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Effect of tetrandrine on morphine dependence in isolated guinea pig ileum[1]

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KEY WORDS calcium channel blockers; tetrandrine; nimodipine; morphine; opioid-related disorders; naloxone; ileum

ABSTRACT

AIM: To evaluate the effects of tetrandrine (Tet) and nimodipine (Nim) on the morphine (Mor) withdrawal response in the isolated guinea pig ileum. **METHODS**: The withdrawal contracture was elicited by addition of naloxone (Nal) (1 $\mu mol \cdot L^{-1}$) to the isolated naive ileum incubated with Mor (3 $\mu mol \cdot L^{-1}$) at 37.5 ℃ for 4 h or to the ileum obtained from Mor-dependent guinea pig. **RESULTS**: When Nim (0.01, 0.05, and 0.1 $\mu mol \cdot L^{-1}$) or Tet (1, 10, and 50 $\mu mol \cdot L^{-1}$) was added 1 min before Nal in the naive ilea bathed in Krebs solution containing Mor, or when the ilea from Mor-dependent guinea pigs were incubated with Nim (0.01, 0.05, and 0.1 $\mu mol \cdot L^{-1}$) or Tet (1, 10, and 50 $\mu mol \cdot L^{-1}$) for 15 min, or when Nim (5 and 10 $mg \cdot kg^{-1}$, ip) or Tet (15 and 30 $mg \cdot kg^{-1}$, ip) was administered *in vivo* to Mor-dependent guinea pigs, the Nal-precipitated withdrawal contracture was significantly decreased in a dose-dependent manner. **CONCLUSION**: Tet and Nim, Ca^{2+} channel blockers, could inhibit the Nal-precipitated Mor withdrawal response in the isolated guinea pig ileum.

INTRODUCTION

The isolated guinea pig ileum has been proved to be a model for the study of opioids dependence[1,2]. Contractile response was elicited when naloxone (Nal) administration to the naive ileum incubated with opioids[1] or to the ileum obtained from opioids-treated guinea pigs[3,4]. These withdrawal responses have pharmacological characteristics similar to the withdrawal signs in the intact animal.

Calcium (Ca^{2+}) plays an important role in some actions of opioids. Ca^{2+}-channel blockers, such as nimodipine (Nim), diltiazem, and verapamil, suppress the morphine (Mor) withdrawal syndrome in rats and mice[5]. The withdrawal contracture precipitated by Nal in the ileum obtained from Mor-dependent guinea pigs is inhibited by Ca^{2+} antagonists[4].

Tetrandrine (Tet), a plant alkaloid isolated from *Stephenia tetrandra* S Moore, is a structurally unique natural Ca^{2+} entry blocker[6]. We have previously demonstrated that Tet suppresses some signs of Mor withdrawal syndrome. In the present study, we investigated the effect of Tet on the Nal-precipitated Mor withdrawal response in the isolated guinea pig ileum, and compared it with Nim.

MATERIALS AND METHODS

Materials Guinea pigs (♂, $n = 25$, weighing 300 – 400 g, Certificate № 01-3056) were purchased from Department of Laboratory Animal Science, Beijing Medical University. Morphine HCl: Shenyang First Pharmaceutical Factory, China; naloxone HCl: Beijing Sihuan Pharmaceutical Factory, China; Nim: Shandong Xinhua Pharmaceutical Factory, China; Tet: Jiangxi Pengze Pharmaceutical Factory, China.

Preparation of ileal segments Guinea pigs were deprived of food for 24 h and killed by cervical dislocation. Pieces of ileum were immediately removed from a point 10 cm nearest to the ileo-caecal

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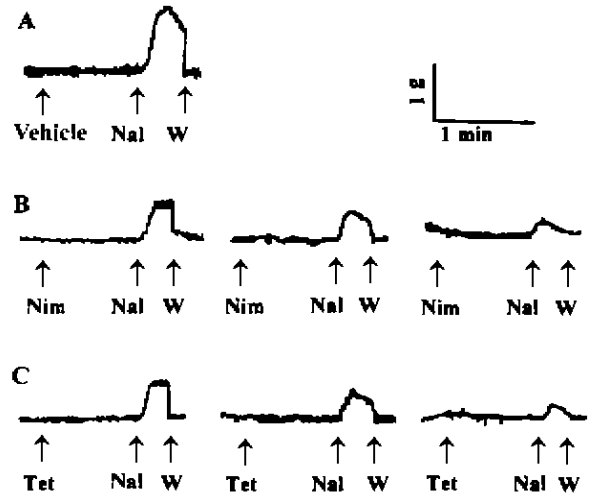
junction. These pieces were placed in Krebs solution, flushed through with the same solution and then divided into the desired numbers of 3 – 4 cm segments. The segments were suspended in Krebs solution (10 mL baths) at 37.5 ℃, aerated with 95 % O_2 + 5 % CO_2. The bath contents were changed at 10 – 15 min intervals. A resting tension of 1 g was applied to each tissue for 15 min before measurement was taken.

