

## Opioid drugs as reinforcing stimuli in rhesus monkeys

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Working in a laboratory at the University of Michigan that was originally designed in the 1960s by Dr. Tomoji Yanagita, we have been evaluating opioid drugs for their ability to maintain behavior in rhesus monkeys. The animals are prepared with intravenous catheters that can be accessed from the outside of the cage. Inside the cage are two response levers and several stimulus lights. When one of the lights is turned on, it signals to the monkey that it can receive an intravenous injection of a centrally acting drug as a consequence of responses on the lever.

Mu opioid agonists, particularly heroin, present serious human abuse problems in the United States and other countries. These drugs also maintain lever responding by monkeys. Kappa opioid agonists do not maintain responding and may be aversive to animals and to humans. Delta opioid agonists also fail to maintain lever responding by monkeys. By using pA<sub>2</sub> analysis we have determined that the same opioid receptor ( $\mu$ ) mediates the analgesic, respiratory depressant, and reinforcing effects of opioid drugs, making it unlikely that abuse liability and analgesic effects can be effectively separated.

It is commonly acknowledged that human heroin addicts take increasing amounts of heroin over time. A similar pattern of increasing opioid intake was shown many years ago in both rats and rhesus monkeys. Although this increasing intake may be the result of tolerance development, it is not clear that the tolerance was to the reinforcing effects, as compared with the rate-suppressing effects, of the opioid. In our studies, we found that daily administration of morphine produced a slight decrease in the potency of morphine and heroin as reinforcers, a much larger decrease in the potency of the partial agonists buprenorphine and nalbuphine, and no change in the potency of alfentanil. This suggests that tolerance to the reinforcing effects of opioids depends on the efficacy of the individual agents.

We have evaluated the ability of a new treatment agent, the partial agonist buprenorphine, to modify the reinforcing effects of alfentanil. Although buprenorphine is an effective reinforcer, it is also able to decrease the potency of alfentanil as a reinforcer

in a dose-related manner. In this model, buprenorphine appears to act as an antagonist to modify alfentanil's reinforcing effects.

Our recent work has concentrated on experiments that can measure the effectiveness of drugs as reinforcers. To do this we have borrowed concepts from economics to evaluate the effects of increased price on the demand for drugs. It is commonly known using various commodities that demand for a product decreases as price increases. This is true using drugs as well when the price is adjusted by changing the number of responses necessary to earn a drug injection. One parameter of demand curves is the Pmax, the price at which the maximum number of responses are made. This can be used as an indicator of the reinforcing effectiveness of a drug. We have found using demand curve analysis that alfentanil is a more effective reinforcer than nalbuphine, a partial mu agonist. Alfentanil is nearly equal in reinforcing effectiveness as cocaine, and more effective than the short-acting barbiturate, methohexital. We have also found that, in our procedures, three opioids with different durations of action but similar onsets of action, fentanyl, alfentanil, and remifentanyl, are nearly equally effective as reinforcers, indicating that onset of action may be more important than duration of action in determining the abuse liability of a drug.

## Anti-depressant activity of delta opioid receptor activation

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Recently, we summarized (Broom et al., 2002) the evidence that supported the proposition that activation of the delta opioid receptor by direct-acting agonists or by enkephalinase inhibitors conveys behavioral effects in forced swimming assays that predict antidepressant-like potential. In addition to this interesting behavioral effect, delta agonists produce a variety of other receptor-mediated effects in rodents, e.g., antinociception and convulsions. We have shown that, although different behavioral effects occur at similar doses, the antidepressant effect is dissociable from other effects of delta agonists. For example, profound tolerance develops to the convulsant effect in mice and rats without a commensurate tolerance to the antidepressant-like effect. In addition, the convulsion can be eliminated by midazolam without interfering with the antidepressant-like effect.

