PHYSIOLOGY

Role of Nitric Oxide in Activity Control of Mechanically Gated Ionic Channels in Cardiomyocytes: NO-Donor Study

V. E. Kazanski, A. G. Kamkin, E. Yu. Makarenko, N. N. Lysenko, P. V. Sutiagin*, Tian Bo, and I. S. Kiseleva

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Whole-cell ionic currents through mechanically gated chnnels (MGC) were recorded in isolated cardiomyocytes under voltage clamp conditions. In unstrained cells, NO donors SNAP and DEA-NO activated MGC and induced MG-like currents. In contrast, in stretched cells with activated MGC, these NO-donors inactivated and inhibited MGC.

Key Words: *isolated cardiomyocytes; whole-cell ionic currents; mechanically gated channels; NO-donors*

Mechanically gated channels (MGC) and especially the cation-nonselective MGC activated during stretching of the cell membrane play the key role in the realization of the mechanoelectric feedback to the heart [4]. Some mechanically gated MG-currents carried out by potassium ions through other channels also contribute to this process [2]. In cardiomyocytes, MGC were extensively studied both under normal [3,5,6] and pathological [8] conditions. However, the possibility of direct pharmacological regulation of MGC conductance was never studied.

Some studies substantiated the hypothesis that NO can be directly involved in the control of MGC conductance. For example, NO affects myocardial contractility and heart rate (HR): in low concentrations, NO-donors increase myocardial contractility and HR, while in high concentrations they exert opposite effects [8,10-12].

Here we used NO-donors for evaluation of the role of NO as a possible regulator of MGC in isolated mouse, rat, and guinea pig cardiomyocytes.

Department of Fundamental and Applied Physiology, *Department of Morphology, Medico-Biological Faculty, N. I. Pirogov Russian State Medical University, Moscow, Russia. ***Address for correspondence:*** kamkin.a@g23.relcom.ru. A. G. Kamkin

MATERIALS AND METHODS

Experiments were carried out on individual cardiomyocytes isolated from the left cardiac ventricle of C57B1 mice, Wistar rats, and guinea pigs. The cardiomyocytes were routinely isolated according to Langendorf technique [4,5]. The isolated cells were placed in an experimental chamber (0.5 ml, 37°C, solution exchange time 15 sec) and perfused with a solution containing (in mM) 150.0 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 20.0 glucose, 5.0 HEPES/NaOH (pH 7.4). The micropipettes were filled with the following solution (in mM): 140.0 KCl, 5.5 $MgCl_2$, 5.0 Na_2ATP, 0.05 EGTA, 10 HEPES/KOH (pH 7.2). After attaching the micropipette to the cell membrane and producing the whole-cell configuration, another micropipette with heat-smoothed tip was placed at a distance of 40 μ from the first one, which also adhered to the membrane. This smoothed micropipette was used to gradually stretch the cell with the help of a MP-285 motorized micromanipulator (Sutter, Novato, CA) equipped with a step motor with the resolution of 0.2 μ [4]. The standard 10-μ stretch was used.

The currents were recorded with a RK-300 patch-amplifier (Biologic, France) and processed with a

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PowerCED system (Cambridge Instruments). To assess the dependence of ionic currents on membrane potential, we used the current-voltage plots (I-V curves). For inactivation of Na_V-channels, the holding potential V_{hp} was set at -45 mV. To exclude the contribution of Ca_V-channels into total ionic current, the membrane potential was clamped initially at 50 mV for 50 msec, thereupon the cells were repolarized from +50 mV to -100 mV at a rate of -100 mV/sec (ramp-like repolarization) [2]. The ionic current is zero in the intersection point of I-V plot with potential axis (abscissa); the corresponding potential is equivalent to diastolic membrane potential V_0 usually measured in the current-clamp mode. The hump in I-V plot at -60 mV is usually explained by I_{K1} current flowing across K_{ir}-channels. At potentials more negative than -20 mV, the membrane currents flow mainly through DG_{K1} channels and cation non-selective MGC-coupled

