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GLUCAGONLIKE PEPTIDE-1(7-36)AMIDE SUPPRESSES GLUCAGON SECRETION AND DECREASES CYCLIC AMP CONCENTRATION IN CULTURED IN-R1-G9 CELLS

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Summary: We previously reported that GLP-1(7-36)amide had glucagonostatic action as well as insulinotropic action in the perfused rat pancreas. In this study, we examined the effect of GLP-1(7-36)amide on glucagon secretion and cAMP concentration in glucagon-secreting cell line, In-R1-G9. GLP-1(7-36)amide (1nM) significantly suppressed glucagon secretion and decreased cAMP concentration in the cells. GLP-1(1-37) did not affect glucagon secretion. It is suggested that inhibitory effect of GLP-1(7-36)amide on glucagon secretion is at least partly mediated by adenylate cyclase system.

Glucagonlike peptide-1(GLP-1)(7-36)amide is a posttranslational processing product of preproglucagon in the mammalian intestine. This peptide has been shown to have a glucose-dependent insulinotropic action in the physiological concentration after nutrient intake(2-3) and thus nominated as a candidate for incretin.

We previously reported that GLP-1(7-36)amide not only stimulated insulin release but also suppressed glucagon secretion in the perfused rat pancreas(4). Drucker has reported that the peptide potentiated the insulin secretion via adenylate cyclase system(1), although the precise mechanism of glucagon suppression has not been elucidated.

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In this study, we examined the effect of GLP-1(7-36)amide on glucagon secretion and cAMP concentration in glucagon-secreting cell line, In-R1-G9, which is one of the clones derived from hamster insulinoma, In-111-R1(5-7).

MATERIALS AND METHODS

(Peptides)
GLP-1(7-36)amide (lot no. 18862) and GLP-1(1-37) (lot no. 9775) were obtained from Peninsula Laboratory (Belmont CA).
(Cell culture)
The In-R1-G9 cells were maintained in plastic culture wells in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS) at 37°C in humidified 5% CO_2 - 95% air. For this study, $5x10^5$ cells were inoculated into culture wells with 1ml of culture medium and were cultured overnight. The following day, the culture medium was removed and the cells were washed twice by changing medium with Hanks' Balanced Salt Solution, then incubated for 1.5, 3, 5, 15, 30 min with a 1ml of test medium. Test medium was RPMI 1640 medium containing 5% FBS, 500μM 3-isobutyl-1-methylxanthine (IBMX), 1000KIU/ml aprotinin and GLP-1(7-36)amide at the concentrations of 0.01-1nM or GLP-1(1-37) at 0.01-1nM. To determine the time course of the response to GLP-1(7-36)amide, 1ml of test medium was removed at various incubation time and stored at -20°C to measure glucagon immunoreactivity(GI) and cAMP. The cells adhered to the plastic wells were treated with trichloroacetic acid and stored at -20°C to measure cAMP.
(Glucagon assay)
GI was measured by the radioimmunoassay procedure as previously described(9) with a minor modification, i.e., use of OAL123, an antiserum specific for the COOH-terminal of the glucagon molecule(8).
(cAMP assay)
The content of cAMP was determined using the radioimmunoassay procedure as previously described (10, 11).
(Data analysis)
The results are expressed as means$\pm$SE of five wells for each experimental condition. Statistical differences were assessed with Student's paired t-test and the differences were considered to be significant at $p<0.05$.

RESULTS

Time course of 1nM GLP-1(7-36)amide action on glucagon secretion was shown in figure 1. GLP-1(7-36)amide reduced glucagon secretion significantly.

