Insufficient adaptive capability of pancreatic endocrine function in dexamethasone-treated ageing rats

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Abstract

This study was aimed at exploring the capability of the pancreatic endocrine adaptive mechanisms of ageing Sprague-Dawley rats to counteract the metabolic challenge induced by the prolonged administration of dexamethasone (DEX) (0·13 mg/kg per day for 13 days). DEX treatment induced peripheral insulin resistance in 3-, 18- and 26-month-old rats, as indicated by the significant and persistent rise of plasma insulin levels in each age group (plasma insulin in 3-, 18- and 26-month-old rats from basal values of $4·3 \pm 0·8$, $4·7 \pm 0·5$ and $5·6 \pm 1·0$ ng/ml (means ± S.E.M.) respectively, rose to $11·9 \pm 1·7$, $29·1 \pm 5·5$ and $27·9 \pm 2·7$ ng/ml respectively, after 9 days of administration). However, plasma glucose concentrations remained unchanged during the treatment in young rats, whereas they increased up to frankly diabetic levels in most 18-month-old and in all 26-month-old animals after a few days of DEX administration. Plasma free fatty acid concentrations increased 2-fold in 3- and 26-month-old rats and 4-fold in 18-month-old rats and could possibly be involved in the glucocorticoid-induced enhancement in insulin resistance, although they showed no significant correlation with glycaemic values.

Incubation of pancreatic islets obtained from treated rats showed that DEX administration increased the insulin responsiveness of islets from not only younger but also older donors. However, in the islets of ageing rats, which already showed an age-dependent impairment of the sensitivity to glucose and other secretagogues, this enhancing effect was clearly attenuated with respect to the younger counterpart. Furthermore, DEX treatment depressed significantly the priming effect of glucose in islets isolated from all the three age groups.

In conclusion, our results show that ageing rats are unable to counteract effectively a prolonged hyperglycaemic challenge as such induced by DEX administration. This homeostatic defect can be ascribed to the age-dependent failure of the endocrine pancreas to provide enough insulin to overcome the aggravation of an antecedent state of increased peripheral insulin resistance.

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Introduction

An age-related alteration of carbohydrate tolerance has been recognized for many years in both humans and rats (De Fronzo 1981, Jackson 1990), but the mechanisms involved have not been fully clarified. Several *in vitro* studies have indicated an impairment in insulin secretion with advancing age, since isolated islets from older rats do not release insulin as rapidly and efficiently as do islets from younger rats (Reaven *et al.* 1979, Sartin *et al.* 1986), despite partial compensation due to the development of islet hypertrophy and/or hyperplasia (Reaven *et al.* 1979, Coordt *et al.* 1995). On the other hand, studies in intact animals and man have generally shown that circulating insulin levels are either unchanged or even increased after a glucose challenge in senescence (Palmer & Ensinck 1975, De Fronzo 1979). Moreover, studies utilizing the euglycaemic clamp technique (De Fronzo 1979, Fink *et al.* 1983, Jackson 1990) indicate that the age-related impairment in glucose disposal may be in large part dependent on the development of peripheral insulin resistance.

Taking into account these results, the present study aimed at exploring whether in aged rats the adaptive mechanisms of pancreatic β-cells could counteract the effects of an additional stimulus leading to increased insulin resistance, such as that induced by glucocorticoid treatment (McMahon *et al.* 1988, Weinstein *et al.* 1995). Furthermore, since glucocorticoids are antagonists of insulin effects on peripheral tissues, including fat, and recently the participation of lipid disregulation in the development of hyperglycaemia in non-insulin dependent diabetes mellitus has been emphasized (Reaven 1995, Unger 1995), we explored also the changes in plasma free fatty acid (FFA) levels of dexamethasone (DEX)-treated rats looking for a possible correlation with glycaemic values.

Finally, we have investigated the effects of the *in vivo* dexamethasone administration on the response of isolated islets to glucose and other secretagogues, in order to compare the *in vitro* functional performances of β-cells from young and old treated animals.