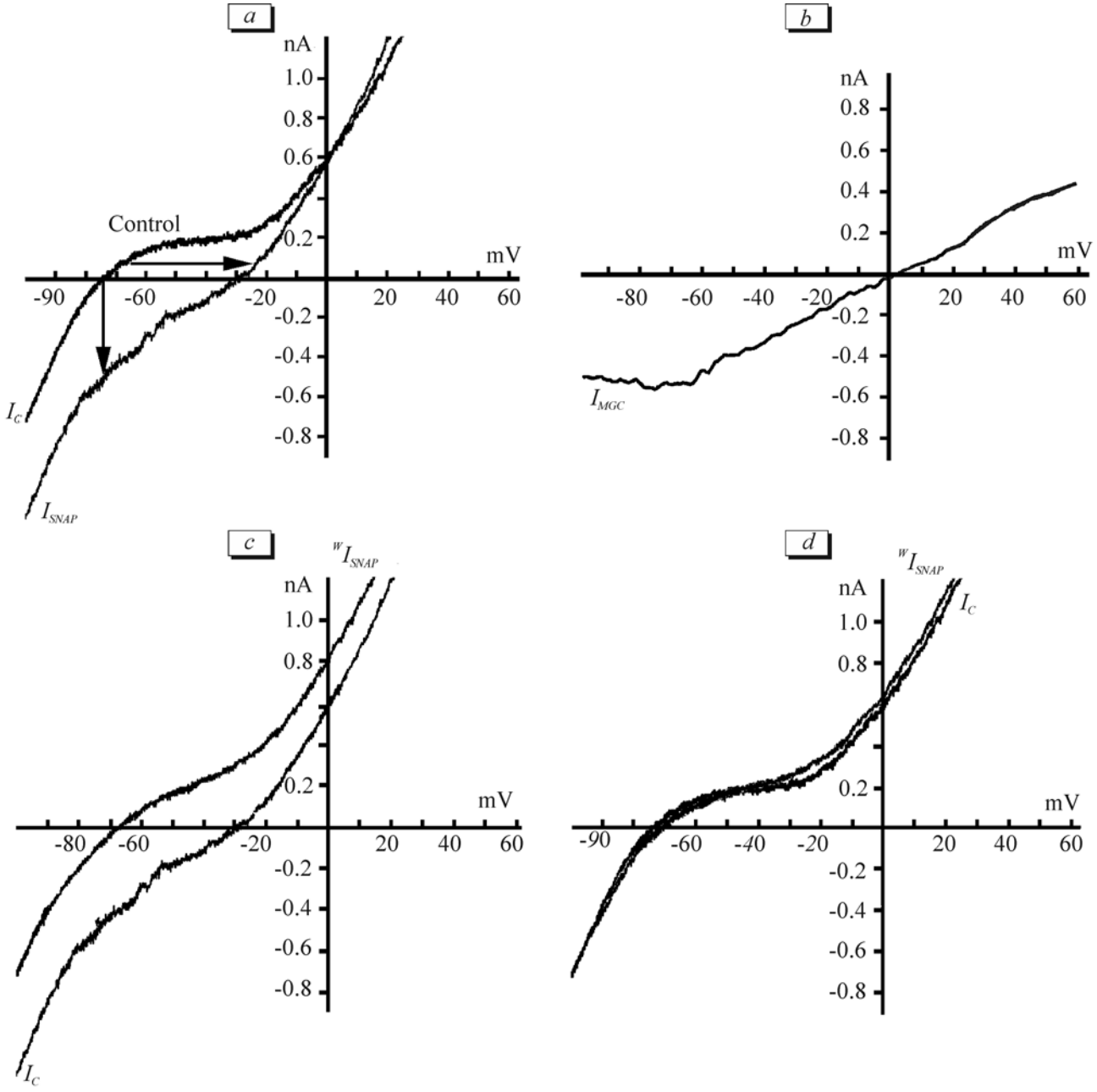

Fig. 1. Current-voltage (I-V) curves of cardiomyocyte membrane during perfusion of unstrained cell from mouse left ventricle with SNAP solution. *a*) control I-V curve (I_C) and I-V curve 2 min after the start of SNAP perfusion (I_{SNAP}). Arrows show directions of the SNAP-induced shift of I-V plot. *b*) I_{MGC} 2 min after the start of SNAP perfusion of stretched cell; *c*) I-V curve after a 2-min washing from SNAP ($^{W}I_{SNAP}$) in comparison with control curve I_C; *d*) I-V curve after a 5-min washing from SNAP ($^{W}I_{SNAP}$) in comparison with control curve I_C.

