

Inhibitory role of GDP on saikosaponin (I) stimulated enzymes secretion and rising of $[Ca^{2+}]_i$ in rat pancreatic acini¹

YANG Wen-Xiu², YU Yi, ZHANG Wen-Zheng, Wang Hui, LI Xiao-Dong
(Department of Biophysics, Nankai University, Tianjin 300071, China);

ZHAO Yu-Ying, LIANG Hong (School of pharmaceutical science, Beijing University, Beijing 100083, China)

R96 A

KEY WORDS saikosaponin (I); guanosine diphosphate; pancreas; cell membrane permeability; secretion; calcium

ABSTRACT

AIM: To study the role of guanosine diphosphate (GDP) on enzyme secretion and rising of $[Ca^{2+}]_i$ in saikosaponin (I) SA(I) stimulated rat pancreatic acini. **METHODS:** Cell membrane of isolated rat pancreatic acini were permeabilized using streptolysin O (SLO). Enzymes secretions were indicated by detecting total protein secretions. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) was measured using Fluo-3 in SPEX spectrofluorimeter. **RESULTS:** The inhibition of GDP on SA(I) stimulated enzymes secretion increased with increasing GDP concentration. There were two peaks in the time course of increase in $[Ca^{2+}]_i$ evoked by SA(I) 10 μ mol/L. After adding GDP 5 mmol/L, $[Ca^{2+}]_i$ rose gradually without the two peaks. In permeabilized acini, the accumulation of enzymes stimulated by SA(I) in 30 min reduced by 57 % compared with intact acini. GDP 5 mmol/L decreased the initial rate of secretion. **CONCLUSION:** Inhibition of GDP on increase in $[Ca^{2+}]_i$ reduces SA(I) stimulated enzymes secretion in pancreatic acini.

INTRODUCTION

It is well established that Ca^{2+} plays a crucial role as an intracellular messenger for the cholecystokinin (CCK) and carbachol stimulated enzymes secretion in pancreatic acini^[1]. CCK-8 activates it receptor coupling G pro-

tein, rises IP_3 , and evokes Ca^{2+} release from endoplasmic reticulum cisterna. Guanosine triphosphate (GTP) enhances the increase in IP_3 and $[Ca^{2+}]_i$ stimulated by CCK-8^[2]. *Bupleurum* is a representative traditional Chinese drug used for treating acute pancreatitis^[3]. Our investigations have indicated that *Bupleurum* and saikoside have significant promoting effects on enzymes secretion in rat pancreatic acini^[3,4], and saikosaponin (I) SA(I) is one of the major active components of *Bupleurum* acting on pancreatic acini^[5]. The characteristics and dynamics of enzymes secretion stimulated by SA(I) have been recently examined^[6]. In this paper, we have employed intact and permeabilized rat pancreatic acini to study the influence of guanosine diphosphate (GDP) on enzymes secretion and change in $[Ca^{2+}]_i$ evoked by SA(I), in order to investigate the mechanism of SA(I) stimulated enzymes secretion.

MATERIALS AND METHODS

Materials Guanosine-5'-diphosphate trisodium salt (GDP), Fluo 3-AM, collagenase IA, ethylene glycol-bis (β -amino-ethyl ether)-N, N'-tetraacetic acid (egtazic acid), and Triton X-100 (all from Sigma Co, USA); streptolysin O (GIBCO Co, USA); trypsin inhibitor (DongFeng Biotechnology Co Ltd, Shanghai); other chemicals were from Tianxiangren Bio Co Ltd (Beijing). Specpure saikosaponin (I) was separated and identified by the School of Pharmaceutical Science, Beijing University.

Preparation of isolated acini Male Wistar rats (200 - 300 g) were from Experiment Animal Center of the Fourth Institute, Academy of Military Medical Sciences (Grade II, No 005). Pancreatic acini were prepared by the method of Kitagawa *et al*^[7].

Permeabilization of pancreatic acini Acini were permeabilized in a permeabilizing PR solution containing final concentrations of SLO 1000 IU/L, egtazic

¹ Project supported by the National Natural Science Foundation of China, No 39770910.

² Correspondence to Prof YANG Wen-Xiu. Phn 86-22-2350-1491. E-mail Yangwenx@public.tpt.tj.cn

Received 2000-07-26 Accepted 2001-03-20

acid 5 mmol/L, MgATP 1 mmol/L, free Mg²⁺ 1 mmol/L, and various concentration of Ca²⁺, which were established by the method of Kitagawa *et al*^[7], and incubated at 37 °C for 10 min.

