During insulin secretion, pancreatic α-cells are exposed to Zn\textsuperscript{2+} released from insulin-containing secretory granules. Although maintenance of Zn\textsuperscript{2+} homeostasis is critical for cell survival and glucagon secretion, very little is known about Zn\textsuperscript{2+}-transporting pathways and the regulation of Zn\textsuperscript{2+} in α-cells. To examine the effect of Zn\textsuperscript{2+} on glucagon secretion and possible mechanisms controlling the intracellular Zn\textsuperscript{2+} level ([Zn\textsuperscript{2+}]\textsubscript{i}), we employed a glucagon-producing cell line (α-TC6) and mouse islets where non-β-cells were identified using islets expressing green fluorescent protein exclusively in β-cells. In this study, we first confirmed that Zn\textsuperscript{2+} treatment resulted in the inhibition of glucagon secretion in α-TC6 cells and mouse islets in vitro. The inhibition of secretion was not likely via activation of K\textsubscript{ATP} channels by Zn\textsuperscript{2+}. We then determined that Zn\textsuperscript{2+} was transported into α-cells and was able to accumulate under both low and high glucose conditions, as well as upon depolarization of cells with KCl. The nonselective Ca\textsuperscript{2+} channel blocker Gd\textsuperscript{3+} partially inhibited Zn\textsuperscript{2+} influx in α-TC cells, whereas the L-type voltage-gated Ca\textsuperscript{2+} channel inhibitor nitrendipine failed to block Zn\textsuperscript{2+} accumulation. To investigate Zn\textsuperscript{2+} transport further, we profiled α-cells for Zn\textsuperscript{2+} transporter transcripts from the two families that work in opposite directions, SLC39 (ZIP, Zrt/Irt-like protein) and SLC30 (ZnT, Zn\textsuperscript{2+} transporter). We observed that Zip1, Zip10, and Zip14 were the most abundantly expressed Zips and ZnT4, ZnT5, and ZnT8 the dominant ZnTs. Because the redox state of cells is also a major regulator of [Zn\textsuperscript{2+}]\textsubscript{i}, we examined the effects of oxidizing agents on Zn\textsuperscript{2+} mobilization within α-cells. 2,2′-Dithiodipiridine (−SH group oxidant), menadione (superoxide generator), and SIN-1 (3-morpholinosydnonimine) (peroxynitrite generator) all increased [Zn\textsuperscript{2+}]\textsubscript{i} in α-cells. Together these results demonstrate that Zn\textsuperscript{2+} inhibits glucagon secretion, and it is transported into α-cells in part through Ca\textsuperscript{2+} channels. Zn\textsuperscript{2+} transporters and the redox state also modulate [Zn\textsuperscript{2+}]\textsubscript{i}.

### Blood Glucose Homeostasis

Blood glucose homeostasis is maintained by appropriate secretion of insulin and glucagon from pancreatic islet β- and α-cells, respectively (1, 2). It is quite clear that when ambient glucose concentrations rise, glucagon secretion is inhibited and insulin secretion is stimulated. The mechanisms that control the suppression of glucagon secretion under high glucose conditions have not been completely agreed upon, but it is likely that α-cells sense and respond to changes in blood glucose through direct and indirect mechanisms (3–6). To date the most likely candidates are glucose itself, paracrine and endocrine factors, and neuronal modulation. Some of the paracrine/endocrine factors that could facilitate the suppression of glucagon secretion include insulin and other factors released upon β-cell exocytosis, including γ-aminobutyric acid and Zn\textsuperscript{2+} (3, 7, 8).

In pancreatic β-cells a fraction of the intracellular Zn\textsuperscript{2+} pool is stored with insulin in intracellular vesicles as a complex of zinc-insulin (9). The concentration of Zn\textsuperscript{2+} in these vesicles is about 20 mm (10, 11). Following exocytosis of the intracellular vesicles, it is likely that the Zn\textsuperscript{2+} released into the extracellular islet space would be transported back into the host cell or into neighboring cells. The effects of β-cell secretory products, Zn\textsuperscript{2+} and insulin, on glucagon secretion are still controversial and may be species-specific. It was shown that Zn\textsuperscript{2+} had inhibitory actions on glucagon secretion from rat islets (8, 12, 13). Recently it was found that switching off pancreatic artery infusions of Zn\textsuperscript{2+} stimulated glucagon secretion in rats (14). Contrary to these experiments, a lack of Zn\textsuperscript{2+} effect on glucagon secretion was observed in mouse islets (15).

In mammalian cells the uptake of Zn\textsuperscript{2+} and its outward transport are regulated by two families of membrane proteins that work in opposite directions. The influx of Zn\textsuperscript{2+} is facilitated by ZIP\textsuperscript{3} (SLC39) proteins (16–20) and by different types of Ca\textsuperscript{2+} channels: dihydropyridine-sensitive Ca\textsuperscript{2+} channels in heart cells (21) and the N-methyl-d-aspartic acid and α-amino-

### Footnotes

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1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Table 1 and Figs. 1 and 2.

2 Supported by a studentship from Canadian Institute of Health Research.

3 The abbreviations used are: ZIP, Zrt/Irt-like protein; GFP, green fluorescent protein; MIP-GFP, mouse insulin promoter-GFP; Znt, Zn\textsuperscript{2+} transporters; VGCC, voltage-gated calcium channel; L-VGCC, L-type VGCC; DiBAC\textsubscript{4}(3), bis-(1,3-dibutylbarbituric acid) trimethine oxonol; TPEN, N,N,N′,N′-tetrakis(2-pyridylmethyl)ethylenediamine; DTPD, 2,2′-dithiodipiridine; SIN-1, 3-morpholinosydnonimine; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazonide; NEM, N-ethylmaleimide; DTE, dithioerythritol; HNE, 4-hydroxynonenal; DIC, differential interference contrast; qPCR, quantitative real time PCR; FITC, fluorescein isothiocyanate; ER, endoplasmic reticulum; TRITC, tetramethylrhodamine isothiocyanate; PI, propidium iodide.
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3-hydroxy-5-methyl-4-isoxazole propionate/kainate-activated Ca$^{2+}$-permeable channels in neurons (22, 23). The efflux of Zn$^{2+}$ or intracellular Zn$^{2+}$ sequestration is promoted by CDF/ZnT (SLC30A) family of transporters, which work in opposition to the ZIP transporters (16–20). In addition, a Na$^{+}$/Zn$^{2+}$ exchange mechanism that is different from both ZnT1 and Na$^{+}$/Ca$^{2+}$ exchanger utilizes the transmembrane electrochemical Na$^{+}$ gradient and mediates extrusion of Zn$^{2+}$ in some cells (24). A Zn$^{2+}$/H$^+$ antiport mechanism, associated with intracellular ZnTs, could be responsible for Zn$^{2+}$ accumulation in intracellular organelles (25). It is likely that ZnT proteins serve as secondary active transporters, using the gradient of other ions to drive the Zn$^{2+}$ transport (20).

