PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2002;8(3):524-527
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

Characteristics and mechanism of enzyme secretion and increase in $[Ca^{2+}]_i$ in Saikosaponin(I) stimulated rat pancreatic acinar cells

Yi Yu, Wen-Xiu Yang, Hui Wang, Wen-Zheng Zhang, Bao-Hua Liu, Zhi-Yong Dong

Yi Yu, Wen-Xiu Yang, Hui Wang, Wen-Zheng Zhang, Bao-Hua Liu, Zhi-Yong Dong, Department of Biophysics, Nankai University, Tianjin, 300071, China
Presented at the Third East Asian Biophysics Symposium, Kyongju, Korea, May 22-26, 2000
Supported by National Natural Science Foundation of China, No. 39770910
Correspondence to: Wen-xiu Yang, Department of Biophysics, School of Physics, Nankai University, Tianjin 300071, China. yangwenx@public.tpt.tj.cn
Telephone: +86-22-23501491 Fax: +86-22-23501490
Received 2001-12-05 **Accepted** 2002-02-19

Abstract

AIM: **This investigation was to reveal the characteristics and mechanism of enzyme secretion and increase in $[Ca^{2+}]_i$ stimulated by saikosaponin(I) [SA(I)] in rat pancreatic acini.**

METHODS: **Pancreatic acini were prepared from male Wistar rats. Isolated acinar cells were suspended in Eagle's MEM solution. After adding drugs, the incubation was performed at 37℃ for a set period of time. Amylase of supernatant was assayed using starch-iodide reaction. Isolated acinar single cell was incubated with Fura-2/AM at 37℃, then cells were washed and resuspended in fresh solution and attached to the chamber. Cytoplasm $[Ca^{2+}]_i$ of a single cell was expressed by fluorescence ratio F340/F380 recorded in a Nikon PI Ca^{2+} measurement system.**

RESULTS: **Rate course of amylase secretion stimulated by SA(I) in rat pancreatic acini appeared in bell-like shape. The peak amplitude increased depended on SA(I) concentration. The maximum rate responded to 1×10^{-5}mol/L SA(I) was 13.1-fold of basal and the rate decreased to basal level at 30min. CCK-8 receptor antagonist Bt_2-cGMP markedly inhibited amylase secretion stimulated by SA(I) and the dose-effect relationship was similar to that by CCK-8. $[Ca^{2+}]_i$ in a single acinar cell rose to the peak at 5min after adding 5×10^{-6}mol/L SA(I) and was 5.1-fold of basal level. In addition, there was a secondary increase after the initial peak. GDP could inhibit both the rate of amylase secretion and rising of $[Ca^{2+}]_i$ stimulated by SA(I) in a single pancreatic acinar cell.**

CONCLUSION: **SA(I) is highly efficient in promoting the secretion of enzymes synthesized in rat pancreatic acini and raising intracellular $[Ca^{2+}]_i$. Signaling transduction pathway of SA(I) involves activating special membrane receptor and increase in cytoplasm $[Ca^{2+}]_i$ sequentially.**

Yi Y, Yang WX, Wang H, Zhang WZ, Liu BH, Dong ZY. Characteristics and mechanism of enzyme secretion and increase in $[Ca^{2+}]_i$ in Saikosaponin(I) stimulated rat pancreatic acinar cells. *World J Gastroenterol* 2002;8(3):524-527

INTRODUCTION

Bupleurum, one kind of traditional Chinese Drug, have a variety of roles in clinical practice. Our pharmacological investigations have indicated Bupleurum has significant promoting effect on enzyme secretion in rat pancreatic acini[1], Saikosides is the compound of the active Saikosaponin component in Bupleurum. The kinetics of enzyme secretion stimulated by Saikosides in a dose-dependent manner can be divided into first phase of high-potency secretion and later phase of low-potency secretion. The accumulation of enzyme secretion stimulated by 200mg/L Saikosides with 30min incubation is 6.5 folds of the basal level[2]. Based on the results of separation, purification and identification for Saikosides[3], we have compared the activities of nine kinds of Saikosaponins. Saikosaponin(I) [SA(I)] has the best effect of promoting secretion. The promoting effect of SA(I) increase in a dose-dependent manner, and the enzyme release stimulated by 5×10^{-5}mol/L SA(I) is 9.5 folds of basal[4,5].

