Effects of Cholinoblockers on Acetylcholine Content in Rat Striatum in Neuroleptic-Induced Parkinsonism

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Correction of neuroleptic-induced parkinsonism in rats with two central cholinoblockers atropine and pentifine (acetylene aminoalcohol synthesized at Institute of Toxicology) were studied by measuring the content of acetylcholine in the striatum. The content of the transmitter secretion was estimated from the content of bound acetylcholine fraction in homogenates of the above-mentioned compartment of the brain. The results indicate that atropine and pentifine in doses equally effectively preventing catalepsy in rats had different effects on acetylcholine secretion in the striatum. Hence, cholinolytics with different pharmacological selective effects differently interact with central muscarine receptor subtypes.

Key Words: *muscarine receptor subtypes; selective activity of muscarine antagonists*

Acetylcholine (AC) and dopamine transmitter imbalance in the nigrostriatal system is the pathogenetic basis for extrapyramidal disorders (EPD) in Parkinson disease of different etiology. According to modern concepts, decrease in the activity of the dopaminergic system is paralleled by hyperfunction of the cholinergic system. Presumably, AC regulates dopamine level in the striatum, which gives grounds to use M-cholinoblockers belonging to different groups of chemical compounds for EPD correction. However, though normally AC functions as a regulator, the relationships between the dopaminergic and cholinergic systems under pathological conditions (decreased dopamine level or sensitivity to it) are little known. Therefore measurements of AC level under conditions of modified status of the dopaminergic system attract special interest.

We studied AC levels in the rat striatum on a model of neuroleptic parkinsonism and during treatment with different cholinoblockers.

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MATERIALS AND METHODS

Experiments were carried out on male albino rats (160-220 g). The content of bound AC fraction in the rat striatum homogenate was measured after treatment with cholinolytics atropine and pentifine and under conditions of neuroleptic-induced parkinsonism (intraperitoneal injection of 10 mg/kg haloperidol).

In experimental series I cholinolytics atropine and pentifine were injected subcutaneously 30 min before isolation of the striatum in doses of 1.230 and 0.023 mg/kg, respectively. According to pharmacological tests, these doses equally effective prevented haloperidol-induced catalepsy in rats [1]. Controls were subcutaneously injected with normal saline 30 min before the study.

In experimental series II (simulation of neuroleptic parkinsonism) cholinolytics were injected in the same doses 30 min before haloperidol injection, 45 min after which the striatum was isolated. Controls were injected (subcutaneously and intraperitoneally) with normal saline according to the same scheme.

Bound AC was measured by biological method [2] using medicinal leech spinal muscle preparation as the test object. The data were processed using Student's *t* test.

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RESULTS

The content of bound AC reflects transmitter level in nerve cells and their terminals and is inversely proportional to its release. First, we measured bound AC level in the striatum after single injection of high doses of haloperidol 45 min before measurements, which induced pronounced EPD in animals.

After parenteral injection of atropine (n=8) the content of bound AC in rat striatum was 6.8±1.7, after pentifine (n=10) it was 20.5±3.3×10^{-7} g/g tissue. Haloperidol (n=6) significantly decreased bound AC level (by 56% of the initial value, 9.2±1.8 *vs.* 21.2± 2.0×10^{-7} g/g tissue, respectively), which was in line with the results of P. De Boer *et al.* [3], who demonstrated that AC content in the striatum dialysate increased by 50% from the basal level after intraperitoneal injection of haloperidol (1 mg/kg).

The aim of the next stage was to select a pair of cholinolytics, which, used in equally effective doses, prevent EPD and have different effects on AC level in the striatum. Atropine was selected as drug No. 1; it is a nonselective cholinergic receptor blocker, *i. e.* injection of atropine equally blocks different types of muscarinic receptors. Pentifine (synthesized at Institute of Toxicology) was selected as drug No. 2; it specifically prevents catalepsy under conditions of experimental parkinsonism, is well tolerated and low toxic [1]. Our data indicate that pentifine possesses high cholinoblocking activity towards M_4 muscarinic receptors, that is, the ratios of the mean effective doses of this drug in pharmacological tests (M_1 — pilocarpine tremor, 75 mg/kg; M_2 — arecoline tremor, 17 mg/kg; M_3 — pilocarpine salivation, 35 mg/kg; M_4 — specific movements of the mandible after injection of 4 mg/kg pilocarpine) are 66:142:40:1 for M_1:M_2:M_3:M_4.

Atropine and pentifine were injected to intact animals in the mean effective doses preventing the development of catalepsy. The level of bound AC decreased by 67% from the initial level (p<0.001) after atropine injection, but no appreciable changes in AC content were observed after administration of pentifine in the mean effective dose.

At the final stage of the study we evaluated the effects of the selected cholinolytics on the content of bound AC in the striatum under conditions of experimental neuroleptic parkinsonism. Injection of atropine for the correction of haloperidol-induced EPD did not normalize the level of bound AC (this parameter remained low). The content of this transmitter after haloperidol and atropine injections did not statistically differ. Injection of pentifine under the same conditions induced a minor decrease in the level of bound AC, statistically not differing from the control (6.1± 3.1 after atropine±haloperidol, n=6, and 14.9±2.4 g/g tissue×10^{-7} after pentifine±haloperidol, n=10).

If we assume the regulatory role of AC in the maintenance of dopamine level, we can expect that equal pharmacological effects (prevention of EPD) are provided by the same level of AC. However, injection of two cholinolytics in doses producing similar pharmacological effect to intact rats demonstrated pronounced differences in the levels of bound AC. Since the decrease in intracellular AC is a manifestation of its increased release, we can hypothesize that AC level largely lost the function of dopamine secretion regulator. The protective effect of cholinoblockers can be explained by their effect on the postsynaptic receptors in the striatum. Prevention of EPD is most likely realized via M_4 muscarinic receptors. This is confirmed by the fact that pentifine, selected as the model drug, is characterized by high pharmacological selective activity towards this subtype. In addition, the concentration of M_4 muscarinic receptors in the striatum is higher than in other brain structures, and M_4 receptors are located on postsynaptic membranes in this anatomical formation [4-6].

On the other hand, differences in AC levels after treatment with two cholinoblockers in doses equally effectively preventing EPD indicates that the regulation of AC secretion in the striatum is mediated by another cholinoreceptor subtype (for example, blocking of presynaptic M_2 muscarinic receptor) [5,7]. However, pentifine is a cholinolytic with the pharmacological selective activity profile characterized by the capacity to block the M_2:M_4 as 142:1. This means that in doses effective mainly for the M_4 subtype the blocking effect of this drug on M_2 subtype is negligible. In concentrations surpassing the selective doses pentifine stimulates AC release in the striatum, similarly to other cholinoblockers. In additional experimental series we showed that injections of pentifine in nonselective doses of 1 and 5 mg/kg decreased the content of bound AC by 50% from its basal level.

Hence, haloperidol decreased the content of bound AC in the striatum. Cholinoblockers atropine and pentifine in doses equally effectively preventing haloperidol-induced catalepsy had different effects on AC level. Regulation of AC level in the striatum and anticataleptic effects of cholinoblockers are presumably mediated by different M-cholinoreceptor subtypes.

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