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离体大鼠肝灌流美托洛尔消除的流量依赖性

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A 摘要 用离体大鼠肝灌流方法，研究了稳态时美托洛尔的代谢模型，灌流量分别为10，20和30 ml·min^{-1}时；流入肝脏的浓度分别为7.6，5.0和3.4 μg·ml^{-1}；流出肝脏的浓度分别为1.2，2.0和2.7μg·ml^{-1}；肝窦平均浓度分别为3.4，3.2和3.0 μg·ml^{-1}. 提示流入和流出浓度呈流量依赖性，与'平行管'模型相符，而与'充分搅拌'模型不符.

关键词 美托洛尔；肝脏；局部灌注法；药物动力学

药代动力学

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Platelet activation by platelet aggregation factor from *Eisenia foelide*[1]

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ABSTRACT A platelet activating factor from earthworm, *Eisenia foelide* (EPAF, 25.9 μmol·L^{-1}), induced human platelet aggregation and 5-HT (maximal release of 89 % at EPAF 74.1 μmol·L^{-1}) was detected during this process. Neither creatine phosphate/creatine phosphate kinase (CP/CPK) nor aspirin completely inhibited the EPAF-induced platelet aggregation. In the presence of fibrinogen, EPAF (55.6 μmol·L^{-1}) induced the aggregation of human platelet which had been thrombin-treated and degranulated. Results indicated that EPAF was a potent platelet agonist and the EPAF-induced platelet aggregation was ADP- and TXA_2-independent.

KEY WORDS platelet activating factor; Oligochaeta; snake venoms; phosphocreatine; creatine kinase; aspirin; serotonin; thromboxane A_2

ADP receptor antagonist and aspirin had been applied to classify strong and weak platelet agonists[1] and aggregation pathway of platelet aggregating factor[2,3]. A platelet aggregating factor from *Eisenia foelide* (EPAF)

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was purified as an acidic protein with an isoelectric point at pH 3.2 and a molecular mass of 27 kDa[4]. The present study explored the biochemical mechanisms of platelet aggregation induced by EPAF.

MATERIALS AND METHODS

EPAF was prepared according to our previous paper[4]. Acidic Phospholipase A_2 ($APLA_2$) was prepared from venom of *Agkistrodon halys* Pallas[5]; o-phthaldialdehyde (OPD), Fluka; Triton X-100, Sigma; thrombin, Zhuhai Biochemical Pharmaceutical Factory, Zuhai, China; fibrinogen, Sigma. Routine reagents were all domestic products of AR grade.

PAM-2 automatic equilibrium platelet aggregating apparatus was from Danyang Electronic Plant, Jiangsu, China.

1 Platelet rich plasma (PRP) Fresh blood collected from humans, rats, and rabbits was mixed with sodium citrate 147 mmol·L^{-1} solution in a 9:1 (vol/vol) ratio and spun at $100\times g$ for 10 min. The uppermost layer was the PRP. The remaining blood was again spun at $800\times g$ for 10 min. The supernatant, the platelet poor plasma (PPP) was used as blank control and diluent to adjust PRP platelet count (2.5×10^{11}/L).

2 Platelet aggregation test Intensity of platelet aggregation was determined by the turbidimetric method[6].

3 Detection of 5-HT by fluorophotometry[7] Sample 0.2 ml was extracted by acidified *n*-butanol in the presence of 0.5 % Triton X-100, then spunned. *L*-cystein and *n*-heptan were added to the supernatant, again spunned. The water phase was transferred, 0.004 % OPD was added, agitated for 5 min, incubated at 100 ℃ for 15 min, then cooled with ice water. The emitted fluorescence at 480 nm (exciting with 365 nm) was detected by fluorospectro-photometer (Hitachi, F-4010).

4 5-HT standard curve PPP 175 μl were added to 25 μl of 5-HT (0, 0.4, 0.8, 1.2, 1.6, 2.0 μg·ml^{-1}) while stirring. 5-HT was determined[7]. Standard curve was plotted by taking the concentrations of 5-HT as the abscissa *vs* A_{480} reading as the ordinate.

