结果：NBP 10 mg·kg^{-1}治疗对缺血重灌注后脑组织中 TXB_2 的产生具有抑制作用，但对 6-keto-$PGF_{1\alpha}$的产生无明显作用．NBP 20 mg·kg^{-1}治疗后、重灌 5 min 缺血脑组织中 TXB_2 和重灌后 30 min 时 6-keto-$PGF_{1\alpha}$含量皆明显减少．NBP 20 或 10 mg·kg^{-1}皆明显提高 PGI_2/TXA_2 的比值．而阿司匹林(20 mg·kg^{-1})除重灌 5 min 提高纹状体 PGI_2/TXA_2 的比值外，在其它时间点上均无提高作用．结论：NBP 提高缺血脑组织中 PGI_2/TXA_2 的比值，可能有利于改善缺血脑组织的微循环状态．

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Nov; 18 (6): 508 - 511

Physostigmine blocked nicotinic acetylcholine receptors in rat sympathetic ganglion neurons[1]

ZHENG Jian-Quan, HE Xiang-Ping, YANG Ai-Zhen, LIU Chuan-Gui[2]
(*Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing 100850, China*)

KEY WORDS sympathetic ganglia; nicotinic receptors; physostigmine; allosteric regulation; patch-clamp techniques

AIM: To study the blocking mechanism of physostigmine (Phy) on nicotinic acetylcholine receptors (NAChR) in sympathetic neurons. **METHODS**: The whole-cell patch-clamp technique was used to observe the effects of Phy on NAChR in the cultured sympathetic neurons from neonatal rat superior cervical ganglia (SCG). **RESULTS**: Phy 5 - 20 μmol·L^{-1} inhibited neuronal NAChR in a concentration-dependent manner and accelerated the desensitization of NAChR. Changing the membrane potential from - 50 to - 90 mV did not affect the blocking effect of Phy. Phy 200 μmol·L^{-1} did not induce any noticeable response in SCG neurons. **CONCLUSION**: Phy blocked NAChR in the sympathetic ganglion neurons by interacting with the allosteric sites out of the binding sites and the open ionic channels of the receptors. Phy did not possess excitative effect on NAChR in SCG neurons.

Physostigmine (Phy) is not only a reversible cholinesterase inhibitor, but also exhibits postsynaptic effects resulting primarily from a direct interaction with nicotinic acetylcholine receptors (NAChR)[1-5]. On muscle[1-3], electric organ[4], and mouse tumor cells[5], the inhibitory effect of Phy on NAChR resulted from blockade of the ionic channels in an open conformation. Apart from the depression, Phy also displayed a direct excited effect on muscular[2,3] and electrical organ NAChR channels even though NAChR were in the desensitization state[4].

Few experiments have been conducted about the blocking effect of Phy on neuronal NAChR. It was only reported that Phy could reduce the depolarized response of rabbit superior cervical ganglia (SCG) neurons to ACh[9]. As the structure and biological characteristics of neuronal NAChR are different from those of muscle NAChR[6], Phy may present diverse pharmacological properties on neuronal NAChR. In this experiment, we used the cultured rat SCG neurons to elucidate the blocking mechanism and analyze the reaction site of Phy on neuronal NAChR.

MATERIALS AND METHODS

Cell culture SCG were isolated from neonatal Wistar rats (1 d). The dissociating method was described in our previous work[7]. Briefly, the ganglia were cut into small pieces and digested with 0.25 % trypsin. The suspension was spun at 500 × *g* for 2 min. The pellet was resuspended in DMEM containing 10 % horse serum. The dissociated neurons were transferred to 35-mm tissue culture dishes and were cultured at 37 ℃ in 95 % O_2 - 5 % CO_2 for 7 - 9 d. In this period,

[1] Project supported by the National Natural Science Foundation of China, No 39130090.
[2] Correspondence of Prof LIU Chuan-Gui.
Phn 86-10-6688-7429, ext 68624.
Received 1996-10-25 Accepted 1997-06-02

the cultured SCG neurons displayed a stable response to NAChR agonists.

Current recording When the gigaseal between the microelectrode and the membrane of the cultured SCG neuron was formed, a swift pulse of suction was applied to rupture the membrane and establish a whole-cell recording configuration[8]. Dimethylphenylpiperazinium (DMPP), one of selective activators of sympathetic ganglion NAChR, was used to activate NAChR. The currents induced by DMPP were amplified by Axopatch-1D. The program of pCLAMP 5.5.1 was used to analyze the recorded signals. The experiments were carried out at 20 - 25 ℃.

