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Morphine-induced analgesic tolerance, locomotor sensitization and physical dependence do not require modification of mu opioid receptor, cdk5 and adenylate cyclase activity

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Abstract

Acute morphine administration produces analgesia and reward, but prolonged use may lead to analgesic tolerance in patients chronically treated for pain and to compulsive intake in opioid addicts. Moreover, long-term exposure may induce physical dependence, manifested as somatic withdrawal symptoms in the absence of the drug. We set up three behavioral paradigms to model these adaptations in mice, using distinct regimens of repeated morphine injections to induce either analgesic tolerance, locomotor sensitization or physical dependence. Interestingly, mice tolerant to analgesia were not sensitized to hyperlocomotion, whereas sensitized mice displayed some analgesic tolerance. We then examined candidate molecular modifications that could underlie the development of each behavioral adaptation. First, analgesic tolerance was not accompanied by mu opioid receptor desensitization in the periaqueductal gray. Second, cdk5 and p35 protein levels were unchanged in caudate-putamen, nucleus accumbens and prefrontal cortex of mice displaying locomotor sensitization. Finally, naloxone-precipitated morphine withdrawal did not enhance basal or forskolin-stimulated adenylate cyclase activity in nucleus accumbens, prefrontal cortex, amygdala, bed nucleus of stria terminalis or periaqueductal gray. Therefore, the expression of behavioral adaptations to chronic morphine treatment was not associated with the regulation of mu opioid receptor, cdk5 or adenylate cyclase activity in relevant brain areas. Although we cannot exclude that these modifications were not detected under our experimental conditions, another hypothesis is that alternative molecular mechanisms, yet to be discovered, underlie analgesic tolerance, locomotor sensitization and physical dependence induced by chronic morphine administration.

Keywords: Mouse; Opioid; Tolerance; Dependence; Neuroadaptation

1. Introduction

Morphine has been used for millennia for its analgesic and rewarding properties and opioids are still the most potent pain killers in clinical use today. Unfortunately, tolerance to morphine-induced analgesia develops with long-term administration (Inturrisi, 2002; Ballantyne and Mao, 2003). Doses must be increased to achieve satisfactory pain relief, but morphine undesirable effects hamper unlimited dose adjustment (Mercadante, 1999). On the other hand, illegal consumption of heroin, the diacetylated derivative of morphine, is a major public health concern, since most heroin users ultimately loose control over their intake and enter a pathological state known as addiction (Robinson and Berridge, 2003; Koob et al., 2004; Hyman, 2005). Chronic opioid exposure also generates physical dependence, which is revealed upon

Abbreviations: Amg, amygdala; BST, bed nucleus of the stria terminalis; CPu, caudate-putamen; cdk5, cyclin-dependent kinase 5; MOR, mu opioid receptor; NAc, nucleus accumbens; PAG, periaqueductal gray; PfC, prefrontal cortex.

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treatment cessation by the emergence of a withdrawal syndrome (Inturrisi, 2002).

At the molecular level, morphine is known to exert its acute pharmacological effects through the activation of the mu opioid receptor (MOR) (Matthes et al., 1996), a G-protein coupled receptor expressed throughout the central nervous system. Conversely, the molecular mechanisms causing the progressive modification of behavioral effects of morphine with chronic exposure are not clearly understood. Current hypotheses on underlying molecular neuroadaptations include regulation of MOR activity (Johnson et al., 2005), intracellular adaptations counteracting MOR chronic activation (Trujillo, 2002; Chao and Nestler, 2004) and more general modifications affecting the whole neuronal circuitry, such as the recruitment of anti-opioid systems (Mollereau et al., 2005), the activation of transcription factors (Chao and Nestler, 2004) or the structural remodeling (Robinson and Kolb, 2004) and functional plasticity of synapses (Williams et al., 2001; Bailey and Connor, 2005).

In the present study, we set up three regimens of chronic morphine exposure, with the aim of modeling human situations relevant either to long-term pain treatment or to addiction in mice. Based on previous evidence, we used distinct morphine doses and injection frequencies to induce analgesic tolerance, locomotor sensitization or physical dependence, where withdrawal could be precipitated by naloxone. Locomotor sensitization, which refers to the gradual increase in locomotor stimulating effects with repeated drug exposure, is commonly employed as a basic assay to study addictive behavior (see Robinson and Berridge, 2001 for review). Noteworthy, whereas the degree of analgesic tolerance (Duttaroy and Yoburn, 1995) and physical dependence (Shaw-Lutchman et al., 2002) parallels the intensity of morphine exposure, locomotor sensitization is rather facilitated by long inter-dose intervals (Kuribara, 1996; Vanderschuren et al., 1997).

