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Sulpiride attenuates ranatensin–M–induced antinociception[1,2]

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ABSTRACT Intracerebroventricular (icv) administration of ranatensin–M (RM), a bombesin–like peptide isolated from the skin of Chinese frog *Rana margaratae*, produced a dose–dependent prolongation in the hot–plate latency in mice. Naloxone 1, 2, or 10 mg · kg^{-1} ip failed to antagonize the effects of RM. However, RM–induced antinociception was attenuated by pretreatment with sulpiride (Sul, 100 mg · kg^{-1}, ip), a selective DA_2 receptor blocker. Sul (100 mg · kg^{-1}, ip) did not affect hot–plate latencies when administered alone. Sch 23390 (0.2 mg · kg^{-1}, ip), a selective DA_1 receptor blocker, did not significantly affect RM–induced antinociception. The results suggest that RM–induced antinociception may be mediated by dopamine neurotransmission within the CNS and that it is mainly the D_2 receptor which was involved in this effects.

KEY WORDS ranatensin–M; bombesin; Sch 23390; sulpiride; dopamine receptor blockers; analgesia

From the skin of Chinese frog *Rana margaratae*, Tang *et al*[1] recently isolated a new octapeptide named ranatensin–M which had a remarkable sequence homology to bombesin. The structure of RM is His–Trp–Ala–Ile–Gly–His–Phe–Met–NH_2. It is the smallest peptide so far among the natural bombesin–like peptides. Binding sites of bombesin in rat brain have been characterized with the highest concentrations of receptors in limbic forebrain and midbrain structures such as the hippocampus, amygdala, hypothalamus, and the periaqueductal gray matter (PAG)[2]. Bombesin has been shown to have various biological effects in mammals[3,4]. Injection of bombesin into PAG of rats has been shown to produce an antinociceptive reaction in the hot–plate test and tail–flick test[5]. It was of interest, therefore, to ascertain whether RM had pain modulation effect. Evidence has suggested that some effects of bombesin on the central nervous system were mediated by central dopamine circuits[6]. Our study was also designed to evaluate the effects of Sch 23390 and sulpiride on the antinociceptive effect of icv RM.

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[2] The peptide was synthesized by PENG Jin–Lin

MATERIALS AND METHODS

Adult, ♀ mice weighing 20 ± SD 2 g were purchased from Shanghai Laboratory Anaimals Center and were group housed in a controlled environment animal facility (12 h light, 12 h dark) with laboratory chow and water available *ad lib*. The mice were housed for at least 1 wk before experiment. Naloxone was purchased from E I du Pont de Nemours & Co, Garden City NY, USA. Sch 23390 and sulpiride (Sul) were from Schering Corp, Bloomfield, NJ USA and Shanghai Tian Feng Pharmaceutical Co, respectively. All drugs were dissolved in saline. Experiments were conducted between 8:00 and 12:00 at ambient temperatures of 22–24℃. Naloxone (1, 2 or 10 mg · kg^{-1}, ip), Sch 23390 (0.2 mg · kg^{-1}, ip) or Sul (100 mg · kg^{-1}, ip) was injected 20 min before icv RM (5 μl). The basic experimental design included 8 groups of mice (8 mice / group): 1) saline (icv), 2) RM (icv), 3) saline (ip) + RM (icv), 4) naloxone (ip) + RM (icv), 5) Sch 23390 (ip) + RM (icv), 6) Sul (ip) + RM (icv), 7) Sch 23390(ip) + saline (icv), and 8) Sul (ip) + saline (icv). Antinociception was assessed by hot–plate test. The temperature of the hot–plate was set

at 55 ± 1℃. An arbitrary cut-off was used to score mice not responding to the noxious stimulus within 40 s. Each mouse was tested every 10 min for 90 min. RM was injected icv after the 3rd reading of the hot-plate latency. A significant increase in the response time for experimental mouse *vs* control one was defined as antinociception. One-way analysis of variance followed by Dunnett's test for multiple comparisons was used.

RESULTS

Dose-response effects of RM-induced analgesia When mice were tested every 10 min after RM icv injection (0.1, 1, or 10 μg / mouse) in the hot-plate test, a dose-dependent increase in the latency was obtained. The effect was most pronounced 20 min after RM injection and appeared to decrease slightly over the course of 1 h (Fig 1).

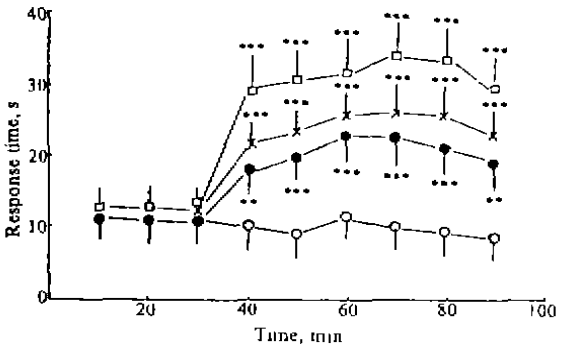

Fig 1. Dose-response effects of RM-induced antinociception in the hot-plate test in mice. Groups of mice (8 mice / group) were treated as follows: (○) saline icv, 5 μl / mouse, (●) RM icv, 0.1 μg / mouse, (×) RM icv, 1 μg / mouse, and (□) RM icv, 10 μg / mouse. $^{**}P<0.05$, $^{***}P<0.01$ *vs* saline.

