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Modulation by nicotine on binding of cerebral muscarinic receptors with muscarinic agonist and antagonist[1]

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KEY WORDS nicotine; nicotinic receptors; muscarinic receptors; cerebral cortex; oxotremorine; quinuclidinyl benzilate

AIM: To study the modulatory effects of nicotine on the binding of brain muscarinic receptors. **METHODS:** The binding of brain muscarinic receptors with the agonist [^{3}H]oxotremorine-M or the antagonist *l*-[^{3}H]QNB was determined in the presence/absence of nicotine. **RESULTS:** Preincubation of the membrane fraction derived from rat cerebral cortex with nicotine 1.0 μmol·L^{-1} led to a decrease in the dissociation constant (K_d) for [^{3}H]oxotremorine-M binding to muscarinic receptors, while the maximal binding value (B_{max}) was unchanged. The K_d value for binding of the muscarinic antagonist *l*-[^{3}H]QNB was concentration-dependently increased by preincubation with nicotine 0.1 nmol·L^{-1} – 10.0 μmol·L^{-1}, with B_{max} unchanged. The effect of nicotine on the K_d for *l*-[^{3}H]QNB binding was prevented by mecamylamine 10 nmol·L^{-1}, but was enhanced by dithiothreitol 10 μmol·L^{-1}, which by itself was also capable of increasing the K_d value. **CONCLUSION:** Nicotine increases the affinity of brain muscarinic receptors for muscarinic agonist, but decreases the affinity of brain muscarinic receptors for muscarinic antagonist.

Nicotine is a highly selective ligand for nicotinic cholinergic receptors. Much attention has been paid to the effects of nicotine on the functions of cholinergic nervous system. Acute and chronic tolerance to nicotinic effects can be developed, and the brain nicotinic receptors can be desensitized by repeated administrations of nicotine[1,2].

The structure, function, and distribution of brain nicotinic and muscarinic receptors differ distinctively, but their endogenous agonist acetylcholine is the same. It is reasonable to assume that there exists a close relationship between the two types of receptors, although this relationship remains obscure. Recently we found that the sensitivity of brain muscarinic receptors for muscarinic agonists was increased, and that of brain nicotinic receptors to nicotinic agonists was decreased by repeated administrations of nicotine. *In vitro* the ability of the muscarinic agonists arecoline and oxotremorine to displace the binding of the muscarinic antagonist *l* -[^{3}H]QNB with brain muscarinic receptors was enhanced by nicotine 1.0 μmol·L^{-1}. This suggested that the affinity of brain muscarinic receptors for their agonists might be increased and/or the affinity of brain muscarinic receptors for their antagonists might be decreased[3-5]. So the effects of nicotine on the binding of cerebral muscarinic receptors to an agonist and an antagonist were studied in these experiments.

MATERIALS AND METHODS

Materials (−)Nicotine hydrogen (+)tartrate, mecamylamine hydrochloride, quinuclidinyl benzilate (QNB), atropine methyl bromide and dithiothreitol were purchased from Sigma Co. *l*-[^{3}H]QNB (1.6 PBq·mol^{-1}) was from Amersham Co. Methyl-[^{3}H]oxotremorine-M acetate (3.2 PBq·mol^{-1}) was from DuPont Co. The 49 type and Whatman GF/C of glass fiber filters were from Hong-guang Co (Shanghai, China) and Whatman Co, respectively.

[^{3}H]Oxotremorine-M binding[1] Wistar rat was decapitated and the cerebral cortex was dissected and homogenized in 10 volumes (w/v) of ice-cold Tris buffer (Tris 50, $CaCl_2$ 2, $MgCl_2$ 1, KCl 5, NaCl 120 mmol·L^{-1}, ascorbic acid 0.1%, pH 7.4). The homogenate was centrifuged at 1000 × *g* at 4 ℃ for 5 min, and the supernatant was recentrifuged twice at 50 000 *g* for 15 min. The pellet was suspended in the Tris buffer. Protein was determined using bovine serum albumin as a standard[3]. The membrane fraction (0.1 mg of protein) was incubated at

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35 ℃ for 30 min in a final 0.5 mL of the Tris buffer containing [^{3}H]oxotremorine-M (0.02 – 10 nmol·L^{-1}). Blanks were determined in the presence of atropine 1.0 μmol·L^{-1}. Samples were filtered through Whatman GF/C filters. The radioactivity was determined by a Beckman 1215 liquid scintillation spectrometer at an efficiency of 36 %.

