

## L-365,260 reversed effect of sincalide against morphine on electrical and mechanical activities of rat duodenum *in vitro*<sup>1</sup>

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**KEY WORDS** sincalide; morphine; acetylcholine; duodenum; electrophysiology; muscle contraction

### ABSTRACT

**AIM:** To study the antagonism of sincalide (CCK-8) to the effect of morphine and its mechanism. **METHODS:** The electrical and mechanical activities of rat duodenum *in vitro* were recorded simultaneously. **RESULTS:** Acetylcholine (ACh, 300 nmol/L) increased the spike potential amplitude (SPA) and the number (SPN) of rat duodenum *in vitro*, followed by an increase of duodenal contraction amplitudes (CA). The SPA, SPN, and CA of duodenum *in vitro* were not obviously affected by injection of morphine (330 nmol/L), but it could selectively inhibit the potentiation of ACh. After administration of CCK-8 (0.7 nmol/L), the SPA, SPN, and CA of duodenal segment did not exhibit obvious changes. But CCK-8 could selectively antagonize the effects of morphine, ie, the SPA and SPN were increased again, followed by an increase of CA. CCK-B receptor antagonist L-365,260 (30 nmol/L) reversed the antagonism of CCK-8 to the effect of morphine. **CONCLUSION:** CCK-8 could selectively antagonize the effect of morphine which inhibited the potentiation of ACh on duodenal activities *in vitro*. The antagonistic effect of CCK-8 on morphine was mainly mediated by CCK-B receptor.

### INTRODUCTION

Sincalide (CCK-8) is a typical brain-gut peptide. Many data showed that CCK-8 was the strongest

endogenous anti-opioid substance up to now. CCK-8 could block morphine analgesia in the rat tail flick test<sup>(1)</sup>. CCK-8 antagonized the analgesic effects of morphine and electroacupuncture (EA), and played an important role in the induction of morphine tolerance and EA tolerance using the behavioral changes and electrophysiologic methods, respectively<sup>(2-5)</sup>. Opioids antagonized the hyperfunction of contraction of guinea pig ileum *in vitro* induced by CCK-8<sup>(6)</sup>. CCK-8 antagonized the effects of morphine-inhibited electrical and contractile activities of rat jejunum *in vitro*<sup>(7)</sup>. But few report about the anti-opioid effect of CCK-8 on the duodenum *in vitro* was found. This paper was to inquire into the antagonism of CCK-8 to the effect of morphine and its mechanism.

### MATERIALS AND METHODS

**Experimental animals** Wistar rats ( $n = 60$ , Grade II, ♂ and ♀, 195-295 g, Animal Department of Provincial Tumor Institute in Heilongjiang, Certificate No 09-2-1 conferred by Medical Experimental Animal Management Committee of Heilongjiang Province) were used.

**Experimental reagents** Tyrode's solution (dissolving NaCl 0.80 g, KCl 0.20 g, CaCl<sub>2</sub> 0.20 g, NaHCO<sub>3</sub> 1.00 g, NaH<sub>2</sub>PO<sub>4</sub> 0.05 g, MgCl<sub>2</sub> 0.10 g, and glucose 1.00 g in 1 L of distilled water), acetylcholine (ACh, 300 nmol/L, Shanghai No 3 Reagent Factory, China), morphine hydrochloride (330 nmol/L, Shenyang First Pharmaceutical Factory, China), CCK-8 (0.7 nmol/L, Squibb, USA), and L-365,260 (30 nmol/L, Merck Sharp and Dohme Researched Laboratories, USA) were used.

**Experimental method** One or two segments of 2 cm duodenum were cut off under pylorus of each rat. The duodenal segments were suspended in a bathtube of Tyrode's solution 50 mL saturated with oxygen at 38 °C. Then the bathtube was maintained at 38 °C in CS-501 superthermostat (Chongqing Experimental Equipment

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Factory). One end of duodenal segment was fixed with resting load 5 g and the other end was connected with a LZ-1 tension transducer (Harbin Tongjiang Transistor Factory) according to longitudinal axis of duodenum. The electrical activities of duodenum were led out by silver adsorptive electrode. The electrical activities, mechanical contraction, and time scale were simultaneously recorded by SJ-41 multipurpose polygraph (Shanghai Medical Electronic Instrument Factory). The parameters were modulated as follows: time constant 0.3 s, high frequency wave filter 30 Hz, electrical gain 3, mechanical gain 4, recording paper velocity 5 mm/s<sup>[7]</sup>.

