EXPERIMENTAL DATA ON THE ACTION OF A NUMBER OF ORGANOPHOSPHOROUS COMPOUNDS ON THE MOTOR FUNCTION OF THE RABBIT'S INTESTINE

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The high biological activity of organophosphorous compounds is one of the reasons for the close study given to their effects on the organism [1, 2, 4, 5, and others]. However, only a comparatively small number of works [2, 3, 7, 8, 10, 11] are concerned with the activity of compounds of this class on intestinal muscle. The study of the sensitivity of the longitudinal and transverse muscles of the intestine to different types of organophosphorous compounds has been entirely neglected. Meanwhile, these questions are not only of theoretical but of practical interest, since the clinician has need of a wider range of preparations acting selectively on individual types of intestinal muscle.

Our problem was to study the effects of organophosphorous compounds, differing in chemical structure and in the character of their activity, on the motor function of the intestine.

Compounds representing 3 groups of preparations were studied: 1) organophosphorous compounds which depress the activity of cholinesterase in vitro and in vivo (pyrophos, thiol isomer of mercaptophos, methylacetophos); 2) preparations possessing a comparatively weak anticholinesterase activity in vitro but which are converted into more effective anticholinesterase agents within the organism (octamethyl, chlorophos, M-81, M-81 Su 3—sulfoxide); 3) substances not affecting cholinesterase either in vitro or in vivo (K-20-35).

According to their chemical structure, the compounds M-81 and M-81 Su 3 belong to the esters of dithiophosphoric acid, the thiol ester of mercaptophos (isosystox), acetophos and methyl acetophos to the esters of thiophosphoric acid, pyrophos and octamethyl to the esters and amides of pyrophosphoric acid, K-20-35 to the esters of urethanephosphoric acid and chlorophos to the esters of phosphoric acid.

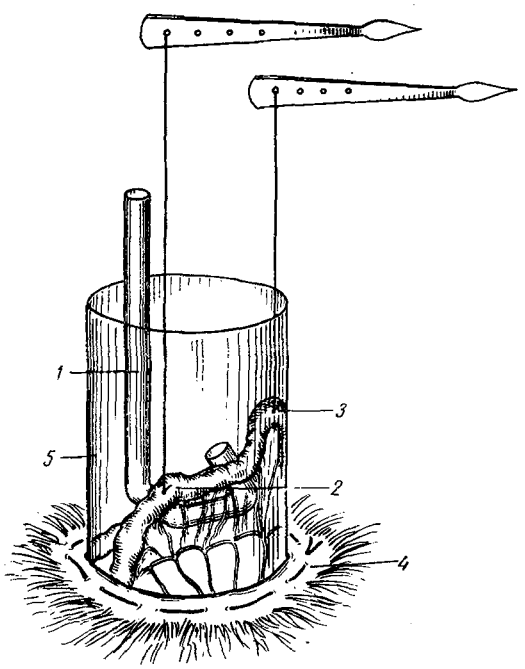
Data on the activity of these compounds on the intestine in limited in the literature to only one of the named compounds, viz. octamethyl [7, 10].

EXPERIMENTAL METHOD

The experiments were carried out on 53 rabbits by a method described by E. N. Speranskaya [9], but with some modification.

The rabbits were narcotized by urethane and an opening (4 × 3 cm) was cut in the middle of the anterior abdominal wall. A portion of the small intestine was attached to the parallel sides of a glass tube which, at the lower end lying on the thoracic surface, had the shape of a letter U (see figure).

For recording the contractions of the transverse muscle a thread was sewn on at one of the places where the intestine was attached to the glass tube and then led to an Engelman lever. For recording the contractions of the longitudinal muscle a second thread was attached 2-3 cm from the 2nd place of attachment, but not to the portion lying



Schematic drawing of the experimental method. 1) U-shaped glass tube for attachment of intestine; 2) place of attachment of thread for recording the contractions of the transverse muscle; 3) place of attachment of thread for recording contractions of longitudinal muscle; 4) suture: 5) glass cylinder.

between the parallel parts of the glass tube, as E. N. Speranskaya suggests. We had the idea that, if the contractions of the longitudinal muscle were recorded at the place where the intestine was fixed to the tube, the strength and character of the contractions might be affected. The vertical part of the glass tube and the threads were led through a hollow, glass cylinder, the lower, flanged edge of which was inserted under the edge of the opening in the abdominal wall. Terod's solution, warmed to 38°, was poured into the abdominal cavity in sufficient quantity to cover the intestine. The whole setup was fixed by clamps and the threads were fastened to Engelman levers. The rabbits were warmed electrically. The recording of the muscle contractions (background) began after allowing 30-50 min for the animal to settle down. The compounds under test were then injected into the aural vein or intramuscularly and the movements of the longitudinal and transverse muscles were recorded on a kymograph.