Materials and Methods

Animals

Experiments were performed on three groups of male Sprague–Dawley rats of 3, 18 and 26 months of age. Animals had free access to food, were kept at 24–25 °C and with 12 h light:12 h darkness. The *Principles of Laboratory Animal Care* (US NIH publication No. 83–25, revised 1985) was followed as well as the recommendations of Italian law for the use of experimental animals (DL No. 116/1992).

Dexamethasone treatment

Rats of each age-group were randomly divided into two subgroups, dexamethasone-treated and controls, which were subjected to a daily s.c. injection of either dexamethasone phosphate (0·125 mg/kg body weight, dissolved in saline) or saline alone respectively, for 13 days. Blood samples were collected from the tail vein of conscious animals just before and 4, 9 and 13 days after the glucocorticoid treatment.

Insulin extraction from the pancreas

The day following the last dexamethasone administration, rats were anaesthetized with 50 mg/kg Nembutal i.p., the pancreas was carefully dissected free of adipose tissue and a substantial amount (300–400 mg) was weighed and homogenized in cold acidified ethanol (150:47:3, v/v; absolute ethanol:H_2O:concentrated HCl) for extraction of insulin as described (Bergamini *et al.* 1991).

Isolation of islets and incubation protocol

The remaining pancreata from each rat of the same group were pooled and used for the isolation of the islets of Langerhans by a modification of the method of Lacy & Kostianovsky (1967), according to the suggestions of Trueheart Burch *et al.* (1984) and using the same procedure in all cases. After a 60-min preincubation period in modified Krebs–Ringer bicarbonate (KRB) buffer containing 0·5% BSA, 10 mM Hepes pH 7·4, and 2·8 mM glucose, groups of seven to ten 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 2-isoketocaproic acid (2-KIC), or 1 mM 3-isobutyl-1-

methyl-xanthine (IBMX). At the end of this first incubation period, the medium was removed for insulin measurement, the islets were washed and incubated for a further 60-min period in 1 ml fresh buffer, containing the same substances as above. Then, the buffer was again collected and 1 ml cold acidified ethanol was added to extract islet insulin content.

Assays

Plasma glucose and FFA levels were measured using commercial kits (Sclavo Diagnostics, Siena, Italy and Boehringer Mannheim Italia SpA, Milan, Italy, respectively).

Insulin was measured by RIA according to Herbert *et al.* (1965), using rat insulin as a standard. The sensitivity and the coefficients of variation of the RIA were as follows: detection limit 0·13 ng/ml, intraassay variation 3·1%, interassay variation 10·2%.

Electron microscopy

Soon after isolation, groups of islets of Langerhans were fixed in phosphate-buffered 2·5% glutaraldehyde, washed, post-fixed in buffered 0·1% osmium tetroxide, dehydrated and finally embedded in Poly/Bed 812 (Polisciences Europe, Eppelheim, Germany) (Sabatini *et al.* 1963). Sections were stained with uranyl acetate and lead citrate (Reynolds 1963) and were observed with a Zeiss 902 microscope. The procedure for electron microscopic morphometry was based on the technique of Weibel (1979).

Statistical analysis

Data are given as means ± S.E.M. Statistical significance was evaluated by ANOVA, followed by Student's *t*-test for unpaired data to assess two by two differences, when appropriate.

Results

Body weight, pancreas weight and pancreatic insulin content

Table 1 shows the body weights, pancreas weights and insulin contents of both the whole pancreas and the isolated islets of Langerhans of the six experimental groups. The administration of DEX for 13 days caused impairment of weight gain in young rats and significant reduction of body weight in aged rats (20 and 35% decrease versus the initial values in 18- and 26-month-old rats respectively).

Concomitantly, in each group of DEX-treated rats, there was a significant decrease of the pancreas weight, which was quantitatively more relevant in 26-month-old (47% reduction) than in 18-month-old (22% reduction) or in young rats (29% reduction).

