Action of Thiamine and Related Compounds on Isolated Clam Heart¹

YASUMI OGURA, JUNKO NARA
AND AKEMI YAMAGUCHI²

*Department of Toxicology and Pharmacology, Institute of
Food Microbiology, Chiba University, Chiba (Post No. 280)*

(Received May 13, 1968)

1. The action of thiamine and related compounds on the isolated heart of clam (*Meretrix meretrix lusoria*) was investigated.
2. S-benzoylthiamine (SBT) and thiamine propyl disulfide (TPD) among these related compounds displayed a very remarkable positive inotropic action, whereas thiamine, acetylthiamine, thiamine diphosphate, thiamine triphosphate, benzoylthiamine monophosphate and ATP did not practically display any clear action.
3. SBT and TPD showed a remarkable antagonistic action against the negative inotropic action of acetylcholine, but have no effect on the action of 5-hydroxytryptamine.
4. The positive inotropic action of SBT and TPD was retained in K-free medium, but it completely disappeared in Na-free or Ca-free medium. When more or less Na⁺ or Ca²⁺ was present in the medium, the action of SBT and TPD appeared as in the normal medium.
5. The positive inotropic action of SBT and TPD, therefore, is believed to be closely related to the transport system of the cellular membrane of the clam heart muscle, in which Na⁺ and Ca²⁺ are involved.

Generally, the clam heart muscle is myogenic and consisted of non-striated muscle unlike mammalian heart muscle (1). It is, therefore, of interest from a view-point of pharmacology and physiology that the clam heart reacts on drugs with a special action. Prosser (2) showed that the heart of *Venus mercenaria*, a clam native to California, was useful in the biological assay of acetylcholine (ACh); Welsh (3), in the biological assay of 5-hydroxytryptamine (5HT); Tanabe *et al.* (4) that the heart of *Macrura sachalinensis*, a shellfish native to Japan, and Aishita *et al.* (5) the heart of *Meretrix lusoria*, a shellfish native to Japan, were useful in

¹ Following abbreviations were used. ACh, acetylcholine; 5-HT, 5-hydroxytryptamine; BOL, 2-bromo(+)-lysergic acid diethylamide; LSD, (+)-lysergic acid diethylamide; TDP, thiamine diphosphate; TTP, thiamine triphosphate; SBT, S-benzoylthiamine; BTMP, benzoylthiamine monophosphate; TPD, thiamine propyl disulfide;

² 小倉保己, 奈良順子, 山口明美

(a) R=C₃H₇ Thiamine propyl disulfide (TPD)

experiment. The shells were opened, the heart was isolated and the heart movement recorded on the smoked paper through a lever. The bath volume was 6 ml and the bath was aerated continuously during experiment. The concentration of drugs used in the experiment was the final concentration. The composition (g/liter) of the artificial sea-water was as follows: NaCl 28.5, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 1.72, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 3.20, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 8.50, KCl 0.8, MgBr_2 0.1. This solution was adjusted to pH 8.2. The experiment was carried out from spring to summer at room temperature (18–26°).

Chemical structures of thiamine and related compounds used in the experiment are shown in Table 1.

RESULTS

1. Proper Action of Thiamine and Related Compounds

Thiamine—It did not cause any action in concentrations lower than 5×10^{-4} M. In a concentration of 3×10^{-3} M it caused a very slight increase in the amplitude from after 3 min, but the increase was only about 20 % even after 30 min and the positive inotropic action was immediately recovered by washing (Fig. 1-A).

FIG. 1 Effect of Thiamine Related Compounds on the Isolated Clam Heart
A: thiamine 3×10^{-3} M, B: TPD 7×10^{-4} M, C: TTP 2.5×10^{-4} M, D: ATP 6×10^{-4} M.

Acetylthiamine — It did not produce any action in concentrations lower than $5 \times 10^{-4} M$. Increase in the amplitude in a higher concentration was almost the same as in the case of thiamine.

Thiamine diphosphate — It did not provoke any action in concentrations lower than $3 \times 10^{-4} M$. A slight increase in the amplitude was noted in a concentration of $7 \times 10^{-4} M$, but no changes were observed in the heart rate (Fig. 1-B). In any case, the positive inotropic action of thiamine, acetylthiamine and TDP was not notable.

