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Modulation of phosphatidylinositol turnover on central nicotinic receptors

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KEY WORDS nicotine; oxotremorine; arecoline; lithium chloride; phosphatidylinositols; nicotinic receptors; convulsions

AIM: To study the modulatory effects of phosphatidylinositol (PI) turnover on nicotinic receptors in CNS, and to study the relationship between brain nicotinic receptors and PI turnover. **METHODS:** Effects of inositol phosphatase inhibitor lithium chloride (LiCl) and muscarinic receptor agonist oxotremorine (Oxo) on nicotine-induced convulsions were investigated in mice. **RESULTS:** The effects of nicotine for producing convulsions were modified by LiCl 2.5 - 10 mmol·kg^{-1}, revealing the convulsive effects of nicotine > 0.8 mg·kg^{-1} were increased by acute pretreatment with LiCl rather than oxotremorine. Mice were given LiCl 5.0 mmol·kg^{-1} once a day for 7 d, the ED_{50} value of nicotine for producing convulsions was increased from 0.58 to 0.97 mg·kg^{-1}, suggesting that the sensitivity of central nicotinic receptors for mediating convulsions was decreased by chronic treatment with LiCl. **CONCLUSION:** The functions of central nicotinic receptors were modulated by PI turnover.

Phosphatidylinositol (PI) turnover is involved in the signal transduction of membrane receptors coupled to GTP binding proteins. Few experiments have been reported to study the relationship between brain nicotinic receptors and phosphatidylinositol turnover.

Inositol levels in rat cerebral cortex, hippocampus, and submaxillary gland rather than striatum, were increased by acutely repeating doses of nicotine, which was prevented by nicotinic receptor antagonist mecamylamine, and was reversed by inositol phosphatase inhibitor lithium chloride (LiCl)[4-6]. Chronic repeating doses of nicotine enhanced muscarinic receptor agonist carbachol-induced inositol monophosphate accumulation in hippocampus rather than cerebral cortex and striatum[2]. It is assumed that there may exist a subtle relationship between central nicotinic receptors and PI turnover.

Central nicotinic receptors were suggested to be responsible for nicotine-induced convulsions in mice[1,7]. The modulatory effects of LiCl or oxotremorine (Oxo), which modified central PI turnover through decreasing the degeneration or stimulating the formation of inositol-1,4,5-triphosphate, on nicotine-induced convulsions were observed, to demonstrate the modulation of phosphoinositide metabolism on the functions of brain nicotinic receptors in these experiments.

MATERIALS AND METHODS

Drugs (-) Nicotine base was purchased from Merck Co; Oxo and LiCl were purchased from Sigma Co. These drugs were dissolved in saline solution. The injection volume was 10 mL·kg^{-1} body weight.

Mice Swiss mice, ♂, weighting 16 - 18 g, were provided by the Laboratory Animal Center of the Academy of Military Medical Sciences.

Nicotine-convulsions Each mouse was placed in a glass jar, diameter 20 cm. After iv injection of nicotine, the intensity of convulsions was determined on a scale of 1 - 7: 1) gustatory movement and scratching; 2) tremor; 3) head bobbing and backward walking; 4) rearing and clonus; 5) falling on its side; 6) whole body jerks; 7) clonic-tonic convulsions, or hindlimb extension and death[7].

Oxo or arecoline induced tremors and salivations Each mouse was placed in a separate jar (15 cm × 15 cm × 15 cm). After sc injection of Oxo or arecoline, mice showed gustatory movement, scratching, head bobbing, rearing, which developed into tremors of the whole body accompanied by lacrimation and salivation. The tremors ended with weakness and immobilization.

Median effective dose Five doses were employed in groups of 5 - 10 mice each, to establish the dose-response relationship. The computer Bliss analysis was applied to determine the median effective doses (ED_{50}) and its 95 % confidence limits (CL_{95}), curve slope (b) and correlation coefficient (r).

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RESULTS

Effects of LiCl and Oxo on nicotine-induced convulsions Mice were pretreated with LiCl 2.5, 5.0, and 10.0 mmol · kg^{-1} sc 30 min before nicotine, the dose-response relationship of nicotine for producing convulsions was modified. The *b* values were increased, but the ED_{50} values were not much altered. The convulsive effects of nicotine 0.8 − 1.0 mg · kg^{-1} (> ED_{50}) were more potent in LiCl-pretreated mice than those in naive mice. This indicated that the sensitivity of central nicotinic receptors to nicotine at the large doses for mediating convulsions was increased by the acute LiCl pretreatment. If the mice were pretreated with LiCl 5.0 mmol · kg^{-1} sc 24-h before nicotine, the dose-relationship of nicotine for producing convulsions remained unchanged. The effects of LiCl on nicotine-induced convulsions were reversible, which disappeared 1 d after sc injection of LiCl.

Mice were pretreated with Oxo 0.05, 0.10, 0.20 mg · kg^{-1} sc 30 min before nicotine, the dose-response relationship of nicotine for producing convulsions was not changed (Tab 1).

