Functional Coupling of Human L-Type Ca\textsuperscript{2+} Channels and Angiotensin AT\textsubscript{1A} Receptors Coexpressed in Xenopus laevis Oocytes: Involvement of the Carboxyl-Terminal Ca\textsuperscript{2+} Sensors

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ABSTRACT

A human recombinant L-type Ca\textsuperscript{2+} channel (\(\alpha_{1C,77}\)) was coexpressed with the rat angiotensin AT\textsubscript{1A} receptor in Xenopus laevis oocytes. In oocytes expressing only \(\alpha_{1C,77}\) channels, application of human angiotensin II (1–10 \(\mu\)M) did not affect the amplitude or kinetics of Ba\textsuperscript{2+} currents (\(I_{\text{Ba}}\)). In sharp contrast, in oocytes coexpressing \(\alpha_{1C,77}\) channels and AT\textsubscript{1A} receptors, application of 1 nM to 1 \(\mu\)M angiotensin gradually and reversibly inhibited \(I_{\text{Ba}}\), without significantly changing its kinetics. The inhibitory effect of angiotensin on \(I_{\text{Ba}}\) was abolished in oocytes that had been preincubated with losartan (an AT\textsubscript{1A} receptor antagonist) or thapsigargin or injected with 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetate, pertussis toxin, guanosine-5'-O-(2-thiodiphosphate, or heparin, suggesting that the recombinant \(\alpha_{1C}\) channels were regulated by angiotensin through G protein-coupled AT\textsubscript{1A} receptors via activation of the inositol trisphosphate-dependent intracellular Ca\textsuperscript{2+} release pathway. Consistent with this hypothesis, no cross-signaling occurred between the AT\textsubscript{1A} receptor and a splice variant of \(\alpha_{1C}\) lacking Ca\textsuperscript{2+} sensors (\(\alpha_{1C,86}\)). The data suggest that the regulation of recombinant L-type Ca\textsuperscript{2+} channels by angiotensin is mediated by inositol trisphosphate-induced intracellular Ca\textsuperscript{2+} release and occurs at the molecular motif responsible for the Ca\textsuperscript{2+} induced inactivation of the channels.

Voltage-gated Ca\textsuperscript{2+} channels are a major route for Ca\textsuperscript{2+} entry into cells in response to stimulation by hormones, neurotransmitters, or drugs. The resulting rise in cytoplasmic free Ca\textsuperscript{2+} triggers a cascade of intracellular signaling events, which underlie a variety of cellular responses, ranging from contraction and secretion to growth and mitogenesis. Therefore, identification of the molecular basis for functional coupling between Ca\textsuperscript{2+} channels and hormone or neurotransmitter receptors may provide critical information on cellular signaling mechanisms.

The cardiac L-type Ca\textsuperscript{2+} channel is composed of the pore-forming \(\alpha_{1C}\) and auxiliary \(\beta\) and \(\delta\) subunits (Catterall, 1995). In an artificial expression system, the \(\alpha_{1C}\),\(\beta\),\(\delta\) complex is sufficient to give rise to Ca\textsuperscript{2+} channels exhibiting all of the major electrophysiological properties observed in vivo. However, functional regulation of the recombinant Ca\textsuperscript{2+} channel remains largely unknown. For example, in cardiac or vascular cells, the \(\alpha_{1C}\) channel is modulated by protein kinase A- and protein kinase C-dependent phosphorylation (McDonald et al., 1994). However, when all three recombinant subunits of the channel are coexpressed in Xenopus laevis oocytes or in eukaryotic systems (Chinese hamster ovary or human embryonic kidney cells), their modulation through phosphorylation is either strongly reduced or essentially absent (Bouron et al., 1995; Zong et al., 1995; Shuba et al., 1997), even though the expressed channels display the same voltage dependence, gating kinetics, unitary conductance, and pharmacological properties as the native \(\alpha_{1C}\) L-type Ca\textsuperscript{2+} channels. These findings demonstrate the complexity of molecular signaling involving the \(\alpha_{1C}\) Ca\textsuperscript{2+} channels; this complexity extends to the largely unexplored area of “cross-talk” between recombinant \(\alpha_{1C}\) channels and hormone receptors that are coexpressed in X. laevis oocytes.

