ELSEVIER

European Journal of Pharmacology 398 (2000) 169–175

ejp

www.elsevier.nl/locate/ejphar

Effects of low-dose $VOSO_4$ on age-related changes in glucose homeostasis in rats

Vincenzo De Tata*, Ettore Bergamini, Maria Bombara, Roberto Lupi, Michela Novelli, Pellegrino Masiello

Dipartimento di Patologia Sperimentale, Biotecnologie Mediche, Infettivologia e Epidemiologia, Università degli Studi di Pisa, Via Roma, 55-Scuola Medica, 56100 Pisa, Italy

Received 19 January 2000; received in revised form 28 March 2000; accepted 31 March 2000

Abstract

The effects of low doses of vanadyl sulfate (0.2 mg/ml in the drinking water) on the age-related impairment of glucose homeostasis in Sprague–Dawley rats were investigated. $VOSO_4$ administration was initiated in 5-month-old animals and lasted 3 months. Thus, in 8-month-old rats, we investigated glucose metabolism in vivo and insulin secretory function in vitro. Results showed that $VOSO_4$ allowed the disposal of an oral glucose load at lower insulin levels than in age-matched controls. No significant changes were found in muscle glucose transporter (GLUT-4) levels or in glycogen content upon $VOSO_4$ treatment. Islets isolated from $VOSO_4$-treated rats released less insulin than control islets, but showed a better preserved sensitivity to secretagogues, in terms of incremental release over basal release, secretory efficiency, and maintenance of the priming effect of glucose. In conclusion, chronic low-dose $VOSO_4$ treatment facilitates insulin action by a mechanism independent of muscle GLUT-4 levels and helps preserve the appropriate sensitivity of β cells to stimuli, thereby preventing age-dependent functional alterations.

Keywords: Aging; Vanadyl sulfate; Insulin action; Insulin release; Islet, isolated

1. Introduction

Aging is characterized by a progressive impairment of glucose tolerance (De Fronzo, 1981) and represents a major risk factor for the development of type 2 diabetes mellitus (Preuss, 1997). This age-related alteration of glucose homeostasis is usually considered to be dependent on either a decline in the insulin secretory capability of pancreatic islets with increasing age (Coordt et al., 1995) or an impairment of the normal sensitivity of peripheral tissues to insulin (Jackson, 1990), or both, although the mechanisms involved have not been fully clarified. Using an oral glucose tolerance test, Klimas (1969) reported that in Sprague–Dawley rats, an impairment of glucose tolerance can develop as early as 6 months of age.

Both vanadate and the vanadyl forms of vanadium have been shown by many investigators to exert insulin-like effects (as reviewed by Fantus and Tsiani, 1998), and we have previously reported that oral vanadyl sulfate administration can rapidly normalize the imbalance of glucose metabolism observed in senescent rats, probably by restoring the ability of the peripheral tissues to utilize circulating insulin efficiently (De Tata et al., 1993). Furthermore, Henquin et al. (1994) have demonstrated that the long-term oral administration of low, non-toxic doses of vanadate can correct the metabolic alterations of animals with mild glucose intolerance and moderate hypoinsulinaemia.

Taking into account these observations, we were prompted to ascertain whether prolonged treatment with low doses of vanadyl sulfate ($VOSO_4$) could retard the onset of age-related changes in glucose metabolism in Sprague Dawley rats, and looked for the underlying mechanisms. Thus, the insulin responsiveness of isolated pancreatic islets was investigated in these rats, as well as the muscle glycogen content and levels of the insulin regulatable glucose transporter GLUT-4.

* Corresponding author. Tel.: +39-50-560-506; fax: +39-50-560-496.
E-mail address: v.detata@ipg.med.unipi.it (V. De Tata).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00270-3

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats were purchased from Harlan–Nossan (Milan, Italy) at 3 months of age, housed under artificial lighting (12-h light-dark cycle) and fed on standard laboratory chow. At 5 months of age, animals were divided into two experimental groups: (1) control rats, receiving tap water; (2) vanadyl-treated rats, receiving tap water supplemented with 0.2 mg/ml vanadyl sulfate ($VOSO_4$). The $VOSO_4$ solution was prepared freshly every other day.

2.2. Oral glucose tolerance test

After 3 months of $VOSO_4$ administration, rats were fasted overnight and given an oral glucose challenge (2 g/kg b.w. as a 40% solution) by syringe and feeding tube. Blood samples were collected from the tail vein of conscious rats at 0, 15, 30, 60, 120 and 180 min after glucose administration. Plasma glucose was measured by the glucose–oxidase method using commercially available kits (Sclavo Diagnostics, Siena, Italy). Plasma insulin was measured by radioimmunoassay according to Herbert et al. (1965), using rat insulin as a standard.

