EXPERIMENTAL STUDY

Dexamethasone-induced insulin resistance and pancreatic adaptive response in aging rats are not modified by oral vanadyl sulfate treatment

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Abstract

Objective: To explore the adaptive response of the endocrine pancreas *in vivo* and *in vitro* and the possible beneficial effect of the insulin-mimetic agent vanadyl sulfate (VOSO_4), using glucocorticoid treatment to increase insulin resistance, in aging rats.

Design and Methods: Dexamethasone (Dex) (0.13 mg/kg b.w.) was administered daily for 13 days to 3- and 18-month old Sprague–Dawley rats and oral VOSO_4 was given from the 5th day. Plasma glucose, insulin and free fatty acids (FFA) concentrations were measured during these treatments and the insulin secretory response of the isolated perfused pancreas was assessed at the end of the experiment.

Results and Conclusions: In both young and aging rats, particularly in the latter, hyperinsulinemia and increased *in vitro* insulin responsiveness to glucose were observed in response to Dex treatment, concomitant with an increase in plasma FFA concentrations. Thus, in glucocorticoid-treated animals, the β -cell adaptive response occurred in both age groups and could possibly be mediated by increased circulating FFA; however, it was insufficient to prevent hyperglycemia in 60% of aging animals. Oral VOSO_4 administration failed to correct Dex-induced alterations in glucose and lipid metabolism, although it influenced *in vitro* β -cell responsiveness to stimuli in aging rats.

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Introduction

Aging is associated with alteration of carbohydrate tolerance, dependent on both impairment of the pancreatic insulin response to physiological stimuli and the development of peripheral insulin resistance (1–3). Studies utilizing the euglycemic hyperinsulinemic clamp technique usually stress the relevance of the diminished insulin sensitivity of target tissues in the development of age-related glucose intolerance (3–5). In contrast, it has been repeatedly reported that the ability of pancreatic β -cells to maintain an insulin secretory function adequate for metabolic demand is impaired with increasing age of the animal (for a review, see (6)).

The relevance of the relative roles of these two factors remains a matter of debate. In an attempt to contribute to clarify the subject, we used glucocorticoid treatment as a means of inducing a further increase in insulin resistance in rats. Recently, we have shown by this approach that in aging, but not in young, rats, dexamethasone (Dex) administration caused an increase in blood glucose to frankly diabetic concentrations notwithstanding a concomitant remarkable increase in

circulating insulin concentrations (7). In the present work, we used the isolated perfused pancreas from 18-month-old and 3-month-old rats to explore the kinetics of insulin secretion after *in vivo* treatment with Dex. Furthermore, as vanadate and vanadyl forms of vanadium have been shown to exert insulin-like effects on glucose metabolism (8), we decided to explore whether administration of oral vanadyl sulfate could correct Dex-induced hyperglycemia or hyperinsulinemia, or both, and possibly influence insulin responsiveness of the perfused pancreas. Oral administration of vanadium has been reported to reduce or normalize hyperglycemia in several animal models of diabetes, including those characterized by insulin resistance (9–12). Finally, because the involvement of plasma lipids has been suggested to play a part in both development of peripheral insulin resistance and β -cell functional changes (13–16), we determined free fatty acids (FFA) plasma concentrations after treatment with Dex, vanadyl sulphate, or both.

The results showed that Dex treatment induced a more severe insulin resistance in older than in younger rats, that, in Dex-treated animals, plasma FFA

concentrations were temporally and quantitatively correlated with plasma insulin, and that, in aging rats, oral VOSO_4 administration was unable to normalize the Dex-induced increase in blood glucose, insulin and FFA concentrations, but modified the β -cell responsiveness to stimuli in the perfused pancreas.

Materials and methods

Animals

Experiments were performed in male Sprague–Dawley rats of 3 and 18 months of age. Animals had free access to food (TEKLAD pelleted diet; Harlan, Milan, Italy), were kept at 24–25°C and subjected to a 12-h light:12-h darkness cycle. The NIH guidelines *Principles of Laboratory Animal Care* (publication No. 83-25, revised 1985) were followed, in addition to the recommendations of Italian law for the use of experimental animals (DL No. 116/1992).

