EFFECTS OF THIAMINE TETRAHYDROFURFURYL-DISULFIDE (TTED) ON THE REFRACTORY PERIOD AND MAXIMUM DRIVING FREQUENCY OF ISOLATED RAT HEART MUSCLE

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Summary
1. Left atrial and papillary muscle isolated from rat heart were suspended in a Magnus' apparatus and influenced by thiamine tetrahydrofurfuryldisulfide (TTFD) added to the medium at concentrations of from $5 \times 10^{-6}$ to $1.6 \times 10^{-4}$ g/ml.
2. Contractile tension of left atrial and papillary muscle driven by electrical stimulation was respectively increased by 27.2 and 55.0% of each control under the most effective conditions at 60 min after addition. Most of the drug effect remained even after the drug removal from medium.
3. The refractory period of these muscles estimated using the modified method of Govier was increased by the drug: the maximum increase in atrial muscle was 106.3% while that in papillary muscle was 38.3% of each control. Most of the drug effect on papillary muscle remained after the drug removal from medium while the effect on left atrial muscle reduced to the similar degree to that seen in the papillary muscle.
4. Maximum driving frequency of left atrial muscle estimated using the modified method of Tanz was decreased by the drug concentrations higher than $4 \times 10^{-5}$ g/ml and the drug effect remained after the drug removal from medium, while the drug effect on papillary muscle was slight at any drug concentration.
5. The drug thiamine used in place of TTFD little influenced the refractory period and the maximum driving frequency of these muscles at the drug concentrations of $10^{-6}$ and $2 \times 10^{-4}$ g/ml.
6. From the results, the effect of TTFD as an antiarrhythmic was discussed.
Since KANNO (1) introduced the positive inotropic and negative chronotropic effect of thiamine tetrahydrofurfuryldisulfide (TTFD) on the spontaneous contractions in isolated guinea pig atria, many investigations have been performed on its pharmacologic action besides its action as a vitamin B₁. The investigations on its pharmacologic action performed seem to be divided into two groups: one treated the positive inotropic effect of TTFD on atrial muscle in comparison with those of the related thiamine disulfide compounds, and the other dealt with its preventive effect on cardiac muscle against extrinsic stimulation or invasion. The latter drug action was pointed out by the authors: the influence of a potassium-free Locke's solution on spontaneous contractions of isolated guinea pig atria and the preventive effect of the drug on this influence (2, 3), the influence of a toxic dose of strophanthin-G on the guinea pig atria and the preventive effect of the drug on this influence (4), and the influence of electrical ectopic stimulation or low temperature on the guinea pig atrial spontaneous contractions and the preventive effect of the drug on this influence (5). From these experimental results, it was assumed that the drug TTFD may have some kind of preventive effect on cardiac muscle against chemical and physical invasions, especially the last result (5) suggested its possible antiarrhythmic action. Then, the effects of TTFD on the refractory period and the maximum driving frequency of cardiac muscle were evaluated in the present experiment.

MATERIALS AND METHODS

Adult rats of both sexes weighing 350 to 450 g were sacrificed by severing the common carotid arteries and the heart was excised. The heart was immersed in Locke's solution saturated with pure oxygen at 30°C and at pH of 7.2 to 7.4 from which a left atrial muscle and two or three papillary muscles were prepared and suspended in a Magnus' apparatus. The medium was the above described Locke's solution. The contraction of the prepared muscle was induced by the electrical stimulation at a supramaximum intensity with a duration of 5 msec for atrial muscle or 10 msec for papillary muscle and a frequency of 1, 2 or 4 cycles per sec. The drug was added to the bathing medium in which the muscle was contracted, and was expected to act rapidly and readily on tissue.

