Shimane J. Med. Sci., Vol.9, pp.21-28, 1985

POSSIBLE ROLE OF PROSTAGLANDINS IN THE REGULATION OF INSULIN RELEASE FROM RAT PANCREATIC ISLETS

(Langerhans' islet/prostaglandins/insulin release)

Yohzi MASAKI, Keiichiro TANIGAWA, Satoshi OHGUNI, Shinichi TSUCHIYAMA, and Shinya NOTE

Department of Internal Medicine, Shimane Medical University, Izumo 693, Japan

(Received June 5, 1985/Accepted July 24, 1985)

To clarify whether prostaglandin synthesis is involved in the regulation of insulin release from isolated rat islets, we performed three different experiments. The direct effects of prostaglandin E_1 and prostacyclin on insulin release were investigated. Neither prostaglandin had any effect in the presence of low and high concentrations of glucose. Indomethacin, aspirin® (acetylsalicylic acid) and flurbiprofen, inhibitors of cyclooxygenase, did not have a significant effect on insulin release evoked by glucose. Exogenous phospholipase A_2 purified from bee venom and melittin stimulated insulin release. A potent and selective inhibitor of phospholipase A_2, mepacrine, inhibited glucose-induced insulin release. The present work indicates that 1) cyclooxygenase does not participate early release of insulin from islet 2) activation of phospholipase A_2 might be involved in insulin stimulus-secretion coupling.

Although prostaglandin (PG) biosynthesis in pancreatic islets has been demonstrated and PGs have been implicated in the regulation of hormone secretion from this organ, controversy exists concerning the modulatory action of PGs in endocrine

function (for reviews, see refs. 1, 2).

In the present study, we have intended to clarify the role of PG in the regulation of insulin release from isolated rat islets. The following were studied: 1) the direct effect of PGE_1 and prostacyclin (PGI_2) on insulin release was tested; 2) the effect of indomethacin, aspirin®(acetylsalicylic acid) and flurbiprofen, potent inhibitors of cyclooxygenase, on glucose-stimulated insulin release was examined; and 3) the precursor of PGs is long-chain, polyunsaturated fatty acid (arachidonic acid) cleaved from phospholipid in the plasma membrane by the activation of endogenous phospholipase A_2 (3). We studied the effect of melittin, a 26-amino acid polypeptide contained in bee venom and an activator of phospholipase A_2 (4), on insulin release. The direct effect of exogenous phospholipase A_2 on insulin release was also studied.

MATERIALS AND METHODS

Islets of Langerhans were obtained from the pancreas of fed, male Sprague-Dawley rats by the collagenase method (5). Standard buffer solution was a Krebs-Ringer Bicarbonate Buffer (KRB) supplemented with 0.25% bovine serum albumin and 3.75 mM glucose. After being preincubated for 45 min at 37°C under a gas phase of 95% O_2 and 5% CO_2, the islets were transfered to an incubation vial containing 0.5 ml of the buffer with and without test substances. The incubation was then performed for 30 min under the same conditions as described for the preincubation. Insulin concentrations in the medium were measured by polyethylene glycol radioimmunoassay (6). Indomethacin and melittin were purchased from Sigma Chemical Co. Bee venom phospholipase A_2 was from Calbiochem-Boehring. Aspirin®(Acetylsalicylic acid) and flurbiprofen were gifts from Shionogi pharmaceutical Co. and Kakenyaku Co., respectively. PGE_1 and prostacyclin were kindly donated by Ono Pharmaceutical Co. Results were expressed as means ± SEM unless stated otherwise. Statistical analysis was performed with the unpaired "Student's" t test.

