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Neurochemical Alterations in Physical Dependence on Butorphanol

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Abstract – This review focuses on findings regarding neurochemical changes in physical dependence on butorphanol, a relatively potent mixed agonist-antagonist opioid analgesic agent that is five times more potent than morphine in antinociceptive effects. The chronic administration of butorphanol induces physical dependence. Withdrawal from such dependence can be reliably precipitated by administration of a narcotic antagonist, e.g., naloxone. Evidence for critical involvement of excitatory amino acid (glutamate), opioid receptors, and phosphorylation of proteins in these phenomena is summarized.

Keywords □ Butorphanol, Physical dependence, glutamate, opioid receptors, phosphotyrosyl proteins

The results of our studies indicate that extracellular fluid (ECF) levels of glutamate are increased in the region of the locus coeruleus 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 glutamate. Thus, direct evidence is available to support an important role of glutamatergic neurotransmission 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 with in a defined brain region, the locus coeruleus.

Results from another set of studies indicate 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. Immunoblotting studies of κ- and μ-opioid receptor proteins substantiate the results obtained from the ligand binding study.

Our laboratory is the first to establish phosphotyrosine proteomic resources to investigate opioid dependence using an animal model. These studies indicate the potential of proteomics as an effective technique for studying tyrosine phosphorylation events during opioid dependence in the brain.

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INTRODUCTION

Butorphanol tartrate is a member of the phenanthrene class of opioids which produces analgesic effects by acting mainly on the κ-opioid receptors (Chang *et al.*, 1983; Dobkin *et al.*, 1976; Lahti *et al.*, 1985). It is a relatively potent synthetic agonist-antagonist opioid analgesic agent (Peachey, 1987; Pircio *et al.*, 1976) and is considered to have lower dependence liability and lower abuse potential than morphine (Peachey, 1987). It has also been reported to have rewarding properties (Mamoon *et al.*, 1995). Cases of abuse involving injectable or inhaled preparations of butorphanol have been reported (Fisher and Glass, 1997; Smith and Davis, 1984).

Butorphanol dependence has been documented in humans (Brown, 1985; Evans *et al.*, 1985) as well as in animals (Horan and Ho, 1991; Pircio *et al.*, 1976; Woods and Gmerek, 1985) following chronic administration of high doses of butorphanol. Studies from our laboratories have demonstrated that butorphanol administration induces dependence through its action on κ-opioid receptors in rats (Feng *et al.*, 1997). Similarly rats subjected to continuous infusion with butorphanol (26 nmol/μl/hr) intracerebroventricularly (i.c.v.) for 72 hours have been shown to develop physical dependence (Jaw *et al.*, 1993 a, b; 1994).

Various behavioral, functional, electrophysiological, biochemical, and molecular studies have suggested possible neuronal systems involved in the development of physical dependence on butorphanol and the expression of many of the signs of withdrawal (Liu *et al.*, 1999; Zhu *et al.*, 1998). The present review summarizes the results obtained from our laboratory regarding neurochemical changes involved in physical dependence on butorphanol.

Glutamate in butorphanol dependence

Several lines of evidence implicated a critical role for excitatory amino acid, and particularly glutamatergic, systems in butorphanol dependence. Microdialysis studies from the laboratories of Aghajanian *et al.* (1994) as well as from our laboratories (Zhang *et al.*, 1994; Feng *et al.*, 1995; Hoshi *et al.*, 1997) have demonstrated that increased extracellular fluid levels of excitatory amino acid neurotransmitters within the locus coeruleus (LC) occur contemporaneously with precipitated withdrawal from dependence on morphine, which acts preferentially as a μ-opioid receptor agonist. The results of our studies (Feng *et al.*, 1995) were the first to demonstrate that ECF levels of glutamate are elevated in the LC during naloxone-precipitated withdrawal from dependence on butorphanol. This provided direct evidence that increases in brain levels of glutamate may represent a general phenomenon of opioid antagonist-precipitated withdrawal from opioid dependence. Such an increase in glutamate efflux is consistent with the tentative conclusion that precipitated withdrawal stimulates release from excitatory amino acid-containing nerve terminals within the LC. This conclusion is supported further by the results of our study (Feng *et al.*, 1995) which demonstrated that withdrawal from butorphanol elicits effects similar to those seen in morphine withdrawal. Furthermore, we have shown that focal injection of glutamate into the LC induced withdrawal signs in butorphanol or morphine-dependent rats, but not in opioid-naïve animals (Tokuyama *et al.*, 1996).

With these interesting findings, we investigated the relative involvement of κ-opioid receptors in the mediation of behavioral and neurochemical responses to withdrawal from butorphanol using *in vivo* microdialysis to detail ECF fluid concentrations of glutamate and aspartate within the LC (Feng *et al.*, 1997). Nor-binaltorphimine, a κ-opioid receptor antagonist, was used to differentiate the withdrawal of butorphanol from that of morphine. Nor-binaltorphimine increased release of glutamate and aspartate in the butorphanol-dependent animals, but not in the morphine-dependent animals. These results indicate a significantly greater participation of κ-opioid receptors in the development of dependence on butorphanol, as opposed to morphine, and identify a differential neurochemical response to butorphanol withdrawal within a defined brain region, the LC. The role of κ-opioid receptors in κ-opioid agonist-induced dependence was also substantiated by a similar study using the relatively pure κ-opioid agonist, U-69,593 (Hoshi *et al.*, 1997).

Data obtained from our laboratory further delineate the role of sub-types of glutamate receptors in butorphanol dependence. An autoradiographic study of NMDA-displaceable [^{3}H]-glutamate binding during butorphanol withdrawal in the rat brain (Jang *et al.*, 1999) showed significant increases of such binding in various brain regions, suggesting that stereospecific binding sites for NMDA on the NMDA receptor/cationic channel complex may play a significant role in butorphanol dependence.

