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Different synaptic mechanisms of long-term potentiation induced by nicotine and tetanic stimulation in hippocampal CA1 region of rats

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KEY WORDS nicotine; long-term potentiation; hippocampus; MK-801; *L*-NAME

ABSTRACT

AIM: To investigate whether long-term potentiation (LTP) induced by nicotine and tetanic stimulation in the hippocampal CA1 region shares different mechanisms**. METHODS**: Extracellular population spikes of the pyramidal cell layer in the hippocampal CA1 region were recorded *in vitro***. RESULTS**: LTP induced by the tetanic stimulation could be facilitated by nicotine 10 μmol/L, meanwhile, the tetanic stimulation did the same effect on LTP induced by nicotine 10 μmol/L. MK-801 10 μmol/L or N^G-nitro-*L*-arginine methyl ester (*L*-NAME) 20 μmol/L blocked LTP induced by tetanic stimulation, but did not inhibit LTP induced by nicotine**. CONCLUSION**: LTP induced by nicotine shares different synaptic mechanisms with LTP induced by tetanic stimulation.

INTRODUCTION

The roles of cholinergic system on learning and memory have been widely studied mainly due to the cholinergic hypothesis of cognitive dysfunction in Alzheimer's disease (AD)[1]. Clinical data also revealed that nicotine ameliorated learning and short-term memory in AD patients[2,3].

Long-term potentiation (LTP), an activity-dependent strengthening of synaptic efficacy evoked by tetanic stimulation, is widely used as a model to investigate the cellular machanisms of learning and memory[4]. LTP elicited by tetanic stimulation requires the release of glutamate from the glutamergic fibres and the activation of the postsynaptic NMDA receptor consequently resulting in Ca^{2+} entry into the postsynaptic neurons[5].

We previously reported that nicotine induced LTP in the hippocampal CA1 region with Ca^{2+}-dependence[6]. It is important to study whether or not LTP induced by nicotine shares different synaptic mechanisms from that by tetanic stimulation, because it would give some clues in studying the mechanisms of nicotine to improve the function of learning and memory.

MATERIALS AND METHODS

Materials Nicotine, MK-801 (an antagonist specific for NMDA receptor) and N^G-nitro-*L*-arginine methyl ester (*L*-NAME) (one of inhibitors specific for NO synthase) were purchased from Sigma. Sprague-Dawley rats (♀♂, 100-120 g, Grade II, Certificate

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No 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 The rats (110-120 g) were anesthetized by inhaling ether. Hippocampal slices (400-μm thickness) were prepared from rats at 0 °C, then incubated in artificial cerebrospinal fluid (ACSF) at 28 °C for at least 90 min. ACSF (in mmol/L: 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; pH 7.4) was saturated with 95 % O_2 and 5 % CO_2 gas mixture. Before recording, the slices were transferred to an interface chamber where the slices were continuously perfused at 1 mL/min with saturated ACSF at 32 °C.

Potentials were recorded extracellularly by use of glass microelectrodes (1-2 MΩ resistance, filled with NaCl 2 mol/L) placed in the pyramidal cell layer of the CA1 region, and stimuli were applied to the Schaffer collateral-commissural pathway through an insulated tungsten wire electrode. The test stimuli (0.017 Hz, with duration of 0.1 ms) adjusted to produce 80 % of the maximal population spike (PS) amplitude were applied. In some experiments, tetanic stimulation (100 Hz, 2 trains, 60 s of interval) was delivered. 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 a X-Y electronic recorder (NIHON KOHDEN, Japan).

Statistical analysis Data were expressed as mean±SD, and analyzed by either paired *t* test in the same group or standard *t* test between two groups ($P<0.05$).

RESULTS

Reciprocal facilitation of LTP by nicotine and tetanic stimulation The effect of nicotine on LTP preestablished by tetanic stimulation was investigated. With test stimuli of giving 80 % of the maximal PS amplitude, tetanic stimulation caused the schaffer collateral LTP (150 %±8 % at the time point of 15 min) compared with baseline ($P<0.01$, Fig 1A). Twenty minutes after tetanic stimulation was delivered, nicotine 10 μmol/L was perfused for 20 min. Nicotine caused further increase of the PS size (163 %±9 % at the time point of 60 min). Nicotine facilitated LTP elicited by tetanic stimulation compared with the values after nicotine exposure and the values at the time point of 15 min ($P<0.01$, paired *t* test, $n=5$).

