sive PAF release from activated leukocytes and platelets might initiate microcirculatory disturbances and induce anaphylactic shock.

The mesenteric microvascular dilation may be a direct action of PAF on vasculature mediated by PAF receptor as the reflex response to systemic hypotension is vasoconstriction. If the most of peripheral microvasculature response to PAF challenge in the way of mesenteric microcirculation, the dilation of microvessels will play a part in PAF-induced systemic hypotension besides the decreased contractability of the heart[3] and the reduced blood volume after iv PAF[1].

Systemic inflammation induces the formation of "white thromboli" in microcirculation because of platelet aggregation. In the present study, "white thromboli" was not observed in the mesenteric microvasculature after PAF administration in rats, indicating that PAF would not activate rat platelets *in vivo*. These results are comparable with those of the previous study *in vitro*[4].

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血小板激活因子对活体大鼠微循环的影响

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提要 应用激光多普勒微循环显微镜定量研究了血小板激活因子(PAF)对大鼠肠系膜微循环的影响. 发现 iv PAF 0.2, 0.4, 0.6 μg / kg 可引起剂量依赖性的动脉血压降低, 肠系膜微动脉和微静脉扩张及血流速度减慢, 白细胞对微静脉壁的粘附性增强. 上述效应可被 PAF 特异性受体拮抗剂 SRI 63-441 阻断.

关键词 血小板激活因子; 微循环; 肠系膜动脉; 肠系膜静脉; 流量计; 大鼠

中国药理学报 *Acta Pharmacologica Sinica* 1990 Jul; **11** (4) : 346-350

蝙蝠葛碱抑制卡西霉素刺激小鼠腹腔巨噬细胞释放血小板活化因子[1]

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Inhibitory effect of dauricine on platelet activating factor released from calcimycin-induced mouse peritoneal macrophages

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ABSTRACT The effects of dauricine (Dau) on the release of platelet activating factor (PAF) from mouse peritoneal macrophages stimulated by calcimycin (A-23187) was studied. The method of sodium [^{3}H] acetate incorporating into macrophages to synthesize PAF was set up for the first time. Calcimycin (0.2 μmol / L) significantly induced mouse peritoneal macrophages to utilize sodium [^{3}H] acetate to synthesize PAF. PAF released from macrophages medium fluid increased as the concentration of sodium [^{3}H] acetate increased. The maximal amount of PAF released from macrophages was attained by incubating macrophages with sodium [^{3}H] acetate (250 μmol / L) and calcimycin (2 μmol / L) over 30 min. Extracted by $CHCl_3$, CH_3OH : H_2O (2 : 2 : 1.8), seperated by thin

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layer chromatography (TLC) and determined by liquid scintillation counting, PAF released was inhibited significantly by Dau both in time (10–30 min) and dose (1–1000 μmol/L) dependent manners. The IC_{50} of Dau for the formation of PAF was 2.5 μmol/L. On the same condition PAF release was also significantly inhibited by quinacrine at 500 μmol/L. The results indicate that Dau is a potent inhibitor of PAF synthesis in mouse peritoneal macrophages.

KEY WORDS platelet activating factor; dauricine; isoquinolines; quinacrine; A–23187 (calcimycin); macrophages

提要 卡西霉素刺激小鼠腹腔巨噬细胞利用氚标记的醋酸钠产生血小板活化因子. 用薄层层析分离，液体闪烁记数仪测定血小板活化因子的放射性活性，证明蝙蝠葛碱在 1–1000 μmol/L 浓度范围内及所试时间范围内，呈剂量及时间依赖性地抑制小鼠腹腔巨噬细胞释放血小板活化因子，其 IC_{50} 为 2.5 μmol/L.

关键词 血小板活化因子; 蝙蝠葛碱; 异喹啉类; 卡西霉素(A–23187); 巨噬细胞; 阿的平

血小板活化因子(platelet activating factor, PAF)是一种具有高度生物活性的内源性脂质[1]. 体内许多病理过程均与 PAF 的作用有关[2–5]. 因此，寻找能抑制 PAF 生成或拮抗 PAF 作用的药物，对于临床上许多疾病的预防或治疗具有重要的现实意义. 银杏甙类具有较强的拮抗 PAF 的作用已有文献报道[6]，磷脂酶 A_2(phosphalipase A_2, PLA_2)抑制剂阿的平(quinacrine, Qui)能抑制 PAF 的生物合成[7]. 蝙蝠葛碱(dauricine, Dau)能抑制血小板及中性白细胞中花生四烯酸代谢的环氧酶及脂氧酶产物[8–9]，提示 Dau 可能抑制 PLA_2. 本文用氚标记的醋酸钠参入小鼠腹腔巨噬细胞合成并释放 PAF 的方法，并以阿的平作对照研究蝙蝠葛碱对 PAF 生物合成的影响.

