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一种对氯乙基可乐定不敏感对5-methyl-urapidil低亲和的新α_1肾上腺素受体亚型

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R965.

摘要 用放射配体结合实验方法发现在大鼠心脏中存在一种新的α_1肾上腺素受体亚型，这种亚型不同于α_{1A}，α_{1B}，α_{1C}及α_{1D}等以往报道的α_1受体亚型，它与α_{1A}一样对WB4101具有高亲和性，但对5-methyl-urapidil呈低亲和，而且对氯乙基可乐定(CEC)不敏感。

关键词 α_1肾上腺素受体；心脏；WB4101；5-methyl-urapidil；氯乙基可乐定

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Opioid, calcium, and adrenergic receptor involvement in protopine analgesia[1]

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ABSTRACT The analgesic effect of protopine (Pro), an alkaloid isolated from *Papaveraceae*, was confirmed by tail-pinch and hot-plate tests when given sc 10—40 mg·kg^{-1}, and 20—40 mg·kg^{-1} inhibited the spontaneous movements of mice. Pro 40 mg·kg^{-1} increased the sleeping rate, prolonged the sleeping duration, and shortened the sleeping latency in mice hypnotized by ip pentobarbital sodium 30 mg·kg^{-1}. Pro 10—40 mg·kg^{-1} did not affect the inflammatory reaction induced by xylene and egg white. An icv injection of Pro 20—200 μg/mouse showed a remarkable analgesic effect in mice. The icv pretreatment of naloxone 2 μg blocked the analgesic effect completely. $CaCl_2$ 40 μg/mouse (icv) or methotrexate 10 mg·kg^{-1}(ip), an agonist of Ca^{2+} channel, showed a complete blockade of the analgesia, while nifedipine 100 mg·kg^{-1}(*po*), a blocker of Ca^{2+} channel, enhanced the analgesic effect. The ip pretreatment of reserpine 4 mg·kg^{-1} reduced the Pro analgesia. Phentolamine 10 mg·kg^{-1}(ip), an α-adrenergic blocker, tended to weaken the analgesia, but propranolol 10 mg·kg^{-1}(ip), a β-blocker, did not affect it. These results suggest that Pro displays its analgesic effect mainly through the opioid and calcium systems and partly through the adrenergic mechanism.

KEY WORDS protopine; analgesia; methotrexate; nifedipine; naloxone; reserpine; phentolamine

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Protopine (Pro), an alkaloid extracted from many species of plants of Papaveraceae, has analgesic[1], anti-platelet aggregation[2-4], and anti-spastic[1,5] effects. In regard to its analgesia, Pro could control the writhing pain induced by acetic acid or by electric stimulation. The present study is to clarify whether Pro has a central analgesic action and how it would be.

MATERIALS AND METHODS

Mice ICR and Kunming strain ♀ mice weighing 20—24 g (Experimental Animal Room of China Pharmaceutical University) were used.

Drugs Pro was isolated from ethanol extracts of *Corydalis* Tuber (*Corydalis ledebouriana* Kar et Kit) in Xinjiang, China. It forms prismatic crystals with mp 208—208.5 ℃ and the same UV and IR absorption as the standard spectra. It was dissolved in 3 % citric acid in saline for use. Mice in the control group were administered equal volume of vehicle. Following drugs were purchased; indomethacin tablets (Ind. Jintan Pharmaceutical Factory, Jiangsu, China), diazepam injection (The People's Pharmaceutical Factory of Tianjin, China), prednisolone injection (The Ninth Pharmaceutical Factory of Shanghai, China), methotrexate injection (MTX, Hangzhou Pharmaceutical Factory, China), nifedipine tablets (Nif, The Fourth Pharmaceutical Factory of Changzhou, China), injection of naloxone hydrochloride (Nal, Beijing Sihuan Pharmaceutical Factory, China), reserpine injection (Hongqi Pharmaceutical Factory of Shanghai Medical University, China), phentolamine injection (Ciba-Geigy, Switzerland), propranolol chloride (The Northeast General Pharmaceutical Factory, Shenyang, China), and pentobarbital sodium (biochemical reagent, The Second Reagent Factory of Shanghai, China).