Dexamethasone and VOSO_4 treatment

Rats of each age group were randomly divided into four experimental subgroups. Group 1 comprised control rats, receiving daily subcutaneous injections of saline for 13 days, group 2 were Dex-treated rats subjected to a daily subcutaneous injection of Dex phosphate (0.125 mg/kg in saline) for 13 days, group 3 Dex– VOSO_4 -treated rats also received vanadyl sulphate (0.5 mg/ml in the drinking water) starting after 4 days of Dex treatment, when the effects of Dex were clearly apparent (7), and group 4 were rats treated with VOSO_4 for 9 days with oral VOSO_4 only. Daily VOSO_4 intake, calculated on the basis of water consumption, averaged 16.0 ± 0.20 and 22.5 ± 0.60 mg in 3- and 18-month-old animals respectively.

Blood samples were collected in the morning (between 09.00 and 10.00 h) from the tail vein of conscious rats, just before and 4, 9 and 13 days after glucocorticoid treatment.

Isolated perfused pancreas preparation

The day after the last Dex administration, rats were anesthetized with 100 mg/kg intraperitoneal sodium pentobarbital. The *in situ* isolated pancreas preparation was a modification of the method of Penhos *et al.* (17). Perfusate was a modified Krebs–Ringer bicarbonate buffer with 4% dextran T40 and 0.25% bovine serum albumin (fraction V), and was equilibrated with 95% O_2 and 5% CO_2 . Flow rate was kept constant at 4 ml/min by a peristaltic pump for the entire perfusion period, which lasted for about 70 min. The first 30 min was always an equilibration period and will not be shown on any graph. Effluent from the portal vein was collected over 1-min time intervals into tubes that were immediately frozen and stored at -20°C

until required for insulin radioimmunoassay. The perfusion procedure consisted of four consecutive periods of 15 min each, with 2.8 mM glucose, 16.7 mM glucose, 2.8 mM glucose and 16.7 mM glucose plus 1 mM isobutylmethylxanthine (IBMX) respectively.

Insulin secretion was calculated as the insulin concentrations in the perfusate (ng/ml) multiplied by flow rate.

Assays

Plasma glucose, FFA and triglycerides concentrations were measured using commercially available kits (Sclavo Diagnostics, Siena, Italy, for glucose; Boehringer Mannheim Italia, SpA, Milan, Italy, for FFA and triglycerides).

Insulin was measured by radioimmunoassay according to Herbert *et al.* (18), using rat insulin as a standard. The sensitivity and the coefficients of variation of the radioimmunoassay were as follows: detection limit 0.15 ng/ml, intra-assay variation 3%, interassay variation 10%.

Statistical analysis

Data are given as means $\pm$ S.E.M. Statistical significance was evaluated by factorial analysis of variance (ANOVA). Two- and three-way designs were used where appropriate, followed by Tukey post-test with multiple comparisons.

Results

Body, pancreas and adrenal gland weights

Table 1 shows body weight, pancreas and adrenal glands weights, in addition to the daily food intake of the four experimental groups of both 3- and 18-month-old rats. Dex administration for 13 days caused impairment of growth in young rats and a significant reduction in body weight in older rats. Food consumption was reduced in response to Dex treatment more in 18-month-old than in 3-month-old rats. In both age-groups of Dex-treated rats, pancreas weight decreased significantly with respect to corresponding untreated controls. Adrenal gland weights were also significantly reduced in Dex-treated rats, testifying to the effectiveness of exogenous Dex administration. Oral VOSO_4 administration did not significantly affect any of the glucocorticoid-induced changes in both young and old rats.

Plasma glucose and insulin concentrations

Table 2 shows the variations in plasma glucose and insulin concentrations during the experimental period and includes additional data from a separate cohort of 3- and 18-month-old Sprague–Dawley rats subjected

Table 1 Body weight, pancreas and adrenal gland weights, and daily food intake in 3- and 18-month-old Sprague–Dawley rats treated with dexamethasone (Dex), vanadyl sulfate (VOSO₄) or Dex plus vanadyl sulfate.

