Oxidized glutathione decreases luminal Ca^{2+} content of the endothelial cell Ins(1,4,5)P_3-sensitive Ca^{2+} store

Philip N. HENSCHKE* and Stephen J. ELLIOTT†

Departments of Pediatrics and Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030, U.S.A.

The model oxidant, t-butyl hydroperoxide (t-buOOH), inhibits Ins(1,4,5)P_3-dependent Ca^{2+} signalling in calf pulmonary artery endothelial cells. Metabolism of t-buOOH within the cytosol is coupled to the oxidation of glutathione. In this study, we investigated whether oxidized glutathione (GSSG) is the intracellular moiety responsible for mediating the effects of t-buOOH on Ca^{2+} signalling. The increase in cytosolic [Ca^{2+}] stimulated by application of 2,5-di-t-butylhydroquinone (BHQ) was used to estimate the luminal Ca^{2+} content of the Ins(1,4,5)P_3-sensitive store in intact cells. Luminal Ca^{2+} content was unaffected by t-buOOH (0.4 mM, 0–3 h) unless intracellular GSSG content was concomitantly elevated. The effect was specific for increased GSSG and was not replicated by depletion of GSH. These results suggest that cytosolic GSSG, produced endogenously within the endothelial cell, decreases the luminal Ca^{2+} content of Ins(1,4,5)P_3-sensitive Ca^{2+} stores. Depletion of internal Ca^{2+} stores by GSSG may represent a key mechanism by which some forms of oxidant stress inhibit signal transduction in vascular tissue. At the plasma membrane, t-buOOH is known to inhibit the capacitative Ca^{2+} influx pathway. Increased intracellular GSSG potentiated the inhibitory effect of t-buOOH on Ca^{2+} influx, thereby providing the first evidence that activity of the capacitative Ca^{2+} influx channel is sensitive to thiol reagents formed endogenously within the cell.

INTRODUCTION

Ins(1,4,5)P_3-stimulated release of Ca^{2+} is regulated in part by the luminal Ca^{2+} content of the Ins(1,4,5)P_3-sensitive store [1,2]. When the store is depleted to less than 30% of steady-state loading levels, sensitivity of the Ins(1,4,5)P_3 receptor increases in proportion to luminal Ca^{2+} content. Furthermore, luminal Ca^{2+} content may regulate the quantal release of Ca^{2+} during stimulation by agonists [3,4]. In addition to modulating Ins(1,4,5)P_3-stimulated Ca^{2+} release, luminal Ca^{2+} also regulates Ca^{2+} permeability at the plasma membrane [5–7]. Depletion of Ins(1,4,5)P_3-sensitive stores activates capacitative Ca^{2+} influx, independent of agonist-stimulated increases in Ins(1,4,5)P_3 [8–12]. Because it regulates both the release of Ca^{2+} from Ins(1,4,5)P_3-sensitive stores and the influx of Ca^{2+} across the plasma membrane, luminal Ca^{2+} appears to play a critically important role in the activation of NO synthase and other Ca^{2+}-dependent signalling systems within non-excitable cells [13].

In endothelial cells, the oxidant t-butyl hydroperoxide (t-buOOH) inhibits the agonist-stimulated release of Ca^{2+} and capacitative Ca^{2+} influx [14,15], two pathways that are regulated by luminal Ca^{2+} content of the Ins(1,4,5)P_3-sensitive store. Metabolism of t-buOOH within the cytosol is coupled to the production of oxidized glutathione (GSSG). Although GSSG oxidizes reduced thiol groups and participates in thiol/disulphide exchange, limited information exists about its physiological effects within intact cells. The biological importance of GSSG rests in the fact that the molecule is produced endogenously during periods of oxidant challenge. Endothelial cells, like most mammalian cells, possess enzymic mechanisms to chemically reduce, export and/or chelate GSSG, suggesting that accumulation of GSSG within the cytosol may be deleterious to cell function. We hypothesized that GSSG might be the intracellular molecular species responsible for the effects of t-buOOH. In the present work we investigate whether GSSG alters the luminal Ca^{2+} content of Ins(1,4,5)P_3-sensitive stores.

We report that increased levels of endogenous GSSG are associated with a decreased luminal Ca^{2+} content of the Ins(1,4,5)P_3-sensitive store. GSSG appears to represent a biologically significant mechanism by which thiol reagents and oxidants inhibit the translocation of Ca^{2+} during stimulation by agonists. Further, we report the first evidence that inhibition of the capacitative Ca^{2+} influx pathway is potentiated in an intracellular redox environment that favours the oxidation of reduced thiol groups.