***l*-[^{3}H]QNB binding**[8] Rat cerebral cortex was homogenized in 10 volumes (w/v) of ice-cold sucrose 0.32 mol·L^{-1}. The homogenate was centrifuged at 1000 · *g* at 4 ℃ for 10 min, and the supernatant was recentrifuged at 20 000 · *g* for 30 min. The pellet (P_2) was suspended in Na/K phosphate buffer 50 mmol·L^{-1}, pH 7.4. The P_2 fraction of brain membranes (0.1 mg protein) was incubated at 35 ℃ for 30 min in 1.0 mL of the buffer containing *l*-[^{3}H]QNB 0.02 – 2.0 nmol·L^{-1}. Specific binding was calculated as the total binding minus that occurring in the presence of *l*-QNB 10 μmol·L^{-1}. Samples were filtered through 49 type glass fiber filters. Counting was as outlined above.

Data analysis The saturation binding parameters were determined by the linear regression analysis of the Scatchard plots. Statistical evaluation of the data was examined by *t* test.

RESULTS AND DISCUSSION

Effects of nicotine on the binding of brain muscarinic receptors with muscarinic agonist The K_d value for [^{3}H]oxotremorine-M saturation binding was decreased in the presence of nicotine 1.0 μmol·L^{-1}. But the B_{max} value was unaltered. This indicated that the affinity of brain muscarinic receptors for the muscarinic agonist was increased, while the amount of receptors was unchanged in the presence of nicotine 1.0 μmol·L^{-1} (Tab 1).

Tab 1. Effects of nicotine on the binding of rat cerebral muscarinic receptors with [^{3}H]oxotremorine-M. ***n* = 4, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.**

Drug, μmol·L^{-1}	K_d, nmol·L^{-1}	B_{max}, nmol/g protein
Control	4.9 ± 2.8	0.38 ± 0.12
Nicotine 1.0	0.4 ± 0.3^b	0.26 ± 0.03^a

The occupation of brain muscarinic receptors by muscarinic agonists can initiate muscarinic responses, and the muscarinic receptor binding with its agonists can be affected either by the receptor isomerization or by the functional status of its effectors[5,9]. On the other hand, the occupation of brain muscarinic receptors by their antagonists can not initiate muscarinic responses, and the muscarinic receptor binding with its antagonists can be affected by the receptor isomerization rather than by the functional status of its effectors. So the effects of nicotine on the saturation binding of brain muscarinic receptors with the antagonist *l*-[^{3}H]QNB were further observed in order to study the allosteric regulation of nicotine toward brain muscarinic receptors.

Effects of nicotine on the binding of brain muscarinic receptors with muscarinic antagonist The K_d values of *l*-[^{3}H]QNB saturation binding were increased, while the B_{max} values were unchanged in the presence of nicotine 1.0 nmol·L^{-1} (Tab 2).

Tab 2. Effects of nicotine on the profiles of *l*-[^{3}H]QNB binding with rat cerebral muscarinic receptors. ***n* = number of samples derived from 20 rats and determined in duplicate.** $\bar{x} \pm s$. **$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* dithiothreitol 10 μmol·L^{-1}; $^gP > 0.05$, $^hP < 0.01$ *vs* nicotine 1.0 μmol·L^{-1}.**

Drug, μmol·L^{-1}	*n*	K_d, nmol·L^{-1}	B_{max}, nmol/g protein
Control	8	0.33 ± 0.05	1.16 ± 0.12
Nicotine: 0.0001	4	0.35 ± 0.04^a	1.12 ± 0.21^f
0.001	8	0.41 ± 0.08^b	1.09 ± 0.23^g
0.01	3	0.51 ± 0.02^c	1.14 ± 0.19^g
0.1	3	0.54 ± 0.02^c	1.23 ± 0.12^c
1.0	8	0.58 ± 0.09^c	1.13 ± 0.20^f
10	4	0.69 ± 0.10^c	1.21 ± 0.21^f
100	4	1.82 ± 0.51^c	1.00 ± 0.15^f
1 000	5	15.66 ± 2.22^c	1.03 ± 0.14^h
Mecamylamine 0.01	3	0.33 ± 0.02^d	1.22 ± 0.13^e
DTT 10	3	0.61 ± 0.08^e	1.04 ± 0.01^h
Mecamylamine 0.01/nicotine 1.0	3	0.35 ± 0.10^{eg}	1.14 ± 0.18^{eg}
Dithiothreitol 10/nicotine 1.0	5	1.64 ± 0.67^{ef}	1.00 ± 0.25^{eh}

The relationship between the concentrations of nicotine (lg mol·L^{-1}) and K_d values (nmol·L^{-1}) of *l*-[^{3}H]QNB binding was linear, $r = 0.9933$, $P < 0.001$. It indicated that affinity of cerebral muscarinic receptors to the muscarinic antagonist was decreased by the presence of nicotine with a

concentration-dependent manner *in vitro*.

