Pergamon

Life Sciences, Vol. 57, No. 7, pp. 729-734, 1995

0024-3205/95 $9.50 + .00

0024-3205(95)00324-X

EFFECT OF GLUTEN EXORPHINS A5 AND B5 ON THE POSTPRANDIAL PLASMA INSULIN LEVEL IN CONSCIOUS RATS

Shin-ichi Fukudome [a], Akira Shimatsu [b], Hiroyuki Suganuma [c] and Masaaki Yoshikawa [c]

[a] Research Control Department, Nisshin Flour Milling Co., Ltd.
Nihonbashi, Chuo-ku, Tokyo 103, Japan
[b] Second Division, Department of Internal Medicine, Faculty of Medicine
Kyoto University, Kyoto 606, Japan
[c] Department of Food Science and Technology, Faculty of Agriculture
Kyoto University, Kyoto 606, Japan

(Received in final form May 22, 1995)

Summary

The effect of exogenous opioid peptides, gluten exorphins A5 and B5, which were isolated from the enzymatic digest of wheat gluten, on the postprandial insulin level were examined in rats. The oral administration of gluten exorphin A5 at a dose of 30 mg/kg w. potentiated the postprandial plasma insulin level and the effect was reversed by naloxone. The administration of gluten exorphin B5 showed a similar effect at a higher dose (300 mg/kg w). Furthermore, intravenous administration of gluten exorphin A5 at a dose of 30 mg/kg w. also stimulated the postprandial insulin release. The fact that orally and intravenously administered gluten exorphin A5 stimulates insulin release suggests that it modulates pancreatic endocrine function by the action after the absorption rather than within the the gastrointestinal tract.

Key Words: opioid, peptide, gluten, exorphin, insulin, glucagon

Exogenous opioid peptides derived from foodstuffs such as wheat gluten and casein have been shown to exert a modulating effects on pancreatic and gastric exo- and endocrine functions (1-7). It has been demonstrated that intragastric administration of the pepsin digest of wheat gluten stimulated the postprandial insulin release in dogs, and its effect was reversed by the opioid antagonist, naloxone (1).

In a previous study, we have isolated five novel opioid peptides, Gly-Tyr-Tyr-Pro-Thr, Gly-Tyr-Tyr-Pro, Tyr-Gly-Gly-Trp-Leu, Tyr-Gly-Gly-Trp and Tyr-Pro-Ile-Ser-Leu from the enzymatic digest of wheat gluten and named them gluten exorphins A5, A4, B5, B4 and C, respectively (8,9). They have structures quite different from the endogenous and exogenous opioid peptides already reported.

In the present study, the effect of gluten exorphins A5 and B5 on the postprandial insulin, glucagon release and blood glucose level were examined in conscious rats.

Corresponding Author: Masaaki Yoshikawa, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan. Fax: (81) (75) 753 6274.

Materials and Methods

All studies were performed in the following catheterized conscious rats. Male Wistar rats weighing 300-400 g were anesthetized with 350 mg/kg b.w. of chloral hydrate. For collecting blood, the jugular vein was catheterized by a polyethylene catheter. After the operation, they were individually caged, kept on a 12-12 h light-dark cycle, and fed standard laboratory chow for 3 days.

In the experiment concerning the oral administration of gluten exorphins, the rats received an intragastric test meal dissolved in saline through a metal sonde according to the following protocols after 3 hours fasting. The first group of rats received glucose at a dose of 2 g/kg w. A second group of rats received glucose at a dose of 2 g/kg w. and gluten exorphin A5 (Gly-Tyr-Tyr-Pro-Thr) or B5 (Tyr-Gly-Gly-Trp-Leu) at a dose of 30 mg/kg w. A third group of rats received 2 g/kg w. glucose, gluten exorphin A5 or B5 at a dose 30 mg/kg w. and naloxone at a dose of 10 mg/kg w. A fourth group of rats received glucose at a dose of 2 g/kg w. and an L-amino acid mixture which was equal to L-amino acids composed of gluten exorphin A5 or B5 at a dose of 30 mg/kg w. A fifth group of rats received glucose at a dose of 2 g/kg w. and gluten exorphin A5 or B5 at a dose of 300 mg/kg w.

