less behavior can be regarded as the result of overproduction of acetylcholine and its cumulation at synaptic level, with the development of synaptic blockade. This hypothesis is confirmed by the fact that injection of drugs lowering the acetylcholine level (atropine, benactyzine) abolishes the signs of aggression, lowers the intensity of the emotional background, and restores the blocked conditioned reflex, whereas arecoline, which releases additional amounts of acetylcholine from the depots [8, 13], potentiates these undesirable effects. Since administration of β-ethyldifacil did not change the rats' behavior, it can be postulated that predominantly the muscarinic rather than the nicotinic cholinergic system is involved in the mechanism of action of cleregil. Since α-methyldopa and pyracetam can partially replace cleregil under the conditions of a stable dissociated state, it can be postulated that not only the cholinergic, but also other mediator systems are involved in the mechanism of development of dissociation.

LITERATURE CITED

1. A. A. Azarashvili, Investigation of Mechanisms of Memory with the Aid of Physiologically Active Compounds [in Russian], Moscow (1981).
2. T. A. Voronina and T. L. Garibova, Byull. Éksp. Biol. Med., No. 1, 49 (1983).
3. R. Yu. Il'yuchenok, Pharmacology of Behavior and Memory [in Russian], Novosibirsk (1972).
4. N. A. Losev, "Pharmacologic analysis of α- and β-derivatives of benactyzine and adiphenine," Author's Abstract of Candidate's Dissertation, Leningrad (1968).
5. N. V. Savateev, in: The Physiological Role of Acetylcholine and the Search for New Drugs [in Russian], Leningrad (1957), pp. 40-49.
6. J. Bures et al., in: Physiologie de l'Hippocampe. Colloque International de la Recherche Scientifique, Paris (1962), pp. 107-241.
7. J. Faure and R. Pierre, Thérepie, 16, 86 (1961).
8. B. Holmstedt, Ann. N.Y. Acad. Sci., 144, 433 (1967).
9. G. V. Koff and T. W. Langfitt, Arch. Int. Pharmacodyn., 164, 272 (1966).
10. D. A. Overton, Psychopharmacology, 76, 385 (1982).
11. D. A. Overton, J. Pharmacol. Exp. Ther., 221, 166 (1982).
12. C. C. Pfeiffer et al., Science, 126, 610 (1957).
13. J. M. J. van Rossum, J. Pharm., 15, 285 (1963).

COMPARATIVE STUDY OF MUSCARINIC ACETYLCHOLINE RECEPTORS OF HUMAN AND RAT CORTICAL GLIAL CELLS

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KEY WORDS: muscarinic acetylcholine receptors; glia; human brain; rats.

For the overwhelming majority of subpopulations of acetylcholine receptors in the vertebrate brain the cellular and subcellular localization has not been established and, in particular, acetylcholine receptors of glial cells have virtually not been studied. However, there is much information on the high level of enzymes for acetylcholine synthesis and breakdown in glial cells. For instance, cholinesterase [7], acetylcholinesterase [1, 4, 9], and cholineacetyltransferase [10] have been found in glial cells. Although data on choline-acetyltransferase are contradictory [11], the presence of enzymes of acetylcholine metabolism in glial cells is indirect evidence of the presence of acetylcholine receptors in these cells

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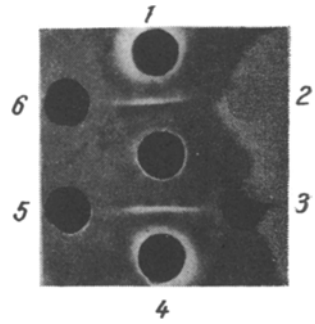

Fig. 1. Immunodiffusion analysis of glial fraction of rat brain for neuron-specific proteins. Central well contains monospecific antiserum to neuron-specific protein 14-3-2 in dilution of 1:8; peripheral wells contain: 1, 4) neuron-specific protein 14-3-2 (1 mg/ml); 2, 3, 5, 6) solubilized glial fraction in serial double dilutions.

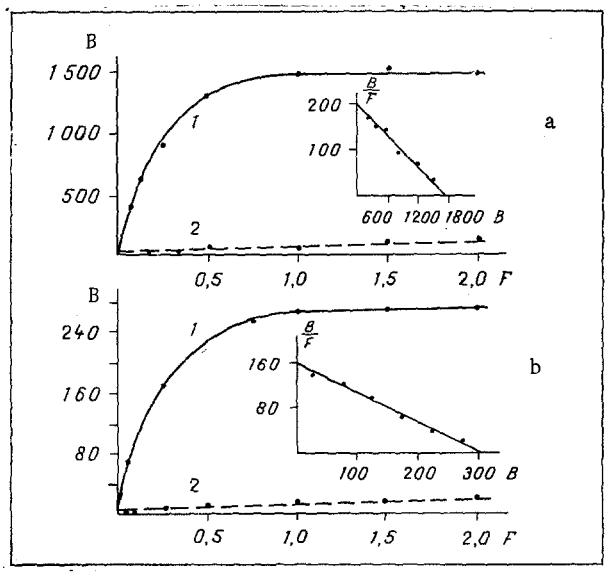

Fig. 2. Specific (1) and nonspecific (2) binding of [^{3}H]-QB (B, in moles/mg protein) by cortical glial cells of rat (a) and human (b) on concentration of ligand (F, in nanomoles). Inset: the same results, shown on Scatchard plot.