MATERIALS AND METHODS

Reagents and solutions

Buthionine-[*S,R*]-sulphoximine (BSO), 1-chloro-2,4-dinitrobenzene (CDNB), glutathione reductase, t-buOOH [70% (w/v) aqueous solution], and 5,5′-dithio-bis-2-nitrobenzoic acid were obtained from Sigma Chemical Co. (St. Louis, MO). 2,5-di-t-butylhydroquinone (BHQ) was purchased from Aldrich Chemical Co. (Milwaukee, WI), and fura-2/AM from Molecular Probes (Eugene, OR). 1,3-Bis-(2-chloroethyl)-1-nitrosourea (BCNU) was obtained from Bristol-Myers Squibb Co. (Evansville, IN).

Hepes-buffered solution (HBS) contained: 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM D-glucose, and

Abbreviations used: BCNU, 1,3-bis-(2-chloroethyl)-1-nitrosourea; BHQ, 2,5-di-t-butylhydroquinone; BSO, buthionine-[*S,R*]-sulphoximine; CDNB, 1-chloro-2,4-dinitrobenzene; CPAEs, calf pulmonary artery endothelial cells; HBS, Hepes-buffered solution; t-buOOH, t-butyl hydroperoxide.

* Present address: Royal Women's Hospital, 132 Grattan St., Carlton, Victoria 3053, Australia.

† To whom correspondence should be addressed at Cardiovascular Research Center, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, U.S.A.

15 mM Hepes, pH adjusted to 7.4 at 37 °C with NaOH. Ca^{2+}-free EGTA buffer contained 0.3 mM EGTA and the same salts as HBS without added $CaCl_2$.

Culture of endothelial cells

Calf pulmonary endothelial cells (CPAEs) were cultured in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) supplemented with 15% (w/v) fetal bovine serum (Hyclone, Logan, UT), 100 μg/ml streptomycin, 100 μg/ml penicillin, 100 μg/ml neomycin and 2 mM glutamine (complete-DME). Endothelial identity of the cell line was confirmed by positive uptake of di-iodoacetyl low-density-lipoprotein [16]. Cultures formed contact-inhibited monolayers typical of endothelial cells. Experiments used cells at passage number 13–15 and 1–2 days post-confluency.

Measurement of $[Ca^{2+}]_i$

CPAEs were suspended in 2.3 ml HBS with fura-2/AM (20 μM) for 30 min at 37 °C [14]. After dilution into 40 ml HBS, cells were incubated for an additional 30 min. The suspension was washed and divided into groups. Aliquots (2 ml) were removed as required and washed twice immediately before measurement of fluorescence. Excitation wavelength alternated between 340 and 380 nm during recording of emission fluorescence at 510 nm. All measurements were corrected for autofluorescence by using cells treated as above except for the absence of fura-2. Calibration of the fluorescent signal was determined on each aliquot by sequential addition of Triton X-100 (0.1%) and EGTA (5 mM). $[Ca^{2+}]_i$ was calculated as previously described [17].

Measurement of $^{45}Ca^{2+}$ efflux

After equilibration of cells with $^{45}Ca^{2+}$ for 15–18 h, the medium was aspirated from the dish, and the monolayer washed 3 times with HBS. Cells were allowed to equilibrate for 5 min, at which point (time zero) sampling of the HBS began. BHQ (final concentration 10 μM) or vehicle was added 1 min after time zero. Aliquots were counted by a standard liquid scintillation technique. Efflux rate constants are reported with their respective correlation coefficients obtained from linear regression analysis.

Modulation of glutathione cycle activity

Cells loaded with fura-2 or equilibrated with $^{45}Ca^{2+}$ were incubated with or without BCNU (75 μM; 20 min) or CDNB (40 μM; 20 min), followed by a wash step and incubation with or without t-buOOH (0.4 mM). In all protocols, the duration of t-buOOH treatment was 0.5 h unless otherwise stated. In some experiments, cells were incubated with or without BSO (0.5 mM; 12–15 h) before loading with fura-2 and treatment with or without t-buOOH. Measurements of $[Ca^{2+}]_i$, $^{45}Ca^{2+}$ flux and glutathione content were made at the completion of the second incubation period.

