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Effects of naloxone on *l*-clausenamide-induced long-term potentiation in dentate gyrus of anesthetized rats[1]

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KEY WORDS naloxone; clausenamide; long-term potentiation; hippocampus; dentate gyrus; synaptic transmission

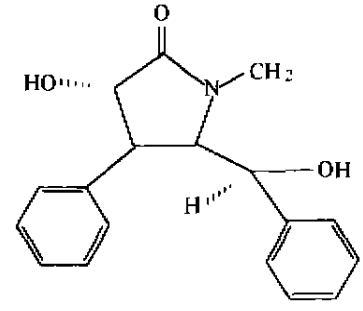
ABSTRACT

AIM: To investigate the mechanisms of *l*-clausenamide-induced long-term potentiation (LTP) in the dentate gyrus of anesthetized rats. **METHODS:** Extracellular recording technique was used to record the population spike (PS) in the dentate gyrus of anesthetized rats. **RESULTS:** Icv injection of naloxone 1 nmol, affecting neither the basal PS amplitude nor the LTP induced by tetanus, reduced the *l*-clausenamide-potentiated LTP only when it was administrated prior to clausenamide. Naloxone 1 nmol (icv), administrated 10 min before *l*-clausenamide, reduced the PS amplitude at 20 min, 55 min, and 90 min after icv injection of *l*-clausenamide 4 nmol from 138 % ± 10 %, 170 % ± 10 %, and 169 % ± 12 % to 111 % ± 7 %, 124 % ± 14 %, and 123 % ± 11 %, respectively. All $P < 0.01$ ($n = 8$). The same dose of naloxone (icv), delivered 10 min after *l*-clausenamide, did not affect the *l*-clausenamide-induced potentiation. **CONCLUSION:** The activation of opioid receptors contributes to the induction of *l*-clausenamide-induced LTP of synaptic transmission in dentate gyrus of anesthetized rats.

INTRODUCTION

Previous studies verified that opioid peptides co-existed with glutamate in the hippocampal neurons and that hippocampal opioids acted at multiple sites to modulate synaptic efficiency[1]. Clausenamide is one of the components isolated from *Clausena lansium* Lour skeels.

l-Clausenamide (Cla), synthetized first in our institute, has been shown to facilitate learning and memory and to improve amnesia impaired by $NaNO_2$ and anisodine in mice[2]. Recently, we demonstrated that Cla induced a long-term potentiation (LTP) of synaptic transmission in the dentate gyrus of anesthetized rats[3].



***l*-Clausenamide**

To elucidate whether the activation of hippocampal opioids receptors was involved in the Cla-induced LTP, we carried out the present study.

MATERIALS AND METHODS

Rats Fifty-six male Wistar rats (200 g ±

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20 g), from the Center of Experimental Animals, Chinese Academy of Medical Sciences were fed lab chow and water *ad lib* and maintained in a thermoregulated environment (19 – 23 ℃) during a 12-h light/dark cycle.

Drugs and drug delivery Cla provided by the Department of Medicinal Synthetic Chemistry in our institute was dissolved initially in Me_2SO to give a stock solution of 0.5 mol·L^{-1}, and then diluted in 0.9 % sodium chloride solution. The corresponding dose of Me_2SO was dissolved in 0.9 % sodium chloride solution for vehicle control. Naloxone (Nal, Sigma Co) was dissolved in 0.9 % sodium chloride solution and kept under 4 ℃. Drug or vehicle injections were delivered via a cannula in the lateral cerebral ventricle, following measurement of the baseline for 30 min from the dentate gyrus of the same hemisphere. The cannula was left in place for 5 min after each drug or vehicle injection. The drugs or vehicle were injected in a 5-μL volume over a 5-min period via a Hamilton syringe.

