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Effects of 3-mercaptopicolinic acid and a derivative of chlorogenic acid (S-3483) on hepatic and islet glucose-6-phosphatase activity

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Abstract

Glucose-6-phosphatase activity was measured in hepatic microsomes and in pancreatic islets from ob/ob mice. In hepatic microsomes vanadate, phlorizin, 3-mercaptopicolinic acid and a derivative of chlorogenic acid (S-3483) inhibited the translocase activity of the enzyme, vanadate in addition inhibited hydrolase activity. In islets, vanadate inhibited both components of the enzyme, phlorizin inhibited only hydrolase activity while 3-mercaptopicolinic acid and compound S-3483 were without effect. Similarly, when islets were incubated with $^{3}H_2O$ and unlabeled glucose, the incorporation of ^{3}H into medium glucose was inhibited by vanadate and phlorizin, but not by 3-mercaptopicolinic acid and S-3483. These findings suggest that, as with glucokinase, different isoenzymes of glucose-6-phosphatase are present in islets and liver.

Keywords: Glucose-6-phosphatase; Islet; Liver; 3-Mercaptopicolinic acid; Chlorogenic acid

1. Introduction

Glucose-6-phosphatase catalyzes the dephosphorylation of glucose-6-phosphate to glucose. The enzyme plays an important role in the regulation of hepatic glucose production (Ashmore and Weber, 1959; Nordlie, 1985; Nordlie and Sukalski, 1985). The activity of hepatic glucose-6-phosphatase is increased in animals rendered diabetic by alloxan treatment (Jakobsson and Dallner, 1968). Overexpression of the glucose-6-phosphatase catalytic subunit in hepatocytes exhibits a metabolic profile resembling that in liver cells from animals and in patients with non-insulin dependent diabetes mellitus (Seoane et al., 1997). Interestingly, the activity of islet glucose-6-phosphatase is markedly increased in diabetic ob/ob mice resulting in an increased rate of glucose cycling (Khan et al., 1990a,b, 1995). Similarly, islet glucose cycling is increased in other animal models of non-insulin dependent diabetes mellitus, e.g., neonatal streptozotocin and Goto–Kakizaki rats (Khan et al., 1990a; Östenson et al., 1993). The neonatal streptozotocin rat is an experimental model of non-insulin dependent mellitus where the animal is injected with streptozotocin neonatally while the Goto–Kakizaki rat is a spontaneously diabetic model (Goto et al., 1975). Overexpression of glucose-6-phosphatase catalytic subunit in INS-1 insulinoma cell line increased the rate of glucose cycling and decreased glucose utilization and glucose-induced insulin secretion (Trinh et al., 1997). This supports our early proposal that an increased rate of glucose cycling contributes to impaired coupling of glucose signal to insulin release (Khan et al., 1992).

We have indirectly measured the activity of hepatic glucose-6-phosphatase in man by determining in vivo the rate of glucose cycling (glucose → glucose-6-phosphate → glucose) (Efendic et al., 1985). The rate was significantly higher in patients with non-insulin dependent diabetes mellitus who were mildly hyperglycemic (Efendic et al., 1988). Furthermore, glucose cycling was increased in patients with acromegaly (Karlander et al., 1986), and thyrotoxicosis (Shulman et al., 1985; Karlander et al., 1989), and in healthy subjects treated with glucocorticoids (Wajngot et al., 1990) suggesting that also in man, an

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augmented activity of hepatic glucose-6-phosphatase is an early feature of impaired glucose tolerance.

A compound decreasing hepatic and/or islet glucose-6-phosphatase activity and hence, decreasing hepatic glucose production and/or increasing insulin secretion could then exert antidiabetogenic action useful in the treatment of patients with non-insulin dependent diabetes mellitus. The 3-mercaptopicolinic acid and derivatives of chlorogenic acid are interesting in this context, since they decrease glucose-6-phosphatase activity in isolated hepatic microsomes and glucose production from perfused rat liver (Bode et al., 1992; Schindler et al., 1994; Foster et al., 1994; Arion et al., 1997; Hemmerle et al., 1997). In the present study, we compared the effects of 3-mercaptopicolinic acid and a derivative of chlorogenic acid, compound S-3483, on liver and islet glucose-6-phosphatase activity in ob/ob mice. In parallel, we investigated the effects of vanadate and phlorizin, known inhibitors of hepatic glucose-6-phosphatase activity (Singh et al., 1981; Arion et al., 1980a).

