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Research Report

Affective and neuroendocrine effects of withdrawal from chronic, long-acting opiate administration

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ABSTRACT

Although the long-acting opiate methadone is commonly used to treat drug addiction, relatively little is known about the effects of withdrawal from this drug in preclinical models. The current study examined affective, neuroendocrine, and somatic signs of withdrawal from the longer-acting methadone derivative l-alpha-acetylmethydol (LAAM) in rats. Anxiety-like behavior during both spontaneous and antagonist-precipitated withdrawal was measured by potentiation of the startle reflex. Withdrawal elevated corticosterone and somatic signs and blunted circadian variations in baseline startle responding. In addition, fear to an explicit, Pavlovian conditioned stimulus (fear-potentiated startle) was enhanced. These data suggest that anxiety-like behavior as measured using potentiated startle responding does not emerge spontaneously during withdrawal from chronic opiate exposure – in contrast to withdrawal from acute drug exposure – but rather is manifested as exaggerated fear in response to explicit threat cues.

1. Introduction

Methadone is a synthetic opiate commonly used to treat drug addiction (Cushman and Dole, 1973; Strain et al., 1999). Methadone has a slower onset and longer duration of action than commonly abuse opiates (e.g., heroin). This pharmacokinetic profile helps to avoid the repeated cycles of euphoria, withdrawal-induced negative emotionality, and heightened stress reactivity associated with shorter-acting opiates, and contributes to methadone's effectiveness in drug addiction pharmacotherapy (Kreek, 1992, 2000).

Owing to substantial individual differences in pharmacokinetics, however, a proportion of methadone-maintained patients do experience withdrawal when the drug is administered on a daily basis (Eap et al., 2002; Hiltunen et al., 1995). Furthermore, methadone users, like other opiate users, show high rates of

Abbreviations: ANOVA, analysis of variance; CRF1, corticotrophin releasing factor-1; CS, conditioned stimulus; HPA, hypothalamic–pituitary–adrenal; LAAM, L-alpha-acetylmethadol; NX, naloxone

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anxiety and depression (Rounsaville et al., 1985), which can be exacerbated by rapid methadone detoxification (Gold et al., 1979). It is therefore important to characterize more fully the negative emotional states produced during withdrawal from long-acting opiates. The current study investigated the consequences of withdrawal from a long-acting opiate in rats on behavioral measures of anxiety, stress hormone responses, circadian rhythms, and somatic signs.

In this study, withdrawal-induced anxiety was indexed by potentiation of the acoustic startle reflex, an established measure of fear in rodents (Davis, 1992). This measure is well suited as a behavioral tool to investigate the negative affective sequelae of opiate exposure for several reasons. First, the startle reflex can be measured repeatedly in the same subjects (Harris et al., 2004), making it suitable for studying within-subject changes of state across time (Beswick et al., 2003; Rounsaville et al., 1985). Second, the anatomical circuit mediating the startle reflex is relatively simple and well characterized (Lee et al., 1996; Nodal and Lopez, 2003), allowing circuitry involved in modulating startle during withdrawal to be more easily identified. Third, an exaggerated startle response is the characteristic of anxiety disorders and drug withdrawal in humans (Dichter and Tomarken, 2008; Krystal et al., 1997; Lang and McTeague, 2009; Orr et al., 2002). Thus, startle has strong utility as a translational measure in anxiety and addiction research.

The startle reflex is potentiated robustly and reliably during withdrawal from acute or intermittent opiate administration, or opiate self-administration (Harris and Gewirtz, 2004; Kalinichev and Holtzman, 2003; Park et al., 2013), and these effects are blocked by anxiolytic drugs (Harris and Gewirtz, 2004; Rothwell et al., 2009). Curiously, however, several studies have reported that withdrawal from chronic, continuous opiate exposure either produces no effect (Fendt & Mucha, 2001) or reduces the startle reflex (Kalinichev and Holtzman, 2003; Mansbach et al., 1992; but see Higgins and Sellers, 1994). In contrast, other measures of anxiety, as well as of dysphoria and anhedonia, are expressed under similar circumstances (Schulteis et al., 1994; Schulteis et al., 1998). This apparent anomaly may be related to the way in which anxiety is elicited in different behavioral paradigms. Where anxiety has been observed during withdrawal from continuous opiate exposure, the behavioral measure has been evoked in the presence of anxiogenic or fear-eliciting stimuli (Fendt and Mucha, 2001; Harris and Aston-Jones, 2003). Hence, the current study examined potentiation of startle in the presence of threat cues (i.e., fear-potentiated startle), as well as in their absence (baseline startle). Anxiety generated under these two conditions may be mediated by different neural substrates (Koob and Zorrilla, 2012).