Firstly, normal electrical and mechanical activities of every segment of duodenum was simultaneously recorded. Then ACh 200  $\mu$ L (25 mg/L) was added quickly into the bathtube by a microinjector. At 60 s, morphine hydro-

chloride 50  $\mu$ L (100 mg/L) was administered. At 120 s and 240 s, 40  $\mu$ L (1 mg/L) of CCK-8 and 60  $\mu$ L (10 mg/L) of L-365,260 were added respectively.

**Statistical analysis** Each value was expressed as  $\bar{x} \pm s$ . All data were analyzed with paired *t*-test.

## RESULTS

**Effects of morphine or CCK-8 on the electrical and mechanical activities of duodenal segments** The electrical and mechanical activities of duodenal segment did not exhibit obvious changes after the injection of morphine (Fig 1A). If CCK-8 injected alone, the activities of duodenal segment did not exhibit obvious changes, indicating no effects of morphine or CCK-8 *per se*.

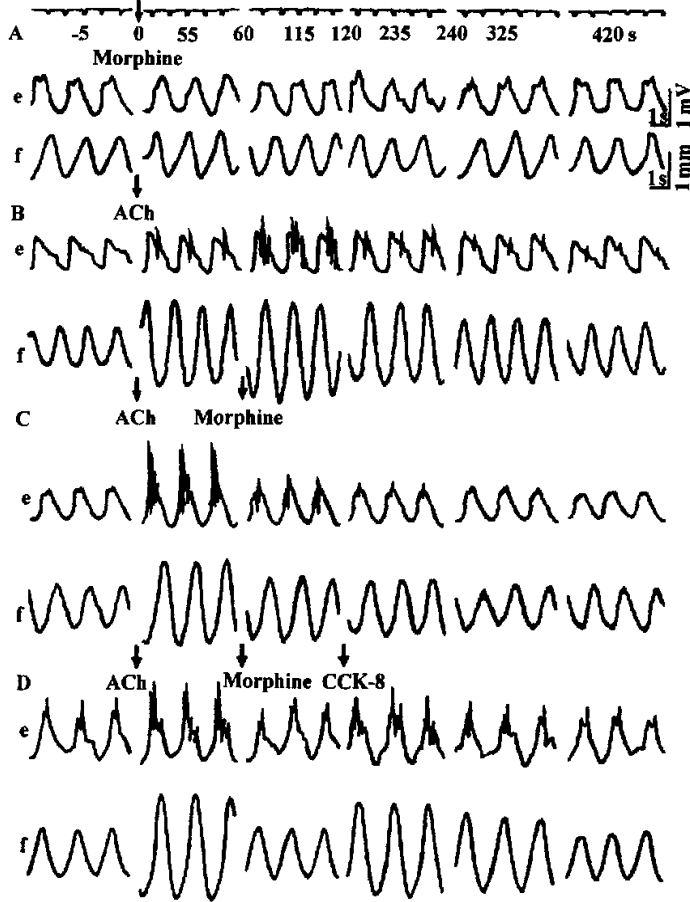


Fig 1. Effects of morphine (A), ACh (B), ACh + morphine (C) or ACh + morphine + CCK-8 (D) on the simultaneous electrical and mechanical activities of rat duodenum *in vitro*. e: electrical activity; f: mechanical contraction;  $\downarrow$ : drug injection.

After injection of morphine or CCK-8, the electrical and contractile changes of 20 duodenal segments did not show significant difference as compared with those before injection ( $P > 0.05$ ).

**Morphine inhibited the potentiation of ACh on the activities of duodenum** As shown in Fig 1B, the spike potential amplitudes (SPA) and the number (SPN) were increased, followed by the increase of the duodenal contraction amplitude (CA) after addition of ACh, showing the enhancement effects of ACh on duodenal activities. At 115 s after injection of ACh, the effects of ACh has been the strongest. At 60 s after addition of ACh, the administration of morphine produced a reduction of the SPA, SPN, and CA, showing that morphine inhibited the excitatory effect of ACh (Fig 1C).

