It can be postulated on the basis of these results that a common property of BDR ligands with an anxiogenic component in the spectrum of their behavioral effects is depression of reciprocal inhibition in the hippocampus.

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N-METHYLCYTISINE: A SELECTIVE LIGAND OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

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The presence of two types of nicotinic acetylcholine receptors (NACR) in the animal brain has now been established. Receptors of the first type interact with the α-neurotoxins of snakes and, with respect to some pharmacologic, chemical, and biochemical properties, they closely resemble neuromuscular NACR [10, 11, 14]. However, there are various circumstances which compel a more critical consideration of the view that the α-neurotoxin-binding components of the brain are NACR. One of the main arguments in support of this approach is the fact that according to electrophysiological data α-neurotoxins do not block cholinergic functions of certain neuronal systems [7, 8]. Accordingly, to study NACR in the CNS, radioactive derivatives of traditional low-molecular-weight ligands have been used more extensively in recent years: acetylcholine, nicotine, and tubocurarine [9, 12, 15]. With their aid nicotine receptors of a second type, insensitive to snake α-neurotoxins and differing in various pharmacologic properties from neuromuscular NACR, have been found in the brain. The authors cited also have described NACR in the optic ganglia of the squid, similar in their pharmacologic properties (in particular, high affinity for cytisine and insensitivity to α-neurotoxins), to the type II nicotinic receptors of the CNS [1, 2, 5]. For the reasons described above, it is interesting to look for new low-molecular-weight ligands capable of interacting selectively with type II NACR.

This paper describes a comparative study of the pharmacologic activity of cytisine and its N-methyl derivatives with respect to neuromuscular NACR of the electric organ of the skate Torpedo marmorata and the nicotinic receptor of the optic ganglia of the squid.

EXPERIMENTAL METHOD

The following substances were used in the experiments: ^{14}C-tubocurarine (^{14}C-TC, 94 Ci/mole) and ^{3}H-methyl iodide (13 Ci/mmole; from Amersham International, England). Cytisine was obtained from the Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek

Brain Research Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow.

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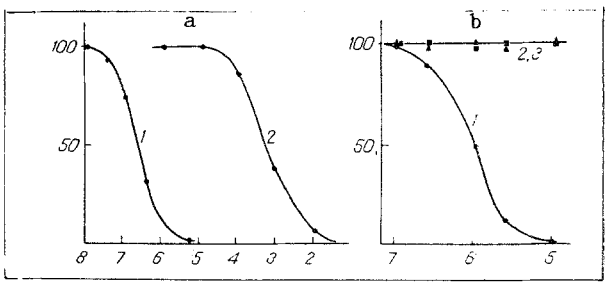

Fig. 1. Dependence of specific binding of ^{3}H-MC with membrane preparations of squid optic ganglia (a) and of specific binding of ^{14}C-TC with membrane preparations of the skate electric organ (b) on concentration of cytisine (1), N-methylcytisine (2), and N,N'-dimethylcytisine (3). Abscissa, concentration (−log); ordinate, specific binding (in %).

SSR. N-methylcytisine, N-^{3}H-methylcytisine (^{3}H-MC), and N,N'-dimethylcytisine were obtained by alkylation of cytisine with cold and radioactive methyl iodide, as described by the writers previously [2].

Membrane preparations of NACR were isolated from the electric organ of the skate T. marmorata, generously provided by Professor B. D. Jankovich (Immunologic Research Center, Belgrade, Yugoslavia), by the method in [13], and from the optic ganglia of the squid Berryteuthis magister, provided by L. M. Epshtein (Pacific Institute of Fisheries and Oceanography, Ministry of Fisheries of the USSR, Vladivostok), as described by the writers previously [1].

Specific binding of ^{3}H-MC and ^{14}C-TC with membrane preparations of NACR was determined by the centrifugation method [5]. Each experiment was repeated three times and the results were presented as the mean value for three independent experiments ± the standard error. Parameters of binding (dissociation constant K_d, maximal number of binding sites B_{max}) for the radioactive derivatives were calculated from a Scatchard plot by means of an HP-97 microcomputer (Hewlett Packard, USA), programmed to approximate the experimental data by the method of least squares. The statistical significance of the parameters was $p > 0.95$ and the standard error of measurement of K_d and B_{max} did not exceed 15%. K_d for complexes for nonradioactive ligands was calculated from the value of IC_{50} by the equation:

$$K_d = IC_{50}(1 + C'/K_d'),$$

where IC_{50} is the 50% inhibition concentration, K_d' the dissociation constant of the radioactive ligand, and C' its concentration in the sample.

