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Effects of age, diet and obesity on insulin secretion from isolated perfused rat pancreas: Response to glucose, arginine and glucagon-like peptide 1 (7-37)

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ABSTRACT. **The insulin secretory responses to glucose, arginine and glucagon-like peptide (GLP)-1 (7-37) have been evaluated from the isolated perfused pancreas of rats with either acquired or genetic obesity, *ie*, a) fed *ad libitum* 14-mo old Sprague-Dawley rats as compared to age-matched animals subjected to two types of dietary restriction (every-other-day feeding, EOD, and 40% restriction, 40% DR), and b) 2.5-mo old genetically obese fa/fa rats as compared to the lean counterpart. In mature fed *ad libitum* rats, the glucose-stimulated insulin release from the perfused pancreas was increased 5-fold by addition of 0.1 nM GLP-1 (7-37). A subsequent challenge with high glucose resulted in an improvement of the first phase of insulin release. In 40% DR rats, a similar pattern of secretion was observed, with the difference of a lower response to arginine than in fed *ad libitum* animals. In EOD rats, the overall secretory performance of the perfused pancreas was approximately 50% lower than in the fed *ad libitum* group, but probably adequate to the reduced weight of the animals. In genetically obese young rats, both the response to GLP-1 (7-37) and the total insulin secretion were higher than in the lean controls. Interestingly, the maximal insulin outputs from the perfused pancreases were observed in both the groups of overweight animals. In conclusion, no impairment in the secretory responsiveness of beta-cells occurs in obese animals. Conversely, at least within the age limits of the present study, the endocrine pancreas develops a compensatory ability to match the augmented insulin demand due to the overweight. In the light of the observed great sensitivity of the isolated perfused pancreas to GLP-1 (7-37), changes in the responsiveness of beta- cells to incretins might be involved in the modulation of the endocrine pancreatic function of obese rats.**

INTRODUCTION

Ageing, overnutrition and obesity are among the major risk factors for the development of Type II diabetes mellitus in man. It has been recognized that in such circumstances both impairment in endocrine pancreatic function and reduction of peripheral insulin sensitivity can play a role in the final metabolic derangement which leads to overt diabetes (1, 2). Which one of these two alterations comes first is still a matter of debate. There are experimental models, such as genetically obese and ventromedial hypothalamus-lesioned rodents, where insulin resistance is not supposed to intervene precociously (3, 4). Indeed, it has been suggested that changes in peripheral effect of insulin may be secondary to the hyperinsulinaemia occurring in these animals due to excess stimulation of endocrine pancreas (3).

In other cases, such as ageing rats with unrestricted food intake, an early reduction in the tissue sensitivity to insulin is likely to precede any other alteration (5).

Despite some information (6-8), there are still unclarified aspects concerning the functional behaviour of the endocrine pancreas in animals with either acquired or genetic obesity. In order to obtain more information on this topic and explore possible mechanisms of functional changes, in the present study the influence of such factors as age, nutrition and overweight on insulin secretion from the isolated perfused rat pancreas has been evaluated in a) Sprague-Dawley mature rats (14-mo old) either with unlimited food

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intake or subjected to two different types of dietary restriction since the age of two months; b) genetically obese young Zucker (fa/fa) rats (2.5-mo old).

In addition to glucose and arginine as stimuli of insulin secretion, glucagon-like peptide 1 (7-37) (GLP-1) was used, since this peptide has been indicated as a possible physiological incretin, enhancing insulin release during food ingestion (9, 10). It was considered worthwhile to check the responsiveness of islet tissue, in these different nutritional and metabolic situations, to concentrations of GLP-1 close to those considered to be physiological after glucose or meal ingestion (9, 11).

MATERIALS AND METHODS

Animals

At the age of two months, male Sprague-Dawley rats, obtained from Nossan (Correzzana, Milano), were divided into three groups and subjected, for 12 months, to the following dietary regimens: A) *ad libitum* feeding (controls); B) every-other-day feeding (EOD), *ie* rats fed *ad libitum* every other day; C) 40% food restriction (40% DR), *ie* rats given 60% only of the daily food intake of group A, calculated on a weekly base. These two types of dietary restriction are commonly used in gerontological research to extend life span and retard age-related deteriorations in mammals (12).

