Role of the adenylate cyclase system in altered insulin release from islets of Langerhans of aging rats

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Abstract. In attempting to understand the causes of the hyperglycaemia observed in aging populations and to determine the mechanism(s) for the diminished in vitro insulin release from islets of Langerhans of older rats, the adenylate cyclase-cyclic AMP system was studied in isolated islets from 12 month old and 2½ month old (control) male rats to determine its role in this altered insulin secretion. Islets of Langerhans were isolated by collagenase digestion and then either incubated in the presence of low or high concentrations of glucose for studies of insulin release or were sonicated and assayed for determinations of activities of adenylate cyclase and phosphodiesterase. Insulin release was identical from islets of 12 month old and 2½ month old rats to 2.8 mM D-glucose, while in the presence of 16.7 mM D-glucose, insulin release was decreased by 33% (P < 0.02) from islets of the older animals. Adenylate cyclase activity was diminished by 60% (P < 0.005) from the 12 month old rats as compared with islets from the 2½ month old controls, while low Kₘ phosphodiesterase activity was similar in islets from both groups of animals. From these studies it appears that the adenylate cyclase-cyclic AMP system may play a role in the altered insulin release from islets of aging rats.

There is an increased incidence of hyperglycaemia in aging human populations (Nilsson et al. 1964; Report of a Working Party 1963; Hayner et al. 1965; O'Sullivan et al. 1971; Joffe et al. 1969). This age-related hyperglycaemia is significant because it is a risk factor in atherosclerosis (Epstein 1967) and it is closely related to the development of maturity onset diabetes mellitus. Possible mechanism(s) for this hyperglycaemia of aging include: 1) altered insulin secretion and 2) altered insulin action. Clinical human studies and in vivo animal studies have not been in agreement as to whether insulin release is altered to glucose stimulation in aging (Andres et al. 1970; Berdanier et al. 1971; Nolan et al. 1973; Dudl & Ensinck 1977; Soerjodibroto et al. 1979; Davidson 1979) and the resulting uncertainty is still not resolved. Recent investigations using isolated islets of Langerhans from aging rats have shown there is diminished glucose-stimulated insulin release when compared with islets from younger animals (Coddling et al. 1975; Gold et al. 1976; Kitahara & Adelman 1979; Reaven et al. 1979), but causes for the observed aging-induced secretory defect have not been elucidated.

As an initial step in understanding the causes for this diminished glucose-stimulated insulin release, we have investigated the possible role of the enzymes of the adenylate cyclase-cyclic AMP system in modulating the insulin release process in aging. The adenylate cyclase-cyclic AMP system is changed in several altered states of glucose-stimulated insulin release. Thus, the increase in insulin release noted in islets after hyperfeeding has been correlated with increased intracellular islet cyclic AMP and adenylate cyclase activity (Howell et al. 1973). The increased insulin release in the late stages of pregnancy is thought to be, at least in part, se-

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secondary to hyperfeeding (Green & Taylor 1974) and is associated with increased cyclic AMP levels (Green et al. 1973) and increased adenylate cyclase and protein kinase activities (Green et al. 1973; Lipson & Sharp 1978). The blunted insulin release in neonatal rodents has been postulated to be in part secondary to immaturity of the adenylate cyclase-cyclic AMP system (Grill et al. 1975).

Thus in this study a simultaneous determination of the enzymes adenylate cyclase and phosphodiesterase was performed on islets from 12 month old and 2½ month old male rats and correlated with studies of insulin release to low and high concentrations of D-glucose in these islets.

Materials and Methods

Collagenase was obtained from Worthington Diagnostics. [α-32P]ATP and [3H]cyclic AMP were purchased from the New England Nuclear Co. All other biochemicals were supplied by the Sigma Chemical Company.

All rats were fed with Purina rodent lab chow 5001 consisting of 56% carbohydrate, 23% protein and 4.5% fat. They were on this diet for 10 days prior to sacrifice and had access to food until 4 h prior to pancreatectomy.

Isolation of rat islets of Langerhans

Male Sprague-Dawley rats 2½ months old (average wt, 216 ± 19 g) and 12 month old (average wt, 472 ± 16 g) were decapitated, the pancreases removed, trimmed free of adipose tissue and minced in Hank's solution. Islets were separated by the collagenase digestion method of Lacy & Kostianovsky (1967) and picked free of exocrine tissue under a dissecting microscope. For each set of determinations, islets which had been subjected to simultaneous identical isolation conditions from two 12 month old or two 2½ month old rats were pooled. The islets were size matched by visual inspection and were taken for insulin release studies with the remainder being sonicated in distilled water and immediately used for the enzyme assays.

