Rat hepatic sodium plus potassium ion-dependent adenosine triphosphatase after treatment with digoxin and thyroxine

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Summary
1. The effect of treating rats with digoxin and thyroxine for 45 days has been studied.
2. Animals fed with digoxin gained significantly more weight than the control animals.
3. Treatment with digoxin, thyroxine or both produced a similar significant increase in the amount of Na\(^+\) + K\(^+\)-dependent adenosine triphosphatase in liver without an additive effect.
4. It is suggested that digoxin resistance in thyrotoxicosis may be related to this similarity in action.

Key words: adenosine triphosphatase, digoxin, thyroxine.

Introduction
For many years it has been apparent that thyrocardiac disease is relatively resistant to treatment with cardiac glycosides (Watson & Gazes, 1973; Smith, 1973; Goldberg, 1970). It is likely that cardiac glycosides have a single pharmacological action, namely inhibition of Na\(^+\) + K\(^+\) dependent adenosine triphosphatase (Post & Sen, 1965), which results in partial blockade of active sodium transport across the cell membrane, and this enzyme is widely regarded as the specific receptor for cardiac glycosides. It has been postulated (Edelman, 1974) that among the many cellular effects of thyroxine, alteration of protein synthesis might result in increased Na\(^+\) + K\(^+\)-ATPase\(^\) activity, and thereby provide a further mechanism for interaction between thyroxine and digoxin. Therefore we have examined their effect, both independently and together, on Na\(^+\) + K\(^+\)-ATPase activity in rat liver.

Method
Twenty-four Sprague–Dawley rats, of average weight 150 g and maintained on a standard diet (Asschem Rat Diet 41B), were divided randomly into four equal groups. Group 1 was fed with thyroxine (0·6 μmol day\(^{-1}\) kg\(^{-1}\) body wt.) mixed in a small amount of moistened crushed standard diet. Group 2 received digoxin (0·5 μmol day\(^{-1}\) kg\(^{-1}\) body wt.); group 3 received both drugs in the same dosage and group 4 were maintained as control rats. After 45 days on this regimen, the rats were killed and blood was obtained by cardiac puncture for estimation of digoxin, protein-bound iodine, plasma and erythrocyte electrolytes. Digoxin assay was performed by radioimmunoassay using a standard kit (Lanoxitest, Wellcome Reagents). Plasma protein-bound iodine estimations were made with a Technicon Autoanalyzer technique (Riley & Gochman, 1964). Samples for measuring plasma and erythrocyte sodium and potassium were prepared in duplicate and flame-photometric estimations carried out ‘blindly’ with an Eppendorf flame photometer. Results are expressed in mmol/kg of erythrocyte mass.

Livers from single rats were perfused, mashed, homogenized and suspended in sucrose (250 mmol/l) and made up to 20 ml. Centrifugation and rewashing (three times) in a Spinco ultracentrifuge.
at 13 000 rev./min allowed removal of nuclear and mitochondrial fragments. The supernatant was centrifuged at 50 000 rev./min for 2 h to sediment the microsomal fraction. The pellets were resuspended in sucrose and centrifuged again and the final volume was adjusted to 5 ml. Determination of protein was carried out by a modification of the Lowry technique (Lowry, Rosebrough, Farr & Randall, 1951).

Assay for ATPase activity was carried out in a medium containing ATP (2 mmol/l), Mg²⁺ (1 mmol/l), Na⁺ (58 mmol/l), K⁺ (8 mmol/l), Cl⁻ (147 mmol/l), CN⁻ (10 mmol/l), EDTA (0·1 mmol/l), Tris buffer (92 mmol/l) (Lamb & Lindsay, 1973). The final osmolality was 313 mosmol/kg and pH was corrected to 7·40 by titration with hydrochloric acid (100 mmol/l) if required. Enzyme and substrate blanks were included in each assay. Non-specific ATPase activity was estimated in a duplicate series with Na⁺ and K⁺ omitted from the assay medium. Choline was added to maintain osmolality. Na⁺ + K⁺-ATPase activity was calculated as the difference between total ATPase and non-specific ATPase values. The addition of thyroxine did not affect the assay and digoxin could not be demonstrated bound to the finally prepared enzyme. Similarly tritiated ouabain added to the liver homogenate was found to be lost during the preparation.

