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COMPARISON OF THE EFFECTS OF PHLOROBUTYROPHENONE DERIVATIVES ON HEART AND LIVER MITOCHONDRIA

ARTO PAKARINEN and LARS RUNEBERG

Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

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Abstract—The effects of desaspidin and flavaspidic acid on the oxidative phosphorylation, ATPase and ATP-P_i exchange of rat heart mitochondria and on ATPase of aged liver mitochondria and microsomes were tested.

Desaspidin and flavaspidic acid inhibit oxidative phosphorylation in heart mitochondria, desaspidin inhibiting at concentrations twenty five to thirty times lower than flavaspidic acid. These substances activate the latent ATPase of fresh heart mitochondria, whereas in higher concentrations they inhibit the native Mg^{2+}-dependent ATPase, flavaspidic acid inhibiting this ATPase at lower concentrations than desaspidin. They also inhibit the ATP-P_i exchange in heart mitochondria, desaspidin inhibiting at lower concentrations. In aged liver mitochondria flavaspidic acid inhibits ATPase at concentrations ten times lower than desaspidin.

The difference between the two ATPases of fresh heart mitochondria with reference to the inhibitory effect of desaspidin and flavaspidic acid is discussed.

IN EARLIER reports it has been shown that desaspidin, flavaspidic acid and some related phlorobutyrophenone derivatives from fern extract uncouple oxidative phosphorylation and inhibit respiration in rat liver mitochondria. Their effect on the latent mitochondrial ATPase is biphasic: stimulation of the ATPase is followed by inhibition when higher concentrations are used. These inhibitory concentrations also inhibit the DNP-stimulated* ATPase, but have no effect on the Mg^{2+}-stimulated ATPase of aged liver mitochondria.[1] The effects of desaspidin on other electron transport-coupled processes have also been studied.[7, 8]

Desaspidin and flavaspidic acid are considered to be muscle and heart poisons.[2, 3] Flavaspidic acid has an inhibitory effect on the Ca^{3+}-activated ATPase of heart homogenate.[3] However, the effects of desaspidin and flavaspidic acid on the heart have not been studied at mitochondrial level. We have now investigated the effects of desaspidin and flavaspidic acid on oxidative phosphorylation of rat heart mitochondria and the effects of these substances on mitochondrial ATPase and ATP–P_i exchange of rat heart in order to evaluate the possible significance of these effects for the toxic action of these antihelmintics. In view of the apparent complexity of the ATPase reactions of muscle mitochondria it was also interesting to compare the effects of these inhibitors on heart mitochondria with the effects obtained on fresh and aged liver mitochondria and microsomes.

MATERIALS AND METHODS

Solutions of ATP, hexokinase, desaspidin and flavaspidic acid were prepared as

* ATP, adenosine-5′-triphosphate; DNP, 2,4,-dinitrophenol; P_i, inorganic orthopnosphate.

described earlier.[1] Rat heart mitochondria were prepared essentially according to Cleland and Slater.[4] The aged liver mitochondria were prepared as described earlier.[1]

For preparation of rat liver microsomes a liver was homogenized in 0·25 M sucrose solution and the homogenate centrifuged at 10,000 *g* for 15 min in a Servall SS-1 centrifuge placed in a cold room at 4°. The supernatant was centrifuged at 100,000 *g* for 60 min in a Beckman Spinco L 50 ultracentrifuge. The microsomes from one liver were suspended in 4 ml ice-cold 0·25 M sucrose.

Mitochondrial oxidative phosphorylation was determined in a Warburg respirometer with 5-ml flasks. The medium contained 40 μmole potassium phosphate buffer, pH 7·4 177 μmole potassium chloride, 8 μmole magnesium chloride, 1 μmole ATP, 30 μmole glucose and 0·75 mg hexokinase, 25 μmole sodium pyruvate plus 5 μmole sodium malate was used as substrate. The mitochondria from one heart were suspended in 0·63 ml 0·25 M sucrose. The final volume of the reaction mixture was 1·0 ml plus 0·1 ml 20% potassium hydroxide in the centre well. The reaction time was 20 min, after 5 min thermoequilibration. The temperature was 30°. Two ml 10 % trichloroacetic acid was used to stop the reaction. Inorganic phosphate was determined essentially according to Ernster *et al.*[5]

Mitochondrial ATPase was determined in a medium containing 500 μmole sucrose 50 μmole Tris buffer, pH 7·4 10 μmole ATP and 8 μmole magnesium chloride in a final volume of 2·0 ml after 250 μl mitochondrial suspension had been added to start the reaction. In experiments in which the amount of mitochondria was varied, 20 μmole ATP was used. The reaction was stopped after 20 min with 3 ml 10 % trichloroacetic acid. The temperature was 30°.

