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Inhibitory Effects of PGE_1 on Glucose Induced Insulin Release in Isolated Islets of Langerhans

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Since prostaglandins are increasingly shown to play an important role on insulin secretion, an evaluation of the PGE_1 action on insulin release by isolated islets of Langerhans has been undertaken. Pancreatic islets were prepared after a modification of the Lacy and Kostianovsky technique and incubated in Hanks solution containing glucose 1.63 or 16.3 mM and/or PGE_1 10^{-5} M. The insulin released was measured after 15, 30 or 60 min of incubation. The results obtained show that PGE_1 does not modify insulin release induced by glucose 1.63 mM (non stimulant concentration) but that this PG significantly diminishes the insulin release induced by glucose 16.3 mM (stimulant concentration) after 15 and 30 min of incubation.

Key words: PGE_1, Insulin release, Islets of Langerhans.

Studies on the role of arachidonic acid metabolites in islets have, in general, followed two major themes: the effects of exogenous prostaglandins and lipoxygenase products on islets function and the effects resulting from inhibition of synthesis of these compounds in the islet.

The majority of *in vivo* studies indicate that prostaglandins inhibit insulin release (6, 14, 17, 19, 20). These findings contrast with several reports of stimulatory effects of prostaglandins on insulin release in the perfused rat pancreas (1, 9, 10, 22) or isolated pancreatic islets (7, 13).

However, other studies demonstrate that in cultured pancreas the inhibition of prostaglandin E synthesis increases glucose-induced insulin release (4, 12). Several reports have also suggested that both stimulation and inhibition of insulin secretion by prostaglandins is possible in the same pancreatic preparation (3, 12).

In view of the growing evidence that prostaglandins play an interesting role in islet-hormone secretion, we have undertaken an evaluation of a biphasic action of prostaglandins E_1 on insulin release.

Insulin was measured by radioimmu-

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noassay techniques and purified rat insulin served as standard.

Materials and Methods

Isolation of islets. — Pancreatic islets were prepared by a modification of the technique of LACY and KOSTIANOVSKY (8) and SHIBATA *et al.* (18). Wistar rats weighing 200-250 g were anesthetized with pentobarbital (50 mg/kg) intraperitoneally prior to the operative procedures, and the abdomen was opened.

The distal end of the common bile duct was clamped adjacent to the duodenum and the acinar tissue was disrupted by injecting 50-60 ml of Hanks solution into the common bile duct. The body and tail of the pancreas were removed, washed in Hanks solution and cut into small pieces with scissors. The tissue was transferred to a conical graduate cylinder, collagenase (1000 U/ml) (18) was added, and the preparation was then incubated for 10-12 min at 37° C. The incubation was finished by adding 10 ml of ice-cold Hanks and the mixture was allowed to settle for 10 min. The supernatant was removed and the sediment containing the islets was resuspended in ice-cold Hanks solution and allowed to settle for another 10 min.

This procedure was repeated four times. The sediment remaining was diluted with Hanks solution, transferred to a glass dish and examined with a dissecting microscope. In order to test the viability of islets, a trypan blue exclusion test was performed.

The islets were aspirated with a micropipette and placed into incubation baskets filled until 1 ml with Hanks solution.

Incubation procedure. — Five islets were placed in each basket, and incubation was subsequently carried out by placing the baskets in a thermostatized bath. The gas phase was 95 % and 5 % CO_2 and the baskets were preincubated at 37° C for 35 min. After preincubation, glucose and/or prostaglandin E_1 were added. Incubation was continued and samples were taken out at 0, 15, 30 and 60 min to measure insulin release.

Solutions and drugs. — Hanks solution has the following composition mM: NaCl 136, KCl 5.36, $CaCl_2$ 1.26, $MgSO_4$ 0.8, Na_2HPO_4 0.33, KH_2PO_4 0.44, and glucose 1.63 or 16.3 mM. $NaHCO_3$ was added until pH = 7.4. Prostaglandin E_1: 5 mg was disolved in 0.5 ml ethanol 95 % and 4.5 ml Na_2CO_3 0.02 %.

