MECHANISM OF THE PHENOMENON OF FIXATION OF ACETYLCHOLINE BY HUMAN BLOOD IN VITRO

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In previous communications [2, 3] we showed that other methods besides enzymic hydrolysis exist for the inactivation of acetylcholine (AC) in the organism, one of which is its fixation by elements of the circulating blood. The phenomenon of AC fixation has been studied by us in a large number of clinically healthy persons and also in patients with various forms of nervous diseases.

It has been found that the proportion of 1 ml of AC added in vitro (10^{-8}), fixed by blood taken from healthy persons during storage for 24 h in a refrigerator, varies from 9 to 49% (mean 29 ± 13%), and the corresponding figures when the blood is taken from patients with a lesion of the diencephalic region are from 70 to 97% (84 ± 8%) and from patients with myasthenia – from 82 to 100% (90 ± 5%).

The results obtained during the study of the phenomenon of AC fixation in patients with other nervous diseases range from 0 to 95%. The mean value differs in each group of patients and depends to a certain degree on the tone and reactivity of the sympathetic and parasympathetic divisions of the autonomic nervous system. It has also been established that the AC fixed by blood may be restored to a free state (reactivation of AC) by incubation for 3 h at 37°.

We have postulated that the fixation of AC by the blood is a compensatory mechanism directed towards inactivation of the surplus of distant-acting AC in the fluid media of the organism. The phenomena of the fixation of histamine and serotonin (histamino- and serotoninopectic effects) have been described in the literature [1, 4, 5, 6]. The existence of a bound form of AC in the organs and tissues has been known for some time, but there is no information regarding the fixation of AC by the blood.

Continuing our investigations, we have studied the mechanisms of the phenomenon of AC fixation and attempted to discover ways of influencing this process.

EXPERIMENTAL METHOD AND RESULTS

In the first place we attempted to determine whether the AC fixation in each individual subject is constant, or whether it may change as a result of various therapeutic procedures. Repeated investigations on healthy persons and patients at intervals of 2-3 months gave uniform results. Neither sex nor age differences could be detected. Consequently, the magnitude of the AC fixation is an individual characteristic of the organism, and is not dependent on change fluctuations in the neurohumoral regulation of functions. As therapeutic procedures, roentgen-ray irradiation of the thymus and operative removal of the gland were performed on patients under the care of L. B. Perel'man. The phenomenon of AC fixation was investigated before and after the course of treatment.

It was found that during a clinical improvement in the state of the patient the AC fixation by the blood falls to the level observed in healthy persons. Only in one case, when no clinical improvement was observed, the AC fixation fell insignificantly after thymectomy. In three patients with myasthenia, investigated once only after thymectomy, the fixation did not exceed 47%, i.e., it lay at the upper limit of normal, whereas very high fixation was characteristic of myasthenia. Hence, with an improvement in the myasthenic patient's condition, the fixation as a rule returned to normal.

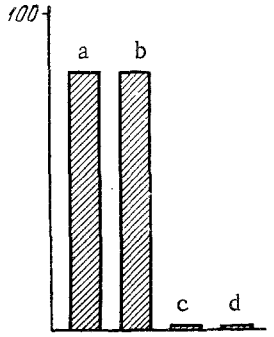

Fig. 1. Fixation of acetylcholine by the blood of a patient with myasthenia (a) by the erythrocytes of the same patient (b), by hemolyzed erythrocytes (c) and plasma (d).

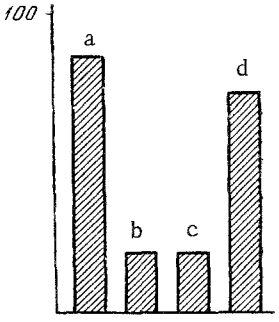

Fig. 3. Fixation of acetylcholine by the blood of a patient with myasthenia (a), by the erythrocytes of a patient with myasthenia mixed with the plasma of a healthy person (b), by the blood of a healthy person (c), and by the erythrocytes of a healthy person mixed with the plasma of a patient with myasthenia (d).