Insulin release studies

Simultaneous experiments were performed on size-matched islets from 2½ month old and 12 month old rats to determine insulin release to low and high concentrations of D-glucose. After a 30 min pre-incubation in the presence of 2.8 mM D-glucose, groups of 20 islets were incubated in plastic 12 × 75 mm tubes containing 0.5 ml of either 2.8 mM or 16.7 mM D-glucose in a modified Krebs-Ringer bicarbonate buffer containing 10 mM Hepes at pH 7.4. Incubations were for 30 min at 37°C in a metabolic shaker bath (80 strokes/min). The incubations were terminated and aliquots of media were assayed for insulin content using rat insulin standards (Hales & Randle 1963).

Adenylate cyclase assay

Basal adenylate cyclase activity was determined in sonicates of islets by the method of Krishna et al. (1968) as described in detail previously (Lipson & Sharp 1978) for islet tissue. Sonicates of freshly isolated islets containing 5–15 µg of protein were allowed to react with [α-32P]ATP in the presence of 10 mM MgCl₂ and an ATP regenerating system for 20 min at 37°C.

Phosphodiesterase assay

Phosphodiesterase activity was measured in the islet sonicates by the method of Brooker et al. (1968), as previously described in detail for islets (Lipson & Sharp 1978). The low Kₘ enzyme was determined in the presence of 0.01 µM [3H]cyclic AMP. Islet protein was kept between 5 and 10 µg/assay, under which conditions linearity of reaction rate was maintained at all substrate concentrations.

Protein determinations

Protein concentrations of islet sonicates were determined by the method of Lowry et al. (1951).

Statistically analysis

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

Results

Islet protein content

The protein content of size matched islets from older and young rats was similar. In 5 paired experiments, the protein content per islet from 12 month old rats was 0.27 ± 0.02 µg, which was similar to the protein content of islets from 2½ month old control rats (0.26 ± 0.02 µg) for equivalent numbers of islets (P < 0.7).

Insulin release studies

Incubations were performed simultaneously on islets from 2½ month old and 12 month old male rats. Basal release of insulin in the presence of 2.8 mM D-glucose was identical for islets of young control and older rats (15.3 ± 2.4 pg insulin released islet⁻¹ min⁻¹ for 2½ month old rats and 15.3 ± 2.0 for year-old animals; n = 6). In response to 16.7 mM D-glucose, insulin release was decreased from islets of the older animals (77.7 ±
Table 1. 
Adenylate cyclase activity in islets from 2½ month old and 12 month old male rats.

<table>
<thead>
<tr>
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<th>Mean ± SEM</th>
<th>Δ ± SEM</th>
<th>P</th>
<th>n</th>
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<tr>
<td>2½ month old</td>
<td>41.2 ± 5.0</td>
<td>24.8 ± 2.7</td>
<td>&lt; 0.005</td>
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<tr>
<td>12 month old</td>
<td>16.4 ± 5.2</td>
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Basal adenylate cyclase activity was determined in sonicates of islets containing 5–15 µg protein which were allowed to react with [α-32P]ATP in the presence of 10 mM MgCl2 and an ATP-regenerating system for 20 min at 37°C. Values are given as picomoles of cyclic AMP synthesized mg⁻¹ islet protein min⁻¹.

5.7 pg insulin released islet⁻¹ min⁻¹ from controls vs 52.0 ± 3.0 for older rats; Δ ± SEM = 25.7 ± 7.2, n = 6, P < 0.02). Thus there was a 33% diminution in glucose-stimulated insulin release from islets of older rats.

Adenylate cyclase activity
Adenylate cyclase activity was determined in the basal state in the islet sonicates. Basal activity from the older animals was 16.4 ± 5.2 pmol cyclic AMP formed mg⁻¹ min⁻¹ which was significantly lower than this activity in younger controls (41.2 ± 5.0, P < 0.005). Thus, there was a 60% decrease in adenylate cyclase activity per mg of islet protein from islets of older animals. The statistical data are shown in Table 1.

Phosphodiesterase activity
The physiologically relevant low Kₘ (high affinity) phosphodiesterase activity was determined in each of the sonicates. The low Kₘ enzyme activity was not significantly different when islet sonicates from older rats were compared with those from younger animals (5.8 ± 0.3 pmol cyclic AMP utilized mg⁻¹ min⁻¹ from islets of 2½ month old rats vs 6.5 ± 0.6 from islets of year-old rats; Δ ± SEM = −0.7 ± 0.6, n = 4, P < 0.4).

Discussion
Hyperglycaemia occurs in aging human populations (Nilsson et al. 1964; Report of a Working Party 1963; Hayner et al. 1965; O'Sullivan et al. 1971; Joffe et al. 1969). This age related phenomenon is of interest because of its close relationship to the development of maturity onset diabetes mellitus and as risk factor in coronary artery disease (Epstein 1967). The causes of this hyperglycaemia have not been fully defined, but alterations in insulin secretion, in tissue sensitivity to insulin or in insulin action could all play a role (Andres & Tobin 1977; Gregerman & Bierman 1974). Clinical human studies of insulin secretion in aging man and in vivo studies in aging rats have shown increased, unchanged or decreased glucose-stimulated insulin release with increasing age (Andres et al. 1970; Berdanier et al. 1971; Nolan et al. 1973; Dudl & Ensink 1977; Soerjodibroto et al. 1979), and it is unclear whether population sampling, differences in adiposity or nutritional state play a role in explaining these differences (Andres & Tobin 1977; Gregerman & Bierman 1974). In order to study directly insulin secretion in aging, isolated islets of Langerhans from older rats have been used as a model system to explore the age related changes in B-cell function (Coddling et al. 1975; Gold et al. 1976; Kitahara & Adelman 1979; Reaven et al. 1979).