2. Materials and methods

2.1. Materials

[1-^{14}C]Glucose-6-phosphate (specific activity 60 mCi/mmol) and tritiated water (specific activity 100 mCi/ml) were purchased from New England Nuclear (Boston, MA). [2-^{3}H]Mannose (15.7 Ci/mmol) was purchased from Amersham (Buckinghamshire, UK). [2-^{3}H]Mannose-6-phosphate was synthesized by the method of Rush and Waechter (1992). Glucose-6-phosphate, mannose-6-phosphate, β-glycerol-phosphate, histone, Triton X-100, HEPES [4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid], PIPES (piperazine-*N*,*N*-bis[2-ethane sulfonic acid]), sodium *ortho*-vanadate(Na_3VO_4), phlorizin (phloretin-2-β-D-glucoside), EDTA (ethylene diamine tetraacetic acid), MES (2[*N*-morpholino] ethane sulfonic acid) and bovine serum albumin (fraction V) were purchased from Sigma (St. Louis, MO). Collagenase was from Boehringer Mannheim, Germany. Amberlite mixed bed resin was purchased from BDH (Poole, UK). The derivative of chlorogenic acid, S-3483, was from Hoechst (Germany). The 3-mercaptopicolinic acid was a gift from Smith Kline and French Laboratories (Swedeland, PA).

Two series of experiments were performed. In the first series of experiments, we investigated the effects of vanadate, phlorizin and S-3483 in hepatic microsomes and isolated islets while in the second series of experiments, the effect of 3-mercaptopicolinic acid was studied.

2.2. Isolation of islets

ob/ob Mice, 10–12-month-old and weighing 45–60 g, were killed by decapitation. Pancreatic tissue was digested with collagenase and islets collected using a glass pipette under stereomicroscopy (Khan et al., 1992). To permeabilize the islets to glucose-6-phosphate and other substances, the islets together with incubation medium were immersed in ethanol-dry ice for 15–20 s. Disrupted islets were prepared by sonication in HEPES buffer (pH 7.4) alone or with histone (0.1%) or Triton X-100 (0.05%) (Khan et al., 1995).

2.3. Preparation of hepatic microsomes

All procedures were performed at 0–4°C. Livers from lean mice, weighing 26–32 g, were homogenized in 250-mM sucrose, 10-mM PIPES buffer (pH 7.4), and the microsomes were isolated by differential centrifugation (DeDuve et al., 1955). A portion of the microsome was diluted to a concentration of 3–5 μg protein/10 μl in the sucrose–PIPES buffer ('intact' microsomes, see below). Disrupted microsomes were prepared by preincubating undiluted microsomes with 1% of Triton for 20 min in ice water and then diluting with sucrose–PIPES, to the same protein concentration as for the intact microsomes. About a 20-fold dilution was required. Microsomes were regarded as 'intact' if the latency of mannose-6-phosphohydrolase activity (measured by the hydrolysis of [^{3}H]mannose-6-phosphate to [^{3}H]mannose, see below) was more than 90%, i.e, less than 10% of the activity exhibited in the absence of Triton that was observed in its presence (Arion et al., 1976).

2.4. Incubations of islets

(1) With [2-^{3}H]mannose-6-phosphate: 15 islets (both permeabilized and sonicated) were incubated in a medium containing 50-mM HEPES (pH 7.4) and 1-mM [^{3}H]mannose-6-phosphate (0.2 μCi), in a total volume of 200 μl for 20 min at 37°C with and without 20-mM β-glycerol-phosphate. Glycerol–phosphate was added to the incubation medium to inhibit non-specific phosphatase activity (Gardner et al., 1993). The incubation was stopped by placing the incubate in ice–water and adding 200 μl of 0.3 M $ZnSO_4$ and after mixing 200 μl of a saturated solution of $Ba(OH)_2$. In three experiments in permeabilized islets incubated in the absence of glycerol–phosphate, only 0.08 to 0.19% of the [^{3}H]mannose-6-phosphate was hydrolyzed to [^{3}H]mannose and in sonicated islets 0.82 to 1.24%. In the presence of glycerol–phosphate, hydrolysis by permeabilized islets ranged from 0.06 to 0.14% and by disrupted islets from 0.89 to 1.29%. These data show that the hydrolysis of mannose-6-phosphate was minimum in permeabilized islets, indicating that the endoplasmic reticulum membrane of the islet was intact. Mannose-6-phosphate cannot be transported through the endoplasmic reticulum membrane by glucose-6-phosphate transporter and therefore, can only be hydrolyzed when the membrane is disrupted. Permeabilized islets were regarded as 'intact' islets

if their mannose-6-phosphatase activity was not more than 15% of the activity in sonicated ('disrupted') islets.