MATERIALS AND METHODS

昆明种小鼠，♀♂不拘，体重 21.5±SD1.5g. Dau 由中国药科大学药化教研室提供，临用前用适量 HCl(1 mol/L)溶解. 再用 pH7.4 的 D–Hanks 液稀释至所需浓度. Qui 由上海寄生虫研究所赠送，临用前用 D–Hanks 液配成所需浓度. sodium[^{3}H] acetate 购自中科院原子能研究所，放射性活性为 7.36 GBq/mmol. PAF 标准品由瑞士 P Hoffmann–La Roche 公司的 P Hadvary 博士惠赠，用无水乙醇配成 1 μg/μl, −20℃保存. 小牛血清白蛋白(BSA)为上海生物制品研究所产品. 卡西霉素(calcimycin, Cal)及 RPMI–1640 为 Sigma 公司产品. 卡西霉素用 DMSO 配成 0.2 mmol/L，临用前稀释成所需终浓度.

Sodium [^{3}H] acetate 的参入及 PAF 的释放 以 D–Hanks 液(pH7.4)灌洗小鼠腹腔，灌洗液经离心沉淀后得细胞沉淀，加入含 10% 小牛血清的 1640–BSA(BSA 占 0.25%)液中制成细胞悬液(1×10^7 细胞/ml)，加入直径 35 mm 培养皿中(1 ml/皿)，置 CO_2 孵箱内贴壁 2 h，再以 D–Hanks 液和 1640 液各洗一次，去除非贴壁细胞，每皿加入 1 ml 1640–BSA 培养液及被试药物，孵育 20 min 后，加入 250 μmol/L 的 sodium [^{3}H] acetate 及终浓度为 2 μmol/L 的 Cal，继续孵育 30 min，取 50μl 培养液测其放射性强度，计算参入%，其余培养液进行提取.

参入% = (参入前 dpm 值−参入后 dpm 值)/参入前 dpm 值

PAF 的提取、分离与测定 巨噬细胞加 sodium [^{3}H]acetate 及 Cal 孵育 30 min 后，倾出全部培养液，离心后按改进的 Bligh 及 Dyer 法[10]提取其中的总脂，提取液用 N_2 流吹干，用 $CHCl_3$:CH_3OH(80:2)溶液 20 μl 溶解残渣，进行薄层层析(TLC)分离. 层析条件:硅胶 G 板(20×5 cm)，样品复溶后线状点样于薄板上，复溶液残渣洗二次，每次 20 μl，重复线状点样，在线状样品旁边，点 5 μl 标准 PAF 液(1μg/μl). 展开剂为 $CHCl_3$:CH_3OH:H_2O (70:15:1)，展开距离为 16 cm，用碘蒸汽显

色，PAF 能与其他高记数脂类明显分开(Fig 1)，将 PAF 标准品对应的色斑刮下后测其放射性强度.

Fig 1. Thin layer chromatography of platelet activating factor released from mouse peritoneal macrophages. The standard PAF was co-chromatographed with samples and was visualised by I_2 vapor. O origin, SF solvent front, P standard PAF.

实验结果以 $\bar{x} \pm SD$ 表示，显著性用 t 检验测定.

RESULTS

Sodium [^{3}H]acetate 的量对巨噬细胞合成 PAF 的影响 用不同量的 sodium[^{3}H]acetate 参入巨噬细胞，PAF 的生成具有明显的量效关系，随着 sodium [^{3}H] acetate 浓度的增加，PAF 的生成也增加. 本文以下实验采用在所试浓度范围内(10 μmol / L – 250 μmol / L)产生 PAF 量最高的 sodium [^{3}H] acetate 浓度即 250 μmol / L.

