EFFECT OF HYDRAZINE DERIVATIVES ON THE CHOLINOREACTIVE STRUCTURES OF THE BRAIN

(UDC 612.826.1.014.46:615.787)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 8, pp. 69-73, August, 1965
Original article submitted April 28, 1964

T. M. Turpaev and S. N. Nistratova have established that the influence of acetylcholine on the cholinoreactive structures of the frog heart is accompanied by a reduction of the reactivity of the thiol groups of the cholinoreactive protein to mercury ions, which is revealed in the form of the "acetylcholine wave" in the mercurimetric titration of a cardiac muscle homogenate. In the opinion of the authors, this peculiarity of the reaction between acetylcholine and the receptor is a specific indicator of cholinoreceptive protein [4].

Using the indicated principle, we detected cholinoreceptive protein in various portions of the brain and demonstrated that the interaction of acetylcholine with this protein is changed under the influence of hydrazine derivatives—tubazide and iprazide (iproniazide).

EXPERIMENTAL PROCEDURE

The experiments were conducted on white rats, weighing 200-250 g. Intact animals and animals that had received subcutaneous injections of iprazide or tubazide (100 mg/kg) 18 h before the experiment were decapitated; the brain was isolated; and homogenates were prepared with constant chilling from the grey matter of the cerebral cortex, thalamus, hypothalamus (suprahypophysary region), midbrain (corpora quadrigemina), left ventricle of the heart, and adrenal medulla. The thiol groups were quantitatively determined in homogenates containing 50 mg of crude tissue by the method of amperometric titration according to Kolthoff [5] in the modification of S. N. Nistratova [3]. Titration was conducted with 0.001 N solution of mercuric chloride in the absence and in the presence of acetylcholine chloride in a concentration of $1 \cdot 10^{-4}$. In the latter case, proserine was added to stabilize the acetylcholine (final concentration $2 \cdot 10^{-5}$).

EXPERIMENTAL RESULTS

Figure 1 shows typical curves of mercurimetric titration of a thalamus homogenate. The titration of the tissue of an intact rat (Fig. 1, 1) is represented by a curve, the horizontal portion of which corresponds to total bonding of the mercury ion, while the point of inflection corresponds to partial bonding. The third portion of the curve corresponds to a directly proportional increase in the diffusion potential, when all the free thiol groups are entirely blocked.

When a thalamus homogenate from an intact rat is titrated in the presence of acetylcholine, the rise in the curve begins considerably earlier (the "acetylcholine wave"), but the total number of thiol groups measured by the volume of the mercuric chloride solution used for titration is unchanged (Fig. 1, 2). The magnitude of the "acetylcholine wave" may be expressed quantitatively by the difference in the millimoles of thiol groups capable of entirely bonding mercury ions in the titration of tissue homogenates in the absence and in the presence of acetylcholine. As can be seen from Fig. 2, A, 1, 2, this difference is statistically reliable for all the titrated tissues, which is evidence of the presence of a cholinoreceptive protein capable of interacting with acetylcholine.

Titration of a rat thalamus homogenate preliminarily prepared by introducing tubazide, in the presence of acetylcholine, reveals that under these conditions the "acetylcholine wave" does not appear (Fig. 1, 3). Analogous

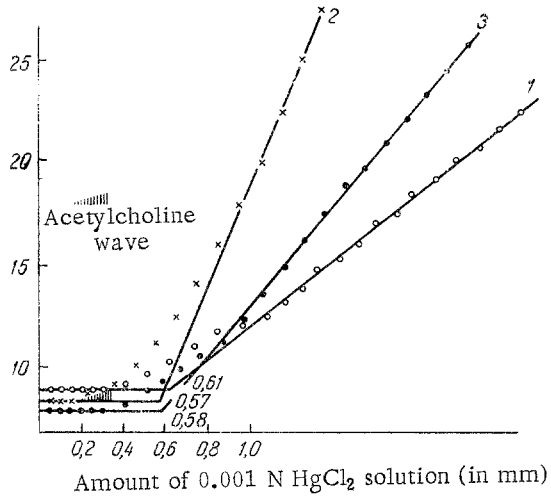

Fig. 1. Curves of mercurimetric titration of SH groups of thalamus homogenates titrated in the absence (1) and in the presence of acetylcholine (2), and thalamus homogenates (titrated in the presence of acetylcholine) of a rat, prepared by preliminary introduction of tubazide (3).

results were observed in the titration of homogenates of the heart and midbrain. The acetylcholine wave also does not appear in the titration of homogenates of these tissues if the animal received preliminary injections of iprazide (Fig. 2, A, I-III). Hydrazides, consequently, hinder the interaction of acetylcholine with the cholinoreactive protein in the tissue of the heart, midbrain, and thalamus. However, they do not disturb the interaction of acetylcholine with the protein that is receptive to it in the adrenal medulla, grey matter of the cerebral cortex, and hypothalamus, where the "acetylcholine wave" appears in the titration of homogenates of these tissues taken from rats preliminarily prepared with iprazide or tubazide (Fig. 2, A, IV-VI).

