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Tungstate stimulates insulin release and inhibits somatostatin output in the perfused rat pancreas

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Abstract

In the rat pancreas, infusion of sodium-tungstate stimulates basal insulin release in a dose-dependent manner. We have studied tungstate's effects on the insulin secretion elicited by various B-cell secretagogues. Somatostatin output was also measured. The study was performed in the perfused pancreas isolated from normal or somatostatin-depleted pancreases as induced by cysteamine pre-treatment. In control rats, tungstate co-infusion (5 mM) potentiated the insulin secretory responses to glucose (2.7-fold; $P<0.01$), arginine (2-fold; $P<0.01$), exendin-4 (3-fold; $P<0.01$), glucagon (4-fold; $P<0.05$), and tolbutamide (2-fold; $P<0.01$). It also inhibited the somatostatin secretory responses to glucose (90%; $P<0.01$), arginine (95%; $P<0.01$), glucagon (80%; $P<0.025$), exendin-4 (80%; $P<0.05$) and tolbutamide (85%; $P<0.01$). In somatostatin-depleted pancreases, the stimulatory effect of tungstate on basal insulin secretion and its potentiation of arginine-induced insulin output were comparable to those found in control rats. Our observations suggest an amplifying effect of tungstate on a common step in the insulin stimulus/secretion coupling process, and would rule out a paracrine effect mediated by the inhibition of somatostatin secretion induced by this compound.

Keywords: Sodium tungstate; Insulin; Somatostatin; Rat pancreas

1. Introduction

Tungstate is an oxyanion which has been shown to exert insulin-mimetic effects in isolated hepatocytes, increasing fructose 2,6-bisphosphate levels, counteracting the effects of glucagon on both fructose 2,6-bisphosphate concentrations and 6-phosphofructo-2-kinase activity, and stimulating glycolytic flux (Dominguez et al., 2003; Fillat et al., 1992).

Oral tungstate treatment partially normalizes hyperglycaemia in streptozotocin-induced diabetic rats (Barberá et al., 1994, 1997, 2001; Fernández-Álvarez et al., 2004). Furthermore, oral administration of homo-polyoxotungstate (Nomiya et al., 2001) or pertungstate treatment (Li et al., 1995) also reduce hyperglycaemia in streptozotocin-treated diabetic mice. On the other hand, tungstate directly stimulates insulin secretion in both isolated rat islets (Barberá et al., 1997) and the perfused rat pancreas (Rodríguez-Gallardo et al., 2000), and chronic tungstate treatment enhances the insulin secretory response to glucose in the perfused rat pancreas (Rodríguez-Gallardo et al., 2000). Both hepatic and pancreatic B-cell effects of tungstate can therefore be considered to interpret its blood glucose lowering effect.

In order to further characterize the acute insulinotropic effect of tungstate, we have studied its effect on the insulin secretion elicited by insulin secretagogues acting on the B-cell via different mechanisms. In a preliminary study, we found that tungstate infusion inhibits arginine-induced somatostatin release. Thus, we investigated the effect of this compound on somatostatin secretion as evoked by various D-cell stimulatory agents. Finally, since a deficit in somatostatin secretion can facilitate insulin release (Marks et al., 1992) we also examined the insulinotropic effect of

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tungstate in a model of pancreatic somatostatin depletion, in pancreases from cysteamine-pretreated donors (Silvestre et al., 1986; Szabo and Reichlin, 1981). The study was performed in the isolated rat pancreas perfused in situ.