Thiamine triphosphate — It caused a very slight decrease (about 10 %) in the amplitude or in the tonus immediately after addition in a concentration higher than $2.5 \times 10^{-4} M$. This action lasted for 5 to 10 min and then it was changed to the amplitude increasing action though it was slight (about 5–10 %). Fig. 2 shows the mean of the typical three cases. There were, however, no changes in the heart rate (Fig. 1-C). Since depression of the amplitude was noted in the early stage with TTP unlike thiamine, acetylthiamine and TDP, ATP in equimolar concentrations were investigated. As shown in Fig. 1-D, a slight increase in the amplitude was noted for 10 min after addition but it was gradually decreased. The extent of decrease was about 20 % even after 30 min. The mechanism of the decreasing action of TTP is unknown at present, though it may be ascribed to triphosphate ester.

S-benzoylthiamine — It did not cause any remarkable action in concentrations lower than $3 \times 10^{-5} M$, but caused increase in the amplitude immediately after addition to a concentration of $3 \times 10^{-4} M$. It gradually increased reaching a peak in 15 to 30 min. This action was very remarkable to give rise to a very slight

FIG. 2 Changes of Time Course in the Action of Thiamine and TTP on the Amplitude of the Isolated Clam Heart

FIG. 3 Action of SBT, BTMP on the Isolated Clam Heart
BT, SBT: $3 \times 10^{-4} M$, BTMP: $6 \times 10^{-4} M$.

FIG. 4 Changes of Time Course in the Action of SBT and BTMP on the Amplitude of the Isolated Clam Heart

TPD. $3.3 \times 10^{-4} M$ 3' 5' 10' 15' 20' 30' W5' W10' W20' W30'

FIG. 5 Action of TPD of Isolated Clam Heart

markable increase in the amplitude immediately after addition reaching a peak in 15 to 30 min in a concentration of $3.3 \times 10^{-4} M$. No striking changes in the heart rate were noted (Fig. 5). These changes resembled closely those of SBT. To reduce the amplitude to the previous level before addition of TPD by washing, 30-40 min was required.

2. Antiacetylcholine Action of SBT and TPD

ACh causes decrease in the amplitude in a low concentration and diastolic stop of the clam heart in a high concentration. Both SBT and TPD caused a very

reduction in the heart rate together with an increase in the amplitude. Occasionally, an appreciable increase in the tonus was noted together with increase in the amplitude (Fig. 3-A). The increased amplitude could be reduced to the previous level before addition of SBT in 20 to 30 min by several times of washing.

Benzoylthiamine monophosphate—

It caused a very slight increase in the amplitude and tonus in a concentration of $2 \times 10^{-4} M$, though the changes were not clear (Fig. 4). The action was not increased by increasing the concentration to $6 \times 10^{-4} M$ and more (Fig. 3-B).

Thiamine propyl disulfide— It did not cause any notable changes in concentrations lower than $3 \times 10^{-5} M$, caused an about 10 % increase in the amplitude in a concentration of $3 \times 10^{-4} M$, a very re-

FIG. 6 Changes of Time Course in the Action of TPD on the Amplitude of the Isolated Clam Heart

FIG. 7 *Inhibitory Action of SBT on the Negative Inotropic Action of ACh*
BT, SBT: 3.3×10^{-4} M.

FIG. 8 *Effect of SBT and TPD on the Dose-response Curve of ACh*

Abscissa: concentration of ACh in log scale.

remarkable antagonistic action against this action of ACh in a concentration higher than 3.3×10^{-4} M and their action was most clear when the increase in the amplitude reached a peak. A cardiac stop caused by the highest concentration of ACh was also inhibited even after appearance of the remarkable positive inotropic action of SBT. Such an action of SBT seemed to be stronger than TPD (Fig. 7). Recovery of the action of ACh after washing was related with the rate of disappearance of the positive inotropic action of SBT or TPD. Such an antiacetylcholine action was not noted in thiamine related compounds other than SBT and TPD.

3. Effect of SBT and TPD on the Action of 5HT

As well known, 5HT causes increase in the contraction of the clam heart, very slightly in a concentration of 10^{-9} g/ml and somewhat remarkably in a concentration of 10^{-8} g/ml. This action of 5HT was not increased or inhibited by pretreatment with SBT or TPD in a concentration higher than 3×10^{-4} M for 15-30 min, and cardiac stop by 5HT in a high concentration also was not affected.