In the study reported here islets from both young and older Sprague-Dawley rats were tested for insulin release characteristics to low, non-stimulatory concentrations of glucose and to high glucose concentrations. Basal insulin release was identical from islets of both groups of animals, but in the presence of high glucose (16.7 mM), insulin release was significantly reduced (33%) in islets from the older rats. These findings are in agreement with those of other investigators using older rats ranging in age from 10 to 24 months (Coddling et al. 1975; Kitahara & Adelman 1979; Reaven et al. 1979).

Possible reasons for the decreased insulin release in aging include: 1) alterations in islet size and/or insulin content in aging, 2) alterations in the activity of the enzymes of the adenylate cyclase-cyclic AMP system or 3) altered ability of islets to respond to or metabolize glucose secondary to decreased activity of the enzymes of the glycolytic pathway.

No significant differences were noted in islet size between older and young rats. Thus we did not find a predominance of large size or small islets from the pancreases of older rats, but there was a continuum of islets of various sizes from both younger and older rats. Recently Kitahara & Adelman (1979) have reported two distinct islet sizes
from older rats, normal size and smaller islets; this latter group had more depressed glucose-stimulated insulin release than did the islets of normal size and when compared with islets from younger controls. Reaven et al. (1979) in their comprehensive morphologic study of islets from aging and young rats, however, found no such population of small islets in their older animals and further showed an increase in both insulin content and B-cell number, with the net effect being an increase in insulin content per B-cell. By size matching our islets in all experiments we have eliminated problems posed if such a subpopulation of smaller islets exists in aging rats. Furthermore such visual size matching seems to be effective in that islet protein content is similar from control and older rats.

Both the adenylate cyclase system and the glycolytic pathway have been shown to be involved in the regulation of insulin secretion in studies of states of altered insulin release. Thus both cyclic AMP content and adenylate cyclase activity in islets have been shown to be increased in hyperfeeding (Howell et al. 1973) and in the late stages of pregnancy (Green et al. 1973; Lipson & Sharp 1978), both states in which glucose-stimulated insulin release is increased over controls. In the blunted glucose-stimulated release seen in foetal and neonatal islets, the cyclic AMP content of the islets has been shown to be reduced when compared with islets from 2 month old rats (Grill et al. 1975). In the decreased insulin release seen in the fasted state, the enzymes of the adenylate cyclase-cyclic AMP system are unchanged (Lipson et al. 1979), however, several enzymes of the glycolytic pathway in islets have been shown to be decreased in activity (Malaissé et al. 1976) and both islets from fasted and fed controls released identical amounts of insulin to glyceraldehyde stimulation suggesting the defect in fasting lies at a rate-controlling point between glucose and the trioses (Lipson et al. 1979).

In attempting to dissect out the cause(s) for the altered insulin release in aging, we investigated the adenylate cyclase-cyclic AMP system in islets from aging rats. It has been shown that increased intracellular cyclic AMP enhances glucose-stimulated insulin release in the presence of high glucose concentrations but not at low, non-stimulatory levels of glucose (Malaissé et al. 1967; Sussman & Vaughn 1967) and further that high glucose levels stimulate islet adenylate cyclase activity and result in increased intracellular levels of cyclic AMP (Grill & Cerasi 1973; Charles et al. 1973; Capito & Hedeskov 1977; Sharp 1979). The diminished insulin release in islets from older rats is consistent with an altered adenylate cyclase system. Our data show a significant decrease of 60% in adenylate cyclase activity while the low K_m (high affinity) phosphodiesterase activity was unchanged from controls. The net effect of these enzyme activities would be a decrease in the intracellular levels of cyclic AMP in the islets of the older rats. Thus the reduction in adenylate cyclase activity could account, at least in part, for the diminished insulin release to elevated concentrations of glucose seen in the islet from aging rats. Further studies are underway on the mode of regulation of adenylate cyclase activity in the islets from the aged animals and to determine if alterations in the enzymes of the glycolytic pathway exercise additional control over insulin release in islets from aging rats.

In conclusion, there appears to be a correlation in islets of aging rats between reduced insulin release to elevated glucose concentrations and a reduction in the adenylate cyclase activity, and it is likely that this diminished adenylate cyclase activity may play a significant role in the decreased insulin release in islets from aging rats.

References


