Report

Autocrine Stimulation of Cardiac Na^+-Ca^{2+} Exchanger Currents by Endogenous Endothelin Released by Angiotensin II

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The goal of the present study was to evaluate the effects of Ang II on the current produced by the Na^+-Ca^{2+} exchanger (I_{NCX}) working in the reverse mode and the possible autocrine role played by the release of endothelin (ET) in these actions. I_{NCX} was studied in isolation in cat cardiac myocytes. Angiotensin II (Ang II) (100 nmol/L) increased I_{NCX} at potentials higher than 0 mV (at +60 mV: 2.07±0.22 pA/pF in control versus 2.73±0.22 pA/pF in Ang II, n=9; $P<0.05$). The increase in I_{NCX} induced by Ang II was prevented by the treatment of the cells with the unspecific blocker of the ET receptors, TAK 044 (1 μmol/L) (at +60 mV: 2.15±0.27 pA/pF in control versus 2.01±0.26 pA/pF in Ang II, n=5, NS). These results show, for the first time, that the effect of Ang II on I_{NCX} is the result of the autocrine actions of ET released by the octapeptide.

Previous experimental evidence indicates that some cardiovascular effects initially thought to be mediated by angiotensin II (Ang II) were, in fact, due to the release of endogenous endothelin (ET). In cultured neonatal rat cardiomyocytes, Ito et al[1] reported inhibition of Ang II–induced hypertrophy with a blocker of ET_A receptors (BQ123) or antisense oligonucleotides directed against mRNA of pre-proET. In rats, the elevation of blood pressure induced by infusion of Ang II was reversed by the ET_A blocker PD 155080.[2] The goal of the present study was to evaluate, in isolated myocytes, the effects of Ang II on the current produced by the NCX (I_{NCX}) working in the reverse mode and the possible autocrine role played by the release of ET in these actions. We present novel evidence that demonstrates, for the first time, that the reverse mode of the cardiac NCX is stimulated by endogenous ET released by the myocytes on Ang II regulation.

Materials and Methods

Cat cardiac myocytes were isolated according to the technique previously described.[3] Animals were provided by a local supplier (San Cayetano, Monte Grande, Argentina) and procedures were performed according to the *Guide for the Care and Use of Laboratory Animals* (US Department of Health and Human Services). The determination of the NCX current (I_{NCX}) was performed under the conditions previously described by Hobai and O'Rourke[4] with some modifications. In some experiments, L-type Ca^{2+} current (I_{Ca}) was recorded. The composition of solutions and other details are described in an expanded Materials and Methods section that can be found in the online data supplement, available at http://www.circresaha.org.

Results and Discussion

Whereas the contribution of the reverse mode of the NCX to basal cardiac contraction is controversial,[5] increases in contractility due to stimulation of this mode of the NCX have been reported to be present after the effects of agonists such as Ang II[6] or ET.[7] Therefore, since the initial goal of the present study was the evaluation of the effects of Ang II on the reverse mode of the NCX, we measured I_{NCX} under recording conditions that favor this mode over the forward mode. Figure 1 shows the inhibitory effects of the NCX blocker KB-R7943 (KBR, 10 μmol/L) on whole-cell currents recorded under conditions of I_{NCX} isolation. Note that KBR blocked both forward and reverse modes of NCX, as it was previously reported for this concentration of the agent.[8] Lower concentrations of KBR were proven to block selectively the reverse mode of NCX.[8] Consistently, no effects of this drug on the forward mode were observed when 5 μmol/L KBR was used (shown in the online data supplement). Ni^{2+} (10 mmol/L), another NCX antagonist, blocked the current registered at +60 mV by 85±5% (n=11). On average, the apparent reversal potentials (E_{rev}) of I_{NCX} were −44.4±2.5 mV (n=6) and −39.5±4.1 mV (n=11) estimated as the zero current potential of the KBR- and Ni^{2+}-sensitive currents, respectively. These values are similar to the I_{NCX} E_{rev} values reported by other authors working in similar conditions to those of the present study.[4] The above experiments allowed us to conclude that the whole-cell currents registered were mainly underlain by the NCX (I_{NCX}).

