Angiotensin II activation of a chloride current in rabbit cardiac myocytes

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1. The effects of angiotensin II (Ang II) on membrane currents were investigated in single ventricular myocytes from the rabbit heart by the whole-cell voltage-clamp method.
2. In the presence of an inhibitor of Ca^{2+} currents (nifedipine at 3 μM or $CdCl_2$ at 0·3 mM) and a β-adrenoceptor blocker (bupranolol at 1 μM), 1 μM Ang II significantly increased the membrane conductance.
3. After elimination of K^+ from external and internal solutions and its replacement by Cs^+, Ang II at 0·1 μM increased an outwardly rectifying current that reached a maximum after about 40 min. The effect was concentration dependent (10^{-9}–10^{-6} M) and was inhibited by saralasin, an antagonist of Ang II receptors.
4. The reversal potential of the Ang II-induced current in the absence of K^+ was compatible with the Cl^- equilibrium potential at various external concentrations of Cl^-.
5. A Cl^- channel blocker, 4,4′-dinitrostilbene-2,2′-disulphonic acid (DNDS, at 5 mM), reversibly decreased the Ang II-induced current.
6. The Ang II-induced current developed when the internal solution contained Ca^{2+} (pCa 7·2 or 7·0) but not when it contained 10 mM EGTA without Ca^{2+}.
7. Besides developing a Cl^- current, Ang II at 1 μM increased the inwardly rectifying K^+ current (I_{K1}) and this effect reached maximum within 3 min.
8. The effect of Ang II on the action potential was biphasic: the duration of the action potential was initially reduced and then it was increased.
9. These results suggest that Ang II induces a Cl^- current that appears likely to modulate the action potential in rabbit ventricular myocytes.

Angiotensin II (Ang II) has a positive inotropic effect, with the generation of inositol 1,4,5-trisphosphate and 1,2-diacylglycerol that results from the hydrolysis of phosphoinositide (PI), in the cardiac muscles of various animals (Lindpaintner & Ganten, 1991; Baker, Booz & Dostal, 1992; Timmermans *et al.* 1993). The role of the hydrolysis of PI in the regulation of cardiac function remains, however, the subject of debate. Characteristics of the Ang II-induced positive inotropic effect are (1) prolongation of isometric twitch contraction (Ishihata & Endoh, 1993) and (2) a wide range of species- and tissue-dependent variations (Baker & Singer, 1988; Moravec *et al.* 1990; Lindpaintner & Ganten, 1991). These characteristics closely resemble those of the activation of other classes of receptors, such as α_1-adrenoceptors (see review by Endoh, 1991), that are also coupled with the hydrolysis of PI in cardiac muscle.

The stimulation of α_1-adrenoceptors decreases K^+ currents, including the transient outward K^+ current (I_{to}) and the inwardly rectifying K^+ current (I_{K1}) in cardiac myocytes (Fedida, Braun & Giles, 1993). Ang II increases the rate of diastolic depolarization and spontaneous discharges in human atrial tissues (Chen, Chang, Chiang, Cheng & Lin, 1991), a result that suggests that membrane currents are affected. Indeed, Ang II can facilitate a Ca^{2+} current (Freer *et al.* 1976; Allen, Cohen, Dhallan, Gaa, Lederer & Rogers, 1988), leading to the elevation of $[Ca^{2+}]_i$ (Kem *et al.* 1991; Xu, Sandirasegarane & Gopalakrishnan, 1993), and a Na^+ current (Nilius, Tytgat & Albitz, 1989; Moorman, Kirsch, Lacerda & Brown, 1989; Benz, Herzig & Kohlhardt, 1992). However, the effects of Ang II on other membrane currents have not been thoroughly investigated. The present study was therefore carried out to examine the effects of Ang II on membrane currents by the whole-cell patch-clamp method in

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single ventricular myocytes isolated from the rabbit heart. The Ang II-induced changes were found to be different from those reported for stimulation of α_1-adrenoceptors. Ang II predominantly activated a Cl^- current, a phenomenon that was characterized in the present study. A preliminary account of this study has been published as an abstract (Endoh, Morita & Kimura, 1993).

METHODS

Preparation of the cells

Single ventricular cells were prepared by a method similar to that described by Yazawa, Kaibara, Ohara & Kameyama (1990). In brief, male rabbits (weighing 1·8–2·8 kg) were anaesthetized with sodium pentobarbitone (65 mg kg^{-1}, I.V.) and their hearts were rapidly removed. Each excised heart was perfused with Tyrode solution for about 5 min on a Langendorff apparatus and then with nominally Ca^{2+}-free Tyrode solution for 5 min. Then the quiescent heart was perfused with Ca^{2+}-free Tyrode solution that contained 0·02% collagenase (Yakult, Tokyo) for 15–20 min. After washing out the collagenase by perfusing with a high K^+–low Cl^- solution (storage solution), the heart was gently agitated to dissociate single ventricular cells in the storage solution. The cells were stored at 4 °C.

Electrophysiological recordings

Single ventricular cells were dispersed in the recording bath under an inverted microscope (TMD; Nikon, Tokyo), and were superfused with the external solution. The temperature of the external solution was kept constant at 36·0 ± 1·0 °C. The whole-cell voltage-clamp method was essentially the same as that described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). Patch pipettes had a tip diameter of 1–2 μm and a resistance of 2–4 MΩ. Currents were recorded with a voltage-clamp amplifier (CEZ-2200; Nihon Kohden, Tokyo) and then filtered with a Bessel-type low-pass filter (24 dB $octave^{-1}$, E3201A; NF, Yokohama, Japan) at 5 kHz for feeding into a computer (PC-9801 RX; NEC, Tokyo).

Ramp clamping was performed with a function generator (FG-121B; NF, Yokohama, Japan) with a pulse range between −120 and +70 mV from a holding potential of −50 mV, and the ramp speed was 190 mV per 250 ms.

