N-n-butyl haloperidol iodide inhibits $\text{H}_2\text{O}_2$-induced $\text{Na}^+/\text{Ca}^{2+}$-exchanger activation via the $\text{Na}^+/\text{H}^+$ exchanger in rat ventricular myocytes

Introducion

Reperfusion of an ischemic myocardium leads to heart dysfunction and cardiomyocyte injury. Such myocardial ischemia/reperfusion (I/R) injury is characterized by impaired blood flow, metabolic dysfunction, contractile dysfunction, dysrhythmias, cellular necrosis, and apoptosis.\(^1\) I/R injury is a complex process involving numerous mechanisms, including cytosolic and mitochondrial $\text{Ca}^{2+}$ overload, release of reactive oxygen species (ROS), acute inflammatory response, and shift in substrate use.\(^2\)

ROS, produced as by-products of oxidative metabolism, are easily managed under normal conditions by reactive oxygen scavengers.\(^3,4\) Several forms of ROS are generated during I/R, including superoxide ($\text{O}_2^-$), $\text{H}_2\text{O}_2$, and the highly reactive hydroxyl radical (-OH), which cause lipid peroxidation and myocardial injury and trigger the contractile dysfunction observed during reperfusion.\(^5,6\) It has also been suggested that the burst in...
ROS upon reperfusion may contribute to Ca\(^{2+}\) overload in cardiomyocytes.\textsuperscript{7} Rothstein et al\textsuperscript{10} and Sabri et al\textsuperscript{10} found that low doses of H\(_2\)O\(_2\) (50 µM, similar to those generated during I/R) cause Ca\(^{2+}\) overload in cultured neonatal rat ventricular myocytes, which is associated with activation of the Na\(^+\)/H\(^+\) exchanger (NHE) in part through extracellular signal-regulated kinase (ERK)-1/2-mediated phosphorylation of NHE-1, the only NHE isofrom in the myocardium. A link between H\(_2\)O\(_2\) and diastolic Ca\(^{2+}\) overload in neonatal rat ventricular myocytes was proposed. Exposure to H\(_2\)O\(_2\) results in the alteration of signaling proteins involved in the mitogen-activated protein kinase (MAPK) pathway, ultimately leading to extracellular signal-regulated kinase (MEK) activation, which then phosphorylates and activates ERK1/2. Activated ERK1/2 subsequently phosphorylates the COOH tail of NHE-1, increasing its exchange activity to elevate intracellular Na\(^+\) concentrations. The resulting rise in intracellular Na\(^+\) decreases the activity of the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX), leading to an increase in diastolic Ca\(^{2+}\) levels.\textsuperscript{9} Therefore, pharmacological approaches to decrease MAPK, NHE-1, and/or NCX activity may ameliorate the alterations in Ca\(^{2+}\) homeostasis that contribute to myocardial tissue injury following I/R.

\textit{N}-n-butyl haloperidol iodide (F\(_2\)), a novel quaternary ammonium salt derivative of haloperidol synthesized in our laboratory, can maintain the effects of coronary artery relaxation without adverse extrapyramidal reactions.\textsuperscript{11} Our previous studies show that F\(_2\) can attenuate myocardial I/R injury, as evidenced by amelioration of hemodynamics and myocardial enzyme activity; reduction in myocardial infarct size, prevention of ventricular arrhythmias, and decreases in myocardial inflammation.\textsuperscript{11–14} The cardioprotective mechanism of F\(_2\) was thought to be associated with calcium-homeostasis maintenance against intracellular Ca\(^{2+}\) overload by inhibiting cardiocyte L-type Ca\(^{2+}\) channels.\textsuperscript{12,13} However, intracellular Ca\(^{2+}\) overload during I/R results primarily from the functional coupling of NHE and NCX. Ischemic hearts develop intracellular acidosis, which activates NHE to extrude H\(^+\) from the cytosol via the reverse mode of NCX, resulting in Ca\(^{2+}\) overload.\textsuperscript{15} It is suggested that the mechanism of F\(_2\) antagonizing myocardial I/R injury might not be only related to suppression of the L-type Ca\(^{2+}\) channel.

In this study, we used rat ventricular myocytes to investigate the effects of F\(_2\) on the MEK/ERK/NHE/NCX signal-transduction pathway involved in H\(_2\)O\(_2\)-induced Ca\(^{2+}\) overload in order to probe the underlying molecular mechanism by which F\(_2\) maintains intracellular calcium homeostasis and antagonizes myocardial I/R injury.

