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Neurochemical Alterations in Physical Dependence on Opioids

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Introduction

Physical dependence on opioids such as morphine and butorphanol is identified, both experimentally and clinically, by the expression of excitatory physiological responses. Hyperactivity of neurons within the locus coeruleus (LC) has become a focal point for the investigations into the genesis of excitation associated with opioid withdrawal. Glutamate, an excitatory amino acid, has been suggested to mediate some component of the increase in neuronal activity (Rasmussen et al., 1990; Tanganelli et al., 1991). The studies conducted in our laboratory support the role of excitatory amino acids within the LC in opioid withdrawal.

Models developed in our laboratories have shown that continuous i.c.v. infusion with morphine or butorphanol can produce physical dependence in the rat (Horan and Ho, 1991; Jaw et al., 1994). The degree of development of dependence on an opioid is quantitated by evaluation of physiological and/or behavioral responses that occur shortly following termination of opioid administration or challenge with an opioid receptor antagonist. Administration of an opioid antagonist can precipitate a more dramatic withdrawal syndrome, when compared with

the "natural" withdrawal syndrome. The use of antagonist-precipitated withdrawal allows behavioral measurements to be restricted to a 30 min period immediately following antagonist administration. The roles of μ-, δ-, and κ-opioid receptors in mediation of physical dependence on morphine or butorphanol had also been systemically investigated using receptor-selective antagonists (Fan et al., 2002). Results obtained demonstrated that different opioid receptors play various roles depending on different classes (either μ - or κ-preferring) of opioid receptor agonist used.

Recently, our studies have developed 2-dimensional electrophoresis (2-DE) methods to perform proteomic analysis of phosphotyrosyl (*p*-Tyr) proteins in the frontal cortex of opioid-dependent rat brains (Kim et al., 2004). When compared to the 2-DE signature of *p*-Tyr protein spots in brains from control rats, the densities of most corresponding spots were increased in opioid dependent rat brains. Additional *p*-Tyr protein spots not found in controls were detected in opioid-dependent rat brains. Of these unique *p*-Tyr proteins, many could be identified as being involved in cell signaling, cell cytoskeleton/differentiation, and intermediary metabolism.

Materials and Methods

All animal use procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 250-275 g were purchased and housed, prior to surgical intervention, in groups of three animals in plastic cages. They were acclimated for 1 wk under controlled conditions of temperature (22 ± 2°C), light cycles (12/12 h light/dark cycle), and humidity (50-55%) with free access to food and water.

Experimental Procedures

In all experiments, rats were rendered physically dependent on opioids by i.c.v. infusion of morphine sulfate (26 nmol/μl/h) or butorphanol tartrate (26 nmol/μl/h) for 72 h through osmotic minipumps (Horan and Ho, 1991). The procedures for conducting the experiments on the role of glutamate in opioid dependence have been described previously (Feng et al., 1997; Tokuyama et al., 1996; Zhang et al., 1994). The experiments performed on binding assays for μ-, δ-, and κ-opioid receptors by autoradiography were conducted as described in the studies of Fan et al. (2002). The study on proteomic analysis of *p*-Tyr proteins was described in the paper of Kim et al. (2004).

Results

1. *Glutamate system in opioid dependence*

Results obtained indicate that extracellular fluid (ECF) levels of glutamate were increased in the region of the LC in naloxone-precipitated morphine- or butorphanol-withdrawal rats (Zhang et al., 1994; Feng et al., 1994). A positive correlation was also noted between the dose of naloxone used to precipitate withdrawal and the ECF level of glutamate. Nor-binaltorphimine, a specific antagonist of the κ-opioid receptor, significantly precipitated withdrawal symptoms and increased glutamate levels in the butorphanol-withdrawal rats but not in the morphine-withdrawal animals (Feng et al., 1997). The direct injection of naloxone or glutamate, i.c.v., also dose-dependently induced withdrawal signs in morphine- or butorphanol-dependent animals. The withdrawal signs precipitated by the glutamate injection were comparable to those precipitated by naloxone, except for the expression of certain specific behaviors and the duration of withdrawal signs. The expression of the withdrawal signs

precipitated by glutamate or naloxone in opioid-dependent animals was completely blocked by pretreatment with MK-801, indicating that opioid withdrawal may be partly mediated through NMDA subtype of glutamate receptors.