The patch-pipettes had a resistance of 1 - 5 MΩ and contained: CsCl 140, HEPES 10, egtazic acid 10 and ATP 2 mmol·L^{-1}. The extracellular solution contained: NaCl 140, KCl 5, $CaCl_2$ 3, $MgCl_2$ 1, HEPES 10, glucose 10, tetrodotoxin 0.001 mmol·L^{-1}.

Phy was purchased from Burroughs Wellcome & Co. DMPP from Sigma Chemical Co.

Applications of drugs Agonists and antagonist were applied by a puff pipette connected to a pressure injector (BH-2, Medical Systems Corp). The puff pipette was consisted of 3 microtubes with a diameter of 5 - 10 μm. The distance between the pipette and the recorded neuron was 20 - 30 μm, and 50 - 60 kPa of N_2 pressure was applied.

Calculation The amplitudes of DMPP-induced currents were measured with the program of pCLAMP 5.5.1. Mean values were given with standard deviation ($\bar{x} \pm s$). The program of Statistics Analysis System (SAS) was used for ANOVA of double factors.

RESULTS

Concentration-dependent inhibition The pipette used to apply drugs was filled with DMPP and different concentrations of Phy. The application time of drugs was 1 s, and the interval was 3 min. When DMPP 100 μmol·L^{-1} was applied to a neuron, an inward current was elicited rapidly (Fig 1).

While the mixture of DMPP and Phy was puffed to the same neuron, the amplitudes of DMPP-induced currents were depressed obviously. The blockade of DMPP-induced currents by Phy was potentiated with the increased Phy concentrations ($F = 5.38$, $P < 0.05$, Tab 1).

Voltage-independent blockage The cell membrane potential was held at −50, −70, and −90 mV, respectively. The current amplitudes induced by DMPP 50 μmol·L^{-1} increased with the hyperpolarization of the membrane potential (Tab 2). The currents decreased remarkably in the presence of

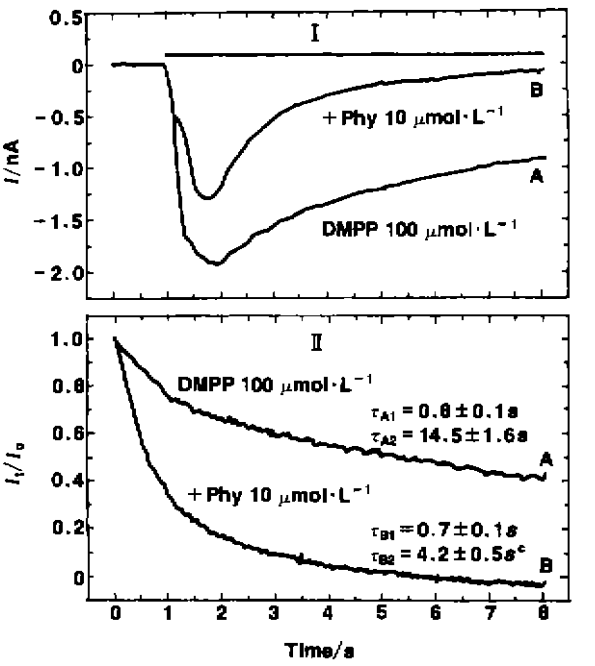

Fig 1. Effect of Phy on decay rate of DMPP-induced currents. I: Recordings from the same neuron. The membrane potential was held at −70 mV. Horizontal bar = time of applying drugs. II: I_0 = top amplitude of DMPP-induced current; I_t = currents. A and B were $\bar{x}$ of 6 experiments. τ_1 and τ_2 = the decay time constants of DMPP-induced current obtained by fitting the double-exponential curve. $^cP < 0.01$ *vs* τ_{A2}.

Tab 1. Inhibitory effect of Phy on DMPP-induced currents. $n = 6$, $\bar{x} \pm s$. ANOVA of double factors: $F = 12.51$, $P < 0.001$; Interaction of double factors: $F = 5.38$, $P < 0.05$; Pairwise comparison of ANOVA: $^cP < 0.01$ *vs* Phy-free.

Phy/ μmol·L^{-1}	DMPP 100 μmol·L^{-1}-induced currents/nA		Inhibition/ %
	Phy-free	+Phy	
5	3.1±0.9	2.6±0.7^c	16±3
10	3.4±1.2	2.2±0.7^c	37±8
20	3.0±0.6	1.7±0.2^c	42±13

Phy 10 μmol·L^{-1} ($F = 5.59$, $P < 0.01$). However, the change of the membrane potentials had no marked influence on the inhibitory effect of Phy ($F = 0.70$, $P > 0.05$).

