Use of in vivo apparent pA₂ analysis in assessment of opioid abuse liability

J. H. Woods, G. Winger and C. P. France

Abuse liability testing of opioid drugs was originally motivated by attempts to separate the analgesic effects of opioids from their likelihood for abuse. It has become apparent that the human population group likely to abuse opioids has little overlap with the population group requiring opioids to treat pain, therefore there is no longer a need to separate these two properties of opioids. This is fortunate, since, as reviewed here by Jim Woods and colleagues, the results of the plethora of studies that have attempted to distinguish these two properties in known opioids strongly indicate that they are inseparable. Evaluation of the abuse potential of novel opioids remains, however, critically important in deciding on governmental restrictions on their accessibility. In addition, opioid abuse liability testing contributes enormously to our understanding of the behavioral mechanism of action of these drugs, and in surprising and helpful ways has increased our appreciation of the various test systems used to garner information about them.

There are few substances in history that could have caused so much misery, and also given so much relief from misery, as opioid drugs. The desire to separate two of the predominant attributes of opioids— their ability to promote drug-seeking behavior and drug taking, and their ability to relieve pain— has been a driving force in much of the research on assessment of the abuse liability of this group of substances. Although it has recently been established that patients receiving or taking opioid drugs for relief of pain are at little risk of becoming opioid abusers¹ even if they control directly the frequency and dose of intravenous delivery, the need to continue careful evaluation of the abuse liability of opioid drugs remains strong. Data obtained from such evaluations are critical for governmental decisions about regulation and control of new opioid drugs. Furthermore, abuse liability testing has provided a continuous source of indispensable information about opioid drugs. Data generated by a variety of techniques for identifying chemicals as opioid-like, and data resulting from the many procedures used to compare these opioids to pharmacological standards, form the basis of our current understanding of the multifaceted pharmacology, biochemistry and neurochemistry of opioid drugs. This article seeks to demonstrate how opioid abuse liability testing continues to expand our knowledge about these compounds. Abuse liability testing involves a number of procedures, each contributing complementary information about the likelihood that a given compound has a risk of being abused. The attributes, described by Stolerman (TIPS, May 1992, pp. 170–176), include measures of physiological dependence capacity, and measures of the reinforcing and discriminative stimulus effects of psychoactive drugs have been applied frequently in investigations of opioid drugs. Some information on evaluation of the analgesic effects of opioid drugs is also included in this article because it is related to the clinical use of opioid drugs, and because it has provided data complementary to measurements directly related to their abuse.

Physiological dependence

Early opioid abuse liability testing was based on the hypothesis

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that the risk of abuse of opioid drugs was linked to their capacity to produce physiological dependence. Thus, it was almost exclusively limited to determining whether various test opioids were able to modify the withdrawal symptoms that developed when morphine-dependent rhesus monkeys or dogs were temporarily deprived of morphine. Medicinal chemists enthusiastically took on the task of synthesizing opioid compounds that might prove incapable of blocking morphine withdrawal and therefore, it was theorized, might have little abuse liability. As a result of this chemical effort, more than 1600 compounds have been synthesized and evaluated in morphine-withdrawn subjects; a considerable number of these have had very interesting and heuristic properties.

Nalorphine, one of the most interesting of the early test drugs, was found to reverse the effects of morphine and actually to induce withdrawal in morphine-dependent monkeys, dogs and humans. It had agonist effects as well, including analgesia, but was quite different from morphine in that it produced dysphoric effects both in patients needing pain relief and in former heroin addicts. Medicinal chemists were eventually able to resolve chemically the dual actions of nalorphine. 'Pure' opioid receptor antagonists, such as naloxone and naltrexone, and a group of non-morphine-like opioid analgesics, were developed. These latter compounds, including initially ketazocine and ethylketocyclazocine, and more recently tifluadom, U50488 and others, were identified as acting on an opioid receptor distinct from that on which morphine acted: this other receptor was given the designation \( \kappa \)-receptor, after the prototype agonist at this site, ketazocine. The designation \( \mu \)-opioid receptor was reserved for drugs with morphine-like actions.

In contrast to morphine-like drugs that attenuated the signs of morphine withdrawal, such as heroin (diamorphine), methadone, fentanyl and alfentanil, drugs with primarily \( \kappa \)-receptor activity did not reverse morphine withdrawal. Physiological dependence developed when \( \kappa \)-receptor agonists were given chronically to monkeys or rats, but the overt withdrawal signs were different from those that occurred in morphine-dependent subjects, and were not reversed by administration of morphine.

