Effects of mu and kappa opioid receptor agonists and antagonists on contraction of isolated colon strips of rats with cathartic colon

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Abstract
AIM: To study the effects of mu and kappa opioid receptor agonists and antagonists on the isolated colon strips of rats with cathartic colon.

METHODS: Cathartic colon model was established by feeding rats with contact laxatives, and effects of mu and kappa opioid receptor agonists and antagonists on electricity-stimulated contraction of isolated colon strips of rats with cathartic colon were observed.

RESULTS: Compared with control group, exogenous mu and kappa agonists inhibited significantly electricity-stimulated contraction of strips of cathartic colon (8.50±0.89 mm, 6.24±0.91 mm, 3.35±0.06 mm vs 11.40±0.21 mm, P<0.01; 8.98±0.69 mm, 6.89±0.71 mm, 4.43±0.99 mm vs 11.40±0.21 mm, P<0.01). In contrast, the exogenous mu antagonist significantly enhanced electricity-stimulated contraction of isolated colon strips (13.18±0.93 mm, 15.87±0.98 mm, 19.46±1.79 mm vs 11.40±0.21 mm, P<0.01), but kappa antagonist had no effect on the isolated colon strips of rats with cathartic colon.

CONCLUSION: Mu and kappa opioid receptors are involved in the regulation of colon motility of rats with cathartic colon.

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INTRODUCTION
The cause and pathogenesis of slow-transit constipation (STC) still remain unknown now[1]. To study the mechanism of STC, more and more attention has been recently paid on the function of numerous neurotransmitters involved in the onset of STC[2-4]. With a rat model of cathartic colon[5,6], we investigated the effects of opioids, inhibitory neurotransmitters, on the electricity-stimulated contraction of isolated cathartic colon strips.

MATERIALS AND METHODS
Materials
Rhubarb and phenolphthalein powders were provided by Chongqing Traditional Chinese Medicine Pharmaceutical Factory and Chongqing Dongfeng Reagent Factory, respectively. Mu and kappa opioid receptor antagonists (Naloxone, Norbni) and agonists (Damgo, U50488H) were purchased from Sigma Co USA.

Fifty Wistar rats of either sex, weighing 230±70 g, were divided randomly into control group (n=10) and cathartic colon group treated with rhubarb (n=20) and phenolphthalein (n=20). Because both rhubarb and phenolphthalein belong to the same kind of contact cathartics, the two groups were considered as one cathartic colon group.

Rats were housed in cage, one per cage under standard laboratory conditions (room temperature, 18-28 °C, relative humidity, 40-80%). Control rats were given soft chows, while the rats in rhubarb group were given chows premixed with rhubarb powder. The initial rhubarb dosage was 200 mg/kg.d, and another 200 mg/kg was added every day until it reached 1000 mg/kg.d for several days until loose stool disappeared. Then, rhubarb was added 200 mg/kg.d again until 2 400 mg/kg.d for 3 mo. The rats in phenolphthalein group were fed chows premixed with an initial dosage of phenolphthalein 200 mg/kg. Its 50% for dosage diarrhea was 1 400 mg/kg.d and its final dosage was 3 200 mg/kg.d.

Method
Rats were killed by head-strike, the abdominal cavity was opened through a median incision, and a 5 cm colon in length from the ileocecum was then quickly dissected and transferred to Krebs solution and rinsed. The Krebs solution contained NaCl, 112.8 mmol; KCl, 5.90 mmol; CaCl, mmol; MgCl, 1.18 mmol; NaHPO, 1.22 mmol; NaHCO, 25.0 mmol; Glu, 11.49 mmol (pH 7.2-7.4). Rinsed colon was scratched off serous membrane and cut into 2 cmx2 cm strips. One end of the strip was fixed on a supporting rod, and the other end was fixed to the tension transducer. Each muscle strip was vertically placed in an organ bath filled with 10 mL Krebs solution maintained at 37 °C and gased with (950 mL/LCO)/(50 mL/LCO). Muscle contraction was activated by electrical field stimulation with a pair of external platinum ring electrodes connected to a square wave stimulator. The electrodes were parallelly placed on each end of the strip, on which continuous electrical stimulations (4 ms in duration, 10 Hz and 70 V in electric pressure) were conducted. The strips were given 1 g initial tensions and equilibrated for 60 min. Isometric contraction was measured with a tension transducer connected to a physiological recorder, and the contraction amplitude was printed on standard chart paper. The direct effects of opioid receptor agonists and antagonists on the contractility of isolated muscle strips were studied by addition of opioid receptor agonists and antagonists to organ bath to make a required solution. The recorded data were depicted into concentration-response curves. To evaluate the response of muscle strips to electrical stimulation, the contraction amplitude was calculated and the average of amplitude was accounted.