Evaluation of analgesic effect in tail-pinch method The analgesic effect was measured at a 15-min interval after the injection of Pro for 90 min by a modified Haffner's method[6], with a cut-off time of 6 s to avoid tissue damage due to prolonged application. The site pinched by forceps was located at the tail root of mice and the latency of biting response to the forceps was measured. The effect of Pro was expressed as a change of response time as well as an area under the curve (AUC) by plotting the increase of response time (s) on the ordinate and time intervals (min) on the abscissa.

Evaluation of analgesic effect in hot-plate method Mice were placed on a hot-plate of 55 ℃. The latency of licking either hind-paw was measured, and those responded between 5 — 30 s were adopted. The analgesic effect was measured at a 15-min interval after Pro injection for 60 min, with a cut-off time of 60 s.

Xylene-induced inflammation The inflammatory reaction was induced on the right ear of mice by painting 30 μl of xylene. Two h later, the thickness of the ear was measured by a micrometer[7].

Egg white-induced inflammation Mice were injected sc into both footpads of the hind limbs using 50 μl of 10 % fresh egg white. Two h later, the thickness of footpads was measured by a micrometer[7].

Count of spontaneous movements of mice The movement activity of mice was checked before drug administration and mice with the similar movability were applied to the experiment. The number of movements of mice was counted during 20 min after the administration of drugs by the multi-purpose instrument for pharmacological and physiological studies with a counter of animal movements (Bengbu Medical College, Anhui, China). The inhibition of movements by drugs was: % inhibition = (counts in control — counts in drug group)/counts in control × 100.

Hypnotizing test Mice were given ip with 30 mg $\cdot kg^{-1}$ of pentobarbital sodium and thereafter the reflexes were scrutinized when turned over. The sleeping rate, latency and duration were measured. The calculation of sleeping rate was: % sleeping rate = number of sleeping mice/number of total mice × 100.

Statistical analysis The results were expressed as $\bar{x} \pm s$. The overall data were assessed by ANOVA. Differences between the individual mean values in different groups were analyzed by Dunnett's test in analgesic experiments, and t test was used in other experiments.

RESULTS

Analgesic effect of Pro In tail-pinch method, one-shot sc injection of Pro 10 — 40 mg $\cdot kg^{-1}$ exhibited an analgesic effect, reaching a peak at 15 min, in a dose-dependent

Tab 1. Analgesic effects of sc Pro and po Ind in tail-pinch and in hot-plate method in ICR mice. $n=8$ mice, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Dose/ mg·kg^{-1}	Response time at 0 min	15 min	30 min	45 min	60 min	75 min	90 min	AUC
Tail-pinch method (100×response time/s)								
Control	68±19	54±19	51±11	92±16	88±49	59±8	58±11	0.2±15
Pro 10	77±19	293±254^b	245±228^b	172±181^a	113±56^a	97±79^a	75±50^a	80±123^a
20	62±18	324±264^b	312±274^b	181±189^a	149±199^a	85±47^a	59±13^a	112±108^b
40	55±14	491±174^c	423±274^c	341±284^a	303±263^a	287±218^b	305±220^b	254±182^a
Ind 100	66±27	413±184^c	187±149^b	178±196^a	178±113^a	175±96^c	133±46^c	125±73^c
Hot-plate method (10×response time/s)								
Conntrol	20±3	23±6	22±6	16±5	15±4			
Pro 10	22±3	27±12^a	30±16^a	22±10^a	28±15^b			
20	21±5	34±16^a	34±17^a	29±16^b	25±16^a			
40	20±3	48±15^c	43±15^c	37±12^c	26±14^a			
Ind 100	23±5	27±9^a	18±5^a	21±10^a	22±17^a			

manner. Ind 100 mg·kg^{-1} *po* also showed a marked analgesic effect. In hot-plate method, a similar analgesia was seen after Pro, while Ind did not show any distinct analgesic effect (Tab 1).