Recently a genome-wide association study identified novel risk loci for type 2 diabetes mellitus (26–28). These loci include regulatory Na$^{+}$/Ca$^{2+}$-permeable channels and four types of VGCC, which could be involved in the importance of Zn$^{2+}$ transport as well as the modulation of intracellular Zn$^{2+}$ in islet cells takes on an important clinical significance. Despite the limited number of articles are dedicated to Zn$^{2+}$ transport (30–33). It was demonstrated that Zn$^{2+}$ accumulation into insulin-secreting $\beta$-cells occurred by the following two pathways: through membrane Zn$^{2+}$ transporters under low glucose conditions, and through L-type voltage-gated Ca$^{2+}$ channels (VGCC) under high glucose (32). To date, Zn$^{2+}$ transport mechanisms have not been studied in other islet cell types, including $\alpha$-cells.

Taking into account the Zn$^{2+}$-rich environment surrounding $\alpha$-cells, it is expected that they should have an effective mechanism for maintaining Zn$^{2+}$ homeostasis. Dysfunction of the transporters responsible for Zn$^{2+}$ export can lead to dramatic changes in cell viability, because an excess of Zn$^{2+}$ can induce oxidative damage, mitochondrial depolarization, and opening of mitochondrial permeability transition pores (34–36). In addition, as stated above, Zn$^{2+}$ released from $\beta$-cells may be a required component of the “off switch” for glucagon secretion. Thus the inhibition of glucagon secretion may require Zn$^{2+}$ transport and the modulation of the cytoplasmic Zn$^{2+}$ level ([Zn$^{2+}$]$_i$). $\alpha$-Cells are electrically active and equipped with different types of channels, including K$_{ATP}$ channels and four types of VGCC, which could be involved in regulating Zn$^{2+}$ as in $\beta$-cells (3, 37). In addition, $\alpha$-cells may also modulate [Zn$^{2+}$]$_i$ through ZnTs and Zips. Finally, it is well known that free Zn$^{2+}$ in cells is modulated by redox state and the level of reactive oxygen species in cells (38, 39), which in turn are regulated by glucose and fatty acid metabolism and $\beta$-oxidation. Thus the redox state of the $\alpha$-cell may also regulate $\alpha$-cell [Zn$^{2+}$].

In this study we showed an inhibitory effect of Zn$^{2+}$ on glucagon secretion in a glucagon-producing $\alpha$-cell line (29). Using the Zn$^{2+}$-selective dye FluoZin-3 AM (32, 40) we studied, for the first time, Zn$^{2+}$ transport in intact mouse pancreatic islet $\alpha$-cells, as well as in the $\alpha$-TC cell line, and showed the possibility of Zn$^{2+}$ transport through both Ca$^{2+}$ channels and Zn$^{2+}$ influx transporters(s). We demonstrated the expression of ZIP and ZnT family of Zn$^{2+}$ transporters in $\alpha$-TC cells, as well as in isolated mouse islets. In addition, we investigated the regulation of [Zn$^{2+}$]$_i$ by oxidizing agents in $\alpha$-cells. Our results suggest that cytoplasmic Zn$^{2+}$ homeostasis in $\alpha$-cells is maintained by a concerted action of Zn$^{2+}$ transporters, Ca$^{2+}$ channels, and the redox state of thiol groups.

**MATERIALS AND METHODS**

**Reagents**—Fluorescent dyes FluoZin-3 AM, bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC$_{4}(3)$), rhodamine 123 (Rh123), MitoTracker Red CMXRos, Hoechst 33342, 3-morpholinosydnonimine (SIN-1) were obtained from Molecular Probes (Eugene, OR). Disparate II (neutral protease) was from Roche Diagnostics. Carbonyl cyanide m-trifluromethoxyphenylhydrazone (FCCP), 2,2'-dithiodipyridine (DTDP), 2-mercaptoypyridine N-oxide (pyrithione), N,N,N',N'-tetraakis-(2-pyridylmethyl)-ethylenediamine (TPEN), nitrendipine, tolbutamide, tetraethylammonium, N-ethylmaleimide (NEM), iodoacetamide, diethioerythritol (DTE), azide, menadione, poly-L-lysine, annexin V-FITC, and propidium iodide from Sigma. 4-Hydroxynonenal (HNE) was from Cayman Chemical Co. ZnSO$_4$ was used in all experiments.

**$\alpha$-TC6 Cells—$\alpha$-TC6 ($\alpha$-TC) glucagon-producing cell line was kindly provided by Dr. Y. Moriyama, Okayama University, Japan. Cells were cultured in Dulbecco’s modified Eagle’s medium with 25 mM glucose, 10% bovine serum, 100 µg/ml penicillin, 100 µg/ml streptomycin, at 37°C and 5% CO$_2$, 95% air. Culture medium was changed every 48 h, and cells were grown in monolayer to 80–90% confluence. The cells were plated onto glass coverslips for fluorescence measurements. Experiments with cells were performed typically 2–3 days after plating.

**Dispersed Islet Cells from Mouse Insulin Promoter-Green Fluorescent Protein (MIP-GFP) Mice**—To identify non-$\beta$ cells we used MIP-GFP mice (CD1 background), in which GFP is specifically expressed in the islet $\beta$-cells. MIP-GFP mice were a gift from Dr. M. Hara, University of Chicago, Chicago, IL (41). Islets were isolated as described previously (32). To obtain dispersed cells, isolated islets were incubated for 10 min in Ca$^{2+}$-free phosphate-buffered solution supplemented with 2 mM EGTA, 3 mM glucose, 100 units/ml penicillin, and 100 µg/ml streptomycin. Islets then were centrifuged and incubated with dispase II, followed by addition of RPMI 1640 medium with 11.1 mM glucose, 10% bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10 mM HEPES, pH 7.4. The suspension was centrifuged, and the pellet was resuspended in the same medium. The cells were plated on 22-mm glass coverslips coated with poly-L-lysine and maintained for 1–3 days at 37°C and 5% CO$_2$, 95% air. We selected GFP-negative (non-green) cells for experiments.