The elevated K_d values of *l*-[^{3}H]QNB binding in the presence of nicotine were prevented by mecamylamine 10 nmol·L^{-1}, which itself had no effects on *l*-[^{3}H]QNB binding parameters. In addition, the *l*-[^{3}H]QNB binding was sensitive to dithiothreitol, and K_d values were also increased by the presence of dithiothreitol 10 μmol·L^{-1}. After the membrane preparation was preincubated with dithiothreitol 10 μmol·L^{-1} at 35 ℃ for 30 min, nicotine 1.0 μmol·L^{-1} further increase the K_d values (Tab 2). These data indicated that the effects of nicotine on K_d values of *l*-[^{3}H]QNB binding could be prevented by the blockade of the membrane nicotinic receptors by mecamylamine, and after the disulphate bound of muscarinic receptors was broken by dithiothreitol, the effect of nicotine still existed.

In summary, the modulatory effects of nicotine on the binding of brain muscarinic receptors with the agonist and antagonist were studied *in vitro*. Nicotine increased the affinity of brain muscarinic receptors for muscarinic agonist, decreased the affinity for muscarinic antagonist, and had no effects of the amount of muscarinic receptors. The allosteric effects of nicotine could be prevented by the nicotinic antagonist mecamylamine, which suggested that the effects of nicotine were indirect, through brain nicotinic receptors.

REFERENCES

1 Bevan P. [^{3}H]oxotremorine-M binding to membranes prepared from rat brain and heart: evidence for subtypes of muscarinic receptor. Eur J Pharmacol 1984; **101**: 101 - 11

2 de Fiebre CM, Collins AC. Decreased sensitivity to nicotine-induced seizures as a consequence of nicotine pretreatment in long-sleep and short-sleep mice. Alcohol 1988; **5**: 55 - 61

3 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; **193**: 265 - 75

4 Marks MJ, Stitzel JA, Collins AC. Time course study of the effects of chronic nicotine infusion on drug response and brain receptors J Pharmacol Exp Ther 1985; **235**: 619 - 28

5 Sokolovsky M, Cohen-Armon M, Egozi Y, Gurwitz D, Henis YI, Kloog Y, *et al*. Modulation of muscarinic receptors and their interaction. Trends Pharmacol Sci 1986; 7 Suppl: 39 - 43.

6 Wang H Modulatory effects of nicotine on muscarinic acetylcholine receptor and its molecular mechanism. Prog Physiol Sci 1994; **25**: 240 - 2.

7 Wang H, Cui WY, Liu CG. The repeated administration of nicotine selectively enhances the sensitivity of muscarinic cholinergic receptors. In: Seredenin SB, Longo V, Gavitaghi G, eds. Biological basis of individual sensitivity to psychotropic drugs. Edinburgh: Graffham Press, 1994: 1441 - 8.

8 Wang H, Liu CG. Increased sensitivity of brain muscarinic receptor to its agonists as a result of repeated nicotine pretreatment in rats, mice and rabbits. In: Adlkofer F, Thurau K, editors Effects of nicotine on biological system. Basel: Birkhauser, 1991: 263 - 72.

9 Wess J, Bonner TI, Dorje F, Brann MR. Delineation of muscarinic receptor domains conferring selectivity of coupling to guanine nucleotide binding proteins and second messenger Mol Pharmacol 1990; **38**: 517 - 23.

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烟碱对大脑皮层毒蕈碱型受体与其激动剂和拮抗剂结合的调节

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关键词 烟碱; 烟碱受体; 毒蕈碱受体; 大脑皮质; 氧颤莫林; 奎纽定二苯羟乙酸 结抗剂

目的: 研究烟碱对脑 M 受体结合性能的调节作用 **方法**: 测定烟碱对脑 M 受体与其激动剂[^{3}H]氧颤莫林-M 和拮抗剂 *l*-[^{3}H]奎纽定二苯羟乙酸(QNB)结合的影响 **结果**: 大鼠大脑皮层膜标本经烟碱预处理后, M 受体与[^{3}H]氧颤莫林-M 特异性结合的平衡解离常数降低, 最大结合量不变; M 受体与 *l*-[^{3}H]QNB 特异性结合的平衡解离常数增高, 最大结合量也不变. 烟碱对 M 受体与 *l*-[^{3}H]QNB特异性结合的影响具有浓度依赖性, 并为 N 拮抗剂美加明对抗, 为二硫苏糖醇增强. **结论**: 烟碱可增强脑 M 受体与其激动剂的亲和力, 降低与其拮抗剂的亲和力.

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