2. Opioid receptors in opioid dependence

Differential changes in opioid receptor bindings in morphine and butorphanol-withdrawal rats have also been observed (Fan et al., 2002). Autoradiographic characterization of binding for brain κ_1-([^{3}H]Cl-977), μ-([^{3}H]DAMAGO), and δ-([^{3}H]DPDPE) opioid receptors was performed in rats undergoing naloxone-precipitated withdrawal from dependence on morphine or butorphanol. Mu-opioid receptor binding decreased in some of the brain regions in naloxone-precipitated withdrawal from morphine, but not butorphanol, while binding for δ-opioid receptors was altered in both withdrawal groups.

During withdrawal from butorphanol, but not morphine, κ_1-opioid receptor binding was increased significantly in these brain regions.

Changes in the levels of the μ-opioid receptor by immunoblotting analysis were observed in 11 of 21 brain regions examined in morphine-withdrawal rats, but only in 3 of 21 in butorphanol-withdrawal rats (Fan et al., 2003). In contrast, immunoblotting analysis of κ-opioid receptors from butorphanol-withdrawal rats showed significant increases in11 of 21 brain regions examined.

3. Phosphotyrosyl proteins in opioid dependence

Similar patterns of protein expression were detected in 2-DE gels of opioid-dependent and saline-treated control rat brains (Kim et al., 2004). However, *p*-Tyr 2-DE images of opioid-dependent rat brains differed from those of controls. All *p*-Tyr protein spots detected in control samples were also detected in both morphine- and butorphanol-dependent samples. The

densities of most matched *p*-Tyr protein spots were increased in opioid-dependent rat brains as compared with the controls. Additional *p*-Tyr protein spots were detected in opioid-dependent rat brains. Fifty *p*-Tyr protein spots were identified in morphine-dependent rat brains and fifty-three *p*-Tyr protein spots were identified in butorphanol-dependent rats. The identified *p*-Tyr proteins are known to be involved in cell signaling, cell cytoskeleton/ differentiations, and intermediary metabolism. Forty-three proteins were identified in rats dependent on both opioids. Five of these proteins, which included GTP-binding proteins, were detected as *p*-Tyr proteins only after chronic administration of opioids.

Conclusions

The results of our studies indicate that ECF levels of Glu are increased in the region of the LC during naloxone-precipitated opioid withdrawal. Furthermore, a positive correlation was noted between the dosage of naloxone used to precipitate withdrawal and the ECF levels of Glu. The study provides direct evidence that glutamatergic neurotransmission may play an important role in acutely precipitated withdrawal from opioids. The data obtained further indicate a significantly greater participation of κ-opioid receptors in the development of butorphanol, rather than morphine dependence and identify a differential neurochemical response to butorphanol withdrawal within a defined brain region, the LC.

Results from another set of studies indicated that κ-opioid receptors were differentially and preferentially altered during withdrawal from dependence on butorphanol, as opposed to morphine. This differed from the lack of effect on μ-opioid receptor binding in many brain regions of naloxone-precipitated butorphanol withdrawal rats. In contrast, both μ- and δ-opioid receptor binding was significantly altered in the brains of naloxone-precipitated morphine-

withdrawal rats. The immunoblotting studies of κ- and μ-opioid receptor proteins also substantiate the results obtained from the ligand binding study.

In the proteomic study, we have been the first to establish phosphotyrosine proteome resources for opioid dependence using an animal model. These studies indicate the potential of proteomics as an effective technique for studying tyrosine phosphorylation events of opioid dependence in the brain.

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