Glucose tolerance was quantitated by using two parameters: ΔG (integrated increase in glycemia over baseline over a period of 180 min after the oral glucose load), and K coefficient (glucose disappearance rate between 15 and 60 min after glucose administration). Insulin secretion during oral glucose tolerance test (OGTT) was quantitated as the incremental insulin values integrated over 180 min after the load (ΔI); the insulinogenic index ($\Delta I/\Delta G$) was also calculated.

2.3. Muscles

Two weeks after the OGTT, i.e. after two additional weeks of $VOSO_4$ treatment, fed animals were anesthetized with Nembutal (50 mg/kg b.w., i.p.) between 9 and 10 am, i.e. close to the circadian peak of muscle glycogen levels (Saubert and Armstrong, 1983). The fast-twitch extensor digitorum longus and the slow-twitch soleus muscles of both sides were removed as quickly as possible and were either weighed and dropped into 30% KOH at 100°C (for glycogen determination) or immediately frozen with aluminium clamps precooled with liquid nitrogen (for GLUT-4 assay). A fragment of liver was also removed for glycogen quantitation.

2.4. Muscle and liver glycogen determination

Muscles and liver were hydrolyzed in boiling KOH solution for 30 min; glycogen was precipitated twice with ethanol and then assayed colorimetrically by the anthrone method (Hassid and Abraham, 1966).

2.5. Measurement of immunoreactive GLUT-4 protein

Portions of muscles (50–100 mg) were homogenized in ice-cold buffer containing 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 1 mM EDTA, and 250 mM sucrose, pH 7.4. Homogenate protein concentration was determined according to Bradford (1976). Aliquots containing 50 μg of protein were solubilized in Laemmli buffer (Laemmli, 1970) containing 2% sodium dodecyl sulfate and electrophoresed on a 10% polyacrylamide resolving gel. After electrophoretic transfer to nitrocellulose sheets (0.45 μm), immunoblotting was performed by incubation with anti-GLUT-4 polyclonal antibody (Biogenesis, UK) diluted 1:500 in phosphate buffered saline (PBS) containing 1% powdered milk for 60 min at 37°C. Nitrocellulose sheets were then washed three times in PBS containing 1% Triton X-100 for 20 min at 22°C and incubated (60 min, 37°C) with ^{125}I-labeled protein A (0.25 μCi/ml, ICN Radiochemicals) in PBS with 1% powdered milk. After washing and air drying, nitrocellulose sheets were autoradiographed at −70°C with Kodak X-Omat AR film with Du Pont intensifying screens for 48 h.

After autoradiography, the immunolabeled bands on the nitrocellulose sheets were excised and counted in a gamma counter (LKB, model 1275 Minigamma). Areas of equal size were excised from unlabeled areas, and their counts were subtracted from total counts. Muscle homogenates from control and $VOSO_4$-treated rats were always processed in adjacent lanes of the same gel to avoid discrepancies in transfer efficiency.

2.6. Isolation of islets and incubation protocol

The pancreas was removed from anesthetized rats, trimmed free of adipose tissue, and minced in Hank's solution. Islets were isolated by a modification of the method of Lacy and Kostianovsky (1967), taking into account the suggestions of Trueheart-Burch et al. (1984). After a 60-min preincubation period in modified Krebs–Ringer bicarbonate (KRB) buffer containing 0.5% bovine serum albumin (BSA), 10 mM HEPES (pH 7.4), and 2.8 mM glucose, batches of 8–10 islets were incubated for 60 min at 37°C in a humidified atmosphere of 5% CO_2 in air in 1 ml KRB–HEPES–BSA buffer containing 2.8 or 16.7 mM glucose, without or with other secretagogues, such as 1 mM 3-isobutyl-1-methylxantine (IBMX) and 10 mM 2-ketoisocaproate (2-KIC). At the end of this first incubation period, the buffer was removed for insulin measurement, the islets were washed, and 1 ml of fresh KRB–HEPES buffer was added, containing the same substances as above, for a further 60-min incubation period. Then, the

medium was again collected for insulin determination. Finally, 1 ml of cold acidified ethanol (150:47:3, v/v; absolute ethanol/H_2O/concentrated HCl) was added to the islets in order to extract their insulin content.

