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# Inhibitory role of GDP on saikosaponin (Ⅰ) stimulated enzymes secretion and rising of $[Ca^{2+}]_i$ in rat pancreatic acini[1]

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**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 μmol/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 protein, rises $IP_3$, and evokes $Ca^{2+}$ release from endoplasmic reticulum cisterna. Guanosine triphophate (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 saikuside 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 chang 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

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acid 5 mmol/L, MgATP 1 mmol/L, free $Mg^{2+}$ 1 mmol/L, and various concentration of $Ca^{2+}$, which were established by the method of Kitagawa *et al*[7], and incubated at 37 ℃ 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 ℃ 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 $[Ca^{2+}]_i$** Isolated acini were incubated with Fluo-3 5 μmol/L at 37 ℃ for 40 min and then washed and resuspended in fresh physiological salt solution containing the agonist. Fluorescence was monitored with SPEX spectrofluorimeter. Then $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ in intact rat pancreatic acini** In Fig 1a, SA(Ⅰ) evoked $[Ca^{2+}]_i$ first rapidly rose to a peak and dropped, then $[Ca^{2+}]_i$ rose again and reached a second peak at 10 min. Finally, $[Ca^{2+}]_i$ declined gradually and maintained itself above the basal value. In $Ca^{2+}$-free medium, at the second step after the first peak, $[Ca^{2+}]_i$ declined gradually to the basal value (Fig 1b). After addition of GDP 5 mmol/L, $[Ca^{2+}]_i$ rose gradually. The two peaks, responding for SA(Ⅰ) 10 μmol/L, disappeared (Fig 1c). At 15 min, the $[Ca^{2+}]_i$ approached the value of $[Ca^{2+}]_i$ as shown in Fig 1a.

**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. $n = 5$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control group. $^fP < 0.01$ *vs* SA(Ⅰ) 10 μmol/L group.**

| SA(Ⅰ) /μmol·L⁻¹ | + GDP /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$^f$ |

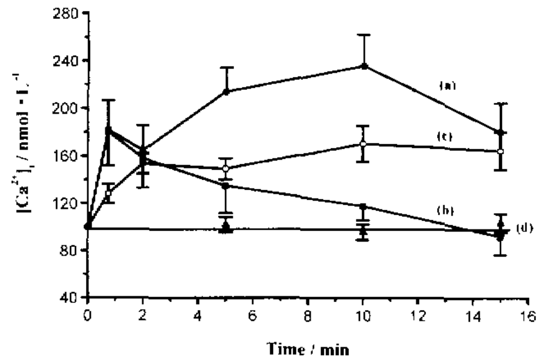


**Fig 1. Effects of GDP on SA(Ⅰ)-evoked increase in $[Ca^{2+}]_i$ in rat pancreatic acini. (a) SA(Ⅰ) 10 μmol/L; (b) SA(Ⅰ) 10 μmol/L & $Ca^{2+}$-free medium; (c) SA(Ⅰ) 10 μmol/L + GDP 5 mmol/L; (d) basal.**

**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.

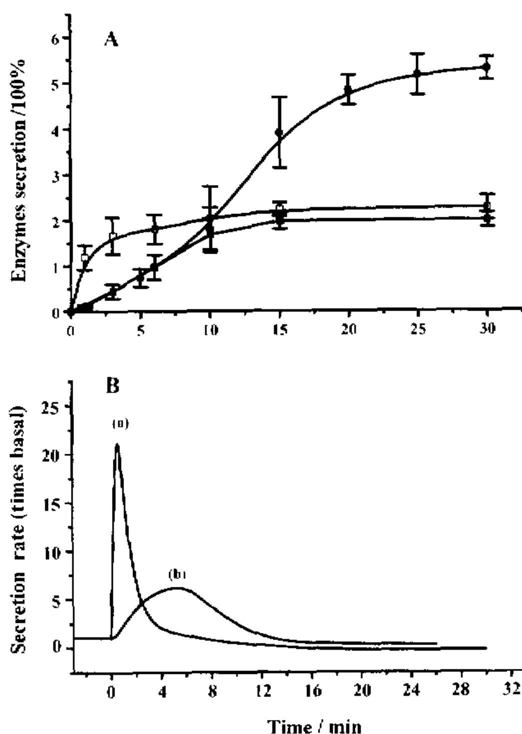


**Fig 2. A) Time course of SA( Ⅰ ) stimulated enzyme secretion in intact and SLO-permeabilized pancreatic acini, ●: SA ( Ⅰ ) 10 μmol/L in intact acini; □: SA( Ⅰ ) 10 μmol/L in SLO- permeabilized acini; ■: SA( Ⅰ ) 10 μmol/L and GDP 5 mmol/L in SLO-permeabilized acini. The value of basal secretion in 30 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 increased 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 inhibites 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 pemeabilized 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.

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## 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]

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**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 μmol/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.

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