Accelerating decay The drug application time was prolonged to 15 s. The DMPP-induced current decayed gradually, even though DMPP was still

Tab 2. Blockage of DMPP-induced currents by Phy. $n = 5$, $\bar{x} \pm s$. ANOVA of double factors: $F = 5.59$, $P < 0.01$; Interaction of double factors: $F = 0.70$, $P = 0.52$; Pairwise comparison of ANOVA: $^cP < 0.01$ *vs* control.

HP/ mV	DMPP 50 μmol·L^{-1}-induced currents/nA		Inhibition /%
	Control	+ Phy 10 μmol·L^{-1}	
−50	0.9±0.3	0.5±0.2^c	49±3
−70	1.6±0.4	0.8±0.2^c	52±6
−90	2.6±0.5	1.1±0.1^c	55±5

applied (Fig 1, Ⅰ). In the presence of Phy 10 μmol·L^{-1}, the currents elicited by DMPP 100 μmol·L^{-1} were depressed from 2.5±0.4 nA to 1.4±0.2 nA ($n = 6$, $P < 0.05$). Phy also accelerated the decay of DMPP-induced currents (Fig 1, Ⅱ). The time constants (τ_2) of the current decay process were obviously shortened from 14.5±1.6 s to 4.2±0.5 s ($P < 0.01$).

No activating effect A high concentration of Phy (200 μmol·L^{-1}) applied for 30 s on 6 neurons did not result in any detectable current.

DISCUSSION

The classic competitive antagonists of NAChR inhibit the receptors by occupying the binding sites of ACh. If the ACh binding site of an NAChR was occupied by agonist competitively, the activities of the excited receptor, including the opening, closing of the channel, and desensitization of the receptor, would not be influenced by the competitive antagonists any more. In this experiment, we found that Phy blocked neuronal NAChR from rat SCG in a concentration-dependent manner, and Phy could speed up the decay rate of the whole-cell currents induced by DMPP. The results implied that Phy could accelerate the desensitization of neuronal NAChR and might not interact with the binding sites of the agonists[10].

When the membrane potentials are hyperpolarized, the force to drive and attract positive ions into the cells throughout the opened receptor channels is potentiated. If an antagonist blocks the open channel of NAChR, its inhibitory effect will increases with the hyperpolarized membrane potential, *ie*, displaying a voltage-dependent property[10,11]. As expected, the amplitudes of DMPP-induced currents were raised with the increased membrane hyperpolarization (Tab 2). However, the blocking effect of Phy was not changed significantly. It was suggested that Phy would not block the open channels of NAChR in SCG neurons[1-3,10,11]. Furthermore, Phy could not activate neuronal NAChR or any other ionic channels, either. The results have shown that a high concentration of Phy did not elicit any detectable currents in SCG neurons.

According to the mechanism, antagonists of NAChR could be divided into 2 groups: non-allosteric and allosteric. The former blocks NAChR by interacting with the binding sites of agonist and the later with the allosteric sites including the open channel[10,11]. Our results have revealed that Phy could not affect the binding site of agonist and the receptor open-channel. So, it was reasonable to deduce that Phy might interact with the allosteric sites rather than the open ionic channels to block NAChR in the sympathetic neurons. As Phy depressed muscular NAChR by blocking the open-channels[1-3], the present results also supported the view that the structural and pharmacological characteristics of neuronal NAChR were different from those of NAChR in skeletal muscle[6].

REFERENCES

1 Albuquerque EX, Akaike A, Shaw K-P, Rickett DL. The interaction of anticholinesterase agents with the acetylcholine receptor-ionic channel complex. Fundam Appl Toxicol 1984; 4: s27 – s33.

2 Aracava Y, Deshpande SS, Rickett DL, Brossi A, Schonenberger B, Albuquerque EX. The molecular basis of anticholinesterase actions on nicotinic and glutamatergic synapses Ann NY Acad Sci 1987; 505: 226 – 55.

3 Shaw K-P, Aracava Y, Akaike A, Daly JW, Rickett DL, Albuquerque EX. The reversible cholinesterase inhibitor physostigmine has channel-blocking and agonist effects on the acetylcholine receptor-ion channel complex. Mol Pharmacol 1985; 28: 527 – 38.

4 Kuhlmann J, Okonjo KO, Maelicke A. Desensitization is a property of the cholinergic binding region of the nicotinic acetylcholine receptor, not of the receptor-integral ion channel. FEBS Lett 1991; 279: 216 – 8.

