

## Devazepide reversed effect of sincalide against morphine on rat jejunal activities<sup>1</sup>

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**KEY WORDS** sincalide; devazepide; morphine; jejunum; electrophysiology; muscle contraction; acetylcholine

### ABSTRACT

**AIM:** To study the antagonism of sincalide to the effect of morphine and its mechanism. **METHODS:** The electrophysiologic and mechanic activities of rat jejunum *in vitro* were recorded. **RESULTS:** Acetylcholine (ACh, 150 nmol · L<sup>-1</sup>) increased the spike potential amplitude (SPA) and the number (SPN) of rat jejunum *in vitro*, followed by an increase of jejunal contraction amplitudes (CA), showing a positive correlation. Morphine 330 nmol · L<sup>-1</sup> inhibited the potentiation of ACh, showing a negative correlation. Sincalide 0.7 nmol · L<sup>-1</sup> antagonized the effects of morphine, i.e., the SPA and SPN were increased again, followed by an increase of CA. CCK-A receptor antagonist devazepide (10 nmol · L<sup>-1</sup>) reversed the antagonism of sincalide to the effect of morphine. **CONCLUSION:** Sincalide antagonized the effect of morphine which inhibited the potentiation of ACh on jejunal activities *in vitro*. The antagonistic effect of sincalide on morphine was mainly mediated by CCK-A receptor.

### INTRODUCTION

Cholecystokinin octapeptide (sincalide) is a typical brain-gut peptide. Sincalide was the strongest

endogenous anti-opioid substance. Sincalide blocked morphine analgesia in the rat tail flick test<sup>[1]</sup>. Sincalide 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>[2,3]</sup>. Morphine and opioid peptides antagonized the hyperfunction of contraction of guinea pig ileum *in vitro* induced by sincalide like peptide<sup>[4]</sup>. Endogenous opioid peptides antagonized the effects of sincalide<sup>[5]</sup>. But few report about the anti-opioid effect of sincalide on the jejunum *in vitro* was found.

This paper was to inquire into the antagonism of sincalide to the effect of morphine and its mechanism.

### MATERIALS AND METHODS

Wistar rats ( $n = 27$ , Grade II, ♂ and ♀, 250 - 350 g, Animal Department of Provincial Tumour Institute in Heilongjiang, Certificate No 09-2-1 conferred by Medical Experimental Animal Management Committee of Heilongjiang Province) were used. Two or three segments of 2 cm jejunum which were in distal 2 cm of duodenojejunal curve of each rat were suspended in Tyrode's solution 50 mL saturated with oxygen at 38 °C. One end of jejunal segment was fixed with resting load 5 g and the other end was connected with a tension transducer according to longitudinal axis of jejunum. The electrophysiological activities of jejunum were led out by silver adsorptive electrode. The electrophysiological activities, mechanical contraction, and time scale were simultaneously recorded by ST-41 multipurpose polygraph; time constant 0.3 s, high frequency wave filter 30 Hz, electrophysiological gain 3, mechanic gain 4, recording paper velocity 5 mm · s<sup>-1</sup><sup>[6]</sup>

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ACh 50  $\mu\text{L}$  ( $150 \text{ nmol} \cdot \text{L}^{-1}$ , Shanghai Third Reagent Factory, China) was added into the bathtub by a microinjector. At 70 s, morphine hydrochloridum 50  $\mu\text{L}$  ( $330 \text{ nmol} \cdot \text{L}^{-1}$ , Shenyang First Pharmaceutical Factory, China) was administered. At 150 s and 250 s, sincalide 40  $\mu\text{L}$  ( $0.7 \text{ nmol} \cdot \text{L}^{-1}$ , Squibb, USA) and devazepide 20  $\mu\text{L}$  ( $10 \text{ nmol} \cdot \text{L}^{-1}$ , Merck Sharp and Dohme Research Laboratories, USA) were added.

Data were expressed as  $\bar{x} \pm s$  and analyzed with paired *t*-test.

## RESULTS

Sincalide antagonized the inhibitory effect of morphine on the effect of ACh. Before ACh, the spike potential amplitudes (SPA) numbers (SPN) of 17 jejunal segments from 17 rats, and their corresponding contraction amplitudes (CA) averaged at ( $0.7 \pm 0.2$ ) mV,  $2.1 \pm 0.6$ , and ( $12 \pm 5$ ) mm, respectively. At 70 s after the injection of ACh, the SPA, SPN, and corresponding CA were increased to ( $1.1 \pm 0.3$ ) mV,  $2.6 \pm 0.9$ , and ( $24 \pm 10$ ) mm, respectively. At 70 s after ACh, morphine was added. The SPA, SPN, and CA were decreased to ( $0.6 \pm 0.2$ ) mV,  $1.9 \pm 0.6$ , and ( $11 \pm 4$ ) mm at 80 s after morphine, respectively. At 100 s after adding sincalide, the SPA, SPN, and CA were increased to ( $1.0 \pm 0.3$ ) mV,  $2.6 \pm 0.6$ , and ( $17 \pm 6$ ) mm, respectively (Fig 1).