In the second part of our study, we employed our mouse behavioral models to investigate putative molecular mechanisms that could underlie the development of tolerance, sensitization and dependence. Specifically, we addressed whether these adaptations involve MOR desensitization, cyclin-dependent kinase 5 (cdk5) regulation and adenylate cyclase superactivation respectively.

MOR desensitization in areas mediating morphine analgesia has been hypothesized to be responsible for decreased morphine efficacy in chronically treated animals (Bailey and Connor, 2005). Periaqueductal gray (PAG) is one of the brain structures critically involved in the analgesic effect of morphine (Heinricher and Morgan, 1999). Accordingly, a decrease in MOR coupling to G proteins (Bohn et al., 2000; Sim-Selley et al., 2000) or to downstream effectors (Noble and Cox, 1996; Bagley et al., 2005a) has been reported in the PAG of animals chronically exposed to morphine or heroin. We therefore examined whether analgesic tolerance is linked to MOR decoupling in the PAG.

Several recent studies have highlighted the potential involvement of cdk5 and its neuronal activator p35 in behavioral adaptations to drugs of abuse (Benavides and Bibb, 2004). In particular, changes in cdk5 and p35 abundance in caudate-putamen (CPu), nucleus accumbens (NAc), amygdala (Amg) and prefrontal cortex (PfC) were reported following chronic psychostimulant administration (Bibb et al., 2001; Scheggi et al., 2004; Chen and Chen, 2005; Wedzony et al., 2005; but see Lu et al., 2003; Hope et al., 2005). Regarding opioids, cdk5 and p35 levels were markedly reduced in PfC of opioid addicts and cerebral cortex of rats treated with escalating doses of morphine (Ferrer-Alcon et al., 2003). We therefore addressed whether morphine-induced locomotor sensitization is associated with altered cdk5 and p35 protein levels in forebrain areas.

A functional up-regulation of the cyclic AMP (cAMP)-dependent signaling pathway in opioid-sensitive neurons has been suggested to develop with prolonged morphine exposure. This counteracting adaptation would be unmasked by opioid abstinence and could play a major role in the withdrawal syndrome (Chao and Nestler, 2004). Accordingly, adenylate cyclase superactivation has been described in locus coeruleus, striatum, Amg and cortex following chronic morphine treatment (Duman et al., 1988; Rasmussen et al., 1990; Terwilliger et al., 1991; Sheu et al., 1995; Valverde et al., 1996; Kaplan et al., 1998; Bohn et al., 2000). It may also occur in the extended amygdala (Shaw-Lutchman et al., 2002), a functional entity connecting the central nucleus of the Amg to the NAc shell through the bed nucleus of the stria terminalis (BST), which plays a role in the aversive aspect of opioid withdrawal (Frenois et al., 2002; Koob, 2003). In the PAG, which is centrally implicated in the expression of many somatic signs of withdrawal (Maldonado et al., 1992; Bozarth, 1994), there is growing electrophysiological evidence for cAMP pathway hyperactivity during opioid withdrawal (Bagley et al., 2005b; Ingram et al., 1998; Jolas et al., 2000). We therefore assessed whether morphine withdrawal is linked to superactivation of adenylate cyclase in a series of brain regions involved in the somatic and affective components of abstinence (Frenois et al., 2002).

To our surprise, we found no change in those three biochemical endpoints, despite the strong behavioral adaptations displayed by the mice.

2. Methods

2.1. Animals

Wild-type mice harboring a hybrid 50% C57BL/6J: 50% 129/SvPas genetic background were used. Mice were housed under standard conditions in a 12 h:12 h dark/light cycle (lights on from 7:00 to 19:00) with free access to water and food. Male and female mice, aged 9–11 weeks old at the beginning of the experiment, were used. Sex and age were matched between treatments. At least 1 week prior to the experiment, mice were transferred from the breeding facility, habituated to the novel housing room and handled. Behavioral experiments were performed between 8:00 and 13:00. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental procedures used in this study were reviewed by and received approval from Strasbourg regional ethics committee (CREMEAS).