

Low dose naltrexone administration in morphine dependent rats attenuates withdrawal-induced norepinephrine efflux in forebrain

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Abstract

The administration of low dose opioid antagonists has been explored as a potential means of detoxification in opiate dependence. Previous results from our laboratory have shown that concurrent administration of low dose naltrexone in the drinking water of rats implanted with subcutaneous morphine pellets attenuates behavioral and biochemical signs of withdrawal in brainstem noradrenergic nuclei. Noradrenergic projections originating from the nucleus tractus solitarius (NTS) and the locus coeruleus (LC) have previously been shown to be important neural substrates involved in the somatic expression of opiate withdrawal. The hypothesis that low dose naltrexone treatment attenuates noradrenergic hyperactivity typically associated with opiate withdrawal was examined in the present study by assessing norepinephrine tissue content and norepinephrine efflux using *in vivo* microdialysis coupled to high performance liquid chromatography (HPLC) with electrochemical detection (ED). The frontal cortex (FC), amygdala, bed nucleus of the stria terminalis (BNST) and cerebellum were analyzed for tissue content of norepinephrine following withdrawal in morphine dependent rats. Naltrexone-precipitated withdrawal elicited a significant decrease in tissue content of norepinephrine in the BNST and amygdala. This decrease was significantly attenuated in the BNST of rats that received low dose naltrexone pre-treatment compared to controls. No significant difference was observed in the other brain regions examined. In a separate group of rats, norepinephrine efflux was assessed with *in vivo* microdialysis in the BNST or the FC of morphine dependent rats or placebo treated rats subjected to naltrexone-precipitated withdrawal that received either naltrexone in their drinking water (5 mg/L) or unadulterated water. Following baseline dialysate collection, withdrawal was precipitated by injection of naltrexone and sample collection continued for an additional 4 h. At the end of the experiment, animals were transcardially perfused and the brains were removed for verification of probe placement. Low dose naltrexone pre-treatment significantly attenuated withdrawal-induced increases of extracellular norepinephrine in the BNST, with a smaller effect in the FC. These findings suggest that alterations in norepinephrine release associated with withdrawal may be attenuated in forebrain targets of noradrenergic brainstem neurons that may underlie reduced behavioral signs of withdrawal following low dose naltrexone administration. Published by Elsevier Inc.

Keywords: HPLC; Microdialysis; Morphine; Naltrexone; Norepinephrine; Withdrawal

Abbreviations: ABC, avidin–biotin complex solution; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; AUC, area under the curve; BNST, bed nucleus of the stria terminalis; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; CREB, cAMP-response element-binding protein; DAB, 3,3-diaminobenzidine tetrahydrochloride; D β H, dopamine-beta-hydroxylase; ED, electrochemical detection; EDTA, ethylenediamine tetraacetic acid; FC, frontal cortex; HPLC, high performance liquid chromatography; LC, locus coeruleus; NTS, nucleus tractus solitarius; PB, phosphate buffer; PKA, protein kinase; TS, Tris–saline buffer.

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1. Introduction

Since their first use in opiate detoxification over 30 years ago, the administration of opiate antagonists (e.g. naloxone and naltrexone) during detoxification has varied either by differences in interval of administration between agonist and antagonist, or by quantity of antagonist administered (Kurland and McCabe, 1976; Mannelli et al., 2004). The closer the antagonist is administered to opiate agonist exposure, the more acute the withdrawal syndrome. Interestingly, the administration of small quantities of naltrexone or naloxone during opiate exposure induces “anti-withdrawal” effects (Shen and Crain, 1997; Mannelli et al., 2004). A better knowledge of the effect of opiate antagonists and their potential

role during the detoxification process may help improve the quality of existing treatments.

Our previous studies have demonstrated that concurrent administration of low dose naltrexone in the drinking water of rats implanted with subcutaneous morphine pellets attenuates behavioral signs of withdrawal and decreases the expression of c-Fos (used as an index of cellular activation in autonomic brain areas (Sagar et al., 1988)) in the noradrenergic locus coeruleus (LC) and nucleus of the solitary tract (NTS) (Mannelli et al., 2004). The LC and the NTS, known to be hyperactive following withdrawal from opiates (Hayward et al., 1990; Stornetta et al., 1993; Beckmann et al., 1995; Chieng et al., 1995; Georges et al., 2000; Mannelli et al., 2004), have previously been shown to be important neural substrates involved in the somatic expression of opiate withdrawal (Nestler et al., 1994; Aston-Jones et al., 1999; Mannelli et al., 2004). In addition, immunoblot analysis of intracellular messengers, cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) and cAMP-response element-binding protein (CREB), demonstrated to be dramatically increased in noradrenergic nuclei following opiate withdrawal (Guitart et al., 1992; Lane-Ladd et al., 1997), showed a decrease in expression (primarily in the NTS) in rats that had received low doses of naltrexone in their drinking water and that were subjected to pharmacological precipitation of opiate withdrawal (Mannelli et al., 2004). Potential explanations of the efficacy of the naltrexone treatment include the possibility that low antagonist dosing may act to facilitate enkephalin release by blocking opioid presynaptic receptors (Ueda et al., 1994). Alternatively, low dose naltrexone may antagonize excitatory opiate receptor functions and unmask the inhibitory effects of opioids (Shen and Crain, 1997). We also reported increases in opioid receptor expression (specifically of the mu subtype) in the NTS following antagonist treatment that may contribute to the attenuation of behavioral expression of withdrawal following low dose naltrexone treatment (Van Bockstaele et al., 2006). Increased expression of mu-opioid receptors may result in increased tonic inhibition of noradrenergic neurons in the NTS that provide noradrenergic innervation to forebrain targets resulting in decreased release of norepinephrine in limbic and cortical targets (Van Bockstaele et al., 2006).

