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Antinociceptive effect of intracerebroventricular injection of a tetrapeptide Asn-Ala-Gly-Ala in rats

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ABSTRACT The antinociceptive effect of intracerebroventricular injection (icv) of Asn-Ala-Gly-Ala (NAGA), a partial sequence of β-lipotropin, was studied in rats. The potassium iontophoresis-induced tail flick was used to measure the pain threshold. The antinociceptive effect of NAGA, which was dose-dependent (icv, 0.03 — 0.24 μmol/rat) and long-lasting (90 min), was reversed by naloxone (icv, 0.26 mg·kg^{-1}) and inhibited by anti-MEK serum (titre: 1:5000, 5 μl) or anti-LEK serum (titre: 1:5000, 5 μl). NAGA-induced antinociception was scarcely affected by anti-β-EP serum (titre: 1:30000, 5 μl) or anti - Dyn A_{1-13} serum (titre: 1:30000, 5 μl). It was suggested that the antinociceptive effect of NAGA may be associated with the release of met-enkephalin and leu-enkephalin in rat brain.

KEY WORDS analgesia; naloxone; endorphins; immune sera; neuropeptides

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The tetrapeptide Asn-Ala-Gly-Ala (NAGA), a sequence of human β-lipotropin$_{14-17}$[1], was isolated from human brain[2]. Intracisternal injection of NAGA to mice produced dose-dependent, long-lasting, and naloxone-reversible antinociceptive effect as evaluted by the hot plate and tail flick methods[3], and the tail-pressure and phenylbenzoquinone-induced writhing tests[4] in mice. In the present study, we report the correlation between NAGA antinociceptive effect and endogenous opioid peptides in rats.

MATERIALS AND METHODS

Rats and drugs Sprague-Dawley ♂ rats (n = 173), weighing 196±s 12 g, were housed at 22±0.5 ℃ with an alternating 12-h light-dark cycle. Food and water were available *ad lib*.

NAGA was synthesized by the conventional solution method[6]. Naloxone hydrochloride was purchased from Sigma. The anti-met-enkephalin (anti-MEK) serum showed <0.01 % cross immunoreactivity with β-endorphin (β-EP) and dynophin A_{1-13} (Dyn A_{1-13}) and 0.44 % with leu-enkephalin (LEK). The anti-LEK serum did not cross immunoreact with β-EP and Dyn A_{1-13}, but about 2.8 % with MEK. The anti-β-EP serum showed no cross-immunoresctivity with MEK, LEK, Dyn A_{1-13}, or Dyn B. The anti-Dyn A_{1-13} serum did not cross-immunoreact with β-EP, MEK, LEK, or Dyn B, and about 1.8 % with Dyn A_{1-17}. The anti-MEK serum, anti-LEK serum, anti-β-EP serum, and anti-Dyn A_{1-13} serum did not show cross-immunoresctivity with NAGA in radioimmunoassay. Other reagents were AR grade.

Intracerebroventricular injection (icv) Rat was anesthtised with sodium pentobarbital (35 mg·kg^{-1}, ip). A stainless steel cannula (od 0.25 mm) was inserted through a guide cannula (od 0.5 mm) positioned stereotaxically L 1.5, A 0.3, H −3.0 mm[6]. The inserted cannula was fixed to the skull with dental cement. The rats were allowed to recover for 1 wk before being used in tail flick test. NAGA and naloxone were injected icv in 5 μl of normal saline (2 μl·min^{-1}). Naloxone (0.26 mg·kg^{-1}) was injected 10 min before NAGA. The antiserum (6 μl) was injected icv 30 min before NAGA.

Tail flick test The potassium iontophoresis induced tail flick to measure the pain thresholds of rats[7]. Pain thresholds were observed for 100 min after icv drugs.

Statistics All data were expressed as $\bar{x}\pm s$ compared by t test. The ED_{50} values were determined by weighted probit analysis method.

RESULTS

Antinociceptive effect of icv NAGA NAGA (0.03, 0.06, 0.12, and 0.24 μmol/rat) icv induced a dose-dependent inhibition of the tail flick response to potassium iontophoresis stimulation, which reached a maximum at 40 min and gradually declined from 50 min to 100 min. The ED_{50} values were 87 pmol/rat with 95 % confidence limits of 67—112 pmol/rat (Fig 1).