EXPERIMENTAL RESULTS

The writers showed previously [5] that specific binding sites for ^{14}C-TC (K_d = 210 ± 30 nM) and for ^{3}H-MC (K_d = 53 ± 9 nM) are present in membrane preparations of squid optic ganglia; the number of these sites is the same, namely 22 ± 3 pmoles/g tissue. Analysis of dependence of inhibition of specific binding of ^{14}C-TC by cytisine, N-methylcytisine, and N,N'-dimethylcytisine, shows that these ligands compete for binding with sites of one type (Fig. 1a). Introduction of one methyl group into the cytisine molecule caused no change in its affinity for NACR of the squid optic ganglia. Values of K_d for N-methylcytisine, calculated from the results of analysis of the data by Scatchard plot for binding the radioactive derivative, and also from the value of IC_{50} for binding of ^{41}C-TC were identical, namely 50 nM. N,N'-Dimethylcytisine also inhibited specific binding of ^{14}C-TC competitively; however, its affinity for the receptor (K_i = 83 μM) was more than three orders of magnitude lower than that of N-methylcytisine.

Cytisine inhibited binding of ^{14}C-TC (Fig. 1b) with NACR of the skate electric organ rather less effectively. K_i of cytisine, calculated on the basis of IC_{50}, was 700 ± 90 nM, which is close to the value of K_i = 350 nM, obtained by other workers (Huho, verbal communication). However, neither N-methylcytisine nor N,N'-dimethylcytisine in concentrations up to 10^{-4} M had virtually any effect on specific binding of ^{14}C-TC. Correspondingly, no specific binding sites for ^{3}H-MC were found in membrane preparations of the skate electric organ.

In the existing view, two functionally important fragments can be distinguished in the composition of molecules of cholinergic ligands: a tertiary or quaternary ammonium group (the "cationic head") and the oxygen atom of the carbonyl group or another nucleophilic atom; however, the affinity of the ligand for the receptor is determined not only by a certain distance between the functionally important atoms, but also by their orientation relative to the recognition site [3, 4]. Correspondingly, in the recognition site of the receptor we can distinguish a binding site for the nucleophilic atom and a binding site for the "cationic head" – the anionic point, which is a spherical cavity corresponding in size to the diameter of the tetramethylammonium ion [4, 6].

The high affinity of cytisine for NACR of the squid optic ganglia and also the fact that this compound is an agonist [6], indicate that its structure is highly complementary to the recognition site of the receptor. Since the cytisine molecule has a rigid spatial structure, introduction of one methyl group ought not to change the distance between the functionally important atoms and their orientation relative to the binding site, but ought to affect only the size of the "cationic head" of the ligand. The equal affinity of cytisine and N-methylcytisine for NACR of the squid optic ganglia is evidence that introduction of one methyl group does not disturb complementarity for the receptor in the region of the anionic point. The introduction of a second methyl group evidently has the result that the cationic head of the ligand does not fit into the anionic point of the receptor. Under these circumstances the orientation of the oxygen atom of the carbonyl group of the ligand may be deflected from the optimal, and collectively this causes a sharp reduction of affinity of N,N'-dimethylcytisine for the receptor. In the case of neuromuscular receptors of nicotinic specificity, the classical representative of which is the NACR of the skate electric organ, steric hindrances in the region of the anionic point arises when only one methyl group is introduced into the cytisine molecule.

It can be concluded on the basis of these results that the recognition sites of cholinergic ligands of neuromuscular receptors and NACR in the CNS, a representative of which is the nicotinic receptor of the squid optic ganglion, differ in the configuration of their anionic points, and they indicate that further study of the pharmacologic properties of N-methylcytisine as a selective agonist of nicotinic specificity with a central type of action would be useful.

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