Prostaglandin E_1 and Collagenase (type V) were purchased from Sigma. All other reagents were of analytical grade.

Insulin assay. — Insulin values were estimated by radioimmunoassay techniques (Serono Insulin kit). Purified rat insulin (Novo Research Institute, Denmark) served as standard.

Presentation of results. — In view of the considerable interexperimental variation, quantitative comparisons have been made between the variables measured within the same experiments. Student's t test was used for unpaired data.

Results

The effects of 10^{-5} M of prostaglandin E_1 on insulin release were tested together with 1.63 mM glucose (non stimulant concentration) in incubations of 15, 30 or 60 min. These effects were compared with the insulin release by 1.63 mM glucose without prostaglandin E_1 (fig. 1). The results obtained show that prostaglandin E_1 does not significantly modify insulin release in these experimental conditions (table I).

To further test the importance of prostaglandin E_1 action on glucose induced insulin release, the effects of 10^{-5} M pros-

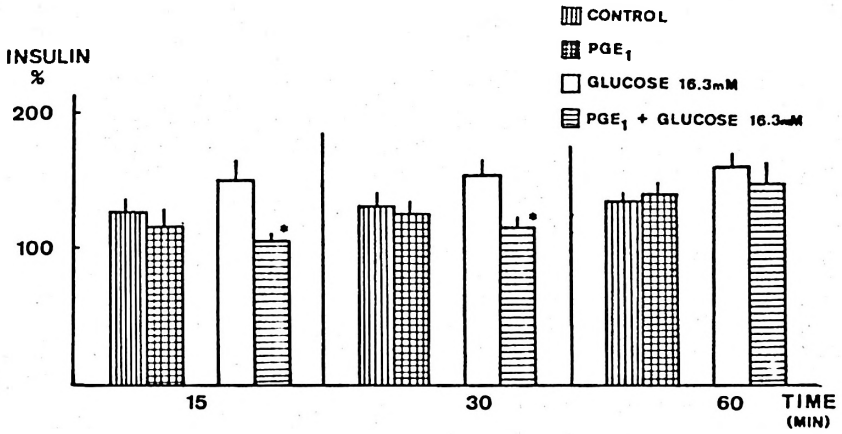

Fig. 1. *Effects of PGE$_1$ (10^{-5}M) on control (glucose 1.63 mM) or glucose induced insulin release (glucose 16.3 mM) in incubations of 15, 30 or 60 minutes.*
Values are expressed as a percentage respect to 0 time and are means of 5 experiments.

taglandin E_1 were tested together with a stimulant concentration of glucose (16.3 mM) which promotes an elevation of insulin release by isolated islets of Langerhans. When the effects of 16.3 mM glucose in the presence or absence of prostaglandin E_1 were compared (fig. 1) it was apparent that the release of insulin significantly diminished in the presence of prostaglandin E_1 but only after 15 and 30 min of incubation time (table I).

After 60 min of incubation in a medium containing 10^{-5} M prostaglandin E_1 and 1.63 mM glucose, no difference was observed with respect to the group of islets incubated with 1.63 mM glucose alone.

Discussion

Contradictory results have been reported concerning the effects of prostaglandins on insulin secretion from isolated pancreatic islets. Both stimulatory (7, 13, 14) and inhibitory (5, 12) effects were reported and therefore it cannot be concluded categorically that prostaglandins are intrinsically either stimulatory or inhibitory since they can have opposite effects

Table I. *Effect of PGE$_1$ on basal or glucose induced insulin release. Results at 15, 30 or 60 min are expressed as a percentage respect to 0 time.*
Values are means ± s.e., number of experiments: 5. * Significant difference $p < 0.05$ from 1.63 mM or 16.3 mM glucose.

