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中国药理学报 *Acta Pharmacologica Sinica* 1990 May; **11** (3): 281–285

维拉帕米、硝苯啶及尼卡地平的抗炎作用[1]

陈蔚如、杨永新、郑慧琴、阿斯亚、刘 发 (新疆医学院药理研究室, 乌鲁木齐 830054, 中国)

Anti-inflammatory effects of verapamil, nifedipine and nicardipine[1]

CHEN Wei-Ru, YANG Yong-Xin, ZHENG Hui-Qin, A Si-Ya, LIU Fa (*Department of Pharmacology, Xinjiang Medical College, Ürümqi 830054, China*)

ABSTRACT Anti-inflammatory effects of calcium antagonists verapamil (Ver), nifedipine (Nif) and nicardipine (Nic) were compared. They all produced significant inhibitions in acute and chronic inflammatory models in a dose-dependent manner. The ED_{30} in the capillary permeability were: Ver 18, Nif 12 and Nic 8 mg/kg, respectively. In the xylene-induced swelling of mouse ears, the ED_{30} were: Ver 39, Nif 14, and Nic 25 mg/kg, respectively. In the carrageenan paw edema of mice, and in the acetic acid-induced pleurisy and cotton granuloma of rats these drugs inhibited inflammatory responses at 5 or 10 mg/kg. Nif showed a greater effect than Ver and Nic on the paw edema, and Ver showed greater effects than Nif and Nic on the pleurisy and granuloma. At 10 mg/kg they depressed the PGE_2 content measured by radioimmunoassay in inflammatory tissues of carrageenan paw edema of mice.

KEY WORDS verapamil; nifedipine; nicardipine; non-steroidal anti-inflammatory agents; prostaglandins E

Received 1989 Apr 21 Accepted 1990 Jan 31
[1]Project supported by the National Natural Science Foundation of China, № 3870891

提要 钙拮抗剂维拉帕米(Ver)、硝苯啶(Nif)及尼卡地平(Nic)ig给药，以剂量依赖方式对多种急慢性炎症模型产生明显抑制作用.降低毛细血管通透性，Nic作用强；抑制炎性肿胀，Nif作用较强；抑制肉芽增生及急性胸膜炎，Ver作用较强. 3种药物还显著降低炎症组织中PGE_2含量.

关键词 维拉帕米；硝苯啶；尼卡地平；非甾体抗炎剂；前列腺素E类

炎症反应的许多环节受Ca^{2+}的调控，包括毛细血管的通透性，炎症介质的产生与释放[1-3]. 本文的目的是观察钙拮抗剂维拉帕米(verapamil, Ver)、硝苯啶(nifedipine, Nif)及尼卡地平(nicardipine, Nic)是否具有抗炎作用.

MATERIALS

小鼠由新疆计划生育研究所动物室提供，大鼠由新疆医学院动物室提供，♀♂兼用. Ver针剂(Finland Orion). Nif及阿司匹林(aspirin, Asp)粉剂(新疆制药厂)，临用前以10%的淀粉配成混悬液，Nic粉剂(南京医药工业研究所)，临用前以蒸馏水配制. 地塞米松针剂

(dexamethason, Dex, 江苏省无锡制药厂). 上述药物均 ig 给药. Evans 蓝(英国 BDH 公司). 角叉菜胶(辽宁省药物研究所). PGE_2 放射免疫测定药盒(中国医学科学院基础所药理室). 液体闪烁仪 LKB-1217/1218 型.

METHODS AND RESULTS

对毛细血管通透性的影响 昆明种小鼠, 体重 20± SD 3 g. ig 给药后 30 min, iv 0.5% Evans 蓝 5 ml/kg, 5 min 后 ip 0.7% 乙酸 10 ml/kg, 30 min 后脱颈椎处死小鼠, 用蒸馏水多次冲洗腹腔, 冲洗液稀释至终容量 10 ml, 按比色法[4]测定 Evans 蓝的渗出量. 结果 3 种钙拮抗剂均剂量依赖性地显著降低毛细血管通透性. 以线性回归求出各药的 ED_{30}, 显示 Nic 作用最强, Nif 居中, Ver 较弱. 3 种药 10 或 20 mg/kg 的作用即超过 Asp 100 mg/kg 的作用. Dex 在此模型中无作用 (Tab 1).

