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Subtypes of central nicotinic receptors involved in learning and memory[1]

CHEN Li-Jun, CHEN Ru-Zhu[2]
(*Department of Pharmacology, Sun Yat-Sen University of Medical Sciences, Guangzhou 510089, China*)

KEY WORDS nicotinic receptors; avoidance learning; hippocampus; long-term potentiation; hexamethonium; bungarotoxin; synaptic transmission

ABSTRACT

AIM: To observe the effects of different subtypes of central nicotinic receptor on learning and memory. **METHODS**: Passive avoidance response, including step-down avoidance and step-through avoidance in mice and long-term potentiation (LTP) in rat hippocampal slices. **RESULTS**: Hexamethonium (C_n) 7 μg/mouse and kappa-bungarotoxin (κ-BTX) 0.6 μg/mouse inhibited the acquisition of avoidance conditioning in mice, and κ-BTX yielded this effect with a dose-response relationship. κ-BTX 1 μmol·L^{-1} suppressed the induction of LTP ($P < 0.05$), but not normal synaptic transmission and maintenance of LTP in rat hippocampal slices. **CONCLUSION**: The subtypes of central nicotinic receptor sensitive to κ-BTX play an important role in learning and memory.

INTRODUCTION

The cholinergic system in the CNS was involved in learning and memory[1,2]. Its research focused on central muscarinic cholinergic receptors, and few reports implicated the role of central nicotinic cholinergic receptors, although nicotine improved behavioral deficits in an animal model of cholinergic dysfunction[3]. Recently, the role of central nicotinic cholinergic receptor in learning and memory drew people's attention because of some interesting findings in the patients with Alzheimer disease (AD). The density of nicotinic receptors but not muscarinic receptor in the brain of patients with AD was decreased compared with the age-opposited control group[4]. Moreover, the changes in densities of subtypes of nicotinic receptors marked by radiolabeled nicotine, kappa-bungarotoxin (κ-BTX) and alpha-bungarotoxin (α-BTX) were different in the brains of patients with AD[5]. It suggested different roles of subtypes of nicotinic receptor in learning and memory and possibility to develop a novel nootropic with high selectivity for the subtype of nicotinic receptor mainly involved in learning and memory. Four antagonists with different selectivities for different subtypes of nicotinic receptor: hexamethonium (C_n), kappa-bungarotoxin (κ-BTX), alpha-bungarotoxin (α-BTX), and dihydro-β-erythroidine (DHβE) were used as tools to explore the different roles of subtypes of nicotinic receptor in learning and memory in this report.

MATERIALS AND METHODS

Chemicals κ-BTX and α-BTX were extracted from crude venom of *Bungarus multicinctus* collected in Zhejiang Province, China[6]. C_n was obtained from Sigma and DHβE was from RBI.

Animals NIH mice (♂♀, 23 g ± 2 g, Grade Ⅱ, Certificate № 26-001 conferred by Medical Animal Management Committee, Guangdong Province), and Sprague-Dawley rats (♂, 130 g ± 20 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. Mice were housed in groups of 10 per cage with free access to food and water for 2 d before the experiment.

Passive avoidance response in mice The

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[2] Correspondence to Prof CHEN Ru-Zhu. Phn 86-20-8733-0561. Fax 86-20-8733-1332. E-mail yujiang@gzsums.edu.cn
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drug effect on the "one-trial" acquisition of a passive avoidance response in mice was measured by a later retention test. The small plastic tube (PE10) was inserted into the cerebral ventricles for 3 mm depth and fixed 3 d before the "one-trial". Then, each mouse was recovered in a box with free access to food and water. On d 3, shortly before their training trial, 2 μL of drug or saline was injected into the ventricles of the brain through the tube. Passive avoidance response included step-through avoidance and step-down avoidance[7]. All mice received drug or saline injections shortly before their training trial. Then, they were given training trial. After 24 h, all mice received one retention trial that duplicated the training-trial procedure. The latency and number of error were used as indices in the step-through model, and the number of error and % of animals with error as indices in the step-down model.

LTP in rat hippocampal slices

Hippocampal slice preparation SD rats were decapitated and the brains were transferred into ice-cold artificial cerebral spinal fluid (ACSF) of the following composition: NaCl 124, KCl 5, KH_2PO_4 1.24, $MgSO_4 \cdot 7H_2O$ 1.3, $NaHCO_3$ 26, $CaCl_2$ 2.4, and *D*-glucose, 10 mmol·L^{-1}. Transverse slices (500-μm thick) were cut with tissue chopper and were incubated in oxygenate (95 % O_2 and 5 % CO_2) ACSF at 34 ℃ for 90 min. At the time of an experiment, slices were transferred on the nylon in a recording chamber (2 mL).