The experiment concerning the intravenous administration of gluten exorphins were carried out according to the following protocol. After 3 hours fasting, rats were intravenously infused saline or gluten exorphin A5 at a dose of 30 mg/kg w. through the jugular vein catheter immediately after orally receiving glucose at a dose of 2g/kg w. through a metal sonde.

All blood samples obtained from the catheter were transferred into chilled tubes containing aprotinin. After centrifugation, the separated plasma was stored at -20°C until time of assay. Plasma insulin and glucagon levels were determined by radioimmunoassay using the kits from Incstar Corporation and Daiichi Radio-Isotope Laboratory, respectively. Glucose was measured by the glucose oxidase method using the GA-1120 autoanalyzer (Kyoto Daiichikagaku, Kyoto).

Gluten exorphins A5 and B5 were synthesized by *t*-Boc method. Naloxone was obtained from Endo Lab. Other reagents used were reagent grade or better.

For statistical comparisons, Student's t-test for paired data was employed and P values of 0.05 or less were considered significant.

Results

Effect of gluten exorphins A5 and B5 on the postprandial plasma insulin, glucagon and glucose levels after the oral administration.

At a dose of 300 mg/kg w., the oral administration of gluten exorphins A5 and B5 significantly potentiated glucose-induced insulin release at 15 min ($P < 0.01$-0.05, vs saline; Fig. 1). The plasma glucagon levels in rats fed gluten exorphins were higher than that in saline at 90 min ($P < 0.01$-0.05; Fig. 1). However, plasma glucose levels did not change significantly among the intragastric test meals.

At a dose 30 mg/kg w., the administration of gluten exorphin A5 only potentiated the postprandial insulin release at 15 min as well as that at a dose of 300 mg/kg w. ($P < 0.05$, vs saline; Fig. 2A).

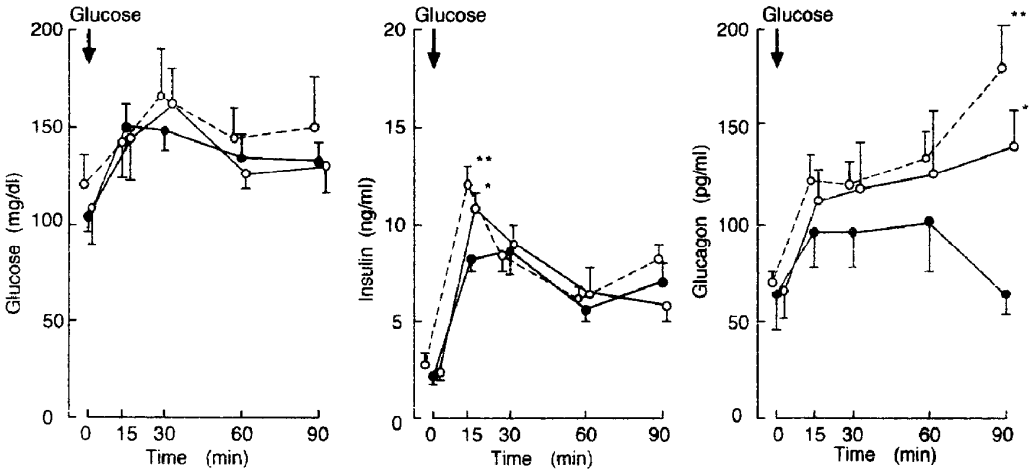


Fig.1

Effect of orally administered gluten exorphin A5 (300mg/kg w.) or B5 (300mg/kg w.) on the plasma levels of postprandial glucose (left), insulin (center) and glucagon (right) in conscious rats (mean±SEM). gluten exorphin A5 (n=5), open circle with solid line; gluten exorphin B5 (n=5), open circle with dotted line; saline (n=5), filled circle (**P<0.01, *P<0.05).

This effect was reversed by the administration of naloxone ($P < 0.05$, vs gluten exorphin A5; Fig. 2A). The administration of the L-amino acid mixture, which had the same composition to gluten exorphin A5, had no effect on the postprandial insulin release ($P < 0.05$, vs gluten exorphin A5). In the above examination concerning gluten exorphin A5 at a dose of 30 mg/kg w., the postprandial glucagon and glucose levels did not change significantly among the intragastric test meals within the 90 min experimental period. On the other hand, the administration of gluten exorphin B5 at a dose of 30 mg/kg w. had no effect on the postprandial insulin level in the absence or presence of naloxone (Fig. 2B). It had no effect on the postprandial glucagon and glucose level within the 90 min experimental period.

