Studies of Immunoreactive Insulin Secretion in NZO Mice *in Vivo*

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Summary. Studies of immunoreactive insulin (IRI) secretion have been performed *in vivo* in NZO mice. After an overnight fast, plasma IRI levels were 2–5 times higher in NZO than in control white mice. The IRI response to arginine, 10 mg/kg IV or isoprenaline, 10 µg/kg IV, was similar in time in the two mice, but IRI increments were greater in the NZO animals. With glucagon 1 µg/kg IV or aminophylline 25 mg/kg IV the initial secretory response was delayed compared to control mice. Glucose 1.0 g/kg IV elicited a markedly attenuated initial response in NZO mice. When IRI responses were related to basal IRI levels, NZO mice showed smaller initial responses (1–5 min) to all agents tested. Later

responses were comparable in the two groups when compared in this way. Diphenylhydantoin, 40 mg/kg administered intraperitoneally 30 min before glucose, 1.0 g/kg IV did not affect glucose-induced insulin secretion in control animals, but abolished it in the NZO mice. These observations suggest the existence of a defect in the IRI release mechanism of the NZO β cell situated at a point in the stimulus secretion coupling mechanism common to all the agents examined.

Key words: Spontaneous diabetes, NZO mice, insulin secretion, islet cell function.

Introduction

The syndrome of obesity and hyperglycemia in NZO mice originally described by Bielschowsky and Bielschowsky [2] is characterised by hyperinsulinemia and increased pancreatic insulin stores [17, 18]. Larkins and Martin [12] reported that glucose, glucagon and tolbutamide, administered intraperitoneally (IP), did not stimulate release of insulin *in vivo* while arginine was able to do so. Because of the important implications of these observations in relation to the hyperinsulinemia of obesity and the regulation of insulin secretion in general, these studies were undertaken using methods previously employed for *in vivo* studies of insulin secretion in rodents [3, 4, 5]

Materials and Methods

Animals

NZO mice from the colony maintained at Prince Henry's Hospital, Melbourne, were used. The progenitors were obtained as a gift from Dr. M. Holmes, Walter and Eliza Hall Institute, Melbourne. Control mice were random-bred white mice from the Prince Henry's Hospital colony and were originally derived from a colony at the Commonwealth Serum Laboratories, Parkville, Victoria. All animals were maintained under standard conditions of lighting, temperature and humidity and fed a commercial chow *ad lib*. Animals were between 4 and 8 months old at the time of study.

Experimental Procedures

Animals were fasted overnight and anaesthetised with pentobarbitone sodium (60 µg/kg, IP, and

30 µg/kg subcutaneously). Insulin secretion was studied in response to glucose, 1.0 g/kg, arginine, 10 mg/kg, glucagon, 1 µg/kg, aminophylline, 25 mg/kg and isoprenaline, 10 µg/kg administered as bolus injections into a tail vein. In one experiment, diphenylhydantoin (DPH), 40 mg/kg was administered IP 30 min before the intravenous injection of glucose. Blood samples were collected from the retro-orbital plexus into heparinised hematocrit tubes, transferred immediately to iced microfuge tubes, centrifuged and the plasma stored at -16°C until assayed.

Assay Procedures

Plasma glucose was determined by a glucose oxidase method [1] on 5 µl aliquots of plasma as described previously [4]. Plasma immunoreactive insulin (IRI) was assayed by the method previously reported [4] with the following modification. At the time of addition of the second antibody, 10 µl of human plasma from which the insulin had been "stripped" by incubation with activated charcoal, was added to the standard curve tubes. This resulted in an equal concentration of plasma in all assay tubes.

Reagents and Chemicals

The mouse insulin standard used in the radioimmunoassay was a gift from Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen, Denmark (Lot No. M20169). Reagents for glucose assays were purchased from Boehringer GmbH, Mannheim, Germany as the GOD-PERID kit. The test substances administered were obtained from the following sources: Glucagon, Eli Lilly and Co., Indianapolis, U.S.A.; Aminophylline (Theophylline ethylene-diamine (81%)), Lancet Pharmaceutical Pty. Ltd., Sydney, Australia; Isoprenaline hydrochloride (isopropyl noradrenaline),

Winthrop Laboratories, Sydney, Australia; L-Arginine monohydrochloride, British Drug Houses Ltd., Poole, England; Diphenylhydantoin, Parke Davis and Company, Detroit, Michigan, U.S.A. Pentobarbitone sodium was purchased from Abbott Laboratories Pty. Ltd., Sydney, Australia.

