THE ACTION OF PHOSPHOTRIOSE, PHOSPHO-ENOLPYRUVIC ACID AND THE PREPARATION ZSC (ZYMOSTIMULATOR CORDIS) ON CARDIAC MUSCLE

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In previous published work carried out jointly by T. M. Tupaev and ourselves [5], it was shown that preparations of "sodium-DPF" — the sodium salt of fructose 1,6-diphosphate in concentrations of 1: 2000 and over, in contrast to the free sugars (glucose and fructose), and also preparations of synthetic glucose 6-phosphate and glucose 1-phosphate, have a prolonged and marked stimulating action on the contraction of the heart muscle of the frog, when weakened by continued work in Ringer's solution.

Subsequently, by means of absorption on carbon, fractionation of the barium salts isolated from the eluate and chromatography of one of the fractions, we were able to isolate from the sodium-DPF preparations, and also from a yeast fermentation mixture, a substance which had a stimulating action on the work of the heart, in a concentration of 10^{-5}, and which we called ZSC (zymostimulator cordis) until its structure has been fully established. A study of the chemical nature of this compound has shown that it closely resembles or is identical with uridinediphosphate (UDP [4]).

Preparations of sodium-DPF, free from ZSC, had no appreciable action on the strength concentration of the isolated frog's heart in the ordinary conditions previously described [5], although clinically they had an antishock action [4].

It appeared of interest to continue the investigation of various phorphorylated products of carbohydrate-phosphorus metabolism in respect to their action on the heart. For this purpose, in the present research we prepared and tested the action on the heart of triose phosphate compounds — products of the aldolase decomposition of DPF and high-energy compounds — phospho-enolpyruvic acid.

EXPERIMENTAL METHOD

Triose phosphate compounds were prepared mainly by the method of Meyerhof and Lohmann [6], by decomposition of DPF (sodium salt) with aldolase. The source of aldolase which was used was a dialyzed extract of rabbit muscle. The triose phosphates were isolated and purified in the form of their barium salts. As in the original work [6], the triose-phosphate phosphorus accounted for about 90% of the total organically combined phosphorus of the preparation: 56.4% of the triose phosphate was in the form of 3-phosphoglyceraldehyde and the remainder phosphodioxyacetone.

Bearing in mind the possibility of contamination of the compounds obtained with highly active substances (for example, of the ZSC or ATP type), we studied the action of the biochemically prepared compounds both before and after treatment with carbon at room temperature to absorb contaminants. The resulting preparation of the barium salt was divided into 3 parts, one of which was not treated with carbon, the second was treated with carbon for one hour at pH 3, and the third, after precipitation of the Ba with sodium

