A POSSIBLE ROLE FOR INSULIN IN THE ALTERED CAPABILITY FOR HEPATIC ENZYME ADAPTATION DURING AGING

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SUMMARY: The pattern of change in the concentration of immunoreactive insulin in portal vein blood during a 7-hour period following intragastric administration of glucose to fasted, male Sprague-Dawley rats is modified both in magnitude and time course of response as rat age increases from 2- to 24-months. This alteration in the control of insulin levels probably is not the consequence of modifications in the availability of administered glucose to the pancreas. A similar modification in the control of insulin secretion also is evident when pancreatic islets isolated from fasted rat donors aged 2- to 24-months are perifused with glucose *in vitro*. This may represent the first demonstration of an altered response of aging animals which is expressed in the same way in an isolated *in vitro* system.

Activity of hepatic glucokinase is markedly reduced in response to 3 days of starvation and is restored nearly to prefasting levels 4 hours following intragastric administration of glucose to young adult rats(1). However, the time required to initiate this enzyme adaptation is progressively delayed in time of onset from 4 to 11 hours as male and female Sprague-Dawley rats age from 2- to 24-months(2). A considerable body of evidence indicates that insulin is required in order for the liver cell to express full responsiveness to glucose in its capability for glucokinase synthesis(e.g., 1,3-5). Therefore, the purpose of the present article is to determine whether or not the pattern of glucose-stimulated secretion of insulin changes during aging. The results provide additional support for our suggestion that changes in the availability of key hormonal factors during aging may contribute to modifications in hepatic enzyme adaptation(2,5,6).

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EXPERIMENTAL

Animals- Male, Sprague-Dawley rats were obtained at 2-, 12- and 24-months of age from a colony maintained for R.C. Adelman at the Charles River Breeding Laboratories. These rats are cesarian-derived and maintained behind a pathogen-defined barrier under rigorously controlled conditions which are described in pending publications. Mean lifespan of these rats is approximately 30-months, their maximal lifespan is approximately 40-months, and at about 24-months of age they are virtually free of detectable gross pathology.

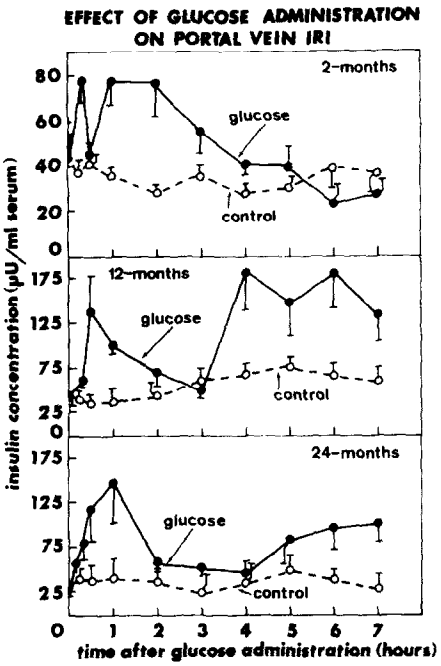
Materials- Guinea pig anti-insulin serum and rabbit anti-guinea pig serum were purchased from Arnel Products; normal guinea pig serum and bovine serum albumin from Miles Laboratories; bovine plasma albumin from Armour Pharmaceutical; type IV collagenase from Worthington; {^{125}I}-labelled porcine insulin from New England Nuclear (100 μc/μg) and from Abbott Laboratories (50 μc/μg); and purified rat insulin from the Novo Research Institute in Copenhagen.

Treatments- Rats were fed ad libitum a pasteurized, sterilized Charles River Chow which is reported to be of constant percent composition and component source. Periods of fasting were begun between 8 and 10 a.m. All fasted rats were provided with drinking water ad libitum. Glucose was administered intragastrically with a feeding needle at 8-10 a.m. and at a dosage of 2.5 mmoles in 1.25 ml of water per 100g of body weight. Serum was collected from portal vein or aortal blood following anesthetization of rats with ether for no longer than 2 minutes.