More recent studies of the effects of opioids in morphine-dependent monkeys have used the technique of drug discrimination. It is possible to train animal subjects to respond by pressing one lever when they are experiencing drug withdrawal (i.e. following administration of an opioid antagonist or upon termination of agonist treatment), and to respond by pressing another lever when they are not experiencing drug withdrawal. Increasing doses of morphine or other \( \mu \)-opioids such as fentanyl, methadone or butorphanol blocked the discriminated withdrawal signs and shifted the responding from the antagonist-appropriate lever to the saline-associated lever. These effects typically mirror those obtained in the earlier preparation where the effects of various opioids on morphine withdrawal were directly observed.

\( \kappa \)-Receptor agonists, including US0488 and ethylketocyclazocine, did not block the discriminative effects of morphine withdrawal. Neither did various non-opioids such as midazolam, ketamine, diazepam or haloperidol. This procedure is considerably more sophisticated in its approach to evaluation of the dependence liability of opioid drugs. Dose-response curves, with dose of opioid drug as the independent variable, and selection of the antagonist-appropriate lever as the dependent variable, can be readily obtained and shifted by prior administration of an antagonist. As shown in Table I, an apparent \( \mathrm{pA}_2 \) value of 7.6 was obtained in experiments in which various doses of quadazocine were given prior to an evaluation of the potency of alfentanil in suppressing naltrexone-associated responding in rhesus monkeys that had been deprived of morphine for 27 hours.

**Self-administration**

The realization that the reinforcing effects of opioids contribute importantly to their abuse led to the development of procedures that measure these effects. Popular current procedures involve preparing experimental animals (typically rats, rhesus monkeys or baboons) with indwelling intravenous catheters and giving them the opportunity to respond on levers and receive remotely delivered intravenous injections of opioid drugs.

A number of opioids have been studied in animal models of intravenous self-administration. The vast majority of opioids with predominant activity at the \( \mu \)-opioid receptor, including heroin, morphine, methadone, codeine, butorphanol, nalbuphine, buprenorphine, fentanyl and alfentanil, maintained self-administration under some conditions. The potency of these \( \mu \)-receptor agonists in maintaining self-administration paralleled their potency in many other assay systems, and suggested a common receptor for each of these actions. For example, there was an excellent correlation \(( r = 0.92) \) between the potencies of various \( \mu \)-opioids in maintaining self-administration and in suppressing the observable signs of morphine withdrawal.

Drugs with actions on the \( \kappa \)-opioid receptor, including ethylketocyclazocine and US0488, did not maintain self-administration behavior in rhesus monkeys. Ethylketocyclazocine was found to

| Table I. Apparent \( \mathrm{pA}_2 \) values for quadazocine used against behaviors induced by different opioid agonists |
|----------------|----------------|----------------|----------------|----------------|
| Agonist        | Analgesia      | Drug discrimination | Reinforcement | Abstinence reversal |
| Allantanil     | 7.6            | 7.9             | 7.6           | 7.6             |
| Morphine       | 8.2            | 7.8             | 7.6           | 7.6             |
| Levorphanol    | 6.1            | 6.3             | 6.4           | 6.4, 5.7        |
| Bremazocine    | 6.4            | 6.4             | 6.4           | 6.4, 5.7        |
| US0488         | 6.4            | 6.4             | 6.4           | 6.4, 5.7        |
| Ethylketocyclazocine | 6.4     | 6.4             | 6.4           | 6.4, 5.7        |

Data from Ref. 31.
maintain lever-pressing in the rat, although U50488 did not. This may suggest that ethylketocyclazocine, but not U50488, has an agonist effect at μ-receptors in the rat. Ketazocine produced a dysphoric effect in humans, quite different from morphine, suggesting that the drug would not be abused in man.

If a test drug is found to maintain responding that leads to its intravenous delivery, in animals experienced in self-administration, this does not necessarily mean that the reinforcing effects of this drug are mediated by a particular opioid receptor. Whether they are or not can be ascertained by establishing whether pretreatment with an opioid antagonist results in a shift to the right of the dose-response curve for the drug required to maintain lever pressing. For example, the reinforcing effects of μ-receptor agonists (e.g. heroin), but not the reinforcing effects of non-opioids (e.g. cocaine), can be antagonized by opioid antagonists. As shown in Table I, the interaction between alfentanil and quadaazocine has been further characterized by obtaining an apparent pA2 value; as indicated by the good correlation between potency in self-administration and in reversing morphine-withdrawal signs, the apparent pA2 value for quadaazocine in shifting these two effects was identical.