ATP–P_i exchange of heart mitochondria was determined essentially as described earlier with liver mitochondria.[1] The ATPase of liver microsomes was determined in a medium containing 500 μmole sucrose, 40 μmole Tris buffer, pH 7·4 10 μmole ATP, 8 μmole magnesium chloride, 200 μmole sodium chloride, 20 μmole potassium chloride and 200 μl microsomal suspension in a final volume of 2·0 ml. Experimental conditions were the same as with determination of mitochondrial ATPase.

RESULTS

Effect of desaspidin and flavaspidic acid on respiration and oxidative phosphorylation of heart mitochondria

The effects of desaspidin and flavaspidic acid are demonstrated in Fig. 1. Desaspidin uncoupled oxidative phosphorylation at concentrations higher than 5×10^{-8} M, when the concentration of mitochondrial protein was 1·25 mg/ml.

Flavaspidic acid also uncoupled oxidative phosphorylation, but higher concentrations were needed. It was effective at concentrations higher than $1{\cdot}5 \times 10^{-6}$ M.

If 50 per cent inhibition is taken as a measure of effectiveness, desaspidin is about thirty times as strong as flavaspidic acid.

Desaspidin and flavaspidic acid did not affect mitochondrial respiration at the concentrations used.

Effect of desaspidin and flavaspidic acid on ATPase of heart mitochondria

Freshly prepared rat heart mitochondria possess an ATPase that we may call the native Mg^{2+}-dependent ATPase. A latent ATPase activity of mitochondria is stimulated by uncoupling agents, including desaspidin and flavaspidic acid.

It can be seen from Fig. 2 that desaspidin stimulated the latent ATPase at concentrations exceeding $1{\cdot}5 \times 10^{-9}$ M, when 0·41 mg mitochondrial protein per ml was used. Concentrations exceeding 5×10^{-8} M inhibited the latent ATPase. Higher desaspidin concentrations ($1{\cdot}5 \times 10^{-6}$–$1{\cdot}5 \times 10^{-5}$ M) kept the mitochondrial ATPase at

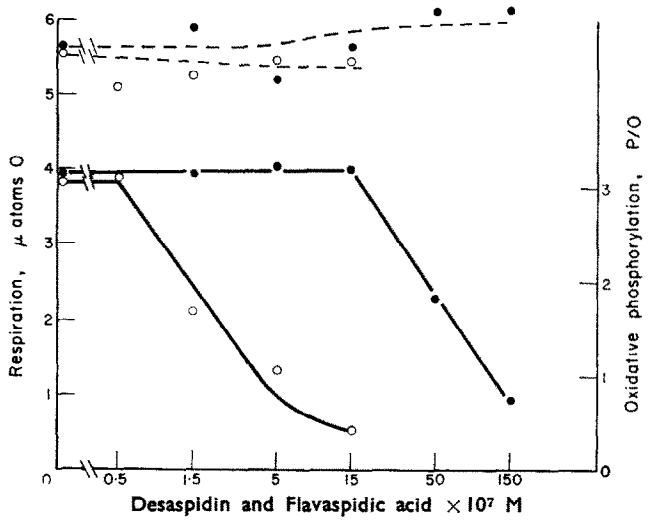

FIG. 1. The effect of desaspidin and flavaspidic acid on respiration and oxidative phosphorylation of heart mitochondria.

The repiration was determined in a Warburg respirometer, as explained under methods. The reaction medium contained 1·25 mg mitochondrial protein. The reaction time was 20 min, after 5 min thermoequilibration. —○— desaspidin, —●— flavaspidic acid, — — — — respiration, μatoms 0, ———— oxidative phosphorylation, P/O.

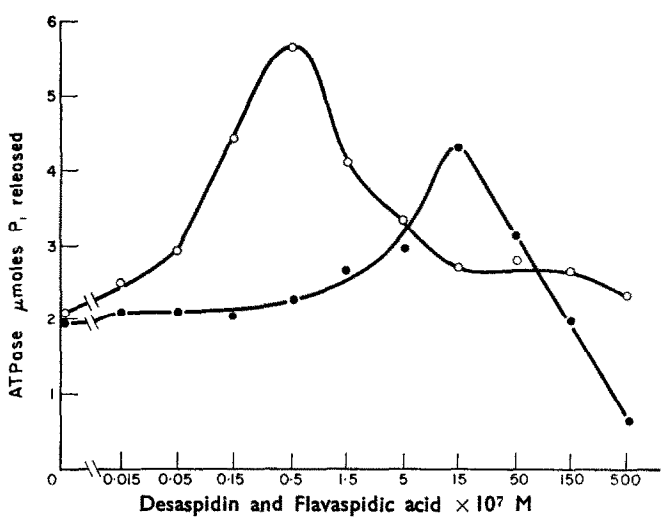

FIG. 2. The stimulating effect of desaspidin and flavaspidic acid on the latent ATPase of heart mitochondria.