Assays- The concentration of insulin was determined by the double antibody radioimmunoassay of Hales and Randle(7), using purified rat insulin as a standard. Radioactivity of samples was measured in a Searle Analytic Model 1195 Automatic Gamma Counter. The concentration of glucose in serum was determined by the method of Nelson-Somogyi(8,9). Pancreatic islets were isolated from 3-day fasted rats aged 2- to 24-months and perifused with glucose in vitro essentially according to the procedures of Lacy and coworkers(10,11), except that collagenase digestion time for tissue from 24-month old rats was increased from 5-6 to 6-7 minutes.

RESULTS

The change in concentration of immunoreactive insulin in serum collected from portal vein blood following intragastric administration of glucose to 3-day fasted rats of the indicated ages is illustrated in Figure 1. Rats were fasted for a period of 3 days prior to administration of glucose in order to reproduce the experimental conditions reported previously to generate delayed initiation of an adaptive increase in hepatic glucokinase activity during aging (2). Several differences in the pattern of insulin response are apparent as these rats age from 2- to 24-months. At 2-months of age, the concentration of insulin increases from 40 to 80 μunits per ml of serum and returns to the original basal level within 30 minutes following administration of glucose. A



1. Effect of Glucose Administration on Insulin Levels in Vivo. The concentration of immunoreactive insulin in serum collected from portal vein blood was determined following intragastric injection of 2.5 mmoles of glucose per 100g of body weight at the indicated times and ages. Lines labelled CONTROL refer to data obtained from rats treated with water containing no glucose. Each value represents the mean ± standard error for 10-12 separate rats.

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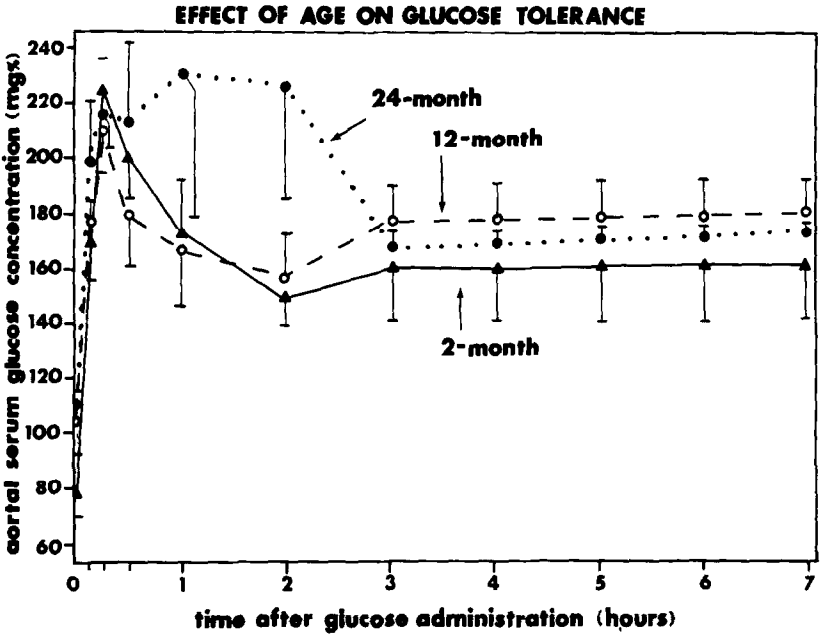
second phase of insulin response is evident at 1 hour, also increasing to a concentration of 80 μunits per ml of serum, and is maintained at that level for another hour, after which it gradually falls to the basal level by approximately 3-4 hours. At 12-months of age, the concentration of insulin increases initially from approximately 40 to 140 μunits per ml of serum within 1 hour following admin istration of glucose, and decreases to the original basal level at 3 hours. The second phase of the insulin response is delayed in time of onset until 3-4 hours, peaks by 4 hours at a concentration of approximately 180 μunits per ml of serum, and is maintained at that level until at least 6-7 hours at which time it may begin to fall. At 24-months of age, the initial phase of the insulin response is very similar to that observed at 12-months. However, the second phase of the



2. Effect of Glucose Administration on Glucose Levels in Vivo. The concentration of glucose in serum collected from aortal blood was determined following administration of glucose exactly as indicated in the legend to Figure 1. Each value represents the mean ± standard error for 6 of the rats treated with glucose for the previous experiment.