Solutions

The compositions of the external and internal solutions used in the experiments are listed in Table 1. For the Cl^--free external solution, NaCl was replaced by an equimolar amount of sodium gluconate. In order to block various channels and exchangers, the following compounds were used: 4-aminopyridine at 1 mM to block I_{to}; either nifedipine at 3 μM or $CdCl_2$ at 0·3 mM to block I_{Ca}; and ouabain at 20 μM to block the Na^+–K^+ pump. The internal concentration of free Ca^{2+} was adjusted by adding $CaCl_2$ to the internal solution that contained 10 mM EGTA. $[Ca^{2+}]_i$ was calculated from the equation of Fabiato & Fabiato (1979) with the correction proposed by Tsien & Rink (1980). As a result, the following concentrations of Ca^{2+} were used: pCa 7·0 (free Ca^{2+}, 1×10^{-7} M: 10 mM EGTA + 4 mM Ca^{2+}); pCa 7·2 (free Ca^{2+}, 6×10^{-8} M: 10 mM EGTA + 3 mM Ca^{2+}); and pCa 8·0 (free Ca^{2+}, 1×10^{-8} M: 10 mM EGTA + 0·6 mM Ca^{2+}). Ang II and saralasin were obtained from the Peptide Institute (Osaka, Japan). All numerical data are expressed as means ± S.E.M.

Liquid junction potentials

The liquid junction potential between the pipette solution and the bathing solution was 4·0 ± 0·2 mV ($n = 3$) and 4·5 ± 0·4 mV ($n = 3$) for the K^+-containing and Cs^+-containing internal solutions, respectively, assuming that 1·5 M KCl in the pipette was not associated with any junction potential with respect to the Tyrode solution. We compensated for the junction potential by shifting the current–voltage (I–V) curve by 4 mV in the negative direction. The junction potentials between the solutions with different Cl^- concentrations in the bath and 1·5 M KCl in the indifferent electrode were 2·7 ± 0·9, 6·2 ± 0·8 and 12·4 ± 0·7 mV ($n = 3$ for each) for 100, 65 and 38 mM Cl^-, respectively. Therefore, the I–V curves were shifted in the negative direction by the corresponding numbers of millivolts.

Table 1. Composition of solutions

	External solution (mM)				Internal solution (mM)			
	Tyrode	K^+ free	Low Cl^-	Low Cl^-, K^+ free	pCa ≫ 8	pCa 8·0	pCa 7·2	pCa 7·2, K^+ free
NaCl	140	140	—	—	—	—	—	—
Sodium gluconate	—	—	140	140	—	—	—	—
Potassium gluconate	—	—	5·4	—	—	—	—	—
Potassium aspartate	—	—	—	—	120	120	120	—
Caesium aspartate	—	—	—	—	—	—	—	120
KCl	5·4	—	—	—	30	30	30	—
CsCl	—	—	—	—	—	—	—	30
$MgCl_2$	1	1	1	1	1	1	1	1
NaH_2PO_4	0·33	0·33	0·33	0·33	—	—	—	—
MgATP	—	—	—	—	4	4	4	4
Hepes	1	1	1	1	5	5	5	5
Glucose	5·5	5·5	5·5	5·5	—	—	—	—
EGTA	—	—	—	—	0·5/1	10	10	10
$CaCl_2$	1·8	1·8	1·8	1·8	n.f.	0·6	3	—

The pH of all external solutions was adjusted to 7·4 with NaOH; pH of pCa 7·2 (K^+ free) internal solution was adjusted to 7·2 with CsOH and that of the other internal solutions was adjusted to 7·2 with KOH; pCa ≫ 8 indicates pCa appreciably larger than 8. n.f., nominally free.

RESULTS

Effects of Ang II in the presence of K^+

Since α_1-stimulation has been shown to decrease I_{K1} in rabbit ventricular myocytes (Fedida, Braun & Giles, 1991), we examined whether Ang II might also affect I_{K1} in a similar manner. Figure 1 shows representative traces obtained by 200 ms square pulses applied between +70 and −110 mV from a holding potential of −80 mV. Superfusion of Ang II at 1 μM for 6 min shifted the holding current in the inward direction and increased the magnitude of the membrane current in response to both hyperpolarizing and depolarizing pulses (Fig. 1*B*). Figure 1*C* shows the Ang II-induced current obtained by subtracting the control current (Fig. 1*A*) from the current in the presence of Ang II (Fig. 1*B*). The difference current was time independent. Figure 1*D* shows isochronal *I–V* relationships determined 100 ms after the onset of the pulses in Fig. 1*A* (control) and *B* (Ang II at 1 μM). The *I–V* relationship of the difference current is illustrated in Fig. 1*E*. The mean *I–V* curve of the difference current crosses the voltage axis at −43·7 ± 5·3 mV ($n = 3$), providing evidence that the difference current is not due to I_{K1} since the equilibrium potential for K^+ was calculated to be −88 mV under the present experimental conditions. These results indicate that the effect of Ang II is different from that of α-adrenoceptor agonists, because Ang II increased a current which was not reversed at E_K but was reversed at a more positive potential than E_K.

