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Modulation of the K^+ Channels Encoded by the Human Ether-a-Gogo-Related Gene-1 (*hERG1*) by Nitric Oxide

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ABSTRACT

The inhibition of nitric oxide synthase by *N*-nitro-L-arginine methyl ester (0.03–3 mM) dose-dependently reduced nitric oxide ($NO^{\bullet}$) levels and enhanced the outward currents carried by human ether-a-gogo-related gene-1 (*hERG1*) K^+ channels expressed in *Xenopus laevis* oocytes, whereas the increase in $NO^{\bullet}$ levels achieved by exposure to L-arginine (0.03–10 mM) inhibited these currents. Furthermore, four $NO^{\bullet}$ donors belonging to such different chemical classes as sodium nitroprusside (1–1000 μM), 3-morpholino-sydnonimine (100–1000 μM), (*Z*)-1-[*N*-(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (NOC-18; 1–300 μM), and *S*-nitroso *N*-acetylpenicillamine (1–300 μM) dose-dependently inhibited *hERG1* outward K^+ currents. By contrast, the $NO^{\bullet}$ donor NOC-18 (0.3 mM) did not affect other cloned K^+ channels such as rat neuroblastoma-glioma K^+ channel 2, rat delayed rectifier K^+ channel 1, bovine ether-a-gogo gene, rat ether-a-gogo-related gene-2, and rat ether-a-gogo-related gene-3. The inhibitory effect of $NO^{\bullet}$ donors on *hERG1* K^+ channels was prevented by the $NO^{\bullet}$ scavengers 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide and hemoglobin. The membrane permeable analog of cGMP, 8-bromo-cGMP (1 mM), failed to reproduce the inhibitory action of $NO^{\bullet}$ donors on *hERG1* outward currents; furthermore, the specific inhibitor of the $NO^{\bullet}$-dependent guanylyl cyclase, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (50 μM), neither interfered with outward *hERG1* K^+ currents nor prevented their inhibition by 0.3 mM NOC-18. Both L-arginine (10 mM) and NOC-18 (0.3 mM) counteracted the stimulatory effect on *hERG1* outward currents induced by the radical oxygen species-generating system $FeSO_4$ (25 μM)/ascorbic acid (50 μM; Fe/Asc). Finally, L-arginine (10 mM) and NOC-18 (0.3 mM) inhibited both basal and Fe/Asc (0.1 mM/0.2 mM)-stimulated lipid peroxidation in *X. laevis* oocytes. Collectively, the present results suggest that $NO^{\bullet}$, both endogenously produced and pharmacologically delivered, may exert in a cGMP-independent way an inhibitory effect on *hERG1* outward K^+ currents via an interaction with radical oxygen species either generated under resting conditions or triggered by Fe/Asc.

Nitric oxide ($NO^{\bullet}$) is a ubiquitous intercellular messenger in vertebrates that plays a crucial role in a wide array of both physiological and pathological processes (Gross and Wolin, 1995). In fact, when produced in appropriate concentrations and times, this gaseous mediator can influence inflammation, vascular tone, memory formation, synaptogenesis, synaptic plasticity, and neuroendocrine secretion. On the other hand, its excessive and/or altered production has been implicated in various pathological states, such as vascular shock, diabetes, neurodegeneration, and stroke (Simonian and Coyle, 1996). Although $NO^{\bullet}$ often exerts its biological effects by binding to the heme group and by activating the soluble form of guanylyl cyclase (Ignarro et al., 1982), in other cases it can directly introduce reversible and irreversible chemical modifications into proteins, causing nitrosylation at the level of tyrosines and thiol-containing amino acids (Darley-Usmar et al., 1995). Furthermore, it should be emphasized that $NO^{\bullet}$ is only one of the redox forms of nitrogen monoxide (NO), because one electron can be withdrawn from or added to $NO^{\bullet}$ to form the nitrosonium ion (NO^+) or the nitroxyl anion (NO^-), respectively, depending on the redox state of the environment (Stamler et al., 1992). Therefore, in close analogy to the array of the redox forms of oxygen, or the so-called

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ABBREVIATIONS: $NO^{\bullet}$, nitric oxide; ROS, radical oxygen species; RNS, radical nitrogen species; $O_2^{\bullet -}$, superoxide anion; $ONOO^-$, peroxynitrite; *rNGK2*, rat neuroblastoma-glioma K^+ channel 2; *rDRK1*, rat delayed rectifier K^+ channel 1; *bEAG*, bovine ether-a-gogo gene; *rERG2* and *rERG3*, rat ether-a-gogo-related gene-2 and -3; *hERG1*, human ether-a-gogo related gene-1; NO_2^-, nitrite; MDA, malondialdehyde; NOS, $NO^{\bullet}$ synthase; L-NAME, *N*-nitro-L-arginine methyl ester; NOC-18, (*Z*)-1-[*N*-(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate; SNAP, *S*-nitroso *N*-acetylpenicillamine; SNP, sodium nitroprusside; SIN-1, 3-morpholino-sydnonimine; PTIO, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide; Hb, hemoglobin; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; Fe/Asc, iron- and ascorbate-containing solution.

radical oxygen species (ROS), the term "radical nitrogen species" (RNS) has been coined for these molecules. Several biological actions of RNS depend on their interaction with ROS, as demonstrated by the reaction of NO$^{\bullet}$ with the superoxide anion ($O_2^{\bullet-}$) to produce peroxynitrite ($ONOO^-$; Beckman and Koppenol, 1996), although other forms of interaction have been proposed (Stamler et al., 1992).

Because several diseases have been related to the oxidative threat posed by the disruption of the homeostatic regulation of oxygen and nitrogen metabolism, the study of the mechanisms mediating the actions of ROS and RNS assumes particular importance. In fact, NO$^{\bullet}$ participates in the early events after ischemia/reperfusion in neuronal cells, exerting both neurodegenerative and neuroprotective responses depending on the stage of evolution of the ischemic process and on the cellular source of NO$^{\bullet}$ (Iadecola, 1997), and in cardiac tissue, NO$^{\bullet}$ mediates the protective effects of ischemic preconditioning against both reversible and irreversible ischemia/reperfusion injury (Takano et al., 1998) and arrhythmias (Bilinska et al., 1996).

In both cardiac and neuronal cells, one of the early events after the ischemic insult is a loss in the ability to control the resting membrane potential (Martin et al., 1994). Because K^+ channels are the main regulators of membrane potential in these tissues, the aim of this study was to investigate whether different classes of K^+ channels primarily expressed in excitable cells could be a target for the molecular actions of NO$^{\bullet}$. Toward this aim, the cDNA clones encoding for various voltage-gated K^+ channels constitutively present in cardiac and/or brain tissues were heterologously expressed, and their possible modulation by endogenously produced or by NO$^{\bullet}$-generating compounds was investigated. *Xenopus laevis* oocytes were chosen as an expression system for these experiments because the two-microelectrode voltage-clamp technique in these cells allows more physiological intracellular conditions to be maintained during the recordings, whereas electrophysiological studies in mammalian cells performed with the whole-cell configuration of the patch-clamp technique, by extensively dialyzing the intracellular compartment, may cause a derangement of the biochemical processes involved in the physiological control of the redox status. In addition, *X. laevis* oocytes have been widely used to characterize the redox modulation of heterologously expressed ion channels (Omerovic et al., 1994; DuVall et al., 1998; Ciorba et al., 1999).

The K^+ channels used in the present investigation were rat neuroblastoma-glioma K^+ channel 2 (*rNGK2*), cloned from a rat brain cDNA library (Taglialatela et al., 1991); rat delayed rectifier K^+ channel 1 (*rDRK1*), cloned from a rat brain cDNA library (Frech et al., 1989) and expressed in both cardiac and neuronal tissues; the bovine isoform of the ether-a-gogo-encoded K^+ channel (*bEAG*; Frings et al., 1998), rat ether-a-gogo-related genes-2 and -3 (*rERG2* and *rERG3*; Shi et al., 1997), exclusively expressed in the nervous system; and human ether-a-gogo related gene-1 (*hERG1*), which plays a crucial role in excitable tissues such as the heart and the brain (Warmke et al., 1994; Taglialatela et al., 1998). In fact, in cardiac tissue, *hERG1* encodes for a K^+ current with the biophysical and pharmacological properties of native cardiac I_{Kr}, one of the main repolarizing currents of the cardiac action potential (Sanguinetti et al., 1995); in addition, mutations affecting the *hERG1* gene are responsible for a life-threatening human cardiac arrhythmia, the long QT syndrome (Curran et al., 1995). In the nervous system, *hERG1* K^+ channels have been implicated in the changes of the resting membrane potential associated with the cell cycle (Arcangeli et al., 1995), in the control of neuritogenesis and differentiation (Faravelli et al., 1996), and in the spike-frequency adaptation (Chiesa et al., 1997) of neuroblastoma cells, although their specific regional and cellular distribution in mammalian brain have yet to be described.

