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BRAIN RESEARCH

Research Report

Suppression of acute morphine withdrawal syndrome by adenovirus-mediated β-endorphin in rats☆

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ABSTRACT

Background: Endogenous β-endorphin (β-EP) in the central nervous system (CNS) is decreased upon opioid addiction. The current study examined whether exogenous β-EP, delivered using an adenoviral vector into the CNS could attenuate morphine withdrawal syndrome in rats. *Methods*: The model of opioid-dependent rats was set up by receiving subcutaneous injection of morphine using an escalating regimen for 6 days (5, 10, 20, 40, 50, 60 mg/kg, three times/day). The adenovirus mediated β-EP gene was constructed based on our previous work. The ilea of opioid-dependent rats were isolated and treated with the supernatant of Ad-NEP. The basic and naloxone-induced (4 μm/l) contractions of dependent ilea were recorded. The Ad-NEP was injected into the left lateral ventricle of the addition rats. The expression of the β-EP gene was verified by radioimmunoassay of the cerebrospinal fluid (CSF) and immunocytochemistry for β-EP. Withdrawal syndrome was evaluated after intraperitoneal injection of naloxone. *Results*: The contractions of dependent ilea were attenuated with supernatant containing β-EP expressed by Ad-NEP. Injection of the Ad-NEP resulted in significant increases in β-EP level in the CSF and β-EP-positive neurons. Rats receiving adenovirus carrying the β-EP gene had significantly less severe withdrawal symptoms upon naloxone challenge. *Conclusions*: Exogenous β-EP mediated by adenovirus could attenuate withdrawal syndrome in morphine-dependent rats.

1. Introduction

Opioid addiction is a chronic, relapsing disease and remains a major public health challenge (Gartry et al., 2009). It is estimated to affect more than 1 million people in North America (Oviedo-Joekes et al., 2009). Risks associated with opioid addiction include infectious diseases, loss of social and economic functioning, and drug-related crimes (Manzoni et al., 2006; March et al., 2006; Miller et al., 2006; Wood et al., 2005).

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Abrupt cessation of chronic opiate use results in a characterized withdrawal syndrome that includes nausea, dysphoria, and anxiety (Liu et al., 2005). Detoxification is a necessary step in treating opioid dependence after cessation of opiate use, usually through the use of pharmacologically equivalent morphine agonists, such as methadone (Leal et al., 2003). This opioid receptor morphine agonist has been used due to its ability to counteract symptoms of withdrawal syndrome, long-lasting effect, and lack of marked reinforcing properties. However, 15 to 25% of the most adversely affected persons do not have a good response to methadone treatment (Johnson et al., 2000). Despite somewhat adequate management of withdrawal syndrome, relapse rates remain unacceptably high (Bisaga and Popik, 2000).

β-Endorphin (β-EP) is an endogenous opioid peptide with potent analgesic effect and its downregulation in the opioid dependent conditions has been reported (Niikura et al., 2008; Van Ree et al., 2000). Previous studies have shown that β-EP level is decreased in blood, spinal cord and brain nuclei in the opioid-dependence models (Gudehithlu et al., 1991; Roth-Deri et al., 2008; Van Reem, 2005). In contrast, glycyl-glutamine, a peptide derived from endogenous β-EP, could attenuate opioid withdrawal symptoms (Cavun et al., 2005).

In the current study, an adenovirus carrying fusion gene of prepropeptide of mouse nerve growth factor (NGF) and human EP was constructed based on our previous work (Xu et al., 2009), the efficacy of adenovirus-mediated β-endorphin (Ad-NEP) in treatment of acute morphine withdrawal syndrome was investigated in rats. The contraction of isolated ileum in opioid-dependent rats was observed after β-EP treatment, and withdrawal behavioral scores of opioid-dependent rats were assessed after intracerebroventricular injection of Ad-NEP.

