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Effects of sodium tungstate on insulin and glucagon secretion in the perfused rat pancreas

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Abstract

Both the direct effect of sodium tungstate on insulin and glucagon secretion in the perfused rat pancreas, and the insulin response to glucose and arginine in pancreases isolated from tungstate-pretreated rats were studied. Infusion of tungstate stimulated insulin output in a dose-dependent manner. The insulinotropic effect of tungstate was observed at normal (5.5 mM), and moderately high (9 mM) glucose concentrations, but not at a low glucose concentration (3.2 mM). Tungstate-induced insulin output was blocked by diazoxide, somatostatin, and amylin, suggesting several targets for tungstate at the B-cell secretory machinery. Glucagon release was not modified by tungstate. Pancreases from chronically tungstate-treated rats showed an enhanced response to glucose but not to arginine. Our results indicate that the reported reduction of glycemia caused by tungstate administration is, at least in part, due to its direct insulinotropic activity. Furthermore, chronic tungstate treatment may prime the B-cell, leading to over-response to a glucose stimulus.

Keywords: Sodium tungstate; Insulin; Glucagon; Pancreas, rat

1. Introduction

Interest in the study of metals as potential antidiabetic agents emerged when vanadate was demonstrated to mimic the effect of insulin on glucose oxidation in rat adipocytes (Shechter and Karlish, 1980) as well as on glucose uptake in skeletal muscle (Dlouha et al., 1981). The classical work by Heyliger et al. (1985), showing that oral vanadate partially normalizes the blood glucose of rats made diabetic with streptozotocin, further stimulated investigations of the metabolic effects of vanadate (review in Shechter, 1990).

Vanadate has also been found to stimulate insulin release directly in incubated as well as in perifused rat islets (Fagin et al., 1987). However, Henquin et al. (1994) have observed that, in mild hypoinsulinemic diabetic rats, while oral vanadate treatment attenuates abnormal glucose tolerance, it does not affect plasma insulin levels.

Studies performed by Fillat et al. (1992) have shown that both molybdate and tungstate exert effects similar to those of vanadate in isolated rat hepatocytes. All these three compounds have insulin-like effects on the glycolytic pathway: they increase basal fructose 2,6-bisphosphate levels, counteract the effects of glucagon on fructose 2,6-bisphosphate concentrations and 6-phosphofructo-2-kinase activity, and stimulate glycolytic flux.

Further work from this group has demonstrated that oral tungstate treatment normalizes glycemia and glucose hepatic metabolism in adult streptozotocin-induced diabetic rats (Barberá et al., 1994) and enhances the insulin response to glucose in islets isolated from both normal and neonatally streptozotocin-induced diabetic rats (Barberá et al., 1997).

We have now studied the direct effect of sodium tungstate on insulin secretion in the isolated perfused rat pancreas as well as the insulin response to both glucose and arginine in pancreas from tungstate-pretreated rats. Glucagon was measured in some experiments.

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2. Methods

Male Wistar rats (200–225 g body weight) fed ad libitum were used as donors. The animals were maintained in accordance with the guidelines established by the European Union (86/609). After anaesthesia of the rat with pentobarbital sodium (50 mg/kg, i.p.), the pancreas was dissected and perfused in situ according to the procedure of Leclercq-Meyer et al. (1976) as adapted in our laboratory (Silvestre et al., 1986). Effluent samples were collected from the portal vein, without recycling, at one-minute intervals (flow rate, 2 ml/min) and frozen at −20° C until the time of assay. The perfusion medium consisted of a Krebs–Henseleit buffer: 115 mM NaCl, 4.7 mM KCl, 2.6 mM $CaCl_2$, 1.19 mM H_2HPO_4, 1.19 mM $MgSO_47H_2O$ and 24.9 mM $HNaCO_3$ (gas phase 95:5, O_2:CO_2; pH 7.4), supplemented with 4% (w/v) dextran T-70 (Pharmacia LKB Biotechnology, Uppsala, Sweden), 0.5% (w/v) Cohn fraction V bovine albumin (Sigma, St. Louis, MO, USA) and glucose (3.2, 5.5 or 9 mM, Sigma).