Drug doses were calculated on the basis that these drugs would theoretically achieve the brain concentration required, assuming the brain volume to be approximately 2 mL[4]. Thus for an estimated brain concentration of Cla 0.5 μmol·L^{-1} or 2.0 μmol·L^{-1}, 5 μL of Cla 200 μmol·L^{-1} or 800 μmol·L^{-1} was injected, respectively. For an estimated brain concentration of Nal 0.5 μmol·L^{-1}, 200 μmol·L^{-1} in a 5 μL injection volume was used.

Surgical procedure Rats were anesthetized with urethane carbamate (1.5 g·kg^{-1}, ip) and placed on a stereotaxic unit (SR-6N, Narishige, Japan).

Three drill holes (1.5 mm in diameter) for an outer guide cannula (0.8 mm outer diameter), a monopolar recording electrode and a bipolar stimulating electrode were sequentially made 0.8 mm, 3.8 mm, and 7.5 mm posterior to bregma and 1.8 mm, 2.5 mm, and 4.2 mm lateral to the mid-line, respectively. The recording electrode, and the stimulating electrode were made from teflon coated stainless steel wires (0.1 mm in diameter). The recording electrode and the stimulating electrode were lowered into the lateral cerebral ventricle, the dentate gyrus granule cell layer, and the perforant path (PP), respectively. The synaptic responses were monitored by a memory oscilloscope (VC-11, Nihon Kohden, Japan). Once the locations of the cannula and electrodes were verified, they would be kept in place for the duration of the experiment.

Measurement of evoked potentials The population spike (PS) amplitude was employed as an indication of the level of excitation of the granular cell population in the dentate gyrus. To obtain this measurement, an evoked response was generated in the dentate gyrus granule cell layer by stimulating the PP at low frequeny (0.033 Hz) with single square wave pulses of 0.15 ms duration. The stimuli were generated by an electronic stimulator (SEN-7203, Nihon Kohden, Japan) and an isolator (SS-202 J, Nihon Kohden, Japan) in a constant current manner. The vertical distance from the minimal point *a* to tangent line *bc* was employed as the amplitude of PS (Fig 1A).

By input/output curve determination the maximal PS amplitude was found, and potentials employed as baseline criteria were evoked at a stimulus intensity which produced 50 % of this maximum.

Tetanisation parameters In the dentate gyrus, LTP was induced by a theta burst stimulation consisting of 10 bursts of 5 pulses (200 Hz frequency, 0.2 ms stimulus duration, 200 ms interburst interval). The stimulus intensity was the same as that used for recordings.

Data collection and analysis For each

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time point, every ten records of evoked responses were averaged. The mean baseline was obtained by averaging the PS amplitudes of 6 time points obtained within 30 min before drug administration or tetanus. The data of each time point was expressed as mean percentage of mean baseline (standard deviation of the mean).

Statistical significance of the difference between means was estimated using the Student's t-test.

RESULTS

Effect of Nal on the basic synaptic transmission and LTP induced by tetanic stimulation Icv injection of Nal 1 nmol (5 μL of 200 μmol · L^{-1}) into the lateral cerebral ventricle did not affect the PS amplitude in the following 90 min (Fig 1A). The tetanic stimulus pattern employed in the present study was efficient to induce LTP, perhaps because this frequency closely approximated to the endogenous hippocampal theta rhythm. In the vehicle group (n = 8), 5 μL of 0.9 % saline was injected into the lateral cerebral ventricle 15 min before tetanus delivery, the PS amplitude at 5 min, 45 min, and 85 min after tetanus were 168 % ± 14 %, 156 % ± 11 %, and 153 % ± 15 %, respectively, all $P < 0.01$ *vs* the baseline value. In the Nal group (n = 8), Nal 200 μmol · L^{-1} 5 μL was injected into the lateral cerebral ventricle 15 min before tetanus delivery, the PS amplitude at 5 min, 45 min, and 85 min were 164 % ± 11 %, 160 % ± 9 %, and 162 % ± 14 %, respectively, all $P > 0.05$ *vs* vehicle (Fig 1B).