The reaction medium contained 0·82 mg mitochondrial protein. —○— desaspidin, —●— flavaspidic acid.

approximately the level of the native ATPase. Still higher desaspidin concentrations inhibited the enzymic activity, reducing it below the level of the native Mg^{2+}-dependent ATPase (Fig. 3).

Flavaspidic acid also stimulated the latent mitochondrial ATPase, but higher concentrations were needed (Fig. 2). Maximal stimulation was obtained with $1{\cdot}5 \times 10^{-6}$ M flavaspidic acid. Higher concentrations strongly inhibited the activity (Fig. 3). The

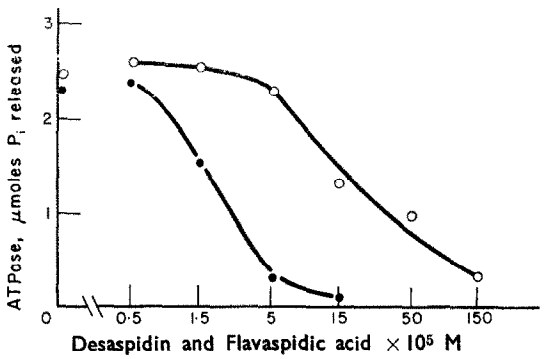

FIG. 3. Inhibitory effect of higher than stimulating concentrations of desaspidin and flavaspidic acid on the ATPase of heart mitochondria.

The reaction medium contained 0·87 mg mitochondrial protein. —○— desaspidin, —●— flavaspidic acid.

activity curve did not form a plateau at the level of the native ATPase, as it did when desaspidin was used.

These results show that desaspidin stimulates and inhibits the latent ATPase at concentrations about thirty times lower than flavaspidic acid, while flavaspidic acid inhibits the enzymic activity below the level of the native Mg^{2+}-dependent ATPase at concentrations about ten times lower than desaspidin.

Titration of heart mitochondria with desaspidin

The phlorobutyrophenones are firmly bound to liver mitochondria, which may thus be titrated with desaspidin.[1]

With heart mitochondria the titration effect is demonstrated in Fig. 4. In this experiment two different amounts of mitochondria were used in identical media. The optimal concentrations of desaspidin for ATPase stimulation were directly proportional to the quantity of mitochondria present.

Effect of desaspidin and flavaspidic acid on ATPase of aged liver mitochondria

Desaspidin at a concentration of about $1{\cdot}25 \times 10^{-4}$ M caused 50 per cent inhibition of ATPase activity of liver mitochondria aged by treatment with desoxycholate (Fig. 5). In this experiment the concentration of mitochondrial protein was 0·28 mg/ml. Flavaspidic acid caused 50 per cent inhibition at about $1{\cdot}6 \times 10^{-6}$ M with the same mitochondrial preparation. Thus flavaspidic acid inhibits the ATPase at concentrations about ten times lower than desaspidin.

Similar results were obtained when mitochondria were aged with desoxycholate as with incubation for 30 min at 37°.

Effect of desaspidin and flavaspidic acid on ATP–P_i exchange of heart mitochondria

Desaspidin and flavaspidic acid inhibited the ATP-P_i exchange in heart mitochondria. Desaspidin was inhibitory at concentrations about ten times lower than flavaspidic acid (Fig. 6). It inhibited ATP–P_i exchange at concentrations higher than 5×10^{-9} M. The concentration of the mitochondrial protein was 0·35 mg /ml.

The inhibition of ATP-P_i exchange reactions parallels the appearance of ATPase activity in the heart mitochondria.

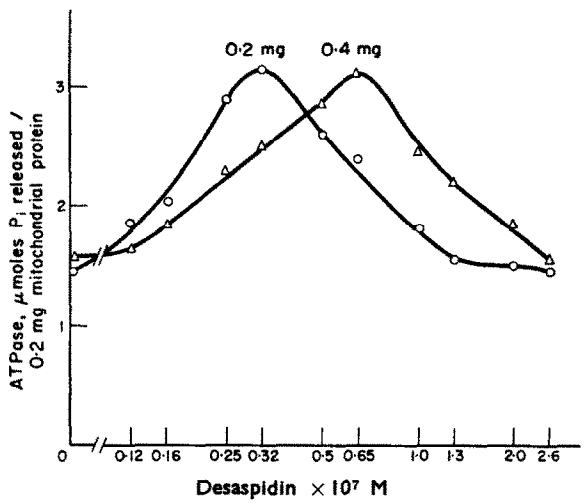

FIG. 4. Titration of heart mitochondria with desaspidin. Each sample contained 0·4 mg or 0·2 mg mitochondrial protein as indicated.

