BIOTRANSFORMATION OF THIAMINE TETRAHYDROFURFURYLDISULFIDE AND ITS EFFECT ON POTASSIUM LOSS IN ISOLATED GUINEA-PIG ATRIA

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In our previous paper,¹ the authors had reported that the reduction in potassium content in isolated guinea-pig atria, kept in a potassium-free medium was prevented by thiamine tetrahydrofurfuryldisulfide (TTFD), added to the medium. In the present experiment, the attempt was to clarify whether or not this drug effect was caused by a biosynthetic thiamine originating from the TTFD. The effect of TTFD was observed in atria which had been treated once with TTFD, then washed out with Locke's solution, and finally subjected to a medium containing a low concentration of potassium ions without TTFD. As this effect seemed to be due to some biosynthetic compound originating from the TTFD, the amounts of thiamine in both the free and the bound forms were chemically estimated. A direct relation, however, could scarcely be found between the effect of TTFD and the amount of biosynthetic thiamine. The effect of TTFD was therefore assumed to be produced by the biotransformation of TTFD in the atria.

EFFECTS of thiamine tetrahydrofurfuryldisulfide (TTFD), differing from those of thiamine²⁻⁵ on the contractions of isolated guinea-pig or rabbit atria have been frequently found, and their interesting character has led to many investigations^{1,5,6} in attempts to clarify whether TTFD reveals these effects as a molecule of TTFD itself, as other compounds modified from TTFD, or as substances biotransformed or biosynthesized from TTFD⁷ in the atrial tissue.

In our previous paper,⁶ it was found that the positive inotropic and negative chronotropic effects, the anti-quinidine effect, and the antagonistic effect against external excess potassium ions were shown not only by TTFD but also by dimethialium propyldisulfide (DMPD), and that these effects of TTFD were assumed to be due to some common characteristic chemical structure

shared with DMPD. It was, thereafter, found¹ that TTFD could prolong the time required to arrest atrial contractions in a potassium free medium and that the drug could prevent the decrease in potassium content and the increase in sodium content in atria induced by this medium, among which effects the former effect could be shown by DMPD, but the latter effect could hardly be shown by DMPD. This latter effect of TTFD suggested that it may be caused by some compound biotransformed or biosynthesized in atrial tissue from TTFD.

Here, the attempt had been to show whether or not the latter effect would be brought about by a thiamine biotransformed or biosynthesized from TTFD in the atrial tissue. For this purpose, TTFD was washed out from the bath fluid after the drug had once affected the atria for a definite time, and then the atria were subjected to a potassium free medium or by a medium containing a lower concentration of potassium ions than that of Locke's solution. And, next, the amount

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Fig.1. and Table I The decrease in potassium content and the increase in sodium content (mEq/Kg) in isolated guinea-pig atria induced by a potassium free medium for one hour, and those in atria pre-treated with TTFD (g/ml) for one hour before affection of this medium (N: each 10).

* In this case TTFD was added to a potassium free medium.

conc. of TTFD
pretreatment

K⁺

Na⁺

0	73.1 ± 6.13	60.0 ± 2.09
10 ⁻⁶	86.1 ± 9.25	58.8 ± 1.84
10 ⁻⁵	93.0 ± 4.03	50.9 ± 4.73
10 ⁻⁴	102.5 ± 1.60 ^a	41.3 ± 6.97 ^b
5 × 10 ⁻⁴	102.9 ± 4.64	40.4 ± 0.63
control	129.9 ± 3.38	45.4 ± 3.39
10 ⁻⁴ *	96.5 ± 2.06 ^c	55.6 ± 4.10 ^d

Each value represents the mean and its standard deviation from 10 cases.

* In this case TTFD was added to a potassium free medium.

Value (a) differs from (c) significantly ($P=0.005$), and (b) does also from (d) significantly ($P=0.005$).

of thiamine in atrial tissue which had been treated with TTFD was chemically estimated, and the gradual decrease in the amount of thiamine was pursued for three hours during which time the atria were again kept in a Locke's solution without TTFD.

