EFFECT OF TOXIC DOSES OF VITAMIN D_2 ON THE DISSOCIATION OF OXIDATIVE PHOSPHORYLATION

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There are reports in the literature that several substances toxic to the living organism may cause dissociation of oxidative phosphorylation. According to the available data [10], vitamin D_2 in a dose of 20,000 i.u. like dihydrotachysterol, a vitamin D_2 analog, inhibits the action of aconitase in vitro, as a result of which processes concerned in the Krebs cycle are depressed.

Experiments on rats have shown [2,3] that vitamin D_2 in toxic doses in vivo increases the accumulation of citric acid in the blood as a result of depression of the aconitase activity in the reaction pyruvic acid → coenzyme A + oxaloacetic acid.

Many authors have shown [5] that K and Mg ions are activators of oxidative phosphorylation. The protective action of KCl and $MgCl_2$ against the toxic effects of certain agents including the vitamin D_2 analog dihydrotachysterol has repeatedly been demonstrated [8,9].

The object of the present investigation was to study the effect of toxic doses of vitamin D_2 on the relationship between oxidation and phosphorylation in the heart and kidney tissues of rats and the action of K and Mg salts when this process is disturbed as a result of the toxic action of this substance.

EXPERIMENTAL RESULTS

Experiments were carried out on male rats with a mean weight of 240 g. The animals were subdivided into three groups. The rats of group 1 received vitamin D_2 in oil daily by mouth in a dose of 300,000 i.u./kg body weight for 5-6 days. The animals of group 2, besides the above doses of vitamin D_2, received KCl and $MgCl_2$ daily by mouth in doses of 0.5 mmole for 5-6 days, and the rats of group 3 (control) received the corresponding dose of vegetable oil. All the animals were kept on the standard vivarium diet.

After 5-6 days the rats were sacrificed, and the rate of oxidative phosphorylation was determined in a mince prepared from heart and kidney tissue by Zaitseva's formula [1]. As phosphate acceptor during determination of the P/O ratio in the heart muscle of the rat, creatine was used, and the intensity of phosphorylation was judged from the formation of creatine phosphate in the course of incubation. When determining the P/O ratio in the kidney tissue of the rat, the phosphate acceptor was a system glucose + hexokinase; the intensity of phosphorylation in this case was judged from the loss of inorganic phosphate during incubation.

The oxygen absorbed by the tissues in the course of incubation (oxidation) was determined by a manometric method in a Warburg's apparatus [4,10]. Altogether 60 rats were investigated.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experimental rats receiving toxic doses of vitamin D_2, the oxidation in the heart muscle was increased by 45% by comparison with this index in the control animals. At the same time, the intensity of phosphorylation in the experimental animals fell by 70% compared with the controls, and for this reason the value of the P/O ratio in the experimental animals was 80% below that in the control. These differences are statistically significant ($P < 0.001$, see table).

The relative intensity of oxidation and phosphorylation in the rats receiving KCl and $MgCl_2$ in daily doses of 0.5 mmole each in addition to toxic doses of vitamin D_2 was close to that observed in the control animals. During investigation of the relative intensity of oxidation and phosphorylation in the kidney tissue of the rats (the renal

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Tissue	Experimental conditions	ΔO, μatoms (per 100 mg fresh tissue)	Difference from control (in %)	P	ΔP, μatoms (100 mg fresh tissue)	Difference from control (in %)	P	P/O	Difference from control (in %)
Heart	Control	2.55 ± 0.062 n = 16		<0.001	4.98 ± 0.23 n = 14		<0.001	1.95 n = 14	
	Vitamin D_2, 300,000 i.u./kg body weight daily for 5-6 days	3.71 ± 0.24 n = 12	+45		1.55 ± 0.153 n = 12	−70		0.42 n = 12	−80
	Vitamin D_2, 300,000 i.u./kg body weight + 0.5 mmole KCl + 0.5 mmole $MgCl_2$ daily for 5 days	2.72 ± 0.121 n = 8		<0.01	4.52 ± 0.103 n = 8		<0.001	1.68 n = 8	
Kidneys	Control	3.79 ± 0.157 n = 11		< 0.01	2.93 ± 0.054 n = 11		<0.001	0.78 n = 11	
	Vitamin D_2, 300,000 i.u./kg body weight daily for 5 days	4.37 ± 0.109 n = 11	+15		1.77 ± 0.045 n = 11	−40		0.44 n = 11	−44

Note: Temperature of incubation 26°. Time of incubation 40 min. Gaseous phase — air.

cortex) it was found that oxidation in the experimental animals (i.e., receiving toxic doses of vitamin D_2) was 15% higher than in the controls. Meanwhile, the intensity of phosphorylation in the experimental series was 40% below that in the control, and the P/O ratio correspondingly was 44% lower in the experimental than in the control animals. The differences are statistically significant ($P < 0.001$).

Hence, in experiments both on the heart and on the kidneys, toxic doses of vitamin D_2 in a dose of 300,000 i.u./kg body weight were found to stimulate oxidation and inhibit phosphorylation, thus producing dissociation of oxidative phosphorylation. Administration of K and Mg salts simultaneously with toxic doses of vitamin D_2 restored the process of oxidative phosphorylation to normal. Changes were also found in the body weight of the experimental and control rats. Whereas the weight of the control animals increased during the 5-6 days of the experiment on the average by 9%, the weight of the control rats fell (on the average by 9.4%). The weight of the rats receiving K and Mg salts together with toxic doses of vitamin D_2 remained almost unchanged.

The results obtained are in agreement with reports in the literature [6] indicating that, when toxic substances act on the living organism, the relative intensity of oxidation and phosphorylation depends on the doses of these toxic substances: with comparatively small doses, dissociating oxidative phosphorylation, the absorption of oxygen in the tissues at first increases, whereas the intensity of phosphorylation falls. With an increase in the dose of the toxic substance, phosphorylation remains at the same low level as with comparatively small doses, but the absorption of oxygen falls.

It may be concluded from these findings that, with the toxic doses of vitamin D_2, used in the investigation, the heart was a more reactive organ than the kidneys: in the heart tissues, the absorption of oxygen was 45% greater in the experimental than in the control animals, but in the kidneys it was only 15% greater, whereas the intensity of phosphorylation in both cases was much lower in the experimental than in the control series.

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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 the first issue of this year.