Inactivation of Vasopressin by Heart Muscle

By

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The inactivation of vasopressin by heart as well as skeletal muscle was investigated in in vitro experiments. Heart muscle can inactivate vasopressin while skeletal muscle only binds some vasopressin. The mechanism of vasopressin inactivation was studied by heat treatment, by determining pH optimum and by time-depency studies.

The inactivation of vasopressin by heart muscle proved to be an active process. The possible role of this phenomenon in coronary vasospasm and in coronary diseases is discussed.

Because of the frequency of coronary diseases every factor causing dilatation or spasm of coronary arteries becomes important. Many investigators have centered around the contribution of the cardiac nerves. Recently the pathogenesis of atherosclerosis has come into the center of interest. The aim of modern biological thinking must evolve to the understanding of the basic biochemical processes that are involved.

Many vasoactive substances are thought to affect cardiac tissue but little is known about the mechanism and duration of their action. In previous studies we studied the actions and the significance of vasopressin under different conditions and its inactivation by different tissues. The results of these experiments and the techniques developed enabled us to study the catabolism of vasopressin in heart muscle.

The present paper compares the inactivation of vasopressin by heart muscle with the action of skeletal muscle.

MATERIAL AND METHODS

Male Wistar rats, weighing between 180 to 200 g, were treated according to a previously described technique. Briefly, they were bled, and slices (70 to 100µ thick) of skeletal and cardiac muscle were obtained immediately after death. Samples of the two muscles were also homogenised. The specimens obtained were suspended in vasopressin ("Pitressin" from Parke Davies) and incubated at 37°C during constant shaking for 60 minutes. After incubation,
the specimens were centrifuged and the supernatant solution was analyzed for vasopressin by bioassay. The amount of vasopressin eliminated by the tissues could be calculated by the difference between the amount added to each suspension and that remaining after incubation.

For the determination of vasopressin two different methods were used:

1. The intravenous rat technique of Ginsburg and Heller\(^6\) with the modification; not only a jugular vein but also the bladder was cannulated on the day before the experiment. We used the special cannulae of Hunter \textit{et al.}\(^3\) which Dr. Hunter kindly put at our disposal. Urine volumes were read at 5 min intervals. The water load was kept at 6 to 8\% of the body weight. Each assay consisted of the intravenous injection of four doses, two of the standard and two of the test material. Each extract was assayed simultaneously on several rats. The volumes injected were always the same for standard and unknown (0.3 to 0.4 ml). The antidiuretic effect of each dose was calculated in terms of \% antidiuresis as described by Ginsburg\(^6\) with the difference that 5 min collecting periods were used. (Percentage antidiuresis = \((V_1-V_2)/V_1\)\times 100, where \(V_1\) = rate of urine secretion during 10 min before the injection of test material, and \(V_2\) = rate of urine secretion during 10 min after the injection.)

2. In order to get a large number of results suitable for statistical analysis, the experiments were also carried out by the intraperitoneal technique of Birnie \textit{et al.}\(^5\) Male albino rats (150 to 180 g) were hydrated by gastric tube three times with 5 ml/100 g body weight of water. After the third dose the volume of urine collected is measured and the test material is injected i.p. (10 to 15 mU of Pitressin or physiologic saline as control). During the following 90 min urine is collected again. The water diuresis of the animals is calculated, e.g., the animal had been given a total of 27 ml of water. Of this, it had excreted 14.8 ml before the test substance was injected. Of this it had excreted 14.8 ml before the test material injected. This means, that 27–14.8=12.2 ml of water were retained. During the following 90 minutes 5.8 ml were excreted. The diuresis was then 5.8\times 100/12.2=47.5 per cent.

Attention must be drawn to the fact, that –in contrary to the intravenous technique–, the results were expressed here in terms of \textit{percentage diuresis} of the test animals: higher values correspond to a smaller hormonal content in the supernatant liquid which, in turn, implies that there was a higher hormonal elimination by the tissue.

\textbf{Quantitative relations:} Because of the different sensitivity of our two assay methods, different amounts of vasopressin were used for each method: In the intravenous method 10 mU of vasopressin was suspended in 20 ml of 0.9\% saline containing 1 g of tissue. From this, 0.2 ml was injected into the test rats.

In the Birnie test 5 to 15 mU of vasopressin result in a strong antidiuresis. Therefore 300 mU of vasopressin was added to 20 ml of saline solution containing
1 g of tissue, and 1 ml of the supernatants were given i.p. to the test animals.