DG_{ns} channels. At potentials more positive than -20 mV, MG potassium currents are carried out predominantly across TRPC6, TREK1 ($K_{2P}2.1$), and TREK2 ($K_{2P}10.1$) channels [1].

The data were processed statistically with ANOVA software and Bonferroni test at $p<0.05$. The results were presented as the mean and standard error of the mean.

RESULTS

In the first series of experiments, we used SNAP (S-nitroso-N-acetylpenicillamine, 100 μM) as NO donor. Figure 1, *a* shows I-V plots of an unstrained cardiomyocyte in the control solution (N-shaped I_C curve with V_0=-85±4 mV, n=8) and after 2-min perfusion of the cell with SNAP solution (I_{SNAP}). SNAP induced MG-like current, which shifted V_0 to -28±4 mV as soon as after 2 min (n=8). This MG-like current is similar to the current flowing across MGC in response to cell stretching by 10 μ. Figure 1, *b* shows the differential current $I_{MGC}=(I_{SNAP}-I_C)=I_{SNAP-C}$ appearing during SNAP perfusion, which is equal to -0.38 nA ($I_{MGC(-45\ mV)}$=-0.39±0.02 nA, E_{rev}=1±1 mV, n=8). Linearity of differential curve I_{SNAP-C} is related to DG_{ns}, while its deviation from the