Materials and Methods

Isolation of *X. laevis* Oocytes. Ovarian lobes were surgically removed from adult female *X. laevis* frogs (Rettili di Schneider, Varese, Italy) and placed in 100-mm Petri dishes containing a Ca^{2+}-free solution consisting of 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM piruvic acid, 100 U/ml penicillin, and 100 μg/ml streptomycin, pH 7.5 with NaOH. After four extensive washes, the oocytes (stages V–VI) were dissociated by collagenase treatment (type IA, 45–80 min at a concentration of 2 mg/ml). Dissociated oocytes were then placed in a Ca^{2+}-containing solution (denominated Solution A) consisting of 100 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM piruvic acid, 100 U/ml penicillin, and 100 μg/ml streptomycin, pH 7.5 with NaOH, and kept in a 19°C incubator for use in experiments the next day.

Measurement of Nitrite Production in *X. laevis* Oocytes. NO$^{\bullet}$ levels in *X. laevis* oocytes were measured by assaying nitrite (NO_2^-) production in these cells according to the Griess reaction, as previously described (Stuehr and Nathan, 1989). Because NO$^{\bullet}$ is known to equilibrate across the cell membrane, its extracellular concentration reflects the intracellular levels of this gaseous mediator. Eight defolliculated oocytes were incubated at 19°C in 500 μl of Solution A (controls) or Solution A plus the substance under investigation in the concentration required by the experimental protocol. According to the experimental protocol, 250 μl of supernatant was removed at specific times (5, 15, 30, and 60 min of incubation) and incubated with 250 μl of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride, and 2% H_3PO_4) for 10 min at room temperature. After this period, the absorbance of the sample was measured at 550 nm. Absolute NO_2^- values were determined using $NaNO_2$ as a standard.

Determination of Lipid Peroxidation in *X. laevis* Oocytes. Lipid peroxidation in *X. laevis* oocytes was determined by assaying the intracellular malondialdehyde (MDA) production by means of the 2-thiobarbituric acid test (Taglialatela et al., 1997) according to previously described procedures (Esterbauer and Cheeseman, 1990).

Molecular Biology and Oocyte Injection. The cloning of *hERG1* (Warmke and Ganetzky, 1994), *bEAG* (Frings et al., 1998), *rDRK1* (Frech et al., 1989), *rNGK2* (Taglialatela et al., 1991), and *rERG2* and *rERG3* (Shi et al., 1997) has already been described. These cDNAs were cloned into the following vectors: pSp64A$^+$ for *hERG1*, pCRII for *bEAG*, and pBluescript for *rDRK1*, *rNGK2*, *rERG2*, and *rERG3*. cDNAs were linearized with *Hin*dIII for *bEAG*, *Eco*RI for *hERG1*, and *Not*I for *rDRK1*, *rNGK2*, *rERG2*, and *rERG3*. cRNAs were transcribed in vitro from linearized cDNAs by means of a commercially available kit (mCAP, Stratagene, La Jolla, CA), using T7 RNA polymerase for *bEAG*, *rDRK1*, *rNGK2*, *rERG2*, and *rERG3* and SP6 RNA polymerase for *hERG1*. RNAs were quantified with the use of the RiboGreen fluorescent kit (Molecular Probes, Eugene, OR) and stored in a stock solution (250 ng/μl) at −20°C in 0.1 M KCl. One day after isolation, *X. laevis* oocytes were microinjected with 46 nl of the respective cRNA stock solution or appropriate dilution.

Electrophysiology: Voltage-Clamp with Two Microelectrodes. At 2 to 10 days after the cRNA microinjection, expressed K^+ currents were measured according to the two-microelectrode voltage-clamp technique with a commercially available amplifier (Warner

OC-725A; Warner Instruments Corp.). Current and voltage electrodes were filled with 3 M KCl and 10 mM HEPES (pH 7.4; ≈1 MΩ resistance). The bath solution contained 88 mM NaCl, 10 mM KCl, 2.6 mM $MgCl_2$, 0.18 mM $CaCl_2$, and 5 mM HEPES, pH 7.5 (ND88). This solution was perfused in the recording chamber at a rate of ≈0.2 ml/min. Data were stored on the hard disk of a 486 PC for off-line analysis. The pCLAMP (version 6.0.2; Axon Instruments, Burlingame, CA) software was used for data acquisition and analysis. Currents were recorded at room temperature. Oocytes that showed signs of membrane deterioration during the experiment (an increase in the holding current at −90 mV > −200 nA) were excluded from the electrophysiological analysis.

Drugs and Statistics. (Z)-1-[*N*-(2-Aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (NOC-18) was obtained from Cayman Chemical (Ann Arbor, MI). 3-Morpholino-sydnonimine (SIN-1) was obtained from Calbiochem (San Diego, CA). *S*-nitroso *N*-acetylpenicillamine (SNAP) and sodium nitroprusside (SNP) were purchased from Sigma Chemical Co. (Milan, Italy), as were all of the other materials not otherwise mentioned. NO· donors were dissolved in the experimental solutions and prepared fresh daily and then stored in light-protected polypropylene tubes; the solutions containing the NO· donors had to be prepared fresh immediately before the execution of the experiments to be effective. They proved ineffective after a few hours (data not shown), a possible consequence of the relatively short half-life of NO· released by these molecules in aqueous solutions. $FeSO_4$ and ascorbate stock solutions (10 and 25 mM, respectively) were prepared daily and stored in light-protected tubes to avoid spontaneous oxidation. Statistical significance between the data was obtained by means of the Student's *t* test. When appropriate, data are expressed as mean ± S.E.

Results

Effects of Modulation of Endogenous NO· Levels on *hERG1* K^+ Channels Heterologously Expressed in *X. laevis* Oocytes. *X. laevis* oocytes injected with cRNA transcribed in vitro from *hERG1* cDNA expressed a K^+-selective current with peculiar biophysical and pharmacological properties. In fact, *hERG1* currents are activated by depolarizing pulses above −60 mV but display pronounced inward rectification at positive potentials (>0 mV; Sanguinetti et al., 1995; Smith et al., 1996). These currents also exhibit rather slow kinetics of activation, carry large inward currents on repolarization to values of membrane potential below the equilibrium potential for K^+ ions, and are selectively blocked by certain second-generation antihistamines and class III antiarrhythmics (Taglialatela et al., 1998). These characteristics have led to the suggestion that *hERG1* K^+ channels represent the main molecular component for the cardiac repolarizing current I_{Kr} (Sanguinetti et al., 1995).

Perfusion of *hERG1*-expressing oocytes for 5 min with extracellular solutions containing increasing concentrations of