Age (months)	Experimental group	Body weight (g)		Food intake (g/day)	Pancreas weight (mg)	Adrenal gland weight (mg)
		Before treatment	After treatment			
3	Control (<i>n</i> = 5)	307±10	343±11	24.1±0.3	1132±62	22.2±3.0
3	Dex (<i>n</i> = 5)	316±9	281±13	21.5±0.5	798±76	13.3±1.4
3	VOSO ₄ (<i>n</i> = 5)	303±12	357±19	23.4±0.5	1259±73	19.2±3.6
3	Dex+VOSO ₄ (<i>n</i> = 5)	333±14	295±14	22.3±0.5	907±50	12.5±1.1
18	Control (<i>n</i> = 5)	740±22	729±18	30.2±0.5	1399±140	24.8±3.2
18	Dex (<i>n</i> = 6)	735±44	580±32	20.5±0.8	1188±50	13.7±0.7
18	VOSO ₄ (<i>n</i> = 4)	728±8	712±6	28.1±0.8	1430±84	17.3±0.9
18	Dex+VOSO ₄ (<i>n</i> = 4)	747±16	555±24	16.7±0.9	973±66	11.5±0.6

Data are means±S.E.M. of the number of rats indicated in parentheses.

Statistical analysis: For *body weight*, two-way ANOVA showed a significant ($P < 0.01$) difference among experimental groups in 18-month-old rats only. In this age-group, Tukey test showed significant ($P < 0.01$) differences between the following groups: Control (C) and Dex; C and Dex+VOSO₄ (DV); Dex and V; V and DV. Three-way ANOVA showed significant ($P < 0.01$) effects of age and age × experimental group. For *food intake and pancreas weight*, two-way ANOVA showed significant ($P < 0.01$) effects of age, experimental group and age × experimental group. Tukey test showed significant ($P < 0.01$) differences between the following groups: C and Dex; C and DV; Dex and V; V and DV. For *adrenal gland weight*, two-way ANOVA showed a significant ($P < 0.01$) difference among experimental groups. Tukey test showed significant ($P < 0.01$) differences between the following groups: C and Dex; C and DV.

to the same *in vivo* experimental procedure. In both young and old control groups, there was no significant change in plasma glucose and insulin concentrations. Dex administration did not modify plasma glucose concentrations in 3-month-old rats, whereas it caused hyperglycemia in 18-month-old animals. During Dex treatment, plasma insulin concentrations increased twofold in young rats, and four- to fivefold in older rats, the increase being already fully apparent on the 4th day of the experimental period. As was apparent from the high variability of glycemic changes, some 18-month-old rats (four of 10) remained normoglycemic throughout Dex treatment, but showed a degree of hyperinsulinemia similar to that of hyperglycemic animals, in agreement with previous results (7).

Oral VOSO₄ administration had no significant effect on plasma glucose and insulin concentrations in both

3- and 18-month-old rats, and was also unable to modify the glucocorticoid-induced changes.

Plasma FFA concentrations

Table 3 shows the variations in plasma FFA concentrations during the experimental period. In both young and old controls, no change in plasma FFA concentrations occurred throughout the experimental period. Dex treatment induced a two- to threefold increase in plasma FFA in young rats and a fourfold increase in older rats, including those that did not develop hyperglycemia. As was the case for hyperinsulinemia, this increase was already maximal on the 4th day after Dex administration. A significant positive correlation was found between plasma FFA and insulin

Table 2 Plasma glucose and insulin concentrations in 3- and 18-month-old Sprague–Dawley rats treated with dexamethasone (Dex), vanadyl sulfate (VOSO₄) or Dex plus vanadyl sulfate.