**CCK-8 antagonized the inhibitory effect of morphine on the effect of ACh** Before injection of ACh, the SPA and SPN of 22 duodenal segments and their corresponding CA averaged at  $(0.70 \pm 0.07)$  mV,  $(2.10 \pm 0.09)$ , and  $(16.8 \pm 0.9)$  mm, respectively. At 60 s after the injection of ACh, the SPA, SPN, and corresponding CA were increased to  $(1.01 \pm 0.09)$  mV,  $(3.13 \pm 0.12)$ , and  $(24.1 \pm 1.2)$  mm, respectively. At this time, morphine was added. The SPA, SPN, and CA were decreased to  $(0.69 \pm 0.06)$  mV,  $(2.09 \pm 0.08)$ , and  $(15.9 \pm 0.9)$  mm at 60 s after injected morphine, respectively. At 120 s after adding CCK-8, the SPA, SPN, and CA were increased to  $(0.89 \pm 0.05)$  mV,  $(3.11 \pm 0.10)$ , and  $(22.8 \pm 0.9)$  mm, respectively. Moreover, all of them showed significant differences ( $P < 0.01$ ) when the latter was compared with the corresponding item of the former (Fig 1D, 2).

**L-365,260 reversed the antagonistic effect of CCK-8 on the effect of morphine** The SPA and SPN of the duodenal segment were increased, followed by an increase of the CA after the injection of ACh, showing the enhancement of duodenal activities. Whereas

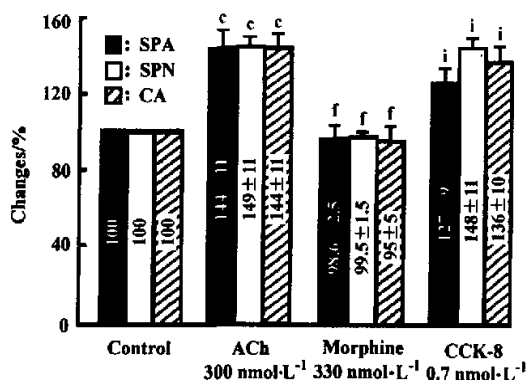


Fig 2. Antagonistic effect of CCK-8 on inhibitory effect of morphine on effect of ACh. Control value (SPA = 0.7 mV, SPN = 2.1, CA = 16.8 mm) as 100%.  $n = 22$  duodenal segments.  $\bar{x} \pm s$ . <sup>c</sup> $P < 0.01$  vs control. <sup>f</sup> $P < 0.01$  vs ACh. <sup>i</sup> $P < 0.01$  vs morphine.

the SPA, SPN, and corresponding CA were reduced by morphine, showing that morphine inhibited the excitatory effects of ACh. At this moment, addition of CCK-8 increased the SPA, SPN, and CA, suggesting an antagonism of morphine effect by CCK-8. On the basis of the above, after CCK-B receptor antagonist L-365,260 was added, the SPA and SPN were decreased again, accompanied by the reduction of CA. It showed that L-365,260 reversed the anti-morphine effect of CCK-8. Moreover, every contraction wave occurred after the beginning of spike potential over the slow potential and the ratio between slow potential and contraction wave was 1:1 (Fig 3).

The statistical analytical results of 25 duodenal segments were shown in Tab 1.

## DISCUSSION

CCK-8 was the first brain-gut peptide found in human. CCK-8 existed in brain and peripheral tissues of

Tab 1. Anti-morphine effects of CCK-8 reversed by L-365,260.  $n = 25$  duodenal segments.  $\bar{x} \pm s$ . <sup>c</sup> $P < 0.01$  vs control. <sup>f</sup> $P < 0.01$  vs ACh. <sup>i</sup> $P < 0.01$  vs morphine. <sup>j</sup> $P < 0.01$  vs CCK-8.

Items	Control (0 s)	ACh 300 nmol·L <sup>-1</sup> (60 s)	Morphine 330 nmol·L <sup>-1</sup> (120 s)	CCK-8 0.7 nmol·L <sup>-1</sup> (240 s)	L-365,260 30 nmol·L <sup>-1</sup> (325 s)
SPA/mV	0.69 ± 0.05	1.00 ± 0.06 <sup>c</sup>	0.67 ± 0.04 <sup>f</sup>	0.87 ± 0.04 <sup>j</sup>	0.55 ± 0.03 <sup>j</sup>
SPN	2.13 ± 0.08	3.07 ± 0.11 <sup>c</sup>	2.06 ± 0.08 <sup>f</sup>	3.06 ± 0.10 <sup>j</sup>	2.09 ± 0.09 <sup>j</sup>
CA/mm	16.8 ± 0.8	24.1 ± 1.2 <sup>c</sup>	15.9 ± 0.8 <sup>f</sup>	22.8 ± 0.9 <sup>j</sup>	15.6 ± 0.8 <sup>j</sup>