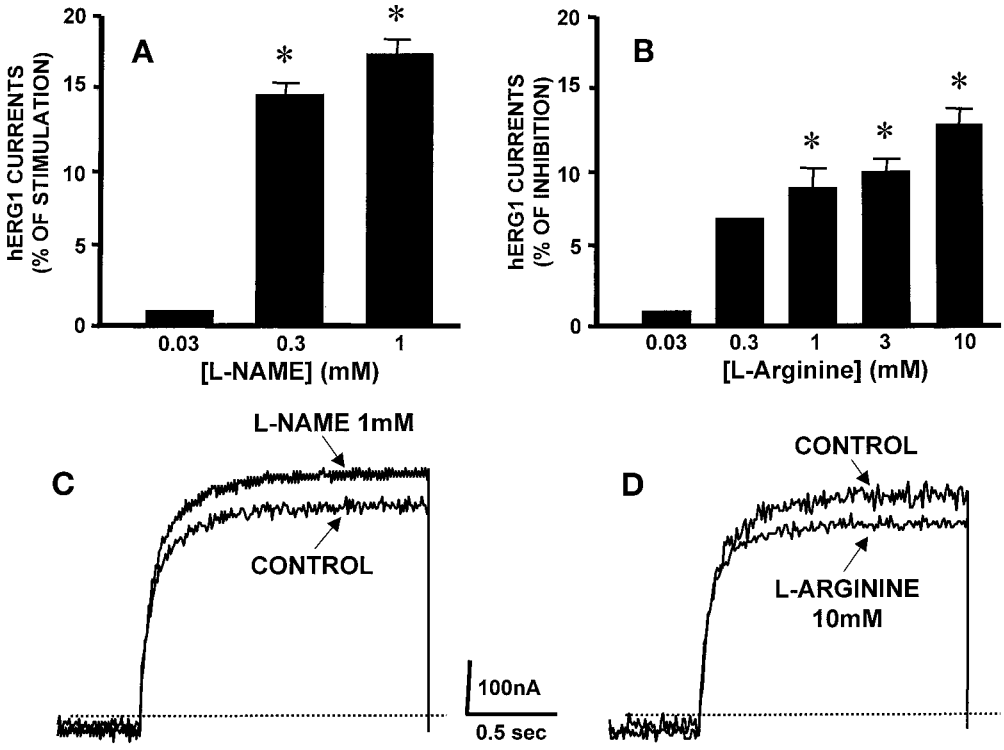

Fig. 1. Effect of the modulation of constitutive NO· production on *hERG1* K^+ currents heterologously expressed in *X. laevis* oocytes. A, dose-response of *hERG1* outward K^+ current stimulation by L-NAME. *hERG1* outward K^+ current was measured at the end of a 1.75-s depolarizing pulse to 0 mV (holding potential, −90 mV; return potential, −100 mV) both under control conditions and after a 5-min superfusion with the indicated L-NAME concentrations and was expressed as percent change versus controls. Each experimental point is the mean ± S.E. of three to eight separate experiments. *Significantly different from control values ($p < .05$). B, dose-response of *hERG1* outward K^+ currents inhibition by L-arginine. *hERG1* outward K^+ current was measured at the end of a 1.75-s depolarizing pulse to 0 mV (holding potential, −90 mV; return potential, −100 mV) both under control conditions and after a 5-min superfusion with the indicated L-arginine concentrations and was expressed as percent change versus controls. Each experimental point is the mean ± S.E. of three to eight separate experiments. *Significantly different from control values ($p < .05$). C, stimulation of *hERG1* outward K^+ currents by 1 mM L-NAME. Two traces of *hERG1* K^+ currents recorded in the same oocyte in control condition and after superfusion for 5 min with 1 mM L-NAME are shown superimposed. Holding potential, −90 mV; 1.75-s test potential to 0 mV; return potential, −100 mV. In C and D, the inward current component elicited on repolarization to −100 mV has been blanked for clarity. D, inhibition of *hERG1* outward K^+ currents by 10 mM L-arginine. Two traces of *hERG1* K^+ currents recorded in the same oocyte under control conditions and after a 5-min superfusion with 10 mM L-arginine are shown superimposed. Holding potential, −90 mV; 1.75-s test potential to 0 mV; return potential, −100 mV.

the NO· synthase (NOS) inhibitor *N*-nitro-L-arginine methyl ester (L-NAME; 0.03–1 mM) caused a dose-dependent enhancement in the outward K^+ currents carried by the heterologously expressed channels (Fig. 1A). Figure 1C shows the outward currents elicited by 1.5-s depolarizing pulses to 0 mV from an holding potential of −90 mV in a *hERG1*-expressing *X. laevis* oocyte, both under control conditions and after a 5-min superfusion with 1 mM L-NAME. On the other hand, the NOS substrate L-arginine (0.03–10 mM) caused a dose-dependent inhibition of the outward K^+ currents carried by the *hERG1* channel, as shown in Fig. 1B. Figure 1D shows the inhibition of the *hERG1* outward K^+ currents by 10 mM L-arginine, using the same experimental protocol of Fig. 1B. The inward currents carried by *hERG1* K^+ channels on membrane repolarization to −100 mV were not modified by either L-NAME or L-arginine (data not shown). Furthermore, the increase in *hERG1* outward K^+ currents induced by the NOS inhibitor L-NAME (1 mM) was reversed if a maximal concentration (10 mM) of the NOS substrate L-arginine was added (Fig. 2A). Figure 2B shows the *hERG1* outward K^+ currents elicited by 1.5-s depolarizing pulses to 0 mV from a holding potential of −90 mV in an *hERG1*-expressing *X. laevis* oocyte under control conditions, after a 5-min superfusion with 1 mM L-NAME, and after an additional 5 min in the presence of 1 mM L-NAME plus 10 mM L-arginine. To establish a more direct link between NO· levels and *hERG1* K^+ channels modulation, we studied NO· levels in *X. laevis* oocytes under various experimental conditions by measuring in these cells the concentration of nitrite (Stuehr and Nathan, 1989), a stable byproduct of NO· metabolism. Figure 3A shows that the incubation of *X. laevis* oocytes for 5 min in the presence of increasing concentrations of L-arginine (0.03–10 mM) caused a dose-dependent increase in nitrite levels, whereas the NOS inhibitor L-NAME (0.03–3 mM) produced a concentration-dependent decrease in endogenous nitrite levels in these cells. Similar results were also obtained at later incubation times (15, 30, and 60 min) with both

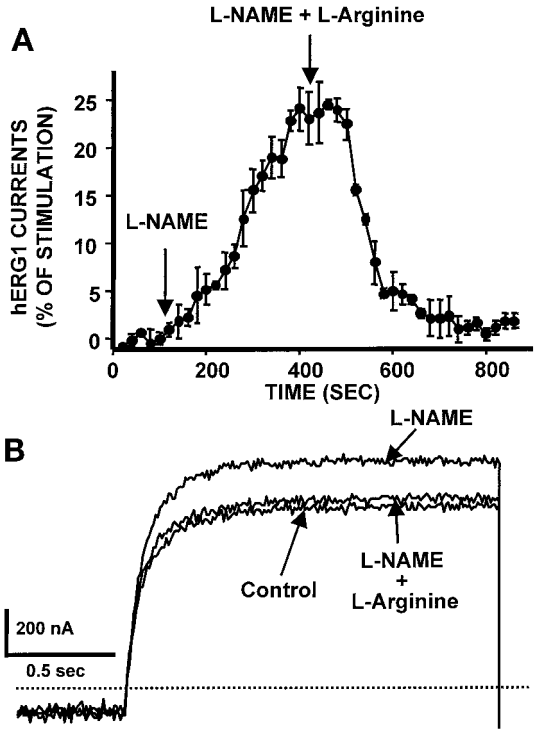

Fig. 2. Reversal of L-NAME-induced stimulation of *hERG1* K^+ currents by L-arginine in *X. laevis* oocytes. A, time course of the reversal of L-NAME-induced stimulation of *hERG1* K^+ currents by L-arginine. *hERG1* outward K^+ current were elicited at 0.05 Hz frequency by means of 1.75-s depolarizing pulses to 0 mV (holding potential, −90 mV; return potential, −100 mV) under both control conditions (5 pulses) and after subsequent superfusion with 1 mM L-NAME or 1 mM L-NAME plus 10 mM L-arginine, as indicated by the respective arrows. The amplitude of the outward K^+ current measured at the end of the depolarizing pulse is expressed as percent of the mean current elicited by the five control pulses. Each experimental point is the mean ± S.E. of four separate experiments. B, reversal of L-NAME-induced stimulation of *hERG1* K^+ currents by L-arginine. Three traces of *hERG1* K^+ currents recorded in the same oocyte in control condition, after superfusion for 5 min with 1 mM L-NAME, and after subsequent 5-min exposure to 1 mM L-NAME plus 10 mM L-arginine are shown superimposed. Holding potential, −90 mV; 1.75-s test potential to 0 mV; return potential, −100 mV. The inward current component elicited on repolarization to −100 mV has been blanked for clarity.

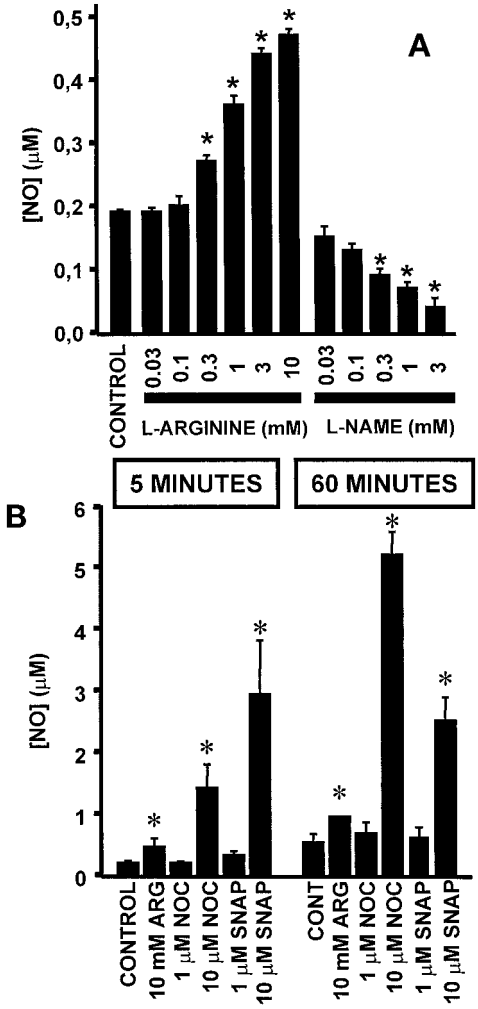

Fig. 3. Modulation of constitutive NO· levels in *X. laevis* oocytes by L-NAME and L-arginine, and comparison with the NO· levels achieved on exposure to NO· donors. A, dose-response for L-arginine-induced stimulation and L-NAME-induced inhibition of constitutive NO· levels in *X. laevis* oocytes. Each experimental point is the mean ± S.E. of three to eight separate experiments performed after 5 min of incubation in each experimental condition. *Significantly different from control values ($p < .05$). B, comparison of the NO· levels reached by L-arginine and NO· donors (SNAP and NOC-18) in *X. laevis* oocytes. Each experimental point is the mean ± S.E. of three to eight separate experiments performed after 5 or 60 min of incubation in each experimental condition. *Significantly different from control values ($p < .05$).

