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**普鲁卡因胺对兔血小板超微结构的影响**


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R97


关键词 普鲁卡因胺；花生四烯酸类；血小板聚集；电子显微镜检查 超微结构



目的：研究普鲁卡因胺(PA)对血小板超微结构的影响．方法：将花生四烯酸(AA)加入经 PA (8.5 - 136 $\mu mol \cdot L^{-1}$)处理的富含血小板血浆(PRP)中，诱导血小板聚集，制备超薄切片，用电镜观察各组血小板超微结构变化．结果：PA 8.5 - 136 $\mu mol \cdot L^{-1}$显著地抑制血小板伪足，α 颗粒，致密颗粒，糖原，开放管道及致密管道系统结构的改变．并存在着剂量依赖关系．结论：PA 对血小板超微结构和释放反应均有显著的抑制作用．


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# Platelet-released ADP stabilizes PAF-induced rabbit platelet aggregation by stabilizing intracellular calcium[1]


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**KEY WORDS** platelet aggregation; platelet activating factor; apyrase; adenosine diphosphate; calcium



**AIM**: To examine whether platelet-released adenosine diphosphate (ADP) would contribute to the stabilization of rabbit platelet aggregation induced by platelet activating factor (PAF). **METHODS**: Rabbit platelet aggregation induced by PAF was measured turbimetrically. ADP release from rabbit platelets stimulated by PAF was determined by HPLC. Intracellular $Ca^{2+}$ was measured using $Ca^{2+}$-sensitive fluorescent indicator Fura 2-AM. **RESULTS**: PAF $\geq$ 1 $nmol \cdot L^{-1}$ induced full platelet aggregation, which did not deaggregate over 5 min after aggregation reached peak. Platelet aggregation was deaggregated in a concentration-dependent manner by subsequent addition of ADP scavenger ATP-diphosphohydrolase (apyrase) at 5 - 100 $mg \cdot L^{-1}$. PAF 3 $nmol \cdot L^{-1}$ stimulated release of ADP (29 % *vs* 6 % of control), and elicited a rapid rise in intracellular calcium ($[Ca^{2+}]_i$) which peaked at approximately 15 s. Then the $[Ca^{2+}]_i$ gradually decayed from 585 ± 80 $nmol \cdot L^{-1}$ within 100 s to a low level (364 ± 82 $nmol \cdot L^{-1}$). Apyrase 100 $mg \cdot L^{-1}$, added 2 min after PAF, reduced $[Ca^{2+}]_i$ to a lower level (171 ± 29 $nmol \cdot L^{-1}$). **CONCLUSION**: Platelet-released ADP stabilizes PAF-induced rabbit platelet aggregation by stabilizing $[Ca^{2+}]_i$ at elevated level.


Platelet aggregation plays an important role in the pathogenesis of thrombosis. Deaggregation appears to take place most readily when the release reaction has not occurred[1]. The effects of the release reaction on the ability of platelets to


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deaggregate have not been studied systematically. It has been speculated that some substances released from the secretary granules may make the aggregation irreversible. Furthermore, the recent observations that apyrase or ticlopidine (a selective inhibitor of ADP-induced platelet function) facilitates the deaggregation of human platelets aggregated by thrombin[2-4], raise the possibility that the released ADP plays a role in *stabilizing platelet aggregation*.

It is well established that $[Ca^{2+}]_i$ is of major importance in activating platelets[5], and that the secretion of ADP is a large positive-feedback loop of platelet activation[6,7]. However, it is not clear whether released ADP and $[Ca^{2+}]_i$ act continually or only at some early special steps. The object of the present study was to examine whether released ADP would be essential for stabilizing PAF-induced platelet aggregation, and if so, whether the stabilizing effect of released ADP on platelet aggregation would be related to the level of $[Ca^{2+}]_i$.

## MATERIALS AND METHODS

**Drugs and reagents** Platelet activating factor (PAF), adenosine 5′-diphosphate (ADP), *Fura 2-AM*, *N-2-hydroxypiperazine-N-*2-ethane sulfonic acid (HEPES), and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. Apyrase was prepared from potatoes[8]. The activity of the preparation was such that the enzyme 1 mg · $L^{-1}$ converted adenosine triphosphate (ATP) 0.25 μmol · $L^{-1}$ to adenosine monophosphate (AMP) at 37 ℃ for 120 s. All concentrations were given as final concentrations after all additions to the platelet suspensions.