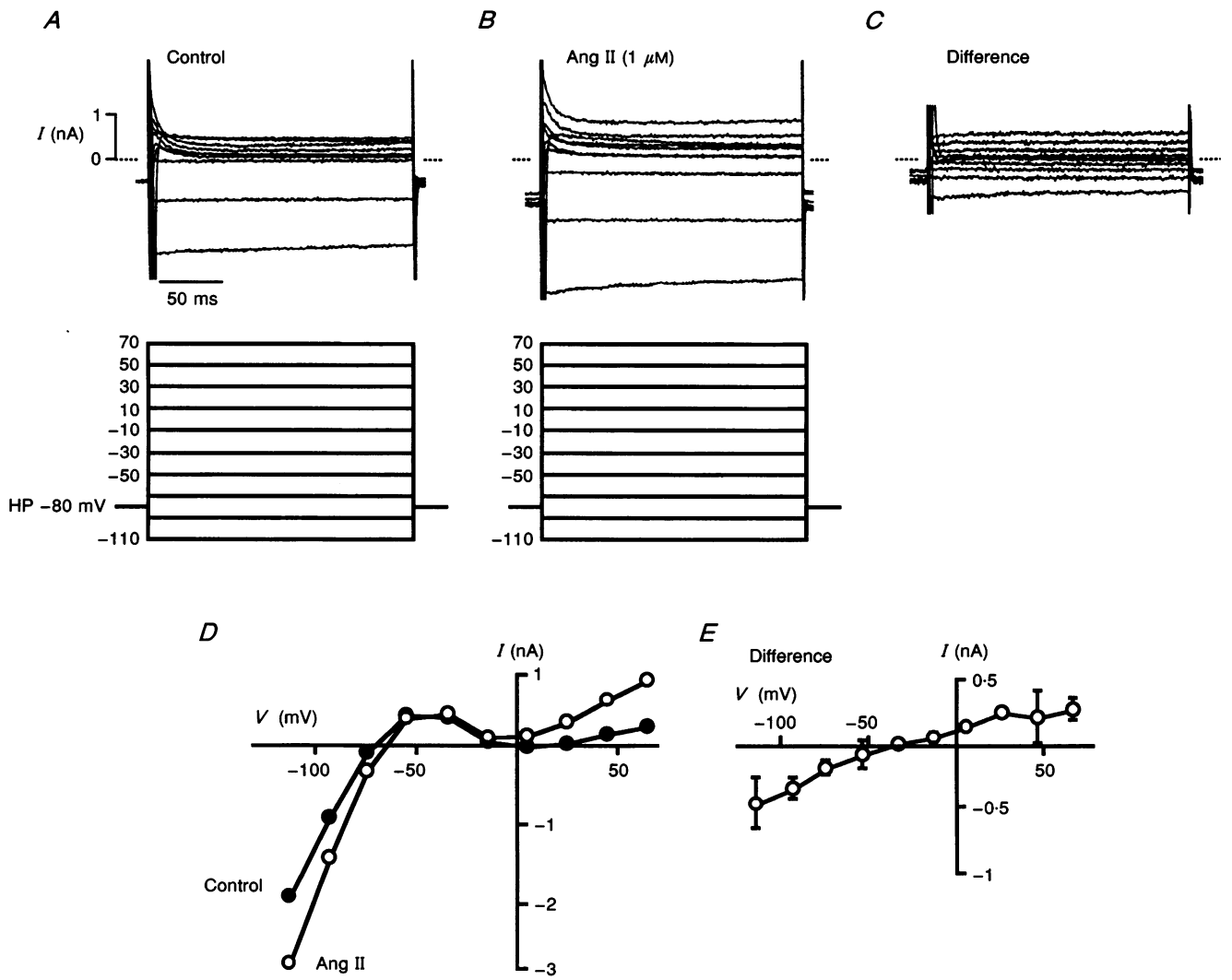

Figure 1. Effects of angiotensin II (Ang II) in the presence of K^+ inside and outside the cell membrane

A, control current in response to the step pulses indicated below. The holding potential was −80 mV. *B*, currents recorded with the same pulse protocol, 6 min after the addition of Ang II at 1 μM. *C*, the net Ang II-induced current obtained by subtracting currents shown in *A* from those in *B*. *D*, *I–V* relationships determined 100 ms after the onset of the square pulses for currents shown in *A* (●) and *B* (○). *E*, *I–V* relationship for the net Ang II-induced current. Each point is the mean ± S.E.M. of values from 3 cells.

Effects of Ang II in the absence of K^+

The reversal potential of the Ang II-induced current indicated that the current was either a non-specific cation current or a Cl^- current. We examined these possibilities by eliminating K^+ from external and internal solutions, replacing K^+ with Cs^+. The internal concentration of Ca^{2+} was set at pCa 7·2 to simulate physiological conditions.

Figure 2*A* shows a typical time course of the change in current in response to superfusion with Ang II at 0·1 μM in the absence of K^+. Ramp pulses were applied every 15 s and a series of square pulses was applied every 10 min. It can be seen from Fig. 2*A* that Ang II progressively increased the current but, in this case, the effect was more prominent in the outward direction. The increase reached a maximum after about 40 min. This response slowly disappeared after washing out Ang II. The extent of recovery was about 40% after washing out Ang II for 60 min (data not shown in figure).

Figure 2*B* and *C* shows currents obtained by square pulses in the control and after 40 min of superfusion with Ang II, respectively. The corresponding *I–V* curves are plotted in Fig. 2*E*. The difference currents are shown in Fig. 2*D* and the corresponding *I–V* curve in Fig. 2*F*. Figure 2*G* shows superimposed *I–V* relationships for the net Ang II-induced current, which were obtained by subtracting the control from the current at the various times indicated. Each *I–V* curve reversed at the same potential. The average reversal potential of the net Ang II-induced current was −34·3 ± 1·3 mV