Sodium [^{3}H]acetate 参入巨噬细胞与最佳孵育时间的选择 将 sodium[^{3}H]acetate 与巨噬细胞孵育不同的时间，计算出参入%，同时算出该时间下 PAF 产生的量(Fig 2). 从图可见，采用 sodium [^{3}H]acetate 参入小鼠腹腔巨噬细胞时，该细胞摄取 sodium [^{3}H]acetate 十分迅速，30 min 后，参入率已达 49.6%，趋于平衡，虽然随着时间的增加，sodium [^{3}H] acetate 参入量也不断增加，但在 30 min 时，PAF 的产量最高，故本文以下实验采用巨噬细胞与 sodium [^{3}H]acetate 孵育 30 min.

蝙蝠葛碱对卡西霉素刺激小鼠巨噬细胞释放 PAF 的影响 巨噬细胞与 sodium [^{3}H] acetate 孵育 30 min 后，培养液经提取、TLC 分离、刮下相应的 PAF 色带，测定其放射性

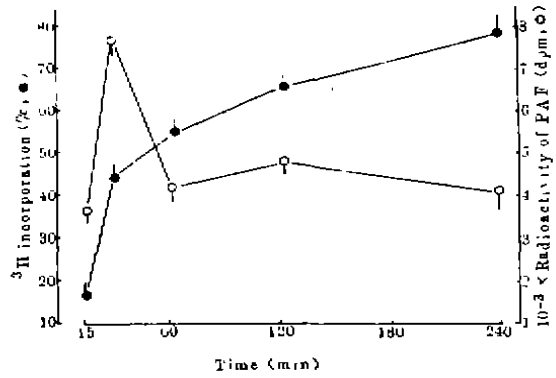

Fig 2. Calcimycin-induced sodium [^{3}H] acetate incorporation into and PAF release from mouse macrophages incubated with sodium [^{3}H] acetate. (O) PAF release, (●) [^{3}H] incorporation, $n = 3$, $\bar{x} \pm SD$.

强度，结果见 Tab 1. 当不用 Cal 刺激时，巨噬细胞产生 PAF 的量较少，仅为刺激后产生的 PAF 量的 1 / 4. Dau 在 1–1000 μmol / L 浓度范围内均能显著抑制小鼠腹腔巨噬细胞产生 PAF.该作用与其浓度呈剂量依赖关系.IC_{50} 为 2.5μmol / L. 在同样的条件下，Qui 在 500 μmol / L 时也能显著抑制小鼠腹腔巨噬细胞产生 PAF.

Tab 1. Effects of dauricine (Dau) on the release of PAF from sodium [^{3}H] acetate labeled mouse macrophages challenged with calcimycin. $^{***}P<0.01$ *vs* calcimycin group.

Group	n	PAF (dpm)	Inhibition(%)
Control	3	1 831 ± 441	
Calcimycin	7	7 022 ± 736	
Calcimycin + Dau (μmol / L)			
1	7	3 927 ± 431***	44.1
10	7	2 955 ± 786***	57.9
100	7	2 596 ± 851***	63.0
1000	7	2 135 ± 265***	69.6
Calcimycin + Qui (μmol / L)			
500	3	4 794 ± 290***	31.7

蝙蝠葛碱抑制卡西霉素刺激小鼠腹腔巨噬细胞释放 PAF 与时间的关系 小鼠巨噬细胞与 sodium [^{3}H] acetate 孵育不同的时间后，培养液经提取、TLC 分离，测定其中相应的

PAF 的放射性强度，结果见 Fig 3. 在所选时间范围内，Dau 在 10 μmol／L 及 100 μmol／L 浓度时均能显著抑制 Cal 刺激的小鼠腹腔巨噬细胞释放 PAF，且随着时间的延长，抑制率也逐渐增加.

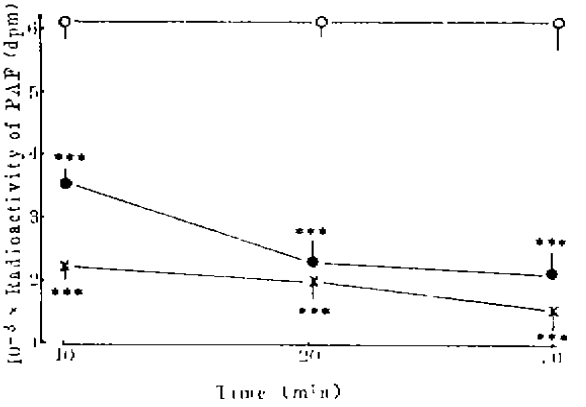

Fig 3. Effects of daurieine on calcimycin-induced PAF release by mouse peritoneal macrophages incubated with sodium [^{3}H] acetate. (O) control, (●) 10 μmol／L, (×) 100 μmol／L. $n=4$, $\bar{x} \pm SD$. $^{***}P<0.01$ vs control.