Table 1 Body weight, pancreas weight and pancreatic insulin content in 3-, 18- and 26-month-old Sprague–Dawley rats treated with DEX for 13 days. Results are means ± S.E.M. of the number of observations indicated in parentheses. DEX was administered s.c. at a daily dose of 0·125 mg/kg

	Treatment	Body weight (g)		Pancreas weight (mg)	Pancreas IRI content (ng/mg)	Islet IRI content (ng/islet)
		Before treatment	After treatment			
Age (months)						
3	Controls	257 ± 10 (9)	347 ± 7§ (9)	1092 ± 41 (9)	231 ± 28 (9)	122 ± 6 (35)
	DEX	285 ± 16 (9)	262 ± 9* (9)	780 ± 22* (9)	423 ± 71* (9)	195 ± 10* (30)
18	Controls	696 ± 26 (7)	710 ± 19 (7)	1541 ± 94 (7)	151 ± 25 (7)	126 ± 8 (35)
	DEX	731 ± 23 (7)	584 ± 18§* (7)	1202 ± 41* (7)	166 ± 44 (7)	128 ± 7 (35)
26	Controls	626 ± 44 (4)	593 ± 49 (4)	1445 ± 159 (4)	273 ± 45 (4)	209 ± 12 (35)
	DEX	623 ± 32 (4)	408 ± 22§* (4)	761 ± 88* (4)	446 ± 110 (4)	312 ± 16* (25)

§$P < 0.01$ versus the initial body weight; *$P < 0.05$, at least, versus the corresponding controls (unpaired Student's *t*-test).

On the other hand, DEX treatment significantly increased the pancreatic insulin content in the young animals only, and the islet insulin content in both 3- and 26-month-old rats ($P < 0.01$).

Electron microscopic morphometry showed no difference between islets isolated from control and DEX-treated rats of each age-group with regard to organelles structure and degree of granulation (data not shown).

Plasma glucose and insulin levels

Figure 1 shows the variations of plasma glucose and insulin levels after 0, 4, 9 and 13 days of treatment with DEX. In all the untreated control groups neither plasma glucose nor insulin levels changed significantly during the experimental period. Plasma glucose levels were not modified by the treatment in 3-month-old rats, whereas 18- and 26-month-old rats developed a marked hyperglycaemia, which, for the latter group, was already significant after 4 days of DEX administration. DEX treatment, on the other hand, rapidly induced a 2-fold increase of plasma insulin levels in young rats, and a 4- to 5-fold increase in older rats. In ageing animals, the increment of both glucose and insulin plasma levels appeared to decline at the end of DEX treatment. It should be noticed that the glycaemic changes of DEX-treated 18-month-old rats were not homogeneous, since three animals out of seven remained normoglycaemic throughout the experiment, whereas the others attained plasma glucose values in the range 375–445 mg/100 ml at the end of the treatment. Independently of glycaemia, all these rats showed a marked hyperinsulinaemia. An interesting positive correlation was found in 18-month-old treated rats between the initial body weight and post-treatment either glycaemic or insulinaemic values ($r = 0.802$ and 0.864 respectively, $P < 0.05$).

Plasma FFA levels

After 13 days of DEX administration, plasma FFA levels increased 2-fold in 3- and 26-month-old rats and 4-fold in 18-month-old animals with respect to basal values (Table 2). In the 18-month-old animal group, the remarkable increase in FFA plasma levels showed little variability and was not correlated to plasma glucose levels ($r = 0.014$, NS). This increase occurred early during DEX treatment and remained stable until the end (plasma FFA concentrations were $1274 ± 237$ µEq/l at 4 days and $1426 ± 160$ µEq/l at 8 days of treatment, i.e. close to the values at day 14 in Table 2).

