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The *in vivo* effect of digitoxin on rat heart phosphatides*

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SUMMARY

Digitoxin was shown to increase the specific activity of heart ventricle phosphatides in rats receiving simultaneously radioactive orthophosphate and digitoxin as compared to control rats not receiving digitoxin. The increase in specific activity was demonstrated in both lecithin and phosphatidyl ethanolamine. The other heart muscle lipids were not investigated. Glycerol and ethanol, which are constituents of the vehicle in which the digitoxin is dissolved, do not alter the specific activity of the heart muscle phosphatides. The vehicle itself (without digitoxin) has some activity.

The biochemical mechanism of action of cardiac glycosides on heart muscle has not been sufficiently investigated. The *in vitro* effect of cardiac glycosides on acetylcholine esterase and adenosine triphosphatase has been discussed by Proctor *et al.* (1), who present the concept that cardiac glycosides may act by inhibiting adenosine triphosphatase with the result that more ATP[1] is available for muscular work. The *in vitro* work of Grisolia (2), who described a potentiating effect of digitoxin on dinitrophenol uncoupling of oxidative phosphorylation, is difficult to reconcile with this hypothesis.

In view of the high caloric value of lipids, the abundant concentration of lipids in heart muscle (3, 4), and the lipophilic nature of cardiac glycosides, it was considered possible that these drugs might increase the turnover of certain lipids with the concomitant increase in ATP production. Experiments were thus carried out in which white rats were given simultaneously P^{32}-labeled orthophosphate and digitoxin, and the specific activity of the total phosphatides, lecithin, and phosphatidyl ethanolamine were determined and compared with those in control rats not receiving digitoxin. These experiments show that digitoxin does produce an increase in the specific activity of the phosphatides. This increase in specific activity was found in both lecithin and phosphatidyl ethanolamine.

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[1] ATP signifies adenosine triphosphate.

METHODS

Experiments 1 and 2: Control rats were injected subcutaneously with 200 μc of P^{32}-orthophosphate contained in 0.5 ml of 0.001 M phosphate buffer pH 7.4. Experimental rats were injected with the same amount of radioactive phosphate and were also injected intraperitoneally with 180 μg of digitoxin (Varick) contained in 0.9 ml of vehicle.[2]

Experiment 3: Rats were given 200 μc of orthophosphate as explained in experiment 1. In addition, these rats were injected intraperitoneally with 0.9 ml of 40% glycerol in water. This experiment was designed to test the effect of glycerol in the vehicle.

Experiment 4: Rats were given 200 μc of orthophosphate as explained in experiment 1. In addition, these rats were injected intraperitoneally with 0.9 ml of 40% ethanol in water. This experiment was designed to test the effect of the alcohol which is in the vehicle.

Experiment 5: Control rats were given 200 μc of radioactive phosphate as explained in experiment 1, and also given 0.9 ml of vehicle intraperitoneally. This vehicle contains all the ingredients in the digitoxin preparation except the digitoxin. Experimental rats were given the same amount of radioactive phosphate and 0.9 ml of digitoxin preparation (vehicle plus 180 μg of digitoxin). This experiment was designed to test the effect of the vehicle.

[2] The vehicle in which the digitoxin is dissolved contains the following: Polyethylene glycol 300, 28.6%; glycerol, 43.8%; benzyl alcohol, 4.0%; ethyl alcohol, 5.0%; and water to make 100%.

TABLE 1. THE EFFECT OF DIGITOXIN ON RAT HEART MUSCLE PHOSPHATIDES

	Specific Activity (cpm/μg of P)						
		Total Phosphatides		Lecithin		Phosphatidyl Ethanolamine	
	No. of Animals	Mean	Standard Error*	Mean	Standard Error*	Mean	Standard Error*
Expt. 1							
Control	3	30.7	3.8	37.7	5.0	29.7	5.8
Digitoxin + vehicle	3	51.3	3.8	52.0	5.0	59.7	5.8
Expt. 2							
Control	5	28.6	2.9	33.8	3.8	22.8	4.5
Digitoxin + vehicle	5	47.4	2.9	44.6	3.8	43.0	4.5
Expt. 3							
Glycerol	5	30.6	2.9	36.2	3.8	27.0	4.5
Expt. 4							
Ethanol	3	30.0	3.8	41.3	5.0	29.3	5.8
Expt. 5							
Control + vehicle	4	39.2	3.2	44.0	4.3	34.5	5.0
Digitoxin + vehicle	5	49.0	2.9	53.0†	5.0	‡	
Expt. 1 + 2							
Controls	8	29.4	2.3	35.2	3.0	25.4	3.5
Expt. 1 + 2 + 5							
Digitoxin + vehicle	13	48.9	1.8	49.2§	2.5	49.2§	3.5

* Standard errors based upon pooled estimates of the standard deviation for each lipid sample.
† Value based upon 4 animals.
‡ Data not included because samples were lost because of an accident.
§ Lecithin mean based upon 12 animals; phosphatidyl ethanolamine mean based on 8 animals.