Tab 1. Effects of verapamil, nifedipine and nicardipine (ig) on increased vascular permeability induced by ip 0.7 % acetic acid in mice. $\bar{x}\pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Drug (mg/kg)	n	Evans blue (μg/ml)	Inhibition (%)	ED_{30} (mg/kg)
Saline	43	3.6±0.9		
Verapamil				
40	19	2.0±0.5***	44.8	
20	14	2.4±0.8***	33.2	18
10	15	2.9±0.9**	19.4	
5	12	3.3±1.0*	6.5	
Nifedipine				
40	15	1.7±0.4***	53.2	
20	15	2.2±0.5***	38.9	12
10	15	2.6±0.6***	27.9	
5	27	3.0±1.0**	14.9	
Nicardipine				
40	10	1.3±0.3***	63.9	
20	10	1.9±0.8***	45.4	8
10	9	2.3±0.7***	34.1	
5	10	2.8±0.6**	22.0	
Aspirin				
100	10	2.4±0.7***	31.6	—
Dexamethasone				
5	16	3.1±1.0*	13.2	—

对小鼠耳壳二甲苯致肿的影响 NIH 小鼠, 体重 22± SD 2 g. ig 给药后 30 min, 每鼠右耳滴 0.03 ml 二甲苯, 致炎 2 h 后脱颈椎处死, 以 8 mm 打孔器取左右耳片称重, 两耳片重量差为肿胀度. 结果 3 种钙拮抗剂, Asp 及 Dex 均显著抑制鼠耳肿胀. ED_{30} 显示, Nif 作用最强, Nic 居中, Ver 较弱. 3 种药在 40 或 80 mg/kg 的作用可超过 Asp 100 mg/kg 作用(Tab 2).

Tab 2. Effects of verapamil, nifedipine and nicardipine (ig) on the swelling of mouse ears induced by xylene. $\bar{x}$ **± SD.** $^{*}P>0.05$, $^{***}P<0.01$.

Drug (mg/kg)	n	Weight increase of ear flap(mg)	Inhibition (%)	ED_{30} (mg/kg)
Saline	25	11.9±1.8		
Verapamil				
80	6	7.1±3.4***	40.7	
40	6	8.3±2.4***	30.2	39
10	6	10.7±1.0*	10.7	
Nifedipine				
80	7	4.2±2.5***	64.7	
40	7	5.6±2.7***	53.3	14
10	7	9.3±2.2***	22.2	
Nicardipine				
80	9	6.2±2.1***	47.9	
40	12	8.9±1.9***	25.7	25
10	10	9.1±1.6***	23.8	
Aspirin				
100	8	7.2±1.6***	39.3	—
Dexamethasone				
5	8	6.1±2.6***	48.7	—

对小鼠足跖角叉菜胶致肿的影响[5] 昆明种小鼠, 体重 20 ± SD 2 g. ig 给药后 30 min, 右足跖 sc 2%角叉菜胶 0.05 ml, 用千分尺测量足跖厚度, 以致炎前后足跖厚度差为肿胀度. 结果 3 种钙拮抗剂, Asp 及 Dex 均显著抑制肿胀, 但前 4 种药起效较快, 于致炎后第 1 h 即开始抑制肿胀, 作用持续 3 h, 而 Dex 于第 2 h 开始显效, 但作用可持续到 4 h 以后(Tab 3).

Tab 3. Effects of verapamil, nifedipine and nicardipine (ig) on the swelling of mouse hind paws induced by carrageenan. $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Drug (mg / kg)	n	Swelling of hind paws (mm) 1	2	3	4h
Saline	25	0.52±0.10	0.57±0.10	0.63±0.10	0.60±0.13
Verapamil					
10	6	0.35±0.09***	0.46±0.11**	0.51±0.12**	0.62±0.09*
5	6	0.42±0.06**	0.47±0.08**	0.52±0.10**	0.52±0.13*
Nifedipine					
10	7	0.29±0.16***	0.36±0.14***	0.41±0.06***	0.52±0.12*
5	7	0.36±0.10***	0.48±0.09**	0.51±0.04***	0.64±0.08*
Nicardipine					
10	7	0.40±0.12**	0.44±0.09***	0.50±0.12***	0.60±0.11*
5	7	0.47±0.10*	0.52±0.12*	0.51±0.04***	0.54±0.05*
Aspirin					
100	7	0.32±0.17***	0.46±0.17**	0.44±0.11***	0.52±0.10*
Dexamethasone					
5	8	0.49±0.12*	0.44±0.09***	0.46±0.09***	0.38±0.18***

对大鼠急性胸膜炎的影响[6] Sprague-Dawley 大鼠，体重 267±32 g. ig 给药后 30 min，在乙醚全麻下，以 2% 醋酸 0.2 ml 注入胸膜腔，致炎后 6 h 测定渗出液体积及白细胞总数。结果显示 3 个钙拮抗剂在减少渗液量同时也减少白细胞游出，而 Dex 仅减少白细胞游出(Tab 4).

