Inhibition by Thiamine Tetrahydrofurfuryl Disulfide (TTFD) of the Arachidonic Acid Cascade-Line Activation as Evidenced in the Heart-Lung Preparation of the Dog

Kazuki MATSUI, Hajime NAKAHARA, Hiroshi WATANABE, Hirokuni TAMATSU, Mikio NAKAZAWA, Yoshito NAKAGAWA, Hirotaha MATSUDA and Shoichi IMAI
Department of Pharmacology, Niigata University School of Medicine, Asahimachi-dori 1 Niigata 951, Japan

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Abstract—The effects of thiamine tetrahydrofurfuryl disulfide (TTFD) on the gradual increase in the coronary blood flow (CBF) inherent in the canine heart-lung preparation were studied. TTFD is a disulfide-type derivative of thiamine reported to have an antinflammatory effect in experimental animals. Since it was found that the substance could reverse the gradual increase in CBF, the possibility that the reversal was brought about through an inhibition of activation of the arachidonic acid cascade-line was tested, examining the effects of this substance on the CBF increase produced by arachidonic acid (AA) and prostacyclin (PGI2). The vasodilator response to AA, which was barely detectable at the start of the experiment at which CBF was at a physiological low level, became potentiated as the gradual increase in CBF occurred, returning to the initial magnitude after TTFD, while the vasodilator response to PGI2 remained essentially unchanged during the entire course of the experiment. It was concluded that TTFD reversed the gradual increase in CBF in the HLP through the inhibition of the arachidonic acid cascade-line activation.

Experiments conducted in this laboratory with canine heart-lung preparations (HLP) demonstrated that the gradual increase in coronary blood flow inherent in the preparation was associated with an accumulation of 6-keto PGF1α in circulating blood and could be reversed by acidic nonsteroidal anti-inflammatory agents such as indomethacin with a decrease in 6-keto PGF1α, indicating that the activation of the arachidonic acid cascade-line was involved in the gradual increase in coronary blood flow (1, 2). Thiamine tetrahydrofurfuryl disulfide (TTFD) is a disulfide-type derivative of thiamine that was reported to produce an antinflammatory effect in experimental animals (3–5). In the present study, the effects of this substance on the gradual increase in coronary blood flow of the HLP were examined. Since it was found that the substance could reverse the gradual increase in coronary blood flow, the possibility that the reversal was brought about through an inhibition of the arachidonic acid cascade-line activation was tested, examining the effects of this substance on the coronary blood flow increase produced by arachidonic acid (AA) and prostacyclin (PGI2).

Materials and Methods
Experiments were performed in the canine heart-lung preparation, the details of which were reported in previous papers (6–8). Beagle dogs of either sex weighing around 10 kg anesthetized with pentobarbital sodium (35 mg/kg) administered intraperitoneally were used as experimental animals. In some experiments, filaria-free mongrel dogs of either sex weighing 9–11 kg were used. The blood obtained from mongrel dogs of either sex weighing around 20 kg
anesthetized with thiopental sodium (20 mg/kg, i.v.) was used to fill up the extracorporeal circuit. Systemic cardiac output was measured with an electromagnetic flowmeter (Statham SP2201) equipped with a cannulating type probe of 6 mm internal diameter. The coronary sinus outflow was led out with a Morawitz cannula, measured with another electromagnetic flowmeter (Statham SP2201) with a cannulating type probe of 2 mm internal diameter and returned to the inferior vena cava. The total coronary blood flow was taken to be 10/6 of the measured coronary sinus outflow. Using an optical device for measurement of the arterio-venous difference of blood oxygen content (A-Vox system), the arterio-venous difference of the oxygen content of the coronary blood was continuously recorded. Myocardial oxygen consumption was calculated by multiplying the total coronary blood flow by the arterio-venous difference of the oxygen content.

Using strain-gauge transducers (Statham P50), the arterial and the right atrial pressures were recorded. To measure the intraventricular pressure, a micro-tip pressure transducer (Millar PC-350) was introduced into the left ventricular cavity via the left atrium. With the aid of an electronic differentiator (Nihon Kohden RPD-5), the first differential of the left ventricular pressure was calculated. Heart rate was counted with a cardiograph (Nihon Kohden RT-5) triggered by R waves of lead II ECG. All these parameters were displayed on linearly recording recorders (Watanabe Sokki Mark III, Graphtec Mark VII).

TTFD and indomethacin were administered into the venous reservoir of HLP. PG12 and AA were administered into the rubber tubing leading to the venous cannula of the preparation over 10 sec.

PG12 was generously supplied by Ono Pharmaceutical Co., Ltd., as Na salt. The stock solution of this substance was prepared by dissolving the salt in cold ethanol and stored at -80°C. Just prior to use, aliquots were taken from this stock solution and diluted with cold Tris-buffer (pH 8.5). AA purchased from the Sigma Chemical Co. was stored under N2 at -20°C in sealed ampoules as a stock solution of 7.7 mg/ml in n-hexane. Just before administration, the required amount of solution was evaporated to dryness in a stream of dry nitrogen at 0°C, and the residue was transformed into the sodium salt with 0.1 M Na2CO3 solution. The aqueous solution of sodium arachidonate was kept in an ice bath. TTFD and thiamine hydrochloride provided from Takeda Chemical Industries, Ltd. was dissolved in saline solution. The stock solution of indomethacin (Merck-Banyu) was prepared with ethanol. Subsequent dilutions were made with Krebs-Henseleit’s solution.