METHOD

Male adult guinea-pigs weighing 250 to 350g were used. After sacrifice by cranial clubbing, the heart was excised and the atria were immediately separated from the heart and suspended on a Magnus' apparatus in a Locke's solution saturated with oxygen at 30 to 31°C. The Locke's solution used consisted of NaCl 9.0g, KCl 0.42g, CaCl₂ 0.24g, NaHCO₃ 0.7g, and glucose 1.0g per litre of distilled water. The atrial contraction were recorded on a smoked drum by means of a spring lever, whereby the auxotonic contraction amplitudes could be obtained. The thus treated atria contracted spontaneously, rhythmically, and continuously for 10 hours or more.

About 30 minutes after setting the preparation when each atrial contraction amplitude had become fairly constant, the experiment was begun. In some experiments, several kinds of modified Locke's solution were used, in which

the potassium concentration was zero or reduced to 1/2, 1/4, 1/10, 1/20, 1/30 and 1/40 of the standard. These reduced solutions are here referred to as "low potassium media" and the solution having no potassium ions is referred to as a "potassium free medium". The reduced potassium ions in these solutions were compensated by the same molar of sodium ions.

In the assay in which the potassium and sodium contents in atrial tissue were estimated, the atria were removed from the bath fluid, weighed in a wet condition, dissolved in 1.0ml of hyamine solution for 24 hours, and their contents were estimated by means of a flame photometer (Coleman 21-type), and they were expressed by mEq/Kg of wet tissue.

In the assay in which the amounts of thiamine in atrial tissue were estimated, the thiochrom method of FUJIWARA and MATSUI⁸ was used, by which the amount of total thiamine and the amount of free thiamine were estimated, and these amounts were expressed by µg/g of wet tissue. The bound thiamine was calculated from these amounts.

The drugs used were thiamine HCl and thiamine tetrahydrofurfuryldisulfide HCl (TTFD), and each concentration of the drug

applied to the medium was expressed in terms of g/ml of bath fluid.

This work was carried out in early summer.

RESULTS

1) The influence of the TTFD with which the

Treatment	control	thiamine 10^{-4}	TTFD 10^{-6}	TTFD 10^{-4}
Locke's solution	110.4 ± 11.23	108.9 ± 7.43	113.0 ± 5.81	117.9 ± 8.01
1/2K	104.1 ± 6.46	106.1 ± 7.77	—	—
1/4K	94.2 ± 9.42	97.8 ± 10.62	—	—
1/10K	83.0 ± 5.91^a	82.8 ± 10.16^b	100.7 ± 12.10^c	104.2 ± 12.12^d
1/20K	79.6 ± 4.29^a	83.0 ± 9.91^b	98.9 ± 25.43^c	101.6 ± 13.52^d
1/30K	80.6 ± 7.16	—	—	89.0 ± 3.43
1/40K	76.3 ± 6.42	79.5 ± 9.65	81.2 ± 15.54	81.5 ± 6.57

Each value represents the mean and standard deviation from 10 cases.

Value (a) differs from (c) and (d) significantly ($P=0.005$), but insignificantly from (b).

Value (a') differs from (c') significantly ($P=0.05$) and from (d') significantly ($P=0.005$), but insignificantly from (b').

Fig. 2. and Table II The decreases in potassium content (mEq/Kg) in isolated guinea-pig atria induced by the media containing low concentrations of K ions for one hour, and the influence of TTFD 10^{-6} , TTFD 10^{-4} or thiamine 10^{-4} (g/ml) on them, when the atria had been affected by the drug for one hour before the affection of the media.

atria had been pretreated on the changes in the potassium and sodium contents in atria kept in a potassium-free medium

In our previous paper,¹ it was shown that TTFD had scarcely any influence on the potassium and sodium contents in atria contracting in a normal Locke's solution, and that TTFD could prevent a decrease in potassium content and an increase in sodium content in atria affected by a potassium free medium to which the TTFD had been added.