Measurement of enzymes secretion from intact and SLO-permeabilized acini Enzymes release from pancreatic acini was determined by measuring protein secretion. Aliquots (1 mL) of GDP or culture medium-treated acini were incubated with SA (I) at 37 °C for 30 min followed by centrifugation (5000 × g) for 15 s. Quantities of protein secreted in the supernatant were determined using G-250. The enzymes secretions were calculated using a computer program.

Measurement of [Ca²⁺]_i Isolated acini were incubated with Fluo-3 5 μmol/L at 37 °C for 40 min and then washed and resuspended in fresh physiological salt solution containing the agonist. Fluorescence was monitored with SPEX spectrofluorimeter. Then [Ca²⁺]_i was assayed by the method of Nathanson *et al*^[8].

Statistical analysis Data were expressed as $\bar{x} \pm s$, and analyzed by *t*-test among experimental groups.

RESULTS

Effects of GDP on enzymes secretion stimulated by SA(I) in intact pancreatic acini In Tab 1, inhibition of GDP on SA(I) stimulated enzymes secretion increased with increasing GDP concentration. Enzymes secretion stimulated by SA(I) 10 μmol/L was 6.4 folds of basal value, and GDP 20 mmol/L decreased its effect by 62 %.

Effects of GDP on SA(I)-evoked rising of

Tab 1. Effects of GDP on SA(I) stimulated enzymes secretion in rat pancreatic acini. The values are the accumulation of 30 min, and basal value was obtained from unstimulated acini. n = 5. $\bar{x} \pm s$. *P < 0.01 vs control group. †P < 0.01 vs SA(I) 10 μmol/L group.

SA(I) + GDP /μmol·L ⁻¹ /mmol·L ⁻¹	Enzyme secretion/mg·L ⁻¹	Times basal/ %
No SA(I) and GDP	12.0 ± 1.5	100
No SA(I) 20.0	11.5 ± 1.7	96 ± 14
10 0	76.8 ± 6.1 ^c	640 ± 51 ^c
10 2.5	65.3 ± 5.1	544 ± 43
10 5.0	33.5 ± 2.4 ^f	279 ± 20 ^f
10 10.0	29.4 ± 1.2 ^f	245 ± 10 ^f
10 20.0	29.0 ± 1.8 ^f	242 ± 15 ^f

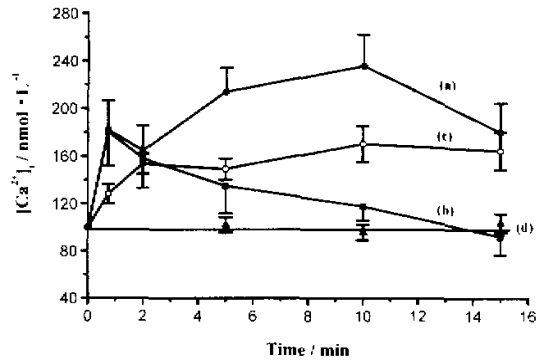


Fig 1. Effects of GDP on SA(I)-evoked increase in [Ca²⁺]_i in rat pancreatic acini. (a) SA(I) 10 μmol/L; (b) SA(I) 10 μmol/L & Ca²⁺-free medium; (c) SA(I) 10 μmol/L + GDP 5 mmol/L; (d) basal.

[Ca²⁺]_i in intact rat pancreatic acini In Fig 1a, SA(I) evoked [Ca²⁺]_i first rapidly rose to a peak and dropped, then [Ca²⁺]_i rose again and reached a second peak at 10 min. Finally, [Ca²⁺]_i declined gradually and maintained itself above the basal value. In Ca²⁺-free medium, at the second step after the first peak, [Ca²⁺]_i declined gradually to the basal value (Fig 1b). After addition of GDP 5 mmol/L, [Ca²⁺]_i rose gradually. The two peaks, responding for SA(I) 10 μmol/L, disappeared (Fig 1c). At 15 min, the [Ca²⁺]_i approached the value of [Ca²⁺]_i as shown in Fig 1a.

Effects of GDP on the kinetics of enzyme secretion in SLO-permeabilized pancreatic acini In SA(I) stimulated intact acinar cells, the accumulation of enzymes release increased speedily from 5 min to 15 min. After 20 min the action of SA(I) disappeared. In permeabilized acinar cells, the accumulation of enzymes secretion increased speedily from 0 – 5 min, then no further increase was observed. The accumulation of enzymes at 30 min reduced by 57 % as compared with intact cells. GDP 5 mmol/L decreased the initial enzymes secretion and increased the longevity of secretory response (Fig 2A).