Interaction between naloxone and RM When naloxone (1, 2 or 10 mg · kg^{-1}, ip) was injected before icv RM, the antinociceptive effect of RM in the hot-plate test was unaffected (Fig 2A) suggesting that

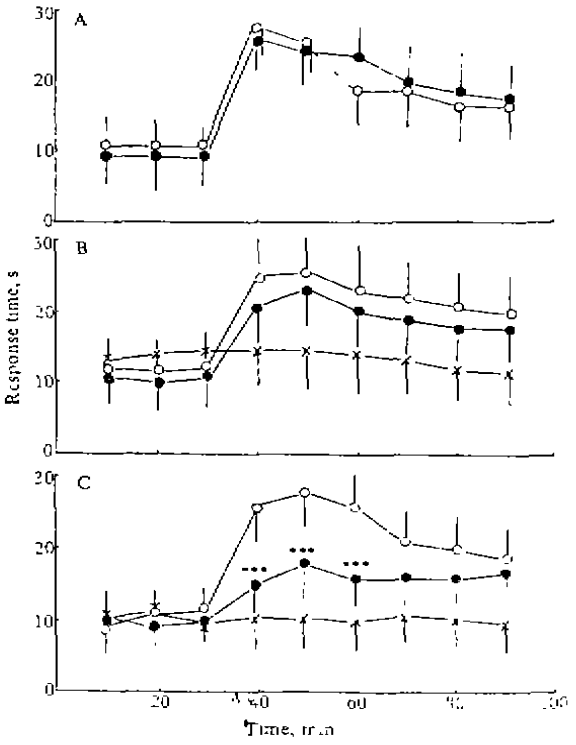

Fig 2. Effect of pretreatment with naloxone (A), Sch 23390 (B) or sulpiride (C) on RM-induced antinociception in the hot-plate test in mice. In (A), groups of mice (8 mice / group) were pretreated with naloxone (10 mg · kg^{-1} ip) (○) or saline (ip) (●) and then icv RM (1 μg / mouse). In (B), treatment categories were as follows: (○) saline (ip) + RM (icv, 1 μg / mouse), (●) Sch 23390 (ip, 0.2 mg · kg^{-1}) + RM (icv, 1 μg / mouse), and (×) Sch 23390 (ip, 0.2 mg · kg^{-1}) + saline. In (C), treatment categories were as follows: (○) saline (ip) + RM (icv, 1 μg / mouse), (●) sulpiride (ip, 100 mg · kg^{-1}) + RM (icv, 1 μg / mouse), and (×) sulpiride (ip, 100 mg · kg^{-1}) + saline. $^{***}P<0.01$ when compared to saline-pretreated control.

RM does not exert its analgesic effect by either releasing endogenous opioids or acting directly through the opioid receptors.

Effects of Sch 23390 or Sul on RM-induced antinociception Sch 23390 (0.2 mg · kg^{-1}, ip) did not affect RM-induced antinociception (Fig 2B) although Sch 23390 (0.2 mg · kg^{-1}, ip) produced a decrease in

mouse locomotor activity, rearing and grooming when given alone as described previously[7]. However, RM-induced antinociception was significantly attenuated by pretreatment with Sul (100 mg · kg^{-1}, ip). Sul did not affect hot-plate latencies when ip alone (Fig 2C).

DISCUSSION

The results suggest that RM-induced antinociception may be mediated by dopamine neurotransmission within the CNS. The suggestion resonates with previous observations. Small doses of apomorphine has been found to increased the tail flick latency in rat when given intrathecally and intravenously[8]. This effect is probably mediated *via* D_2 receptors since the D_2 agonist LY171555 had a similar effect whereas the D_1 agonist SK&F 38393 was inactive. Furthermore the D_2 blocker Sul blocked the effects of apomorphine and LY171555. Evidence has suggested that the dopamine agonists are primarily influencing nociceptive process at the spinal level[9] and that the dopaminergic innervation of the spinal cord probably originates from the A11 periventricular cell group[10]. Although our results suggest that it is mainly the D_2 receptor which was involved in the antinociceptive effect of RM, further studies are needed to determine whether RM induces antinociceptive effect by activating the descending spinal dopamine system.

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舒必利拮抗绿臭蛙肽引起的镇痛作用

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提要 从绿臭蛙(Rana Margaratae)中分离到的绿臭蛙肽(ranatensin-M)脑室内注射后可引起镇痛. 纳络酮不能对抗绿臭蛙肽引起的镇痛作用，表明它不通过体内阿片系统起作用. 绿臭蛙肽引起的镇痛作用可以被选择性多巴胺受体 D_2 拮抗剂—舒必利对抗，但选择性多巴胺受体 D_1 拮抗剂--Sch 23390 对上述镇痛作用无明显影响. 结果表明中枢神经系统中多巴胺系统 D_2 受体可能参与绿臭蛙肽的镇痛作用.

关键词 绿臭蛙肽；铃蟾肽；Sch 23390；舒必利；多巴胺受体阻滞剂；镇痛