Cholecystokinin (CCK) and Carbachol (CCh) are two important secretagogues for enzyme secretion from pancreatic acini. The promoting response associated with CCK-8 is in a dose-dependent manner, and high concentrations of CCK have submaximal amylase release. Furthermore, the relative potency of CCK to mobilize intracellular Ca^{2+} is dependent on the concentration of CCK-8. At low concentration of CCK-8 (10^{-12}mol/L-10^{-11}mol/L), it induces a series of transient increase in $[Ca^{2+}]_i$ termed "Ca^{2+} oscillations", and high concentration of CCK-8 evokes the single peak of $[Ca^{2+}]_i$[6-8]. Cellular signaling transduction pathway of increase in $[Ca^{2+}]_i$ evoked by CCK-8, involves activating G protein coupled receptors by CCK-8 binding its receptor, producing inositol 1,4,5-triphosphate(IP_3) and diacylglycerol(DAG) by activating the phospholipase C (PLC), and releasing Ca^{2+} from endoplasmic reticulum (ER) by activating the IP_3 receptor[9-14]. Increase in $[Ca^{2+}]_i$ evoked by CCk-8 and CCh not only result from ER, but from influx of extracellular Ca^{2+}[15-17]. Recently, it was shown that both activated PKC and elevated intracellular Ca^{2+} mediate activation of NF-κB and mob-1 expression by supraphysiological CCK[18,19]. Our investigations have indicated Saikosides is also a significant Ca^{2+}-mobilizing secretagogue. The kinetics of $[Ca^{2+}]_i$ induced by Saikosides varied with two peaks, and promotion of Saikosides on pancreatic exocrine could be correlative with the kinetics of $[Ca^{2+}]_i$. The effect of 200mg/L Saikosides was reduced by 35% and the second peak of $[Ca^{2+}]_i$ dramatically declined in Ca^{2+}-free medium[2]. These results showed that increase in $[Ca^{2+}]_i$ evoked by SA(I) include both Ca^{2+} release from the intracellular Ca^{2+} pool and subsequent influx of Ca^{2+} through plasmic membrane.

In order to elucidate the characteristics and mechanism of enzyme secretion and increase in $[Ca^{2+}]_i$ in SA(I) stimulated rat pancreatic acinar cells, here we report an analysis in rate kinetics of SA(I) stimulated amylase secretion, Characteristics of ligand-receptor binding and kinetics of $[Ca^{2+}]_i$ evoked by SA(I) in a single cell as well as the effects of guanosine-5'-diphosphate trisodium salt (GDP) on amylase secretion and $[Ca^{2+}]_i$.

MATERIALS AND METHODS

Materials

Cholecystokinin-8(CCK-8), Carbachol(CCh), Dibutyrlguanosine-3',

5'-cyclic monophosphate (Bt_2-cGMP), Guanosine-5'-diphosphate trisodium salt(GDP), Fura2-AM, Collagenase IA, soybean trypsin inhibitor were from Sigma Chemical Co(St. Louis, MO); Other chemicals were from Tianxiangren Bio. Co.Ltd.(Beijing, China). Saikosaponin I was separated and identified by the School of Pharmaceutical Science, Beijing University.

Methods

Preparation of isolated acini Isolated pancreatic acini were prepared by the method of Duan from male Wistar rat (200-250g) that had been fasted overnight[20]. Individual Acini were prepared by 0.3g/L collagenase digestion at 37℃ and purified by centrifugation through the Eagle's MEM containing 4% bovine serum albumin. The isolated acini were then allowed to resuspend at 37℃ for 30min in HEPES-buffered Ringer solution supplemented with 11.1mmol/L glucose, minimal Eagle's amino acids medium and 0.1g/L soybean trypsin inhibitor and were through gas with 1000mL/L O_2.

Measurement of amylase release Acini were suspended in Eagle's MEM solution. After adding the drug, the incubation was performed at 37℃ for a set period of time. Then the supernatant was removed and assayed for amylase. Amylase release was expressed at the ratio of the content of amylase released into the medium during the incubation to the total content. The total amylase content was estimated by measuring the amylase activity present in the acini broken at the beginning of the incubation.