(2) With [1-^{14}C]glucose-6-phosphate: 15 islets (both permeabilized and sonicated) were incubated in a medium containing 50-mM HEPES (pH 7.4), 4-mM [1-^{14}C]glucose-6-phosphate (0.5 μCi) and 100 μM of vanadate or 1 mM of phlorizin or 10 μM of S-3483 or without any addition in a total volume of 200 μl. Incubation was for 20 min at 37°C. For the experiments with 3-mercaptopicolinic acid, the islets were preincubated either with 1 or 2 mM of 3-mercaptopicolinic acid or without any addition in HEPES buffer (50 mM), pH 7.4, at 25°C for 60 min. The islets were then incubated in the presence of same concentration of 3-mercaptopicolinic acid with [1-^{14}C]glucose-6-phosphate as described above.

(3) With $^{3}H_2O$: 15 islets were incubated in 100 μl of Krebs-bicarbonate buffer, pH 7.4, with 0.5% bovine albumin, 5.5-mM glucose and 2.5 mCi of $^{3}H_2O$ in the presence of either 100 μM of vanadate or 1 mM of phlorizin or 10 μM of S-3483 or 3-mercaptopicolinic acid (1 or 2 mM). The vials with the islets and incubation medium were placed in a glass scintillation flask, sealed and gassed for 2 min with 95% O_2: 5% CO_2. After 2 h incubation at 37°C, 100 μl of 10% perchloric acid was injected into the vial. Then 2 mg of glucose was added, the supernatant was neutralized with KOH, and potassium perchlorate was removed by centrifugation. The supernatant was passed through mono-bed resin and the effluent freeze-dried. The residue was dissolved in water and evaporated. Solution in water and evaporation were repeated twice more to assure the removal of $^{3}H_2O$. The residue was then dissolved in water and glucose was isolated by high performance liquid chromatography, using an Aminex HPX-87P carbohydrate analysis column (Bio-Rad Hercules, CA) with H_2O at 85°C as eluant.

2.5. Incubations with hepatic microsomes

(1) With [1-^{14}C]glucose-6-phosphate: hepatic microsomes were incubated for 10 min at 37°C in a medium containing 4-mM glucose-6-phosphate, [1-^{14}C]glucose-6-phosphate (0.1 μCi), 2–5 μg of microsomal protein, 50-mM sodium acetate in a total volume of 50 μl. Microsomes were also incubated with the addition of 100-μM vanadate or 1-mM phlorizin or 10 μM of S-3483 or without any addition. For 3-mercaptopicolinic acid experiments, before incubations, intact microsomes were preincubated at 25°C and disrupted microsomes at 15°C with 1 or 2 mM of 3-mercaptopicolinic acid or without any additions for 60 min (Foster et al., 1994). Incubation was stopped by placing the tubes in ice–water and adding of 50 μl of 0.3 M $ZnSO_4$ and after mixing 50 μl of a saturated solution of $Ba(OH)_2$.

(2) With [2-^{3}H]mannose-6-phosphate: microsomes were incubated in an identical manner as with [1-^{14}C]glucose-6-phosphate except that [2-^{3}H]mannose-6-phosphate was added to the incubate instead of [1-^{14}C]glucose-6-phosphate.

2.6. Measurement of enzyme activity

Glucose-6-phosphatase activity in the incubations with islets and microsomes was taken as the amount of glucose-6-phosphate hydrolyzed to glucose, traced with ^{14}C. After stopping the incubation, 2 mg of glucose was added to the incubate which was then mixed by vortexing and centrifuged for 2 min in an Eppendorf centrifuge (10 000 $\times$ g). The supernatant was passed through the mono-bed resin. The effluent was evaporated to dryness. The residue was dissolved in 1 ml of water and 500 μl was counted for radioactivity. Glucose concentration in the remainder was determined using a glucose oxidase method (Glucose Analyzer, model 23A, Yellow Springs Instrument, Yellow Springs, OH).

