BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 May; 17 (3): 230 - 232

Effects of tetrandrine, Fura 2-AM, and Bay k 8644 on platelet-activating factor release from rat peritoneal macrophages stimulated by lipopolysaccharides

SONG Shu-Qiang, HU You-Mei
(*Department of Pharmacology, Third Military Medical University, Chongqing 630038, China*)

KEY WORDS calcium channel blockers; tetrandrine; Fura 2-AM; Bay k 8644; lipopolysaccharides; macrophages; platelet-activating factor

AIM: To study the relationship between the calcium and the release of platelet-activating factor (PAF) from rat peritoneal macrophages (PMØ) stimulated by lipopolysaccharides (LPS). **METHODS:** The effects of tetrandrine (Tet), Fura 2-AM, and Bay k 8644 on the PAF release from rat PMØ was investigated by the bio-assay of PAF. **RESULTS:** LPS stimulated PMØ to release PAF, without increasing intracellular Ca^{2+} of PMØ, Tet at 0.1, 1.0, 10, 100 μmol·L^{-1} and Fura 2-AM at 0.01, 0.1, 1.0, 10 μmol·L^{-1} could dose-dependently decrease the release of PAF (9.8 ± 1.2, 6.5 ± 1.6, 4.7 ± 0.8, 3.4 ± 0.4 μg·L^{-1} and 9.2 ± 1.7, 5.2 ± 1.3, 3.7 ± 0.4, 3.2 ± 0.3 μg·L^{-1}, respectively, no drugs 11.8 ± 1.2 μg·L^{-1}), Bay k 8644 at 1.0, 5.0, 10 μmol·L^{-1} could dose-dependently increase the release of PAF (13.2 ± 1.7, 16.2 ± 1.4, 17.6 ± 1.5 μg·L^{-1}), and the effects of Tet and Fura 2-AM were completely or partly reversed by Bay k 8644 at 5.0 μmol·L^{-1}. **CONCLUSION:** Although LPS did not increase intracellular Ca^{2+} of PMØ, intracellular Ca^{2+} was necessary for PAF release from rat PMØ stimulated by LPS.

Platelet-activating factor (PAF) is a potent bioactive analog of phosphatidyl choline which plays an important role in respiratory and circulatory failure caused by endotoxemia. The control of PAF released by acetyltransferase sensitive to a calcium-dependent protein kinase and stimulated phosphoinositide metabolism were shown in neutrophils and macrophages[1]. Bacterial lipopolysaccharides (LPS) increased breakdown of phosphatidylinositol 4, 5-bisphosphate, but did not rapidly increase intracellular levels of calcium[2,3]. It is not clear whether the release of PAF from LPS-stimulated peritoneal macrophages (PMØ) has relationship with intracellular Ca^{2+}. Tetrandrine (Tet) is the active principle of *Stephania tetrandra* S Moore which has immunosuppressive[4] and calcium antagonist properties[5], Fura-2 is a Ca^{2+} fluorescence indicator which can chelate intracellular Ca^{2+}[6], and Bay k 8644 is a calcium channel agonist[7]. We investigated the effects of Tet, Fura 2-AM, and Bay k 8644 on the release of PAF to study the relationship between the calcium and the release of PAF from PMØ stimulated by LPS.

Received 1994-01-12 Accepted 1995-05-11

MATERIALS AND METHODS

Drugs LPS (*E Coli* 0111: B_4, Sigma). PAF (Sigma). Minimum Essential Medium (MEM, Gibco). Tetrandrine (Tet, Jinhua Pharmaceutical Factory, Zhejiang). Bay k 8644 (Sigma). Fura 2-AM (Institute of Materia Medica, Chinese Academy of Medical Sciences, 9105). Tris-Tyrode's bovine serum albumin (BSA): pH 7.4, KCl 2.62, $MgCl_2 \cdot 6H_2O$ 1.0, NaCl 137, $CaCl_2 \cdot 2H_2O$ 1.30, Tris 10, glucose 5.6 mmol·L^{-1}, BSA 0.25 %.