Figure 2 shows the effect of varying concentration of GLP-1(7-36)amide and GLP-1(1-37) on glucagon secretion. GLP-1(7-36)amide suppressed glucagon secretion in dose-dependent manner and the suppression was statistically significant at the concentration of

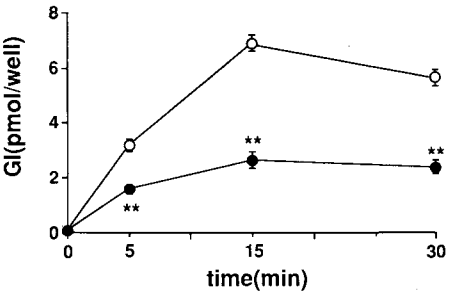


Figure 1. Time course of the effect of 1nM GLP-1(7-36)amide(●) on glucagon secretion from In-R1-G9 cells. Control experiments(o) were incubated without the peptide. Values are means ± SE; n=5. **p<0.001 vs. control.

1nM. GLP-1(1-37) did not affect glucagon secretion at any concentration examined.

The time course of cAMP concentratin in In-R1-G9 cells and in the culture medium were shown in figure 3. The concentration of cAMP in the cells rose rapidly and reached a plateau until 30 min, while cAMP in the medium increased.

Figure 4 shows the effect of varying concentration of GLP-1(7-36)amide on intracellular cAMP concentration in In-R1-G9 cells. GLP-1(7-36)amide at 1nM decreased cAMP concentration significantly.

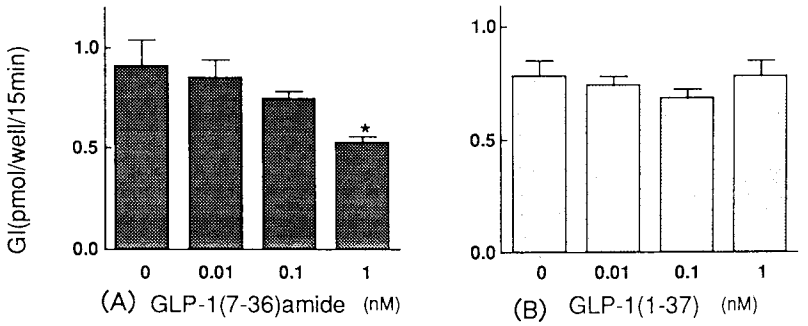
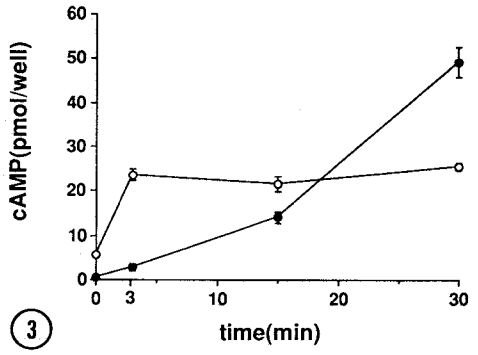


Figure 2. Effect of GLP-1(7-36)amide (A) and GLP-1(1-37) (B) on glucagon secretion from In-R1-G9 cells. Cells were incubated with GLP-1(7-36)amide or GLP-1(1-37) at the concentrations of 0.01, 0.1, 1nM for 15 minutes. Control experiments were incubated without the peptide. Values are means ± SE; n=5. *p<0.05 vs.control.



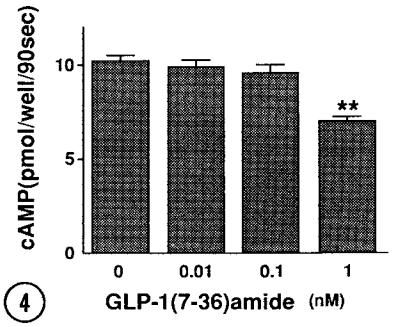


Figure 3. Time course of intracellular (o) and extracellular (•) cAMP concentrations in In-R1-G9 cells in the culture medium. Culture medium contained 500μM IBMX. Values are means $\pm$ SE;n=5.

Figure 4. Effect of GLP-1(7-36)amide on intracellular cAMP concentration in In-R1-G9 cells. Cells were incubated with GLP-1(7-36)amide at the concentrations of 0.01, 0.1, 1nM for 90 seconds in the presence of 500μM IBMX. Control experiments were incubated without the peptide. Values are means $\pm$ SE ;n=5. **p<0.001 vs.control.

