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海马胞外钙不依赖去甲肾上腺素释放及蛋白激酶C的调制作用

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摘要 在细胞外无钙时, 佛波醇基酯能加强3,4-二氨基吡啶、藜芦定或哇巴因所诱发的去甲上腺素(NE)释放, 但对莫能星(Mon)诱发的NE释放无作用. 河豚毒素能阻断前3种物质诱发的NE释放, 但对Mon诱发的释放无作用. 钙螯合剂BAPTA-AM能抑制这4种物质诱发的NE释放. 结果提示蛋白激酶C仅调制由膜去极化因素诱发的NE释放.

关键词 去甲肾上腺素; 氨基吡啶; 藜芦定; 哇巴因; 莫能星; 蛋白激酶C

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Pentoxifylline attenuates platelet activating factor-induced permeable edema in isolated perfused guinea pig lungs[1]

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ABSTRACT The effect of pentoxifylline (Pen) on platelet activating factor (PAF)-induced pulmonary injury was studied in isolated guinea pig lungs perfused with cell-free Tris buffered Ringer solution. PAF (1.0 nmol·L^{-1}) increased lung weight and pulmonary filtration coefficient (K_f), which indicated the formation of high permeable pulmonary edema. Pen (0.5 and 1.0 mmol·L^{-1}) markedly attenuated the PAF-induced increment of lung weight and vascular permeability, but not the increment of pulmonary capillary pressure and venous resistance. There was no correlation between the severity of lung edema and the number of leukocytes in the perfusates. These results suggest that Pen has direct anti-permeability effect on pulmonary microvessels.

KEY WORDS pentoxifylline; pulmonary edema; platelet activating factor

Pentoxifylline [1-(5-oxohexyl)-3, 7-dimethylxanthine, Pen] can increase intracellular cAMP by inhibiting cyclic nucleotide phosphodiesterase[1] and attenuate the formation of high permeable pulmonary edema in several models of acute lung injury including sepsis after *Escherichia coli* administration[2,3]. The ability of Pen to reduce lung injury was attributed to its inhibitory effects on neutrophil functions[4,5].

Platelet activating factor (PAF) is a potent lipid mediator involved in endotoxin-induced acute lung injury[6]. Our previous study showed that PAF increased albumin flux across cultured pulmonary endothelial cell

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monolayers and caused permeable edema of isolated rat lung perfused with cell-free solution[7,8]. The present study was to investigate whether Pen could prevent PAF-induced permeable pulmonary edema in isolated guinea pig lungs perfused without neutrophils.

MATERIALS AND METHODS

Synthetic PAF (L-α-phosphatidylcholine, β-acetyl-γ-*O*-hexadecyl) purchased from Sigma Chemical Co was dissolved in perfusate containing 0.25 % bovine serum albumin. Pentoxifylline was obtained from Shanghai No 17 Pharmaceutical Factory (Lot № 910912).

Isolated lungs Guinea pigs ♀, ♂, $n = 26$, weighing 338±*s* 44 g were anesthetized with ip sodium pentobarbital 30 mg·L^{-1}. The isolated, ventilated, perfused lungs were prepared[9]. The lungs were ventilated with room air at a tidal volume of 3 ml and a rate of 60 per min. The pulmonary circulation was rinsed free from blood with Tris buffered Ringer solution containing 0.5 % bovine serum albumin. The system was closed for recirculation of perfusion at 12 ml·min^{-1} with a roller pump. Pen (0.5 or 1.0 mmol·L^{-1} of final concentration) and/or PAF (1.0 nmol·L^{-1} of final concentration given 5 min after Pen pretreatment) were added to the perfusate after 10-min stable perfusion.

Pulmonary artery pressure and lung weight were continuously monitored using a PT 14M pressure transducer (Gaolian Transducer Co, Shanghai) and a force displacement strain gauge transducer (№ 634 Institute, Department of China Aviation and Aerospace Indostry, Beijing). Mean pulmonary capillary pressure and filtration coefficient (K_f) were determined[9]. The vascular resistances were calculated:

$$R_a = (P_a - P_{cl})/Q$$
$$R_v = (P_{cl} - P_v)/Q$$
$$R_t = R_a + R_v$$

where R_a, R_v, and R_t are the arterial, venous, and total vascular resistances, respectively, and Q is the perfusate flow rate. Relative lung water content was measured after perfusion and expressed as (wet lung weight − dry lung weight)/wet lung weight.

RESULTS

Lung weight increment and relative lung water content In control group there was no evident increase in lung weight (Fig 1), which remained unchanged for at least 1 h. PAF treatment increased lung weight by 2.44 ± 0.97 g at 15 min when compared with control group (0.20±0.21 g, $P<0.01$). The relative lung water content was increased after PAF treatment (Tab 1). Pretreatment with Pen markedly reduced pulmonary edema caused by PAF (Fig 1, Tab 1) in a concentration-dependent manner. Pen alone had no significant influence on lung weight and relative lung water content.

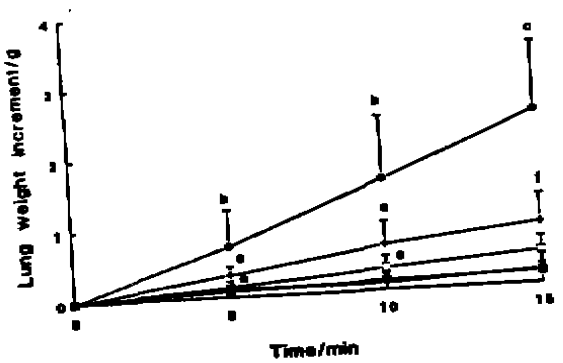

Fig 1. Effect of pentoxifylline (Pen) on platelet activating factor-induced increment of lung weight in isolated lungs. The weight of isolated lungs in 5 groups before perfusion was 3.05±0.38 g. (○) control, n =6 guinea pigs; (●) PAF (1.0 nmol·L^{-1}), n=5; (+) Pen (0.5 mmol·L^{-1}) + PAF (1.0 nmol·L^{-1}), n=5; (□)Pen (1.0 mmol·L^{-1}) + PAF (1.0 nmol·L^{-1}), n=5; (■) Pen (1.0 mmol·L^{-1}), n=5. $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs control; $^dP<0.05$, $^eP<0.01$ vs PAF.

