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Effects of thienorphine on the contraction of isolated ureter and bladder of guinea pigs

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ABSTRACT

Opioid analgesics are widely used in moderate to severe pain including renal colic. Morphine is believed to cause spasm of ureter and affect the bladder contractions. Thienorphine is a partial opioid agonist that is a good candidate for the treatment of opioid dependence and pain. This study examined the effects of thienorphine on the guinea pig isolated ureter and bladder. The contractile amplitude of isolated ureter induced by KCl (40 mM) was not influenced by thienorphine or buprenorphine, whereas morphine increased the amplitude of the isolated ureter. Thienorphine, buprenorphine or naloxone concentration-dependently antagonized the isolated ureter contraction induced by morphine. Thienorphine (1.0–32.0 μM) or buprenorphine (1.0–32.0μM) had no effects on the spontaneous or acetylcholine (Ach) induced contractions of isolated bladder, but decreased the amplitude of the contractions of isolated bladder at 100 μM concentration. Morphine (0.1–3.2 mM) concentration dependently increased the spontaneous movement and Ach (1 μM) induced contractions of isolated bladder. The mRNA levels of μ receptor in the ureter and bladder was as the same as that in the frontal cortex. In comparison, the mRNA levels of κ receptor, δ receptor and N/OFQ receptor was fewer than that in the frontal cortex. In summary, thienorphine has little influence on the guinea pig isolated ureter and bladder compared with morphine, which may result in a lack of adverse renal colic effects.

1. Introduction

Renal colic as a result of urolithiasis is a common cause of severe acute pain. Approximately 1–10% of the population is estimated to suffer from kidney colic at least once during their lifetime (Shokeir et al., 2002). Many drugs can be used to relieve renal colic: non-steroid anti-inflammatory agents (NSAIDs), opioid analgesics, and loco-regional anesthesia, et al. NSAIDs inhibit prostaglandin release and have been shown to be effective for the renal colic. However, the side effects including renal failure and gastrointestinal bleeding of NSAIDs are well recognised (Holdgate and Pollock, 2004). Opioids have significant analgesic effects on moderate to severe pain. However, the utility of opioid agonists is limited by a number of well-known side effects. There are concerns over drug dependency and drug seeking behaviour presenting as renal colic. Peripherally, opioids act directly upon ureteral smooth muscles and increase isotonic contraction tone and length to produce ureteral spasm (Zabihi and Teichman, 2001). Therefore, the opioids such as morphine, codeine, and their derivates are second-choice therapy for renal colic. Opioid receptors play a major role in the inhibition of overactive bladder by tibial nerve stimulation (TNS) (Tai et al., 2012). Further study indicates that activation of μ receptor and κ receptor is essential for producing TNS inhibition (Zhang et al., 2015). Morphine can decrease the spontaneous release of Ach from the guinea-pig ileum and inhibit the electric-evoked contraction of myenteric plexus-longitudinal muscle from the guinea-pig ileum (Szerb,1982). In addition to changing neurotransmission in the TNS inhibitory pathway, opioids might directly changing the contraction of bladder. It is yet not known, what effects of the three opioid receptors subtypes, namely receptor, κ receptor or δ receptor, are involved in this process.

Thienorphine, N-cyclopropylmethyl-7-[1-(R)-1-hydroxy-1-methyl-3-(thien-2-yl)propyl]-6,14-*endo*-ethanotetrahydro-oripavine, is a partial opioid agonist with long-lasting antinociceptive effect and high oral bioavailability lasting antinociceptive effect and high oral bioavailability compared with its analogue buprenorphine (Liu et al., 2005). Due to its slow dissociation from the μ receptor, the risk of the development of drug dependence and analgesic tolerance is lower with thienorphine than with full μ opioid agonists (Yu et al., 2006,2014). Thienorphine might have wider application for the treatment of pain and opioid dependence. In vitro, Thienorphine has little influence on the guinea pig isolated sphincter of Oddi, choledochus, gallbladder and ileum. Thienorphine moderately inhibits intestinal transit, and has less influence

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on the guinea pig bile flow compared with morphine and buprenorphine (Zhou et al., 2013, 2014).

Maggi and Meli (1984) reported that 40 mM KCl induced the rhythmic contraction on guniea pig ureter. Ca^{2+} channel blocker reduced both the frequency and the amplitude of KCl-induced ureter rhythmic contraction. There is no report about what effects are induced by the opioids on the 40 mM KCl induced-rhythmic contraction of ureter and which opioids receptor subtype exist in guinea pig ureter smooth muscle (Maggi and Meli, 1984). Therefore, in the present study, the effects of thienorphine on the contraction of 40 mM KCl induced ureter or bladder were analyzed in vitro.

2. Material and methods

2.1. Material

2.1.1. Animals

Male or female guinea pigs (300–500 g) were obtained from Beijing Animal Center (Beijing, China). Animals were housed in a temperature controlled room (25 ± 1 °C) and maintained on a 12-h/12-h light/dark cycle. Animals had free access to food and water. Animal care and procedures were strictly in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and this study was approved by the Animal Care Committee of Beijing Institute of Pharmacology and Toxicology.