Glutathione content

To determine glutathione content, cells were washed twice, scraped from the culture dish, and suspended in a mixture of perchloric acid (1 M) and EDTA (2 mM) with or without *N*-ethylmaleimide (50 mM). The suspension was acid-precipitated, microcentrifuged at 13000 *g* (2 min) and the supernatant stored at −20 °C until glutathione measurement. GSH and GSSG contents were measured by anion-exchange HPLC coupled to an in-line recycling post-column reaction [18]. Parallel determinations of cell protein content were performed with a commercial assay kit (Bio-Rad, Richmond, CA).

ATP content

ATP content was determined with the firefly luciferase bioluminescence assay (Sigma Chemical Co., St. Louis, MO) and quantified from a linear standard curve relating luminescence intensity to the concentration of ATP. An aliquot of each cell lysate sample was stored at −20 °C for later measurement of protein content.

Data analysis

Data are expressed as means ± S.E.M. Statistical significance was assigned when the probability of α-error was more than 0.05 with Student's two-tailed unpaired *t*-test. Bonferroni corrections were applied for multiple comparisons.

RESULTS

GSH depletion and luminal Ca^{2+} content

CDNB (40 μM; 20 min), a co-substrate for glutathione *S*-transferase, rapidly depleted cells of glutathione to undetectable levels, but had no effect on cell viability, as determined by ATP content (Table 1). Inhibition of glutathione synthesis *de novo* by incubation of cells with BSO (0.5 mM) had a similar but smaller effect on GSH content.

The increase in $[Ca^{2+}]_i$ upon addition of BHQ was used to estimate microsomal luminal Ca^{2+} content [11,12,15]. The BHQ-stimulated increase in $[Ca^{2+}]_i$ in cells depleted of GSH was identical to that observed in control cells (Figure 1a). Next we determined whether depletion of GSH would decrease the filling state of internal Ca^{2+} stores during oxidant stress. We have previously shown that 0.4 mM t-buOOH, during incubations of 3 h, does not alter the luminal Ca^{2+} content of Ins(1,4,5)P_3-sensitive Ca^{2+} stores [15]. This was confirmed in the present study, as indicated by the cumulative data shown in Figure 2. Luminal Ca^{2+} content was unaffected by incubation of cells with both CDNB and t-buOOH (Figure 2a). Similar results were obtained when cells were depleted of GSH by BSO (not shown). Thus the depletion of reducing equivalents supplied by GSH does not lead to a depletion of Ins(1,4,5)P_3-sensitive Ca^{2+} stores during oxidant stress.

Table 1 Glutathione content and cell viability

Cells were incubated in the absence or presence of BSO (0.5 mM), CDNB (40 μM, 20 min) or BCNU (75 μM, 20 min) before incubation with or without t-buOOH (0.4 mM, 30 min). Cell contents of total and oxidized glutathione and ATP were determined as described in the Materials and methods section. All values represent means ± S.E.M. ($n = 7$–14). n.d., not detectable. * $P < 0.001$ with respect to control.

Incubation condition	GSH (nmol GSH equiv. per mg protein)	GSSG (nmol GSH equiv. per mg protein)	ATP (nmol per mg protein)
Control	12.4 ± 1.6	0.08 ± 0.01	55 ± 3
t-buOOH	9.5 ± 3.9	0.12 ± 0.01	52 ± 2
BSO	3.8 ± 1.4	n.d.	53 ± 1
BSO, t-buOOH	3.2 ± 1.2	n.d.	54 ± 2
CDNB	n.d.	n.d.	50 ± 2
CDNB, t-buOOH	n.d.	n.d.	51 ± 3
BCNU	4.0 ± 0.8	0.12 ± 0.01	50 ± 3
BCNU, t-buOOH	3.4 ± 0.3	0.22 ± 0.01*	53 ± 2