Insulin secretion from isolated islets

Figure 2 shows the results of the stimulation by various secretagogues of the islets of Langerhans isolated from control and DEX-treated rats of 3, 18 and 26 months of age. At 2·8 mM glucose, the effect of age on basal insulin secretion (as percentage of the islet insulin content) was significant ($F = 38.7$, $P < 0.01$) and influenced significantly the effect of DEX treatment ($F_{interaction} = 10.5$, $P < 0.01$). In the islets from 18-month-old untreated rats, the insulin secretion was significantly stimulated by glucose, but, at variance with the young controls, the other secretagogues (IBMX and 2-KIC) were unable to potentiate the
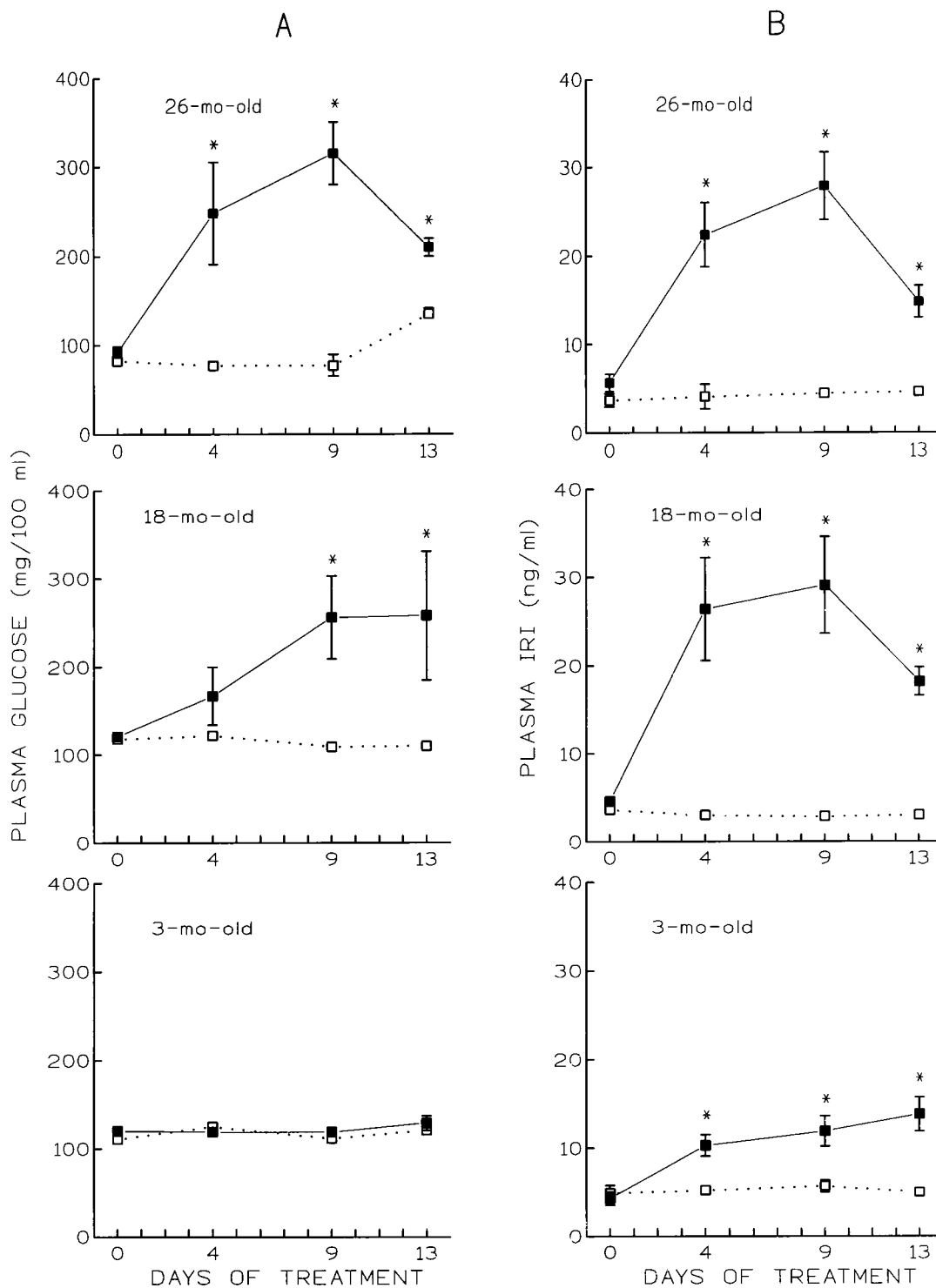
Figure 1 Plasma glucose (A) and insulin (B) concentrations in control (□) and dexamethasone-treated (■) Sprague–Dawley rats of various ages. Data are expressed as means ± S.E.M. of nine, seven and four cases for 3-, 18- and 26-month-old rats, respectively. *P<0·05, at least, versus age-matched control rats (unpaired Student's *t*-test).