Genetically obese "Zucker" (fa/fa) rats of 2.5 months of age and age-matched lean (fa/+) controls were kindly provided by Professor Bernard Jeanrenaud, Laboratoires de Recherches Métaboliques, Geneva, Switzerland.

All pancreas preparations were obtained from animals in the fed state, including EOD rats which were utilized at the end of the 24-hr period of feeding.

Pancreas perfusion

Pancreas was isolated and perfused according to Penhos *et al* (13), with minor modifications as described in detail elsewhere (14). The pancreas was perfused with a modified Krebs-Ringer buffer containing 12.5 mM Hepes, 121 mM NaCl, 4.8 mM KCl, 1.0 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.6 mM $NaHCO_3$, 40 g/l Dextran T-40, 2.5 g/l human serum albumin and 2.8 mM glucose. The perfusing buffer was continuously gassed with a mixture of 95% O_2 and 5% CO_2, pH 7.4. Flow rate was kept constant at 5 ml/min. An equilibration period of 30 min, during which no sample was taken, was followed by a 120-min period of variable stimulatory conditions (see below),

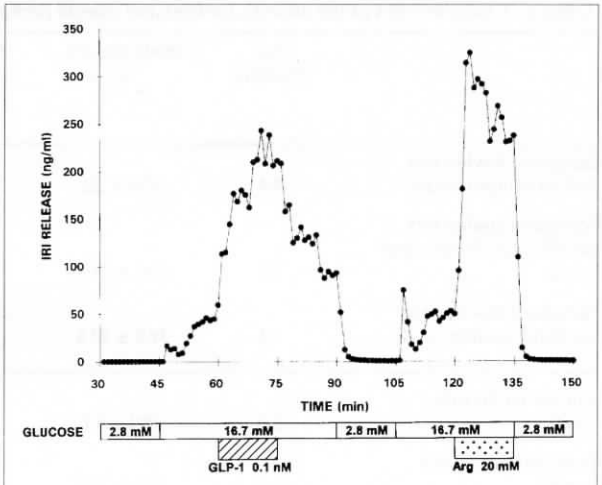

Fig. 1 - *Insulin secretion from the isolated perfused pancreas of 14-mo old Sprague-Dawley rats fed* ad libitum, *in response to glucose, glucagon-like peptide 1 (7-37) [GLP-1] and arginine. Results are given as mean of 6 observations. IRI = immunoreactive insulin.*

during which the pancreatic effluent was collected in 30-sec aliquots from a catheter in the portal vein. The tubes were immediately chilled and stored at -20°C until assayed for determination of insulin. The perfusion protocol consisted of eight consecutive periods of 15 min each with the following conditions: 2.8 mM glucose; 16.7 mM glucose; 16.7 mM glucose plus 0.1 nM GLP-1; 16.7 mM glucose; 2.8 mM glucose; 16.7 mM glucose; 16.7 mM glucose plus 20 mM arginine; 2.8 mM glucose. For Zucker rats, the perfusion was limited to the first five periods. Usually, at the end of the perfusion, the pancreas of Sprague-Dawley rats was removed, weighed and homogenized in cold acidified ethanol (0.7 M HCl:ethanol, 1:3 v/v) for extraction of insulin. The supernatant of the centrifuged homogenate was stored at -20°C until assayed for insulin content.

Assays

Plasma glucose was measured by the glucose-oxidase method using commercially available kits. Insulin was measured by radioimmunoassay according to Herbert *et al* (15), using rat insulin as standard.