Samples were prepared in duplicate and the reaction was started by the addition of 0·2 ml of liver extract to 1·8 ml of incubation medium. After incubation for 1 h at 37°C, the reaction was terminated by the addition of 2·0 ml of trichloroacetic acid (0·8 mol/l). A portion (1·5 ml) of molybdate reagent (sodium molybdate, 70·4 mmol/l, NaCl, 2·24 mmol/l) was added to each tube, the contents were mixed and 4·0 ml of ethyl acetate was added. The tube was capped and agitated vigorously for 60 s. After separation of the two layers, the top layer was removed and stored in a sealed tube. The extinction of all samples was determined spectrophotometrically at 310 nm against ethyl acetate blanks. Standards containing inorganic phosphate were treated with molybdate and the resultant phosphomolybdate was taken up in ethyl acetate. Ouabain was obtained as ouabain glycoside (Laboratory Nativelle Ltd, Paris, France) and prepared fresh from stock.

Results

After receiving digoxin for 45 days, the rats in group 2 had gained significantly more weight than those in the other groups (P<0·05 from control). The animals fed with thyroxine only (group 1) were, on the average, lighter than their controls.

| TABLE 1. Effect of digoxin and thyroxine treatment on body weights and liver Na⁺ + K⁺-ATPase from adult rats |
|---|---|---|---|---|
| Group 1 (thyroxine) | Group 2 (digoxin) | Group 3 (thyroxine and digoxin) | Group 4 (control) | Significance |
| Weight (g) Initial | 141·7± 3 | 149·7± 3·2 | 156·5± 2·0 | 150± 5·4 | NS |
| Final | 362·7± 14* | 448·5± 10** | 406·5± 6·9* | 414·7± 12·2** | *P<0·05 **P<0·05 |
| Plasma protein-bound iodine (nmol/l) 1377± 126* | | | | 298± 11 |
| Plasma digoxin (nmol/l) | 14·0± 2·5 | 11·0± 1·87 | | **P<0·001 (from control) NS |
| Liver Na⁺ + K⁺-ATPase (mmol/l) | 2·3± 0·25* | 2·35± 0·34* | 2·70± 0·34* | 1·41± 0·22 |
| + Ouabain (100 μmol/l) | 1·03± 0·18 | 1·21± 0·21 | 1·29± 0·24 | 0·65± 0·10 |
| Inhibition (%) | 55 | 52 | | 54 |
| NS | | | | |
although the mean weights did not significantly differ (Table 1). The group fed with both digoxin and thyroxine gained almost exactly the same amount of weight as the control group but were significantly heavier than the group fed with thyroxine alone, and lighter than the group fed with digoxin alone. The serum protein-bound iodine was 298 ± 11 nmol/l in the control group, a value well within the expected normal and significantly below that of groups 1 and 3, which were fed with thyroxine. The serum digoxin concentrations in groups 2 and 3 were not significantly different, being well into the toxic range for the human. Plasma and erythrocyte electrolyte concentrations did not differ between groups after 45 days of therapy.

The addition to the diet of digoxin, thyroxine or the combination of both drugs induced a significant increase in the activity of Na\(^+\) + K\(^+-\)ATPase obtained from the liver (Table 1). Both drugs increased the amount of the enzyme which could be inhibited by ouabain at a concentration of 0.1 nmol/l, although the percentage of the total inhibited did not change. It is of considerable interest that the effects of those drugs in combination were not additive. In fact, the value obtained in group 3 (2.75 ± 0.35 μmol h\(^-1\) mg\(^-1\)) was not significantly greater than that in groups 1 and 2.