**Preparation of washed rabbit platelet (WRP) suspensions** New Zealand white rabbits ($n = 30$, $2.2 \pm s\ 0.3$ kg) were provided by the Animal Center of Hu-nan Medical University (Grade I, Certificate № 001). Rabbit platelets were prepared[9]. The final suspending medium was HEPES-buffered Tyrode solution (pH = 7.35) containing HEPES 10 mmol · $L^{-1}$, 0.25 % BSA, and with (for aggregation and release studies) or without $Ca^{2+}$ 1 mmol · $L^{-1}$ (for $[Ca^{2+}]_i$ measurement).

**Platelet aggregation and deaggregation** Platelet aggregation and deaggregation were studied in an aggregation module (Danyang Institute of Electrical Research, Suzhou, China). Maximal change in light transmission was assumed to represent maximal platelet aggregation[1].

**Measurement of platelet ADP** Platelet ADP content was measured by HPLC[10]. Released ADP was determined in cell-free supernatants of WRP suspensions incubated at 37 ℃ for 2.5 min with saline or PAF 3 nmol · $L^{-1}$ with stirring. Isocratic elution was performed with a mobile phase consisting of $KH_2PO_4$ 200 mmol · $L^{-1}$, adjusted to pH 6.0 with $NH_4OH$. The buffer ran at a rate of 0.4 mL · $min^{-1}$ for 45 min. Nucleotide were detected at 254 nm using a spectrophotometric detector (Lambda-Max Model 490, Waters). Results were expressed as μmol/$10^{11}$ platelets.

**Measurement of $[Ca^{2+}]_i$** $[Ca^{2+}]_i$ was measured using Fura 2-AM with a spectrofluorophotometer (RF-5000, Shimadzu) at $\lambda_{ex}$ = 340 and 380 nm and $\lambda_{em}$ = 500 nm, according to the method of Grynkiewicz G *et al*[11].

**Statistical analysis** Data were presented as $\bar{x} \pm s$. Paired $t$ test or ANOVA was employed for statistical analysis.

## RESULTS

**Effect of apyrase on platelet aggregation and deaggregation** PAF at threshold concentration of 10 pmol · $L^{-1}$ induced slight and reversible aggregation. The platelets were maximally aggregated by addition of PAF (1, 3, 10, or 30 nmol · $L^{-1}$) to WRP suspensions, which peaked at 2 min after addition of PAF and did not deaggregate for 5 min after peak. The platelet aggregation was deaggregated by subsequent addition of apyrase 100 mg · $L^{-1}$ to remove released ADP. The degree of deaggregation was decreased as the dose of PAF increased. Platelets aggregated by PAF 3 nmol · $L^{-1}$ were deaggregated by addition of apyrase in a dose-dependent manner to platelet suspensions on the peak of aggregation (Fig 1).

When the platelets were preincubated with apyrase 100 mg · $L^{-1}$, the degree of inhibition caused by apyrase was decreased as the dose of PAF increased. Reversible aggregation occurred

