Dose Effects of Cholecystokinin and Acetylcholine on Phosphorus Compounds and Secretory Responses in Isolated Perfused Pancreas of Rat

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Abstract Phosphorus nuclear magnetic resonance (^{31}P NMR) spectroscopy was used to study energy supply for protein secretion in the isolated perfused rat pancreas. Stimulation with cholecystokinin (CCK-8) increased fluid secretion and protein output. With 10 pM of CCK-8, the tissue contents of ATP, inorganic phosphate (P_i), and creatine phosphate (PCr) remained unchanged. With 100 pM of CCK-8, which induced the maximum response in fluid secretion and protein output, P_i increased slightly, ATP and PCr remained almost unchanged. A high dose of CCK-8 (1 nM) suppressed the fluid and protein secretory rates, decreased ATP, and increased P_i significantly, but PCr showed a tendency of increase. Significant changes in ATP and P_i occurred on withdrawal of CCK-8 (1 nM), suggesting activation of ATP hydrolysis for recovery from secretory suppression. During stimulation with 0.1, 0.3, and 3.0 μM of acetylcholine, the similar dose-dependent response was observed in the secretion and the phosphorus metabolism. The present study demonstrated that cytosolic energy metabolism for secretory responses in the pancreas is low and the Lohmann reaction showed less contribution than in the salivary gland. The findings suggest that the energy supply for protein secretion may be cytosolic diffusion of ATP and that the Lohmann reaction system may contribute to synthesis and storing of secretory protein at resting state.

Key words: ^{31}P NMR, perfused pancreas, cholecystokinin, acetylcholine, protein secretion.

Exocrine secretion includes a fluid secretion based on electrolyte transport and a protein release. Both processes require respiratory energy. A scanning organ photo-spectroscopy revealed an activation of mitochondrial oxidative phosphorylation during secretion by pancreas (MATSUMOTO and KANNO, 1988). Energy supply to fluid secretion has been intensively studied in the perfused mandibular salivary gland by phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy during stimulation with various doses of acetylcholine (ACh) (MURAKAMI *et al.*, 1988). They demonstrated the occurrence of a functional compartmentation even a small distance between mitochondrial and cytosolic creatine phosphokinase (CPK) system sites in the acinar cell of rat mandibular gland, during stimulation with high dose of ACh.

The knowledge on the energy supply for a protein release is still limited. In the pancreatic acinar cell, cholecystokinin-(26-33) (CCK-8) and ACh stimulate a fluid secretion and a digestive enzyme release, and the pancreas is useful to study energetics for a protein release. In the isolated perfused rat pancreas, ^{31}P NMR study revealed that ATP and creatine phosphate (PCr) remained unchanged during exocrine secretion increased by $0.1\ \mu\text{M}$ ACh (MATSUMOTO *et al.*, 1988), suggesting that the metabolic pathway through the Lohmann reaction system could supply ATP to compensate for the energy consumption during a mild secretory stimulation.

To obtain further information on energy supply to a protein release, we have examined the effects of different doses of CCK-8 and ACh on phosphorus compounds, fluid secretion, and protein release in the isolated perfused rat pancreas. The results suggest that CPK system could be compartmentalized with high doses of CCK-8 and ACh, but the Lohmann reaction is less important in the protein release than in fluid secretion.

MATERIALS AND METHODS

Isolation and perfusion. Male Wistar rats (Shizuoka Laboratory Animal Center), weighing 250–300 g, were anesthetized by an intraperitoneal injection of pentobarbital sodium (Nembutal, 50 mg/kg body weight). The pancreatic tissue, weighing 500–900 mg, was surgically isolated for vascular perfusion as described previously (MATSUMOTO *et al.*, 1988) and placed in a 10 mm diameter of NMR tube, and perfused through the coeliac artery, at the rate of 2 ml/min, with the aid of a Cole-Palmer peristaltic pump. Experiments were performed at 25°C , since 1) our preparations maintained secretory abilities for 4 h at 25°C , 2) tissue levels of high-energy phosphorus compounds of PCr and ATP were maintained constant at least for 2 h at 25°C (MATSUMOTO *et al.*, 1988), 3) 1-h stimulation is required to observe the sustained secretory response, and 4) we could not accommodate the isolated perfused pancreas such a long period at 37°C .