Detection of cytosolic free Ca^{2+} concentration According to the method of Cui et al[21] briefly, isolated acini were incubated with 5×10^{-6}mol/L Fura-2/AM at 37℃ for 40min and then washed and resuspended in fresh physiological salt solution. Isolated acini were attached to Sykus-Moor perfusion chamber and continuously superfused by a buffer medium containing agonist at 1ml/min. Stimulus was introduced by changing perfusion buffer containing relevant chemicals. Inlet perfusion buffer was pre-warmed to 37℃ in a water bath. Fluorescence ratios of f340/f380 were recorded in a Nikon PI Ca^{2+} measurement system, with a pin-hole size of 0.5 focused onto the apical portion of a single acinar cell within acinar formation or formations, and a Nikon neutral density filter (#8) was placed in the excitation light path.

Statistical analysis Values of each group were expressed as $\bar{x}\pm s$. Group comparison was performed using student's *t* test. $P<0.05$ was considered significant.

RESULTS

Rate dynamics of amylase secretion in SA(I) stimulated pancreatic acini

Under the different stimulation of 1×10^{-6}mol/L, 5×10^{-6}mol/L and 1×10^{-5}mol/L SA(I) in pancreatic acini, the amylase release accumulation from acini within 37℃ incubation at different time were detected. Rate course of amylase release was obtained by differential analytics of enzyme accumulation with a computer program (Figure 1). The kinetics of rate-time effect could be divided into two phases,named by increasing phase and declining phase. With the concentration of SA(I) increasing, secreting rate rose more quickly and reached the higher peak, then it fell into basal level soon. The maximum rates of amylase secretion stimulated by 1×10^{-6}mol/L, 5×10^{-6}mol/L and 1×10^{-5} mol/L SA(I) were 3.2-fold,7.4-fold,and 13.1-fold of basal,corresponding time at 16.5min, 14.5min, 12.5min. Those data indicated that though the SA(I) stimulated enzyme release obviously, the main promoting effect of SA(I) took place in the 20min after adding the drug, and promoting action disappeared at 30min.

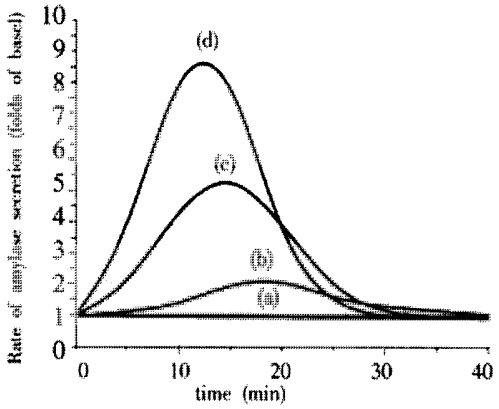

Figure 1 Rate kinetics of amylase secretion stimulated by SA(I) in the rat pancreatic acini. The results were expressed at the ratio of amylase secretion during the incubation to the basal secretion. a: basal value was standardized as 1, b: 1×10^{-6}mol/L SA(I), c: 5×10^{-6}mol/L SA(I), d: 1×10^{-5}mol/L SA (I). The values represented the $\bar{x}\pm s$ from four independent experiments.

Dose-response of inhibition of receptor antagonist on amylase secretion stimulated by SA(I)

In order to explore whether SA(I) stimulated signal was transduced through the effect of membranous receptor, the effects of CCK-8 receptor antagonist Bt_2-cGMP and CCh receptor antagonist atropine on SA(I) stimulated amylase secretion had been studied. In Table 1, enzyme secretion induced by SA(I) was markedly depressed by Bt_2-cGMP. The inhibitory effects of Bt_2-cGMP on action of SA(I) and CCk-8 had similar characteristics of dose-effect relationship. Amylase secretion stimulated by secretagogues decreased within the medium containing 1×10^{-7}mol/L Bt_2-cGMP ($^aP<0.05$). With increasing concentration of Bt_2-cGMP, the inhibition was enhanced ($^bP<0.01$). 1×10^{-4}mol/L Bt_2-cGMP decreased action of SA(I) and CCK-8 by 31.3% and 34.4% respectively.