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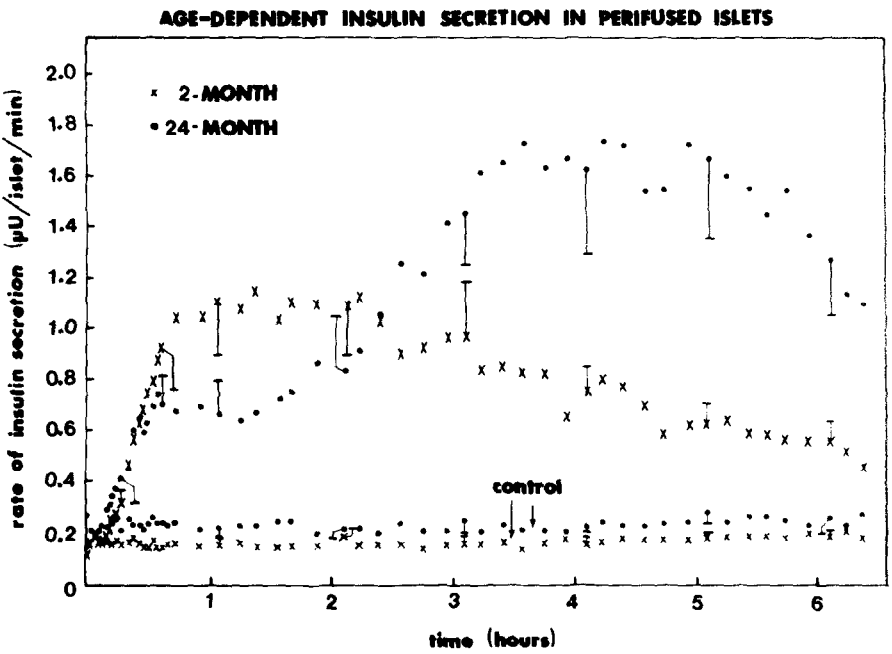
response is delayed even further in time of onset until 5-6 hours, and a level of approximately 100 μunits per ml of serum is maintained for at least 6-7 hours. A control experiment, in which identical volumes of water containing no glucose were administered intragastrically to 3-day fasted rats of the indicated ages, indicates that the stress of handling and/or treatment in the absence of administered glucose exerts a negligible effect on the concentration of immunoreactive insulin.

Illustrated in Figure 2 are the levels of aortal blood glucose which bathe the pancreas in response to intragastric administration of glucose, exactly as described for the preceding experiment. The level of blood glucose increases at similar rates from 80-100 mg to 220 mg per 100 ml of serum within 15 minutes following administration of glucose at each age. At 2- and 12-months of age, the concentration of glucose decreases to approximately



3. Glucose-Stimulated Section of Insulin in Vitro. The rate of secretion of immunoreactive insulin into the medium was determined following perifusion with glucose of islets isolated from 3-day fasted rats of the indicated ages. Two lots of 50 islets were collected from each pancreatic digest. One of these lots was perifused with Krebs-Ringer bicarbonate buffer, pH 7.4, containing 30 mg of glucose per 100 ml of medium for the entire experiment, and the obtained data are labelled CONTROL. The second lot of islets was perifused with the same buffer for 40 minutes after which the concentration of glucose was raised immediately to 300 mg per 100 ml of medium (indicated as ZERO TIME). The rate of insulin secretion was calculated from the concentration of immunoreactive insulin determined in serially collected samples of the perifusate. Values represent the mean ± standard error for separate lots of islets isolated from 8 2-month and 6 24-month old rats. Only representative standard errors are presented for the sake of artistic convenience.

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160 mg per 100 ml of serum at 30-60 minutes, and is maintained at that level from 1 to at least 7 hours following administration of glucose. At 24-months of age, the concentration of glucose also decreases to a plateau of approximately 160 mg per 100 ml of serum, although onset of the decrease may be delayed more than 2 hours. A control experiment identical to that described immediately above indicates that the stress of handling and/or treatment in the absence of administered glucose also exerts a negligible effect on the concentration of glucose throughout at least 7 hours (data not shown).