The perfusate (bubbled continuously with 95% O_2 /5% CO_2 at 25°C) contained 117 mM NaCl, 5.6 mM KCl, 25 mM NaHCO_3 , 1 mM NaH_2PO_4 , 1 mM MgCl_2 , 2.5 mM

CaCl_2 , 5 mM glucose, and 5% (w/v) Dextran T70 (Pharmacia, Uppsala, Sweden). The solution was buffered at pH 7.4 with 10 mM N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid (HEPES; Sigma, St. Louis, MO).

Pancreatic exocrine secretion was stimulated with synthetic CCK-8 (gift from Prof. N. Yanaihara, Shizuoka Prefectural University, Shizuoka, Japan) or acetylcholine chloride (Sigma, St. Louis, MO) for 60 min. Pancreatic juice was collected from the duodenal end of the pancreatic duct, and its protein concentration was determined by the method reported by LOWRY *et al.* (1951) with bovine serum albumin (Sigma, St. Louis, MO) as a standard.

^{31}P NMR spectroscopy. The ^{31}P NMR spectra were obtained with a WM-360 wb NMR spectrometer (Bruker) operated at 145.8 MHz using a 10 mm broadband tunable probe. For the kinetic measurement, a radiofrequency pulse of 5 μs (15° pulse) was followed by a relaxation delay of 0.3 s, and 1,000 transients of free induction decay were accumulated for 5 min. Proton decoupling was not used. The Fourier transform was applied to free induction decay with 15 Hz line broadening for improvement of the signal-to-noise ratio of the spectrum. A picture-analyzing system (MOP AM03, Kontron) was used to measure the area beneath the resonant peak corresponding to each phosphorus compound. The tissue contents of phosphorus compounds were expressed as percentages of the resting values. The tissue pH was calculated from the chemical shift of inorganic phosphate resonance by the previous reported equation assuming pK' as 6.85 (SEO *et al.*, 1983).

Statistics. Results are expressed as means $\pm$ S.E. of several experiments. Comparison between two means was executed using appropriate *t*-test with $p < 0.025$ as the level of significance.

RESULTS

Secretory responses to pancreatic secretagogue

Removal of the duodenum from the pancreas made it possible to record ^{31}P NMR spectra only from the pancreatic tissue (MATSUMOTO *et al.*, 1988). In addition, this preparation allowed ACh or CCK-8 to stimulate a markedly larger increase in secretory responses than previous preparations with duodenum. To evaluate the secretory abilities of the present preparation, fluid secretion and protein output were measured at 10 pM, 100 pM, and 1 nM CCK-8 (Fig. 1).

Both fluid secretion and protein release showed a two-phase response, initial and sustained secretions. With 10 pM CCK-8, protein output rose gradually to a plateau level ($87 \pm 15 \mu\text{g}/(\text{min} \cdot \text{g wet weight})$, $n=4$) at 30–35 min after start of stimulation). Juice flow increased gradually to a plateau level ($3.1 \pm 0.5 \mu\text{l}/(\text{min} \cdot \text{g})$ at 30–35 min). With 100 pM CCK-8, protein output rose rapidly to a maximum of $243 \pm 18 \mu\text{g}/(\text{min} \cdot \text{g wet weight})$ ($n=4$) at 5–10 min and then declined to $94 \pm 17 \mu\text{g}/(\text{min} \cdot \text{g})$ at 55–60 min. Juice flow rose rapidly to a maximum of $5.0 \pm 0.9 \mu\text{l}/(\text{min} \cdot \text{g})$ ($n=4$) at 5–10 min, and then declined to $3.2 \pm 0.6 \mu\text{l}/(\text{min} \cdot \text{g})$ at 55–60 min. With 1 nM CCK-8, protein output rose to a maximum of $326 \pm 81 \mu\text{g}/$

Fig. 1. Time course of changes in pancreatic juice flow and protein output during continuous perfusion of the isolated rat pancreas with 10 pM (left), 100 pM (middle), and 1 nM CCK-8 (right). Each value represents the mean $\pm$ S.E. of four glands. The open horizontal bars indicate the period of CCK-8 stimulation.