Age (months)	Experimental group	Plasma glucose (mg/100 ml)				Plasma insulin (ng/ml)			
		Day 0	Day 4	Day 9	Day 13	Day 0	Day 4	Day 9	Day 13
3	Control (<i>n</i> = 8)	123±3.6	124±2.5	120±3.1	123±2.5	3.4±0.3	3.2±0.3	3.4±0.4	3.3±0.4
3	Dex (<i>n</i> = 10)	144±4.9	140±5.0	136±4.2	121±3.2	3.0±0.3	5.4±0.7	6.9±1.1	8.4±1.7
3	VOSO ₄ (<i>n</i> = 8)	134±3.6	137±6.3	128±3.9	127±4.5	4.0±0.4	3.0±0.2	2.4±0.3	3.0±0.7
3	Dex+VOSO ₄ (<i>n</i> = 8)	138±5.2	134±6.3	123±4.2	126±3.5	3.0±0.3	6.0±0.4	5.5±0.2	4.3±0.2
18	Control (<i>n</i> = 10)	114±3.7	120±2.7	108±1.7	109±4.5	3.6±0.4	3.0±0.3	2.8±0.3	3.1±0.2
18	Dex (<i>n</i> = 10)	122±5.5	167±16	236±39	250±42	5.0±0.5	23±4.5	26±4.3	19±1.3
18	VOSO ₄ (<i>n</i> = 8)	115±3.9	119±3.4	117±2.7	110±4.6	3.8±0.5	3.8±0.4	3.2±0.4	2.5±0.3
18	Dex+VOSO ₄ (<i>n</i> = 10)	112±3.2	153±17	261±40	234±43	4.1±0.5	13±2.0	23±4.7	20±2.7

Data are means±S.E.M. of the number of rats indicated in parentheses, pooled from two separate experiments.

Statistical analysis: For *plasma glucose*, two-way ANOVA showed a significant ($P < 0.01$) difference among experimental groups in 18-month-old rats only. In this age-group, Tukey test showed significant ($P < 0.01$) differences between the following groups: Control (C) and Dex; C and Dex+VOSO₄ (DV); Dex and V; V and DV. For *plasma insulin*, two-way ANOVA showed a significant ($P < 0.01$) difference among experimental groups in both young and old rats. In both age-groups, Tukey test showed significant ($P < 0.01$) differences between the following groups: C and Dex; C and DV; Dex and V; V and DV. For both *plasma glucose and insulin*, three-way ANOVA showed significant ($P < 0.01$) effects of age and age × experimental groups.

Table 3 Plasma FFA concentrations in 3- and 18-month-old Sprague–Dawley rats treated with dexamethasone (Dex), vanadyl sulfate (VOSO₄) or Dex plus vanadyl sulfate.

Age (months)	Experimental group	Plasma FFA ($\mu\text{Eq/l}$)			
		Day 0	Day 4	Day 9	Day 13
3	Control ($n = 5$)	350 $\pm$ 61	330 $\pm$ 73	348 $\pm$ 63	346 $\pm$ 43
3	Dex ($n = 5$)	450 $\pm$ 14	964 $\pm$ 36	980 $\pm$ 133	874 $\pm$ 131
3	VOSO ₄ ($n = 5$)	361 $\pm$ 53	369 $\pm$ 40	376 $\pm$ 35	421 $\pm$ 80
3	Dex+VOSO ₄ ($n = 5$)	429 $\pm$ 28	946 $\pm$ 47	1077 $\pm$ 95	994 $\pm$ 50
18	Control ($n = 4$)	289 $\pm$ 44	302 $\pm$ 50	315 $\pm$ 60	331 $\pm$ 33
18	Dex ($n = 6$)	340 $\pm$ 45	1288 $\pm$ 174	1412 $\pm$ 110	1285 $\pm$ 113
18	VOSO ₄ ($n = 4$)	333 $\pm$ 78	350 $\pm$ 60	439 $\pm$ 55	734 $\pm$ 101
18	Dex+VOSO ₄ ($n = 4$)	445 $\pm$ 98	1261 $\pm$ 19	1451 $\pm$ 275	1574 $\pm$ 298

Data are means $\pm$ S.E.M. of the number of rats indicated in parentheses.

Statistical analysis: Two-way ANOVA showed a significant ($P < 0.01$) difference among experimental groups in both young and old rats. In both age-groups, Tukey test showed significant ($P < 0.01$) differences between the following groups: Control (C) and Dex; C and Dex+VOSO₄ (DV); V and DV. The difference between C and V was significant in 18-month-old rats only. Three-way ANOVA showed a significant effect of age and age $\times$ experimental groups.

concentrations in Dex-treated rats ($r = 0.60$, $P < 0.01$) and in Dex+VOSO₄-treated animals.