2. Methods

Male Wistar rats (200–225 g body weight) from our inbred colony, fed ad libitum, were used as donors. In some experiments, somatostatin depletion was induced by treatment with cysteamine (Szabo and Reichlin, 1981). Cysteamine HCl (Sigma-Aldrich Química S.A., Spain), dissolved in distilled water, was administered in a single dose (300 mg/kg b.w.) via a gastric tube 22–24 h before experiments. Control rats were given distilled water. 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 as previously described (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 $MgSO_4 \cdot 7H_2O$ 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-Aldrich) and glucose (5.5 mM) (Sigma-Aldrich). Solutions of sodium tungstate (Merck, Darmstadt, Germany) in normal saline were prepared daily, immediately before experiments. When added to the perfusate, the final concentration was 5 mM. 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 sidearm cannula. Stimulation of hormone secretion was induced by: 1) increasing perfusate glucose concentration (from 5.5 to 9 mM or from 5.5 to 16.6 mM); 2) infusion of 10 mM L-arginine hydrochloride (Sigma-Aldrich); 3) infusion of 10 nM exendin-4 (Amylin Pharmaceutical Inc., San Diego, CA, USA); 4) infusion of 30 nM glucagon (Peninsula Labs.). 5) infusion of 0.1 mM sodium tolbutamide (Sigma-Aldrich). Insulin and somatostatin were analyzed by radioimmunoassay (Harris et al., 1978; Herbert et al., 1965; Yallow and Berson, 1960). Anti-pig insulin serum (I8510, Sigma-Aldrich) and rat insulin standards (Novo Nordisk, Denmark) were employed. Anti-somatostatin serum (80C) was kindly donated by R.H. Unger (University of Texas, Health Sciences Center, Dallas, TX, USA). The interassay variations were 5.3% and 6.4% for insulin and somatostatin, respectively, and the intra-assay variations were 4% and 4.5%, respectively. Experiments were randomized and all samples for each series of experiments were analyzed within the same assay. To determine total pancreatic hormone content, pancreases subjected to perfusion were removed and frozen at −70 °C for future extraction, a procedure that was performed with an acid–alcohol mixture (82.5% ethanol, 0.77 N H_2SO_4) after homogenization of the glandular tissue by sonication. Results are expressed as the mean±standard error of the mean (S.E.M.). Hormone response was calculated as the integrated area of 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 by the Kolmogorov–Smirnov test. Differences between values were tested for significance by repeated-measures analysis of variance for each group and by Student's *t*-test for unpaired samples.

3. Results

The effects of sodium tungstate infusion on the insulin and somatostatin secretory responses to glucose are depicted in Fig. 1. As shown in Fig. 1A, infusion of sodium tungstate potentiated the first phase of the insulin secretory response to an increase in perfusate glucose concentration from 5.5 to 9 mM (incremental area — from 0 to 5 min: 84±3, Mean±S.E.M., vs. 31±11 ng/5 min in controls; $P<0.01$), without significantly affecting the second-phase insulin secretory response. In order to stimulate D-cell secretion, a high glucose concentration (an increase from 5.5 to 16.6 mM) was employed (Fig. 1B). Tungstate co-infusion blocked glucose-induced somatostatin output (incremental area: 80±109 vs. 808±246 pg/10 min; $P<0.01$).

Fig. 2 demonstrates the effect of tungstate on the insulin and somatostatin secretory responses to a high arginine concentration (10 mM). At this concentration, arginine behaves as a potent

