Insulin release in aging: the role of glyceraldehyde

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Abstract. Glucose-stimulated insulin release is decreased from pancreatic islets of older rats. The mechanism for this and its relationship to the hyperglycaemia of aging remain to be fully elucidated. We have recently shown that adenylate cyclase activity is reduced in islets from older rats and this, might, in part, account for the decreased insulin release. It has been shown, however, that islet glucose oxidation is also reduced in aging. In an attempt to gain further insight into the biochemistry of this age-related decrease in insulin release, we investigated D-glyceraldehyde-stimulated insulin secretion. Simultaneous experiments were performed on islets from 2 and 15 month old rats to determine insulin release in response to 2.8 mM or 16.7 mM D-glucose or 2.8 mM D-glucose + 2.5, 5.0, 7.5, 10.0 or 14.4 mM D-glyceraldehyde. Islets were incubated for 30 min at 37°C in a metabolic shaker bath and the media were then assayed for insulin by conventional radioimmunoassay. Insulin secretion in response to 2.8 mM D-glucose was similar in the two groups of islets (9.8 ± 0.9 pg insulin islet⁻¹min⁻¹ from old vs 8.9 ± 1.0 from the young control rats, P < 0.2). Insulin release was diminished by 32% from islets of older rats in the presence of 16.7 mM D-glucose compared with islets from young control rats (84.0 ± 5.6 pg insulin islet⁻¹min⁻¹ from older animals vs 123.5 ± 10.7 from controls, P < 0.001). In the presence of 2.8 mM D-glucose + all concentrations of D-glyceraldehyde used, insulin release was similar from islets of older and young rats. From these results, it appears that the diminished insulin release from islets of older rats involves not only changes in the adenylate cyclase system, but also may involve changes in glucose metabolism at a rate-limiting step in stimulus-secretion coupling before the metabolism of the trioses.

The hyperglycaemia of aging, which is frequent in older human populations, is of importance because it is a risk factor in coronary artery disease and because of its close relationship to the development of maturity onset diabetes mellitus (Nilsson et al. 1964; Report of a Working Party 1963; Joffe et al. 1969; O'Sullivan et al. 1971; Epstein 1967). The possible causes of this hyperglycaemia of aging include: 1) altered insulin biosynthesis and release, 2) altered insulin sensitivity and 3) altered insulin action. Clinical human studies and in vivo animal studies have not been in agreement as to the role of altered insulin secretion in the hyperglycaemia of aging (Andres et al. 1970; Berdanie et al. 1971; Nolan et al. 1973; Dudl & Ensink 1977; Soerjodibroto et al. 1979; DeFronzo 1979; Davidson 1979). Recently studies on an animal model of aging, the older male Sprague-Dawley rat, have shown that there is diminished glucose-stimulated insulin release from isolated pancreatic islets of Langerhans of these older animals as compared with younger controls (Coddling et al. 1975; Kitahara & Adelman 1979; Reaven et al. 1979; Lipson et al. 1981a,b; Reaven & reaven 1980; Gold et al. 1981). To date studies to determine the biochemical changes in the

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islet responsible for the diminished glucose-stimulated insulin release have shown alteration in the islet adenylate cyclase system (Lipson et al. 1981a,b), in islet glucose utilization (Reaven & Reaven 1980) and in islet insulin biosynthesis and handling (Gold et al. 1981) of older rats.

Changes in the islet adenylate cyclase-cyclic AMP system and glucose metabolism may play a significant role in regulating glucose-stimulated insulin release in several states of physiologically altered secretion. Thus the enhanced insulin secretion from islets of rats in the late stages of pregnancy has been correlated with increase intracellular cyclic AMP levels and increased activities of adenylate cyclase and protein kinase (Green et al. 1973; Lipson & Sharp 1978). The blunted insulin release from islets of late foetal and neonatal rodents has been postulated to be secondary to both immaturity of the adenylate cyclase-cyclic AMP system and to decreased islet glucose metabolism and undeveloped glycolytic enzyme activities (Grill et al. 1975; Agren et al. 1976). The diminished insulin secretion in the fasting state has been shown not to involve alteration in activities of the enzymes of the adenylate cyclase-cyclic AMP system (Lipson et al. 1979), but to involve decreased activity in enzymes responsible for islet glucose oxidation (Malaisse et al. 1976).

In this study, as a further step in defining the mechanism(s) for the reduced insulin secretion seen in islets from older rats, we chose to study the effect of the three carbon glucose metabolite, D-glyceraldehyde, on insulin secretion.

