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Secrets of the opium poppy revealed

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Abstract

Studies concerning drugs of abuse have made major contributions in defining the circuitry, as well as cellular and molecular substrates that underlie certain behaviors. Opiate drugs for example, have revealed important insights concerning pain perception and reward. Up to the late 1960s, opiate drugs were suspected to work by mysteriously perturbing lipid membrane structure. We now know the following: the sequence and neuroanatomy of the G-protein coupled receptors that mediate opiate effects; that many proteins interact with opioid receptors such as G-protein sub-unit combinations, G-protein receptor kinases, arrestins and calmodulin; that many signaling molecules are modulated by opioid receptors, including ion channels, kinase cascades and adenyl cyclase. More than 20 different peptides, excised from three precursor proteins by specific proteases, have been shown to be endogenous ligands for opioid receptors. Revealing the molecules of the endogenous opioid system has inspired efforts for developing new opioid analgesics with the hope of minimizing abuse potential. This article will detail the current rationale for searching for less-addictive opiate analgesics and speculate on the future of drug abuse research in furthering our understanding of neural plasticity and the underpinnings of addictive behavior.

Keywords: Morphine; Opioid signaling; Receptor type

1. Introduction

Substance abuse, like many psychiatric illnesses, requires an environmental trigger—in this case, a drug. The assumption is that if drug supplies could be halted, addiction disorders would be less prevalent in society. However, it is difficult to predict the behavioral outcome of those susceptible to addictive disorders that do not indulge. It could be argued that drug abuse may actually be protective for society, focusing the reward system on an exogenous substance rather than more primal behaviors such as mating and survival, which the circuitry probably evolved to promote. Despite rigorous efforts to eradicate illicit opium-derived drugs, they remain readily obtainable. As soon as one source is quenched, another seems to emerge. According to the National Survey on Drug Use and Health (NSDUH), the prevalence of lifetime heroin use among youths aged 12–17 increased from 0.1% in 1995 to 0.4% in 2002. Over the same seven-year time period, the lifetime use rate doubled among youths aged 18–25, reaching 1.6% in 2002. The Drug Abuse Warning Network (DAWN) which monitors drug-related emergency room visits, reported 93,518 visits related to heroin in 2002, which was up from 63,158 in 1994. Prescription opiates such as morphine, oxycontin and vicodin are also significantly abused and addictive use is increasing, as society demands its library of pharmaceuticals to include effective drugs for pain suppression. In 2002, according to DAWN there were 119,185 emergency room visits as a result of opiate therapeutics, up from 44,518 visits in 1994. Nearly 50,000 of the emergency room visits in 2002 were associated with oxycodone or hydrocodone (e.g. oxycontin, percocet and vicodin), and this is probably an underestimate since in the approximately 42,000, visits to the emergency room, the opiate pharmaceutical was not recorded. These statistics underline the caution needed when prescribing opiate analgesics for pain. Results

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from the 2002 NSDUH survey also suggested that non-medical pain reliever use is increasing. For young adults, 6.8% reported misuse of an opiate pain medication in 1992 and 22.1% in 2002. Clinicians working with abusing populations paint an extremely bleak picture for opiate addicts, including the destructive drug-focused behaviors, the high rates of incarceration resulting in disruption of relationships and social structure and the associated diseases such as AIDS and hepatitis C virus as a consequence of needle sharing and risky sexual behaviors. Clearly opiate abuse is on the rise, and with the low cure rate of opiate addicts (for heroin addicts relapse is close to 100%) it can be assumed that opiate addiction will continue as a medical problem, undaunted by the current punitive repercussions, financial burden and poor prognosis.

