LOCALIZATION OF CHOLINERGIC SYSTEMS IN VARIOUS PARTS OF THE BRAIN, ADRENAL MEDULLA, AND MYOCARDIUM OF RATS

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Besides the view that cholinergic systems are mainly localized in the mesencephalic reticular formation of the brain stem [5, 15, 17], the opinion has also been expressed that they are distributed diffusely in the central nervous system [13], and in particular, that cholinergic structures are also present in the cerebral cortex [10, 14].

On the basis of quantitative differences in the action of muscarine-like cholinolytics (benactyzine, metamizil, scopolamine), and the nicotine-like cholinolytics (pachycarpine, adiphenine, caramiphen) on the activation reaction caused by stimulation of the reticular formation by an electric current or by administration of cholinomimetic drugs, many investigators have concluded that the cholingergic systems of the reticular formation are structures of muscarine-sensitive type [3, 4, 6, 7]. In the cerebral cortex [2, 16], the mesencephalic reticular formation, thalamus, caudate nucleus and hypothalamus [1] it is considered that mainly muscarine-like cholinergic systems are present.

All the investigators cited above studied the localization of cholinergic structures in the central nervous system using electrophysiological and pharmacological methods of analysis.

In the present investigation an attempt was made at a biochemical approach to the problem of localization of the cholinergic structures in the brain and some other tissues.

The choline-receptive substance of the frog's myocardium is a protein containing free sulfhydryl groups. During the action of a thiol poison (mercuric chloride) inactivation of the choline-receptive protein and corresponding loss of sensitivity of the heart to acetylcholine are observed. In the presence of acetylcholine the ability of the thiol groups of the choline-receptive protein to react with mercury ions is depressed, and this is seen as the "acetylcholine wave" during mercurimetric titration of tissue homogenates [11]. The "acetylcholine wave" is found only during titration of homogenates from organs sensitive to acetylcholine, and it may be used as a test for choline-receptive protein [9, 12].

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 200-250 g. After decapitation of the animals the brain, adrenals, and heart were extracted. With constant cooling, homogenates were prepared from the gray matter of the cerebral cortex, thalamus, hypothalamus (the suprahypophyseal part), the mesencephalon (the corpora quadrigemina), the left ventricle of the heart, and the medulla of the adrenals. The thiol groups in the homogenates containing 50 mg of fresh tissue were estimated quantitatively by the method of amperometric titration [8] with a 0.001 N solution of mercuric chloride.

Mercurimetric titration was carried out in the absence and presence of acetylcholine chloride in a final concentration of $1 \cdot 10^{-4}$ (using neostigmine $2 \cdot 10^{-5}$ as stabilizer), and also in the presence of adiphenine and substances known to cause selective excitation or paralysis of muscarine-like (arecoline, benactyzine) or nicotine-like (nicotine, benzohexonium) cholinergic structures.

Besides experiments on the brain of intact rats, titration was also carried out in the presence of acetylcholine on tissue homogenates of rats receiving a subcutaneous injection of iproniazid or isoniazid in a dose of 100 mg/kg 18 h before the experiment. Each series of experiments was carried out on 4-7 rats.

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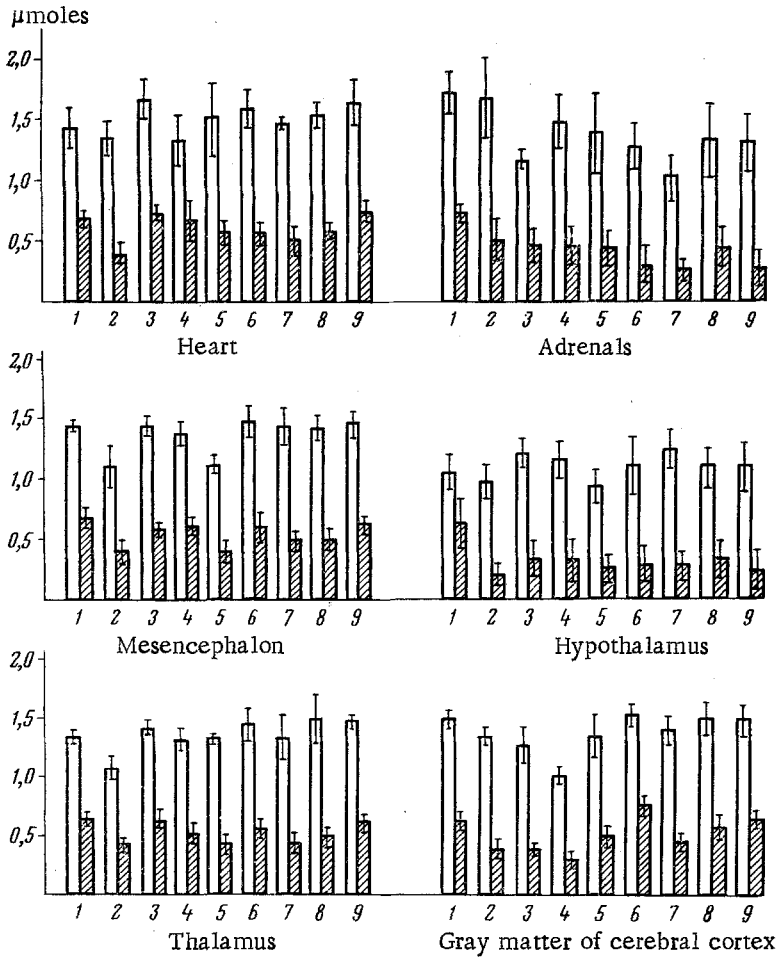