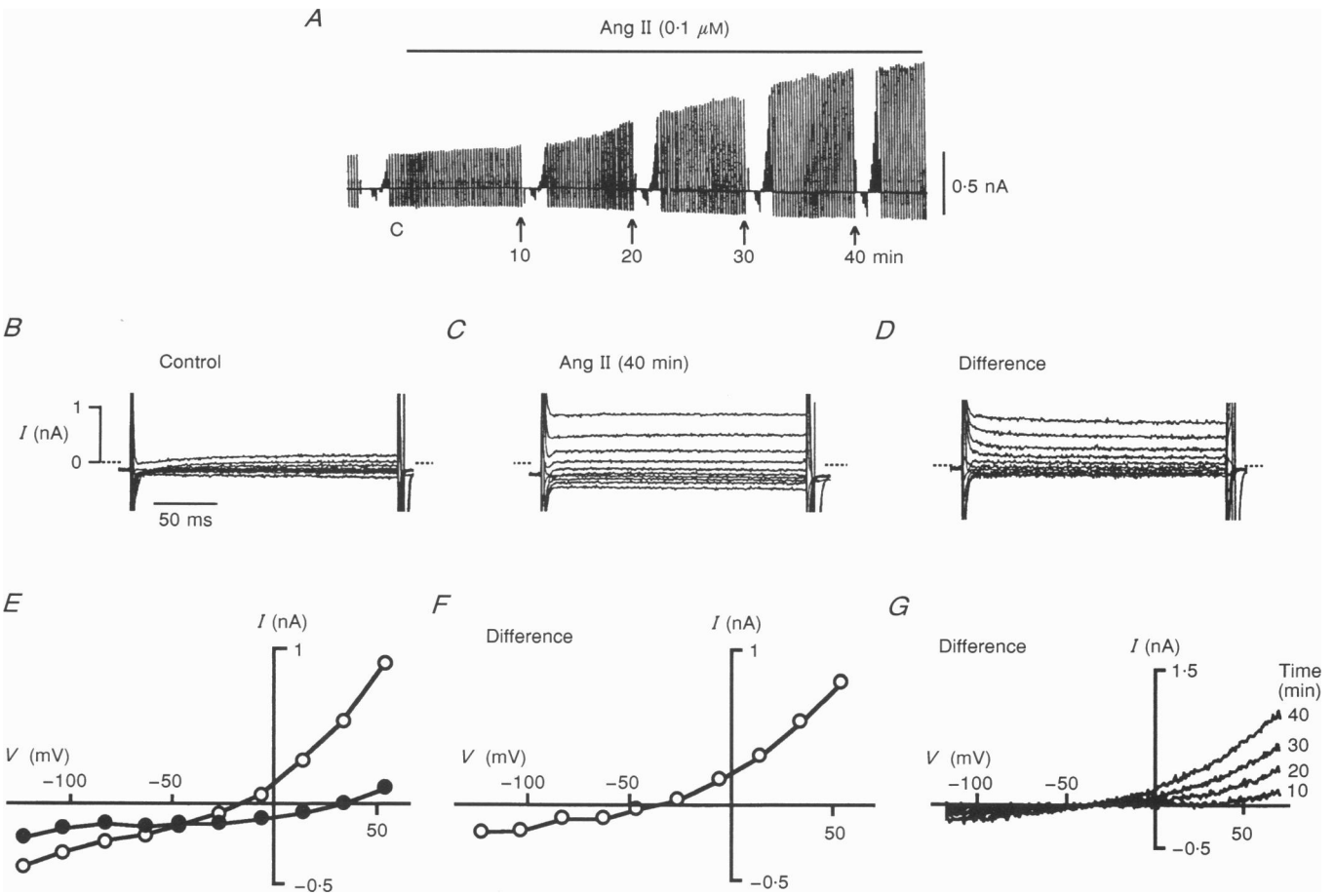

Figure 2. Effects of Ang II in the absence of K^+

External K^+ was omitted, and internal K^+ was replaced by Cs^+. *A*, the time course of the change in current during superfusion with Ang II at 0·1 μM. C, control. Ramp pulses were applied every 15 s and a series of square pulses was applied at 10 min intervals. The holding potential was −50 mV. Approximately 40 min was required for the current to reach a maximum steady state. *B*, control currents obtained with 200 ms square pulses with 20 mV steps from a holding potential of −50 mV. *C*, currents recorded with the same protocol but after 40 min of superfusion with Ang II at 0·1 μM. *D*, the Ang II-induced current, obtained by subtracting the current shown in *B* from that shown in *C*. *E*, isochronal *I–V* curves determined 100 ms after the onset of the square pulses for the currents shown in *B* (●) and *C* (○). *F*, *I–V* relationship for the Ang II-induced current shown in *D*. *G*, *I–V* curves for the net Ang II-induced current, recorded at 10 min intervals, as indicated by arrows in *A*. The control current at time 0 was subtracted from each current.

($n = 12$), which was close to the E_{Cl} of −38 mV obtained under our experimental conditions. This result suggests that the Ang II-induced current was more likely to have been a Cl^- current than a non-specific cation current, which would be expected to reverse at 0 mV.

Effects of Ang II in the absence of Cl^-

Ang II induced a current in the absence of K^+. However, the I–V curves obtained in the absence and in the presence of K^+ were different. In the absence of K^+, the current had an outward rectifying property while, in the presence of K^+, the outward rectification was not marked. In this latter case, the current tended to rectify in the inward direction. Furthermore, the average reversal potential of the Ang II-induced current was slightly more positive in the absence than that in the presence of K^+. These observations suggest that the Ang II-induced current has not only a Cl^- component but also a K^+ component.

Therefore, we next studied the effect of Ang II in the absence of external Cl^-, replacing Cl^- with gluconate. As shown in Fig. 3*A*, Ang II increased the inward current and the effect of Ang II was maximal within 3 min. Figure 3*B* and *C* shows the current traces obtained in response to