Mice were pretreated with LiCl 2.5, 5.0, 10.0 mg · kg^{-1} sc 30 min before nicotine, the dose-response relationship of Oxo for producing tremors was modified. The values of ED_{50} were decreased. The effects of oxotremorine at ED_{50} for producing tremors were more potent in LiCl-pretreated mice than in naive mice. If the mice were pretreated with LiCl 10.0 mmol · kg^{-1} sc 24 h before Oxo, the dose-response relationship of Oxo for producing tremors was unaltered either. The modulatory effects of LiCl in Oxo-induced tremors were also reversible, and disappeared 1 d after sc injection of LiCl (Tab 1).

Effects of repeated doses of LiCl on nicotine-induced convulsions Mice were pretreated with 5 mmol · kg^{-1} sc once a day for 7 d, 30 h after the last sc injection of LiCl, the mice were challenged with nicotine, the ED_{50} value of nicotine for producing convulsions was increased from 0.58 to 0.97 mg · kg^{-1}. The convulsive effects of nicotine were decreased by the chronic pretreatment with LiCl. On the other hand, the ED_{50} value of arecoline for producing salivations was increased from 3.86 to 5.70 mg · kg^{-1}; The effects of arecoline at ED_{50} producing tremors and salivations were also decreased by the chronic pretreatment with LiCl (Tab 2).

DISCUSSION

Inositol-1,4,5-triphosphate (IP_3) is a key

Tab 1. Effects of pretreatment with LiCl or Oxo on nicotine-induced convulsions or Oxo-induced tremors in mice. △ **Interval between pretreatment and nicotine or Oxo.** $^aP>0.05$, $^bP<0.05$, $^cP<0.01$, *vs* **control.**

Drug	Pretreatment Dose	Interval/h△	ED_{50} (CL_{95}/mg · kg^{-1})	*b*	*r*
Nicotine-convulsions					
Control	−	−	0.64 (0.57 − 0.71)	7.2	0.96
LiCl/mmol · kg^{-1}	2.5	0.5	0.59 (0.45 − 0.73)^a	14.5^c	0.99
	5.0	0.5	0.62 (0.53 − 0.71)^a	10.5^b	0.99
	10.0	0.5	0.71 (0.61 − 0.81)^a	12.5^b	0.98
	5.0	24	0.77 (0.63 − 0.91)^a	7.2^a	0.94
Oxo/mg · kg^{-1}	0.05	0.5	0.57 (0.43 − 0.71)^a	7.0^a	0.96
	0.10	0.5	0.59 (0.42 − 0.76)^a	5.6^a	0.86
	0.20	0.5	0.67 (0.55 − 0.79)^a	8.2^a	0.94
Oxotremorine-tremors					
Control	−	−	0.112 (0.096 − 0.128)	8.1	0.99
LiCl/mmol · kg^{-1}	2.5	0.5	0.074 (0.065 − 0.083)^c	15.4^c	0.99
	5.0	0.5	0.077 (0.056 − 0.098)^c	5.6^b	0.98
	10.0	0.5	0.051 (0.038 − 0.064)^c	6.2^a	0.95
	10.0	24	0.114 (0.093 − 0.135)^a	7.2^a	0.95

Tab 2. Effects of repeated doses of LiCl for 7 d on nicotine-induced convulsions or arecoline-induced tremors and salivations in mice. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **control.**

Pretreatment	$ED_{50}(CL_{95})$/mg·kg^{-1}	b	r
Nicotine convulsions			
Control	0.58 (0.48 − 0.68)	6.0	0.94
LiCl	0.97 (0.80 − 1.14)^c	5.7^a	0.99
Arecoline tremors			
Control	3.88 (3.48 − 3.92)	9.3	0.97
LiCl	4.34 (3.12 − 5.56)^a	4.6^b	0.94
Arecoline salivations			
Control	3.86 (3.44 − 4.28)	8.6	0.99
LiCl	5.70 (4.27 − 7.13)^b	5.6^a	0.98

intracellular messenger, which is produced from polyphosphatidylinosite decomposition, and is broken down into inositol and phosphates. The later process is catalyzed by inositol phosphatase, and can be inhibited by LiCl[3]. In the acute experiment, the decomposition of IP_3 was inhibited by the pretreatment with LiCl, consequently, the central effects of IP_3 accumulated by muscarinic agonist Oxo and mediating tremors were enhanced. Similarly, the convulsive effects of nicotine at the large doses were also enhanced.

In the chronic experiment, LiCl was chronically injected to deplete the inositol of brain and submaxillary gland, which resulted in a decrease in the formation of polyphosphatidylinositides. Finally, the accumulation of IP_3 stimulated by Oxo was prominently decreased. That was the reason why the sensitivity of muscarinic receptors to Oxo for producing tremors and salivations was decreased by the chronically repeating doses of LiCl. In the same experimental conditions, the sensitivity of central nicotinic receptors to nicotine for producing convulsions was also decreased.

