

Activation of p42/44 mitogen-activated protein kinase pathway in long-term potentiation induced by nicotine in hippocampal CA1 region in rats¹

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KEY WORDS nicotine; hippocampus; long-term potentiation; mitogen-activated protein kinase; Western blotting

ABSTRACT

AIM: To investigate the relationship between activation of p42/44 mitogen-activated protein kinase (MAPK) pathway and hippocampal long term potentiation (LTP) induced by nicotine in area CA1. **METHODS:** Extracellular recording of population spike (PS) was performed within the pyramidal cell layer of hippocampal area CA1 *in vitro*; Western blot analysis was employed to detect the active phosphorylated state and the total protein expression of p42/44 MAPK. **RESULTS:** PD98059 concentration-dependently (25 $\mu\text{mol/L}$, 50 $\mu\text{mol/L}$) attenuated the induction of LTP induced by nicotine 10 $\mu\text{mol/L}$; both p42 and p44 MAPK were activated with their total protein expression increasing in CA1 subregion in response to LTP induced by nicotine. **CONCLUSION:** Activation of p42/44 MAPK pathway is required for hippocampal LTP induced by nicotine.

INTRODUCTION

Over the past 15 to 20 years, the long-lasting synaptic transmission enhancement, known as long-term potentiation (LTP) has been believed to be a key to understand the cellular and molecular mechanisms by which memories are formed and stored⁽¹⁾. The hippocampal LTP has been widely studied and much progress has been made to

elucidate the mechanisms underlying its induction⁽²⁾.

It is well accepted that the induction of LTP by the tetanic stimulation requires synaptic activation of post-synaptic *N*-methyl-*D*-aspartate (NMDA) receptors. The subsequent rise of intracellular Ca^{2+} is critical for initiating a complicated signal transduction in LTP induction. On the other hand, the researches which focus on biochemical pathway activated by Ca^{2+} implicated that several protein kinase including protein kinase C (PKC), cyclic adenosine 3', 5'-monophosphate (cAMP)-dependent protein kinase (PKA), the tyrosine kinase Src, and mitogen-activated protein kinase (MAPK) contribute to LTP induction. Meanwhile, roles of these protein kinases have been found to depend on their phosphorylation process⁽³⁾.

MAPK is a group of serine/threonine dual specificity protein kinases which can be activated by growth factors and have been shown to be involved in cellular proliferation and differentiation in classical studies⁽⁴⁾. The components of MAPK cascade are abundant expressed in post-mitotic neurons of the developed nervous system⁽⁵⁾. A set of studies have shown that this cascade plays a fundamental role in memory consolidation⁽⁶⁾. Moreover, activation of the MAPK cascade has been recently found necessary in synaptic plasticity in both area CA1 of rat hippocampus and neurons of *Aplysia*^(7,8).

We previously reported that the subtypes of central nicotinic receptors sensitive to κ -BGT played an important role in learning and memory⁽⁹⁾. At the same time, we demonstrated that the hippocampal long term potentiation in area CA1 could be induced by nicotine⁽¹⁰⁾. To further understand the action of nicotinic acetylcholine receptors (nAChR) in learning and memory, we utilized the compound PD98059⁽¹¹⁾, an specific inhibitor of MEK (MAPK kinase) to identify the role of MAPK cascade in LTP induction by nicotine in area CA1 of hippocampus.

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MATERIALS AND METHODS

Materials (-)-Nicotine and Me₂SO were purchased from Sigma Chemical Co; PD98059, phosphop44/42 MAP kinase (Thr202/Tyr204) antibody kit was purchased from New England Biolab Co. Sprague-Dawley rats (♂ ♀, 100–120 g) were obtained from the Experimental Animal Center of the Sun Yat-sen University of Medical Sciences (Grade II, Certificate No 99A005 conferred by Medical Animal Management Committee, Guangdong Province).