**Glucagon Secretion Assay—$\alpha$-TC cells were plated onto 24-well plates and cultured for 48 h in Dulbecco’s modified Eagle’s medium with 1-glutamine supplemented with serum, streptomycin, and penicillin at 37°C. Culture medium was aspirated, and cells were preincubated for 15 min with buffer containing (mM) 130 NaCl, 5 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 5 NaHCO$_3$, 10 HEPES, pH 7.4, supplemented with 20 mM glucose. Buffer was then aspirated, and cells were incubated in 0.5 ml of buffer with 1 or 20 mM glucose with or without different concentra-
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Measurements of Cells Simultaneously Stained with FluoZin-3 AM, MitoTracker Red CMXRos, and Hoechst 33342—α-TC cells were loaded with 2 μM FluoZin-3 AM for 30 min in incubation buffer in the presence of 2 mM glucose at 37 °C and 5% CO₂, 95% air. Then 1 μM of mitochondria-staining dye MitoTracker Red and 2 μM of DNA-specific dye Hoechst 33342 were added, and cells were incubated for 20 min. Cells were washed in incubation buffer, transferred to the chamber on the microscope stage, and maintained at 36–37 °C. Excitation wavelengths for MitoTracker Red and Hoechst 33342 were 540 and 350 nm, respectively, and emission was measured using 660-nm band pass filter and 550-nm beam splitter for MitoTracker Red and 465-nm band pass filter and 400-nm beam splitter for Hoechst 33342.

Confocal Fluorescent Measurements—Confocal imaging was performed using a Zeiss LSM510 laser scanning microscope. Coverslips with α-TC cells co-loaded with FluoZin-3 AM (2 μM) and MitoTracker Red (500 nM) or FluoZin-3 AM and endoplasmic reticulum (ER)-staining dye FM 4-64 (2 μM) were transferred into the chamber on the stage of the microscope fitted with a 40 × 0.75 water immersion objective. For FluoZin-3 AM and MitoTracker Red, as well as for FluoZin-3 AM and FM 4-64 excitations, the 488- and 514-nm argon laser lines were used, and emissions were acquired using FITC and TRITC set of filters, respectively. The images were analyzed using Zeiss LSM Image software.

Measurements of NAD(P)H Fluorescence—Changes in NAD(P)H redox state of α-TC were observed at perfusion of cells with incubation buffer in the presence of 1 mM glucose at 37 °C. The excitation wavelength for NAD(P)H autofluorescence was 360 nm, and emission was measured using 465-nm band pass filter and 400-nm beam splitter. To assess the maximal signal (maximal NAD(P)H reduction) in cells, the mitochondrial electron transport chain was inhibited by azide.

Assessment of α-TC Cells Apoptosis and Necrosis—For apoptosis and necrosis measurements, cells plated on glass coverslips were incubated at 37 °C during 1 h with different concentrations of Zn²⁺ in incubation buffer containing 1 mM glucose. Then coverslips were simultaneously incubated with annexin V-FITC (0.4 μg/ml) and propidium iodide (PI, 1 μM) for 20 min at room temperature in darkness in incubation buffer with 1 mM glucose. Coverslips were washed in the same buffer, transferred to an open chamber on the microscope stage, and maintained at 36–37 °C. The fluorescence of annexin V-FITC was excited at 480 nm and emission measured with 525-nm band pass filter using a 505-nm beam splitter. The PI fluorescence was excited at 540 nm and emission measured with a 660-nm band pass filter using a 550-nm beam splitter. Percent of apoptosis and necrosis was calculated as the ratio of annexin V or PI-positive cells to all cells in the field of view. For these experiments ~400–500 cells in the field of view were analyzed per coverslip.

ZIP and ZnT mRNA Expression Analysis in α-TC6 Cells—Total RNA was isolated from α-TC cells, mouse islets (FVB background), and whole mouse brain using TRIzol Reagent (Invitrogen) according to the manufacturer’s instruction. The extracted total RNA was treated with rDNase I (Ambion, Houston, TX). One μg of the isolated RNA was reverse-transcribed
using Moloney murine leukemia virus reverse transcriptase according to the manufacturer’s instructions (Invitrogen). The resulting cDNA was used for amplification in quantitative real time PCR (qPCR). qPCR was performed as described previously (32). Primers were designed using Primer Express version 2.0 software (Applied Biosystems, Foster City, CA). Primer sequences are indicated in supplemental Table 1. 10 ng of α-TC6 cDNA per well was used as the template for amplification. The real time PCR protocol employed was as follows: heat activation of polymerase at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s. Readings were carried out on an ABI Prism® 7900HT Sequence Detection System (Applied Biosystems) and compared against a standard curve created from mouse genomic DNA by serial dilutions. Data were normalized to mouse 32% higher than at 20 mM glucose (Fig. 1A).

RESULTS

Effect of Zn2+ on Glucagon Secretion—Pancreatic α-cells are exposed to a large amount of Zn2+ when insulin is secreted from β-cells. To test the effect of Zn2+ on glucagon secretion in α-TC cells, we incubated α-TC cells with 1 or 20 mM glucose in the presence of 5, 10, and 20 μM Zn2+ (see “Materials and Methods”). As seen in Fig. 1A, at low glucose, glucagon secretion was decreased by 26.2% (p < 0.05, n = 9), 50.3% (p < 0.005, n = 9), and by 29.4% (p < 0.01, n = 9) with 5, 10, and 20 μM Zn2+, respectively. At high glucose conditions, glucagon secretion was reduced by 45.5% (p < 0.01, n = 9), by 58.3% (p < 0.005, n = 9), and by 36.6% (p < 0.05, n = 9) with 5, 10, and 20 μM Zn2+, respectively. Glucagon secretion at 1 mM glucose was 32% higher than at 20 mM glucose (p < 0.01, n = 9) (Fig. 1A).