Results

Fig. 1 shows the response of plasma IRI to glucose 1.0 g/kg IV. Initial plasma IRI is higher in NZO than control mice. After glucose administration, the control

Fig. 1. Values for plasma IRI and glucose in NZO and control mice after glucose 1.0 g/kg IV. (Mean $\pm$ SEM)

mice respond with an immediate, marked release of insulin. The NZO mice in contrast have a lesser initial response and at one minute, the plasma IRI levels are significantly higher in the control mice ($p < 0.05$). Plasma IRI levels progressively decline in NZO mice after the initial response while in NZO animals the elevation of plasma IRI is maintained for at least two hours. This occurs despite the fact that plasma glucose levels are not significantly different in the two groups at this time.

In contrast with the impaired initial response to glucose, NZO mice respond to arginine (Fig. 2) with an immediate release of insulin which is quantitatively greater than in the controls. With administration of isoprenaline also (Fig. 3), IRI release in NZO mice is similar in time compared to controls reaching both peak plasma values at 5 min. Quantitatively, NZO mice release greater amounts of insulin than do controls.

With both glucagon (Fig. 4) and aminophylline (Fig. 5) administration, a delayed initial release is observed in NZO compared to white mice. Thus, the peak plasma IRI levels are seen at 5 min in NZO

Fig. 2. Arginine, 10 mg/kg IV administered to NZO and control mice: Plasma IRI and glucose levels. (Mean $\pm$ SEM)

animals while in the controls an immediate short-lived response is seen with the one minute values being the highest. After aminophylline, the plasma IRI levels in NZO mice remain substantially elevated above basal throughout the study.

In Table 1, the responses of plasma IRI to the various test agents are compared as percentage elevations above basal (taken as 100%). When expressed in this manner, it is evident that the initial insulin release is deficient in the NZO mice in response to all the agents while later release is comparable in the two groups.

Fig. 6 shows the results of an experiment in which DPH was administered IP 30 min before glucose, 1.0 g/kg IV. Following DPH, plasma IRI rose in both NZO and control mice. Glucose induced IRI release was unaffected in the controls but was abolished in the NZO animals.

Discussion

These studies present further evidence for a defective insulin secretory mechanism in the inbred mouse strain NZO. The abnormalities of β -cell function

insulin. The present results, while confirming the presence of defective insulin release to a variety of known insulin secretagogues suggest that there is a relative, not an absolute, inability on the part of these agents to cause insulin release. The reasons for the differences between Larkins and Martin's results and those in the present study may relate to the differences in experimental protocols especially in the route of administration of the test substances.

Relatively few kinetic studies of insulin release *in vivo* in obese hyperglycemic rodents have been published [3, 4, 5, 12, 21]. In *obob* mutants and DBM mice before they become grossly hyperglycemic, it

Fig. 3. Responses of plasma IRI and glucose to isoprenaline, 10 µg/kg IV. (Mean $\pm$ SEM)

in this strain appear to be twofold. First, basal insulin secretion is elevated. In the present study, fasting plasma IRI values in NZO mice were two to five times greater than in controls. Second, the ability of the β -cells to respond rapidly to an acute insulinotropic stimulus appears impaired. Elevated plasma IRI levels in NZO mice have been reported by Sneyd [17] and by Stauffacher *et al.* [18] as well as by Larkins and Martin [12]. The latter authors have reported the only kinetic studies to date on IRI release in this strain. They found that glucose, glucagon and tolbutamide administered IP all failed to elicit IRI release. Arginine, however, caused marked release of

Fig. 4. Plasma IRI and glucose levels in NZO and control mice after administration of glucose, 1 µg/kg IV. (Mean $\pm$ SEM)

was considered [3, 4, 21] that IRI release was quantitatively excessive though temporally normal. The same applied to the induced syndrome of goldthio-glucose obesity [3, 4]. However, on re-examination of the data, if these responses are considered in relation to the basal IRI levels, insulin responses to stimuli may be considered deficient in all these syndromes. The *acomys cahirinus* appears to have a generalised defect of insulin secretion [3, 5]: despite the presence of obesity, basal plasma IRI levels are low and IRI release is deficient in both absolute and relative terms.

