108-15 hybrid cells has proved to be a convenient model system from which much has been learned about the long-term regulation of receptors. Because of the similarity of chronic phenomena in this system to opioid tolerance/dependence in whole animals, it is attractive to postulate that the latter are based on similar processes. Some support for this idea is provided by studies showing that opioids inhibit adenylate cyclase in some regions of the brain and that down-regulation of opioid receptors may occur during chronic opioid treatment.

However, no studies have yet demonstrated that the receptors responsible for analgesia regulate this second messenger system. Furthermore, whereas the chronic administration of opioids can induce down-regulation of opioid receptors, this down-regulation is not correlated with the development of tolerance. Thus, questions remain as to the relevance of the molecular processes underlying the chronic actions of opioids in NG cells to narcotic addiction in whole animals.

It also should be emphasized that, even if these processes were demonstrated and shown to correlate closely with tolerance/dependence development, it would still remain to elucidate their relationship with the latter. Whole animal phenomena such as analgesia undoubtedly are mediated by multicellular networks, which to some extent have properties that transcend those of their individual members. Recent evidence that tolerance might be based on such a collective response was reported by Williams and North (1983). They found that acute administration of opioids either to whole animals or to slices of the locus coeruleus (LC) induced a hyperpolarization in the LC neurons. In animals chronically treated with morphine, a high degree of tolerance to this response was seen, but the tolerance was much lower in cells of isolated LC. Apparently, connections of these cells with other neurons are vital to the full development of tolerance.

Another set of studies that illustrates the critical importance of multicellular interactions in opioid tolerance/dependence was reported by Yeung and Rudy (1980). In agreement with others, they found that morphine could induce analgesia when injected either intracerebroventricularly or intrathecally. When administered to both locations simultaneously, however, the ED50 was far lower than that necessary for injection at one site. This synergistic relationship indicates that opioid action involves interaction between spinal and supraspinal centers.

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