	TIME (min)			
	0	15	30	60
Glucose 16.3 mM	100	125.0 ± 7.1	131.4 ± 7.5	134.7 ± 4.1
PGE$_1$ + Glucose 16.3 mM	100	118.4 ± 6.3	127.5 ± 9.1	137.8 ± 6.9
Glucose 16.3 mM	100	146.8 ± 15.8	149.0 ± 12.7	153.3 ± 9.1
PGE$_1$ + Glucose 16.3 mM	100	112.2 ± 5.4*	118.7 ± 7.9*	146.3 ± 14.8

depending upon how the islet is stimulated. It is interesting to note that many investigators have studied total insulin secretion over at least one hour (7, 15) and do not normally distinguish between effects on insulin secretion of arachidonic acid metabolites themselves and their effects on glucose-induced insulin secretion.

The purpose of the present work was to investigate the effect of prostaglandin E_1 on basal insulin release, as compared to insulin secretion induced by glucose and its modification by prostaglandin E_1.

The results of our studies have shown that adding prostaglandin E_1 produces an inhibition of glucose-induced insulin release in isolated islets of Langerhans. This inhibition was observed after 15 and 30 min of incubation in the presence of prostaglandin E_1.

However, 60 min after the prostaglandin E_1 addition, no significant differences were found between insulin concentrations released by islets incubated in presence or absence of prostaglandin E.

These data could explain the contradictory results obtained *in vitro* when prostaglandin action on insulin secretion was studied. Most studies report data that reflect insulin secretion in one hour (7, 15) and found that prostaglandin enhances insulin release in isolated pancreatic islets. This does not contradict the results obtained in the present work, but the initial decrease in the insulin secretion showed at 15 and 30 min could go unnoticed in studies covering a time period of at least one hour.

It is important to recall that in most tissues, prostaglandins are rapidly metabolized and exert their action in a short period of time. Consequently, it is clearly possible that after one hour of incubation, prostaglandin E_1 is metabolized and its effects are therefore not detected.

As mentioned above, it is also important to distinguish between the effect of prostaglandin E_1 itself on insulin secretion and its effect on glucose-induced insulin secretion. Present data have clearly shown that under the influence of prostaglandin E_1, islet activity is characterized by an apparent inability to respond to glucose signals, and the secretory response is the same at high or low glucose concentration. The reason for emphasizing these points is that the major defect in insulin secretion in diabetics involves glucose as a stimulus and includes a lack of insulin response to an intravenous glucose challenge. Consequently, the concept has emerged that there may be a specific problem with recognition of glucose by the pancreatic islets. In this regard, it is worthwile recalling from the information provided that prostaglandins E could have an interesting role in the pathogenesis of Diabetes mellitus (2, 11, 16).

WAITZMAN *et al.* (21) found a higher concentration of prostaglandins in blood from diabetic patients, compared with blood from non-diabetics, and METZ (11) after observing that prostaglandins inhibit insulin release in response to glucose, and that rat pancreatic cell cultures synthesize and release prostaglandins E, suggests that glucose can stimulate the production of an inhibitor of insulin secretion by the beta-cell, probably prostaglandins, thus completing a classic negative feed-back loop.

These evidences and our results suggest that defects on insulin release in diabetes, could therefore be due, in part, to an excessive production of PGs, that involve a failure in the beta-cells to respond to glucose signals.

Resumen

Se estudia la acción de la PGE_1 sobre la liberación de insulina en islotes de Langerhans aislados siguiendo la técnica de Lacy y Kostianovsky. Se incuban en solución de Hanks con glucosa 1,63 mM ó 16,3 mM y/o PGE_1 10^{-5} M. La insulina liberada

se mide a los 15, 30 y 60 min de incubación. La PGE_1 no modifica la liberación de insulina inducida por glucosa 1,63 mM, concentración no estimulante, pero sí la disminuye significativamente a 16,3 mM concentración estimulante tras 15 ó 30 min de incubación.

Palabras clave: PGE_1, Liberación de insulina, Islotes de Langerhans.

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