2.7. Total insulin content of the pancreas

Fragments of the pancreas removed before islet isolation were homogenized in cold acid–ethanol mixture for extraction of insulin, as detailed elsewhere (Bergamini et al., 1991).

3. Results

Table 1 shows that the 3-month administration of 0.2 mg/ml $VOSO_4$ in the drinking water, initiated in 5-month-old Sprague–Dawley rats, caused no significant change in body or pancreas weight with respect to that of controls. No alteration in food and water intake occurred during the experimental period (data not shown). As calculated on the basis of water consumption, the daily intake of vanadium averaged 6.3 ± 0.07 mg/day in treated animals. After $VOSO_4$ treatment, the islet insulin content was slightly lower than in controls, whereas there was no change in the insulin content of the whole pancreas. Low-dose $VOSO_4$ treatment caused no significant changes in basal plasma and insulin levels with respect to those of control rats during the experimental period (data not shown).

Fig. 1 shows the results of an oral glucose tolerance test performed in 8-month-old rats, either untreated or treated with $VOSO_4$ for 3 months, in comparison with the same test performed in untreated younger (3-month-old) animals. In 8-month-old untreated animals, the intragastric administration of glucose (2 g/kg b.w.) caused a rapid increase of plasma glucose and insulin levels, which both peaked 15 min after the load, and then gradually decreased at a slower rate than in normal young animals (indeed, the 60-min K coefficient was significantly ($P < 0.05$) reduced with respect to that of younger animals, as shown in

Table 1
Effect of a 3-month administration of $VOSO_4$ (0.2 mg/ml in the drinking water) on body and pancreas weight and on pancreatic and islet insulin content of Sprague–Dawley rats
Data are means ± S.E.M. of the number of observations indicated in parentheses. For islet insulin content, the mean ± S.E.M. was calculated from the pooled values measured at the end of the incubations (five replicas for each stimulus, obtained in two separate experiments for each group of rats). IRI, immunoreactive insulin.

	Control rats	$VOSO_4$-treated rats
Body weight (g)	648 ± 18.0 ($n = 6$)	608 ± 21.9 ($n = 8$)
Pancreas weight (g)	1339 ± 47.1 ($n = 6$)	1349 ± 70.2 ($n = 8$)
Pancreas IRI content (μg/pancreas)	244 ± 35.2 ($n = 6$)	230 ± 47.0 ($n = 8$)
Islet IRI content (ng/islet)	191 ± 12.8 ($n = 50$)	158 ± 7.4[a] ($n = 50$)

[a] $P < 0.05$ versus controls (Student's t-test).

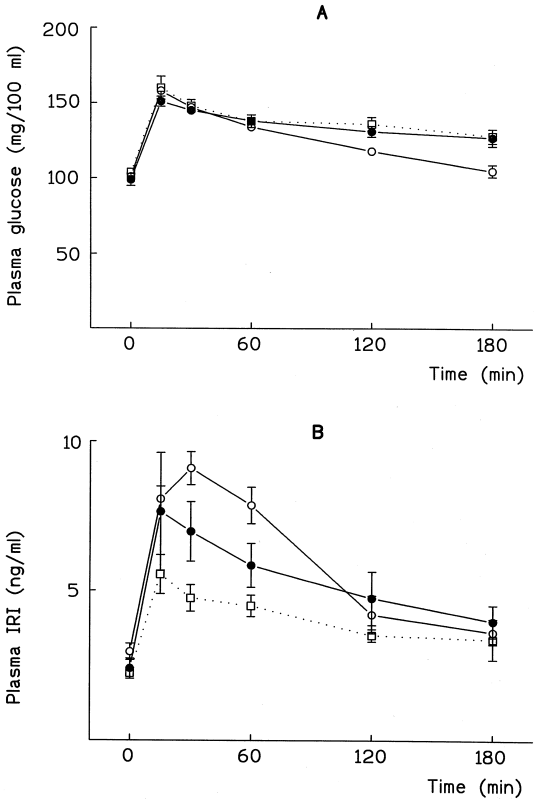

Fig. 1. Plasma glucose (A) and insulin (B) levels during an oral glucose tolerance test (2 g/kg b.w.) performed in 8-month-old untreated (●) and $VOSO_4$-treated (□) rats. For comparison, oral glucose tolerance test was performed also in untreated 3-month-old animals (○). Data are means ± S.E.M. of 6–10 observations.

