Effect of Prooxidants on Insulin Secretion by the Isolated Rat Pancreas

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B cells of the Langerhans islets are known to be sensitive to organic peroxides and reactive oxygen species [3,6]. Free radicals exert a direct cytotoxic influence on the Langerhans islets and participate in the mechanism of alloxan and streptozotocine diabetes and damage caused by pancreatropic viruses [6,8]. Protein and nonprotein SH groups are crucial for insulin secretion [1]. Modification of SH groups due to activation of lipid peroxidation is probably one of the factors affecting insulin secretion.

The aim of the present study was to investigate the effect of prooxidants on insulin secretion in the isolated rat pancreas.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 280-320 g maintained on a standard diet. The pancreas preparation was obtained as described elsewhere [4]: briefly, the pancreas was removed under urethane anesthesia (1 g per kg body weight) by separating the duodeno-pancreatic complex from the colon, stomach, and spleen. The isolated pancreatic preparation was placed on a thermostatically controlled platform. Afferent and efferent cannulas were inserted into *a.celiaca* and *v.porta*, respectively, and equilibrium perfusion was performed during 20 min with the following medium: 4.4 mM KCl, 2.3 mM $CaCl_2 \times 2H_2O$, 1.5 mM KH_2PO_4, 29.3 mM $NaHCO_3$, 11.28 mM NaCl, 1.2 mM $MgSO_4 \times 7H_2O$, 4.5 mM glucose, and dextran T-40 (4%). The basal and stimulated insulin secretion was assessed with glucose concentrations of 4.5 and 16.7 mM. For constant pH maintenance and oxygenation the medium was aerated with a 95% O_2 and 5% CO_2 mixture. The measurement and precise control of the temperature were performed with an electronic thermometer. Perfusion pressure (30-35 mm Hg) was created by a peristaltic pump (Peripump D, type 5187, Hungary), the rate of the perfusion flow being 4 ml/min. Reagents were introduced into the perfusion medium by means of a fine pipette. The outflowing perfusion medium was collected into plastic tubes, snap frozen and stored at -18°C. Viability of the preparation was assessed by the rate of glucose-stimulated insulin secretion and by the duodenal motility [4]. The concentration of insulin in the perfusion medium was determined using an RIO-INS-PG-^{125}I kit with some modifications. Peroxidation was induced by infusing the

TABLE 1. Effect of Tert-Butyl Hydroperoxide and $FeSO_4$ on TBARS Content and the Rate of Glutathione Release in Isolated Rat Pancreas ($M \pm m$)

Index	Control	Prooxidant
TBARS, nmol/g	130±15 (n=6)	230±25 (n=6)
GSH, nmol/g	4.4±0.2 (n=8)	1.1±0.1 (n=8)
GSSG, nmol/g	2.3±0.2 (n=8)	2.2±0.2 (n=8)
GSH/GSSG ratio	1.9	0.5

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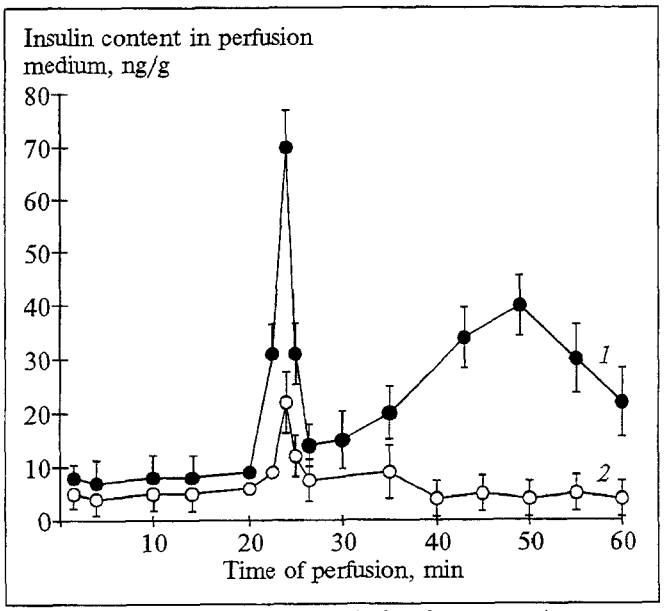

Fig. 1. Insulin secretion in the isolated pancreas in response to addition of glucose (16.7 mM). *a*) glucose concentration in perfused medium 4.5 mM; *b*) glucose concentration in perfused medium 16.7 mM; *1*) control; *2*) after preperfusion with tert-butyl hydroperoxide (10^{-4}) and $FeSO_4$ (10^{-4}).

mixture of prooxidants: tert-butyl hydroperoxide and $FeSO_4$ in a final concentration of 10^{-4} M. The content of thiobarbituric acid-reactive substances (TBARS) in the pancreas was measured spectrophotometrically [8]. Glutathione in the perfusion medium was measured using ortho-phthaldialdehyde with a Hitachi 650 spectrofluorometer (Japan) [5]. Statistical processing of the results was performed on an IBM PC using Statgraf software.