Effects of Pro on inflammation induced by xylene on ear and by egg white in footpad Pro 10—40 mg·kg^{-1} injected sc twice (2 h before and immediately after induction of inflammation) did not show any inhibitory activity, while prednisolone 10 mg·kg^{-1} im and Ind 100 mg·kg^{-1} *po* showed a strong inhibition, against inflammation induced by either xylene or egg white (Tab 2).

Tab 2. Effects of Pro, Prednisolone (Pre) and Ind on xylene-, and egg white-induced inflammations in ICR mice. $n=8$ mice, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

	Dose/ mg·kg^{-1}	$10^3\times$Ear swelling/mm	$10^3\times$Footpad swelling/mm
Control		43±20	323±138
Pro	10	not done	326±57^a
	20	49±30^a	360±72^a
	40	31±27^a	193±42^b
Pre	10	24±11^b	233±94^a
Ind	100	11±14^c	not done

Effect of Pro on spontaneous movements Pro 20 and 40 mg·kg^{-1} sc markedly inhibited the spontaneous movements, and so did diazepam 10 mg·kg^{-1} ip (Tab 3).

Tab 3. Effect of sc Pro and ip diazepam on spontaneous movements in Kunming mice. $n=8$ mice, $\bar{x}\pm s$. $^cP<0.01$ *vs* control.

	Dose/ mg·kg^{-1}	Count of movements during 20 min	Inhibition/ %
Control		111±24	—
Pro	20	45±23^c	60
	40	47±22^c	57
Diazepam	10	27±15^c	76

Effect of Pro on pentobarbital-induced sleep Mice were injected sc Pro 20 and 40 mg·kg^{-1} 30 min prior to ip pentobarbital sodium 30 mg·kg^{-1}. Three of the 16 mice fell asleep in the control group. Pro showed a dose-dependent increase of the sleeping rate, in which 4/16 and 12/16 mice were in sleeping state after Pro 20 and 40 mg·kg^{-1}, respectively. Pro also showed a tendency to shorten the sleeping latency and remarkably prolonged the sleeping duration. Pro itself did not show any hypnotizing effect (Tab 4).

Tab 4. Effect of sc Pro on pentobarbital-sleep in Kunming mice. $n=16$ mice, $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$ *vs* control.

	Dose/ mg·kg^{-1}	Sleeping rate/%	Sleeping latency/min	Sleeping duration/min
Control		19	14±3	18±11
Pro	20	25	15±12^{a}	31±7^{a}
	40	75	10±4^{a}	40±23^{b}

Analgesic effect of Pro by icv injection Pro (1, 2, and 20 g·L^{-1}, pH 6) was injected icv 10 μl/mouse at 2 mm from either side of the midline on a line drawn through the anterior base of the ears. Pro showed a dose-dependent analgesic effect, reaching a peak at 15 min after Pro 20 and 200 μg/mouse, which descended more rapidly in the 20 μg than in the 200 μg group (Fig 1).

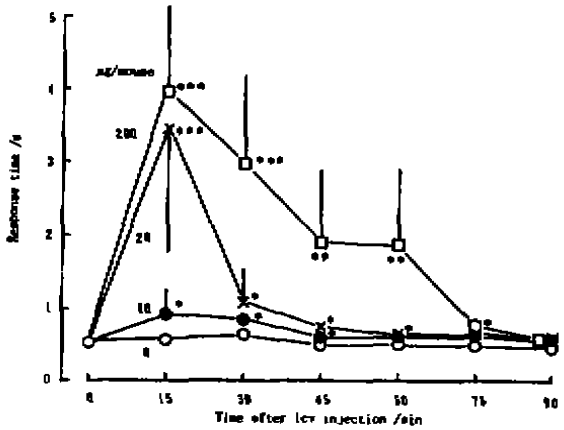

Fig 1. Analgesic effect of icv Pro. $n=8-10$ mice, $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

Blockade of Pro-analgesia by Nal pretreatment Mice received icv pretreatment with Nal 200 mg·L^{-1} (10 μl/mouse) 10 min prior to the sc injection of Pro 40 mg·kg^{-1}. The analgesic effect of Pro was blocked completely, while Nal itself did not show any analgesic activity (Fig 2).