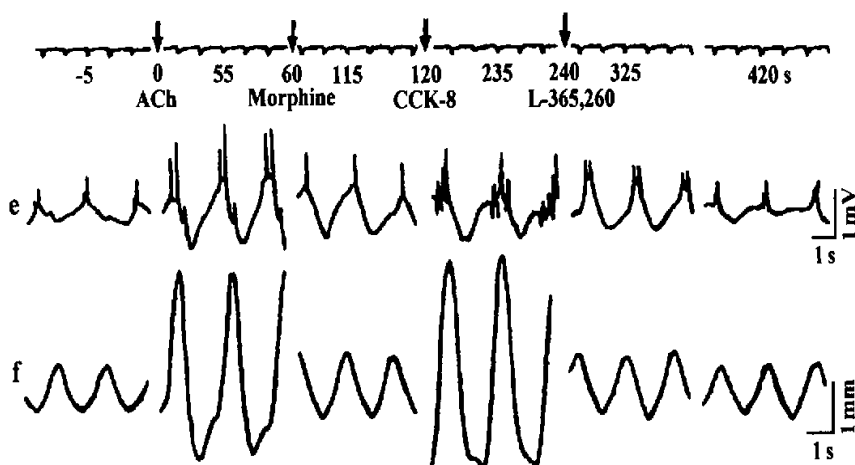


Fig 3. Influence of L-365,260 on anti-morphine effect of CCK-8. e: electrical activity; f: mechanical contraction.

animals and human<sup>[8]</sup>. In the studies of the relationship between CCK-8 and opioid peptides, it was found that CCK-8 played an important role in the modulation of pain sensation, acting as an anti-opioid substance<sup>[9,10]</sup>. So did CCK-8 in the regulation of gastrointestinal motility function<sup>[11,12]</sup>. Previous works indicated that an antagonistic interaction might occur between CCK and opioid peptides<sup>[2,3]</sup>. The experimental results demonstrated that ACh could increase the SPA, SPN, and CA of rat duodenum *in vitro*. Morphine on the activities of duodenal segments was not obviously affected, but it could selectively inhibit the potentiation of ACh. CCK-8 *per se* did not show any effect, but could selectively antagonize the effects of morphine which inhibited the potentiation of ACh to rat duodenum *in vitro* with the electrical and mechanical activities. The conclusion was similar to those previous reports<sup>[6,7,13]</sup>.

Recent receptor binding studies have confirmed the existence of 2 distinct CCK receptor subtypes, ie, CCK-A and CCK-B receptors, which were presented in both brain and peripheral tissues<sup>[14]</sup>. L-365,260 was considerably more potent in inhibiting CCK binding to brain-type receptor (CCK-B) than to peripheral-type receptor (CCK-A)<sup>[15]</sup>. This results showed that L-365,260 could reverse the antagonism of CCK-8 to the effect of morphine, therefore it was inferred that CCK-B receptor participated in the anti-morphine effect of CCK-8.

The present work firstly demonstrated that CCK-8 could antagonize the elimination of morphine on the

potentiations of ACh to duodenal activities, and these effects were mediated by CCK-B receptor. It is suggested that CCK-like peptides and opioid substances together with cholinergic system could regulate the gastrointestinal activities, and provide a new experimental basis for further research in the clinical treatment of the intestinal motility disturbances.

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## L-365,260 翻转辛卡利特对抗吗啡对大鼠离体十二指肠电与机械活动的影响<sup>1</sup>

R916 A

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**关键词** 辛卡利特; 吗啡; 乙酰胆碱; 十二指肠; 电生理学; 肌收缩

**目的:** 研究辛卡利特(CCK-8)的抗吗啡作用及其作用机制. **方法:** 采用了同步描记大鼠离体十二指肠电与机械活动的方法. **结果:** 乙酰胆碱(ACh, 300 nmol/L)可使大鼠离体十二指肠峰波振幅增大、数目增多, 其收缩幅度随之增大, 两者呈正相关. 吗啡(330 nmol/L)对离体十二指肠峰波的振幅、数目和收缩幅度均无明显影响, 但能选择性抑制ACh对十二指肠段电与机械活动的加强作用, 呈负相关. CCK-8 (0.7 nmol/L)本身对十二指肠段的电与收缩活动均无明显影响, 但能选择性对抗吗啡的作用, 即峰波振幅、数目再次增加, 收缩幅度也随之增加. 在此基础上, CCK-B受体拮抗剂L-365,260 (30 nmol/L)能翻转CCK-8的抗吗啡作用. **结论:** CCK-8能对抗吗啡抑制ACh加强十二指肠活动的作用, 推测该作用是通过CCK-B受体实现的.

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