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Effect of CCK and Carbachol on Enzyme Secretion From The Isolated Pancretic Acinar Cells of Rats fed Heated or Raw Soybean Diet

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CCK와 Carbachol이 익힌 대두와 생대두를 먹인 쥐에서 분리한 췌장세포의 외분비기능에 미치는 영향

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□ 국 문 초 록 □

CCK(Cholecystokinin)와 carbachol(carbamylcholine chloride)이 췌장의 효소분비 능력을 촉진시킨다는 사실은 잘 알려져 있다. 생대두식이를 먹인 쥐에서 췌장세포의 hypertrophy현상이 두드러지게 나타나고 소화효소의 과다분비 현상이 나타나므로 이번 실험에서는 익힌 대두와 생대두를 먹인 후 정상적인 췌장세포와 hypertrophy가 일어난 췌장세포에 CCK와 carbachol로 자극을 준 후 효소분비 능력을 비교하였다. 췌장세포에서 분비되는 단백질 분해효소들이 세포자체에 미치는 영향을 최소한으로 줄이기 위하여 췌장의 acinar cell을 분리한 후 cell column을 만들어 superfusion technique에 의해 stimulus-enzyme secretion coupling 방법을 사용하였다.

분리한 췌장세포를 CCK와 carbachol로 자극을 주었을때 chymotrypsin분비는 생대두를 먹인 쥐의 세포에서 익힌 대두를 먹인 군보다 높게 나타났고 amylase의 분비는 chymotrypsin과는 달리 익힌 대두를 먹인 쥐의 세포에서 생대두를 먹인 군보다 높게 나타났다. 이번 실험의 결과는 지금까지 논쟁중에 있는 췌장의 acinar cell에서의 여러가지 효소분비 비율은 항상 일정한 것이 아니라 자극의 종류에 따라 효소분비량의 구성비율이 달라진다는 것을 알려주고 있다.

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INTRODUCTION

In vitro secretory studies have been performed with pancreatic slices[1], fragmented pancreas[2], pancreas lobules[3], and freshly dispersed acinar cells[4,5] as well as with isolated acini[6,7]. Although in vitro incubations allow simultaneous examination of many conditions, they entail certain disadvantages. Tissues or cells are constantly exposed to their secretory products, which accumulate to the medium throughout the incubation. These secreted products are rich in proteolytic enzymes which could directly damage the secretory tissue. Although these problems can partially be avoided by the addition of trypsin inhibitors to the incubation medium, such additions complicate the conditions. To avoid the above problems, a model system was developed by Guderley and Heisler[8], in which freshly dispersed pancreatic acinar cells were suspended with gel filtration beads in a column and then superfused. Cell column superfusion systems, designed to monitor hormone release, were used by Lowry[9] to study pituitary cells and by others[10,11] for pituitary and adrenal cortical cells. The use of cell column perfusion system for the study of stimulus-enzyme secretion coupled with exocrine pancreas offered several advantages: (1) the continuous removal of secreted enzyme minimizes the deleterious effect on cell, (2) by washing out the contents of broken cells prior to collecting the enzymes secreted in response to a stimulus, one can be assured that the collected proteins originating from broken cells, (3) the sequential monitoring of the column perfusate allowed temporal analysis of secretagogue action, and (4) each column served as its own control, therefore a comparison between basal and stimulated secretory rates can be made for each column and comparison between the secretory profiles for different columns can also be made.

The peptide hormone CCK, as well as cholinergic agents such as acetylcholine and carbamylcholine, are known to stimulate enzyme secretion from the pancreas in vivo and in vitro[12,13,14]. It was reported that CCK stimulates the growth of the exocrine pancreas[15,16]. Repeated injection (sc) of CCK into rats 3 times per day for 1 week produced pancreatic adaptation similar to that observed with trypsin inhibitor or high protein[17,18]. Green and Lyman[19] postulated a theory as regards the mechanism by which trysin inhibitors influence pancreatic enzyme biosynthesis in rats fed SBTI(Soybean Trypsin Inhibitor). According to Green and Lyman[20] and Ihse et al.[21], trypsin in the upper part of the intestine exerts a negative feedback regulation on pancreatic secretion. The secretagogue effect of the inhibitors has been related to stimulation of increased release and/or synthesis of a humoral factor such as CCK[21]. Therefore, it was suggested that stimulation of the pancreas by endogenous CCK, released by protein or TI(Trypsin Inhibitor) in the intestine, was the event which lead to pancreatic adaptation[22].

This experiment was designed to determine whether or not there is a difference in the response of acinar cells to the secretagogues, CCK and carbachol(carbamylcholine chloride), when pancreases of rats were already adapted to the diet containing heated or raw soybean.