In Table 2, atropine was observed to depress amylase secretion stimulated by CCh ($^bP<0.01$). 1×10^{-5}mol/L atropine eliminated the promoting effect of CCh,but had no apparent inhibition on the that of SA(I) at 1×10^{-8}mol/L$^{-1}\times10^{-5}$mol/L atropine range ($P>0.05$).

Table 1 Effect of different concentrations of Bt2-cGMP on amylase secretion stimulated by SA(I) and CCK-8

Bt^a-2-cGMP concentration (mol/L)	Amylase release (% of total)	
	1×10^{-9}mol/L CCk-8	5×10^{-6}mol/L SA(I)
Control	19.5±1.6	31.0±2.1
1×10^{-7}	17.8±1.9^a	27.4±2.5^a
1×10^{-6}	15.6±2.0^b	25.6±1.4^b
1×10^{-5}	14.0±1.2^b	23.1±1.8^b
1×10^{-4}	12.8±1.7^b	21.2±0.9^b

Rat pancreatic acini were incubated with either 1×10^{-9}mol/L CCk-8 or 5×10^{-6}mol/L SA(I) in adding different concentrations of Bt_2-cGMP medium. After 30min, the incubation was stopped and the content of amylase in the supernatant was assayed. The values represented the $\bar{x}\pm s$ from 4 separate experiments. $^aP<0.05$, $^bP<0.01$ *vs* control.

Table 2 Effect of different concentrations of atropine on amylase secretion stimulated by SA(I) and CCh

Atropine concentration (mol/L)	Amylase release (% of total)	
	1×10^{-5}mol/L CCh	5×10^{-6}mol/L SA(I)
Control	15.2±1.3	30.8±3.3
1×10^{-8}	11.6±1.6^b	27.9±11.4
1×10^{-7}	10.5±1.2^b	25.1±7.4
1×10^{-6}	8.8±1.3^b	27.5±6.9
1×10^{-5}	8.3±1.6^b	25.5±6.2

Rat pancreatic acini were incubated with either 1×10^{-5}mol/L CCh or 5×10^{-6} mol/L SA(I) in adding different concentration of atropine medium. After 30min, the incubation stopped and the content of amylase in the supernatant was assayed. The values represent the $\bar{x}\pm s$ from 4 separate experiments. $^aP<0.05$, $^bP<0.01$ *vs* control.

Dynamics of SA(I)and CCK-8 evoked $[Ca^{2+}]_i$ in single pancreatic acinar cell

The time courses of SA(I)and CCK-8 evoked $[Ca^{2+}]_i$ in a single pancreatic acinar cell were shown in Figure 2. 1×10^{-9}mol/L CCK-8 induced a monophasic $[Ca^{2+}]_i$ spike resulting in an increase of 4.2 folds from basal and declined to basal speedily. 5×10^{-6}mol/L SA(I) evoked $[Ca^{2+}]_i$ gradually increased within 5min after addition of SA (I), and peak of $[Ca^{2+}]_i$ was 5.0 folds of basal. Unlike CCK, $[Ca^{2+}]_i$ rose again after falling, caused a diphasic Ca^{2+} spike.

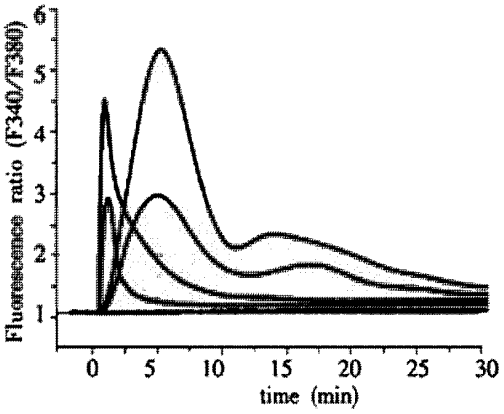

Figure 2 Kinetics of SA(I) and CCK-8 evoked $[Ca^{2+}]_i$ and effects of GDP in a single rat pancreatic acinar cell. $[Ca^{2+}]_i$ were expressed by Fluorescence ratio F340/F380. a. 1×10^{-9}mol/L CCk-8; b. 1×10^{-9}mol/L CCk-8 + 5×10^{-3}mol/L GDP; c. 5×10^{-6}mol/L SA(I); d. 5×10^{-6}mol/L SA(I)+5×10^{-3}mol/L GDP; e. Base. Time 0 is determined by the time of adding drug.

