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A comparison of the effects of digoxin, ouabain and milrinone on naloxone-precipitated withdrawal syndrome in mice

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ABSTRACT

Modulation of Na^+, K^+-ATPase activity by acute and chronic opiates has been established for many years. However, the effects of digoxin, a putative inhibitor of Na^+, K^+-ATPase, on naloxone-precipitated morphine withdrawal syndrome are unknown. In the present study, a digoxin dose-response curve was conducted to observe the effects on naloxone-precipitated withdrawal and locomotor activity in mice. Higher doses of digoxin (1.0 and 2.5 mg/kg) inhibited locomotor activity and naloxone-precipitated withdrawal jumping and weight loss, while lower doses of digoxin (0.1 and 0.25 mg/kg) inhibited withdrawal weight loss precipitated by naloxone without affecting locomotor activity and naloxone-precipitated withdrawal jumping. To explore the possible mechanisms underlying this behavior, another Na^+, K^+-ATPase inhibitor ouabain, which does not cross the blood brain barrier, and another cardiotonic drug milrinone, a non-inhibitor of Na^+, K^+-ATPase, were also included in the present study. Both milrinone and ouabain inhibited, in a dose-dependent manner, naloxone-precipitated weight loss while neither affected naloxone-precipitated withdrawal jumping nor locomotor activity in mice. These results indicate that both the cardiotonic effects and central inhibition of Na^+, K^+-ATPase contribute to the inhibitory effects of digoxin on morphine withdrawal syndrome in mice.

1. Introduction

Although opiates are widely used analgesics, repeated administration of these scheduled drugs result in physical dependence. This major side effect of opiate administration limits its clinical utility, and most importantly, contributes to opioid addiction that may cause social problems relevant to its non-clinical use (Bailey and Connor, 2005). So far, only a few drugs to treat opiate dependence are commercially available as patients may suffer from insufficient treatment (Lobmaier et al., 2010; Veilleux et al., 2010). Therefore, development of new drugs and strategies for the treatment of opiate dependence is needed.

Opiate dependence is manifested by characteristic withdrawal syndrome of multiple aversive behavioral and physiological signs in a wide variety of animal species (Alcantara et al., 2011; Emmett-Oglesby et al., 1990). In humans, opiate withdrawal syndrome includes cognitive (e.g., drug craving), affective (e.g., anxiety), and behavioral (e.g., twitches) signs (Maldonado et al., 1996). In mice, withdrawal symptoms include compulsive jumping, diarrhea, rearing, ptosis, tremor, piloerection, and weight loss. Compulsive jumping and weight loss are two indicators to measure the severity of opiate withdrawal (Bhargava, 1994; Papaleo and Contarino, 2006). The mechanism involved in opiate dependence has been heavily investigated and most attention has been drawn to the cyclic Adenosine 3,5-monophosphate—Protein Kinase A (cAMP-PKA) pathway (Cao et al., 2010; Olson et al., 2005; Self et al., 1998). Acute opiate administration decreases cAMP, while chronic administration increases cAMP signaling and precipitated withdrawal further increasing cAMP expression (Shen et al., 2000; Wu et al., 2006). The overshoot of cAMP mediates many behavioral and physiological signs and plays an important role in opiate dependence.

The activity of Na^+, K^+-ATPase was found to be modulated by opiates both in vitro and in vivo (Desaiah and Ho., 1977; Horvath et al., 2003; Masocha et al., 2002; Nishikawa et al., 1990). It was recently found that opiates regulate the activity of Na^+, K^+-ATPase through the cAMP-PKA pathway (Wu et al., 2006). Na^+, K^+-ATPase is an active transport system of sodium and potassium, which maintains the electric membrane potential and controls multiple essential cellular functions (Bagrov et al., 2009; Xie, 2003). Na^+, K^+-ATPase plays an important role in resting potential and opiate withdrawal is accompanied with increased neuronal firing (Nestler, 2004; Nestler and Aghajanian, 1997). We hypothesize that modulation of the

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activity of Na^+, K^+-ATPase might affect opiate withdrawal syndrome.

Ouabain and digoxin are cardiotonic drugs that have been used for cardiac failure for hundreds of years (Riganti et al., 2011). Pharmacological studies demonstrate that these cardiotonics drugs are inhibitors of Na^+, K^+-ATPase. In the present study, we investigated its effects on opiate withdrawal syndrome. Milrinone is another cardiotonic drug that has no effect on the activity of Na^+, K^+-ATPase. Initially, we used milrinone as a control drug to compare its effect with ouabain and digoxin. Interestingly, we found that all of these drugs alleviated naloxone-precipitated withdrawal weight loss in mice. We also studied the effects of these drugs on locomotor activity and postulated the possible mechanisms involved.