The coupling of \(\alpha_{1C}\) Ca\textsuperscript{2+} channels with angiotensin II AT\textsubscript{1} receptors has attracted much attention. For example, the L-type Ca\textsuperscript{2+} channel blockers verapamil, diltiazem, and nifedipine have been shown to block angiotensin II-mediated vascular contraction in vivo in humans (Andrawis et al., 1992). Activation of AT\textsubscript{1} receptors seems to be associated with both immediate contractile and long term growth responses in vascular smooth muscle and cardiac myocytes (Baker et al., 1992; Sadoshima and Izumo, 1993; Miyata and Haneda, 1994). Supporting the possibility of interactions between the G protein-coupled AT\textsubscript{1} receptors (Anand-Srivastava, 1983; Ohya and Sperelakis, 1991) and voltage-activated Ca\textsuperscript{2+} channels is the regulation of neuronal (Scott and

ABBREVIATIONS: PTX, pertussis toxin; IP\textsubscript{3}, inositol trisphosphate; BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N',N",N"-tetraacetate; GDP\textsubscript{bs}, guanosine-5'-O-(2-thiodiphosphate; \(I_{\text{Ca,Ap}}\), Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} current; \(I_{\text{Ba}}\), Ba\textsuperscript{2+} current; \(I_{\text{Cl}}\), Cl\textsuperscript{-} current; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
Dolphin, 1987) and cardiac (Yatani et al., 1987) L-type Ca2+ channels by PTX-sensitive or insensitive G proteins. Similar interactions have been suggested for angiotensin II activation of L-type Ca2+ currents in rat portal vein myocytes (Macrez-Lepretre et al., 1996) and T-type Ca2+ currents in adrenal zona glomerulosa cells (Lu et al., 1996).

In this study, we have used the X. laevis oocyte expression system to study the functional coupling between recombinant rat AT1A receptors and splice variants of recombinant human \( \alpha_1C \) Ca2+ channels with or without the molecular motif responsible for Ca2+-dependent inactivation of the channel. We show that heterogeneously expressed Ca2+ channels and AT1A receptors are functionally coupled via the G protein/IP3-mediated Ca2+ signaling cascade. Additionally, we report that the molecular locus for the angiotensin-induced modulation of the \( \alpha_1C \) Ca2+ channel is independent of permeation of Ca2+ through the pore and is confined to the carboxyl-terminal cytoplasmic motif (positions 1572–1651), which contains multiple Ca2+ sensors of the channel.

Materials and Methods

Preparation of mRNAs. Template \( \alpha_{1C,77} \) (Soldatov et al., 1995) and \( \alpha_{1C,86} \) (Soldatov et al., 1995) cDNAs were linearized by digestion with BamHI. Capped transcripts were synthesized in vitro with T7 RNA polymerase, using the mRNA cap kit (Stratagene, La Jolla, CA). mRNAs were dissolved in water (0.5 \( \mu \)g/\( \mu \)l). Rat angiotensin AT1A receptor (Murphy et al., 1991) transcripts were kindly provided by Kathryn Sandberg (Georgetown University).

Oocyte preparation and injection. Mature female X. laevis frogs were purchased from Xenopus I (Ann Arbor, MI). Clusters of oocytes were defolliculated by shaking for 2 hr at room temperature in 25 ml of medium containing 82.5 mM NaCl, 2 mM KCl, 1 mM MgCl2, 5 mM HEPES, pH 7.5 (adjusted with NaOH), and 0.2% collagenase A (Boehringer Mannheim, Indianapolis, IN). Oocytes were injected with 50–100 nl of collagenase A (Boehringer Mannheim, Indianapolis, IN). Oocytes were defolliculated by shaking for 2 hr at room temperature (21°). Mature female X. laevis oocytes of cRNAs coding for auxiliary \( \alpha_1C \) and \( \alpha_1C \)-subunits gave rise to the expression of well defined, slowly inactivating currents through Ca2+ channels 2–3 days after the injection of cRNAs (Soldatov et al., 1995). With Ba2+ as a charge carrier, step depolarization to +20 mV from a holding potential of −90 mV activated a slowly inactivating, L-type

Results

Coexpression of the \( \alpha_{1C,77} \) channel with the AT1A receptor allows regulation of Ca2+ channels by angiotensin. Coinjection into X. laevis oocytes of cRNAs coding for the conventional \( \alpha_{1C,77} \) channel and auxiliary \( \beta_1 \) and \( \alpha_2 \delta \) subunits gave rise to the expression of well defined, slowly inactivating currents through Ca2+ channels 2–3 days after the injection of cRNAs (Soldatov et al., 1995). With Ba2+ as a charge carrier, step depolarization to +20 mV from a holding potential of −90 mV activated a slowly inactivating, L-type