Effect of Nal on the synaptic transmission in the presence of Cla In the Me_2SO group (n = 8), 5 μL 0.9 % saline containing Me_2SO 56 nmol was injected into the lateral cerebral ventricle (the second arrow), the PS were not affected in the following 95 min. In the vehicle group (n = 8), the PS amplitude at 20 min, 55 min, and 90 min after icv injection

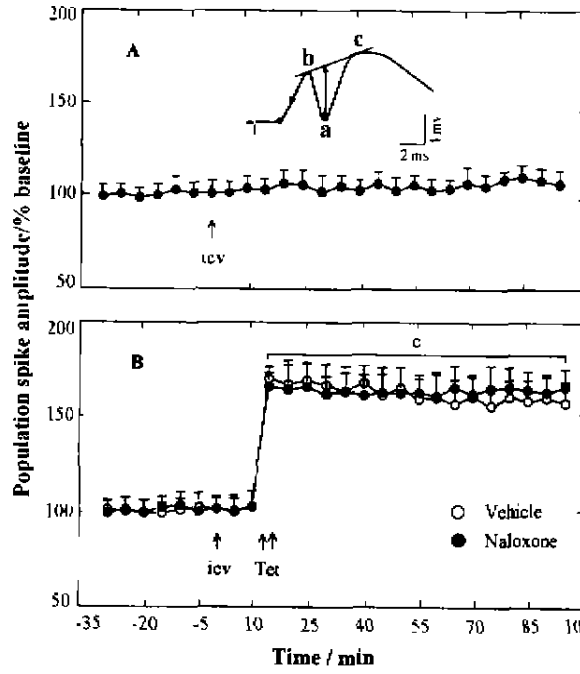

Fig 1. Effects of naloxone 200 μmol · L^{-1} 5 μL on the basal synaptic transmission (A) and LTP induced by tetanus (B) in the dentate gyrus of anesthetized rats. A) No tetanus was delivered after naloxone administration. B) Tetanus (Tet) was delivered 10 min after naloxone or vehicle (0.9 % sodium chloride 5 μL) administration. n = 8 rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* baseline.

(the second arrow) of Cla 4 nmol (5 μL of 800 μmol · L^{-1}) were 138 % ± 10 %, 170 % ± 10 %, and 169 % ± 12 %, respectively, all $P < 0.01$ *vs* Me_2SO group. In the Nal + Cla group (n = 8), Nal 1 nmol (5 μL of 200 μmol · L^{-1}, the first arrow) was injected into the lateral cerebral ventricle 10 min before Cla (the second arrow), the PS amplitude at 20 min, 55 min, and 90 min after icv injection of Cla 4 nmol were 111 % ± 7 %, 124 % ± 14 %, and 123 % ± 11 %, respectively, all $P < 0.01$ *vs* vehicle group. In the Cla + Nal group (n = 8), the sequence of drug administration was the reversal of that in Nal + Cla group, the PS amplitude at 20 min, 55 min, and 90 min after Cla administration were 126 % ± 9 %, 171 % ± 10 %, and 167 % ± 14 %, respectively, all $P > 0.05$

vs vehicle group (Fig 2).

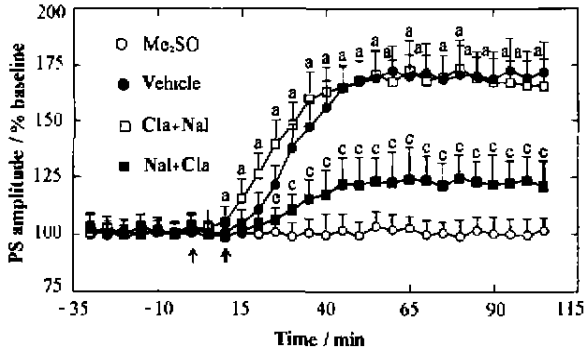

Fig 2. Effects of naloxone 200 μmol·L^{-1} 5 μL on synaptic transmission in the presence of Cla 800 μmol·L^{-1} 5 μL. $n = 8$ rats. $\bar{x} \pm s$. $^{a}P > 0.05$, $^{c}P < 0.01$ *vs* vehicle group.