The refractory period of the prepared muscle was determined using the modified method of GOVIER (6). Basal stimulation was given to the muscle at a supramaximum intensity with a duration of 5 msec for atrial muscle or 10 msec for papillary muscle and a frequency of 1, 2 or 4 cycles per sec. Test stimulation, under the same conditions as those of the basal stimulation, was given at from 10 to 500 msec after each corresponding basal stimulation. If the time interval between the two kinds of stimuli was shorter than the normal refractory period for the tested muscle, contractile response corresponding to the test stimulation would be absent. When the muscular contractile responses corresponding to the test
stimulations began to appear during gradual increase of the time intervals, the estimated time interval was determined to represent the refractory period of the tested muscle.

The maximum driving frequency of cardiac muscle to the electrical stimulation was estimated using the modified method of TANZ (7). The stimulation was given to muscle at a supramaximum intensity and a duration of 5 msec for atrial muscle or 10 msec for papillary muscle, and its frequency was increased from 1 to 13 cycles per sec at each 15 sec of time intervals. The maximum driving frequency of the tested muscle was defined by the stimulation frequency at which the muscle contractile responses corresponding to each stimulation began to lose the correspondence of one to one.

Usually, after setting of the preparation in a Magnus' apparatus, electrical stimulation was given to the preparation at a frequency of 2 cycles per sec for 30 min, within which time most of the contraction in preparation became to equilibrium. After the equilibrium, the tested drug was added to the medium, and at further 60 min after the drug addition, the contractile tension, the refractory period or the maximum driving frequency of the preparation was estimated. The drug effect only at one concentration was tested in each muscle preparation, and the volume of drug solution added never exceeded 1% of the bath volume. The drug concentration in the medium was expressed in terms of g/ml and the drug effect was represented by percentage or percent change of the control value. When the control value was tested, the same volume of Locke's solution as that of the drug solution was added.

Electrical stimulators (Nihon-kohden MSE-3R and JM, Tokyo) were used, and the contractile tension of the stimulated muscle was isometrically recorded by means of strain gauges and pen-writing oscillographs (Shinko-tsushin UL-2 and AS-3A, Tokyo). The tested drugs were thiamine hydrochloride (Takeda) and thiamine tetrahydrofurfuryldisulfide hydrochloride (TTFD, Takeda), which were initially dissolved in distilled water and then diluted in Locke's solution. Locke's solution used consisted of NaCl 9.0 g, KCl 0.42 g, CaCl₂ 0.24 g, NaHCO₃ 0.7 g and glucose 1.0 g per liter of distilled water.

RESULTS

Influence of TTFD on the contractile tension of the electrically driven left atrial and papillary muscle

The change of contractile tension in left atrial muscle induced by TTFD at the concentration range of from $5 \times 10^{-6}$ to $1.6 \times 10^{-4}$ was less than 30% of the control, though the degree of the change was varied by the alteration of electrical stimulation frequency at 1, 2 and 4 cycles per sec. The TTFD effect on the contractile tension of papillary muscle was more remarkable than the drug effect on that of atrial muscle: the increase was $52.1 \pm 10.5$, $55.0 \pm 8.9$ and $47.6 \pm 7.0\%$
(n=each 8) of the control at respective drug concentrations of $2 \times 10^{-5}$, $4 \times 10^{-5}$, and $8 \times 10^{-5}$, when the muscle was driven by a frequency of 4 cycles per sec. The drug at the same concentrations, however, decreased the tension dose-dependently when the muscle was driven by a frequency of one cycle per sec. Generally, the influence of the stimulation frequency on the drug effect was more remarked in papillary muscle than in atrial muscle (Fig. 1).

In this experiment, the drug effect on the rate and contractile tension of

![Graphs showing the influence of TTFD on the contractile tension of left atrial and papillary muscle.](image)

**Fig. 1.** Influence of TTFD on the contractile tension of left atrial and papillary muscle isolated from rat heart and driven by electrical stimulation at supramaximum intensity with a duration of 5 msec for atrial muscle or 10 msec for papillary muscle and with a frequency of 1, 2 or 4 cycles per sec. The drug effect was estimated at 60 min after addition to the medium. Each point is a mean from values in 8 preparations.

