**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 insulino-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 VOSO₄ 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. 83-23, revised 1985) were followed, in addition to the recommendations of Italian law for the use of experimental animals (DL No. 116/1992).

Dexamethasone and VOSO₄ 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–VOSO₄-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 VOSO₄ for 9 days with oral VOSO₄ only. Daily VOSO₄ 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). Perfusionate was a modified Krebs–Ringer bicarbonate buffer with 4% dextran T40 and 0.25% bovine serum albumin (fraction V), and was equilibrated with 95% O₂ and 5% CO₂. 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±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 VOSO₄ 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
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. Dexamethasone did not modify plasma glucose concentrations in 3-month-old rats, whereas it caused hyperglycemia in 18-month-old animals. During Dexamethasone 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 Dexamethasone 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. Dexamethasone treatment induced a twof- 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 Dexamethasone administration. A significant positive correlation was found between plasma FFA and insulin concentrations.

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**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.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Experimental group</th>
<th>Body weight (g)</th>
<th>Food intake (g/day)</th>
<th>Pancreas weight (mg)</th>
<th>Adrenal gland weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control (n = 5)</td>
<td>307±10</td>
<td>343±11</td>
<td>24.1±0.3</td>
<td>1132±62</td>
</tr>
<tr>
<td>3</td>
<td>Dex (n = 5)</td>
<td>316±9</td>
<td>281±13</td>
<td>21.5±0.5</td>
<td>798±76</td>
</tr>
<tr>
<td>3</td>
<td>VOSO₄ (n = 5)</td>
<td>303±12</td>
<td>357±19</td>
<td>23.4±0.5</td>
<td>1259±73</td>
</tr>
<tr>
<td>3</td>
<td>Dex+VOSO₄ (n = 5)</td>
<td>333±14</td>
<td>295±14</td>
<td>22.3±0.5</td>
<td>907±50</td>
</tr>
<tr>
<td>18</td>
<td>Control (n = 5)</td>
<td>740±22</td>
<td>729±18</td>
<td>30.2±0.5</td>
<td>1399±140</td>
</tr>
<tr>
<td>18</td>
<td>Dex (n = 6)</td>
<td>735±44</td>
<td>580±32</td>
<td>20.5±0.8</td>
<td>1188±50</td>
</tr>
<tr>
<td>18</td>
<td>VOSO₄ (n = 4)</td>
<td>728±8</td>
<td>712±6</td>
<td>28.1±0.8</td>
<td>1430±84</td>
</tr>
<tr>
<td>18</td>
<td>Dex+VOSO₄ (n = 4)</td>
<td>747±16</td>
<td>555±24</td>
<td>16.7±0.9</td>
<td>973±66</td>
</tr>
</tbody>
</table>

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; and 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; and V and DV. For adrenal gland weight, two-way ANOVA showed a significant (P < 0.01) difference among experimental groups. Tukey test showed significant differences (P < 0.01) between the following groups: C and Dex; C and DV; and V and DV.

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**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.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Experimental group</th>
<th>Plasma glucose (mg/100 ml)</th>
<th>Plasma insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 4</td>
</tr>
<tr>
<td>3</td>
<td>Control (n = 8)</td>
<td>123±3.6</td>
<td>124±2.5</td>
</tr>
<tr>
<td>3</td>
<td>Dex (n = 10)</td>
<td>144±4.9</td>
<td>140±5.0</td>
</tr>
<tr>
<td>3</td>
<td>VOSO₄ (n = 8)</td>
<td>134±3.6</td>
<td>137±6.3</td>
</tr>
<tr>
<td>3</td>
<td>Dex+VOSO₄ (n = 8)</td>
<td>138±5.2</td>
<td>134±6.3</td>
</tr>
<tr>
<td>18</td>
<td>Control (n = 10)</td>
<td>114±3.7</td>
<td>120±2.7</td>
</tr>
<tr>
<td>18</td>
<td>Dex (n = 10)</td>
<td>122±5.5</td>
<td>167±16</td>
</tr>
<tr>
<td>18</td>
<td>VOSO₄ (n = 8)</td>
<td>115±3.9</td>
<td>119±3.4</td>
</tr>
<tr>
<td>18</td>
<td>Dex+VOSO₄ (n = 10)</td>
<td>112±3.2</td>
<td>153±17</td>
</tr>
</tbody>
</table>

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; and 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; and V and DV. For both plasma glucose and insulin, three-way ANOVA showed significant (P < 0.01) effects of age and age × experimental groups.

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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.
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 \textit{in vitro} and \textit{in vivo} (for a review, see (8)). Indeed, the administration of vanadium corrects hyperglycemia in both streptozotocin-diabetic rats (19–21) and genetically diabetic \textit{db/db} mice (22). Although the mechanism of action of vanadium has not been fully clarified, many of its \textit{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 \textit{ob/ob} mice and obese hyperinsulinemic \textit{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. Hyperlipidemia appears to be unresponsive to the concomitant increase in circulating insulin (and to exogenous VOSO₄), 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 hyperlipidemia 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 VOSO₄, 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 VOSO₄-treated animals. Indeed, VOSO₄ 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 VOSO₄ (41).

Surprising was the observation that, in aging rats, the combined in vivo treatment with Dex and VOSO₄ 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 VOSO₄, 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 hyperlipemic 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.

References


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