Drug discrimination

Establishing a drug as a discriminative stimulus in animal subjects has been compared to asking humans about the subjective effects of a drug. Hence, this evaluation is also an important aspect of a drug’s potential for abuse. Many opioid drugs have been used in several animal species to establish a drug discrimination. In most species tested, μ-agonists such as morphine, fentanyl, methadone, codeine, alfentanil, nalbuphine and etorphine all had discriminative effects in common with other similar agonists. The order of potency in producing discriminative effects was similar to the order of potency in producing other behavioral effects. Figure 1, as an example, demonstrates the correlation between the potency of μ-agonist compounds as discriminative stimuli and their potency in maintaining self-administration behavior.

κ-Receptor agonists did not produce morphine-like discriminative stimulus effects in monkeys or rats. They did substitute for each other, however. Ethylketocyclazocine, ketazocine, cyclazocine, U50488, tifluadom, nalorephine and bremaezocine are all drugs with identified κ-receptor agonist activities that produced ethylketocyclazocine-like or nalorephine-like discriminative effects in rhesus monkeys. On the other hand, methadone and morphine did not have discriminative stimulus effects in rhesus monkeys trained with nalorephine.

Opioid antagonists shifted the dose-response curve for the discriminative stimulus effects of both μ- and κ-receptor agonists to the right, indicating the opioid nature of these effects. The dose of antagonist necessary to shift the discriminative effects of κ-receptor agonists typically was different from the dose necessary to shift the discriminative effects of μ-receptor agonists. Table I illustrates this difference: the apparent pA2 value of quadaazocine in antagonizing the discriminative stimulus effects of three μ-receptor agonists was more than an order of magnitude greater than the apparent pA2 of quadaazocine in reversing the discriminative stimulus effects of three κ-receptor agonists. A similar difference in apparent pA2 values has been found using naloxone to reverse either ethylketocyclazocine or morphine.

Analgesia

Studies of the ability of various opioids to produce analgesia in animal models have complemented work that has been primarily focused on evaluation of the abuse liability of drugs. Although there are hundreds of studies of the analgesic effects of opioids in rodents using several different types of behavioral measures, those most relevant to the work described above have used rhesus monkeys as subjects. The latency with which the monkey removes its tail from a heated water-bath indicates the amount of analgesia produced by the drug (tail-flick or -withdrawal assay).

Traditional μ-opioid agonists such as morphine, alfentanil, nalbuphine and buprenorphine increased the latency of the tail-withdrawal response in the monkey. Traditional κ-opioid agonists such as ethylketocyclazocine, tifluadom and U50488 were also effective in this assay. As has been found in tests of the discriminative effects of μ- and κ-receptor agonists, larger doses of naloxone and quadaazocine were necessary to antagonize the anal-
Receptor-related issues

The interaction between drugs and opioid receptors is more complicated and more interesting than simply whether observed effects can be attributed to either a μ- or a κ-receptor. In the μ-receptor system, for example, there are intriguing examples of drugs that are fully effective in some conditions, yet have little effect, or even antagonist effects, in other conditions.

Nalbuphine, for example, was fully effective in attenuating morphine withdrawal when a discriminative stimulus measure of withdrawal was used, but it precipitated abstinence when directly observable effects in morphine-dependent monkeys were recorded. Similarly, nalbuphine produced full analgesia in the monkey tail-withdrawal assay using 50°C water, but did not produce analgesia when 55°C water was used. At the higher water temperature, nalbuphine antagonized the effects of antinociceptive stimuli in this assay [Walker, E. (1989) PhD Thesis].

Nalbuphine had full morphine-like discriminative stimulus effects when the training dose of morphine was relatively small, but had only partial morphine-like discriminative stimulus or antagonist effects (A. M. Young, pers. commun.) when the training dose was larger. One interpretation of these results is that different amounts of stimulation (i.e., agonist efficacy) are needed to produce an effect under all these conditions. Where a drug has limited efficacy, it might not produce a full effect under all test conditions. Nalbuphine thus appears to be a low-efficacy μ-receptor agonist.

The data also indicate that different assay systems are differentially sensitive to μ-agonist stimulation, a situation that has been noted with in vitro assays as well. Furthermore, within a single assay system, it is possible to ‘adjust’ the sensitivity of the system by increasing the response requirement in measures of reinforcing stimulus effect, by increasing the dose of the training drug in measures of discriminative stimulus effect, by making animals more dependent in measures of morphine withdrawal attenuation, or by increasing the temperature of the water in an analgesia assay, the efficacy requirements of the system appear to be increased. Thus, the efficacy of the drug, the efficacy requirements of the assay, and the number of spare receptors combine to determine whether an agonist or antagonist effect of a low-efficacy agonist are observed. Characterization of agonists according to their relative efficacies provides yet another theoretically, as well as empirically, important dimension for describing the pharmacology of opioids.