[8]. The possible presence of acetylcholine receptors in cultures of chick embryonic brain astroblasts has recently been demonstrated by radioligand analysis [13]. The aim of the present investigation was a comparative study of muscarinic acetylcholine receptors in human and rat glial cells.

EXPERIMENTAL METHOD

[^{3}H]Quinuclidinyl-benzylate ([^{3}H]-QB) (31 Ci/mmole, from "Amersham Corporation," England), atropine, platiphylline, decamethonium, carbamylcholine, tubocurarine (from "Sigma, West Germany), nicotine (base; from "Serva," West Germany) were used; the remaining reagents were from "Soyuzkhimreaktiv" (USSR) and were of the chemically pure grade.

The glial cell fraction was obtained from the cerebral cortex of rats weighing 130-140 g and from the frontal pole of the postmortem brain from men aged 60-70 years by the method in [5]. The purity of the glial preparations was verified immunochemically on the basis of the content of neurospecific proteins. Methods of isolation of neurospecific proteins and of preparation of antibodies were described previously [3].

Specific binding of [^{3}H]-QB with the preparation of glial cells was carried out in Ringer's solution for warm-blooded animals as described previously [2]. Binding of [^{3}H]-QB, inhibited by 10^{-5} M atropine or platiphylline, was considered to be specific. Protein was de-

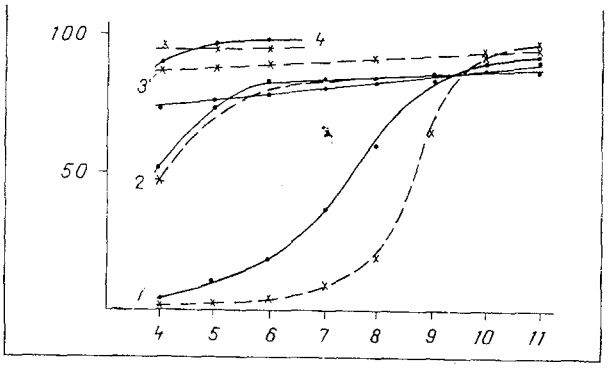

Fig. 3. Specific binding of [^{3}H]-QB with cortical glial cells of rat (broken line) and human (continuous line) brain as a function of concentration of atropine (1), nicotine (2), D-tubocurarine (3), and decamethonium (4). Abscissa, log F (in M); ordinate, B (in %). Remainder of legend as to Fig. 2.

termined by the method in [12]. The radioactivity of the samples was measured on a "Mini-Rack" counter (from LKB, Sweden) in "Unisolve-100" scintillator (from "Koch-Light," England). The counting efficiency in the channel for [^{3}H] was 49%. The experimental data were subjected to statistical analysis by a modified program on an HP-97 calculator (from "Hewlett-Packard," USA).

EXPERIMENTAL RESULTS

Since the existence of muscarinic acetylcholine receptors in the glial cells could be explained by contamination with neurons, the content of neurospecific proteins 14-3-2 and S-100 in the glial preparations was studied by the method of double immunodiffusion in agar gel. Individual proteins and monospecific rabbit antisera against these proteins were used as the test system for each neurospecific protein. As will be clear from Fig. 1, the glial fraction of rat brain in serial dilutions does not exhibit downward bending of the precipitation zones of the test system toward protein 14-3-2. This is evidence of the absence of protein 14-3-2, a specific protein of neurons [3], in the glial fraction and it can be deduced that this fraction contained no neurons.

The next step was to study the pharmacologic properties of muscarinic acetylcholine receptors of glial cell membranes. As Fig. 2 shows, the number of receptors, determined from specific binding of the antagonist with muscarinic specificity [^{3}H]-QB, in rat glial cells was about five times higher than in human glial cells. [^{3}H]-QB bound more firmly with acetylcholine receptors of the glia of the human brain; the dissociation constant KD was 2.0 and 9.1 nM for glial receptors of the human and rat brain, respectively.