Rat PMØ isolation Wistar rats (160 ± *s* 15 g) were decapitated. Twenty mL of MEM medium with HEPES 25 mmol·L^{-1} containing gentamycin sulfate 1 mg·L^{-1} (4 ℃) was injected into the peritoneal cavity, the abdomen was massaged several times and the fluid was aspirated. The cells were collected by centrifugation (500 × *g*, 4 ℃, 5 min), washed twice with the MEM, and then the cells were suspended in a special medium. The cells viability was > 95 % as determined by trypan blue exclusion, the PMØ in the cells were > 90 % as determined by morphology and Wright's staining.

$[Ca^{2+}]_i$ in PMØ A modified Fura-2 method was used[8].

Cultivation of PMØ and extraction of PAF The PMØ were suspended in the MEM with 0.25 % BSA at the concentration of 2×10^9 cells·L^{-1}. The cell suspension was incubated at 37 ℃ in an incubator containing 5 % CO_2 atmosphere for 2 h. Nonadherent cells were removed. The drugs were added and the PMØ were incubated for another 15 min. The PMØ were stimulated with LPS (5 mg·L^{-1}) for

60 min. The supernatant was collected and PAF was extracted[9] as follows: adding 1 mL acetone to each quantum of supernatent at −20 ℃, mixing for 30 s and incubating at 4 ℃ for 10 min before centrifuging at 1560 · g for 5 min; to the second supernatent was added an equal volume of chloroform at −20 ℃, mixed for 30 s and centrifuged at 1560 × g for 5 min. The organic layer was collected and evaporated to dryness by vacuum. The residues were kept at −20 ℃ before use.

Washed rabbit platelet Washed rabbit platelet was collected from blood of New Zealand white rabbits ($2.8 \pm s$ 0.3)[10]. The platelets were suspended in Tris-Tyrode's BSA at a concentration of $3 \times 10^{11} \cdot L^{-1}$.

Bio-assay of PAF PAF was assayed in the aggregation of washed rabbit platelet with an agglutometer[10].

RESULTS

Effect of LPS on $[Ca^{2+}]_i$ of PMØ Basal levels of $[Ca^{2+}]_i$ in nonstimulated PMØ were 178 ± 32 nmol · L^{-1}. After stimulation by LPS (5 mg · L^{-1}) for 0.5, 1, 2 min, $[Ca^{2+}]_i$ of PMØ were 190 ± 27, 196 ± 19, 192 ± 35 nmol · L^{-1}, respectively (n = 6). There were no statistical difference between nonstimulated and stimulated PMØ.

PAF released from PMØ stimulated by LPS PAF was released from PMØ under the stimulation of LPS and it was not synthesized without LPS stimulation. The released PAF reached the peak at LPS 5 mg · L^{-1} after 60 min incubation (Tab 1).

Tab 1. The release of PAF from PMØ stimulated by LPS in various concentration and time (n = 3).

Time/min (LPS 5 mg · L^{-1})	PAF/ μg · L^{-1}	LPS/ mg · L^{-1} (Time 60 min)	PAF/ μg · L^{-1}
0	0	0	0
20	0.21 ± 0.03	0.5	0.60 ± 0.06
30	1.2 ± 0.3	1.0	3.43 ± 0.24
40	3.25 ± 0.27	5.0	11.6 ± 1.3
50	8.0 ± 1.2	10.0	12.4 ± 1.7
60	11.4 ± 1.6		
70	8.4 ± 1.0		

Effect of drugs on the release of PAF Tet and Fura 2-AM decreased the release of PAF in a dose-dependent manner, Bay k 8644 increased that. Bay k 8644 by itself, however, did not cause the release of PAF from PMØ. Under the same condition, the positive control drug mepacrine (Mep) also inhibited the release of PAF. In the presence of Bay k 8644 5.0 μmol · L^{-1}, the effects of Tet and Fura 2-AM were completely or partly reversed (Tab 2).

Tab 2. Effect of Tet, Fura 2-AM, and Bay k 8644 on the release of PAF from PMØ in the presence of LPS 5 mg · L^{-1}. n = 6, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* LPS group; $^fP < 0.01$ *vs* LPS + Bay k 8644 group.