Various measures of the abuse liability of opioid drugs, in conjunction with measures of their analgesic effects, indicate that μ-opioids produce their effects through a different receptor than that responsible for the effects of κ-opioid drugs, and the same μ-receptor underlies each of the measures related to abuse liability as well as to the analgesic effects of μ-opioid drugs. Current information, therefore, indicates that the dependence-producing, reinforcing stimulus, discriminative stimulus, and analgesic effects of morphine-like drugs cannot be separated on a pharmacodynamic basis. Needless to say, a subtype of μ-opioid receptor may eventually be discovered that mediates a subset of these effects. Thus, it remains possible that analgesia will one day be separated from factors contributing to abuse of these drugs.

Evaluation of the abuse liability of opioid drugs clearly does not involve a simple ‘yes’ or ‘no’ answer from a single behavioral assay system. The sensitivity of any assay system will vary depending on the parameters used in that system, and the effect of any test compound will depend on the sensitivity of the test system as well as on the efficacy of the drug under investigation. Therefore, a broad approach to behavioral evaluation of opioid drugs, in which test drugs can be compared with many other drugs in a wide range of assay systems, seems most able to provide information necessary for forecasting their abuse potential.

References

Cytokines and neuropathology

Maria Cristina Morganti-Kossmann, Thomas Kossmann and Sharon M. Wahl

Inflammatory processes in the brain require the cooperation of immuno-competent cells and glial cells, which communicate by secreting bidirectional mediators. Resident cells within the nervous system can synthesize and secrete inflammatory cytokines, as well as neuropeptides, contributing to the response within the CNS to injury or immunological challenge. Although the mechanisms of cell activation and immune interaction are poorly understood, accumulating evidence implicates these pathways in neuropathogenesis, as described here by Sharon Wahl and colleagues. For example, in the acquired immune deficiency syndrome (AIDS), HIV-1-induced nervous system dysfunction and dementia are associated with the presence of infiltrating leukocytes and the release of inflammatory cytokines. Defining the pathways of cytokine dysregulation and neurotoxicity invoked by the infiltrating leukocytes, as well as the contribution of the neural cells themselves, may help to identify mechanisms of intervention in this and other debilitating CNS diseases.

Recent evidence suggests that bidirectional communication occurs between cells of the nervous and immune systems. The basis for this communication is the release of soluble molecules or cytokines by immunocompetent cells, as well as hormone products of the neuroendocrine system. Not only are these systems integrated under normal physiological conditions, but the aberrant regulation of one system, by the cells and products of the other, may be responsible for the development of pathological conditions. Integrity of the blood-brain barrier and alterations of this barrier in brain pathology also influence interactions between these two systems.

In diseases of the nervous system caused by viral and bacterial infections, autoimmune diseases or after traumatic injury, there may be infiltration of cells of the immune system, including T and B lymphocytes and mononuclear phagocytes, and release of cytokines may occur. Moreover, under certain conditions, resident cells within the nervous system, particularly astrocytes and microglia, may function as immunocompetent cells. Astrocytes were originally considered to function primarily in a support capacity for neurons, but have recently been shown to perform a variety of functions, many of which overlap with those of microglia, the resident macrophages of the CNS.

Cytokine expression in brain pathology

Participation of astrocytes in the host response to immunological challenge, and expression of macrophage-like phenotype and function by these cells, is a focus of the emerging field of neuroimmunology. As a dominant cell type within the brain, astrocytes have the capacity to proliferate, form the glial scar in astroglisis, phagocytose, and secrete mediators and cytokines central to the inflammatory process, when appropriately challenged. One of these cytokines, interleukin 1 (IL-1), was first described as a factor produced by macrophages with the ability to promote T-cell proliferation. Subsequently, IL-1 has been shown to be produced by a wide range of cell types, and to exhibit a variety of functions. Within the nervous system, the synthesis of IL-1 has been attributed to activated astrocytes and microglia in disease (Table I), while in normal brain, IL-1 is reportedly associated with neurons that also express IL-1 receptors. By increasing adhesion of leukocytes to endothelial cells, IL-1 may promote recruitment of leukocytes through the blood-brain barrier, favoring the onset of an inflammatory process in the CNS.

Another cytokine with multifunctional properties found within the CNS is tumor necrosis factor \( \alpha \) (TNF-\( \alpha \)), initially identified by its cytotoxic effects on tumor cells. In culture, glial cells and microglia stimulated with lipopolysaccharide release a cytotoxic factor that can be specifically inactivated by anti-TNF-\( \alpha \). Moreover, TNF-\( \alpha \) also operates by...