One goal, or rather dream, of opioid research has been the development of opiate drugs that are analgesic yet lack abuse potential, tolerance and withdrawal. Given the statistics for opiate pharmaceutical abuse, a non-addictive opiate would clearly fulfill an important medical niche. Over the years, I have vacillated between considering a non-addictive opiate analgesic as pie in the sky or an intriguing possibility. The reason for my vacillating opinions has been the continuing emergence of knowledge of how opioid drugs trigger cellular signaling cascades that eventually lead to analgesia and addiction. I will frame this article with discoveries, including those from our NIDA Center, The Center for Study of Opioid Receptors and Drugs of Abuse (CSORDA), that have defined the molecules of the endogenous opioid system and rekindled hope for more sophisticated opiate drug development. I will also speculate that recent and future substance abuse research will provide critical insight to our understanding of plasticity mechanisms in the brain by studying cellular and molecular reorganization that accompanies altered behaviors as a result of drug taking.

2. Revealing the endogenous opioid system

Parallel with the demonstration that opiate drugs interact with receptor binding sites was the concept of multiple opioid receptors. This notion first emerged following pharmacological analyses of different opiate drugs in dogs. In early studies, a series of behavioral effects and cross-tolerance among drugs were the tools used to differentiate receptor types (Martin et al., 1976). The discovery of the endogenous opioid peptides, methionine and leucine–enkephalin, provided an entirely new pharmacophore on which to design opioid ligands. As the pharmacology became more sophisticated, with binding assays, bioassays and second messenger assays, the concept of multiple opioids receptors became firmly established. Multiple opioids receptors gave an exciting possibility that by restricting opioid drugs to one, or a select combination of opioids receptors, this would provide an opiate analgesic without addictive potential.

Pharmacological studies of multiple opioid receptors clearly defined three receptor types, the mu, delta and kappa receptors. Although in vivo experiments suggested a multiplicity of sub-types (Pasternak, 1993), these three receptor types were undisputed. During the decade spanning the mid-1970s to the late 1980s, more than 20 different endogenous opioid peptides were identified and shown to possess differential affinity for the three opioid receptor types. All unequivocal mammalian opioid peptides have an N-terminal enkephalin sequence (Tyr-Gly-Gly-Phe-Met/Leu), with many peptides containing a C-terminal extension which modulates receptor selectivity and susceptibility to degradation by extracellular proteases (reviewed by Weber et al., 1983). The endogenous opioids are derived from three opioid protein precursors by selective proteolytic cleavages predominantly at basic and paired basic residues. Proopiomelanocortin contains beta-endorphin, a potent mu and delta opioid receptor agonist and shares the precursor protein with adrenocorticoptrophic hormone, a critical pituitary hormone for coordination of stress responses. Proenkephalin contains multiple repeats of the enkephalin sequence, seven in the human proenkephain precursor, and depending on the proteolytic processing many different opioid peptides can be generated. Finally, prodynorphin contains three leucine–enkephalin core opioid sequences and analogous to proenkephalin, differential processing leads to multiple opioid peptides. Each opioid peptide precursor has a unique pattern of expression, with proopiomelanocortin transcripts restricted to the pituitary, the arcuate nucleus of the hypothalamus and some cells in the nucleus of the solitary tract, whereas both proenkephalin and prodynorphin have a considerably more expansive distribution (Akil et al., 1984). Still unclear is the biological significance of the multiplicity of endogenous opioids and if differences in receptor selectivity and stability against extracellular proteases tell the entire story. In addition to the cornucopia of endogenous opioids peptides, synthetic chemists were generating both peptide and alkaloid ligands with various selectivities and efficacies at the different receptor types. The ligand armory for opioids receptors has become staggering.

Hope that selective ligands for either mu, delta or kappa receptors could be the key in designing non-addictive opioid analgesics was thwarted by adverse side effects of agonists at all three receptors types. Animal models concluded that, though analgesic, delta agonists induced seizures and kappa agonists were dysphoric and possibly hallucinogenic (reviewed by Bodnar and Hadjimarkou, 2003). Mu receptors were