Devazepide reversed the antagonistic effect of sincalide on the effect of morphine. The SPA and SPN of the jejunal segment were increased, followed by an increase of the CA after the injection of ACh, showing the enhancement of jejunal activities. The SPA, SPN, and corresponding CA were reduced by morphine, showing that morphine inhibited the excitatory effects of ACh. At this moment, addition of sincalide increased the SPA, SPN, and CA, suggesting an antagonism of morphine effect by sincalide. After devazepide, the SPA and SPN were decreased again, accompanied by the reduction of CA. It showed that devazepide reversed the anti-morphine effect of sincalide. 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 2).

The results of 43 jejunal segments ( $n = 27$  rats)

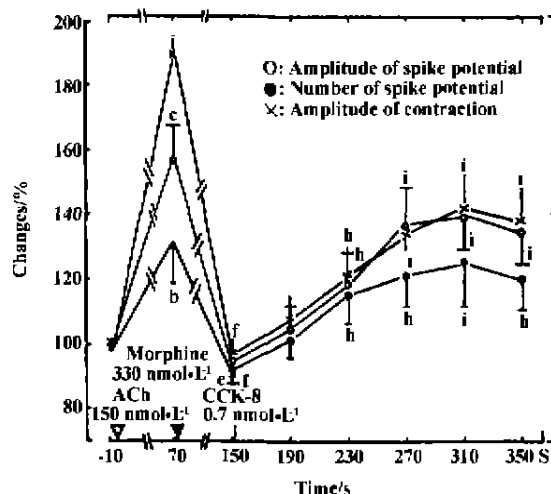


Fig 1. Antagonistic effect of sincalide on inhibitory effect of morphine on effect of ACh. Control value (SPA = 0.7 mV, SPN = 2.1, CA = 12.1 mm) as 100%.  $n = 17$  jejunal segments.  $\bar{x} \pm s$ .  $^bP < 0.05$ ,  $^cP < 0.01$  vs control.  $^eP < 0.05$ ,  $^fP < 0.01$  vs ACh.  $^hP < 0.05$ ,  $^iP < 0.01$  vs morphine.

were shown in Tab 1.

## DISCUSSION

Sincalide was the first brain-gut peptide found in human. Sincalide existed in brain and peripheral tissues of animals and human<sup>[7]</sup>. Previous works indicated that an antagonistic interaction might occur between CCK and opioid peptides. The experimental results demonstrated that sincalide *per se* did not show any effect, but could selectively antagonize the effects of morphine which inhibited the potentiation of ACh to rat jejunum *in vitro* with the electrophysiological and mechanical activities. The conclusion was similar to those previous reports<sup>[4,5]</sup>.

Recent receptor binding studies have confirmed the existence of 2 distinct CCK receptor subtypes, ie, CCK-A and CCK-B receptors were presented in both brain and peripheral tissues<sup>[8]</sup>. Devazepide was considerably more potent in inhibiting CCK binding to peripheral-type receptor (CCK-A) than to brain-type receptor (CCK-B)<sup>[9]</sup>. This results showed that devazepide could reverse the antagonism of sincalide to the effect of morphine, therefore it was inferred that CCK-A receptor participated in the anti-morphine effect of sincalide.

The present work firstly demonstrated that

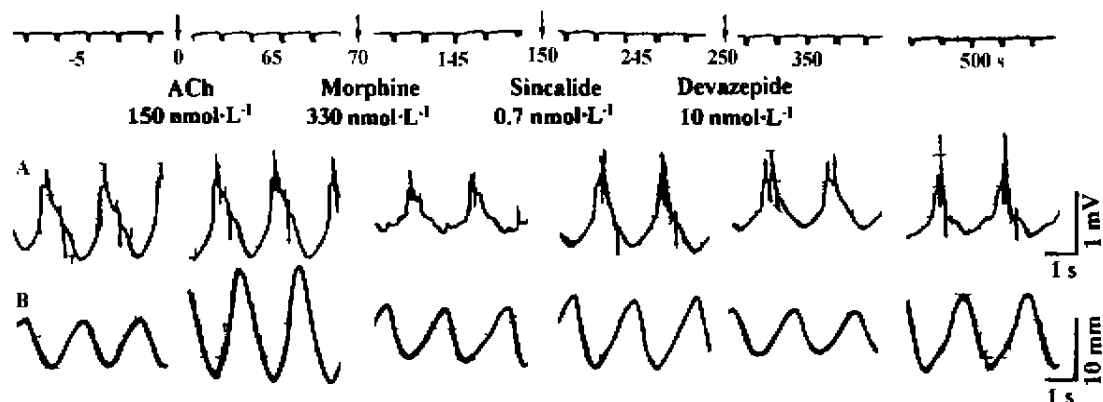


Fig 2. Influence of devazepide on anti-morphine effect of sincalide. A) electrophysiologic activity. B) mechanical contraction.