Considering that in animals prepared by the introduction of hydrazides, the "acetylcholine wave" does not appear in the titration of homogenates of the cardiac muscle in the presence of acetylcholine, while in the titration of homogenates of the adrenal medulla it is detected under the same conditions, we should assume that hydrazides hinder the reaction of acetylcholine with the protein receptive to it by an influence on the M-cholinoreactive structures, rather than the N-cholinoreactive structures.

Thus, we may assume a localization of the M-cholinoreactive structures in the thalamus and in the midbrain, while the N-cholinoreactive structures are evidently localized in cells of the cerebral cortex and in the hypothalamic region. These hypotheses coincide in general with the data obtained by the method of pharmacological analysis [1].

The influence of hydrazides on the cholinoreactive structures of certain divisions of the brain may be due to their direct action or may be the result of the accumulation of catecholamines and serotonin in the brain tissue. The results of the following series of experiments, conducted in vitro, eliminate the latter hypothesis.

It was found that tubazide, added directly to the homogenate of the heart and midbrain 15 min before its titration in the presence of acetylcholine, eliminates the "acetylcholine wave" (Fig. 2, B, 5), while the addition not only of tubazide, but also of serotonin to the homogenate has no effect upon the indicated action of tubazide (Fig. 2, B, 6, 7). Thus, the influence of hydrazides on the interaction of acetylcholine with the protein receptive to it, detected in the titration of homogenates of the heart and midbrain of rats that received preliminary injections of tubazide or iprazide, can be reproduced in vitro.

In addition, the interaction of acetylcholine with the cholinoreceptive protein is not disturbed (Fig. 2, B, 8, 9) if mercurimetric titration of homogenates of the heart and midbrain is conducted after the introduction of serotonin or adrenalin into the homogenate in a final concentration of $2 \cdot 10^{-4}$. In view of the possible decomposition of these amines by the tissue homogenates (absence of inhibitors of monoaminooxidase of O-methyltransferase in the homogenates), the experiment was conducted in 2 parallel samples, one of which was used for the titration, while the 2nd was used for biological determination of amines after the titration of the 1st sample had been complete. For this purpose, the homogenate of the heart of midbrain (20 μg/kg) was injected into the vein of narcotized rats, which also received injections of atropine and hexonium. Injection of homogenate into such rats was accompanied by an increase in the arterial pressure, which under conditions of atropinization and hexonium blockage of the N-cholinoreactive structures of the adrenals and ganglia may be due to the presence of serotonin or adrenalin in the homogenate (Fig. 3).

Thus, serotonin and adrenalin do not prevent the detection of the "acetylcholine wave," but hydrazine derivatives—tubazide and iprazide—disturb the process of interaction of acetylcholine with the cholinoreactive protein in homogenates of the heart, midbrain, and thalamus in rats, without, however, affecting the cholinoreactive structures of the adrenals, cerebral cortex, and hypothalamus. These data permit us to assume that the effect of inhibitors of monoaminooxidase, in particular, iprazide, may be made up of functional changes due to the decrease in the monoaminooxidase activity [2, 6-8] and effects that are not related to blockage of this enzyme.