In the present study, the hypothesis that low dose naltrexone treatment in morphine dependent rats attenuates forebrain noradrenergic hyperactivity usually associated with pharmacological precipitation of withdrawal (Crawley et al., 1979; Swann et al., 1982; Grasing et al., 1997; Fuentealba et al., 2000) was tested. First, we measured (from morphine dependent rats that received low dose naltrexone or unadulterated water) tissue content of norepinephrine from two forebrain regions that are targeted by brainstem noradrenergic nuclei: the bed nucleus of the stria terminalis (BNST) that receives noradrenergic projections primarily from the NTS (Dunn and Williams, 1995; Delfs et al., 2000) and the frontal cortex (FC) that receives afferents primarily from the LC (Robbins, 1984; Aston-Jones, 1985). We also examined tissue samples from the amygdala, a region that receives noradrenergic innervation from both the LC and NTS (Robbins, 1984; Aston-Jones, 1985; Dunn and Williams, 1995; Delfs et al., 2000), and cerebellum, which receives noradrenergic

afferents solely from the LC. Subsequently, norepinephrine efflux in the FC and BNST was assessed using high performance liquid chromatography (HPLC) with electrochemical detection (ED).

2. Methods

2.1. Animals

Male, Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 225–250 g at the start of experiments were used for these studies. All procedures were approved by the Institutional Animal Care and Use Committee at Thomas Jefferson University and are in compliance with the guidelines of the National Institutes of Health.

2.2. Immunoperoxidase labeling of D β H

Tissue sections through the forebrain of one ($n=1$) rat were processed for the immunocytochemical detection of the noradrenergic synthesizing enzyme, dopamine-beta-hydroxylase (D β H). The rat was deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and transcardially perfused with 200 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4). The brain was extracted, and 30- μ m-thick coronal sections through the BNST were cut using a Vibratome and collected into chilled 0.1 M phosphate buffer. Tissue sections were incubated in 1% sodium borohydride solution for 30 min and then rinsed in 0.1 M phosphate buffer and 0.1 M Tris–saline buffer (pH 7.6). Sections were blocked in 0.5% bovine serum albumin in 0.1 M Tris–saline buffer for 30 min and rinsed extensively in 0.1 M Tris–saline buffer. Tissue sections were incubated in a mouse anti-D β H (1:1000) primary antibody made in 0.1 M Tris–saline buffer containing 0.1% bovine serum albumin at room temperature overnight on a rotary shaker. Next, tissue sections were rinsed in 0.1 M Tris–saline buffer and then incubated in biotinylated donkey anti-mouse IgG (Vector Laboratories, Burlingame, CA; 1:200) made in 0.1 M Tris–saline buffer containing 0.1% bovine serum albumin for 30 min. Tissue was rinsed in 0.1 M Tris–saline buffer, incubated in an avidin–biotin complex solution (Vector Laboratories) for 30 min and then washed again in 0.1 M Tris–saline buffer. A peroxidase color reaction was obtained by exposing the tissue sections to a solution containing 22 mg of 3,3-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO) in a 0.1-M Tris–saline solution containing 0.05% hydrogen peroxide for 6 min. Tissue was rinsed with 0.1 M Tris–saline buffer, followed by 0.1 M phosphate buffer. Sections were mounted onto gelatin-coated microscope slides and the distribution of D β H containing fibers within the BNST was photographed. The distribution of D β H fibers in this region was compared to a rat brain atlas (Paxinos and Watson, 1986) and aided in the appropriate selection of coordinates for microdialysis probe placement (data not shown).

2.3. Drug treatment for tissue analysis and high pressure liquid chromatography for microdialysis

For norepinephrine tissue analysis, fifteen rats were used. Ten rats were implanted subcutaneously with two slow release

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