THE ACTION MECHANISM OF CERTAIN ANTICHOLINESTERASE PREPARATIONS

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In 1934, Walker [12] showed that physostigmine, a strong cholinesterase inhibitor, caused a marked improvement in the condition of myasthenia patients. A number of anticholinesterase preparations are now being used to treat this disease (Prostigmin, proserine, Mestinone, Oxamysil et al.). The action mechanism of these substances mainly consists in their anticholinesterase effect. According to the anticholinesterase theory, acetylcholine insufficiency and neuromuscular block in myasthenia are relieved by the administration of preparations which depress cholinesterase activity and thereby mobilize acetylcholine.

Works have recently appeared, however, which give reason to suppose that the cholinesterase inhibition effected by anticholinesterase preparations is not the sole mechanism of their antimyasthenic effect. For example, it has been shown experimentally on a nerve-muscle preparation that neostigmine retains its effect on muscle even after strong DFP inhibition of cholinesterase [6, 11]. The administration of DFP to myasthenia patients caused marked cholinesterase inhibition, but only a mild therapeutic effect [10].

Wilson and co-workers [13] compared the dynamics of change in muscular strength with the inhibition of true and false cholinesterase (TCE and FCE) effected by physostigmine. They discovered that the changes in muscular strength and in FCE corresponded exactly. The change in TCE did not, however, correspond with either of the other two indices.

Guanidine has a clear antimyasthenic effect although it does not possess anticholinesterase properties. Moreover, caffeine and the antihistamines, which are cholinesterase inhibitors, are known to have no specific antimyasthenic effect, and quinine, which also inhibits cholinesterase, actually impairs the condition of myasthenia patients. There are, therefore, many contradictory facts which do not conform with the anticholinesterase theory.

An important role in the genesis of myasthenic disorders has been recently ascribed to disturbance of the sympathetic nervous system. There are indications that proserine normalizes the transmission of stimulation in the synapses of this system [1]. A. M. Utevskii and co-workers have shown that the trophic influence of the sympathetic nervous system is realized through the metabolism of adrenalin and adrenalin-like substances [7]. L. B. Perel'man and É. Sh. Matlina [5] established that the improvement in the clinical condition of myasthenia patients resulting from the administration of proserine is attended by an increased content of adrenalin-like substances (ALS) in the blood. An increase in the amount of noradrenalin excreted with the urine was observed by É. Sh. Matlina and V. M. Prikhozhan [3] in myasthenia patients treated systematically with anticholinesterase preparations.

The association of the anticholinesterase with the antimyasthenic properties of anticholinesterase preparations is based on a study of short-acting substances of the Prostigmin type. In order to gain a more exact notion of the interrelationship between these properties, we made a parallel study of proserine, a short-acting preparation, and Oxamysil, a long-acting preparation synthesized in 1958 at the All-Union Institute of Pharmaceutical Chemistry (synonyms – Myssuran, Mytelasa, Win-8077). Our purpose was to compare the dynamics of the clinical condition of myasthenia patients administered these anticholinesterase drugs with the changes in the FCE, TCE and ALS content in the blood.

EXPERIMENTAL METHODS

Dynamic observation of the latter indices according to the existing methods meant that large quantities of blood would have to be taken repeatedly from a vein. Such intervention is extremely traumatic, especially in the case of patients with a severe disease like myasthenia. We therefore modified the established methods of determining FCE, TCE and ALS. In these microdeterminations, FCE and TCE were determined in 0.1 ml of blood taken from a finger, and ALS was determined in another 0.1 ml of blood.

TCE and FCE were determined by the method of S. R. Zubkova and N. V. Pravdich-Neminskaya as modified by É. Sh. Matlina and V. M. Prikhozhan [4]. The level of FCE and TCE activity was expressed in milligrams of acetylcholine destroyed by each cholinesterase in 0.1 ml of plasma or erythrocytes, incubated at 37° for 30 min.

ALS was determined in the blood by É. Sh. Matlina's modification of Show's method [2].

We observed 30 myasthenia patients who had received proserine and Oxamysil. The dynamics of the clinical condition and humoral changes under the influence of proserine and Oxamysil were studied in 17 patients. The investigations were done on an empty stomach. Each patient was investigated at least 12 hrs after his last dose of the anticholinesterase preparations. After blood had been taken from a finger, the patient was given 10 mg Oxamysil internally or 1.5 ml of a 0.05% proserine solution subcutaneously. These doses were used because they have been found to be optimal for the majority of myasthenia patients. Fifteen, 30, 60, 120, 180 and 240 min after administration of the preparation, the changes in clinical condition were recorded, and blood was taken for the TCE, FCE and ALS determinations (the first blood specimen was taken after 30 min in the patients given Oxamysil).

EXPERIMENTAL RESULTS

Dynamics of Clinical Condition. The change in the muscular strength of the hand (determined by dynamometer) paralleled the general dynamics of the clinical condition (we investigated patients with a generalized form of myasthenic disturbances). We will therefore present only the dynamometric data.