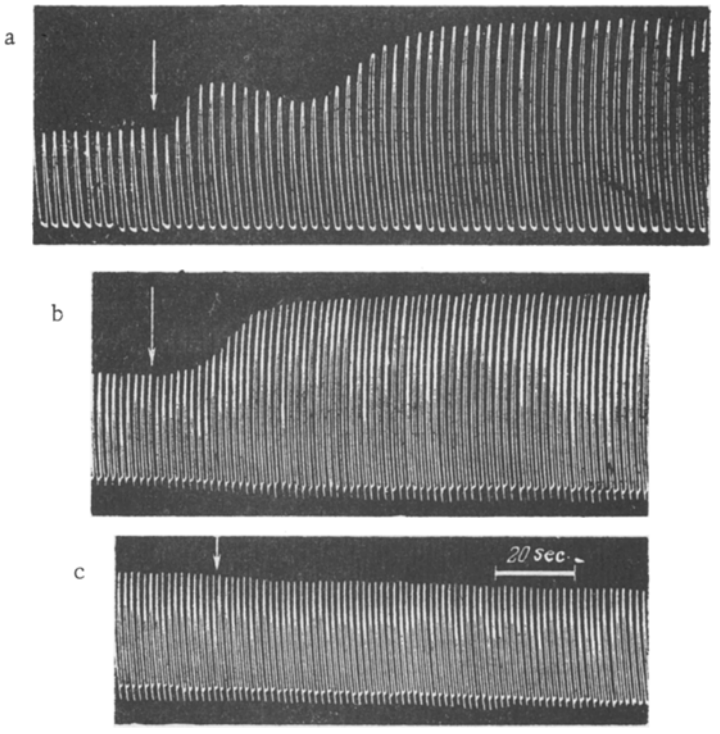

Fig. 1. The action of the triose phosphate preparations in a concentration of 0.05% on the frog's heart. a) Before treatment with carbon; b) after treatment with carbon at pH 9; c) after treatment with carbon at pH 3. The arrow indicates the moment of injection of the solution of the preparations into the cannula.

sulfate, was treated with carbon at pH 9 (also for one hour). For the biological trials we prepared basic 1% solutions of the sodium salt of the triose phosphates (untreated with carbon, and treated at pH 3 and pH 9), from which less concentrated solutions were prepared by the addition of suitable amounts of diluent.

Phospho-enolpyruvic acid (PEP) was prepared by biochemical and chemical methods.

Biochemically, PEP was prepared by the method of V. A. Belitser and T. V. Saenko [2] from the sodium salt of 3-glycerophosphoric acid, which is converted by the action of the yeast enzymes phosphoglyceromutase and enolase into PEP. The raw material — 3-glycerophosphoric acid (GPA)-was prepared by Ostern and Guthke's modification of Neuberg and Kobel's method, taking into consideration the findings of Belitser and Karlina [1] and Severin and Meshkova [3], by oxidation of triose phosphates (formed from DPF) with acetaldehyde in the presence of sodium fluoride. GPA was isolated in the form of the acid barium salt with two molecules of water of crystallization. The product contained 91-93 % of the crystalline hydrate of the barium salt of GPA (ignoring hygroscopic moisture).

PEP, obtained biochemically from GPA, was isolated in the form of the barium-silver salt, which was then converted into the barium and sodium salt. The biochemically prepared PEP, as in the experiments with triose phosphates, was used either untreated with carbon or treated with carbon at pH 3 and pH9.

PEP was synthesized chemically by Ohlmeyer's method, by phosphorylation of pyruvic acid in anhydrous quinoline by means of phosphorus oxychloride. Synthetic PEP was isolated in the form of the crystalline barium-silver salt. After two or three recrystallizations a preparation was obtained containing about 92% of the barium-silver salt of PEP (ignoring hygroscopic moisture). All the phosphorus in the preparation was organic and belonged to the PEP, since it was completely split off with iodine.

For the biological trials, the barium-silver salt of the synthetic PEP was converted, by the addition of Na_2SO_4 and HCl (with subsequent neutralization), into the sodium salt, which was prepared in the form of an

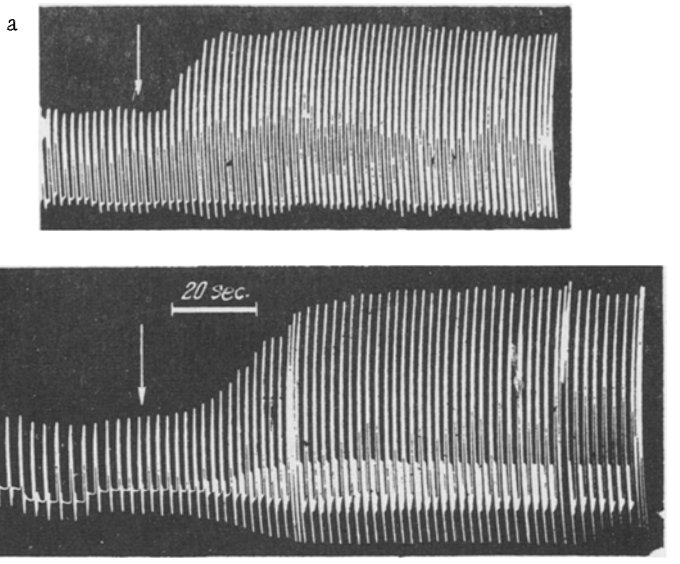

Fig. 2. The action on the frog's heart of eluates from carbon after treatment of triose phosphates with carbon at pH 3 (a), and of a solution of ZSC in concentration of 10^{-5} (b).