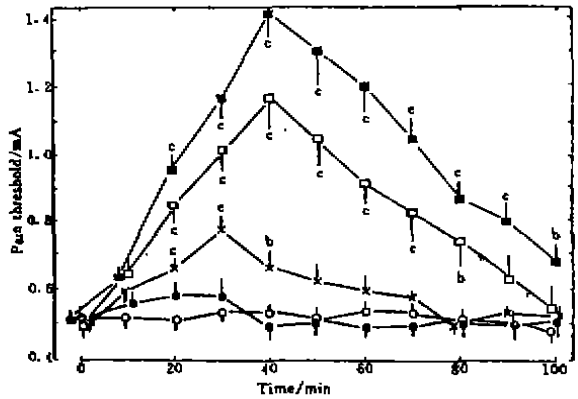

Fig 1. Antinociceptive activity of NAGA. **0 (○), 0.03 (●), 0.06 (×), 0.12 (□), 0.24 (■) μmol.** n=9. $^bP<0.05$, $^cP<0.01$ *vs* **control.**

Effect of naloxone on NAGA antinociception The antinociceptive activity of NAGA (0.12 μmol/rat, icv) was reversed by pretreatment with naloxone 0.26 mg·kg^{-1}, icv. Naloxone itself had no significant effect on pain thresholds of rats (Fig 2).

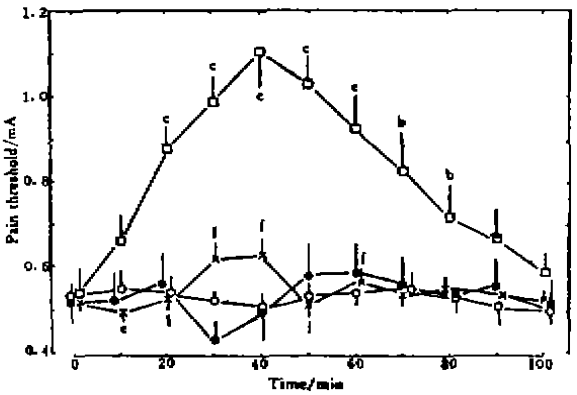

Fig 2. Effects of naloxone (Nal) on NAGA antinociception. **(○) control, (●) Nal, (×) NAGA + Nal, (□) NAGA.** n=9. $^bP<0.05$, $^cP<0.01$ *vs* **control.** $^eP<0.05$, $^fP<0.01$ *vs* **NAGA.**

Effect of opioid peptide anti-sera on NAGA antinociception Pre-treatment with anti-MEK serum (titre: 1:5000) or anti-LEK serum (titre: 1:5000) inhibited the antinociceptive effect induced by NAGA (0.12 μmol/rat, icv). Pretreatment with anti-β-EP serum (titre: 1:30000) or anti-Dyn A_{1-13} serum (titre: 1:30000) failed to block the antinociceptive activity of NAGA. Normal rabbit serum had no significant effect on the antinociceptive activity of NAGA. The anti-MEK serum, anti-LEK serum, anti-β-EP, and anti-Dyn A_{1-13} serum *per se* had no significant effect on pain thresholds of rats (Fig 3 and Fig 4).

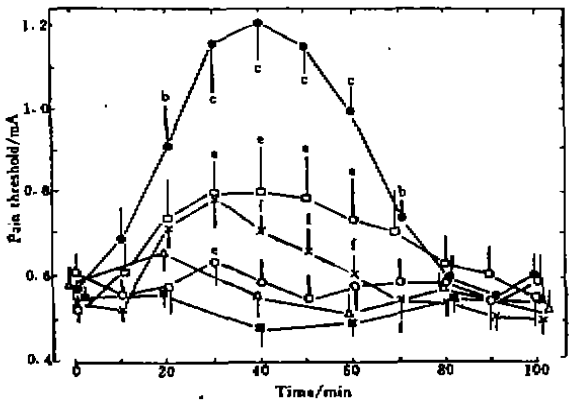

Fig 3. Effects of anti-MEK serum or anti-LEK serum on NAGA antinociception. (○) **control**, (●) **normal rabbit serum + NAGA**, (×) **anti-LEK serum + NAGA**, (□) **anti-MEK serum + NAGA**, (■) **anti-LEK serum**, (△) **anti-MEK serum.** $n=9-10$. $^bP<0.05$, $^cP<0.01$ *vs* **control.** $^eP<0.05$, $^fP<0.01$ *vs* **normal rabbit serum + NAGA group.**