Similarly, the effect of tetanic stimulation on LTP induced by nicotine was observed. Nicotine 10 μmol/L was firstly given to elicit stable expression of LTP in the CA1 region (149 %±8 % at the time point of 35 min) compared with baseline ($P<0.01$, Fig 1B). Forty-five minutes after nicotine exposure, tetanic stimulation was delivered. Tetanic stimulation also further increased the PS size (178 %±10 % at the time point of 60 min) compared with the values after tetanic stimulation delivering and the values at the time point of 35 min

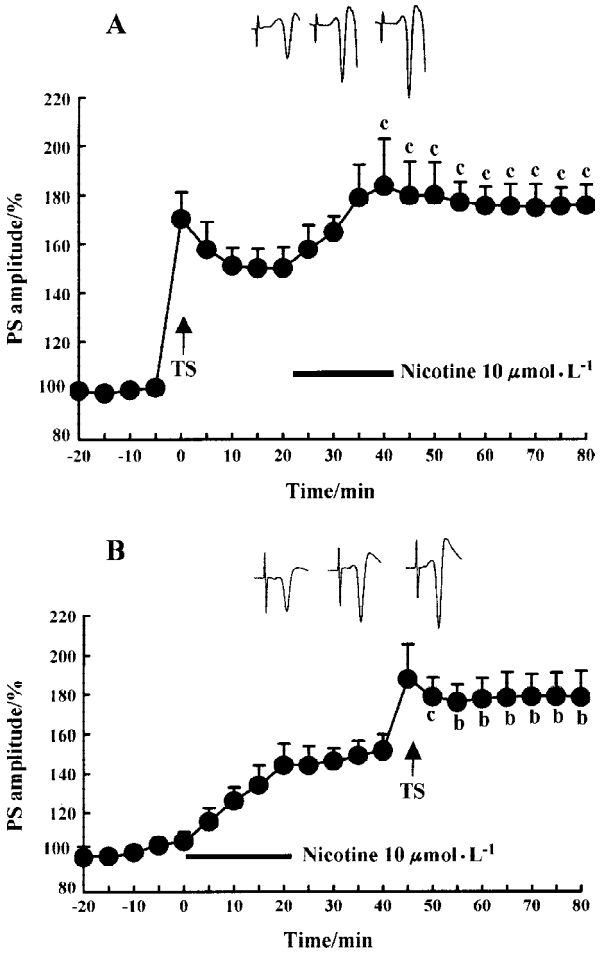

Fig 1. Nicotine and tetanic stimulation (TS) reciprocally facilitated LTP by each other. Calibration: 5 ms, 1 mv. A) Nicotine 10 μmol/L further facilitated LTP induced by tetanic stimulation. $n=5$. Mean±SD. $^cP<0.01$ *vs* the values at the time point of 15 min. Insets, representative original traces at baseline, 15 min and 50 min after TS delivering are shown, respectively. B) Tetanic stimulation further facilitated LTP induced by nicotine 10 μmol/L. $n=5$. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* the values at the time point of 35 min. Insets, representative original traces at baseline, 35 min and 60 min after nicotine application are shown, respectively.

(P<0.05, paired t test, n=5).

Effects of MK-801 on LTP induced by nicotine MK-801 10 μmol/L incubated with the hippocampal slice for 2 h inhibited completely the induction of LTP by tetanic stimulation (113 %±8 % at 30 min after tetanic stimulation delivering) compared with the control (160 %±14 % at 30 min after tetanic stimulation delivering) (P<0.01, standard t test, n=5) (Fig 2A).

The slice was incubated with MK-801 10 μmol/L for 2 h, then transferred to the interface chamber. Nicotine 10 μmol/L together with MK-801 10 μmol/L was perfused for 20 min. At the presence of MK-801, nicotine still induced a pronounced potentiation of the PS amplitude (154 %±8 % at 30 min after nicotine exposure) (Fig 2B). The difference was not statistically significant compared with the group only treated with nicotine (150 %±6 % at 30 min after nicotine exposure) (P>0.05, standard t test, n=5).

Effects of *L*-NAME on LTP induced by nicotine *L*-NAME 100 μmol/L inhibited the baseline spike (data not shown here), while *L*-NAME 20 μmol/L did not. *L*-NAME 20 μmol/L completely abolished LTP induced by tetanic stimulation (97 %±9 % at 30 min after tetanic stimulation delivering) compared with control (161 %±14 % at 30 min after tetanic stimulation delivering) (P<0.01, standard t test, n=3) (Fig 3A).