In a first series of experiments, we studied the direct effect of sodium tungstate on insulin and glucagon secretion at a constant glucose concentration (5.5 mM). The effect of tungstate on insulin secretion was also examined at 3.2 and 9 mM glucose. Solutions of sodium tungstate

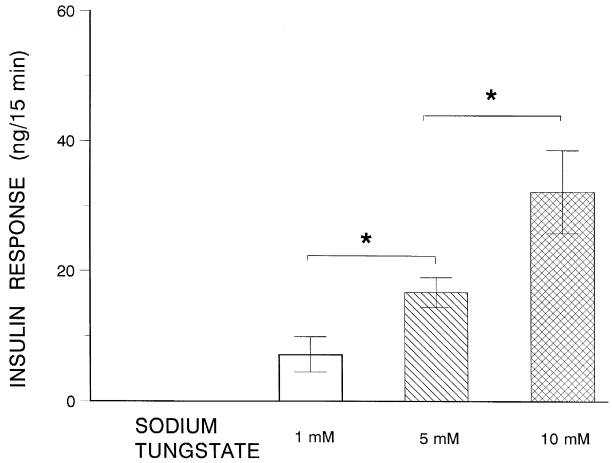

Fig. 2. Incremental insulin responses to sodium tungstate: insulin secretion in the rat pancreas perfused at 5.5 mM glucose; 1 mM tungstate, $n = 5$; 5 mM tungstate, $n = 7$; 10 mM tungstate, $n = 5$. Means ± S.E.M. Statistical analysis by unpaired Student's t-test: $^{*}P < 0.05$.

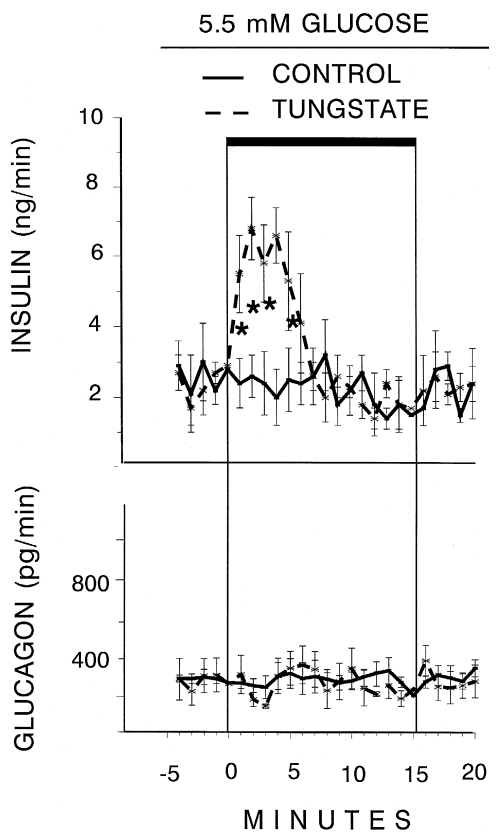

Fig. 1. Effect of 5 mM sodium tungstate on insulin (upper panel) and glucagon (lower panel) secretion in the rat pancreas perfused at 5.5 mM glucose. Solid lines correspond to control experiments: infusion of 5.5 mM glucose alone (upper panel: $n = 3$; lower panel: $n = 4$). Broken lines correspond to tungstate experiments: from 0 to 15 min, tungstate infusion (upper panel: $n = 5$; lower panel: $n = 4$). Means ± S.E.M. Unpaired Student's t-test between tungstate and control experiments at a given time: $^{*}P < 0.05$.