Rate course of SA(I) stimulated enzymes release was obtained by a differential analysis of accumulation with the help of a computer program (Fig 2B). In permeabilized acini cells, maximal secretion rate was observed soon after adding the SA(I), then the rate declined speedily. Inhibited by GDP 5 mmol/L, the peak of rate dropped and shifted from 30 s to 5 min, but declined gradually.

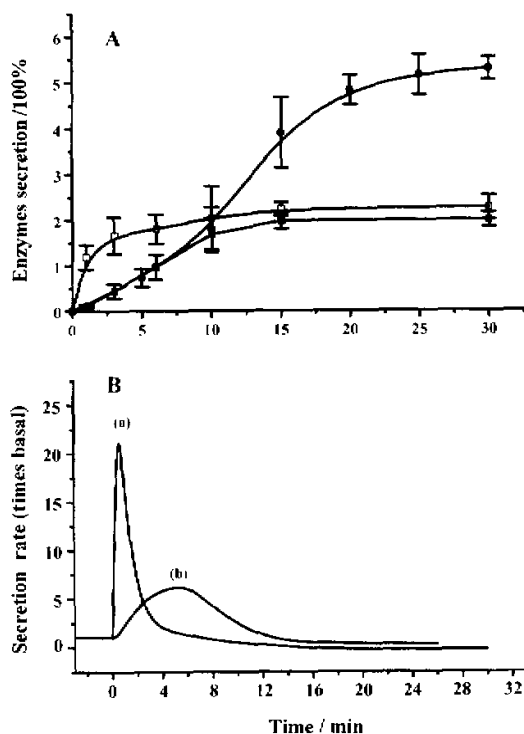


Fig 2. A) Time course of SA(I) stimulated enzyme secretion in intact and SLO-permeabilized pancreatic acini, ●: SA(I) 10 μmol/L in intact acini; □: SA(I) 10 μmol/L in SLO-permeabilized acini; ■: SA(I) 10 μmol/L and GDP 5 mmol/L in SLO-permeabilized acini. The value of basal secretion in 30 min was taken as 100%. $n = 5$. $\bar{x} \pm s$. B) Effects of GDP on rate course of SA(I) stimulated enzyme secretion in SLO-permeabilized pancreatic acini. (a): SA(I) 10 μmol/L; (b): SA(I) 10 μmol/L and GDP 5 mmol/L.

DISCUSSION

In this present study, the results indicate that the kinetics of enzymes secretion stimulated by SA(I) is closely related to a rise in $[Ca^{2+}]_i$ in intact acinar cells. After adding SA(I), the $[Ca^{2+}]_i$ rose in two peaks resulting in significant increase in enzymes secretion. In Ca^{2+} -free buffer, the second peak of $[Ca^{2+}]_i$ was lost. This indicates that the second peak of $[Ca^{2+}]_i$ depends on the influx of extracellular Ca^{2+} and suggest that the first peak of $[Ca^{2+}]_i$ is generated by Ca^{2+} release from intracellular Ca^{2+} pool. When the acinar cells were permeabilized, the enzymes secretion stimulated by SA(I) significantly decreased at 30 min, but significantly in-

creased from 0–5 min. It is known that SLO-induced plasma membrane permeabilization makes cytosolic contents, such as GTP and small G proteins, to diffuse outside from the cell, and the activity of G protein reduces remarkably^[9,10]. Enzymes secretion is mainly induced by the diffusion inside from extracellular free Ca^{2+} ^[11]. GDP displaces GTP from the GTP-binding proteins thereby deactivating them, and inhibits enzymes secretion^[12]. In our experiments, when adding GDP, the peaks of $[Ca^{2+}]_i$ stimulated by SA(I) were lost in intact acinar cells and enzymes secretions were inhibited in intact and permeabilized acinar cells. These results indicate that the inhibition of GDP on the increase in $[Ca^{2+}]_i$ results in the decrease of SA(I) stimulated enzymes secretion.