L-arginine and L-NAME (data not shown). These observations suggest that NO$^{\bullet}$ is constitutively produced in *X. laevis* oocytes and that this production can be pharmacologically modulated by exposing the cells either to the NO$^{\bullet}$ metabolic precursor L-arginine or to the NOS inhibitor L-NAME. Figure 3B shows a comparison between the levels of NO$^{\bullet}$ achieved in *X. laevis* oocytes on their exposure to the highest concentration of L-arginine (10 mM) and those obtained with the two NO$^{\bullet}$ donors NOC-18 and SNAP (1 and 10 μM for both compounds). The results show that after both 5 and 60 min of incubation, 10 mM L-arginine induced an increase in NO$^{\bullet}$ levels that was comparable with that achieved with concentrations of the NO$^{\bullet}$ donors between 1 and 10 μM. These measurements also show that the kinetics of NO$^{\bullet}$ release from NOC-18 were significantly slower than those of SNAP. In fact, NO$^{\bullet}$ levels after 60 min of incubation with NOC-18 were about 4 times higher than those achieved after 5 min, whereas similar NO$^{\bullet}$ values were obtained at the same time points with SNAP.

Effects of Four Different NO$^{\bullet}$ Donors on K$^+$ Currents Carried by *hERG1* and Other Cloned K$^+$ Channels Heterologously Expressed in *X. laevis* Oocytes. The observation that exogenously added NO$^{\bullet}$ donors, particularly at concentrations of >10 μM, can achieve NO$^{\bullet}$ levels much higher than those obtained with L-arginine prompted us to investigate the effects of four NO$^{\bullet}$ donors belonging to different chemical classes (SNAP, NOC-18, SNP, and SIN-1) on the K$^+$ currents recorded in *X. laevis* oocytes on heterologous expression of *hERG1* and of other cloned K$^+$ channels. Perfusion of *hERG1*-expressing oocytes for 5 min with SNP (1 mM), SIN-1 (1 mM), NOC-18 (0.3 mM), and SNAP (0.3 mM) inhibited the outward K$^+$ currents at all the potentials tested between −40 and +40 mV (Fig. 4A). By contrast, the inward K$^+$ currents carried by *hERG1* K$^+$ channels were unaffected by the perfusion with NO$^{\bullet}$ donors. In fact, these inward K$^+$ currents, when expressed as a percent of their respective control values, were 103.7 ± 1.7% ($p > .05$, $n = 8$) for 1 mM SNP, 99.6 ± 1.4% ($p > .05$, $n = 6$) for 1 mM SIN-1, 104.4 ± 1.7% ($p > .05$, $n = 7$) for 0.3 mM NOC-18, and 96 ± 3% ($p > .05$, $n = 7$) for 0.3 mM SNAP. The inhibitory effect of NO$^{\bullet}$ donors on the outward K$^+$ currents seemed to be highly specific for *hERG1* channels. In fact, when NOC-18 (0.3 mM) was perfused on *X. laevis* oocytes expressing the other K$^+$ channels, *rNGK2*, *rDRK1*, *bEAG*, *rERG2*, and *rERG3*, no modulatory effect could be detected (Fig. 4B). In addition to NOC-18, SNAP (0.3 mM) failed to affect the K$^+$ currents carried by *rNGK2*, *rDRK1*, *bEAG*, *rERG2*, and *rERG3* (data not shown). Figure 5 shows the dose-responses for the inhibition of the outward I_{hERG1} at the peak value of 0 mV by the four NO$^{\bullet}$ donors. The results obtained show that SNP, SNAP, and NOC-18 induced an inhibition of the outward *hERG1* K$^+$ current that reached a plateau value of 30 to 40% of the total outward current. On the other hand, concentrations of >1 mM SIN-1 could not be studied because 3 mM SIN-1 exerted toxic effects on *X. laevis* oocytes (data not shown). The IC_{50} values for the outward I_{hERG1} inhibition were between 5 and 8 μM for SNP, SNAP, and NOC-18, whereas SIN-1 was ≈50 times less potent. The lower inhibitory potency of SIN-1 on I_{hERG1} inhibition compared with other NO$^{\bullet}$ donors might be related to the ability of SIN-1, in addition to releasing NO$^{\bullet}$, to spontaneously decompose and yield superoxide anions ($O_2^{\bullet -}$) that might reduce the half-life of the NO$^{\bullet}$ formed (Southam and Garthwaite, 1991) or exert opposite effects on *hERG1* outward currents (Taglialatela et al., 1997), as further discussed later. It should be emphasized that the extent of inhibition of *hERG1* outward K$^+$ currents induced by IC_{50} values of NOC-18 and SNAP (approximately 15–20% of inhibition) is comparable with that achieved with 10 mM L-arginine and that, interestingly, these concentrations of the NO$^{\bullet}$ donors released an amount of NO$^{\bullet}$ that was comparable with that achieved with the maximal concentration of the NO$^{\bullet}$ precursor, as previously shown in Fig. 3B.

Biophysical Mechanism of NO$^{\bullet}$-induced Inhibition of *hERG1* Outward K$^+$ Currents. The selective modulation exerted by NO$^{\bullet}$ on the outward K$^+$ currents carried by *hERG1* channels, with no modification of the inward currents, was already suggestive of an interference of this gaseous mediator with the peculiar inactivation mechanism characteristic of *hERG1* channels. This rapid and voltage-dependent inactivation process reduces the conductance at positive potentials (>0 mV), therefore being responsible for the inward rectification occurring within this voltage range (Sanguinetti et al., 1995; Smith et al., 1996). To study the

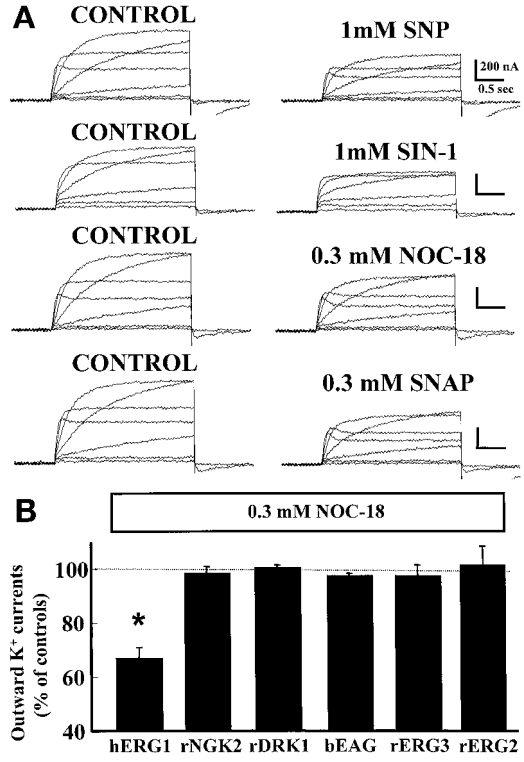

Fig. 4. Selective effect of four NO$^{\bullet}$ donors on K$^+$ currents elicited by *hERG1* channels heterologously expressed in *X. laevis* oocytes. A, inhibition of *hERG1* outward K$^+$ currents by 1 mM SNP, 1 mM SIN-1, 0.3 mM NOC-18, and 0.3 mM SNAP. K$^+$ currents were recorded in the same oocyte under control conditions and after superfusion with the indicated NO$^{\bullet}$ donor. Holding potential, −90 mV; 1.75-s test potentials from −80 mV to +40 mV (except for SIN-1, in which the steps were from −80 to +20 mV) in 20-mV steps; return potential, −100 mV. The inward current component elicited on repolarization to −100 mV has been blanked for clarity. B, selective modulation of *hERG1* K$^+$ channels by 0.3 mM NOC-18. The percentage variation of the outward K$^+$ current induced by 5-min perfusion with 0.3 mM NOC-18 ($I_{NOC-18}/I_{control}$) at voltages that fully activated the conductance for each K$^+$ channel (0 mV for *hERG1* and *rERG3*; +40 mV for *bEAG*, rNGK2, and rDRK1, and +20 mV for *rERG2*) are reported. Each experimental point is the mean ± S.E. of three to eight separate experiments. *Significantly different from control values ($p < .05$).

