Antigen-induced Contraction of Guinea Pig Isolated Thoracic Aorta

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ABSTRACT **OBJECTIVE:** To investigate vascular anaphylaxis and examine the role of mast cell-associated mediators during vascular anaphylaxis. **METHOD:** The guinea pigs were actively sensitized with ovalbumin. The thoracic aorta (TA) were isolated and cut into ring segments of about 3 mm, and suspended in 10 ml organ baths filled with oxygenated Krebs-Henselit (K-H) solution. We characterized the kinetics of and determined the mediators involved in antigen-induced contractions of TA. **RESULTS:** Ovalbumin (OA) induced contractions of TA, which reached maximum amplitude by 4-min, and delayed to about 50 % of maximum by 9-min. By 30-min, the tension was within (8 ±2) % max. Diphenhydramine (Dip) delayed the onset of contraction and decreased the peak of response. Famotidine (Fam) did not alter antigen-induced contractions, but partially reversed effects of Dip. Ketanserin (Ket) or Indomethacin (Ind) or/and Caffeic acid (Caf) significantly inhibited the contraction of TA. Incubated with Ket, Caf, Ind and Dip, the contraction of antigen-induced was almost inhibited. **CONCLUSIONS:** These results suggested that histamine, 5-HT, and metabolite of AA mediate antigen-induced contraction of TA.

KEY WORDS Anaphylaxis, Antigen, Thoracic aorta, Guinea pig

抗原诱导豚鼠离体胸主动脉的变态收缩反应

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摘要 目的:研究血管过敏变态反应并观察血管变态反应过程中肥大细胞释放介质的影响。方法:迅速截取卵蛋白致敏豚鼠的胸主动脉环(约 3 mm)悬挂于 10 ml 氧饱和的克-亨氏液浴槽中,记录不同试剂对抗原诱导的胸主动脉收缩反应影响。结果:抗原(卵蛋白)引起的胸主动脉收缩于 4 min 达到最大,9 min 约恢复一半,至 30 min 时基本恢复到基线,约为最大值的(8 ±2) %;苯海拉明对抗原引起的血管收缩反应有延迟作用并显著降低其最大收缩峰值,而法莫替丁不影响抗原对血管的收缩作用,但可部分减弱苯海拉明的作用;5-HT_2 受体抑制剂,酮色林、环加氧酶抑制剂,吲哚美锌和脂氧合酶抑制剂,咖啡酸对抗原诱导的血管收缩均有显著的抑制作用;当苯海拉明、法莫替丁、酮色林、吲哚美锌和咖啡酸一起孵育胸主动脉环时,抗原诱导的血管收缩作用基本被完全抑制。结论:上述结果表明,组胺、5-HT 和花生四烯酸代谢物对抗原诱导的胸主动脉血管收缩反应均有调节作用。

关键词 过敏反应;抗原;胸主动脉;豚鼠

Systemic anaphylaxis is accompanied with severe disturbances of cardiovascular and respiratory function. However, the cardiovascular events are concealed by respiratory systemic changes including sinus tachycardia, atrioventricular, conduction block etc. It was regarded that the respiratory system was the primary target system and the cardiovascular system was the secondary system during the system anaphylaxis (James H. zavecz and Roberto Levi, 1977). Recent studies (Laurie J, Kelly, Bradley, *et al*.1993) showed that heart was recognized not only as a secondary target organ for mediators released from the lung but also as the primary target organ in systemic hypersensitivity reactions. The cardiovascular event is caused by the anaphylaxis mediators. Previous studies on isolated guinea-pig trachea and pulmonary artery (PA) have demonstrated that the contractile

responses to antigen result from the action of at lease two of these substances, histamine and SRS-A. There are few papers in which experiments have carried out taking account of the thoracic aorta anaphylaxis.

The present study was designed to investigate vascular anaphylaxis and examine the role of mast cell-associated mediators during vascular anaphylaxis.

