A STUDY ON THE CARDIAC ACTION OF THIAMINE DERIVATIVES IN ISOLATED GUINEA PIG ATRIA (I)

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Received for publication June 22, 1964

During the investigation of atrial anaphylaxis, positive inotropic effects of various drugs have been compared with that of the anaphylactic manifestation in the isolated guinea pig atria, it was observed that thiamine derivatives increased the contractile force of the atrium. Thiamine propyl disulfide (TPD) and thiamine tetrahydrofurfuryl disulfide (TTFD) are allthiamine derivatives discovered by Fujiwara (1), having a marked vitamin B₁ action on the process of glucose and lipid metabolism in the body because these compounds readily penetrate the living tissue. Clinically these drugs are used for the treatment, not only of vitamin B₁ deficiency but also, of various heart diseases, postoperative intestinal paralysis etc, and their effects have been reported (2-4). However, nothing more than effect of these drugs on energy metabolism of the cardiac muscles has been known. Very few investigation has been attempted to elucidate the nature of their clinical effectiveness. Katsura (5) has reported the increased amplitude of the isotonic contraction and prolongation of the repolarization phase of the action potential of cardiac muscle fibers in the isolated toad heart by TPD. H. Simonnet *et al.* (6) have made a comparative study of pharmaco-dynamic action of thiamine and TPD but they could not obtain any findings to explain the effect of TPD.

In the present study, attempts were made to describe a positive inotropic action of the thiamine derivatives and to compare their influences on transmembrane action potential pattern, potassium contracture and Ca uptake with those of other drugs that act inotropically in guinea pig atria *in vitro*.

METHODS AND MATERIALS

Male and female guinea pigs weighing from 200 to 300 g were used throughout the experiment. The atrium was quickly removed from exsanguinated guinea pigs and placed in Ringer's solution composed of NaCl 154 mM, KCl 5.6 mM, CaCl₂ 2.2 mM, glucose 5 mM, NaHCO₃ 5.95 mM per liter, with aerated 95% O₂ and 5% CO₂ in 30 to 32°C. And isometric recordings of contractility with an initial load of 600 mg were carried out by using a strain gage transducer of unbonded type (Shinkoh Communication Co., Ltd.: UL-10-120) in connection with a conventional dynamic strain amplifier (DS6-P) and a pen recorder. Preparations of electrically driven left atrium and of naturally

beating whole atria were used in the experiment. Electric drive was carried out by a rectangular pulse of 5 msec duration, at supramaximal voltage, 120 pulses per minute.

Transmembrane action potential of proper atrial fiber was led through a suspension ultra-micro-glass electrode of 15 to 50 MΩ from an atrium pinned horizontally on a cork block in 80 ml rectangular acryl resin bath. The simultaneous observation of the transmembrane action potential and contractile tension was done on a dual beam oscilloscope and recorded continuously on a pen recorder. The method was described in details in the previous papers (7, 8). The other experimental procedures will be described with the results.

FIG. 1

Thiamine derivatives used in the experiment are: thiamine propyl disulfide, thiamine tetrahydrofurfuryl disulfide, thiamine hydrochloride (Takeda Chemical Industries Co., Ltd.), thiamine 8-methyl-6-acetyldihydrothioctate disulfide (TATD) (Fujisawa Pharmaceutical Co., Ltd.) and their chemical structure are shown in Fig. 1. Thiol compounds are listed in Table 1.

RESULTS

I. Rate of beat and contractile tension of the atrium

a) The action on naturally beating atrium

1) TPD, TTFD and thiamine HCl: When TTFD was administered in a final concentration of 0.125 mg/ml into bath after a naturally beating atrium reached an equilibrium state, the contractile tension was enhanced at the maximum in 5 to 15 minutes and continued over two hours. The average increase was 1.6 times. The beat rate increased temporarily after administration of TPD in some instances, but gradually decreased at time goes on. The rate of decrease was 20 per cent in 20 minutes, 30 per

FIG. 2A. Isometric record of contraction, proceeded right to left, numbers indicated the beat in every 20 sec.

cent in 40 minutes and 40 per cent in 100 minutes. No further decrease was observed (Fig. 2 A, B). The larger the given dose is, the stronger action resulted up to 0.375 mg/ml, but no further increase was found beyond that range. On the contrary, when an extremely large amount (1.25 mg/ml) was applied, the beat rate decreased immediately and the contractile force also decreased followed by transient increase (Fig. 3). Finally the cardiac arrest occurred. These reactions were also produced by the use of TPD.