DISCUSSION

This study demonstrates that GLP-1(7-36)amide suppresses glucagon secretion and also decreases cAMP concentration in In-R1-G9 cells. Full-sequence GLP-1(1-37) had no effect on glucagon secretion at all. In-R1-G9 cells secrete little quantities of insulin and no somatostatin(5). Thus inhibitory effect of GLP-1(7-36)amide on glucagon secretion is directly exerted without the paracrine effects of insulin and somatostatin in this cell line. It has been reported that intravenous infusions of GLP-1(7-36)amide decreased glucagon concentration in human being(3). We previously reported that GLP-1(7-36)amide suppressed glucagon secretion in isolated perfused rat pancreas(4). However the mechanism of inhibition of the peptide on pancreatic A cells was still unclear. Our present results show that the effect of GLP-1(7-36)amide on glucagon secretion would be mediated by specific receptors associated with adenlate cyclase system.

It has been reported that glucagon secretion from In-R1-G9 cells is stimulated markedly by amino acids and is not affected

by glucose(5). Glucagon secretion is also stimulated by theophilin, 12-o-tetradecanoyl phorbol 13-acetate and calcium ionophore. Therefore protein kinase A, protein kinase C and calcium possibly play important roles in glucagon secretion at least in this cell line(6-7).

On the other hand, it has been shown recently that binding to the receptors of GLP-1(7-36)amide resulted in an increase in both insulin release and cAMP concentration in another cell line, RINm5F(12). This indicates that the effect of GLP-1(7-36)amide is mediated by the adenylate cyclase system.

The reason why GLP-1(7-36)amide exhibited the inhibitory effect on the cAMP production in this In-R1-G9 cell line is not clear. In-R1-G9 may retain the characteristics of insulinoma-derived cell as RINm5F in respect of the GLP-1(7-36)amide receptor-adenylate cyclase system. This effect could be due to interaction of GLP-1(7-36)amide with specific receptors associated with the adenylate cyclase system. The coupling systems of GLP-1(7-36)amide receptor and the adenylate cyclase still awaits further investigation in the respective cells.

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REFERENCES

1. Drucker D.J., Philippe J., Mojsov S., Chick W.L., and Habener J.F.(1987) Proc Natl Acad Sci USA. 84,3434-8.

2. Holst J.J., Ørskov C., Vagn Nielsen O., and Schwartz T.W.(1987) FEBS Lett. 211,1169-1174.

3. Kreymann B., Williams G., Ghatei M.A., and Bloom S.R. (1987) Lancet. 5,1300-1303.

4. Komatsu R., Matsuyama T., Namba M., Watanabe N., Itoh H., Kono N., and Tarui S.(1989) Diabetes. 38,902-905.

5. Takaki R., Ono J., Nakamura M.,Yokogawa Y., Kumae S., Hiraoka T., Yamaguchi K., Hamaguchi K., and Uchida S.(1986) In Vitro. 22,120-126.

6. Ono J., Kumae S., Sato Y., and Takaki R.(1986) Diabetes Res Clin Pract. 2,29-34.

7. Ono J., Yamaguchi K., Okeda T., Asano T., and Takaki R.(1988) Diabetes Res Clin Pract. 4,203-207.

8. Nishino T., Kodaira T., Shin S., Imagawa k., Shima k., Kumahara Y., Yanaihara C., and Yanaihara N.(1981) Clin Chem. 27,1690-1697.

9. Namba M., Matsuyama T., Itoh H., Imai Y., Horie H., and Tarui S.(1986) Regul Pept. 15, 121-128.

10. Kimura H., Thomas E., and Murad F.(1974) Biochim Biophys Acta. 343,519-524.

11. Mashita K., Kawamura S., Kishino B., Kimura H., Nonaka K., and Tarui S.(1982) Endocrinology. 110, 1023-29.

12. Göke R. and Conlon J.M.(1988) J Endocrinol. 116,357-362.