MATERIALS AND METHODS

Fourteen weanling male rats(Wistar strain) weighed about 50 grams were divided into two groups and fed experimental diets containing heated or raw soybean as a protein source. Compositions of experimental diets are given in Table 1 and 2. After one week

Table 1. Composition of heated soybean (HS) diet and raw soybean (RS) diet

Ingredient (% of diet)	HS	RS
Heated soy flour	18.3	-
Raw soy flour	-	19.1
Glucose	15.0	15.0
Fiber	5.0	5.0
Corn oil	5.5	5.5
Lard	2.5	2.5
Dextrin	46.46	45.665
DL-methionine	0.03	0.035
Vitamin mix*	2.0	2.0
Mineral mix*	5.0	5.0
Choline chloride	0.2	0.2

*Compositions of vitamin and mineral mixtures are given in Table 2.

Table 2. Compositions of vitamin and mineral mixtures

Vitamin Mixture Provides (amount/kg diet)			
Thiamin HCl	20 mg	Vitamin B_{12}	50 mcg
Riboflavin	20 mg	Vitamin A acetate	20,000 IU
Niacin	90 mg	Vitamin D_3	2,200 IU
Pyridoxine HCl	20 mg	Menadione sodium bisulfite	20 mg
D-calcium pantothenate	60 mg	DL-α tocopherol acetate	50 IU
Folic acid	4 mg	Ascorbic acid	900 mg
D-biotin	0.4 mg	I-inositol	200 mg
Mineral Mixture Provides (as noted % or mg/kg diet)			
Calcium	0.75%	Copper	15 mg
Phosphorus	0.45%	Sulfur as SO_4	12 mg
Potassium	0.46%	Fluoride	5.0 mg
Sodium	0.20%	Cobalt	3.2 mg
Chloride	0.20%	Chromium	3.0 mg
Magnesium	0.065%	Iodine	0.6 mg
Manganese	65 mg	Molybdenum	0.8 mg
Iron	60 mg	Selenium	0.2 mg
Zinc	40 mg		

of feeding experimental diet, one rat from each group was killed weekly. Animals were fasted overnight before sacrifice. Pancreases were removed, weighed and acinar cells were isolated by enzymatic digestion and mechanical dispersion using the method of Kondo and Schulz[23] as modified by Chauvelot et al[24]. Two cell columns were prepared from the cell preparation of each rat according to Guderley and Heisler[8]. The secretagogue, CCK

and carbachol, was administered to each column after an equilibration period of 90 min. Stimulus was applied for 20 min and followed by readministration of the initial buffer to monitor the return to basal secretory rates. Concentration of CCK(10^{-9}M) and carbachol(10^{-5}M) were chosen from the results of Peikin et al.[25] and Gardner and Jackson[26], respectively.

Secretion was monitored by measuring the amylase and chymotrypsin activity in the prefusate fraction. Trypsin activity was not determined because soybean trypsin inhibitor was added in the medium during the cell isolation procedures. Amylase activity was determined by the method of Bernfeld[27]. Chymotrypsin activity was determined by the method of Bundy[28]. Stimulatory effect of CCK or carbachol on amylase and chymotrypsin secretion was estimated by the ratio of the area under the peak of the cells from RS(Raw Soybean) fed rats over those from HS(Heated Soybean) fed rats. The area under the peak was measured by using Planimeter(Compensating Polar Planimeter, Keuffel & Esser Co. Model No. 62-0000).

Statistical significance between two groups was determined using a paired t-test for the enzyme activity and Student's t-test for the pancreas size[29].

RESULTS AND DISCUSSION

Pancreatic enlargement was apparent in group RS(0.62 ± 0.10% body weight) compared to group HS(0.42 ± 0.13%). Difference in pancreatic size between two groups was statistically significant($p < 0.05$).

Typical curves of the enzyme activity in the perfusate fraction from the cell column