The specific activity of [1-^{14}C]glucose-6-phosphate in the incubate was calculated from the quantity of glucose-6-phosphate in the incubate (pmol) and the amount of ^{14}C in that glucose-6-phosphate. The dpm in the 2-mg glucose added as carrier after stopping the incubation was calculated from the ^{14}C activity (dpm) in the effluent from the ion exchange column and the amount of glucose in the effluent, usually about 1.5 mg. The pmol of glucose-6-

Table 1
Effects of vanadate, phlorizin and S-3483 on glucose-6-phosphatase activity in intact (translocase) and disrupted (hydrolase) hepatic microsomes ($n = 5$) and in permeabilized (translocase) and sonicated (hydrolase) pancreatic islets ($n = 7$)

	Hepatic microsomes Enzyme activity (nmol/mg protein per min)		Pancreatic islets Enzyme activity (pmol/islet per min)	
	Translocase	Hydrolase	Translocase	Hydrolase
Control	185 ± 21	422 ± 31[a]	11.65 ± 1.16	19.58 ± 2.51[d]
Vanadate	65 ± 14[b]	68 ± 10[c]	6.66 ± 0.92[e]	8.26 ± 1.20[f]
Phlorizin	99 ± 11[b]	406 ± 30	10.30 ± 0.85	12.74 ± 1.96[g]
S-3483	77 ± 15[b]	404 ± 20	12.58 ± 1.88	18.57 ± 238

Results are expressed as the mean ± S.E.M.; [a] $P < 0.001$ translocase vs. hydrolase in hepatic microsomes (control); [b] $P < 0.001$ control vs. compound in hepatic microsomes (translocase); [c] $P < 0.001$ control vs. vanadate in hepatic microsomes (hydrolase); [d] $P < 0.01$ translocase vs. hydrolase in islets (control); [e] $P < 0.001$ control vs. vanadate in islets (translocase); [f] $P < 0.001$ control vs. vanadate in islets (hydrolase); [g] $P < 0.01$ control vs. phlorizin in islets (hydrolase).

Table 2
Effect of 3-mercaptopicolinic acid (MPA) on glucose-6-phosphatase activity in intact (translocase) and disrupted (hydrolase) hepatic microsomes ($n = 5$) and in permeabilized (translocase) and sonicated (hydrolase) pancreatic islets ($n = 5$)

	Hepatic microsomes Enzyme activity (nmol/mg protein per min)		Pancreatic islets Enzyme activity (pmol/islet per min)	
	Translocase	Hydrolase	Translocase	Hydrolase
Control	207 ± 19	416 ± 21[a]	9.70 ± 1.38	20.62 ± 2.19
MPA (1 mM)	78 ± 11[b]	429 ± 44	9.59 ± 1.32	19.05 ± 2.67
MPA (2 mM)	81 ± 14[b]	420 ± 43	9.55 ± 1.79	19.65 ± 1.82

Results are expressed as the means ± S.E.M.; [a]$P < 0.001$ translocase vs. hydrolase in hepatic microsomes (control); [b]$P < 0.001$ control vs. compound in hepatic microsomes (translocase).

phosphate hydrolyzed to glucose was then calculated by dividing the dpm in the 2 mg of glucose by the specific activity of [1-^{14}C]glucose-6-phosphate in dpm/pmol after subtracting the dpm in the 2 mg of glucose when an identical incubation was done except for the absence of islets or microsomes. Mannose-6-phosphatase activity in incubates with islets and microsomes was taken as the amount of mannose-6-phosphate hydrolysis to mannose traced with ^{3}H. The procedure was analogous to that for glucose-6-phosphatase, but using [2-^{3}H]mannose-6-phosphate rather than [1-^{14}C]glucose-6-phosphate.

In the experiments with permeabilized islets dpm in glucose averaged 2.1% of dpm added in [1-^{14}C]glucose-6-phosphate and with sonicated islets 3.5%. In the experiments with intact microsomes, the dpm in glucose was 0.05% and with disrupted, i.e, Triton treated microsomes the average was 0.18%. Without islets, glucose in the incubate contained 0.04% of the added dpm and without microsomes 0.004%. With intact microsomes, there was 0.08% in mannose and with disrupted microsomes the average was 1.42%. Without islets or microsomes on incubation, there was 0.05% in mannose. In islets incubated with $^{3}H_2O$, total dpm in glucose was calculated from the amount of glucose recovered and the dpm in glucose after ion-exchange chromatography. When $^{3}H_2O$ was incubated without islets, there was no radioactivity in glucose.