All the data were expressed as the mean±S.E. Statistical analyses were conducted using Student’s t-test. P values less than 0.05 were considered to be significant.

**Results**

Up to the dose of 100 mg, TTFD was without effects on any parameters of the canine HLP when it was given to the preparation during the period during which the coronary blood flow remained still at a physiological low level. In contrast, the gradual increase in coronary blood flow inherent in this preparation was reversed with this compound. Thiamine hydrochloride did not produce such an effect. Figure 1 depicts the effects of 100 mg of this substance given at a time when coronary blood flow attained a steady high level around 1 hr after set-up of the preparation (the peak increase in coronary blood flow) on the various parameters of HLP. The most remarkable change was a decrease in coronary blood flow. Though not significant, there was a slight increase in the myocardial oxygen consumption. Right atrial pressure tended to rise slightly with a tendency for systemic output to decrease slightly. As shown in Fig. 2 with a representative record and in Fig. 3 with mean values, there was an augmentation of the vasodilator response to AA at the time of peak increase in coronary blood flow, while the vasodilator response to PG12 remained essentially unchanged, indicating a greater conversion of this substance to a vasodilator PG or PGs (as shown in the later section of this paper, the coronary blood flow increase induced by AA in this preparation could be abolished by indomethacin). After 100 mg of TTFD, the gradual increase in coronary blood...
flow inherent in HLP was significantly reduced. At this time, the vasodilator response to PG12 was significantly augmented and that to AA showed a tendency to be potentiated.

**Fig. 1.** Effects of TTFD (100 mg) on the canine heart-lung preparation. TTFD was administered at a time when the coronary blood flow attained a maximum steady level as a result of the gradual increase inherent in this preparation. △SOP: changes in systemic cardiac output, △RAP: changes in right atrial pressure, △HR: changes in heart rate, △Cor F: changes in coronary blood flow, △O2 Cons: changes in myocardial oxygen consumption. All the changes are expressed as % of the value just before administration of TTFD. Numbers in the parentheses are the number of experiments. **Significantly different from the values before administration of TTFD (P<0.01.**

**Fig. 2.** A representative record showing the changes in coronary vasodilator responses to prostacyclin (PG12) and arachidonic acid (AA) that occurred during the course of gradual increase in coronary blood flow (Cor F) and the effects of TTFD.

After 200 mg of TTFD, which produced an almost complete reversal of the increase in coronary blood flow, the vasodilator response to PG12 tended to remain augmented, though not significantly, while the augmentation of the increase in coronary blood flow by AA
observed at the peak increase in coronary blood flow was no longer observed. In Fig. 3 are also shown the relative magnitude of the increase in coronary blood flow induced by AA and PGI$_2$ designated as AA/PGI$_2$, as a measure of the possible conversion of AA to PGI$_2$. The ratio showed a tendency to become greatest at the time of the peak increase in coronary blood flow. After 100 mg of TTFD, there was a tendency for the ratio to become smaller. The ratio became significantly lower after 200 mg of TTFD than that at the time of the peak increase in coronary blood flow.

For comparison, the effects of indomethacin are depicted in Fig. 4 with a representative record. The reversal of an increase in coronary blood flow, and the modifications of the effects of AA and PGI$_2$ produced by this compound were essentially the same as those produced by TTFD. As is evident from Fig. 4, the increase in coronary blood flow produced by AA was no longer observed after treatment of the preparation with indomethacin. PGI$_2$ produced similar increases in coronary blood flow irrespective of whether it is administered at the start of the experiments when coronary blood flow was still at a physiological low level or at the time of the peak increase in coronary blood flow.

Discussion

In the present experiments, AA produced an increase in coronary blood flow that could be abolished by indomethacin, indicating the conversion of this substance into a vasodilator PG or PGs. We may tentatively suppose the product to be PGI$_2$, for it is now well established that PGI$_2$ is a major metabolite of the arachidonic acid cascade-line in the heart. On this assumption, we calculated the relative magnitude of increase in coronary blood flow induced by AA and PGI$_2$ (AA/PGI$_2$ ratio) as an index representing the magnitude of possible conversion of AA to PGI$_2$. It was found that the AA/PGI$_2$ ratio was significantly greater at the time of the peak increase in coronary blood flow than at the start of the experiments. The finding is consistent with our previous reports (1, 2) that the gradual increase in coronary blood flow inherent in HLP is associated with the augmented production of PGI$_2$ from AA. TTFD produced a reversal of this increase in coronary blood flow, and at the same time abolished the augmented production of PGI$_2$ from AA as was evidenced by a significantly lower AA/PGI$_2$ ratio obtained after 200 mg of this compound. After 100 mg TTFD and the first treatment of 200 μg indomethacin, the vasodilator response to AA was not abolished. This may have resulted from an incomplete inhibition of prostaglandin biosynthesis and the augmentation of vasodilator response to PGI$_2$ (Fig. 3). According to Smith and Lands (9), the inhibition of prostaglandin biosynthesis by indomethacin proceeds rather slowly. The antiinflammatory activity of TTFD has been reported in several papers (3–5).
We ourselves have found that the substance could produce an inhibition of the carrageenin-induced edema in the rat paw (K. Matsui et al., unpublished observations). In view of the findings of the present experiments, it may be natural to suppose that the antiinflammatory activity of TTFD is due to an inhibition of arachidonic acid cascade-line activation. This interesting possibility merits further study.

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