5 EPAF induced release of 5-HT[8] After 220 μl of PRP were incubated at 37 ℃ for 5 min, 30 μl of EPAF were added and incubated for 6 min, and then spun immediately at $1000\times g$ for 5 min. The supernatant was assayed fluorophotometrically. 5-HT concentration ($C_{5\text{-HT}}$) was determined on the standard curve. The $C_{5\text{-HT}}$ in human platelets was 619 ± 282 ng/10^9 platelets[8]. The % of 5-HT released = $(175\times 2.5\times C_{5\text{-HT}})/(619\times 10^4)\times 100$ %.

6 Preparation of degranulated platelet suspension (DPS)[9] Human PRP suspension was incubated at 37 ℃ for 5 min, thrombin (0.3 u·ml^{-1}) and edetic acid (0.2 mmol·L^{-1}) was added, agitated and continue to incubate for 6 min, then spunned ($1000\times g$) for 10 min. The platelet pellet was washed twice by Teng Solution, then suspended in Teng solution containing 0.25 % bovine serum albumin. The count of platelet was adjusted to 2.5×10^{11}/L. DPS was then used to detect the EPAF induction of aggregation.

RESULTS

1 Effect of EPAF on platelet aggregation EPAF induced human PRP aggregation, which was directly proportional to the concentration of EPAF added. EPAF induced a maximal aggregation (76 %) of human PRP at 37.0 μmol·L^{-1} (Fig 1). The minimal concentration of EPAF necessary to aggregate human PRP was 25.9 μmol·L^{-1}. EPAF (37.0 μmol·L^{-1}) also induced aggregation of PRP from rats and rabbits, with aggregation rates of 40 % and 23 %, respectively. Thus,

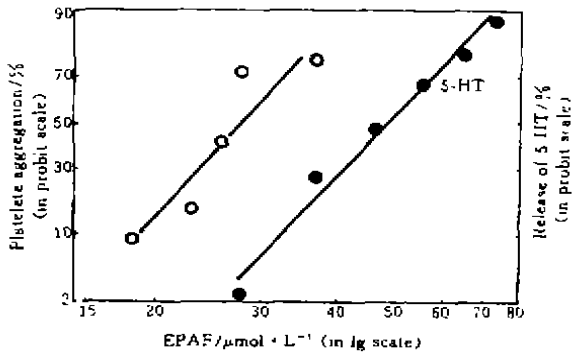

Fig 1. EPAF induced human platelet aggregation and release of 5-HT.

EPAF was most sensitive to human platelets.

EPAF induced the aggregation of PRP prepared with heparin (5 u·ml^{-1}) as anticoagulant to a maximal aggregation of 46.4 % at a final concentration of 55.6 μmol·L^{-1}.

2 Inhibition of $APLA_2$ on human PRP aggregation induced by EPAF $APLA_2$ (100 μg·ml^{-1}) was added to human PRP and incubated at 37 ℃ for 2 min. After EPAF was added to a final concentration of 55.6 μmol·L^{-1}, aggregation of platelets was determined. $APLA_2$ inhibited the EPAF-induced human platelet aggregation as the inhibition rate reached 60 % (Fig 2 A).