Table 2 FFA plasma levels in Sprague–Dawley rats of various ages, treated with DEX (0·125 mg/kg per day) for 13 days. Results are means ± S.E.M. of the number of observations indicated in parentheses

	FFA (μEq/l)	
	Before treatment	After treatment
Age (months)		
3	410 ± 38 (9)	799 ± 155 (7)
18	324 ± 49 (7)	1315 ± 137 (7)
26	247 ± 40 (4)	656 ± 37 (4)

ANOVA showed that the effect of DEX treatment was significant ($F=56\cdot6$, $P<0\cdot01$), as well as the effect of age ($F=7\cdot33$, $P<0\cdot01$), and the interaction ($F=5\cdot09$, $P<0\cdot05$).

stimulatory effect of the sugar. Finally, in the islets from the 26-month-old untreated rats, the response to all stimuli was weak. DEX treatment caused generally an increase of insulin release from the isolated islets of all age-groups in the presence of both glucose alone and glucose plus IBMX or 2-KIC: this enhancing effect was relevant in young islets and clearly attenuated in islets taken from 18- and 26-month-old rats (the effect of the interaction age × DEX treatment was significant for all three stimuli). Additional experiments performed in DEX-treated 18-

month-old rats showed no difference in the insulin secretory response of islets isolated from either normoglycaemic or hyperglycaemic animals (data not shown).

As reported in Table 3, the insulin release from isolated islets of young controls during the second incubation period (see Materials and Methods) was significantly enhanced by the repeated stimulation with glucose alone or glucose plus IBMX. In the case of older islets, only the combination of 16·7 mM glucose and 1 mM IBMX retained this ability, in agreement with previous results (Bombara *et al*. 1995). In the islets of DEX-treated animals of all age groups, the time-dependent potentiation of insulin release did not occur and occasionally significantly less insulin was released during the second incubation than during the first.

Discussion

One of the main results of the present study is the observation that in ageing rats, at variance with young animals, the adaptive mechanisms of the endocrine pancreas are inadequate to overcome the glucocorticoid-induced insulin resistance, so that hyperglycaemia ensues.