Materials and Methods

Isolation of pancreatic islets

Pancreases were removed from male Sprague-Dawley rats, 2 months (average weight 175 g) and 13 months (average weight 540 g) of age. Islets of Langerhans were isolated by the collagenase digestion method of Lacy & Kostianovsky (1967). For each set of insulin release studies, islets were isolated under identical conditions from 2 and 13 month-old rats (Lipson et al. 1981b).

Insulin release studies from rat islets

Simultaneous experiments were performed on islets from 2 and 13 month-old male rats to determine insulin release in response to low and high concentrations of D-glucose and to D-glyceraldehyde. Islets from older and younger rats were pre-incubated for 30 min and then groups of 10 size-matched islets were picked and incubated in 0.5 ml in plastic 12 × 75 mm tubes containing either 2.8 mM D-glucose, 2.8 mM D-glucose + 2.5, 5.0, 7.5, 10.0 or 14.4 mM D-glyceraldehyde, or 16.7 mM D-glucose for 30 min at 37°C in a metabolic shaker bath (80 strokes/min) (Lipson et al. 1981b). The incubation medium was a modified Krebs-Ringer bicarbonate buffer containing 10 mM Hepes, pH 7.4. After the incubations were terminated, aliquots of the media were assayed for insulin content by conventional radioimmunoassay (Herbert et al. 1965) using rat insulin standards.

Statistical analysis

Tests for significance were performed using Student's t-test.

Results

Insulin release studies were performed simultaneously on islets from older and young rats. In the presence of 2.8 mM D-glucose, basal insulin release was similar from islets of both 2 and 13 month-old rats (9.8 ± 0.9 pg insulin released islet⁻¹min⁻¹ in older animals compared with 8.9 ± 1.0 in young controls, P < 0.2; see Fig. 1). In response to 16.7 mM D-glucose, insulin release from controls was 123.5 ± 10.7 pg insulin islet⁻¹min⁻¹, a 13-fold increase in insulin release over basal conditions. Insulin release was diminished by 32% in islets of 13 month-old rats as compared with islets from young controls (84.0 ± 5.6 pg insulin islet⁻¹min⁻¹, P < 0.001). When islets from young controls and older rats, however, were exposed to 2.8 mM D-glucose + various concentrations of D-glyceraldehyde ranging from 2.5 to 14.4 mM, insulin release was similar between islets from 13 and 2 month-old rats (P-values as follows: 2.5 mM, P < 0.6; 5.0 mM, P < 0.2; 7.5 mM, P < 0.5; 10.0 mM, P < 0.8; 14.4 mM, P < 0.8; see Fig. 1). Furthermore in the presence of both 7.5 mM and 10.0 mM D-glyceraldehyde and 2.8 mM D-glucose, insulin release from islets of older animals was significantly higher than that produced by 16.7 mM D-glucose alone (84.0 ± 5.6 pg insulin islet⁻¹min⁻¹ released to 16.7 mM D-glucose compared with 100 ± 4.8 to 7.5 mM D-glyceraldehyde + 2.8 mM D-glucose (P < 0.001) and compared with 109.3 ± 3.9 to 10 mM D-glyceraldehyde + 2.8 mM D-glucose (P < 0.001)). Thus maximal insulin secretory ability from islets of older rats was not achieved by 16.7 mM D-glucose.
Insulin release from batches of 10 size-matched islets from 2 and 13 month-old male rats. After an initial 30 min pre-incubation, islets were size-matched and picked into batches of 10 and then exposed to either 2.8, 16.7 or 2.8 mM D-glucose + 2.5, 5.0, 7.5, 10.0 and 14.4 mM D-glyceraldehyde for 30 min. Results were expressed as picograms of insulin released islet⁻¹min⁻¹ ± SEM. The number of experiments under each condition is in brackets.

Fig. I.

**Discussion**

The role of altered insulin secretion in the hyperglycaemia of aging has been a topic of some controversy and attempts are now being made to understand its importance using an animal model for aging, the older male Sprague-Dawley rat. Insulin release from isolated islets of these older rats and young controls is similar at low non-stimulatory concentrations of glucose. At elevated concentrations of glucose, however, insulin release from older rats is diminished between 32 and 50% as compared with that from younger rats (Reaven et al. 1979; Lipson et al. 1981a,b; Reaven & Reaven 1981). Several potential mechanisms for this age-related decreased insulin secretion have been investigated. Thus it has been shown that: 1) biosynthesis of pro-insulin in response to glucose and the size of the glucose responsive insulin secretory pool are decreased in aging (Gold et al. 1981), 2) islet adenylate cyclase-cyclic AMP system is altered in aging and 3) islet glucose oxidation is diminished in aging.