![Fig. 1](https://example.com/fig1.png) Effect of angiotensin on Ca2+ currents. A, Representative traces of \( I_{Ba} \) through \( \alpha_{1C,77} \) channels, elicited by stepwise depolarization to +20 mV from a holding potential of −90 mV, before (○) and 4 min after (□) application of 1 \( \mu \)M angiotensin. B, Traces of \( I_{Ba} \) through \( \alpha_{1C,77} \) channels coexpressed with AT1A receptors, recorded before (○) and 1.5, 2, and 3 min after application of 1 \( \mu \)M angiotensin. C, Time dependence and reversibility of the angiotensin effect on \( I_{Ba} \) through \( \alpha_{1C,77} \) channels coexpressed with AT1A receptors (□). \( I_{Ba} \) amplitudes were measured in response to 250-msec test pulses to +20 mV, applied at 30-sec intervals, and were normalized to maximal \( I_{Ba} \) in the absence of angiotensin. Arrows, times of application of bath solutions containing the indicated concentrations of angiotensin. D, Current-voltage relationships for \( I_{Ba} \) through \( \alpha_{1C,77} \) channels coexpressed with AT1A receptors, before treatment (○) and 5 min after treatment with 1 \( \mu \)M angiotensin (□), and after a 10-min perfusion with bath medium (□), obtained using the same oocyte as in B and C (□). Experiments were performed at room temperature (21°C).
Fig. 3B shows that 1 μM angiotensin (0.1–1 μM) inhibited I_{Ba} by ~54% (n = 12), in a time- and concentration-dependent manner (Table 1). The suppressive effect of angiotensin developed within 3–4 min of the hormone exposure, but the effect slowly (20–30 min) reversed even in the presence of the hormone. Fig. 1C shows that 57.6% inhibition of I_{Ba} by 1 μM angiotensin reversed spontaneously and washout of the hormone did not accelerate the recovery of the current (Fig. 2). In the presence of angiotensin, I_{Ba} recovered by 90.2 ± 4.0% (n = 7) within 20–30 min. The voltage dependence of I_{Ba} at the peak of the hormone effect was often shifted by approximately +10 mV (Fig. 1D). These results suggest that the time course of the hormone effect is not critically dependent on the continued presence of the hormone.

Fig. 3A illustrates the concentration dependence of the angiotensin effect on α_{1C,77} channels. Under our experimental conditions, the maximal inhibitory effect (~60% suppression) was reached with 1 μM angiotensin. In none of the cells tested (n = 12) did the inhibitory effect on I_{Ba} exceed 60%. The estimated IC_{50} value for angiotensin was 33 ± 8 nM (n = 4), with a Hill coefficient of approximately 0.85.

Angiotensin failed to suppress the Ca^{2+} channels in the presence of the reversible AT_{1A} receptor antagonist losartan. Fig. 3B shows that 1 μM losartan had no effect by itself on I_{Ba} in an oocyte coexpressing AT_{1A} receptors and α_{1C} channels but completely blocked the angiotensin effect. Replacement of losartan-containing solution with one containing 1 μM losartan reversed even in the presence of the hormone. Taken together, these data suggest that the suppression of I_{Ba} through α_{1C,77} channels by angiotensin is mediated through the direct interaction of angiotensin with AT_{1A} receptors.

**Angiotensin activates a transient I_{Cl}.** The rapid application of the hormone in Cl~−-free solutions was often but not always accompanied by activation of a large, transient, inward current lasting ~2 min. The activation of this inward holding current, measured at ~90 mV in Cl~−-free extracellular solution (Fig. 4, lower), preceded the decrease in I_{Ba}. This current had properties similar to those previously identified (Hartzell, 1996; Gomez-Hernandez et al., 1997) for I_{Cl(Ca)}. During the activation of I_{Ba}, I_{Cl(Ca)} often exhibited decreased inactivation kinetics, producing large, slowly decaying, tail currents (Fig. 4, upper, traces 2 and 3). Interestingly, the angiotensin-induced, transient suppression of I_{Ba} outlasted the activation of I_{Cl(Ca)} by 2–3 min (Fig. 4), suggesting either different affinities of Ca^{2+} channels and Ca^{2+}-activated Cl~− channels for Ca^{2+} or differences in the spatial distribution of the two channels with respect to the intracellular Ca^{2+} pools. Lower affinity of I_{Cl(Ca)} for activation by Ca^{2+}, compared with Ca^{2+}-induced inactivation of Ca^{2+} channels, and variations in the Ca^{2+} contents of intracellular Ca^{2+} pools of the oocytes might be partly responsible for the variations in the magnitude of I_{Cl} in different oocytes.

