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Impairment of the priming effect of glucose on insulin secretion from isolated islets of aging rats

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Abstract. The time-dependent potentiation (TDP) of insulin release or priming effect exerted by glucose was evaluated in the islets of Langerhans of mature and old rats. Islets isolated from 12- and 26-month-old male Sprague-Dawley rats and incubated for two consecutive 60-min periods in the presence of various stimulating agents were unable to enhance their insulin responsiveness significantly during the second incubation period and showed other abnormalities in their sensitivity to secretagogues compared with islets from 3-month-old animals. The priming action of glucose plus arginine or isobutylmethylxanthine (IBMX) was not observed in islets from 12-month-old rats, but surprisingly, islets from senescent rats showed a restoration of the beta-cell memory in the presence of IBMX. Interestingly, the islets isolated from 2-month-old animals previously exposed to an intravenous glucose load in vivo released approximately twice as much insulin as the islets taken from fed rats not subjected to the load. This potentiation exerted by the intravenous glucose administration was reduced but not abolished in the islets of glucose-intolerant, 12-month-old rats. In conclusion, the glucose TDP of insulin secretion is impaired in islets of mature and old rats, confirming an early loss of sensitivity of beta-cells to secretagogues during aging.

Key words: Insulin secretion – Aging – Time-dependent potentiation – Priming effect – Rat islets

Introduction

It is well established that prior glucose stimulation of pancreatic beta-cells amplifies the insulin secretory response to a second glucose challenge [1–4]. This effect has been referred to as the time-dependent potentiation (TDP) or priming effect of glucose. The phenomenon has been extensively studied and characterized [3, 5, 6], but the underlying mechanism is not entirely understood.

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Apart from glucose, other secretagogues, either metabolizable or not, can elicit a TDP memory in beta-cells [7–11], which improves the insulin response to subsequent stimuli.

This priming effect has been observed in humans [4, 12] and rats [3, 13], but not in mice [14], and is lacking in diabetic rats [15, 16] and dexamethasone-treated rats [15]. At present, little is known about the occurrence of the TDP of insulin release in the islets of aging rats, which on the other hand have altered functional capabilities in vitro [17–19]. Indeed, a decrease in the responsiveness to glucose and to other insulin secretagogues is already apparent in the islets of 12-month-old rats [19] and occurs simultaneously with the development of islet hyperplasia and/or hypertrophy [20].

Therefore, the present study was undertaken firstly to study the TDP of glucose and other secretagogues in the isolated islets of mature and old rats and secondly to investigate the effect of an in vivo glucose load on the insulin response to a subsequent in vitro stimulation of the isolated islets.

Materials and methods

Animals

Male Sprague-Dawley rats were purchased from Nossan, Milan, at 8 weeks of age and housed under artificial lighting (12 h light-dark cycle) until used for the study. Tap water and standard laboratory chow were given ad libitum. All experiments were conducted on non-fasted animals and started between 0900 and 1100 h. The 'Principles of laboratory animal care' (NIH publication No. 83-25, revised 1985) were followed, as well as all the recommendations of Italian law for the use of experimental animals (DL No. 116, 1992).

Experimental protocols

In a first set of experiments, rats of various ages were anaesthetized with an intraperitoneal injection of 50 mg/kg body weight sodium pentobarbital. The pancreas was removed, trimmed free of adipose tissue and minced in Hank's solution. Islets were isolated by a modification of the method of Lacy and Kostianovsky [21], according

to the suggestions of Trueheart Burch et al. [22], who warned against the utilization of too harsh procedures for the isolation of islets from older rats, to prevent damage. In particular, the pancreatic minces from both young and old rats were subjected to collagenase digestion (2 mg/ml), carried out in test tubes by vigorous manual shaking at 37°C for 6 or 7 min depending on the degree of digestion as judged by inspection. Occasionally, the digests of pancreas from old donors required a few additional shakings. The digests were washed four times with cold Hank's solution, and islets were picked out by hand under stereomicroscope observation. Groups of 7–10 islets were placed in each incubation well; very small or very large islets were discarded as were those surrounded by undigested material, which was found more frequently in digests from old animals. The overall time required for the isolation was slightly more than 1 h on average. After a 60-min preincubation period in modified Krebs-Ringer bicarbonate (KRB) buffer containing 0.5% bovine serum albumin, 10 mM *N*-2-hydroxy-ethylpiperazine-*N*^{1}-2-ethanesulphonic acid (Hepes) (pH 7.4) and 2.8 mM glucose, islets were incubated for 60 min at 37°C in a humidified atmosphere of 5% CO_2 in air in 1 ml KRB-Hepes buffer containing 2.8 or 16.7 mM glucose, this latter alone or plus 20 mM arginine or 1 mM 3-isobutyl-1-methylxanthine (IBMX). At the end of this first incubation period, the medium was removed for insulin measurement, the islets were washed with buffer, and 1 ml of fresh KRB-Hepes buffer was added, containing the same substances as above, for a further 60-min incubation period. At the end of this second incubation, the medium was again collected for insulin determination. Finally, 1 ml of cold acidified ethanol (0.7 M HCl:ethanol, 1:3 v/v) was added to the islets in order to extract their insulin content.