considered to mediate all the classical beneficial and non-beneficial effects of opiate drugs. This was subsequently verified in mu opioid receptor null mice (reviewed by Gaveriaux-Ruff and Kieffer, 2002), which show no morphine-analgesia, no morphine-place preference, no morphine changes in gut motility and no naloxone-precipitated withdrawal from morphine-treated mice. Furthermore, supraspinal delta-analgesia appears compromised in mu knockout mice either suggesting crosstalk of mu and delta ligands in vivo or dependence on delta function by mu receptors. It should be emphasized that relating findings regarding opioid effects from rodents to humans has an important caveat concerning the differential distribution of opioid receptors among species (Peckys and Landwehrmeyer, 1999). For example, the kappa opioid receptors appear substantially more widely distributed in human than in rodent. In the case of delta receptors, humans have a considerably more selective localization in the dorsal horn of the spinal cord than rodents or non-human primates (Mennicken et al., 2003) with the implication that delta ligands may be more effective analgesics in humans. It remains unclear if in humans, delta agonists are seizure promoting and if so, whether partial agonists could still prove to be efficacious analgesics.

Identification of the nature of opioid receptors was not straightforward. However, by the end of 1992, our NIDA Center at UCLA and Brigitte Kieffers' group in Strasbourg had independently reported clones for the mouse delta opioid receptor using very similar approaches. (Evans et al., 1992; Kieffer et al., 1992). Cloning of the mu and kappa opioid receptors followed soon after, since they were highly homologous to the delta receptor (reviewed by Kieffer, 1995; Zaki et al., 1996). As predicted, opioid receptors are seven transmembrane G-protein coupled receptors and unremarkable when compared with other receptors in the same family, with the exception of the large transcript size (over 10 kb for the mu and delta opioid receptors). There was much speculation that more than three opioid receptor genes would be discovered and provide targets that may differentiate the in vivo effects of opioid drugs. However, despite considerable efforts, no other opioid receptors genes were identified besides the Opioid Like Receptor (ORL-1 or Op4) and this was not considered a classical opioid receptor since it did not bind classical opiate ligands such as naloxone. Furthermore, ORL-1 had a separate endogenous ligand, namely Orphanin FQ, that was derived from a protein precursor different from the opioid peptides (reviewed by Zaki and Evans, 1998). Although not a classic opioid receptor, some opiate drugs that are routinely used in the clinic such as buprenorphine do bind to ORL-1 receptors and recent research from our center has shown that this may have clinical significance, since ORL-1 receptor interaction modifies the antinociceptive properties of buprenorphine markedly, at least in some rodent models (Lutfy et al., 2003).

Cloning defined the family of opioid receptors which enabled the detailed anatomical analysis of opioid receptors (Mansour et al., 1995 and references therein), the analysis of opioid receptor transcripts, the development of new opioid receptor cell lines, structure–activity studies and the generation of receptor knockout animals. However, the cloning did not provide the diversity anticipated from the in vivo heterogeneity in opiate responses. Many of us searched for other mechanisms to create receptor diversity from single genes such as RNA editing or differential splicing. Mu receptor alternative splicing at the very C-terminus has been observed in rodent receptors and clearly this could provide heterogeneity in opioid receptor molecules and modify receptor functioning (reviewed by Wei et al., 2004).

3. Receptor trafficking

Ligand-regulated receptor trafficking is an area of research that has blossomed since the molecular characterization of G-protein coupled receptors. The analysis of the opioid receptors has been particularly insightful given the variety of alkaloid and peptide ligands, including agonists, partial agonists, antagonists and inverse agonists. The finding that many opioid agonists, including the endogenous opioids, can induce mu receptor internalization whilst morphine does not, resulted in much speculation on the potential for some opioid drugs to elude certain regulatory mechanisms (reviewed by Kieffer and Evans, 2002; von Zastrow et al., 2003). However, heterogeneity of the cellular environment of mu opioid receptors has proven critical for distinction among agonists and in the case of mu receptors internalization by morphine has been observed in dendrites but not cell bodies (Haberstock-Debic et al., 2003). Furthermore, increasing levels of proteins associated with receptor internalization such as beta-arrestin and G-protein receptor kinase 2 can markedly enhance internalization of morphine in vitro (Whistler and von Zastrow, 1998). The concept that agonist-induced internalization, and the susequent loss of surface receptors, could be a model for tolerance has focused experiments of internalization upon desensitization. However, the in vivo pharmacology indicates that opioid agonists that are efficient or inefficient at internalization or even up-regulate surface mu receptors, as in the case of buprenorphine, can all induce profound tolerance (reviewed by Evans et al., 2000). It appears if there are any correlations, the lack of internalization promotes tolerance, potentially by prolonging signaling and allowing other downstream adaptive