**The IP_{3}/Ca^{2+} signaling pathway is involved in channel regulation by angiotensins.** Ca^{2+} stores in X. laevis oocytes are known to be regulated through the activation of IP_{3}-sensitive Ca^{2+} release channels (Berrendr and Irvine, 1989; Putney et al., 1989). These channels are thought to be involved in receptor-mediated Ca^{2+} signaling, and their activation is known to evoke I_{Cl(Ca)} in oocytes (Yao and Parker, 1993; Hartzell, 1996). Consistent with this idea, in oocytes bathed in Barth’s solution and expressing only AT_{1A} receptors, a transient (2–3-min) I_{Ba} was activated upon rapid application of angiotensin (data not shown). To further characterize the steps in the regulation of recombinant α_{1C} channels by AT_{1A} receptors, when coexpressed in oocytes, we probed the various steps of the IP_{3}-mediated Ca^{2+} signaling cascade by inhibiting the G proteins, blocking the IP_{3} receptor, and interfering with the rise in intracellular Ca^{2+} levels.

**Release of intracellular Ca^{2+} mediates the angiotensin-induced effects.** The depletion of intracellular Ca^{2+} stores by overnight incubation of oocytes with 10 μM thapsigargin (Thastrup et al., 1990) completely abolished the effect of 1 μM angiotensin on I_{Ba} (Fig. 6, A and B). No significant difference in the amplitude of I_{Ba} in control and thapsigargin-incubated oocytes was observed (Table 1). Similarly, oocytes injected with Ca^{2+} buffers failed to respond to angiotensin. Fig. 6, C and D, shows data recorded from an oocyte that was injected with 50 nl of 94 mM Cs_{2}BAPTA solution 30 min before measurements of I_{Ba}. The data (n = 4) showed

**TABLE 1**

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Drug</th>
<th>Average amplitude of I_{Ba} (μA)</th>
<th>Inhibition of I_{Ba} (%)</th>
<th>n*</th>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Angiotensin</td>
<td></td>
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<tr>
<td>α_{1C,77}</td>
<td>None</td>
<td>−1.64 ± 0.33</td>
<td>−1.57 ± 0.30</td>
<td>3 ± 3</td>
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<tr>
<td>α_{1C,77} + AT_{1A}</td>
<td>None</td>
<td>−1.57 ± 0.29</td>
<td>−0.72 ± 0.15</td>
<td>54 ± 4</td>
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<tr>
<td>α_{1C,77} + AT_{1A}</td>
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<td>−0.43 ± 0.19</td>
<td>−0.42 ± 0.19</td>
<td>0</td>
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<tr>
<td>α_{1C,77} + AT_{1A}</td>
<td>PTX</td>
<td>−1.24 ± 0.29</td>
<td>−1.23 ± 0.29</td>
<td>0</td>
</tr>
<tr>
<td>α_{1C,77} + AT_{1A}</td>
<td>GDPβS</td>
<td>−1.01 ± 0.31</td>
<td>−1.03 ± 0.33</td>
<td>0</td>
</tr>
<tr>
<td>α_{1C,77} + AT_{1A}</td>
<td>Thapsigargin</td>
<td>−2.57 ± 0.40</td>
<td>−2.60 ± 0.42</td>
<td>0</td>
</tr>
<tr>
<td>α_{1C,77} + AT_{1A}</td>
<td>BAPTA</td>
<td>−0.54 ± 0.09</td>
<td>−0.55 ± 0.09</td>
<td>0</td>
</tr>
<tr>
<td>α_{1C,77} + AT_{1A}</td>
<td>Heparin</td>
<td>−1.18 ± 0.24</td>
<td>−1.12 ± 0.22</td>
<td>4 ± 2</td>
</tr>
</tbody>
</table>

* n, number of tested oocytes.
that signaling between $\alpha_{1C,77}$ channels and AT1A receptors in response to 0.1–1 $\mu$M angiotensin was completely suppressed.