Our data confirm that dexamethasone-induced insulin resistance is associated with increased circulating insulin levels as a result of a compensatory response of pancreatic β-cells (Bonner-Weir *et al*. 1981). In our experiments, plasma insulin concentrations had already increased significantly after 4 days of treatment in all age-groups and
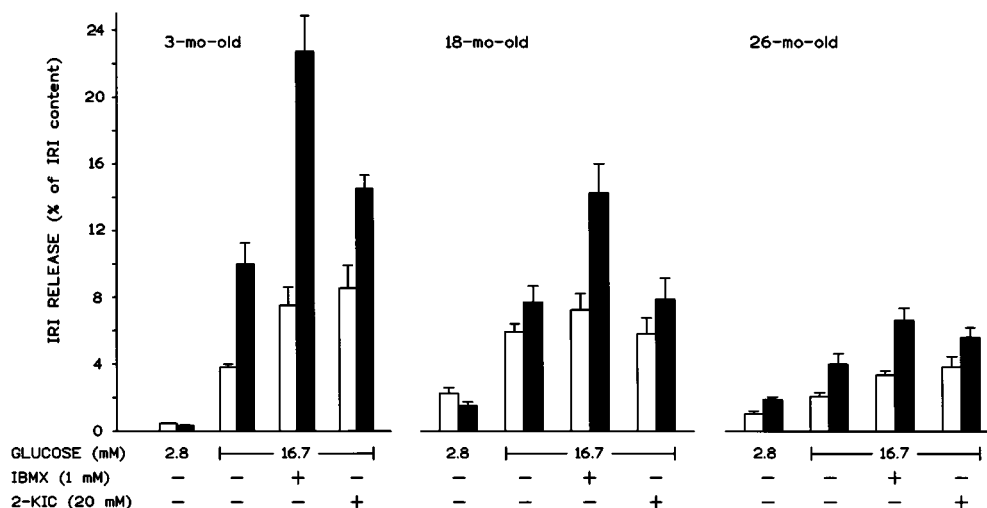
Figure 2 Insulin release from isolated islets of 3-, 18- and 26-month-old control (□) and dexamethasone-treated (■) rats. Islets were incubated for 60 min in the presence of the indicated secretagogues. Means ± S.E.M. of five observations. ANOVA showed that the effect of dexamethasone was significant ($P<0\cdot01$) for the stimulation with 16·7 mM glucose alone ($F=31\cdot6$) or in combination with IBMX ($F=64\cdot1$) or 2-KIC ($F=20\cdot1$); the effect of age was significant ($P<0\cdot01$) for all the incubation conditions ($F=38\cdot7$; $F=24\cdot5$; $F=30\cdot4$; $F=27\cdot4$; for 2·8 mM glucose, 16·7 mM glucose alone or in combination with IBMX or 2-KIC, respectively); the interaction was also significant ($P<0\cdot05$ at least) in all cases ($F=10\cdot5$; $F=6\cdot11$; $F=11\cdot0$; $F=4\cdot11$; respectively).

Table 3 Insulin release from isolated islets of control and DEX-treated rats of various ages during the second 60-min incubation period in the presence of the indicated stimuli. Results are means ± S.E.M. of four or five incubations for each group of islets. To facilitate comparisons between 1st and 2nd incubation, changes versus 1st incubation are also indicated

	Treatment	16·7 mM glucose		16·7 mM glucose+1 mM IBMX	
		IRI release during 2nd incubation (% of IRI content)	Changes vs 1st incubation (%)	IRI release during 2nd incubation (% of IRI content)	Changes vs 1st incubation (%)
Age (months)					
3	Control	5·5 ± 0·2*	+43	15·7 ± 1·6*	+106
	DEX	9·7 ± 0·9	−0·5	15·6 ± 1·2*	−31
18	Control	3·5 ± 0·5*	−41	14·2 ± 1·4*	+93
	DEX	6·6 ± 1·0	−13	13·9 ± 1·8	−2·7
26	Control	2·3 ± 0·3	+8·3	7·1 ± 0·6*	+112
	DEX	2·7 ± 0·6	−34	4·7 ± 0·7	−30

*$P<0.05$ (at least) versus the corresponding values of the 1st incubation period (Fig. 2).

remained high on the following days. However, whereas a moderate hyperinsulinaemia is sufficient to prevent any increase in plasma glucose levels in young rats, even a remarkable increase in circulating insulin appears inadequate to maintain normoglycaemia in most of older animals. Our results point out that the age of 18 months may be critical for the development of the age-related failure of the adaptive mechanisms regulating glucose homeostasis. In fact, differently from 26-month-old rats which became all markedly hyperglycaemic following DEX administration, in the 18-month-old group, three out of seven animals remained normoglycaemic throughout the treatment. Apparently, this discrepancy was due to differences neither in insulin secretory response nor in lipid disregulation, since circulating insulin and FFA levels increased similarly in both hyper- and normoglycaemic animals of this age-group. It is worth noticing that our results confirm that the antilipolytic effect of insulin is blunted by DEX treatment (Venkatesan *et al*. 1996).