Extracellular recording and stimulation Extracellular recordings were obtained from the CA1 synaptic area using a 4 – 5 MΩ glass microelectrode filled with NaCl 2 mol·L^{-1}. The Schaffer collateral-commissural fibers were stimulated using a concentric bipolar electrode and 200 μs constant current pulses at an intensity sufficient to evoke a 50 % maximal population spike amplitude (PSA). Stimuli were delivered every 20 s. LTP was produced by an electric tetanus using the same current pulses administered for 100 Hz, 2 s. Before the tetanus, population spike evoked by single pulse was recorded 15 min as baseline. The drugs were dropped into recording chamber.

Statistical analysis Data were expressed as $x \pm s$ and compared with *t*-test.

RESULTS

Effects of κ-BTX, α-BTX, C_6, and DHβE on the acquisition of avoidance conditioning in mice Mice were divided at random into control group (NS 2 μL/mouse, icv), κ-BTX group (κ-BTX 0.6 μg/mouse, icv), α-BTX group (α-BTX 1.0 μg/mouse, icv), C_6 group (C_6 7 μg/mouse, icv), and DHβE group (DHβE 9 μg/mouse, icv). Intraventricular injection of κ-BTX increased the number of error, in both models (control: 0.3 ± 0.6, κ-BTX group: 1.7 ± 1.5, $P < 0.05$ in step-through model, and control: 0.8 ± 0.9, κ-BTX: 2.8 ± 1.6, $P < 0.01$ in step-down model) and shortened the latency in step-through model (control: 262 s ± 64 s, κ-BTX group: 128 s ± 105 s, $P < 0.01$). Intraventricular injection of C_6 also obviously increased the number of errors in both models (control: 0.3 ± 0.6, C_6 group: 1.3 ± 1.1, $P < 0.01$ in step-through model, and control: 0.8 ± 0.9, C_6 group: 1.6 ± 1.0, $P < 0.05$, in step-down model). While intraventricular injection of α-BTX and DHβE did not markedly affect the number of errors and latency in both models. It suggested that learning and memory acquisition was inhibited by κ-BTX and C_6 (Tab 1).

Tab 1. Effects of κ-BTX, α-BTX, C_6, and DHβE on acquisition of avoidance response in mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Compound	Step-down test		Step-through test	
	№ of errors	% of error	Latency/s	№ of errors
NS	0.8 ± 0.9	54 %	262 ± 64	0.3 ± 0.6
κ-BTX	2.8 ± 1.6^c	100 %^{b}	128 ± 105^c	1.7 ± 1.5^b
α-BTX	1.2 ± 1.0	69 %	230 ± 90	0.7 ± 0.8
C_6	1.6 ± 1.0^b	100 %^{b}	196 ± 93	1.3 ± 1.1^c
DHβE	0.9 ± 1.2	50 %	232 ± 99	0.4 ± 0.6

Moreover, κ-BTX did this effect with a good dose-response relationship (Tab 2).

Effects of κ-BTX and DHβE on LTP in rat hippocampal slices LTP was induced and recorded in the CA1 area by a single electric shock after applying a tetanus of 100 Hz for 2 s to the Schaffer-commissural fibers. The amplitude of the population spike after the tetanus (100 Hz, 2 s) was increased by > 200 %, compared with the baseline. LTP phenomenon lasted

Tab 2. Dose-effect relationship of κ-BTX inhibiting acquisition of avoidance response in mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$.

Dose μg	n	Step-down test Latency/s	Step-down test No of errors	Step-through test Latency/s	Step-through test No of errors
0.05	9	101 ± 69	1.1 ± 0.9	252 ± 102	0.3 ± 0.5
0.20	9	99 ± 71	1.4 ± 1.8	194 ± 115	1.0 ± 1.0
0.60	9	31 ± 29	2.3 ± 1.0	90 ± 91	1.2 ± 0.6
r	27	−0.4754^b	0.3780	−0.6380^c	0.4040^b

for > 2 h.

κ-BTX 1 μmol · L^{-1} (final concentration), when applied 10 min before the tetanus, suppressed the induction of LTP ($P < 0.05$), not normal synaptic transmission. DHβE 10 μmol · L^{-1} (final concentration) did not inhibit the induction of LTP ($P < 0.05$) (Fig 1A).