Effects of $CaCl_2$, MTX, and Nif on Pro-analgesia $CaCl_2$ 4 g·L^{-1} (10 μl/mouse), MTX 10 mg·kg^{-1}, and Nif 100 mg·kg^{-1} were used for the icv, ip, and *po*, respectively, to mice 10 min before sc Pro 40 mg·kg^{-1}. $CaCl_2$ and MTX blocked the effect of Pro almost completely, while Nif showed an obvious enhancement of the Pro analgesia. These 3 drugs themselves did not show any analgesic activity (Fig 2).

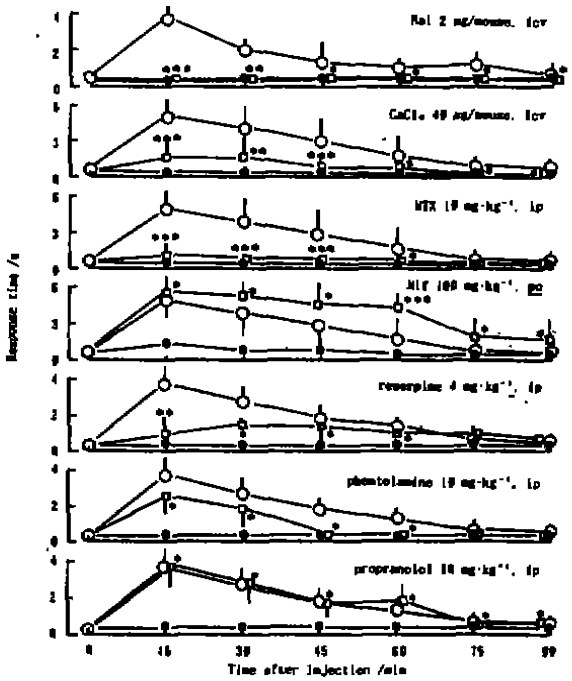

Fig 2. Effects on sc Pro (40 mg·kg^{-1}) analgesia in mice after premedications. ○: saline+Pro, ●: premedication+saline, □: premedication+Pro. $n=8$ mice, $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* saline+Pro.

Effects of reserpine, phentolamine, and propranolol on Pro-analgesia Reserpine, phentolamine, and propranolol were given ip to mice before the analgesic test by injecting Pro 40 mg·kg^{-1}. Pretreatment with reserpine 4 mg·kg^{-1} 4 h before Pro caused a remarkable lessening of the analgesic activity of Pro. Given 30 min prior to Pro, phentolamine 10 mg·kg^{-1} tended to inhibit the analgesia but propranolol 10 mg·kg^{-1} did not.

Reserpine, phentolamine, propranolol *per se* exhibited no analgesic activity (Fig 2).

DISCUSSION

In both tail-pinch (mechanical stimulation) and hot-plate (thermal stimulation) methods, Pro exhibited a remarkable analgesic effect. There are evidences that the central transmission of mechanical and thermal nociceptive information is regulated by different neuronal mechanisms such as opioid and monoamine systems[8,9]. The present study, thus, suggested a possible participation of manifold mechanisms in the Pro-analgesia.

However, Pro affected neither the xylene-induced ear swelling nor the egg white-induced footpad reaction. This finding suggested that Pro may display its analgesic activity through different mechanisms from that by antipyretic analgesics because the latter such as indomethacin usually has an anti-inflammatory effect in addition to its analgesic effect. On the other hand, Pro showed a markedly sedative activity of inhibiting the spontaneous movements of mice as diazepam did. To the pentobarbital sodium-induced sleep, the alkaloid showed distinct activities of enhancing the sleeping rate and prolonging the sleeping duration without causing hypnotizing effect by itself at the dosages used. These evidences indicated a possibility that the analgesic activity of Pro was effected via a central mechanism. Supports had been obtained through the following experiments. Namely, an icv administration of Pro resulted in a remarkable analgesic effect, which 10 times more potent *vs* sc administration. In addition, the Pro analgesia was blocked completely by naloxone, a specific blocker of opiate receptor, indicating the participation of opioid system in the analgesia production.