As soon as 15 min after the administration of proserine, 80% of the cases showed a clear increase in muscular strength; the maximal increase was observed after 30 min in 50% of the cases and after 60 min in 40%. Strength began to decline after 120 min in 50% of the cases. The increase in muscular strength varied from 4 to 22 kg.

All changes in muscular strength effected by Oxamysil were much slower to develop than those induced by proserine. An increase in muscular strength was observed after 30 min in 64% of the cases, and after 60 min in the other 36%. The muscular effect was maximal after 120 min in 72% of the cases, and after 180-240 min in the rest. In 75% of the patients, strength began to decline after 240 min, and in the rest of the patients, later. The increase in muscular strength ranged from 2 to 20 kg.

TCE Dynamics. In the 18 myasthenia patients investigated, the original level of TCE activity ranged from 0.76 to 1.76 (arithmetic mean – 1.26). The TCE activity in the patients observed was the same as it was in 20 healthy subjects.

Proserine administration depressed the activity of the enzyme in every case; this inhibition was first apparent after 15 min in 88% of the cases and was maximal after 60 min in 50% of the cases and after 120 min in the rest. In 40% of the cases, reactivation of the enzyme began after 180 min, while no reactivation was evident even after 240 min in 50% of the patients.

All TCE changes occurred later with the administration of Oxamysil than with the administration of proserine. Inhibition of the enzyme was observed after 60 min in 90% of the patients and, in a majority of cases (70%), was not maximal until the 240th minute.

FCE Dynamics. The original FCE level in the myasthenia patients ranged from 0.26 to 0.9 (arithmetic mean – 0.52). FCE, like TCE, activity was the same as in the 20 healthy people.

Proserine caused FCE inhibition after 15 min in 80% of the cases; maximal inhibition was observed after 60 min in 60% of the patients. In 50% of the cases, reactivation occurred after 180 min – after 240 min in the rest.

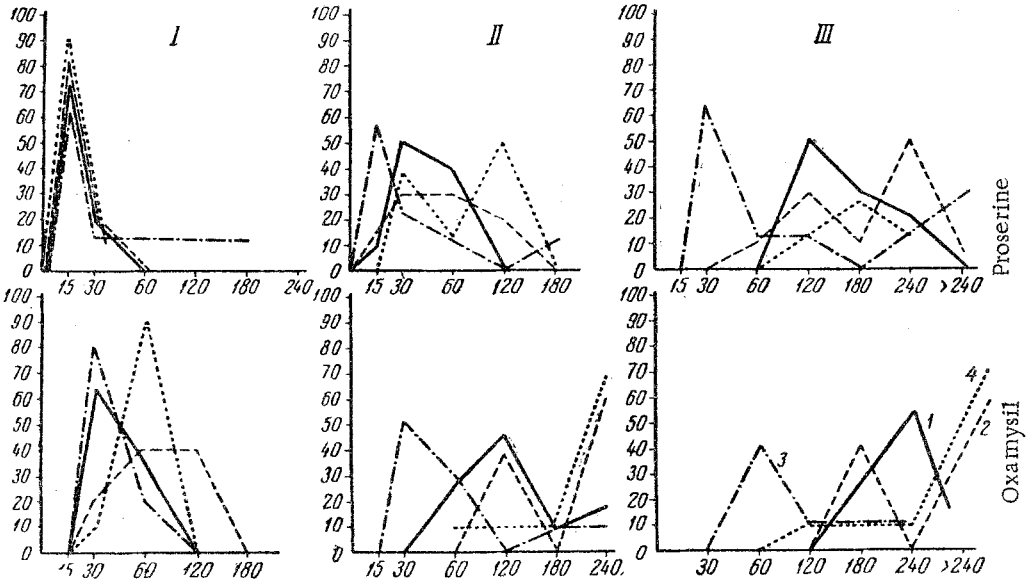
With Oxamysil administration, the changes in FCE were slower to appear. FCE inhibition was first observed after 60 min in 60% of the cases and became maximal after 120 min (40% of the patients) and 240 min (60%). One should note that the inhibition of the enzyme was regularly preceded (in 80% of the cases) by an increase in FCE activity observed after 30 min.

ALS Dynamics. The original ALS level in the myasthenia patients (18) ranged from 1 to 17.5 mg % (arithmetic mean – 6.8 mg %). In the 20 healthy people examined as the control, the ALS content ranged from 1.4 to 15 mg % (arithmetic mean – 7.3 mg %). Therefore, the ALS content of the blood was within the normal range in the majority of the myasthenia patients, which confirms the results we obtained earlier [5].

After proserine administration, an increase in the ALS content was observed after 15 min in 62% of the patients and was maximal at this time in 55% of the patients.

An increase in the ALS content was observed 30 min after the administration of Oxamysil in 80% of the patients; this increase was maximal in 80% of the cases after 30-60 min.

Therefore, the administration of proserine and Oxamysil leads to an increase in muscular strength and ALS content as well as inhibition of FCE and TCE.



