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Long-term potentiation induced by nicotine in CA_1 region of hippocampal slice is Ca^{2+}-dependent[1]

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KEY WORDS nicotine；hippocampus；long-term potentiation；calcium；nifedipine；tharpsigargin

ABSTRACT

AIM：To observe the effects of Ca^{2+} on hippocampal long-term potentiation (LTP) induced by nicotine in CA_1 region of rat hippocampal slice. **METHODS**：Extracellularly recorded population spikes (PS) of the pyramidal cell layer in the hippocampal CA_1 region *in vitro*. **RESULTS**：Nicotine 1 μmol·L^{-1} induced LTP in the hippocampal CA_1 region. It did not induce LTP in CA_1 region when Ca^{2+} was removed from artificial cerebrospinal fluid (ACSF). Nifedipine 1 and 10 μmol·L^{-1} partly inhibited LTP induced by nicotine，and thapsigargin 1 and 10 μmol·L^{-1} completely inhibited LTP induced by nicotine. **CONCLUSON**：LTP induced by nicotine in hippocampal CA_1 region is Ca^{2+}-dependent. Both Ca^{2+} influx and Ca^{2+} release participate in the induction of LTP.

INTRODUCTION

Hippocampal long-term potentiation (LTP)，a long-lasting increase in the efficacy of synaptic transmission，is assumed to underlie the plastic changes associated with learning and memory and is assumed to be a cellular mechanism of learning and memory[1]. The facilitation of synaptic transmission which contributes to LTP in hippocampal CA_1 cells requires the convergence of a transient elevation in intracellular Ca^{2+} with transmitter binding to cell-surface receptors. This temporal convergence of Ca^{2+} and G-protein-stimulated second-messenger cascades synergistically stimulates several classes of serine/threonine protein kinases，which in turn modulate receptor function or cell excitability through the phosphorylation of ion channels，resulting in LTP[2].

There has been an increase in interest regarding the important role of neuronal nicotinic acetylcholine receptors (nAChRs) in memory modulation and regarding the potential role of nAChRs in the treatment of Alzheimer's disease (AD)[3]，but the studies are still inconclusive. On finding that nicotine induced LTP in CA_1 region of hippocampal slices，in present experiments，we used hippocampal slices *in vitro* to study the role of Ca^{2+} on LTP induced by nicotine.

MATERIAL AND METHODS

Chemicals Nicotine，nifedipine，and thapsigargin were obtained from Sigma. Sprague-Dawley rats (♂♀，100 – 120 g，Grade Ⅱ，Certificate № 26-001 conferred by Medical Animal Management Committee，Guangdong Province) were obtained from the Experimental Animal Center of Sun Yat-sen University of Medical Sciences.

Methods Hippocampal slices (400-μm thick) were prepared at 0 ℃ and incubated in artificial cerebrospinal fluid (ACSF) at 28 ℃ for at least 90 min. ACSF (NaCl 124，KCl 3.4，KH_2PO_4 1.2，$MgSO_4 \cdot 7H_2O$ 1.7，$NaHCO_3$ 25，$CaCl_2$ 2.4，glucose 10 mmol·L^{-1}；pH 7.4) was previously saturated with 95 % O_2 + 5 % CO_2. Before recording，slices were transferred to an interface chamber where slices were continuously perfused at 1 mL·min^{-1} with saturated ACSF at 33 ℃. Nicotine，nifedipine，and thapsigargin were added into the perfusate solution.

Potentials were recorded extracellularly by the use of glass microelectrodes (1 – 2 MΩ resistance，filled with NaCl 2 mol·L^{-1}) placed in the pyramidal cell layer of CA_1 region，stimuli being applied to the Schaffer collateral-commissural pathway through a bipolar，insulated tungsten wire electrode. Test stimuli (0.017 Hz，0.1 ms

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width) adjusted to give 80 % of maximal population spikes (PS) amplitude were applied. Potentials were fed through a microelectrode amplifier (MEZ-7101；Nihon Kohden, Japan) to a dual-beam memory oscilloscope (VC-10；Nihon Kohden, Japan) and recorded by an X-Y electronic recorder (Nihon Kohden, Japan).

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed by t-test.

RESULTS

LTP induced by nicotine After the potentials became steady for 15 min, nicotine was added into ACSF and was perfused for 20 min. The mean levels of potentiation at 50 min after nicotine exposure were 125 % ± 5 %, 158 % ± 10 %, and 166 % ± 8 % of the baseline with the concentrations of 0.1, 1, and 10 μmol·L^{-1}, respectively. Nicotine 1 and 10 μmol·L^{-1} induced more marked LTP which were markedly different in magnitude from that induced by nicotine 0.1 μmol·L^{-1} ($P < 0.05$). Therefore, nicotine induced LTP of the hippocampal CA_1 region lasted for at least 65 min, and at least nicotine 1 μmol·L^{-1} was required to produce a maximal effect ($P > 0.05$ between the groups of nicotine 1 and 10 μmol·L^{-1}) (Fig 1).