effect of NO· on this fast inactivation process, we used a voltage protocol that took advantage of the kinetic difference existing between the fast recovery from inactivation and the slower channel deactivation occurring at hyperpolarized potentials. The voltage protocol was the following: the channels were first opened and inactivated by a 2-s depolarizing pulse at 0 mV, recovered from inactivation by means of 25-ms conditioning pulses from −120 to +60 mV, and finally depolarized again to +20 mV. Even though the duration of the conditioning pulses using this protocol might not have been long enough to be considered steady state, longer conditioning pulses could not be used due to the occurrence of substantial channel deactivation, particularly at negative potentials (Smith et al., 1996). Perfusion with the long-lasting NO· donor NOC-18 (0.3 mM) caused a -14.4 ± 1.7 mV ($n = 5, p < .05$ versus controls) shift of the *hERG1* voltage dependence of inactivation (Fig. 6). On the other hand, the activation properties of the *hERG1* K^+ channels were unaffected by 0.3 mM NOC-18 (Fig. 6). Even if the −14-mV shift of the *hERG1* inactivation curve induced by NOC-18 could be in principle accounted for by changes in deactivation kinetics (particularly at very negative potentials), it should be emphasized that the channel deactivation proceeds very slowly at around the midpoint potential of the steady-state inactivation curve (≈−60 mV), having a time constant of several seconds. Therefore, the NOC-18-induced changes in the inactivation process could not be accounted for by a slight modification of the deactivation kinetics. The product of steady-state activation and inactivation curves gives the steady-state current-to-voltage relationship. Figure 6 (inset) shows this product for both controls and NOC-18-treated groups. The results indicate that the negative shift of the steady-state inactivation curve of *hERG1* channels induced by NOC-18, in the absence of any modification of the steady-state activation curve, qualitatively reproduced the 40% inhibition of the current-to-voltage relationship of the outward *hERG1* K^+ currents.

Effect of NO· Scavengers on NO· Donor-Induced *hERG1* K^+ Current Inhibition and Possible cGMP-Mediated Effect of NO· on Outward *hERG1* K^+ Currents. Figure 7A shows that 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO, 0.3 mM), a stable radical compound that has been shown to exert a potent scavenging action against NO· via a radical-radical reaction (Akaike et al., 1993), was also able to prevent the inhibitory action exerted by NOC-18 (0.3 mM), SNAP (0.3 mM), and SIN-1 (1 mM) on outward *hERG1* K^+ currents. Furthermore, the effects of NOC-18 (0.3 mM) were also prevented by the simultaneous presence of 10 μM hemoglobin (Hb), a heme-containing protein that, among various radical species, is known to bind and inactivate NO· (Yu, 1994). This suggests a direct participation of NO· in this biological phenomenon.

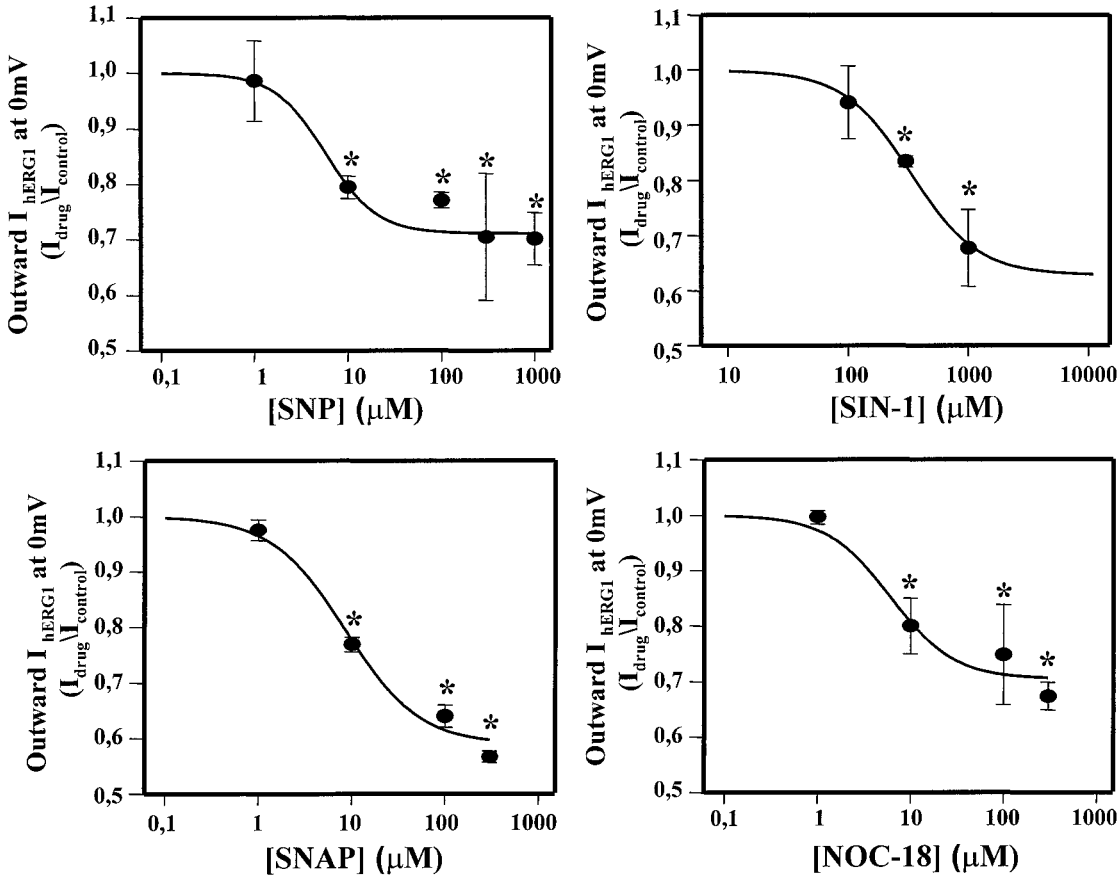

Fig. 5. Dose-response curves for *hERG1* K^+ channels inhibition by NO· donors in *X. laevis* oocytes. The outward *hERG1* K^+ currents recorded at the end of depolarizing pulses to 0 mV after the exposure to different drug concentrations were normalized to the control value and expressed as a function of each drug concentration. The solid lines represent the fits of experimental data to the following binding isotherm: $y = \max/(1 + X/IC_{50})^n$, where X is the drug concentration and n is the Hill coefficient. Fitted values for n were between 1.1 and 1.5. Each point is the mean ± S.E. of three to six determinations. *Significantly different from control values ($p < .05$).

It is widely recognized that NO· can affect cellular functions by exerting both cGMP- and non-cGMP-mediated effects (Gross and Wolin, 1995). Considering that the K^+ channels belonging to the *ERG* subfamily have a consensus sequence for cyclic nucleotides in their C terminus (Warmke and Ganetzky, 1994) and that the functional role played by cyclic nucleotides in the modulation of ERG K^+ channels is currently unknown, it was thought to be relevant to investigate whether cGMP mediated the inhibitory effects exerted by NO· on *hERG1* K^+ channels. To this aim, *hERG1*-expressing *X. laevis* oocytes were perfused with 1 mM 8-bromo-cGMP, a membrane-permeable cGMP analog that mimics the intracellular actions of cGMP in many cellular systems (Brüggemann et al., 1993). After a 20-min incubation time, no significant change on *hERG1* outward K^+ currents (Fig. 7B), as well as on any biophysical property of the channel (voltage dependence of activation, kinetics of activation and deactivation, inward rectification), was detected. Furthermore, the effects of a specific inhibitor of the NO·-dependent guanylyl cyclase, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ; Garthwaite et al., 1995), on both resting and NOC-18-inhibited outward *hERG1* K^+ currents were evaluated. Perfusion with 50 μM ODQ failed to affect resting outward *hERG1* K^+ currents and, more importantly, did not prevent their inhibition by 0.3 mM NOC-18 (Fig. 7B).