METHODS

Male and female guinea pigs (400 ~ 500g) were actively sensitized by intraperitoned injection of OA (1 %, 0.5 ml) on day 1, 3 and 5. During the first 21 days after the last injection, guinea pigs were killed by a blow on the head. The thoracic aorta immediately was isolated and placed in oxygenated K-H solution (4 ℃), containing (millimolar concentration): 118 NaCl; 5.4 KCl; 1.0 Na_2HPO_4; 1.2 $MgSO_4 \cdot 7H_2O$; 1.9 $CaCl_2$; 25.0 $NaHCO_3$ and 11.1 Glucose, adjusted to a pH of 7.4. All connective tissues and adhering fat were carefully removed. The TA was cut into rings 3-4 mm in length. The tissues were placed in 10-ml organ baths containing K-H solution maintained at 37 ℃ and bubbled with pure oxygen. The arterial rings were suspended in the baths with tethers of suture attached to small stainless steel loops carefully placed through the rings to minimize damage to the endothelium. Tissue contractions were recorded with FD-2 (Made in Chengdu, China). Each tissue was equilibrated for 90 min. The load applied to the thoracic aorta was adjusted to 2g.

Dip (10^{-6} M), Fam (10^{-4} M), Ket (3×10^{-6} M), Ind (10^{-6} M) and/or Caf (10^{-4} M) were added to the organ baths 30 min before the addition of OA (10^{-2} mg/ml) The contractions induced with OA (at time 0) were recorded for 30 min.

RESULTS

OA (10^{-2} mg/ml) induced contractions of TA tings, which reached maximum amplitude by 4-min (peak: Con, 1.601 ±0.267, n = 8) and decayed to about 50 % of maximum by 9-min. By 30-min, the tension was within (8 ±2) % of the maximum in TA.

Effect of histamine receptor antagonists. The H_1-receptor antagonist Dip (10^{-6} M) delayed the onset of contraction by 1 min, and decreased the peak of response by 58 %. The amplitude was delayed 2 min to maximum. Incubation with the H_2 receptor antagonist Fam (10^{-4} M) partially reversed the effect of Dip on the TA. The peak response was greater than obtained by incubation with Dip alone (Compared with Dip, t-test, $P < 0.05$) 〖(peak: Con, 1.601 ±0.267, n = 8; Dip, 0.707 ±0.183, n = 10; Dip and Fam, 0.971 ±0.308, n = 10)〗. The thoracic aorta rings incubated with Fam alone, responses were not significantly affected (Compared with Con, t-test, $P > 0.05$) (peak: Fam, 1.587 + 0.133, n = 10)[fig 1].

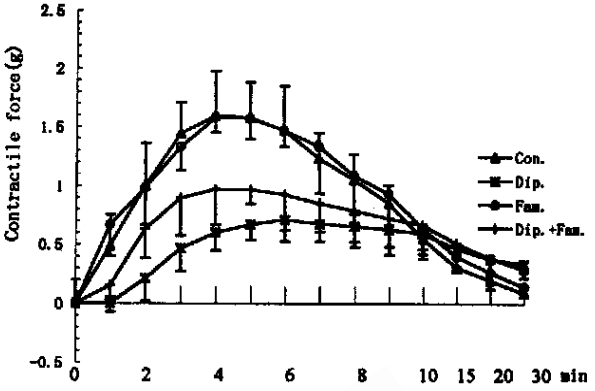

Fig 1. Effect of diphenhydramine or/and famotidine on OA-induced contraction of guinea pig TA (Con, n = 8; Dip, n = 10; Fam, n = 10; Dip and Fam, n = 10).

Effects of Ind and caf. Pretreatment with Ind, which inhibits the cycloxygenase, had significant inhibition. The peak amplitude was inhibited about 30 % (Compared with Con, t-test, $P < 0.05$) 〖peak: Con, 1.680 ±0.348, n = 8; Ind, 1.200 ±0.361, n = 10〗. The lipoxygenase inhibitor Caf (10^{-4} M) had significant inhibition on the peak amplitude of the antigen-induced response of the TA. The maximum was decreased 31 % (Compared with Con, t-test, $P < 0.05$) 〖peak: Caf, 1.150 ±0.307, n = 10〗. Combination with Ind and Caf, the peak amplitude of the antigen-induced response of the TA decreased 36 % of maximum (Compared with Con, t-test, $P < 0.01$) 〖peak: Caf and Ind, 1.030 ±0.352, n = 10〗[fig 2].