### Materials and Methods

#### Materials

F\(_2\) (synthesized by our lab and identified by the Shanghai Organic Chemistry Institute of the Chinese Academy of Sciences; purity greater than 98%) was prepared as a 0.1 M stock solution in dimethyl sulfoxide and diluted to the desired concentration with extracellular solution before each experiment. HEPES (4-[2-hydroxyethyl]-1-piperazine-ethanesulfonic acid), CsCl, 1,2-bis(2-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid (BAPTA), ouabain, nifedipine, ryanodine, epidermal growth factor (EGF), angiotensin (Ang) II and 5-(N,N-dimethyl)-amiloride (DMA) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). U0126 (1,4-diamino-2,3-dicyano-1,4-bis[3-aminophenylmercapto]butadiene) was from Merck Millipore (Billerica, MA, USA), 2,7-bis(2-carboxyethyl)-5(6)-carboxyfluorescein-acetoxyethyl ester (BCECF-AM), and Pluronic® F127 were from Thermo Fisher Scientific (Waltham, MA, USA). Anti-MEK, anti-phosphorylated (p)-MEK, anti-ERK, and anti-p-ERK antibodies were from Cell Signaling Technology (Danvers, MA, USA), anti-NCX from Santa Cruz Biotechnology Inc., (Dallas, TX, USA), anti-NHE-1 antibody from Abcam (Cambridge, UK), anti-β-actin from Sigma-Aldrich, and secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Ig)G) from BosterBio (Pleasanton, CA, USA).

#### Isolation of ventricular myocytes

Adult male Sprague Dawley rats (180–250 g) were obtained from the Laboratory Animal Breeding and Research Center (Shantou, People’s Republic of China). All experiments were conducted in strict accordance with the \textit{Guide for the Care and Use of Laboratory Animals} published by the US National Institutes of Health (publication 85-23, revised 1996).\textsuperscript{16} The protocol was approved by the Medical Animal Care and Welfare Committee of Shantou University Medical College (permit SUMC2010-093). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Single ventricular myocytes were isolated by an enzymatic dissociation method described previously.\textsuperscript{17,18} Single ventricular myocytes were harvested after filtration through a nylon mesh (pore size 200 mm).

#### I\(_{\text{NCX}}\) recording

Myocytes were perfused with extracellular solution (140 mM NaCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 0.33 mM
NaH$_2$PO$_4$, 10 mM glucose, 10 mM HEPES, 0.02 mM ouabain, 0.01 mM nifedipine, 2 mM CsCl, and 0.01 mM ryanodine, pH 7.2) at a rate of 1 mL/minute in a recording chamber. Patch pipettes were forged from 1.5 mm diameter glass capillaries with a two-stage microelectrode puller (pp-830; Narishige, Tokyo, Japan). The pipette resistance was 2–3 MΩ when filled with the pipette solution (20 mM NaCl, 20 mM BAPTA, 10 mM CaCl$_2$ [free Ca$^{2+}$ concentration of 226 nM], 120 mM CsOH, 3 mM MgCl$_2$, 50 mM aspartic acid, 5 mM Mg-adenosine triphosphate, and 10 mM HEPES, pH 7.2). NCX current ($I_{\text{NCX}}$) was recorded by a tight-seal whole-cell voltage clamp with the use of an Axopatch™ 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). The electrode capacitance was maximally compensated by use of the amplifier. No compensation was made for membrane capacitance or series resistance.

For recording $I_{\text{NCX}}$, the extracellular solution contained ouabain, nifedipine, Cs$^+$, and ryanodine to block Na$^+$/K$^+$ pump current, $I_{\text{Ca}}$, and Ca$^{2+}$ release channels of the sarcoplasmic reticulum, respectively. $I_{\text{NCX}}$ was induced by ramp-voltage pulses from a holding potential of −60 mV to +60 mV, and then hyperpolarizing to −150 mV before ramping back to the holding potential at a rate of 600 mV/second. The descending limb (from +60 to −150 mV) was plotted as the current–voltage ($I$–$V$) relationship without capacitance compensation. $I_{\text{NCX}}$ was measured as the Na$^{2+}$-sensitive current that could be selectively inhibited by 5 mM NiCl$_2$.