Statistical analysis

The statistical analysis of data was performed by using both non parametric and parametric tests. In particular, for each 15-min perfusion period, non para-

Table 1 - *Characteristics of the animals utilized and overall performances of isolated perfused pancreata.*

	Age (months)	Body weight (g)	Glycaemia (mg/dl)	Insulinaemia (ng/ml)	Total IRI output in 105 min perfusion (μg)	Total pancreatic IRI content after perfusion (μg)
Sprague-Dawley rats fed *ad libitum* (n=6)	14	574 ± 22	120 ± 5	2.4 ± 0.3	25.8 ± 3.6	628 ± 66
Sprague-Dawley rats on 40% food restriction (n=5)	14	456 ± 20§	103 ± 9	1.6 ± 0.3	20.0 ± 7.0	570 ± 79
Sprague-Dawley rats on EOD feeding (n=4)	14	365 ± 21§	123 ± 13	2.5 ± 0.5	14.8 ± 1.5§	781 ± 39
Zucker (fa/fa) rats (n=4)	2.5	280 ± 11	116 ± 4	16.5 ± 1.4	23.0 ± 2.0	——
Lean (fa/+) controls (n=3)	2.5	235 ± 9*	108 ± 3	1.2 ± 0.2**	15.1 ± 1.7*	——

Mean ± SEM of the number of observations indicated. § $p < 0.05$ *vs* fed *ad libitum* Sprague-Dawley rats; * $p < 0.05$, ** $p < 0.01$ *vs* Zucker rats. IRI = immunoreactive insulin.

metric multiple comparisons (Kruskal-Wallis test) were made in the case of Sprague-Dawley animals to check for inter-group differences, whereas the Wilcoxon-Mann-Whitney U-test was applied for Zucker rats. When significant differences ($p < 0.05$) were found, then unpaired Student's *t* test was performed to make two-by-two comparisons. The level of significance obtained by Student's t test is indicated in the Table and Figures.

RESULTS

In *ad libitum* fed Sprague-Dawley rats (Fig. 1), 16.7 mM glucose caused a biphasic insulin release and 0.1 nM GLP-1 produced a further 5-fold enhancement of this glucose-induced insulin release. This response to GLP-1, which showed a kind of biphasic pattern with a higher second phase, declined in a stepwise fashion upon withdrawal of the peptide, yet remaining above the rate of secretion observed during the previous glucose stimulation, and finally dropped back to basal values with 2.8 mM glucose. The renewed stimulation with high glucose resulted in a 3-fold enhancement of the first phase of insulin release compared to that occurring during the first glucose challenge. Upon addition of 20 mM arginine, a marked potentiation of insulin secretion occurred promptly, showing a biphasic profile.

In 40% DR rats (Fig. 2), a similar secretory profile was obtained. However, in these rats, the potentiation of glucose-stimulated insulin release by arginine was less marked than in controls.

In EOD rats (Fig. 3), the temporal pattern of the secretory response was close to that of controls, but the amount of secreted insulin was clearly reduced.

When considering the integrated values of insulin secretion during each individual perfusion period (Fig. 4), it can be noticed that the overall secretory performance of perfused pancreas of *ad libitum* fed rats is not significantly different from that of 40% DR rats (with an exception for arginine stimulation), while being approximately twice that of EOD rats. With respect to GLP-1 stimulation, there was no significant change in insulin output in 40% DR rats and a significant reduction ($p < 0.05$) in EOD rats, in comparison to the *ad libitum* fed animals. Nonetheless, in all three experimental groups, GLP-1 proved to be a very strong potentiator of glucose-stimulated insulin release. Upon its removal, insulin release remained at high levels in the presence of 16.7 mM glucose in all groups but especially in EOD and 40% DR animals.

In lean young rats, glucose challenge led to a typical biphasic insulin response from the isolated perfused pancreas and GLP-1 elicited a further enhanced rate of hormone output, which declined slowly upon discontinuation of the peptide (Fig. 5). The age-matched genetically obese rats used in the study had an average body weight increase of 20% relative to the lean

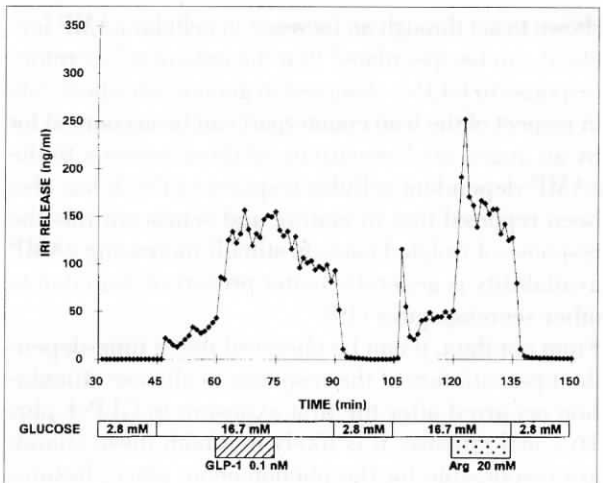

Fig. 2 - *Insulin secretion from the isolated perfused pancreas of 14-mo old Sprague-Dawley rats subjected to 40% food restriction, in response to glucose, glucagon-like peptide 1 (7-37) [GLP-1] and arginine. Results are given as mean of 5 observations.*
IRI = immunoreactive insulin.