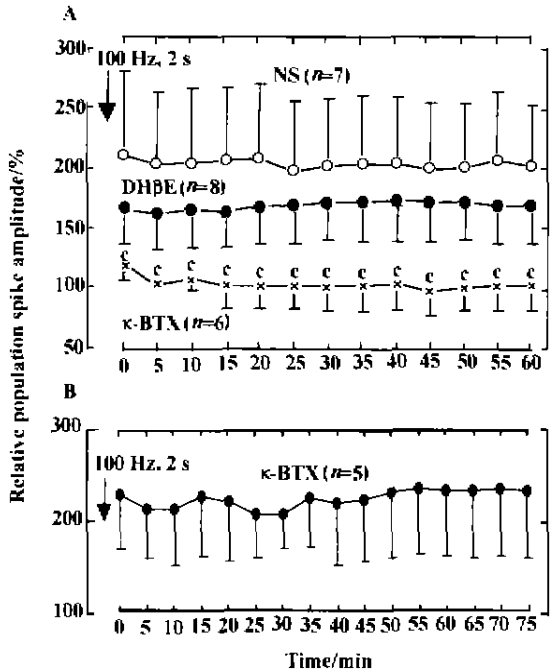

Fig 1. Effects of κ-BTX, DHβE on LTP in rat hippocampal slices. A) κ-BTX 1 μmol · L^{-1}, DHβE 10 μmol · L^{-1} were applied 10 min before the tetanus. B) κ-BTX 1 μmol · L^{-1} was applied 20 min after the tetanus. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **control.**

However, LTP induced after the tetanus was not affected by κ-BTX 1 μmol · L^{-1} (final concentration) applied 20 min after the tetanus (Fig 1B). These results showed that κ-BTX could interfere with the formation of LTP, but not normal synaptic transmission and maintenance of LTP in rat hippocampal slices.

DISCUSSION

Oliverio[8] reported that mecamylamine, a nicotinic antagonist, inhibited the acquisition of avoidance conditioning. We observed that hexamethonium, a nicotinic antagonist, with lower selectivity, inhibited the acquisition of passive avoidance response. It suggested that central nicotinic receptor be associated with learning and memory.

There are several subtypes of central nicotinic receptor, such as the subtype sensitive to κ-BTX, the subtype sensitive to α-BTX, and the subtype sensitive to DHβE. Molecular-cloning studies have identified several genes encoding α and β subunits of the nicotinic acetylcholine receptor[9].

We found that κ-BTX inhibited the acquisition of passive avoidance conditioning and it yielded this effect with a dose-response relationship. It showed that the subtype of central nicotinic receptors sensitive to κ-BTX was involved in learning and memory.

LTP was a phenomenon proposed by Bliss[10] in 1973, which is about a prolonged enhancement in synaptic efficacy after brief high-frequency stimulation of several afferent pathways in the hippocamps. It is believed to be a cellular model of learning and memory[11]. In this report, we found that κ-BTX interfered with the formation of LTP, but not normal synaptic transmission and maintenance of LTP in the hippocampal slices. It has been thought that excitatory amino acid-glutamate released from presynapse, activates NMDA receptor in the postsynapse and leads to the generation of LTP[12]. Recent studies showed that generation of LTP also involved a series of the second cellular messengers, such as Ca^{2+}, protein kinase C, cAMP, NO, *etc*[13]. It seems that in the generation of LTP, the activation of NMDA receptor induces LTP. Then LTP is maintained by a series of the second messengers. κ-BTX depressed the induction of LTP in the CA1 area of hippocampal slice in our experiment possibly by blocking the subtype of central nicotinic receptors sensitive to κ-BTX in the presynapse membrane, reducing the release of glutamate from presynapse membrane, and unactivating NMDA receptor. Some studies supported it, Sugaya[15] dis-

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covered that κ-BTX 1 μmol · L^{-1} decreased the electrically evoked releases of [^{3}H]-ACh from rat frontal cortex slices, they also found high density binding sites of [^{125}I]-κ-BTX in the hippocampal CA3 area. Another guess is that κ-BTX depressed the induction of LTP by blocking postsynapic nicotinic receptor. Recent research showed that central nicotinic receptor was similar to NMDA receptor, a kind of gate-operated calcium channels[14]. It suggested that activating the central nicotinic receptor might induce LTP.

These results implicated that the subtypes of central nicotinic receptors sensitive to κ-BTX played an important role in learning and memory.

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学习和记忆中的中枢烟碱受体亚型[1]

陈丽君，陈汝筑[2]
（中山医科大学药理教研室，广州 510089，中国）

关键词 烟碱受体；回避学习；海马；长时程增强；六烃季铵；银环蛇毒素；突触传递

目的：观察不同的中枢烟碱受体亚型在学习、记忆中的作用. **方法**：小鼠被动回避性反应试验，包括跳台试验和避暗试验，大鼠海马脑片 CA1 区长时程突触增强（LTP）效应. **结果**：六烃季铵（C_6）和 Kappa-银环蛇毒素（κ-BTX）明显抑制小鼠被动回避条件反应的获得，且 κ-BTX 的作用有量效关系. κ-BTX 1 μmol·L^{-1}抑制大鼠海马脑片 CA1 区 LTP 形成（$P<0.05$），但不影响正常突触传递，也不影响 LTP 维持. **结论**：中枢烟碱受体与学习记忆有关，对 κ-BTX 敏感的中枢烟碱受体亚型在学习记忆中起重要作用.

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