(min·g) ($n=4$) at 5–10 min, and then declined to $34 \pm 10 \mu\text{g}/(\text{min} \cdot \text{g})$ at 55–60 min. Juice flow also rose to a level of $4.1 \pm 0.3 \mu\text{l}/(\text{min} \cdot \text{g})$ at 5–10 min, and then decreased to a level of $0.6 \pm 0.1 \mu\text{l}/(\text{min} \cdot \text{g})$ at 55–60 min. Washout of CCK-8 raised both protein output and juice flow, significantly compared with those at the end of stimulation.

The rate of fluid secretion is very small in the pancreas compared with the mandibular salivary gland. In the isolated perfused pancreas stimulated with 100 pM CCK-8, the initial fluid secretion (0–10 min) was 1/26 of that by the mandibular gland stimulated with $1 \mu\text{M}$ ACh and the sustained fluid secretion (50–60 min) was 1/15 of that by the mandibular gland at 24°C (MURAKAMI *et al.*, 1988). On the other hand, the protein secretory rate (30–50 min) by the pancreas was $115 \mu\text{g}/(\text{min} \cdot \text{g})$ and several folds larger than that by the rabbit mandibular gland at 37°C ($37.5 \mu\text{g}/(\text{min} \cdot \text{g})$), stimulated with $0.8 \mu\text{M}$ ACh; CASE *et al.*, 1980).

³¹P NMR spectroscopy

The above observation on secretory responses reflects that the energy consumption for protein secretion is relatively high in the pancreas in respect to the mandibular salivary gland. Dose effect on ³¹P NMR spectra of the mandibular gland suggested that the Lohmann reaction system plays an important role to supply energy for fluid secretion (MURAKAMI *et al.*, 1988). To assess how the Lohmann reaction works for protein output, the phosphorus compounds in the perfused pancreas were measured during stimulation with 10 pM, 100 pM, and 1 nM CCK-8, as shown in Fig. 2.

Fig. 2. Time course of changes in creatine phosphate (PCr), ATP, inorganic phosphate (P_i), and tissue pH in the isolated perfused rat pancreas during continuous stimulation with 10 pM (left), 100 pM (middle), and 1 nM CCK-8 (right). Each value represents the mean $\pm$ S.E. of four experiments, expressed as percentages of the respective resting level. The large and small asterisks indicate significant increase ($p < 0.025$) and decrease ($p < 0.025$), respectively. The open horizontal bars indicate the period of CCK-8 stimulation.

Comparing with the rat mandibular gland, the pancreas showed small changes in ^{31}P NMR spectra. During stimulation with 10 pM CCK-8, the tissue contents of PCr, ATP, and inorganic phosphate (P_i) showed no significant changes from the resting control levels. With 100 pM CCK-8, the tissue contents of ATP and PCr were almost unchanged, but P_i showed a slight rise. With 1 nM CCK-8, ATP decreased and P_i increased, which consisted of two phases; an initial transient decrease to $72 \pm 4\%$ ($n=4$) at 5–10 min after start of stimulation, then recovered to the resting level (10–45 min), and a subsequent decrease to $80 \pm 3\%$ ($n=4$) at 45–60 min (Fig. 2). P_i increased gradually and significantly to 163% during the stimulation. By washout of 1 nM CCK, P_i increased more markedly than that observed during stimulation, whereas, PCr showed no significant change during the stimulation.

In addition, small unpredictable changes were observed as follows. With 10 pM

CCK-8, PCr decreased gradually to ca. 50% at 30 min from start of washout of CCK-8, and the tissue pH showed an initial transient drop. With 100 pM CCK-8, P_i showed a transient decrease to 80% at 5–10 min which was followed by a gradual increase to 147% during sustained stimulation. After washout of CCK-8, ATP decreased by ca. 20% of control. With 1 nM of CCK-8, tissue pH rapidly increased to 7.33 ± 0.01 pH ($n=4$) and remained at the increased level during and even after the stimulation. Since the changes in ^{31}P NMR were so small and the timings of individual response were varied, the time courses of phosphorus compounds included large standard errors of means.