Opioid receptors in butorphanol dependence

Earlier studies from our laboratory have suggested that κ-opioid receptors preferentially mediate the development of physical dependence on butorphanol and its withdrawal syndrome. Both nor-binaltorphimine and naltrindole (a δ-opioid receptor selective antagonist) have been shown to precipitate withdrawal signs similar to those precipitated by the non-selective opioid receptor antagonist, naloxone, in butorphanol-dependent rats. β-Funaltrexamine, a μ-opioid receptor selective antagonist, failed to precipitate withdrawal in butorphanol-dependent rats. This preferential involvement of the κ-opioid receptor is unique to butorphanol (and perhaps other selective κ-opioid receptor agonists) and is not seen or is much less prevalent in morphine dependence. Thus, nor-binaltorphimine blocked the development of physical dependence on butorphanol when given prior to or during butorphanol administration, whereas nor-binaltorphimine fail to block development of morphine dependence in a similar treatment protocol.

Further studies also indicated significant alterations in κ_1-opioid receptor binding in several brain regions within the forebrain, diencephalon, midbrain, and pons in rats that had been rendered dependent on butorphanol and in which withdrawal was precipitated by naloxone (Fan *et al.*, 2002b). In contrast, no changes could be found in μ-opioid receptor binding while changes in δ-receptor binding were less marked and occurred in a smaller set of brain regions (Fan *et al.*, 2002b). Significant regionally specific changes in the binding of κ_1, κ_2, and total κ-opioid receptor among brain regions of butorphanol-dependent rats were also demonstrated (Fan *et al.*, 2002a). These binding data could be explained by either increased binding

affinity or from increases in total binding of κ_1- and κ_2-opioid receptors (Fan *et al.*, 2002b). A subsequent immunoblotting analysis substantiated the findings that naloxone-precipitated withdrawal from physical dependence on butorphanol was associated with regionally specific changes in levels of brain κ-opioid receptor proteins (Fan *et al.*, 2003a). The study indicated that increases in the levels of the κ-opioid receptor protein occur as a major response to naloxone-precipitated withdrawal from physical dependence on butorphanol.

Competitive binding assays with the selective κ-opioid antagonist, nor-binaltorphimine, were performed in brain sections taken from rats infused i.c.v. with butorphanol (Fan *et al.*, 2003b). The IC_{50} values for displacement of [^{3}H]CI-977 (for κ_1-opioid receptors) and [^{3}H]bremazocine (in the presence of DAMGO, DPDPE, and U-69,593, for κ_2-opioid receptors) binding by nor-binaltorphimine were significantly decreased in both butorphanol-dependent and butorphanol-withdrawal groups in all brain regions examined, when compared with those of the controls. Both in rats that were butorphanol-dependent and in those undergoing butorphanol withdrawal, IC_{50} values for binding of nor-binaltorphimine to brain κ_1- and κ_2-opioid receptors were 5-30-fold lower than in controls, indicating the development of a greater degree of sensitivity to antagonist binding. These data are similar to the finding that κ_1-opioid receptors became supersensitive to binding of the selective antagonist, nor-binaltorphimine, in the spinal cord of rats made dependent on butorphanol following 3-day intrathecal infusions (Wongchanapai *et al.*, 1998a, b).

Phosphotyrosyl proteins in butorphanol dependence

Comparative brain proteomic analysis has been shown to provide expression profiles of proteins and their posttranslational modifications in specific brain regions (Edgar *et al.*, 1999). Proteomic analysis of dynamic phosphorylation events and identification of phosphoproteins in specific brain regions from butorphanol-dependent animals was hypothesized to provide novel and unique markers for identifying butorphanol dependence. Therefore, proteomic analysis of phosphotyrosyl proteins in butorphanol-dependent rats was performed.

The relatively high density of opioid receptors in brain frontal cortex and the need for sufficient tissue to carry out two-dimensional electrophoresis/mass spectrometry (2-DE/MS) analysis led to selection of this brain region of our study (Kim *et al.*, 2004). A 2-DE map of proteins expressed in the brain frontal cortex from butorphanol-dependent rats was established. Alterations in tyrosine phosphorylation of frontal cortical proteins induced by chronic butorphanol administration were also determined using immunoblotting and proteomic analysis. Phosphotyrosyl (p-Tyr) proteins were detected by immunoblotting with an anti-p-Tyr monoclonal antibody. To improve the resolution of p-Tyr proteins in 2-DE gels, three different narrow-range immobilized pH gradient (IPG) strips, as well as a broad-range IPG strip, were examined. The protein spots showing predominant changes in tyrosine phosphorylation compared to those in saline-treated control samples were identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Our results revealed that similar patterns of protein expression were detected by 2-DE in brain frontal cortex of butorphanol-dependent and saline-treated control rats. All 65 phosphotyrosyl (p-Tyr) protein spots detected in pH 3-10 phosphotyrosine 2-DE of control rat brains were detected in butorphanol-dependent rat brains. However, the densities of most p-Tyr protein spots were increased in butorphanol-dependent rat brains compared to saline-treated control samples. Eighteen additional p-Tyr protein spots were detected in pH 3-10 2-DE images from butorphanol-dependent rat brains. Immobilized pH strips with three different narrow pH ranges were examined to improve the resolution of p-Tyr proteins in 2-DE gels. Fifty-three p-Tyr protein spots were identified as proteins known to be involved in cell cytoskeleton, cell metabolism, and cell signaling. This proteomic approach can provide useful information for understanding the complex mechanism of butorphanol dependence *in vivo*.

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