Here, the atria were treated with TTFD in a Locke's solution for one hour, then were kept in a Locke's solution without TTFD for a half hour, and then immersed in a potassium free medium without TTFD. This process is referred to as "pretreatment with TTFD".

As shown in Fig. 1 and Table I, the potassium content decreased and the sodium content increased in atria kept in a potassium free medium for one hour, but the changes of these contents were influenced by the pretreatment with TTFD in concentrations of 10^{-6} , 10^{-5} , 10^{-4} and 5×10^{-4} . The changes in potassium and sodium contents in atria induced by the media were reduced in proportion to the TTFD concentrations with which the atria had been pretreated. This preventive effect of the TTFD 10^{-4} was more remarkable than the effect of the same concentration of TTFD added to a potassium free medium. The latter effect of TTFD had been already reported in our previous paper¹ and was reexamined for the sake of the former effect

TABLE III THIAMINE CONTENTS ($\mu\text{g/g}$ TISSUE) IN ATRIA WHICH HAD CONTRACTED IN LOCKE'S SOLUTION CONTAINING THIAMINE OR TTFD IN EACH CONCENTRATION OF 10^{-4} g/ml FOR ONE HOUR

Treatment	thiamine			TTFD			control		
	thiamine in atria			thiamine in atria			thiamine in atria		
	total	free	bound	total	free	bound	total	free	bound
	39.83	39.83	0.00	493.93	470.14	23.79	6.08	1.09	4.99
	46.68	44.16	2.52	571.76	550.12	21.64	6.58	0.82	5.76
	45.96	44.19	1.77	556.92	549.92	7.00	6.91	1.43	5.48
	—	—	—	474.54	441.54	33.00	—	—	—
mean	44.2	42.7	1.4	524.3	502.9	21.4	6.5	1.1	5.4
$\pm$ S.D.	3.76	2.51	1.29	47.31	55.61	10.77	0.41	0.30	0.39

Thiamine in the bound form was calculated from the amounts of total thiamine and of thiamine in the free form.

TABLE IV THE AMOUNT OF TOTAL THIAMINE ($\mu\text{g/g}$ TISSUE) IN ATRIA WHICH HAD CONTRACTED IN THE LOCKE'S SOLUTION CONTAINING TTFD (g/ml) FOR ONE HOUR AND ITS DECREASE IN THE AMOUNT WHEN THE ATRIA WERE THEREAFTER KEPT IN A TTFD-FREE LOCKE'S SOLUTION FOR ONE OR THREE HOURS

Treatment	control	TTFD 10^{-6}	TTFD 10^{-5}	TTFD 10^{-4}
with TTFD for one hour	6.4 ± 1.26	12.0 ± 1.46	66.9 ± 1.27	564.9 ± 12.77
1 hour after wash out of TTFD		9.3 ± 0.75	54.8 ± 2.45	478.3 ± 10.21
3 hours after wash out of TTFD		7.1 ± 0.70	50.6 ± 0.95	449.4 ± 17.14

Each value represents the mean and standard deviation from 5 cases.

of TTFD in this experiment (the latter effect is marked with * in Fig. 1 and Table I).

2) *The changes in potassium contents in atria pretreated with TTFD or thiamine and kept in media containing concentrations of potassium ions lower than the Locke's solution*

The decrease in potassium contents in atria kept in low potassium media were examined. These results, as controls, were compared with the ones obtained in the experiments in which the atria were pretreated with TTFD 10^{-6} or 10^{-4} and with thiamine 10^{-4} . As shown in Fig. 2 and Table II, a proportional relation was observed between the potassium content in atria and the potassium concentrations in the media surrounding the atria, and the influence of low

potassium media may be assumed to be fairly antagonized by the pretreatment of atria with TTFD 10^{-4} and 10^{-6} among which the effect of 10^{-4} was stronger than that of 10^{-6} though a remarkable difference could scarcely be observed between them. On the other hand, thiamine 10^{-4} had scarcely any effect on the potassium content of atria influenced by the lower concentrations of potassium ions in the media.