Illustrated in Figure 3 are the patterns of insulin secretion observed when pancreatic islets are isolated from 3-day fasted rat donors aged 2- to 24-months, and perifused with glucose in vitro. In islets from fasted 2-month old rats, the rate of secretion of insulin into the medium begins to increase at approximately 3-5 minutes following changeover in the glucose concentration from 30 to 300 mg per 100 ml of perifusion medium, increases as much as 4- to 7-fold at a nearly linear rate by approximately 30 minutes, persists at that enhanced rate of secretion for an additional 1-2 hours, and gradually approaches the basal rate of insulin secretion at 6-7 hours. In islets from fasted 24-month old rats, a 2-phase response is evident in which the bulk of glucose-stimulated secretion of insulin occurs several hours later than that observed in islets isolated from younger rats. Initially, the rate of secretion of insulin into the medium begins to increase also at 3-5 minutes following changeover in the glucose concentration from 30 to 300 mg per 100 ml of perifusion medium, increases 2.5- to 4-fold at a nearly linear rate, and persists at the enhanced rate of secretion for approximately 1 hour. At 1.5 hours, a second increase occurs in the rate of secretion of insulin, as great as 6- to 10-fold above basal levels for a period of several hours, after which the rate of insulin secretion begins to decrease at 5-6 hours. Control experiments, in which islets from each age group were perifused for 7 hours in the presence of 30 mg of glucose per 100 ml of perifusion medium, indicate a constant low rate of secretion into the medium. The low rate of insulin secretion in these control experiments probably attests to the comparable viability of islets prepared from donors of different ages.

DISCUSSION

The present data provide additional support for the concept that impairments in the capability for hepatic enzyme adaptation during aging reflect modifications in the availability of crucial hormones(12,13). Glucose-stimulated increases in the circulating level of immunoreactive insulin and in the activity of hepatic glucokinase(2) are progressively modified during aging. In contrast,

hepatic responsiveness to insulin *in vivo*(5) and *in vitro*(14) is not altered during aging. Similar data were reported for the role of corticosterone in stress-induced alterations in the activity of hepatic tyrosine aminotransferase (5,6,15-19). Interpretation of the modified insulin response must await evaluation of at least the following: 1) differential influence of growth, obesity and aging; 2) relative distribution of various hormone precursors, metabolites, and genetic variants; and 3) the relative importance of each phase of the insulin response to the subsequent elevation in hepatic glucokinase activity.

Availability of glucose to the pancreas following its intragastric administration is not altered in any obvious way that might account for the pattern of insulin secretion at different ages. The similarity in the insulin response up to 2 hours following exposure to a high level of blood glucose in 12- and 24-month old rats, as well as in adult and aging humans(20), is striking. Presumably, the maintenance of elevated glucose levels at 160 mg per 100 ml of serum for at least 7 hours reflects in part the pharmacological dosage of administered glucose, originally intended as a stimulus for hepatic enzyme adaptation. Whether changes in the availability of other endogenous effectors of insulin secretion, i.e., amino acids, various hormones, etc., contribute to the modified insulin response at different ages remains to be seen. However, detection of the late insulin response to glucose *in vitro* in islets isolated from aged rat donors is consistent with a pancreatic origin for its delayed time of onset.

The late insulin response to glucose is a previously unreported phenomenon which may relate to a similar response to a standard diet administered to young adult humans(21). Failure of others to detect the response previously probably relates to the relatively unusual set of experimental conditions. These include monitoring of glucose treatment at later times than generally were studied; administration of a continuous 7-hour challenge by a high level of blood glucose; the use of 3-day fasted animals; and collection of serum from portal vein blood. This delayed insulin response to glucose both *in vivo* and in isolated pancreatic islets is the first reported example of a response

which characterizes intact aging animals that also can be detected the same way in an *in vitro* system isolated from donors of respective ages. Thus, at least certain fundamental expressions of aging may be localized within the pancreatic islets, and perhaps, even within the beta cells.

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