**Measurement of intracellular pH and NHE activity**

Intracellular pH (pH$_i$) was measured by monitoring the fluorescence of the pH-sensitive dye BCECF.$^{9,10}$ Myocytes placed in a petri dish were loaded with BCECF by incubation for 15 minutes in the dark at room temperature with the acetoxyethyl ester form (BCECF-AM, 2 μM) in modified Krebs solution (135 mM NaCl, 5.9 mM KCl, 1.5 mM CaCl$_2$, 1.2 mM MgCl$_2$, 11.5 mM glucose, 11.6 mM HEPES, pH 7.4) supplemented with 0.1% bovine serum albumin and 0.02% Pluronic F127. The cells were then washed three times and incubated for an additional 45 minutes in fresh Krebs solution in the presence or absence of the MEK inhibitor U0126 (5 μM). BCECF fluorescence was recorded using confocal microscopy (FluoView FV1000; Olympus, Tokyo, Japan). A ratio of fluorescence emitted at 515 nm from excitation at 490 nm to that at 440 nm was converted to intracellular pH$_i$ using the nigerinic high-K$^+$ protocol of Thomas et al.$^{21}$

NHE activity was measured by monitoring the recovery rate from rapid acidification using the NH$_4$Cl prepulse technique.$^{21,22}$ After determination of basal pH$_i$, cells were exposed to Krebs solution containing 25 mM NH$_4$Cl for 5 minutes to cause rapid alkalinization as NH$_3$ diffused into the cells and titrated intracellular H$^+$. Then, perfusion with Na$^+$-free Krebs solution (Na$^+$ isosmotically replaced with N-methylglucamine) removed NH$_4^+$ from the external medium to cause a rapid decrease in pH$_i$. There was no recovery from this acid load in the absence of Na$^+$. pH$_i$ recovered when the perfusate was switched to an Na$^+$-containing Krebs solution. This Na$^+$-dependent recovery was operationally defined as NHE activity. To quantify the rate of pH$_i$ recovery, the slope of a straight line fitted to the initial 60 seconds after the onset of recovery was measured.$^{10}$

**Western blotting**

Total protein extracts were prepared from cells using cell-lysis buffer containing a protease-inhibitor cocktail (aprotinin, leupeptin, pepstatin A, and phenylmethylsulfonyl fluoride). The protein concentration was determined by a Bradford protein-assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Equal amounts of total protein (40 μg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (10%), followed by electrophoretic transfer to nitrocellulose membranes (GE Healthcare UK Ltd., Little Chalfont, UK). The blots were incubated with primary antibody (rabbit antirat) at 4°C overnight, followed by secondary antibody (horseradish peroxidase-conjugate goat antirabbit IgG) for 2 hours at room temperature. The bound antibodies were detected by the use of a SuperSignal Western blotting kit (Thermo Fisher Scientific). Densitometric analysis of protein bands was performed with Quantity One® software (version 4.5.2; Bio-Rad Laboratories Inc.).

**Statistical analysis**

All values are presented as means ± standard error of the mean. Statistical analysis was carried out using paired Students’s $t$-tests or one-way analysis of variance followed by the Student–Newman–Keuls test, with $P<0.05$ considered statistically significant.

**Results**

$F_2$ inhibits the H$_2$O$_2$-induced increase of $I_{\text{NCX}}$

Currents were recorded when myocytes were perfused in sequence with the control extracellular solution, and solutions containing H$_2$O$_2$ (100 μM), H$_2$O$_2$ + F$_2$ (0.1, 1.0, or 10 μM),
and NiCl₂ (5 mM) for 10 minutes, respectively. Bidirectional outward and inward $I_{\text{NCX}}$ were induced by 1 mM Ca²⁺ and 140 mM Na⁺ in the external solution, and 20 mM Na⁺ and 226 mM free Ca²⁺ in the pipette solution. Under these ionic conditions, the reversal potential of $I_{\text{NCX}}$ with a 3Na⁺:1Ca²⁺ stoichiometry was calculated to be −65 mV at room temperature according to the equation $E_{\text{NCX}} = 3E_{\text{Na}} - 2E_{\text{Ca}}$. Figure 1A illustrates the $I-V$ relation of control myocytes (a), and myocytes exposed to H₂O₂ (b), H₂O₂+0.1, 1.0, or 10 µM F₂ (c–e), and NiCl₂ (f). The net Ni²⁺-sensitive currents all crossed the voltage axis at about −65 mV (Figure 1B), confirming that the Ni²⁺-sensitive currents were $I_{\text{NCX}}$. Both outward and inward $I_{\text{NCX}}$ increased after perfusion with 100 µM H₂O₂. F₂ diminished the increase of $I_{\text{NCX}}$ in a concentration-dependent manner, with reverse-mode NCX being greater than forward-mode inhibition (Figure 1C).