FIG. 2 B

A decrease in the contractile force appeared after administration of a large amount of B_1 -HCl (1.25 mg/ml), but not at all in the moderate amount (0.125 mg/ml). The beat rate was decreased by B_1 -HCl in the moderate amount. The action of B_1 -HCl is apparently different from that of TPD and TTFD, and suggests that tension increase might be due to the disulfide chain of these derivatives.

FIG. 3. Isometric record of contraction, proceeded right to left, numbers indicated the beat in every 20 sec.

2) *TATD*: TATD (9, 10) is known as a B₁ derivative which has a -S-S- chain in the molecule and easily permeable into the body tissues. The administration of this compound in the final concentration of 0.125 mg/ml increased the contractile force and decreased the beat rate. The tension increase was 40 per cent and returned to the control level after 30 minutes, but the decrease of the beat rate persisted. When the bath was washed with Ringer's solution, the contractile force returned to the normal, although the beat rate was still low. The next administration of TATD induced a lesser effect on the contractility. The tachyphylaxis observed in the cardiac muscle contractility shows the pharmacological difference of TATD from TPD and TTFD by disulfide chain.

b) The action on the electrically driven atrium

The administration of TTFD (0.125 mg/ml) to the electrically driven atrium produced a marked increase in the contractile force, about 1.6 times in maximum and returned to the normal level during 30 to 40 minutes after the administration. The repetitive administration of TTFD resulted the same reaction of enhanced contractile force in every occasion. No tachyphylaxis was seen in the contractile force produced by TTFD. TPD showed the same action as found in TTFD. But B₁-HCl (0.125 mg/ml) caused no increase in contractile force.

II. Comparison with thiol compounds

TPD is split at the -S-S- chain in the body producing propyl mercaptan (11), but the final fate in the living body is still unknown. This naturally gives rise to a question of possible pharmacological action of this propyl mercaptan in cardiac muscle contractility because the positive inotropic action of TPD is ascribed to the disulfide chain. So, the effect of thiols on the contractile tension and the beat rate of the atrial preparation were examined. Thiol compounds used in the experiment are in Table I.

Among the five compounds, dimercaprol increased the contractile tension and decreased the beat rate. The change by dimercaprol is similar to the effect of TPD

TABLE I. Thiol compounds used in the experiment, and their action on the beat rate and the contractile tension in guinea pig atria.

Compounds		Beat rate	Contractile tension
Ethyl mercaptan*	C_2H_5SH	(0)	(—)
n-Butyl mercaptan*	$CH_3(CH_2)_3SH$	(—) s	(—)
n-Propyl mercaptan*	$CH_3(CH_2)_2SH$	(—) s	(—)
Dithioglycol*	$SH(CH_2)_2SH$	(—) m	(—)
Dimercaprol**	$HO-CH_2-\underset{\cdot}{\underset{ }{CH}}-SH$ CH_2SH	(—) s	(+)

(0) : no effect, (—)s : decreased slightly,

(—)m : decreased moderately, (+) : increased.

* : obtained from Tokyo Kasei Co., Ltd.,

** : from Daichi Pharmaceutical Co., Ltd.

and of TTFD, but this disappeared in 40 minutes and inclined to be depressed. Although the contractile tension is not enhanced by the monothiols and dithioglycol, mercaptoradical released in the living cell seems to be related to the increase of the contractile force of the heart muscle.

III. Effects on the atria pretreated with DCI or atropine

Exposure of guinea pig atria to DCI in the concentration of 0.1 mg/ml for 10 to 20 minutes effectively antagonize the positive inotropic and chronotropic actions of adrenaline and noradrenaline (12, 13). TTFD and TPD increased similarly the contractile tension even in the atria pretreated by DCI in such a way. And the negative chronotropic action of the compounds was shown in the atrium pretreated with atropine sulfate (0.1 μ g/ml) same as in the normal one. From these findings it is suggested that the action of the thiamine derivatives is not related to the autonomic innervation and tissue catecholamines but probably due to primary action on cardiac muscle fibers.

IV. Transmembrane action potential of proper atrial fibers

The prolongation of the repolarization phase of the action potential was found at administration of TTFD (Fig. 4) and TPD (0.125 mg/ml). This characteristic change was observed also both with TATD (0.125 mg/ml) and moderate large amount of B_1 -HCl (0.4 mg/ml), but in the latter contractile tension was decreased (Fig. 5). As to the influence of dimercaprol on the action potential (Fig. 6), there was observed a marked shortening of repolarization phase in contrast to the action of TTFD. The change in contour of the action potential by the thiamine derivatives is different from adrenaline, histamine and serotonin (14) and digitalis glycoside (15, 16).