Studies of chronic opiate dependence in rodents typically precipitate a sudden state of withdrawal using an opiate antagonist such as naloxone. Since withdrawal in humans is not typically induced through administration of an opiate antagonist, measures of "spontaneous" withdrawal after cessation of drug treatment may have greater direct relevance to clinical psychopathology (Rothwell et al., 2009). To broaden the applicability of our results, both spontaneous and precipitated withdrawal paradigms were used in these studies. Furthermore, we measured plasma levels of the steroidal stress hormone corticosterone and used startle to assess alterations in circadian rhythms. Both are common outcome measures of opiate withdrawal (e.g., Becker et al., 2008; Beswick et al., 2003; Li et al., 2010) and depression (for a review, see Kronfeld-Schor and Einat, 2012).

Chronic opiate treatment in these studies was achieved using L-alpha-acetylmethydol (LAAM) rather than methadone. Methadone has a half-life in rats of approximately 3 h (Misra et al., 1973). In contrast, the active metabolites of LAAM have a half-life of more than 24 h in rats, more closely resembling the half-life of methadone in humans (Henderson et al., 1977). Therefore, although no longer available for clinical purposes in the United States (US Food and Drug Administration, 2013), LAAM was used to maintain a pattern of opiate exposure that more closely mirrors the time course of drug exposure in methadone-treated patients. We also used two procedures for inducing dependence. The first was closely based on our previous studies with LAAM (Hamilton et al., 2005), whereas the second resembled the chronic, continuous opiate delivery procedures used with other opiates (Fendt and Mucha, 2001; Kalinichev and Holtzman, 2003). Using these approaches, our results suggest that withdrawal from a long-acting opiate induces a negative affective state, but one that is distinct from the state induced during withdrawal from intermittent opiate exposure.

2. Results

2.1. *Experiment 1: precipitated withdrawal following 21 days of LAAM*

2.1.1. *Weight change during induction of dependence*

Rats administered LAAM gained weight more slowly than water-treated rats. In Experiment 1, there were a significant effect of LAAM treatment on weight gain ($F(1, 39)=15.13$, $p=0.000$), a significant effect of day of treatment ($F(10, 390)=104.83$, $p=0.000$), and a LAAM treatment × day interaction ($F(10, 390)=23.15$, $p=0.000$). Follow-up t-tests revealed that LAAM-treated rats gained less weight than water-treated rats during the last half of drug treatment (Fig. 1). LAAM produced a similar suppression of weight gain in Experiments 2–5 (data not shown).

2.1.2. *Withdrawal measures*

Startle was not affected by LAAM and naloxone treatment ($p=0.83$; Table 1). There was no potentiation of the startle response above the Water+0.5 NX group in any of the other groups during withdrawal.

In contrast, there was a naloxone dose-related increase in the incidence of diarrhea (Table 1). Rats administered the higher doses of naloxone displayed diarrhea more frequently than rats administered lower doses of naloxone or saline following LAAM or rats administered water and a low dose of naloxone ($\chi^2(6, N=41)=25.93$, $p=0.000$). Moreover, Table 1 shows that there was a naloxone dose-related increase in weight loss during withdrawal ($F(6, 34)=4.86$, $p=0.001$). Follow-up Fisher's PLSD tests indicated that rats administered LAAM plus any of the three highest doses of naloxone lost more weight than rats treated with water and a low dose of naloxone.