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STUDIES ON THE DISTRIBUTION OF ADENOSINETRIPHOSPHATASE

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For a considerable time our interest has been focused on the enzymatic activity of the heart and other organs under the influence of digitalis-like glycosides. One of these enzymes is adenosinetriphosphatase (ATPase).

Our previous work, directed by the late Dr. A. G. Mulder (1951:95) had demonstrated a significant decrease in the adenosinetriphosphate (ATP) content of dog hearts *in situ* in experimentally induced pathological states as compared with normal dog hearts *in situ*. It is conceivable that the decreased mechanical efficiency observed in the hearts examined in these cases might be correlated with the decreased ATP levels found. It is also possible that at least a part of the mechanism of action of the cardiac glycosides might reside in their effect on the enzyme system which regulates the level of ATP in heart muscle.

The ATPase is responsible for the hydrolysis of ATP in the muscular tissue. Within the scope of our present knowledge it would seem that the balance between the ability of the carbohydrate metabolism to produce ATP and the activity of ATPase is the principal factor governing the level of ATP in the myocardium.

It has been shown by Barron and Singer (1945) that ATPase is a sulfhydryl critical enzyme. This property of the enzyme affords a mechanism by which the cardiac glycosides may inhibit the ATPase, thus preserving the ATP in the tissue. Digitalis-like glycosides contain an unsaturated lactone ring at C-17 in the steroid moiety of the molecule, the integrity of which is essential for cardiac activity. The presence of c r i t i c a l sulfhydryl groups in ATPase and the presence of a sulfhydryl reactive unsaturated lactone ring in the cardiac glycosides presents the possibility of a glycoside-produced oxidation and/or complexing of ATPase sulfhydryl, yielding inhibition of the enzyme and preservation of ATP. Such an inhibition would foster increased efficiency of cardiac contraction by the sparing of ATP.

Our preliminary experiments seem to indicate that administration of calculated therapeutic doses of digitoxin to dogs results in a rise of the a d e n o s i n e p o l y p h o s p h a t e (APP) content of the heart, as compared with the APP levels in control undigitalized dog hearts.

We have shown (Proctor, *et al., in press*) in another series of experiments employing the method of Ackerman and Potter (1941) that the complex formation between digitoxin and ATPase is reversible. Analyses of digitoxin ATPase inhibition, according to the method of Hunter and Downs (1945), indicate that the inhibition is substrate com-

petitive and is therefore subject to regulation by the substrate concentration. A postulation which would involve preservation of ATP by virtue of cardiac glycoside inhibition of ATPase due to a sulfhydryl mechanism must countenance the existence of the sulfhydryl critical enzymes which are normally involved in ATP syntheses. It would appear that a selective inhibition by sulfhydryl reaction with specific enzymes such as ATPase, to the exclusion of such action on other enzymes, is indeed possible in the case of the cardiac glycosides.

Taking all these hypotheses into consideration, we have undertaken a series of in vitro studies of the distribution of ATPase activity in the heart, muscle, kidney, liver, brain, and the gut, as a part of the preliminary work involved in our research.

Methods

ATPase activity of the tissue homogenates was determined by a method like that used by Bell, Carr and Krantz (1952) to determine ATPase activity in arterial walls. The method of Griswold, Humeller, and McIntyre (1951) was used to determine the phosphate liberated. Phosphate spectrophotometer readings were taken at 660 mμ wavelength rather than at 450 mμ wavelength which they used. Modifications in the homogenate levels for the tissues had to be made to effect measurable enzyme action within limits imposed by the enzyme levels available. We have found 2% homogenates of all organs most suitable for our work. We have also substituted NaCl-glycine buffer at pH 7.0 for the veronal buffer used by Bell and co-workers, so as to exclude any possible effect due to the barbiturate.

Rabbits were sacrificed by a blow to the head. Dogs were anesthetized with pentobarbital, 29 mg./kg. body wt., intraperitoneally. After sacrificing the animals, the organs were removed and placed in ice-cold water. Weighed tissue was then homogenized, in cold sand with a previously chilled mortar and pestle, and diluted to volume. This homogenate was then centrifuged for 5 minutes at 590 x G to yield a distinctly turbid supernatant fluid. This was then kept in a constant temperature water bath at 37° C. for 30 minutes. Meanwhile the reaction mixture was prepared, consisting of 1.2 ml. 0.1 M NaCl-glycine buffer, 1 ml. of 0.0096 M diNaATP, 0.5 ml. 0.032 M $CaCl_2$, and 1.1 ml. of water. To this, 1 ml. of the 2% homogenate was added at the end of the incubation period. The mixture was well mixed and again placed in the water bath, this time for 15 minutes. The enzymatic reaction was stopped with 0.2 ml. of 0.1 M Na acetate saturated with ammonium sulfate and kept at 0° C. for 10 minutes. After filtration, to 2 ml. of the filtrate, 3 drops of concentrated NH_4OH and 1 ml. of Mg mixture were added, and the solution kept in the refrigerator overnight to precipitate the phosphate. On the following day the determination of the phosphate liberated was concluded.