Tab 1. Effects of ACh, morphine, sincalide, and devazepide on electrophysiologic and contractile activities of 43 jejunal 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>l</sup>P < 0.01 vs sincalide.

Items	Control (0 s)	ACh 150 nmol·L <sup>-1</sup> (70 s)	Morphine 330 nmol·L <sup>-1</sup> (150 s)	Sincalide 0.7 nmol·L <sup>-1</sup> (250 s)	Devazepide 10 nmol·L <sup>-1</sup> (400 s)
SPA/mV	0.72 ± 0.21	1.0 ± 0.3 <sup>c</sup>	0.57 ± 0.24 <sup>f</sup>	0.9 ± 0.4 <sup>i</sup>	0.6 ± 0.3 <sup>l</sup>
SPN	1.8 ± 0.5	2.3 ± 0.6 <sup>c</sup>	1.8 ± 0.5 <sup>f</sup>	2.3 ± 0.7 <sup>i</sup>	1.8 ± 0.5 <sup>l</sup>
CA/mm	11 ± 4	22 ± 8 <sup>c</sup>	12 ± 5 <sup>f</sup>	19 ± 7 <sup>i</sup>	12 ± 6 <sup>l</sup>

sincalide could antagonize the elimination of morphine on the potentiations of ACh to jejunal activities, and these effects were mediated by CCK-A receptor. It is suggested that CCK-like peptides and opioid substances together with cholinergic system could regulate the gastrointestinal activities.

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### 地伐西匹翻转辛卡利特对抗吗啡对大鼠空肠活动的作用<sup>1</sup>

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**关键词** 辛卡利特; 地伐西匹; 吗啡; 空肠;  
电生理学; 肌肉收缩; 乙酰胆碱

**目的:** 研究辛卡利特(sincalide)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空肠  
电生理与机械活动的方法。 **结果:** 乙酰胆碱  
(ACh)可使大鼠离体空肠峰波振幅增大、数目增  
多, 其收缩幅度随之增大, 两者呈正相关。 相反  
地, 吗啡能抑制 ACh 的加强作用, 呈负相关。 辛  
卡利特能对抗吗啡的作用, 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-A 受体拮抗剂地伐西匹能翻转辛卡利特的抗  
吗啡作用。 **结论:** 辛卡利特对抗吗啡抑制 ACh 加  
强空肠活动的作用。 推测该作用是通过 CCK-A 受  
体实现的。

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## 关于申报评选 1999 年第三届 中国药理学会 Servier 青年药理学工作者奖的通知

经中国药理学会与法国 Servier 研究院商定, 现将 1999 年第三届中国药理学会 Servier 青年药理学工作者奖申报评选工作的通知:

### 1 获奖候选人条件:

- 1.1 中国药理学会会员;
- 1.2 年龄在 37 岁(1962 年 10 月 31 日以后出生)以下;
- 1.3 在国内从事药理学研究并取得优秀成绩, 不包括在国外做过的工作;
- 1.4 从获奖之日起至少在国内工作一年以上。

### 2 报名及评选程序:

- 2.1 符合上述条件的青年药理学工作者接到通知后可向各地区或直辖市评选负责人(名单列后)报名, 报名截止日期为 1999 年 7 月 31 日(以邮戳为准)。报名时需报送以下材料:(1)个人简历, 中英文各一份;(2)未发表的论文一篇, 全文用中文或英文撰写均可, 但摘要和图表须用英文撰写;(3)二位专家(教授或相当职称者)的推荐信, 并经单位同意及加盖公章。
- 2.2 初审各地评选负责人(或学会)组织专家对本地区申报材料进行评审, 评选出 3 名候选人(西南、重庆可多报 1-2 名), 将其材料及本地区专家组评审意见及排名顺序, 于 1999 年 8 月底前报送中国药理学会办公室(100050 北京先农坛街 1 号中国药理学会孙静霞收)。
- 2.3 终审: 由中国药理学会组织专家评审, 选出 12-16 名候选人送 Servier 法方专家评审。9 月由中法两国评委共同评出 8 名获奖者。

### 3 颁奖:

1999 年 10 月在西安“中国药理学会世纪之交学术会议”期间举行颁奖活动, 将发给每位获奖者荣誉证书和壹万元奖金。

附: 全国各地区评选负责人名单:

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上海	王永铭	200032 上海医科大学药学院药理教研室
天津	刘昌孝	300193 国家医药管理局天津药物研究院
西北	白元让	710061 西安医科大学药理教研室
西南、重庆	包定元	610041 成都华西医科大学基础医学院药理室
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