As to the morphine analgesia, many reports[10,11] have indicated the implication of Ca^{2+} in the function of modulating the pain in central nervous system. In our study, we also found that both icv preinjection of $CaCl_2$ and ip MTX, an agonist of Ca^{2+} channel, completely antagonized the analgesic effect of Pro, while Nif, a blocker of Ca^{2+} channel, showed a distinct enhancement of the effect of Pro. These results revealed a similarity between Pro and morphine analgesia of which both the action could be modulated by the calcium system, suggesting that a change of Ca^{2+} content in the brain may affect the activity of morphine as well as that of Pro. Moreover, it has been reported that Pro showed an inhibitory effect against the contraction of rabbit blood vessels and guinea pig intestines induced by various factors through inhibiting the release of Ca^{2+} in cells without affecting the generation of PGI_2[5]. Such characteristics of Pro may be relevant to its analgesic effect.

On the other hand, it is generally accepted that the catecholaminergic function plays an important role in the production of morphine analgesia. Here, we found that in animals pretreated with reserpine, known to be capable of exhausting the monoamine mediators, the analgesic effect of Pro was significantly suppressed. Phentolamine, an adrenergic α-blocker, but propranolol, a β-blocker, also tended to reduce the Pro analgesia. Thus, we cannot exclude the possibility of the mediation of catecholaminergic mechanism in the production of Pro analgesia, in addition to those of opioid and calcium mechanisms.

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阿片、钙及肾上腺素受体与普罗托平的镇痛

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摘要 小鼠尾根部加压及热板法证实，普罗托平10—40 mg·kg^{-1}，有显著镇痛作用，抑制小鼠自发活动，促进戊巴比妥钠的催眠，但无抗炎作用。icv 20—200 μg/鼠时镇痛显著。纳洛酮、$CaCl_2$和MTX可阻断，Nif则增强其镇痛。利血平和酚妥拉明对其镇痛有抑制或抑制趋势，而普奈洛尔无影响。因此，其镇痛作用主要系阿片及钙机制，部分通过肾上腺素能机制。

关键词 普罗托平；止痛；甲氨蝶呤；硝苯地平；纳洛酮；利血平；酚妥拉明

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Effects of direct lytic factors from southern Chinese cobra venom on Ca^{2+} movement in rabbit aorta strip[1]

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ABSTRACT The purified direct lytic factors (DLF) from southern Chinese cobra (*Naja naja atra*) venom induced a contractile response in Ca^{2+}-free Krebs' solution and a further increase in the tension following a subsequent addition of Ca^{2+} into bath. After depletion of intracellular Ca^{2+} pool by phenylephrine, DLF failed to induce any contractile response. In $^{45}Ca^{2+}$ experiments, DLF increased both $^{45}Ca^{2+}$ release and $^{45}Ca^{2+}$ influx. Procaine 2 mmol·L^{-1} decreased the DLF induced $^{45}Ca^{2+}$ release and $^{45}Ca^{2+}$ influx by 67±23% and 46±32%, respectively. Nifedipine and varapamil 1 mmol·L^{-1} markedly inhibited the contractile response and the $^{45}Ca^{2+}$ influx induced by DLF. These results suggest that DLF induces extracellular Ca^{2+} entry through voltage dependent Ca^{2+} channel and Ca^{2+} release from the intracellular Ca^{2+} pool which is sensitive to phenylephrine.

KEY WORDS thoracic aorta; calcium; direct lytic factors; procaine; phenylephrine; nifedipine; verapamil

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