**U0126 and DMA inhibit H₂O₂-induced $I_{\text{NCX}}$ increases**

To confirm the involvement of the MAPK pathway and the NHE in H₂O₂-induced NCX activation, we tested the effects of U0126, a highly selective inhibitor of MEK, and DMA, an NHE inhibitor, on the H₂O₂-induced increase in $I_{\text{NCX}}$. We initially determined the minimal effective concentrations that completely blocked H₂O₂-induced MEK activation and NHE-1 activity, and used those concentrations to examine the roles of MEK and NHE in F₂-mediated inhibition of H₂O₂-mediated induction of $I_{\text{NCX}}$ activity. Results showed that perfusion of 5 µM U0126 for 10 minutes, which alone did not affect $I_{\text{NCX}}$, significantly inhibited the H₂O₂-induced increase in $I_{\text{NCX}}$ at 60 mV by 81.13%±3.63% and at −150 mV by 93.64%±4.52% (n=5) (Figure 2A and B). In contrast, perfusion of 20 µM DMA for 10 minutes only inhibited the H₂O₂-induced increase by 39.98%±3.00% at 60 mV, and by 32.42%±1.78% at −150 mV (n=5) (Figure 2C and D). This result indicates that the H₂O₂-induced increase in $I_{\text{NCX}}$ was primarily mediated by the MEK/MAPK pathway, and partially through activation of NHE-1.

**F₂ inhibits H₂O₂-induced MEK/ERK activation and EGF-induced $I_{\text{NCX}}$ increases**

To investigate whether F₂ modulates MEK activity, we examined the effect of F₂ on H₂O₂-induced and EGF-induced MEK/ERK activation. As shown in Figure 3A, H₂O₂ (100 µM) and EGF (50 ng/mL) led to a significant increase in the level of phosphorylated MEK and ERK, and 1 µM F₂ inhibited both H₂O₂-induced and EGF-induced MEK and ERK activation. We then observed the effect of F₂ on the $I_{\text{NCX}}$ increase induced by EGF. $I_{\text{NCX}}$ was increased by EGF, and treatment with 1 µM F₂ resulted in a significant reduction in EGF-induced $I_{\text{NCX}}$ rise at 60 mV by 72.88%±5.76% and at −150 mV by 71.14%±3.19% (n=8) (Figure 3B).

**F₂ inhibits H₂O₂-induced and Ang II-induced NHE activity**

To investigate the effects of F₂ on NHE activity, we examined its effects on H₂O₂-induced and Ang II-induced, Na⁺-dependent recovery from acid load in rat ventricular myocytes. The mean resting pH of ventricular myocytes in bicarbonate-free Krebs solution at room temperature was 7.48±0.13 (n=10). The addition and removal of NH₄Cl from the external medium caused a rapid rise and decrease in pH. Cells were unable to recover from this acid load in Na⁺-free medium. Reintroduction of Na⁺ led to a rapid recovery of pH that approached resting values (Figure 4A). This Na⁺-dependent recovery was completely blocked by DMA (25 µM) (Figure 4B). Exposure to

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**Figure 1** Effect of F₂ on the H₂O₂-induced increase in $I_{\text{NCX}}$.

**Notes:** (A) $I-V$ curves of control (a), or in the presence of 100 µM H₂O₂ (b), H₂O₂+F₂ (0.1, 1.0 or 10 µM) (c–e) and 5 mM NiCl₂ (f). Inset: ramp-pulse protocol. (B) $I-V$ curves of net Ni²⁺-sensitive currents, obtained by subtracting the corresponding $I-V$ curves in (A). (C) Concentration–response relationships of the inhibitory effect of F₂ on $I_{\text{NCX}}$. Outward currents were measured at +60 mV, inward currents were measured at −150 mV (n=9 cells/group). *P<0.05 outward currents versus inward currents.

