BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Jul; 19 (4): 383 - 386

Effects of 5-HT released from platelets on thrombin-induced aggregation and ATP release in rabbit platelets *in vitro*

LI Bai-Yan[1], LI Wen-Han
(*Department of Pharmacology, Harbin Medical University, Harbin 150086, China*)

KEY WORDS serotonin; arachidonic acids; heptanoic acids, thromboxane A_2; S-145; methysergide; thrombin; blood platelets; platelet aggregation

AIM: To study the effects of arachidonic acid (AA)-induced endogenous serotonin (5-HT) release on platelet aggregation and ATP release by thrombin (Thr). **METHODS**: Platelet aggregation and release reaction were quantified by light transmission in platelet-rich-plasma (PRP) and the amount of ATP in medium. The effects of endogenous 5-HT were evaluated by the filtration of content in cuvette A (content A) containing endogenous 5-HT into cuvette B in which Thr-induced aggregation was observed in the absence/presence of {(±)-5 (Z)-7-[3-endophenylsulfonylamino [2.2.1] bicyclohept-2-exo-yl]heptanoic acid, sodium salt} (S-145) or/and methysergide (Met). **RESULTS**: (1) AA 100 and 200 $\mu mol \cdot L^{-1}$ induced aggregation and ATP release in cuvette A. When the aggregation reached a peak, the content A directly caused platelet aggregation in cuvette B, and it was inhibited by S-145 100 $nmol \cdot L^{-1}$, Met 30 $\mu mol \cdot L^{-1}$, and inhibited more potently by S-145 + Met. (2) In the presence of S-145 100 $nmol \cdot L^{-1}$ in cuvette B, aggregations by Thr 0.1 and 0.3 $IU \cdot L^{-1}$ were enhanced ($P < 0.01$) by the filtrate, while Thr 0.5 $IU \cdot L^{-1}$-caused ATP release was suppressed ($P < 0.01$) without the effect on aggregation. Preincubation with S-145 and Met, the effects of the filtrate on aggregation and ATP release were abolished. (3) By prolongation of the time intervals between filtration and addition of Thr, the aggregation was enhanced and ATP release was reduced. **CONCLUSION**: Endogenous 5-HT was released from activated platelet and plays, in turn, a role in the regulation of platelet aggregation by the superimposition of cytosolic-free calcium ($[Ca^{2+}]_i$) and the feedback loop to regulate release reaction and calcium.

[1] Correspondence to Dr LI Bai-Yan. Phn 86-451-667-1354. Fax 86-451-666-9491. E-mail liby@ems.hrbmu.edu.cn
Received 1997-08-25 Accepted 1998-01-13

Platelets are closely related to thromboxane A_2 (TXA_2) from the phospholipid of platelet membrane, epoprostenol (Epo) from endothelial cells, and serotonin (5-HT) and adenosine diphosphate (ADP) released from dense granules during aggregation. Both TXA_2 and 5-HT from platelets themselves have crucial roles in the regulation of platelet aggregation and release reaction as they are activated. Exogenous 5-HT enhanced ADP-, epinephrine-, and low dose of a stable analog of TXA_2 (STA_2)-induced aggregation[1-3] via synergistic mechanisms[3,4]. Platelet exogenous 5-HT also suppressed high dose of STA_2-induced release reaction without any change in the magnitude of aggregation due to the reduction of STA_2-induced intracellular Ca^{2+} mobilization by the pretreatment of exogenous 5-HT with washed rabbit platelets[5]. Therefore, the filtrate experiments were performed to demonstrate whether or not the endogenous 5-HT possessed the same effects on platelet aggregation and release reaction as exogenous 5-HT did in rabbit platelet *in vitro*.

MATERIALS AND METHODS

Agents Luciferase-luciferin (Chrono-Log Corp, Havertown PA) and ATP (Sigma Co, St Louis) were used in the experiment of release reaction[6]. 5-HT (Wako Pure Chemicals, Osaka) and methysergide (Met, Sandoz Pharmaceutical Co, Tokyo) were stored in Tris-buffer saline at 4 ℃. Thr was provided by Parke-Davis Div of Warner-Lambert Co, Morris Plains NJ. S-145 {(±)-5 (Z)-7-[3-endophenylsulfonylamino [2.2.1] bicyclohept-2-exo-yl] heptanoic acid, sodium salt}[7] was kindly gifted by Shionogi Pharmaceutical Co, Osaka, dissolved and diluted with buffered saline before use. Arachidonic acid (AA, Sigma Co, St

Louis) was dissolved in stock solution, stored at −20 ℃ and diluted with HEPES-buffered saline prior to use.