Ballard and Schaffer[9] reported that Ang II or ET increased the activity of the NCX in canine cardiac sarcolemmal vesicles. Thus, we next examined whether Ang II was able to affect I_{NCX} in isolated cat cardiac myocytes. Figure 2A depicts the time course of the effect of Ang II (100 nmol/L) on the current recorded at +60 mV in a single myocyte. Ang II induced a gradual increase in the current that reaches steady-state values after 5 minutes of administration of the peptide. Average data of the current recorded at +60 mV in the absence and presence of Ang II are shown in Figure 2C. This current increased by 24.7±4.9% (n=26) in the presence of Ang II. The Ang II type 1 receptor (AT_1) antagonist losartan (1 μmol/L) reversed the effects of Ang II (data not shown).

The outward current recorded at +60 mV is mostly but not totally I_{NCX} because a small remnant current is present after the effects of the NCX blockers Ni^{2+} or KBR. Thus, the possibility that the Ni^{2+}-insensitive current could be affected by Ang II was evaluated. No increase in outward current was induced by Ang II (100 nmol/L) in the presence of Ni^{2+} (10 mmol/L) (0.75±0.31 pA/pF in Ni^{2+} and 0.78±0.36 pA/pF in Ni^{2+} plus Ang II, n=5; NS), indicating that the

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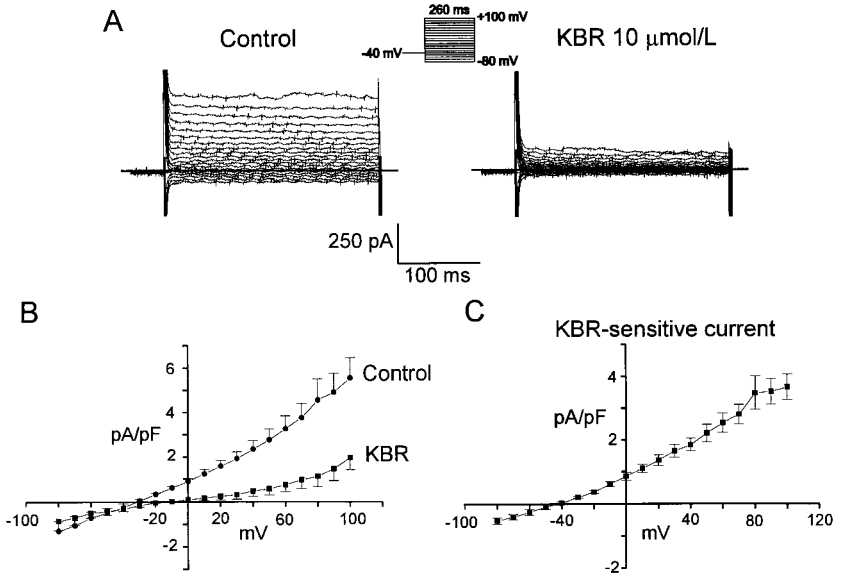

Figure 1. Characterization of I_{NCX}. A, Families of representative whole-cell currents evoked by depolarizing pulses between −80 to +100 mV from a holding potential of −40 mV, recorded in a myocyte in the absence and presence of KBR (10 μmol/L). Pulses (260 ms in duration) were delivered at 0.1 Hz. B, Voltage dependence of the average current density measured in 6 myocytes exposed to KBR (10 μmol/L). C, Average current-voltage relations of the KBR-sensitive currents, representing I_{NCX} (n=6).

current affected by Ang II is most likely I_{NCX}. Moreover, to confirm that Ang II affects I_{NCX}, we studied the effects of this peptide on the KBR-sensitive currents. Figure 2D shows average current density-voltage relations obtained in 9 myocytes exposed to 10 μmol/L KBR after having obtained the increase in the whole-cell current by Ang II. Ang II induced a significant increase in outward currents at potentials positive to 0 mV. KBR blocked the currents recorded after Ang II to the same extent of those recorded in the absence of the peptide (Figure 1). Figure 1E shows the effects of Ang II on the average current-voltage relation for the KBR-sensitive currents, corresponding to I_{NCX}, obtained by subtracting the currents in KBR to those recorded in both control and in Ang II. Ang II significantly increased I_{NCX} at potentials positive to 0 mV. At +60 mV, I_{NCX} increased by 38.7±14.6% in the presence of Ang II (n=9).