**Abbreviations:** F₂ N-n-butyl-hexadecyl imidazole; $I_{\text{NCX}}$: current of Na⁺/Ca²⁺ exchanger; $I-V$: current–voltage; H₂O₂ hydrogen peroxide; NiCl₂ nickel chloride; Ni²⁺ nickel ion.
Ang II at a low concentration stimulates NHE-1 activity (F) and NHE activity in the presence of Ang II (G). These results suggest that F exerts its cardiovascular protective effects by blocking NHE activity.

**F inhibits Ang II-induced I_{NCX} increases**

Ang II at a low concentration stimulates NHE-1 activity to elevate intracellular Na+ levels, which reverses NCX activity and leads to I_{NCX} increases. We observed that 1 nM Ang II increased outward I_{NCX} at 60 mV by 26.92±4.40% and inward I_{NCX} at -150 mV by 14.26±2.95% (n=5), consistent with a prior report. Addition of 1 μM F2 resulted in a significant reduction in the Ang II-induced I_{NCX} rise at 60 mV by 62.27±3.42% and at -150 mV by 46.19±3.36% (n=5) (Figure 5), consistent with a role for F2 in blocking NHE activation.

**Effects of F2 on the protein expression of NHE and NCX**

Exchanger activity is regulated by changes in protein expression and by phosphorylation of existing exchangers or a closely associated modulatory protein. Therefore, we examined the effects of F2 on the protein expression of NHE and NCX. The results showed that the total protein expression of NHE and NCX did not change after myocytes were treated with F2.
with H$_2$O$_2$, EGF, and Ang II for 30 minutes, and that F$_2$ had no significant effect on the total protein expression of either NHE or NCX (Figure 6).

**Discussion**

The present study describes the effects of F$_2$ on the H$_2$O$_2$-induced signal-transduction pathway for $I_{\text{NCX}}$ increase in rat ventricular myocytes. F$_2$ can inhibit the signal-transduction pathway involved in H$_2$O$_2$-induced $I_{\text{NCX}}$ increase at multiple sites.

Excess ROS production and intracellular Ca$^{2+}$ overload play a prominent role in I/R injury. Moreover, there is a reciprocal interaction between excess ROS production and accumulation of cytosolic and mitochondrial Ca$^{2+}$ due to the cross talk between ROS and Ca$^{2+}$. Excess ROS production and intracellular Ca$^{2+}$ overload play a prominent role in I/R injury. Moreover, there is a reciprocal interaction between excess ROS production and accumulation of cytosolic and mitochondrial Ca$^{2+}$ due to the cross talk between ROS and Ca$^{2+}$.
Haloperidol inhibits Na\(^+\)/Ca\(^{2+}\)-exchanger activation

(ERK, Jun N-terminal kinase [JNK], p38),\(^{38}\) which are activated during ischemia, and to a greater extent on reperfusion.\(^{39,40}\) Activated ERK1/2 leads to phosphorylation and activation of NHE-1,\(^{9,41}\) and this may contribute to a feed-forward activation loop (Ca\(^{2+}\) → ROS → ERK → more Na\(^+\) → more Ca\(^{2+}\)), enhancing Ca\(^{2+}\) overload in I/R injury.\(^{37}\) The ability to disrupt this vicious cycle will exert beneficial effects on recovery from I/R injury. In this study, we
Figure 5 Effect of F₂ on Ang II-induced \( I_{\text{NCX}} \) increase.

Notes: (A) \( I-V \) curves of control (a), or in the presence of 1 nM Ang II (b), Ang II + F₁ (1 μM) (c), and 5 mM NiCl₂ (d). (B) \( I-V \) curves of net Ni²⁺-sensitive currents, obtained by subtracting the corresponding \( I-V \) curves in (A).

Abbreviations: \( F₂ \): N-butyhaloperidol iodide; Ang, angiotensin; \( I-V \), current–voltage; NiCl₂, nickel chloride; Ang II, angiotensin II; \( I_{\text{NCX}} \), NCX current; Ni²⁺, nickel ion.

demonstrated that F₂ can inhibit \( \text{H}_2\text{O}_2 \)-induced increase in NCX activity through inhibiting both MEK/ERK activation and NHE activity, blocking intracellular Ca²⁺ overload to protect against myocardial I/R injury.