Effect of proserine and Oxamysil administration on muscular strength, adrenalin-like substances and false and true cholinesterase in myasthenia patients. Abscissa axis: time of investigation (in min); ordinate axis; number of subjects (in percent). I) Time effect began; II) period of maximal changes; III) time effect began to abate. Top three graphs show proserine administration, bottom three – Oxamysil. I) Muscular strength; 2) FCE; 3) ALS; 4) TCE.

It was necessary to establish the sequence in which these changes occurred in order to better understand certain aspects of the action mechanism of these preparations. The times at which the effect became evident, the changes were maximal and the effect began to wear off were taken into account. The data obtained were depicted graphically as Gaussian distribution curves (see figure).

Proserine is known to be a short-acting preparation. All changes invoked by its administration occur comparatively rapidly. Increased muscular strength and ALS content and inhibition of FCE and TCE were observed after only 15 min in the majority of cases. The period when proserine's effect is maximal and the time the effect begins to wear off can be used to differentiate these indices in time. The first maximal changes were observed in the ALS content, the next in muscular strength and FCE activity and the last, in the TCE activity. The effect began to wear off in the following sequence: ALS, muscular strength, FCE and finally, TCE.

Analysis of the data giving the period of maximal effect and the time the effect began to abate led us to conclude that the increase in muscular strength and ALS content does not run parallel to the inhibition of the cholinesterases. This conclusion would be better founded if it were based on data giving the time of the initial, as well as the maximal changes. But because proserine is a fast-acting preparation and all the initial changes in the experimental indices were so close together in time, it was difficult to determine their sequence and impossible to judge the importance of cholinesterase inhibition and increased ALS content in the mechanism relieving the myasthenic symptoms.

Unlike proserine, Oxamysil is a long-acting preparation with an effect which is slow to develop. Therefore, the changes induced by this drug were easier to follow. In 64% of the cases, an increase in muscular strength was observed 30 min after the administration of Oxamysil, before any inhibition of FCE, and even TCE in a number of cases, was detected. The maximal increase in muscular strength (after 120 min) considerably preceded maximal cholinesterase inhibition, which was observed the 240th minute when muscular strength had already begun to decline. These data make it possible to conclude that the antimyasthenic effect of Oxamysil, perhaps that of proserine as well, is not wholly due to its anticholinesterase properties. After studying the effect of Oxamysil on a frog's rectus muscle, Karczmar [9] came to an analogous conclusion about this preparation.

An increase in the ALS content of the blood was observed in the majority of patients given proserine and Oxamysil. When Oxamysil was the drug administered, this increase preceded the initial and maximal FCE and TCE inhibition. In the case of proserine, the maximal changes in the ALS content and the beginning of its decrease also occurred before the changes in activity of both cholinesterases. These data indicate that the increase in ALS content does not depend on inhibition of the cholinesterases.

We also found that the initial increase in ALS content in a number of cases and the maximal in the majority of patients given Oxamysil occurred before the corresponding changes in muscular strength. In the case of proserine, the ALS content and muscular strength showed the same relationship as far as the period of maximal effect and the time that the effect began to fail. These data give reason to propose that one aspect of the action of proserine and Oxamysil is an activating effect on the sympathetic nervous system. In all probability, this activation is one of the features which promotes normalization of muscular activity in myasthenia patients.

The activation of FCE which we observed 30 min after the administration of Oxamysil surprised us. This phenomenon may be connected with the activating effect of Oxamysil in small doses, as it is gradually absorbed from the gastrointestinal tract. (Hardegg's works [8] have shown that small doses of certain anticholinesterase preparations have an activating effect on cholinesterase).

We also investigated the dynamics of muscular strength and the cholinesterases under the influence of Oxamysil administration in five more patients. Blood was taken from a vein, and the cholinesterases were determined by the method of S. R. Zubkova and N. V. Pravdich-Neminskaya. These investigations also showed a different sequence of changes in muscular strength and in the activity of the cholinesterases.

The data obtained indicate that one cannot attribute the antimyasthenic effect of Oxamysil and proserine solely to their anticholinesterase action. Since both Oxamysil and proserine are, like acetylcholine, quaternary ammonium compounds, they could exert a direct effect on the cholinergic synapses and the end-plate receptors of a contractile substrate. Under these conditions, activation of the sympathetic nervous system could result from stimulation of the cholinergic synapses of the sympathetic nervous system.

More attention must be given the hunt for antimyasthenic drugs without anticholinesterase properties. The use of these drugs sharply reduces the muscarine side effects and prevents the cholinergic crises which complicate the course of myasthenia in patients given anticholinesterase agents.

SUMMARY

An inquiry was made into the time correlation of changes occurring in the muscular power with humoral shifts (adrenalin-like substances, pseudo and true cholinesterase) in myasthenia patients subjected to the administration of anticholinesterase preparations: briefly acting proserin and oxamysil – a preparation with a prolonged effect. There was no complete parallelism in the time of appearance of the antimyasthenic and anticholinesterase shifts. A rise of adrenalin-like substances in these conditions precedes the rise of the muscular power and cholinesterase inhibition, which may point to the activating effect of proserin and oxamysil on the sympathetic nervous system.

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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 this issue.