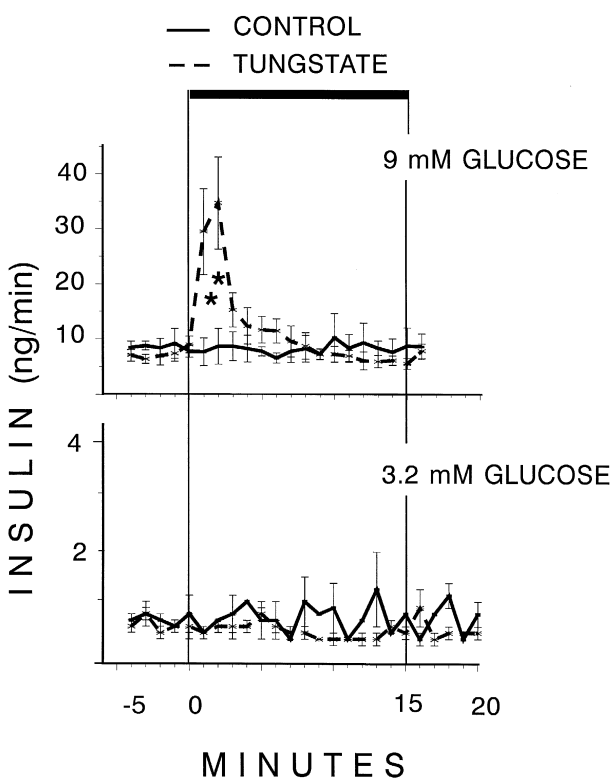

Fig. 3. Effect of 5 mM sodium tungstate on insulin secretion in the rat pancreas perfused at different constant glucose concentrations. Upper panel: Solid line corresponds to control experiments: infusion of 9 mM glucose alone ($n = 3$). Broken line corresponds to tungstate experiments: infusion of 9 mM glucose, plus tungstate (from 0 to 15 min) ($n = 5$); Lower panel: Solid line corresponds to control experiments: infusion of 3.2 mM glucose alone ($n = 3$). Broken line corresponds to tungstate experiments: infusion of 3.2 mM glucose, plus tungstate (from 0 to 15 min) ($n = 4$). Means ± S.E.M. Unpaired Student's t-test between tungstate and control experiments at a given time: $^{*}P < 0.05$.

(Merck, Darmstadt, Germany) in normal saline were prepared daily, immediately before experiments. When added to the perfusate, the final concentration was 1, 5 or 10 mM, as indicated in the corresponding figures. After a 35-min equilibration period, baseline samples were collected for 5 min and, at zero time, normal saline with or without tungstate was infused through a side-arm cannula. Sodium tungstate was likewise tested in the presence of known inhibitors of insulin secretion: diazoxide (300 μM; Sigma), 14-somatostatin (10 nM; Peninsula Laboratories, Europe), and rat amylin (1 nM; Amylin Pharmaceuticals). In another series of experiments, we examined the capacity of the B-cell subjected to tungstate pre-infusion to be stimulated by glucose. For this purpose, 5 mM tungstate was infused for 15 min at 5.5 mM glucose and, after a wash-out period of 10 min, insulin secretion was stimulated by increasing the glucose concentration to 9 mM for 10 min. In control perfusions, the same glucose stimulus was applied.

We also investigated the insulin responses to glucose and arginine in pancreases obtained from rats chronically treated with tungstate. Sodium tungstate was dissolved in tap water (2 mg/ml). The animals, according to their water intake (24.3 ± 0.5 ml/day), received a dose of 48.6 ± 1 mg/day. This treatment was carried out for 8 days. During this period, the fluid and food intake as well as the body weight of the animals were measured daily. Insulin release was stimulated by increasing the glucose concentration (from 5.5 to 9 mM), and by 5 mM arginine.