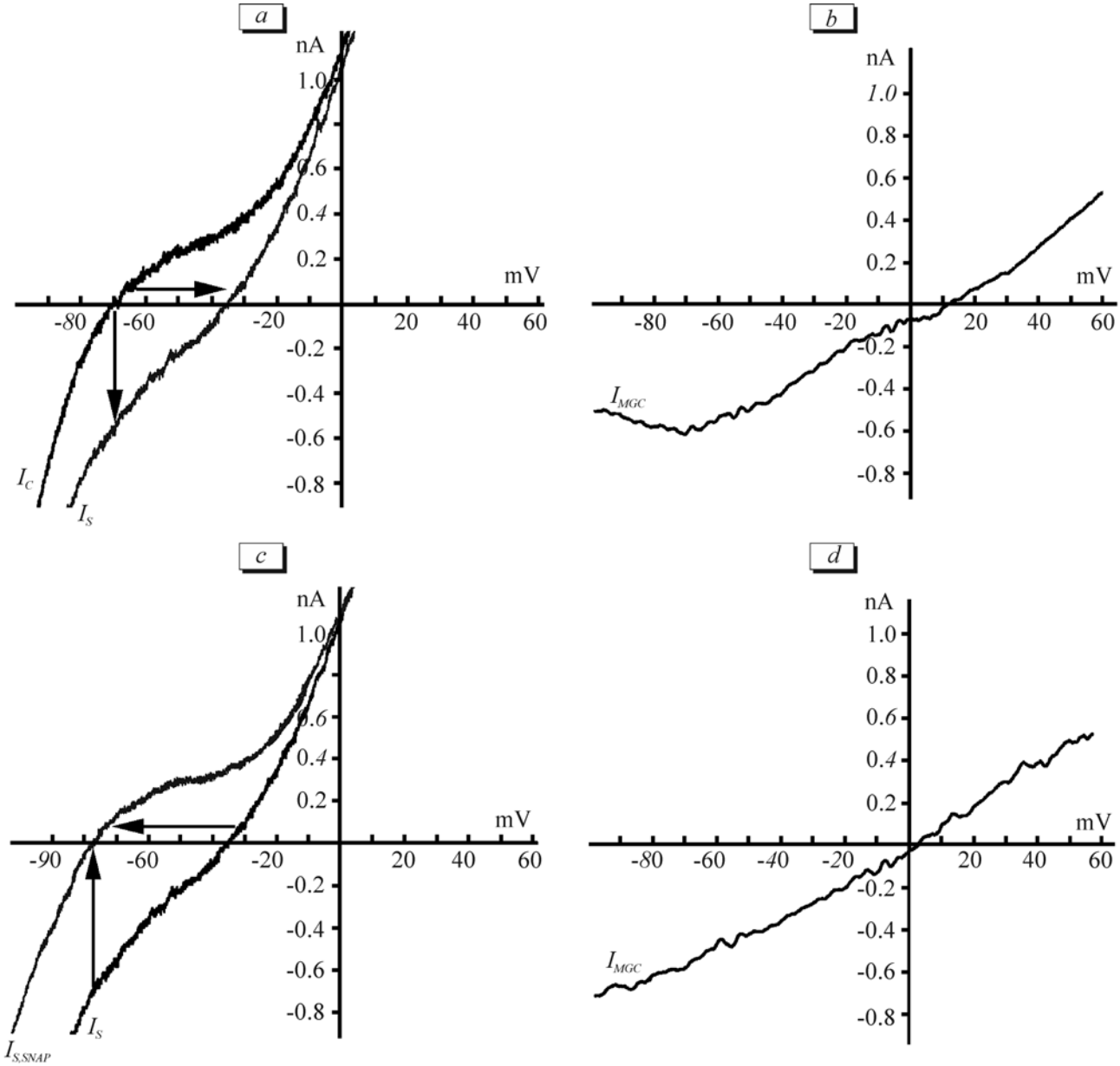

Fig. 2. Inhibitory effect of SNAP on MGC-currents in stretched cardiomyocyte. *a*) control I-V curve (I_C) and I-V curve of the deformed cell (I_S). Arrows show directions of stretch-induced shift of I-V plot; *b*) differential current I_{MGC}, activated by stretching the cell (I_{S-C}); *c*) modification of I-V curve of the deformed cell (I_S) by SNAP perfusion for 3 min ($I_{S,SNAP}$). Arrows show directions of SNAP-induced shift of I-V plot of the deformed cell (I_S); *d*) differential current I_{MGC}, activated by stretching the cell before SNAP perfusion for 3 min.

straight line can result from mechanically induced deactivation of G_{K1} channels. Washing the cell with normal solution rapidly (within 2 min) reduced the effect of SNAP (Fig. 1, *c*), and in 5 min the I-V plot ($^{W}I_{SNAP}$) virtually coincided with the control curve (Fig. 1, *d*). Since perfusion with SNAP in the absence of mechanical deformation of the cell induced MG-like current, we believe that the presence of NO is critical for MGC, and elevation of exogenous NO is sufficient for activation of these channels.

At the next stage, preliminary stretched cell was perfused with SNAP solution. Figure 2, *a* shows the control current I_C and the ionic current of stretched membrane I_S. Evidently, prior to stretching, I-V curve was N-shaped (V_0=-85±4 mV, *n*=8). Mechanical deformation shifted the curve to more positive potentials (equivalent to depolarization under current clamp conditions or in unclamped cell) and changed V_0 to -40+5 mV (*n*=8). The differential current $I_{MGC}=I_{S-C}$ was -0.46 nA ($I_{MGC(-45mV)}$=-0.42±0.02 nA, E_{rev}=8±2 mV, *n*=8, Fig. 2, *b*). Thus, deformation increases MG-current, which would depolarize the cell free from the voltage clamp conditions. Similarly to the previous case, deviation of I_{S-C} from the straight line is considered