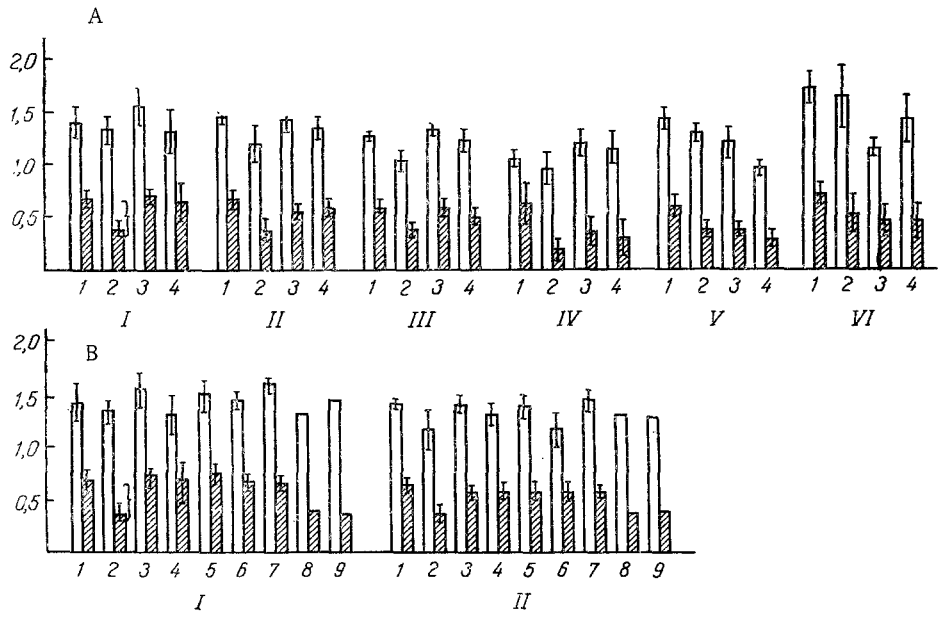

Fig. 2. Total amount of titratable thiol groups (unshaded columns) and amount of SH groups that entirely bond mercury ions (shaded columns) in homogenates of various tissues (I-VI). A) Experiments with preliminary administration of hydrazides to the animals; B) experiments with introduction of the investigated substances directly into the tissue homogenates: I) heart; II) midbrain; III) thalamus; IV) hypothalamic region; V) grey matter of the cerebral cortex; VI) adrenal medulla; 1) thiol groups are titrated in homogenates of tissues of intact rats in the absence of acetylcholine; 2) the same in the presence of acetylcholine; 3) thiol groups are titrated in the presence of acetylcholine in tissue homogenates from rats prepared by the injection of iprazide; 4) the same in rats prepared by injection of tubazide; 5) thiol groups are titrated in the presence of acetylcholine in tissue homogenates from intact rats 15 min after addition of tubazide to the homogenate; 6) the same 15 min after addition of tubazide and serotonin to the homogenate; 7) thiol groups are titrated in the presence of acetylcholine in tissue homogenates from rats prepared by preliminary injection of iprazide 12 min after addition of serotonin to the homogenate; 8) thiol groups are titrated in the presence of acetylcholine in tissue homogenates from intact rats 12 min after addition of serotonin into homogenate; 9) the same 12 min after addition of adrenalin into the homogenate. The brace denotes the value of the "acetylcholine wave."

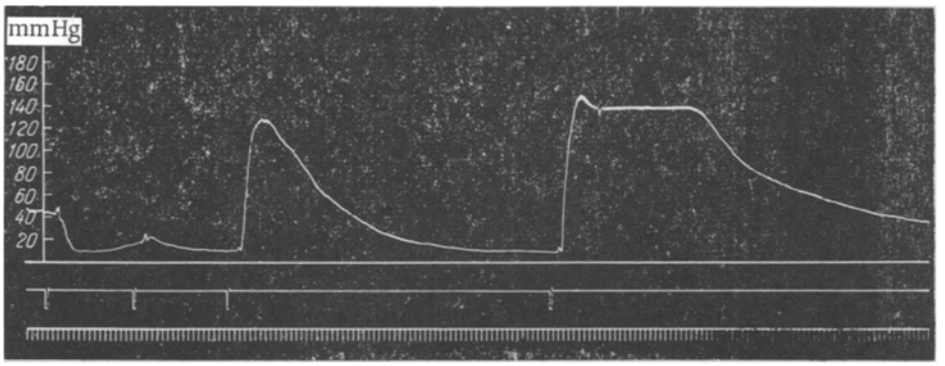

Fig. 3. Recording of the arterial pressure in the abdominal aorta of a narcotized rat. Significance of the curves (top to bottom): arterial pressure; zero line; indication of introduction (left to right) of: benzohexonium (2.5 mg/kg); atropine (2.5 mg/kg); homogenate of quadrigemina (0.1 ml/kg); adrenalin (20 μg/kg); time marking (5 sec). Weight of rat 260 g.

SUMMARY

The mercurimetric titration of homogenates of the heart, the cerebral substance of the adrenal glands, the grey matter of the brain cortex, thalamus, hypothalamus (suprahypophyseal region), the midbrain (quadrigeminal tubers) of rats carried out in the presence of acetylcholine ($1 \cdot 10^{-4}$) reveals an "acetylcholine wave" pointing to the presence in these tissues of cholinoreactive protein capable of interaction with acetylcholine. Hydrazine derivatives—tubazide and iprazide (iproniazide)—preliminarily injected to rats and directly injected into the homogenate interfere with the detection of the acetylcholine wave during titration in the presence of acetylcholine of the homogenates of the heart, midbrain and thalamus, but do not disturb the interaction of acetylcholine with the recipient protein in the homogenates of the cerebral substance of the adrenal glands, hypothalamus, and brain cortex. The capacity of hydrazides to interfere with the interaction between acetylcholine and cholinoreceptive protein is not caused by the accumulation of catecholamines or serotonin in the brain or heart tissue, because the amines themselves, when injected into the homogenate ($2 \cdot 10^{-4}$) do not interfere with the detection of the "acetylcholine wave."

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