REFERENCE

- 1 Yoshiyuki A, Toshinart K, Tsukoshi Y, Yoshimitsu O, Hajime N. Effects of calcium-channel blockers on cytosolic free calcium and amylase secretion in rat pancreatic acini. *Pharmacol Toxicol* 1991; 68: 83–7.
- 2 John A, Williams JA, George T, Blevins JR. Cholecystokinin and regulation of pancreatic acinar cell function. *Physiol Rev* 1993; 73: 709–23.
- 3 Chen XQ, Yang WX, Xu WS. Stimulative action of *Bupleurum chinensis* on amylase secretion from pancreatic acini and its dependence on Ca^{2+} . *Chin J Integrated Tradit West Med* 1997; 17: 211–2.
- 4 Yang WX, Yu Y, Yao HQ, Zhao YY, Liang H. Kinetics of enzymes secretion and $[Ca^{2+}]_i$ in pancreatic acini stimulated by Saikosides. *Chin Sci Abstr [papers]* 1999; 5: 1185–6.
- 5 Yang WX, Yu Y, Yao HQ, Wang H, Zhao YY, Liang H. Comparative research of enzymes secretion in pancreatic acini stimulated by saikosaponins. *Chin Sci Abstr [papers]* 1999; 5: 1529–30.
- 6 Yang WX, Yu Y, Zhao YY, Liang H, Wang H, Zhou J. The characteristics of saikosaponin-stimulated pancreatic acini protein secretion and effects of $[Ca^{2+}]_i$. *Acta Scientiarum Naturalium Universitatis Nankaiensis* 2000; 33: 41–4.
- 7 Kitagawa M, Williams JA, de Lisle RC. Amylase release from streptolysin O-permeabilized pancreatic acini. *Am J Physiol* 1990; 259: G157–64.
- 8 Nathanson MH, Padfield PJ, O'Sullivan AJ, Burgstahler AD, Jamieson JD. Mechanism of Ca^{2+} wave propagation in pancreatic acinar cells. *J Biol Chem* 1992; 267: 18118–21.
- 9 Brown AM, O'Sullivan AJ, Gomperts BD. Induction of exocytosis from permeabilized mast cells by the guanosine triphosphatases Rac and Cdc42. *Mol Biol Cell* 1998; 9: 1053–63.
- 10 Rosales JL, Ernst JD. GTP-dependent permeabilized neutrophil secretion requires a freely diffusible cytosolic protein. *J Cell Biochem* 2000; 80: 37–45.
- 11 Padfield PJ, Panesar N. Ca^{2+} -dependent amylase secretion

from SLO-permeabilized rat pancreatic acini requires diffusible cytosolic proteins. *Am J Physiol* 1995; 269: G647-52.

- 12 Padfield PJ, Ding TG, Jamieson JD. Ca^{2+} -dependent amylase secretion from pancreatic acinar cells occurs without activation of phospholipase C linked G-proteins. *Biochem Biophys Res Commun* 1991; 174: 536-41.

GDP 对柴胡皂甙(I)促大鼠胰腺腺泡酶分泌和升高 $[Ca^{2+}]_i$ 的影响¹

杨文修², 余奕, 张文正, 王辉, 李晓东
(南开大学生物物理系, 天津 300071, 中国);

赵玉英, 梁鸿

(北京大学药学院, 北京 100083, 中国)

关键词 柴胡皂甙(I); 鸟苷二磷酸; 胰腺; 细胞膜渗透性; 分泌; 钙

目的: 研究鸟苷二磷酸(GDP)对柴胡皂甙(I) [SA(I)]刺激大鼠胰腺腺泡酶分泌和胞内钙离子升高的影响。**方法:** 分离大鼠胰腺腺泡细胞, 用链球菌溶血素-O(SLO)通透细胞膜, 检测腺泡分泌蛋白量标志腺泡酶分泌功能。用钙离子荧光指示剂 Fluo-3和荧光光谱测定胞内钙离子浓度。**结果:** GDP可抑制SA(I)促胰腺腺泡的酶分泌作用, 抑制作用随剂量增加而加强; SA(I) 10 $\mu\text{mol/L}$ 以双峰值为特征使 $[Ca^{2+}]_i$ 显著上升; GDP 5 mmol/L, 导致 $[Ca^{2+}]_i$ 逐步升高且两个峰值消失。细胞通透以后, 与正常细胞相比, SA(I)刺激的 30 min 酶分泌的累积量下降了 57%; GDP 5 mmol/L 使 SA(I)刺激的早期酶分泌速率进一步降低。**结论:** GDP 通过抑制细胞 $[Ca^{2+}]_i$ 的升高抑制了 SA(I) 刺激的胰腺腺泡酶分泌作用。

(责任编辑 吕静)