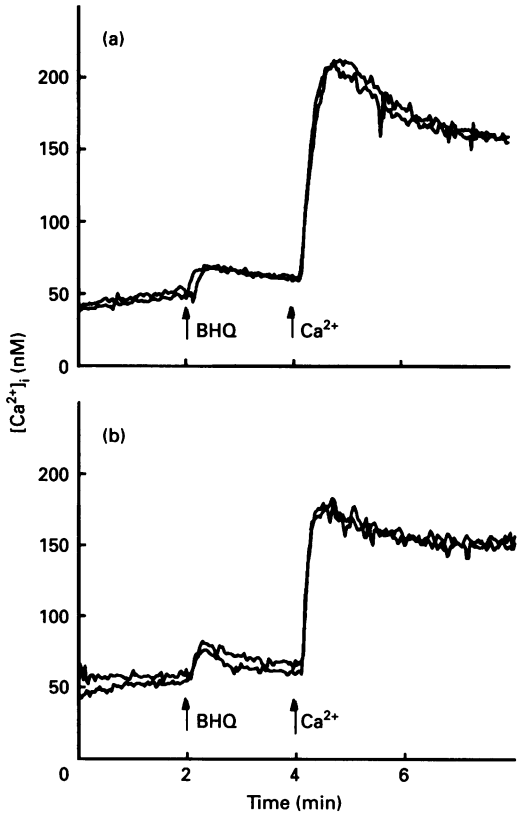

Figure 1 Luminal Ca^{2+} content and capacitative influx

(**a**) Fura-2-loaded cells were incubated with (upper trace) or without (lower trace) CDNB (40 μM, 20 min), washed, and resuspended in Ca^{2+}-free EGTA buffer. BHQ (10 μM) and $CaCl_2$ (2 mM) were added at 2 and 4 min, respectively. Traces are representative of 10 independent experiments. CDNB had no effect on the BHQ-stimulated increase in $[Ca^{2+}]_i$ or on BHQ-stimulated Ca^{2+} influx. (**b**) As for (**a**) except that cells were incubated with (upper trace) or without (lower trace) BCNU (75 μM, 20 min). BCNU had no effect on the BHQ-stimulated increase in $[Ca^{2+}]_i$ or on BHQ-stimulated Ca^{2+} influx.

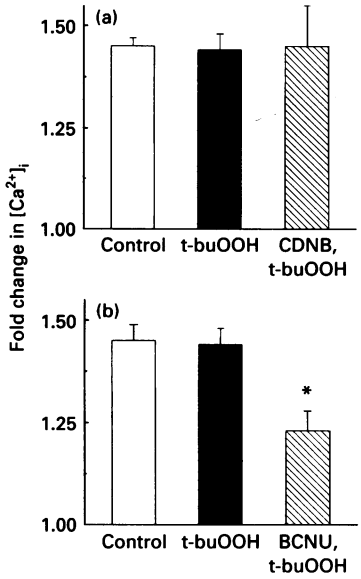

Figure 2 Response to BHQ

(**a**) Fura-2-loaded cells were incubated with or without CDNB (40 μM; 20 min) before a wash step and incubation with or without t-buOOH (0.4 mM, 0.5 h). At the completion of the second incubation period, cells were washed and resuspended in Ca^{2+}-free/EGTA buffer. Shown are the values (means $\pm$ S.E.M.; $n = 4$) for the increases in $[Ca^{2+}]_i$ stimulated by BHQ (10 μM). Data represent control cells (open column), cells treated with t-buOOH (filled column), and cells treated with CDNB and t-buOOH (shaded column). Cells treated with CDNB alone did not differ from controls (see Figure 1). (**b**) As for (**a**), except that cells were incubated with or without BCNU (75 μM; 20 min) before incubation with or without t-buOOH (0.4 mM, 0.5 h). Data represent control cells (open column), cells treated with t-buOOH (filled column), and cells treated with BCNU and t-buOOH (shaded column). Cells treated with BCNU alone did not differ from controls (see Figure 1). * $P < 0.01$ with respect to t-buOOH alone.

GSSG and luminal Ca^{2+} content

The next series of experiments aimed at determining whether increased GSSG is associated with decreased luminal Ca^{2+} content. BCNU (75 μM; 20 min), an irreversible inhibitor of GSSG-reductase, increased [GSSG] to 0.12 ± 0.01 nmol per mg protein, but this level of GSSG did not affect the response of $[Ca^{2+}]_i$ to BHQ (Figure 1b). When BCNU-treated cells were incubated with t-buOOH, however, [GSSG] increased significantly to 0.22 ± 0.01 nmol per mg protein, a result consistent with production of GSSG in the absence of GSSG-reductase activity. Under this condition, BHQ stimulated an increase in $[Ca^{2+}]_i$ of 1.23 ± 0.05-fold, compared with increases of 1.45 ± 0.04-fold in controls and 1.44 ± 0.04-fold in cells treated solely with t-buOOH (Figure 2b).