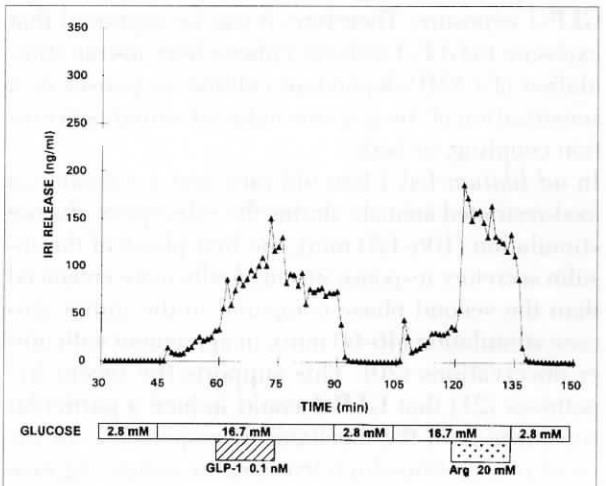

Fig. 3 - *Insulin secretion from the isolated perfused pancreas of 14-mo old Sprague-Dawley rats subjected to EOD feeding, in response to glucose, glucagon-like peptide 1 (7-37) [GLP-1] and arginine. Results are given as mean of 4 observations.*
IRI = immunoreactive insulin.

controls and showed normoglycaemia and hyperinsulinaemia (Table 1). In these animals, the effect of glucose on insulin output from the perfused pancreas was not significantly different, in terms of both secretory pattern (Fig. 5) and total amount (Fig. 6), from that occurring in controls. However, GLP-1 caused a marked potentiation of glucose-stimulated insulin release, which was significantly higher ($p < 0.05$) than the response observed in lean animals. The elevated secretion rate was maintained even upon withdrawal of the peptide and a delayed return of insulin secretion back to basal values was also observed when 16.7 mM glucose was discontinued (Figs. 5 and 6).
In Table 1, where the body weights and the plasma glucose and insulin levels of the different groups of rats are also given, it can be seen that the total insulin output from the perfused pancreas was higher in the mature fed *ad libitum* Sprague-Dawley rats than in the food-restricted animals and was higher as well in the young genetically obese rats compared to the lean controls.
Changes in the overall insulin release from the perfused pancreas of EOD and 40% DR rats were not due to alteration of pancreatic insulin content.

DISCUSSION

The results of our perfusion studies indicate clearly that the functional performances of the endocrine pancreas of both 14-mo old Sprague-Dawley rats with unrestricted diet and genetically obese euglycaemic young rats were not impaired, since the secretory response to various stimuli was always prompt and even larger than that observed in age-matched food-restricted and lean animals, respectively. Actually, these observations are in line with previous ones (6, 16) and fully compatible with the hypothesis that the endocrine pancreas of these animals is able to provide the compensatory effort needed for the maintenance of glucose homeostasis. Nevertheless, as the animals age further, this compensatory ability may be partially lost and either glucose intolerance or hyperglycaemia may ensue.
Our data confirm also the effectiveness of GLP-1 in potentiating glucose-stimulated insulin release in perfused pancreas at concentrations close to those found in human plasma after oral glucose or a mixed meal (9, 11). The potent insulinotropic action exerted by GLP-1 in our experiments further supports the idea of the potential usefulness of this peptide in the treatment of non-insulin dependent diabetic patients, which is currently under investigation (17).
All the animals studied, both young and mature, were exquisitely sensitive to the effect of the peptide, although to a different extent. Since GLP-1 has been