responses to occur within the cell or in connected cells (reviewed by von Zastrow et al., 2003).

4. Receptor cross-talk

The concept of opioid receptor cross-talk, in particularly between mu and delta opioid receptors, has been with the field for many years and stems from a number of pharmacological observations, mostly in vivo (reviewed by Zaki et al., 1996). Two recent studies in which CSORDA was involved illustrate the potential importance of receptor signaling cross-talk on the outcome of receptor activation. The first is the modulation of insulin receptor kinase signaling cascades by mu receptors, a study principally from the laboratory of Dr. Roby Polakiewicz (Li et al., 2003). Insulin binding activates an insulin receptor tyrosine kinase activity resulting in tyrosine phosphorylation of a number of proteins including Insulin Receptor Substrates (IRS proteins) and Shc. IRS proteins phosphorylated by insulin receptors complex with many other signaling proteins, including PI3Kinase, which results in Akt phosphorylation and activation, contributing to glucose uptake. Insulin receptor signaling also activates Mitogen Activated Protein kinase (MAPK or Erk1/2) via a Ras-mediated pathway involving SOS, Grb2 and Shc. Activation of mu receptors has a dramatic inhibitory effect on subsequent activation of both Akt and MAPK via insulin receptors. Opioid receptor activation results in serine phosphorylation of IRS both in vitro and in vivo, at a site inhibiting tyrosine phosphorylation by the insulin receptor and thus diminishes the ability of insulin to activate PI3Kinase and consequently Akt. Opioid inhibition of MAPK signaling by the insulin receptor undoubtedly occurs by an entirely different mechanism and can be attributed to serine phosphorylation of the insulin receptor itself, which weakens the formation of a complex between IR, Shc, and Grb2. Since inhibitors of the MAPK pathway block opioid-induced serine phosphorylation of both IRS and IR, it appears that opioid receptors modulate insulin signaling via the MAPK cascade. This demonstrates receptor cross-talk among very different receptors.

The second example of signaling cross-talk concerns mu and delta receptors (Charles et al., 2003). Some cells, including the anterior pituitary cell line GH3, exhibit spontaneous oscillations in intracellular calcium levels. In GH3 cells expressing only mu receptors, selective mu agonists inhibit spontaneous calcium signaling via inhibition of calcium influx, activation of potassium channels and adenyl cyclase inhibition. However, in cells expressing both mu and delta receptors, mu agonists have a PKC-mediated excitatory effect on spontaneous calcium signaling. Several possible mechanisms could explain this altered mu-receptor mediated signaling, one attractive hypothesis is the formation of a mu delta receptor oligomer. An exciting area of regulation of G-protein coupled receptors that has recently emerged is the potential for receptors to form homo and heterooligomers (reviewed by Rios et al., 2001). In the case of the GABA-B receptor, expression of two distinct G-protein coupled receptors ($GABA\text{-}B_{(1A)}$ and $GABA\text{-}B_{(2)}$) is required for activity by GABA. Evidence that many other G-protein coupled receptors also form oligomers comes from many different biochemical, pharmacological and biophysical experiments. There is now a strong evidence for opioid receptor homo and heterooligomerization and importantly, implications for altered pharmacology. A second explanation for the altered mu signaling in GH3 expressing delta receptors is that delta receptors have constitutive activity and signal, albeit at a low efficacy, in the absence of an agonist (Milligan et al., 1997). This constant low-level activity of delta receptors could modify the composition and/or activity of mu receptor signaling complexes.