Radiotracer flux analysis provides a second method by which microsomal Ca^{2+} content can be estimated in intact cells [12]. Isotope released from internal Ca^{2+} stores by BHQ is detected in the extracellular compartment and measured. Under control conditions, basal and BHQ-stimulated initial rate constants were $(9.0 \pm 2.3) \times 10^{-2}$ per min ($r = 0.99$) and $(15.7 \pm 1.5) \times 10^{-2}$ per min ($r = 0.99$), respectively. Thus 83 ± 2% and 52 ± 1% of isotope present at time zero remained associated with the cells 4 min after the respective additions of vehicle or BHQ (Figure 3). In cells treated with t-buOOH (1 h), basal and BHQ-stimulated rate constants were $(8.5 \pm 0.7) \times 10^{-2}$ per min ($r = 0.99$) and $(12.6 \pm 1.3) \times 10^{-2}$ per min ($r = 0.99$) respectively (for each, P not significant with respect to control). Likewise, BCNU alone had no effect on $^{45}Ca^{2+}$ efflux (not shown). However, in cells treated with both BCNU and t-buOOH, the BHQ-stimulated efflux rate constant was $(7.2 \pm 4.8) \times 10^{-2}$ per min ($r = 0.99$). Inhibition of BHQ-stimulated $^{45}Ca^{2+}$ efflux under this condition provides further evidence that accumulation of GSSG during oxidant stress depletes the Ins(1,4,5)P_3-sensitive store of releasable Ca^{2+}.

The relationship between time-dependent oxidant effects and glutathione status were investigated by treating cells with t-buOOH after preincubation with vehicle, CDNB or BCNU. Figure 4 indicates that even when incubation of CDNB-treated cells with t-buOOH is extended to 2 h and beyond, little effect on the response of $[Ca^{2+}]_i$ to BHQ is observed. This finding is again consistent with the conclusion that GSH depletion is not associated with decreased luminal Ca^{2+} content during the early stages (0–2 h) of oxidant stress. By contrast, t-buOOH time-dependently attenuated the $[Ca^{2+}]_i$ response to BHQ in BCNU-treated cells (Figure 4).

GSSG and capacitative Ca^{2+} influx

Addition of Ca^{2+} to cells whose microsomes have been depleted of Ca^{2+} by BHQ provides an estimate of capacitative Ca^{2+} influx [10,12]. We have previously used this technique to show that t-buOOH inhibits capacitative Ca^{2+} influx in CPAEs [15]. In the present study, addition of Ca^{2+} increased $[Ca^{2+}]_i$ 2.6 ± 0.3-fold in

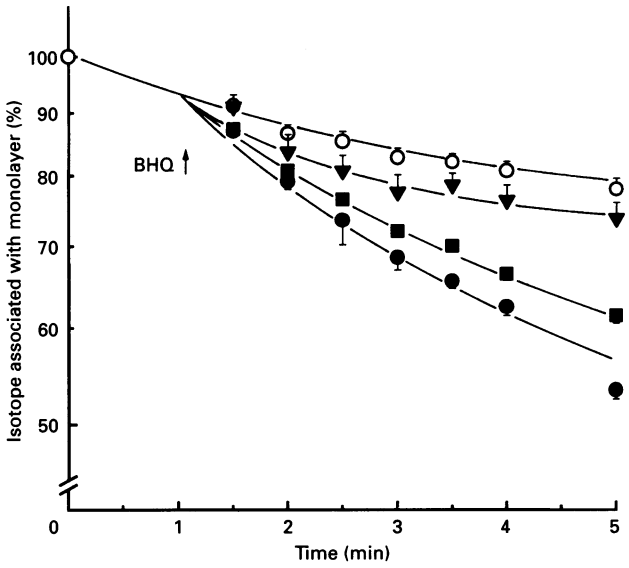

Figure 3 GSSG and inhibition of BHQ-stimulated $^{45}Ca^{2+}$ efflux

Intracellular GSSG was increased by incubating BCNU-treated cells with t-buOOH. In the experiment shown, monolayers of CPAEs were equilibrated for 15–18 h with $^{45}Ca^{2+}$ before incubation with or without BCNU (75 μM) for 20 min. After a wash procedure, cells were treated with t-buOOH (0.4 mM) for 1 h. Efflux of $^{45}Ca^{2+}$ was determined in the absence (○, basal efflux) or presence (▼, BCNU and t-buOOH; ■, t-buOOH; ●, control) of BHQ, added at the time indicated. Basal efflux did not vary between groups, and for clarity is shown only for control cells. Basal and stimulated efflux from cells incubated with BCNU alone (i.e., in the absence of t-buOOH) did not differ from the control, and for clarity is not shown. Data represent means ± S.E.M. ($n = 4$).