Group	PAF/μg · L^{-1}
None	0
LPS	11.8 ± 1.2
LPS + Tet 0.1 μmol · L^{-1}	9.8 ± 1.2[b]
LPS + Tet 1.0 μmol · L^{-1}	6.5 ± 1.6[c]
LPS + Tet 10 μmol · L^{-1}	4.7 ± 0.8[c]
LPS + Tet 100 μmol · L^{-1}	3.4 ± 0.4[c]
LPS + Fura 2-AM 0.01 μmol · L^{-1}	9.2 ± 1.7[c]
LPS + Fura 2-AM 0.1 μmol · L^{-1}	5.1 ± 1.3[c]
LPS + Fura 2-AM 1.0 μmol · L^{-1}	3.7 ± 0.4[c]
LPS + Fura 2-AM 10 μmol · L^{-1}	3.2 ± 0.3[c]
LPS + Bay k 8644 0.5 μmol · L^{-1}	12.7 ± 1.3[a]
LPS + Bay k 8644 1.0 μmol · L^{-1}	13.2 ± 1.7[c]
LPS + Bay k 8644 5.0 μmol · L^{-1}	16.2 ± 1.4[c]
LPS + Bay k 8644 10.0 μmol · L^{-1}	17.6 ± 1.5[c]
Bay k 8644 5.0 μmol · L^{-1}	0
LPS + Mepacrine 100 μmol · L^{-1}	4.4 ± 0.8[c]
LPS + Bay k 8644 5.0 μmol · L^{-1}	16.5 ± 1.1
LPS + Bay k 8644 + Tet 0.1 μmol · L^{-1}	12.2 ± 0.9[af]
LPS + Bay k 8644 + Tet 1.0 μmol · L^{-1}	10.2 ± 0.9[af]
LPS + Bay k 8644 + Tet 10 μmol · L^{-1}	9.2 ± 0.6[bf]
LPS + Bay k 8644 + Tet 100 μmol · L^{-1}	5.2 ± 0.5[cf]
LPS + Bay k 8644 + Fura 2-AM 0.01 μmol · L^{-1}	10.7 ± 1.1[af]
LPS + Bay k 8644 + Fura 2-AM 0.1 μmol · L^{-1}	6.5 ± 1.7[cf]
LPS + Bay k 8644 + Fura 2-AM 1.0 μmol · L^{-1}	4.7 ± 0.4[cf]
LPS + Bay k 8644 + Fura 2-AM 10 μmol · L^{-1}	4.2 ± 0.5[cf]

DISCUSSION

In the extracting procession of PAF, it was possible that Tet was co-extracted with PAF, which inhibited the platelet aggregation at a concentration of 12.5 − 100 mg · L^{-1}[11], but in our experiment, the maximal concentration of Tet was 100 μmol · L^{-1} and its final concentration in platelets suspension was 6.6 mg · L^{-1}, even if Tet was compeletly extracted, Tet has little effect on the platelet

aggregation at this concentration, the assay of PAF was reasonable.

PAF can be produced and released by various cells. The results indicated that LPS stimulated the rat PMØ to release PAF, but did not rapidly increase $[Ca^{2+}]_i$ of PMØ. The intracellular calcium chelator Fura 2-AM and the calcium antagonist Tet dose-dependently decreased the release of PAF, the calcium channel agonist Bay k 8644 increased the release of PAF and completely or partly reversed the effect of Tet and Fura 2-AM, although Bay k 8644 by itself did not cause the release of PAF. It suggested that the release of PAF was affected by the increase or decrease of intracellular $[Ca^{2+}]_i$, and the intracellular $[Ca^{2+}]_i$ was necessary for the release of PAF from rat PMØ stimulated by LPS, although LPS did not increase intracellular $[Ca^{2+}]_i$ of PMØ.