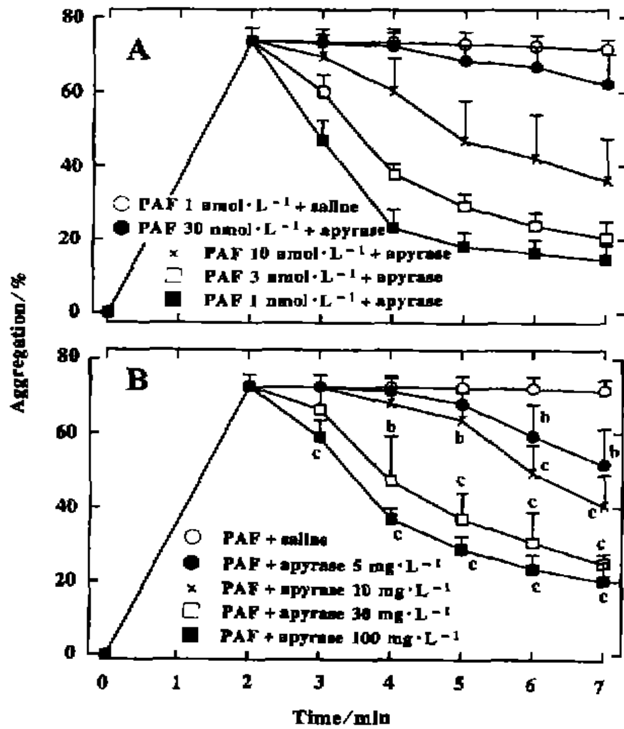


**Fig 1.** Effect of apyrase on the deaggregation of washed rabbit platelets aggregated by PAF. PAF added at 0 min, apyrase (or saline) added at 2 min. A) Effect of apyrase 100 $mg \cdot L^{-1}$ on the deaggregation of platelets aggregated by PAF (1, 3, 10, and 30 $nmol \cdot L^{-1}$). $n = 6$ independent experiments from 6 rabbit. $\bar{x} \pm s$. B) Dose-dependent effect of apyrase on the deaggregation of platelets aggregated by PAF 3 $nmol \cdot L^{-1}$. $n = 6$ independent experiments from 6 rabbit, $\bar{x} \pm s$.
$^bP < 0.05$, $^cP < 0.01$ *vs* saline control.

more readily at low concentrations of PAF (Fig 2).

**PAF-induced ADP release from rabbit platelet** The basic rabbit platelet ADP content was 4.4 ± 0.6 $\mu mol/10^{11}$ platelets $n = 7$ independent experiments from 7 rabbit. PAF 3 $nmol \cdot L^{-1}$ stimulated release of ADP (1.3 ± 0.3 $\mu mol/10^{11}$ platelets *vs* 0.26 ± 0.08 $\mu mol/10^{11}$ platelets of saline control, $n = 7$ independent experiments from 7 rabbit, $P < 0.01$).

**Effect of apyrase on $[Ca^{2+}]_i$** In the presence of $[Ca^{2+}]_0$ 1 $mmol \cdot L^{-1}$, PAF 3 $nmol \cdot L^{-1}$ induced the rise of $[Ca^{2+}]_i$ of Fura 2-AM-loaded rabbit platelets. The $[Ca^{2+}]_i$ rose from a resting level (141 ± 22 $nmol \cdot L^{-1}$) to a peak (585 ± 80 $nmol \cdot L^{-1}$) at approximately 15 s and thereafter decayed to a rather high sustained level (364 ± 82 $nmol \cdot L^{-1}$) within 100 s, and remained steady for at least 10 min. When apyrase was added 2 min after PAF, $[Ca^{2+}]_i$ was decreased rapidly to a lower level (171 ± 29 $nmol \cdot L^{-1}$). When platelets were preincubated with apyrase 100 $mg \cdot L^{-1}$, the increase of $[Ca^{2+}]_i$ in response to PAF 3 $nmol \cdot L^{-1}$ was inhibited from 585 ± 80 $nmol \cdot L^{-1}$ to 207 ± 85 $nmol \cdot L^{-1}$ (Fig 3).

## DISCUSSION

The present experiments showed that the maximal aggregation and $[Ca^{2+}]_i$ increase in the sustained plateau phase first reached at almost the same time, ie 2 min after PAF, and then both kept steady for over 5 min. Moreover, removal of released ADP with apyrase caused rapid deaggregation and decrease in $[Ca^{2+}]_i$.

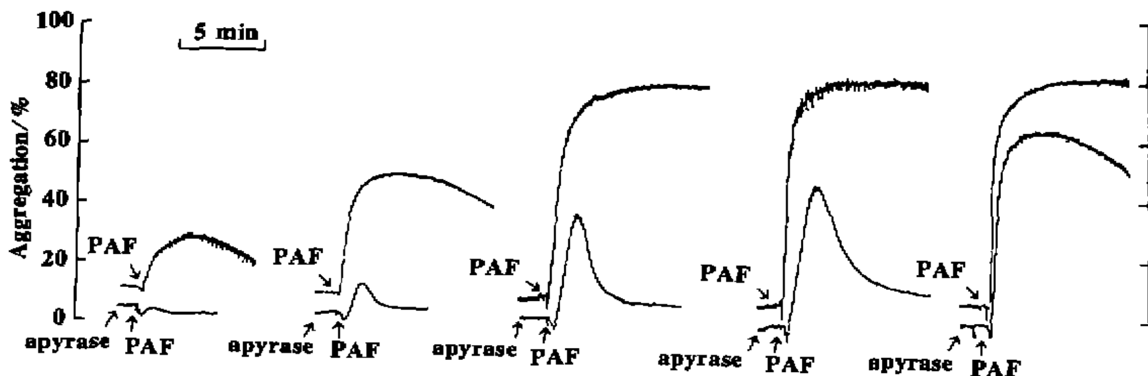


**Fig 2.** Effects of apyrase on PAF-induced rabbit platelet aggregation. Washed platelets were incubated with apyrase 100 $mg \cdot L^{-1}$ at 37 ℃ for 30 s, and then exposed to PAF 0.1 (a), 0.3 (b), 1 (c), 10 (d), 100 (e) $nmol \cdot L^{-1}$. Representatives of 7 independent experiments from 7 rabbit.