4. Action of SBT and TPD in K-free Solution

Even when the clam heart was placed in K-free solution, the positive inotropic action of SBT was produced without being inhibited, it often accompanied the augmentation of the tonus.

FIG. 9 Action of SBT in K-free Solution
BT, SBT: 3.3×10^{-4} M.

5. Effect of Ca Ion on the Action of SBT and TPD

Both SBT and TPD caused a positive inotropic action in 1/3 Ca containing solution (the content of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in normal artificial sea-water was reduced to 1/3) (Fig. 10). In Ca-free solution (prepared by eliminating Ca^{2+} from normal artificial sea water), however, the movement of the clam heart was remarkably depressed in the amplitude and heart rate and arrhythmia was induced at times. The positive inotropic action of SBT and TPD on the clam heart under such a condition could not be noted (Fig. 11).

FIG. 10 Action of SBT in 1/3 Ca-containing Solution
BT, SBT: 3×10^{-4} M.

FIG. 11 Action of SBT and TPD in Ca-free Solution
BT, SBT: $3 \times 10^{-4} M$, TPD: $3.3 \times 10^{-4} M$.

6. Effect of Na Ion on SBT and TPD

In 1/3 Na containing solution or in Na-free solution, the movement of the clam heart was depressed in the amplitude and in a short time resulted in cardiac stop, but when 2/3 Na containing solution was used, apparent changes in the cardiac movement were not noted.

FIG. 12 Action of TPD and SBT in 2/3 Na-Containing Solution
TPD: $3.3 \times 10^{-4} M$, BT: SBT $3 \times 10^{-4} M$.

After cardiac stop produced in 1/3 Na containing solution or in Na-free solution, both SBT and TPD could not produce their positive inotropic action. In 2/3 Na containing solution, however, they caused positive inotropic action as in normal artificial sea-water (Fig. 12). TPD often caused increase in the tonus as shown in Fig. 12-A.

DISCUSSION

The effect of seven compounds related to thiamine on the clam heart was investigated. A very strong positive inotropic action was noted in SBT and TPD. This action did not appear in Na-free or Ca-free solution, but appeared in K-free solution. This action was also completely retained in 1/3 Na containing solution and 2/3 Ca containing solution. Such a phenomenon seems to be similar to that of excitability of the smooth muscle as like taenia coli or skeletal muscle of crayfish (16). This may be related to the fact that the heart of *Meretrix meretrix lusoria* is myogenic and consisted of non-striated muscle cells.

Nakazawa *et al.* (17) found that both TPD and TTFD caused prolongation of the repolarization phase and inhibition of Ca influx in the heart muscle cell of the guinea-pig by means of electrophysiological method, but thiamine did not possess such action. Misu *et al.* (18) also reported almost similar results. Kanno (19) and Nakazawa *et al.* (17) reported that TPD and thiamine tetrahydrofurfuryl disulfide caused the positive inotropic action in the isolated guinea-pig atria. Katsura *et al.* (20) also recognized the same results in the toad heart. These results are closely similar to those obtained in our experiment of the clam heart.

There is, however, a great difference in the reactivity to drugs between the heart of vertebrate and that of clam. In the case of the latter, the negative inotropic action of ACh is not antagonized by curare, pentamethonium and atropine, not increased by eserine, but antagonized by only mytolon and tetraethylammonium (21,10). Luduena *et al.* (11) suggested that such an antiacetylcholine action of mytolon was not related to its ganglion blocking action, curare-like action or ACh-esterase inhibiting action. The antiacetylcholine action of SBT and TPD observed in the present experiment, therefore, may not be the antagonism to the ACh receptor. Moreover, such an action of SBT and TPD is not caused by their action on the 5HT receptor, because the action of 5HT is not enhanced or inhibited by SBT and TPD. It also can be shown by the facts that SBT do not possess any 5HT-like action in vertebrates and invertebrates and that they are not antagonized by 5HT antagonists. The positive inotropic action of SBT and TPD, therefore, is believed to be closely related to the excitability of the cell membrane in which Na^+ and Ca^{2+} play an important role.