In a second experiment, two groups of rats, aged 2 and 12 months, were administered intravenous glucose (1.5 g/kg b.w. as a 40% solution) under sodium pentobarbital anaesthesia. Blood samples were collected from the tail vein at 0, 10, 30 and 50 min after glucose administration for glucose and insulin determinations. Fifty minutes after the load, the animals were killed, and islets of Langerhans were isolated as described. Following a 60-min preincubation period in KRB-Hepes buffer with 2.8 mM glucose, groups of 7–10 islets were incubated for a single 60-min period in the presence of the above-mentioned secretagogues and also of 20 mM 2-ketoisocaproate (2-KIC) and 30 mM KCl. Age-matched rats of the same litter were injected with saline and used as donors of control islets.

Assays

Plasma glucose was measured by the glucose-oxidase method using commercially available kits (Glucinet, Sclavo Diagnostici, Siena, Italy). Plasma insulin, either released into the buffer or extracted from the islets at the end of the incubation period(s), was measured by radioimmunoassay (RIA) according to Herbert et al. [23], using rat insulin as a standard. The minimum detectable insulin level was 0.13 ng/ml in the assay, and the intra-assay and inter-assay variations were 3.1% and 10.2%, respectively.

Chemical reagents

Arginine, 2-KIC and IBMX were purchased from Sigma, St. Louis, Missouri, USA. Collagenase used for islet digestion was obtained from Serva Feinbiochemica GmbH, Heidelberg, Germany. ^{125}I-Insulin was kindly provided by Prof. R. Navalesi, Cattedra di Malattie del Ricambio, University of Pisa. All reagents were of analytical grade.

Expression of results and statistical analysis

Insulin release from isolated islets has been expressed as percentage of the islet insulin content. Data are given as mean±SEM. Statistical significance was evaluated by Student's *t*-test for unpaired data and in some cases by analysis of variance (ANOVA).

Results

In the rats of the present study, body weight increased by approximately 50% between 3 and 12 months of age, remaining substantially unchanged at 26 months (Table 1). Plasma glucose concentrations did not change with aging, whereas plasma insulin concentrations showed a significant increase (Table 1).

Table 2 shows the results of the stimulation by various secretagogues of islets of Langerhans isolated from rats of different ages, during two consecutive 60-min static incubations in KRB-Hepes buffer. In the islets from 3-month-old rats, the insulin release during the first incubation period was stimulated by 16.7 mM glucose and potentiated in the presence of 1 mM IBMX. In this experiment the addition of 20 mM arginine caused only a slight further increase in insulin release. As expected, previous exposure to glucose resulted in an enhancement of the insulin response to a subsequent glucose challenge, when compared with either a timed control (islets simultaneously exposed to glucose for the first time) or with the first priming response to the sugar. This priming effect was also observed in the presence of additional secretagogues such as IBMX or arginine.

When islets were isolated from 12-month-old rats, the overall insulin responsiveness to glucose and other secretagogues was reduced, whereas the basal insulin release was increased in comparison with younger animals. Interestingly, in these mature animals, the priming effect of glucose disappeared, since no significant enhancement of insulin release occurred during a repeated exposure to the sugar alone or in association with other stimulating agents. When the islets taken from senescent rats (26-month-old) were subjected to the same incubation protocol, the pattern of secretory response to the stimuli was similar to that found in the islets of 12-month-old animals, with a relevant exception. Indeed, a weak response to secretagogues was observed, and previous exposure to glucose alone and to glucose plus arginine failed to improve insulin release during a subsequent stimulation. However, unlike what occurred in the islets of 12-month-old rats, a significant TDP of insulin release was observed in the presence of glucose plus IBMX (Table 2).