PTX-sensitive G proteins and IP$_3$ receptors mediate the angiotensin-induced effects. AT$_1A$ receptors in mammalian cells are known to be coupled to G proteins (Lu et al., 1996). In X. laevis oocytes coexpressing AT$_1A$ receptors and Ca$^{2+}$ channels, we probed the functional manifestation of G protein coupling. In oocytes that had been preinjected with 50–100 nl of GDP$\beta$S (10 mM), angiotensin (1 $\mu$M) failed to produce significant inhibitory effects on I$_{Ba}$ (Fig. 6, C and D; Table 1). Because the effect of angiotensin was also blocked in parallel experiments with microinjection of 50–100 nl of PTX (5 $\mu$g/ml) (Fig. 6, C and D; Table 1), the coupling between the recombinant AT$_1A$ receptors and $\alpha_{1C,77}$ Ca$^{2+}$ channels seemed to be mediated through the activation of endogenous G proteins of the $G_i$ type.

To directly examine the involvement of IP$_3$-sensitive Ca$^{2+}$ release channels, oocytes coexpressing $\alpha_{1C}$ channels and AT$_1A$ receptors were injected with 50 nl of 10 $\mu$M heparin (known to block IP$_3$ receptors) (Guillemette et al., 1989) 30–60 min before the experiment. Fig. 6, E and F, shows that, in heparin-injected oocytes, angiotensin failed to produce its Ca$^{2+}$ channel-suppressive effect on either the current-voltage relationship (Fig. 6E; Table 1) or the time course of I$_{Ba}$ (Fig. 6F).

Therefore, it seems that the release of Ca$^{2+}$ via the IP$_3$ signaling pathway mediates the angiotensin-induced suppressive effect on I$_{Ba}$. We examined whether the effect of the hormone was directly related to the content of intracellular Ca$^{2+}$ stores. Fig. 5 shows data from an experiment in which, after inhibition and recovery of I$_{Ba}$ in the presence of 1 $\mu$M angiotensin and a 10-min washout in Ba$^{2+}$-containing Ringler’s solution, the reapplication of 1 $\mu$M angiotensin failed to produce any effect on I$_{Ba}$. However, after 5 min of incubation of the oocyte in normal Ca$^{2+}$-containing Barth’s solution, the response of Ca$^{2+}$ channels to 1 $\mu$M angiotensin partially

Fig. 2. Failure of angiotensin to suppress I$_{Ba}$ through $\alpha_{1C,86}$ channels coexpressed with AT$_1A$ receptors. A, Representative traces of I$_{Ba}$ elicited by stepwise depolarization to +20 mV, from a holding potential of −90 mV, recorded before (●) and 5 min after (○) application of 1 $\mu$M angiotensin. B, Time dependence of the effect of 1 $\mu$M angiotensin added to the external Ba$^{2+}$ solution (arrow) on I$_{Ba}$ through the $\alpha_{1C,86}$ channels. C, Averaged current-voltage relationships (n = 3) determined before (●) and 5 min after (○) application of 1 $\mu$M angiotensin. In B and C, the amplitudes of I$_{Ba}$ were normalized to maximal I$_{Ba}$ in the absence of angiotensin.

Fig. 3. A, Concentration-response relationship for the angiotensin effect. Angiotensin, at the indicated concentrations, was applied to an oocyte coexpressing $\alpha_{1C,77}$ channels and AT$_1A$ receptors. I$_{Ba}$ was measured at +20 mV, after 5 min of equilibration. The averaged concentration dependence clearly shows saturation of the effect at 1 $\mu$M angiotensin. The curve was normalized to the maximal effect and then fit by the function $I = I_{max}/(1 + (IC_{50}/[A]^n))$, where $I$ is the normalized I$_{Ba}$ amplitude, IC$_{50}$ is the concentration of angiotensin producing 50% inhibition of I$_{Ba}$, [A] is the concentration of angiotensin in the bath solution, and $n$ is the Hill coefficient. The regression coefficient was 0.992. Values are means ± standard errors of four oocytes. B, Time course of the effect of 1 $\mu$M losartan and/or 1 $\mu$M angiotensin on peak I$_{Ba}$ evoked by stepwise depolarization to +20 mV from a holding potential of −90 mV, in an oocyte expressing $\alpha_{1C,77}$ channels and AT$_1A$ receptors. Horizontal bars, times at which losartan and/or angiotensin was applied to the oocyte. Inset, traces of I$_{Ba}$ recorded at 1 (●), 4 (□), 8 (■), and 15 (○) min in this experiment.
recovered. This finding is consistent with the idea that, during incubation in Barth's solution, IP$_3$-sensitive intracellular Ca$^{2+}$ stores are replenished with Ca$^{2+}$ by entry of Ca$^{2+}$ through depletion-activated Ca$^{2+}$ channels (Zwiebach and Lewis, 1995; Lepple-Wienhues and Cahalan, 1996; Parekh and Penner, 1997) or $\alpha_{1C,77}$ Ca$^{2+}$ channels, making it possible for angiotensin to induce Ca$^{2+}$ release.