Hippocampal slice preparation and field potential recording Transverse hippocampal slices 400-μ thick prepared from SD rats at 0 °C were incubated in artificial cerebrospinal fluid (ACSF; NaCl 124.0, KCl 3.0, MgSO₄ · 7H₂O 1.3, NaH₂PO₄ 1.25, NaHCO₃ 22.0, CaCl₂ 2.5, D-glucose 10.0 mmol/L; pH 7.4) at 28 °C ± 1 °C. ACSF was pre-saturated with 95 % O₂ and 5 % CO₂. PD98059 was dissolved in Me₂SO and diluted into ACSF to gain the desired final concentration of 25 μmol/L and 50 μmol/L. Slices were preincubated in PD98059 25 μmol/L and 50 μmol/L or 0.25 % Me₂SO for 45 min to 1 h prior to recording. After incubation, slices were placed on an interface chamber and perfused with ACSF or modified solution (nicotine was added to give the final concentration of 10 μmol/L) at the speed of 0.7 mL/min in 32 °C ± 0.5 °C water bath.

For extracellular field recording of the Schaffer collateral synapses in area CA1, stimuli was applied to the Schaffer collateral-commissural pathway through a bipolar insulated wire electrode, and the glass microelectrode (0.6–1.5 MΩ, filled with NaCl 2 mol/L) was placed in the pyramidal cell layer of area CA1. Test stimuli was adjusted to elicit a population spike (PS) that was 80 % of the maximum response. Nicotine added in artificial cerebrospinal fluid (ACSF) was perfused into chamber for 35 min, then only ACSF was continuously perfused. In some control group, slices were treated with two trains of high frequency stimulation (100 Hz; interval = 1 min). Slices were stored in liquid nitrogen at -170 °C for Western blot analyses of phospho-p44/42 MAPK or p44/42 MAPK 20 min after nicotine or high frequency stimulation was given. The PS was monitored for at least 15 min to ensure a stable baseline. The PS amplitude was recorded by X-Y recorder (RIKADENKI, Japan) every 5 min throughout the test.

Western blot analysis The CA1 subregion, which was cut from individual slice stored in liquid nitrogen, was sonicated (3 W; 1 min) in ice-cold buffer

(Tris-HCl 50 pH 7.5, NaCl 50, egtazic acid 10, edetic acid 5, PMSF 1 mmol/L; leupeptin 20, aprotinin 4 mg/L). Following sonication, the soluble extract was obtained after centrifugation at 10 000 × g (4 °C). Protein concentration in the soluble fraction was then measured using a Bradford assay⁽¹²⁾, with bovine serum albumin as the standard. Equivalent amounts of protein for each sample were resolved in 12 % SDS-polyacrylamide gel, and electrophoresis was operated for 45 min at 200 V. The separated protein on gel was transferred to PVDF membrane at 100 V for 60 min. Membranes were blocked for 3 h in TBST buffer (Tris-HCl 50 pH 7.5, NaCl 150 mmol/L; Tween-20 0.3 %) containing 5 % non-fat milk at room temperature. They were then incubated overnight at 4 °C with an antiserum that recognized phospho-p44/42 MAPK or p44/42 MAPK. This is followed by 1 h incubation with anti-rabbit IgG conjugated to horseradish peroxidase at room temperature, and then the blots were visualized using the Enhanced Chemiluminescence (ECL) Western blot detection kit (NEB). Half-quantitative analysis of immunoreactivity were measured by the IBAS image analysis system in terms of calculating absorbance and area of each blot.

Statistical methods The data were normalized with average baseline values or control. They were expressed as $\bar{x} \pm s$ and analyzed by *t*-test.

RESULTS

PD98059 dose-dependently attenuated the induction of LTP in area CA1 When nicotine was applied, LTP can be elicited to present a stable population spike which lasts for at least 60 min and its value reaches 140 %–150 % compared with average baseline PS. However, only a slight increase of PS in slices treated with PD98059 50 μmol/L was recorded compared with the slices treated with nicotine alone (At *t* = 100 min, the mean PS value was 114 % ± 7 % of baseline response, *n* = 7, *P* < 0.01). On the other hand, when PD98059 25 μmol/L was applied, the potentiation was attenuated to a lower level compared with the slices treated with nicotine alone (At *t* = 100 min, the mean PS value was 134 % ± 8 % of baseline response, *n* = 5, *P* > 0.05) (Fig 1). These data suggested that PD98059 inhibited LTP induced by nicotine.

p42 and p44 MAPK were all activated in area CA1 when LTP was induced by nicotine Western blot analysis of the area CA1 subregions revealed the marked increase in phosphorylated immunoreactivity of