The cause of elevated basal IRI levels in NZO mice

is uncertain. Crofford and Davis [6] reported a 4–5 fold decrease in insulin sensitivity in NZO compared to control mice. Stauffacher *et al.* [18] have presented evidence for a relative insulin resistance in the muscle tissues of this strain and have suggested that hyperinsulinemia may be a compensatory phenomenon. Gates *et al.* [9] on the other hand have shown that the implantation of islets derived from normal mice into NZO animals results in a decrease in plasma insulin levels in the recipients. They have suggested that the NZO strain lacks a pancreatic factor normally acting to inhibit insulin secretion. The effectiveness of arginine as an insulin secretagogue in NZO mice may be relevant since Felig *et al.* [8] have demonstrated raised levels of certain amino acids in the plasma of

greater IRI release in NZO mice than controls while the temporal pattern is the same as in control animals. By contrast, initial IRI release with glucose is deficient in NZO mice in absolute as well as relative terms. With glucagon or aminophylline, initial release appears delayed in the NZO animals with peak values being seen at 5 min compared with one minute in controls. It is possible that with these agents the true peak occurred between one and 5 min and was therefore not detected. It seems probable that a defect exists in the final common pathway of release for all stimuli. This could be related to either the storage or release of insulin. It has been suggested that different insulin secretagogues may cause release from different “pools” within the β -cell [22]. In addition, it is possible

Table 1. % Increments of plasma IRI in NZO and control mice in response to various secretagogues

Min	0	1	5	15	30	45	60	120
Glucose, 1.0 g/kg IV								
Control	100	3762	2593	1519		425		
NZO	100	467	457	283		467		
Glucose, 1.0 g/kg IV								
Control	100	1070	684		223		238	215
NZO	100	255	200		251		232	
Arginine, 10 mg/kg IV								
Control	100	2548	222	95		204		
NZO	100	1233	317	817		210		
Glucagon, 1 μ g/kg IV								
Control	100	2000	640	220		220		
NZO	100	340	389	207		200		
Isoprenaline, 10 μ g/kg IV								
Control	100	732	2536	465		366		
NZO	100	184	605	234		171		
Aminophylline, 25 mg/kg IV								
Control	100	525	183	146		100		
NZO	100	327	404	288		327		

obese humans. Data on the plasma amino acid levels in NZO mice are not available. Islet mass and pancreatic insulin content are known to be elevated in NZO mice [18]. However, it is not clear whether the elevated basal insulin levels stem from an increased number of β -cells all secreting at a normal rate, or if the individual β -cells have an increased rate of secretion. Such information may aid in the understanding of the impaired acute insulin release seen after administration of various secretagogues.

The analysis of the data as presented in Table 1 indicates that, relative to the basal levels of IRI, the initial release of insulin is defective with all the agents studied. However, it is evident that the response to some agents is more markedly affected than to others. In absolute terms, arginine and isoprenaline cause

that abnormalities occur on the stimulus side of the stimulus secretion coupling mechanism, since the various secretagogues are affected to different degrees. For instance, arginine is more effective both in absolute and relative terms than glucose when compared to the response in control mice. Glucagon, isoprenaline and aminophylline are considered to stimulate insulin secretion by increasing β -cell cyclic AMP levels and the adenylyl cyclase system is considered to be an important component of the mechanism whereby glucose causes insulin release [10, 14, 16, 19, 20]. Gates *et al.* [9] have reported that adenylyl cyclase activity is the same in isolated islets of NZO mice and control mice after incubation with glucose, glucagon or tolbutamide. This could imply that the defect in initial insulin release lies beyond the generation of

cyclic AMP or that other, noncyclic AMP dependent mechanisms are abnormal in NZO mice.

Larkins [11] has reported studies of IRI release in isolated islets of NZO mice. These studies showed responses similar to those observed *in vivo*. Basal insulin secretion was greater from NZO islets. The threshold for a significant release of insulin with glucose was higher in NZO mice than controls and there was a delayed pattern of release. Glucagon and tolbutamide failed to elicit a response in NZO mice at

has been reported to inhibit glucose induced IRI release to a greater degree than in normal controls [13]. The basis of its action is unclear; however, it may interfere with an already defective receptor mechanism for glucose. It appears that further studies of insulin secretion in NZO mice are indicated, since an understanding of the basis for the observed abnormalities may be an important step in the study of the control of insulin secretion in maturity onset diabetes.

Fig. 5. Responses of plasma IRI and glucose to aminophylline, 25 mg/kg IV. (Mean $\pm$ SEM)

concentrations which were effective in control animals, whereas NZO islets were more sensitive to arginine. It was further shown that the response to glucose in NZO mice was greatly increased by theophylline. Despite the demonstration of these abnormalities *in vitro*, it remains uncertain as to whether they represent primary defects within the β -cell or are secondary to some longstanding metabolic abnormality *in vivo*.

NZO mice therefore exhibit certain characteristics of insulin secretion which are comparable to those seen in human obese diabetics. The basal levels of plasma IRI are elevated and the early phase of IRI release, is diminished and/or delayed. Later phase insulin release and therefore total insulin released, is excessive in absolute terms. The effect of DPH is also similar to that seen in obese humans where it

Fig. 6. Effect of intraperitoneal diphenylhydantoin (DPH) on the IRI response to glucose in NZO versus white mice. (Mean $\pm$ SEM)

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