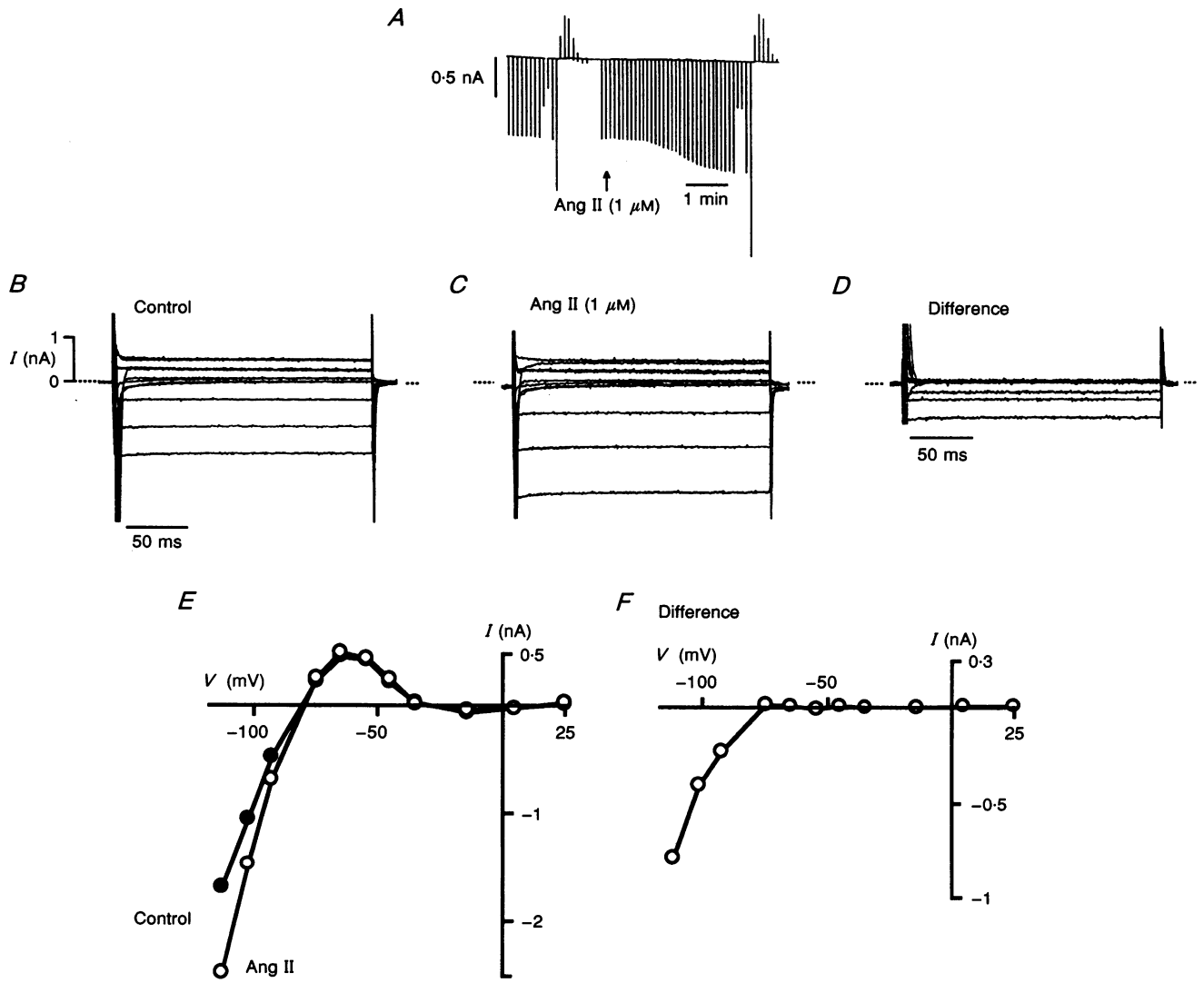

Figure 3. Effects of Ang II in the absence of external Cl^-

External Cl^- was replaced with gluconate in the presence of internal and external K^+. *A*, the time course of the changes in current during superfusion with Ang II at 1 μM. Square pulses from −80 to −100 mV were applied at 7 s intervals. *B*, control currents. The voltage was changed to −110, −100, −90, −70, −60, −50, −40, −30, −10, 10 and 30 mV in steps from a holding potential of −80 mV. *C*, currents recorded with the same pulse protocol but in the presence of Ang II at 1 μM. *D*, the net Ang II-induced current obtained by subtracting the current shown in *B* from that shown in *C*. *E*, I–V relationships determined 100 ms after the onset of the pulses for the currents shown in *B* (●) and *C* (○). *F*, I–V curve for the net Ang II-induced current shown in *D*. The I–V curve crosses the voltage axis at approximately −80 mV and has a negative slope, an indication that this current is I_{K1}.

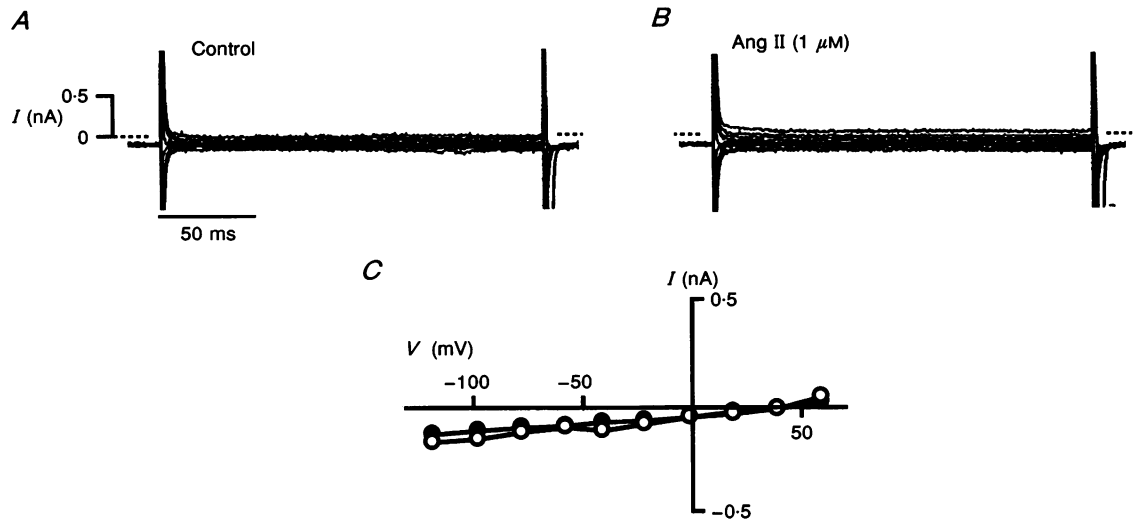

Figure 4. Effects of Ang II in the absence of external Cl^-, external K^+ and internal K^+
External K^+ was omitted, and internal K^+ was replaced by Cs^+. External Cl^- was totally replaced by an equimolar amount of gluconate. *A*, control current. The pulse range was between −120 and +60 mV from a holding potential of −50 mV. *B*, the currents obtained with the same protocol 11 min after application of Ang II at 1 μM. *C*, *I–V* curves in the absence (●) and in the presence of Ang II (○).

square pulses and Fig. 3*D* shows the difference currents. Figure 3*E* and *F* shows *I–V* curves for the original currents and the difference currents, respectively. The *I–V* relationship for the Ang II-induced current in the absence of Cl^- indicates that it was most likely I_{K1}, since it reversed near E_K (−80 mV), its inward component showed a prominent inward-directed rectification, and the outward component, although tiny, had a negative slope. Similar results were obtained in two other cells, indicating that Ang II increased I_{K1}.

Effects of Ang II at various concentrations of Cl^-

We attempted to identify the Ang II-induced current component that appeared in the absence of K^+, by varying the external concentration of Cl^-. First, the external Cl^- was totally replaced by gluconate in the absence of K^+. As shown in Fig. 4, the current hardly increased even in the presence of Ang II at 1 μM.

This result suggested that the components of the Ang II-induced current were carried by Cl^- and K^+. We examined the influence of various external concentrations of Cl^- to determine whether the reversal potential of the current would shift as predicted theoretically for a Cl^- current. In the presence of Cl^- at 38 mM in the internal solution, the external concentration of Cl^- was changed to 38, 65, 100 and 145·6 mM by replacing Cl^- with gluconate. The reversal potential of the Ang II-induced current was determined by use of ramp pulses.