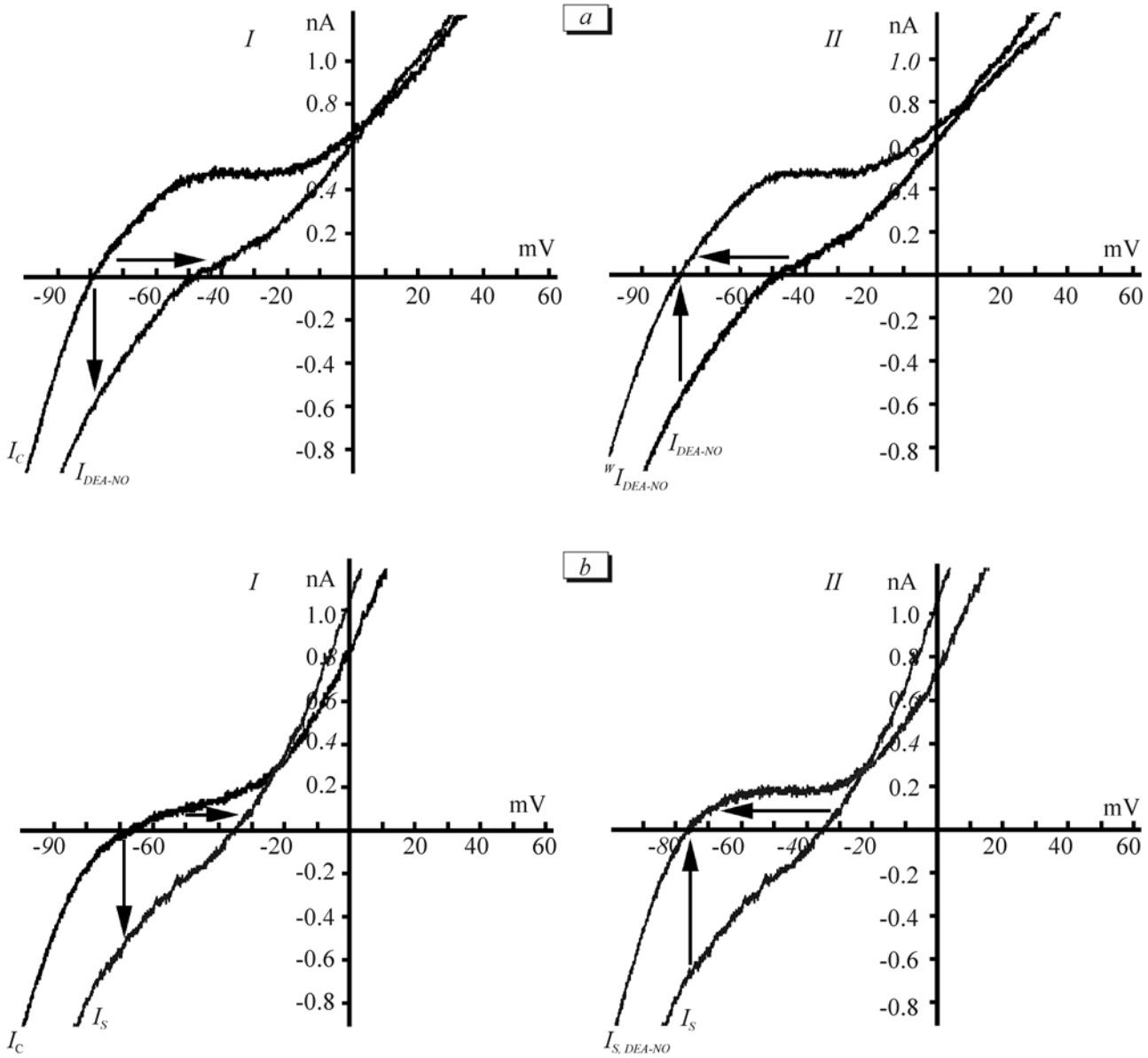

Fig. 3. Effect of DEA-NO on MGC-currents in control and stretched (10 μ) cardiomyocyte. *a*: I) control I-V curve (I_C) and I-V curve recorded 2 min after perfusion of undeformed cell with DEA-NO (I_{DEA-NO}). Arrows show directions of donor-induced shift of I-V plot; *a*: II) I-V curve after a 2-min washing from DEA-NO ($^{W}I_{DEA-NO}$) in comparison with I-V curve recorded during perfusion of undeformed cell with DEA-NO (I_{DEA-NO}). Arrows show directions of DEA-NO induces shift of I-V plot. *b*: I) control I-V curve (I_C) and I-V curve of the deformed cell (I_S). Arrows show directions of stretch-induced shift of I-V plot; *b*: II) I-V curve of the deformed cell (I_S) and I-V curve recorded 3 min after perfusion of the deformed cell with DEA-NO ($I_{S,DEA-NO}$). Arrows show the direction of I_S shift of the deformed cell by DEA-NO.

as indication on mechanically induced deactivation of G_{K1} channels. The next step in this experiment was replacement of normal perfusion solution washing the preliminary stretched cell for SNAP solution. Despite maintained deformation of the cell, SNAP returned I-V curve ($I_{S,SNAP}$) to nearly initial shape (Fig. 2, *c*) with the peculiar N-like meander (V_0=-87±4 mV, *n*=8). In 3 minutes, the differential current $I_{MGS}=I_{S\text{-}S,SNAP}$ was 0.53 nA ($I_{MGC(-45\ mV)}$=0.48±0.05 nA). Thus, SNAP perfusion of a deformed cell inhibited MG-current by blocking MGC.

