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Enhancement of cardiac performance of isolated working guinea pig heart by rabbit platelet and involvement of histamine

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ABSTRACT Alterations of cardiac performance by washed rabbit platelets were investigated in isolated working guinea pig hearts. Enhanced cardiac functions emerged about 5 s after intracardiac injection of platelet suspension ($6\times10^8-10\times10^8$ cells · ml^{-1}). The LVP, $+dP/dt_{max}$, $-dP/dt_{max}$, and HR were increased by 30%, 73%, 46%, and 13% ($P<0.05$ or 0.01), respectively, and recovered about 10 min later. In the range of $1\times10^8-70\times10^8$ cells · ml^{-1}, platelet suspension strengthened the cardiac performance in a concentration-dependent manner. The supernatant demonstrated positive inotropic and chronotropic effects as well, quite similar to those of the suspension. *Cimetidine* (Cim, 1 $\mu mol \cdot L^{-1}$) *reduced the cardiotonic* effect of the platelet. Mast cell of the isolated guinea pig heart was proven to be in an activated state upon 10^8 cells · ml^{-1} platelet challenge by electron microscopy. Histamine content in platelet suspension measured by HPLC was shown to be $2.6\pm s$ 0.7 μg per 10^8 platelets. It is concluded that histamine, released from platelets and cardiac mast cells, was a key factor in the cardiac alterations.

KEY WORDS histamine; cimetidine; blood platelets; heart; hemodynamics; mast cells; electron microscopy

Little attention has been directed toward the non-thrombotic properties or prethrombotic events of the platelet[1] and involvement of histamine. In this study, for the first time the washed rabbit platelet suspension was used to perfuse the isolated working guinea pig heart. Contrary to other platelet-perfused isolated heart models[2-4], the isolated guinea pig heart displayed an obvious change in cardiac performance. As histamine has long been recognized as a cardiotonic agent, cimetidine (Cim), an H_2 antagonist, was utilized in the isolated heart pretreatment. Ultrastructural examination of the mast cell in isolated heart and histamine analysis of the platelet suspension by HPLC were also carried out.

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MATERIALS AND METHODS

Platelet suspension The washed rabbit platelet suspension was prepared according to the method of Ardlie *et al*[5] with some modifications. Briefly, the rabbit blood was collected into 3.8% trisodium citrate (1 : 9, vol : vol) and centrifuged at $270\times g$ for 10 min at 25 ± 1℃. Platelet rich plasma (PRP) was centrifuged 3 times at $950\times g$ with intermediate washings in Ca^{2+}-free Tyrode solution (pH 7.35, Hepes-buffered) containing 0.35% bovine serum albumin (BSA, Sigma) and ethylene glycotetraacetic acid 0.5 mmol · L^{-1} (EGTA, Shanghai Chemical Reagent Factory). The platelet was suspended in BSA-Tyrode solution and counted, and its activity was tested with a dual-channel aggregometer (DAM-1, Jiangsu).

Isolated working heart Guinea pigs, weighing $315\pm s$ 30 g, were supplied by the animal center of Hunan Medical University. Cardiac function parametera were recorded on an 8-channel Polygraph (RM-6000, Nihon Kohden) as described previously[6].

Histamine analysis The analysis method was from that of Rönnberg *et al*[7] with a little adaptation. Briefly, it was performed by HPLC (Waters 510) in connection with a fluorospectrophotometer (RF-5000, Shimadzu). The fluorescence was monitored at 445 nm with excitation at 350 nm. Histamine was derivatized to a *fluorogenic compound o-phthaldialdehyde* (OPA, Fluka). Peaks were separated on a Spherisorb C_{18}, 5 μm particle size, reversed-phase column 250 × ID 4.6 mm (Dalian Institute of Chemical Physics, Chinese Academy of Sciences). The mobile phase consisted of 45% methanol and 55% acetate buffer, pH 4.50. The flow rate was 0.7 ml · min^{-1}.

Histamine standards (histamine dihydrochloride, Sigma) were run daily to ascertain the consistency of elution time and the sensitivity of the assay. The elution time of histamine-OPA was 9 min. The detection limit for histamine was 3.75 pmol by this assay. S : N = 3 : 1. Detailed description of methodology was published elsewhere[8].