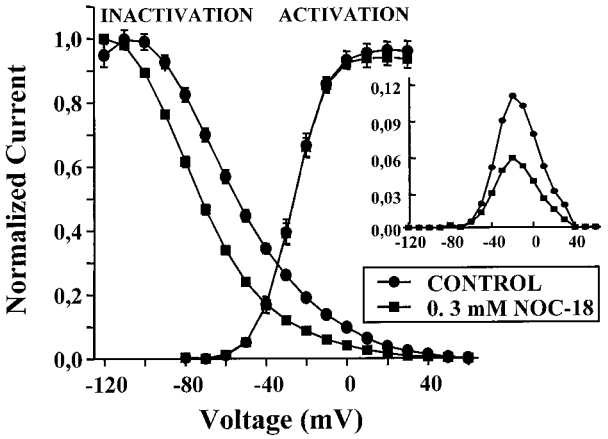

Fig. 6. Modulation of *hERG1* channel activation and inactivation by NOC-18 in *X. laevis* oocytes. Steady-state inactivation and activation curves of *hERG1* K^+ channels in control condition and on NOC-18 exposure. The same *hERG1*-expressing oocytes were recorded in control condition and after 5-min exposure to 0.3 mM NOC-18. For the inactivation curves, the following protocol was used: holding potential −90 mV, 1.75-s depolarizing steps to 0 mV, 25-ms conditioning pulses from −120 to +60 mV in 10-mV increments, and 200-ms test potential to +40 mV. The currents were measured on extrapolation of the outward current elicited by the +20-mV test pulse to time 0′ and plotted versus the membrane voltage of the conditioning pulses. The experimental data were fitted to the following form of the Boltzmann equation: $gK_v = \max/(1 + \exp(V_{1/2} - V)/k)$, where V is the test potential, $V_{1/2}$ is the half-activation potential, and k (or kT/ze) is the slope of the conductance-to-voltage relationship. The values for $V_{1/2}$ for the inactivation curve were -54.8 ± 1.4 ($n = 5$) and -71.2 ± 1.3 ($n = 5$) in controls and NOC-18 conditions, respectively ($p < .05$). The values for $V_{1/2}$ for the activation curve were, respectively, -26.7 ± 1.7 ($n = 3$) and -27 ± 1.5 ($n = 3$) in controls and after NOC-18 exposure ($p > .05$). Inset, product of the fitted steady-state activation and inactivation curves at each potential.

Effect of Endogenous or Pharmacologically Delivered NO· on the Increase in *hERG1* Outward K^+ Currents and on Membrane Iron- and Ascorbate-Containing Solution (Fe/Asc)-Induced Lipid Peroxidation. Recent evidence from our laboratory has shown that ROS can specifically modulate *hERG1* K^+ channels (Taglialatela et al., 1997). In fact, an enhancement of ROS production, achieved by activation of the Fenton reaction in the presence of Fe/Asc (Yu et al., 1994), increases *hERG1* outward K^+ currents. On the other hand, a decrease in ROS levels induced by ROS scavengers can prevent the increase in *hERG1* outward K^+ currents determined by oxidative stress and inhibits the same K^+ currents under resting conditions. The occurrence of this modulation by ROS on *hERG1* K^+ channels prompted us to investigate whether the effects of NO· may have been interpreted as a consequence of its interaction with ROS. For this purpose, we studied the effects of the NO· donor NOC-18 (0.3 mM) on *hERG1* outward K^+ currents activated by oxidative stress. As shown in Fig. 8, A and B, 0.3 mM NOC-18 completely counteracted the stimulatory effect of the Fe/Asc ROS-generating system. Similar effects could

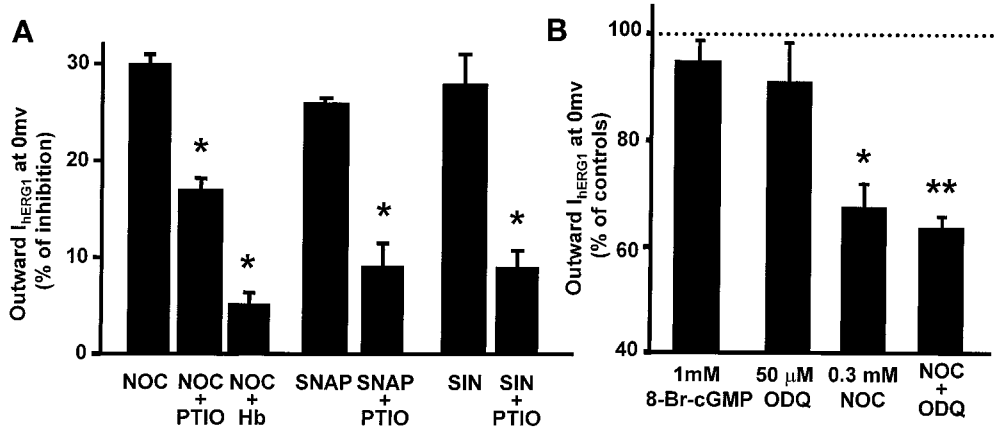

Fig. 7. Effect of the NO·-scavenging compounds PTIO and Hb, as well as of 8-bromo-cGMP and ODQ, on resting and NO· donor-induced inhibition of I_{hERG1} in *X. laevis* oocytes. A, reversal of NO· donor-induced inhibition of *hERG1* K^+ currents by the NO· scavengers PTIO and Hb. The percentage of variation of the *hERG1* outward K^+ current at the end of a 0-mV depolarizing pulse caused by 5-min perfusion with 0.3 mM NOC-18, 0.3 mM NOC-18 plus 0.3 mM PTIO, or 0.3 mM NO·C-18 plus 10 μM Hb are reported. Each experimental point is the mean ± S.E. of three to eight separate experiments. *Significantly different from the NO· donor-treated group ($p < .05$). B, lack of effect of 8-bromo-cGMP and ODQ on *hERG1* outward K^+ currents. The currents measured at the end of 2-s depolarizing pulses to 0 mV in each experimental condition (20-min perfusion with 1 mM bromo-cGMP; 5-min perfusion with 50 μM ODQ, 0.3 mM NOC-18, or 50 μM ODQ plus 0.3 mM NOC-18) were normalized and expressed as percent of the respective control value. Each point is the mean ± S.E. of four to nine determinations in separate cells. *Significantly different from control values ($p < .05$). **NOC + ODQ group is not significantly different from the NOC group ($p > .05$).

also be detected when different NO• donors such as SNAP (0.3 mM) or SIN-1 (1 mM) were used (data not shown). In addition, the pharmacological modulation of endogenous NO• levels achieved by L-arginine perfusion was able to counteract the increase in *hERG1* outward K^+ currents elicited by Fe/Asc perfusion. In fact, Fig. 9A shows the outward currents elicited by depolarizing pulses to 0 mV in a *hERG1*-expressing oocyte subsequently exposed to the following experimental conditions: control, 25/50 μM Fe/Asc (5 min), 25/50 μM Fe/Asc plus 10 mM L-arginine (5 min), and washout (10 min). The time course of the same experiment averaged in four different cells is reported in Fig. 9B. To more directly test the hypothesis that NO•, either endogenously produced or exogenously released from NO• donors, might interfere with the oxidating process promoted by Fe/Asc, we measured the effects of the pharmacological modulation of endogenous NO• levels in *X. laevis* oocytes on resting and Fe/Asc-enhanced intracellular MDA production, a direct index of lipid peroxidation. The results obtained showed that both L-arginine (10 mM) and the NO• donor NOC-18 (0.3 mM) inhibited resting MDA production (Fig. 10). By contrast, L-NAME (1 mM), which decreased endogenous NO• levels in *X. laevis* oocytes (Fig. 3A), enhanced MDA content in the same cells, and this enhancement, in accordance to the results of the electrophysiological experiments shown in Fig. 2, was reversed by the simultaneous presence of L-arginine (10 mM). Furthermore, both L-arginine (10 mM) and NOC-18 (0.3 mM) completely counteracted the stimulatory effect of Fe/Asc (0.1/0.2 mM) on the levels of the lipid peroxidation product. Similar results were obtained using other NO• donors (SNAP and SIN-1; data not shown).

Discussion

The results of the present study show that the outward K^+ currents carried by *hERG1* channels heterologously expressed in *X. laevis* oocytes are inhibited by both endogenously produced and pharmacologically delivered NO•. The existence of a tonic modulation of outward *hERG1* K^+ currents by NO• is revealed by the observation that L-NAME, a specific inhibitor of NOS, potentiated depolarization-activated outward *hERG1* K^+ currents by reducing NO• levels, whereas L-arginine inhibited them by increasing endogenous NO• production. The extent of *hERG1* channel inhibition by L-arginine reached a maximal value of ≈15% of the control current, whereas NO• donors such as SNP, SIN-1, NOC-18, and SNAP, which were able to produce higher concentrations of NO• compared with L-arginine, exerted a stronger inhibition of outward *hERG1* K^+ currents. In fact, the dose-response analysis of the effect of NO• donors revealed that the degree of maximal inhibition of *hERG1* K^+ channels was ≈30 to 40%.