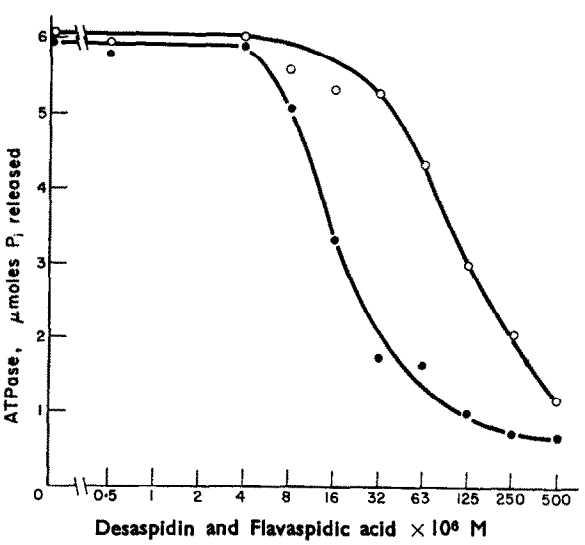

FIG. 5. Inhibition of the ATPase of aged liver mitochondria with desaspidin and flavaspidic acid. Liver mitochondria were aged with desoxycholate treatment and 0·56 mg mitochondrial protein was used. —○— desaspidin, —●— flavaspidic acid.

Effect of desaspidin and flavaspidic acid on ATPase of liver microsomes

Desaspidin and flavaspidic acid did not affect the ATPase of liver microsomes at the concentrations used (5×10^{-7} to 5×10^{-4} M) when the concentration of the microsomal protein was 0·6 mg/ml.

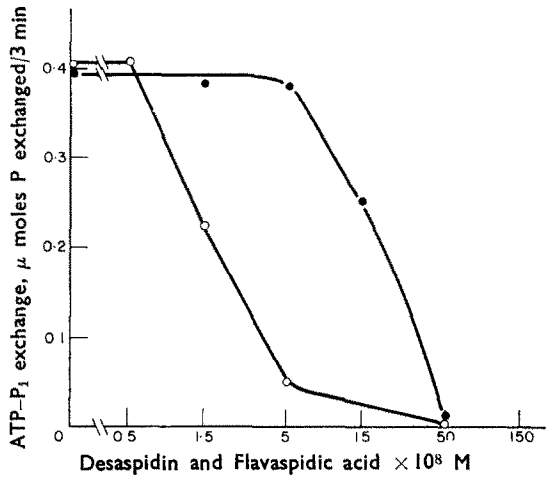

FIG. 6. Inhibition of the ATP–P_i exchange of heart mitochondria with desaspidin and flavaspidic acid. 0·70 mg mitochondrial protein was used. —○— desaspidin, —●— flavaspidic acid.

DISCUSSION

Desaspidin and flavaspidic acid uncouple oxidative phosphorylation in heart mitochondria as in liver mitochondria. In the heart respiration is less sensitive to inhibition by phlorobutyrophenone derivatives in relation to their uncoupling effect than in the liver. Since desaspidin is a more potent uncoupler than flavaspidic acid in the heart as well as in the liver, these results are in accordance with the proposed site of action of desaspidin.[1] In the liver maximal stimulation of mitochondrial ATPase was obtained with 0·7–0·9 μmole desaspidin/g mitochondrial protein,[1] whereas in the heart only about 0·12 μmole desaspidin was needed/g mitochondrial protein (Fig. 2). This might contribute to the cardiotoxic effect of these agents. It could also be interpreted as showing that there are about 6–8 times as many desaspidin binding sites per unit weight of protein in liver mitochondria as in heart mitochondria.

Both heart and liver mitochondria possess a latent ATPase, which is activated by uncoupling agents, including desaspidin and flavaspidic acid. In addition, freshly prepared heart mitochondria exhibit a native Mg^{2+}-dependent ATPase, which is absent in fresh liver mitochondria. The difference between the two ATPases of fresh heart mitochondria with reference to their sensitivity to the inhibitory effect of desaspidin and flavaspidic acid is demonstrated by the present results. Desaspidin stimulates and inhibits the uncoupler-activated ATPase and inhibits ATP–P_i exhange as well as oxidative phosphorylation at lower concentrations than flavaspidic acid, while the reverse is true for the inhibition of the native Mg^{2+}-dependent ATPase. In this respect the ATPase of aged liver mitochondria resembles the native ATPase of heart mitochondria. Thus the native Mg^{2+}- dependent ATPase of heart mitochondria appear to have fewer reaction steps in common with oxidative phosphorylation than the uncoupler-activated ATPase of these preparations have. The native ATPase of muscle

mitochondria has indeed been proposed to be completely unrelated to oxidative phosphorylation.[6]

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