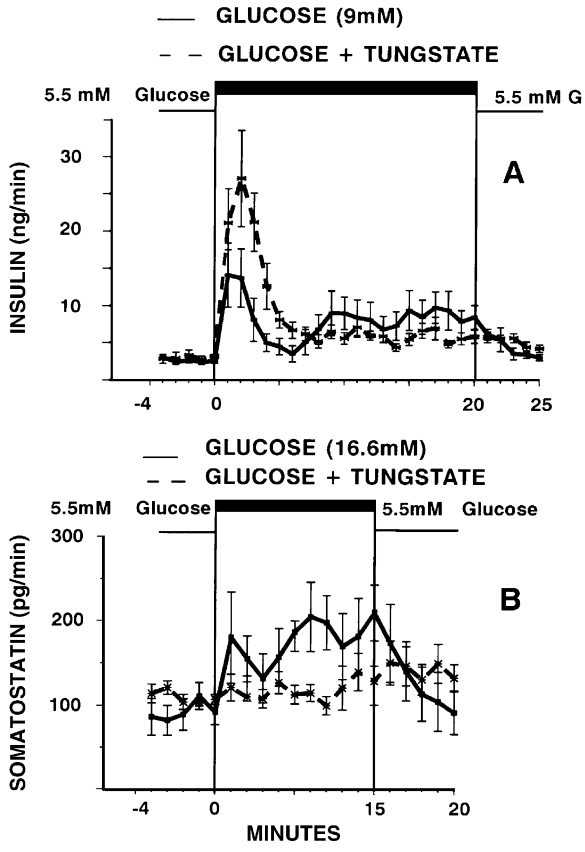

Fig. 1. Effect of sodium tungstate (5 mM) on the insulin (A) and somatostatin (B) responses to glucose. Solid lines correspond to control experiments: Fig. 1A, from 0 to 20 min glucose infusion (an increase from 5.5 to 9 mM ($n=5$). Fig. 1B, from 0 to 10 min glucose infusion (an increase from 5.5 to 16.6 mM) ($n=5$). Broken lines correspond to tungstate experiments: Fig. 1A, from 0 to 20 min glucose+tungstate infusion ($n=6$). Fig. 1B, from 0 to 10 min glucose+tungstate infusion ($n=6$). Mean±S.E.M.

secretagogue of both B-and D-cells. During tungstate co-infusion the first phase of arginine-induced insulin secretion was markedly increased (incremental area: 330±36 vs. 170±35 ng/5 min in controls; $P<0.01$) while the late phase was reduced. Tungstate abolished the somatostatin secretory response to this amino acid (incremental area: 75±18 pg/15 min vs. 1520±320 pg/20 min in control experiments; $P<0.01$).

Tungstate infusion also potentiated the first phase of insulin secretion induced by both exendin-4 (incremental area: 262±74 vs. 77±22 ng/5 min in controls; $P<0.05$; Fig. 3A) and glucagon (incremental area: 87±15 vs. 21±7 ng/15 min in controls; $P<0.05$; Fig. 4A). In these experiments, tungstate blocked the somatostatin secretory responses to exendin-4 (incremental area: 127±26 vs. 725±26 pg/15 min in controls; $P<0.05$; Fig. 3B) and to glucagon (incremental area: 335±331 vs. 1559±208 pg/10 min in controls; $P<0.025$; Fig. 4B).

Fig. 5 shows the insulin and somatostatin secretory responses to 0.1 mM tolbutamide. Co-infusion of sodium tungstate potentiated tolbutamide-induced insulin release throughout the experimental period without affecting the insulin secretory pattern (incremental area: 203±50 vs. 95±16 ng/20 min in controls; $P<0.01$) and inhibited tolbutamide-induced somatostatin secretion (incremental area: 224±207 vs. 1518±223 pg/20 min in controls; $P<0.01$).

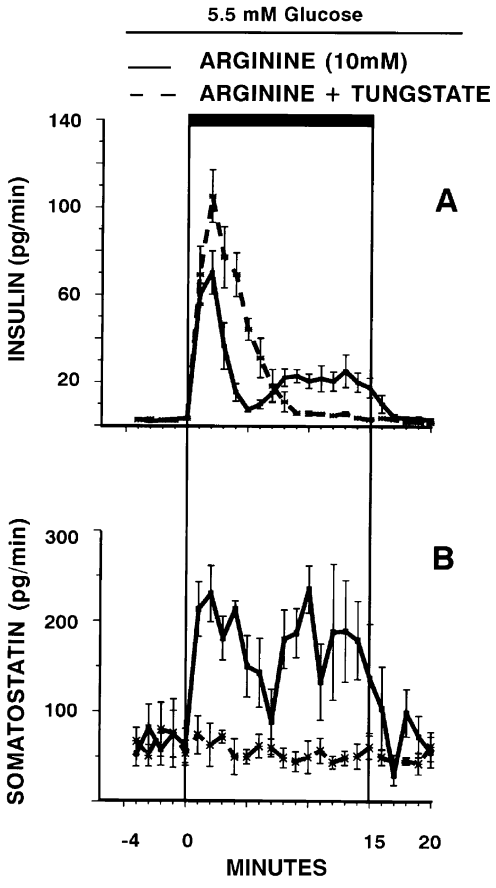

Fig. 2. Effect of sodium tungstate (5 mM) on the insulin (A) and somatostatin (B) responses to 10 mM arginine. Solid lines correspond to control experiments: from 0 to 15 min arginine infusion ($n=5$). Broken lines correspond to tungstate experiments: from 0 to 15 min, arginine+tungstate infusion ($n=6$). Mean±S.E.M.

