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STUDIES ON THE DISTRIBUTION OF ACETYLCHOLINESTERASE

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In many respects the action of cardiac glycosides on the conduction system of the heart is very similar to that elicited by acetylcholine. For example, the glycosides cause generalized slowing of ventricular conduction and atrio-ventricular nodal delay with little or no action on sino-auricular nodal tissue. The action of acetylcholine is exactly analogous.

Since our main interest is directed toward the study of the effect of cardiac glycosides on the enzyme systems of the heart and other important organs of the body, and since the question is still posed as to whether digitalis acts on the heart directly, through the vagus, or both, we have included in our studies experiments to show the effect of digitoxin on acetylcholinesterase (AcHase). Wood and Moe (1942:16) have demonstrated that digitalis causes release of intracellular potassium. An analogous release of intracellular potassium is caused by acetylcholine These findings suggest a possibility of cardiac glycoside anti-acetylcholinesterase (AcHase) activity. Since AcHase, like adenosinetriphosphatase (ATPase), contains sulfhydryl groups believed to be critical for its activity, it is possible that the cardiac glycoside could function with reference to the AcHase in a manner similar to that which has been postulated for ATPase, (Rebar, Tigerman and Proctor, 1955: 104-107). Such inhibition of AcHase would decrease the lability of endogenous heart acetylcholine, would increase its effective concentration, and thus might afford part of the explanation for the slowed conduction time seen in hearts under the influence of cardiac glycosides.

The work presented here is a preliminary study, conducted *in vitro*, because it was felt that such conditions afforded the best possibility for testing. We have felt it advisable to include the relative acetylcholinestrease activity in the heart, muscle, liver, kidney, brain, and gut of the rabbit and the dog, as in the case of ATPase. The literature yielded very little information on this activity, the only report found being that of Glick and his co-workers, (1939:31) who studied the AcHase activity in the organs of the swine.

Methods

From a study of the variations of the enzyme-substrate mixture necessary to obtain optimal results, we have found that the concentrations of the homogenate had to vary for the different organs of both experimental species. A 0.012 M solution of acetylcholine bromide (AcHBr) was found to be the best concentration of the substrate solution. Thus, for the heart we have

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

Experiment number	Heart	Muscle	Liver	Brain	Kidney	Gut
1	0.068	0.025	0.820	4.000	0.025	.....
2	0.046	0.025	0.900	2.340	0.025	0.279
3	0.068	0.016	0.940	3.880	0.062	0.620
4	0.054	0.032	0.656	3.360	0.016	0.082
5	0.049	0.019	0.918	4.050	0.011	0.032
6	0.082	0.041	0.574	3.060	0.025	0.164
Average	0.061 ± .005	0.026 ± .004 t=5.1	0.801 ± .062 t=11.9	3.450 ± .272 t=8.0	0.027 ± .007 t=3.6	0.235 ± .109 t=1.7

[1] Expressed as units of AcHase, defined as μM AcH hydrolyzed by 1 mgm. of tissue in 30 minutes.

used a 30% homogenate, for the muscle and kidney 50%, for the liver 10%, and for the brain and gut 5%. Rabbits were sacrificed by a blow to the head. Dogs were anesthetized with pentobarbital, given intraperitoneally at a dose of 29 mg./kg. body wt.

The excised organs were placed immediately in ice-cold water. The sample was then homogenized with sand in a mortar and pestle and diluted to a definite volume. The homogenate was centrifuged for 5 minutes at 590 x G, and the supernatant used for further determination.

To 0.5 ml. homogenate, kept previously for 30 minutes in a water bath at 37° C. in order to equilibrate, we added 0 5 ml. 0.24 M $MgCl_2$, 1 ml. of 0.45 M NaCl, and 1 ml. of 0.075 M $NaHCO_3$. One ml. of 0.012 M AcHBr was added to this mixture which was incubated for 30 minutes in a water bath at 37° C. to have sufficient time for the enzymatic reaction to take place. The reaction was stopped by the addition of 0.1 ml. of 50% tricholoracetic acid. The mixture was filtered and 1 ml. of the filtrate was used for the colorimetric determination of AcHase according to the Hestrin method (1949:261).

RESULTS

We have expressed our results as AcHase units which are μM acetylcholine hydrolyzed by 1 mgm. of tissue in 30 minutes.

The results obtained with rabbits are listed in Table 1. Data from each experiment listed by number present the findings from the organs of an individual animal. It can be seen that AcHase activity in the brain is highest, as one would expect, followed by the activity in the liver, gut, heart, kidney, and muscle in that order. Upon statistical analysis one finds a significant difference between the activity in the heart and all other organs except the gut. By close examination of the results in the gut, we can see large variations, which explain the low "t" value. More experiments are necessary to estimate whether the apparent difference in activity is statistically significant.

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

Experiment number	Heart	Muscle	Liver	Brain	Kidney	Gut
1	0.054	0.057	0.820	0.360	0.082	0.240
2	0.043	0.026	0.650	0.450	0.041	0.041
3	0.082	0.049	1.600	0.490	0.098	0.210
4	0.054	0.026	1.200	0.240	0.073	0.240
5	0.095	0.049	1.400	0.450	0.057	0.210
Average	0.065 ± .009	0.041 ± .006 t=1.9	1.130 ± .170 t=2.0	0.390 ± .045 t=7.0	0.070 ± .010 t=.30	0.180 ± .038 t=2.2

[1] Expressed as units of AcHase, defined as μM AcH hydrolyzed by 1 mgm. of tissue in 30 minutes.

Data in Table 2 show the results in the dog. Each experiment lists the findings from a given animal. It is clear that the results in the dog follow the same general pattern as that found in the rabbit. Exceptions are the statistically significant difference in AcHase activity of the gut when compared to that of the heart and the virtually equal activity found in the kidney and the heart.

DISCUSSION

There is some difference in the AcHase activity of some of the analogous organs of the two species studied. In the case of the kidney and the muscle, the level of activity in the dog is higher than that in the rabbit, while in the case of the brain, just the opposite is true. In each instance the difference is statistically significant. Results in swine, obtained by Glick and his co-workers (1939:31) are in the range found by us in the rabbit and the dog.

We feel that this preliminary study is of great aid to us in studying further the effect of the glycosides on this enzyme. We have shown (Proctor, *et al., in press*) that the digitoxin inhibition of AcHase results from either irreversible or pseudo-irreversible union of the digitoxin with the enzyme. Under such conditions the inhibitor virtually "titrates" the enzyme, and the degree of inhibition obtained depends upon the concentration of the enzyme. Thus we feel that the knowledge of the distribution of AcHase activity of the various organs will be definitely of value in future work. Such distribution of AcHase as we have found might imply that a given amount of digitoxin could bring about a pharmacologically significant degree of AcHase inhibition in the heart, without exerting sufficient effect on the enzyme at other critical sites such as the brain, gut, etc. to manifest observable pharmacological change.

SUMMARY

We have found some significant difference in AcHase activity between some of the analogous organs of the dog and the rabbit. Rabbit brain has higher AcHase activity than dog brain. In the case of the kidney and the muscle just the converse is true. AcHase distribution

is such that the activity of the brain, the gut, and the liver surpasses by far that of heart, kidney, and muscle. The relationship of this finding to cardiac glycoside action has been discussed.

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