Male rats (200 to 225 g) were used in all experiments. The rats were sacrificed 24 hours after injection. This time interval was chosen because it represents a plateau region of the specific activity time curve (5). Total lipid extracts were made of the heart ventricles. The lipids were extracted with methanol-ether and then washed three times with isotonic saline. The total lipids were dissolved in isoamyl alcohol-benzene 1/1 (v/v) at a concentration of 40 mg/ml. Appropriate aliquots were taken for the determination of the specific activity of the total phosphatides and for quantitative paper chromatography. The latter method was used to determine the specific activity of lecithin and phosphatidyl ethanolamine. The quantitative paper chromatography has been described previously (6). The data of these experiments are summarized in Table 1.

RESULTS

The data in Table 1 demonstrate that in three different experiments the digitoxin preparation markedly increases the specific activity of the total rat heart ventricle phosphatides. Furthermore, the data show that this increase occurs in both lecithin and phosphatidyl ethanolamine. The effect is greater with phosphatidyl ethanolamine than with lecithin.[3]

Since the vehicle in which the digitoxin is dissolved contains several compounds, it was desirable to test some of these compounds and the vehicle independently. The results of these experiments, which are also given in Table 1, show that glycerol and ethanol had no effect on the turnover of the heart phosphatides, but that the vehicle did produce some stimulation. The total digitoxin preparation, however (vehicle plus digitoxin), produced the greatest effect. When the differences among the means are compared with their standard errors, it can be seen that digitoxin does increase the rate of incorporation of radioactive phosphorus in the rat heart phosphatides.

[3] It was not ascertained whether the plasmalogen or diester fraction of these phosphatides was stimulated. Our analysis (unpublished data) shows that 8% of the lecithin and 23% of the phosphatidyl ethanolamine in rat heart is of the plasmalogen form.

DISCUSSION

These experiments support the hypothesis that, in part, the action *in vivo* of digitoxin on heart muscle is to increase the turnover[4] of certain phosphatides. This increased turnover may be coupled with ATP production and hence yield energy for muscle work. It remains to be determined whether digitoxin affects the other lipids of heart muscle. It also remains to be determined at what time interval the effect of digitoxin is most pronounced.

Digitoxin has its therapeutic action on the failing heart and gives little or no physiological response on the normal heart.[5] The experiments reported in this paper were done on normal rats. The difficulty in producing artificial heart failure in rats did not allow the same study on such rats.

The reason why digitoxin is more effective on the failing heart than on the normal heart may be related to the rate of ATP production. If the rate of ATP production in the normal heart is just sufficient or in excess of its demands, then any further increase in its production would have no effect on the animal. On the other hand, if the ATP production is less than the required amount for a normal functioning heart (as might prevail in the failing heart), then an increase in its production would have a marked effect on the animal. It may be possible that the increased incorporation of isotope into the phosphatides is associated with an increased rate of oxidation of these lipids. This increased oxidation might yield more ATP by the usual metabolic pathway for fatty acid oxidation in the mitochondria.

The effect observed in these experiments may also be considered to involve membrane phosphatides and hence a permeability process might be implicated rather than ATP production. The increased incorporation may be associated with the transport of a specific cation or substrate across the cell membrane.

[4] It is assumed that the increase in specific activity of the phosphatides means an increased turnover rate, and hence both the rate of synthesis and degradation are increased. It is also possible that the digitoxin stimulates the uptake of inorganic phosphate or increases the specific activity of phosphorylcholine and phosphorylethanolamine or their respective cytidine nucleotide derivatives. Furthermore, the turnover of the phosphorylated base and the diglyceride moieties of the glycerol phosphatides may or may not be the same.

[5] The therapeutic dose of digitoxin given to humans is between 1.2 to 1.6 mg/day on the first day, and thereafter 0.1 to 0.2 mg/day. The dose given to the rats in this experiment on a weight basis may appear to be in excess, but it is difficult to extrapolate from the human to the rat.

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