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# Monitor peptide gene expression is increased by exogenous CCK in the rat pancreas and in a rat pancreatic acinar cell line (AR4-2J)

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Monitor peptide (CCK-releasing peptide) mRNA increased on the administration of CCK in rat pancreas and the AR4-2J pancreatic cell line. Subcutaneous injection of CCK into rats at 8 h intervals increased the level of monitor peptide mRNA in the pancreas. Concomitant injection of CCK antagonist CR-1409 strongly decreased it. The monitor peptide mRNA was also increased by CCK in AR4-2J cells and was decreased by the antagonist. These findings suggest that the plasma CCK induced by prolonged intake of a high protein diet may be responsible for the adaptative increase in the monitor peptide as well as exocrine proteases in the pancreas.

Monitor peptide; mRNA level; Cholecystokinin (CCK); CCK antagonist; Pancreas; AR4-2J

## 1. INTRODUCTION

The monitor peptide (CCK-releasing peptide), originally found in the bile-pancreatic juice of rats [1], is an essential luminal factor for pancreatic enzyme secretion via CCK-release in rats [2,3]. The infusion of the monitor peptide into the lumen induces hypersecretion of CCK and a subsequent increase in the level of pancreatic enzyme secretion [2].

Previously, we reported that both the concentration in zymogen granules and mRNA level of the monitor peptide as well as pancreatic proteases in the rat pancreas increased in response to prolonged high protein intake [4]. Although the mechanism underlying the increase in the monitor peptide caused by high protein intake remains unclear, it is important to elucidate the mechanisms underlying the adaptative increase in exocrine proteases due to continuous high protein intake. There is growing evidence that CCK is involved in the adaptative increase in pancreatic proteases in response to prolonged high protein intake [5], therefore, CCK may also induce an increase in the monitor peptide level in the pancreas.

The stimulatory effect of CCK on the monitor peptide level in the pancreas was examined. Repeated subcutaneous injection of CCK increased the monitor peptide mRNA level in the rat pancreas and it was suppressed by the CCK-antagonist, CR-1409. To demonstrate the direct effect on pancreatic acinar cells, AR4-2J, a pancreatic acinar cell line, was used.

*Abbreviation:* CCK, cholecystokinin.

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## 2. MATERIALS AND METHODS

### 2.1. *In vivo experiment*

Male Wistar rats (Oriental Bioservice, Kyoto), each weighing 180–200 g, received subcutaneous injections of 1 μg/kg body weight of CCK-8 (Peptide Institute Inc., Osaka) dissolved in saline containing 12% (w/v) hydrolyzed gelatin (Sigma) at 8 h intervals for 5 days. A specific CCK receptor antagonist, CR-1409, was injected at a dose of 5 mg/kg subcutaneously 30 min before each CCK injection. The CR-1409 was provided by Rotta Research Laboratory (Italy). Pancreases were sampled before CCK injection (0 h), and then 4, 10, 24, 48, 72 and 120 h after starting the CCK injections. Total RNA was extracted by the AGPC method [6], denatured and passed through nylon filters. The mRNA was quantified by means of slot-blot hybridization. $^{32}$P randomly primed monitor peptide cDNA and a $^{32}$P-5′-end-labeled oligonucleotide specific to the monitor peptide were used as probes [4]. The rat chymotrypsinogen B and amylase mRNA levels were also determined using the corresponding cDNAs, which were cloned in our laboratory, as probes [7,8]. The conditions for hybridization and the determination of bound radioactivity with a densitometer were the same as given previously [4]. The profile of the increase in the monitor peptide concentration in zymogen granules was confirmed to be the same as that of mRNA in the pancreas [4].

### 2.2. *In vitro experiment*

The AR4-2J cell line [9] was purchased from Dainippon Seiyaku (Osaka). The cells were maintained at 37°C under a 5% $CO_2$/95% air atmosphere in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum. To determine whether or not the cells express the monitor peptide mRNA, poly(A)$^+$ RNA was prepared and analyzed by Northern blotting. For quantitative determination of the change in the mRNA level of the monitor peptide caused by CCK, AR4-2J cells were seeded into 35 mm dishes at an approximate density of 3–4 × $10^4$/cm$^2$. Five days after plating, the cells were about 80% confluent, and the culture medium was replaced by medium (0 h) containing CCK-8 (10 nM) or CCK-8 plus CR-1409 (100 μM), each

