物的解毒处理能力. 方法: 制备肾上腺、肝亚细胞组分. 测定谷胱甘肽转移酶(GST)、还原酶、过氧化物酶. 结果: GST在胎肾上腺微粒体、线粒体、胞浆中含量分别是肝各亚细胞组分中的373 %、270 %和167 %. 肾上腺微粒体GST活性与细胞色素P-450、与氨基比林脱甲基酶活性皆呈正相关. 肾上腺线粒体谷胱甘肽还原酶、过氧化物酶分别是肝线粒体中的506 %和482 %. 结论: 胎肾上腺有比胎肝更大的解毒能力. 提示胎肾上腺兼有药物代谢器官的功能.

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Dual effects of 5-hydroxytryptamine on stable analogue of thromboxane A_2-induced aggregation and release reaction in rabbit platelets[1]

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KEY WORDS platelet aggregation; adenosine triphosphate; calcium; serotonin; thromboxane A_2

AIM: To study the effects of 5-hydroxytryptamine (5-HT) on stable analogue of thromboxane A_2 (STA_2)-induced platelet shape, aggregation, and release reaction. **METHODS**: Platelet shape change and aggregation were quantified by the light transmission through platelet-rich plasma (PRP). Release reaction was evaluated by the amount of ATP in the medium and cytosolic-free Ca^{2+} was measured by fluorescent imaging. **RESULTS**: (1) STA_2 0.3 - 3 $\mu mol \cdot L^{-1}$-induced shape change followed by aggregation. When STA_2 1 or 3 $\mu mol \cdot L^{-1}$ was added to PRP, the release reaction was occurred. Pretreatment of PRP with 5-HT 3 $\mu mol \cdot L^{-1}$, the shape change by STA_2 was abolished and the aggregation by STA_2 0.3 $\mu mol \cdot L^{-1}$ was enhanced ($P < 0.01$), STA_2 1 or 3 $\mu mol \cdot L^{-1}$-induced aggregation was not affected, but the release reaction was partially suppressed ($P < 0.01$). (2) STA_2 0.3 $\mu mol \cdot L^{-1}$-induced $[Ca^{2+}]_i$ elevation was further increased by 5-HT pretreatment, but the $[Ca^{2+}]_i$ mobilizations by STA_2 3 $\mu mol \cdot L^{-1}$ was decreased by 5-HT, especially the peak level. (3) The aggregation without release reaction was increased from 3.4 ± 2.1 to 25.6 ± 1.8 % ($P < 0.01$) with 10 s interval and the enhancement was declined with the prolongation of the intervals. The aggregation with release reaction was not affected by changing the intervals, but the release reaction was decreased in the same treatment. **CONCLUSION**: The dual effects of 5-HT on STA_2-induced aggregation and release reaction and the molecular mechanism of this effect was probably through the regulative action of 5-HT on $[Ca^{2+}]_i$ mobilization by STA_2.

Blood platelet plays an important role directly through its functions or indirectly due to some active substances or cytokines released during platelet activation, for example, adenosine diphosphate (ADP), 5-hydroxytryptamine (5-HT), calcium ion (Ca^{2+}), platelet activating factor (PAF), and some enzymes. In addition, the platelet is the most important store of 5-HT, as a local circulating regulator it can be released from dense granules of platelets upon suitable stimulation, and enhanced the aggregations by ADP and epinephrine[1-3] but the effects of 5-HT on release reaction are still uncleared. In the present research, a stable analogue of thromboxane A_2 (STA_2) was selected since it caused both aggregation and release reaction, which relied on the concentration of STA_2. Furthermore, the effects of 5-HT on STA_2-induced platelet shape change, aggregation and release reaction were observed separately, and cytosolic-free Ca^{2+} ($[Ca^{2+}]_i$) was also evaluated in washed single

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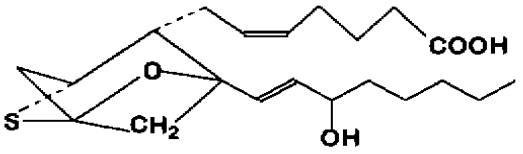
platelet loaded with Fura 2-AM by fluorescent imaging technique.