The observation that the four NO• donors SNP, SIN-1, NOC-18, and SNAP, which are chemically dissimilar and whose only common denominator is their ability to release NO•, all inhibit *hERG1* outward K^+ current suggests that this modulation is mediated by NO•, rather than being a consequence of the activation of other NO•-independent mechanisms. This view seems to be confirmed by the reversal of the inhibitory effect exerted by NO• donors on *hERG1* K^+ channels by the NO• scavengers PTIO and Hb. Furthermore, evidence that increasing or reducing endogenous NO• levels with the precursor or the synthesis inhibitor reproduced effects on *hERG1* K^+ currents that are the same or the opposite of the NO• donors, respectively, reinforces the hypothesis that NO• is the mediator of *hERG1* modulation exerted by these compounds.

In addition, the present study shows that the inhibitory effect of NO• on *hERG1* K^+ channels appeared to be highly selective for this K^+ channel subtype. In fact, other cloned voltage-dependent K^+ channels only distantly related to *hERG1*, such as *rNGK2*, *rDRK1*, and *bEAG*, or belonging to the same gene family of *hERG1*, such as *rERG2* and *rERG3*, do not display any sensitivity to the modulation by NO• donors.

The inhibition of *hERG1* outward K^+ currents induced by NO• in the present study can be accounted for by several

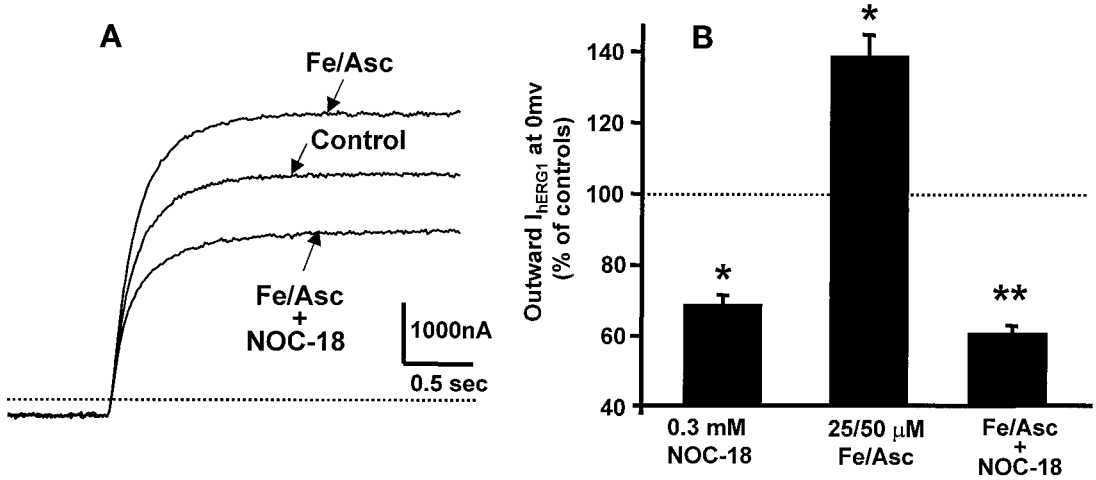

Fig. 8. NOC-18 prevents the stimulation of the *hERG1* outward K^+ currents by ROS in *X. laevis* oocytes. A, representative current traces of the effects of Fe/Asc and Fe/Asc plus NOC-18 on *hERG1* outward currents. The same oocyte was subsequently perfused for 5 min with control solution, Fe/Asc (25 μM/50 μM), and Fe/Asc (25/50 μM) plus NOC-18 (0.3 mM). A single HERG1 outward currents trace in response to a 0-mV depolarization in each experimental condition is shown. B, effect of NOC-18, Fe/Asc, and Fe/Asc plus NOC-18 on *hERG1* outward K^+ currents. Reported is the percentage of variation induced by the indicated experimental conditions (5-min perfusion) on the *hERG1* outward current measured at the end of a depolarizing pulse to 0 mV. Each point is the mean ± S.E. of four to nine determinations in separate cells. *Significantly different from control values ($p < .05$).). **Fe/Asc + NOC-18 group is significantly different from the controls and Fe/Asc groups ($p > .05$).

biophysical mechanisms: 1) changes in the gating voltage dependence, 2) decreased single-channel conductance, and 3) a reduction in the number of functional channels. The results of the present study suggest that NO• interferes with the first of these mechanisms because it causes a −14-mV shift in the voltage dependence of inactivation without any significant change in the current kinetics or steady-state voltage dependence of activation. This evidence suggests that when NO• levels are increased, *hERG1* channels enter into a nonconductive inactivated state on membrane depolarization. On the other hand, the fact that NO• does not inhibit the inward component of *hERG1* K^+ currents is due to the previously described hyperpolarizing shift in the voltage dependence of inactivation. In fact, at hyperpolarized potentials where inward currents are recorded, *hERG1* K^+ channels undergo a fast recovery from inactivation, thus preventing any effect of the NO• at these negative values of membrane potential. This biophysical mechanism has considerable analogies with that involved in the inhibitory action of NO• donors on neuronal voltage-dependent Na^+ channels (Li et al., 1998).

Despite the presence of a consensus domain for cyclic nucleotide binding in *hERG1* C-terminal sequence (Warmke and Ganetzky, 1994), the inhibitory effects exerted by NO• donors on *hERG1* K^+ channels seem not to be the consequence of a NO•-dependent activation of the soluble form of guanylyl cyclase and of an increased production of intracel-

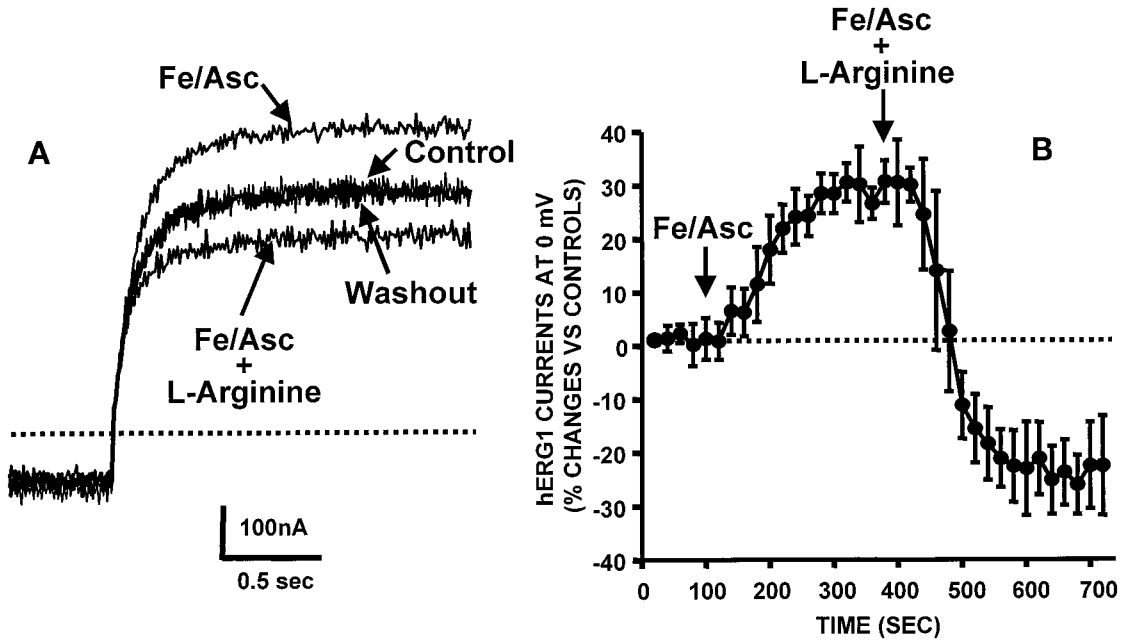

Fig. 9. L-Arginine prevents the stimulation of the *hERG1* outward K^+ currents by ROS in *X. laevis* oocytes. A, representative current traces of the effects of Fe/Asc and Fe/Asc plus L-arginine and washout on *hERG1* outward currents. The same oocyte was subsequently perfused for 5 min with control solution, Fe/Asc (25/50 μM), and Fe/Asc (25/50 μM) plus L-arginine (10 mM), and washout. A single *hERG1* outward current trace in response to a 0-mV depolarization in each experimental condition is shown. B, time course of the reversal by L-arginine of the Fe/Asc-induced stimulation of *hERG1* K^+ currents. *hERG1* outward K^+ current were elicited at 0.05-Hz frequency by means of depolarizing pulses to 0 mV in control conditions (five pulses), after superfusion Fe/Asc (25/50 μM), and after subsequent perfusion with Fe/Asc (25/50 μM) plus L-arginine (10 mM), as indicated by the respective arrows. The amplitude of the outward K^+ currents measured at the end of depolarizing pulse is expressed as percent of the mean current elicited by the five control pulses. Each experimental point is the mean ± S.E. of four separate experiments.