Correlation between dose-dependent secretory responses and ATP level

Secretory responses to CCK-8 at higher doses consisted of two phases. Dose-response relations between secretion and ATP were obtained separately, during initial secretion (at 0–30 min from the start of stimulation) and during sustained secretion (at 30–60 min from the start of stimulation), as shown in Fig. 3.

During initial secretion, ATP tends to decrease gradually depending on the dose of CCK-8. At the supramaximal dose (1 nM) of CCK-8, the fluid secretion was not suppressed so much and the protein release was evoked as well as at the

Fig. 3. Correlation between tissue content of ATP and secretory responses induced by different doses of CCK-8 in the isolated perfused rat pancreas. Each value in ATP level represents the mean $\pm$ S.E. of four experiments. Open and closed columns represent the mean $\pm$ S.E. of total amount of pancreatic juice flow and protein output obtained during the first (left columns) and the second (right columns) half of the 60-min stimulation with different doses of CCK-8 as indicated below.

Fig. 4. Correlation between tissue content of ATP and secretory responses induced by different doses of ACh in the isolated perfused rat pancreas. Each value in ATP level represents the mean $\pm$ S.E. of five experiments for 0.1 μ M ACh or four experiments for 0.3 and 3.0 μ M ACh. Open and closed columns represent the mean $\pm$ S.E. of total amount of pancreatic juice flow and protein output obtained during the first (left columns) and the second (right columns) half of the 60-min stimulation with different doses of ACh as indicated below.

maximal dose (100 pM).

During sustained stimulation, ATP gradually decreased depending on the dose of CCK-8 and a significant decrease of ATP was observed at 1 nM of CCK-8 ($86 \pm 2\%$ of the resting level, $n=4$). The fluid secretion and the enzyme release by the pancreas were markedly suppressed at 1 nM of CCK-8. The amounts of protein output and juice flow at 1 nM of CCK-8 were one-third and one-fourth of those at 100 pM of CCK-8 which induced the maximal secretory responses.

Similar dose-response relations were obtained in the pancreas stimulated with ACh at 0.1, 0.3, and 3.0 μ M (Fig. 4). Content of ATP decreased during both initial and sustained secretions by the higher doses of ACh (0.3 and 3.0 μ M). With 3 μ M ACh, ATP decreased significantly to ca. 80% of the resting level. During the initial secretion by 3.0 μ M of ACh, the protein output and juice flow were suppressed to one-fourth and one-fifth of those at 0.3 μ M ACh. This dose of ACh induced the maximal secretory responses in spite of decrease in ATP content. During the sustained secretion by 3.0 μ M ACh, the protein output and juice flow decreased to one-sixth and one-seventh of those at 0.3 μ M ACh.

DISCUSSION

Energy supply for protein release. In the rat exocrine pancreas, almost 80% of the total cells are acinar cells (KEMPEN *et al.*, 1977), and its main role is a secretion of enzyme proteins. The discharge of enzyme proteins by the pancreatic acinar cell is believed to depend on respiration (JAMIESON and PALADE, 1971), and the synthesis and intracellular transport of these proteins also require respiratory energy (JAMIESON and PALADE, 1968). More than 90% of the total metabolic energy is produced by the mitochondria in the rat pancreatic acinar cell (BAUDUIN *et al.*, 1969). However, the knowledge of precise mechanisms to supply energy for protein release is still limited.

For muscle contraction, high-energy phosphates are transferred in the form of PCr across the cytosol to the site of ATP utilization. The PCr molecule has a higher degree of diffusibility than that of ATP (KUSHMERICK and PODOLSKY, 1969; YOSHIZAKI *et al.*, 1982). That is known as an "energy shuttle system" (MEYER *et al.*, 1984). From studies of ACh-dose effects on phosphorus compounds in the mandibular salivary gland, it was demonstrated that PCr was supplied energy to activation of Na^+/K^+ ATPase during fluid secretion (MURAKAMI *et al.*, 1988). Based on functional compartmentation of Lohmann reaction system during high-dose ACh stimulation, they suggested that the intracellular movement of electrolytes (as well as PCr) could be delayed and cause a suppression of fluid secretion.