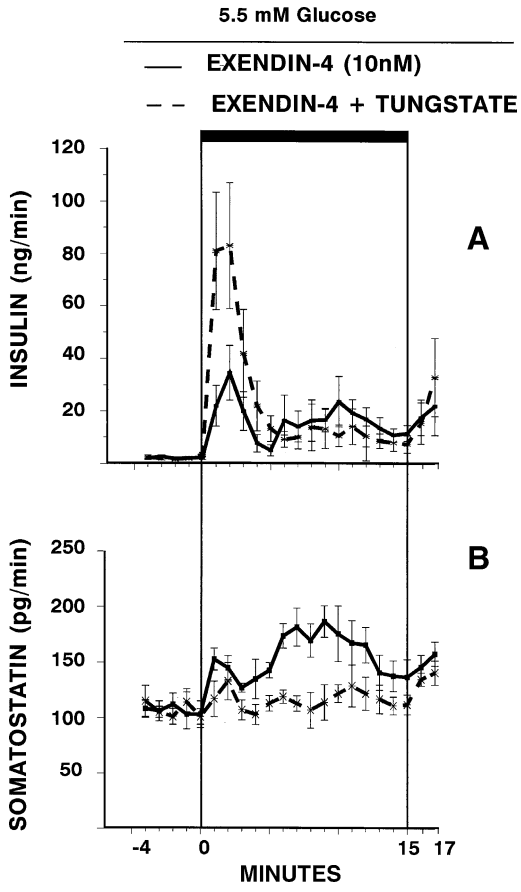

Fig. 3. Effect of sodium tungstate (5 mM) on the insulin (A) and somatostatin (B) responses to 0.1 mM tolbutamide. Solid lines correspond to control experiments: from 0 to 20 min tolbutamide infusion ($n=7$). Broken lines correspond to tungstate experiments: from 0 to 20 min, tolbutamide+tungstate infusion ($n=5$). Mean±S.E.M.

Finally, we studied the insulinotropic effect of tungstate in cysteamine-treated rats. Cysteamine treatment induced pancreatic somatostatin depletion (79±8 vs. 295±37 ng/g wet tissue; $P<0.01$) without significantly affecting pancreatic insulin content (33±3 vs. 35±2 μg/g wet tissue; $P=0.15$). As shown in Fig. 6B, in pancreases from cysteamine-treated rats, somatostatin secretion was not stimulated by arginine. In these somatostatin-depleted pancreases, potentiation of arginine-induced insulin secretion by tungstate (Fig. 6A, incremental areas: 302±36 vs. 172±15 ng/5 min in controls; $P<0.01$) was comparable to that found in untreated rats (Fig. 2A, incremental areas: 302±36 and 330±36 ng/5 min, respectively). Furthermore, in cysteamine-treated rats, tungstate stimulation of basal insulin secretion was comparable to that observed in untreated rats (incremental areas: 21±4.1 vs. 19±4.4 ng/5 min, respectively; $P=0.7$) (Fig. 7).

4. Discussion

The foregoing results demonstrate that, as previously described (Rodríguez-Gallardo et al., 2000), in the perfused pancreas from normal rat, infusion of tungstate induces a prompt, short-lived insulin secretory response, comparable