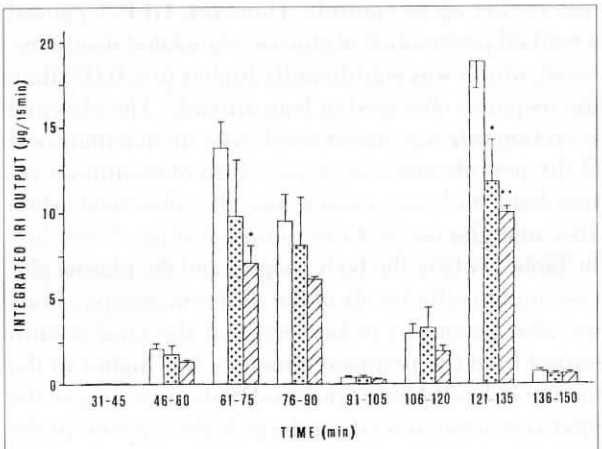

Fig. 4 - *Integrated insulin output from the isolated perfused pancreas of 14-mo old Sprague-Dawley rats subjected to* ad libitum *feeding (□), 40% food restriction (▨) and EOD feeding (▨), during eight consecutive 15-min periods.*
Experimental protocol: 31-45 = 2.8 mM glucose; 46-60 = 16.7 mM glucose; 61-75 = 16.7 mM glucose + 0.1 nM GLP-1; 76-90 = 16.7 mM glucose; 91-105 = 2.8 mM glucose; 106-120 = 16.7 mM glucose; 121-135 = 16.7 mM glucose + 20 mM arginine; 136-150 = 2.8 mM glucose.
*Results are given as mean ± SEM of 4-6 observations. * p < 0.05, ** p < 0.01 vs fed* ad libitum *rats.*
IRI = immunoreactive insulin.

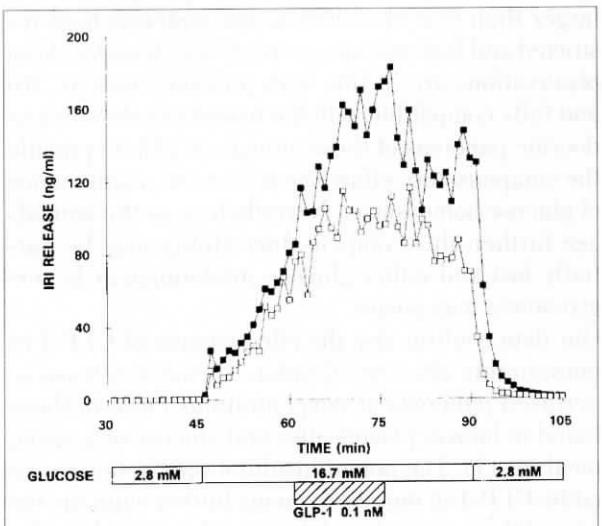

Fig. 5 - *Insulin secretion from the isolated perfused pancreas of 2.5-mo old genetically obese Zucker fa/fa rats (■) and lean controls (□), in response to glucose and glucagon-like peptide 1 (7-37) [GLP-1]. Results are given as mean of 3-4 observations.*
IRI = immunoreactive insulin.

shown to act through an increase in cellular cAMP levels, it can be speculated that the enhanced secretory response to GLP-1 observed in genetically obese rats in respect of the lean counterpart can be accounted for by an augmented sensitivity of these animals to the cAMP-dependent cellular responses (18). It has also been reported that in mature and senescent rats the response of isolated islets to stimuli increasing cAMP availability is generally better preserved than that to other secretagogues (19).

From our data, it can be observed that a time-dependent potentiation of the response to glucose stimulation occurred after the first exposure to GLP-1 plus 16.7 mM glucose. It is likely that both these stimuli are responsible for the phenomenon, since, besides glucose, it has been shown that GLP-1 (7-36) amide alone, at the same concentration used here, can exert a priming effect in the isolated perfused rat pancreas (20).

It should also be noticed that upon removal of GLP-1 from the perfusion medium, glucose-stimulated insulin release declined slowly in a stepwise fashion, remaining however higher than that observed before GLP-1 exposure. Therefore, it can be supposed that exposure to GLP-1 induces either a long-lasting stimulation of cAMP-dependent cellular responses or a sensitization of the glucose-induced stimulus-secretion coupling, or both.