RESULTS

PGE_1 or prostacyclin was added to the medium at concentrations ranging from 10^{-7}M to 10^{-5}M. No significant

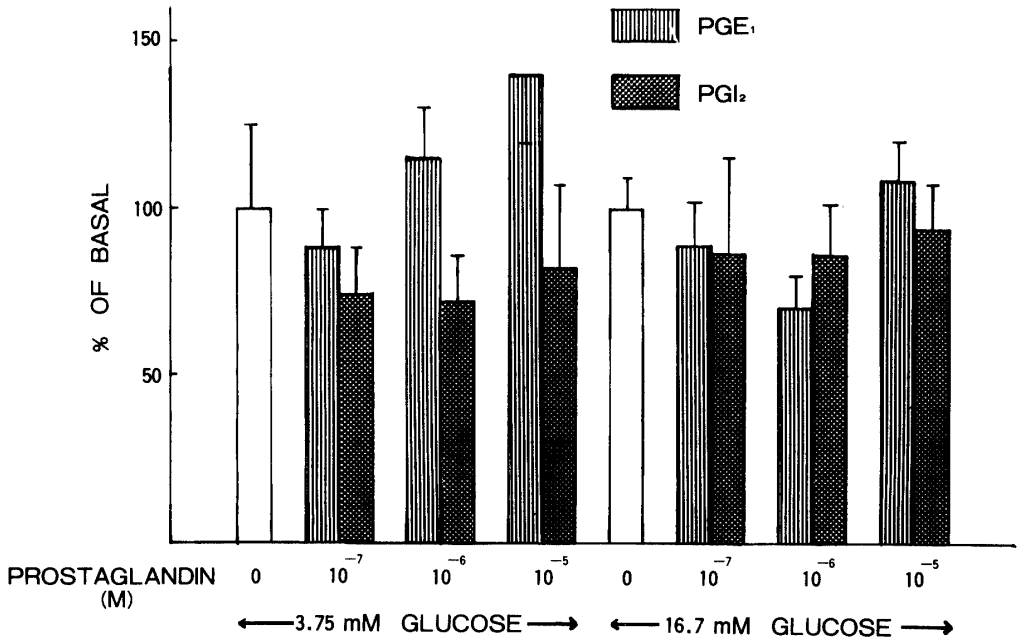

Fig.1. Effect of PGE_1 and Prostacyclin on insulin release. The amounts of insulin release were 13.3 ± 1.9 or 346.7 ± 31.2 μU/islet/30 min in the presence of either 3.75 or 16.7 mM glucose alone, respectively. Results were expressed as % of control. Each column and bar represents the mean ± SEM of 8 experiments.

Table I. EFFECT OF VARIOUS AGENTS UPON INSULIN RELEASE EVOKED BY 16.7 mM GLUCOSE.

Agent	Insulin release[a] (n)
Nil	203.2 ± 23.5 (7)
Indomethacin (M)	
$2x10^{-5}$	220.9 ± 18.0 (7)
$2x10^{-4}$	233.7 ± 33.4 (8)
$2x10^{-3}$	278.5 ± 22.4 (8)
Aspirin® (Acetylsalicylic acid)(M)	
$5x10^{-5}$	242.6 ± 13.3 (6)
$5x10^{-4}$	212.0 ± 28.1 (6)
$5x10^{-3}$	252.8 ± 16.9 (6)
Flurbiprofen (M)	
$1x10^{-6}$	198.2 ± 28.1 (6)
$1x10^{-5}$	204.1 ± 31.8 (6)
$1x10^{-4}$	223.2 ± 19.4 (6)

[a]Mean values (±SEM) are expressed as microunits per 30 min/islet.

