Study Reports Tottori Univ Col Med Care Technol : 16, 1~8, 1991.

Effects of atropine and propranolol on glucose- and arginine-induced insulin release in rats

Tadasu IKEDA, Izumi OHTANI,* Katsumi FUJIYAMA*, Tazue HOSHINO,* Yasushi TANAKA*, Tatsuo TAKEUCHI* and Hiroto MASHIBA*

Tottori University College of Medical Care Technology,
and *The First Department of Internal Medicine, Tottori University
School of Medicine, Yonago 683, Japan

Several observations lead to support the suggestion that the pancreatic beta-cell responds in different ways to an oral ingestion and an intravenous infusion of nutrients[1~3]. It is well known fact that insulin concentration in plasma provoked by a certain amount of orally ingested glucose is several times higher than that provoked by intravenously infused glucose, which is injected by a corresponding amount for blood concentration (incretin effect)[1~3]. The insulin response to a meal is of the result from composite neural and endocrine input to the islets. Both of the sympathetic and parasympathetic nervous systems are known to affect on the secretion of insulin[4~6], and blockades of sympathetic or parasympathetic nervous systems (beta-adrenergic blockade or atropine) have been reported to reduce the insulin response after oral or intravenous glucose[7~11]. However, the effect of the autonomic nervous system on insulin secretion after oral or intravenous ingestion of nutrients remains to be fully elucidated. In this study, the effect of an administration of atropine or propranolol into rats on insulin secretion after intraduodenal or intravenous infusion of glucose or arginine was investigated to elucidate the possible role of the autonomic nervous system in insulin response.

Materials and methods

Animals

Male Wistar albino rats weighing approximately 200g were provided through this study. After fasting overnight, the rats were anesthetized with intraperitoneal pentobarbital sodium (30mg/kg), then being exposed to bilateral femoral veins.

Intravenous (IV) infusion of glucose or arginine

Glucose (0.5g/kg in a 20% solution) or L-arginine (0.5g/kg in a 20% solution) was infused into the femoral vein, and blood was drawn from the contralateral femoral vein at given times.

Intraduodenal (ID) infusion of glucose or arginine

After anesthesia, the abdomen was opened. Then the equal amount (0.5g/kg in a 20% solution) of glucose or L-arginine was infused into the duodenum through a polyethylene cannula, and blood was drawn from the femoral vein at given times.

Atropine or propranolol pretreatment

In some groups of the experiments, atropine sulphate (1mg/kg, Tanabe Seiyaku, Osaka, Japan) or propranolol hydrochloride (0.5mg/kg, ICI Pharma, Osaka, Japan) was injected subcutaneously 30 min before the administration of the inducers.

Measurements

Blood glucose concentration was measured by a glucose oxidase method. Insulin and glucagon were analysed by respective radioimmunoassays[12, 13], of which sensitivities were estimated to be 2μU/ml for insulin and 20pg/ml for glucagon. Intra- and interassay coefficients of variation were 5 and 10% for insulin and 7 and 15% for glucagon, respectively.

Statistics of data

The data were expressed as the mean ± SD. Analysis of variance and two-tailed Student's non-paired t test were applied.

Results

Basal levels of blood glucose and plasma insulin were not altered by the atropine or propranolol administration. While basal plasma glucagon level was not altered by atropine but raised slightly by propranolol (Figs. 1 and 2).

Responses of blood glucose, plasma insulin and plasma glucagon to the glucose ingestion

Figure 1 shows variations of three kinds of deduction from the glucose infusion. In a case of ID ingestion, blood glucose was increased in the similar pattern in spite of the presence of atropine or propranolol. While increase of plasma insulin was in somewhat different pattern from that in blood glucose, and at a 30min point after ID ingestion, insulin reached to the peak values of 80 ± 13, 67 ± 15, and 50 ± 15μU/ml in control, atropine and propranolol groups, respectively. In the middle left figure, at 30 and 60 min points, plasma insulin level was significantly reduced under the propranolol pretreatment. Plasma glucagon was suppressed in the similar