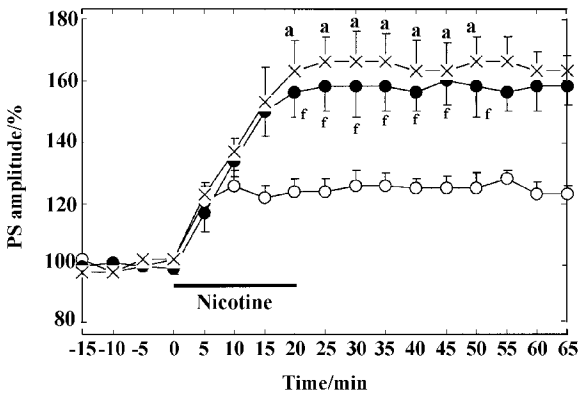

Fig 1. LTP induced by nicotine in the CA_1 region of the hippocampal slice. (○) nicotine 0.1 μmol·L^{-1}, (●) nicotine 1 μmol·L^{-1}, (×) nicotine 10 μmol·L^{-1}. $n = 5$. $\bar{x} \pm s$. $^aP > 0.05$ *vs* nicotine 1 μmol·L^{-1}. $^fP < 0.01$ *vs* nicotine 0.1 μmol·L^{-1}.

Test stimuli failed to evoke the increase in PS amplitude in 3 of 10 slices treated with nicotine 1 and 10 μmol·L^{-1}. The same intensity of test stimuli was delivered in all the following experiments.

Effect of nicotine on PS in the Ca^{2+}-free ACSF Slices were divided into control and experimental groups. In slices from control group incubated in Ca^{2+}-containing ACSF for 1 h, nicotine 1 μmol·L^{-1} induced hippocampal LTP (Fig 2 Ⅰ). The role of Ca^{2+} on LTP induced by nicotine was observed in slices from experimental group (Fig 2 Ⅱ).

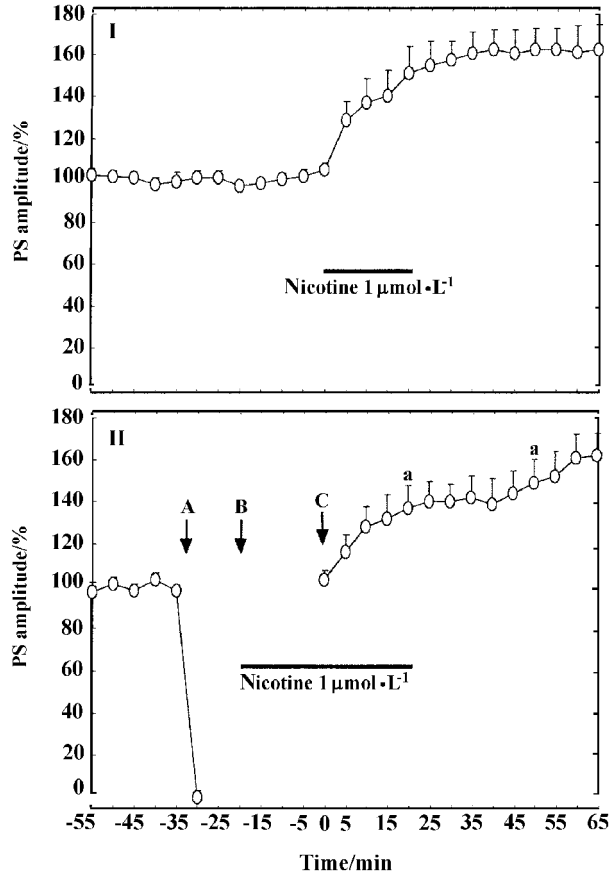

Fig 2. LTP induced by nicotine necessitated Ca^{2+} in ACSF. Ⅰ: Control. Ⅱ: Experimental group. A) removing Ca^{2+} from ACSF; B) adding nicotine 1 μmol·L^{-1} into ACSF; C) adding Ca^{2+} into ACSF. $n = 5$. $\bar{x} \pm s$. $^aP > 0.05$ *vs* control.

The test stimuli evoking the normal PS was dependent on the presence of Ca^{2+} in ACSF. When Ca^{2+} was removed from ACSF, spikes disappeared (Fig 2 Ⅱ A). After nicotine was added in Ca^{2+}-free ACSF, test stimuli still did not elicit spikes and did not induce LTP in CA_1 region (Fig 2 Ⅱ B). After perfusion with ACSF containing Ca^{2+} (2.4 mmol·L^{-1}), the normal spikes reappeared and the capacity of nicotine to induce LTP was restored (Fig 2 Ⅱ C) ($P > 0.05$).

