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EFFECT OF TOLBUTAMIDE ON THE RESPIRATION OF HEART MUSCLE MITOCHONDRIA

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ABSTRACT

Tolbutamide, one of the hypoglycemic sulphonylurea drugs was found to decrease the respiratory control index of beef heart mitochondria as well as that of rat liver mitochondria.

The effect of the drug on beef heart mitochondria was more pronounced than on liver mitochondria in the range of concentrations of 30-80 mg%. It was of interest to find that DNP release of mitochondrial respiration was not observed after the addition of 100 mg% tolbutamide.

These findings were discussed in relation to the side effect of this drug.

INTRODUCTION

Tolbutamide* is extensively used for the treatment of diabetes mellitus, since it was known to stimulate the release of insulin from the pancreas. Recently, however, it was reported that cardiovascular mortality rate was 12.7% for tolbutamide treated patients whereas it was 4.9% for patients receiving placebo[1]. This result suggested that the effect of this drug on the vascular system needed examination, in addition to the known effects on gastric intolerance, skin reaction and liver damage. In connection with these undesirable effects, the report of Beer and Schepper on the uncoupling effect of this drug on oxidative phosphorylation of isolated rat liver mitochondria may be pertinent[2]. Assuming that the uncoupling effect is an indication of toxicity, the effect needs to be studied in detail not only on rat liver mitochondria but also on the vascular system and on other organs. The present paper describes the effect of this drug on heart muscle mitochondria when compared to the effect observed on liver mitochondria. The detailed exami-

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* Tolbutamide: N-4-Methylbenzenesulfonyl-N'-butylurea.

nation of the mode of uncoupling effect on liver mitochondria will appear elsewhere (Katsumata and Hagihara, in press).

MATERIALS AND METHODS

Beef heart mitochondria were prepared according to the method of Hatefi *et al.*[3] by treatment of the homogenate with Nagarse (Teikoku Chemical Industry Co., Ltd.). The final pellet of mitochondria was suspended (60 mg of protein per ml) in a solution which contained 0.21 M mannitol, 0.07 M sucrose, and 0.1 mM EDTA[4]. Rat liver mitochondria were freshly prepared as described by Hogeboom[5] using the same mixture employed for suspending beef heart mitochondria. Oxygen consumption was measured polarographically using a Clark type oxygen electrode (Beckman Co., Ltd.) at 25°C. The reaction medium contained, 0.3 M mannitol, 10 mM KH_2PO_4, 10 mM KCl, 2.5 mM $MgCl_2$ and 0.25 mM EDTA in 20 mM Tris-HCl, pH 7.4 and 12 mg mitochondrial protein

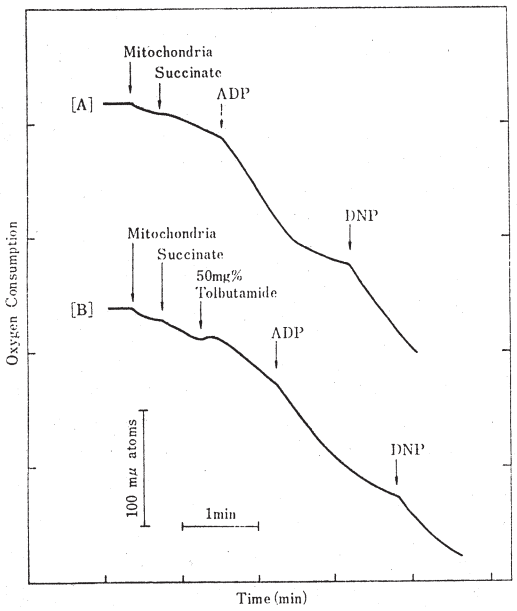

FIG. 1. The effect of tolbutamide on beef heart muscle mitochondrial respiration. Oxygen uptake was measured with a Clark type oxygen electrode. The reaction medium contained 0.3 M mannitol, 10 mM KH_2PO_4, 20 mM Tris-HCl (pH 7.4), 10 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM EDTA, 12 mg mitochondrial protein, 10 mM sodium succinate, 0.2 mM ADP and 20 μM DNP. Total volume was 5 ml, and pH of the complete system was adjusted to 7.4. Beef heart mitochondria were prepared from beef heart muscle as described in the text.

A: Control; B: 50 mg% tolbutamide (in ethanol) was added.

in a final volume of 5 ml. Mitochondrial protein was determined by the biuret method[6]. Tolbutamide was purchased from Hochst Co., Ltd., Germany, and was dissolved in absolute ethanol. Final concentration of tolbutamide was 10, 30, 50, 80, and 100 mg%. One hundred mg% tolbutamide is equivalent to 3.8 mM. Concentration of ethanol used in the control experiment was 0.2, 0.6, 1.0, 1.6 and 2.0% which corresponded to the various amounts of ethanol used for dissolving tolbutamide. Tolbutamide or ethanol was added to the reaction medium before the addition of mitochondria, followed by sodium succinate and ADP as shown in Fig. 1. ADP (0.2 mM) was used for the determination of respiratory control index (RCI). RCI was calculated by the method of Chace and Williams (1956). Finally 20 μM dinitrophenol (DNP) was added and respiratory release by DNP was examined.