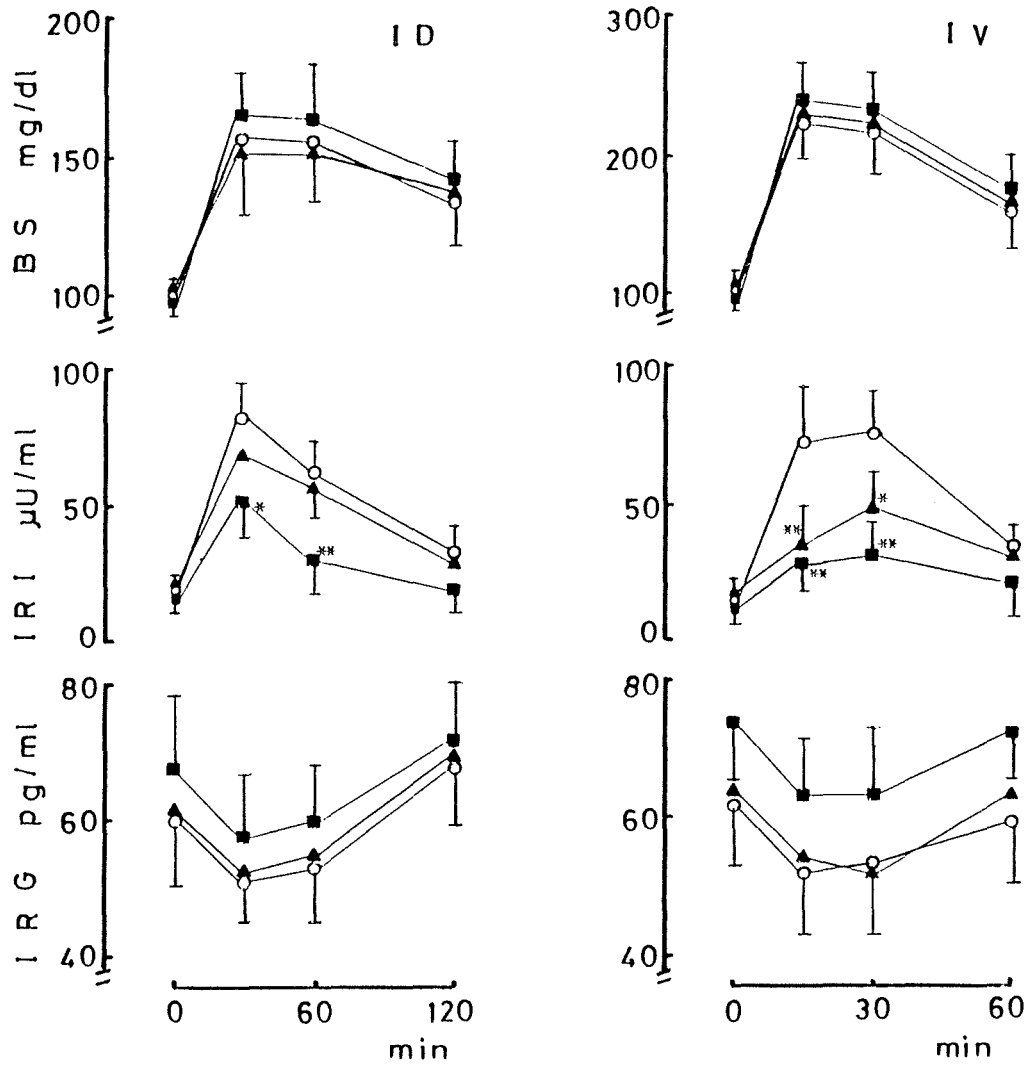

Fig. 1 Blood glucose (BS), plasma insulin (IRI) and plasma glucagon (IRG) responses to the glucose ingestion in rats. Bars represent SD, ID, intraduodenal ingestion (left side), IV; intravenous ingestion (right side), ○ control group (n=7), ■ propranolol-treated group (n=7), ▲ atropine-treated group (n=7), $^{*}p<0.05$ and $^{**}p<0.02$, points significantly different from control.

manner regardless of the blockades.

In another case of IV ingestion, blood glucose and plasma glucagon showed more or less similar variations as in the case of ID ingestion. Besides, plasma insulin was increased, and the peak values at a 30 min point after IV ingestion were 75 ± 16, 47 ± 15, and 32 ± 11 μU/ml at 30 min in control, atropine and propranolol groups, respectively. In the middle right figure, at 15 and 30 min points, plasma in-

sulin level was extremely reduced under the propranolol or even atropine.

Responses of blood glucose, plasma insulin and glucagon to the arginine ingestion

Figure 2 shows variations of three kinds of deduction from the arginine infusion. Blood glucose was slightly increased by the ID arginine but its level remained lower by the IV arginine, regardless of the blockades. Plasma insulin was slightly increased by ID or IV arginine but the blockades were not available. As compared ingestive routes, arginine was again slightly more inducible to plasma insulin by IV than ID route. Plasma glucagon was increased by arginine through ID and IV routes, regardless of the blockades, though the extent of its release was still weak.

