EXPERIMENTAL BIOLOGY

Typifying Presynaptic M-Cholinergic Receptors in Different Structures of Rat Brain

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Pre- and postsynaptic M-cholinergic receptors are typified by radioligand analysis with selective cholinoblockers by comparing ligand binding in homogeneous and synaptosomal fractions of different structures of rat brain. It is found that presynaptic receptors of the hemispheres belong to M_3 subtype and those of the brain stem are probably of M_4 subtype.

Key Words: *presynapsis; muscarinic cholinergic receptors; heterogeneity*

The effect of cholinotropic drugs on the tonicity of cholinergic innervation depends on the sum of its influences at the pre- and postsynaptic levels. Therefore, the type of pre- and postsynaptic M-cholinergic receptors (M-ChR) in different structures of the central nervous system (CNS) is important for understanding the mechanisms of action of selective cholinergic agonists and antagonists and for synthesis and screening of highly specific cholinergic drugs.

In the present study we used the synaptosome fraction as an object enriched with presynaptic M-ChR in comparison with homogenate of the correspondent brain structure.

Our objective was to demonstrate the possibility of using the proposed approach for typifying pre- and postsynaptic M-ChR in the brain hemisphere and stem.

MATERIALS AND METHODS

Experiments were carried out on female rats weighing 180-220 g. The synaptosomal fraction was isolated by the classical method [1,2]. The affinity of cholinergic antagonists for M-ChR was determined by the method of radioligand analysis using ^{3}H-quinuclidinyl benzilate (Amersham) with a specific activity of 37 Ci/mmol. The experimental procedure was based on the method described elsewhere [3]. The selective cholinoblockers pirenzepine and hexahydroxyladiphenidol were resynthesized, and SL-2, a tetra-amine M_2-selective cholinoblocker, was synthesized at the Institute of Toxicology. These cholinoblockers were used as test ligands. Radioactivity was measured in a Mark-III scintillation counter using the standard dioxane scintillator. Data processing, calculation of competitive inhibition constant (K_i), and percentage of receptors with high (B_h) and low (B_l) affinity for the test ligands were performed by nonlinear regression analysis using specially designed software.

RESULTS

Table 1 shows the selectivity profiles of the chosen M-cholinoblockers.

Homogenates of brain hemispheres, myocardium, and salivary glands, i.e., structures with predominating M_1-, M_2-, and M_3-ChR, respectively, served as test tissues. The constants characterizing the binding of the cholinoblockers to the prevailing population of M-ChR are listed in Table 1. Their

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TABLE 1. Profiles of Selective Test Ligands

Preparation	Brain hemispheres (M_1-ChR)	Myocardium (M_2-ChR)	Salivary glands (M_3-ChR)
		K_i, M	
Pirenzepine	3.5×10^{-8}	1.3×10^{-6}	0.72×10^{-6}
SL-2	1.4×10^{-6}	1.7×10^{-7}	1.6×10^{-5}
Hexahydroxyladiphenidol	1.8×10^{-7}	2.7×10^{-7}	3.7×10^{-8}

values indicate that pirenzepine is an M_1-cholinoblocker, SL-2 is an M_2-selective ligand, and hexahydroxyladiphenidol is an M_3-selective cholinolytic agent. We then compared the bindings of these cholinoblockers in homogenates and synaptosomal fractions of the brain hemispheres and stem. Synaptosomal fractions were regarded as an object enriched with presynaptic M-ChR in comparison with homogenate of the correspondent structure. The data are summarized in Table 2.

Eighty percent of M-ChR in homogenate prepared from the hemispheres and 30% of M-ChR in the stem homogenate have a high affinity for the M_1-cholinoblocker pirenzepine. In the synaptosomal fractions isolated from the hemispheres and stem, the content of pirenzepine-sensitive M_1-ChR declined, while the content of low-affinity receptors which does not belong to M_1 type rose. Thus, as the number of presynaptic receptors in the total population increased, the proportion of M_1-ChR decreased, indicating that the presynaptic receptors of the hemispheres and stem are not M_1-ChR. The M_2-selective blocker SL-2 showed the maximum affinity for 16% of receptors in the hemisphere homogenate and for >90% of receptors in the stem homogenate. The synaptosomal fraction of the hemispheres does not contain the high-affinity M_2-component. Obviously, the presynaptic receptors of brain hemispheres do not belong to M_2 subtype. The binding of SL-2 in the synaptosomal fraction of the stem is single-populational, which is related to the proportions of M-ChR subtypes in this structure, degree and profile of the selectivity of the preparation, and the sensitivity of radioligand analysis. In the stem homogenate, SL-2 exhibited the maximal binding, which is consistent with the preparation's affinity for M_2-ChR. In the synaptosomal fraction, the binding dropped by an order of magnitude, suggesting that the stem presynaptic receptors do not belong to M_2 subtype. The binding of the M_3-selective blocker hexahydroxyladiphenidol in the homogenate and synaptosomal fraction of the hemispheres showed a decrease in the content of low-affinity M-ChR, which do not belong to M_3 subtype, and an increase in the content of M_3-ChR, which are highly sensitive to hexahydroxyladiphenidol. The number of M_3-ChR in the stem presynaptosomal fraction drops in comparison with that in homogenate, indicating that presynaptic receptors of the stem do not belong to M_3 type.

Thus, our findings indicate that presynaptic M-ChR of the rat brain stem belong neither to M_1-, nor to M_2-, or M_3-subtype. They are probably M_4-cholinergic receptors. Bearing in mind that M_1- and M_3-ChR are coupled to the phosphoinositide and M_2- and M_4-ChR to the adenylate cyclase system of second messenger systems, the results obtained indicate that different second messenger systems are involved in the regulation of acetylcholine secretion in brain hemispheres and stem. The fact that pre- and postsynaptic M-ChR in different brain structures belong to different types is an essential prerequisite

TABLE 2. Binding of Selective Cholinoblockers to M-ChR in Homogenates and Presynaptic Fraction of Different Structures of Rat Brain

Preparation		Brain hemispheres		Stem	
		K_i, M			
		B_h (%)	B_l (%)	B_h (%)	B_l (%)
Pirenzepine	homogenate	8.2×10^{-8} (76)	1.3×10^{-6} (22)	2.8×10^{-8} (33)	5.2×10^{-7} (65)
	synaptosomes	8.7×10^{-8} (53)	1.3×10^{-6} (37)	8.9×10^{-9} (15)	7.9×10^{-7} (82)
SL-2	homogenate	1.0×10^{-7} (16)	1.3×10^{-6} (76)	2.0×10^{-7} (94)	-
	synaptosomes	-	0.86×10^{-6} (86)	-	1.1×10^{-6} (93)
Hexahydroxyladiphenidol					
	homogenate	2.1×10^{-8} (4)	1.8×10^{-7} (96)	6.4×10^{-9} (31)	1.2×10^{-7} (69)
	synaptosomes	1.3×10^{-8} (11)	1.5×10^{-7} (89)	7.8×10^{-9} (11)	2.7×10^{-7} (88)

for the screening of highly selective cholinergic drugs that selectively modify the tonicity of parasympathetic innervation in different CNS structures.

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