2. Materials and methods

2.1. Animals

Female and male kunming mice, weighing 18–22 g, were obtained from the Experimental Center of Henan Province. The animals were fed ad libitum and were group-housed in a room with a controlled ambient temperature (22 ± 2 °C), humidity ($50 \pm 10\%$), and a 12 h light/dark cycle (lights on 08:00 to 20:00). Animals were acclimated to the housing conditions and handled for 3–4 day before experiments. All experiments were performed during the light phase. The experimental procedures were approved by the local Committee on Animal Care and Use. Every effort was made to minimize the number and suffering of animals in the following experiments.

2.2. Drugs and chemicals

Morphine hydrochloride was purchased from Shenyang First Pharmaceutical Factory (China). Naloxone Hydrochloride injection was purchased from Beijing Sihuan Pharmaceutical Holding Group LTD (China). Digoxin was kindly provided by Dr. Hong-min Liu (Zhengzhou University, China). Digoxin was suspended, using ultrasound, in 0.1% Carmellose Sodium (CMC-Na). Ouabain was purchased from Sigma-Aldrich Company (USA). Milrinone was obtained from Lunan Pharmaceutical Group (China). Morphine, Ouabain, and Milrinone were dissolved in saline. Morphine was injected subcutaneously (s.c.). Naloxone, ouabain and milrinone were injected intraperitoneally (i.p.). Digoxin was administered intragastrically (i.g.). The doses of all drugs are expressed as weight of the base. All drugs were administered in a volume of 20 ml/kg.

2.3. Locomotor activity

The spontaneous locomotor activity was recorded automatically with a Small Animal Locomotion Recording Apparatus (Shandong Academy of Medical Sciences, China), which consisted of eight boxes. In each box there was one pyroelectric infrared sensor 10 cm above the floor. The sensor detected the movements of the mice through infrared radiation. The apparatus recorded only the gross movements of the mice, whereas small movements such as gnawing or grooming could not be counted.

2.4. Induction of morphine dependence and naloxone-precipitated withdrawal

Morphine solutions were freshly made prior to each injection. The animals were rendered dependent on morphine using a method previously described (Mohammed et al., 2004). Mice were injected with morphine s.c. twice daily at 09:00 and 16:00 for 4 consecutive days. There was a 10 mg/kg daily increase in the doses of morphine, from 30 mg/kg on the first day to 60 mg/kg on the fourth day. On the fifth day, 140.0 mg/kg of morphine was injected at 9:00 only. A separate group of animals were administered saline (instead of morphine). Four hours later, the test drug or saline was administered and 60 min later each mouse was weighed and injected i.p. with naloxone (5.0 mg/kg). Immediately after the injection of naloxone, animals were placed individually in a glass beaker and the number of jumps (all feet losing contact with the ground) was observed continuously for 30 min. The percentage of weight loss was evaluated 30 min after naloxone administration.

2.5. Experimental protocols

2.5.1. Effects of acute administration of digoxin, ouabain, and milrinone on naloxone-precipitated morphine withdrawal signs in mice

In this experiment, the morphine-dependent groups were administered either digoxin (vehicle, 0.1, 0.25, 1.0, or 2.5 mg/kg, i.g., $n=9$–11 per group), ouabain (vehicle, 0.16, 0.32, or 0.64 mg/kg, i.p. $n=9$–10 per group) or milrinone (vehicle, 2.0, 4.0, or 8.0 mg/kg, i.p. $n=8$–9 per group), while the saline-treated group was administered vehicle,1 hour prior to the administration of naloxone (5.0 mg/kg, i.p.).

2.5.2. Effects of acute administration of digoxin, ouabain, and milrinone on locomotor activity

Naive mice were put into the test boxes 60 min after administration of digoxin (vehicle, 0.1, 0.25, 1.0, or 2.5 mg/kg, i.g. $n=8$–10 per group), ouabain (vehicle, 0.16, 0.32, or 0.64 mg/kg, i.p. $n=8$–10 per group) or milrinone (vehicle, 2.0, 4.0, or 8.0 mg/kg, i.p. $n=7$–8 per group) treatment. Locomotor counts were recorded every 15 min for 120 min.

2.6. Statistical analysis

The data were presented as the means ± S.E.M. All the experiments were evaluated by one-way analysis of variance (ANOVA) and a post hoc least significant difference (LSD) test for multiple comparisons at a minimum significance level of $P<0.05$. All statistical analyses were performed using SPSS for Windows (SPSS 15.0) software.

3. Results

3.1. Effects of acute administration of digoxin on naloxone-precipitated morphine withdrawal signs in mice

After administration of naloxone (5.0 mg/kg, i.p.), the morphine-dependent group demonstrated a pronounced withdrawal syndrome including jumping, diarrhea and significant body weight loss in a period of 30 min. This was not observed in the control group (saline+vehicle+naloxone) ($P<0.001$). Compared with the vehicle group, the higher doses of digoxin (1.0 and 2.5 mg/kg) significantly decreased jumping in a dose-dependent manner ($P<0.05$; $P<0.01$, respectively), while the lower doses of digoxin (0.1 and 0.25 mg/kg) had no significant effect on jumping behavior (Fig. 1A). All doses of digoxin significantly attenuated the percentage of weight loss in morphine-dependent mice ($P<0.05$) (Fig. 1B).