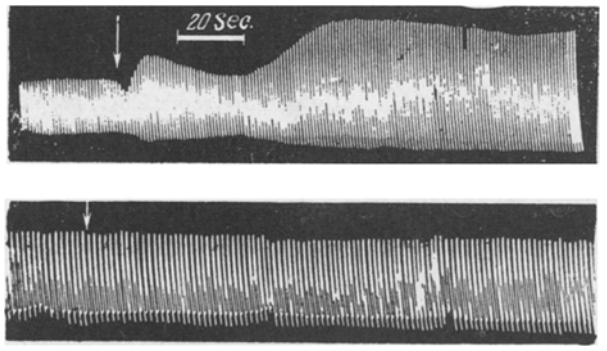

Fig. 3. The action of biochemically prepared PEP, in a concentration of 0.1%, on the frog's heart. a) Before treatment with carbon; b) after treatment with carbon at pH 3.

original 1% solution, as in the experiments with the biochemically prepared PEP. Before the experiment, all the test substances were diluted with Ringer's solution to the required concentrations.

EXPERIMENTAL RESULTS

Neutral solutions of the sodium salts of the triose phosphates were tested on the frog's heart, isolated by Straub's method, the contractions of which were greatly depressed in amplitude by prolonged working (1-2 days) in a humid chamber at a temperature of 2-4°.

The triose phosphates which were not treated with carbon, in concentrations of 0.001 and 0.01%, had no or a comparatively weak action on the contraction of the heart muscle. In concentrations of 0.05-0.1%, however, a very obvious effect was observed (Fig. 1, a): the amplitude of the cardiac contractions was sharply increased and this action lasted about 30 minutes.

Both by the character of its action (the presence of three phases — an initial rise, then a fall in the amplitude of the contractions and a subsequent prolonged increase in the amplitude of the cardiac contractions), and by the magnitude of the effective doses, the triose phosphate preparations untreated with carbon closely resembled the previously studied DPF preparations, also untreated with carbon [5], although they were slightly inferior to then in respect to the action of small doses.

The triose phosphate preparations treated with carbon at pH 9 possessed practically the same intensity of activity as the untreated preparations, but the character of their action was altered: the three-phase character had disappeared and was replaced by an even increase in the amplitude of the contractions (Fig. 1, b).

The triose phosphate preparations treated with carbon at pH 3 were completely inactive (Fig. 1,c).

In order to discover to what extend the triose phosphates were preserved when treated as described above in an alkaline or acid medium, analyses were made of the triose phosphate content (as alkaline-labile and total phosphorus). As was to be expected, the triose phosphate content of the preparations treated with carbon at pH 9 had fallen by approximately 35-40% of the initial value. In the preparations treated at pH 3, and having completely lost their activity, no fall in the triose phosphate content was observed.

These facts led to the conclusion that the action on the heart (under these conditions of testing) was a property not of the triose phosphates themselves, but of the contaminants absorbed on the carbon.

Since the triose phosphate preparations were obtained from DPF, it was natural to asume that they might contain primarily the same contaminants as were present in the DPF, including ZSC, especially as in the subsequent work there was no treatment which would completely remove ZSC.

For confirmation of the hypothesis that ZSC was present in the triose phosphate preparations after adsorption of contaminants on carbon at pH 3, the carbon was treated with water alkalized with NaOH to pH 9, as was done in the isolation of ZSC. The eluate obtained was tested on the heart after neutralization, and showed high activity with a monophasic rise in the amplitude of the cardiac contractions (Fig. 2 a).

Finally, by means of the method described above, triose phosphates were prepared from DPF which had been preliminarily treated by our method [4] to free it from ZSC. These triose phosphates were found to be inactive.