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dissolved in PBS, and was changed daily. Total RNA was extracted from the cultured cells before the addition of CCK (0 h), and then 12, 24, 48 and 72 h after the addition of CCK. mRNA was quantified using the reverse transcription-polymerase chain reaction (RT-PCR) method [10]. One $\mu$g of total RNA was reverse transcribed to obtain cDNA with a downstream primer specific to the monitor peptide. PCR was performed with 2 out of the 10 $\mu$l of the cDNA as a template with Heat-TUFF DNA polymerase (Clontech, USA). A combination of upstream and downstream primers was used to detect the monitor peptide mRNA. In preliminary experiments, various PCR conditions were examined with different amounts of total RNA to find the conditions with which different amounts of monitor peptide mRNA proportionally affect the yield of the PCR product. The following conditions were adopted in the present study: 22 cycles performed with a PROGRAM TEMP CONTROL SYSTEM PC-700 (ASTEC, Fukuoka) at 94°C for 1 min, 60°C for 2 min, 72°C for 3 min and 72°C for 6 min at the end with 10 pmol of each pair of primers and 1.5 mM $MgCl_2$. The specificity of the amplification was verified by showing the size fragments of PCR products after enzymatic digestion and by Southern blotting with the monitor peptide or pancreatic secretory trypsin inhibitor specific oligonucleotides as probes [4]. The PCR products were quantified by DNA slot-blot hybridization.

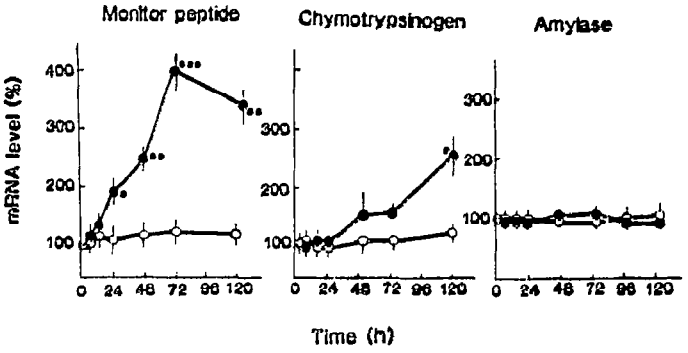


Fig. 1. Effect of CCK on the monitor peptide, chymotrypsinogen B and amylase mRNA levels in rat pancreas. Pancreatic mRNA was determined in saline (containing hydrolyzed gelatin) (○) and CCK-treated (●) animals by slot-blot hybridization with the corresponding cDNAs as probes. Values obtained were expressed as percentages of those of 0 h. Data shown represent means ± S.D. for 4–5 animals for each time point. Significant difference from saline-treated animals: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

## 3. RESULTS AND DISCUSSION

CCK exerts a trophic action on the pancreas [11]. In the present in vivo study, CCK induced marked pancreatic growth, which was suppressed by the CCK antagonist, CR-1409 (data not shown). Therefore, the result of CCK administration to rats in the present study was similar to that reported previously [11].

The CCK-induced change in the monitor peptide mRNA in the rat pancreas was determined by slot-blot hybridization. As shown in Fig. 1, the monitor peptide mRNA level in rats, which was determined using the monitor peptide cDNA as a probe, increased after 24 h CCK administration and reached a maximal level after 72 h. An identical result was obtained using a monitor peptide specific oligonucleotide as a hybridization probe (data not shown). The chymotrypsinogen B and amylase mRNAs were determined as reliable indicators of the pancreatic response to CCK. An increase in chymotrypsinogen B mRNA was observed at 120 h after starting the CCK injections. Amylase mRNA did not change throughout the experiment. The results obtained with the two pancreatic enzymes were compatible with those obtained previously [5]. As Fig. 2 shows, concomitant injection of CR-1409 with CCK blocked the increase in the monitor peptide mRNA significantly, which indicates that the increase in the monitor peptide mRNA in the rats was caused by the exogenous injection of CCK.