Experimental protocol The washed rabbit platelet was adjusted to within the range of $6\times10^8-10\times10^8$ cells · ml^{-1}, and first tested for its activity with the method of platelet aggregation induced by platelet-activating factor (PAF, Sigma) 1 μmol · L^{-1}. Only the suspensions showing normal platelet activities were used for the experiments: 1) The isolated hearts ($n=8$) were challenged by the platelet suspension alone or with pretreatment of Cim 1 μmol · L^{-1}. The effect of the supernatant of the platelet suspension was examined as well ($n=4$). The possibility of platelet concentration effect ($n=4$) was explored within a gradient of platelet concentrations 1×10^8, 3×10^8, 1×10^9, 3×10^9, and 7×10^9 ml^{-1}. 2) The suspension ($n=6$) was deproteinized by perchloric acid 0.4 mol · L^{-1} [9] and then analyzed by HPLC. 3) The mast cell of the right atrium was observed under a transmission electron microscope (H-600, Hitachi) since it is abundant in histamine. Procedures for fixing, embedding, and sectioning followed those of routine tissue preparation.

Statistical analysis Data were expressed as $\bar{x}\pm s$, some were expressed as percent change over control, and evaluated with paired t test.

RESULTS

Effects of platelet suspension on isolated hearts and the antagonism of cimetidine An obvious enhancement phase, which appeared after 5±1 s of platelet suspension administration, was seen in the isolated hearts ($n=8$). The LVP, $+dP/dt_{max}$, $-dP/dt_{max}$, and HR were increased by 30%, 73%, 46%, and 13% ($P<0.01$) over the control group, respectively. Then it was followed by a prolonged negative inotropic phase. After 10±5 min, the isolated hearts recovered. The enhancing effect of platelets was largely antagonized by Cim 1 μmol · L^{-1} by decreases of LVP, $+dP/dt_{max}$, $-dP/dt_{max}$, and HR by 18%, 32%, 21%, and 6% ($P<0.05$ or 0.01), respectively (Tab 1). The supernatant exerted a similar effect as the suspension did ($P>0.05$) as LVP, $+dP/dt_{max}$, $-dP/dt_{max}$, and HR increased by 18%, 59%, 55%, and 26%, respectively. Concentration-response relationship of platelet on isolated guinea pig hearts was shown in Fig 1.

Tab 1. Effects of platelets (6×10^8 · ml^{-1}) on the isolated working guinea pig hearts and antagonistic effect of cimetidine 1 μmol · L^{-1}. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control.

	Control	Platelets	Cim +platelets
LVP, kPa	13.2±2.8	17±5[c]	14±3[b]
$+dP/dt_{max}$, kPa·s^{-1}	270±60	480±164[c]	330±93[b]
$-dP/dt_{max}$, kPa·s^{-1}	210±60	300±94[c]	240±68[a]
HR, bpm	221±12	249±21[c]	233±22[a]

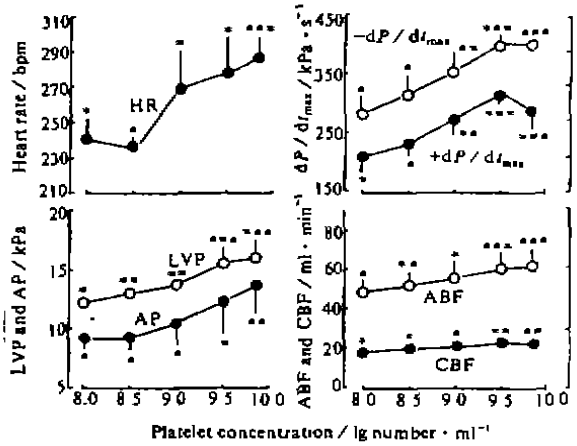

Fig 1. Effect of washed rabbit platelet suspension on isolated guinea pig heart. $n=4$, $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ vs control.

Effects of platelet suspension on mast cell morphology Ultrastructural changes of mast cell and surrounding capillaries of the isolated heart challenged with the platelet suspension were shown in Fig 2 (Plate 1). The mast cell

was activated upon challenge, and the cytosolic granules showed degranulation and exocytosis.

Quantitation of histamine in platelet suspension The derivatization product in the platelet suspension displayed a single peak on the chromatogram. The peak appeared constantly at 9 min which was coincident with that of histamine standard (Fig 3). Histamine content in the platelet suspension was 2.6±0.7 μg (23±6 nmol) per 10^9 platelets.