In the next experiment, we tested DEA-NO, another NO donor (2-(N,N-Diethylamino)-diazenolate-2-oxide diethylammonium salt, 250 μM). Figure 3, *a* shows I-V plots of unstretched cardiomyocyte in control solution (N-shaped I_C curve with V_0=-86±5 mV, *n*=9) and 2 min after the onset of perfusion of the cell with DEA-NO solution ($I_{DEA\text{-}NO}$). The MG-like current induced by DEA-NO donor in unstretched membrane corresponds to MG-current evoked by a 10 μ stretch of the cells. The differential current $I_{MGC}=I_{DEA\text{-}NO\text{-}C}$ was -0.46 nA ($I_{MGC(-45\ mV)}$=-0.43±0.03 nA, E_{rev}=-3±2 mV, *n*=9). Washing the cell with normal solution for 2 min completely eliminated the effect of DEA-NO (Fig. 3, *a*).

In the next series of experiments, DEA-NO was applied to preliminary stretched cardiomyocytes. Figure 3, *b* shows the control current I_C and the current recorded in the deformed cell (I_S). Prior to deformation, I-V curve (I_C) was N-shaped (V_0=-83±4 mV, *n*=9). Deformation of the cell shifted I-V plot to more positive potentials (V_0=-38±3 mV, *n*=9) which would depolarize the unclamped cell. The differential current $I_{MGC}=I_{S\text{-}C}$ was -0.31 nA ($I_{MGC(-45\ mV)}$=-0.38±0.04 nA, E_{rev}=-15±3 mV, *n*=9). Thus, deformation increased MG-current and would depolarize the unclamped cell. A 3-min perfusion with DEA-NO of preliminary stretched cell resulted in the membrane current ($I_{S,DEA\text{-}NO}$), which differed from previously recorded current in the deformed cell (Fig. 3, *b*). Despite maintained deformation, DEA-NO returned I-V curve ($I_{S,DEA\text{-}NO}$) almost to the initial plot I_C with characteristic N-like inflexion (V_0=-87±3 mV, *n*=9). In 3 min, the differential current $I_{MGC}=I_{S\text{-}S,DEA\text{-}NO}$ was -0.34 nA ($I_{MGC(-45\ mV)}$=-0.40±0.03 nA, E_{rev}=-14±4 mV, *n*=9). Thus, application of DEA-NO to the deformed cell inhibited MG-current by blocking MGC.

Therefore, both NO donors tested in this study (SNAP and DEA-NO) activated MG-like current in the unstretched cardiomyocytes. This effect is reversible, since washing eliminated it. In contrast, both this donors inhibited MG-current in the preliminary stretched cardiomyocytes. These data accord with the dose-dependent biphasic NO effect on myocardial contractility and the heart rate [8,10-12]. Similar data were obtained on the cardiomyocytes isolated from the left ventricle of mice, rats, and guinea pigs.

Since directivity of NO donor effects depended on NO concentration, we hypothesize that perfusion of cardiomyocytes with exogenous NO-donors opens MGC and evokes MG-like currents. Such MG-currents are induced by stretching of the cells and are accompanied by elevation of endogenous NO concentration. It is suggested that in both cases, the concentration of NO is rather low, and under such condition NO plays the role of MGC opener. However, if NO concentration increased in the cell during its deformation, the use of NO-donors elevates it still further, which probably results in inhibition of MGC. Since there are no data on MGC structure in cardiomyocytes, our hypothesis is bases on the analogies with the potential-gated currents such as $I_{Ca\text{-}L}$, which are also subjected to the dose-dependent effects of NO-donors [7].

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