Effects of GDP on dynamics of amylase secretion rate and $[Ca^{2+}]_i$ induced by SA(I)in pancreatic acini

Figure 3 illustrated the effects of GDP on rate change of amylase release stimulated by SA(I). GDP could inhibit the rate of amylase secretion stimulated by 5×10^{-6}mol/L SA(I), whose rate-time curve of amylase secretion was similar to that of SA(I) in configuration, but addition of 5×10^{-3}mol/L GDP decreased the maximal rate by 54%. These data suggested GDP mainly inhibited high-potency phase of amylase secretion stimulated by SA(I).

In the kinetic experiments of $[Ca^{2+}]_i$ induced by SA(I) and CCK-8 in a single pancreatic acinar cell(Figure 2), addition of 5×10^{-3}mol/L GDP inhibited 1×10^{-9}mol/L CCK-8-induced $[Ca^{2+}]_i$ peak amplitude by 39%, with similar dynamic characteristics of $[Ca^{2+}]_i$. Addition of 5×10^{-3}mol/L GDP caused diphasic spike of $[Ca^{2+}]_i$ induced by 5×10^{-6} mol/L SA(I) decrease either, this resulted in a 44% decrease of initial Ca^{2+} peak, but a secondary increase in $[Ca^{2+}]_i$ still appeared.

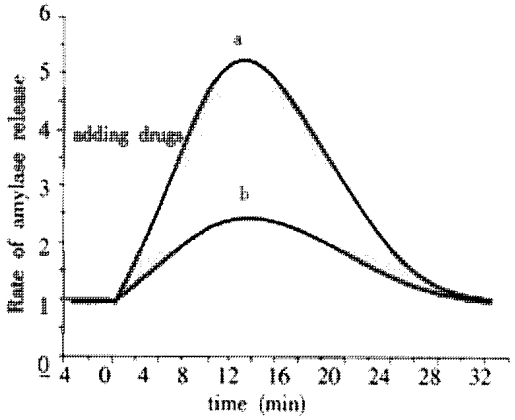

Figure 3 Inhibitory of GDP on rate course of SA(I) stimulated amylase secretion in rat pancreatic acini (base secretion rate is normalized as 1.0). a.5×10^{-6}mol·L^{-1} SA(I); b. 5×10^{-6}mol·L^{-1} SA(I)+5×10^{-3}mol·L^{-1} GDP. The data points represent from four separate experiments

DISCUSSION

A series of investigation for amylase secretion stimulated by Bupleurum and its effective components show that they have the rapid and high-potency ability of promoting pancreatic acini enzyme secretion and the validity of reversing functional disorder of pancreas exocrine. From the analysis of rate kinetics of amylase secretion stimulated by SA(I) (Figure 1), within 15min following adding SA (I), the rate of amylase secretion increased rapidly, then gradually decreased and returned to basal level at 30min. It indicates that different from CCK to enhance the synthesis of protein[22,23], the main effect of SA(I) is to hasten secretion of the exocrine protein synthesized and accumulated in granules.

To determine whether the stimulating signal of SA(I) on acinar cells is transduced by binding to the receptor on the cellular membrane, is the first task in the investigation of its mechanism of action. It is known that physiological functions of CCK-8 and CCh are mediated by specific types of receptors now termed the CCK_A and M_3 receptors. CCK_A and M_3 receptors belong to the receptors of G protein-coupled superfamily which transduce the stimulating signal by generation of intracellular second messengers, primarily IP_3 and DAG[9-11,14,24]. We compared the effects of CCK-8 and CCh receptor antagonist, Bt_2-cGMP and atropine, on amylase secretion caused by SA(I) (Table1, 2), the results showed that atropine had no detectable influence on the action of SA(I). Moreover, the dose-effect relationships of inhibition of Bt_2-cGMP on SA(I) and CCK-8 were similar. The results suggested that interaction between SA(I) and its membrane receptor initiated intracellular signaling transduction. The receptor of SA(I) has similar characteristics as that of CCK-8. To clarify the detail machinery of receptor of SA(I) will require further study.