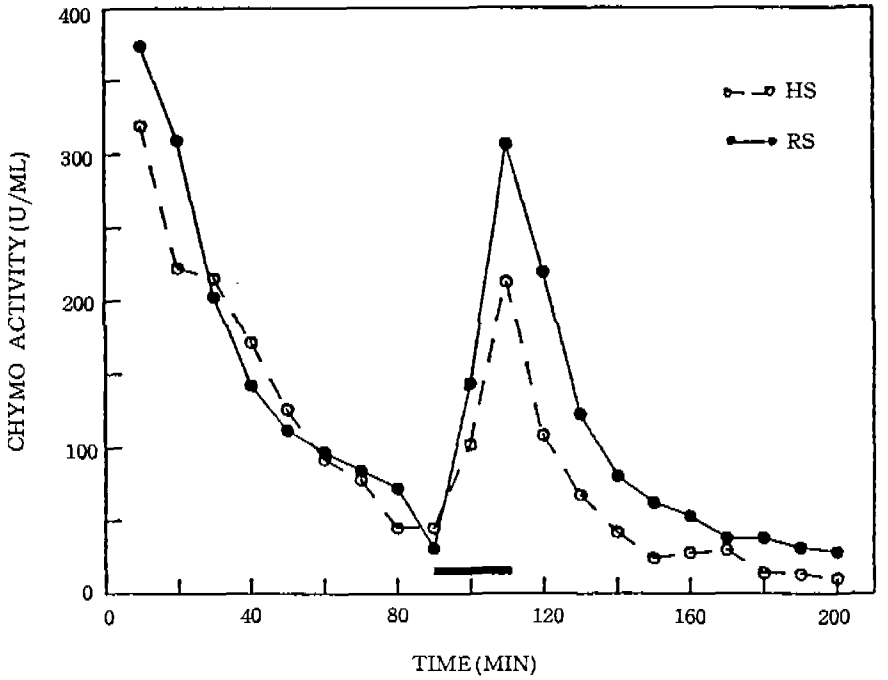

Fig. 1. Chymotrypsin activity of perfusate fraction of column of cells isolated from rats fed HS or RS diet when CCK was used as secretagogue.
▬ Period when secretagogue was added to column.

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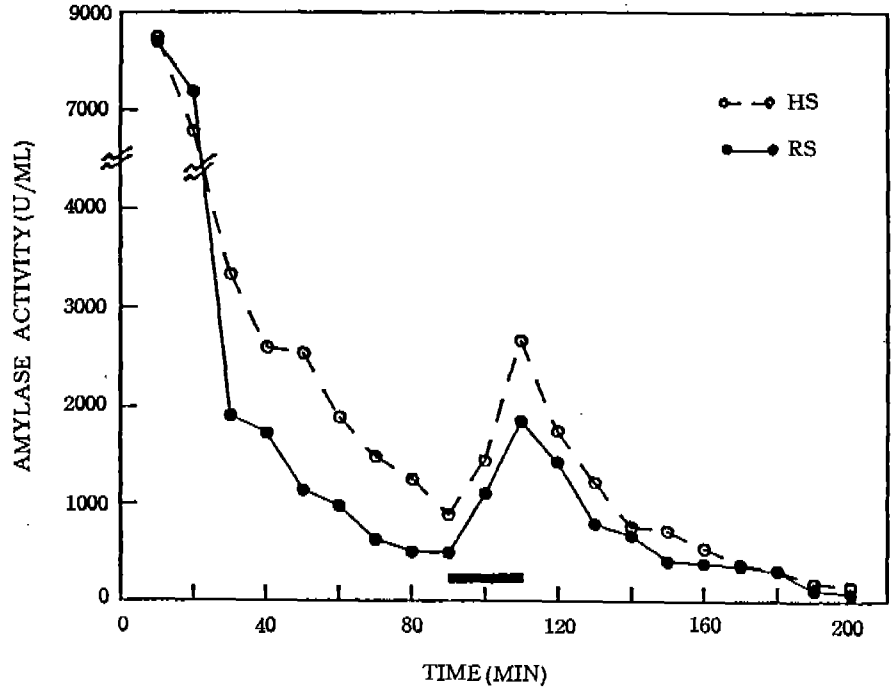

Fig. 2. Amylase activity of perfusate fraction of column of cells isolated from rats fed HS or RS diet when CCK was used as secretagogue.
▬ Period when secretagogue was added to column.

of RS or HS fed rat pancreas with CCK stimulation are shown in Fig. 1 and 2. The first 90 minutes was the equilibration period to wash out all the broken cells. When the basal secretion level was reached, secretagogue, CCK or carbachol was introduced for 20 minutes. After stimulation original buffer was perfused for 90 minutes until the basal secretion level was reached. By using the ratio of stimulation peak area between RS and HS fed rats, error due to the different basal lines could be eliminated.

