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Mepyramine inhibits platelet activating factor-induced rabbit platelet aggregation: role of intracellular histamine[1]

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KEY WORDS platelet aggregation; platelet activating factor; pyrilamine; histamine

AIM: To study the possible role of intracellular histamine (HA) in platelet activating factor (PAF)-induced platelet activation. **METHODS:** Washed rabbit platelet suspension was used to test the inhibitory effect of mepyramine (Mep, an H_1 receptor antagonist) on PAF-induced platelet aggregation. The thromboxane B_2 (TXB_2) generation was measured by radioimmunoassay and the intracellular calcium ($[Ca^{2+}]_i$) concentration was determined by the specific fluorescence indicator Fura-2. **RESULTS:** Mep >100 $\mu mol \cdot L^{-1}$ generated a concentration-dependent inhibition on PAF-induced aggregation, with an IC_{50} value of 162 (95 % confidence limits: 114 - 232 $\mu mol \cdot L^{-1}$). Cimetidine, an H_2 receptor antagonist, even up to 400 $\mu mol \cdot L^{-1}$ had no effect on it. Exogenous HA (10 $\mu mol \cdot L^{-1}$) and H_1 receptor agonist, 2-thiazolylethylamine had no energetic effect. α-Fluoromethylhistidine, an inhibitor of histidine decarboxylase, did not inhibit platelet responses. However, in platelets permeabilized with saponin (8 - 10 $mg \cdot L^{-1}$), exogenous HA attenuated the inhibitory effect of Mep to about 50 % at a concentration of 50 $\mu mol \cdot L^{-1}$. Preincubation of platelets with Mep (100 or 200 $\mu mol \cdot L^{-1}$) resulted in an inhibition on TXB_2 generation and $[Ca^{2+}]_i$ elevation induced by PAF. **CONCLUSION:** Platelets activated by PAF is associated with an intracellular HA synthesis and release via a common pathway of TXB_2 generation and the rise of $[Ca^{2+}]_i$.

Platelet activating factor (PAF)-induced aggregation is associated with activation of phospholipase C, resulting in the cleavage of phosphotidylinositol-4, 5-biphosphate (PIP_2), and also depending on the synthesis of thromboxane B_2 (TXB_2)[1]. The role of intracellular histamine (HA) on platelet activation was found recently[2,3]. Intracellular HA mediates platelet aggregation in response to various agonists[4,5]. However, the role of intracellular HA on PAF-induced aggregation is not clear, though PAF is one of the most powerful platelet activation agent. The present study was undertaken to investigate the role of intracellular histamine in PAF-induced platelet activation.

MATERIALS AND METHODS

Preparation of platelet suspension Washed rabbit platelets were prepared[6]. The platelet count was adjusted to $4 \times 10^{11} \cdot L^{-1}$ in modified Tyrode/buffer containing 0.25 % bovine serum albumin (BSA).

Platelet aggregation measurement Platelet suspensions (250 μL) containing $CaCl_2$ 1 $mmol \cdot L^{-1}$ were homogenized in siliconized tubes and aggregation was monitored photometrically using a dual channel aggregometer (Type DAM-1) at 37 ℃.

Measurement of TXB_2 generation A radioimmunoassay for TXB_2 was used[7].

Determination of $[Ca^{2+}]_i$ Washed rabbit platelets were loaded with Fura 2-AM 1.25 $\mu mol \cdot L^{-1}$ at 37 ℃ for 40 min, and then washed twice. The platelets were treated with Mep (100 and 200 $\mu mol \cdot L^{-1}$) in the presence of $CaCl_2$ 1 $mmol \cdot L^{-1}$. The fluorescence was monitored by a spectrofluorometer (RF-5000, Shimadzu, Japan) with λ_{ex} 340 nm and 380 nm at 0.5 HZ, λ_{em} at 485 nm. The concentration of calcium was calculated by the formula[8]:

$$[Ca^{2+}]_i = K_d(R - R_{min})/(R_{max} - R) \cdot V$$

R_{max} is the maximal ratio of Fura-2 fluorescence excited at 340 nm when the suspension was lysed with 0.1 % Triton X-100, resulting in the exposure of Fura-2 to Ca^{2+} 1 $mmol \cdot L^{-1}$; R_{min} is recorded after the addition of egtazic acid and Tris Base. V is the ratio of fluorescence of Triton X-100 and egtazic acid, K_d value (224 $nmol \cdot L^{-1}$) is the dissociation constant of the Fura-2-Ca^{2+} complex.