Lipacidemia was not modified by oral VOSO₄ administration in 3-month-old rats, whereas it showed a moderate significant increment in 18-month-old rats at the end of the treatment. VOSO₄ treatment did not affect the glucocorticoid-induced changes in plasma FFA concentrations.

Plasma concentrations of triglycerides determined in the same plasma samples showed a slight increase in Dex-treated rats of both ages, which achieved statistical significance in the 18-month-old group only ($171 \pm 12 \mu\text{Eq/l}$, compared with $125 \pm 10 \mu\text{Eq/l}$ in controls, $P < 0.05$) and was not substantially modified by VOSO₄ administration.

Insulin secretion from the isolated perfused pancreas

Glucose-stimulated insulin output from the isolated perfused pancreas of control and Dex-treated rats 3 months of age is shown in Fig. 1A. In both groups the typical biphasic pattern of insulin release was observed, with a first phase peaking at 3 min after the change in glucose concentration. Quantitatively, glucose-stimulated insulin output was not significantly different between the two groups (as also indicated by the integrated area-under-the-curve (AUC) data, illustrated in the insert in Fig. 1), although release of insulin from the perfused pancreas of Dex-treated rats was consistently greater with respect to control rats for each time-point. Insulin secretion from the perfused pancreas of control and Dex-treated rats 18 months of age is shown in Fig. 1B. Both the first and the second phase of insulin release were attenuated in control animals compared with those in corresponding young rats (Fig. 1A). Dex treatment significantly enhanced the insulin secretory response to glucose in the pancreas of old rats, as also shown by the integrated AUC data

(Fig. 1, insert). It should also be noted that the output of insulin in the presence of non-stimulating glucose concentrations was much greater in older Dex-treated animals than in controls.

Oral VOSO₄ administration to either untreated or Dex-treated young rats did not appear to influence substantially either the secretory kinetics (Fig. 1C) or the overall output (insert) of the perfused pancreas. In contrast, in older animals, VOSO₄ administration caused a remarkable increase in both phases of glucose-stimulated insulin secretion with respect to age-matched controls (Fig. 1D and insert). In Dex-treated older animals, the concomitant VOSO₄ administration, while not affecting the increase in basal insulin secretion, caused a glucose-stimulated insulin secretion that was evidently blunted with respect to that observed in age-matched rats given either substance alone (Fig. 1D and insert).

The addition of 1 mM IBMX to 16.7 mM glucose, for a subsequent stimulation, markedly potentiated both phases of insulin release (not shown), again eliciting responses of different extents from the perfused pancreas of different animal groups. In both 3- and 18-month-old rats, these variations in the secretory responses followed substantially the same pattern as those observed during the first stimulation with glucose alone (not shown).

Discussion

The present work confirms that a prolonged Dex administration (0.125 mg/kg per day for 13 days) causes a significant increase in plasma glucose concentrations in many, but not all, 18-month-old rats, despite a concomitant marked augmentation in circulating insulin. In contrast, in all young (3-month-old) Dex-treated rats a moderate hyperinsulinemia was sufficient to prevent any increase in plasma glucose concentrations.

Figure 1 Glucose (G)-stimulated insulin secretion from the isolated perfused pancreas of 3-month (mo)-old (A and C) and 18-month-old (B and D) control (○), Dex-treated (●), VOSO_4 -treated (△) and Dex+ VOSO_4 -treated (▲) rats. Pancreas perfusions were performed after 13 days of Dex and 9 days of VOSO_4 treatment. Data are means $\pm$ S.E.M. of five observations for 3-month-old rats and four to six observations for 18-month-old rats. The insert shows the integrated insulin output in response to 16.7 mM glucose, calculated from the data of panels A–D and expressed as means $\pm$ S.E.M. □, Control rats; ▨, Dex-treated rats; ▩, VOSO_4 -treated rats; ▤, Dex+ VOSO_4 -treated rats. Statistical analysis: two-way ANOVA showed a significant ($P < 0.01$) difference between experimental groups; Tukey test showed significant differences ($P < 0.01$) between Dex and controls, and between VOSO_4 and controls.

Thus the degree of glucocorticoid-induced insulin resistance is much greater in older than in young rats.