It has been shown by others and us that locomotor activity is increased by delta agonists, and this effect is associated with evidence from conditioned place preference for rewarding effects of these compounds (Spina et al., 1998). We (Gomez et al., Submitted) have carried out a study in rats of self-injection with a delta agonist following prior experience with, in different groups, milk or milk and nomifensine conditioning. Though the delta agonist produced a transient increase in responding, when substituted for either milk or nomifensine, it failed to sustain responding indicative of a positive reinforcing effect in the rat. This finding supports earlier research by us in rhesus monkeys suggesting that delta agonists fail to maintain reinforced responding (Negus et al., 1994).

Delta agonists have been shown to interfere with learning under certain circumstances in primates and rodents (Pakarinen et al., 1995). We have carried out a study to examine if the reduced immobility could be due to a learning deficit produced by the delta agonist. We compared a delta agonist directly with scopolamine, a drug with well-documented effects on acquisition processes. The compounds were quite different in their behavioral profiles; the delta agonist induced reductions in immobility at doses that produced very little effect on acquisition while the reverse was the case with scopolamine (Jutkiewicz et al., In press).

Many of the medically approved antidepressants and non-drug approaches to treating depression have in common (Duman, 2002) the ability to induce slowly an upregulation of brain-derived neurotrophic factor (BDNF). We have recently shown that delta agonists will upregulate BDNF; its regulation appears not to be dependent upon convulsant effect and is different from other compounds that have antidepressant activity (Torregrossa et al., 2003) in that the effect occurs with the first dose.

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## **Substance P (SP) and its bioactive fragment SP(1-7) in relation to opioid tolerance and withdrawal.**

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The neuroactive undecapeptide substance P (SP; Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub>) is widely distributed in the central nervous system (CNS). Tissue levels of SP is regulated by several enzymes, one of which is substance P endopeptidase (SPE), known to release the bioactive N-terminal fragment SP(1-7) from the parent compound (Persson et al., 1995). SP preferentially acts on the neurokinin-1 (NK-1) receptor, which is highly expressed in CNS areas that are critical for the regulation of pain influx, affective behavior and stress. Moreover, earlier studies have implicated the substance P (SP) system in opioid reward and dependence. It is thus shown that the NK-1 receptor has a potential role in opioid reward (Murtra et al., 2000). It is further demonstrated that SP potentiates the intensity of the reaction to opioid withdrawal, an effect which is counteracted by the heptapeptide fragment SP(1-7). In recent years we have particularly focused our investigation of the SP system on SP(1-7) and SPE. We observed a concomitant increase in the level of the heptapeptide and SPE, as well, in brain areas related to opioid reward during naloxone precipitated withdrawal (Zhou et al., 1998, 2001). A significant correlation between several signs of opioid withdrawal and the SPE activity was found. Recently, we investigated the effect of SP(1-7) on the expression of the dopamine receptors D1 and D2 receptor mRNA in the brain of morphine dependent male rats (Zhou et al., 2003). We also examined the effect of SP(1-7) on dopamine release using a microdialysis technique. In the first experiment the peptide was infused through an implanted cannula aimed to the lateral ventricle 30 min before a naloxone challenge in rats, previously treated with twice daily injections of morphine for 7 days. The animals were decapitated 4h after naloxone injection. The result indicated that SP(1-7) induced an alteration in the expression of the receptor gene transcripts in nucleus accumbens and frontal cortex (Zhou et al., 2003). In the microdialysis experiment the probe was inserted into the nucleus accumbens. HPLC combined with electrochemical detection was used to quantitate dopamine and its metabolite DOPAC. It was found that the SP fragment produced a significant increase in both dopamine and DOPAC in this particular brain area (Zhou and Nyberg, 2002). It was concluded that SP(1-7) may elicit an increased release of dopamine and thereby counteract the withdrawal reaction. This findings together with other observations showing that SP(1-7) opposes the effect of SP suggest neuropeptide conversion as an important pathway in neuromodulation (Hallberg and Nyberg, 2003).

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