Platelet aggregation and filtrate experiment Platelet-rich-plasma (PRP) and platelet-poor-plasma (PPP) were prepared according to the previous method[8]. The platelet aggregation was quantified by the light transmission[9] and the release reaction was assayed by the amount of ATP in the medium[6]. AA 100 and 200 $\mu mol \cdot L^{-1}$ were used to activate platelet in cuvette A with PRP 250 μL, the platelet aggregation % and the amount of ATP in the medium were quantified simultaneously. When the aggregation reached the peak level, the content A was transferred immediately into the cuvette B with PRP 250 μL through a filter and the total volume in cuvette B was 450 ± 13 μL, $n = 45$ after filtration, and the aggregation in cuvette B was recorded in the absence or presence of S-145 and/or Met, antagonists of TXA_2 and 5-HT receptors, preincubated for 10 min before the filtration in the treated group. The content A was replaced by the same volume of HEPES-buffered saline[8] in control group and filtered into cuvette B. In following experiments, Thr 0.1, 0.3, and 0.5 $IU \cdot L^{-1}$ were added to cuvette B after filtration.

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

RESULTS

Platelet aggregation in cuvette A AA 100 and 200 $\mu mol \cdot L^{-1}$ aggregated the blood platelets, the aggregation % being 49 ± 11 and 72 ± 9, respectively. This aggregation was accompanied with release reaction, the levels of ATP in the medium were 0.47 ± 0.26 and 0.64 ± 0.21 $\mu mol \cdot L^{-1}$.

Platelet aggregation in cuvette B When the aggregation reached the maximal level by AA 100 and 200 $\mu mol \cdot L^{-1}$ in cuvette A, the content A was filtered into cuvette B and caused platelet aggregations. These effects were almost or partially inhibited by the preincubation of PRP with S-145 or Met, and more potently inhibited by preincubation with S-145 + Met (Tab 1).

Thr-induced aggregation in cuvette B For understanding the effects of endogenous 5-HT easily and removing the effect of ADP release,

Tab 1. AA-induced aggregation in cuvette A as control, and the effects of content A on PRP in cuvette B without inhibitor as content A, in the presence of S-145, methysergide (Met), and S-145 + Met. $n = 12 - 14$ **preparations from 6 rabbits.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **content A.**

Groups	AA 100 $\mu mol \cdot L^{-1}$	AA 200 $\mu mol \cdot L^{-1}$
Control	49 ± 11	72 ± 9
Content A	32 ± 7	46 ± 8
S-145 100 $nmol \cdot L^{-1}$	1.8 ± 2.1^c	18 ± 5^c
Met 30 $\mu mol \cdot L^{-1}$	14 ± 5^b	27 ± 6^b
S-145 + Met	0 ± 0	11 ± 6^c

AA 100 $\mu mol \cdot L^{-1}$ was used to aggregate platelets in cuvette A in the following experiments with Thr.

In the presence of S-145 100 $nmol \cdot L^{-1}$ in control group, Thr-induced aggregation was concentration-dependent in cuvette B, in the treated group, Thr 0.1 and 0.3 $IU \cdot L^{-1}$-induced aggregation was enhanced by the content A ($P < 0.01$), and Thr 0.5 $IU \cdot L^{-1}$-induced aggregation was not affected, but ATP release was suppressed ($P < 0.01$). In the presence of S-145 + Met 30 $\mu mol \cdot L^{-1}$ in cuvette B, the effects of enhancement and reduction of Thr-induced aggregation and ATP release by the content A disappeared (Fig 1).

