

Effects of 7-nitroindazole on long-term potentiation induced by *l*-clausenamide and high-frequency stimulation in rat hippocampus *in vivo*¹

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KEY WORDS indazoles; nitric-oxide synthase; clausenamide; long-term potentiation; neuronal plasticity; hippocampus; dentate gyrus; arginine

ABSTRACT

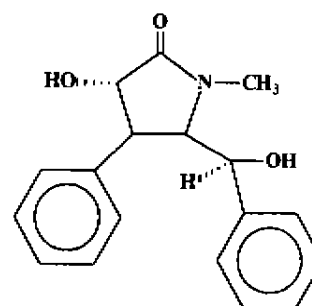
AIM: To study the antagonistic effect of selective neuronal nitric-oxide synthase (nNOS) inhibitor 7-nitroindazole on the long-term potentiation (LTP) induced by *l*-clausenamide (Cla) in rat hippocampus *in vivo*. **METHODS:** Population spike (PS) of evoked potentials was determined by extracellular recording technique in the hippocampal dentate gyrus (DG) of anesthetized rats. **RESULTS:** 7-Nitroindazole 2 nmol icv blocked the induction of LTP elicited by high-frequency (100 Hz) stimulation or Cla 5 nmol icv ($P < 0.01$), and *L*-arginine 225 mg·kg⁻¹ ip prevented the action of 7-nitroindazole ($P < 0.01$). **CONCLUSION:** Nitric oxide produced by nNOS plays a role in the induction of Cla-induced LTP in hippocampus.

INTRODUCTION

The synaptic plasticity including long-term potentiation (LTP), long-term depression (LTD), and metaplasticity was the cellular basis of learning and memory^[1,2]. Since Bliss and Lomo found the phenomenon of LTP in 1973, the exact cellular and molecular mechanism of synaptic plasticity is still unknown. A recent hypothesis was that the retrograde

messengers such as nitric oxide (NO) were involved in the synaptic plasticity^[3,4]. For elucidating the possible role of NO in the synaptic plasticity, many nitric-oxide synthase (NOS) inhibitors were used in cultured neurons, hippocampal slices, and anesthetized, freely moving or gene-knockout animals⁵. There were some paradox results in those experiments, and one reason for this was that they used different NOS inhibitors.

Clausenamide is one of the components isolated from *Clausena lansium* (Lour) Skeels. *l*-Clausenamide (Cla), first synthesized in our institute, had been shown to facilitate learning and memory and to improve amnesia impaired by NaNO₂ and anisodine in mice^[6]. Our laboratory found that Cla induced LTP in the dentate gyrus of anesthetized rats^[7]. The present study aimed to observe whether the activation of nNOS was responsible to the Cla-induced LTP.



l-Clausenamide

MATERIALS AND METHODS

Rats Male Sprague-Dawley rats ($n = 48$, 200–300 g, Grade II, Certificate No 07, the Administrative Commission of Medical Experimental Animals of Beijing, from the Center of Experimental Animals,

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National Institute for the Control of Pharmaceutical and Biological Products) were fed lab chow and water *ad lib* and housed (19–23 °C) under a 12-h light/dark cycle.

Drugs Cla, provided by Prof HUANG Liang (Department of Medicinal Synthetic Chemistry) was dissolved with Me₂SO (Sigma, USA) to give a stock solution of 0.5 mol·L⁻¹, and diluted with 0.9 % NaCl solution. 7-Nitroindazole (7-NI, Sigma, USA) was dissolved in Me₂SO (0.1 mol·L⁻¹) and diluted to the required concentration with 0.9 % NaCl solution. The corresponding dose of Me₂SO was dissolved in 0.9 % NaCl solution for vehicle control. Drug or vehicle injections were delivered via a cannula inserted through the outer guide cannula that was placed in the lateral cerebral ventricle. After measuring the baseline for 25 min from the dentate gyrus of the same hemisphere, the cannula was left in place for 5-min after each injection. The drugs or vehicle were injected in a 5 μL volume over a 5-min period through a Hamilton syringe.

Drug doses were calculated on the basis that these drugs would theoretically achieved the brain concentration required, assuming the brain volume to be approximately 2 mL^[8]. Estimated final brain concentration of Cla 2.5 μmol·L⁻¹ and 7-NI 1 μmol·L⁻¹ was used. The vehicle or 7-NI was injected into the lateral cerebral ventricle at 15 min before the use of HFS or Cla. L-Arginine 225 mg·kg⁻¹ ip was given 5 min before application of 7-NI.

