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THE EFFECT OF VB_1 AND ITS DERIVATIVES ON THE RESPIRATION OF THE ISOLATED RAT LIVER MITOCHONDRIA

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ABSTRACT

The effect of thiamine and its various derivatives on the respiratory control of rat liver mitochondria was examined. Except TDP all other thiamine derivatives tested were shown to damage mitochondrial respiration. In these experiments DCET and TATD are shown to be strong inhibitors. It was noted that thiamine and its derivatives except TDP have aldehyde group in their chemical structure. Because formaldehyde and acetalhyde are inhibitors to the respiration of the isolated rat liver mitochondria, the aldehyde group in chemical structure is speculated to have some relation to their action on liver mitochondria. Further studies are necessary concerning the mechanism of the inhibitory actions of thiamine derivatives.

INTRODUCTION

Many thiamine derivatives are at present widely used in Japan by clinicians in place of thiamine. Except thiamine diphosphate, thiamine derivatives used in Japan have an aldehyde group in their chemical structure. It is well known that formaldehyde and acetaldehyde are inhibitors to the respiration by isolated rat liver mitochondria[1]. The effect of thiamine derivatives on the mitochondrial functions must be an interesting problem to be studied. Muraoka has reported that TPD and TTFD decreased respiratory control of the isolated rat liver mitochondria. He suspected that Pi in the reaction medium and SH group in their chemical structure have some relation to this effect[2].

In the present report we examined the effect of thiamine and its seven derivates including TPD and TTFD on the respiration of isolated rat liver mitochondria and we recognized that all derivatives except TDP impaired

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Thiamine: B_1

TDP : Thiamine diphosphate
TPD : Thiamine propyldisulfide
TTFD : Thiamine tetrahydrofurfuryl disulfide
BUTDS : *o*-butyroyl thiamine disulfide
BTMP : *s*-benzoyl thiamine monophosphate
DCET : *o, s*-diethoxycarbonyl thiamine
TATD : Thiamine methyl-6-acetyl-dihydrothioacetate disulfide

mitochondrial respiration. We discnssed its results in relation to their chemical structure.

MATERIALS AND METHODS

Preparation of Mitochondria

Rat liver mitochondria were freshly prepared as described by Hogeboom[3]. 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. Mitochondrial protein was determined by the biuret method[4].

Reagents

Hydrochloride salts of thiamine, TDP, TPD and TTFD were donated by Takeda Co., Ltd., BUTDS by Tanabe Co., Ltd., BTMP by Sankyo Co., Ltd., DCET by Shionogi Co., Ltd., and TATD by Fujisawa Co., Ltd. in Japan, Thiamine, TDP, TPD and TTFD were dissolved in distilled water, BUTDS, DCET and TATD were dissolved in distilled HCl, BTMP was dissolved in distilled NaOH.

Measurement of Oxygen Uptake

Oxygen consumption was measured polarographically using 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 EDTA in 5 mM Tris-HCl, pH 7.4 and 12 mg mitochondrial protein in a fiinal volume of 5 ml. Various concentrations of thiamine and it's derivatives were added to the reaction medium before the addition of mitochondria and then the pH of this system was adjusted to 7.4 by Tris-HCl buffer. Sodium succinats (10 mM) was used as substrate. ADP (0.2 mM) was used for the determination of respiratory control index (RCI). RCI was calculated by the method of Chance and Williams[5]. Finally 20 μM dinitrophenol (DNP) was added and respiratory release by DNP was examined.

RESULTS

Table 1 shows the effect of various concentrations of thiamine and it's derivatives on the rate of respiration of isolated rat liver mitochondria. Various concentrations of TDP did not show appreciable effect on mitochondrial respiration. On the contrary, 500 mg, 1000 mg% thiamine, 500 mg% TTFD and 250 mg% BUTDS decreased RCI by less than 3.3.

Higher concentrations of TTFD and BUTDS decreased clearly RCI of liver mitochondria, 1000 mg% BUTDS increased state 4 respiration and decreased state 3 respiration showing a decrease of respiratory control.

Fig. 1 shows the effect of thiamine, TDP, TTFD and BUTDS on the oxidation of succinate by the isolated liver mitochondria.

TABLE 1 Effect of Thiamine, TDP, TTED, and BUTDS on the Respiration of Isolated Mitochondria

Additions (mg%)		Respiration		RCI
		State 4	State 3	
None		27.5	115.5	4.2
Thiamine	100	27.5	115.5	4.2
	250	28.0	106.4	3.8
	500	27.5	96.3	3.3
	1000	28.0	92.4	3.3
TDP	100	27.5	110.0	4.0
	250	27.5	107.3	3.9
	500	28.0	103.9	3.7
	1000	27.5	99.0	3.6
TTFD	100	27.5	101.8	3.4
	250	27.5	93.5	3.4
	500	22.5	69.8	3.1
	1000	22.5	60.8	2.7
BUTDS	100	27.5	101.8	3.7
	250	28.0	72.8	2.6
	500	28.0	58.8	2.1
	1000	35.0	56.0	1.6

Thiamine and its derivatives were added to the reaction medium before the addition of mitochondrial protein.