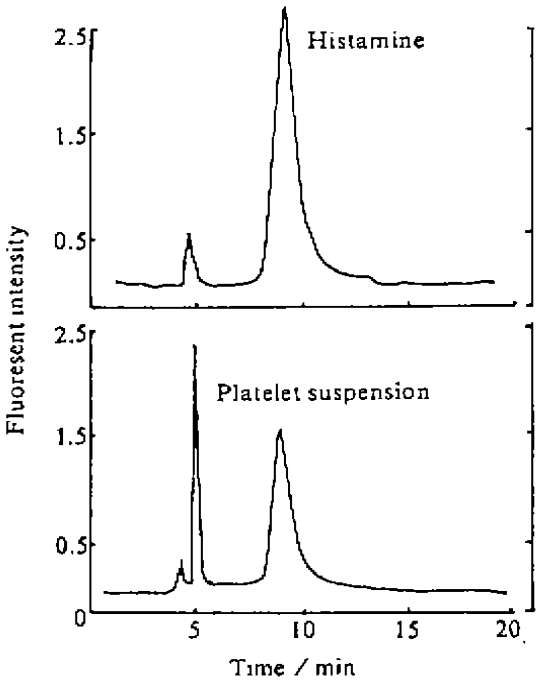

Fig 3. Chromatogram of standard histamine and platelet suspension sample derivatized with OPA.

The platelet sample presented an elution peak at 9 min simultaneously with that of histamine.

DISCUSSION

Histamine was proven to possess properties of positive inotropism and chronotropism[10]. In our experiment, Cim, an H_2 receptor blocker, reduced the enhancing effect of the platelets on the isolated heart, while pyrilamine, an H_1 blocker, did not. HPLC analysis verified the existence of histamine in the rabbit platelet suspension up to 23 ± 6 nmol per 10^9 cells. If a mean platelet volume of 6.0 μm^3 [11] was assumed, 23 nmol of histamine per 10^9 platelet yielded a cytoplasmic histamine of 3.8 mmol · L^{-1}. The cardiac mast cell was activated upon platelet challenge as the cytosolic granules presented degranulation and/or exocytosis. Therefore, histamine is one of the candidates capable of eliciting the cardiac alterations.

Histamine synthesis and secretion were likely independent of platelet aggregation in the case of washed rabbit platelet and it was supported by PAF aggregation test. Newly synthesized intracellular histamine played a role in collagen-mediated platelet activation and it may facilitate the secretion response of platelet granules[12]. Therefore, intrinsic histamine could promote aggregation of the platelets possibly as a second messenger.

In conclusion, the washed rabbit platelets enhanced the cardiac performance of isolated working guinea pig hearts in a concentration-dependent manner, which could partly be ascribed to the effects of histamine originated in platelets and/or the myocardial mast cells.

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家兔血小板对离体豚鼠作功心脏的增强效应及组胺的参与

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摘要 用洗涤家兔血小板悬液和其上清灌注离体豚鼠作功心脏，心脏作功浓度依赖性增强。西咪替丁(Cim) 1 μmol·L^{-1}显著抑制增强相。经HPLC测出血小板悬液每10^9血小板含组胺2.6±0.7 μg。电镜显示受血小板攻击的心脏肥大细胞处于活动期。结果表明，心功能加强与血小板及心脏肥大细胞释放的组胺有关。

关键词 组胺；西咪替丁；血小板；心脏；血液动力学；肥大细胞；电镜

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Chlorpromazine attenuated electroacupuncture analgesia in conscious rabbits[1]

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ABSTRACT By measuring the defense behavior in response to the noxious stimulation induced by potassium iontophoresis on ear-lobe skin of concious rabbit, chlorpromazine (CPZ) (0.5 mg·kg^{-1}, iv) induced hyperalgesia, whereas it significantly attenuated electroacupuncture analgesia (EAA) efficacy. Monoamines and their metabolites in cerebrospinal fluid (CSF) were measured by high pressure liquid chromatography with electrochemical detector (HPLC-ECD) while the attenuation effect of CPZ on EAA was observed. CPZ markedly enhanced 3,4-dihydroxyphenylacetic acid (DOPAC) ($P<0.05$) and homovanillic acid (HVA) ($P<0.01$) contents in CSF both in the presence and absence of electroacupuncture. CPZ attenuated EAA with elevations of either DOPAC or HVA concentration in CSF. There was a positive cor-

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