Determination of dependence Guinea pigs were divided into several groups: naive guinea pigs; non-dependent guinea pigs; Mor-dependent guinea pigs; Mor-dependent and treated with Nim or Tet guinea pigs. Tissues obtained from naive guinea pigs were bathed in Krebs solution containing Mor (3 μmol·L^{-1}) for 4 h. The withdrawal contracture was precipitated by addition of Nal (1 μmol·L^{-1})[11]. Nim (0.01, 0.05, and 0.1 μmol·L^{-1}) or Tet (1, 10, and 50 μmol·L^{-1}) were added 1 min before Nal. Guinea pigs were made Mor-dependent by sc injection of increasing doses of Mor tid for 6 days according to the following dose every day: 5, 10, 20, 40, 50 mg·kg^{-1}. Mor was given only once on the morning of the sixth day. Two h after the last dose of Mor, guinea pigs were killed and the ileum was removed for the *in vitro* study. Tissues obtained from Mor-dependent guinea pigs were bathed in Krebs solution containing Mor (0.3 μmol·L^{-1}). These preparations were incubated for 15 min with Nim (0.01, 0.05, and 0.1 μmol·L^{-1}) or Tet (1, 10, and 50 μmol·L^{-1}). Then the Nal-induced contracture was evaluated. In the group of Mor-dependent and treated with Nim or Tet guinea pigs, Nim (5, 10 mg·kg^{-1}, ip) or Tet (15, 30 mg·kg^{-1}, ip) was administered 1 h before killing. After equilibrating for 15 min, the withdrawal response was measured. All the *in vitro* withdrawal was precipitated by addition of Nal (1 μmol·L^{-1}) to the organ bath. The volume of drugs applied to the bath is 0.1 mL.

Statistics Responses in the presence of Nal which were obtained from different preparations were compared with their control responses by *t* test.

RESULTS

Naive ileum incubated with Mor Segments of ileum, taken from the same naive guinea pigs, were incubated in Krebs solution containing Mor (3 μmol·L^{-1}) at 37.5 ℃ for 4 h. The withdrawal contracture was elicited by challenge with Nal (1 μmol·L^{-1}). The precipitated withdrawal contracture of ileum was suppressed dose-dependently by addition of Nim (0.01, 0.05, and 0.1 μmol·L^{-1}) or Tet (1, 10, and 50 μmol·L^{-1}) 1 min before challenge with Nal (Fig 1). Nal could not elicit contracture in the ileum incubated with vehicle. Application of vehicle did not affect the Nal-precipitated withdrawal contracture.



Drug/μmol·L^{-1}	Withdrawal contracture/g
Control	1.03 ± 0.16
Nim 0.01	0.69 ± 0.16[b]
Nim 0.05	0.47 ± 0.12[c]
Nim 0.1	0.13 ± 0.10[c]
Tet 1	0.78 ± 0.21[a]
Tet 10	0.63 ± 0.14[c]
Tet 50	0.10 ± 0.06[c]

Fig 1. Effects of Nim or Tet on Nal (1 μmol·L^{-1})-precipitated withdrawal contracture in the ileum incubated with Mor (3 μmol·L^{-1}). A: Control. B: Addition of Nim (0.01, 0.05, and 0.1 μmol·L^{-1}) 1 min before Nal. C: Addition of Tet (1, 10, and 50 μmol·L^{-1}) 1 min before Nal. D: Results of the experiments. $\bar{x} \pm s$. W: Wash. $n = 3$-4 guinea pigs. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs* control group.