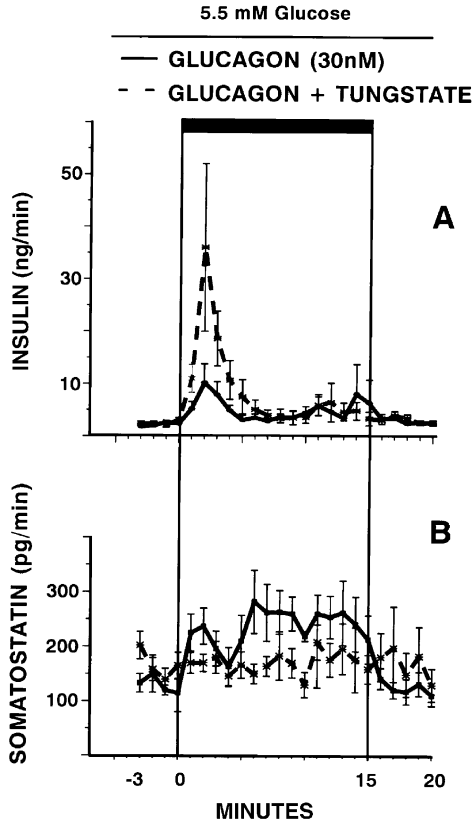

Fig. 4. Effect of sodium tungstate (5 mM) on the insulin (A) and somatostatin (B) responses to 30 nM glucagon. Solid lines correspond to control experiments: from 0 to 15 min glucagon infusion ($n=5$). Broken lines correspond to tungstate experiments: from 0 to 15 min, glucagon+tungstate infusion ($n=5$). Mean±S.E.M.

to the early phase of glucose-induced insulin secretion suggesting an effect on the readily available insulin pool.

The transient stimulatory effect of tungstate on basal insulin secretion is comparable to that elicited by some anions that have been shown to induce alkalinization of B-cells (Juntti-Berggren et al., 1991; Lindstrom and Shelin, 1986). Thus, the possibility that the insulinotropic action of tungstate might be a result of its effect on intracellular pH could also be considered. In fact, alkalinization of muscle intracellular pH has been proposed to mediate the effects of vanadate on system A amino acid transport and on glycolysis in soleus muscle (Muñoz et al., 1992), and biological similarities exist between tungstate and vanadate. It should be pointed out that the stimulatory effect of alkalinization on insulin secretion has been observed both at high and low glucose concentration (Best, 1992; Juntti-Berggren et al., 1991, 1994; Lindstrom and Shelin, 1986). We demonstrated previously that the insulinotropic effect of tungstate was observed at 5.5 and 9 mM glucose, but not at 3.2 mM glucose (Rodríguez-Gallardo et al., 2000). Thus, the stimulatory effect of tungstate on basal insulin secretion does not seem to be mediated by intracellular pH changes. On the other hand, an increase in pH has also been associated with an increase in glucagon secretion (Rebolledo and Gagliardino, 1983) and, in our pancreas system, tungstate failed to significantly modify glucagon output (Rodríguez-Gallardo et al., 2000, and data not shown).

Besides its direct insulinotropic effect, sodium tungstate co-infusion markedly potentiated the insulin secretory responses to a number of B-cell secretagogues, such as glucose, arginine, exendin-4, glucagon and tolbutamide, acting on the B-cell via different mechanisms. The potentiating effect of tungstate was mainly observed during the first phase of the insulin secretory responses to these secretagogues. Data in animals and humans support a crucial physiological role of the first-phase insulin secretion in postprandial glucose homeostasis (Aizawa et al., 2002; Bruce et al., 1988; Caumo and Luzi, 2004; Del Prato et al., 2002; Kahn et al., 2001). Thus, if the loss of early insulin release plays a major role in the pathogenesis of post-prandial hyperglycaemia, therapeutic intervention capable of restoring it should be able to improve glucose tolerance in type 2 diabetic patients.

Little is known about the mechanism by which sodium tungstate stimulates insulin secretion. Jonas and Henquin (1996) have reported that tungstate, as well as vanadate,

