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Acetylcholine contents and muscarinic receptor levels in frontal cortex, corpus striatum, and hippocampus of reserpinized rats and mice

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ABSTRACT The acetylcholine (ACh) levels in rat and mouse frontal cortex increased 155% and 124%, respectively, 24 h after ip reserpine 3 mg · kg^{-1}. Striatal ACh contents, however, were diminished by 47% in rats and 80% in mice. ACh contents elevated 50% and scopolamine (Scop) depleted the ACh by 47% in mouse striatum 12 h following reserpine. Receptor binding assay showed that 24 h after reserpine the B_{max} of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding to muscarinic receptors increased in frontal cortex (by 33% in rats, by 30% in mice) and decreased in striatum (31% in rats, 26% in mice). In mouse hippocampus the ACh contents, B_{max}, and affinity of muscarinic receptors lowered 63%, 19%, and 26%, respectively. But these changes were not seen in rat hippocampus.

KEY WORDS reserpine; scopolamine; quinuclidinyl benzilate; acetylcholine, muscarinic receptors; frontal lobe; corpus striatum; hippocampus

Clinical and experimental data showed that a dynamic interaction existed between monoamines and acetylcholine (ACh) in brain. Anticholinergic drugs are useful in early stage of Parkinson's disease which is principally characterized by degeneration of the nigro-striatal dopaminergic system. The studies of postmortem brain from Parkinsonian patients discovered that the muscarinic receptor B_{max} increased in cortex[1,2]. Mania and depression may be due to a relative cholinergic deficit and excess, respectively. Rats receiving a high dose of reserpine developed a syndrome of "paradoxical" hyperactivities, which was completely abolished by a series of anticholinergic agents[3], and blocked the effects of oxotremorine-induced increases in striatal ACh[4]. The present investigation was to determine the changes of ACh contents and muscarinic receptor levels in different brain regions after depletion of monoamines by reserpine.

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MATERIALS AND METHODS

Wistar ♂ rats weighing 230 ± *s* 30 g and Kunming ♂ mice weighing 28 ± 3 g were obtained from the Academy of Traditional Chinese Medicine (Beijing) and housed in laboratory at 17-20℃ for 7 d. The animals were decapitated between 09 : 00 and 11 : 00 h. The brain, frontal cortex, striatum, and hippocampus were dissected out on ice.

[^{3}H]Quinuclidinyl benzilate ([^{3}H]QNB, 251.4 TBq · mol^{-1}) was purchased from Amersham, UK. [^{3}H]ACh (73.63 TBq · mol^{-1}), ACh, ACh antibody were obtained from the Academy of Military Medical Sciences, Beijing. Scopolamine (Scop) was bought from Merck. Reserpine was purchased from Qiaoguang Pharmaceutical Factory, Guangzhou. GF/49 filters and microporous filtering films (0.45 μm in pore diameter) were bought from Hongguang Paper Mill, Shanghai.

Measurement of ACh Brain regions were homogenized in 9.3 vol of ice-cold $HClO_4$ 0.1 mol · L^{-1}, and centrifuged at 8500 × *g* for 3 min. ACh concentrations in the supernatant were determined using a radioimmunoassay procedure[5], and calculated as described previously[6].

Preparation of M receptors 20% Homogenates (wt/vol) were prepared in Na-K phosphate buffer 50 mmol · L^{-1}, pH 7.4 and then centrifuged at 15 000 × *g* for 10 min. The pellet was resuspended and washed

twice with 1 : 20 (wt / vol) buffer mentioned above. The final pellet was resuspended at a concentration of 100 mg · ml^{-1} and frozen at −20℃ until being used in binding assay. Protein content in homogenates was estimated by a colorimetric method[7].

M receptor binding assay The homogenates (100 μg protein) were incubated with various concentrations of [^{3}H]QNB at 37℃ for 30 min in 240 μl of the phosphate buffer indicated above. Non-specific binding of the ligand was examined in the presence of atropine 1 μmol · L^{-1}. The binding reaction was stopped by addition of 0.5 ml ice-cold buffer and followed by vacuum filtration through glassfiber filters. The filters were dried at 80 ℃ and the bound radioactivity was counted by liquid scintillation counter. The density (B_{max}) and apparent dissociation constant (K_d) of [^{3}H]QNB specific binding to muscarinic receptors in rat or mouse brain were calculated from Scatchard analysis.