Figure 5 shows the relationship between the reversal potential of the Ang II-induced current and the external concentration of Cl^-. Increasing the external concentration of Cl^- shifted the reversal potential in the negative direction and each value was close to the values of E_{Cl} calculated from the Nernst equation. The calculated values of E_{Cl} were 0, −12·2, −25·7 and −35·8 mV and the measured values of the reversal potential were −15·1 ± 1·9,

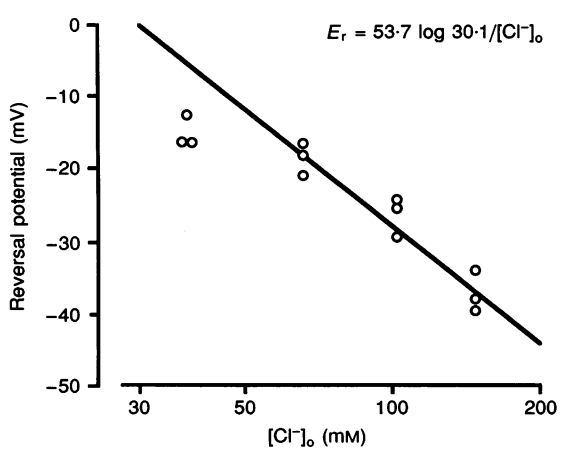

Figure 5. Dependence of the reversal potential (E_r) of the Ang II-induced current on the external concentration of Cl^- ($[Cl^-]_o$)
Ordinate, reversal potential of the Ang II-induced current. Abscissa, external concentration of Cl^- on a logarithmic scale. The internal solution contained 38 mM Cl^-. The values at 38 mM Cl^- were not included in the fitting of the line because the junctional potential may have had a major influence and may have shifted the values from those expected from the linear relationship.

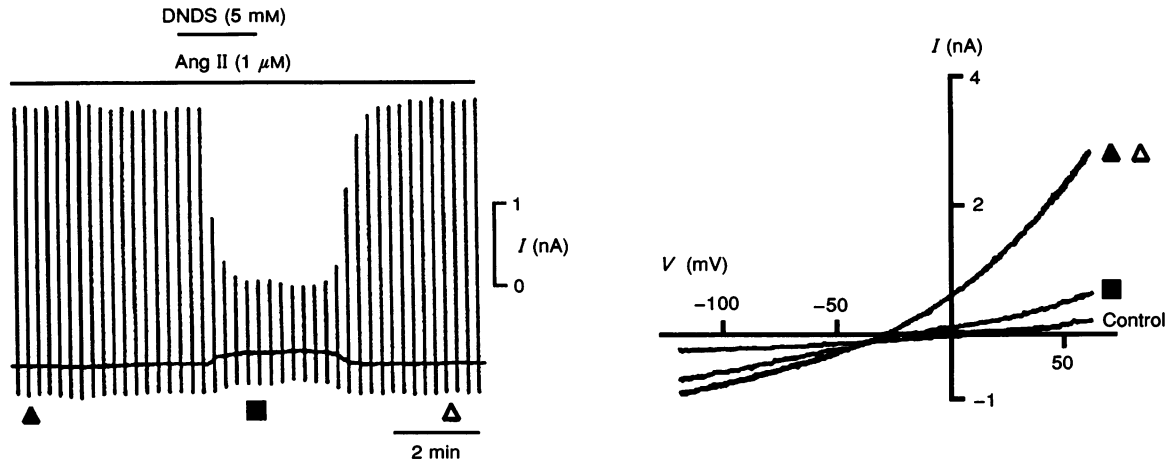

Figure 6. Effects of DNDS on the Ang II-induced current

A, actual recording of the current after the Ang II-induced response had reached a maximum. Regular ramp pulses were given at 15 s intervals. Application of DNDS decreased the current dramatically and the effect was rapidly reversed upon washing out DNDS. *B*, *I–V* relationships recorded from the control before application of Ang II (not shown in *A*) and after application of Ang II (▲), at the time when the effect of DNDS was maximal (■), and after washing out DNDS (△). Ang II was present at 1 μM throughout the experiment. Symbols correspond to those in *A*.

$-18{\cdot}5 \pm 2{\cdot}1$, $-26{\cdot}0 \pm 2{\cdot}2$ and $-37{\cdot}4 \pm 2{\cdot}5$ ($n = 3$, in each case) for 38, 60, 100 and 145·6 mM Cl^-, respectively. A linear relationship was observed between the logarithm of the concentration of Cl^- ($\geqslant$60 mM) and the reversal potential. A tenfold change in the concentration of Cl^- shifted the reversal potential by 53·7 mV, an indication that the Ang II-induced current was a Cl^- current.

Sensitivity to a Cl^- channel blocker

Figure 6 shows the effects of a Cl^- channel blocker, DNDS, on the Ang II-induced current after it had reached a maximum, 40 min after the application of Ang II. The Ang II-induced current was reduced within 1 min by the application of DNDS at 5 mM. The percentage reduction was $84{\cdot}0 \pm 4{\cdot}5\%$ ($n = 3$) at +70 mV and $20{\cdot}7 \pm 2{\cdot}5\%$ at −120 mV. Thus, the outward component was reduced more than the inward component. The inhibition of the Ang II-induced current was completely reversible. This result also supports our hypothesis that the Ang II-induced current was a Cl^- current.

Effects of an antagonist of Ang II receptors

We tested the effect of saralasin a specific antagonist of Ang II receptors on the Ang II-induced current. Figure 7 shows the time course of the development of current in the absence ($n = 6$) and in the presence ($n = 3$) of saralasin. The ordinate indicates the ratio of the developed current to the control. The ratio was apparently lower in the presence of saralasin. This result confirms that Ang II induced the current by activation of Ang II receptors.

Dependence on the concentration of Ang II of the Ang II-induced current

The concentration–response curve for the Ang II-induced current was obtained for a range of concentrations of Ang II from 10^{-9} to 3×10^{-6} M. The current was measured approximately 40 min after administration of Ang II, when a maximum steady-state effect was apparent. One concentration of Ang II was tested per cell. In order to compare the results from different cells, the current density

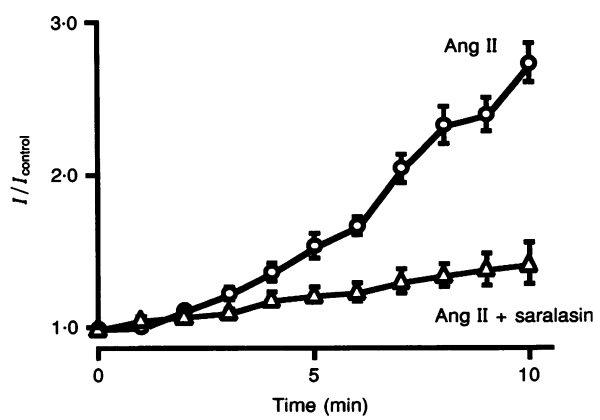

Figure 7. Effects of saralasin on the time course of the Ang II-induced current

The time course of the Ang II-induced current in the absence (○; $n = 6$) and the presence (●; $n = 3$) of saralasin at 1 μM. The ordinate is a ratio of the Ang II-induced current to the control.