The results obtained in an experiment involving the AR4-2J cell line were the same as those obtained in the in vivo experiment. The AR4-2J cell line was established from an azaserine-induced rat pancreatic adenocarcinoma [9]. This cell line has been reported to express exocrine enzymes [12]. Recently, Estival et al. [13] reported that 10 nM caerulein, which is an analogue of CCK, increased the chymotrypsinogen B mRNA in AR4-2J cells. We first examined whether or not the monitor peptide was expressed in this cell line. As shown in Fig. 3, a signal was detected on Northern blotting with the monitor peptide cDNA as a probe. However, as reported previously [4], the cDNA probe could not distinguish the monitor peptide mRNA from that of the pancreatic secretory trypsin inhibitor because of their high nucleotide sequence homology. No hybridization signal with the monitor peptide specific oligonucleotide probe was observed for the cell line. Then, we performed RT-PCR with the monitor peptide specific primers: downstream primer, 5'CTTGCTAAACATTATGGTAGCCC3'; and upstream primer, 5'CCTAATTGCCCTAAGCAAATTATG3'. The RT-PCR product yielded hybridized with the monitor peptide specific oligonucleotide (Fig. 3), but not with the

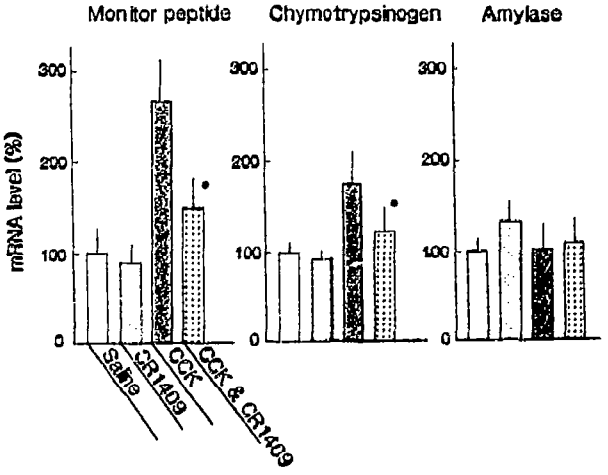


Fig. 2. Effect of a CCK antagonist (CR-1409) on the monitor peptide, chymotrypsinogen B and amylase mRNA levels in rat pancreas. The mRNA levels in the pancreas at 120 h after starting the CCK injections are expressed as percentages of that in the saline-treated animals. Data shown represent means ± S.D. for 4–5 animals. Significant difference from CCK-treated animals: $^{*}P < 0.05$.

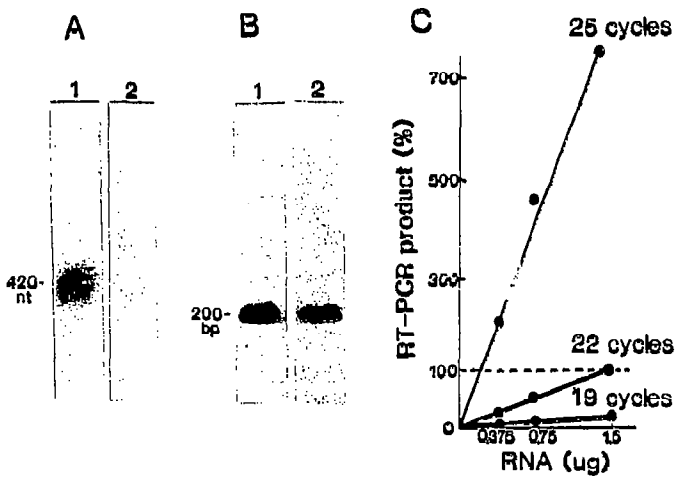


Fig. 3. (A) Northern blot analysis of the monitor peptide mRNA in AR4-2J cells. Poly(A)$^+$ RNA (20 $\mu$g) was hybridized with [$^{32}$P]cDNA of the monitor peptide (lane 1) or with a $^{32}$P-labeled monitor peptide specific oligonucleotide [4] (lane 2). The size of the monitor peptide mRNA is indicated. (B) Southern blot analysis of PCR products: lane 1, cDNA amplified by PCR from the monitor peptide cDNA clone [4] as a template; lane 2, cDNA amplified by RT-PCR from total RNA of the AR4-2J cells. The specific primers for the monitor peptide described in the text were used for amplification. The amplified DNAs were hybridized with the $^{32}$P-monitor peptide specific oligonucleotide [4]. The size of the amplified cDNA of the monitor peptide is indicated on the left. (C) The linear relationship between the amount of applied RNA (0.375, 0.75 and 1.5 $\mu$g) and the corresponding RT-PCR products after various PCR cycles. The RT-PCR products were passed through a nylon filter and then hybridized with the $^{32}$P-monitor peptide specific oligonucleotide [4]. Bound radioactivity was determined by scanning the autoradiograph with a densitomer. Values are expressed as percentages of the density of 1.5 $\mu$g of RNA after 22 cycles.