5 Wachtel RE. Physostigmine block of ion channels activated by acetylcholine in BC3H1 cells. Mol Pharmacol 1993; 44: 1051 – 5.

6 Sargent PB. The diversity of neuronal nicotinic acetylcholine receptors. Annu Rev Neurosci 1993; 16: 403 – 43.

7 Zheng JQ, He XP, Liu CG. Effect of extracellular calcium on desensitization of nicotinic receptors in cultured rat sympathetic neurons. Chin J Pharmacol Toxicol 1996; 10: 8 – 11
8 Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patchclamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981; 391: 85 – 100.
9 Mo N, Dun NJ, Karczmar AG. Facilitation and inhibition of nicotinic transmission by eserine in the sympathetic ganglia of the rabbit. Neuropharmacology 1985; 24: 1093 – 101.
10 Léna C, Changeux J-P. Allosteric modulations of the nicotinic acetylcholine receptor. Trends Neurosci 1993; 16: 181 – 6.
11 Changeux J-P, Revah F. The acetylcholine receptor molecule: allosteric sites and the ion channel. Trends Neurosci 1987; 10: 245 – 50.

毒扁豆碱阻断大鼠交感节神经元烟碱乙酰胆碱受体[1]

郑建全，何湘平，杨爱珍，刘传绩[2]

（军事医学科学院毒物药物研究所，北京 100850，中国）

关键词 交感神经节；烟碱受体；毒扁豆碱；变构调节；膜片箝技术

目的：研究毒扁豆碱阻断交感节神经元烟碱受体的作用机理。**方法**：以培养的新生大鼠颈上交感节神经元为标本，使用全细胞膜片箝技术，观察毒扁豆碱对交感节烟碱受体选择性激动剂 DMPP 诱发电流的影响。**结果**：毒扁豆碱（5 – 20 μmol·L^{-1}）以浓度依赖性方式抑制 DMPP 诱发电流，促进诱发电流的衰减，其抑制作用没有电压依赖性，毒扁豆碱 200 μmol·L^{-1} 不能激活烟碱受体。**结论**：与骨骼肌烟碱受体相比，交感神经元烟碱受体表现出不同的药理学特性。毒扁豆碱通过作用于变构位点抑制交感神经元烟碱受体，不影响其开放的离子通道和激动剂结合位点。

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Nov; 18 (6): 511 – 514

Elevation of an endogenous inhibitor of nitric oxide synthase in diabetic rat serum[1]

XIONG Yan, LU Rong, LI Yuan-Jian[2], DENG Han-Wu
(*Department of Pharmacology, Hunan Medical University, Changsha 410078, China*)

KEY WORDS arginine; experimental diabetes mellitus; blood glucose; malondialdehyde; acetylcholine; thoracic aorta

AIM: To study the endogenous inhibitor of NO synthase N^G,N^G-dimethyl-arginine (DMA) in the diabetic rat serum. **METHODS**: In streptozocin-induced diabetic rats, the serum DMA level and endothelium-dependent vasorelaxation to acetylcholine (ACh) were determined. **RESULTS**: The serum DMA concentration was increased in the diabetic rats compared with their age-matched controls (5.4 ± 1.0 *vs* 0.7 ± 0.3 μmol·L^{-1}, $P < 0.01$). The serum malondialdehyde (MDA) level was also increased in the diabetic rats compared with controls (2.5 ± 0.3 *vs* 1.5 ± 0.1 μmol·L^{-1}, $P < 0.01$). Vasodilator response to ACh was impaired in diabetic thoracic aortas, which was improved by preincubation with *L*-arginine 1 mmol·L^{-1}. **CONCLUSION**: Hyperglycemia elevated the endogenous DMA content, which contributed to attenuated endothelium-dependent vasorelaxation in streptozocin-induced diabetic rats.

Nitric oxide (NO), besides regulating vascular tone, possesses a protective effect on endothelial cells and an inhibitory modulation of vascular smooth muscle cell proliferation[1]. NO is synthesized from *L*-arginine by NO synthase in endothelial cells, and *L*-arginine analogues such as N^G,N^G-dimethyl-arginine (DMA), which is present in blood of both human and

[1] Project supported by the National Natural Science Foundation of China. No 39400163 and Ministry of Public Health. No 96Q – 020.
[2] Correspondence to Prof LI Yuan-Jian.
Phn 86-731-447-4411, ext 2704. Fax 86-731-447-1339.
Received 1997-01-08 Accepted 1997-06-12