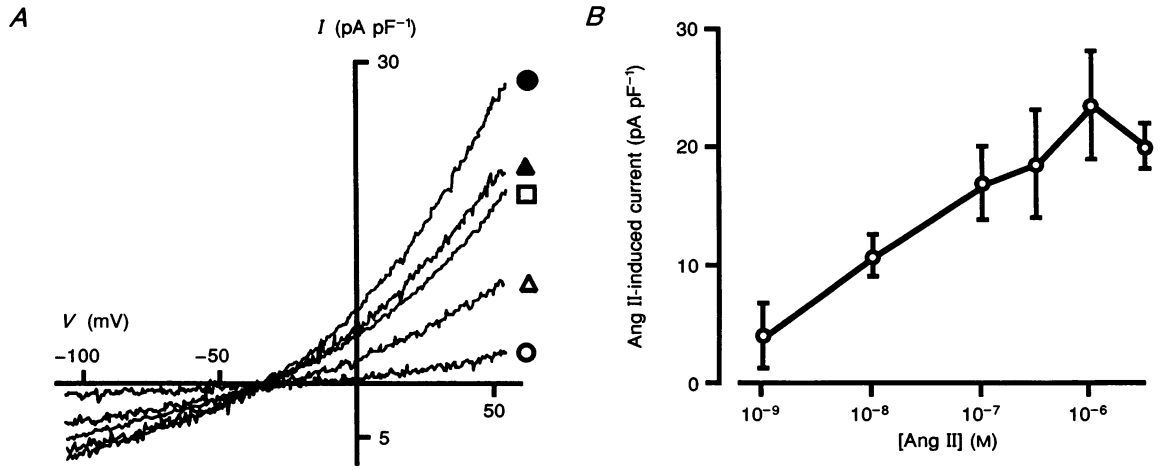

Figure 8. *I–V* curves and a concentration–response curve for Ang II-induced currents

A, steady state *I–V* curves for the net Ang II-induced currents determined when the effect was maximal. Each *I–V* curve was obtained from a different cell. The magnitude of the current was determined about 40 min after the addition of Ang II. *B*, a concentration–response curve for the Ang II-induced current. The magnitude of the current was determined at +60 mV when the Ang II-induced effect had reached a maximum steady level. Mean ± S.E.M. of results from 3 to 5 cells were plotted for each concentration of Ang II. [Ang II]: ○, 10^{-9} M; △, 10^{-8} M; □, 10^{-7} M; ▲, 3×10^{-7} M; ●, 10^{-6} M. The concentration of Ang II for the half-maximal effect was approximately 3×10^{-8} M.

was determined by dividing the current by the capacitance of the cell. Figure 8*A* shows superimposed *I–V* relationships for the net currents induced by 10^{-9}–10^{-6} M Ang II that were obtained by this experimental protocol. All the Ang II-induced currents crossed the axis at about −36 mV.

In Fig. 8*B*, steady-state current densities determined at +60 mV are plotted against the concentration of Ang II. Ang II from 10^{-9} M to 10^{-6} M increased the current in a concentration-dependent manner. The concentration–response curve reached a peak at 10^{-6} M

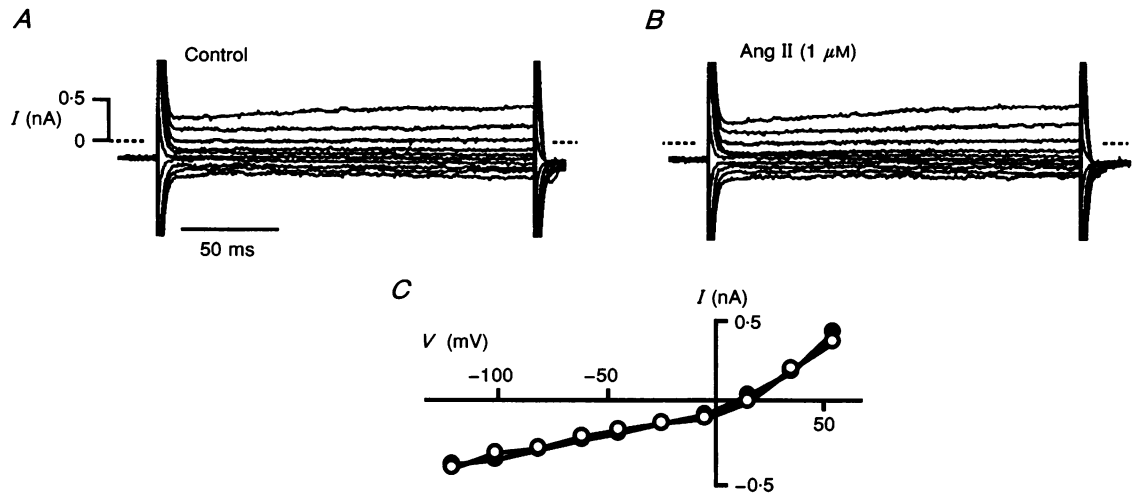

Figure 9. Effects of decreases in the intracellular concentration of Ca^{2+} on the Ang II-induced current

The pCa of the pipette solution was 8·0. *A*, control current for the range of voltages between −120 and +60 mV with a holding potential of −50 mV. *B*, currents recorded 7 min after application of Ang II at 1 μM. *C*, isochronal *I–V* relationships determined 100 ms after the onset of the square pulses for the currents shown in *A* (●) and *B* (○). *A* and *B* are superimposable, an indication that Ang II does not induce a current at pCa 8·0.