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Experimental protocol The inhibitory effect of Mep and cimetidine (Cim) were studied with 1-min preincubation before the addition of PAF. Exogenous HA and 2-thiazolylethylamine (2-THEA) were also added in platelet suspension before PAF to study their effects. α-Fluoromethylhistidine (α-FMH), a time-dependent irreversible histidine decarboxylase (HDC) inhibitor, was added 1 min before the addition of PAF. Platelets, permeabilized with saponin (8 – 10 mg·L^{-1}), were used to study the effect of exogenous HA. The concentration of saponin was carefully titrated for each individual experiment. After 2 min of saponin exposure, platelets did not result in spontaneous aggregation and produced 13.4 ± 6.9 % release of lactate dehydrogenase into the supernatant with the time course, indicating that saponin at such a concentration produced a platelet permeabilization without altering membrane receptor functions[9]. The reversal effect of Mep by HA to permeabilized platelet was calulated:

$$\text{Rate of reversal} = \frac{\%\ \text{aggregation (Mep + PAF + HA)} - \%\ \text{aggregation (Mep + PAF)}}{\%\ \text{aggregation PAF} - \%\ \text{aggregation (Mep + PAF)}}$$

Drugs and reagents Synthetic PAF, HA·HCl, Mep, Cim, 2-THEA, BSA, Triton-X-100, egtazic acid, and Fura 2-AM were purchased from Sigma Chemical Co. α-FMH was a gift from Merck Sharp & Dohme Research Laboratories.

Statistics Results were expressed as $\bar{x} \pm s$. The difference between 2 means was evaluated using t-test or one-way ANOVA.

RESULTS

Effect of HA antagonists on PAF-induced platelet aggregation Mep and Cim at 1 – 10 μmol·L^{-1} had no effect on PAF-induced platelet aggregation. Mep 100 and 400 μmol·L^{-1} resulted in a concentration-dependent inhibition of aggregation, IC_{50} value for Mep was 162 (95 % confidence limits: 114 – 232 μmol·L^{-1}). No effect was observed with Cim at 400 μmol·L^{-1} (Fig 1).

Effect of HA, 2-THEA, and α-FMH on PAF-induced aggregation HA (100 μmol·L^{-1}) had no synergetic effect on PAF-induced aggregation (Tab 1). Preincubation with α-FMH 1, 40, 80, and 120 μmol·L^{-1} for 1 min also gave a negative result (Tab 2).

Attenuation of the inhibitory effect of Mep by HA in permeabilized platelets Addition of HA (25 – 200 μmol·L^{-1}) to saponin-permeabilized platelets quickly after PAF, attenuated the effect of Mep on aggregation to about 50 % at a concentration of 50

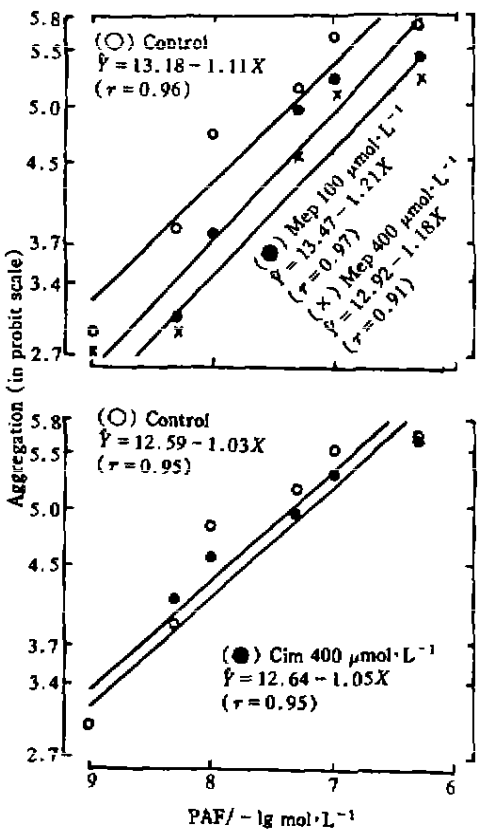

Fig 1. Effect of Mep and Cim on PAF-induced aggregation in rabbit platelets. $n = 5$, $\bar{x} \pm s$.