We next examined whether the Ang II–induced increase in I_{NCX} observed in the present study was produced by release of endogenous ET. For this purpose, we pretreated the cells with the nonselective blocker of ET receptors, TAK 044 (1 μmol/L). This blocker did not affect basal current (at +60 mV: 2.59±0.15 pA/pF in control versus 2.45±0.12 pA/pF in TAK 044-treated myocytes, n=13; NS). Figure 3A shows the time course of effects of Ang II in the presence of TAK 044 on the current registered at +60 mV. In the presence of the ET receptor blocker, Ang II failed to induce enhancement of this current. On average, no effects of Ang II were observed in the presence of TAK 044 on the current recorded at +60 mV (Figure 3C) or at any of the tested voltages (Figure 3D). In addition, no effects of Ang II in the presence of TAK 044 were observed on the Ni^{2+}-sensitive currents, representing I_{NCX} (at +60 mV:

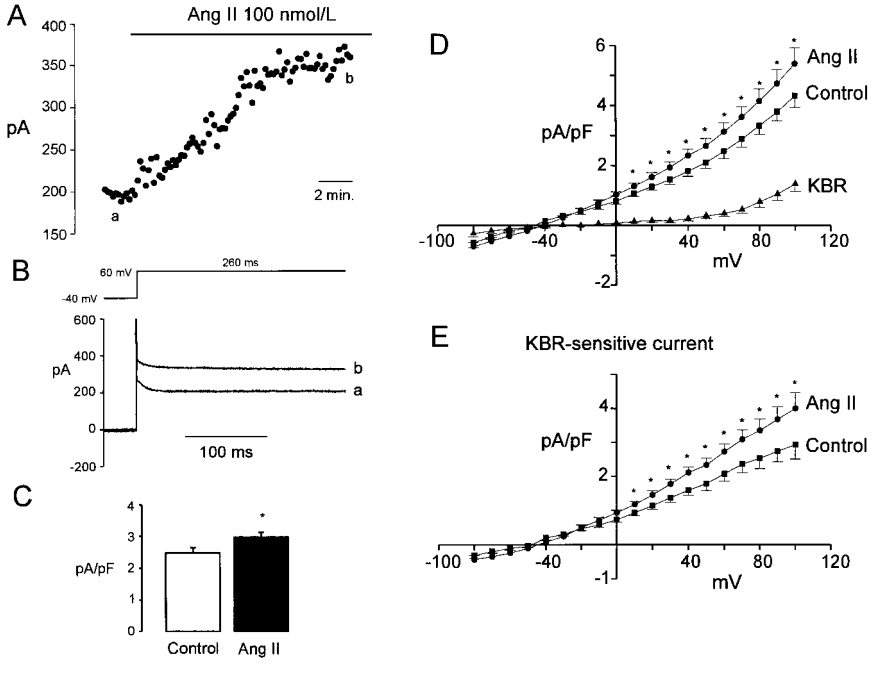

Figure 2. Ang II effects on I_{NCX}. A, Time course of effects of Ang II on the current registered at +60 mV from a holding potential of −40 mV, recorded in a single myocyte. Pulses were delivered at 0.1 Hz. B, Representative traces of whole-cell currents corresponding to the points indicated in panel A. C, Average data of current density recorded at +60 mV before and after the addition of Ang II (100 nmol/L) (n=26). D, Ang II (100 nmol/L) and KBR (10 μmol/L) effects on average current-voltage relations evoked by the voltage protocol of Figure 1 (n=9). E, Ang II effects on average current-voltage relations for the KBR-sensitive current, corresponding to I_{NCX} (n=9). *Ang II statistically different from control.