Electrophysiological recording The rats were anesthetized with urethane 1.5 g·kg⁻¹ ip in the duration of all experiments. Surgical preparation and electrophysiological recording were performed as described^[7].

Data collection and analysis Each time point which averaged 5 records of evoked responses was measured every 5 or 15 min. The mean baseline was obtained by averaging the PS amplitude of 5 time points within 25 min before drug administration or tetanus. The data were expressed as mean percentage of mean baseline ± s and compared with *t*-test.

RESULTS

Basic synaptic transmission Population spike (PS) was elicited at the control test frequency of

0.033 Hz for 105-min to determine effects of 7-NI and the vehicle on the baseline of PS amplitude (Fig 1).

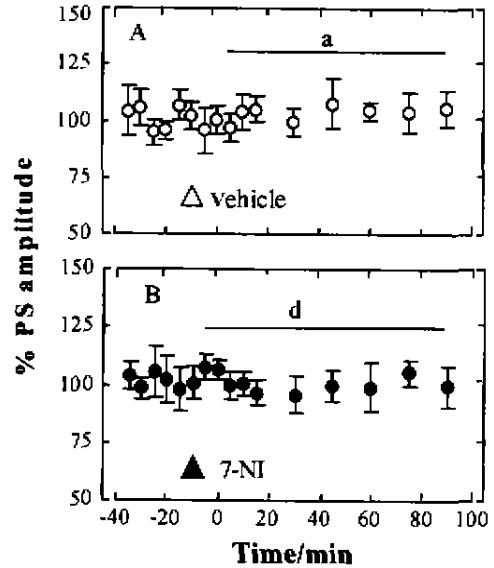


Fig 1. Effects of vehicle and 7-NI on baseline PS amplitude in DG of hippocampus. A) The vehicle did not affect the baseline PS amplitude significantly over the duration of the experiments ($n = 6$, $^*P > 0.05$). B) The 7-NI (2 nmol, icv) did not affect the baseline PS amplitude significantly over the duration of the experiments ($n = 6$, $^*P > 0.05$).

The amplitude of the PS did not change significantly after the vehicle over 105-min recording period. For example, the PS amplitude at 10, 30, 60, and 105-min after the vehicle administration measured 96 % ± 10 %, 105 % ± 6 %, 107 % ± 12 %, 106 % ± 7 %, respectively ($n = 6$, $P > 0.05$). The change of PS amplitude was not obvious after 7-NI (2 nmol, icv). They measured 107 % ± 5 %, 96 % ± 6 %, 99 % ± 7 %, and 99 % ± 9 % at 10, 30, 60, and 105 min, respectively. There was no significant difference between two groups ($n = 6$, $P > 0.05$). (Fig 1).

LTP induced by HFS 7-NI inhibited the induction of LTP induced by HFS (100 Hz) after 15-min application of vehicle. The values of PS amplitude were 234 % ± 13 %, 190 % ± 15 %, 185 % ± 15 %, and 188 % ± 15 % at 10, 30, 60, and 90 min after tetanic stimuli ($n = 6$). This LTP was prevented by 7-NI ($n = 6$, $P < 0.01$). The PS

amplitude measured $93\% \pm 6\%$ at 10 min, $109\% \pm 10\%$ at 30 min, $107\% \pm 11\%$ at 60 min, and $101\% \pm 7\%$ at 90 min. (Fig 2)

LTP induced by Cla 7-NI inhibited the induction of LTP induced by Cla. After 15 min of administration of vehicle, Cla (5 nmol, icv) resulted in an enhancement of synaptic transmission $147\% \pm 7\%$, $167\% \pm 9\%$, $178\% \pm 11\%$, and $196\% \pm 14\%$ at 10, 30, 60, and 90 min, respectively, indicating that LTP was induced. 7-NI (2 nmol, icv) 15 min before Cla inhibited the synaptic enhancement ($n = 6$, $P < 0.01$). The PS amplitude changed to $110\% \pm 14\%$, $115\% \pm 9\%$, $112\% \pm 6\%$, and $113\% \pm 10\%$ at 10, 30, 60, and 90 min, respectively. (Fig 2)