Insulin and glucagon were analyzed by radioimmunoassay (Yalow and Bergson, 1960; Herbert et al., 1965; Faloona and Unger, 1974). Anti-pig insulin serum (I8510, Sigma) and rat insulin standards (Novo Nordisk, Denmark) were used. Anti-glucagon serum (04A) was kindly donated by R.H. Unger (University of Texas, Health Sciences Center, Dallas, TX, USA). All samples for each series of experiments were analyzed within the same assay. Results are expressed as the means ± S.E.M. Hormone response was calculated as the integrated area under the curve above the mean preinfusion level (average of all the baseline levels for each perfusion) using the trapezoidal method. The normal distribution of our data was demonstrated with the Kolmogorov–Smirnov test. Differences between values were tested for significance with analysis of variance and the Student's *t*-test for unpaired samples.

3. Results

As shown in Fig. 1 (upper panel), incorporation of 5 mM tungstate into the perfusate was followed by a prompt increase in insulin release (incremental area: 16.5 ± 2.5 ng/15 min; $F(15,60) = 10.5$, $P < 0.01$). Glucagon secretion (Fig. 1, lower panel) was not affected by tungstate.

As illustrated in Fig. 2, the insulin secretion elicited by sodium tungstate was dose-dependent: 7.2 ± 2.7 ng/15 min at 1 mM tungstate; 16.7 ± 2.3 ng/15 min, at 5 mM; 32.3 ± 6.4 ng/15 min, at 10 mM. Differences between values were statistically significant among all three series of perfusions ($P < 0.05$).

Fig. 3 (upper panel) shows that, in pancreases perfused at 9 mM glucose, tungstate infusion markedly stimulated insulin release (incremental area: 73.3 ± 16 ng/15 min;

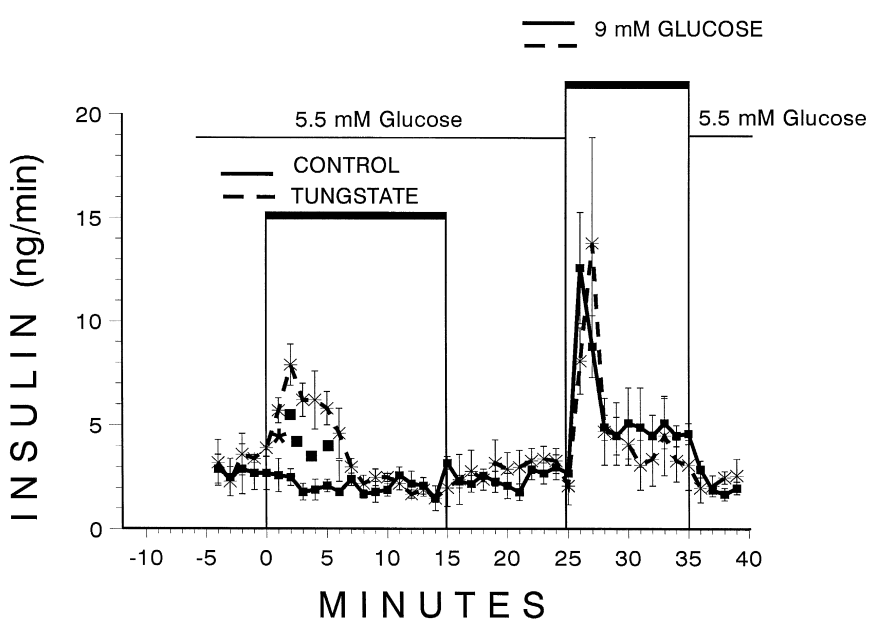

Fig. 4. Effect of previous sodium tungstate (5 mM) infusion on the insulin response to 9 mM glucose in the perfused rat pancreas. Solid line corresponds to control experiments: 5.5 mM glucose infusion until min 25; from 25 to 35 min, 9 mM glucose infusion ($n = 5$). Broken line corresponds to tungstate experiments: from 0 to 15 min tungstate infusion at 5.5 mM glucose; from 15 to 25 min, wash-out period (5.5 mM glucose alone); and from 25 to 35 min, 9 mM glucose infusion ($n = 4$). Means ± S.E.M. Unpaired Student's *t*-test between tungstate and control experiments at a given time: $^{*}P < 0.05$; ■ $P < 0.01$.