Prolongation of time intervals between filtration and addition of Thr The time

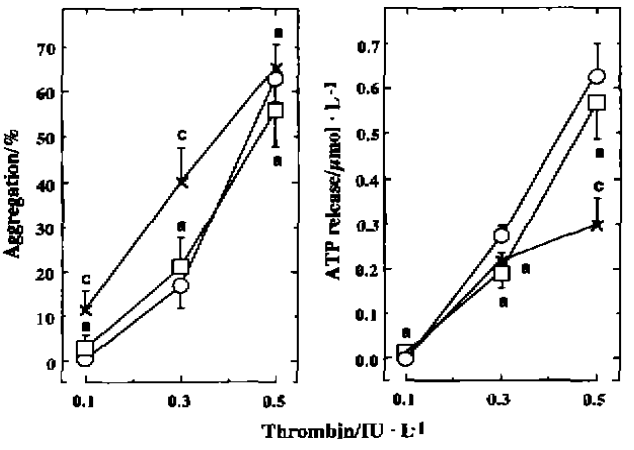

Fig 1. Effects of content A on Thr 0.3 $IU \cdot L^{-1}$-induced aggregation and ATP release in cuvette B in the presence of S-145 100 $nmol \cdot L^{-1}$. ○: Saline control; ×: content A; □: content A + Met 30 $\mu mol \cdot L^{-1}$. $n = 9 - 11$ **preparations from 6 rabbits.** $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* **Thr only.**

intervals between filtration and the addition of Thr were 10, 30, 60, and 120 s. In the presence of S-145 in cuvette B, Thr 0.3 IU·L^{-1}-induced less potent aggregation was enhanced ($P < 0.01$, Fig 2A), ATP release was slightly increased ($P > 0.05$, Fig 2B), and Thr 0.5 IU·L^{-1} induced ATP release was reduced ($P < 0.01$, Fig 2B) without effect on its aggregation by the prolongation of the time intervals between filtration and the addition of Thr. When the PRP was pretreated with S-145 + Met in cuvette B, the enhancement of aggregation and the reduction of ATP release by the content A were completely inhibited ($P > 0.05$ *vs* 0 s, Fig 2A, B).

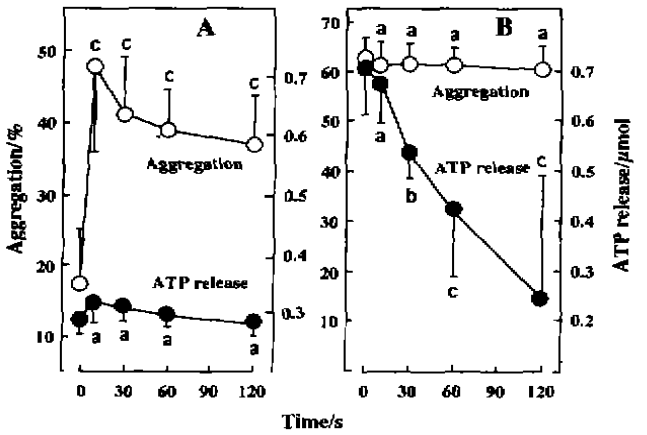

Fig 2. Effects of changing the time intervals between the filtration and the addition of Thr 0.3 IU·L^{-1}(A) and 0.5 IU·L^{-1}(B) on aggregation in cuvette B in the presence of S-145 100 nmol·L^{-1}. $n = 11 - 14$ preparations from 6 rabbits. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* 0 s.

DISCUSSION

5-HT is the richest in the dense granules of blood platelets and released to blood stream during platelet aggregation activated by stimuli such as AA, Thr, collagen, and platelet-activating factor, etc, but the functional significances of released 5-HT are not fully understood.

To investigate the effects of AA-induced endogenous 5-HT on platelet aggregation and release reaction, the filtrate experiment was performed in this study. AA induced aggregation with ATP release, and the aggregation in cuvette B induced by content A was inhibited by S-145 or Met respectively, these results demonstrated that the content A contained TXA_2 formed and endogenous 5-HT released. ADP was also released with endogenous 5-HT and TXA_2 formation, since the pretreatment of PRP with S-145 + Met did not completely abolished the aggregation in cuvette B by the content A with AA 100 μmol·L^{-1}. So, AA 100 μmol·L^{-1} was selected for activating platelets in cuvette A to avoid the effect of ADP.