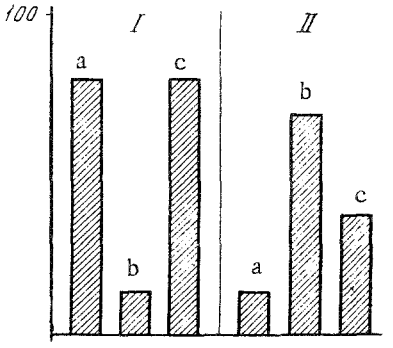

Fig. 2. I) Fixation of acetylcholine by the blood of a patient with myasthenia (a), with the addition of potassium chloride (b), and by washed erythrocytes with the addition of potassium chloride (c). II) fixation of acetylcholine by the blood of a healthy person (a), with the addition of calcium chloride (b), and with washed erythrocytes with the addition of calcium chloride (c).

To elucidate the mechanism of AC fixation, 5 series of experiments were conducted.

1. Relationship between Fixation Effect, AC Concentration, and Blood Dilution

To two samples of blood from the same subjects AC was added in concentrations of $1 \cdot 10^{-8}$ (sample 1) and $1 \cdot 10^{-9}$ (sample 2). In all subjects the percentage of AC fixed in sample 2, in which its concentration was lower, was greater than that in sample 1. Consequently, with a reduction in the amount of AC added, a proportionally greater part of it passed into the bound state.

Simultaneously with the determination of the fixing power of whole blood, investigations were made of the fixation of AC by blood diluted with Ringer's solution in proportions of 1:1, 1:2, and 1:3, the concentration of added AC being constant. It was found that the fixation fell in a linear relationship to the dilution of blood.

2. Importance of Individual Blood Elements for Fixation of AC

The fixing power of the erythrocytes, plasma, and hemolyzed erythrocytes was studied. For this purpose patients with a relatively high initial AC fixation were selected and the fixation of AC by centrifuged and washed erythrocytes, plasma, and erythrocytes hemolyzed with distilled water was investigated (Fig. 1). The fixation of AC by the erythrocytes was the same as its fixation by whole blood. After incubation, AC fixed by erythrocytes changed to the free form. The plasma of the same patients did not fix AC. Hemolyzed erythrocytes from patients with a high initial AC fixation in whole blood also failed to fix added AC.

3. Effect of Potassium Ions on Fixation of AC by Whole Blood and Washed Erythrocytes

Since potassium is known to influence certain stages of AC metabolism, for instance by increasing the ratio $\frac{\text{free AC}}{\text{fixed AC}}$, we studied the AC fixation phenomenon after addition of 0.1 ml of 2% KCl solution (2 ml to 8 ml of blood)

to the blood samples and the washed erythrocytes. In all patients with a high AC fixation, the addition of KCl led to a considerable lowering of the AC fixation (Fig. 2, I), and in some cases to its total suppression. Addition of KCl to washed erythrocytes did not change the AC fixation by the erythrocytes in 8 experiments, and in two it was lowered only very slightly. Consequently, potassium ions suppress or diminish the fixation of AC by erythrocytes only in the presence of the nonfixing part of the blood – the plasma.

4. Effect of Calcium Ions on AC Fixation by Whole Blood and Washed Erythrocytes

Investigations were conducted on healthy persons, and on patients with rheumatic encephalitis, a dienecephalic syndrome, arachnoiditis, and myotonia, and on patients with myasthenia in a state of remission after thymectomy. The subjects were selected in this way in order to study the effect of calcium ions on the AC fixation by the blood of persons with normal and high fixation values. In all the subjects the AC fixation after addition of 0.1 ml of 2% $CaCl_2$ solution was higher than initially (Fig. 2, II). A particularly important finding was that in patients with myasthenia, in whom a clinical remission after thymectomy was accompanied by a significant fall in the AC fixation (usually to normal), the addition of $CaCl_2$ caused a considerable increase in fixation, sometimes to the level determined before thymectomy.

The addition of $CaCl_2$ to the washed erythrocytes caused little or no increase in the AC fixation. A significant increase was observed in only 4 patients with myasthenia undergoing thymectomy, but it did not reach the level found after the addition of $CaCl_2$ to whole blood. Hence, calcium ions increase the fixation of AC by erythrocytes in the absence of plasma, but only in patients with myasthenia treated by thymectomy.