9,11-Epithio-11,12-methano-thromboxane A_2

MATERIALS AND METHODS

Reagents A stable analogue of STA_2, kindly provided by Ono Pharmaceutical Co of Japan, were dissolved in ethanol and stored in a freezer. It was diluted with Tris-buffer saline prior to use. Other reagents were the same as previous study[4].

The preparation of platelet-rich-plasma (PRP), platelet-poor-plasma (PPP), washed platelet in rabbits from Department of Experimental Animals, Harbin Medical University, and the Fura-2 loading, the measurements of platelet aggregation, release reaction, and $[Ca^{2+}]_i$ in single washed platelets were according to our previous research[5].

Platelets with different densities have different responses to thrombin[5,6]. The largest group and higher density of platelets was selected especially for the measurement of $[Ca^{2+}]_i$.

Statistical analysis Results were expressed as $\bar{x} \pm s$ and compared with t-test.

RESULTS

Decrease in light transmission (DLT)

STA_2 0.3 $\mu mol \cdot L^{-1}$ induced DLT, so called platelet shape change, and small reversible aggregation without release reaction. STA_2 1 and 3 $\mu mol \cdot L^{-1}$ also caused a transient DLT before its aggregation. The pretreatment of PRP with 5-HT 3 $\mu mol \cdot L^{-1}$, which induced DLT only in rabbit[1], abolished the DLT by STA_2 (Tab 1).

Egtazic acid 3 $mmol \cdot L^{-1}$ did not affect STA_2-induced DLT (Tab 2).

Aggregation and release reaction In rabbit PRP, STA_2 1 and 3 $\mu mol \cdot L^{-1}$ induced DLT and subsequently aggregation with release reaction which were not inhibited by indomethacin 3 $\mu mol \cdot L^{-1}$, but inhibited by egtazic acid (Tab 2). When 5-HT 3 $\mu mol \cdot L^{-1}$ was administrated before, STA_2 0.3 $\mu mol \cdot L^{-1}$-induced aggregation was enhanced ($P < 0.01$), and STA_2 1 and 3 $\mu mol \cdot L^{-1}$-caused aggregations were not changed, while, the release reactions were partially suppressed ($P < 0.01$, Tab 1).

Tab 1. Effects of 5-HT 3 $\mu mol \cdot L^{-1}$ on STA_2-induced platelet shape change, aggregation, release reaction in PRP ($n = 13 - 17$ preparations) from 6 – 8 rabbits. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ vs STA_2.

STA_2 ($\mu mol \cdot L^{-1}$)	5-HT	Shape change (% of light transmission change)	Aggregation (% of light transmission change)	Release reaction (ATP $\mu mol \cdot L^{-1}$)
0.3	0	9.9 ± 1.3	1.6 ± 0.9	0
0.3	3	1.1 ± 0.7[c]	12.3 ± 1.2[c]	0[a]
1.0	0	8.9 ± 1.4	35 ± 7	0.09 ± 0.03
1.0	3	1.1 ± 0.8[c]	38 ± 7[a]	0.047 ± 0.027[c]
3.0	0	9.1 ± 2.6	49 ± 9	0.14 ± 0.03
3.0	3	1.0 ± 0.5[c]	50 ± 4[a]	0.054 ± 0.015[c]

Tab 2. Effects of indomethacin (Ind) and egtazic acid (EGTA) on STA_2-induced platelet shape change, aggregation, release reaction in PRP ($n = 12 - 24$ preparations) and $[Ca^{2+}]_i$ in washed single platelet ($n = 28 - 44$ cells) from 6 rabbits. $\bar{x} \pm s$. $^cP < 0.01$ vs STA_2.