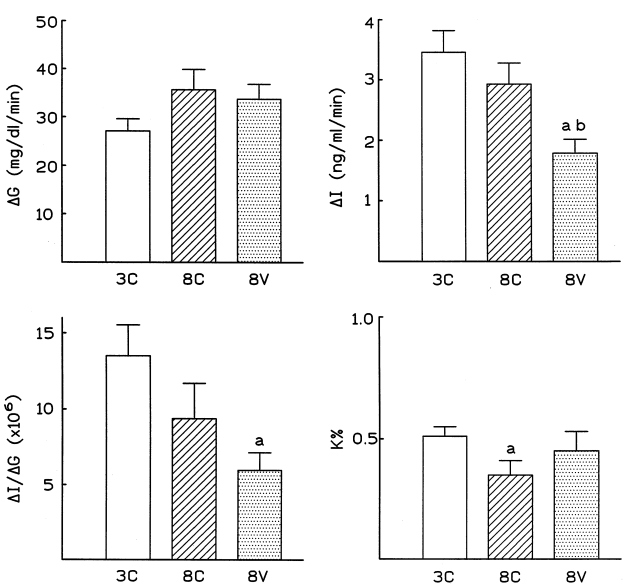

Fig. 2. Integrated increase in plasma glucose (ΔG) and insulin (ΔI) levels, insulinogenic index ($\Delta I/\Delta G$), and glucose disappearance rate (K%) during oral glucose tolerance test in 3-month-old untreated (□), 8-month-old untreated (◪), and 8-month-old $VOSO_4$-treated (dotted square) rats. Data are means ± S.E.M. of 6–10 observations. [a] $P < 0.05$, at least, versus young untreated animals; [b] $P < 0.05$ versus age-matched untreated controls (Student's t-test for unpaired data).

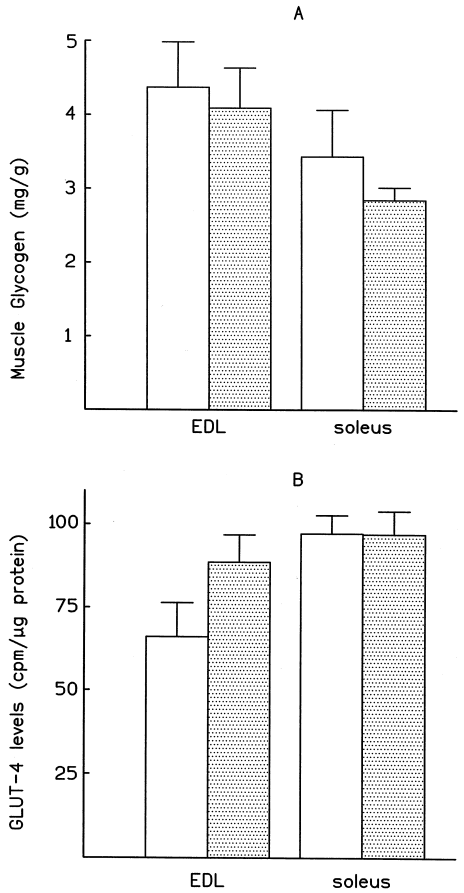

Fig. 3. Glycogen (A) and GLUT-4 protein (B) levels in extensor digitorum longus (EDL) and soleus muscles of control (□) and $VOSO_4$-treated (dotted square) 8-month-old rats. GLUT-4 levels were determined by immunoblotting and expressed as counts per minute per unit of protein (see Section 2). Data are means ± S.E.M. of six observations. Statistical analysis (ANOVA) showed for glycogen: difference between muscles $F = 4.5$, $P < 0.05$; effect of treatment $F = 0.7$, NS; interaction $F = 0.09$, NS; for GLUT-4: difference between muscles $F = 6.2$, $P < 0.05$; effect of treatment $F = 2{,}1$, NS; interaction $F = 1.7$, NS.

Fig. 2). In $VOSO_4$-treated animals, post-loading glycemic values were not very different from those observed in age-matched controls, but the rate of glucose disappearance from the bloodstream increased slightly during the first 60-min period after the load, so that the K coefficient in these $VOSO_4$-treated rats was not significantly different from that of young controls (Fig. 2). Furthermore, in $VOSO_4$-treated rats, for each time point, plasma insulin levels were consistently lower than in either age-matched or younger untreated controls. As a consequence, while differences in ΔG, K and $\Delta I/\Delta G$ between control and $VOSO_4$-treated 8-month-old rats did not achieve statistical significance, ΔI value was significantly ($P < 0.05$) lower in $VOSO_4$-treated animals (Fig. 2).