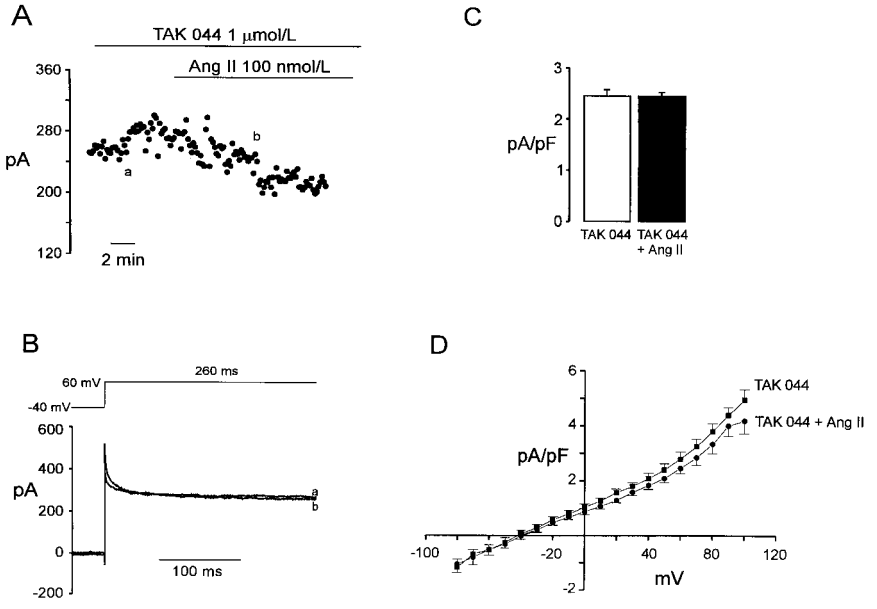

Figure 3. Inhibition of Ang II effects on I_{NCX} by the ET receptor antagonist TAK 044. A, Time course of effects of Ang II in the presence of TAK 044 on the current registered at +60 mV from a holding potential of −40 mV, recorded in a single myocyte. Pulses were delivered at 0.1 Hz. B, Representative traces of whole-cell currents corresponding to the points indicated in panel A. C, Average data of current density recorded at +60 mV before and after the addition of Ang II (100 nmol/L) to the solution containing TAK 044 (1 μmol/L) (n=13). D, Ang II effects in the presence of TAK 044 on average current-voltage relations evoked by the same voltage protocol of Figure 1 (n=7).

2.15±0.27 pA/pF in TAK 044 versus 2.01±0.27 pA/pF in Ang II plus TAK 044, n=5; NS).

To rule out the possibility that the ET receptor blocker TAK 044 is acting on the Ang II receptors, we performed experiments in which the L-type calcium current (I_{Ca}) was measured in the absence and presence of Ang II (100 nmol/L) with or without TAK 044 (1 μmol/L) in the extracellular solution. We have recently reported that Ang II enhances I_{Ca} via activation of AT_1 receptors in cat cardiac myocytes.[3] Under the recording conditions of the present study, the peak I_{Ca} recorded at 0 mV increased by 23±8% (n=5) after 10 minutes in the presence of Ang II. A similar increment in I_{Ca} after 10 minutes of Ang II was observed in the presence of TAK 044 (21±7%, n=5). These results suggest that this ET receptor blocker did not unspecifically bind to AT_1 receptors and that the endogenous ET released by Ang II did not affect I_{Ca}. The previously reported failure of ET-1 to affect I_{Ca} when standard whole-cell configuration is used can account for the last result.[10]

Taken together, the above results allow us to suggest that in cardiac myocytes the stimulation of I_{NCX} induced by Ang II is, in fact, mediated by endogenous ET released by Ang II. The major finding of this study is the demonstration at the cardiac single-cell level of the existence of an autocrine mechanism in the heart involving the myocyte as the source and target of ET. Additional observations and extended discussion of the results are presented in the online data supplement.

Acknowledgments

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