As mentioned in our previous papers[25], the rising dynamics of mean intracellular $[Ca^{2+}]_i$ induced by SA(I) in rat pancreatic acinar cells could produce two peaks. When pancreatic acini were incubated in Ca^{2+}-free medium, in the second step, $[Ca^{2+}]_i$ did not rise and fell to the basal level gradually, and the second peak disappeared. The finding demonstrated the Ca^{2+} release from intracellular Ca^{2+} pool resulted in the first $[Ca^{2+}]_i$ peak, and second $[Ca^{2+}]_i$ peak depending on the extracellular Ca^{2+} influx in sequence. In the present experiments of single acinar cell $[Ca^{2+}]_i$ was concordant with our previous report, 1×10^{-9}mol/L CCK-8 could cause a large transient increase in $[Ca^{2+}]_i$. But it is different from CCK-8 that the change of $[Ca^{2+}]_i$ caused by SA (I) had relatively slow rate and higher peak value. In addition, there was a secondary increase after the initial peak of $[Ca^{2+}]_i$. The data suggested SA(I) had a different mechanism from that of CCK inducing Ca^{2+} release from ER and might be through more signaling tranduction pathways to initiate the intracellular calcium mobilization and subsequent extracellular calcium influx. So the intracellular increase in $[Ca^{2+}]_i$ caused by SA(I) could maintain a longer time course. These mechanisms correlated with high-potency effect of SA(I) on pancreatic exocrine. It is now known that the changes of intracellular calcium play a key role in many functions of cells. Recently, several reports showed that the spatio-temporal patterns of the intracellular calcium carried the Ca^{2+} signals to regulate gene expression and cell differentiation[26-29]. The specificity of Ca^{2+} signal is somewhat more acute in polarized secretory cells such as pancreatic acinar cells[30-32]. These researches suggest that such pattern of Ca^{2+} signal induced by SA(I) in our present studies may offer specific signal to modulate enzymes secretion in pancreatic acinar cells.

In addition, GDP could cause obvious decrease of amylase secretion and increase in $[Ca^{2+}]_i$ induced by SA(I). GDP mainly inhibited the early peak of $[Ca^{2+}]_i$ and high-potency phase of secretion stimulated by SA(I). The decrease of $[Ca^{2+}]_i$ anticipated the inhibition of amylase secretion in sequence (Figure 2,3). Several

investigators had reported that the increase in intracellular level of GDP could cause the inhibition of G-protein activity in pancreatic acini[33-35]. These G-proteins, including both ras-like small GTP-binding proteins and heterotrimeric G-proteins, had important role on the release of intracellular calcium and amylase secretion stimulated by secretagogues[14, 36-40]. More experimental data are required whether intracellular signal of stimulatory effect of SA(I) transducing to down stream through activation of G protein coupling receptor.

In summary, the results presented in this study prove that SA(I) has high-potency in stimulating the amylase secretion in rat pancreatic acini and its main effect is to promote exocytosis of enzymes synthesized by the cells. The transmembrane signal of SA(I) is transduced through interaction with its membrane receptor. Subsequently, $[Ca^{2+}]_i$ is increased by intracellular Ca^{2+} release and extracellular Ca^{2+} influx, so as to enhance the function of cellular enzyme secretion.