Tab 1. Effect of HA (100 μmol·L^{-1}) and 2-THEA (100 μmol·L^{-1}) on PAF-induced aggregation. $n = 5$, $\bar{x} \pm s$. $^aP > 0.05$ *vs* control.

PAF /nmol·L^{-1}	Aggregation/% Control	HA + PAF	2-THEA + PAF
100	66 ± 10	65 ± 11^a	67 ± 7^a
50	56 ± 7	57 ± 4^a	55 ± 12^a
10	32 ± 7	37 ± 13^a	38 ± 13^a
5	13 ± 7	15 ± 8^a	18 ± 6^a
1	2.5 ± 1.8	1.6 ± 1.9^a	2.5 ± 1.6^a

Tab 2. Effect of α-FMH on PAF (10 nmol·L – 1)-induced aggregation. $n = 3$, $\bar{x} \pm s$. $^aP > 0.05$ *vs* control.

α-FMH /μmol·L^{-1}	Aggregation/% Control	α-FMH + PAF
1	59 ± 7	54 ± 4^a
40	42 ± 6	40 ± 3^a
80	60 ± 4	56 ± 7^a
120	39 ± 3.2	38 ± 4^a

μmol · L^{-1} (Fig 2). But in intact cells, no attenuation was seen.

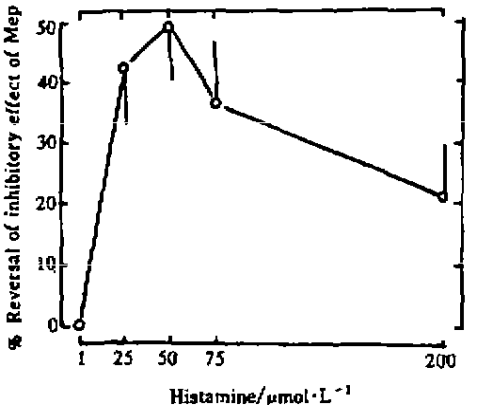

Fig 2. HA reversal of the inhibitory effects of Mep 200 μmol·L^{-1} on PAF-induced aggregation in saponin-permeabilized platelets. $n = 4$, $\bar{x} \pm s$.

***Effect of* Mep on PAF-induced TXB_2 generation** Pretreatment with Mep (100 or 200 μmol ·L^{-1}) before PAF (7.5 nmol · L^{-1}) decreased PAF-induced TXB_2 generation level (Tab 3).

Tab 3. Pretreatment with Mep inhibited PAF-stimulated platelet TXB_2 production. $n = 4$, $\bar{x} \pm s$.
$^aP > 0.05$, $^cP < 0.01$ *vs* control; $^fP < 0.01$ *vs* PAF.

	TXB_2 (ng/10^8 platelet)
Control	0.53 ± 0.10
PAF (7.5 nmol·L^{-1})	6.24 ± 2.37^c
Mep (100 μmol·L^{-1}) + PAF	0.37 ± 0.19^a
Mep (200 μmol·L^{-1}) + PAF	0.23 ± 0.02^f

Effect of Mep on PAF-induced $[Ca^{2+}]_i$ elevation PAF 2 nmol · L^{-1} to Fura-2-loaded platelets in the presence of Ca^{2+} 1 mmol · L^{-1} elicited a rapid rise in $[Ca^{2+}]_i$ which thereafter declined slightly higher than the resting value. Pretreatment with Mep inhibited this effect (Tab 4).

Tab 4. Preincubation with Mep inhibited PAF-induced $[Ca^{2+}]_i$ elevation. Control: $[Ca^{2+}]_i$ before PAF. $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$ *vs* control, $^bP < 0.05$ *vs* PAF.