Fig. 3 shows that the chronic administration of low doses of $VOSO_4$ was unable to induce significant variations of muscle glycogen levels in the fast-twitch extensor digitorum longus or in the slow-twitch soleus. Likewise, chronic administration of $VOSO_4$ did not modify the levels of immunoreactive GLUT-4 protein in either muscle (indeed, the small increase observed in the extensor digitorum longus muscle of treated rats did not achieve statistical significance) (Fig. 3). Furthermore, no significant difference in liver glycogen content was found between $VOSO_4$-treated and untreated rats (46.5 ± 2.50 and 46.6 ± 6.43 mg/g, respectively).

Fig. 4 shows the insulin release from islets isolated from control and $VOSO_4$-treated rats during two consecutive 60-min static incubations in KRB–HEPES buffer containing various secretagogues known to act through different mechanisms. In islets taken from control rats, the insulin release during the first incubation was significantly stimulated by either 16.7 mM glucose or 10 mM 2-KIC, and significantly potentiated by the addition of either IBMX or 2-KIC to 16.7 mM glucose. A similar pattern of secretory response to the various stimuli was observed in islets taken from vanadyl-treated animals, but the amount of insulin released was consistently lower (by 25–30% in the average). However, it should also be noticed that in islets from treated rats, basal insulin release was lower, so that the incremental secretion after exposure to secretagogues was comparable to that of control islets.

During a second exposure of islets from control rats to stimuli, in most cases no further enhancement of insulin release with respect to that of the first incubation occurred, with the exception of the islets incubated in the presence of 16.7 mM glucose plus 2-KIC. In islets from vanadyl-

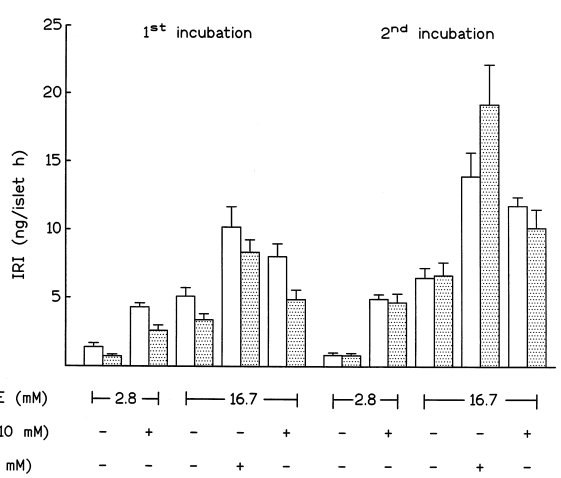

Fig. 4. Insulin release from isolated islets of control (□) and $VOSO_4$-treated (dotted square) 8-month-old rats during two subsequent 60-min incubation periods in the presence of the indicated secretagogues. Data are means ± S.E.M. of 10 observations, pooled from two separate experiments. IRI, immunoreactive insulin. Statistical analysis (ANOVA 2×2×5) showed that differences in the effects of secretagogues ($F = 95.6$) and incubations ($F = 48.2$) were significant (for both $P < 0.01$), whereas the effect of $VOSO_4$ treatment was not significant ($F = 2.63$, NS). The interactions treatment × incubation ($F = 7.01$) and secretagogues × incubation ($F = 9.41$) were significant ($P < 0.01$); the interactions treatment × secretagogues ($F = 4.18$) and treatment × incubation × secretagogues ($F = 1.79$) were not significant.

treated rats, a clear-cut improvement of insulin responsiveness to all stimuli was observed during the subsequent incubation.

4. Discussion

Vanadium and its derivatives are potent insulino-mimetic agents, whose effects include stimulation of glucose oxidation and transport in isolated adipocytes and skeletal muscle (Clausen et al., 1981; Marti et al., 1998), enhanced glycogen synthesis in the liver (Tolman et al., 1979), inhibition of hepatic gluconeogenesis (Ramasarma and Crane, 1981) and protein degradation (Seglen and Gorden, 1981) as well as alleviation of some symptoms of experimental diabetes in the rat (Heyliger et al., 1985; Shisheva et al, 1994). 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 (Verma et al., 1998).