Effect of nifedipine on LTP induced by nicotine After PS were steadily elicited for 15 min, nifedipine 1 or 10 μmol·L^{-1} was perfused for 30 min. Ten minutes after nifedipine was added, nicotine 1 μmol·

L^{-1} was added into ACSF for 20 min. Nifedipine 1 or 10 μmol·L^{-1} inhibited LTP induced by nicotine ($P < 0.05$) (Fig 3). The inhibition produced by nifedipine 1 and 10 μmol·L^{-1} was not significantly different ($P > 0.05$). This inhibitory action of nifedipine was not complete because nicotine still increased the PS amplitude to 133 % ± 10 % and 145 % ± 9 % at 50 min after nicotine was given in the presence of nifedipine 1 or 10 μmol·L^{-1}, respectively.

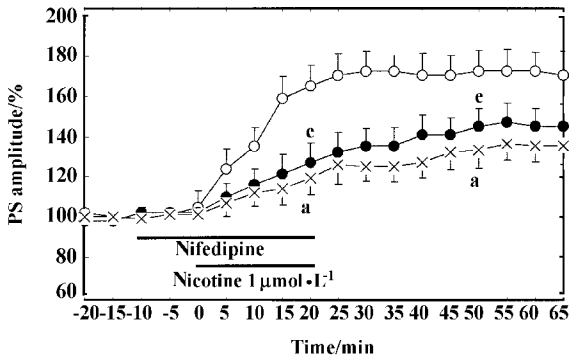

Fig 3. Partial inhibition by nifedipine of LTP induced by nicotine. (○) nicotine 1 μmol·L^{-1} (control), (●) nicotine 1 μmol·L^{-1} + nifedipine 1 μmol·L^{-1}, (×) nicotine 1 μmol·L^{-1} + nifedipine 10 μmol·L^{-1}. $n = 5$. $\bar{x} \pm s$. [a] $P > 0.05$ *vs* nicotine 1 μmol·L^{-1} + nifedipine 1 μmol·L^{-1}. [c] $P < 0.05$ *vs* nicotine 1 μmol·L^{-1}.

Effect of thapsigargin on LTP induced by nicotine Tharpsigargin 1 or 10 μmol·L^{-1} was applied for 10 min. Then, nicotine 1 μmol·L^{-1} was perfused for 20 min. Tharpsigargin 1 or 10 μmol·L^{-1} markedly inhibited LTP caused by nicotine ($P < 0.01$) (Fig 4).

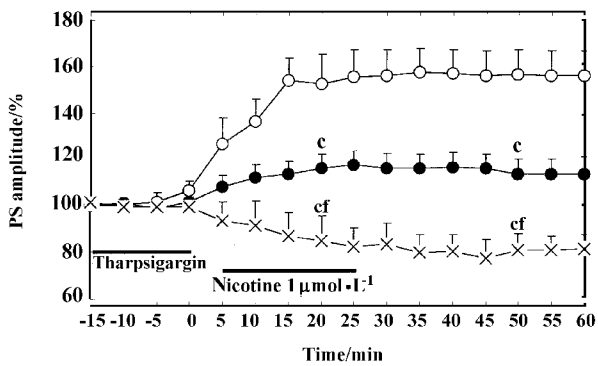

Fig 4. Tharpsigargin completely inhibited LTP induced by nicotine. (○) nicotine 1 μmol·L^{-1} (control), (●) nicotine 1 μmol·L^{-1} + thapsigargin 1 μmol·L^{-1}, (×) nicotine 1 μmol·L^{-1} + thapsigargin 10 μmol·L^{-1}. $n = 5$. $\bar{x} \pm s$. [c] $P < 0.01$ *vs* nicotine 1 μmol·L^{-1}. [f] $P < 0.01$ *vs* nicotine 1 μmol·L^{-1} + thapsigargin 1 μmol·L^{-1}.

The mean levels of potentiation recorded at 50 min after nicotine exposure were 114 % ± 7 % and 81 % ± 8 % of the baseline in the presence of thapsigargin 1 or 10 μmol·L^{-1}, respectively. Tharpsigargin 10 μmol·L^{-1} had more marked inhibitory action than thapsigargin 1 μmol·L^{-1} ($P < 0.01$).

Tharpsigargin 1 or 10 μmol·L^{-1} had no effect on baseline. But, when nicotine 1 μmol·L^{-1} was delivered following thapsigargin 10 μmol·L^{-1}, the PS amplitudes were conversely decreased compared with baseline spikes ($P < 0.01$).