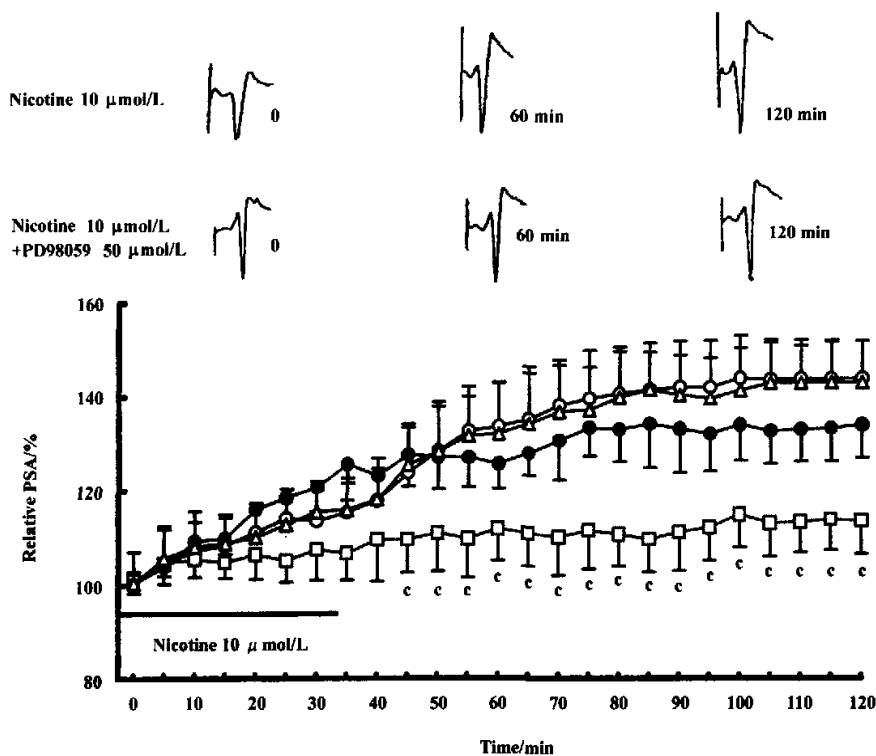


Fig 1. PD98059 markedly attenuated the induction of LTP induced by nicotine in area CA1. (○) nicotine 10 $\mu\text{mol/L}$, (●) nicotine 10 $\mu\text{mol/L}$ + PD98059 25 $\mu\text{mol/L}$, (△) nicotine 10 $\mu\text{mol/L}$ + Me₂SO 0.25%, (□) nicotine 10 $\mu\text{mol/L}$ + PD98059 50 $\mu\text{mol/L}$. $n = 7$. $\bar{x} \pm s$. * $P < 0.01$ vs nicotine 10 $\mu\text{mol/L}$.

p42 and p44 MAPK with nicotine application (p42 MAPK: 299% \pm 34% of control, p44 MAPK: 238% \pm 34% of control, $n = 6$, $P < 0.01$, Fig 2). Meanwhile, the immunoreactivity of p44 MAPK induced by nicotine treated with PD98059 50 $\mu\text{mol/L}$ was 29% \pm 6% of control, and that of the group treated with PD98059 25 $\mu\text{mol/L}$ was 67% \pm 14% of control, $n = 6$, $P < 0.01$, Fig 2). For p42 MAPK, the immunoreactivity of the group treated with PD98059 50 $\mu\text{mol/L}$ was 39% \pm 5% of control ($n = 6$, $P < 0.001$, Fig 2), and that of the group treated with PD98059 25 $\mu\text{mol/L}$ was 102% \pm 10% of control ($n = 6$, $P > 0.05$, Fig 2). These results strongly suggested that nicotine could stimulate MAPK cascade and induce LTP while PD98059 inhibited the LTP induced by nicotine by blocking activation of MAPK cascade.

Both p42 and p44 MAPK total protein expression increased in area CA1 in response to LTP induced by nicotine. The immunoreactivity of p42 MAPK total protein was 190% \pm 8% of control;

and that of p44 MAPK was 176% \pm 11% of control. ($n = 6$, $P < 0.01$, Fig 3). At the same time, with nicotine application, a negative correlation between MAPK protein expression and the dose of PD98059 could be seen. The immunoreactivity of p44 MAPK total protein of the group treated with PD98059 25 $\mu\text{mol/L}$ was 78% \pm 8% of control, and that of the group treated with PD98059 50 $\mu\text{mol/L}$ was 66% \pm 10% of control ($n = 6$, $P < 0.01$, Fig 3). For p42 MAPK total protein, the immunoreactivity of the group treated with PD98059 25 $\mu\text{mol/L}$ was 91% \pm 5% of control ($n = 6$, $P < 0.01$, Fig 3), and that of the group treated with PD98059 50 $\mu\text{mol/L}$ was 78% \pm 3% of control ($n = 6$, $P < 0.01$, Fig 3). These data give evidence for a protein expression of p42 and p44 MAPK increasing during induction of LTP by nicotine.