Similar changes were observed in isolated mouse islets (Fig. 1B). This result is in agreement with the role of glucose as one of the physiological inhibitors of glucagon secretion. As seen in Fig. 1 in α-TC cells, the inhibitory effect of 10 μM Zn2+ on glucagon secretion is greater than that of 20 μM Zn2+. Experiments using an expanded concentration range showed that a further increase in Zn2+ concentration led to a further decline of the inhibition (supplemental Fig. 1A). To investigate the possible effect of Zn2+ treatment on apoptosis or necrosis, we incubated α-TC cells for 1 h with buffer supplemented with 1 mM glucose in the presence of various Zn2+ concentrations and measured cell viability (supplemental Fig. 1B). The analysis of fluorescence intensity of annexin V and PI showed that compared with control, treatment with 10 μM Zn2+ had no effect on the necrotic processes, whereas at 20, 50, and 100 μM Zn2+ necrosis was significantly increased (supplemental Fig. 1B). None of the Zn2+ concentrations used had any significant effect on the degree of apoptosis (not shown). Based on these results, it is likely that the mechanism underlying the α-TC secretory response to high concentrations of Zn2+ involves necrosis. We hypothesize that the increased glucagon secretion (compared with 10 μM Zn2+) in the presence of 20, 50, or 100 μM Zn2+ could be explained by an interplay between a direct inhibitory effect of Zn2+ and injury/partial destruction of cells resulting in the release of intracellular glucagon.

Fluorescent Visualization of Zn2+ Accumulation in α-TC Cells—Our previous results showed the partial co-localization of FluoZin-3 and a mitochondrial marker in β-cells (32). In this study α-TC cells stained simultaneously with FluoZin-3 AM and the mitochondrial marker dye MitoTracker Red (Fig. 2A, B).
Regulation of Zn$^{2+}$ Transport in $\alpha$-Cells

**A**

Fluorescent visualization in $\alpha$-TC cells. A, detection of Zn$^{2+}$ in $\alpha$-TC cell mitochondria (panels i) and ER (panels ii) using confocal microscopy. Coverslips with cells were simultaneously loaded with 2 $\mu$M FluoZin-3 AM and 500 nM MitoTracker Red (panels i) or with 2 $\mu$M FluoZin-3 AM and 2 $\mu$M FM4-64 (panels ii) (see "Materials and Methods") and then washed twice without dyes and transferred to the open chamber with incubation medium with 1 mM glucose at room temperature. B and C, Zn$^{2+}$ detection in $\alpha$-TC cells at low (B) and high (C) glucose conditions using conventional microscopy. Coverslips with cells were simultaneously loaded with 3 $\mu$M FluoZin-3 AM, 1 $\mu$M MitoTracker Red, and 2 $\mu$M Hoechst 33342, then washed twice without dyes, and transferred to the open chamber with incubation medium at 37 °C. B, corresponding DIC, fluorescent and merged images at 1 mM glucose concentration before and after 15 min of exposure to 10 $\mu$M Zn$^{2+}$. C, corresponding DIC, fluorescent and merged images at 20 mM glucose concentration before and after 15 min of exposure to 10 $\mu$M Zn$^{2+}$. The corresponding line profiles of FluoZin-3 fluorescence intensity along the line drawn in DIC images at 1 mM (B) and 20 mM (C) glucose conditions before and after 10 $\mu$M Zn$^{2+}$ addition are shown in the right panels. Fluorescence was normalized to background (100%).

**B**

+10 $\mu$M Zn$^{2+}$

1 mM glucose

**C**

+10 $\mu$M Zn$^{2+}$

20 mM glucose

FIGURE 2. Fluorescent Zn$^{2+}$ visualization in $\alpha$-TC cells. A, detection of Zn$^{2+}$ in $\alpha$-TC cell mitochondria (panels i) and ER (panels ii) using confocal microscopy. Coverslips with cells were simultaneously loaded with 2 $\mu$M FluoZin-3 AM and 500 nM MitoTracker Red (panels i) or with 2 $\mu$M FluoZin-3 AM and 2 $\mu$M FM4-64 (panels ii) (see "Materials and Methods") and then washed twice without dyes and transferred to the open chamber with incubation medium with 1 mM glucose at room temperature. B and C, Zn$^{2+}$ detection in $\alpha$-TC cells at low (B) and high (C) glucose conditions using conventional microscopy. Coverslips with cells were simultaneously loaded with 3 $\mu$M FluoZin-3 AM, 1 $\mu$M MitoTracker Red, and 2 $\mu$M Hoechst 33342, then washed twice without dyes, and transferred to the open chamber with incubation medium at 37 °C. B, corresponding DIC, fluorescent and merged images at 1 mM glucose concentration before and after 15 min of exposure to 10 $\mu$M Zn$^{2+}$. C, corresponding DIC, fluorescent and merged images at 20 mM glucose concentration before and after 15 min of exposure to 10 $\mu$M Zn$^{2+}$. The corresponding line profiles of FluoZin-3 fluorescence intensity along the line drawn in DIC images at 1 mM (B) and 20 mM (C) glucose conditions before and after 10 $\mu$M Zn$^{2+}$ addition are shown in the right panels. Fluorescence was normalized to background (100%).
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In glucose concentration from low to high did not have any significant effect on the rate of Zn\(^{2+}\) accumulation. Switching from high to low glucose (Fig. 3C) also had little effect on the rate of Zn\(^{2+}\) influx.

Depolarization of \(\alpha\)-TC cells with KCl resulted in an increase of the Zn\(^{2+}\) accumulation rate. Fig. 3D and pie chart i demonstrated the effect of KCl on the rate of Zn\(^{2+}\) influx following low glucose treatment. In \(\alpha\)-TC cells (75 cells in four independent experiments), the increase in the rate of Zn\(^{2+}\) accumulation because of perfusion with KCl was observed in 57 cells (76%). In 14 cells (18.7%) KCl did not change the rate of Zn\(^{2+}\) accumulation, and in 4 cells (5.3%) treatment led to a decrease. As seen in Fig. 3B, treatment with KCl following high glucose also resulted in increased Zn\(^{2+}\) influx in \(\alpha\)-TC cells. In 50 investigated cells (four independent experiments), we observed an increased rate of Zn\(^{2+}\) accumulation in 32 cells (64%), no effect in 14 cells (28%), and a decrease in 4 cells (8%). Fig. 3B, pie chart ii, illustrates these data.

To determine whether the conditions used above resulted in changes of \(\Delta \Psi_{p}\) in \(\alpha\)-cell, we used the potential-sensitive anionic dye DiBAC\(_{4}\). The initial level of DiBAC\(_{4}\) fluorescence was monitored at low glucose conditions, and stimulation with high glucose led to some decrease in DiBAC\(_{4}\) fluorescence (hyperpolarization) (Fig. 3E). Further perfusion with 30 mM KCl resulted in a fluorescence increase, reflecting depolarization of these cells (Fig. 3E). It is of note that fast increases in intracellular Zn\(^{2+}\) because of treatment with Zn\(^{2+}\)/pyrithione also caused depolarization of both the plasma and mitochondrial membrane (Fig. 3G). Perfusion with FCCP caused more complete depolarization of mitochondria (Fig. 3G).