**Discussion**

Our results confirm that oral feeding of either digoxin or thyroxine to rats produced an increased activity of Na\(^+\) + K\(^+-\)ATPase in liver. The addition of both thyroxine and digoxin to the diet did not result in any further increase in the amount of enzyme isolated. Acute inhibition of the sodium pump enzyme system by cardiac glycosides has been extensively studied, with much interest devoted to the mechanism linking this known effect to the inotropic effects on cardiac muscle. It has been suggested that inhibition of the sodium transport system results in an increased sodium concentration. Sodium/calcium exchange is thereby stimulated, increasing the concentration of free calcium within the cell. Recent work has indicated that the rise in intracellular sodium produced by partial inhibition of the Na\(^+-\)K\(^+\) transport will result in an increased production of Na\(^+\) + K\(^+-\)ATPase, in other words, an increase in the density of sodium pump sites on the membrane (Boardman, Lamb & McCall, 1972). It would appear this is an adaptive change by cells to an insult to their internal environment, in an attempt to restore the *status quo*. In most instances they never quite do so and a new steady state is reached, with a higher intracellular sodium and the cells pumping sodium out more vigorously (Boardman *et al.*, 1972). The production of these fresh pump sites requires protein synthesis and is a form of enzyme induction (Lindsay, 1974). Chronic administration of digoxin would then lead to the production of new sodium pump sites by enzyme induction secondary to the rise in intracellular sodium concentration (Lamb & Newton, 1973). Such a mechanism is confirmed by our present experiments.

The effects of thyroxine on cell metabolism, on the other hand, are more varied, but one bears remarkable similarity to the pharmacological action of cardiac glycosides. In hyperthyroid animals, early studies indicated that the increased tissue respiration was related to an increased activity of the sodium pump (Ismail-Beigi & Edelman, 1970, 1971). In fact, almost all of the extra heat generated could be accounted for by the increment in pump activity and the energy released by production of inorganic phosphate from ATP. Further investigation has been centred around the mechanism of action of thyroxine on pump activity and this is still not clear. This action could occur at any one of several points in the sodium-exchange mechanism (Edelman, 1974). For example, increased diffusion of sodium into the cell might stimulate pump activity or, alternatively, a direct action on the enzyme system might result in faster pump activity. A change in the stoichiometry of the pump might mean more ATP hydrolysed per unit of sodium extruded from the cell, or the action of thyroxine might increase the amount of ATP available by stimulating oxidative phosphorylation in mitochondria. The final alternative, and one which is most relevant to our results, would require an increased number of sodium pump sites, either by a direct action on their production rate or indirectly through a rise in intracellular sodium concentration. We have confirmed such an increase in sodium pump sites exhibited as an increased activity of the enzyme Na\(^+\) + K\(^+-\)ATPase.

It is interesting to speculate on interaction of digoxin and thyroxine since in the long term both have the same effect on the sodium pump, but presumably by differing mechanisms. There is no...
information whether or not thyroxine affects digoxin binding per se, although it must increase the total number of cell membrane binding sites (since digoxin binds to Na\(^+\)K\(^+\)-ATPase). Assuming no effect on the affinity of digoxin binding, then in thyrotoxic patients a higher concentration of digoxin should be required to saturate the same fraction of sites, perhaps explaining some increased resistance, at a cellular level.

Although both digoxin and thyroxine produce more Na\(^+\)K\(^+\)-ATPase independently, together they do not exhibit an additive effect and it is this fact which may be of relevance when digoxin resistance in hyperthyroidism is considered.

There are therefore both cellular and haemodynamic explanations for digoxin resistance in thyrotoxicosis but the contribution of each is still in doubt.

Care must be taken, as always, in drawing conclusions about the human situation from experiments in rats, which are less sensitive than man to the effects of digoxin. In addition, other mechanisms for interaction exist. The altered haemodynamic status of the thyrotoxic patient must contribute to digoxin resistance and it has been suggested that plasma digoxin concentrations 1 week after digitalization are lower in thyrotoxic patients (Croxson & Ibbertson, 1975), a secondary effect of increased glomerular filtration.

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