5. Opioid receptors as multiple complexes

Many convergent areas of receptor research including signaling, oligomerization and trafficking now visualize membrane receptors not as isolated units in the membrane but as dynamic complexes with many interacting proteins. Analysis of the NMDA receptor has been illustrative of the complexity with to date more than 180 anticipated protein partners (Grant, 2003). Clearly not all are partners with the NMDA receptor at the same time and many are competitive and their interaction dependent on numerous other factors such as the activation state of the receptor. It is anticipated that like NMDA receptors, opioid receptor complexes will also be extensive. Known proteins directly interacting with opioid receptors include other G-protein coupled receptors, G-proteins, G-protein coupled receptor kinases, arrestins and calmodulin (Wang et al., 1999). Many of these proteins in turn complex with other proteins. For example, the activated G-proteins interact with ion channels, various kinases, adenyl cyclase and regulators of G-protein signaling (RGS proteins). Additionally, arrestins can interact directly with clatherin, and calmodulin interacts with many proteins including several kinases.

At any one point, an opioid receptor complex generated would likely depend upon many factors including:

(a) The individual cellular proteome. The complex formed will be dictated by the levels of expression of individual proteins with ability to either directly interact with opioid receptors, modify other

proteins that can interact (e.g. by phosphorylation, alternative splicing, etc.) or compete for interacting proteins.

(b) The cellular compartment of the receptor i.e. endosome, extracellular membrane, secretory vesicle, dendrite or cell body. Each cellular compartment will have a different set of targeted proteins for interaction with the receptor.

(c) The activation state of the receptor. The activation state is likely the major influence upon the molecular architecture of the opioid receptor complex, mediating the interacting proteins in close proximity such as G-proteins, kinases and arrestins.

(d) The local history of the receptor and its environment. The recent activation of the receptor will clearly influence the complex as evidenced by agonist-regulated trafficking. Activation of other receptors in close proximity could also have a pronounced effect on the complex formed e.g. as a result of activated kinases, analogous to the effects of opioid receptor activation on insulin signaling as described above.

(e) Oligomerization with other receptors. Oligermerization could influence the binding of ligands as well as the proteins associated in the receptor complex.

Opioid receptor complexes could be enormously diverse both within the same cell and between different cell-types. Some of the complexes may be formed prior to ligand occupation (e.g. oligomeric forms of the receptor) and some may require agonist occupation (e.g. G-protein coupling and beta-arrestin binding). The nature and diversity of receptor complexes will presumably become clearer as proteomic approaches progress and have the required sensitivity to detect this diversity.

Considering the receptor as a component of a heterogeneous large complex begs the question of whether different agonists have the ability to promote or recognize different receptor complexes, and if so, is there possibility for ligand directed signaling. In the case of activation of mu receptors by morphine or DAMGO, it is clear that the morphine-occupied receptor ends up significantly less phosphorylated than the DAMGO-occupied receptor. This differential phosphorylation has been attributed to the activity of G-protein receptor kinases. The phosphorylated receptor appears a better substrate for beta-arrestin binding which in turn promotes clatherin-mediated endocytosis. Clearly this is suggestive of two different ligands promoting different receptor complexes. Although no signaling differences between DAMGO and morphine have been consistently reported other than efficacy of GTPgamma S binding, it is assumed that as we understand more about the functioning of these opioid receptor signaling complexes, differences will indeed emerge.

Returning to the question of whether it is pie in the sky to hope for less-addictive opioid drugs, I would answer no. My assumption is that the opioid receptor complexes formed in different cell-types and for different signaling pathways can be agonist-selective. Given that one particular signaling pathway may be critical for analgesia and another for plasticity, drugs distinguishing signaling complexes could be the key to developing less-addicitive opiates. Complex discrimination by an agonist is likely one of many potential mechanisms that could make a ligand a partial agonist and it is partial agonists at the mu receptor that perhaps should be carefully assessed for their addictive versus analgesic potential.