FIG. 4. Effects of TTFD on the transmembrane action potentials of the proper atrial fiber (solid line) and contractile tension (broken line). Arrows indicate changes after the administration.

FIG. 5. Effects of thiamine HCl on the transmembrane action potentials of the proper atrial fiber (solid line) and contractile tension (broken line). Arrows indicate changes after the administration.

FIG. 6. Effects of BAL on the transmembrane action potentials of the proper atrial fiber (solid line) and contractile tension (broken line). Arrows indicate changes after the administration.

V. Potassium contracture

When bath solution was replaced with 160 mM KCl solution, a slow development of tension which is called 'potassium contracture' will be resulted as reported in details

TABLE 2

Item	Dose/ml	Contractile tension	⁴⁵ Ca		K-contracture, agent added in			Change in A.P.-duration
			Uptake	Release	Depolarization During	Driven state After	Ca depleted state	
Adrenaline	0.01 μ g	+38%	+ 55%	$\pm$	0	0	+10%	0 nothing or slight prolongation
Histamine	1.0 μ g	+64	+112	+	0	0	+10	0
Serotonin	1.0 μ g	+84	+ 41	$\pm$	0	0	+10	0
Nicotine	40 μ g	+58	+ 32	$\pm$	0	0	+10	0
ACh	0.01 μ g	-72	+ 97	++	0	0	+20	0
Digitamin	20 λ	+44	+ 76	$\pm$	0	0	+10	0 slight shortening
TTFD	0.125 mg	+62	- 19		0	0	0	0
Caffeine	1.0 mg	+58	- 12	$\pm$	+30	+20	+30	+500 to 1000%
CaCl ₂	0.32 mg	-82	- 21*	-	+20	+20	+20	
BaCl ₂	0.53 mg	+79	- 10	$\pm$	+20	+20	+20	
Anaphylaxis	—	+78	+ 96	++	+10	0	+40	0 prolongation
Egg albumin	20 μ g	0	0	++	0	0	0	0 nothing

Electrically driven left atria of guinea pigs were used throughout, in 31–32°C Ringer's solution under 120 stimuli per min.
(0 : no effect, + : increased slightly, ++ : increased markedly, — : decreased. *total uptake of Ca is appeared as 158.4%).

in the previous paper (17). In order to observe effects on the potassium contracture TTFD and TPD were applied in the following ways: a) with KCl-solution, b) in the completed potassium contracture state (depolarized state), c) in the reconditioning interval in which the atria were electrically driven in Ringer's solution, at 120 per minute, d) in Ca depleted state induced by soaking in Ca free Ringer's solution in the reconditioning interval. Procedures of the potassium contracture are quite same with previous report (17). Whenever TTFD and TPD were applied in the mentioned four states, no change was observed in the tension development of the contracture.

As previously reported (Table 2) (14) addition of caffeine, Ba and Ca to KCl-solution enhanced the tension development of the potassium contracture for about 10 per cent, and the tension increased up to 500 to 1,000 per cent in Ca depleted state, while the application of adrenaline, histamine, serotonin, digitalis or acetylcholine produced no effect on the potassium contracture. However, magnitude of the contracture was enhanced by adrenaline, histamine and the others applied during the reconditioning interval prior to the contracture. These findings indicate that mode of action of TTFD and TPD on the potassium contracture distinctly differs from the above drugs.

VI. Rate of Ca-uptake

In order to estimate the rate of Ca-uptake in the atrium, electrically driven atria were incubated in Ringer's solution containing approximately $2 \mu\text{C}$ per ml in the presence or absence of TTFD and TPD (0.125 mg/ml) for 10 minutes. At the end of the incubation the preparations were repeatedly washed for a total period of 10 minutes with normal Ringer's solution. Atria were then blotted on filter paper, weighed and homogenized in distilled water (one ml for 20 mg tissue). An aliquot of the homogenate were evaporated in planchette under heating lamp, and radioactivity was counted with gas flow counter.

Rate of Ca uptake was depressed 18.4 per cent by TTFD (0.125 mg/ml) and 24.6 per cent by TPD (0.125 mg/ml). As already reported (Table 2) (14), Ca-uptake was markedly increased by adrenaline, serotonin and digitalis glycoside as well as by acetylcholine, but it is depressed by caffeine and Ba.