REFERENCES

1 Chen XQ, Yang WX, Xu WS, Yang F, Kong D, Wu XZ. Stimulative action of bupleurum chinensis on amylase secretion from pancreatic acini and its dependence on Ca^{2+}. *Zhongguo Zhongxiyi Jiehe Zazhi* 1997;17:211-212

2 Yang WX, Yu Y, Yao HQ, Zhao YY, Liang H. Kinetics of enzyme secretion and $[Ca^{2+}]_i$ in pancreatic acini stimulated by Saikasides. *Zhongguo Xueshu Qikan Wenzhai (Keji Kuaibao)* 1999;5:1185-1186

3 Liang H, Zhao YY, Qi HY, Huang J, Zhang RY. A new saikosaponin from bupleurum DC. *Yaoxue Xuebao* 1998; 33:37-41

4 Yang WX, Yu Y, Yao HQ, Wang H, Zhao YY, Liang H. Comparative research of enzyme secretion in pancreatic acini stimulated by saikosaponins. *Zhongguo Xueshu Qikan Wenzhai (Keji Kuaibao)* 1999;5: 1529-1530

5 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+}. *Nankai Daxue Xuebao* 2000;33:41-44

6 Tsunoda T, Stuenkel EL, Williams JA. Oscillatory mode of calcium signaling in rat pancreatic acinar cells. *Am J Physiol* 1990; 258: C147-C155

7 Williams JA, Blevins GT. Cholecystokinin and regulation of pancreatic acinar cell function. *Physiol Rev* 1993;73:709-723

8 Giovanna T, Zhang BX, Xu X, Muallem S. Compartmentalization of Ca^{2+} signaling and Ca^{2+} pools in pancreatic acini. *J Bio Chem* 1994;269: 29621-29628

9 Muallem S, Beeker TG. Relationship between hormonal, GTP and Ins(1,4,5)P_3-stimulated Ca^{2+} uptake and release in pancreatic acinar cells. *Biochem J* 1989;263:333-339

10 Yasuhiro T, Chung O. The regulatory site of functional GTP binding protein coupled to the high affinity cholecystokinin receptor and phospholipase A2 pathway is on the G-β subunit of Gq protein in pancreatic acini. *Biochem Biophys Res Com* 1995;211:648-655

11 Berridge MJ. Inositol trisphosphate and calcium signaling. *Nature* 1993;361:315-325

12 Takashi K, Shunsuke S, Shinji K, Toyohiko H, Chohei S, Junji K. Cholecystokinin receptor occupation and cholecystokinin-induced calcium mobilization in the early phase in rat pancreatic acini. *Biochem Biophys Acta* 1991;1094:231-237

13 Xu X, Zeng WZ, Muallem S. Regulation of inositol 1,4,5-trisphosphate-activated Ca^{2+} channel by activation of G protein. *J Bio Chem* 1996; 271:11737-11744

14 Verspohl EJ, Herrmann K, Imclvement of G proteins in the effect of Carbachol and Cholecystokinin in rat panceatic islets. *Am J Physiol* 1996;271:E65-E72

15 Hurley TW, Ronald WB. Regulating transient and sustained changes of cytosolic Ca^{2+} in rat pancreatic acini. *Am J Physiol* 1990; 258:C54-C61

16 Stephen JP, Mari SSP. Cyclic GMP mediates the agonist-stimulated increase in plasma membrane calcium entry in the pancreatic acinar cell. *J Bio Chem* 1990; 265: 12846-12853

17 Anna G, Stephen P. Nitric oxide production regulates cGMP formation and calcium influx in pancreatic acinar cells. *Am J Physiol* 1994; 266:G350-G356

18 Han B, Logsdon C. CCK stimulates mob-1 expression and NF-κB activation via protein kinase C and intracellular Ca^{2+}. *Am J Physiol Cell Physiol* 2000; 278: C344-C351

19 Gukovsky I, Gukovskaya AS, Blinman TA, Zaninovic V, Pandol SJ. Early NF-kappaB activation is associated with hormone-induced pancreatitis. *Am J Physiol* 1998; 275:G1402-G1414

20 Duan RD, Wagner ACC, Yule DI, Williams JA. Multiple inhibitory effects of genistein on stimulus-secretion coupling in rat pancreatic acini. *Am J Physiol* 1994; 266: G303-G310

21 Cui ZJ, Kanno T. Cholecystokinin analog JMV-180-induced intracellular calcium oscillations are mediated by inositol 1,4,5-trisphosphate in rat pancreatic acini. *Zhongguo Yaoli Xuebao* 2000;21: 377-380