Effect of gluten exorphin A5 on the postprandial plasma insulin after the intravenous administration.

To examine whether the transit of gluten exorphin A5 in gastrointestinal tract is a necessary step for the stimulation on insulin release, the peptide was administered intravenously. At a dose 30 mg/kg w., the intravenous administration of gluten exorphin A5 potentiated the postprandial insulin release at 15 min by the same degree as that of the oral administration of gluten exorphin A5 ($P < 0.05$, vs saline; Fig.3). Plasma glucose levels did not change significantly in response to gluten exorphin A5 within the 90 min experimental period.

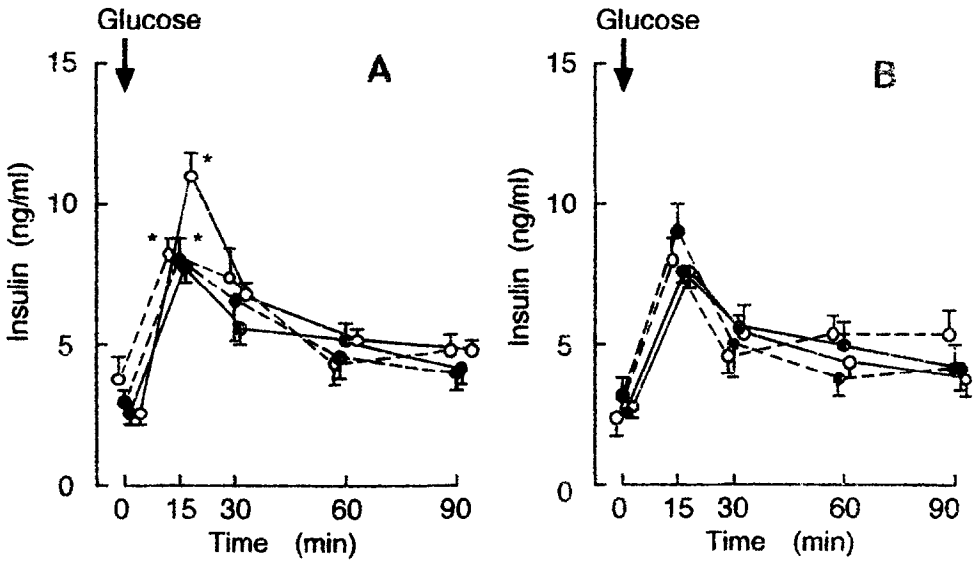
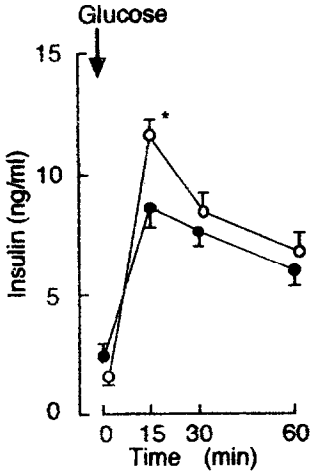


Fig.2
Effect of orally administered gluten exorphin A5 (30mg/kg w.) (A) or B5 (30 mg/kg w.) (B) on the plasma levels of postprandial insulin in conscious rats (mean ± SEM).
(A) gluten exorphin A5 (n=10), open circle with solid line; gluten exorphin A5 + naloxone (n=6), filled circle with dotted line; L-amino acid mixture (n=5), open circle with dotted line or saline (n=11), filled circle with solid line (*P<0.05).
(B) gluten exorphin B5 (n=9), open circle with solid line; gluten exorphin B5 + naloxone (n=5), filled circle with dotted line; L-amino acid mixture (n=5), open circle with dotted line; saline (n=11), filled circle with solid line (*P<0.05).

Fig.3
Effect of intravenously administered gluten exorphin A5 (30mg/kg w.) on the plasma levels of postprandial insulin in conscious rats (mean ± SEM). gluten exorphin A5 (n=5), open circle with solid line; saline (n=5), filled circle with solid line (*P<0.05).