L-Arginine reversed the action of 7-NI on the LTP induction L-Arginine $225\text{ mg} \cdot \text{kg}^{-1}$ ip was injected 5 min before 7-NI. In the HFS-groups, the PS amplitude measured $166\% \pm 8\%$, $152\% \pm 9\%$, $149\% \pm 12\%$, and $147\% \pm 6\%$ at 10, 30, 60, and 90 min (Fig 3A, $n = 6$). This synaptic transmission increased significantly compared with the 7-NI + HFS group ($n = 6$, $P < 0.01$) but decreased vs the vehicle + HFS group ($n = 6$, $P < 0.01$). In the

Cla (5 nmol, icv) groups, the PS amplitude of rats injected with L-arginine ($225\text{ mg} \cdot \text{kg}^{-1}$, ip) and 7-NI (2 nmol, icv) was higher than that of rats injected with only 7-NI ($n = 6$, $P < 0.01$) during the recording period, and recovered the level to that of the vehicle group at 10, 15, 75, and 90 min ($n = 6$, $P < 0.05$) (Fig 3B).

DISCUSSION

The present results showed clearly that 7-NI prevented the induction of LTP induced by HFS or Cla, and L-arginine reversed those effects. Because 7-NI is a potent nNOS selective inhibitor and L-arginine is the substrate used by NOS for NO production, it is clear that NO synthesized by nNOS was involved in the Cla-induced synaptic enhancement.

The possibility that NO may be a retrograde messenger in synaptic plasticity has excited considerable interest. Being a membrane permanent retrograde signal, NO produced by the post-synaptic neuron may back to the presynaptic neuron and is absorbed by the haem group of an NO-sensitive guanylate cyclase. Production of cGMP then evokes an increase in release

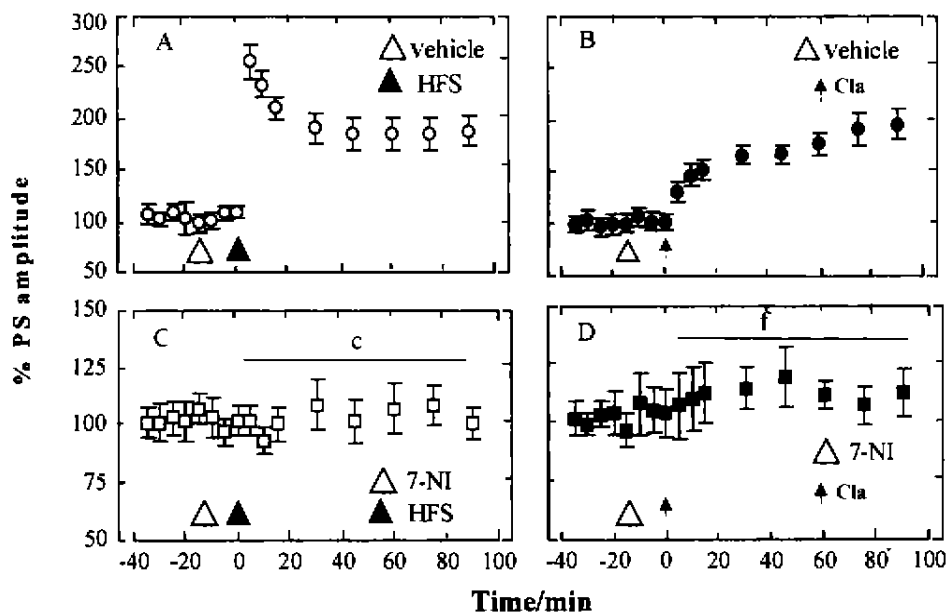


Fig 2. Effects of vehicle and 7-NI on induction of LTP in DG of hippocampus. A) LTP induced by HFS (100 Hz) after the vehicle injection. B) LTP induced by Cla (5 nmol, icv) after the vehicle injection. C) LTP induced by HFS (100 Hz) was blocked by the 7-NI (2 nmol, icv) ($n = 6$, $P < 0.01$). D) LTP induced by Cla (5 nmol, icv) was blocked by the 7-NI (2 nmol, icv) ($n = 6$, $P < 0.01$).