DISCUSSION

In the electrically driven (2 cps) atrium the effect of TTFD and TPD to enhance contractile tension of the cardiac muscle disappears in 40 minutes. The beat rate in naturally beating atrium slows gradually down to 60 per cent. The long-sustaining contractile tension in the naturally beating atrium may be related to the decrease of the beat rate. On the other hand, unpublished data of our laboratory have demonstrated an increased contractile tension with a decreased beat as temperature of the bath is lowered in a range between 38°C to 20°C or 23°C . And a modification of contractile tension with change in beating frequency, the stair-case phenomenon, was also reported by Niedergerke (18), Vaughan Williams (19), and others (20). Brinks and Koch-Weeser (21) have also studied the effects of changes in rate and rhythm upon myocardial con-

tractility and discussed that the term 'stair-case' has been used to refer to the interval-strength relationship. It is clear anyway that the change of the rate of beat influences contractile tension of the cardiac muscle fiber. The action of TTFD (TPD will not be mentioned because it has a same action) resembles to this phenomenon, and it seems as if the decrease in the rate is the cause of increased contractile tension. However, the increase of the tension is only temporary with TATD which has a similar action in spite of decreased state of the rate. Therefore, it is likely that TTFD not only reduced the rate, but also increases the force of contraction for a long time. The effect of TTFD on contractile tension and beat rate of the cardiac muscle is similar to the action of dimercaprol in small quantity, except that the action of the latter is shorter. Besides, their action potentials differ distinctly from each other. Dimercaprol markedly shortens the duration in repolarization phase, whereas TTFD, like TATD and B_1 -HCl, prolongs it. It is likely that thiamine in the living cells might plays a role in the negative chronotropic action and in the prolongation of the repolarization phase.

Although propyl mercaptan is derived from TPD in the living tissues, which has been already proved by Itogawa (11) on ^{35}S labeled TPD, this thiol derivative applied exogenously is not responsible for the increase of the contractile force. However, it is undeniable that mercaptoradical cloven in living cells is likely to induce the increase of contractile force of the cardiac muscle.

The fact that TTFD does not affect potassium contracture of the cardiac muscle under any condition suggests the unique action of this compound. Ineffectiveness of the compound applied in the depolarized state of cell membrances possibly means that TTFD does not act directly on contractile mechanism of muscle fibers. TTFD is as ineffective as adrenaline or digitalis when given with KCl, although the latters enhance the contracture only when administered during the electrically driven state. Therefore, the action of TTFD distinctly differs from adrenaline or digitalis, and also it is entirely different from caffeine, Ba and Ca which may act directly on contractile mechanism or alternatively to alter the relation between depolarization and contraction (17, 18). Furthermore, TTFD differs from adrenaline-histamine group or digitalis (Table 2) in its inhibition of Ca-uptake.

It is concluded that the positive inotropic action of TTFD is quite different from any other cardiotonic drugs and may not associate directly with contractile mechanism of cardiac muscle fibers. But some further investigations are needed to explain the role of the contractility in decreased beat rate and prolongation of repolarization phase of action potential caused by the thiamine derivatives. And also it is remained to be investigated whether the negative chronotropic action is caused by intracellular invasion of thiamine or to change in metabolism.

SUMMARY

The effect of thiamine hydrochloride and its derivatives on cardiac contractile force, beat rate, transmembrane action potential, potassium contracture and rate of Ca-uptake

was studied. The results obtained are summarized as follows:

1. TPD and TTFD (0.125 mg/ml) increase contractile tension for 60 per cent and decrease the beat rate for 40 per cent. These effects last longer than 2 hours, whereas B_1 -HCl has no such action.

2. In the electrically driven (2 cps) left atrium, contractile tension is similarly enhanced by TPD and TTFD, but the effect does not last long (approximately 30 minutes).

3. TATD (0.125 mg/ml) increases contractile tension for 40 per cent and reduces the beat rate, but the tension enhancing effect is rather short and causes tachyphylaxis.

4. Dimercaprol (50 μ g/ml) has the effect similar to TTFD. It increases contractile tension and reduces the beat rate, but the effect is short.

5. Repolarization phase of the transmembrane action potential is prolonged by TPD, TTFD, TATD and B_1 -HCl, but shortened by dimercaprol.

6. Potassium contracture of the left atrium is never influenced by TPD or TTFD.

7. ^{45}Ca influx is inhibited slightly by TPD and TTFD.

8. The mechanism of positive inotropic action of TPD and TTFD is entirely different from hitherto known cardiotonic agents such as digitalis, adrenaline and caffeine.

Acknowledgment : The authors are indebted to Takeda Chemical Industries Ltd., Osaka, and Fujisawa Pharmaceutical Industries Ltd., Osaka, for the thiamine derivatives used in the study.

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