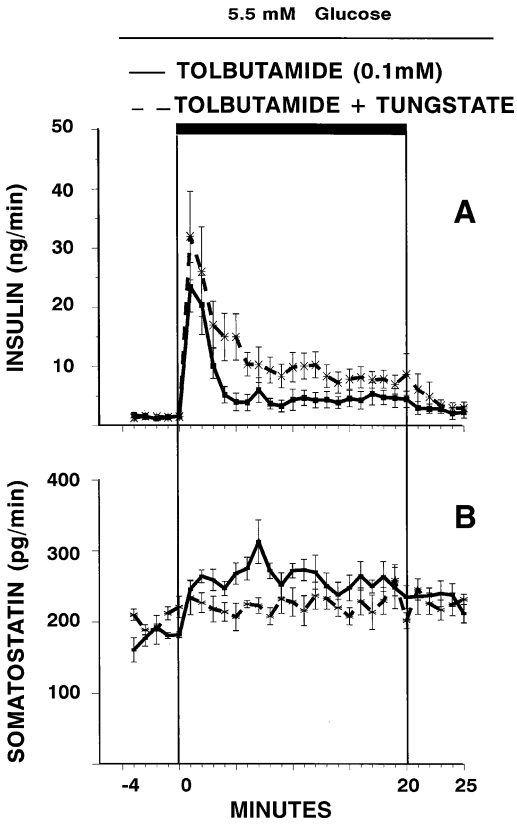

Fig. 5. Effect of sodium tungstate (5 mM) on the insulin (A) and somatostatin (B) responses to 10 nM exendin-4. Solid lines correspond to control experiments: from 0 to 15 min exendin-4 infusion ($n=6$). Broken lines correspond to tungstate experiments: from 0 to 15 min, exendin-4+tungstate infusion ($n=6$). Mean±S.E.M.

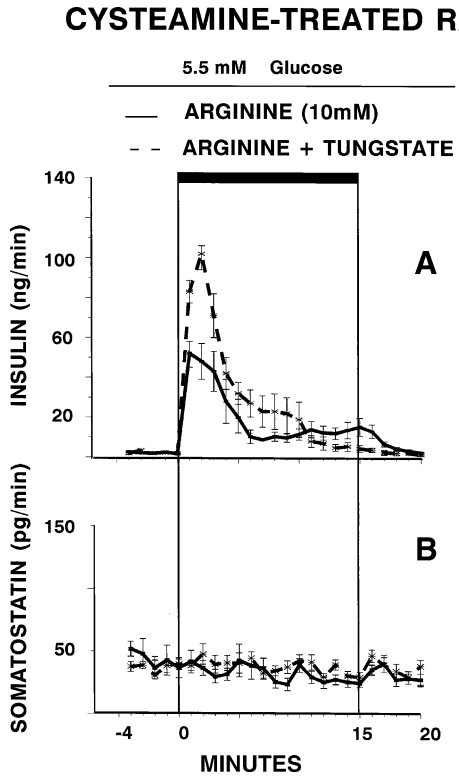

Fig. 6. Effect of sodium tungstate (5 mM) on the insulin (A) and somatostatin (B) responses to 10 mM arginine on cysteamine-treated rats. Solid lines correspond to control experiments: from 0 to 15 min arginine infusion ($n=6$). Broken lines correspond to tungstate experiments: from 0 to 15 min, arginine+tungstate infusion ($n=6$). Mean±S.E.M.

stimulates inositol phosphate production in isolated mouse islets, a pathway that mediates the activation of the insulin releasing mechanism by some secretagogues. In agreement with this observation, it has recently been shown that, in cultured pancreatic BRIN-BDII cells, tungstate treatment increases the insulin secretory response to a protein kinase C activator, phorbol 12-myristate 13-acetate (Liu et al., 2004).

On the other hand, as has been demonstrated for vanadate (Morita et al., 1994; Tan et al., 1999), tungstate activates adenylate cyclase in a number of tissues (Hwang and Ryan, 1981) although, to date, there is no evidence of it having this effect on the B-cells. In our pancreas system, tungstate stimulated insulin secretory responses to glucagon and to exendin-4, secretagogues known to increase B-cell cAMP concentration by activation of the pancreatic adenylate cyclase activity (Berggren et al., 1992; Drucker et al., 1987). A plausible mechanism of the activation of the adenylate cyclase/cAMP system by tungstate might be the blockade of tungstate-induced insulin secretion by somatostatin previously observed in our laboratory (Rodríguez-Gallardo et al., 2000). Somatostatin has been shown to bind and activate an inhibitory G_i protein of the adenylate cyclase system and to reduce cAMP levels in B-cells (Sharp, 1996).