Ileum obtained from Mor-dependent guinea pigs Addition of Nal (1 μmol·L^{-1}) to the organ bath induced a contractile response in the ileum obtained from Mor-treated guinea pig. Ileal segments

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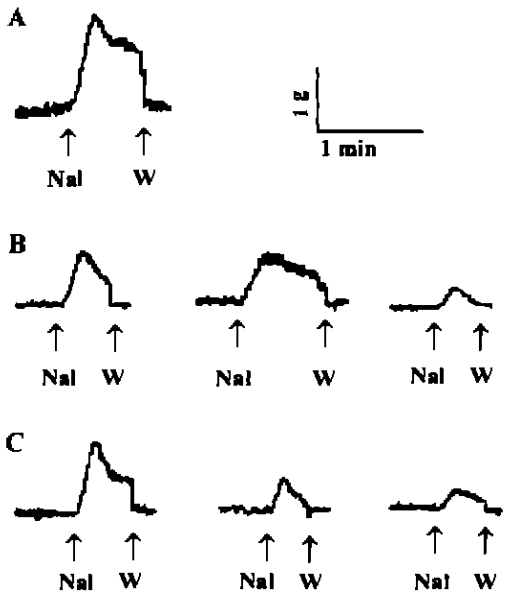
from guinea pigs treated chronically with vehicle did not respond to Nal.

When Mor-dependent preparations were incubated for 15 min before addition of Nal with Nim (0.01, 0.05, and 0.1 $\mu mol \cdot L^{-1}$) or Tet (1, 10, and 50 $\mu mol \cdot L^{-1}$) respectively, the contractile response induced by Nal was inhibited in a dose-dependent manner (Fig 2). Vehicle did not affect the contractile response.

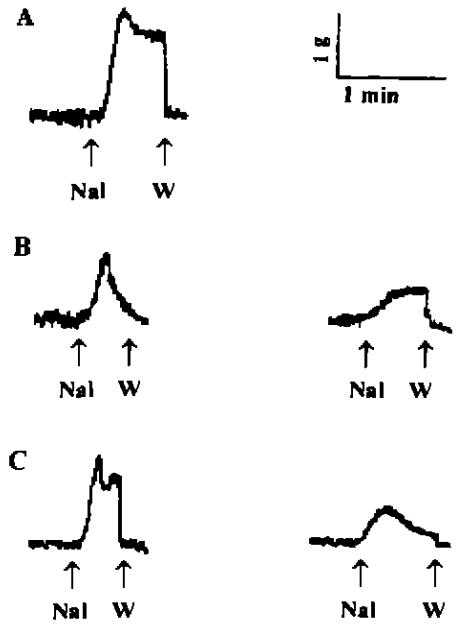


D

Drug/$\mu mol \cdot L^{-1}$	Withdrawal contracture/g
Control	1.75 ± 0.18
Nim 0.01	0.92 ± 0.44[b]
Nim 0.05	0.71 ± 0.07[c]
Nim 0.1	0.29 ± 0.07[c]
Tet 1	1.38 ± 0.33[a]
Tet 10	0.54 ± 0.19[c]
Tet 50	0.21 ± 0.07[c]

Fig 2. Effects of incubation with Nim or Tet for 15 min on the Nal (1 μmol · L^{-1}) precipitated withdrawal contracture in the ileum obtained from Mor-dependent guinea pigs. A: Control. B: Preincubation with Nim (0.01, 0.05, and 0.1 μmol · L^{-1}). C: Preincubation with Tet (1, 10, and 50 μmol · L^{-1}). D: Results of the experiments. $\bar{x} \pm s$. W: Wash. $n = 3 - 4$ guinea pigs. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs* control group.

The withdrawal contracture induced by Nal was also decreased in the ileum obtained from Mor-dependent guinea pig pretreated with Nim (5, 10 mg · kg^{-1}) or Tet (15, 30 mg · kg^{-1}) 1 h before killing (Fig 3).



D

Drug/$\mu mol \cdot L^{-1}$	Withdrawal contracture/g
Control	1.75 ± 0.18
Nim 5	1.08 ± 0.14[c]
Nim 10	0.63 ± 0.13[c]
Tet 15	1.41 ± 0.12[b]
Tet 30	0.82 ± 0.16[c]

Fig 3. Effects of Nim or Tet pretreatment 1 h before killing on the Nal (1 μmol · L^{-1}) precipitated withdrawal contracture in the ileum obtained from Mor-dependent guinea pigs. A: Control. B: Pretreatment with Nim (5 and 10 mg · kg^{-1}, ip). C: Pretreatment with Tet (15 and 30 mg · kg^{-1}, ip). D: Results of the experiments. $\bar{x} \pm s$. W: Wash. $n = 3 - 4$ guinea pigs. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs* control group.