According to the results of the above acute and chronic experiments, it is reasonable to suggest that the sensitivity of central nicotinic receptors to nicotine for producing convulsions can be modulated by PI turnover.

Both LiCl and Oxo can potentiate the central effects of IP_3, why could the convulsive effects of nicotine not be modulated by Oxo? As we known, the effects of Oxo are specific on brain muscarinic receptor-mediated PI turnover, but those of lithium chloride are nonspecific on some brain receptor-stimulated PI turnover. The structures, functions and distributions of muscarinic receptors are different from those of nicotinic receptors in brain. In this experiment, convulsions induced by excitation of central nicotinic receptors could only be affected by the pretreatment with lithium chloride, suggesting that not all factors which could affect brain PI turnover, could modify the functions of brain nicotinic receptors.

It is concluded that the functions of central nicotinic receptors were modulated by PI turnover.

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磷脂酰肌醇代谢对中枢烟碱受体功能的调节

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关键词 烟碱; 氧颤莫林; 槟榔碱; 氯化锂; 磷脂酰肌醇; 烟碱受体; 惊厥

目的: 研究磷脂酰肌醇代谢对中枢烟碱受体功能的调节作用, 以分析脑烟碱受体与磷脂酰肌醇代谢之间的关系. **方法**: 在小鼠上观察肌醇磷酸酶

抑制剂氯化锂对烟碱诱发惊厥作用的影响. 结果：氯化锂 2.5－10 mmol·kg^{-1}预处理后，烟碱诱发小鼠惊厥的量效关系发生变化，在高于半数效量的剂量下，烟碱诱发惊厥的作用显著增强. 但氧颤莫林 0.05－0.20 mg·kg^{-1}预处理后，烟碱诱发小鼠惊厥的量效关系无显著变化. 在小鼠上每日注射一次氯化锂 5.0 mmol·kg^{-1} 7 d后，烟碱诱发惊厥的作用显著减弱，半数效量由 0.58 增至 0.97 mg·kg^{-1}. 结论：磷脂酰肌醇代谢可调节中枢烟碱受体的功能.

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Antagonistic effects of extract from leaves of *Ginkgo biloba* on glutamate neurotoxicity

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KEY WORDS cultured cells; neurons; calcium; cerebral cortex; arcuate nucleus; glutamates; *Ginkgo biloba*; ginkgolide B; quercetin

AIM: To determine whether the extract of leaves of *Ginkgo biloba* L (EGb) and several active constituents of EGb have protective effects against glutamate (Glu)-induced neuronal damage. **METHODS:** Microscopy and image analysis of nucleus areas in the arcuate nuclei (AN) of mice were made. The neuronal viability in primary cultures from mouse cerebral cortex was assessed using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] staining and the intracellular free calcium concentration ($[Ca^{2+}]_i$) of single neuron was measured using Fura-2. **RESULTS:** EGb (2.5 mg·L^{-1}) and its constituent ginkgolide B (Gin B, 2 mg·L^{-1}) protected the neuronal viability against Glu-induced injury, and prevented the Glu-induced elevation in $[Ca^{2+}]_i$. EGb (3 － 10 mg·kg^{-1}) attenuated the decrease of nucleus areas in arcuate nuclei induced by Glu (1 g·kg^{-1}, sc). **CONCLUSION:** EGb and Gin B prevent neurons from Glu neurotoxicity through reduction of the rise in $[Ca^{2+}]_i$.

Glutamate neurotoxicity (GNT) participated in the neuron loss associated with a number of neurodegenerative diseases, eg, Alzheimer's disease and Huntington's disease[1,2]. To investigate the GNT and anti-GNT drugs, a primary cell culture system derived from fetal mouse neocortex was used. *In vivo* studies were to detect the effects in arcuate nucleus in hypothalamus, which were not fully protected by the blood-brain barrier (BBB) in immature animals[3].

Overactivation of Glu receptors caused an excessive influx of Ca^{2+} into the neuron, which resulted in activation of lipase, protease, and protein kinase C, subsequently led to the generation of fatty acids, free radicals, and ultimately neuronal death. Ca^{2+} overloading and loss of Ca^{2+} homeostasis might play an important role in GNT[4,5].

The extract of leaves of *Ginkgo biloba* L (EGb) contained 24 % of flavonoid glycosides, the aglycon of which was a flavonol (including quercetin, kaempferol, and isorhamnetin), 6 % of terpene lactones (including ginkgolides A, B, C, J, and bilobalide), and 70 % of other substances (proanthocyanidins, organic acids, sugars, *etc*)[6]. EGb had protective effects on GNT in a dose-dependent manner in both cultured mouse cortical neurons and cultured human hippocampal neurons[7]. This study was to investigate whether EGb or some of its constituents protected neurons against GNT *in vitro* and *in vivo*.

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