The present experiment was planned to examine whether energy for protein secretion is supplied by Lohmann reaction system. The hypothesis was based on three evidences: 1) Existence of Lohmann reaction system in the pancreatic acinar cell was confirmed by detection of PCr by ^{31}P NMR spectroscopy (MATSUMOTO *et al.*, 1988). 2) Enzyme protein secretions by the pancreas were suppressed at high dose of CCK-8 and cholinergic agonist, as well as fluid secretion (KANNO *et al.*, 1984). 3) The relative work for protein secretion in the total secretory activity is much larger in the pancreas than in the salivary gland stimulated with ACh. The third evidence was confirmed quantitatively in the present preparation.

The changes in phosphorus compounds (decreases in ATP and PCr, increase in P_i) were very small even with higher doses of secretagogues (Fig. 2), in comparison with the salivary gland (MURAKAMI *et al.*, 1988). This finding suggests two possibilities: 1) a small energy demand for secretion or 2) an effective supply of phosphorus energy compounds with onset of secretion. The former is preferable because of the following observations. Since the perfusion rate was 2 ml/min for 500–900 mg pancreas, the expected oxygen supply to the pancreas is calculated as ca. $70 \mu\text{l}/(\text{g} \cdot \text{min})$. The oxygen consumption (Q_{O_2}) in the perfused rat pancreas was increased by $1.3\text{--}3.3 \mu\text{l}/(\text{g} \cdot \text{min})$ with $0.1\text{--}10 \mu\text{M}$ ACh at 37°C (KANNO *et al.*, 1983). The Q_{O_2} of the salivary gland was increased by $15\text{--}72 \mu\text{l}/(\text{g} \cdot \text{min})$ with $0.1 \mu\text{M}\text{--}1 \text{ mM}$ ACh at 24°C (MURAKAMI *et al.*, 1988), although the resting Q_{O_2} of both glands was similar. These suggest that the energy supply for protein secretion could

be smaller than that for fluid secretion.

The content of PCr in the pancreas was almost unchanged during stimulation with different doses of secretagogues (Fig. 2), suggesting a small contribution of Lohmann reaction system to protein secretion. With lower doses, ATP levels were unchanged (Figs. 3 and 4). ATP supply could be so effective to compensate the consumed ATP. Under well-oxygenated condition, the dose response of Q_{O_2} and mitochondrial redox response were almost linear during stimulation with CCK-8 (MATSUMOTO and KANNO, 1988) and ACh (KANNO *et al.*, 1983), suggesting that mitochondrial oxidative phosphorylation can be normally activated for ATP synthesis with different doses of secretagogues. With higher doses, ATP decreased during initial secretion and decreased significantly during sustained secretion. In the mandibular gland stimulated with $1\text{ }\mu\text{M}$ ACh, ATP decreased by 20%, PCr decreased by 30%, and P_i increased by 80% (MURAKAMI *et al.*, 1988), whereas, in the pancreas, the decrease of ATP was ca. 20% with 1 nM CCK-8 or $3\text{ }\mu\text{M}$ ACh (Figs. 3 and 4) and, PCr and P_i showed only small changes. These observations suggest that the Lohmann reaction system could have less contribution to an energy supply for the protein release by the pancreas than that for the fluid secretion by the salivary gland. ATP could diffuse across cytosol from mitochondria to the unknown sites of ATP hydrolysis for protein secretion.

Role of Lohmann reaction in the pancreas. Existence of Lohmann reaction in the pancreas has been proved as described above. What is a role of Lohmann reaction in the pancreas? Energy supply for fluid secretion could be one of the roles of Lohmann reaction. The fluid secretion is small but important in the pancreas, which is maintained by activation of $\text{Na}^+\text{-K}^+$ pumps during stimulation with CCK-8 and cholinergic agonist (HOOTMAN *et al.*, 1983). Another role of Lohmann reaction is suggested during resting state by the finding that hypoxia caused a rapid decrease in PCr and a subsequent gradual decrease in ATP (MATSUMOTO *et al.*, 1988). During unstimulated resting condition, the pancreatic secretions decreased to 1/25–1/50 of the maximum secretory rate (Fig. 1). Therefore the energy demand during resting state could be originated from 1) synthesis of secretory proteins, 2) storing process of secretory protein, and 3) maintenance of basic cell condition itself.