In *ad libitum* fed 14mo old rats, and even more in food-restricted animals, during the subsequent glucose stimulation (106-120 min), the first phase of the insulin secretory response was markedly more enhanced than the second phase compared to the initial glucose stimulation (46-60 min), in agreement with other observations (20). This supports the recent hypothesis (21) that GLP-1 could induce a particular sensitization of the mechanisms responsible for the rapid phase of insulin release, *eg* by enhancing exocytosis of secretory granules in response to glucose through cAMP-dependent phosphorylation of elements of the exocytotic machinery (22).

On the top of the second glucose stimulation, 20 mM arginine elicited in all cases a biphasic maximal insulin response which fell abruptly down to basal values as soon as the isolated perfused pancreas was exposed again to non-stimulatory glucose concentrations, thereby indicating a still very good condition of our pancreas preparation after a long perfusion.

In 40% DR rats, the pattern of insulin secretion from the isolated perfused pancreas was generally similar

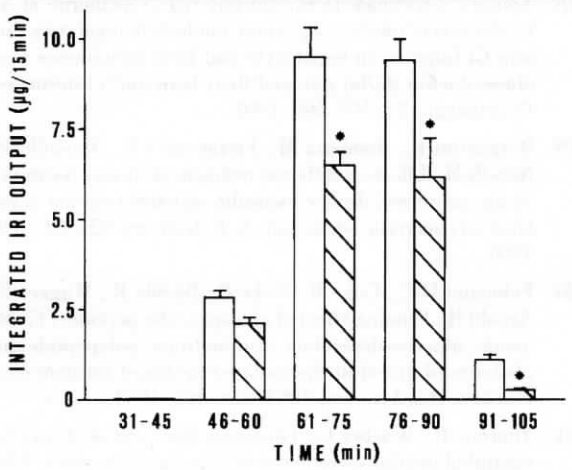

Fig. 6 - *Integrated insulin output from the isolated perfused pancreas of 2.5-mo old Zucker fa/fa rats (□) and lean controls (▨), during five consecutive 15-min periods.*
Experimental protocol: 31-45 = 2.8 mM glucose; 46-60 = 16.7 mM glucose; 61-75 = 16.7 mM glucose + 0.1 nM GLP-1; 76-90 = 16.7 mM glucose; 91-105 = 2.8 mM glucose.
*Results are given as mean ± SEM of 3-4 observations. *p<0.05 vs Zucker rats.*
IRI = immunoreactive insulin.

to that obtained in fed *ad libitum* animals, with the exception of a less marked response to arginine. In EOD rats, the overall response was approximately 50% lower than in the fed *ad libitum* group, although the temporal pattern of secretion was the same. However, the reduced amount of secreted insulin in EOD rats was probably adequate to the reduced weight of these animals. Indeed, if we calculate the total insulin output per unit of animals' body weight, we find no difference among fed *ad libitum*, EOD and 40% DR rats (see Table 1). Interestingly enough, it can also be noticed that the maximal total insulin outputs from the perfused pancreas were observed in the animals with overweight (mature fed *ad libitum* Sprague-Dawley rats and young genetically obese rats).

On the whole, our results suggest that a compensatory ability of the endocrine pancreas develops to match augmented insulin demand due either to the acquired overweight or the genetically determined obesity syndrome. The dietary restrictions used in this study modify the pancreatic secretory performances to levels probably adequate to the reduced metabolic needs of the animals, thus confirming a previous report about another type of caloric restriction (6). Among the mechanisms involved in the modulation of the endocrine pancreatic response, changes in either the glucose-competence or the sensitivity of beta-cells to incretins, such as GLP-1, should be taken into account. Indeed, it has been shown that sub-populations of beta-cells require pre-exposure to GLP-1, or other agents elevating intracellular cAMP, to become glucose-responsive (23). Therefore, one can speculate that GLP-1 and other incretins, whose production is related to food intake, may represent modulatory factors of the glucose-competence of sub-populations of beta-cells, as they are able to affect not only insulin release but also insulin biosynthesis through cAMP-mediated activation of the insulin gene (21).

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