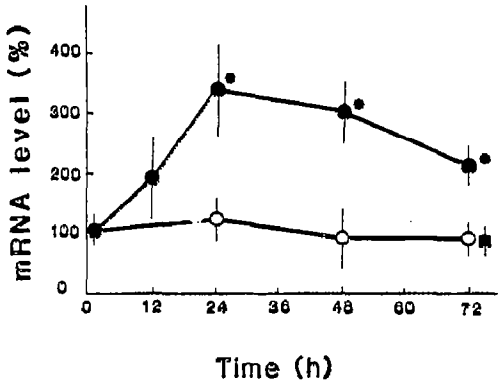


Fig. 4. Effects of CCK and a CCK antagonist on the monitor peptide mRNA level in AR4-2J cells. mRNAs were amplified from PBS- (○), CCK- (●) and CCK plus antagonist-treated cells (■) by means of RT-PCR and the resulting PCR products were quantified by slot-blot hybridization. Values are expressed as percentages of that of 0 h. Data represent means ± S.D. for 3–6 separate dishes. Significant difference from PBS-treated dishes: $^*P < 0.05$.

pancreatic secretory trypsin inhibitor specific oligonucleotide [4]. In addition, 88 and 112 bp fragments, which were expected from the monitor peptide cDNA sequence, were observed by *Bsm*I digestion of the RT-PCR products. *Eco*T22I, which digests the pancreatic secretory trypsin inhibitor cDNA but not the monitor peptide cDNA, did not digest the RT-PCR products. This is the first report of cultured cells expressing the monitor peptide mRNA. A linear relationship was revealed between the amount of applied RNA and the corresponding PCR product up to 25 cycles (Fig. 3). Fig. 4 shows that the addition of CCK to the medium to a final concentration of 10 nM led to an increase in the monitor peptide mRNA level. The increasing profile in the cell study was similar to that observed in an in vivo experiment. In the presence of CR-1409 in the medium, the monitor peptide mRNA was not increased by CCK. These results indicate that exogenous CCK causes an increase in the monitor peptide mRNA level in both rats and a cell culture, which suggests that CCK is responsible for the adaptative increase in the monitor peptide in the pancreas during prolonged high protein diet intake.

In conclusion, we propose a cycle in which the plasma CCK increases the monitor peptide in the pancreas, which in turn induces a high plasma CCK level. Such a cycle may be involved in the adaptative increase in the pancreatic exocrine proteases on prolonged high protein intake. This cycle can slow down when the pancreatic proteases reach a level sufficient for protein digestion, because the monitor peptide in the lumen is easily digested by large amounts of proteases.

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## REFERENCES

[1] Fushiki, T., Fukuoka, S. and Iwai, K. (1984) Biochem. Biophys. Res. Commun. 118, 532–537.
[2] Iwai, K., Fukuoka, S., Fushiki, T., Kodaira, T. and Ikei, N. (1986) Biochem. Biophys. Res. Commun. 136, 701–706.
[3] Iwai, K., Fushiki, T. and Fukuoka, S. (1988) Pancreas 3, 720–728.
[4] Tsuzuki, S., Fushiki, T., Kondo, A., Murayama, H. and Sugimoto, E. (1991) Eur. J. Biochem. 199, 245–252.
[5] Renaud, W., Giorgi, D., Iovanna, J. and Dagorn, J.C. (1986) Biochem. J. 235, 305–308.
[6] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[7] Bell, G.I., Quinto, C., Craik, C.S. and Rutter, W.J. (1984) J. Biol. Chem. 259, 14265–14270.
[8] MacDonald, R.J., Crerar, M.M., Swain, W.F., Pictet, R.L., Thomas, G. and Rutter, W.J. (1980) Nature 287, 117–122.
[9] Jessop, H.W. and Hay, R.J. (1980) In Vitro 16, 212.
[10] Makino, R., Sekiya, T. and Hayashi, K. (1990) Technique 2, 295–301.
[11] Löser, C.H.R., Fölsch, U.R., Sahelijo-Kroun, P. and Creutzfeldt, W. (1989) Eur. J. Clin. Invest. 19, 448–458.
[12] Swarovsky, B., Steinhilber, W., Scheele, G.A. and Kern, H.F. (1988) Eur. J. Cell Biol. 47, 101–111.
[13] Estival, A., Pradel, P., Wicker-Planquart, C., Vaysse, N., Puigserver, A. and Clemente, F. (1991) Biochem. J. 279, 197–201.