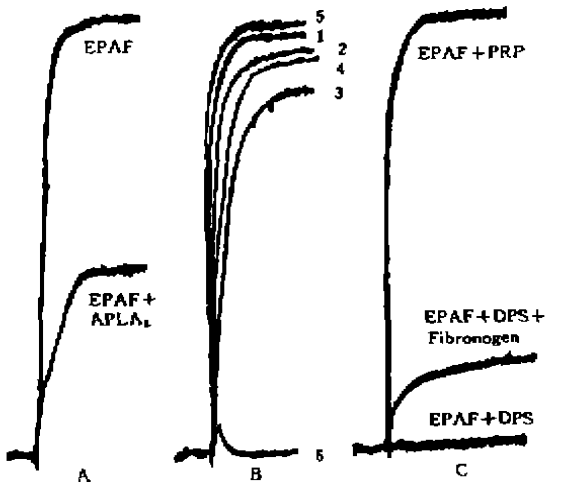

Fig 2. EPAF (55.6 μmol·L^{-1})-induced human platelet aggregation. A) Inhibition by $APLA_2$ from the snake venom of *Agkistrodon halys* Pallas. B) Influence of CP/CPK (5 mmol·L^{-1}/10 u·ml^{-1}) and aspirin (10 mmol·L^{-1}). 1. EPAF; 2. EPAF + aspirin; 3. EPAF + CP/CPK; 4. EPAF + CP/CPK + aspirin; 5. ADP 6. ADP + CP/CPK. C) EPAF-induced degranulated human platelet aggregation.

3 EPAF induced 5-HT release from human PRP EPAF induced concentration-dependently human PRP to release 5-HT, was also related to PRP aggregation. The concentration of EPAF that caused maximal (89%) release and 50 % release of 5-HT were 74.1 μmol·L^{-1} and 46.3 μmol·L^{-1}, respectively (Fig 1).

4 Inhibition of aspirin and CP/CPK to EPAF-induced human platelet aggregation CP/CPK 5 mmol·L^{-1}/10 u·ml^{-1}, were added to human PRP. After incubating at 37 ℃ for 1 min, ADP 150 μmol·L^{-1} and EPAF 55.6 μmol·L^{-1} were added. No aggregation was detected when ADP was added, whereas little inhibition was detected in EPAF induction with an aggregation rate of 68 %. To test the aspirin inhibition, human PRP was incubated with aspirin 1, 5 and 10 mmol·L^{-1} at 37℃ for 2 min did not show prominent effect on EPAF-induced human platelet aggregation. When both aspirin (10 mmol·L^{-1}) and CP/CPK were added, EPAF still induced aggregation of human PRP (Fig 2 B).

5 EPAF-induced aggregation of degranulated human platelet EPAF (final concentration 55.6 μmol·L^{-1}) induced the aggregation of DPS in the presence of 0.3 % fibronogen. But compared to the untreated platelet, the intensity of the aggregation caused by DPS was greatly reduced. The aggregation of DPS was not induced by EPAF in the absence of fibrinogen (Fig 2 C).

DISCUSSIONS

EPAF-induced platelet aggregation was not inhibited by either ADP receptor antagonist or aspirin, so EPAF should be considered as a strong platelet agonist[1]. Also, EPAF-induced platelet aggregation was different from those induced by ADP and collagen or arachidonic acid and TXA_2. The activating pathway of EPAF was ADP- and TXA_2-independent[2,3]. EPAF induced the degranulated platelet aggregation further demonstrated that EPAF-induced platelet aggregation was not fully dependent on platelet releasing actions.

EPAF-induced platelet aggregation was

not inhibited by heparin, and thus it was independent of thrombin. $APLA_2$ from venom of *Agkistrodon halys* Pallas inhibited the platelet aggregation induced by ADP and collagen, but did not inhibit the platelet aggregation induced by arachidonic acid (in press). $APLA_2$ inhibition of EPAF-induced platelet aggregation indicated that the platelet aggregation pathway of EPAF was different from that of arachidonic acid.

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赤子爱胜蚓血小板聚集因子对血小板的活化作用

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摘要 赤子爱胜蚓血小板聚集剂(EPAF, 25.9 $\mu mol \cdot L^{-1}$) 能诱导人血小板聚集. EPAF 为 74.1 $\mu mol \cdot L^{-1}$ 时引起 5-羟色胺最大释放 (89%). ADP 受体拮抗剂(CP/CPK)和阿司匹林均不能抑制 EPAF 引起的人血小板聚集反应; EPAF(55.6 $\mu mol \cdot L^{-1}$)能诱导凝血酶处理后脱颗粒人血小板产生聚集, 表明 EPAF 诱导的血小板聚集不依赖于 ADP 和 TXA_2, 是一种强血小板激动剂.

关键词 血小板激活因子; 寡毛目; 蛇毒; 磷酸肌酸; 肌酸激酶类; 阿司匹林; 血清素; 血栓素 A_2