$F(15,60) = 8.3$, $P < 0.05$). However (Fig. 3, lower panel), tungstate infusion did not modify the insulin output at a low (3.2 mM) glucose level.

In order to explore the functional viability of the B-cell previously perfused with tungstate, we tested the insulin response to glucose in pancreases pre-infused with this salt (Fig. 4). As expected, tungstate infusion stimulated insulin output (peak at 2 min: 7.1 ± 0.8 ng/min vs. basal value:3.3 ± 0.5 ng/min, $P < 0.05$; $F(15,45) = 11.28$; $P < 0.01$). After a 10-min wash-out period, the insulin response to an increase in glucose concentration, from 5.5 to 9 mM, was comparable to that observed in control experiments (peaks: 13.8 ± 5 vs. 12.6 ± 2.3 ng/min; incremental areas: 29 ± 1.1 vs. 29 ± 8 ng/10 min).

Fig. 5 shows that the insulinotropic effect of 5 mM sodium tungstate was abolished by 300 μM diazoxide (upper panel) and by 10 nM somatostatin (middle panel). Amylin, at 1 nM, (lower panel) also inhibited the tungstate-induced insulin release (incremental areas: tungstate, 15 ± 2 ng/10 min; tungstate + amylin, 4.8 ± 2.9 ng/10 min, $P < 0.05$).

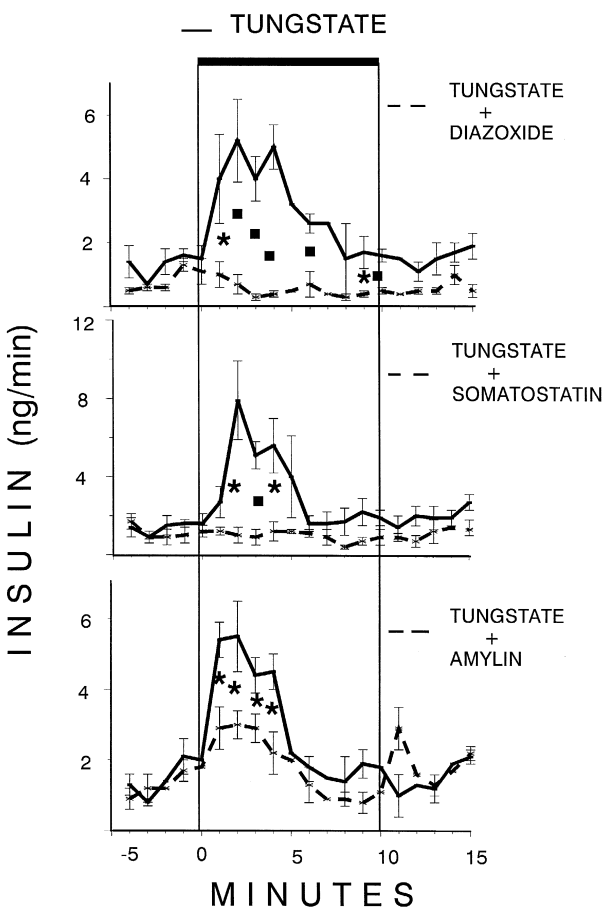

Fig. 5. Effect of 300 μM diazoxide (upper panel), 10 nM 14-somatostatin (middle panel) and 1 nM amylin (lower panel) on the insulin response to 5 mM sodium tungstate in the rat pancreas perfused at 5.5 mM glucose. Solid lines correspond to tungstate experiments: from 0 to 10 min, tungstate infusion (upper and middle panels: $n = 3$; lower panel: $n = 4$). Broken lines correspond to: upper panel: from 0 to 10 min, tungstate plus diazoxide infusion ($n = 4$); middle panel: from 0 to 10 min, tungstate plus 14-somatostatin infusion ($n = 3$); lower panel: from 0 to 10 min, tungstate plus amylin infusion ($n = 4$). Means ± S.E.M. Unpaired Student's *t*-test between tungstate and control experiments at a given time: * $P < 0.05$; ■ $P < 0.01$.