As shown in Table 3, chymotrypsin secretion was higher in group RS than in group HS with both kinds of secretagogue, CCK and carbachol. In contrast to chymotrypsin, amylase secretion was higher in group HS than in group RS with both kinds of secretagogue. This result suggested that chymotrypsin and amylase secretion from the acinar cells is not parallel with CCK and carbachol stimulation. A non-parallel secretion of amylase and chymotrypsinogen from the pancreas stimulated by oleic acid was also observed by Dagorn et al.[30]. They found that the stimulation had induced a non-parallel response in the rates of synthesis of amylase and chymotrypsinogen. Macleod et al.[31] also demonstrated the non-parallel synthesis of amylase and chymotrypsinogen when pancreas was stimulated with SBTI. They reported that compared to the control group, rats fed SBTI incorporated more label into chymotrypsinogen and less into amylase. Dagorn and Mongeau[32] studied the rate of biosynthesis of amylase, lipase and chymotrypsinogen in rat pancreas after intravenous injection of CCK associated with secretin. This pancreatic stimulation resulted in non-parallel variations of the rates of biosynthe-

Table 3. Ratio of enzyme secretion from the isolated acinar cells of the rats fed RS and HS diet with CCK or carbachol stimulation

Week of experiment	Chymotrypsin (RS/HS)		Amylase (RS/HS)	
	CCK stimulation	Carbachol stimulation	CCK stimulation	Carbachol stimulation
1	1.08	1.81	0.27	0.38
2	0.55	2.24	0.19	0.78
3	1.36	1.23	0.70	0.46
4	1.00	1.25	0.36	0.28
5	2.27	1.17	0.86	0.80
6	3.48	2.20	0.31	0.57
7	1.01	0.73	0.19	0.51
Mean± SD	1.39± 1.15	1.52± 0.57	0.41± 0.26	0.54± 0.19
p value	$p<0.1$	$p<0.05$	$p<0.05$	$p<0.05$

sis of the enzymes in that chymotrypsinogen, amylase and lipase products were increased by 63, 26 and 10%, respectively. These results suggested that the non-parallel response of pancreas to various stimulus could be demonstrated in in vivo and in vitro systems.

SUMMARY

The effects of the hormonal factor (CCK) and neural factor(carbachol) on the exocrine function of the pancreas were studied in this experiment. A superfusion technique was used for in vitro study of stimulus-secretion coupling in isolated pancreatic acinar cells from the rats fed heated or raw soybean diet. Chymotrypsin secretion was higher in cells from the raw soybean group than in those from the heated soybean group with both kinds of stimulants(CCK and carbachol), whereas, amylase secretion was higher in the heated soybean group than in the raw soybean group. This indicated that chymotrypsin and amylase secretion from the acinar cells are not parallel with CCK and carbachol stimulation.

REFERENCES

1) Kempen, H.J.M., Depont, J.J.H. H.M. & Bonting, S.L.: *Rat pancreatic adenylate cyclase. Ⅳ. Effect of hormones and other agents on cyclic AMP level and enzyme release. Biochim. et Biophys. Acta. 496:65-76, 1977.*

2) Heisler, S., Fast, D. & Tenenhouse, A.: *Role of Ca^{2+} and cyclic AMP in protein secretion from rat exocrine pancreas. Biochim. et Biophys. Acta. 279:561-572, 1972.*

3) Haymorvits, A. & Scheele, G.A.: *Cellular cyclic nucleotides and enzyme secretion in the pancreatic acinar cell. Proc. Nat. Acad. Sci. 73:156-160, 1976.*

4) Amsterdam, A. & Jamieson, J.D.: *Studies on dispersed pancreatic exocrine cells. Ⅰ. Dissociation technique and morphologic characteristics of separated cells. J. Cell Biol. 63:1037-1056, 1974.*

5) Amsterdam, A. & Jamieson, J.D : *Studies on dispresed pancreatic exocrine cells. Ⅱ. Functional characteristics of separated cells. J. Cell Biol. 63:1057-1073, 1974.*

6) Logsdon, C.D. & Williams, J.A.: *Epidermal gr-*

owth factor binding and biological effects on mouse pancreatic acini. Gastroenterology: 85: 339-345, 1983.

7) Ludford, J.M., Tulamo, B.R.: *β-Adrenergic and muscarinic receptors in developing rat parotid glands. J Biol. Chem. 255: 4619-4627, 1980.*

8) Guderley, H. & Heisler, S.: *A model system for the study of stimulus-enzyme secretion coupling in rat pancreatic acinar cells. Can. J. Physiol. Pharmacol. 58: 965-973, 1980.*

9) Lowry P.J.: *A sensitive method for the detection of corticotropin releasing factor using a perfused pituitary cell column. J. Endocrin. 62: 163-164, 1974.*

10) Mulder, G.H. & Smelik, P.G.: *Superfusion system for the study of the sites of action of glucocorticoids in rat hypothalamus-pituitary-adrenal system in vitro. I. Pituitary cell superfusion. Endocrinology. 100: 1143-1152, 1977.*