Only p42, but not p44 MAPK was activated in area CA1 when LTP was induced by high frequency stimulation. Western blot analysis of CA1 subregions revealed that phosphorylated immunoreactivity

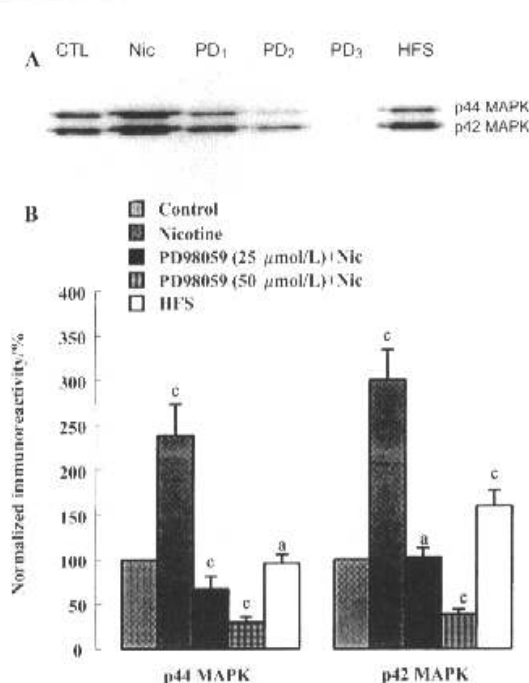


Fig 2. Activation of p44 and p42 MAPK during induction of LTP. **A:** Representative Western blots of individual area CA1 subregions from control (CTL), nicotine-treated (Nic), high frequency stimulation-treated (HFS) slice and slices treated with PD98059 50 $\mu\text{mol/L}$ (PD₄) and nicotine in the presence of PD98059 25 $\mu\text{mol/L}$ (PD₁) or PD98059 50 $\mu\text{mol/L}$ (PD₂). **B:** Normalized phosphorylated p44 and p42 MAPK immunoreactivity. $n=6$. $\bar{x} \pm s$. ^a $P > 0.05$, ^c $P < 0.01$ vs control.

of p42 MAPK increased with high frequency stimulation, but no considerable change of p44 MAPK could be observed (p42 MAPK: 159% \pm 18% of control, $n=6$, $P < 0.01$; p44 MAPK: 94% \pm 10% of control, $n=6$, $P > 0.05$, Fig 2). This result is consistent with which was reported by English and Sweatt^[8]. Furthermore, in our observation, only a slight increase of p42 MAPK total protein immunoreactivity could be detected (p42 MAPK: 108% \pm 6% of control, $n=6$, $P < 0.01$; p44 MAPK: 94% \pm 9% of control, $n=6$, $P > 0.05$, Fig 3).

DISCUSSION

Genetic and pharmacological experiments have recently demonstrated several protein kinases cascades in LTP and memory formation. In present studies, the MAPK pathway, which is usually associated with cell