State 3 and State 4 respiration were expressed as mμ atoms 0 per minute per ml.

Mitochondrial protein concentration was 12 mg. 10 mM sodium succinate, 0.2 mM ADP were used.

Total volume was 5 ml and pH of the reaction system was adjusted to 7.4.

As shown in Fig. 1, 1000 mg% TDP did not produce appreciable effect on the respiration of mitochondria. 1000 mg% TTFD, thiamine and BUTDS decreased state 3 respiration and also decreased RCI. 1000 mg% BUTDS increased state 4 respiration. Addition of 20 μM DNP causes respiratory release of respiration by rat liver mitochondria with thiamine. TDP, 1000 mg% BUTDS and TTFD decreased apparently the respiratory release of 20 μM DNP.

Table 2 shows effect of other derivatives of thiamine on the respiration of isolated rat liver mitochondria. 100 mg% BTMP, 100 mg% TPD and 50 mg% DCET and 50 mg% TATD decreased state 3 respiration decreasing RCI. Higher concentrations of BTMP, TPD, DCET, TATD clearly decreased the state 3 respiration and decreased RCI of the rat liver mitochondria.

Fig. 2 shows the effect of 500 mg% TPD, 100 mg% BTMP, 150 mg% DCET and 100 mg% TATD on the respiration of isolated rat liver mitochondria and also on the respiratory release of DNP. They were shown to be inhibitors to the isolated rat liver mitochondria and to decrease the respiratory release

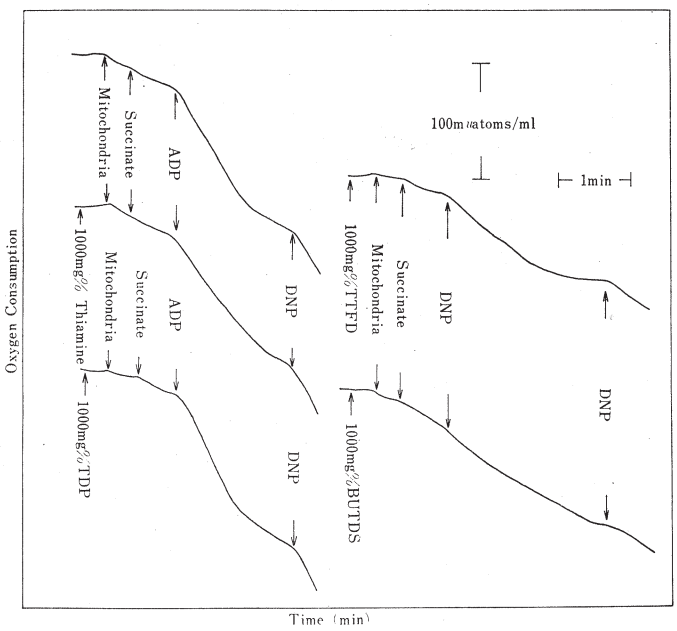

FIG. 1. 1000 mg% thiamine, 1000 mg% TDP, 1000 mg% TTFD and 1000 mg% BUTDS were added respectively to the reaction medium before the addition of mitochondria.

pH of this system was adjusted to 7.4 by Tris-HCl buffer. 10 mM sodium succinate, 0.2 mM ADP and 20 μM DNP were added as indicated in figure. Oxygen consumption was measured polarographically using Clark type oxygen electrode (Beckmann Co., Ltd.) at 25°C. Final volume of this system was 5 ml and mitochondrial protein was 12 mg.

TABLE 2. Effect of Some Derivatives of Thiamine on the Respiration of Isolated Mitochondria

Additions (mg%)		Respiration		RCI
		State 4	State 3	
None		27.5	115.5	4.2
BTMP	100	27.5	77.0	2.8
	250	27.5	57.8	2.1
	500	25.0	40.0	1.6
	1000	25.0	28.0	1.1
TDP	50	27.5	93.5	3.4
	100	27.5	93.5	3.0
	250	27.5	52.3	1.9
	500	27.5	30.0	1.1
DCET	25	27.5	99.0	3.6
	50	27.5	85.3	3.1
	100	26.0	59.8	2.3
	150	25.5	28.0	1.1

TABLE 2. (*Continued*)

Additions (mg%)		Respiration		RCI
		State 4	State 3	
TATD	25	22.5	81.0	3.6
	50	22.5	58.5	2.6
	75	20.0	38.0	1.9
	100	20.0	25.0	1.1

Experimental condition was the same as described in Table 1. As thiamine derivatives, BTMP, TPD, DCET and TATD were used.