Pulmonary vascular filtration coefficient There was an increase in K_f 15 min after PAF treatment (Tab 1). Pretreatment with Pen attenuated the PAF-induced increment of K_f, which indicated decrement of pulmonary vascular permeability. There was a close correlation between the increment of pulmonary vascular permeability and lung weight 15 min after perfusion. The relative lung water content and vascular permeability had no

Tab 1. Effects of pentoxifylline (Pen) on platelet activating factor (PAF)-induced changes of pulmonary filtration coefficient (K_f), relative lung water content (LWC), and number of leukocytes in perfusates. $n = 5-6$ guinea pigs, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control. $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ vs PAF.

Pen mmol·L^{-1}	PAF nmol·L^{-1}	K_f(ml·min^{-1}·kPa^{-1}/g dry lung)	LWC (%)	$10^{-5}\times$Leukocytes·ml^{-1}
0	0	0.05±0.04	82.1±3.4	0.87±0.37
0	1.0	0.41±0.17^c	89.0±4.3^b	1.41±0.59^d
0.5	1.0	0.16±0.03^{be}	86.5±1.0^{bd}	1.03±0.27^d
1.0	1.0	0.09±0.03^{af}	84.0±2.6^{ad}	1.54±1.37^d
1.0	0	0.06±0.04^a	85.3±1.6^a	1.41±0.79^d

correlation with the number of leukocytes in perfusates after 15 min perfusion.

Pulmonary vascular pressure and resistance There were no significant changes in pulmonary artery pressures among 5 groups before and after PAF and/or Pen treatment. However, PAF treatment increased pulmonary capillary pressure and venous vascular resistance from the baseline value of 0.77 ± 0.49 kPa and 1120±244 mN·s·m^{-5} to 1.06±0.33 kPa and 1267±161 mN·s·m^{-5}, respectively, after 15-min perfusion (Fig 2). Pretreatment with Pen had no effect on the increment of capillary pressure and venous resistance caused by PAF addition. Neither capillary pressure nor vascular resistance changed in lungs of the control and those perfused with Pen alone.

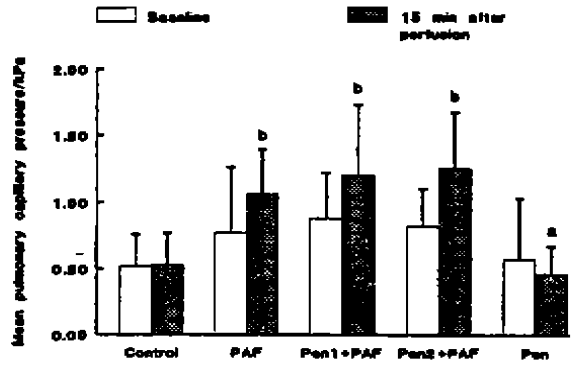

Fig 2. Effect of pentoxifylline (Pen1, 0.5 mmol·L^{-1}; Pen2, 1.0 mmol·L^{-1}) on platelet activating factor (PAF, 1.0 nmol·L^{-1})-induced changes of mean pulmonary capillary pressure in isolated lungs. $n=5-6$ guinea pigs. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ vs baseline and control.

DISCUSSION

The results showed that PAF significantly increased lung weight and lung water content as well as vascular filtration coefficient, which indicates that pulmonary edema induced by PAF is mainly due to increased vascular permeability. However, the contribution of increased capillary pressure cannot be excluded as PAF could slightly increase pulmonary capillary pressure by contracting venous vessels. These results agree to our previous study on isolated perfused rat lungs[8].

Our results also demonstrated that Pen attenuated pulmonary edema in isolated guinea pig lungs perfused with cell-free solution. Although leukocytes could not be rinsed absolutely free from the pulmonary vasculature, there was no correlation of the severity of lung edema with the leukocyte number in perfusates, which suggest that the effect of Pen to reduce lung injury in this model is not primarily on leukocytes.

Many studies showed that agents that increase intracellular cAMP such as β-receptor agonists and aminophylline could inhibit high-permeability edema induced by various inflammatory mediators[10,11]. Our preliminary study also revealed that isoproterenol signifi-

cantly inhibited PAF-induced cellular morphologic changes and intercellular gap formation in cultured bovine pulmonary endothelial cells, and that Pen reduced PAF-induced hyperpermeability of endothelial monolayers (unpublished data). Together with the present results, it is suggested that Pen has direct vascular anti-permeability effects by increasing endothelial cAMP level and inhibiting intercellular gap formation.

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己酮可可碱减轻血小板激活因子致离体豚鼠肺通透性水肿

R965.2

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摘要 血小板激活因子(PAF，1 nmol·L^{-1})可使离体灌注豚鼠肺重量和微血管液体滤过系数明显增加，己酮可可碱(Pen，0.5和1.0 mmol·L^{-1})对此有明显抑制作用，但对PAF引起的肺毛细血管压和静脉阻力的增高无明显影响。各组动物肺水肿的程度与灌注液中白细胞数无相关关系，提示Pen有直接抗肺血管通透性的作用。

关键词 己酮可可碱；肺水肿；血小板激活因子