In order to determine whether enzymatic activity was involved in vasopressin elimination by the tissues, a series of experiments was performed in which the tissues (slices of homogenates) were boiled for one or two minutes prior to incubation in the vasopressin solution. In other tests the tissue preparations were kept at 56°C for 30 minutes, after cooling, vasopressin was added in the specified amount.

A second test, the effect of pH on the disappearance rate of vasopressin, was performed by using phosphate buffer, from 5.6 to 8.2 pH. The tissues were incubated with vasopressin dissolved in the various buffers, and after centrifugation the resultant supernatant solution was adjusted to pH 7.0 immediately before injection into the rats.

Time-dependency studies were also performed to confirm the enzymatic nature of the process under study. Fresh preparations of skeletal and heart muscle were incubated for 1.20, 40, 60 and 90 minutes intervals.

RESULTS

The inactivation of vasopressin by heart and skeletal muscle is indicated by the data presented in Fig. 1. The rats receiving the superantant solution from the non-heated heart vasopressin suspension excreted much of their fluid supply, proving small vasopressor activity, that is a marked inactivating capacity for vasopressin. With the skeletal muscle less vasopressin disappeared indicating a lesser inactivation.

The same was revealed by the intravenous method (Fig. 2). The antidiuresis in % with the supernatants of heart muscle is smaller than with that of skeletal muscle.

The statistical evaluation of our data is given in Table I. Both the Wilcoxon and the Student tests were employed. There proved to be a significant deviation between heart and skeletal muscle at a level of significance of 0.01.

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<th>Table I. Statistical Evaluation of Our Data Gained by the Birnie Method</th>
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$\alpha = 0.05$ $t_{[42]} = 4.845$ $p < 0.1\%$

$\alpha = 0.05$ $t_{[42]} = 4.56$ $p < 0.1\%$
Prior heat denaturation of the heart tissue resulted in a decrease in the amount of vasopressin eliminated during the incubation procedure. There was no difference in water excretion between the rats receiving supernatant from the unheated skeletal muscle-vasopressin and those receiving the supernatant from the preheated skeletal muscle-vasopressin incubation mixture. Since the heat treatment lowered the inactivating capacity of the heart muscle, it was assumed that the mechanism of inactivation incorporated some enzyme-dependent activity.

Further proof for this contention was obtained by repeating the experiments at different pH levels (Fig. 3). The pH maximum for the elimination of vasopressin by heart muscle was found to be at pH 6.8. On the contrary the change in pH did not influence the ability of skeletal muscle to remove vasopressin.

During the first minute of the time-dependency studies there was a decrease
Fig. 2. The effect of the supernatant solutions in the intravenous rat test 6. Effect of incubating vasopressin with heart as well as skeletal muscle homogenates for 60 min at 37°C C.I.V. injections of 0.2 ml/rat. 

a) 100 µU vasopressin, b) 100 µU vasopressin after incubating with heart muscle c) 100 µU vasopressin after incubating with skeletal muscle, d) 100 µU vasopressin. The numerals below the responses indicate % antidiuresis.

Fig. 3. Vasopressin inactivation at different pH values. Birnie test. Vasopressin was incubated for 60 min at 37°C with tissue homogenates in phosphate buffers. Result of 5 experiments each.

in vasopressin activity with both heart and skeletal muscle (Fig. 4). Subsequently there was a gradual semilogarithmic decrease in vasopressin activity in the supernatant solutions obtained from the heart suspensions. No further elimination was observed in the supernatant solution of skeletal muscle.
DISCUSSION

It is generally accepted that vasopressin is identical with antidiuretic hormone. The hormone has the kidney as its site of action in certain animals. However, in man and other higher animals, it also has extrarenal activity.\textsuperscript{1,2,4,5,12,21,22} Vasopressin has a physiological action on vascular tone in lower animals.\textsuperscript{20,21} It is conceivable that vasopressin may be released during stress along with other vasoactive substances and these may be important in the pathological reaction of the coronary arteries during stress.

The data presented indicate that heart muscle is capable of inactivating vasopressin, presumably by an enzymatic process. The inactivating capacity of heart muscle suggests that there is a local protective mechanism against vasoressor activity induced by vasopressin. Further studies are needed to determine whether the development of coronary disease may be the result of a defect in the inactivating mechanism.

Acknowledgment

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References

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