DISCUSSION

The tetrapeptide NAGA was shown to display antinociceptive effect in mice and rabbits[2,3,4]. In the present study, our chemically synthesized NAGA exhibited a dose-dependent blockade of the tail flick response in rats and the antinociceptive effect of NAGA was

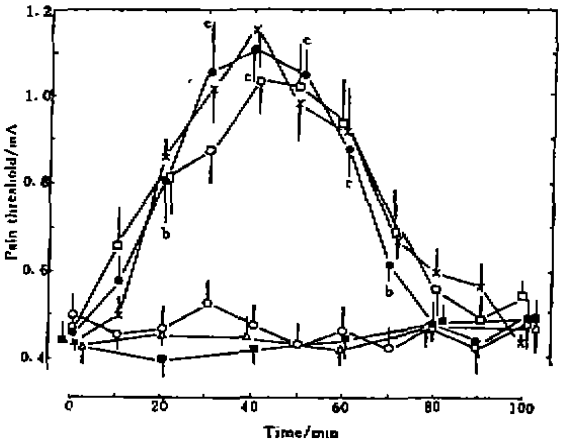

Fig 4. Effects of anti-β-EP serum or anti-Dyn A_{1-13} serum on NAGA antinociception. (○) **control**, (●) **normal rabbit serum + NAGA**, (×) **anti-Dyn A_{1-13} serum + NAGA**, (□) **anti-β-EP serum + NAGA**, (■) **anti-Dyn A_{1-13} serum**, (△) **anti-β-EP serum.** $n=9$. $^bP<0.05$, $^cP<0.01$ *vs* **control.**

almost completely reversed by naloxone. Thus, NAGA action is presumed to be mediated by endogenous opioid system. The antinociception induced by opiates has been suggested to be revealed through their action on mu-, delta-, and kappa-receptors[8,9]. NAGA did not significantly affect the actions of opioid peptides on the electrically evoked twitches of the myenteric plexus-longitudinal muscle strips of the guinea pig ileum, submucous plexus-longitudinal muscularis mucosae of the guinea pig esophagus and strips of the mouse vas deferens, it may neither act directly on mu-, kappa-, and delta-opiate receptors nor inhibit the met-enkephalin, leu-enkephalin and dynorphin A_{1-13} degradation enzymes[5].

The present results indicated that the antinociceptive effect induced by NAGA was significantly antagonized by pretreatment with anti-MEK serum or anti-LEK serum, but pretreatment with anti-β-EP serum or anti-Dyn A_{1-13} serum had no significant effect on the

antinociception induced by NAGA. The anti-MEK serum, anti-LEK serum, anti-β-EP serum and anti-Dyn A_{1-13} serum did not show cross-immunoreactivity with NAGA in radioimmunoassay (unpublished data). These results suggested that the antinociception induced by NAGA might be associated with the release of met-enkephalin and leu-enkephalin in rat brain.

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大鼠脑室注射四肽 Asn-Ala-Gly-Ala 引起的镇痛作用

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摘要 icv 四肽 Asn-Ala-Gly-Ala (NAGA)能引起大鼠痛阈持续升高，并呈剂量依赖关系(0.03—0.24 μmol/rat)。这一作用可被纳洛酮(icv. 0.26 mg·kg^{-1})翻转。icv 甲硫氨酸脑啡肽抗血清或亮氨酸脑啡肽抗血清，能抑制 NAGA 引起的镇痛作用；icv 强啡肽 A_{1-13}抗血清或 β-内啡肽抗血清不影响 NAGA 引起的镇痛作用。结果提示 NAGA 的镇痛作用可能与大鼠脑内甲硫氨酸脑啡肽和亮氨酸脑啡肽的释放有关。

关键词 镇痛；纳洛酮；内啡肽类；免疫血清；神经肽