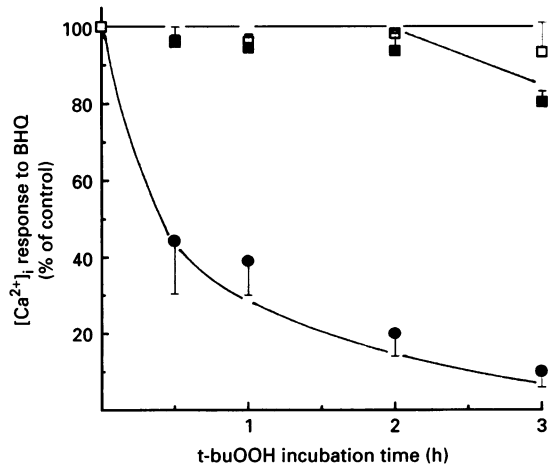

Figure 4 Response to BHQ as a function of t-buOOH incubation time

Shown are the BHQ-stimulated increases in $[Ca^{2+}]_i$ observed in t-buOOH-treated cells after preincubation with vehicle (□), CDNB (■) or BCNU (●). The average change in $[Ca^{2+}]_i$ (1.41 ± 0.02-fold; $n = 36$) stimulated by addition of BHQ to control cells in Ca^{2+}-free EGTA buffer was calculated and assigned a value of 100%. Plotted values represent the BHQ-stimulated change in $[Ca^{2+}]_i$ (means ± S.E.M.; $n = 3$ or 4) after incubation under the various conditions compared with the control (100%).

control cells, and 1.8 ± 0.1-fold in cells treated with t-buOOH ($P < 0.05$ with respect to control). By contrast, Ca^{2+} increased $[Ca^{2+}]_i$ 1.5 ± 0.04-fold in cells treated with BCNU and t-buOOH ($P < 0.05$ with respect to t-buOOH alone). BCNU itself had no effect on capacitative Ca^{2+} influx (Figure 1). In control experiments, the addition order of BHQ and Ca^{2+} was reversed, such that Ca^{2+} was added to the Ca^{2+}-free EGTA external buffer in the absence of BHQ. The Ca^{2+}-stimulated increase in $[Ca^{2+}]_i$ was unaffected by preincubation of cells with BCNU (results not shown).

DISCUSSION

The major finding in this study is that elevated levels of GSSG, produced endogenously within the endothelial cell during oxidant stress, are associated with depletion of the Ins(1,4,5)P_3-sensitive Ca^{2+} store. To increase cellular GSSG, cytosolic GSSG-reductase was inhibited by BCNU [19], a compound that selectively blocks the reduction of GSSG formed during metabolism of t-buOOH. BCNU does not alter the activity of other cytosolic enzymes, and the brief time-frame of the present experiments limits the potential for non-specific effects of the compound. Indeed, BCNU itself had no effect on Ca^{2+} flux, as shown in Figure 1.

In the present study, depletion of GSH failed to alter the luminal Ca^{2+} content of the Ins(1,4,5)P_3-sensitive Ca^{2+} store. This finding is not related to the rate of depletion of GSH because similar results were obtained whether GSH was depleted acutely by CDNB or more slowly by inhibition of *de novo* synthesis. Even in the presence of the exogenous oxidant, t-buOOH, depletion of GSH had little or no effect on the filling state of the Ins(1,4,5)P_3-sensitive Ca^{2+} store. Because the formation of radical species derived from t-buOOH is favoured by depletion of GSH [20,21], these data therefore offer evidence that free-radical species do not deplete Ins(1,4,5)P_3-sensitive stores of releasable Ca^{2+}.