DISCUSSION

The Nal-precipitated withdrawal contracture in the ileum has been used as an indication of opioid dependence, since their actions in the enteric nervous system are thought to mimic those in the central nervous system[1,2]. In the present study, when the naive

ileum incubated in Krebs solution containing Mor withdrawal for 4 h, addition of Nal induced withdrawal contracture. The withdrawal contracture was also induced by addition of Nal to the ileum obtained from guinea pig treated chronically with Mor. These results agree to other reports[1,3].

Studies have demonstrated that acute Mor administration reduces the Ca^{2+} content, on the other hand, chronic Mor administration increases the Ca^{2+} content in brain and during Nal-precipitated withdrawal the elevated Ca^{2+} levels returns toward control values[7]. Such important redistribution of Ca^{2+} is probably a key event for the neurochemical and behavioral expressions of the Mor syndrome. Ca^{2+}-channel blockers suppress, and Ca^{2+} agonists enhance some behavioral actions of Mor withdrawal syndrome[5]. The peripheral mechanisms are also possible involved in this action, since Ca^{2+} channel binding sites have been identified in intestinal smooth muscle, and verapamil can antagonize Ca^{2+}-induced contractures in intestinal smooth muscle *in vitro*[8]. In the ileum obtained from Mor-dependent guinea pigs, Ca^{2+} antagonists could inhibit the Nal-precipitated withdrawal contracture[4]. These results indicated an important role of Ca^{2+} in the opioids dependence.

Tet, a bis-benzylisoquinoline alkaloid derived from the Chinese medicinal herb *Stephania tetrandra*, is a putative Ca^{2+} entry blocker. King *et al*[6] suggested that Tet can inhibit L-type Ca^{2+} channel activity by interacting at the benzothiazepine-binding sites of the Ca^{2+} entry blocker receptor complex. Another study[9] indicated that Tet inhibited both T- and L-type calcium channel currents. To our knowledge, however, no information is available on the effect of Tet on Mor dependence. We engaged in studying this effect. We have previously demonstrated that Tet suppressed some signs of Mor withdrawal syndrome in mice and rats. The present study showed that Tet could inhibit the Nal-precipitated withdrawal contracture in the naive ileum incubated with Mor. When Tet was administered *in vivo* to Mor-dependent guinea pigs, or when Tet was added *in vitro* to the ileum from Mor-dependent guinea-pigs, the Nal-precipitated withdrawal contracture was also significantly decreased in a dose-dependent manner. Similar to other study[4], Nim also suppressed the withdrawal contracture. These findings provide evidence to support the peripheral anti-withdrawal effects of Ca^{2+} antagonists and the inhibitory effects of Ca^{2+} antagonists on opioid withdrawal syndrome.

Taken together with previous observations, our results indicate the inhibitory effect of Tet on Mor withdrawal response and its possible therapeutic role in opioid addiction.

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粉防己碱对吗啡在离体豚鼠回肠中依赖性的作用[1]

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关键词 钙通道阻滞剂；粉防己碱；尼莫地平；吗啡；阿片类有关的紊乱；纳洛酮；回肠

目的：研究粉防己碱（Tet）和尼莫地平（Nim）对吗啡在离体豚鼠回肠中戒断性反应的影响. **方法**：戒断性收缩由纳洛酮（1 $\mu mol \cdot L^{-1}$）加入已在含吗啡（3 $\mu mol \cdot L^{-1}$）的 37.5 ℃ Krebs 液中孵育 4 h 的离体豚鼠回肠或加入从吗啡依赖豚鼠中取得的回肠引起. **结果**：离体豚鼠回肠在含吗啡的 Krebs 液中孵育 4 h，给 Nim（0.01、0.05 和 0.1 $\mu mol \cdot L^{-1}$）或 Tet（1、10 和 50 $\mu mol \cdot L^{-1}$）抑制其戒断性收缩. Nim 和 Tet 体内或体外给药都能抑制吗啡依赖豚鼠离体回肠的戒断性收缩. **结论**：钙拮抗剂 Nim 和 Tet 抑制吗啡在离体豚鼠回肠的戒断性收缩.

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