Sodium tungstate also potentiates the insulin secretory responses to glucose and tolbutamide, substances known to induce insulin secretion by closing ATP-dependent K^+ channels, depolarizing the B-cell membrane, opening voltage-dependent Ca^{2+} channels and, in turn, increasing the intracellular calcium concentration (Ashcroft et al., 1984; Flatt, 2003; Henquin et al., 1994). In agreement with a possible interference of tungstate with ATP-dependent K^+ channels, we demonstrated previously that the direct insulinotropic effect of tungstate on basal insulin secretion was blocked by diazoxide, a well known agonist of this K^+ channel type (Rodríguez-Gallardo et al., 2000). It should be pointed out that vanadate, which is chemically related to tungstate, depolarizes muscle fibre membranes (Dlouha et al., 1981). Likewise, tungstate treatment has been shown to increase the insulin secretory responses induced by KCl and by calcium (Liu et al., 2004).

Membrane depolarization and the subsequent opening of L-type calcium channels has been shown to be the mechanism by which L-arginine stimulates insulin secretion (Sener et al., 2000; Smith et al., 1997; Thams and Capito, 1999). In our pancreas model, the biphasic insulin secretory response to arginine is not observed when this amino acid is infused at low concentration (2.5 mM) (Silvestre et al., 2000, 2003). We have found that sodium tungstate potentiates the first phase of the insulin secretory response to arginine while reduces the late phase. To interpret this reduction it can be speculated that an interaction between this amino acid (a cation) and tungstate (an anion) would reduced the concentration of arginine reaching the B-cell.

Taken together, our results demonstrate that sodium tungstate behaves as a general insulin stimulatory agent. This observation suggests an effect of tungstate on a common step in the insulin stimulus/secretion coupling process and/or in the exocytotic machinery.

The direct effect of tungstate on insulin secretion might be responsible for the chronic effect of oral tungstate observed in normal and streptozotocin-induced diabetic rats.

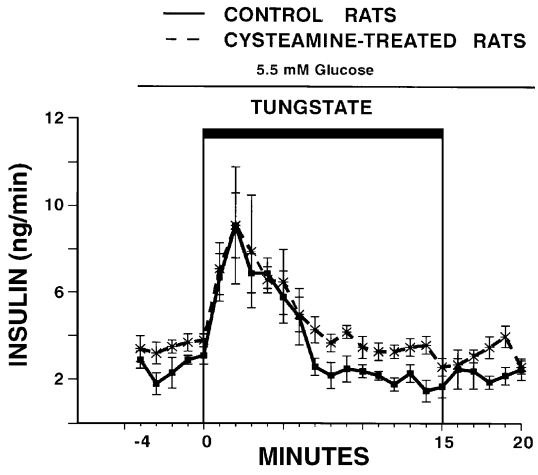

Fig. 7. Effect of 5 mM sodium tungstate infusion (from 0 to 15 min) on basal insulin release in perfused pancreas isolated from normal control rats (solid lines; $n=5$) and from cysteamine-treated rats (broken lines; $n=7$). Mean±S.E.M.

In normal rats, oral tungstate treatment potentiates the insulin secretory response to glucose (Rodríguez-Gallardo et al., 2000). In neonatal streptozotocin-diabetic rats, oral tungstate administration over a one-month period normalizes blood glucose levels (Barberá et al., 1997, 2001; Fernández-Álvarez et al., 2004) and increases the insulin level (Barberá et al., 1997; Fernández-Álvarez et al., 2004) and islet insulin content (Fernández-Álvarez et al., 2004). However, this potentiating effect of tungstate treatment was not observed in a diabetic animal model in which, besides streptozotocin, rats received nicotinamide, an antioxidant agent (Fierabracci et al., 2002).