One of the aims of the present study was to investigate whether VOSO_4 administration could correct Dex-induced hyperglycemia and improve insulin sensitivity in aging rats. Both the vanadate and the vanadyl forms of vanadium have been shown by many investigators to have insulin-like effects on glucose metabolism both *in vitro* and *in vivo* (for a review, see (8)). Indeed, the administration of vanadium corrects hyperglycemia in both streptozotocin-diabetic rats (19–21) and genetically diabetic *db/db* mice (22). Although the mechanism of action of vanadium has not been fully clarified, many of its *in vivo* insulin-like effects have been attributed to its ability to restore peripheral tissue sensitivity to circulating insulin (23).

Quite surprisingly, our results showed that oral VOSO_4 , given from the 5th day after the beginning of Dex treatment, was unable to modify glucocorticoid-induced

hyperglycemia and hyperinsulinemia in old rats. As far as we know, this is the first model system in which VOSO_4 does not show glucose-decreasing effects. The combination of hyperglycemia and hyperinsulinemia occurring in our experimental conditions can also be found in other animal models with severe insulin resistance, such as diabetic *ob/ob* mice and obese hyperinsulinemic *fa/fa* rats; in both these cases, vanadate was reported to be effective in correcting glucose metabolism, with reductions in blood glucose and insulin concentrations (10, 24, 25), most probably through a partial restoration of muscle insulin sensitivity (26). Thus, vanadium appears to be able to bypass the insulin resistance of these genetic syndromes, but not that induced by glucocorticoid treatment in old animals, which most probably have already developed an age-dependent reduction in peripheral insulin sensitivity (2). From our results on body and pancreas weights, it can be also argued that VOSO_4 treatment

is ineffective in counteracting the catabolic effects of Dex. However, this is not surprising, as vanadium does not always mimic the anabolic action of insulin (27).

As regards the alterations of lipid metabolism observed in our experimental animals, it should be noted that the increase in plasma FFA concentrations induced by Dex treatment in both age groups (albeit larger in older than in young rats) was remarkable, occurred early and remained quite stable during the experimental period. Hyperlipacidemia appears to be unresponsive to the concomitant increase in circulating insulin (and to exogenous VOSO_4), not only in old but also in young rats, in which a threefold increase in FFA plasma concentrations was observed without any increase in plasma glucose. Therefore, it appears that glucocorticoids counteract the antilipolytic action of insulin more effectively than other insulin-regulated processes. As regards the mechanisms underlying β -cell adaptation to the Dex-induced changes in insulin sensitivity, our data support the idea that the increased plasma FFA concentrations, while contributing to the induction or aggravation of peripheral insulin resistance (13), may mediate insulin hypersecretion, either directly (14, 16) or by favoring triglyceride synthesis in β cells and subsequent generation of lipid signaling molecules through lipolysis (15). The hyperlipacidemia achieved in Dex-treated rats was in the range of FFA concentrations (less than 1.5–2.0 mM) that have been suggested to exert insulinotropic effect on β -cells (14, 16), and are temporally coincident and quantitatively correlated with circulating insulin concentrations. Thus, in Dex-treated Sprague–Dawley rats, increased plasma FFA concentrations may be the mediators of both peripheral insulin resistance and β -cell sensitization for compensatory hyperfunction, as has been suggested to occur in genetically obese Zucker rats before the onset of hyperglycemia (14). It should also be considered that *in vivo* glucocorticoid treatment may influence the leptin–insulin axis (28, 29). In this context, we did indeed observe a relevant reduction in food consumption and body weight in old Dex-treated rats, thus confirming previous results obtained with much larger doses of glucocorticoids (30), but we did not measure the animals' leptin concentrations, which have been reported to increase in man in response to short-term Dex treatment (29). Further studies are required to clarify the complex mechanisms responsible for these changes.