Table 1. Body weight, plasma glucose and insulin levels, and islet insulin content of Sprague-Dawley rats of 3, 12 and 26 months of age

Age (months)	Body weight (g)	Plasma glucose (mg/dl)	Plasma IRI (ng/ml)	Islet IRI content (ng/islet)
3	365 ± 4 (5)	124 ± 4 (5)	4.2 ± 0.7 (5)	137 ± 8 (34)
12	542 ± 31* (5)	120 ± 5 (5)	6.7 ± 1.5 (5)	170 ± 7* (41)
26	592 ± 49* (4)	131 ± 9 (4)	8.2 ± 0.8* (4)	196 ± 12* (35)

Results are expressed as mean ± SEM of the number of observations indicated in parentheses. * $P<0.01$ versus the corresponding values of 3-month-old rats (unpaired Student's *t*-test)
IRI, Immunoreactive insulin

Table 2. Insulin release from isolated islets of 3-, 12- and 26-month-old rats during two consecutive 60-min incubations

Incubation conditions		IRI release (% of IRI content)		
		3 months	12 months	26 months
2.8 mM Glucose	1st h	0.47 ± 0.03	0.58 ± 0.06	1.05 ± 0.16
Idem	2nd h	0.35 ± 0.09	0.44 ± 0.08	0.93 ± 0.10
2.8 mM Glucose	1st h	0.46 ± 0.05	0.48 ± 0.04	0.92 ± 0.09
16.7 mM Glucose	2nd h	3.17 ± 0.36**	0.66 ± 0.12	1.02 ± 0.05
16.7 mM Glucose	1st h	3.81 ± 0.19	1.34 ± 0.10	2.08 ± 0.25
Idem	2nd h	5.47 ± 0.18**	1.54 ± 0.18	2.17 ± 0.23
16.7 mM Glucose + 1 mM IBMX	1st h	7.51 ± 1.12	2.54 ± 0.32	3.35 ± 0.26
Idem	2nd h	12.51 ± 1.57*	2.92 ± 0.26	7.80 ± 0.78**
16.7 mM Glucose +20 mM Arginine	1st h	4.37 ± 0.23	1.94 ± 0.18	3.67 ± 0.39
Idem	2nd h	6.54 ± 0.44**	2.24 ± 0.20	3.13 ± 0.66

Results are expressed as mean ± SEM of 4-5 observations. * $P<0.05$, ** $P<0.01$ vs the corresponding 1st incubation period (unpaired Student's t-test). The statistical significance of the effects of IBMX was confirmed by analysis of variance (effect of age, $P<0.01$; effect of incubation period, $P<0.01$; interaction age-incubation period, $P<0.05$)
IBMX, Isobutylmethylxanthine

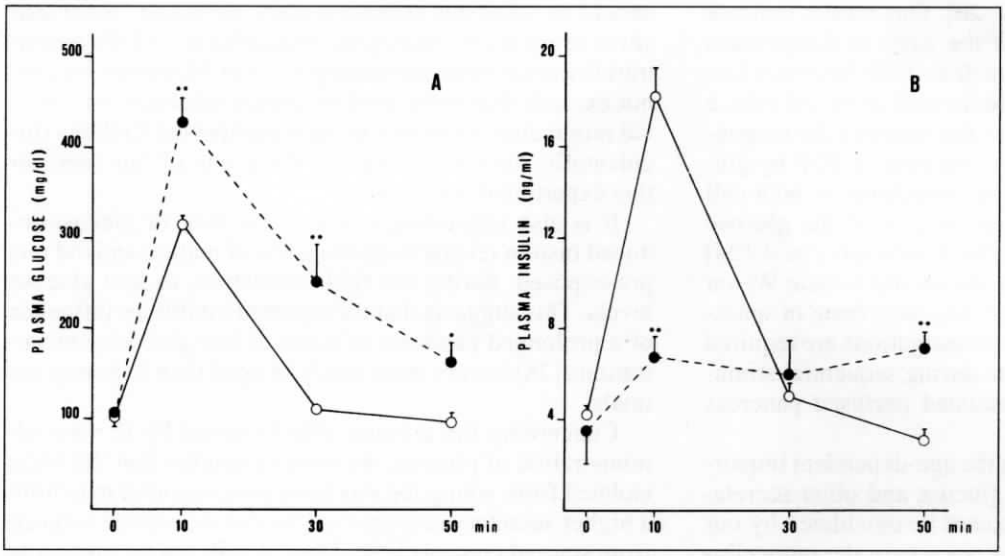

Fig. 1A, B. Plasma glucose (**A**) and insulin (**B**) levels in 2-month-old (○) and 12-month-old (●) rats subjected to an intravenous glucose load. Mean±SEM of 5 observations. The symbols * and ** indicate significant differences between the two groups of rats evaluated by unpaired Student's t-test ($P< 0.05$ and $P< 0.01$, respectively)

Also, in both mature and senescent rats, the addition of 16.7 mM glucose to islets pre-exposed to non-stimulating concentrations of the sugar failed to stimulate insulin release significantly. In contrast, in younger islets, 16.7 mM glucose elicited the same secretory response when added either in the first or in the second incubation period.