2.1.2. Materials

Thienorphine HCl and buprenorphine HCl were synthesized in Beijing institute of pharmacology and toxicology (Liu et al., 2005). Naloxone and acetylcholine (Ach) were purchased from Sigma (St. Louis, MO). NaCl, KCl, $CaCl_2$, KH_2PO_4, $NaHCO_3$, $MgSO_4$ and glucose were provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Morphine was produced by Qinghai Pharmaceutic Factory (Xining, China).

2.2. Methods

2.2.1. Isolated tissue preparation

Guinea pigs of either sex (300 − 500 g) were stunned and decapitated. The ureter together with the bladder were quickly removed and placed in Kreb's solution (KCl 4.69 mM, $CaCl_2$ 2.52 mM, KH_2PO_4 1.18 mM, $MgSO_4$ 1.22 mM, $NaHCO_3$ 25.0 mM, NaCl 118.06 mM, Glucose 10.0 mM, pH =7.4) bubbled continuously with 95% O_2 and 5% CO_2.

After removal of fat, blood and mucosa, the whole ureter was cut into approximately 15 mm segments. The ureter strips were suspended in 10 ml organ bath. The bladder was cut longitudinally into two or three muscle strips (8 × 3 mm) and the strips were mounted in the organ bath with a volume of 10 ml.

The preparations were connected to an isotonic force transducer linked to eight channel organ baths (Meiyi Ltd., Nanjing, China) containing Krebs' solution at 37 °C and bubbled with 95% O_2 and 5% CO_2. The longitudinal ureter and strips of bladder preparations were suspended under 1.0 g tension for 1 h equilibration with washes every 15 min before exposure to drugs.

2.2.2. Isometric tension measurement of isolated ureter after thienorphine treatment

Contractions of the ureter were measured using an isometric transducer connected to Medlab-U/4CS (Medlab6, Meiyi Ltd., Nanjing, China). The contractile amplitude of ureter was measured over a 5–10 min period, both in rest state and during chemical treatment. The actions of thienorphine, buprenorphine and morphine were evaluated by testing their effects upon a 40 mM KCl – induced rhythmic contraction. Only those tissue preparations which responded to KCl (40 mM) and produced contractions of more than 0.2 g tension were selected. Opioid-containing solutions were prepared immediately before the experiment. The tissue preparations were treated with morphine (10^{-5}–3.2×10^{-3} M), thienorphine (10^{-6}–10^{-4} M), or buprenorphine (10^{-6}–10^{-4} M) for 10 min at each concentration. In the first experimental group, after KCl addition, the ureter pretreated with thienorphine, buprenorphine or naloxone for 10 min and then treated with morphine. In the second experimental group, after KCl addition, the ureter pretreated with morphine for 10 min and then treated with thienorphine, buprenorphine or naloxone. The contractile response of ureter to the chemicals was expressed as change in amplitude%= strips contractions after opioids treatment/muscle contractions after KCl (40 mM) or morphine (1.0 mM) treatment × 100%.

2.2.3. Isometric tension measurement of isolated bladder muscle strips after thienorphine treatment

Contractions of the bladder muscle strips were measured using an isometric transducer connected to Medlab-U/4CS. At the start of each experiment, a maximum response to Ach (10^{-6} M) was obtained in each tissue to confirm its suitability. After washing the preparation, the chemicals were added to the organ bath for 10 min after the second contraction with Ach was obtained. The area under the curve (AUC) of the contractile muscle was measured over a 5–10 min period, both in rest state and during chemical treatment.

The muscle strips were treated with morphine (10^{-5}–3.2×10^{-3} M), thienorphine (10^{-6}–10^{-4} M), or buprenorphine (10^{-6}–10^{-4} M) for 10 min at different concentration. The contractile response of bladder muscle strips to the chemicals was expressed as change in AUC (amplitude)%= muscle contractions after treatment/ muscle contractions before treatment × 100%.

2.2.4. mRNA expression levels for μ, κ, δ and N/OFQ receptor in ureter and bladder of guniea pigs

The relative mRNA expression levels for μ, κ, δ and N/OFQ receptor were quantified by SYBR Green-Based Quantitative PCR (polymerase chain reaction). Total RNA was extracted from ureter and bladder of the guniea pigs using the RNeasy Mini kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). First-strand cDNA was synthesized using 1 μg RNA, 5 × first strand buffer (Invitrogen, Karlsruhe, Germany), 1 μl Oligo(dT)18 primer (10 mM), 2 μl dNTPs (10 mM), 1 μl MML-V (200 U/μl), 1 μl ribonuclease inhibitor (40 U/μl), and nuclease-free water to a total of 20 μl. Reverse transcription reactions were performed for 1 h at 40 °C, then 10 min at 70 °C. Afterward, SYBR green-based quantitative (q) PCR was performed using an ABI PRISM 7300 Sequence Detection System (Applied Biosystems, Foster City, CA) with the primers in Table 1. All experiments were performed in duplicate and repeated at least three times. Three controls aimed at detecting genomic DNA contamination in the RNA sample during the RT or qPCR reactions were always included: an RT mixture without reverse transcriptase, an RT mixture including the enzyme but no RNA as the negative control (reaction mixture without cDNA template). The data were collected and analyzed using OneStep Software (ABI). Relative quantification was performed using the comparative threshold (CT) method (ΔΔCT) after determining reference CT values for β-Actin and the target gene.

2.3. Statistical analysis

Statistical and curve-fitting analyses were performed using PRISM 5.0 (GraphPadSoftwareInc., LaJolla, CA, USA). The data were