Glycation of insulin in a cultured insulin-secreting cell line.

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One of the many consequences of hyperglycaemia associated with diabetes is the non-enzymatic glycosylation (glycation) of proteins. Glycation can affect blood proteins [1], structural proteins [2], functional proteins [3] as well as peptide hormones [4]. Glycation can lead to alterations in conformation and/or function of both structural and functional proteins [5-6].

Recent studies in our laboratory have demonstrated significant increases in percentage glycated insulin and proinsulin in pancreatic extracts of hyperglycaemic animal models of diabetes and lean mouse islets cultured in hyperglycaemic conditions [7]. Other investigations have shown that human insulin is glycated preferentially at the amino terminal Phe[1] site of the B-chain [8].

The present study was initiated to examine the characteristics of intracellular glycation of insulin in pancreatic B-cells using the glucose-sensitive insulin-secreting BRIN-BD11 cells which is produced by electrofusion of RINm5F cells with New England mouse islets cultured in hyperglycaemic conditions [7]. In addition, the effects of inhibitors of glycation and the secretion of glycated insulin were investigated. For comparative purposes, the characteristics of insulin glycation were examined in vitro by incubation of insulin in phosphate buffered saline (pH 7.4) containing glucose and glucose metabolites for 24h.

Glycated and non-glycated insulin in cell extracts were separated by glycopel B affinity chromatography columns, comprised of 1 ml m-amino-phenyl boronic acid, prior to quantification by radioimmunoassay [10].

Glycation of insulin was significantly increased after culture for 72h with 33.3 mM or 11.1 mM glucose (p<0.01) compared with 5.6 mM or 1.4 mM (18.8 ± 2.5 %, 10.9 ± 1.5 %, 3.4 ± 2.1 and 3.3 ± 1.0 % respectively, n=6). Glycation of insulin was time-dependent following culture at 33.3 mM glucose for 2-72h reaching 16.6 ± 3.2 % by 2h (p<0.01) compared to 5.6 mM. Cellular glycation of insulin was decreased by 66-80 % by co-culture with established inhibitors of glycation (10 mM vitamin C, 22 mM aminoguanidine or 0.5 mM acetylsalicylic acid) at 33.3 mM glucose.

The secretion of glycated insulin after culture of BRIN-BD11 cells for 48h at 33.3 mM glucose was confirmed in acute 60 min test with 25 mM K⁺ (9.0 ± 0.5%) or 10 mM L-alanine (11.5 ± 0.4%). Exposure of insulin for 24h in vitro to 0-110 mM glucose resulted in a stepwise increase in the glycation of insulin to 16.5 ± 0.2 % which was substantially less than that observed following culture of BRIN-BD11 cells for 24h at the lower concentration of 33.3 mM glucose (24.9 ± 4.2%). However the level of in vitro glycation was increased by 39-43% (p<0.001) when insulin was exposed to glucose-6-phosphate or glyceraldehyde-3-phosphate alone compared with equimolar concentrations of glucose.

These observations indicate that intracellular glycation of insulin is dependent on both time of exposure and glucose concentration. Glycated insulin is readily secreted from stimulated pancreatic B-cells at glucose concentrations likely to be encountered in diabetes. Furthermore, the extent of intracellular glycation of insulin in B-cells following exposure to hyperglycaemic conditions can be reduced by the use of the inhibitors of glycation. Since glycated insulin exhibits decreased biological potency [11], these observations suggest that glycation of insulin in the pancreatic B-cells during synthesis and storage may contribute to B-cell dysfunction and pathophysiology of NIDDM [12].

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