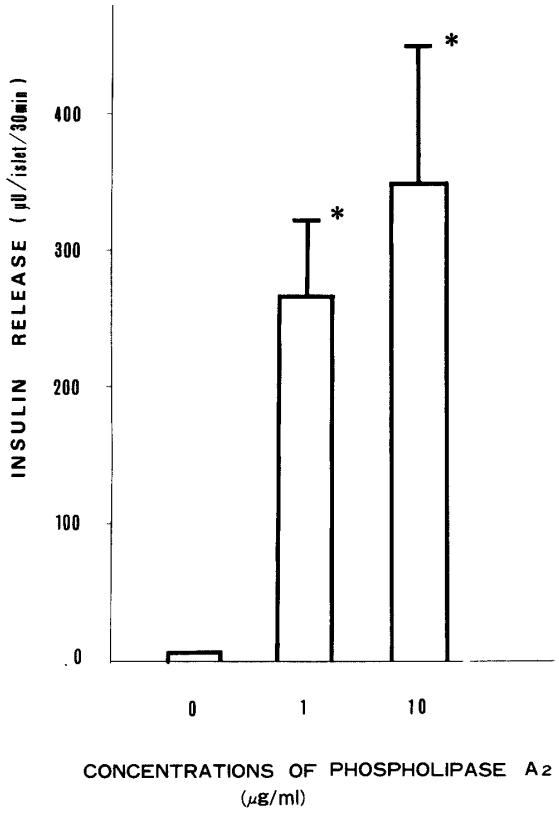

Fig.2. Effect of phospholipase A_2 on insulin release from the isolated islets incubated in 3.75 mM glucose. Each column and bar represents the mean ± SEM of 8 observations. *P < 0.001 versus islets incubated in the absence of phospholipase A_2.

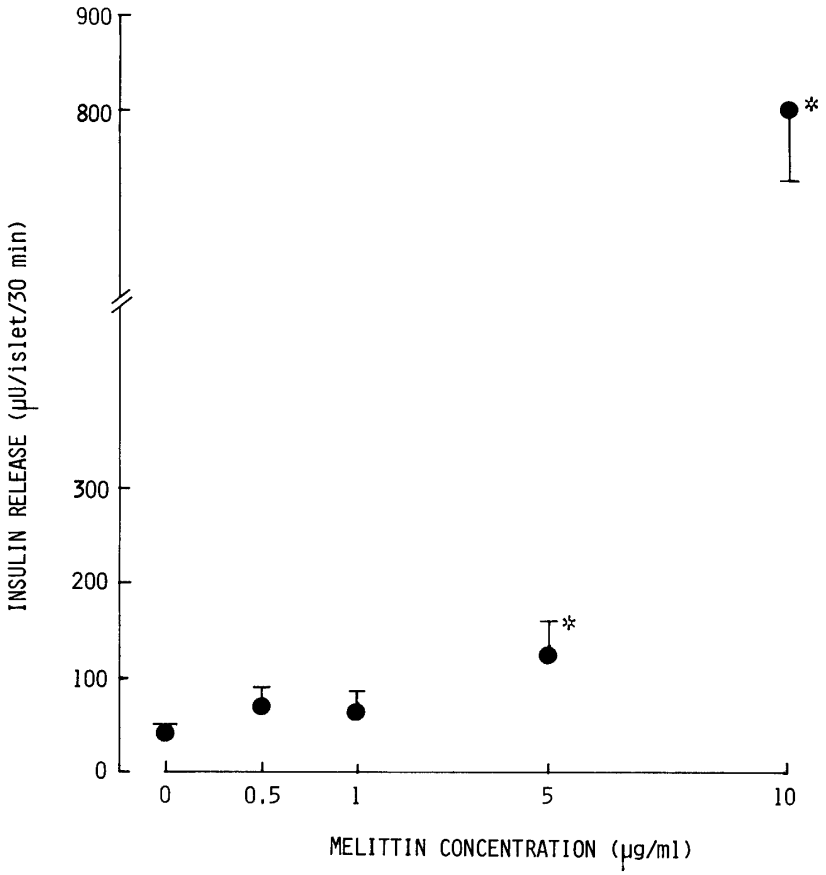

Fig.3. Effect of melittin on insulin release from the isolated islets incubated in 3.75 mM glucose. Results are presented as mean values ± SEM for 10-13 experiments. *P < 0.01 relative to absence of melittin.