5. The Phenomenon of AC Fixation by a Mixture of Fixing Erythrocytes and Plasma from Nonfixing Blood or a Mixture of Nonfixing Erythrocytes and Plasma from Fixing Blood

In two series of experiments erythrocytes of patients with myasthenia with a high AC fixation were mixed with plasma from healthy persons with low AC fixation (sample 1), and erythrocytes of healthy persons with low AC fixation were mixed with plasma from patients with myasthenia and high AC fixation (sample 2). In each experiment the initial level of fixation by blood of a healthy subject (sample 3) and by blood from a patient with myasthenia (sample 4) was determined. In all cases the AC fixation in sample 1 was much lower than in sample 4. The AC fixation in sample 2 was much higher than in sample 3. Hence, the high fixing power of the erythrocytes of the patients with myasthenia was depressed by the plasma of the healthy person, while the low fixing power of the erythrocytes of the healthy person was increased by the plasma of the patient with myasthenia (Fig. 3).

It may be concluded from these findings that human blood, in vitro, has the power to fix a definite amount of added AC. If the AC concentration in the blood is lowered, the percentage undergoing fixation is increased correspondingly. If the blood is diluted, the fixation falls in accordance with a linear relationship.

It may be regarded as established that the elements fixing AC are unchanged (nonhemolyzed) erythrocytes. Plasma itself does not have the power to fix added AC.

The fact that potassium and calcium influence the AC fixation phenomenon shows that AC fixation is a complex biochemical process in which a part is played by the part of the blood not possessing fixing power – the plasma. The fact that the depressant action of potassium and the stimulant action of calcium on AC fixation are revealed principally in whole blood, i.e., erythrocytes in the presence of plasma, suggests the presence of a factor regulating AC fixation. The existence of a regulating factor in the plasma is confirmed by the results of a series of experiments involving crossed activation and depression of AC fixation by plasma of patients with myasthenia (in the former case) and of healthy persons (in the latter case). Under the influence of "foreign" plasma, the effect of AC fixation by the erythrocytes is modified and becomes close in magnitude to the AC fixation of the blood from which the plasma was obtained. It may be postulated on reasonably good grounds that the plasma of a healthy person contains an "anti-fixing factor" activated by potassium ions, and the plasma of patients with certain nervous diseases (myasthenia, lesions of the diencephalic region) contains a "fixing" factor, activated by calcium ions.

Thymectomy and roentgenotherapy of the thymus in patients with myasthenia, which give good clinical results, cause significant changes in AC fixation, which becomes closer to normal. The addition of potassium depresses the AC fixation by the blood of patients with myasthenia. The addition of calcium to the blood of patients with myasthenia, investigated during a period of clinical improvement following thymectomy, causes an increase in fixation to the initial high level.

The results show that the blood of healthy persons and of patients with various nervous diseases is capable of fixing added AC in vitro. Fixation is carried out by the erythrocytes, but its level is controlled by a certain factor contained in the plasma. The addition of potassium ions to the blood depresses, and of calcium ions stimulates AC fixation. We suggest that AC fixation by the blood in vitro characterizes the state of the autonomic nervous system, and is largely determined by the concentration of sympathetic and parasympathetic metabolites, hormones, and electrolytes in the blood.

SUMMARY

The phenomenon of acetylcholine binding with human blood was described by the authors in 1961. At low temperature the blood of a healthy individual is found to bind within 24 h 29-13% of an added 1 ml acetylcholine in a 10^{-9} dilution. The amount of acetylcholine mixed by the blood of patients suffering from various diseases varied within a wide range. The highest degree of binding (up to 100%) was noted in myasthenia and in some forms of diencephalic lesions. The binding phenomenon probably reflects the state (reactivity and tone) of the parasympathetic nervous system.

A study of the mechanisms of the phenomenon demonstrated that the binding capacity was confined to erythrocytes. Blood plasma contains a factor regulating the binding phenomenon. Hemolyzed erythrocytes fail to bind acetylcholine. Addition of potassium ions to the blood decreases its binding properties, whereas addition of calcium ions stimulates binding.

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