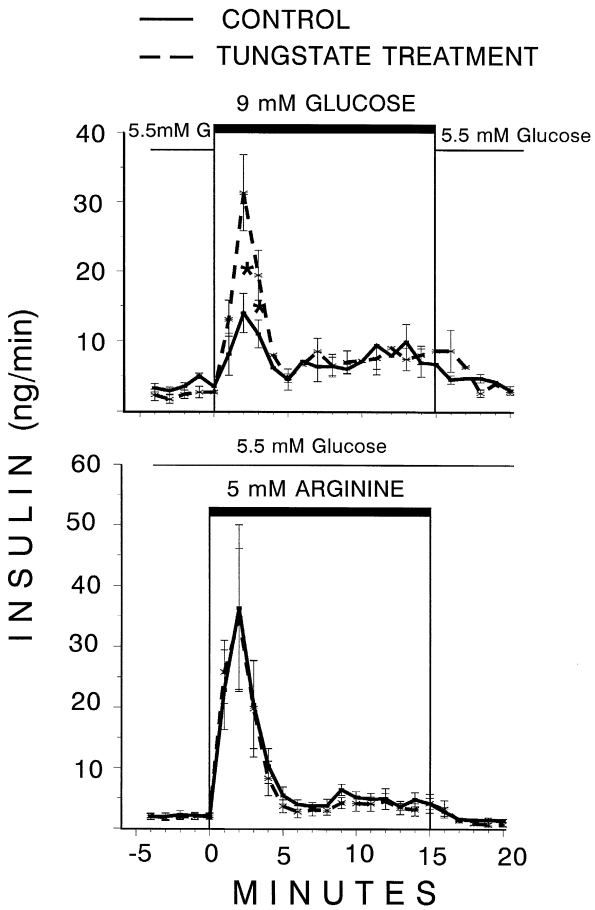

Fig. 6. Effect of previous oral sodium tungstate treatment (48.6 ± 1 mg/day for 8 days) on the insulin responses to increasing (from 5.5 to 9 mM) the glucose concentration (upper panel) and to arginine (lower panel) in the isolated perfused rat pancreas. Upper panel: from 0 to 15 min, 9 mM glucose infusion. Solid line: untreated control rats ($n = 5$). Broken line: tungstate-treated rats ($n = 5$). Lower panel: from 0 to 15 min, 5 mM arginine infusion. Solid line: untreated control rats ($n = 5$). Broken line: tungstate-treated rats ($n = 5$). Means ± S.E.M. Unpaired Student's *t*-test between tungstate and control experiments at a given time: * $P < 0.05$.

Fig. 6 (upper panel) shows that pancreases isolated from rats treated with tungstate for 8 days had an insulin response to glucose greater than that of pancreases from untreated animals. This potentiation only affected the first phase (0–5 min) of insulin output (peaks: 31 ± 5 vs. 14 ± 3 ng/min, $P < 0.025$; incremental areas: 60 ± 6 vs. 20 ± 6 ng/5 min, $P < 0.01$). As depicted in Fig. 6, lower panel, tungstate treatment did not modify the insulin response to arginine.