Discussion

The present study demonstrates that the intragastric or intravenous administration of gluten exorphins A5 and B5 influence postprandial insulin secretion in rats. The oral administration of gluten exorphin A5 at a dose of 30 or 300 mg/kg w. rapidly potentiated the postprandial glucose-induced increase in insulin release. In addition, its intravenous administration at a dose of 30 mg/kg w. also showed the same effect. The oral administration of gluten exorphin B5 stimulated the

postprandial insulin release at a dose of 300 mg/kg w., however, the effect was not recognized at a dose of 30 mg/kg w. Though the opioid activity of gluten exorphin B5 is higher than that of gluten exorphin A5 in the GPI (guinea pig ileum) and MVD (mouse vas deferens) assays (8), it might be that gluten exorphin B5 was more rapidly hydrolyzed compared to gluten exorphin A5 *in vivo*.

The effect of gluten exorphin A5 on the insulin secretion in the intragastric administration is mediated via opioid receptors as shown by the antagonistic effect of naloxone. The peak glucose level did not change significantly in the presence or absence of gluten exorphin A5. This means elevation of the insulin level by gluten exorphin A5 is not a secondary effect due to the increase in glucose level. Similar results have been reported in the action of the other exogenous opioid peptides such as gluten hydrolyzate or ß-casomorphins on the insulin release (1,2). It has been also reported that the stimulatory effect of enkephalins on insulin secretion was rapid and transient (10). The increase in the insulin level by gluten exorphin A5 might be short to reduce the postprandial glucose level. On the other hand, it has been reported that the infusion of ß-endorphin increased plasma insulin and glucagon levels and decreased plasma glucose level in type 2 diabetic patients (11-13). In this regard, it is of interest whether the postprandial insulin release and glucose level in type 2 diabetic model animals might be modulated by gluten exorphin A5.

The mechanism of modulation of the secretory action of pancreatic hormones by exogenous opioid peptides is still not clear (1-5). However, it has been guessed whether the modulation might be exerted at the level of the opioid receptors within the gastrointestinal tract, thereby influencing the hormonal and/or neural signal between the gut and islets of Langerhans, or at the level of islets of Langerhans after absorption from gut (2). In addition, some studies have suggested that small peptides can be absorbed intact from the gastrointestinal tract after oral administration (14-15). From the result that both intragastric and intravenous administration of gluten exorphin A5 influenced postprandial insulin secretion, it is suggested that the potentiation might be caused at the level of islets of Langerhans after its absorption from the gut rather than at the level of opioid receptors within the gastrointestinal tract. It is also possible that the effect of gluten exorphin A5 might be mediated primary via the action to somatostatin, calcium ions or cAMP in islets such as that of endogenous opioid peptides, enkephalins and ß-endorphin (16-19). On the other hand, it has been reported that μ-receptor agonists such as ß-casomorphins had stimulatory effects on insulin and glucagon secretion, while ∂-receptor agonists such as Leu-enkephalin had inhibitory effects and all these effects were antagonized by naloxone in dogs (2-5). In this regard, it is of interest that gluten exorphin A5 which is rather selective for ∂-receptors showed the stimulatory effect on insulin secretion in rats (8).

Schusdziarra et al. reported that the pepsin digest of wheat gluten having opioid activity stimulated glucagon release and the effect was not reversed by naloxone in dog (1). In the present study, the administration of gluten exorphins A5 or B5 at a higher dose (300 mg/kg w.) elevated the postprandial glucagon release in rats. However, it is not clear whether it is due to the opioid activity or not.

Gluten exorphin A5 has an important N-terminal Gly which is essential for the opioid activity and has a significantly different structure from other opioid peptides ever reported. The gluten exorphin A5 sequence is found 15 times in the primary structure of glutenin. In addition, the release of this peptide from wheat gluten is accomplished by the concerted actions of pepsin and microbial neutral proteases such as thermolysin (8). Therefore, it is not certain whether gluten exorphin A5 itself was responsible for the increase in the postprandial plasma insulin level after oral ingestion of pepsin digest of gluten in dogs as reported by Schusdziarra et al (1). It is also possible that the larger fragments containing gluten exorphin A5 sequence, which exists in the pepsin digest

of wheat gluten, potentiates the postprandial insulin release. The present study shows that oral or intravenous administration of gluten exorphins A5 and B5 can influence the regulation of postprandial insulin release.

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