In several non-endothelial cell lines, compounds that react with protein thiol groups alter Ins(1,4,5)P_3-stimulated Ca^{2+} flux [22–24]. Direct addition of GSSG to permeabilized rat hepatocytes attenuates Ins(1,4,5)P_3-stimulated Ca^{2+} release and decreases the rate of microsomal Ca^{2+} re-uptake [25]. By contrast, more recent evidence indicates that GSSG has no effect on the size of the Ins(1,4,5)P_3-sensitive Ca^{2+} pool in permeabilized rat hepatocytes [26]. This result, which differs from that of the present study, might be explained by methodological differences between the study of intact endothelial cells and that of permeabilized hepatocytes. Subtle changes in protein folding within permeabilized hepatocytes, for example, might limit the interaction between exogenously added GSSG and thiol groups on Ins(1,4,5)P_3-operated Ca^{2+} channels and/or microsomal Ca^{2+} pumps. It is further possible that differences between endothelial cells and hepatocytes are not simply methodological in nature, because hepatocyte microsomal Ca^{2+}-ATPase seems more sensitive to t-buOOH than does endothelial cell Ca^{2+}-ATPase [15,25,27].

It is uncertain precisely how increased levels of GSSG are associated with decreased luminal Ca^{2+} content of endothelial cell Ins(1,4,5)P_3-sensitive stores. GSSG might form one or more mixed disulphides with microsomal Ca^{2+}-ATPase, leading to inhibition of Ca^{2+}-pump activity. In this way, inhibition might result from the reaction of GSSG with thiol groups at Ca^{2+}- or ATP-binding sites or with thiol groups that are removed from the active binding sites yet are still critically important for pump function. Alternatively, GSSG might promote the formation of disulphides with the Ins(1,4,5)P_3-receptor/Ca^{2+} channel complex, leading to decreased Ins(1,4,5)P_3 binding and/or increased Ca^{2+} leakage. The finding that endogenously produced GSSG alters Ins(1,4,5)P_3-dependent Ca^{2+} pools in vascular endothelial cells suggests that GSSG might regulate or modulate the function of other proteins and enzymes within this cell type. This concept

was first proposed by Gilbert [28] when he showed that rabbit muscle phosphofructokinase activity was modulated by GSH/GSSG redox status. In the present work, the effects of GSSG are not simulated by GSH depletion, suggesting that GSSG itself is the primary mediator of decreased luminal [Ca^{2+}].

We have previously shown that t-buOOH inhibits capacitative Ca^{2+} entry in vascular endothelial cells [15]. The molecular mechanism responsible for this effect has not yet been identified. However, capacitative Ca^{2+} influx is inhibited at a time when t-buOOH has no effect on the luminal Ca^{2+} content of Ins(1,4,5)P_3-sensitive stores [15], suggesting that the oxidant either directly inhibits the influx channel or inhibits its mechanism of activation. Two general mechanisms have been proposed to account for the communication between the Ins(1,4,5)P_3-sensitive store and the capacitative Ca^{2+} influx pathway: an intracellular messenger molecule released along with microsomal Ca^{2+} may activate Ca^{2+} influx [29], or a *trp*-like protein may provide a physical link between internal store and plasma membrane [30]. Oxidant-inhibition of the mechanism responsible for activation of capacitative influx might therefore be explained by oxidation of a messenger molecule or oxidation of a structural protein.

We postulated that inhibition of capacitative influx by t-buOOH is due to oxidation of thiol-containing residues. Increased intracellular GSSG, itself an endogenous thiol oxidant, clearly potentiated the inhibitory effect of t-buOOH on capacitative influx. This result suggests that the capacitative Ca^{2+} influx channel or the mechanism responsible for its activation possesses thiol-containing residues that are functionally important for Ca^{2+} influx.

In summary, we report that GSSG depletes the Ins(1,4,5)P_3-sensitive Ca^{2+} store of releasable Ca^{2+}. The level of GSSG necessary to deplete internal stores is readily achieved when cytosolic GSSG-reductase is inhibited in the face of oxidant stress. In addition, GSSG potentiates the effect of t-buOOH on capacitative Ca^{2+} influx. To our knowledge, these are the first pieces of evidence that GSSG produced endogenously within endothelial cells during oxidant stress modulates Ca^{2+} flux by altering luminal Ca^{2+} content and that capacitative Ca^{2+} entry is sensitive to thiol-reactive oxidants.

We thank Ms. Naiyi Shi for assistance and Dr. Hiram F. Gilbert for helpful discussions and assistance in the measurement of cell glutathione. This work was supported by Grant HL02595 from the National Institutes of Health and by a grant from the Wyeth Pediatric Research Fund. S.J.E. is the recipient of an NIH Clinical Investigator Award.

Received 12 June/25 July 1995; accepted 2 August 1995

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