Additionally, we performed a similar study in primary \(\alpha\)-cells to look at the Zn\(^{2+}\) transporting characteristics. It is known that pancreatic islets contain four types of cells (\(\alpha\)-, \(\beta\)-, \(\delta\)-, and PP-cells), and the dominant are \(\beta\)-, \(\alpha\)-, and \(\delta\)-cells with following distribution in rodents: \(\beta\)-cells, 75–80%; \(\alpha\)-cells, 10–18%, and \(\delta\)-cells, 5–10% (54–58). We used dispersed islet cells isolated from MIP-GFP mice, where \(\beta\)-cells are GFP-positive. In our study the smallest GFP-negative dispersed islet cells in each field of view were chosen for fluorescent measurements, and the responses of these cells were evaluated. We took into account that two types (\(\alpha\)- and \(\delta\)-cells) of GFP-negative cells are possible and that the mean diameter of \(\alpha\)-cells (10.6 \(\mu\)m) is smaller than \(\delta\)-cells (11.8 \(\mu\)m) (59, 60). Fig. 4 shows representative experiments reflecting the rate of Zn\(^{2+}\) accumulation in primary \(\alpha\)-cells both at low and high glucose conditions, as well as at treatment with KCl. A depolarizing concentration of KCl changed the rate of Zn\(^{2+}\) accumulation in most cells. In 17 investigated GFP-negative cells (five independent experiments) treated with KCl following low glucose incubation, we observed an increased rate of Zn\(^{2+}\) accumulation in 11 cells (64.7%), no effect in 5 cells (29.4%), and decreased Zn\(^{2+}\) influx in 1 cell (5.9%) (Fig. 4A). In 12 investigated GFP-negative cells (three independent experiments) treated with KCl following high glucose, increased rate of Zn\(^{2+}\) accumulation was observed in 10 cells (83.3%), no effect in 1 cell (8.3%), and decreased Zn\(^{2+}\) influx in 1 cell (8.3%) (Fig. 4B). Pie charts in Fig. 4 indicate the percentage of cells responding to KCl. Similar to our observations in \(\alpha\)-TC cells, switching from high to low glucose did not
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**FIGURE 3.** Time-dependent kinetics of Zn\(^{2+}\) accumulation, plasma membrane (\(\Delta\Psi_m\)) and mitochondrial membrane (\(\Delta\Psi_m\)) potential changes in \(\alpha\)-TC cells. A, kinetics of Zn\(^{2+}\) uptake in \(\alpha\)-TC cells. The averaged trace \(\pm\) S.E. of FluoZin-3 AM loaded cells from randomly chosen separately delineated cells (\(n = 17\)) of the same experiment is presented. Inset shows all traces. As a control Zn\(^{2+}\) ionophore pyrithione and a membrane-permeable Zn\(^{2+}\) chelator TPEN were added. As a base line (100%) here and in subsequent figures, the mean level of fluorescence during the initial 100 s was taken. In right panel the corresponding DIC and fluorescent images of cells at different times of the experiment are shown. B, kinetics of Zn\(^{2+}\) transport in \(\alpha\)-TC cells after switching from low (1 mM) to high (20 mM) glucose. C, kinetics of Zn\(^{2+}\) transport in \(\alpha\)-TC cells after switching from high (20 mM) to low (1 mM) glucose. D, kinetics of Zn\(^{2+}\) transport in \(\alpha\)-TC cells after depolarization with KCl followed by low glucose perfusion. Dashed lines show the slopes of FluoZin-3 fluorescence changes, which reflect the relative rate of Zn\(^{2+}\) accumulation in cells. Pie charts show the percent of \(\alpha\)-TC cells that responded to KCl treatment followed by low (panel i) or high (panel ii) glucose perfusion (see text). E, changes of \(\Delta\Psi_m\) in \(\alpha\)-TC cells at low and high glucose conditions. At the end of the experiment KCl was added for complete plasma membrane depolarization. F, effect of pyrithione-induced fast Zn\(^{2+}\) influx into \(\alpha\)-TC cells on \(\Delta\Psi_m\). G, effect of pyrithione-induced fast Zn\(^{2+}\) influx into \(\alpha\)-TC cells on \(\Delta\Psi_m\). At the end of experiment FCCP was added for mitochondrial depolarization. The averaged traces \(\pm\) S.E. of \(n = 16\) (B), 12 (C), 21 (D), 12 (E), 19 (F), and 15 (G) cells are shown. In all cases the traces are representative of at least three independent experiments. Bars indicated perfusion with corresponding reagents.

Change the rate of Zn\(^{2+}\) accumulation (Fig. 4C). Because the mean diameter of \(\alpha\)- and \(\delta\)-cells is distinguished only by a 10% difference, one is never completely sure that all traces presented in Fig. 4 belong to \(\alpha\)-cells. However, taking into account that the average abundance of \(\alpha\)-cells (10–18%) is significantly higher than that of \(\delta\)-cells (5–10%), we can assume that the majority of traces presented in Fig. 4 reflect the \(\alpha\)-cell responses.

**Effect of \(K_{\text{ATP}}\) Channel and Selective and Nonselective Ca\(^{2+}\) Channels Inhibitors on Zn\(^{2+}\) Accumulation**—Previously it was shown that a \(K_{\text{ATP}}\) channel antagonist increased and L-VGCC inhibitors decreased Zn\(^{2+}\) influx in \(\beta\)-cells under high glucose- and KCl-depolarized conditions (32). In contrast to \(\beta\)-cells the \(K_{\text{ATP}}\) channel inhibitor tolbutamide had no measurable effect on the rate of Zn\(^{2+}\) influx, despite tolbutamide-induced depolarization of \(\alpha\)-cells both at low and high glucose (not shown). We also did not observe any visible inhibitory effect of nitrendipine, a dihydropyridine L-VGCC inhibitor, on the rate of Zn\(^{2+}\) influx in \(\alpha\)-TC cells either at low or high glucose (not shown).