Fig. 1. Total quantity of titratable thiol groups (in μmoles/100 mg tissue, unshaded columns) and quantity of SH-groups (in μmoles/100 mg tissue) completely binding mercury ions (shaded columns) in homogenates of various rats' tissues. 1) Titration in tissue homogenates of intact rats; 2) the same in the presence of acetylcholine ($1 \cdot 10^{-4}$); 3) titration in the presence of acetylcholine in tissue homogenates of rats prepared by administration of iproniazid; 4) the same in rats prepared by administration of isoniazid; 5) titration in tissue homogenates of intact rats in the presence of arecoline ($2 \cdot 10^{-4}$); 6) the same in the presence of nicotine ($2 \cdot 10^{-4}$); 7) in the presence of benactyzine ($2 \cdot 10^{-4}$); 8) in the presence of adiphenine ($2 \cdot 10^{-4}$); 9) in the presence of benzohexonium ($2 \cdot 10^{-4}$).

EXPERIMENTAL RESULTS

The results obtained are shown in Fig. 1. The difference between the heights of the second and first shaded columns determines the size of the "acetylcholine wave." This wave was revealed during mercurimetric titration of homogenates of all the studied tissues (Fig. 1, 1 and 2) carried out in the presence of acetylcholine, and this demonstrates the presence of choline-receptive protein in these tissues.

The choline-receptive protein discovered in this way may react, despite the acetylcholine, with arecoline, benactyzine, and adiphenine. In the presence of these substances an "acetylcholine-like" wave appeared: an "arecoline," "benactyzine," and "adiphenine" wave. When homogenates of the gray matter of the cerebral cortex were titrated, only an "adiphenine" wave could be detected (Figs. 1, 5, 7, and 8).

Meanwhile, mercurimetric titration of tissue homogenates carried out in the presence of nicotine and benzohexonium revealed an "acetylcholine-like" wave—a "nicotine" and "benzohexonium" wave—only in the homogenates of the adrenal medulla and the hypothalamus. In the other tissues no "nicotine" or "benzohexonium" wave was found.

Effect of Acetylcholine and Several Other Substances on the Course of Mercurimetric Titration of Homogenates of Certain Rats' Tissues

Homogenate	Presence or absence of "acetylcholine wave" during mercurimetric titration in the presence of						Effect of hydrazides (iproniazid and isoniazid) on "acetylcholine wave"*
	acetylcholine	arecoline	benactyzine	adiphenine	nicotine	benzohexonium	
Ventricle of the heart	+	±	+	+	±	−	abolishes it
Adrenal medulla	+	+	+	+	+	+	no change
Mesencephalon	+	+	+	+	−	−	abolishes it
Suprahypophyseal region of the brain	+	+	+	+	+	+	no change
Thalamus	+	+	+	+	−	−	abolishes it
Gray matter of cerebral cortex	+	±	+	−	−	−	no change

*See Figs. 1, 3, and 4.

Legend: + statistically significant presence, - absence of "acetylcholine" or "acetylcholine-like" wave, ± the difference of the means indicating the presence of an "acetylcholine" or analogous wave lies at the borderline of significance (P = 0.05).