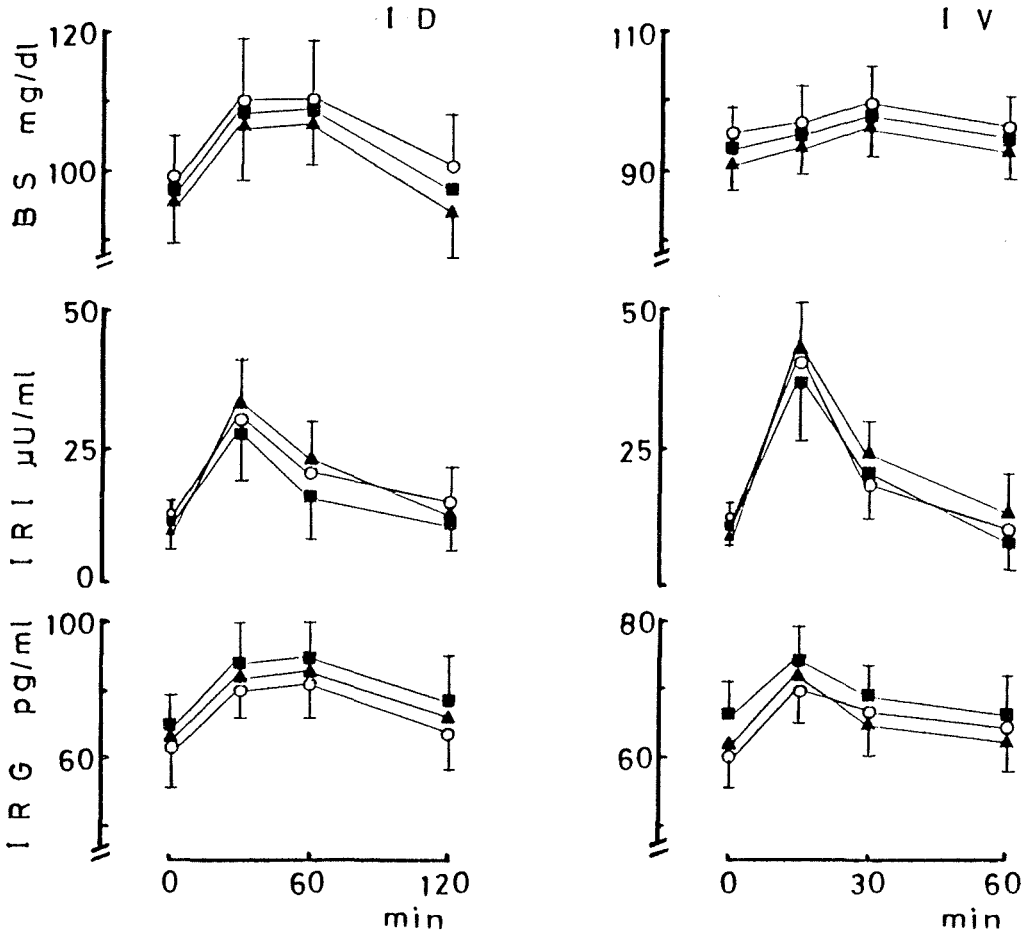

Fig. 2 Blood glucose, plasma insulin and plasma glucagon responses to the arginine ingestion.

See legends at the footnote of Fig. 1.

Discussion

In the intravenous glucose infusion test, insulin concentration in rat plasma was significantly reduced by an administration of propranolol. It has been reported that glucose-induced insulin release in men is reduced by the propranolol administration[9, 10], while Robertson and Porte[14] could detect no alteration in the insulin response in men to intravenous glucose during propranolol infusion. Acetylcholine has been known to enhance glucose-induced insulin release[4, 5]. The administration of a cholinergic blockade, atropine, was sure to significantly inhibit this time intravenous glucose-induced insulin release in rats. Henderson et al.[7], however, have reported that insulin release brought about by intravenous glucose is not altered by atropine (1mg) in men. While, Daniel et al.[15] have reported that atropine inhibits insulin release as stimulated by intravenous glucose in rhesus monkeys. These contradictions may be due to the difference of species. In rats so far, both cholinergic and beta-adrenergic blockades may inhibit the release of intravenous, glucose-induced insulin.

Because atropine or propranolol might alter the gastric emptying rate and subsequent glucose absorption, glucose was administered directly into the duodenum instead of oral infusion. Atropine hardly reduced insulin secretion in intraduodenal glucose ingestion. Because the quantity of insulin secreted after the oral glucose administration is affected by gastrointestinal factors[1~3], insulin secreted this time after an intraduodenal glucose may be almost due to influence by insulinotropic intestinal factors (incretin effect), rather than due to the glucose stimulation. Atropine may have not affected the incretin effect and thus an inhibitory effect of atropine on glucose-induced insulin release may be overcome by the incretin effect in the intraduodenal glucose infusion test. Henderson et al.[7] have reported that insulin release after oral glucose is significantly diminished by atropine in men. This discrepancy may be due to the difference of the route of administration, because atropine is a strong inhibitor of gastric emptying.