To verify whether other types of Ca\(^{2+}\) channels could be involved in Zn\(^{2+}\) transport across the plasma membrane, we treated cells with the nonselective Ca\(^{2+}\) channel blocker, Gd\(^{3+}\) partially blocked Zn\(^{2+}\) entry under both low (Fig. 5A) and high glucose (Fig. 5B) conditions (by 25 ± 3%, \(n = 3\), \(p < 0.05\) and 24.6 ± 6.4% \(n = 4\), \(p < 0.05\), respectively). Nitrendipine did not significantly attenuate Zn\(^{2+}\) influx in KCl-depolarized \(\alpha\)-TC cells (16.5 ± 7.2%, \(n = 3\), \(p = 0.15\); Fig. 5C). However, an inhibitory effect of Gd\(^{3+}\) on Zn\(^{2+}\) accumulation in KCl-treated cells was observed (23 ± 2.5%, \(n = 4\); \(p < 0.05\)) (Fig. 5D). These findings indicated that Zn\(^{2+}\) transport in \(\alpha\)-cells under physiological conditions is not associated with L-VGCC but rather with other types of Ca\(^{2+}\) channels. Based on experiments with several organic Ca\(^{2+}\) channel antagonists, it is suggested that the effects of...
Gd$^{3+}$ on Zn$^{2+}$ channels in cortical neurons may be direct and inhibitory in nature (61). Gd$^{3+}$ has been shown to inhibit Zn$^{2+}$ transport and Zn$^{2+}$-induced depolarization in kidney cells, and the inhibition does not depend on the activation of Cl$^{-}$ or Na$^{+}$ channels (62). Thus, in this study, it cannot be ruled out that Gd$^{3+}$ directly affects the Zn$^{2+}$ transport pathway(s). It is of note that in our study, H9251-TC cells can exhibit spontaneous oscillations of intracellular Ca$^{2+}$ ([Ca$^{2+}$]$_{i}$) both at low and high glucose. Treatment with Gd$^{3+}$ and nitrendipine caused the inhibition of spontaneous Ca$^{2+}$ oscillations (not shown), which indicated the presence of L-VGCC and other types of VGCCs.

Expression of ZIP and ZnT Family of Zinc Transporters in H9251-TC6 Cells, Mouse Islets, and Mouse Brain—Our observations illustrated that Gd$^{3+}$ partially inhibited Zn$^{2+}$ influx both at low and high glucose (Fig. 5, A and B), suggesting other possible Zn$^{2+}$ transport mechanisms are active, including plasma membrane Zn$^{2+}$ transporters. To begin to address this possibility, we used qPCR to evaluate the expression of zinc influx (Zip) and efflux (ZnT) transporter genes in the α-TC cells. We examined the expression level of the KATP channel Kir6.2 subunit (KCNJ11), which is present in α-TC cells (3, 37), for comparison/reference. Zip1, Zip3, Zip10, and Zip14, as well as ZnT1, ZnT4, ZnT5, ZnT6, ZnT7, and ZnT8 transcripts are expressed in α-TC cells (Fig. 6A). Of all the transporter genes expressed, Zip1 and Zip14 were the most abundant influx transporters, and ZnT4, ZnT5, and ZnT8 were the dominant efflux transporters (Fig. 6A). We also investigated the level of Zip and ZnT transporter genes in mouse islets (Fig. 6B). These data indicated the presence of several transporter genes where the predominant efflux transporters were ZnT5 and ZnT8 (Fig. 6B). Our observation of the expression of ZnT8 gene in mouse islets was in line with recent studies, which showed the localization of ZnT8 in β-cell secretory vesicle membranes (29, 63); however, our results suggest that this gene is also expressed in the α-cell. Furthermore, our immunohistochemical data showed expression of ZnT8 protein in both insulin and glucagon positive dispersed mouse islet cells (Fig. 6D). Mouse brain was used as a positive control for expression of both Zip and ZnT transporter genes (Fig. 6C).

Effect of Zn$^{2+}$ on K$\text{ATP}$ Currents in α-Cells—To determine whether Zn$^{2+}$ was inhibiting glucagon secretion via an effect on
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The K\(_{ATP}\) channel, we examined whether extracellularly applied Zn\(^{2+}\) had any effect on K\(_{ATP}\) channel current density in mouse islet \(\alpha\)-cells. Because capacitance of cells is proportional to the size of cells, \(\alpha\)-cells were identified by their small size (capacitance \(<4\) pF) and the presence of Na\(^{+}\) current when depolarized from a holding potential of \(-70\) mV (6). K\(_{ATP}\) current measured in these cells was very small under the recording conditions applied, and 10 \(\mu\)M Zn\(^{2+}\) was found to have no significant effect on the current density (Fig. 7). As was mentioned above, in these experiments we anticipate that the majority of cells recorded would be \(\alpha\)-cells.

**Mobilization of Intracellular Zn\(^{2+}\) Pool and Zn\(^{2+}\) Accumulation After Treatment with \(-\text{SH} Group\) Modifiers**—It is known that excess Zn\(^{2+}\) in cells is buffered by binding to cysteine, histidine, or glutamate residues of metallothioneins, one of the major intracellular Zn\(^{2+}\) stores in most of cells (64). To examine the intracellular protein-bound Zn\(^{2+}\) pool, we treated cells with a variety of compounds that affect Zn\(^{2+}\) binding by causing oxidative/nitrosative stress or by chemical modification of \(-\text{SH} \) groups in the proteins.

The redox status of the cells is known to play a vital role in the regulation of different cellular processes. We investigated the effect of oxidative modification of \(-\text{SH} \) groups on the change of the intracellular Zn\(^{2+}\) profile. A small amount (10 \(\mu\)M) of \(-\text{SH} \) group oxidant DTDP led to a significant increase of the FluoZin-3 fluorescence level, indicating elevation of free intracellular Zn\(^{2+}\) in \(\alpha\)-TC cells (Fig. 8A) or in GFP-negative dispersed mouse islet cells (supplemental Fig. 2A). Other oxidizing compounds menadione (generator of superoxide) (Fig. 8B) or SIN-1 (generator of peroxynitrite by simultaneous production of nitric oxide and superoxide) (Fig. 8C) also elevated FluoZin-3 fluorescence, further supporting mobilization of intracellular Zn\(^{2+}\) by redox state. Products of lipid peroxidation can trigger multiple signaling cascades. HNE, a reactive aldehyde product of lipid peroxidation, which itself increases reactive oxygen species production, can form adducts with proteins containing histidine, cysteine, or lysine residues (65, 66). As seen in Fig. 8D, treatment of \(\alpha\)-cells with HNE also led to mobilization of intracellular Zn\(^{2+}\). The cells treated with the above oxidizing reagents were loaded with cell-impermeable dye PI, and no fluorescence increase was detected (not shown), which indicated the maintenance of cell membrane integrity during the time of experiments.