For evaluating the effects, the ratios between the contractions were measured over equal time intervals before and after the administration of the compounds.

Before the beginning of the experiment and at various periods following the injection of the organophosphorous compounds, blood samples were taken for determination of the cholinesterase activity. The activity in the erythrocytes and in the serum were determined separately [12].

The water-soluble preparations (pyrophos, octamethyl, chlorophos, and K-20-35) were used in concentrations of 0.01-20% in water. Of the remaining compounds, the original 10% or 50% alcoholic solutions were diluted with distilled water to concentrations of 0.1-1.0%. For studying the effects of the preparations on the muscular movements of the intestine, doses of 0.01-0.2 LD_{50} were injected. In certain experiments the doses were increased until toxic effects were obtained. In a number of experiments in which there was no response to the original dose, the injection was repeated.

Doses producing the maximal effects were determined.

EXPERIMENTAL RESULTS

Esters of Dithiophosphoric Acid

M-81 (11 experiments) was injected in doses of 1-15 mg/kg. The minimum dose producing the greatest effect was 5 mg/kg. The effect was expressed by an increase in the amplitude of the oscillations of the longitudinal muscle by 2-8.5 (average 4.5) times and of the transverse muscle by 1.2-6 (average 2.5) times. The effect set in 15-20 min after the injection and lasted for 1-1½ h. The activity of the cholinesterase in the serum was reduced by 20-50% and in the erythrocytes by 5.8-31.9%.

M-81 Su 3 (sulfoxide) (four experiments) was tested in doses of 0.3-9.3 mg/kg. The maximal effects were given by 0.3-0.6 mg/kg. A large increase in the tonus of the intestine was observed after the injection. The amplitude of the oscillations of the longitudinal muscle increased by 2.6-8.3 (average 4.6) times and of the transverse muscle by 2-2.5 (average 2.2) times. The action of the preparation set in after 15-20 min and continued for about an hour. The activity of cholinesterase in the erythrocytes and serum was reduced by not more than 17.7%.

Esters of Thiophosphoric Acid

ISOSYSTOX (4 experiments) was injected in doses of 0.5-4.75 mg/kg. The dose bringing about maximal effects was equal to 1-2.5 mg/kg. An increase in the contractions of the intestine set in 2-3 sec after the injection of

the preparation, the amplitude of the contractions of the longitudinal muscle increasing by 1.7-7 (average 2.5) times and of the transverse muscle by 2-8 (average 3) times. The tonus of the intestine and the frequency of the contractions were almost unchanged. The effects lasted for 20-40 min. The activity of cholinesterase in the serum was reduced by 58.7% and in the erythrocytes by 51.0%.

ACETOPHOS (4 experiments) was used in doses of 0.5-25 mg/kg. A dose of 25 mg/kg was lethal to the rabbits but a dose of 2-2.5 mg/kg produced the maximal effects. The action of the preparation set in after 2-4 min and continued for 20-25 min and was made evident by a moderate increase in the tonus and by an increase in the oscillations of the longitudinal muscle by 1.5-2.5 (average 2) times and of the transverse muscle by 2-2.5 (average 2.3) times. The cholinesterase activity of the serum was reduced by 61.9% and that of the erythrocytes by 49.8%.

METHYLACETOPHOS (4 experiments) was tested at doses of 5-100 mg/kg. The maximal effect was observed at 15 mg/kg which increased the contractions of the longitudinal muscle by 2-5 (average 2.5) times and of the transverse muscle by 1.3-6.6 (average 2.7) times. The action lasted 35-40 min. The reduction in the cholinesterase activity of the serum was 42.5% and of the erythrocytes by 46.6%.

It was noted that the injection of acetophos (2.5 mg/kg) and methylacetophos (30 mg/kg) on a background in which the motor function of the intestine was depressed by a cholinolytic (atropine or tropazine in doses of 20 mg/kg) brought about a reduction in the contractions of the longitudinal and transverse muscles.

Esters and Amides of Pyrophosphoric Acid

PYROPHOS (7 experiments) was injected in doses of 0.05-1.5 mg/kg. The maximal effects were given by doses of 0.3-0.45 mg/kg. The action of the preparation set in after a few seconds and lasted until the end of the experiment (1-1$^1/_2$ h). The contractions of the longitudinal muscle were increased by 2-5 (average 3) times and of the transverse muscle by 1.7-4 (average 1.6) times. As a rule, the tonus of the intestine was raised and the contractions assumed the character of rhythmic, undulating waves. A dose of 0.45 mg/kg led to a reduction in the cholinesterase activity of the erythrocytes by 23% and of the serum by 43.2%.