Thr elicited platelet aggregation and ATP release in rabbit PRP and both exogenous[3] and endogenous 5-HT did not induce visible platelet aggregation in the same preparation. These results suggested that endogenous 5-HT enhanced the aggregation by Thr 0.1 – 0.3 IU·L^{-1} without or with slightly increase in ATP release might be due to the superimposition of cytosolic-free calcium mobilization $[Ca^{2+}]_i$[3]. The potentiations and enhancements of aggregation by exogenous 5-HT were similarly observed with ADP[1,3], epinephrine[2,4], and TXA_2 analogue (STA_2)[5]. Thr 0.5 IU·L^{-1} caused full aggregation with ATP release, under this situation, the aggregation was not further enhanced by released 5-HT, but ATP release was reduced, suggesting that a negative feedback loop may exist to limited aggregation through reduction of release reaction, which can be activated by full aggregation, and endogenous 5-HT plays an important role in that loop by lowering release reaction including adenosine diphosphate which was considered to be closely associate with the stabilization of $[Ca^{2+}]_i$ level and platelet deaggregation[5,11]. So, we can draw the conclusion that endogenous 5-HT was released from activated platelet and plays, in turn, a role in the regulation of platelet aggregation by the superimposition of $[Ca^{2+}]_i$ and the feedback loop to regulate release reaction and calcium related to platelet aggregation.

REFERENCES

1 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.

2 De Clerk F, Xhonneux B, de Chaffoy de Courcelles D. Functional expression of the amplification reaction between serotonin and epinephrine on platelets. J Cardiovasc Pharmacol 1988; 11 (Suppl 1): S1 – S5.

3 Li BY, Bai Y, Li WH. Enhancement of ADP-induced aggregation by 5-HT in rabbit platelets. Acta Pharmacol Sin 1998; 19: 58 – 62.
4 De Chaffoy de Courcells 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.
5 Li BY, Zhou YC, Li WH. Dual effects of 5-hydroxytryptamine on stable analogue of thromboxane A_2-induced aggregation and release reaction in rabbit platelets. Acta Pharmacol Sin 1998; 19: 171 – 4.
6 Feinman RD, Lubowsky J, Charo I, Zabinski MP. The lumi-aggregometer: a new instrument for simultaneous measurement of secretion and aggregation. J Lab Clin Med 1977; 90: 125 – 9.
7 Hanasaki H, Arita H. Characterization of a new compound, S-145, as a specific TXA_2 receptor antagonist in platelets. Thromb Res 1988; 50: 365 – 76.
8 Li BY, Bai Y, Makoto K, Li WH. Effects of MK-447 on thrombin-induced aggregation, secretion of ATP and $[Ca^{2+}]_i$ mobilization in rabbit platelets. Acta Pharmacol Sin 1994; 16: 108 – 13.
9 Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; 194: 927 – 9.
10 Mitchell JRA, Sharp AA. Platelet clamping *in vitro*. Br J Haematol 1964; 10: 78 – 83.
11 Cattaneo M, Canciani MT, Lecchi A, Kinlough-Rathbone RL, P ackham MA, Mannucci PM, *et al*. Released ADP stabilizes thrombin-induced human platelet aggregates. Blood 1990; 75: 1081 – 6.

兔血小板释放的内源性 5-HT 对凝血酶诱导的血小板聚集和 ATP 释放的体外影响

李柏岩[1]，李文汉
（哈尔滨医科大学药理教研室，哈尔滨 150086，中国）

关键词：血清素；花生四烯酸类；庚酸类；血栓素 A_2；S-145；美西麦角；凝血酶；血小板；血小板聚集

目的：研究内源性血清素 5-HT 对凝血酶(Thr)介导的血小板聚集和 ATP 释放的影响. **方法**：以透光法和介质中 ATP 的量评价聚集和释放反应. 采用滤过实验观察内源性 5-HT 的作用. **结果**：(1) 花生四烯酸(AA)诱导 A 管聚集和 ATP 释放，其内容物使 B 管 PRP 聚集，被 S-145 或美西麦角(Met)抑制. (2) S-145 预处理，A 管内容物增强 Thr 0.1 – 0.3 IU·L^{-1}的聚集，抑制 Thr 0.5 IU·L^{-1}的 ATP 释放($P < 0.01$)，B 管内预置 S-145 + Met，上述作用被消失. (3) S-145 存在下，延长滤过到加入 Thr 的间隔，A 管内容物使 Thr 的聚集/释放反应进一步增强/减弱. **结论**：血小板内源性 5-HT 增强其聚集反应，并通过调节释放反应和钙发挥对血小板的反馈调节.