It is of interest to analyse the action of thiamine and related compounds on the clam heart from the view-point of chemical structure-activity relationship. The positive inotropic action becomes appreciable when the thiazole nucleus is fissured as in SBT and TPD no distinct difference in the action is noted between SBT and TPD. It can be shown that the pyrimidine nucleus not necessarily plays an important role to increase the contractile force by the fact that thiamine and acetylthiamine have no remarkable positive inotropic action.

When these compounds are phosphorylated, the original action is reduced or disappears. For example, BTMP, a monophosphate of SBT, does not provoke the positive inotropic action as shown in SBT. Such a phenomenon also could be recognized between thiamine and TDP or TTP. These results correspond well with the fact that phosphorylation caused decrease in or disappearance of the recurarisation inducing action and histamine releasing action of thiamine related compounds as already reported by the authors (15).

The present experiment was carried out with preparation placed in a medium of high pH as 8.3 unlike in the case of isolated organs of warm-blooded animals. In our experiment of the isolated guinea-pig atria, both SBT and TPD caused decrease in the amplitude when pH was below 7.0, while they caused increase when pH was higher than 7.0 (not published). According to Satoh (22), the rat's liver tryptophan pyrrolase activity was enhanced by TPD and TTFD when pH was higher than 8.0. In view of these facts, the positive inotropic action of SBT and TPD on the clam heart may be ascribable to the dependability to pH.

ACKNOWLEDGEMENT

We should like to express our thanks to Sankyo Co. Ltd. for the offer of acetylthiamine hydrochloride, TTP, SBT, and BTMP, and to Takeda Chemical Industries, Ltd. for the offer of thiamine, TDP and TPD.

REFERENCES

1. Prosser, C. L., *Comparative Animal Physiology*, p. 76, W. B. Saunders Co., (1962).
2. Prosser, C. L., *Biol. Bull.*, **78**, 72 (1940).
3. Welsh, J. H., *Arch. Exp. Path. Pharmacol.*, **219**, 23 (1953).
4. Tanabe, T., and Sato, M., *Seitai-no-Kagaku*, **4**, 135 (1952).
5. Aishita, H., Segawa, T., and Takagi, H., *Folia pharmacol. japon.*, **61**, 16 (1965).
6. Ogura, Y., Mori, Y., and Watanabe, Y., *J. Pharmac. exp. Ther.*, **154**, 456 (1966).
7. Ogura, Y., and Nara, J., *Folia pharmacol. japon.*, **61**, 126 (1965).
8. Ogura, Y., and Nara, J., *Ann. Rept. Inst. Food Microbiol. (Chiba Univ.)*, **17**, 45 (1964).
9. Ogura, Y., and Tamura, J., *Ann. Rept. Inst. Food Microbiol. (Chiba Univ.)*, **20**, 95 (1967).
10. Florey, E., and Merwin, H. J., *Nervous Inhibition*, p. 13, Pergamon Press, 1961.
11. Luduena, F. P., and Brown, T. G. Jr., *J. Pharmac. exp. Ther.*, **105**, 232 (1952).
12. Welsh, J. H., and Taub, R., *Brit. J. Pharmacol.*, **8**, 327 (1953).
13. Greenberg, M. J., *Brit. J. Pharmacol.*, **15**, 375 (1960).
14. Cheymol, J., Bourillet, F., Levassort, C. et Kerp, L., *Arch. int. Pharmacodyn.*, **3**, 36 (1957).
15. Ogura, Y., *Abstract of XIX Japanese Pharmaceutical Congress*, p. 150, (1964).
16. Ogura, Y., Watanabe, Y., and Mori, Y., *Folia pharmacol. japon.*, **60**, 128 (1964).
17. Nakazawa, Y., and Ueno, A., *Jap. J. Pharmacol.*, **15**, 10 (1965).
18. Misu, Y., Takaori, S., and Shimamoto, K., *Jap. J. Pharmacol.*, **15**, 257 (1965).
19. Kanno, M., *Jap. J. Pharmacol.*, **16**, 327 (1966).
20. Katsura, H., and Murai, J., *Vitamins*, **30**, 262 (1964).
21. Faenge, R., *Pharmacol. Rev.*, **14**, 281 (1962).
22. Satoh, T., *Jap. J. Pharmacol.*, **18**, 373 (1968).