RESULTS

ACh contents At 12 h after ip reserpine (3 mg · kg^{-1}), the striatal ACh in mice increased 50%, but ACh in cortex and hippocampus remained unchanged. At 24 h after ip 3 mg · kg^{-1}, reserpine increased the ACh levels of the frontal cortex by 155% in rats, 124% in mice, and the whole brain by 40% in mice, but decreased the ACh concentrations of striatum by 47% in rats and 80% in mice. In hippocampus the ACh contents did not show any change in rats but lowered 63% in mice (Tab 1, Tab 2).

M receptors At 12 h after reserpine (3 mg · kg^{-1}, ip) the B_{max} of [^{3}H]QNB binding to M receptors did not change in mouse brain. However, at 24 h following reserpine, the B_{max} of [^{3}H]QNB binding to receptor in the frontal cortex increased 33% in rats and 30% in mice, and the striatum decreased 31% in rats and 26% in mice (Tab 1, Tab 2).

Tab 1. Acetylcholine and muscarinic receptors in mouse brain 12 h after ip reserpine 3 mg · kg^{-1}. $n=7-9$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* saline.

Brain regions	Acetylcholine, pmol / mg brain	[^{3}H]QNB binding parameters: B_{max}, fmol / mg protein	[^{3}H]QNB binding parameters: K_d, nmol · L^{-1}
Frontal cortex			
Saline	8.6 ± 1.0	780 ± 18	0.45 ± 0.10
Reserpine	9.3 ± 0.9*	834 ± 38*	0.82 ± 0.15***
Striatum			
Saline	16.1 ± 2.2	1 121 ± 40	0.96 ± 0.24
Reserpine	24 ± 6***	1 066 ± 35*	0.91 ± 0.29*
Hippocampus			
Saline	13.3 ± 1.4	712 ± 34	0.71 ± 0.20
Reserpine	13.8 ± 1.9*	763 ± 35*	0.71 ± 0.15*

Tab 2. Acetylcholine contents and [^{3}H]QNB binding to muscarinic receptors in reserpinized (ip 3 mg · kg^{-1}, 24 h) rat and mouse brain. $n=7-10$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* saline.

Brain regions	Acetylcholine, pmol / mg brain	[^{3}H]QNB binding parameters: B_{max}, fmol / mg protein	[^{3}H]QNB binding parameters: K_d, nmol · L^{-1}
Rat frontal cortex			
Saline	5.5 ± 1.9	1 083 ± 65	0.042 ± 0.04
Reserpine	14 ± 5***	1 437 ± 79***	0.181 ± 0.08***
Rat striatum			
Saline	12.3 ± 2.6	1 327 ± 80	0.21 ± 0.09
Reserpine	6.5 ± 2.5***	914 ± 52***	0.20 ± 0.07*
Rat hippocampus			
Saline	9.4 ± 2.7	753 ± 19	0.10 ± 0.02
Reserpine	9.8 ± 2.5*	767 ± 29*	0.10 ± 0.05*
Mouse whole brain			
Saline	22 ± 4	761 ± 44	0.54 ± 0.04
Reserpine	31 ± 7**	1 084 ± 70***	0.68 ± 0.06*
Mouse frontal cortex			
Saline	8.5 ± 2.2	506 ± 27	0.30 ± 0.07
Reserpine	19 ± 5***	660 ± 40***	0.42 ± 0.09*
Mouse striatum			
Saline	35 ± 12	1 243 ± 90	1.10 ± 0.12
Reserpine	7 ± 3***	926 ± 30***	1.00 ± 0.08*
Mouse hippocampus			
Saline	19 ± 9	776 ± 20	0.65 ± 0.09
Reserpine	7 ± 3**	626 ± 28***	0.48 ± 0.05*

Scop-induced alterations in striatal ACh

The mice that received reserpine (3 mg · kg^{-1}, ip) were treated with ip Scop 2 mg · kg^{-1} at 12 h after injection of reserpine. Then the mice were killed 30 min after injection of Scop. Results showed that Scop reduced the ACh contents by 47% in reserpinized mouse striatum and did not alter the ACh levels in this region of control (Tab 3).