Groups	Shape change (% of light transmission change)	Aggregation (% of light transmission change)	Release reaction (ATP $\mu mol \cdot L^{-1}$)	$[Ca^{2+}]_i$/ $nmol \cdot L^{-1}$
STA_2 3 $\mu mol \cdot L^{-1}$	−9.9 ± 0.5	54 ± 11	0.19 ± 0.03	266 ± 24
Ind 3 $\mu mol \cdot L^{-1}$	−10.6 ± 0.7	0[c]	0[c]	
EGTA 1 $mmol \cdot L^{-1}$	−9.6 ± 1.3	21 ± 8[c]	0.11 ± 0.03[c]	
EGTA 3 $mmol \cdot L^{-1}$	−10.3 ± 1.5	0.6 ± 0.8[c]	0.07 ± 0.02[c]	172 ± 29[c]

Effects of time interval between additions of 5-HT and STA_2 STA_2 was added at 10, 30, 60, and 120 s after the addition of 5-HT. STA_2 0.3 $\mu mol \cdot L^{-1}$-induced aggregation was enhanced by 5-HT pretreatment and this enhancement was reduced with prolongation of the time intervals between additions of 5-HT and STA_2 (the same result was seen with ADP 0.3 $\mu mol \cdot L^{-1}$). In the case of STA_2 3 $\mu mol \cdot L^{-1}$, the aggregation was not influenced, but the release reaction was reduced by prolongation of that intervals (Fig 1).

$[Ca^{2+}]_i$ mobilization In single washed platelet, STA_2 0.3 – 3 $\mu mol \cdot L^{-1}$ caused a concentration-dependent increase in $[Ca^{2+}]_i$ from resting level 82 ± 3 $nmol \cdot L^{-1}$ to 98 ± 9, 187 ± 5 and 266 ± 24 nmol $\cdot L^{-1}$, respectively. The peak value was seen at 20 s after stimulation with STA_2 3 $\mu mol \cdot L^{-1}$ and rapidly returned to about 200 nmol at 40 s after stimulation and

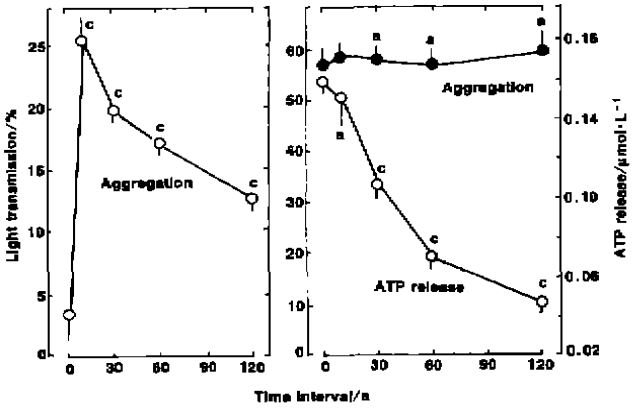


Fig 1. Effects of time interval between the addition of 5-HT and that of STA_2 on aggregation and release reaction by STA_2. 5-HT was administrated at 10, 30, 60, and 120 s before STA_2. $n = 8 - 12$ preparations from 6 rabbits. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* 0 s.

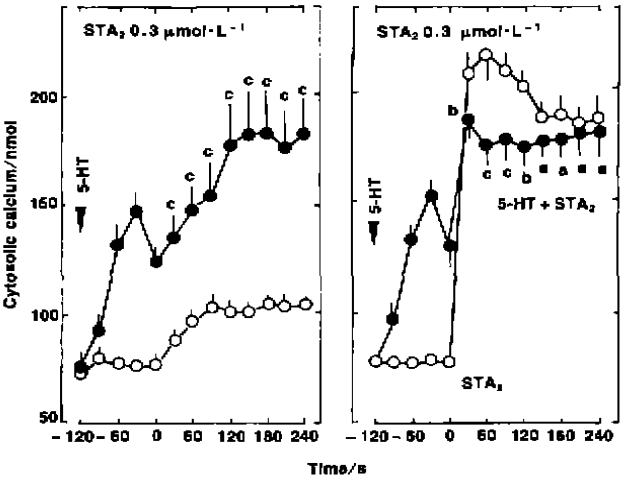


Fig 2. Effects of 5-HT on $[Ca^{2+}]_i$ mobilizations by STA_2. 5-HT was added 2 min before the addition of STA_2. $n = 34 - 41$ cells from at least 6 rabbits. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* STA_2.

then maintained this level during 2 min. STA_2-induced $[Ca^{2+}]_i$ mobilization was partially inhibited by extracellular egtazic acid 3 mmol·L^{-1} which inhibited STA_2 3 μmol·L^{-1}-induced aggregation completely and the release reaction was partially inhibited, but the DLT was not affected (Tab 2).