A study of the ultraviolet light absorption of the eluate from the carbon used in treatment of the triose phosphates showed the presence of uridine derivatives, since the peak at 260 mμ fell sharply after treatment with bromine.

The action of the triose phosphate preparations on the isolated heart was thus due primarily to the presence of ZSC as a contaminant, but this does not, of course, exclude the posibility of other active contaminants, on the investigation of which we are at present engaged.

Fig. 2 shows the kymogram of the action on the heart of chromatographically homogeneous ZSC, prepared by the method described previously [4], in a concentration of 10^{-5}. A noteworthy feature is the monophasic character of the action — an even increase in the amplitude of the contractions-which distinguishes it sharply from ATP, which is characterized by a three-phase action.

The change in the character of the action of the triose phosphate preparations treated with carbon at pH 9 (disappearance of three-phase character) may evidently be accounted for by adsorption of a substance with a three-phase action (perhaps ATP on the carbon, or by destruction of some contaminant at this pH.

This problem is being studied at the present time. The three-phase character of the action of the triose phosphate preparations is evidently the sum of the actions of ZSC, with a monophasic action, and of a substance with a three-phase action (probably ATP).

The monophasic character of action of the eluate obtained by treatment with carbon at pH 9 (after treatment of triose phosphates with this carbon at pH 3) is evidently explained by removal from the carbon of ZSC alone, and retention on the carbon of the substance with a three-phase action, for example ATP, which possesses more basic properties than the ZSC.

Neutral solutions of preparations of the sodium salt of PEP, obtained by a biochemical method, showed a constant and persistent three-phase effect on the contractions of the isolated heart, like that of the triose phosphates, in concentrations of 0.05% and over (Fig. 3, a). Biochemically prepared PEP, treated with carbon at pH 9, possessed the same degree of activity; the preparation treated with carbon at pH 3 was inactive (Fig. 3,b).

Analysis of the two preparations showed that treatment at pH 3 and pH 9 causes only a slight fall or practically none at all (within limits of 10%) in the content of PEP. The eluate from the carbon used in the treatment of the PEP at pH 3 possessed high activity on the heart.

PEP prepared by chemical synthesis, in a concentration of 0.05%, was found to be inactive; higher concentrations (above 0.1%) of the synthetic PEP, just as of that prepared biochemically, depressed the activity of the heart*.

* We express our deep gratitude to T. M. Turpaev for advice in the physiological testing of our preparations.

All these phenomena may evidently be explained just as in the case of the triose phosphates: the activity of the biochemically prepared PEP is primarily due to the action of ZSC as a contaminant, present in the yeast.

It seems to us that a fact of great interest is that PEP itself (synthetic, or prepared biochemically and purified), being a high-energy compound, had no stimulating action on the contraction of the cardiac muscle under these conditions of testing.

In this connection it must be remembered that recently Moos and Lorand [7], in a study of the action of synthetic PEP on a far simpler system — fibers of the psoas muscle treated with glycerol — showed that PEP causes relaxation of the fibers after the action of ATP.

SUMMARY

Preparations of the sodium salt of triose phosphate, obtained by biochemical means, as well as those of the sodium salt of phospho-enolpyruvic acid (PEP) obtained both biochemically and by chemical synthesis were studied in respect to their action upon the fatigued frog's heart (by Straub's method). Synthetic PEP was found to be inactive, while triose phosphate and biochemically obtained PEP exercise a stimulating effect on the heart similar by its character and its effective dose to the action of the sodium salt of fructose-1,6-diphosphate (DPF).

Treatment of triose phosphate preparations (obtained biochemically) and those of PEP with charcoal at various pH, and their subsequent examination along with that of the eluted fluid (wash off) were investigated. It was demonstrated that the action of the preparations on the fatigued heart is, at least in its main part, associated with the presence of the low quantities of zymostimulator cordis (ZSC) as admixture. ZSC is a uridine derivative close or identical to uridinediphosphate (UDP) formerly isolated by the authors from yeast and sodium-DPF preparations.

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* See English translation.