![Graphs showing the influence of TTFD on the rate and contractile tension of spontaneous contractions in isolated rat right atrium.](image)

**Fig. 2.** Influence of TTFD on the rate and contractile tension of spontaneous contractions in isolated rat right atrium. The drug effect was estimated at 60 min after addition to the medium. Each point and its standard error were calculated from values in 6 preparations.
spontaneous contractions in isolated rat right atrium was examined for reference (Fig. 2). The drug effect on this tension was more than the effect on the above electrically driven left atrium a little, but the effect on papillary muscle was much more than the effect on either right or left atrium, and the drug effective concentration on papillary muscle was lower than those on each atrium.

The influence of TTFD on atrial and papillary muscle remained at the almost same degree even after the drug removal from the medium.

**Influence of TTFD on the refractory period of left atrial and papillary muscle**

The effect of TTFD on the refractory period of left atrial and papillary muscle was varied by the alteration of basal stimulation frequency at 1, 2 and 4 cycles per sec. TTFD at concentrations of $8 \times 10^{-5}$ and $1.6 \times 10^{-4}$ increased the refractory period of left atrial muscle by $47.6 \pm 12.6$ and $106.3 \pm 16.1\%$ ($n=$each 10) of the control, respectively, when the basal stimulation was given to the muscle at a frequency of 4 cycles per sec. When the frequency was used at 1 or 2 cycles per sec, the drug effect was seen to be weaker even at these drug concentrations: the increase was less than 40% of the control at most. The papillary muscle refractory period was increased by the drug concentrations of $8 \times 10^{-5}$ and $1.6 \times 10^{-4}$, but the increase, also, was less than 40% of the control (Fig. 3).

When TTFD was removed from medium after the drug application for 60 min, the increase in papillary muscle refractory period was seen at the similar degree to those before the drug removal, while the drug effect on left atrial muscle was reduced by the removal to less than 40% of the control (Fig. 4).

![Fig. 3](image-url)

*Fig. 3. Influence of TTFD on the refractory period of isolated rat left atrial and papillary muscle estimated by means of Govier's method. The refractory period of the muscle estimated was varied by the alteration of basal stimulation frequency at 1, 2 or 4 cycles per sec. The drug effect was examined at 60 min after addition to the medium. Each point is a mean from values in 10 preparations.*
Fig. 4. Influence of TTFD pretreatment on the refractory period of isolated rat left atrial and papillary muscle estimated by means of GOVIER's method. After the muscle was influenced by the drug in the medium for 60 min, the drug was removed from the medium and then the muscle refractory period was examined. The estimated refractory period of the muscle was varied by the alteration of basal stimulation frequency at 1, 2 or 4 cycles per sec. Each point is a mean from values in 8 preparations.

Fig. 5. Influence of TTFD on the maximum driving frequency of isolated rat left atrial and papillary muscle estimated by means of TANZ's method. The drug effect was examined at 60 min after addition to the medium. Each point and its standard error were calculated from values in 8 preparations.

**Influence of TTFD on the maximum driving frequency of left atrial and papillary muscle**

TTFD at concentrations of $4 \times 10^{-6}$, $8 \times 10^{-6}$ and $16 \times 10^{-6}$ decreased the maximum driving frequency of left atrial muscle by $54.6 \pm 7.0$, $53.9 \pm 6.9$ and $57.6 \pm 8.0\%$ ($n=each\ 8$) of the control, respectively, and the drug influence remained even
Fig. 6. Influence of TTFD pretreatment on the maximum driving frequency of isolated rat left atrial and papillary muscle estimated by means of Taniz's method. After the muscle was influenced by the drug in the medium for 60 min, the drug was removed from the medium and then the muscle maximum driving frequency was examined. Each point and its standard error were calculated from values in 8 preparations.

The maximum driving frequency of papillary muscle was little influenced by TTFD even at high concentration as $16 \times 10^{-5}$ (Figs. 5 and 6).