The preincubation of washed platelet with 5-HT 3 μmol·L^{-1}, STA_2 0.3 μmol·L^{-1}-induced $[Ca^{2+}]_i$ mobilization was enhanced. STA_2 3 μmol·L^{-1}-induced $[Ca^{2+}]_i$ increase was partially suppressed by the same treatment (Fig 2).

DISCUSSION

STA_2 was selected to activate the platelet, because STA_2-induced shape change, aggregation, and release reaction were closely related to its concentration, and they were not associated with the formation of thromboxane, but due to directly binding to its receptor[6].

The effect of 5-HT on shape change by STA_2 was similar to that on ADP[1]. That means that before the shape change by 5-HT reversed, the addition of STA_2 could not induce shape change again and directly induced aggregation, suggesting that platelet shape changes by different agents might be through the similar intracellular mechanisms, but via different receptors (Tab 3). With egtazic acid, the results suggested that the shape change was related to intracellular Ca^{2+} release, the extracellular Ca^{2+} was required for aggregation, and both intra- and extracellular Ca^{2+} might be needed for release reaction since egtazic acid only partially inhibited STA_2-induced release reaction and $[Ca^{2+}]_i$ mobilization by STA_2.

In the experiments of 5-HT with ADP[1] and 5-HT

Tab 3. Effects of 5-HT on the platelet shape changes induced by thrombin, STA_2, arachidonic acid (AA), collagen, and ADP. $n = 11 - 23$ preparations from 6 – 9 rabbits. $\bar{x} \pm s$.

Agents	Platelet shape change (%) in the presence of 5-HT (μmol·L^{-1})						
	Control	0.01	0.03	0.1	0.3	1	3
Thrombin 0.3 U·L^{-1}	11.9 ± 2.6	11.2 ± 2.9	10.4 ± 2.4	8.5 ± 2.1	6.0 ± 1.9	2.9 ± 1.1	0.9 ± 0.4
STA_2 3 μmol·L^{-1}	10.2 ± 2.7	10.2 ± 2.1	9.9 ± 1.9	9.4 ± 2.2	5.9 ± 1.9	2.0 ± 1.1	0.2 ± 0.5
AA 200 μmol·L^{-1}	9.8 ± 1.9	9.9 ± 1.3	10.0 ± 1.2	9.5 ± 1.5	5.2 ± 2.0	1.4 ± 0.9	0.5 ± 0.6
Collagen 5 mg·L^{-1}	8.0 ± 1.1	7.7 ± 1.6	7.8 ± 1.9	6.2 ± 1.8	3.8 ± 2.1	0.9 ± 0.6	0.1 ± 0.3
ADP 3 μmol·L^{-1}	7.8 ± 1.2	7.9 ± 0.8	7.6 ± 0.6	6.3 ± 0.6	4.2 ± 0.7	1.1 ± 0.4	0.04 ± 0.19

with STA_2[7], the evidences indicated that the increase in the rate of Ca^{2+} influx might be so important for platelet aggregation and release reaction. The rate of $[Ca^{2+}]_i$ elevation by 5-HT, ADP, and lower concentration of STA_2 was slowly and the aggregation was usually weak and reversible. Once aggregation occurred with release reaction by higher concentration of STA_2 or thrombin[4,7,8], the slope of $[Ca^{2+}]_i$ mobilization was very sharp, suggesting that a large amount of $[Ca^{2+}]_i$ accumulation might be needed to activating the intracellular enzyme system involved in release reaction. In addition, the enhancement of aggregations were declined with the prolongation of the intervals, which had a similar time course of inositol triphosphate formation[1].