Our results show that acute exposure of cardiac myocytes to 100 μM \( \text{H}_2\text{O}_2 \) causes the \( I_{\text{NCX}} \) to increase, along with a rapid activation of MEK and an increase in NHE activity. The \( \text{H}_2\text{O}_2 \)-induced \( I_{\text{NCX}} \) increase was blocked almost completely by the MEK inhibitor U0126, but only partly by the NHE inhibitor DMA (Figure 2), indicating the \( I_{\text{NCX}} \) increase was primarily mediated by the MEK MAPK pathway and partially through activation of NHE, consistent with prior reports.²,²⁵ Furthermore, the \( \text{H}_2\text{O}_2 \)-induced increase in NHE activity was abolished by pretreatment with the MEK inhibitor U0126 (Figure 4E), suggesting that MAPKs act upstream of NHE in \( \text{H}_2\text{O}_2 \)-induced \( I_{\text{NCX}} \) increase. The present study shows that F₂ blocks MEK activation-induced by not only \( \text{H}_2\text{O}_2 \) but also EGF (Figure 3A), suggesting that F₂ directly inhibits MEK activation.

Dyck et al found an increase in steady-state levels of NHE-1 messenger ribonucleic acid in chronic ischemia in rat myocardium, suggesting that increased activity is due to an increase in protein expression.¹¹ However, in our experiments, acute exposure to \( \text{H}_2\text{O}_2 \) caused a rapid activation of NHE and NCX activity in the absence of changes in total NHE and NCX. The most likely explanation is that the exposure to \( \text{H}_2\text{O}_2 \) in our experiment was too short for changes in protein expression, indicating that posttranslational modification rather than gene expression played the major role in the rapid time course for regulation of exchanger activity. Unfortunately, we could not detect phosphorylation of NHE-1 and NCX due to the absence of antibodies for phospho-NHE-1 and phospho-NCX, which was a limitation of this study.

NHE activation increases \( I_{\text{NCX}} \) through increasing intracellular Na⁺ concentration. NCX is one of the major mechanisms for regulating intracellular Ca²⁺ concentration in cardiac myocytes. Under physiological conditions, the Na⁺/Ca²⁺ exchanger operates in forward mode, extruding Ca²⁺ from the cell to maintain intracellular Ca²⁺ homeostasis. Conversely, during I/R, a large burst of ROS contributes to Ca²⁺ loading via activation of the NCX Ca²⁺-influx mode, which accelerates intracellular Ca²⁺ overload.⁴² \( \text{H}_2\text{O}_2 \) increases NCX activity, leading to Ca²⁺ overload via activation of the MEK/ERK/NHE pathway.⁴³ Our previous studies demonstrate that F₂ blocks L-type Ca²⁺ channels and protects the activity of sarco/endoplasmic reticulum Ca²⁺-adenosine triphosphatases to attenuate Ca²⁺ overload against I/R injury in cardiac myocytes.¹²,¹³,¹⁸,⁴⁴ We now show an additional mechanism for F₂ in the regulation of calcium homeostasis, demonstrating that F₂ inhibits both MEK activation and NHE activity to diminish \( \text{H}_2\text{O}_2 \)-induced \( I_{\text{NCX}} \) increase, but we do not rule out inhibition by F₂ on NCX activity. Figure 7 illustrates the possible signaling pathways from \( \text{H}_2\text{O}_2 \) to NCX and the target of F₂ action.

In conclusion, we demonstrate an additional mechanism by which F₂ can alleviate intracellular Ca²⁺ overload, and thus protect against myocardial I/R injury. F₂, a novel quaternary ammonium salt derivative of haloperidol with a different chemical structure from classical Ca²⁺-channel antagonists, seems like an undesirable drug due to its broad, nonspecific
effects, but the study of its structure–function relationship may help to develop new drugs for the treatment of ischemic heart disease.

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Disclosure

The authors report no conflicts of interest in this work.
**Figure 7** Scheme of possible signaling pathways for NCX activation by $\text{H}_2\text{O}_2$ and the target of $F_2$ action.

**Abbreviations:** NCX, $\text{Na}^+/\text{Ca}^{2+}$ exchanger; $\text{H}_2\text{O}_2$, hydrogen peroxide; $F_2$, N-n-butyl haloperidol iodide; NHE, Na+/H+ exchanger; ERK, extracellular signal-related kinase; MEK, extracellular signal-regulated kinase.

**References**


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