DISCUSSION

用标记的 lyso-PAF 及 sodium [^{3}H] acetate 参入胞膜合成 PAF 是文献中常用的方法[7]. 但用 sodium[^{3}H] acetate 参入巨噬细胞合成 PAF 尚未见文献报道. 在一般的生物方法测定巨噬细胞产生 PAF 的文献中，PAF 的最高产率在细胞孵育 45-60 min 之间[11]. 本文用 sodium [^{3}H] acetate 参入小鼠腹腔巨噬细胞观察 PAF 的合成，发现细胞与 Cal 及 sodium [^{3}H] acetate 孵育 30 min 时 PAF 产率最高.

本实验观察到 Dau 能显著地抑制 Cal 刺激小鼠腹腔巨噬细胞释放 PAF，且随剂量的增加及时间的延长其抑制作用也增强. 有报道 Dau 具有抗炎镇痛作用[12]，可能与其具有抑制 PAF 生成作用有关. 由于体内 PAF 的合成与 PLA_2 密切相关，抑制 PLA_2 活性可能导致 PAF 合成减少. 本实验确实观察到了典型的 PLA_2 抑制剂 Qui 能抑制小鼠腹腔巨噬细胞产生 PAF，但由于 PAF 在体内的合成过程较复杂，不仅需要 PLA_2，还有许多中间过程，特别是还需要乙酰基转移酶这一限速步骤[13]，因此 Dau 作用于哪一环节抑制 PAF 的生物合成，尚待研究.

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Effects of galanin on electrical activity of pancreatic islet cells

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ABSTRACT Intracellular microelectrode technique was used to investigate the effect of galanin on the electrical activity of β cells of Langerhans islets of mice. Galanin 0.15 and 0.3 μmol / L in the perfusion medium inhibited the basic electrical activity induced by glucose 5.5, 11.1 and 20 mmol / L in decreasing the frequency and amplitude of the spikes. In some cells, during galanin inhibition there was a hyperpolarization after the spike. Verapamil (30, 60 and 90 μmol / L), a blocker of voltage- dependent Ca channels, blocked dose-dependently the electrical activity induced by glucose and attenuated the depolarization induced by KCl 50 mmol / L. Galanin 0.3 μmol / L also attenuated the depolarization induced by KCl 50 mmol / L, similar to the effect of verapamil. The results suggest that the effect of galanin on inhibition of the electrical activity of β cell might be due to blocking of voltage-dependent Ca^{2+} channels.

KEY WORDS microelectrodes; galanin; verapamil; islands of Langerhans; peptides

Our previous study reported that galanin inhibited insulin secretion from cultured rat islets[1]. Peptide hormones or neurotransmitters are capable of modulating the activity of a variety of ionic channels. For example, β-adrenergic agonists activate Ca^{2+} channels in myocardial cells [2], somatostatin inhibits Ca^{2+} current in pituitary cell lines[3], and GABA induces inhibition of voltage-dependent Ca^{2+} channels in chicken dorsal root ganglion cells [4]. Insulin secretion is clearly related to the electrical activity generated by the insulin-secreting cell and voltage-dependent Ca^{2+} channels which play a key role in this electrical activity.

This study was to determine the effect of galanin on the electrical activity of mouse β cells, and to study which type of channel regulated by this peptide known to alter insulin secretion.

MATERIALS AND METHODS

Preparation Mice, ♀, ♂, weighing 22.1 ± SD 2.8 g were anesthetized with ip urethane 1 g / kg,. A piece of pancreas was excised and fixed to a 1 cm^2 black rubber in the perfusion chamber. The pancreatic membrane and some acinar tissues covering the islets were then removed, so as to let the islets exposed. The preparation , usually took about 15 min and was perfused with glucose-containing Krebs solution at 37 ℃. The volume of the chamber was 1.8 ml, and the perfusion flow rate was 4-5 ml / min.

Solutions Krebs solution was conti-

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