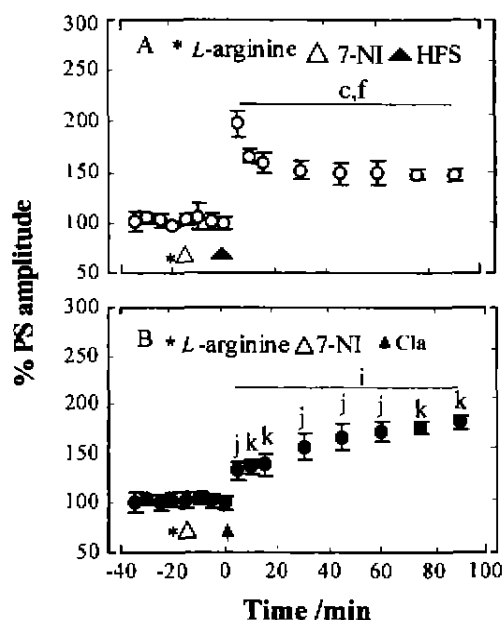


Fig 3. *L*-Arginine attenuated the action of 7-NI on induction of LTP. A) *L*-Arginine (225 mg·kg⁻¹, ip) prevented the effect of 7-NI (2 nmol) on the LTP induced by HFS ($n = 6$, $^cP < 0.01$). The PS amplitude did not reach the level of vehicle + HFS group ($n = 6$, $^iP < 0.01$). B) *L*-Arginine (225 mg·kg⁻¹, ip) prevented the effect of 7-NI (2 nmol, icv) on the LTP induced by Cla (5 nmol, icv) ($n = 6$, $^iP < 0.01$). It changed the PS amplitude to the level of vehicle + Cla group at 10, 15, 75, and 90 min ($^jP > 0.05$, $^kP < 0.05$).

of neurotransmitter or other synaptic activities⁽⁹⁾. There are three forms of NOS in the brain including endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). 7-NI is a potent inhibitor of NOS which exhibits selectivity for the nNOS without increasing blood pressure⁽¹⁰⁾. There are differences in the sensitivity of different hippocampal pathways to NOS inhibitors even with the same brain region and there is different neurochemical mechanism in different hippocampal region^(11,12). Although the NOS inhibitors blocked the induction of LTP in many studies *in vitro*, some NOS inhibitor was failed to block the LTP *in vivo*. *L*-N^G-Nitroarginine methyl ester derivative (*L*-NAME) had no effect, but Ciaran Doyle *et al* found that 7-NI 30 mg·kg⁻¹, ip could prevent the LTP induction in CA1 *in vivo*^(13,14). The present study found the same result in DG *in vivo*, which

supported the view that selective nNOS inhibitor blocked the induction of LTP *in vivo*. In our experiment, action of *L*-arginine on the inhibition of 7-NI provided an explanation that 7-NI inhibited NO production and then prevented the induction of LTP. Cla was found to improve learning and memory in the amnesia animal. It also enhanced the basic synaptic transmission and the magnitude of LTP induced by HFS^(6,7). In the present study, 7-NI was found to block the induction of LTP by Cla, which supports the previous results and suggests that one of the nootropic mechanism of Cla is affecting the retrograde messenger NO in the brain and then resulting in the synaptic transmission efficacy enhancement.

In conclusion, NO especially produced by neuronal nitric-oxide synthase contributed to the Cla-induced LTP.

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7-硝基吲唑对在体大鼠海马 L-黄皮酰胺和
高频电刺激诱发长时程增强的影响¹

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关键词 吲唑类; 一氧化氮合酶; 黄皮酰胺; 长时程增强; 神经元可塑性; 海马; 齿状回; 精氨酸

目的: 研究选择性 nNOS 抑制剂 7-硝基吲唑(7-NI) 在 L-黄皮酰胺所致在体大鼠海马长时程增强(LTP)中的拮抗作用。 方法: 用细胞外记录技术记录麻醉大鼠齿状回中诱发动作电位的群峰电位(PS)。 结果: 7-硝基吲唑(7-NI 2 nmol, icv)阻断高频电刺激(100 Hz)和 L-黄皮酰胺(5 nmol, icv)诱导的 LTP($n=6, P<0.01$), L-精氨酸(225 mg·kg⁻¹, ip)能逆转 7-NI 的这种作用($n=6, P<0.01$)。 结论: nNOS 产生的 NO 参与了 L-黄皮酰胺所致海马 LTP 的诱导过程。

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