	$[Ca^{2+}]_i$/nmol·L^{-1}
Control	109 ± 11
PAF	434 ± 20
Before Mep (100 μmol·L^{-1}) + PAF	140 ± 12^a
Mep (100 μmol·L^{-1}) + PAF	254 ± 28^b
Before Mep (200 μmol·L^{-1}) + PAF	149 ± 20^a
Mep (200 μmol·L^{-1}) + PAF	225 ± 29^b

DISCUSSION

In the present study, We demonstrated that Mep inhibited platelet aggregation induced by PAF at a higher concentration than those obtained in myocardial or vascular endothelium from functional H_1-response, which were generally regarded to bind to the high affinity site of HA. A low affinity site exists in human platelets[3]. Our results showed that IC_{50} value observed for Mep was almost identical to that previously reported for phorbol 12-myristate 13-acetate (PMA)-induced platelet aggregation and mast cell response[5,10]. Its anti-platelet aggregation action did not correlate with its potency as H_1-antagonist, suggesting that there is a low HA binding site different from traditional H_1 and H_2 receptor.

In intact cells, exogenous HA and 2-THEA showed no synergetic effect in PAF-induced platelet activation, whereas in saponin-permeabilized platelets where signal transduction and membrane responses were preserved[11], HA attenuated Mep inhibitory effect. It is reasonable to speculate that the site of action of HA is intracellular, possibly associated with HA synthesis or release during platelet activation. α-FMH was reported to inhibit HA and platelet aggregation in parallel in human platelets[2,3], but in our observation, it failed to inhibit PAF-induced rabbit platelet aggregation even with high dose. The possible explanation is due to species difference. The storage of HA in rabbit platelets is much higher than that in human platelets, α-FMH inhibits the production of HA, but cannot influence cytosolic HA already present in the cell granules.

The inositol phosphate cycle and the mobilization of Ca^{2+} play a major role in H_1 and PAF-receptor mediated responses[1,10]. In our study, preincubation platelets with Mep reduced the $[Ca^{2+}]_i$ elevation and TXB_2 generation. This inhibitory effect of Mep is correlated to the inhibition of $[Ca^{2-}]_i$ mobilization. Whereas

calcium mobilization is strongly implicated in the mediation of platelet subsequent intracellular signals and many functional changes induced by PAF. It facilitates arachidonate release by activation of phospholipase A_2, which is then metabolized to TXB_2. These results indicate that intracellular HA is involved in the TXB_2 generation and $[Ca^{2+}]_i$ elevation that act as a common pathway on platelet activation. To prove this hypothesis, further experiments are undertaken by using DPPE [N-N-diethyl-2-[4-(phenylmethyl)-phenoxy] ethanamine ·HCl], a specific intracellular HA H_1 receptor antagonist, and quantification of intracellular HA.

In summary, our finding suggested that a low affinity HA site exist in rabbit platelets, PAF induced platelet activation is associated with an intracellular HA synthesis or release *via* a common pathway of TXB_2 generation and elevation in $[Ca^{2+}]_i$.

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美吡拉敏抑制血小板激活因子诱导的兔血小板聚集：细胞内组胺的作用[1]

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关键词 血小板聚集；血小板激活因子；美吡拉敏；组胺

目的：研究细胞内组胺在 PAF 诱导血小板活化中的作用。**方法**：采用血小板聚集及放免法测定 TXB_2，Fura-2 负载测细胞内钙。**结果**：美吡拉敏高于 100 μmol·L^{-1}抑制 PAF 诱导的聚集，而西咪替丁在 400 μmol·L^{-1}亦无效，组胺及 2-thiazolylethylamine 也无协同作用。但在皂素（8 – 10 mg·L^{-1}）通透的血小板，组胺可减弱美吡拉敏的抑制效应，美吡拉敏还可抑制 PAF 引起的 TXB_2 产生和细胞内钙增加。**结论**：PAF 诱导的血小板活化伴随有细胞内组胺的合成或释放通过 TXB_2 产生和细胞内钙增加这一共同通路。