L-NAME 20 μmol/L was perfused for 40 min, and at 20 min after *L*-NAME was delivered, nicotine 10 μmol/L was delivered for 20 min. The PS size increased gradually and reached to the maximal response at 30 min after nicotine exposure (146 %±16 % at 30 min after nicotine exposure) (Fig 3B). LTP induced by nicotine was not inhibited significantly by the existence of *L*-NAME, compared with the group only treated with nicotine (149 %±12 % at 30 min after nicotine exposure) (P>0.05, standard t test, n=5).

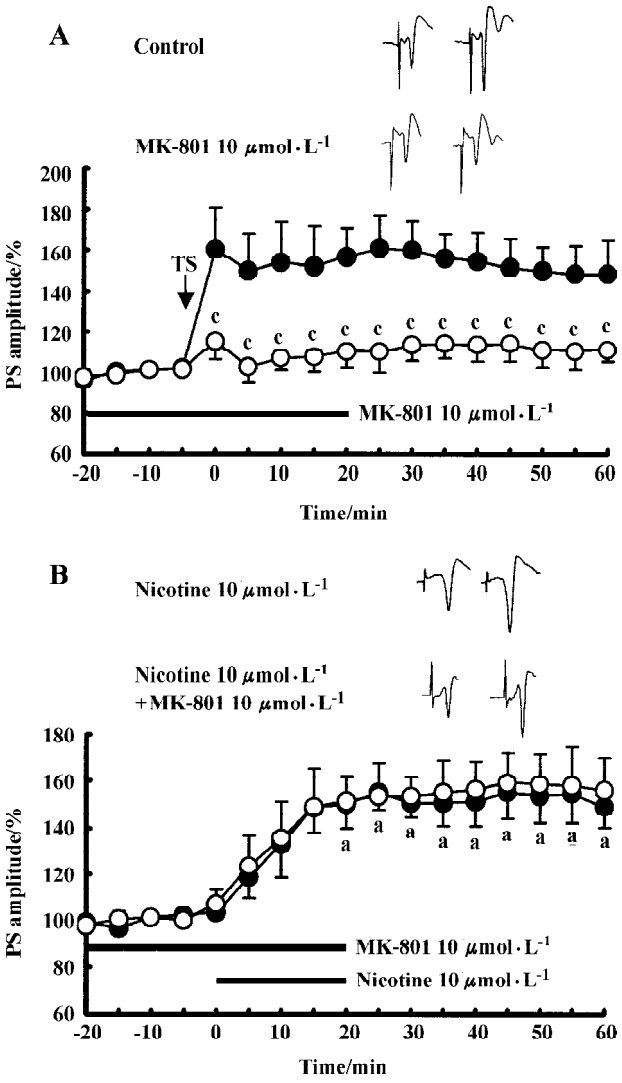

Fig 2. The different effect of MK-801 on LTP induced by tetanic stimulation (TS) and nicotine. Calibration: 5 ms, 1 mv. A) MK-801 10 μmol/L markedly inhibited LTP evoked by tetanic stimulation. (●) control, (○) MK-801 10 μmol/L. n=5. Mean±SD. ^{c}P<0.01 *vs* control group. Insets, representative original traces at baseline and 30 min after TS delivering are shown, respectively. B) MK-801 10 μmol/L had no effect on LTP induced by nicotine 10 μmol/L. (●) nicotine 10 μmol/L, (○) nicotine 10 μmol/L+MK-801 10 μmol/L. n=5. Mean±SD. ^{a}P>0.05 *vs* nicotine group. Insets, representative original traces at baseline and 30 min after nicotine application are shown, respectively.

DISCUSSION

After LTP induced by tetanic stimulation became stead, nicotine further increased the PS size. Similarly, tetanic stimulation also further potentiated LTP induced by nicotine in the CA1 region. These suggested that LTP induced by nicotine and tetanic stimulation could share different mechanisms.

Electrophysiological experiments disclosed some roles of the neuronal nicotinic acetylcholine receptors (nAChR) on the glutamergic fibres. Application of nanomolar concentrations of nicotine to the synapse between the neurons of the medial habenula nucleus and the interpeduncular nucleus enhanced the fast excitatory transmission of the glutamatergic synapses by activation of presynaptic nAChR containing the alpha 7 subunit[7]. Submicromolar concentrations of nicotine prompted the release of glutamate by activating the presynaptic nAChR containing the alpha 7 subunit on the terminal of the mossy fibres in the hippocampal CA3