11) Vernes, I., Mulder, G.H. & Smelik, P.G.: *Superfusion system for the study of the sites of action of glucocorticoids in rat hypothalamus-pituitary-adrenal system in vitro I. Hypothalamus-pituitary cell-adrenal cell superfusion. Endocrinology 100: 1153-1159, 1977.*

12) Harper, A.A.: *Hormonal control of pancreatic secretion. Handbook Physiol., Section 6. 2: 969-995, 1967.*

13) Schramm, M.: *Secretion of enzyme and other macromolecules. Annu. Rev. Biochem. 36: 307-320, 1967.*

14) Jorpes, J.E. & Mutt, V.: *Secretion and cholecystokinin (CCK) In: Secretion, CCK, PZ and Gastrin. Jorpes, J.E. & Mutt, V. eds. Springer-Verlag New York, Inc., N.Y. pp. 1-177, 1973.*

15) Mainz, D.L., Black, O. & Webster, P.D.: *Hormonal control of pancreatic growth. J. Clin. Invest. 52: 2300-2304, 1973.*

16) Solomon, T.E., Petersen, H., Elashoff, J. & Grossman, M.I.: *Interaction of caerulein and secretion on pancreatic size and composition in rat. Am. J. Physiol. 235, E714, 1978.*

17) Snook, J.T.: *Factor in whole-egg protein influencing dietary induction of increases in enzyme and RNA levels in rat pancreas. J. Nutr. 97: 286-294, 1969.*

18) Rothman, S.S. & Wells. H.: *Enhancement of pancreatic enzyme synthesis by pancreozymin. Am. J. Physiol. 213: 215-218, 1967.*

19) Green, G.M. & Lyman, R.L.: *Chymotrypsin inhibitor stimulation of pancreatic enzyme secretion in the rat. Proc. Soc. Exp. Biol. Med. 136: 649-654, 1971.*

20) Ihse, I., Lilja, P. & Lundquist, I.: *Feedback regulation of pancreatic enzyme secretion by intestinal trypsin in man. Digestion, 15: 303-308, 1977.*

21) Khayambashi, M. & Lyman, R.L.: *Secretion of rat pancreas perfused with plasma from rats fed soybean trypsin inhibitor. Am. J. Physiol. 217: 647-651, 1969.*

22) Green, G.M., Olds, B.A., Matthews, G. & Lyman, R.L.: *Protein as a regulator of pancreatic enzyme secretion in the rat. Proc. Soc. Exp. Biol. Med. 142: 1162-1167, 1973.*

23) Kondo, S. & Schulz, I.: *Calcium ion uptake in isolated pancreas cells induced by secretagogues. Biochim. et Biophys. Acta. 419: 76-92, 1976.*

24) Chauvelot, L., Heisler, S., Huot, J. & Gagnon, D.: *Prostaglanding and enzyme secretion from dispersed rat pancreatic acinar cells. Life. Sci. 25: 913-920, 1979.*

25) Peikin, S.R., Rottman, A.J., Batzri, S. & Gardner, J.D.: *Kinetics of amylase release by dispersed acini prepared from guinea pig pancreas. Am. J. Physiol. 235: E743-749, 1978.*

26) Gardner, J. & Jackson, M.J.: *Regulation of amylase release from dispersed pancreatic acinar cells. J. Physiol. 270: 439-454, 1977.*

27) Bernfeld, P.: *Amylases, α and β. In: Methods in Enzymology Vol I. Colowick, S.B. and Kaplan, N.O. eds. Acad. Press. pp. 149-158, 1955.*

28) Bundy, H.F.: *Chymotrypsin-catalyzed hydrolysis of N-acetyl- and N-benzoyl-L-tyrosine-*

p-nitroanilides. Arch. Biochem & Biophys. 102:416-422, 1963.

29) Li, J.C.R.: *Statistical Inference* I. *Edwards Brothers, Inc., Michigan. Chapter 8, and Chapter 26, 1969.*

30) Dagorn, J.C., Paradis, D. and Morisset, J. : *Non-parallel response of amylase and chymotrypsinogen biosynthesis following pancreatic stimulation: A possible explanation for observed non parallelism in pancreatic secretion. Digestion, 15:110-120, 1977.*

31) Macleod, E.P., Derbenwick J. P. and Snook. J.T.: *Comparison of pancreatic changes induced in rats by soybean and egg-white trypsin inhibitor fed with different levels of nitrogen. J. Nutr. 102:469-478, 1972.*

32) Dagorn, J. & Mongeau, R.: *Different action of hormonal stimulation on the biosynthesis of three pancreatic enzymes. Biochim. et Biophys. Acta. 498:76-82, 1977.*