changes in the insulin release were found at either low (3.75 mM) and high (16.7 mM) glucose concentrations (Fig. 1). Neither indomethacin, aspirin® (acetylsalicylic acid) nor flurbiprofen produced any significant effect on insulin release evoked by 16.7 mM glucose (Table I). At the concentration of 1 mM of indomethacin glucose-induced insulin release appeared to be enhanced. Figure 2 illustrates the stimulatory effect of phospholipase A_2 on insulin release in the presence of 3.75 mM glucose. Insulin secretion from the islets incubated with 0.5 µg/ml phospholipase A_2 was approximately 10 fold higher than that of control (7.5 ± 0.6 vs. 92.7 ± 15.5 µU/islet/30min $P < 0.001$). Both 1 µg/ml and 10 µg/ml phospholipase A_2 caused a further increase in insulin release. Melittin was a potent stimulator of insulin release and the result confirmed previous observation (7) (Fig. 3). A significant effect of melittin on insulin secretion was observed at a concentration of 5 µg/ml (Fig. 3). The effect of exogenous phospholipase A_2 was much more potent than melittin at the concentration of less than 5 µg/ml. On the other hand, the addition of 50 µM mepacrine resulted in a marked decrease in insulin secretion evoked by glucose (395.0 ± 69.8 vs. 258.8 ± 46.7 µU/islet/30min $P < 0.05$).

DISCUSSION

Both PGE_1 and prostacyclin were without significant effect on insulin release from isolated rat pancreatic islets at 3.75 mM and 16.7 mM glucose concentrations. These results accord with previous findings by Vance *et al.* (8) and Rossini *et al.* (9) Johnson *et al.* found an enhanced insulin release by PGE_1 in the presence of 1.0 mM theophylline (10). They proposed that stimulation of adenylate cyclase was the mechanism through which PGs augmented insulin release. Probably differences in the experimental models may be responsible for the inconsistencies in the data.

It is well known that most anti-inflammatory agents, such as indomethacin, aspirin® (acetylsalicylic acid) and flurbiprofen, decrease PG synthesis via cyclooxygenase. Therefore in the second step of experiments we examined the effect of these drugs on the insulin release. None of these drugs affected glucose-induced insulin release significantly. Addition of high concentration of indomethacin showed a tendency to enhance

insulin release, but insulin release was markedly stimulated by indomethacin, when islets were incubated in the absence of glucose (data not shown). Thus a toxic effect on islet function can not be excluded as an explanation of such increases in insulin secretion. These findings may support the observation that lipoxygenase pathway is involved in insulin stimulus-secretion coupling in the beta cell rather than cyclooxygenase pathway (11). In contrast to our present results, Dunlop *et al.* recently reported that both acetylsalicylic acid and indomethacin augmented insulin release in a dose-dependent fashion from neonatal islets (12). They have also indicated that other nutrients, excluding glucose, failed to respond to inhibitors of cyclooxygenase. Morgan *et al.* have demonstrated that flurbiprofen enhanced glucose-stimulated insulin release, but the islets were incubated for 20 hrs in their study (13). Such observations could not explain the possible role of PGs in initiating the process of insulin release from adult pancreatic islets.

Phospholipase A_2 has been shown to liberate arachidonic acids from membrane phospholipids and thus provide the substrates for PGs synthesis. The enzyme was found in rat pancreatic islet (14) and phospholipase A_2 stimulated glucagon release from the isolated guinea pig pancreatic islets (15). Therefore it was of interest to determine if phospholipase A_2 affects insulin release from islets of rat pancreas. The results presented here clearly showed that bee venom phospholipase A_2 stimulated insulin release. According to a recent study by Fujimoto *et al.* exogenous phospholipase A_2 (Naja naja venom) stimulated prostaglandin E_2 production in a dose-dependent manner in rabbit kidney and enhanced the release of unsaturated fatty acids from the medulla slices (16). A similar study using isolated islets of rat pancreas is now in progress in our laboratory.