Tab 4. Effects of verapamil, nifedipine and nicardipine (ig) on the acute pleurisy induced by acetic acid in rats. $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

	Dose (mg/kg)	n	Exudation volume(ml)	$10^{-6}\times$ leucocytes
Saline	—	10	2.3±0.3	12.9±3.6
Verapamil	10	6	0.8±0.2***	3.9±1.2***
Nifedipine	10	6	1.7±0.6**	8.1±2.2**
Nicardipine	10	6	1.0±0.4***	5.0±1.3***
Dexamethasone	5	9	1.8±0.9*	7.6±4.3***

对棉球肉芽肿增生的影响[4] Sprague-Dawley 大鼠，体重 203±35 g，乙醚全麻，无菌操作，两侧腋下作切口，皮下各植入 20 mg 消毒棉球一个. ig 给药 qd ×6 d. 于 d 7 处死大鼠，剥离肉芽肿组织、肾上腺及胸腺. 肉芽组织于 90℃ 烘 1 h 称重，结果所用的药物均显著抑制肉芽增生. 3 种钙拮抗剂中 Ver 的作用较强. 3 药均不明显影响肾上腺重量，Ver 40 mg / kg 显著减轻胸腺重量. Dex 使肾上腺及胸腺明显萎缩(Tab 5).

Tab 5. Effects of verapamil, nifedipine and nicardipine (ig) on the weight of granuloma, adrenal and thymus in rats. $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Drug (mg / kg)	n	Weight (g / kg body wt) Granuloma	Adrenal	Thymus
Saline	10	1.00±0.21	0.17±0.04	1.20±0.32
Ver				
40	6	0.47±0.10***	0.16±0.05*	0.67±0.09***
10	6	0.60±0.08***	0.14±0.03*	1.30±0.20*
Nif				
40	6	0.60±0.28***	0.21±0.04*	1.30±0.42*
10	6	0.66±0.21***	0.26±0.11*	1.35±0.57*
Nic				
40	6	0.64±0.26***	0.24±0.09*	0.99±0.26*
10	6	0.71±0.12**	0.18±0.07*	1.60±0.73*
Dexa				
5	6	0.16±0.07***	0.11±0.03**	0.12±0.02***

对小鼠足跖致炎组织 PGE_2 含量的影响[5] 昆明种小鼠，体重 21 ±2 g. 给药量 3 种钙拮抗剂均为 10 mg / kg，Asp 100 mg / kg 角叉菜胶致炎，方法同前. 于致炎后 3 h 将肿胀足跖剪下置液氮冰冻，精确称取 50 mg 组织，按 PGE_2 放射免疫测定法制备样品和测定[7]. 结

果 3 种钙拮抗剂和 Asp 均显著降低 PGE_2 含量(pg/mg 湿重).生理盐水组: 55± 6 (n=12); Ver 组为 33 ± 4(n=8); Nif 组为 37 ± 5 (n=8); Nic 组: 34± 4 (n=11); Asp 组为 38 ± 5 (n=6).与生理盐水组比较, 各给药组均 $P<0.01$.

DISCUSSION

3 种钙拮抗剂对多种急慢性炎症模型具有抑制作用, 并显示与剂量相关, 说明在炎症反应的复杂过程中, 可能有许多环节受 Ca^{2+}的调节. 钙拮抗剂可以通过阻断细胞的 Ca^{2+} 摄取, 降低细胞 Ca 含量, 或其它作用方式从多个环节阻滞炎症过程.

在急性炎症时,血管透透性的升高主要是由于血管内皮细胞连结部位裂隙形成所致[8]. 当内皮细胞内 Ca^{2+}增多时, 内皮细胞收缩形成裂隙[9]. 一些致炎介质如组胺、5-羟色胺、缓激肽以及钙离子载体卡西霉素(A23187)能够增加 Ca^{2+}进入内皮细胞, 导致血管通透性增加[1,10], 因此, 钙拮抗剂有可能通过阻断内皮细胞的 Ca^{2+}摄取而降低血管通透性. 这可能是它们抑制炎性肿胀及胸腔渗液的机理之一.