DISCUSSION

Behavioural experiments in animal and human studies disclose that nicotinic agonists improve performance on a variety of memory tasks and that nicotinic antagonists such as mecamylamine impair memory function[4]. The cellular mechanisms of nicotine regarding enhancing of the memory functions have evoked many scientists' interest. Some studies have reported the relationship between neuronal nAChRs and LTP. GTS-21, a nicotinic agonist, could facilitate LTP caused by tetanus in CA_1 region of rat's hippocampus[5]. Hamid *et al*[6] have reported that a challenge dose of nicotine (0.4 mg·kg^{-1}) produced a long-lasting potentiation of field excitatory postsynaptic potentials (EPSPs) evoked in the dentate gyrus by stimulation of the medial perforant path in urethane-anaesthetized rats primed 4 wk earlier with 7-d injections of nicotine (0.8 mg·kg^{-1}·d^{-1}). In our experiments, with test stimuli which evoked 80 % maximal PS amplitude, nicotine caused long-lasting increases of the PS amplitude in the CA_1 region, and at least nicotine 1 μmol·L^{-1} was required to produce the maximal excitatory response.

Nicotine did not induce the hippocampal LTP in Ca^{2+}-free ACSF and the capacity of nicotine to induce LTP was restored when Ca^{2+} was added into ACSF, revealing that LTP induced by nicotine is dependent on the presence of Ca^{2+}. Neuronal nAChRs, including α-bungarotoxin sensitive subtypes and α-bungarotoxin insensitive subtypes, have a high relative permeability to Ca^{2+} compares with other ligand-gated ion channels[7]. In CA_3 region of hippocampal slice, nicotine 0.5 μmol·L^{-1} increases the concentration of Ca^{2+} in mossy-fibre presynaptic terminals and enhances glutamatergic synaptic transmission[8]. Obviously, Ca^{2+} influx into postsynaptic CA_1 neurons through neuronal nAChRs is the main

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pathway which increases the intracellular Ca^{2+} concentrations.

Nicotine increases the intracellular Ca^{2+} concentrations in cultured hippocampal neurons, and this response is dependent on the presence of extracellular Ca^{2+} and is blocked by $CdCl_2$, suggesting that the increases in the intracellular Ca^{2+} concentrations are due to activation of voltage-gated Ca^{2+} channels[9]. L-type voltage-gated Ca^{2+} channels, visualized using a monoclonal antibody, are located in the cell bodies and proximal dendrites of hippocampal pyramidal cells and are clustered in high density at the base of major dendrites[10]. The partly inhibiting effect of nifedipine, an L-type voltage-gated Ca^{2+} channels blocker, on LTP induced by nicotine suggests that Ca^{2+} might enter into postsynaptic neurons only through L-type Ca^{2+} channels and not through neuronal nAChRs. Perhaps, activation of postsynaptic nAChRs provides the level of postsynaptic depolarization which is a prerequisite for the opening of L-type voltage-gated Ca^{2+} channels. Ca^{2+} influx into postsynaptic CA_1 neurons through both neuronal nAChRs and L-type Ca^{2+} channels is necessary for the induction of LTP.

Calcium entry into postsynaptic CA_1 neurons of hippocampal slices might further increase the Ca^{2+} concentration through Ca^{2+}-induced Ca^{2+} release from intracellular stores[11]. Tharpsigargin, which depleted intracellular Ca^{2+} stores, completely inhibited LTP induced by nicotine indicating that increase in intracellular Ca^{2+} released from Ca^{2+} stores also contributes to the induction of LTP.

Hence LTP induced by nicotine in CA_1 region of the hippocampal slice provides a powerful evidence that nicotine enhances the efficacy of synaptic transmission. Further studies regarding LTP induction by nicotine would be beneficial to understand the roles of nAChRs on learning and memory.

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烟碱诱导的海马脑片 CA_1 区长时程增强呈钙离子依赖性[1]

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关键词 烟碱；海马；长时程增强；钙；硝苯地平；tharpsigargin

目的：观察钙离子在烟碱诱导的大鼠海马脑片 CA_1 区长时程增强中的作用. 方法：细胞外记录离体海马脑片 CA_1 区锥体细胞层群体峰电位. 结果：至少烟碱 1 $\mu mol \cdot L^{-1}$ 可诱导海马 CA_1 区长时程增强形成. 移去脑脊液中的钙离子，烟碱不能诱导 CA_1 区长时程增强形成. 硝苯地平 1 与 10 $\mu mol \cdot L^{-1}$ 部分抑制而 Tharpsigargin 1 与 10 $\mu mol \cdot L^{-1}$ 完全抑制烟碱诱导的长时程增强形成. 结论：烟碱诱导的海马 CA_1 区长时程增强呈钙离子依赖性，胞外钙内流和胞内钙释放都参与了长时程增强形成.

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