Table 3
Effects of vanadate, phlorizin and S-3483 (A) and 3-mercaptopicolinic acid (MPA) (B) on the incorporation of ^{3}H from $^{3}H_2O$ into medium glucose in isolated islets

A		B	
	dpm		dpm
Control	13163 ± 1816	Control	11319 ± 702
Vanadate	8408 ± 1080[a]	MPA (1 mM)	9509 ± 949
Phlorizin	8684 ± 817[a]	MPA (2 mM)	8990 ± 1293
S-3483	12484 ± 1555		

Results are expressed as the means ± S.E.M. of five experiments; [a]$P <$ 0.05 control vs. vanadate and control vs. phlorizin. Intact islets (25) were incubated in Krebs-bicarbonate buffer (pH 7.4) with 11 mM glucose, 1 mCi $^{3}H_2O$ and with or without addition of compound for 2 h at 37°C. Studies were performed in duplicate. Glucose from the incubate was separated as described in Section 2.

Activities are expressed per islet or per μg of microsomal protein. Protein concentration in microsomes was determined using the Bio-Rad method (Bradford, 1976). Results are expressed as means ± S.E.M. Differences between groups were tested for significance using Student's *t*-test for unpaired data.

3. Results

In intact hepatic microsomes, glucose-6-phosphatase was significantly inhibited by vanadate, phlorizin and S-3483 (Table 1). Disruption of microsomes increased the enzyme activity ($P < 0.001$). Vanadate also inhibited the enzyme activity in disrupted microsomes, while phlorizin and S-3483 were without effect. In separate experiments, 3-mercaptopicolinic acid significantly inhibited the enzyme activity in intact microsomes and had no effect in disrupted microsomes (Table 2).

In permeabilized islets, glucose-6-phosphatase activity was significantly inhibited by vanadate, while phlorizin and S-3483 had no effect (Table 1). Disruption of islets in the presence of either histone or Triton, or without any addition to the incubation medium, increased the enzyme activity to the same degree (control 12.0 ± 1.3; histone 27.9 ± 2.1; Triton 25.6 ± 4.4; without any addition 25.4 ± 2.6 pmol/islet per min, $n = 4$). In disrupted islets, the enzyme activity was inhibited by vanadate and phlorizin, while S-3483 had no effect. The 3-mercaptopicolinic acid was without effect on glucose-6-phosphatase activity both in permeabilized and disrupted islets irrespective of its concentration (Table 2).

In experiments with $^{3}H_2O$, vanadate and phlorizin inhibited the incorporation of ^{3}H into glucose while S-3483 had no effect (Table 3). The 3-mercaptopicolinic acid was also without effect.

4. Discussion

Hepatic glucose-6-phosphatase is a multicomponent enzyme consisting of at least four components: a glucose-6-P specific transporter which mediates the entry of the hexose

phosphate from the cytosol into the lumen of the endoplasmic reticulum, a non-specific phosphohydrolase located on the luminal surface of the membrane, and two other transport systems to mediate rapid and reversible fluxes of the hydrolytic products, inorganic phosphate and glucose (Arion et al., 1976). Mannose-6-phosphate is dephosphorylated by glucose-6-phosphatase, but is not transported into the lumen of intact endoplasmic reticulum vesicles by the glucose-6-phosphate transporter (Arion et al., 1980b). Thus, the latency of mannose-6-phosphatase activity can be used to determine the intactness of the endoplasmic reticulum vesicles. In hepatocytes, the latency of mannose-6-phosphatase was only 58% (Jorgenson and Nordlie, 1980). In our permeabilized islet preparations, the latency of mannose-6-phosphatase was 85–90% which is very close to 90% or more reported in hepatic microsomal preparations (Arion et al., 1980b). While histone and Triton have been reported to have different effects on disrupted hepatic microsomes (Arion and Canfield, 1993), we found no difference in the enzyme's activity both with glucose-6-phosphate and mannose-6-phosphate as substrate, whether the islets were sonicated alone or with Triton or histone.