Since a reduction in peripheral insulin sensitivity has been reported to develop quite early during aging, already leading to glucose intolerance in 6-month-old rats (Klimas, 1969), we explored whether this age-related alteration could be corrected by chronic treatment with low doses of oral $VOSO_4$. For this purpose, 5-month-old rats were given 0.2 mg/ml $VOSO_4$ in the drinking water for 3 months and were then subjected to an oral glucose tolerance test. The amount of administered $VOSO_4$ (approximatively 6 mg daily intake) corresponded to the minimal dose reported to be both effective on glucose metabolism in diabetic rats (Shechter, 1990) and safe. Indeed, our data on food and water intake and body weight of $VOSO_4$-treated rats in this study are indicative of the absence of any toxic or unwanted side effect, including anorexia. In both control and treated rats, the body weight gain during the experimental period, as well as the variability observed at 8 months of age, was consistent with the typical growth curve of this rat strain.

Partially confirming the results of Klimas (1969), 8-month-old untreated rats showed a slight alteration in post-loading glucose disposal with respect to that of 3-month-old animals, as indicated by the small but significant reduction in the K coefficient. In $VOSO_4$-treated rats there was a slight increase in the K value, which became not significantly different from that of younger animals and most importantly, this improvement occurred in the presence of lower circulating insulin levels. Actually, the low-dose $VOSO_4$ treatment used in this study was effective in preventing the onset of the age-related alteration in glucose homeostasis and confirms the beneficial influence of the metal on insulin action. Because of the peripheral insulino-mimetic effect of vanadium, it is likely that during $VOSO_4$ administration pancreatic β cells, which are subjected to low insulin demand, are in a functional resting state. However, it cannot be excluded that pancreatic accumulation of vanadium occurs, despite the low dosage, as has been reported upon chronic oral administration of $VOSO_4$ (Cadène et al., 1997) and that the accumulated vanadium exerts an inhibitory effect on glucose-stimulated insulin secretion.

With regard to the possible mechanism of action of vanadium in the periphery, our data show that chronic treatment with low doses of $VOSO_4$ caused no change in GLUT-4 protein levels, suggesting that this factor is not involved in the beneficial effect of the compound. Nevertheless, since under our experimental conditions we measured only total GLUT-4 muscle levels, we cannot exclude at present that $VOSO_4$ enhanced the insulin-mediated translocation of this glucose transporter (Lund et al., 1994). We should mention that vanadate is reported to reverse the reduction in muscle GLUT-4 total protein levels in STZ-diabetic rats (Strout et al., 1990). Our data also show that neither muscle nor liver glycogen content was modified by $VOSO_4$ treatment. In a previous study, we showed that $VOSO_4$ administration for a few days did not modify muscle glycogen content in old, fed rats, but influenced its accumulation upon refeeding after fasting (De Tata et al., 1993). It is worth noticing that the small but significant differences found between the two types of muscles (soleus had lower glycogen and higher GLUT-4 levels than extensor digitorum longus) were in agreement with previous reports (Villa Moruzzi et al., 1981; Rodnick et al., 1990).

These results indicate that although glucose disposal in skeletal muscles, which are responsible for most of the insulin-stimulated clearance of glucose from blood (De Fronzo et al., 1981), was not substantially modified by the chronic treatment with low doses of $VOSO_4$, it was driven by lower levels of circulating insulin than those measured in untreated animals. These findings are in general agreement with those reported by Henquin et al. (1994), who showed that oral vanadate at low doses ameliorated glucose homeostasis in moderately diabetic rats without increasing plasma insulin levels. Moreover, Brichard et al. (1989) showed that, in obese hyperinsulinemic rats, vanadate was able to improve glucose utilization at lower plasma insulin levels.

Recently, Cadène et al. (1997), using isolated perfused pancreas preparations, obtained evidence of an in vitro direct pancreatic insulinotropic action of vanadyl sulfate. However, in the same study, the in vivo chronic exposure of adult rats to $VOSO_4$ resulted in a decreased in vitro responsiveness of perfused pancreas to glucose. Taking these findings into account, we were interested to find out whether chronic treatment with $VOSO_4$ at the low dose used in the present study could modify the age-related decline in ''in vitro'' insulin responsiveness of the islets of Langerhans, whose onset occurred at an age of 6–9 months in previous studies (Bergamini et al., 1991).