Results

The ATPase units have been expressed as micrograms P liberated by 1 mg. of tissue in 15 minutes.

TABLE 1.—Distribution of ATPase Activity in the Rabbit.[1]

Experiment number	Heart	Muscle	Liver	Brain	Kidney	Gut
1	0.760	2.100	1.120	0.370	2.400	1.100
2	0.300	2.800	1.425	0.475	0.325	0.850
3	0.725	1.875	1.475	0.725	2.100	4.175
4	0.470	2.400	0.970	0.770	0.520	2.070
5	0.625	2.750	1.600	0.475	2.300	0.500
Average	0.576 ±.085	2.384 ±.180 t=9.0	1.318 ±.114 t=5.3	0.563 ±.077 t=0.1	1.529 ±.433 t=2.5	1.739 ±.150 t=4.3

[1] Expressed as units of ATPase defined as mcg. P liberated by 1 mgm. of tissue in 15 minutes.

TABLE 2.—Distribution of ATPase Activity in the Dog.[1]

Experiment number	Heart	Muscle	Liver	Brain	Kidney	Gut
1	0.702	1.500	2.100	1.075	3.725	0.600
2	0.950	2.220	1.800	0.970	0.320	2.100
3	1.275	1.725	2.050	1.000	3.400	1.150
4	0.800	2.420	4.400	1.200	4.500	2.900
5	1.575	1.825	2.375	1.400	2.125	2.300
Average	1.060 ±.161	1.938 ±.167 t=3.7	2.545 ±.211 t=2.9	1.169 ±.080 t=0.6	2.814 ±.748 t=2.3	1.810 ±.400 t=1.7

[1] Expressed as units of ATPase defined as mcg. of P liberated by 1 mgm. of tissue in 15 minutes.

The results obtained with rabbits are listed in Table 1. Data from each experiment listed by number present findings from the organs of an individual animal. From Table 1 it can be seen that with the exception of the brain, all organs have higher ATPase activity than the heart. It seems that the brain and the heart show about the same ATPase activity.

Data in Table 2 show the results in the dog. Each experiment lists the findings for a given animal. Examination of the data reveals that the distribution pattern observed in the dog is similar to that observed in the rabbit. However, in the dog, the heart, brain, and liver show greater ATPase activity *per se* than in the rabbit. The apparent difference in the kidney is not statistically significant. More experiments of this type are still in progress.

DISCUSSION

The results of this preliminary study have been of great interest to us in a consideration of the hypothesis on the mechanism of cardiac glycoside action which we have recently advanced (Proctor, *et al.*, *in press*). Based on findings previously reported, the hypothesis postulates a

digitalis glycoside induced inhibition of ATPase, the degree of which is regulated by the ratio of ATP concentration to enzyme level. To ascertain whether or not our postulates meet the test presented by the relative organ specificity of action on the heart, exerted by the therapeutic doses of cardiac glycosides, determination of the ATPase levels and ATP content of the various mammalian organs is necessary. Extra-cardiac ATPase levels equal to or greater than that found for the heart would support our hypothesis, and therefore the experiments reported here tend to sustain our original contentions. None-the-less, it should be pointed out that the final conclusion relative to this portion of the mechanism cannot be arrived at until the corresponding organ ATP levels have been determined. This is true even though digitoxin inhibition of ATPase has been shown to be competitive in type, regulated by substrate concentration and effected to the highest degree in the heart.

Summary

The ATPase activity of heart, skeletal muscle, brain, gut, liver, and kidney of the rabbit and dog has been determined. The distribution pattern for the enzyme is similar in each species. No species difference has been found in the case of the gut, skeletal muscle, and kidney. The ATPase activity of heart, brain, and liver is greater in the dog than in the rabbit. In both animals all the organs except the brain are significantly greater than the heart in ATPase activity. The ATPase activity of the brain is essentially equal to that of the heart. The findings have been discussed in relationship to the action of cardiac glycosides on the heart.

Acknowledgments

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Literature Cited

Ackerman, W. W., and V. R. Potter. 1949. Enzyme inhibition in relation to chemotherapy. Proc. Soc. Exper. Biol. and Med., 72: 1-9.

Barron, E. S. G., and T. P. Singer. 1945. Studies on biological oxidations. Jour. Biol. Chem., 157: 221-238.

Bell, F. K., C. J. Carr, and J. C. Krantz. 1952. Determination of inorganic phosphate produced by arterial enzymatic action. Anal. Chem., 24: 1184-1185.

Griswold, B., F. L. Humeller, and A. R. McIntyre. 1951. Inorganic phosphate esters in tissue extracts. Anal. Chem., 22: 192-194.

Hunter, A., and C. E. Downs. 1945. The inhibition of arginase by amino acids. Jour. Biol. Chem., 157: 427-445.

Mulder, A. G., *et al.* 1951. Effect of experimental coronary occlusion on phosphate fractions of the dog heart. Fed. Proc., 10: 94-95.

Proctor, C. D., *et al.* A hypothesis for a mechanism of cardiac glycoside action. Ann. N. Y. Acad. Sci., *in press.*