On the other hand, insulin release by intraduodenal glucose was suppressed by propranolol administration. This result agreed with the report of Imura et al.[8], which the insulin response to oral glucose was impaired during concomitant infusion with propranolol in men. Glucose absorption in rats was not altered by propranolol, though Flaten et al.[16] have reported that propranolol inhibits release of the gastric inhibitory polypeptide (GIP, most probable candidate of incretin) after intraduodenal glucose in men. These suggest that propranolol may inhibit insulin release by intraduodenal glucose probably through the mechanism of

the decreased GIP secretion and/or inhibitory effect on some insulinotropic intestinal hormone(s). Further studies are needed to explain well this phenomenon.

Plasma insulin level after intravenous arginine was slightly higher than that after intraduodenal arginine. Raptis et al.[17] and Moxley et al.[18] have independently reported that the insulin response to amino acids after oral or intraduodenal infusion is greater than that after intravenous infusion in men. While, Nogowski et al.[19] have reported that arginine is four times more effective when given in rabbits by the intravenous way than by the oral one. These differences may depend on species applied and/or methods examined. In this study, neither atropine nor propranolol did affect insulin secretion by intraduodenal or intravenous arginine, suggesting that cholinergic and beta-adrenergic systems may have no important roles in arginine-induced insulin secretion.

In conclusion, it may be able to draw that both cholinergic and beta-abrenergic mechanisms may have important roles in insulin secretion induced by intravenous glucose not by intravenous arginine, and that the beta-adrenergic mechanism, but not the cholinergic one, may affect insulin release in rats by oral glucose.

Summary

Effects of atropine or propranolol on insulin or glucagon release in rats by secretagogues, glucose or L-arginine, were investigated. In highly sensitive measurement, the insulin release by the intravenous ingestion of glucose was sure to be extremely blocked in the presence of either atropine or propranolol, while by the intraduodenal ingestion it was blocked not by atropine but by propranolol. On the contrary, the insulin release in response to L-arginine by both of the ingestive routes was not blocked at all. Besides, the glucagon release by glucose or L-arginine was not altered at all.

These findings postulate the possible role of the autonomic nervous system to release of the pancreatic hormones; (1) cholinergic mechanism my be involved in insulin release by IV glucose, (2) beta-adrenergic mechanism may be involved in insulin release by IV and ID glucose, and (3) autonomic nervous system may not be involved in both of insulin and glucagon responses to L-arginine.

References

1) McIntyre, N., Holdworth, C. D. and Tnrner, D. S., J. Clin. Endocrinol. Metab., **25**, 1317-1326, 1965.

2) Creutzfeldt, W., Diabetologia, **16**, 75-85, 1979.
3) Unger, R. H. and Eisentraut, A. M., Arch. Intern. Med., **123**, 261-266, 1969.
4) Iversen, J., Diabetes, **22**, 381-387, 1973.
5) Kaneto, A. and Kosaka, K., Endocrinology, **95**, 676-681, 1974.
6) Day, J. L., Metabolism, **24**, 987-996, 1975.
7) Henderson, J. R., Jefferys, D. B., Jones, R. H. and Stanley, D., Acta Endocrinol., **83**, 772-780, 1976.
8) Imura, H., Kato, Y., Ikeda, M., Morimoto, M. and Yawata, M., J. Clin. Invest., **50**, 1069-1079, 1971.
9) Cerasi, E., Luft, R. and Efendic, S., Acta Endocrinol., **69**, 335-346, 1972.
10) Raptis, S., Dollinger, H., Chrissiku, M., Rothenbuchner, G. and Pfeiffer, E. F., Europ. J. Clin. Invest., **3**, 163-168, 1973.
11) Flaten, O., Sand, T. and Myren, J., Acta Physiol. Scand., **113**, 415-416, 1981.
12) Yalow, R. S. and Berson, S. A., J. Clin. Invest., **39**, 1157-1175, 1960.
13) Nishino, T., Kodaira, T., Shin, S., Imagawa, K., Shima, K., Kumahara, Y., Yanaihara, C. and Yanaihara, N., Clin. Chem., **27**, 1690-1697, 1981.
14) Robertson, R. P. and Porte, D., J. Clin. Invest., **52**, 870-876, 1973.
15) Daniel, P. M. and Henderson, J. R., Acta Endocrinol., **78**, 736-745, 1975.
16) Flaten, O., Scand. J. Gastroenterol., **16**, 641-645, 1981.
17) Raptis, S., Dollinger, H. C., Schröder, K. E., Schleyer, M., Rothenbuchner, G. and Pfeiffer, E. F., New Engl. J. Med., **288**, 1199-1202, 1973.
18) Moxley, R. T., Lockwood, D. H., Amatruda, J. M., Tobin, J.D. and Pozefsky, T., Diabetes, **27**, 78-84, 1978.
19) Nogowski, L. and Nowak, K. W., Horm. Metab. Res., **18**, 730-733, 1986.

(Received February 20, 1991)