Reactive oxygen species have been associated with the impairment of B-cell function in type 2 diabetes (Green et al., 2004; Robertson et al., 2004). In fact, as compared with many other cell types, the B-cell may be at high risk for oxidative damage and has an increased sensitivity to apoptosis (Robertson et al., 2004). Recently, it has been proposed that the increases in reactive oxygen species production and oxidative stress might be implicated in glucose toxicity (Fridlyand and Philipson, 2004; Wu et al., 2004). Reactive oxygen species production associated with hyperglycaemia disrupts glucose-stimulated insulin release in pancreatic B-cells (Green et al., 2004; Sakai et al., 2003). It has been shown that tungstate can prevent hepatic injury induced by compounds generating reactive oxygen species and, thus, causing oxidative stress (Pawa and Ali, 2004). Therefore, besides a direct effect of tungstate on insulin secretion, the chronic insulinotropic effect of this compound observed in diabetic rats might also be mediated by a preventive effect of tungstate against pancreatic injury induced by oxidative stress. Interestingly, it has recently been shown that oxovanadium complex exerts a protective effect on the pancreatic antioxidant status in streptozotocin-induced diabetic rats (Ramachandran et al., 2004).

The insulinotropic effect of tungstate reinforces the antidiabetic effects of this substance observed in animal models of diabetes (Barberá et al., 1994, 1997, 2001; Dominguez et al., 2003; Fernández-Álvarez et al., 2004; Fierabracci et al., 2002). However, because of the tissue accumulation and potential toxicity derived from chronic administration of tungstate compounds, the pharmacological use of tungstate in the treatment of diabetes is not necessarily exempt from concern. Tungstate is chemically related to vanadate, a substance that has also been shown to have insulin-like effects (Fillat et al., 1992; Shechter, 1990) and to stimulate insulin release (Fagin et al., 1987). In fact, vanadium salts are the most widely studied insulinomimetic oxyanions, although toxic effects, including dehydration, diarrhea and increases in serum urea and creatinine have been reported in diabetic rats (Domingo et al., 1991; Domingo, 2002). Early investigations in cats, rabbits, dogs, mice, and rats showed that tungstate was less toxic than vanadate when given intravenously (Domingo, 2002). Furthermore, in a long-term study, oral tungstate treatment of streptozotocin-diabetic rats was not associated with toxic effects (Barberá et al., 2001) and treated diabetic rats had a higher long-term survival rate than their untreated counterparts. Therefore tungstate does not seem to have the negative effects associated with vanadate treatment (Barberá et al., 2001; Domingo, 2000, 2002; Fernández-Álvarez et al., 2004). Several facts suggest that the tungstate effects on insulin secretion we have found were not due to toxicity. First, after a wash-out period, pancreases preexposed to tungstate secreted amounts of insulin in response to a subsequent glucose bolus comparable to control, not tungstate preexposed, pancreases (Rodríguez-Gallardo et al., 2000). Furthermore, the fact that the insulinotropic effect of tungstate is transient, is dose-dependent and was inhibited by known inhibitors of insulin release (Rodríguez-Gallardo et al., 2000) argues against the presence of “leaky” or irreversibly damaged islets.

Little is known about the influence of tungstate on the secretion of the other pancreatic hormones. In a previous study, we showed that tungstate failed to modify glucagon release at a constant glucose concentration (Rodríguez-Gallardo et al., 2000). Similarly, tungstate infusion did not significantly modify the glucagon secretory responses to glucose or to arginine (data not shown). As of this writing, there is no information on the effect of tungstate on somatostatin secretion. We have now demonstrated that, in the isolated perfused rat pancreas, tungstate behaves as a potent inhibitor of somatostatin secretion. Infusion of sodium tungstate blocked arginine-induced somatostatin release and markedly reduced the somatostatin secretory responses elicited by glucose, tolbutamide, exendin-4 and glucagon.

Somatostatin has been shown to be an effective inhibitor of insulin secretion in a number of experimental conditions (Marks et al., 1992); thus, under suppression of somatostatin output, an increase in the secretory responses to insulin could be expected. However, in somatostatin-depleted pancreases, the insulinostatic effect of tungstate was comparable to that found in normal untreated-rats. Therefore, the absence of somatostatin release did not alter the normal insulin secretory pattern induced by tungstate.

In summary, our results support a direct stimulatory effect of sodium tungstate on B-cell secretion that is not paracrine mediated by the concomitant inhibition of D-cell secretion induced by this salt.

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