A Ca$^{2+}$-insensitive $\alpha_{1C,86}$ Ca$^{2+}$ channel coexpressed with the AT$_{1A}$ receptor is not modulated by angiotensin. To examine a molecular motif possibly involved in angiotensin-mediated modulation of Ca$^{2+}$ channels, a recently described Ca$^{2+}$ channel isoform ($\alpha_{1C,86}$) lacking the Ca$^{2+}$ sensors responsible for Ca$^{2+}$-induced modulation (Soldatov et al., 1997) was coexpressed with AT$_{1A}$ receptors in X. laevis oocytes. In contrast to the effect of angiotensin on the $\alpha_{1C,77}$ channel (Figs. 1, 3, and 4), the $\alpha_{1C,86}$ channel was insensitive to modulation by angiotensin (Table 1). Fig. 2A demonstrates that neither the amplitude nor the kinetics of $I_{Ba}$ were significantly changed in the presence of angiotensin. There was often a 5–15% increase in the amplitude of $I_{Ba}$ (Fig. 2B), which resembled the small increase of $I_{Ba}$ observed in oocytes expressing $\alpha_{1C,77}$ without the AT$_{1A}$ receptor (Fig. 1C). Interestingly, the voltage dependence of $I_{Ba}$ through $\alpha_{1C,86}$ channels was also reversibly shifted to more positive potentials in the presence of 1 $\mu$M angiotensin (Fig. 2C), in a manner similar to that observed for $\alpha_{1C,77}$ (Fig. 1D). This shift might be the result of additional screening effects of the released Ca$^{2+}$ on the plasma membrane cation-binding sites. The absence of angiotensin effects in oocytes coexpressing $\alpha_{1C,86}$ with AT$_{1A}$ receptors suggests that the Ca$^{2+}$ sensors of the Ca$^{2+}$ channel are critical in mediating the suppressive effect of angiotensin on the channel.

![Figure 4](image-url) **Fig. 4.** Time course of the development of angiotensin effects on $I_{Ba}$ and $I_{Cl(Ca)}$ in oocytes coexpressing AT$_{1A}$ receptors and $\alpha_{1C,77}$ channels. **Lower**, time dependence of the effect of angiotensin on the holding current, $I_{Cl(Ca)}$ [■], measured at ~90 mV and on $I_{Ba}$ [□] measured at ~20 mV. **Arrow**, time when 1 $\mu$M angiotensin was applied to the oocyte. **Upper**, traces of $I_{Ba}$ recorded at the times indicated (numbers in lower).

Our results show conclusively that, in the oocyte expression system, human recombinant $\alpha_{1C}$ Ca$^{2+}$ channels can be modulated by angiotensin through AT$_{1A}$ receptors via the G protein-dependent, IP$_3$-activated Ca$^{2+}$ release system. Inhibition of any of the key steps in the IP$_3$-dependent Ca$^{2+}$ signaling pathway, including blockade of AT$_{1A}$ receptors (by losartan), G proteins (by GDPβS or PTX), or IP$_3$ receptors (by heparin) and depletion of intracellular Ca$^{2+}$ stores (by thapsigargin or BAPTA), eliminated the suppressive effect of angiotensin on the Ca$^{2+}$ channels. The hormone-induced transient increase of the intracellular Ca$^{2+}$ concentration also activated Ca$^{2+}$-dependent Cl$^-$ channels (Hartzell, 1996; Gomez-Hernandez et al., 1997), which was monitored in our experiments as the transient increase in the holding current at ~90 mV (Fig. 4). The Ca$^{2+}$-dependent outward Cl$^-$ flux (inward $I_{Cl}$) seems to produce sufficient increases in membrane conductance to cause slowing of the inactivation kinetics of $I_{Ba}$ and development of slowly deactivating "tails" (Fig. 4).