RESULTS

The perfusion of the pancreas with a medium containing 4.5 mM glucose was accompanied by a low level of the basal insulin secretion accounting for 1 ng/g tissue/min. Increasing the glucose concentration to 16.7 mM resulted in a rapid elevation of the insulin secretion to 70 ng/g 2-3 min after the addition of the stimulator. The rapid phase of secretion switched after 5-8 min to the slow phase of hormone release, the rate of secretion at this time being 40 ng/g (Fig. 1). These results are in conformity with the data reported by other investigators, who observed the characteristic biphasic glucose-induced release of the hormone in the isolated pancreas.

The perfusion of the pancreas with the medium containing tert-butyl hydroperoxide and $FeSO_4$ in a final concentration of 10^{-4} for 20 min resulted in a twofold elevation of the TBARS content (Table 1). The rate of basal insulin secretion under these conditions did not change. The addition of glucose in a concentration of 16.7 mM after preperfusion with prooxidants led to a less pronounced stimulation of insulin secretion during the first phase (20 ng/g) and to a marked decrease of the hormone release during the second phase (Fig. 1)

Thus, the preperfusion with prooxidants activated free-radical lipid peroxidation and reduced the sensitivity of the pancreatic islets to glucose, the major inductor of secretion.

The stimulus-secretion coupling in the B cells of the Langerhans islets is known to depend on the redox state of the membrane thiols, which is largely determined by the ratio of reduced (GSH) to oxidized (GSSG) glutathione [1]. Moreover, SH groups are the most probable target for lipoperoxides, and so the oxidation of SH groups may influence the release of oxidized glutathione from the B cells and, probably, the proportion between the reduced to oxidized forms of the tripeptide [2,7,10]. Indeed, without tert-butyl and $FeSO_4$ the ratio between the outflow rates of the reduced and oxidized forms is 1.9, whereas the addition of prooxidants lowered this ratio to 0.5 (Table 1). The overall decrease of the rate of glutathione release in response to prooxidants is probably due to the formation of mixed disulfides and depletion of the GSH pool in the cell [2].

Thus, under conditions of LPO activation the rate of glucose-stimulated insulin secretion in the isolated rat pancreas is decreased with a simultaneous shift of the proportion between the reduced and oxidized forms in the glutathione system, which modulate B-cell sensitivity to glucose. This may represent one of the mechanisms of the development of hypoinsulinemia in states of the organism characterizing by LPO activation.

REFERENCES

1. H. P. T. Ammon and M. N. Mark, *Cell. Biochem. Funct.*, **3**, № 3, 157 (1985).
2. G. Bellomo, F. Mirabelli, P. Richelmi, and G. Finardi, *Hum.Toxicol*, **8**, № 2, 152 (1989).
3. K. Grankvist and S. L. Marklund, *Int. J. Biochem.*, **18**, № 2, 109 (1986).
4. G. M. Grodsky and H. Heldt, *Meth. Diabet. Res.*, **1**, Pt. B, 137 (1984).
5. P. I. Hissin and R. Hilf, *Analyt. Biochem*, **74**, 214 (1976).
6. W. J. Malaisse, *Biochem. Pharmacol.*, **31**, № 22, 3527 (1982).
7. N. Masaki, M. E. Kyle, and J. L. Farber, *Arch. Biochem*, **269**, № 2, 390 (1989).
8. D. Pipeleers and M. Van de Wincel, *Proc. Nat. Acad. Sci. USA*, **83**, № 14, 5267 (1986).
9. Y. Yagi, M. Matsuda, and K. Yagi, *Experientia*, **32**, № 7, 905 (1976).
10. A. S. Yalcin, A. Killing, G. Gulcan, *et al.*, *Biochem. Arch.*, 5, 147 (1989).