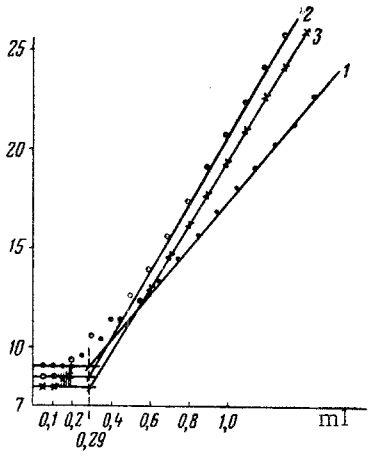

Fig. 2. Curves of mercurimetric titration of thiol groups in homogenates of the hypothalamus with 0.001 N solution of $HgCl_2$. 1) Control; 2) in the presence of arecoline ($2 \cdot 10^{-4}$); 3) in the presence of nicotine ($2 \cdot 10^{-4}$).

Curves of mercurimetric titration of homogenates of the suprahypophyseal region of the rats' brain are given in Fig. 2. In the presence of arecoline (2) and of nicotine (3) the course of the titration curve was modified, showing an "acetylcholine wave" (the shaded parts of the curve), due to interaction of the arecoline and nicotine with choline-receptive protein.

If it assumed that choline-receptive protein, which is capable of interacting with arecoline, may also react with nicotine or benzohexonium, it still is not clear why nicotine and benzohexonium react only with the choline-receptive protein of the hypothalamus, the adrenal medulla, and possibly the heart, although protein capable of reacting with arecoline was found in all the investigated tissues (see table). It is evident that two types of choline-receptive protein exist. One of them, capable of reacting with nicotine and benzohexonium, is found in the adrenal medulla and the hypothalamic region of the brain in rats, but is absent from the other investigated tissues.

It may be concluded from the results taken as a whole that the cholinergic structures in the muscle of the left ventricle of the heart, the mesencephalon, and the thalamus of rats are structures of muscarine-sensitive type. The hypothalamus and adrenal medulla contain two types of choline-receptive proteins. The specific effect of the cholinergic substances is evidently due, not only to the presence of the choline-receptive protein, but also to its inclusion in the excitable structures of the cells.

LITERATURE CITED

1. S. V. Anichkov and Yu. S. Borodkin, In the book: Abstracts of Proceedings of the 10th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], Moscow–Leningrad, Vol. 1 (1964), p. 62.
2. A. V. Val'dman, In the book: Current Problems in the Pharmacology of the Reticular Formation and Synaptic Transmission [in Russian], Leningrad (1963), p. 281.
3. P. P. Denisenko, Farmakol. i Toksikol., No. 1, 8 (1962).
4. P. P. Denisenko, Vestn. Akad. Med. Nauk SSSR, No. 3, 48 (1962).
5. R. Yu. Il'yuchenok and M. D. Mashkovskii, Fiziol. Zh. SSSR, No. 11, 1352 (1961).
6. R. Yu. Il'yuchenok and R. U. Ostrovskaya, Farmakol. i Toksikol., No. 6, 643 (1962).
7. R. Yu. Il'yuchenok and R. B. Matveeva, Farmakol. i Toksikol., No. 5, 525 (1963).
8. S. N. Nistratova, In the book: Thiol Compounds in Medicine [in Russian], Kiev (1959), p. 89.

9. S. N. Nistratova and T. M. Turpaev, Biokhimiya, No. 1, 171 (1959).
10. G. D. Smirnov and R. Yu. Il'yuchenok, Fiziol. zh. SSSR, No. 10, 1141 (1962).
11. T. M. Turpaev, The Mediator Function of Acetylcholine and the Nature of the Choline-Receptor [in Russian], Moscow (1962).
12. T. M. Turpaev, S. N. Nistratova, and E. M. Roitburg et al., Fiziol. zh. SSSR, No. 4, 502 (1964).
13. F. B. Bradley, In the book: The Reticular Formation of the Brain [Russian translation], Moscow (1962), p. 119.
14. J. E. Desmedt and G. la Grutta, J. Physiol. (London), Vol. 136 (1957), p. 20.
15. K. A. Exloy, G. Fleming, and A. D. Espelien, Brit. J. Pharmakol., Vol. 13 (1958), p. 485.
16. V. Fliris, G. Morocutti, and G. F. Ayala, Boll. Soc. ital. Biol. Sper., Vol. 38 (1962), p. 407.
17. F. Rinaldi and H. E. Himwich, Arch. Neurol. Physchiat. (Chic.), Vol. 73 (1955), p. 396.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