DISCUSSION

It has been demonstrated that enkephalins and dynorphins, coexisting with glutamate in the perforant path fibers and the granule cells in dentate gyrus respectively, are only released by high-frequency stimulation and have opposite effects on the induction of LTP induced by tetanic stimulation[1]. This was consistent with the result in the present study that Nal affected neither the basal PS amplitude nor the LTP induced by high-frequency stimulation.

LTP consists of two phases: induction and maintenance. As shown in Fig 2, the PS amplitude in vehicle group began to increase 10 min after Cla delivery and almost reached its maximum 25 min later. This meant that the maintenance of Cla-induced LTP began 35 min after Cla administration. In Nal + Cla group, Nal began to show its inhibitory effects at the time point 15 min after Cla delivery or 25 min after Nal administraton, suggesting that activation of opioid receptors participate at least in the induction of Cla-induced LTP and that Nal had diffused and reached its effective concentration in the perforant fiber-dentate granule cell pathway within 25 min after its delivery. Thus Nal in Cla + Nal group should begin to show its effects 35 min after Cla administration, because it was delivered 10 min after Cla. That was to say, Nal would only affect the maintenance of Cla-induced LTP in Cla + Nal group if possible. But results in the present study showed that the PS amplitudes at all time points 35 min after Cla administration in Cla + Nal group were not affected compared to that at the corresponding time points in vehicle group, indicating that the activation of opioid receptors did not contribute to the maintenance of Cla-induced LTP.

It was verified that activation of the μ-receptors or δ-receptors facilitated the induction of LTP at the synapses on the granule cells[5,6]. In contrast, activation of κ-receptors blocked the induction of LTP at the perforant-path synapses[7]. Because the opioids have little effect on the basal synaptic transmission, it seems less possible that Cla potentiated the synaptic transmisssion by suppressing the activity of κ-receptors. In view of the facts that Cla increases the intracellular Ca^{2+} concentration and the release of glutamate from synaptosomes and that opioids coexist with glutamate in the hippocampal neurons, we suppose that Cla may cause the release of opioids even when the afferent fibers receive no high-frequency stimulation and then the released opioids increase the excitability of granule cells by activating the μ-receptors or δ-receptors.

In conclusion, the activation of opioid receptors is involved in the Cla-induced long-term potentiation in the dentate gyrus of anesthetized rats with contribution to its induction.

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纳洛酮对 *l*-黄皮酰胺诱导的麻醉大鼠海马齿状回长时程增强的影响[1]

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关键词 纳洛酮；黄皮酰胺；长时程增强；海马齿状回；突触传导

目的：研究 *l*-黄皮酰胺诱导麻醉大鼠海马齿状回长时程增强（LTP）的机制。**方法与结果**：细胞外记录麻醉大鼠海马齿状回诱发电位。脑室注射纳洛酮 1 nmol 对其基础突触传递活动和强直刺激诱导的 LTP 无影响。在 *l*-黄皮酰胺之前 10 min 脑室注射同剂量的纳洛酮，其群峰电位（PS）的幅值于脑室注射 *l*-黄皮酰胺 4 nmol 后 20 min，55 min 和 90 min 分别从 138 % ± 10 %、170 % ± 10 % 和 169 % ± 12 % 降为 110 % ± 7 %，124 % ± 14 % 和 123 % ± 11 %。$P < 0.01$（$n = 8$）。在 *l*-黄皮酰胺之后 10 min 给予同剂量的纳洛酮对 PS 无影响。**结论**：阿片受体的激活参与了 *l*-黄皮酰胺对麻醉大鼠海马齿状回 LTP 的诱导过程。

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