Specific binding of [^{3}H]-QB (Fig. 3) is not inhibited by nicotine, D-tubocurarine, and decamethonium for both human and rat glial cells, evidence that the pharmacologic properties of the muscarinic acetylcholine receptors of the glia are similar to those of neuronal receptors of brain synaptosomes and they distinguish glial receptors from muscarinic receptors of the insect brain [6], which are sensitive to decamethonium and tubocurarine. It will also be clear from Fig. 3 that displacement of [^{3}H]-QB from rat glial receptors by atropine is observed at lower concentrations than in the case of glial receptors of the human brain, in agreement with the value of KD for complexes of receptors with [^{3}H]-QB. It will also be clear that the curve of displacement of [^{3}H]-QB by atropine in the case of human glial cells is more sloping, an indirect indication of the presence of several populations of muscarinic receptors.

The use of the method of radioimmune binding of [^{3}H]-QB with human and rat glial cell membranes thus demonstrated the presence of a muscarinic acetylcholine receptor in the glial cells. Acetylcholine receptors of human cells have higher affinity for QB than receptors of rat glial cells. By their pharmacologic properties acetylcholine receptors of the glia of the mammalian brain differ from receptors of the insect brain and are similar to the receptor systems of synaptosomes of the animal brain.

LITERATURE CITED

1. V. N. Anders et al., in: Functions of the Neuroglia [in Russian], Tbilisi (1976), p. 31.
2. V. P. Demushkin and N. V. Khramtsov, Biokhimiya, No. 1, 425 (1981).
3. S. D. Zaiko et al., Biokhimiya, 43, 1825 (1978).
4. I. V. Torskaya, in: Functions of the Neuroglia [in Russian], Tbilisi (1976), p. 30.
5. C. Blomstrand and A. Hamberger, J. Neurochem., 16, 1401 (1969).
6. Y. Dudai, Trends Biochem. Sci., 4, 40 (1979).
7. M. S. Ghandour et al., J. Neurochem., 36, 195 (1981).
8. C. Hebb and A. Silver, in: Neuropathology. Methods and Diagnosis, Boston (1970), p. 665.
9. K. Hemminki et al., Int. J. Neurosci., 4, 219 (1972).
10. K. Hemminki, Int. J. Neurosci., 5, 87 (1973).
11. G. B. Peterson et al., Dev. Biol., 34, 119 (1973).
12. G. B. Peterson, Anal. Biochem., 2, 346 (1977).
13. H. Ropke and K. Maderspach, Brain Res., 232, 208 (1982).

PRESYNAPTIC CONTROL OF DOPAMINE RELEASE BY β-PHENYLETHYLAMINE

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KEY WORDS: local perfusion of the brain; [^{3}H]dopamine; release; β-phenylethylamine.

It has been suggested on the basis of existing experimental data that endogenous [7, 11] β-phenylethylamine (β-PEA) can modulate the efficiency of dopaminergic synaptic transmission [4, 6, 15] through its action on the presynaptic [4, 8] and postsynaptic [3] parts of the dopaminergic synapse. The presynaptic action of β-PEA has been shown to include both potentiation (facilitation) of dopamine (DA) release and, although admittedly to a much lesser degree, inhibition of DA reuptake by dopaminergic endings [4].

It was accordingly decided to study the effect of extracellular ions (Ca^{++}, Na^{++}) on the β-PEA releasing effect, dependence of this effect on the membrane potential of dopaminergic endings, and the participation of dopamine presynaptic autoreceptors in the realization of the effects of β-PEA on DA release.

EXPERIMENTAL METHOD

The method of local perfusion of the brain by a push-pull cannula system, described by the writers previously, with simultaneous recording of brain electrical activity from the region of perfusion [2] was used. Experiments were carried out on noninbred male albino rats weighing 300-350 g, anesthetized with pentobarbital (40 mg/kg). During the preliminary operation a guide tube with the recording electrode (AP = −1.5; L = 2.5; V = 5) was inserted into the neostriatum and stimulating electrodes (AP = 4.5; L = 2; V = 8.5) were inserted into the substantia nigra stereotaxically, using the atlas of Fifkova and Marsala. The parameters of stimulation were threshold strength for evoked potential generation in the region of the neostriatum (30 V, 0.1 msec). By means of a microsyringe, [^{3}H]-DA hydrochloride ([^{3}H]-DA) in a dose of 3-4 μl, $1.25 \cdot 10^{-9}$ M (specific activity 3.2 Ci/mmole, from Amersham Corporation, England) was injected through the guide tube into the region destined for perfusion. After a 30-min period of application necessary for uptake of the exogenous DA by the cells, perfusion of that region of the brain began. The push-pull cannula was inserted into the neostriatum through the guide tube as far as the level of the tip of the recording electrode. An artificial CSF of the following composition was used for perfusion (in mM): NaCl 126.5, KCl 2.4, $CaCl_2$ 1.1, KH_2PO_4 0.5, $NaHCO_3$ 27.5, $MgSO_4$ 1.1, glucose 5.9 [12], with the addition of the monoamine oxidase inhibitor iproniazid (10^{-6} M). Before the experiment the perfusion fluid was

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