Our results give useful information and deserve some comment. Firstly, the isolated islets of $VOSO_4$-treated rats

had an insulin content and a basal insulin release that were lower than those of islets from untreated age-matched animals and closer to those of younger animals (Bergamini et al., 1991). However, the slight difference in islet insulin content was insufficient to modify the insulin content of the whole pancreas. Actually, it should be remembered that, in aging animals, both islet insulin content and basal insulin release are usually increased and this increase is interpreted as an adaptation process to a prolonged and sustained metabolic demand due to unlimited food intake (Adelman, 1989; Bergamini et al., 1991). Secondly, the insulin secretion of the islets isolated from $VOSO_4$-treated rats in response to glucose and other secretagogues was moderately reduced with respect to that of controls, in substantial agreement with the results obtained for the perfused pancreas of rats treated chronically with a higher dose (0.75 mg/ml of drinking water) (Cadène et al., 1997). However, the incremental percentage increase in insulin release over basal values was similar in the islets isolated from control and vanadyl-treated rats. Therefore, the functional behavior of islets isolated from $VOSO_4$-treated rats appears well preserved in terms of sensitivity to stimuli and efficiency of secretion (also on the basis of their insulin content). The maintenance of the physiological performance of these islets is also testified by the preservation of the time-dependent potentiation of insulin release or the priming effect of glucose during the second glucose challenge, a well-known feature of healthy pancreatic beta cells (Grill et al., 1978). Indeed, we have recently shown that the loss of the priming effect of glucose could be considered an early marker of the age-dependent impairment of islet responsiveness to glucose (Bombara et al., 1995). Thus, our data indicate that chronic administration of low-dose $VOSO_4$ may be advantageous for a longer preservation of the functional characteristics of pancreatic beta cells, probably by delaying their age-dependent secretory impairment.

In conclusion, our results show that chronic oral administration of low doses of $VOSO_4$ to Sprague–Dawley rats appears to favor peripheral glucose disposal at lower circulating levels of insulin than in age-matched controls, thereby preventing the onset of mild age-related changes in glucose homeostasis. This beneficial peripheral effect is not dependent on changes in muscle GLUT-4 levels. Furthermore, by lowering chronic insulin demand on pancreatic β cells, $VOSO_4$ treatment helps preserve the exquisite sensitivity of these cells to glucose and non-glucose secretagogues independently of other age-dependent compensatory changes such as increased cell size or insulin content.

Acknowledgements

This study was supported by grants from MURST and CNR, Rome. The skillful technical assistance of Mr. E. Taccini is gratefully acknowledged.

References

Adelman, R.C., 1989. Secretion of insulin during aging. J. Am. Geriatr. Soc. 37, 983–990.

Bergamini, E., Bombara, M., Fierabracci, V., Masiello, P., Novelli, M., 1991. Effect of different regimens of dietary restriction on the age-related decline in insulin secretory response of isolated rat pancreatic islets. Ann. N. Y. Acad. Sci. 621, 327–336.

Bombara, M., Masiello, P., Novelli, M., Bergamini, E., 1995. Impairment of the priming effect of glucose on insulin secretion from isolated islets of aging rats. Acta Diabetol. 32, 69–73.

Bradford, M.M.A., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye binding. Anal. Biochem. 72, 248–254.

Brichard, S.M., Pottier, A.M., Henquin, J.C., 1989. Long term improvement of glucose homeostasis by vanadate in obese hyperinsulinemic fa/fa rats. Endocrinology 125, 2510–2516.

Cadène, A., Mongold, J.J., Gross, R., Poucheret, P., Masiello, P., Roye, M., Ribes, G., Serrano, J.J., Cros, G., 1997. Vanadyl differently influences insulin response to glucose in isolated pancreas of normal rats after in vivo exposure. Eur. J. Pharmacol. 318, 145–151.

Clausen, T., Andersen, T.L., Sturup-Johansen, M., Petkova, O., 1981. The relationship between the transport of glucose and cations across cell membranes in isolated tissues: XI. The effect of vanadate in 45Ca-efflux and sugar transport in adipose tissue and skeletal muscle. Biochim. Biophys. Acta 646, 261–267.

Coordt, M.C., Ruhe, R.C., McDonald, R.B., 1995. Aging and insulin secretion. Proc. Soc. Exp. Biol. Med. 209, 213–222.

De Fronzo, R.A., 1981. Glucose intolerance and aging. Diabetes Care 4, 493–501.

De Fronzo, R.A., Jacot, E., Jequier, E., Maeder, E., Wahren, J., Felber, J.P., 1981. The effect of insulin on the disposal of intravenous glucose. Diabetes 30, 1000–1007.

De Tata, V., Novelli, M., Cavallini, G., Masiello, P., Gori, Z., Bergamini, E., 1993. Beneficial effects of the oral administration of vanadyl sulphate on glucose metabolism in senescent rats. J. Gerontol. 48, B191–B195.

Fantus, I.G., Tsiani, E., 1998. Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects. Mol. Cell. Biochem. 182, 109–119.