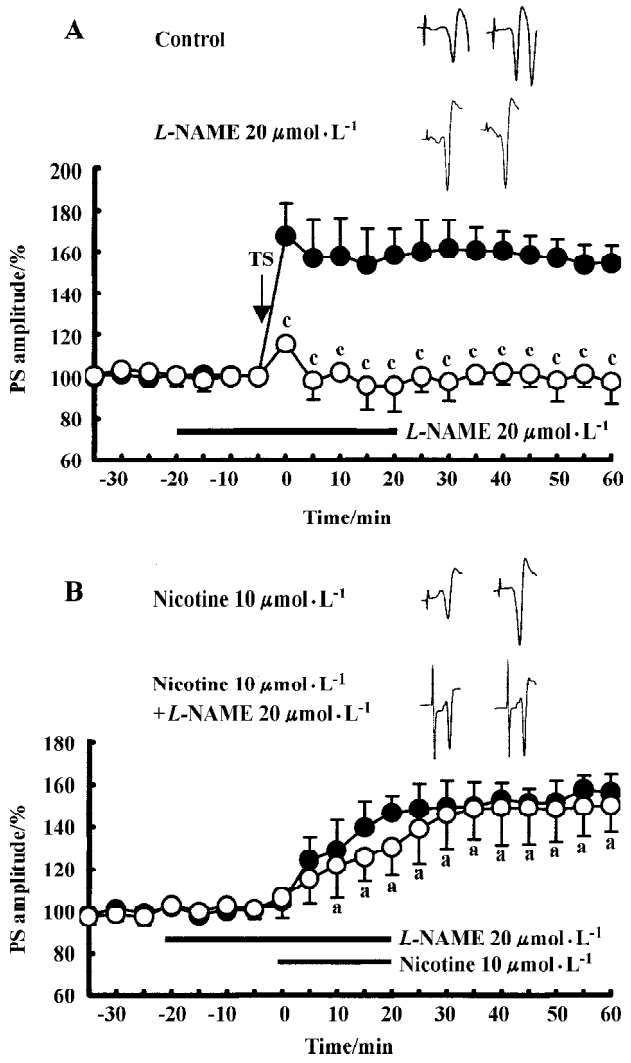

Fig 3. The different effect of N^G-nitro-*L*-arginine methyl ester (*L*-NAME) on LTP induced by tetanic stimulation (TS) and nicotine. Calibration: 5 ms, 1 mv. A) *L*-NAME 20 μmol/L completely abolished LTP induced by tetanic stimulation. (●) control, (○) *L*-NAME 20 μmol/L. *n*=3. Mean±SD. $^cP<0.01$ *vs* control group. Insets, representative original traces at baseline and 30 min after TS delivering are shown, respectively. B) *L*-NAME 20 μmol/L did not inhibit LTP induced by nicotine 10 μmol/L. (●) nicotine 10 μmol/L, (○) nicotine 10 μmol/L+*L*-NAME 20 μmol/L. *n*=5. Mean±SD. $^aP>0.05$ *vs* nicotine group. Insets, representative original traces at baseline and 30 min after nicotine application are shown, respectively.

region[8]. Those results intrigued us great interests whether glutamate released from the glutamergic fibres by activation of presynaptic neuronal nAChR participated in LTP induced by nicotine. LTP induced by nicotine was not inhibited by MK-801, showing that it did not require the activation of NMDA receptor. Earlier reports also showed that NMDA receptor-independent LTP in hippoacmpal CA1 region was induced in the presence of 2-amino-5-phosphonovaleric acid by high frequency (200 Hz) tetanic stimulation and suppressed by nifidipine, an antagonist of L-type Ca^{2+} channel[9].

LTP caused by tetanic stimulation in the hippocamal CA1 region was originally postsynaptic, but it was related to the enhancement of the release of transmitters from the presynaptic fibres[10]. Nitric oxide (NO) may act as a candidate retrograde messenger during the induction of LTP, which was produced in the postsynaptic neurons and acted on the presynaptic terminals to increase the release of transmitters[11]. LTP induced by nicotine was not obviously affected by the existence of *L*-NAME, which suggested that NO was not involved in the induction of LTP by nicotine. Some experiments also suggested that nicotine protected cultured cortical neurons against glutamate-induced cytotoxicity by reducing formation of NO[12]. Considering that the activation of the presynaptic nAChRs resulted in the entry of Ca^{2+} into the presynaptic terminals and the increase of the release of transmitters[7], the potentiation of synaptic transmission prompted by nicotine was probably independent of postsynaptically derived retrograde messenger.

Since LTP induced by nicotine did not require the activation of NMDA receptor and NO synthase, it was confirmed that LTP induced by nicotine shared different mechanisms at least in the synaptic level with LTP by tetanic stimulation. Further studies about LTP induced by nicotine would be beneficial to understand intensively the roles of the neuronal nAChR in learning and memory.

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