In conclusion, the platelet shape changed by different agents occurred due to the similar intracellular mechanisms. 5-HT possessed dual effects on STA_2-induced aggregation and release reaction, the rate of $[Ca^{2+}]_i$ elevation seemed to be closely associated with aggregation and release reaction. The enhancement of 5-HT on STA_2-induced aggregation was probably attributed to the $[Ca^{2+}]_i$ superimposition of calcium influx by STA_2 based on intracellular calcium release by 5-HT, but the mechanisms of suppression of STA_2-stimulated release reaction by 5-HT could not be explained with these data.

REFERENCES

1 Li BY, Bai Y, Li WH. Enhancement of ADP-induced aggregation by 5-hydroxytryptamine in rabbit platelets. Acta Pharmacol Sin 1998; 19: in press.

2 de Chaffoy de Courcelles D, Roevens P, Van Belle H, De Clerck F. The synergistic effect of serotonin and epinephrine on the human platelet at the level of signal transduction. FEBS Lett 1987; 219: 283 - 8.

3 Vanags DM, Rodgers SE, Duncan EM, Lloyd JV, Bochner F. Potentiation of ADP-induced aggregation in human platelet-rich plasma by 5-hydroxytryptamine and adrenaline. Br J Pharmacol 1992; 106: 917 - 23.

4 Li BY, Qiao GF, Sun JP, Gao YR, Li WH. Inhibitory effects of ONO-3708 and S-145 on shape change and aggregation of rabbit platelets induced by STA_2 Acta Pharmacol Sin 1996; 17: 345 - 8.

5 Li BY, Bai Y, Li GZ, Li WH, Katori K, Wang YP. Effects of MK-447 on thrombin-induced aggregation, secretion of ATP, and $[Ca^{2+}]_i$ mobilization in rabbit platelets. Acta Pharmacol Sin 1995; 16: 108 - 13.

6 Li BY, Li WH, Bai Y. Relationship between platelet density and platelet aggregation, ATP release, and cytosolic-free calcium mobilization in rabbits. Acta Pharmacol Sin 1995; 16: 367 - 72.

7 Li BY, He SZ, Li WH. Heterogeneity of human platelet density subpopulations in aggregation, secretion of ATP, and cytosolic-free calcium concentration. Acta Pharmacol Sin 1996; 17: 152 - 5.

8 Majima M, Li BY, Huang A, Katori M. Calcium mobilization in density subpopulations of rabbit washed platelets. Int J Tissue React 1995; 17: 51 - 60.

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5-HT 对 STA_2 介导的血小板聚集和释放反应的双重影响[1]

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关键词 血小板聚集; 腺苷三磷酸; 钙; 血清素; 血栓素 A_2

目的: 研究 5-HT 对 STA_2 血小板聚集和释放反应的影响及可能的分子机制. **方法**: 以透光法, 介质中 ATP 含量及荧光图像法评价血小板变形, 聚集反应和 $[Ca^{2}]_i$ 水平. **结果**: (1) 5-HT 预处理可消除 STA_2 的血小板变形, STA_2 0.3 $\mu mol \cdot L^{-1}$的聚集增强, 1 - 3 $\mu mol \cdot L^{-1}$的聚集不变, 释放反应抑制. (2) 5-HT 预处理增加 STA_2 0.3 $\mu mol \cdot L^{-1}$的 $[Ca^{2+}]_i$, 降低 3 $\mu mol \cdot L^{-1}$的 $[Ca^{2+}]_i$ 降低. (3) 延长加入 5-HT 和 STA_2 的间隔, STA_2 0.3 $\mu mol \cdot L^{-1}$的聚集增强, 3 $\mu mol \cdot L^{-1}$的聚集不变、释放反应抑制. **结论**: 5-HT 对 STA_2 介导的聚集和释放反应有双重影响. 对 STA_2 $[Ca^{2+}]_i$ 的调节可能是上述反应的分子机制.