REFERENCES

1 Hanahan DJ. Platelet-activating factor: a biologically active phosphoglyceride. Annu Rev Biochem 1986; 483-509.

2 Prpic V, Weiel JE, Somers SD, DiGuiseppi J, Gonias SL, Pizzo SV, *et al*. Effects of bacterial lipopolysaccharide on the hydrolysis of phosphatidylinositol 4, 5-bisphosphate in murine peritoneal macrophages. J Immunol 1987; 139: 526-33.

3 Drysdale BE, Yapundich RA, Shin ML, Shin HS. Lipopolysaccharide-mediated macrophage activation: the role of calcium in the generation of tumoricidal activity. J Immunol 1987; 138: 951-6.

4 Seow WK, Ferrante A, Goh DB, Chalmers AH, Li SY, Thong YH. *In vitro* immunosuppressive properties of the plant alkaloid tetrandrine. Int Arch Allergy Appl Immunol 1988; 85: 410-5.

5 Fang DC, Jiang MX. A new calcium antagonist of Chinese medicinal origin: tetrandrine. J Hypertens 1986; 4 Suppl: S150-2.

6 Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985; 260: 3440-50.

7 Presuss KC, Gross GJ, Brooks HL, Warltier DC Slow channel calcium activators, a new group of pharmacological agents Life Sci 1985; 37: 1271-8.

8 Guan YY, Duan DY, Wu JX, Sun JJ. Effects of cardiotoxine from Southern Chinese Cobra Venom on the cytoplasmic calcium ion concentration of lacrimal acinar cells isolated from rat studied with Fura-2 fluorescence technique. Chin Pharm Bull 1990; 6: 296-8.

9 Stenton SC, Court EN, Kingston WP, Goadby P, Kelly CA, Duddridge M, *et al*. Platelet-activating factor in bronchoalveolar lavage fluid from asthmatic subjects. Eur Respir J 1990; 3: 408-13.

10 Wykle RL, O'Flaherty JT, Thomas MJ. Platelet-activting factor. Methods Enzymol. 1988; 163: 45-54.

11 Qian YM, Huang YH. Effects of tetrandrine on rabbit platelet aggregation, thromboxane A_2 generation and calmodulin activity. Acta Pharmacol Sin 1989; 10: 61-5.

230-232

粉防己碱，Fura 2-AM 和 Bay k 8644 对脂多糖刺激大鼠腹腔巨噬细胞释放血小板活化因子的影响

宋述强，胡友梅

（第三军医大学药理教研室，重庆 630038，中国）

关键词 钙通道阻滞剂；粉防己碱；Fura 2-AM；Bay k 8644；脂多糖；腹腔巨噬细胞；血小板活化因子

R P65.1 R972

目的：研究钙与脂多糖（LPS）刺激大鼠腹腔巨噬细胞（PMØ）释放血小板活化因子（PAF）的关系. **方法**：通过 PAF 的生物测定法，观察了粉防己碱（Tet）、Fura 2-AM 和 Bay k 8644 对 LPS 刺激 PMØ 释放 PAF 的影响. **结果**：LPS 刺激 PMØ 释放 PAF，但并不使其细胞内钙增高，Tet 在 0.1，1.0，10，100 μmol·L^{-1}和 Fura 2-AM 在 0.01，0.1，1.0，10 μmol·L^{-1}时降低 LPS 刺激的 PMØ 释放 PAF（分别为 9.8±1.1，6.5±1.6，4.7±0.8，3.4±0.4，9.2±1.7，5.2±1.3，3.7±0.4，3.2±0.3 μg·L^{-1}，无药物时 11.8±1.2 μg·L^{-1}），Bay k 8644 在 1.0，5.0，10 μmol·L^{-1}时能增加 LPS 刺激的 PAF 释放能增加 LPS 刺激的 PAF 释放（分别为 13.2±1.7，16.2±1.4，17.6±1.5 μg·L^{-1}），并且 Bay k 8644 在 5.0 μmol·L^{-1}时能全部或部分逆转 Tet 和 Fura 2-AM 对 PAF 释放的抑制作用 **结论**：尽管 LPS 并不明显增加巨噬细胞内钙，但细胞内钙对 LPS 刺激的 PAF 释放是必要的