Influence of thiamine on the refractory period and the maximum driving frequency of left atrial and papillary muscle

Thiamine used in place of TTFD gave little influence on either refractory period or maximum driving frequency of left atrial and papillary muscle at the drug concentrations of $10^{-6}$ and $2 \times 10^{-4}$; their changes induced by the thiamine were less than 10% of each control at most.

DISCUSSION

SHINOZAKI et al. (5) have pointed out that thiamine tetrahydrofurfuryldisulfide (TTFD) can increase the electrical intensity when the arrhythmic contractions were induced by the threshold electrical stimulation in spontaneous contractions of isolated guinea pig atria. As the report suggested a possible antiarrhythmic action of TTFD, the drug effect on the refractory period and on the maximum driving frequency of atrial and papillary muscles were tested in the present experiment. The rat cardiac muscle was used here, though that of guinea pig was dealt in our previous experiments (2–5), because it was found the papillary muscle of guinea pig could be easily fatigued by repeated stimulation while that of rat can continued to respond without fatigue.
TTFD was found to increase the refractory period of atrial muscle by more than 100% and to decrease its maximum driving frequency by more than 50% of each control, but the effective concentration of the drug was so high as $8 \times 10^{-5}$ and $1.6 \times 10^{-4}$ g/ml. Then, the drug effect may be not so strong even if the drug is effective as an antiarrhythmic. The result supports the finding of SHINOZAKI et al. (5) in which, also, the drug effect was shown at its high concentration such as $10^{-4}$ g/ml. However, the result obtained by them lasted almost completely after the drug removal from the medium while our result on the refractory period of atrial muscle was found to be largely reduced by the drug removal. On the other hand, the maximum driving frequency of atrial muscle was decreased by the drug at concentrations higher than $4 \times 10^{-2}$ g/ml, and the effect remained after the drug removal from the medium. Then, this drug effect rather than that on atrial muscle refractory period seems to be more similar to the result of SHINOZAKI et al. (5).

The authors (8) have compared the antiarrhythmic drug effect to prolong the refractory period of guinea pig atrial muscle in ajmaline, cocaine, diphenylhydantoin, lidocaine, procainamide, propranolol, quinidine and trimetazidine at concentrations at which they suppress the rate and contractile tension in atrial spontaneous contractions by a given degree. If the TTFD effect is evaluated by the similar manner, TTFD can prolong the refractory period of atrial muscle by more than 100% at its concentration at which the drug not only does not suppress the contractile tension but also even increases it, and at which the drug suppresses the rate by less than 30% of the control at most. When the TTFD effect is compared with those of the above eight kinds of antiarrhythmics, TTFD is assumed to be the safest drug of them to prolong the atrial muscle refractory period, if the difference of the experimental animals may be allowed.

Although KANNO (1) emphasized the positive inotropic effect of TTFD on atrial spontaneous contractions, the TTFD effect on papillary muscle contractile tension was found to be stronger than that on atrial muscle and the effective drug concentration on papillary muscle was lower than that on the atrial muscle. If the augmentation of contractile tension in cardiac muscle were to be an important effect of TTFD, the drug effect on papillary muscle would be more important than that on atrial muscle.

Regarding the experimental technique, the TTFD effect on the refractory period in cardiac muscle was found to have little relation to that on the maximum driving frequency, though TANZ (7) has assumed a drug effect on the refractory period of cardiac muscle from the effect on its maximum driving frequency. The drug effects on both function of cardiac muscle would be different from each other.

It would require the succeeding investigations to decide which frequency of electrical stimulation given to the cardiac muscle is most suitable to assume the drug effect on the muscle acting in a living animal, when the drug effects on contractile tension and on refractory period of cardiac muscle estimated are varied by the alteration of stimulation frequency at 1, 2 and 4 cycles per sec.
As the effects thus shown by TTFD were little seen when thiamine was used in place of TTFD, the effect of TTFD on the refractory period and the maximum driving frequency of cardiac muscle is assumed to be its proper pharmacologic action different from that of the drug thiamine. It is a future problem whether the TTFD effect found in this study relates to its action as a vitamin B₁ or not.

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