The present study clearly indicates that the variable effects of glucocorticoid treatment on blood glucose concentrations are not only genetically determined as suggested by Ogawa *et al*. (1992), but also dependent on acquired factors such as age, overweight or both. Taking into account the hypothetical involvement of abundant lipid availability in the pathogenesis of hyperglycaemia in insulin resistant states such as obesity (Unger 1995), it is possible that the elevation of blood glucose in older rats could be mediated, at least in part, by the DEX-induced rise in circulating FFA levels, which interferes with glucose uptake and utilization particularly in muscles (Venkatesan *et al*. 1987, Groop *et al*. 1991, Roden *et al*. 1996). However, besides the reported failure of inhibitors of FFA oxidation in improving muscle glucose uptake (Venkatesan *et al*. 1996), it should be stressed that in our study the putative unfavourable effect of elevated plasma

FFA levels occurred only in rats which were likely to be already insulin resistant because of advanced age and/or overweight. Indeed, it is of interest that DEX-treated 26-month-old rats became hyperglycaemic despite a modest increase in FFA circulating levels and that in 18-month-old animals post-treatment glucose levels were positively correlated with the initial body weight, but not with plasma FFA concentrations.

Glucocorticoid-induced insulin resistance is usually associated with a compensatory increase in β-cell mass (Ogawa *et al*. 1992) and pancreatic insulin content (Bonner-Weir *et al*. 1981). Our data on pancreatic and islet insulin stores fully confirm this latter observation for DEX-treated young rats only.

Stimulation of insulin release in isolated islets from aged untreated rats highlights a diminished responsiveness to glucose alone or in combination with other secretagogues, in agreement with previous observations (Reaven *et al*. 1979, Bergamini *et al*. 1991, Inoue *et al*. 1997). This reduced response was particularly remarkable in the islets from the oldest control group. However, it should be stressed that the secretory response was enhanced by DEX treatment not only in islets of young rats, in agreement with Malaisse *et al*. (1967) and Wang *et al*. (1994), but also in islets of older animals, although to a lesser extent. Therefore, despite the possibility of an adverse influence of a glucose-rich environment, a phenomenon known as glucose toxicity (Weir & Leahy 1994), the hyperglycaemic state of the older animals in our experimental conditions does not appear to be harmful for islet function both *in vivo* and *in vitro*, at least within the time limits of our study. Nevertheless, the hypothesis that prolonged hyperglycaemia might unfavourably affect the extent of β-cell compensatory response cannot be ruled out.

The present data confirm our previous observation that the time-dependent potentiation of glucose effect (i.e. the

enhancement of insulin secretion in response to a second glucose stimulus) is impaired in ageing animals (Bombara *et al.* 1995). Interestingly, DEX treatment interfered with the priming effect of glucose in the islets from all age-groups, likely as a consequence of the large insulin release from the β-cells during the first incubation. Similar results were obtained by Grill & Rundfeldt (1986) in the perfused pancreas of DEX-treated young rats.

In conclusion, our results show that the adaptive mechanisms regulating glucose homeostasis weaken in ageing rats and become unable to efficiently counteract a metabolic hyperglycaemic challenge such as that represented by the prolonged administration of DEX. In this age-related alteration of glucose homeostasis two functionally distinct aspects can be identified. On one side, the elevation of plasma glucose and FFA levels in older DEX-treated rats in spite of the concomitant increase in circulating insulin, clearly indicates a reduction of the insulin sensitivity of the peripheral tissues, which most likely is the result of the synergistic effects of DEX treatment and antecedent age- and/or weight-related alterations. On the other hand, the '*in vitro*' experiments with the isolated pancreatic islets reveal an age-related impairment of the maximal secretory capabilities of β-cells, which probably makes these cells unable to provide sufficient compensation to prevent alteration of glucose homeostasis.

Acknowledgements

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