Grill, V., Adamson, U., Cerasi, E., 1978. Immediate and time-dependent effects of glucose on insulin release from rat pancreatic tissue. Evidence for different mechanisms of action. J. Clin. Invest. 61, 1034–1043.

Hassid, W.Z., Abraham, S., 1966. Chemical procedure for analysis of polysaccharides. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in Enzymology vol. 8 Academic Press, New York, pp. 34–37.

Henquin, J.C., Carton, F., Ongemba, L.N., Becker, D.J., 1994. Improvement of mild hypoinsulinaemic diabetes in the rat by low non-toxic doses of vanadate. J. Endocrinol. 142, 552–556.

Herbert, V., Lau, K.S., Gottlieb, C.W., Bleicher, S.J., 1965. Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. 25, 1375–1384.

Heyliger, C.E., Tahiliani, A.G., McNeill, J.H., 1985. Effect of vanadate on elevated blood glucose and depressed cardiac performance in diabetic rats. Science 227, 1474–1477.

Jackson, R.A., 1990. Mechanisms of age-related glucose intolerance. Diabetes Care 13 (Suppl. 2), 9–19.

Klimas, J.E., 1969. Oral glucose tolerance during the life-span of a colony of rats. J. Gerontol. 23, 31–34.

Lacy, P.E., Kostianovsky, M., 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16, 35–39.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lund, F., Flyvberg, A., Holman, G.D., Larsen, F.S., Pedersen, O., Schmitz, O., 1994. Comparative effects of IGF-I and insulin on the glucose transporter system in rat muscle. Am. J. Physiol. 267, E461–E466.

Marti, L., Morin, N., Enrique-Tarancon, G., Prevot, D., Lafontan, M., Testar, X., Zorzano, A., Carpene, C., 1998. Tyramine and vanadate synergistically stimulate glucose transport in rat adipocytes by amine oxidase-dependent generation of hydrogen peroxide. J. Pharmacol. Exp. Ther. 285, 342–349.

Preuss, H.G., 1997. Effect of glucose/insulin perturbations on aging and chronic disorders of aging: the evidence. J. Am. Coll. Nutr. 16, 397–403.

Ramasarma, T., Crane, F.L., 1981. Does vanadium play a role in cellular regulation? Curr. Top. Cell. Regul. 20, 247–301.

Rodnick, K.J., Holloszy, J.O., Mondon, C.E., Janos, D.E., 1990. Effects of exercise training on insulin-regulatable glucose-transporter protein levels in rat skeletal muscle. Diabetes 39, 1425–1429.

Saubert, C.V., Armstrong, R.B., 1983. Hormonal influence on diurnal glycogen rhythms in rat skeletal muscles. J. Exp. Biol. 102, 285–293.

Seglen, P.O., Gorden, P.B., 1981. Vanadate inhibits protein degradation in isolated rat hepatocytes. J. Biol. Chem. 256, 7699–7701.

Shechter, Y., 1990. Insulin-mimetic actions of vanadate: possible implications for future treatment of diabetes. Diabetes 39, 1–5.

Shisheva, A., Ikonomov, O., Shechter, Y., 1994. The protein phosphatase inhibitor, pervanadate, is a powerful antidiabetic agent in streptozotocin-treated rats. Endocrinology 134, 507–510.

Strout, H.V., Vicario, P.P., Biswas, C., Saperstein, R., Brady, E.J., Pilch, P.F., Berger, J., 1990. Vanadate treatment of streptozotocin diabetic rats restores expression of the insulin-responsive glucose transporter in skeletal muscle. Endocrinology 126, 2128–2132.

Tolman, E.L., Barris, E., Burns, M., Pansini, A., Partdridge, R., 1979. Effects of vanadium on glucose metabolism in vitro. Life Sci. 25, 1159–1164.

Trueheart-Burch, P., Berner, D.K., Leontire, A., Vogin, A., Matschinsky, B.M., Matschinsky, F.M., 1984. Metabolic adaption of pancreatic tissue in aging rats. J. Gerontol. 39, 2–6.

Verma, S., Cam, M.C., McNeill, J.H., 1998. Nutritional factors that can favourably influence the glucose/insulin system: vanadium. J. Am. Coll. Nutr. 17, 11–18.

Villa Moruzzi, E., Bergamini, E., Gori Bergamini, Z., 1981. Glycogen metabolism and the function of fast and slow muscles of the rat. Pfluegers Arch. 391, 338–342.