NAD(P)H autofluorescence was used to monitor the changes in cellular redox state following oxidant treatment. We found that perfusion of \(\alpha\)-TC cells with 20 \(\mu\)M DTDP led to a drop in NAD(P)H fluorescence, indicating the oxidation of pyridine nucleotides (not shown). NEM, a sulfhydryl alkylating agent that covalently modifies cysteine residues in proteins, also significantly increased intracellular Zn\(^{2+}\) mobilization in both \(\alpha\)-TC cells (Fig. 8E) and GFP-negative dispersed mouse islet cells (supplemental Fig. 2B). Similarly, iodoacetamide, another alkylating agent for cysteine and histidine residues in proteins, also mobilized intracellular Zn\(^{2+}\) (Fig. 8F).

The comparison of Fig. 8, B and C with E and F, shows the difference in the maximal values of FluoZin-3 fluorescence after treatment with various compounds. This diversity reflects the ability of compounds used to extract Zn\(^{2+}\) from intracellular Zn\(^{2+}\) stores, as well as the duration of treatment.

Our data suggest that these reagents extracted Zn\(^{2+}\) from Zn\(^{2+}\)-containing metallothioneins or other possible Zn\(^{2+}\) stores. Because this extractable Zn\(^{2+}\) reflects a pool of total Zn\(^{2+}\) present in the cells, these results demonstrated a significant amount of bound Zn\(^{2+}\) in this cell type. Thus, bound Zn\(^{2+}\) reflects another significant pool that is highly modulated by redox state and potentially oxidative stress. To further examine this, we investigated whether the removal of oxidants or alkylating agents as well as a shift in redox state to more reduced conditions resulted in a decrease in [Zn\(^{2+}\)\_]. As seen in Fig. 8G, the DTDP-stimulated increase in [Zn\(^{2+}\)\_], was reversible. The fast increase in [Zn\(^{2+}\)\_] was observed (Fig. 8G) if cells were
perfused with 20 μM DTDP. The removal of the −SH group oxidant from the perfusion medium led to restoration of the initial FluoZin-3 fluorescence during ~10 min (Fig. 8G). Similar effects were observed during the treatment of α-TC cells with or SIN-1 (not shown). Removal of the −SH group modifier (NEM, 15 μM) resulted in a decrease in the rate of [Zn²⁺] accumulation but not to the restoration of the initial fluorescence level (not shown), possibly indicating the higher affinity of NEM to its target. We also used the thiol-reducing agent DTE to study the effect of −SH group reduction on [Zn²⁺]. Experiments showed that perfusion of α-TC (Fig. 8H) with DTE (100 μM) following exposure to DTDP (20 μM) led to the dramatic
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![Diagram of Zn\(^{2+}\) Transport in α-Cells](image)

**FIGURE 7. Effect of Zn\(^{2+}\) on K\(_{ATP}\) current in mouse islet α-cells.** Extracellularly applied Zn\(^{2+}\) (10 μM) had no effect on K\(_{ATP}\) current density. Results represent the average ± S.E. of 9 cells.

during depolarization of cells with KCl (Fig. 5D). These findings indicate that Zn\(^{2+}\) is transported into α-cells in part through dihydropyridine-insensitive Ca\(^{2+}\) channels. Four types of VGCC (L-, T-, N-, and R-type) and tetrodotoxin-sensitive voltage-gated Na\(^{+}\)-channels have been observed in α-cells and thus could be involved in Zn\(^{2+}\) transport (3, 37). Further studies are required to examine if other ion channels are involved.

As stated, the mechanism of the inhibition of glucagon release by Zn\(^{2+}\) is unclear. It was suggested that Zn\(^{2+}\) inhibits glucagon secretion in rat α-cells because of activation of the K\(_{ATP}\) channel resulting in hyperpolarization of cells (12). However, the activation of the K\(_{ATP}\) channel by Zn\(^{2+}\) was not found in mouse α-cells (70). In these studies both groups used different protocols for measuring K\(_{ATP}\) channel modulation. Our results are in agreement with previous findings in mouse; we see no effect of Zn\(^{2+}\) on α-cell K\(_{ATP}\) current, suggesting that under the conditions studied this mechanism is not involved in Zn\(^{2+}\) modulation of glucagon secretion. The inhibition of secretion therefore appears to require transport as part of the mechanism (Fig. 7).

In addition to ion channels, another possible pathway of Zn\(^{2+}\) transport is through plasma membrane Zn\(^{2+}\) transporter(s). Although there are some data on Zn\(^{2+}\) influx transporters in the pancreas, information about Zip transporters in α- and β-cells is limited. Zip1 transcripts were previously found in human (71), but not mouse, pancreas (72). The expression of Zip5 mRNA in the whole mouse pancreas was also observed (73, 74). Our previous work using quantitative real time PCR demonstrates a number of Zip transcripts in mouse insulinoma β-cells (MIN6), mouse islets, and mouse brain (32). In this work we have identified in glucagon-producing α-TC cells the expression of a number of genes of the ZIP and ZnT families (Fig. 6A). The most abundantly expressed Zip transporter genes in α-TC cells were Zip1, Zip10, and Zip14 (Fig. 6A). The expression profile of Zip transporters in mouse islets (Fig. 6B) and mouse brain (Fig. 6C) mainly confirms our previous results (32).

Unlike Zip transporters, the Zn\(^{2+}\) efflux transporters (ZnT) in pancreatic β-cells are better characterized. Among the ZnT family of transporters, the ZnT8 was recently detected in insulin-secreting INS-1 cells and human pancreatic islets and was co-localized with insulin in these cells, suggesting they are in vesicular membranes (29, 63). Our results identified the expression of ZnT1, ZnT4, ZnT5, ZnT6, ZnT7, and ZnT8 transporter genes in α-TC cells (Fig. 6A) and mouse islets (Fig. 6B), with ZnT4, ZnT5, and ZnT8 showing the highest level of expression in α-TC cells and ZnT5 and ZnT8 in islets. The high level of ZnT5 in α-TC cells (Fig. 6A) and islets (Fig. 6B) is in agreement with a previous report, in which ZnT5 was found to be abundantly expressed in pancreas (75). Our data demonstrated that ZnT8 was not exclusively a β-cell Zn\(^{2+}\) transporter. It is noteworthy that others have shown that ZnT8 was also expressed in adipose tissue with comparable abundance as ZnT1, ZnT5, and ZnT7 (76). The role and localization of ZnT8 in α-TC cells are not clear. As stated above, in β-cells ZnT8 is thought to be localized to the insulin secretory granule, and overexpression studies in β-cells suggests it functions to modulate [Zn\(^{2+}\)]i and regulate (enhance) insulin secretion (29, 63). It is important to