The present study confirms previous findings demonstrating that vanadate, phlorizin, S-3483 and 3-mercaptopicolinic acid inhibit translocase activity of hepatic glucose-6-phosphatase and that vanadate in addition inhibits the hydrolase component of the enzyme (Singh et al., 1981; Bode et al., 1992; Schindler et al., 1994; Arion et al., 1997; Hemmerle et al., 1997; Foster et al., 1994). In addition to chlorogenic acid, phlorizin and 3-mercaptopicolinic acid, there are also a number of other inhibitors which specifically inhibit the hepatic translocase activity of the enzyme. They include picolinic acid, 2-mercaptonicotinic acid (Bode et al., 1992), *N*-bromoacetylethanolamine (Foster et al., 1996), diethyl pyrocarbonate (Arion et al., 1984), certain stilbene disulfonate derivatives (Zoccoli and Karnovsky, 1980) and fatty-Acyl-CoA esters (Fulceri et al., 1992). In parallel to the inhibition of hepatic translocase activity in isolated microsomes, both 3-mercaptopicolinic acid and chlorogenic acid exert inhibitory effects on gluconeogenesis and glycogenolysis in perfused rat liver (Bode et al., 1992; Hemmerle et al., 1997). Interestingly, both S-3483 and 3-mercaptopicolinic acid had similar inhibitory effect on translocase activity of hepatic glucose-6-phosphatase, 62% with 3-mercaptopicolinic acid and 58% with S-3483) although the concentration of S-3483 was 100–200 times lower than that of 3-mercaptopicolinic acid. S-3483 is the most potent inhibitor of the chlorogenic acid family, since several fold higher concentrations of chlorogenic acid and its other derivatives were required to achieve the similar inhibitory effects on the translocase activity of hepatic glucose-6-phosphatase (Hemmerle et al., 1997; Arion et al., 1997).

The novel finding is that the compound S-3483 and 3-mercaptopicolinic acid do not effect translocase and hydrolase activities of islet glucose-6-phosphatase, while they inhibit translocase activity of the hepatic enzyme. Also, the compounds had no effect on the rate of incorporation of ^{3}H into medium glucose when islets were incubated with $^{3}H_2O$. Incorporation of ^{3}H from $^{3}H_2O$ into glucose provides a measure of glucose-6-phosphatase activity in the intact, untreated (no sonication, no freezing) islet cells. That incorporation of ^{3}H occurs in equilibration of the glucose-6-phosphate with fructose-6-phosphate and then its hydrolysis. In agreement with the present findings, we have previously demonstrated the inhibitory effect of S-3483 on translocase activity in hepatic microsomes and no effect on islet microsomes (Khan et al., 1994). These findings imply that as in the case of glucokinase (Magnuson et al., 1989), two isoenzymes of glucose-6-phosphatase are present in the liver and islets.

In patients with non-insulin dependent diabetes mellitus fasting blood glucose concentrations are highly correlated with hepatic glucose production (DeFronzo, 1988). Hence, liver plays a crucial role in the development of hyperglycemia. Presently, we confirm that the derivatives of chlorogenic acid or 3-mercaptopicolinic acid have potential to decrease hepatic glucose production by normalizing glucose flux through glucose-6-phosphate due to the inhibition of translocase activity. These compounds may be of interest when considering the future treatment of non-insulin dependent diabetes mellitus provided that they are not toxic and that they do not decrease hepatic glucose production below the level which is necessary to provide enough glucose for the brain. The complete absence of hepatic glucose-6-phosphatase activity results in severe hypoglycemia as in the case of glycogen storage disease (Cori and Cori, 1952).

Neither chlorogenic acid nor 3-mercaptopicolinic acid cannot be used to evaluate whether the inhibition of glucose-6-phosphatase and glucose cycling in islets in animal models of non-insulin dependent diabetes mellitus improves impaired insulin release and restores glucose tolerance. It remains to be established whether inhibition of islet glucose-6-phosphatase activity constitutes a rational target for the development of drugs for the treatment of non-insulin dependent diabetes mellitus. In conclusion, our results show inhibitory effects of S-3483 and 3-mercaptopicolinic acid on the hepatic but not on the islet enzyme suggesting that islet glucose-6-phosphatase has different properties than hepatic glucose-6-phosphatase. We have no adequate explanation for an inhibitory effect of phlorizin on glucose-6-phosphatase in sonicated, but not in permeabilized islets, even though phlorizin inhibited the incorporation of ^{3}H into glucose by intact islets.

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