It must be mentioned that rats treated for 8 days with tungstate showed an increase in body weight (29.6 ± 2.9 g;

$n = 36$) significantly lower than that found in control rats (52.6 ± 2.9 g; $n = 35$). The initial food intake was comparable in the two groups (tungstate-treated: 20.6 ± 0.7 g/day; controls: 20 ± 0.5 g/day). Three days after the start of tungstate administration, the treated rats ate less than did the controls (18.3 ± 0.3 vs. 20.8 ± 0.4 g/day; $P < 0.01$). This difference persisted throughout the period of tungstate administration (at day 8: 18.2 ± 0.7 vs. 20.3 ± 0.6 g/day; $P < 0.05$). The mean water intake in the tungstate-treated group ranged between 22.3 ± 1 (initial value) and 28.3 ± 1 ml/day throughout the experimental period, with no statistically significant difference at any day.

4. Discussion

The foregoing results demonstrate that, in the perfused pancreas from normal rats, infusion of tungstate induces a prompt, short-lived insulin response, comparable to the early phase of glucose-induced insulin secretion. The insulinotropic effect of tungstate is dose-dependent and takes place at normal (5.5 mM) and moderately high (9 mM) glucose concentrations but not at a low glucose level (3.2 mM). Glucagon release was not affected by tungstate.

Direct infusion of tungstate (5 mM) into the pancreas does not seem to be toxic to the B-cell, since it does not alter the insulin response to a subsequent glucose stimulus.

In agreement with results of previous studies with incubated rat islets (Barberá et al., 1997), pancreases from rats chronically treated with tungstate show a greater insulin response to glucose than do those from control rats. This potentiating effect, however, is not observed when the B-cell is stimulated by arginine.

The perfused rat pancreas, while a suitable model to characterise the insulin secretory pattern elicited by tungstate, does not allow direct investigation of the detailed mechanism of action of this substance. The lack of influence of tungstate on glucagon release would rule out mediation of the insulinotropic effect of tungstate by an A-cell paracrine effect. The observation that tungstate-induced insulin secretion is blocked by diazoxide, an activator of K^+-ATP channels (Trube et al., 1986), would suggest interference of tungstate with this pathway. The insulinotropic effect of tungstate is also inhibited by somatostatin, an insulin blocker that, besides activating K^+-ATP channels, decreases adenylate cyclase activity and possibly interferes with the exocytotic process itself (Berggren et al., 1992). It is of note that tungstate has been found to activate adenylate cyclase in a number of tissues (Hwang and Ryan, 1981) although so far there is no information about such an effect on the pancreatic islets. Jonas and Henquin (1996) have recently reported that tungstate, as well as vanadate, stimulates inositol-phosphate production in isolated mouse islets, a pathway which mediates the activation of the insulin releasing mechanism by some secretagogues such as the 26–33 fragment of cholecystokinin (CCK-8) (Florholmen et al., 1989). In our pancreas model, amylin, an inhibitor of CCK-8-induced insulin release (Salas et al., 1995), behaves as an effective blocker of the insulinotropic effect of tungstate. Thus, it may be considered that tungstate could also enhance insulin output by increasing phospholipid turnover within the B-cell.

The results reported here do not pertain to an animal model comparable to human type 2 diabetes. Extensive toxicological studies are needed to determine whether tungstate can be considered as a possible antidiabetic agent. It is worthy of mention that our series of rats treated with tungstate for 8 days showed a decrease in body weight gain with a concomitant slight reduction in food intake. Barberá et al. (1994) found that tungstate treatment was followed by a parallel decrease in both food intake and body weight whilst in another study, this group reported no statistically significant effect on these parameters (Barberá et al., 1997). These authors have also observed diminished water intake after tungstate administration, in contrast with our results. The study of larger groups of treated animals will clarify these points.

Our observations indicate that the reported reduction of glycemia caused by tungstate administration (Barberá et al., 1997) is due, at least in part, to its insulinotropic effect. Interestingly, the finding that tungstate infusion does not stimulate insulin output at a low glucose level suggests that this compound would be ineffective during hypoglycaemia. Concerning the potential antidiabetic effect of tungstate, the observed lack of effect of this salt on arginine-induced insulin release does not seem to be a major drawback, since the insulin response to aminogenic stimulus is maintained in mild type 2 diabetes (Pfeifer et al., 1981).