Statistical Analysis
Results were expressed as mean±SE. Differences were
analyzed by Student t test. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Electricity-stimulated contractile response of isolated colon strips**

The contractile response of most colon strips (about 70\%) showed a typical sinusoid curve, while about 30\% strips demonstrated wild and irregular waves. In the cathartic colon group, the decrease of contraction amplitude was about 27.43\% of that in control group.

**Effect of mu opioid receptor agonists on electricity-stimulated contractile response of cathartic colon strips**

The mu opioid receptor agonist, Dmago, caused a concentration-dependent inhibition of electricity-stimulated contraction of cathartic colon strips. Dmago solutions (0.05 \( \mu \)mol/L, 0.10 \( \mu \)mol/L, 1.00 \( \mu \)mol/L) could induce a significant inhibition of the contractile response, which showed that the amplitude of muscle strip contraction was significantly reduced (\( P < 0.01 \)) in the presence of Dmago (Table 1).

**Effect of kappa opioid receptor agonists on electricity-stimulated contractile response of cathartic colon strips**

The mu opioid receptor antagonist, naloxone, could induce a concentration-dependent elevation of electricity-stimulated contraction of cathartic colon strips. Each naloxone concentration (0.05 \( \mu \)mol/L, 0.10 \( \mu \)mol/L, 1.00 \( \mu \)mol/L) induced a significant elevation of the contractile response, which showed that the contraction amplitude was significantly elevated (\( P < 0.01 \)) in the presence of naloxone (Table 2).

**Effect of kappa opioid receptor antagonists on electricity-stimulated contractile response of cathartic colon strips**

Nobin is a highly-selective kappa opioid receptor antagonist, which did not show any evident effect on the electricity stimulated contraction of colon strips of rats with cathartic colon. Norbin concentration (0.05 \( \mu \)mol/L, 0.10 \( \mu \)mol/L, 1.00 \( \mu \)mol/L) induced a significant inhibition of the contractile response (\( P < 0.01 \)) (Table 3).

**DISCUSSION**

Opioids have extensive distributions and potent effects on the gastrointestinal tract\(^{[11,12]}\). Contractility studies also indicated that opioids, in combination with mu, kappa and delta opioid receptor agonists, could inhibit motor activity of the gastrointestinal tract by suppression of excitatory neurotransmitter release\(^{[13-15]}\). Our study manifested that exogenously added opioid receptor agonists (mu, kappa) inhibited the contractility of colon strips of rats with cathartic colon, which showed a significant reduction of contraction amplitude as compared to the basic contraction with no agonists. The inhibitory effects were negatively correlated with concentrations. In contrast, mu receptor antagonists elevated electricity stimulated contraction of cathartic colon in rats. However, kappa receptor antagonists had no effect. The results suggested that mu and kappa opioid receptor might play an important role in the regulation of gastrointestinal motility in rats. It also further approved that opioids could slow down the propulsive peristalsis performed by nerves and muscles in colon and played a very important role in the onset and pathologic process of STC.

Kreek hypothesized that the changes of opioids activity were the important etiological factor, and STC patients could be successfully treated with naloxone, a mu opioid receptor antagonist. The present study was designed to investigate the effects of opioid agonists and antagonists on isolated cathartic colon muscle strips, it provided a new fundamental theory on the STC treatment with opioid receptor antagonists. It also indicated that the studies on the subtype and binding site of opioid receptors, as well as the overall research in clinic, would provide new methods for STC therapies, and also benefit the clarification of the pathogenesis of STC. In conclusion, mu and kappa opioid receptors are involved in the regulation of colon motility of rats with cathartic colon.

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