It is intriguing to note that, although the hormone suppressed the amplitude of $I_{Ba}$ by releasing intracellular Ca$^{2+}$, the kinetics of the current was not significantly accelerated (Fig. 1B), as might have been expected from a comparison of Ca$^{2+}$ and Ba$^{2+}$ current traces recorded in the oocyte expression system (e.g., Fig. 2A in the report by Soldatov et al., 1998). One possible explanation for this result is that pore-permeating Ca$^{2+}$ and intracellularly released Ca$^{2+}$ may regulate the $\alpha_{1C}$ channel activity by targeting different molecular sites (Ca$^{2+}$ sensors) associated with the channel. Similar dual modulation of Ca$^{2+}$ channel kinetics by intracellular Ca$^{2+}$ was first observed in dorsal root ganglion neurons (Morad et al., 1988). In that case, photorelease of caged Ca$^{2+}$...
(10–50 μM) strongly suppressed the Na⁺ current through the channel, without affecting the kinetics of its inactivation. In support of the idea of dual modulation, we recently reported that a segment (positions 1572–1651) of the cytoplasmic carboxyl-terminal tail of α1C,77 contains two separate Ca²⁺ sensors (molecular determinants for the Ca²⁺-dependent inactivation of the channel) (Soldatov et al., 1998). The identified Ca²⁺ sensors may differentially contribute to the Ca²⁺-induced inactivation of the channel, because they may be selectively targeted by permeating versus cytoplasmic Ca²⁺ because of their specific locations with respect to the pore. Consistent with this idea, the α1C,86 channel, which lacks Ca²⁺ sensors in the carboxyl-terminal tail and does not show Ca²⁺-dependent inactivation, conducts Ca²⁺ and Ba²⁺ with comparable kinetics (Soldatov et al., 1997) and is insensitive to angiotensin-mediated increases in intracellular Ca²⁺ concentrations (Fig. 6; Table 1). Because segment 1572–1651 is the only molecular motif modified in the α1C,86 channel, 

Fig. 6. Molecular steps in the modulation of IBa by angiotensin. Two sets of pairs of averaged current-voltage relationships (A, C, and E) and time-dependent relationships for the angiotensin effect (B, D, and F), as well as representative current traces, were recorded before (●, ▲) and 5 min after (○, △) application of 1 μM angiotensin. Arrows, times at which angiotensin (AT₁) was applied. A and B, Examination of the role of intracellular Ca²⁺ release. Oocytes were incubated overnight with 10 nM thapsigargin (A, ●, ○, n = 3; B, □) or injected 30–60 min before measurements with 50 nl of 94 mM Cs₄BAPTA (A, ●, ○, n = 6; B, □) or 5 μg/ml PTX (C, ▲, △, n = 5; D, ■). E and F, Examination of the involvement of IP₃ receptors. Oocytes were injected 30–60 min before measurements with 50 nl of 10 mM heparin (molecular weight, 3000) (E, ○, △, n = 4; F, □). We also show the response of a control oocyte, coexpressing α1C,77 channels and AT₁A receptors (E, ●, ▲, △, n = 6; F, ■, □) to angiotensin before the interventions described in A–F.
compared with the \( \alpha_{1C,77} \) channel, we conclude that this locus is largely responsible for the angiotensin-induced modulation of the \( \alpha_{1C,77} \) channel coexpressed with the AT\(_{1A} \) receptor. This modulation takes place when Ba\(^{2+} \) is the charge carrier through the channel and is apparently independent of permeation of Ca\(^{2+} \) through the pore.

Our data on the differential modulation of Ca\(^{2+} \) channels by pore-permeating Ca\(^{2+} \) and Ca\(^{2+} \) released in the cytosol might indicate critical steps in cross-signaling between the angiotensin receptor and IP\(_3\)\(^{3-} \) gated Ca\(^{2+} \) stores. Such dual control adds to the complexity of the mechanisms of cross-talk between Ca\(^{2+} \) channels and G protein-coupled receptors and may be of fundamental physiological significance, considering that signaling may take place in confined cellular microdomains.

**Acknowledgments**

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