6. Mechanisms of plasticity

Opiate administration leads to major changes in behaviors such as tolerance, sensitization, craving and withdrawal. Presumably, changes in neuronal circuitry underlying these drug behaviors share mechanisms utilized in adapting to other environmental inputs, such as stress. As has been articulated elsewhere, the questions now being addressed in many areas of substance abuse research parallel closely those in other areas of plasticity research such as learning and memory. In recent years, substance abuse research has begun to focus on neural plasticity at multiple levels in attempts to identify the key changes in cells that correlate with drug behaviors. At the molecular and cellular levels, several signaling pathways, including the cAMP cascade and transcription factors such as deltaFosB, have been identified as possible players in the circuitry mediating drug-induced behaviors (reviewed by Nestler, 2004). A series of elegant studies have determined changes in the number of physical aspects of neurons as a result of chronic drug administration. In the case of opiate administration, changes in the number of dendritic spines in cortex and hippocampus have been observed (Robinson et al., 2002). Importantly, alterations in spine densities were observed one month following the drug treatment regimen, suggestive of a mechanism for long-term plasticity.

For many years opioid research has been modeling opioid adaptations such as tolerance and withdrawal in cells that contain opioid receptors. However, the brain is made up of circuits and networks that rely on each other for a behavioral outcome and clearly, adaptive processes could occur at any point in the network besides cells with opioid receptors. A recent study from CSORDA investigated morphine modulation of MAPK activation in the mouse brain (Eitan et al., 2003). MAPK is strongly implicated in synaptic plasticity as evidenced by numerous behavioral and electrophysiological studies (reviewed by Thomas and

Huganir, 2004). Acute morphine administration was shown to induce prolonged MAPK phosphorylation and thus activation in many areas of cortex. However, co-localization experiments showed the phospho-MAPK to be predominantly in cells that do not contain mu opioid receptors. This result implicates cells other than those containing mu opioid receptors as potentially important targets for plasticity changes underlying opiate-induced behaviors. A recent paper addressing the role of MAPK in the actions of cannabinoids provides strong evidence that the MAPK cascade is indeed involved in plasticity associated with tolerance (Rubino et al., 2004). Using Ras-GRF1 knock out mice, in which cannabinoids do not induce ERK activation, tolerance to THC analgesic and hypolocomotor activity was abolished. The studies of MAPK activity following systemic opiate and cannabinoid treatment show many parallels. Both studies show tolerance to MAPK phosphorylation in many brain areas after repeated exposure and both show pronounced instatement of agonist-induced MAPK activation in the hippocampus, after repeated agonist exposure. The instatement of morphine-induced MAPK activation after repeated morphine was in processes within the CA3 region of the hippocampus, an area implicated in mu opioid receptor dependent learning and memory processes (Meilandt et al., 2004). Such experiments are focusing research on specific areas of brain and indeed specific neuronal types to elucidate changes that may underlie drug-induced behaviors.

7. Summary

Over the last four decades, since the inception of NIDA, secrets of how opiate drugs target and regulate cells have been revealed. The molecular components of the endogenous system, including the endogenous opioid peptides and their receptors, have been mapped and signaling pathways elucidated. Mouse knockout studies have clearly identified the mu receptor as the principle target for opiate analgesia and reward. With our current understanding, we now must consider opioid receptors as heterogeneous protein complexes, each complex exhibiting a potentially unique pharmacology. Whether there is hope for ligands that distinguish signaling complexes and perhaps favor activation of selective mureceptor-mediated behaviors remains unknown but a likely outcome of our current knowledge. With regard to the future of opiate research, I consider the most exciting area is the analysis of adaptive mechanisms in the CNS leading to drug behaviors. The robust changes that acute and chronic administration of opiates incur on discrete behaviors are providing insights that will undoubtedly be relevant for normal adaptive responses to the environment. The challenge will not be in the finding of individual changes in molecules and circuitry as a result of drug administration, but in assembling the myriad of altered processes to explain behavior.

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