It is worth noticing that the islet insulin content, measured at the end of the incubation periods, increased by 24% in 12-month-old and by 43% in 26-month-old rats, over that of young controls (Table 1).

Figure 1 shows the abnormal profiles of plasma glucose (A) and insulin (B) levels after an intravenous glucose load in 12-month-old rats compared with younger ones. In older rats, the glucose load induced a higher peak and a slower clearance of plasma glucose, as well as a lower peak in plasma insulin, than in younger rats. Moreover, both glucose and insulin plasma values did not return to basal levels within 50 min after the load.

Figure 2 shows the insulin secretion by islets isolated from 2-month-old (A) and 12-month-old (B) animals, subjected to the above-mentioned glucose load, compared with that by islets isolated from age-matched controls without any load. Interestingly, the islets isolated from 2-month-old rats previously exposed to the glucose load in vivo released, for each stimulus, approximately twice as much insulin as the islets taken from rats not subjected to the load.

Also, in the islets isolated from 12-month-old rats after the glucose load, the priming effect exerted by the intravenous glucose administration was present, reduced somewhat in comparison with that in younger rats, but not abolished.

Discussion

Stimulation of insulin release in isolated islets exerted by glucose alone or in the presence of various other secreta-

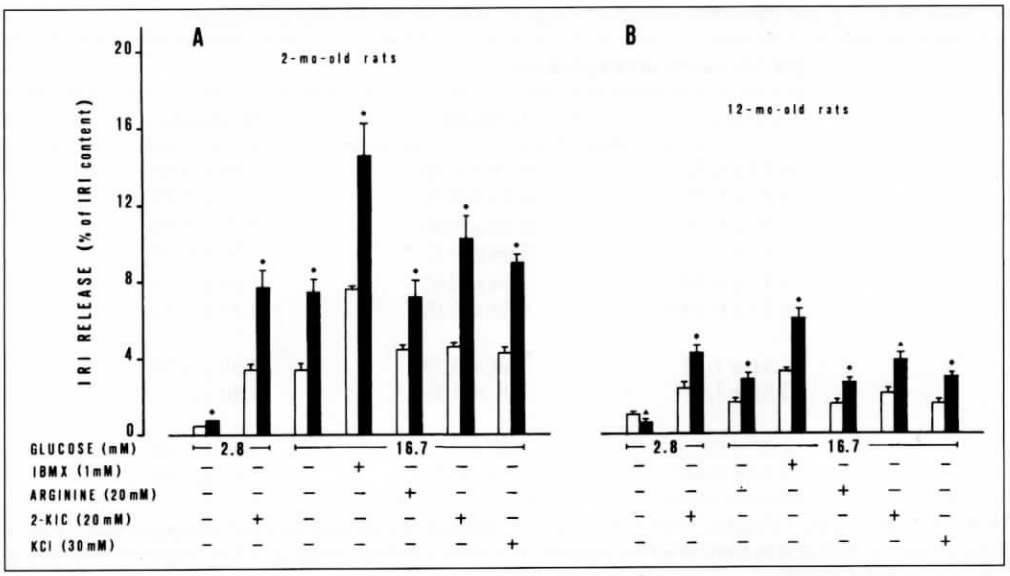

Fig. 2A, B. Insulin release from isolated islets of 2-month-old (**A**) and 12-month-old (**B**) rats subjected to an intravenous glucose load (*solid bars*) compared with age-matched controls without any load (*open bars*). Islets were isolated from rats killed 50 min after i.v. glucose administration (1.5 g/kg b.w.) and incubated, like those isolated from control rats, for 60 min in the presence of the indicated substances. Mean±SEM of 5 observations. ▲ $P< 0.05$, $^{*}P< 0.01$ vs the corresponding controls without glucose load (unpaired Student's *t*-test). *IBMX*, Isobutylmethylxanthine; *2-KIC*, 2-ketoisocaproate

gogues highlights a diminished islet sensitivity in both mature and old rats compared with young ones, in agreement with previous observations [17–20]. Our results indicate clearly that a typical feature of the islets of Langerhans isolated from young animals, such as TDP, becomes lost during aging. Indeed, in mature as well as in old rats, a second exposure to glucose does not improve the responsiveness of the islets. Therefore, the loss of TDP by glucose can be included among the alterations of beta-cell function occurring during aging. A lack of the glucose priming effect has been reported by Tsuchiyama et al. [24] in the perfused pancreas of 10-month-old female Wistar rats, but no previous observation has been done in senescent animals. Certainly, further investigations are required on the kinetics of insulin release during sequential stimulations in perifused islets or isolated perfused pancreas from over 2-year-old rats.