**DISCUSSION**

In pancreatic β-cells Zn\(^{2+}\) is co-secreted with insulin so it is anticipated that islet cells are exposed to high levels of Zn\(^{2+}\) especially under high glucose conditions. It is possible that α-cells possess an effective Zn\(^{2+}\) influx system that in part regulates glucagon secretion as well as other important cellular processes. α-Cells also likely have an effective efflux system to protect from excess Zn\(^{2+}\) influx, because high levels of Zn\(^{2+}\) lead to cell damage and death (34, 36). Thus Zn\(^{2+}\) likely influences α-cell function at many different levels requiring an effective means to maintain Zn\(^{2+}\) homeostasis. The [Zn\(^{2+}\)]i, in cells may be determined by a number of mechanisms as follows: Zn\(^{2+}\) accumulation through the Ca\(^{2+}\) channels, ZIP plasma membrane import transporter(s), Zn\(^{2+}\) efflux catalyzed by ZnT export transporter(s), and redox state of cells. Our data suggest all four modes of regulation are possible in the α-cell. A defect in any one of these mechanisms may result in changes of [Zn\(^{2+}\)]i, leading to α-cell dysfunction.

The effects of glucose and Zn\(^{2+}\) on glucagon secretion remain uncertain at the present time. In intact rat and mouse islets increased glucose decreases glucagon release (15, 67, 68). Contrary to this, glucose stimulates glucagon release in isolated rat pancreatic α-cells (13). In glucagonoma cell lines InR1-G9 (69) and α-TC1-9 (15), an increase in the glucose concentration led to a decrease in glucagon secretion. Our results in this study showed that glucose caused the inhibition of glucagon secretion (Fig. 1) in both an α-cell line and isolated islets. However, Zn\(^{2+}\) reduced glucagon secretion both at low and high glucose conditions suggesting its effects are not entirely glucose-dependent (Fig. 1). Our experiments also demonstrated that Zn\(^{2+}\) accumulated in α-cells both at low and high glucose conditions (Fig. 2, B and C, Fig. 3, B and C, and Fig. 4, A and B). Unlike β-cells (32), treatment with tolbutamide or nitrendipine did not have any visible effect on the rate of Zn\(^{2+}\) accumulation under the conditions studied. The nonselective Ca\(^{2+}\) channel blocker Gd\(^{3+}\) moderately attenuated Zn\(^{2+}\) accumulation both at low and high glucose conditions (Fig. 5, A and B), as well as
reiterate that polymorphisms in the ZnT8 gene link ZnT8 transporter to T2D (26, 29), and this pathological association may involve effects on both α- and β-cell function. It will be important to determine the compartmental distribution of α-cell ZnTs in future studies and further assess their function in islet cells.

The reversible intracellular release of Zn\(^{2+}\) in response to redox changes (Fig. 8, A–G) indicated that a significant level of Zn\(^{2+}\) is bound to metallothioneins or other Zn\(^{2+}\) stores. Although oxidation of thiol groups released Zn\(^{2+}\), their reduction or the removal of oxidizing agents restored the Zn\(^{2+}\) binding capacity of metallothioneins or other Zn\(^{2+}\) stores (Fig. 8, G and H), thus modulating [Zn\(^{2+}\)]. However, these experiments do not exclude the participation of Zn\(^{2+}\) efflux transporters in restoration of [Zn\(^{2+}\)] with respect to changes in redox, because both ZIP and ZnT contain histidine-rich motifs in loop domains located in the cytoplasm (17, 20). Therefore, α-cell [Zn\(^{2+}\)], may be modulated by redox state through effects on histidine/cysteine residues of metallothioneins and specific Zn\(^{2+}\) transporters.

Recently, a vicious cycle of Zn\(^{2+}\)-dependent injury was suggested in brain cells (77). This cycle includes oxidative stress-induced Zn\(^{2+}\) release from metallothioneins, followed by mitochondrial uptake of Zn\(^{2+}\) leading to organelle dysfunction and mitochondrial reactive oxygen species generation, which in turn caused further Zn\(^{2+}\) release from metallothioneins. The increase in [Zn\(^{2+}\)], activates cytotoxic mitochondrial Zn\(^{2+}\) sequestration and apoptosis (77). A similar scenario could also take place in islet α-cells in the presence of high glucose or free fatty acid levels, termed glucolipotoxicity, a condition characterized by increased reactive oxygen species production (78–80).

Our experiments showed that a significant increase in [Zn\(^{2+}\)] led to mitochondrial depolarization (Fig. 3G). One could speculate that processes leading to perturbations of Zn\(^{2+}\) homeostasis could be through a disturbance in the function of Zn\(^{2+}\) efflux transporter(s), including ion channels. In this case, a shift to the reduced state of cells, leading to Zn\(^{2+}\)

FIGURE 8. Effect of treatment with -SH group oxidant and blockers on the level of intracellular Zn\(^{2+}\). Increase of intracellular Zn\(^{2+}\) level in α-TC cells after treatment with oxidants (A) DTDP, (B) menadione, (C) SIN-1, (D) HNE, -SH group blockers (E) NEM, and (F) iodoacetamide. G, reversible changes of intracellular Zn\(^{2+}\) after cyclic addition and removal of DTDP. H, effect of treatment with reducing agent DTE on intracellular Zn\(^{2+}\) level. The averaged traces ± S.E. of n = 13 (A), 17 (B), 12 (C), 14 (D), 18 (E), 17 (F), 16 (G), and 14 (H) cells are shown. In all cases the traces are representative of at least three independent experiments.
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binding and a decrease in [Zn$^{2+}$], could decrease the damage caused by excess Zn$^{2+}$ and may have a protective role. Our experiments with the thiol-reducing agent DTE (Fig. 8H), which led to a more reduced intracellular environment and decreased [Zn$^{2+}$], support such a conclusion.

In summary the data presented demonstrate that Zn$^{2+}$ accumulates in glucagon-producing α-cells under low and high glucose conditions through both dihydroxypropyl-insensitive Ca$^{2+}$ channels and other Zn$^{2+}$-transporting mechanisms. [Zn$^{2+}$], in α-cells is therefore likely regulated by Ca$^{2+}$-channels and Zn$^{2+}$ transporters as well as through the redox sensitivity of thiol groups.

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