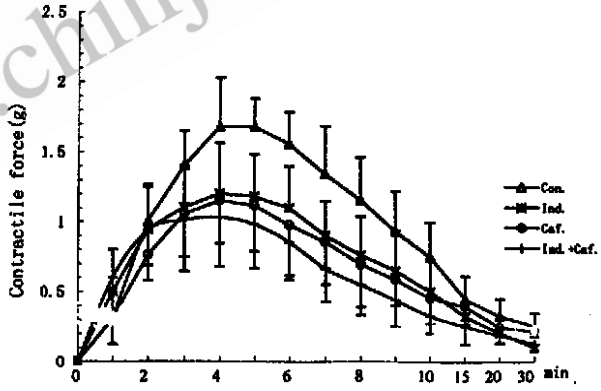

Fig 2. Effect of Indonmethacin or/and caffeic acid on OA-induced contraction of guinea pig TA (Con, n = 8; Ind, n = 10; Caf, n = 10; Ind and Caf, n = 10).

Effect of Ket. Pretreatment with Ket, which inhibitor the 5-HT_2 receptor, significantly decreased the amplitude of the antigen-induced response of the TA (Compared with Con, t-test, $P < 0.01$) 〖(peak: Con, 1.714 ±0.289, n = 8; Ret, 1.116 ±0.366, n = 10〗. The contraction amplitude was reduced 35 %[fig 3].

Effects of Ket, Caf, Ind and Dip. When tissues were incubated with Ket, Caf, Ind and Dip for 30 min, the contraction of antigen-induced was almost abolished. The peak amplitude was re-

duced 85 % (Compared with Con , *t*-test , $P < 0.001$) 〖peak : Con , 1 .714 ±0 .289 , $n = 8$; Ket , Caf , Ind and Dip ,0 .257 ± 0 .064 , $n = 10$〗[fig 3] .

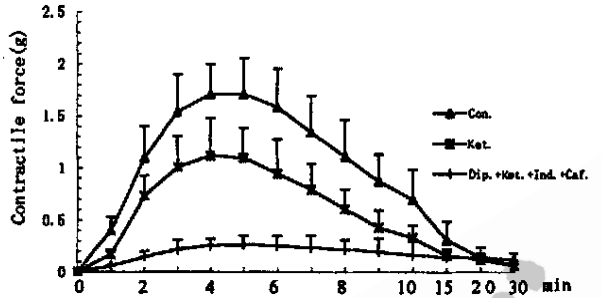

Fig 3 . Effect of ketanserin or Dip , Ket , Caf and Ind on OA-induced contraction of guinea pig TA(Con , $n = 8$; Ket , $n = 10$; Dip , Ket , Ind and Caf , $n = 10$) .

DISCUSSION

A number of mediators are known to being released from airways and PA during immediate hypersensitivity reactions , including histamine , sulfidopetides (LTs) and prostaglandins (Laurie J ,Kelly ,Bradley ,*et al* ,1993)

In this study , we examine the role of mediators in the thoracic aorta isolated from the anaphylactic guinea pigs . Pretreatment with Dip , an H_1 receptor antagonist delayed the onset and significantly reduced the magnitude of the contraction . Pretreatment with Fam , an H_2 receptor antagonist did not alter antigen-induced contraction , but partially reversed the effect of Dip on the TA , as previously shown by others (Hand , J . M , J . A . Will , C .K .BUCKNER , 1982) . These results suggest that H-receptor stimulation modulate the response to antigen induced contraction of TA .

Ind , which inhibits the cyclooxygenase , inhibited the response of antigen-induced contraction in the TA , The result suggests that contractile prostaglandin' s may also be released during the response to antigen .Incubation with Caf , an inhibitor of lipoxygenase , the peak amplitude of response of antigen was significantly decreased . The result demonstrates that LTs also appears to play a role in the antigen response . Incubation with Caf and Ind further inhibited contractile response , the effect suggests that Caf and Ind are coordinate with each other .

Ret , which is the inhibitor of 5- HT_2 receptor , reduced the maximum amplitude . 5- HT appears to contribute to antigen-induced contraction .

Incubation with Dip , Caf , Ind and Ket , antigen-induced contraction almost abolished . The result demonstrates that the contraction of antigen-induced on the TA are modulated by a number of pharmacologically active substances , where histamine is more prominent , particularly during the initial phase of the contrition .

In summary , histamine , 5- HT , leukotriedes and prostaglandins modulate the response of the antigen-induced in the thoracic aorta .

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