The mechanisms underlying the age-dependent impairment of the priming effect of glucose and other secretagogues on the isolated islets cannot be elucidated by our data. However, we can state that the size of the intracellular insulin pool plays no crucial role for the priming phenomenon, since in older animals the islet insulin content is greater than in younger ones (24% increase in 12-month-old and 43% increase in 26-month-old over young animals, as shown in Table 1). In any case, the insulin released in response to glucose represents only a small fraction of the total insulin in the beta-cell. Similar conclusions have been raised by Borg et al. [25], on the basis of morphological studies on the redistribution of secretory granules within the beta-cells.

It has been suggested that the metabolism of glucose plays a key role in TDP of insulin release [3], and it has been thought that the lack of glucose priming is linked to a decreased islet metabolic capacity [24]. A reduced glucose oxidation was observed indeed by some investigators in the islets from old animals [26], but was not found by others [27]. Our data, showing that TDP is partially preserved in the islets from mature animals pre-exposed to glucose in vivo, also suggest that other still unknown factors could be involved.

As regards the restoration of TDP in islets of 26-month-old rats stimulated in vitro with glucose plus IBMX, it should be noted that senescent islets are usually more sensitive to agents increasing the intracellular cAMP concentration than to other secretagogues [19]. However, we cannot exclude that some kind of animal selection for survival might also contribute to such unexpected findings (incidentally, the survival rate of the group of rats used for this experiment was 24%).

It is also interesting to quote the lack of glucose-induced insulin release from the islets of mature and old rats pre-exposed, during the first incubation, to low glucose levels. This suggests that the reported inhibitory influence of a prolonged exposure of islets to low glucose concentrations [28] occurs more easily in aged than in young animals.

Concerning the priming effect exerted by in vivo administration of glucose, we must remember that the islets isolated from young fed rats have been reported to exhibit a higher secretory response to glucose than those isolated from starved controls [29]. Thus, it may not be surprising that the glucose 'pulse' determined by the intravenous glucose load in young fed animals is able to ameliorate the islet responsiveness to a subsequent stimulation in vitro. The reason why this phenomenon occurs to some extent in mature rats also, in contrast to the inability of islets from such donors to be primed in vitro, is unclear, perhaps related to the more physiological way of priming induction.

For a general interpretation of the results of the present study, it should also be taken into account that the age-related increase in body weight of our Sprague-Dawley rats (but this occurs in other strains, too) is paralleled by an increase in adipose mass, as estimated by the weight of their epididymal fat pads (V. De Tata, personal communication). The development of obesity and the usually concomitant reduction of peripheral tissue sensitivity to insulin in aging animals could undoubtedly contribute to the alteration of glucose tolerance, as confirmed by the post-loading profiles of plasma glucose and insulin in 12-month-old rats, shown in Fig. 1. On the other hand, it is unlikely that obesity by itself could be responsible for the age-related im-

pairment in TDP, since the phenomenon also occurs in mature female rats weighing much less than males [24].

As a final comment, we think that the occurrence of TDP can be considered an index of the exquisite sensitivity of beta-cells to physiological stimuli. Conversely, the attenuation or the loss of TDP may represent a reliable sign of a still mild impairment of the islet responsiveness. For example, the loss of TDP is the only indication of the partial damage induced by in vitro exposure of the islets to streptozotocin in the presence of large doses of the protective agent 3-aminobenzamide [30]. A loss of TDP occurs also in neonatally streptozotocin-induced diabetic rats as well as in dexamethasone-treated rats and has been attributed in both cases to excessive demand on beta-cell performance [15]. In this latter perspective, in our senescent rats fed ad libitum throughout their life span, the long-lasting stimulation of beta-cells due to excessive metabolic challenge could lead as well to the loss of TDP, simultaneously with or even preceding other signs of functional alterations.

In line with this interpretation and with the observation that the priming effect of glucose can be restored in diabetic rats after fasting or insulin therapy [15, 31], future research is warranted to explore the effect of dietary restriction on TDP in islets of senescent animals.

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