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References

Barberá, A., Fernández-Álvarez, J., Truc, A., Gomis, R., Guinovart, J.J., 1997. Effects of tungstate in neonatally streptozotocin-induced diabetic rats: mechanism leading to normalization of glycaemia. Diabetologia 40, 143–149.

Barberá, A., Rodríguez-Gil, J.E., Guinovart, J.J., 1994. Insulin-like actions of tungstate in diabetic rats. Normalization of hepatic glucose metabolism. J. Biol. Chem. 269, 20047–20053.

Berggren, P.O., Rorsman, P., Efendic, S., Östenson, C.G., Flatt, P.R., Nilsson, T., Arkhammar, P., Juntti-Berggren, L., 1992. Mechanisms of action of entero-insular hormones, islet peptides and neural input on the insulin secretory process. In: Flatt, P.R. (Ed.), Nutrient Regulation of Insulin Secretion. Portland Press Res. Monogr. Portland Press, London, pp. 289–318.

Dlouha, H., Teisinger, T., Vyskocil, F., 1981. The effect of vanadate on the electrogenic Na^+/K^+ pump, intracellular Na^+ concentration and electrophysiological characteristics of mouse skeletal muscle fiber. Physiol. Bohemoslov. 30, 1–10.

Fagin, J.A., Ikejeri, K., Levin, S.R., 1987. Insulinotropic effects of vanadate. Diabetes 36, 1448–1452.

Faloona, G.R., Unger, R., 1974. Glucagon. In: Jaffe, B.M., Behrman, H.R. (Eds.), Methods of Hormone Radioimmunoassay. Academic Press, New York, pp. 317–330.

Fillat, C., Rodríguez-Gil, J.E., Guinovart, J.J., 1992. Molybdate and tungstate act like vanadate on glucose metabolism in isolated hepatocytes. Biochem. J. 282, 659–663.

Florholmen, J., Malm, D., Vonen, B., Burhol, P.G., 1989. Effect of cholecystokinin on the accumulation of inositol phosphates in isolated pancreatic islets. Am. J. Physiol. 257, G865–G870.

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, 555–561.

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

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

Hwang, P.L., Ryan, R.J., 1981. Tungstate stimulates adenylate cyclase. Endocrinology 108, 435–439.

Jonas, J.C., Henquin, J.C., 1996. Possible involvement of a tyrosine kinase-dependent pathway in the regulation of phosphoinositide metabolism by vanadate in normal mouse islets. Biochem. J. 315, 49–55.

Leclercq-Meyer, V., Marchand, J., Leclercq, R., Malaisse, W.J., 1976. Glucagon and insulin release by the in vitro perfused rat pancreas. Diabete Metab. 2, 57–65.

Pfeifer, M.A., Halter, J.B., Porte, D. Jr., 1981. Insulin secretion in diabetes mellitus. Am. J. Med. 70, 579–588.

Salas, M., Silvestre, R.A., García-Hermida, O., Fontela, T., Rodríguez-Gallardo, J., Marco, J., 1995. Inhibitory effect of amylin (islet amyloid polypeptide) on insulin response to non-glucose stimuli. Study in the perfused rat pancreas. Diabete Metab. 21, 269–273.

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

Shechter, Y., Karlish, S.J.D., 1980. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. Nature 284, 556–558.

Silvestre, R.A., Miralles, P., Moreno, P., Villanueva, M.L., Marco, J., 1986. Somatostatin insulin and glucagon secretion by the perfused rat pancreas from the cysteamine-treated rats. Biochem. Biophys. Res. Commun. 134, 1291–1297.

Trube, G., Rorsman, P., Ohno-Shosaku, T., 1986. Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic beta-cells. Pfluegers Arch. 407, 493–499.

Yalow, R.S., Bergson, S.A., 1960. Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39, 1157–1175.