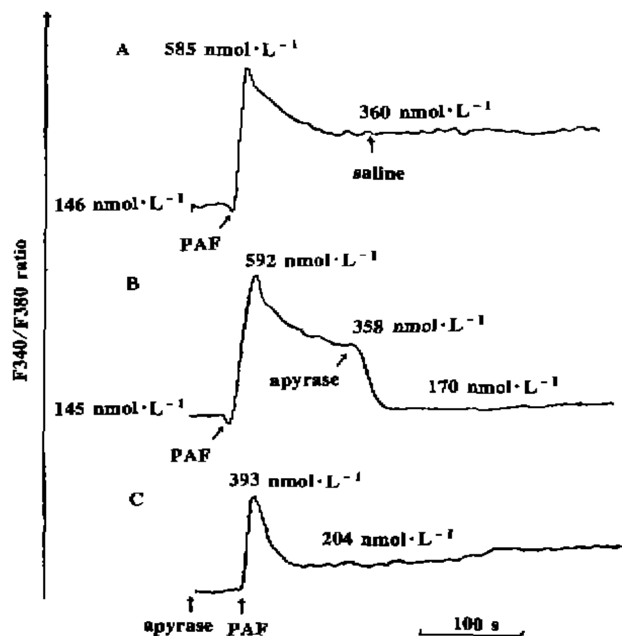


**Fig 3.　Effects of apyrase on the kinetics of $[Ca^{2+}]_i$ alteration induced by PAF 3 $nmol \cdot L^{-1}$.　Representative of 6 independent experiments from 6 rabbit.**

The results indicated that an elevated steady $[Ca^{2+}]_i$ level was related to stabilizing PAF-induced rabbit platelet aggregation and that released ADP stabilized PAF-induced rabbit platelet aggregation by stabilizing $[Ca^{2+}]_i$ at elevated level.

The studies[2] showed that apyrase only enhanced deaggregation caused by combinations of inhibitors, but the present experiments showed that apyrase itself caused rapid deaggregation.

This difference may be due to the fact that PAF is a much weaker secretory agonist than thrombin[6].　The present observation that PAF 3 $nmol \cdot L^{-1}$ induced full aggregation but only caused almost 29 % release of ADP content also supported PAF as a weak secretory agonist.　In conclusion, our results demonstrate for the first time that platelet-released ADP stabilizes PAF-induced platelet aggregation by stabilizing $[Ca^{2+}]_i$.

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## 血小板释放的 ADP 通过稳定细胞内钙浓度而稳定 PAF 诱导的兔血小板聚集[1]


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R331.143 R592.1

**关键词**　血小板聚集；血小板激活因子；PAF 腺苷三磷酸双磷酸酶类；腺苷二磷酸；钙


**目的**：研究释放的 ADP 对 PAF 诱导的兔血小板聚集的稳定作用．**方法**：以比浊法测血小板聚集；用 HPLC 测 ADP；以荧光法测细胞内钙（$[Ca^{2+}]_i$）．**结果**：PAF≥1 $nmol \cdot L^{-1}$时，均引起不可逆性的兔血小板聚集．PAF 3 $nmol \cdot L^{-1}$能诱导血小板释放 ADP、并诱导$[Ca^{2+}]_i$升高，$[Ca^{2+}]_i$在 15 s 左右达到最高，在随后的 100 s 内逐渐降到一个平台．而在聚集达顶峰时（PAF 之后 2 min）加 ADP 清除剂腺苷三磷酸双磷酸酶（apyrase）则能使$[Ca^{2+}]_i$迅速降低到一个更低的平台，并能呈剂量依赖性地引起解聚．**结论**：血小板释放的 ADP 通过稳定血小板细胞内钙浓度从而稳定 PAF 诱导的兔血小板聚集．