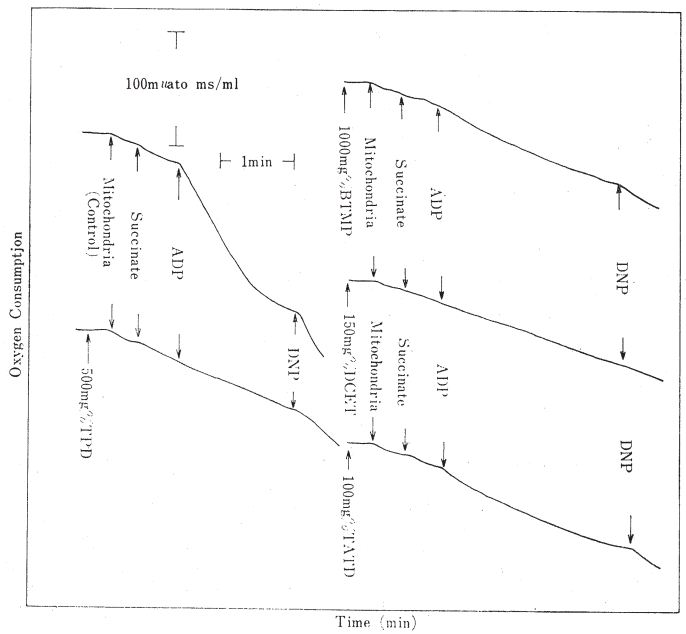

FIG. 2. Experimental conditions were same as mentioned in Fig. 1, except the use of different thiamine of derivatives. In this experiment, 500 mg% TPD, 1000 mg% BTMP, 150 mg DCET and 100 mg% TATD were used.

of DNP.

DISCUSSION

The difference in the effect of thiamine and it's derivatives on the mitochondrial respiration are very interesting. Except for TDP, large amounts of thiamine and all thiamine derivatives tested in the present study were shown to apparently decrease RCI, decreasing state 3 respiration. Thiamine derivatives can inhibit respiration of liver mitochondria by a lesser amount than by thiamine. TDP had no effect on the respiration of rat liver mitochondria.

1000 mg BUTDS increased state 4 respiration and decreased the state 3 respiration resulting in decrease of RCI. It has been reported that acetaldehyde and of formaldehyde are inhibitors to the isolated rat liver mitochondria. It was noted that thiamine and it's derivatives except TDP have an aldehyde group in their chemical structure.

Aldehyde group in thiamine and it's derivatives may have some relation to their action on liver mitochondria, because acetaldehyde is a well known inhibitor to liver mitochondria.

Although some relation between aldehyde group in thiamine and it's derivatives and inhibitory action is suspected, the fact that formaldehyde and actaldehyde were reported to have no relation to respiratory release induced by DNP shows that further study is needed concerning the relation between aldehyde group and thiamine and it's derivatives. Whether the action of thiamine and it's derivatives on the mitochondrial respiration is also demonstrated *in vivo* or not is important to be resolved. Suzuoki *et al.*[6] reported that TTFD which was intra-venously administured to rats did not have any effect on the oxidation of succinate and oxidative phosphorylation of the rat liver mitochondria. Considering the concentration of thiamine and it's derivatives impairing the respiration of mitochondria, the *in vivo* effect of thiamine and it's derivatives on the liver mitochondria can be ruled out, but it must be heeded not to use clinically higher doses of thiamine derivatives.

SUMMARY

Thiamine derivatives except TDP are shown to damage mitochondrial respiration. Among them DCET and TATD are strong inhibitors of mitochondrial respiration. Because they have an aldehyde group in their chemical structure, except TDP, the aldehyde group is speculated to have some relation to their action on liver mitochondria.

REFERENCES

1) Buskirk, T. T. V., and Frisell, W. R., Inhibition of formaldehyde of energy transfer in rat liver mitochondria, *Achieves, Biochem. Biophys.*, **1960**, 132, 130.
2) Muraoka, S., *Vitamine* (Japan), **1965**, 39, 293.
3) Hogeboom, G. H., Fractionation of cell component of animal tissues, Method in Enzymology, Academic Press Inc., New York, **1**, 16, 1955.
4) Layne, E., Spectrophotometric and turbidimetric methods for measuring protein, Method in Enzymology, Academic Press Inc., New York, **3**, 447, 1957.
5) Chance, B. and Williams, G., The respiratory chain and oxidative phosphorylation, *Advance in Enzymology*, **17**, 65, 1956.
6) Suzuoki, Z., Tsuda, M., Fujita, T., and Taketomi, S., Effect of thiamine tetrahydrofurfuryldisulfide on SH-related enzyme systems in the rat, *J. Takeda Res. Lab.*, **30** (1), 110, 1971.