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Possible involvement of nitric oxide in arginine-induced analgesia[1]

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ABSTRACT Intracerebroventricular injection (icv) of l-arginine (Arg, 0.5, 5.0, 50 μg) produced a dose-dependent prolongation in the hot-plate latency in mice. A similar result was obtained when nitroprusside (0.1, 1.0, 10 μg, icv) was given. Naloxone (2 mg · kg^{-1}, ip) failed to antagonize the effects of Arg or nitroprusside. But Arg-induced antinociception was attenuated by N^G-monomethyl-arginine (NMMA, 10 μg). 8-Bromoguanosine 3' : 5' cyclic monophosphate (bromo-cGMP, 50 μg) also produced an antinoceptive effect. The results suggest that Arg induces an analgesia possibly via activation of nitric oxide-cGMP pathway.

KEY WORDS arginine; nitroprusside; nitric oxide; guanosine cyclic monophosphate; naloxone; analgesia

Kyotorphin is an analgesic dipeptide (Tyr-Arg) isolated from bovine brain[1]. It is synthesized in the brain by kyotorphin synthetase from tyrosine (Tyr) and arginine (Arg)[2]. Arg (icv), but not Tyr, also produces an analgesic effect in the mouse[3]. However, the mechanism of the Arg-induced analgesia is not clear although it was proposed that Arg (icv) induced an analgesia possibly via an increase in kyotorphin levels in the brain[3]. Since Arg is the substrate precursor of nitric oxide (NO) and NO, as a messenger molecule, may play a part in neuronal communication in the brain[4-7], we examined the possible involvement of NO in the Arg-induced analgesic effect in mice.

MATERIALS AND METHODS

Adult, ♀ mice, weighing 20±*s* 2 g, from Shanghai Laboratory Animal Center were group housed in a controlled environment animal facility (12-h light, 12-h dark) with laboratory food and water *ad lib* for at least 1 wk before experiment. Tyr, Arg, N^G-monomethyl-*l*-arginine (NMMA) and 8-bromoguanosine 3': 5'-cyclicmonophosphate (bromo-cGMP) were purchased from Sigma, USA. Sodium nitroprusside (Nit) was made by Wuhan Pharmaceuticals, China. Naloxone (Nal) was bought from Du Pont, USA. All chemicals were dissolved in saline. Experiments were conducted between 08:00 and 12:00 at ambient temperatures of 22—24℃. Nal (2 mg · kg^{-1}, ip) was injected 20 min before icv Arg (0.5, 5.0, or 50 μg) or Nit (0.1, 1.0, or 10 μg). The experiment consisted of 7 groups (8 mice/group): 1) saline icv; 2) Arg icv; 3) Nit icv; 4) Nal ip + Arg icv; 5) Nal ip + Nit icv; 6) NMMA icv + Arg icv; and 7) bromo-cGMP, icv. Antinocicepotion 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. Arg, Nit, or bromo-cGMP 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 ($P<0.05$ or 0.01) was defined as antinociception. One way analysis of variance followed by Dunnett's test for comparison for multiple comparisons was used.

RESULTS

Antinociceptive effects of Arg, Nit, and bromo-cGMP When mice were tested every 10 min after Arg icv injection (0.5, 5.0, 50 μg/mouse) in the hot-plate test, a dose-dependent increase in the latency was found. The effect was most pronounced at 10 min after Arg injection and decreased slightly afterward (Fig 1A). A similar result was obtained when Nit (0.1, 1.0, 10 μg/mouse, icv) was given (Fig 1B). Bromo-cGMP (5,

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50 μg, icv) also produced an antinociceptive effect (Fig 1C).

