

EFFECT OF THE SAPONIN OF *ACHYRANTHES ASPERA* ON THE PHOSPHORYLASE ACTIVITY OF RAT HEART

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Summary: Phosphorylase activity of isolated perfused rat heart has been determined before and following perfusion of saponin of *Achyranthes aspera* or adrenaline.

Adrenaline or saponin, in concentrations which stimulated the mechanical activity of the heart, had little effect on the total phosphorylase activity but the activity of phosphorylase *a* was increased.

INTRODUCTION

Hess and Haugaard (5) have reported that the positive inotropic effect of adrenaline and aminophylline on rat heart is associated with an increase in phosphorylase activity. It was reported earlier that the saponin of *Achyranthes aspera* causes a marked increase in the force of contraction of heart and this effect was comparable to that produced by adrenaline (4). In the present study the effect of saponin on the phosphorylase activity of perfused rat heart has been investigated and compared with that of adrenaline.

MATERIALS AND METHODS

Albino rats weighing between 100-120 g kept on synthetic diet were used for the study. The animals were killed by decapitation and the heart was quickly removed and weighed. A cannula was introduced into the aorta and the heart was perfused with oxygenated Ringer-Locke solution maintained at $38^{\circ} \pm 0.5^{\circ}\text{C}$. The drugs used were adrenaline bitartrate (2×10^{-7} g), and saponin (1×10^{-5} g), dissolved in Ringer-Locke solution.

Phosphorylase activity was measured by the method of Cori and Illingworth (2) as follows:— At the time of maximum contraction hearts were separated from the cannula, and ground in a glass homogeniser with sand and 2 ml of freeze cold 0.001 M EDTA-0.02 M sodium fluoride per 100 mg of tissue. The heart suspension was centrifuged for 5 min at 5000 rpm and 0.1 ml of the supernatant fluid was incubated at 30°C with 0.1 ml substrate mixture with and without AMP.

Inorganic phosphate was determined before and after the incubation and the activity was expressed in phosphorylase units according to the method of Cori *et al* (1). Units of phosphorylase = $1000 \times K = 1000 \times \frac{1}{t} \times \text{Log} \frac{x_e}{x_e - x}$, where $t = 5$ min, and $x_e = \%$ of the initial

TABLE I: Effect of adrenaline and the saponin of *Achyranthes aspera* on the phosphorylase activity/g tissue of isolated perfused rat heart.

Control			Adrenaline			Saponin		
A	B	B/A x 100	A	B	B/A x 100	A	B	B/A x 100
Units Phosphorylase + adenylic acid	Units Phosphorylase no adenylic acid		Units Phosphorylase + adenylic acid	Units Phosphorylase no adenylic acid		Units Phosphorylase + adenylic acid	Units Phosphorylase no adenylic acid	
84.8	51.2	60.5	80.8	68.0	84.1	68.0	51.0	75.3
68.0	34.4	50.6	68.0	51.2	75.3	68.2	51.2	75.3
68.0	34.4	50.6	68.0	61.6	90.5	75.1	54.4	72.8
51.2	32.4	63.2	71.0	58.1	81.8	78.1	60.1	76.9
60.0	30.4	50.6	75.1	55.2	73.5	68.0	53.1	78.0
84.8	51.2	60.5	—	—	—	—	—	—
80.8	50.1	62.0	—	—	—	—	—	—
68.0	34.4	50.6	—	—	—	—	—	—
68.0	30.4	44.7	—	—	—	—	—	—
Mean 70.4± ±SE 3.8	38.7± 3.1	54.8± 2.2	72.6± 2.4	58.8± 2.9	81.0± 3.1	71.5± 2.1	53.9± 1.6	75.6± 1.7
Increase in B/A x 100 with adrenaline 26.24, t=6.7, P<0.01						Increase in B/A x 100 with saponin 20.8; t=7.9 P<0.01		

glucose-1-phosphate which is reacting at equilibrium. The value of 85% was used in calculation. x = the % of initial glucose-1-phosphate which is reacting after 5 min.

The relative amounts of phosphorylase *a* and *b* were calculated from the expression (5).

$$\frac{\text{Units of phosphorylase without AMP}}{\text{Units of phosphorylase with AMP}} \times 100$$

RESULTS

The results of various experiments with and without adrenaline or saponin are shown in Table I.

It can be seen from the Table that in the hearts stimulated by adrenaline or saponin, the phosphorylase activity in the presence of AMP was almost the same as in the case of control. However, the activity in the absence of AMP, was increased after adrenaline or saponin perfusion. The ratio of activity without AMP to the activity with AMP was significantly more in hearts perfused with adrenaline and saponin as compared to controls.

DISCUSSION

The enzyme phosphorylase, catalyses the reaction ; glycogen + inorganic phosphate = glucose-1-phosphate and is believed to exist in two forms. Phosphorylase *a* is active in the absence of AMP and phosphorylase *b* is active only in the presence of AMP (3). Adrenaline is known to activate the enzyme which catalyses the formation of active phosphorylase and its action on the heart is probably related to this effect (5). In the present study the saponin of *Achyranthes aspera* which exerts a stimulant action on the myocardium has been found to stimulate the phosphorylase activity of the heart and its effect is comparable to that of adrenaline. Its mode of action may therefore be similar to that of adrenaline or it may be acting by releasing adrenaline bound in the heart cells. The possibility that the increase in phosphorylase activity is a sequence of the increased heart activity rather than a direct effect of the drug cannot also be ruled out completely.

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REFERENCES

1. Cori, C.F. , G. T. Cori and A. A. Green. Crystalline muscle phosphorylase III. *Kinetics. J. Biol. Chem.*, **151:39**, 1943.
2. Cori, G. T. and B. Illingworth. The effect of epinephrine and other glycogenolytic agents on the phosphorylase *a* content of muscle. *Biochem. biophys. Acta.*, **21: 105**, 1956.

3. Green A. A. and G. T. Cori. Crystalline muscle phosphorylase. I. Preparation, properties and molecular weight. *J. Biol. Chem.*, **151**: 21, 1943.
4. Gupta, S.S., A. W. Bhagwat and A. K. Ram. Cardiac stimulant activity of the saponin of *Achyranthes aspera*. *Ind. J. Pharmac.*, 1971 (in press).
5. Hess, M. E. and N. Haugaard. The effect of epinephrine and aminophylline on the phosphorylase activity of perfused contracting heart muscle. *J. Pharmac. Exp. Ther.*, **122**: 169, 1958.