Tab 3. Mouse striatum acetylcholine 30 mim after Scop and 12 h after reserpine. $n=10$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* **saline.** $^{+++}P<0.01$ *vs* **Scop.**

Scopolamine	Reserpine	Acetylcholine, pmol / mg brain
0	0	18.9 ± 2.9
0	3 mg · kg^{-1}	24 ± 5***
2 mg · kg^{-1}	0	18.1 ± 2.2*
2 mg · kg^{-1}	3 mg · kg^{-1}	12.8 ± 1.5^{***+++}

DISCUSSION

Because endogenous serotonin and dopamine exerted an inhibitory effect on ACh release from cholinergic interneurons of striatum[8-10], in the present study it was found that the ACh contents in mouse striatum were different at 12 and 24 h after ip reserpine. These might be explained by the mechanism of reserpine-induced depletion of neurotransmitters. At the beginning, owing to storage impairment, the dopamine and serotonin escaped from vesicles and entered the synaptic clefts, then they inhibited the regulation of striatal cholinergic neurons so that ACh release was inhibited and contents were increased in this brain region. Finally, when catecholamines and serotonin in the striatum were depleted by reserpine, the inhibition of ACh release was no longer perceptible. Thus ACh release increased and contents decreased in the striatum.

Although it has been found that the ACh contents and M receptor numbers showed an interaction of regulation[11-15], the present data revealed that the changes of muscarinic receptor up- or down-regulation might be influenced by the decrease or increase in ACh release in mice and rats receiving reserpine, respectively. All these results brought to a conclusion that the cholinergic neurotransmitter and receptors in brain can be controlled by catecholamines and / or serotonin in central nervous system.

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223–226

利血平化大、小鼠额叶、纹状体和海马的乙酰胆碱及毒蕈碱受体

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提要 ip 利血平 3 mg · kg^{-1}、24 h 后大、小鼠皮层 ACh 分别增加 155%和 124%，M 受体 B_{max} 数增加、亲和力降低. 纹状体 ACh 减少，M 受体 B_{max} 数下降、亲和力不变. 在海马，利血平化小鼠的 ACh、M 受体 B_{max} 数及其 K_d 值均减少. ip 利血平后 12 h，小鼠纹状体 ACh 升高 50%，并加强了 Scop 对该部位 ACh 含量降低的作用.

关键词 利血平；东莨菪碱；二苯羟乙酸奎宁酯；乙酰胆碱；毒蕈碱受体；额叶；纹状体，海马

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Protective effect of cycloprotobuxine–A against cardiac arrhythmias induced by ouabain

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ABSTRACT Cycloprotobuxine–A (CPB–A) 1–4 mg · kg^{-1} iv increased the dose of ouabain required to induce ventricular arrhythmias in guinea pigs. At the equitoxic doses (1/50 LD_{50}), CPB–A was more potent than cyclovirobuxine–D and amiodarone. Pretreatment with reserpine (5 mg · kg^{-1} ip), vagotomy or pithing spinal cord did not prevent the action of CPB–A, which indicate that the protective effect of CPB–A may be due to its direct action on myocardium without the involvement of nervous system. In isolated guinea pig ventricular muscles, CPB–A 3 μmol · L^{-1} consistently decreased the amplitude of oscillatory afterpotentials (OAP) and blocked triggered activity elicited by ouabain. At 30 μmol · L^{-1}, CPB–A abolished the appearance of OAP. It seems that one of the mechanisms for the anti–arrhythmic action of CPB–A was a decrease in the amplitude of OAP.

KEY WORDS arrhythmia; electrophysiology; myocardium; ouabain; cycloprotobuxine–A; amiodarone

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