Next, a set of experiments was performed to test the effect of melittin, an activator of phospholipase A_2, on insulin release. The result indicated that melittin is a potent stimulator of insulin release(Fig. 3). Thus phospholipase A_2 may play some role in regulating hormone secretion from the islets. However, at present we do not have convincing evidence about the effect of melittin on insulin release in relation to prostaglandin synthesis. Further experiments will be needed to elucidate the mechanisms involved.

ACKNOWLEDGEMENTS

The authors are grateful to Drs. T. Fujii and A. Tamura, Kyoto College of Pharmacy, for their generous gift of phospholipase A_2 and to Mrs. A. Kawakami for her skillful secretarial assistance.

REFERENCES

1) Robertson, R.P.(1979) Prostaglandin as modulators of pancreatic islet function. Diabetes, 28, 943-948
2) Robertson, R.P. and Stewart, A.M. (1979) Sounding board. Prostaglandins, the glucoreceptor, and diabetes. N. Engl. J. Med., 301, 1446-1447
3) Vogt, W. (1978) Role of phospholipase A_2 in prostaglandin formation. In Advances in Prostaglandin and Thromboxane Research. (Galli, C., ed) pp. 89-95, Raven Press, New York
4) Grandison, L. (1984) Stimulation of anterior pituitary prolactin release by melittin, an activator of phospholipase A_2. Endocrinology, 114, 1-7
5) Lacy, P.E. and Kostianovski, M. (1967) Method for the isolation of intact islets of Langerhans from the pancreas. Diabetes, 16, 35-39
6) Desbuquios, S. and Auerbach, G.D. (1971) Use of polyethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassay. J. Clin. Endocrinol., 33, 732-738
7) Morgan, N.G. and Montague, W. (1984) Stimulation of insulin secretion from isolated rat islets of Langerhans by melittin. Bioscience Reports, 4, 665-671
8) Vance, J.E., Buchanan, K.D., and Williams, R.H. (1971) Glugagon and insulin release, influence of drugs affecting the autonomic nervous system. Diabetes, 20, 78-82
9) Rossini, A.A., Led, J.B., and Frawley, T.F. (1971) An unpredictable lack of effect of prostaglandins on insulin release in isolated rat islets. Diabetes, 20 (supple 1), 374
10) Johnson, D.G., Fujimoto, W.Y., and Williams, R.H. (1973) Enhanced release of insulin by prostaglandins in isolated pancreatic islets. Diabetes, 22, 658-663
11) Metz, S.A., Murphy, R.C., and Fujimoto, W. (1984) Effects on glucose-induced insulin secretion of lipoxygenase- derived metabolites of arachidonic acid. Diabetes, 33, 119-124

12) Dunlop, M., Christanthou, A., Fletcher, A., Veroni, M., Woodman, P., and Larkins, R. (1984) Effects of inhibitors of eicosanoid synthesis on insulin release by neonatal pancreatic islets. Biochem. Biophys. Acta, 713, 581-588

13) Morgan, R.O. and Pek, S.B. (1984) Role of arachidonate lipoxygenase and cyclooxygenase products in insulin and glucagon secretion from rat pancreatic islets. Metabolism, 33, 928-935

14) Laychock, S.G. (1982) Phospholipase A_2 activity in pancreatic islets is calcium-dependent and stimulated by glucose. Cell calcium, 3, 43-54

15) Luyckx, A.S. and Lefebvre, P.J. (1980) Prostaglandins (PGS) and glucagon release: effect of phospholipase A_2 and indomethacin on isolated islets. Diabetes, (supple 2) 29, 106A

16) Fujimoto, Y., Akamatsu, N., Hattori, A., and Fujita, T. (1984) Stimulation of prostaglandin E_2 synthesis by exogenous phospholipase A_2 and C in rabbit kidney medulla slices. Biochem. J., 218, 69-74