In our experiments we also addressed the question of the secretory capabilities of isolated perfused pancreas in rats treated with Dex or VOSO_4 , or both, investigated at the end of the treatment. We observed that the glucose-stimulated insulin output from the perfused pancreas of 18-month-old untreated rats was markedly reduced in comparison with that in younger rats, in agreement with previous reports (31, 32). Dex treatment appears to increase the secretory performance of

the isolated pancreas in both young and aging rats, but, at variance with the results obtained with isolated islets (7), this enhancing effect is more relevant and attains statistical significance only in the case of older rats. It is worth noticing that the basal release of insulin from the perfused pancreas was increased in Dex-treated older rats only. Such an increase has often been reported in animals with low peripheral insulin sensitivity, and it is considered to be secondary to enhanced demand on β -cell secretion (33).

Complex and controversial results have been obtained by various authors when insulin secretion was measured *in vitro* after the treatment of experimental animals with glucocorticoids, as in the present study. Augmented (34–36), unchanged (37, 38) or decreased (33, 39) insulin secretion has been reported in either perfused pancreas or isolated islets. These differences are not easily interpretable and appear to depend on the dose and duration of glucocorticoid administration, the strain of the animals, and the stimulus used *in vitro*. From this point of view, we would suggest that the Dex dosage used in our study was too low to induce a direct inhibitory effect on pancreatic β cells, such as that seen when pancreatic islets were directly incubated with Dex *in vitro* (40), so that the adaptive stimulatory effect on insulin secretion prevailed and was particularly strong in aging rats, probably reflecting the extent of *in vivo* compensatory requirements. On the basis of our functional data *in vitro*, we would also point out that the hyperglycemic state of the Dex-treated old rats does not appear to be harmful for β -cell function, as the insulin secretory response of the perfused pancreas was not significantly different between normoglycemic or hyperglycemic Dex-treated rats (not shown).

Another interesting aspect of our results is the secretory performance of the perfused pancreas of VOSO_4 -treated animals. Indeed, VOSO_4 treatment (0.5 mg/ml in the drinking water for 9 days), ineffective in younger rats, in older animals led to a relevant increase in glucose- and glucose plus IBMX-stimulated insulin release, which largely surpassed the age-dependent impairment in secretory responsiveness. To our knowledge, such an effect has been previously reported in the perfused pancreas only in response to *in vitro* exposure to VOSO_4 (41).

Surprising was the observation that, in aging rats, the combined *in vivo* treatment with Dex and VOSO_4 caused a drastic loss of the glucose-induced insulin stimulatory effect seen in the perfused pancreas of animals treated with either substance alone. This *in vitro* effect was in contrast with the observed increased circulating insulin concentrations, which were not very different from those occurring in rats treated with Dex alone. It should be stressed that, in the perfused pancreas of rats treated with both Dex and VOSO_4 , the basal insulin secretion was increased, as it was for rats treated with Dex alone. As a result, the incremental glucose-stimulated insulin output over basal values was

little for the first phase and almost negligible for the second phase (Fig. 1D), suggesting that an interference with glucose metabolism had occurred by unknown mechanisms that require further studies in order to be clarified. We may just speculate that if in the β cells of hyperlipidemic Dex-treated rats, triglyceride synthesis and subsequent lipolysis are implicated in the generation of lipid signaling molecules enhancing glucose-stimulated insulin secretion, then the concomitant exposure to vanadium, which has been reported to exert an antilipolytic action (42), could interfere with this mechanism. Such a hypothesis is in line with the fact that, in our Dex plus VOSO₄-treated rats, a subsequent glucose stimulation in the presence of a cAMP agonist, which might also activate β -cell triacylglycerol lipase (43), resulted in a significantly greater insulin release than in age-matched controls (not shown).

In conclusion, the results of the present study, while confirming that the adaptive capability of endocrine pancreas is preserved in aging rats, indicate that the compensatory oversecretion of insulin, which could be mediated by increased circulating FFA concentrations, may be sufficient or not to match Dex-induced alterations in glucose metabolism, probably depending on the magnitude of pre-existing age-related insulin resistance. Furthermore, in aging rats, oral VOSO₄ treatment for several days is unable to overcome glucocorticoid-induced changes *in vivo*, but modifies the responsiveness of perfused pancreas in an apparently contrasting fashion (improvement of the age-related impairment of glucose-stimulated insulin release in control rats; suppression of the enhanced response to glucose in Dex-treated animals). Further studies are required to elucidate the mechanisms responsible for these findings.

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