3) *The amount of thiamine in atria which were treated or pretreated with TTFD or thiamine*

A total amount of thiamine and an amount of thiamine in a free form in atria were chemically estimated, after treatment for one hour with TTFD 10^{-6} , 10^{-5} and 10^{-4} or with thiamine 10^{-4} in a Locke's solution. And, next, the total

amount of thiamine in the atria, which had spontaneously contracted in a Locke's solution for one or three hours after the above pretreatment with TTFD 10^{-6} , 10^{-5} or 10^{-4} were estimated. The results obtained are shown in Table III and IV. More thiamine was found in atria which had been affected by TTFD 10^{-4} than in those which had been affected by TTFD 10^{-5} or 10^{-6} . But, the amount of thiamine in the atria affected by TTFD 10^{-6} was less than that in the atria affected by thiamine 10^{-4} . And, the estimated total amount of thiamine was found to consist largely of thiamine in a free form. Each amount of this biosynthetic thiamine from TTFD was proportional to the concentration of TTFD with which the atria had been affected, and it reduced in a small amount even one or three hours after the removal of TTFD from the bath fluid.

DISCUSSION

The effect of TTFD, which could prevent the decrease in potassium content and the increase in sodium content in atria which had been kept in a potassium free medium containing the drug, has already been reported by the authors.¹ As this effect was scarcely shown by DMPD, this kind of the drug-effect led us to the speculation that this effect may be caused by TTFD transported into atrial tissue. And this speculation developed to a second speculation that a large amount of thiamine biosynthesized from TTFD in atrial tissue may play a role in the manifestation of the above preventive effect of TTFD on the changes in atria induced by potassium free media.

In order to realize the first speculation, the atria were once treated with TTFD in a Locke's solution, they were kept in a Locke's solution without TTFD, and then they were affected by a potassium free medium or by the low potassium media. If TTFD acts after transportation in atrial tissues, the above preventive effect of TTFD would be shown even in a latter case. As may shown in experiment 1, this first speculation proved to be fact. And this effect of the TTFD with which the atria had been pretreated was more remarkable than that of the TTFD added to the potassium free medium later on. As the thiamine used as a drug promoted the increase in the atrial sodium content induced by the medium,¹ the thiamine in the medium may have had a reverse action to the thiamine being in the atrial tissue in relation to the preventive effect on the change in sodium content in atria induced

by a potassium free medium.

The next experiment was carried out to realize the second speculation; the thiamine assumed to be biosynthesized from TTFD in atrial tissue was chemically estimated in experiment 3. Actually, large amounts of thiamine were found in atria after they had been affected by TTFD in its high concentrations and the amounts were proportional to the TTFD concentrations in the media. And, in experiment 1, the decrease in potassium content in atria induced by a potassium free medium was prevented by TTFD in proportion to the concentration of the drug with which the atria were pretreated. From these experimental results, it seems to be reasonable to assume that a large amount of thiamine biosynthesized in atrial tissue from TTFD could prevent the potassium loss in atria induced by a potassium free medium.

But, this assumption may be denied because of the following facts: although the preventive effect of TTFD 10^{-6} on the potassium loss in atria induced by the media containing low concentrations of potassium ions was stronger than that of thiamine 10^{-4} when the atria were pretreated with each drug, the amount of thiamine in atria affected by TTFD 10^{-6} was much less than that in the atria affected by TTFD 10^{-4} but it was also less than that in the atria affected by thiamine 10^{-4} . These results may show that the amount of thiamine found in atrial tissue does not always play a role in the preventive effect of TTFD in the potassium loss in the atria.

It was true that a large amount of thiamine was found in atrial tissue which had been affected by TTFD at a high concentration, but the preventive effect of TTFD on a potassium loss in atria may be due not so much to the thiamine biosynthesized in atria from TTFD but to the change in permeability for potassium ions through the atrial membrane developed in the process of transportation of TTFD.

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