在大鼠用角叉菜胶造成的炎症模型中, PGE_2 是主要炎症介质之一[11,12], 3 种钙拮抗剂降低小鼠足跖角叉菜胶致炎组织中 PGE_2 含量, 说明它们的抗炎作用和抑制炎症介质 PGE_2 的产生有关. PGE_2 是花生四烯酸环氧酶途径的主要代谢物, 而使花生四烯酸从膜磷脂释放的磷脂酶 A_2 则是一种 Ca^{2+}和钙调蛋白依赖的酶[13,14]. 在小鼠腹腔巨噬细胞, 卡西霉素促进花生四烯酸代谢物包括 PGE_2 的释放, 而钙拮抗剂维拉帕米则抑制卡西霉素的作用[15]. 推测在炎症过程中, 3 种钙拮抗剂降低 PGE_2 的产生是由于它们阻断 Ca^{2+}进入细胞而抑制花生四烯酸代谢的结果.

3 种钙拮抗剂对大鼠肾上腺重量无明显影响,且在多种炎症模型中与 Dex 的表现不同.而与 Asp 的作用类似, 提示它们的抗炎作用属于非甾体类抗炎剂. 在 3 种钙拮抗剂之间, 各药的作用强度不同, 说明 3 种药物对不同组织和细胞的选择性有差别. 本工作表明 3 种钙拮抗剂在 5 或 10 mg/kg 时对急慢性炎症即可产生明显抑制作用.

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中国药理学报 *Acta Pharmacologica Sinica* 1990 May; 11 (3) 285-288:

中性粒细胞对肥大细胞组胺释放的影响[1]

王瑶生、卞如濂、周汉良 (浙江医科大学药理教研室, 杭州 310006, 中国)

Effects of neutrophils on histamine release from mast cells

WANG Yao-Chen, BIAN Ru-Lian, ZHOU Han-Liang (*Department of Pharmacology, Zhejiang Medical University, Hangzhou 310006, China*)

ABSTRACT To determine whether neutrophils (NP) contribute to immediate anaphylaxis, the effects of NP on histamine release from mast cells (MC) were studied and changes in the NP morphology were investigated. When rat peritoneal MC and pleural NP collected from actively sensitized rats were mixed and challenged with antigen (trichosanthin), histamine release from MC was significantly increased. This promotive effect of NP on antigen-induced histamine release from MC was dosage-dependent. The observation of scanning and transmission electron microscopy showed that NP were activated and secretion was resulted. It is suggested that NP may be activated in immediate anaphilaxis and secrete some mediators which could promote histamine release from MC. Quercetin may inhibit the promotive effect of the NP on histamine release and the secretion of the NP while ketotifen and isoproterenol have no influence on this promotive effect.

KEY WORDS neutrophils; mast cells; histamine; quercetin; trichosanthin; electron microscopy

提要 中性粒细胞 (NP) 可以促进抗原诱导的致敏大鼠的肥大细胞(MC)组胺释放. 其促进作用与 NP 的数量呈依赖关系. 同时, NP 形态上表现出活性增强及分泌反应. 提示 NP 可以通过释放某些介质促进 MC 组胺释放. 槲皮素可以抑制 NP 对 MC 组胺释放的促进作用以及 NP 形态的变化.

关键词 中性粒细胞; 肥大细胞; 组胺; 槲皮素; 天花粉蛋白; 电子显微镜检查

中性粒细胞 (neutrophil, NP) 与迟发型哮喘反应及气道高反应性有密切关系[1]. 但是, 对 NP 在速发型变态反应中的作用了解甚少. 本文观察 NP 对肥大细胞 (mast cell, MC) 组胺释放的影响及槲皮素 (quercetin) 等药物对于 NP 有关功能的影响. 这将有助于了解 NP 在速发型变态反应中的作用. 进一步阐明过敏性哮喘的发病机理. 为寻找防治哮喘的有效药物提供依据.

MATERIALS AND METHODS

Sprague-Dawley 大鼠, ♀♂兼用, 体重 201 ± SD 13 g (本校动物科). 角叉菜胶 (carrageenin, 辽宁药物研究所), 天花粉蛋白 (trichosanthin, 上海有机化学研究所), 槲皮素 (上海试剂二厂), 酮替芬(ketotifen, 上海第十六制药厂), 盐酸异丙肾上腺素(isoproterenol, 上海信谊制药厂).

大鼠致敏 Sprague-Dawley 大鼠, 每批 10 只, 共 8 批. 每只脚掌注射 5 mg/ml 天花粉蛋白悬液 0.1ml(悬混于氢氧化铝 4%凝胶

Received 1988 Dec 29 Accepted 1990 Jan 3
[1]Project supported by the National Natural Science Foundation of China, № 3861201