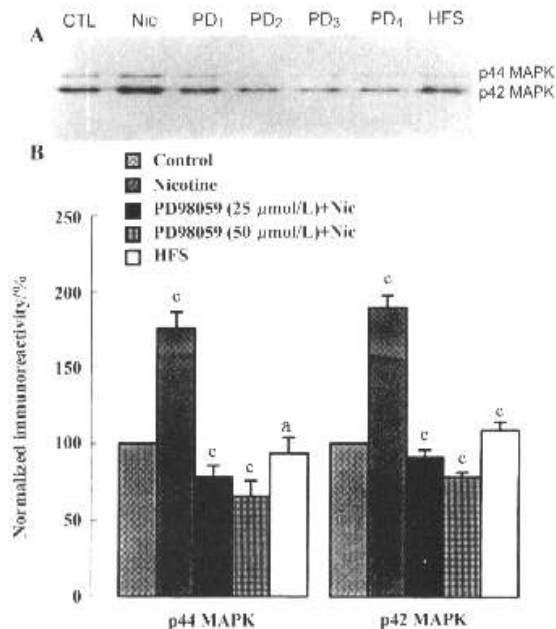


Fig 3. Alteration of total p44 and p42 MAPK protein expression during induction of LTP. **A:** Representative anti-MAPK Western blots of individual area CA1 subregions from Control (CTL), Nicotine-treated (Nic), HFS-treated (HFS) slice and slices treated with PD98059 25 $\mu\text{mol/L}$ (PD₃) or 50 $\mu\text{mol/L}$ (PD₄) alone and nicotine in the presence of PD98059 25 $\mu\text{mol/L}$ (PD₁) or PD98059 50 $\mu\text{mol/L}$ (PD₂). **B:** Normalized total protein of p44 and p42 MAPK immunoreactivity. $n=6$. $\bar{x} \pm s$. ^a $P > 0.05$ vs control, ^c $P < 0.01$ vs control.

proliferation and differentiation is also involved in behavior and neuroplasticity. In Aplysia gill-withdrawal reflex, the *ras*-dependent MAPK cascade is found to play an essential role in the long term, but not the short term facilitation process^[13]. In addition, in the rodent hippocampus, inhibition of the MAPK pathway impairs the induction of LTP which is induced by high frequency stimulation^[8]. Furthermore, we previously reported that LTP can be elicited by nicotine with certain test stimuli in hippocampal area CA1^[10]. In this study, we hypothesized that MAPK were also involved in LTP induced by nicotine. We firstly observed that PD98059, a selective inhibitor of the MAPK cascade, concentration-dependently attenuated the induction of LTP and blocked MAPK activation when nicotine was applied. Secondly, we discovered both p42 and p44 MAPK were markedly activated in response to LTP induction. This finding is different from the results of English who reported that p42 MAPK but not p44 MAPK was activated when high fre-

quency stimulation was applied^[8]. Therefore, our results gave evidence to implicate that p42 and p44 MAPK pathway played an important role in signal transduction of LTP induced by nicotine. Moreover, p44 MAPK is believed to play a different role in high frequency stimulation- and nicotine-induced LTP. It is also interesting to investigate whether the other MAPK pathway such as p38 or JNK takes part in LTP induction.

The other observation is about p42 and p44 MAPK total protein expression during LTP induction. Our Western blot analysis provided the first demonstration of increasing total protein expression of p42 and p44 MAPK accompanied with their activation in response to LTP induction. Although there were some studies showed changes in hippocampal gene expression associated with the LTP induction^[14], our observation is intriguing for the specific LTP phenomenon that can be induced by nicotine.

It is important to note that with nicotine applied, the duration before a stable population spike presentation was longer than that we reported previously^[10]. We considered that it was due to a larger interface chamber and lower perfusion speed used in our research. However, It is shown that the amplitude of PS and lasting duration of LTP were as same as our previous results.

In conclusion, p42 and p44 MAPK are necessary for signal transduction in hippocampal LTP induced by nicotine in area CA1. To determine the downstream effectors of MAPK would be beneficial to understand the mechanisms of synaptic plasticity as well as to elucidate the roles of nAChR on learning and memory.

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在烟碱诱导的大鼠海马 CA1 区长时程增强形成中 p42/44 促细胞分裂剂活化的蛋白激酶通路被激活¹

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关键词 烟碱; 海马; 长时程增强; 促细胞分裂剂活化的蛋白激酶; 蛋白质印迹

目的: 研究 p42/44 MAPK 通路在烟碱诱导大鼠海马 CA1 区长时程增强(LTP)形成中的作用. **方法:** 细

胞外场电位记录离体海马脑片 CA1 区锥体细胞层群体峰电位; 蛋白质印迹检测 p42/44 MAPK 磷酸化程度及其总蛋白表达. **结果:** PD98059 25 $\mu\text{mol/L}$ 和 50 $\mu\text{mol/L}$ 呈剂量依赖性抑制烟碱(10 $\mu\text{mol/L}$)诱导大鼠海马 CA1 区 LTP 的形成; 在烟碱诱导 LTP 形成的海马 CA1 区组织内 p42 和 p44 MAPK 磷酸化均明显增强并有 p42 和 p44 MAPK 总蛋白表达量的增加. **结论:** p42/44 MAPK 通路参与烟碱诱导大鼠海马 CA1 区 LTP 形成的信号转导过程.

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