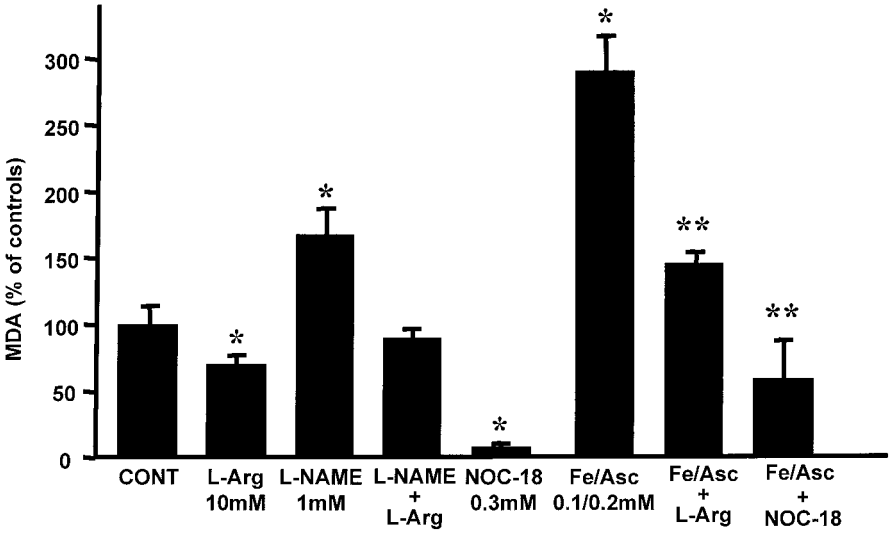

Fig. 10. Effects of L-arginine, L-NAME, and NO• donors on resting and Fe/Asc-induced lipid peroxidation in *X. laevis* oocytes. Each experimental point is the mean ± S.E. of 4 to 16 determinations performed in triplicate. *Significantly different from control values ($p < .05$). **Fe/Asc plus L-arginine and Fe/Asc plus NOC-18 groups are significantly different from the Fe/Asc group ($p < .05$). The MDA content in control group was 6.7 ± 0.9 pmol/mg protein/2 h.

lular cGMP. In fact, the membrane-permeable cGMP analog 8-bromo-cGMP (Brüggemann et al., 1993) did not reproduce the inhibitory modulation of outward *hERG1* K^+ currents observed with the NO• donors and the NO• synthesis precursor. Furthermore, the specific inhibitor of the NO•-dependent guanylyl cyclase ODQ (Garthwaite et al., 1995) failed to affect *hERG1* outward K^+ currents and was unable to prevent their inhibition induced by NOC-18. On the other hand, several of the described effects exerted by NO• or NO• donors on various ion channels, including the *N*-methyl-D-aspartate subtype of glutammate receptors in striatal and cerebellar neurons (Manzoni et al., 1992), the calcium-activated K^+ currents in ciliary ganglia neurons (Cetiner and Bennett, 1993), the L-type Ca^{2+} channels in cardiac myocytes (Campbell et al., 1996), and the tetrodotoxin-sensitive and -insensitive neuronal Na^+ channels (Li et al., 1998), appear to be at least in part independent of changes in intracellular cyclic nucleotide levels.

The cGMP-independent signals of NO• have been typically grouped under the broad heading of "redox" effects (Stamler et al., 1997) to emphasize the fact that depending on the redox state of the environment, various RNS can be produced with different biological actions and potential toxicity (Stamler et al., 1992). Recent results from our laboratory suggest that *hERG1* K^+ channels are influenced by the cell redox status (Taglialatela et al., 1997). In fact, an enhancement of ROS production promoted by oxidative conditions increases *hERG1* outward K^+ currents, whereas a decrease in ROS levels achieved by ROS scavengers (catalase, in particular) can inhibit these K^+ currents and prevent their increase induced by oxidating conditions. The analogies between the actions of ROS scavengers and of NO• at the level of *hERG1* channels are evident in several respects: 1) both ROS scavengers and NO• selectively inhibit *hERG1* channels, with no effect on any other cloned K^+ channel investigated; 2) they both specifically modulate the outward, and not the inward, current component; and 3) the maximal degree of channel modulation is similar with ROS scavengers and with increased NO• levels (≈30–40% of inhibition). The analogies of ROS scavengers and of NO• actions in the modulation of *hERG1* K^+ currents prompted us to investigate whether NO• could interfere with ROS levels in resting redox conditions or during oxidative stress (Yu et al., 1994). The fact that NO• completely counteracted the enhancement of *hERG1* outward K^+ currents elicited by oxidative stress suggested an interaction between RNS and ROS. This hypothesis was confirmed by the results showing that L-arginine and NO• donors can inhibit lipid peroxidation occurring in oxidating conditions. Therefore, it seems likely that in the present experimental model, NO• may exert potent antioxidant effects (Kanner et al., 1991; Yu et al., 1994). This interplay between RNS and ROS in the modulation of *hERG1* K^+ channels seems to not be restricted to conditions of oxidative stress but also to play a relevant role in resting conditions. In fact, a reduction in endogenous NO• levels with the synthesis inhibitor L-NAME increased resting lipid peroxidation products and potentiated outward of *hERG1* K^+ currents, whereas the increase of endogenous NO• levels achieved by the NO• synthesis precursor L-arginine inhibited both resting lipid peroxidation and *hERG1* K^+ channel activity. Altogether, the present results emphasize the possible physiological role exerted by NO• and ROS levels in the control of the activity of this K^+ channel. The antioxidant effects of NO• have also been demonstrated in other experimental systems; in fact, NO• has been shown to block lipid peroxidation induced by hypoxantine-xantine oxidase and peroxynitrite in phosphatidylcholine liposomes (Rubbo et al., 1994), to inhibit low-density lipoprotein oxidation (Goss et al., 1999), and to prevent superoxide anion production by neutrophils (Clancy et al., 1992). These antioxidant properties of NO• have been interpreted as a consequence of its ability to interact with hydroxyl, alkoxyl, and peroxyl radicals, thus terminating lipid radical chain propagation reactions (Rubbo et al., 1994); in addition, the ability of physiological concentrations of NO• to inhibit lipid peroxidation in vivo has been recently proposed (O'Donnell et al., 1997). Furthermore, the antioxidant effects of NO• have been implicated in the reported ability of NO• donors to protect against cellular damage and cytotoxicity from reactive oxygen species in V79 Chinese hamster lung fibroblasts (Wink et al., 1993). Finally, although NO• has been reported to exert both protective and destructive effects in pathological events associated with ischemia/reperfusion, the inhibition of oxidant-related mechanisms promoted by the stimulation of endogenous NO• production or by the exogenous administration of NO• has been proposed to explain the possible neuroprotection afforded by NO• (Iadecola, 1997). On the other hand, although the most likely explanation for the *hERG1* K^+ channel modulation by NO• described in the present study seems to involve its ability to interfere with ROS action, which in turn influences the activity of the K^+ channels, the existence of additional mechanisms by which NO• directly interferes with the *hERG1* K^+ channel protein cannot be completely ruled out.

The described NO•-dependent modulation of the outward K^+ currents mediated by *hERG1* channels has been presently studied in an amphibian heterologous expression system, which allows the recording of macroscopic K^+ currents in isolation without perturbing the intracellular environment. Nevertheless, the present results showing that the interplay between ROS and RNS is crucial in determining the amount of outward K^+ current flowing through *hERG1* K^+ channels may have considerable relevance for the understanding of the early events leading to ischemia-related toxicity in excitable tissues, which are accompanied by characteristic changes in membrane potential mainly controlled by K^+ channels. On the basis of the present results, further work will be needed to clarify the involvement of *hERG1*-encoded K^+ channels as targets of ROS and RNS in the pathophysiology of cardiac and brain ischemia and to evaluate the possible protective effect exerted by available drugs that are able to modulate their function.

Acknowledgments

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