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REFERENCES

- BAUDUIN, H., COLIN, M., and DUMONT, J. E. (1969) Energy sources for protein synthesis and enzymatic secretion in rat pancreas *in vitro*. *Biochim. Biophys. Acta*, **174**: 722–733.
CASE, R. M., CONIGRAVE, A. D., NOVAK, I., and YOUNG, J. A. (1980) Electrolyte and

- protein secretion by the perfused rabbit mandibular gland stimulated with acetylcholine or catecholamines. *J. Physiol. (Lond.)*, **300**: 467–487.
- HOOTMAN, S. R., ERNST, S. A., and WILLIAMS, J. A. (1983) Secretagogue regulation of Na^+ - K^+ pump activity in pancreatic acinar cells. *Am. J. Physiol.*, **245**: G339–G346.
- JAMIESON, J. D. and PALADE, G. E. (1968) Intracellular transport of secretory proteins in the pancreatic exocrine cell. IV. Metabolic requirements. *J. Cell Biol.*, **39**: 589–603.
- JAMIESON, J. D. and PALADE, G. E. (1971) Condensing vacuole conversion and zymogen granule discharge in pancreatic exocrine cells: Metabolic studies. *J. Cell Biol.*, **48**: 503–522.
- KANNO, T., MATSUMOTO, T., KAWAHARA, J., HATTORI, C., SAITO, T., KATO, Y., and EGUCHI, J. (1984) Extracellular calcium responsible for the secretory response of the exocrine pancreas to cholecystokinin. In: *Secretion: Mechanisms and Control*, ed. by CASE, R. M., LINGARD, J. M., and YOUNG, J. A., Manchester University Press, Manchester, pp. 181–186.
- KANNO, T., SAITO, A., and IKEI, N. (1983) Dose-dependent effects of acetylcholine stimulating respiratory chain and secretion of isolated perfused rat pancreas. *Biomed. Res.*, **4**: 175–186.
- KEMPEN, H. J. M., DEPONT, J. J. H. M., and BONTING, S. L. (1977) Rat pancreas adenylate cyclase, V. Its presence in isolated rat pancreatic acinar cells. *Biochim. Biophys. Acta*, **496**: 521–531.
- KUSHMERICK, M. J. and PODOLSKY, R. J. (1969) Ionic mobility in muscle cells. *Science*, **166**: 1297–1298.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265–275.
- MATSUMOTO, T. and KANNO, T. (1988) Optical absorbance changes induced by CCK-8 under limited O_2 supply in isolated perfused rat pancreas. *Am. J. Physiol.*, **254**: C727–C734.
- MATSUMOTO, T., KANNO, T., SEO, Y., MURAKAMI, M., and WATARI, H. (1988) Phosphorus nuclear magnetic resonance in isolated perfused rat pancreas. *Am. J. Physiol.*, **254**: G575–G579.
- MEYER, R. A., SWEENEY, H. L., and KUSHMERICK, M. J. (1984) A simple analysis of the “phosphocreatine shuttle.” *Am. J. Physiol.*, **246**: C365–C377.
- MURAKAMI, M., SEO, Y., and WATARI, H. (1988) Dissociation of fluid secretion and energy supply in rat mandibular gland by high dose of ACh. *Am. J. Physiol.*, **254**: G781–G787.
- SEO, Y., MURAKAMI, M., WATARI, H., IMAI, Y., YOSHIKAWA, K., NISHIKAWA, H., and MORIMOTO, T. (1983) Intracellular pH detection by a ^{31}P -NMR technique. The second dissociation constant of phosphoric acid in biological system. *J. Biochem.*, **84**: 729–734.
- YOSHIKAWA, K., SEO, Y., NISHIKAWA, H., and MORIMOTO, T. (1982) Application of pulsed-gradient ^{31}P NMR on frog muscle to measure the diffusion rates of phosphorus compounds in cells. *Biophys. J.*, **38**: 209–211.