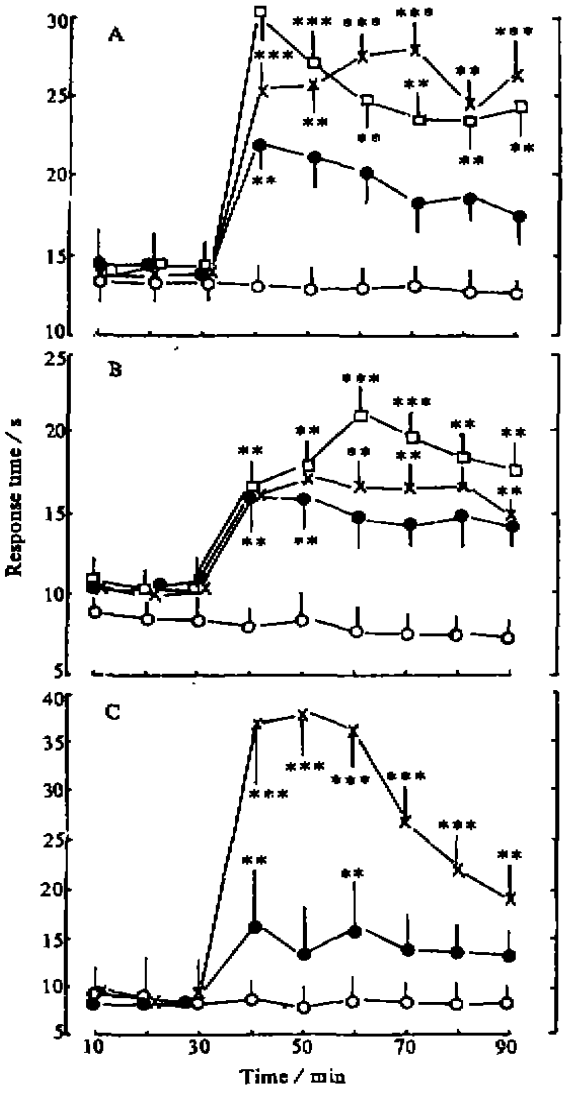

Fig 1. Effects of *l*-arginine-, nitroprusside-, or 8-bromoguanosine 3',5'-cyclic monophosphate (bromo-cGMP)-induced antinociception in hot-plate test in mice (8/group). In 1A, mice were injected icv saline (○), *l*-arginine 0.5 μg (●), 5 μg (×), or 50 μg (□). In 1B, mice were injected icv saline (○), nitroprusside 0.1 μg (●), 1.0 μg (×), or 10 μg (□). In 1C, mice were injected icv saline (○), bromo-cGMP 5 μg (●), or 50 μg (×). **$P<0.05$, ***$P<0.01$ *vs* saline.

Effects of Nal or NMMA on Arg-induced antinociception When Nal (2 mg·kg^{-1}, ip) was injected before icv Arg (5 μg/mouse) or Nit (1 μg/mouse), the antinociceptive effect of Arg or Nit was unaffected. But Arg-induced antinociception was attenuated by pretreatment with NMMA (10 μg, which did not significantly affect the hot-plate latency. icv) (Fig 2).

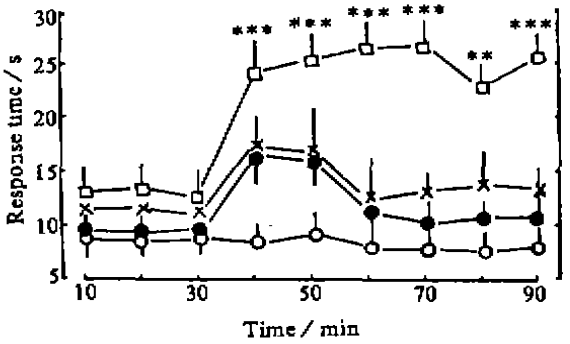

Fig 2. Effect of pretreatment with N^G-monoethyl-*l*-arginine (NMMA) on *l*-arginine-induced antinociception in hot-plate test in mice (8/group). Mice were injected icv saline (○), NMMA (10 μg) alone (●) or pretreated with icv NMMA (10 μg, ×) or saline (□) then injected icv *l*-arginine 5 μg. **$P<0.05$, ***$P<0.01$ *vs* saline pretreated control.

DISCUSSION

Our results that Arg induced antinociception confirms the previous findings by Ueda *et al*[3]. However, with regard to the mechanisms of antinociceptive effect of Arg, we found that Nal did not block the analgesic effect of Arg suggesting that Arg did not exert its analgesic effect, in our case, by releasing endogenous opioid. Our finding that NMMA, an inhibitor of NO synthase, attenuated the Arg-induced analgesic effect suggested that the Arg-induced analgesia was mediated through NO produced from Arg in the brain. In support of this hypothesis, we found that Nit, a nitrovasodilator which generated NO[6],

also induced an antinoci ceptive effect. Activation of soluble guanylyl cyclase is the main mechanism of action of NO in both the vascular and nervous systems[9]. Our results that bromo-cGMP induced analgesic effect further support the suggestion that the NO-cGMP pathway is involved in the mechanism of Arg-induced analgesia.

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一氧化氮可能参与精氨酸引起的镇痛

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摘要 小鼠脑室内注射精氨酸(0.5，5.0，50 μg icv)或硝普钠(0.1，1.0，10 μg icv)均引起镇痛. 该作用不被纳洛酮(2 mg·kg^{-1} ip)对抗，但 N^G-甲基精氨酸(50 μg icv)可对抗精氨酸引起的镇痛作用. 8-溴鸟苷环一磷酸(50 μg icv)也引起镇痛. 结果提示精氨酸引起的镇痛作用有一氧化氮-cGMP 参与.

关键词 精氨酸；一氧化氮；鸟苷环一磷酸；镇痛；纳洛酮；硝普钠

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