22 Bragado MJ, Groblewski GE, Williams JA. Regulation of protein synthesis by cholecystokinin in rat pancreatic acini involves PHAS-I and the p70 S6 kinase pathway. *Gastroenterology* 1998; 115:733-742

23 Bragado MJ, Tashiro M, Williams JA. Regulation of the initiation of pancreatic digestive enzyme protein synthesis by Cholecystokinin in rat pancreas *in vivo*. *Gastroenterology* 2000; 119:1731-1739

24 Talkad VD, Potto RJ, Metz DC, Turner RJ, Fortune KP, Bhat ST, Gardner JD. Characterization of the three different states of the cholecystokinin(CCk) receptor in pancreatic acini. *Biochem Biophys Acta* 1994;1224:103-116

25 Yang WX, Yu Y, Zhang WZ, Wang H, Li XD, Zhao YY, Liang H. Inhibitory role of GDP on saikosaponin(I) stimulated enzyme secretion and rising of $[Ca^{2+}]_i$ in rat pancreatic acini. *Acta Pharmacol Sin* 2001;22: 669-672

26 Bootman MD, Lipp P, Berridge MJ. The organisation and functions of local Ca^{2+} signals. *J Cell Sci* 2001; 114: 2213-2222

27 Dotmentsch RE, Xu K, Lewis RS. Calcium oscillation increase the efficiency and specificity of gene expression. *Nature* 1998; 392: 933

28 Kummer U, Olsen LF, Dixon CJ, Green AK, Bornberg-Bauer E, Baier G. Switching from simple to complex oscillations in calcium signaling. *J Biophys* 2000; 79:1188-1195

29 West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornkauser JM, Shaywitz AJ, Takasa MA, Tao X, Greenberg ME. Calcium regulation of neuronal gene expression. *Proc Natl Acad Sci USA* 2001; 98:11024

30 Xu X, Zeng WZ, Diaz J, Muallem S. Spacial compartmentalization of Ca^{2+} signaling complexs in pancreatic acini. *J Biol Chem* 1996; 271: 24684-24690

31 James W. Putney, Pharmacology of Capacitative Calcium Entry. *Mol Interv* 2001; 1: 84-94

32 Shuttleworth TJ. Intracellular Ca^{2+} signalling in secretory cells. *J Exp Biol* 1997; 200: 303-314

33 Padfield PT, Ding TG, Jamieson TD. Ca^{2+}-dependent amylase secretion from pancreatic acinar cells occurs without activation of phospholipase C linked-G-proteins. *Biochem Biophys Res commum* 1991;174:536-541

34 Gasman S, Chasserot-Golaz S, Popoff MR, Aunis D, Bader MF. Involvement of Rho GTPases in calcium-regulated exocytosis from adrenal chromaffin cells. *J Cell Sci* 1999; 112: 4763-4771

35 Rosales J, Ernst JD. GTP-dependent permeabilized neutrophil secretion requires a freely diffusible cytosolic protein. *J Cell Biochem* 2000; 80:37-45

36 Ohnishi H, Mine T, Shibata H, Ueda N, Tsuchida T, Fujita T. Involvement of Rab4 in regulated exocytosis of rat pancreatic acini. *Gastroenterology* 1999; 116:943-952

37 Dabrowsk A, Vanderkuuar JA, Carter-Su C, Williams JA. Cholecystokinin stimulates formation of Shc-Grb2 complex in rat pancreatic acinar cells through a protein kinase C-dependent mechanism. *J Bio chem* 1996;271:27125-27129

38 Ohnishi H, Samuelson LC, Yule DI, Ernst SA, Williams JA. Overexpression of Rab3D enhances regulated amylase secretion from pancreatic acini of transgenic mice. *J Clin Invest* 1997;100: 3044-3052

39 Padfield PJ, Panesar N. The two phases of regulated exocytosis in permeabilized pancreatic acini are modulated differently by heteratrimeric G Proteins. *Biochem Biophys Res Com* 1998;245:332-336

40 Nozu F, Tsunoda Y, Ibitayo AI, Bitar KN, Owyang C. Involvement of RhoA and its interaction with protein kinase C and Src in CCK-stimulated pancreatic acini. *Am J Physiol* 1999;276: G915-G923

Edited by Wu XN