OCTAMETHYL (3 experiments) was applied in doses from 3-33 mg/kg. The maximal effects were noted at 3-5 mg/kg. The preparation did not act immediately, but 20-40 min after injection. The oscillations of the longitudinal muscle increased by 2-9.5 (average 4.8) times and of the transverse muscle by 1.6-2.5 (average 1.3) times. The tonus of the intestine and the frequency of the contractions did not change. The effects continued for 20-40 min.

Esters of Urethanephosphoric Acid

K-20-35 (3 experiments), applied in doses of 1000-2500 mg/kg, produced no action either on the movements of the intestine or on the activity of cholinesterase.

Esters of Phosphoric Acid

CHLOROPHOS (7 experiments) was injected both intramuscularly and intravenously in doses of 25-300 mg/kg. Intramuscular injection of the preparation produced only a small increase in the contraction of the longitudinal muscle (by 2.5 times). A greater effect was obtained by intravenous injection in doses of 25-30 mg/kg. At the same time, the contractions of the longitudinal muscle increased by 2-7 (average 5.1) times and those of the transverse muscle by 2.5-12 (average 4.8) times. The effects set in after 5-8 min and continued for 30-40 min. Doses of 25-30 mg/kg led to a reduction in the cholinesterase activity of the serum by 38.4% and of the erythrocytes by 66.9%. Doses of 100 mg/kg and above produced obvious symptoms of poisoning.

The results of the experiments carried out showed that all the compounds which depressed the activity of cholinesterase increased the muscular movements of the intestine to some extent or other. The action of the active compounds set in after periods which varied from a few seconds to 3-8 min after injection. The effects of the activating compounds were manifest over a period of 15-40 min.

It was evident that the action of the compounds examined depended not only (not so much for some compounds) on a reduction of cholinesterase activity but on their immediate effect on the cholinoreactive system of the intestine. Thus, in a number of experiments, it was noticed that methylacetophos, M-81 and M-81 Su 3 increased the muscle movements of the intestine at doses which reduced the enzyme activity to only a small extent. Furthermore,

the injection of acetophos and methylacetophos on a background depression of muscle action by a cholinolytic (atropine or tropazine) caused new waves of intestinal contractions.

Each of the compounds, differing in chemical structure, had a different effect on the groups of intestinal muscles; the pyrophosphates, esters of dithiophosphoric and phosphoric acids had a marked influence on the longitudinal and less on the transverse muscles. On the other hand, the esters of thiophosphoric acid strongly increased the contractions of the transverse muscles.

For peristaltic movement of the chyme through the intestine not only is an increase in the strength of the contractions important but also the relation between the intensities of the contractions of the 2 groups of muscles. In addition, the contractions of the longitudinal muscle should predominate over those of the transverse muscle. In the opposite case the contractions resemble rhythmic segmentation and this would delay the advance of the chyme through the intestine. According to the degree to which peristalsis was increased, the best preparations proved to be M-81, M-81 Su 3, pyrophos and octamethyl. The ratios between the average increases in the amplitude of the contractions of the longitudinal and transverse muscles confirm this fact. These ratios were: M-81, 4.5 : 2.5; M-81 Su 3, 4.6 : : 2.2; pyrophos, 3:1.6; octamethyl, 4.8 : 1.3. It should be noted that, of these preparations, M-81 Su 3 reduced the activity of cholinesterase least of all.

SUMMARY

Investigations have been made on the effects on the muscles of the rabbit's intestine of compounds which reduce the activity of cholinesterase: a) in vitro and in vivo (pyrophos, isosystox, methylacetophos); b) slowly in vitro but more actively in vivo (octamethyl, chlorophos, M-81, M-81 Su 3) and c) neither in vitro nor in vivo (K-20-35).

From the results of the experiments it was determined that the substances in the first group began to cause an increase of the intestinal contractions in periods ranging from several seconds to 3-8 min; the compounds of the 2nd group in periods from 15-40 min and the preparations of the 3rd group had no effect on the intestinal motor functions.

It was noted that the esters of thiophosphoric acid (isosystox, acetophos, methylacetophos) exerted a stronger action on the transverse muscles and pyrophos, octamethyl, chlorophos, M-81 and M-81 Su 3 had a stronger action on the longitudinal muscles. According to their capacity for increasing peristalsis, the best preparations were M-81, M-81 Su 3, pyrophos, and octamethyl.

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