2. Results

2.1. *β-Endorphin expressed by Ad-NEP on ilea of opioid dependent rats*

The concentration of β-EP in the supernatant of HEK 293 cells was 7575.0±156.5 pg/ml detected by radioimmunoassay, which suggested that β-EP could be successfully expressed by the virus. The β-EP in the incubated solution of the Ad-NEP group was 378.8±7.8 pg/ml detected by radioimmunoassay. No β-EP was detected in the Ad-Null group. As shown in Figs. 1 and 2, the basic contraction force in the isolated ilea of opioid dependent rats was increased when compared to that of the naive rats

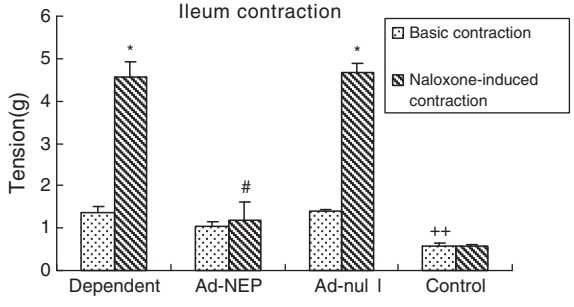

Fig. 1 – Naloxone-induced contraction. $^{++}P<0.01$ VS other three groups before naloxone intervention. $^{*}P<0.01$ VS baseline contraction. $^{\#}P<0.01$ VS dependent group and Ad-null group after naloxone intervention.

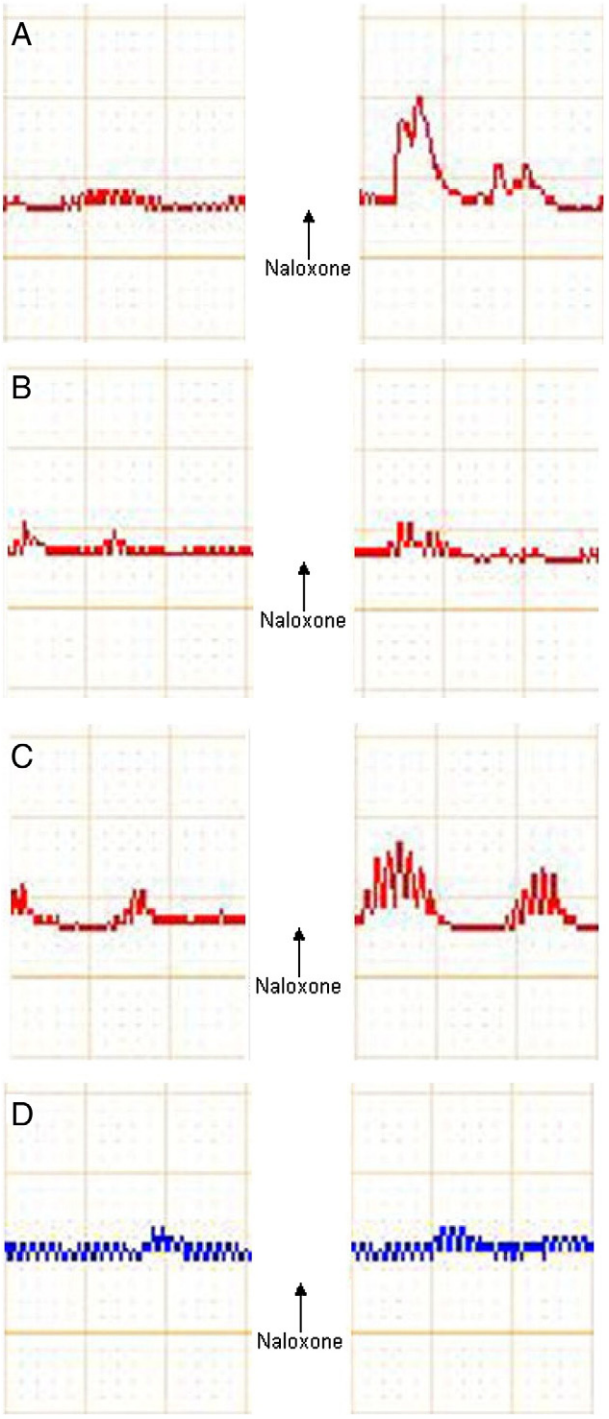

Fig. 2 – Naloxone-induced withdrawal contraction. Tetanic contraction was showed in dependent goup (A) or Ad-null group(C) after naloxonewas added (4 μmol/L). In Ad-NEP group (B), β-endorphin significantly inhibited the contractile response to naloxone. Naloxone had no effect on control group (D).