We have previously demonstrated that the adenylate cyclase-cyclic AMP system may play a role in this altered release (Lipson et al. 1981a,b). We have found that islet adenylate cyclase activity is significantly diminished in islets from older rats while basal phosphodiesterase and protein kinase activities remain at similar levels to those from young controls (Lipson et al. 1981b). Thus the presumed effect is from diminished cyclic AMP content (Lipson et al. 1981b) and is in accord with other tissues studied in the aging process (Williams & Thompson 1973; Krall et al. 1981) and with the observed insulin secretory characteristics of islets from older rats. The adenylate cyclase-cyclic AMP system has been implicated as responsible, at least in part, for changes in insulin release in other physiologically altered states of secretion. Hence, in late pregnancy, when glucose-stimulated insulin release is enhanced, cyclic AMP levels, adenylate cyclase and protein kinase activities are all increased (Green et al. 1973; Lipson & Sharp 1978), while in islets from foetal rats where insulin release is diminished, cyclic AMP levels are decreased (Grill et al. 1975).

Glucose oxidation by islets from older rats is decreased compared with that from younger animals (Reaven & Reaven 1980). This finding is of significance because of the hypothesis that glucose must first be metabolized prior to its being able to stimulate insulin secretion (Malaisse et al. 1976).
When the insulin secretagogue, D-glyceraldehyde, was used, however, islets from both fasted and fed animals released insulin in a biphasic manner and in identical amounts (Lipson et al. 1979). Evidence in favour of this finding was the report that while fasting decreased the insulin release response to glucose, it did not decrease the response to certain other stimulators, among which was glyceraldehyde (Levy et al. 1976). In one report on islets from fasted rats, however, the effect of D-glyceraldehyde on insulin release was reduced by 58% (Wolters et al. 1977). The study of Lipson et al. (1979) when coupled with those showing that several islet glycolytic enzymes have reduced activity in fasted rats (Malaisse et al. 1976) suggests that the defect in insulin secretion in fasting occurs at a rate controlling point between glucose and the trioses (Lipson et al. 1979). A similar situation exists in islets from foetal and neonatal rodents where glucose-stimulated insulin secretion is also diminished. Not only is islet cyclic AMP content decreased but also D-glyceraldehyde has been shown to equalize insulin release of foetal islets with those of controls (Grill et al. 1975; Agren et al. 1976).

In this study it has been shown that insulin release in the presence of a low glucose concentration (2.8 mM) from islets of young controls and older rats is similar, while release to 16.7 mM D-glucose is reduced by 32% in islets from older rats compared with controls in static incubations. In response to 2.8 mM D-glucose + 2.5, 5.0, 7.5, 10.0 and 14.4 mM D-glyceraldehyde, however, insulin release was similar from islets of the young and the old rats. These findings make it likely that there is a defect in insulin secretion at a rate controlling point between glucose and the trioses (Malaisse et al. 1979). Of note is the fact that insulin secretion to 14.4 mM D-glyceraldehyde of islets from both young and old rats is less than that which occurs with either 7.5 or 10.0 mM D-glyceraldehyde. Unlike insulin release to glucose where increasing glucose concentrations eventually lead to a plateau level of insulin secretion which does not decline at higher concentrations, increases in the dose response curve to glyceraldehyde occur up to about 10-15 mM at which point a pronounced inhibition of insulin release occurs (Helmman et al. 1974; Zawalich et al. 1978). The reason for this decline in insulin secretion at higher glyceraldehyde concentrations has been ascribed to a decline of intracellular adenosine triphosphate at high concentrations of glyceraldehyde (Ashcroft et al. 1973).

The possibility exists that in the islet selection and size-matching process, a selection has taken place from different parts of the young and old islet population. These subgroups might behave differently to the various secretory stimuli since there is an increase in B-cells and insulin secretory granules in islets from the older rats (Reaven et al. 1979). This potential subgrouping was found not to be the case (Reaven et al. 1982) since islets from different parts of pancreases from older rats consistently have an insulin secretory defect to glucose when compared with islets from different pancreatic areas of younger rats when expressed on a per islet volume basis.

From these and previous studies, the diminished insulin release from islets of older rats involves not only changes in the adenylate cyclase system, but also may involve a rate-limiting step in the stimulus-secretion coupling in glucose metabolism before the metabolism of the trioses. The relative impact of these has yet to be defined.

References


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