RESULTS

Oxygen uptake of beef heart muscle mitochondria is reproduced in Fig. 1 A, showing a typical state 3-4-3 transition as well as the release of respiration by DNP.

Upon addition of tolbutamide, state 3 respiration was decreased while state 4 respiration was increased, resulting in the decrease in RCI. The result is typically demonstrated in Fig. 1 B, which shows the oxygen uptake when 50 mg% tolbutamide was added. This tendency became clear on increasing the amount of tolbutamide, and RCI reached 1 when 100 mg% tolbutamide was added.

The relation between the amount of tolbutamide and the respiration rate is represented in Table 1. As can be seen from the table, the addition of 10 mg% tolbutamide did not show an appreciable effect on mitochondrial respiration, but the effect became clear when the amount of tolbutamide was increased beyond 30 mg%. Even though a small effect was observed with ethanol, the solvent for tolbutamide, the effect was negligible when compared with that of tolbutamide.

Table 2 shows the effect of tolbutamide on the rate of respiration of isolated rat liver mitochondria. As can be seen from the table, the effect of tolbutamide on the rate of respiration of liver mitochondria was similar to that observed when heart muscle mitochondria were used. It was noticed that the effect seemed to be less on liver mitochondria than on heart mitochondria when the values of RCI were compared, though in both cases it became 1 if the drug concentration reached 100 mg%. It should be noted that the release of respiration by DNP was no longer observed when both liver and heart mitochondria were treated by tolbutamide.

TABLE 1. The Effect of Tolbutamide on Respiration Rate of Isolated Heart Muscle Mitochondria

Additions		Respiration (mμ atoms O/min./ml)		
Ethanol (%)	Tolbutamide (mg%)	State 4	State 3	RCI
None	None	30	120	4.0
0.2	None	31.3	109.5	3.5
0.2	10	32.6	100.1	3.1
0.6	None	32.5	112.7	3.5
0.6	30	37.6	82.5	2.2
1.0	None	30.1	105	3.5
1.0	50	40.4	75.8	1.9
1.6	None	31.2	104.5	3.3
1.6	80	50.5	65	1.3
2.0	None	32.5	104.3	3.2
2.0	100	56	56	1.0

Oxygen uptake was measured polarographically with a Clark type oxygen electrode (Beckman Co., Ltd.) at 25°C. The reaction medium contained 0.3 M mannitol, 10 mM KH_2PO_4, 20 mM Tris-HCl, pH 7.4, 10 mM KCl, 2.5 mM $MgCl_2$ 0.25 mM EDTA. 12 mg of heart muscle mitochondrial protein, 10 mM sodium succinate and 0.2 mM ADP which were added successively. Tolbutamide was dissolved in ethanol at the concentration indicated in the table. Tolbutamide in ethanolic solution or ethanol was added to the reaction mixture before the addition of heart muscle mitochondria. Total volume was 5 ml and pH of the reaction medium was adjusted to 7.4.

TABLE 2. The Effect of Tolbutamide on Respiration Rate of Isolated Rat Liver Mitochondria

Additions		Respiration (mμ atoms O/min./ml)		
Ethanol (%)	Tolbutamide (mg%)	State 4	State 3	RCI
None	None	15.1	84.9	5.64
0.2	None	17.0	86.5	5.10
0.2	10	16.2	89.9	5.56
0.6	None	17.0	89.9	5.30
0.6	30	22.5	82.4	3.66
1.0	None	15.1	87.3	5.80
1.0	50	26.3	84.9	3.22
1.6	None	21.6	82.4	3.82
1.6	80	37.4	65.0	1.73
2.0	None	22.5	81.0	3.59
2.0	100	45.0	45.0	1.00

The reaction medium and the measurement of oxygen consumption were the same as those of Table 1, except for mitochondrial preparation.

DISCUSSION

Beer and Scheppers have reported that 5 mM tolbutamide uncoupled the oxidative phosphorylation of rat liver mitochondria and postulated that this was related to the hypoglycemic action of the drug. Taking into account the fact that some adverse reaction of this drug was anticipated (The University Group Diabetes Program, 1970)[1], the uncoupling effect of this drug on oxidative phosphorylation should be considered when examining the toxic effect of this drug. Since the effect on the vascular system was emphasized, our present study was directed to the effect of this drug on the respiration of heart muscle mitochondria.

In addition, our attention was focused on a detailed examination of the effect of this drug at a number of concentrations. The effect was dependent on concentrations in the range of 30-80 mg%, when heart mitochondria were used. Since it was reported that the blood concentration of this drug reached 20 mg% upon administration *per os* of 1.5 g of the drug to diabetic patient per day, 30 mg% adopted in the present *in vitro* experiment may be close to the concentration achieved in blood during treatment[7]. Accordingly, the present data should be considered when cardiovascular side effects of this drug are discussed.

SUMMARY

1. Tolbutamide was found to decrease the RCI of beef heart mitochondria as well as that of rat liver mitochondria.

2. The effect on heart mitochondria was more pronounced than on liver mitochondria.

3. The effect was dependent on the concentration of the drug in the range of 30-80 mg%.

4. The ability of DNP to release mitochondrial respiration was no longer observed after the treatment with this drug.

The effect was discussed in relation to the cardiovascular side effects of this drug.

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