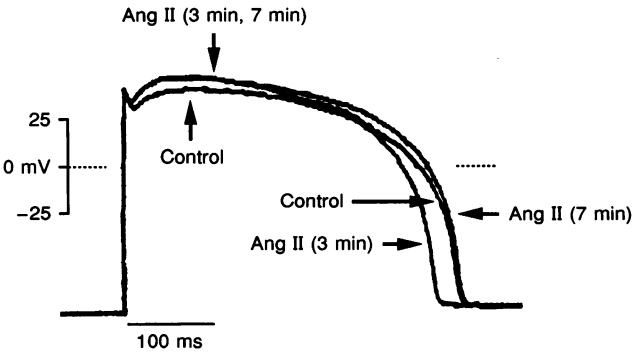

Figure 10. Effects of Ang II on the action potential in a rabbit ventricular cell
Action potentials were elicited by suprathreshold current pulses of 2 ms duration at 1 Hz. Superimposed action potentials from the control, and 3 and 7 min after application of Ang II at 1 μM. Ang II first shortened the duration of the action potential and then prolonged it. The height of the plateau was increased by Ang II.

Ang II. The EC_{50} value was about 3×10^{-8} M. This finding also confirmed that the increase in the membrane current was induced by stimulation of Ang II receptors.

Effects of intracellular Ca^{2+} on the Cl^- current

So far we have described the results of experiments that were performed with an internal solution at pCa 7·2. At an early stage of this series of experiments, we found that when the intracellular level of Ca^{2+} was low, namely with 1 mM EGTA but no added Ca^{2+} in the internal solution, the Cl^- current did not develop in six out of seven cells examined. Decreasing the level of EGTA from 1 to 0·5 mM in a nominally Ca^{2+}-free internal solution restored the effect of Ang II. Therefore, we made a quantitative comparison of the effects of internal Ca^{2+} on the Ang II-induced effect by reducing the level of internal Ca^{2+} to pCa 8·0. As shown in Fig. 9, Ang II did not elicit a current at pCa 8·0 in three out of three cells. Ang II never failed to develop a current at pCa 7·2 ($n > 40$) or at pCa 7·0 ($n = 3$). This result suggests that Ang II required intracellular Ca^{2+} for induction of a Cl^- current.

Effects of stimulation of Ang II receptors on the action potential

Figure 10 shows the effects of Ang II at 1 μM on the action potential. Administration of Ang II produced an increase in the height of the plateau and a biphasic change in the duration of the action potential, namely an initial decrease that reached minimum in about 1 min, which was followed by a steady increase. The increase of the amplitude of the action potential became maximal within 3 min. Even when we monitored the action potential for 7 min, the effect of Ang II on the duration of the action potential was not maximal. In this series of experiments, the clamp did not last for more than 7 min for unknown reasons so that we were unable to record the effects of Ang II for longer times. Similar results were obtained in two other cells.

DISCUSSION

We have shown that stimulation of Ang II receptors induces a Cl^- current in rabbit ventricular myocytes. In addition, we found that Ang II increases the inwardly rectifying K^+ current (I_{K1}), and that this effect develops much earlier than the increase in the Cl^- current in the same preparation. There have been several previous reports to indicate that Ang II affects various classes of ion channels and ion exchangers, including Ca^{2+} currents, Na^+ currents (Benz *et al.* 1992), K^+ currents and the Na^+–H^+ exchanger (Vallega, Canessa, Berk, Brock & Alexander, 1988) in cardiac, vascular and neuronal cells. In mesangial cells from the rat kidney, Ang II stimulated a Ca^{2+}-activated Cl^- current (Okuda, Yamashita & Kurokawa, 1986). However, this current developed maximally immediately after application of Ang II, indicating involvement of a different signal transduction mechanism for Ang II from that in the heart. To date, no effects of Ang II on a Cl^- current or on I_{K1} have been described in cardiac myocytes.

In the rabbit heart, an Ang II-induced positive inotropic effect was reported as early as 1974 (Bonnardeaux & Regoli, 1974). However, there have been only a few reports of electrophysiological studies of the effects of Ang II in the rabbit heart. Ang II was demonstrated to induce spontaneous action potentials in partially depolarized preparations of rabbit atria (Freer *et al.* 1976), results that suggested augmentation of the Ca^{2+} current. Whole-cell clamp data from rabbit atrial cells showed that Ang II increased the Ca^{2+} current and decreased the delayed K^+ current (Bkaily *et al.* 1988). An increase in the Ca^{2+} current was also reported in bundles of calf Purkinje fibre (Kass & Blair, 1981) and neonatal rat heart (Allen *et al.* 1988). However, in the rabbit ventricular cells, Ikenouchi, Bridge, Lorell, Zhao & Barry (1992) failed to detect an increase in the Ca^{2+} current or in the intracellular concentration of Ca^{2+}, although the motion of cells was augmented by Ang II.

Differences between stimulation of Ang II receptors and of α_1-adrenoceptors

It is evident that Ang II-induced changes in membrane currents, as determined in the present study, are markedly different from those induced by stimulation of α_1-adrenoceptors even though both activation of Ang II receptors (Ishihata & Endoh, 1993) and α_1-receptors (Endoh, Hiramoto, Ishihata, Takanashi & Inui, 1991) enhance the hydrolysis of PI in the rabbit ventricular myocardium. Considering that the concentration- and time-dependent changes in the rate of hydrolysis of PI that is induced by these two classes of receptors are similar and that both types of receptor are associated with a positive inotropic effect, our present findings for membrane currents are rather unexpected. It became clear during the present study that hydrolysis of PI, accelerated by activation of different classes of receptors, leads to modulation of different types of ion channel, although the way in which such segregation is achieved in the cell is unknown. Namely, even though the positive inotropic effect induced by Ang II closely resembles that induced by stimulation of α_1-adrenoceptors, it is not necessarily true that the mechanisms of signal transduction to the membrane ion channels are identical.

The Cl^- current induced by Ang II

In cardiac myocytes, various classes of Cl^- currents have been found to date. They include the currents that are activated by cAMP-dependent protein kinase (Harvey, Clark & Hume, 1990; Horie, Hwang & Gadsby, 1992), by external ATP (Matsuura & Ehara, 1992), and by stretching (Hagiwara, Masuda, Shoda & Irisawa, 1992). Most of these currents have been investigated in the absence of K^+ and are reported to rectify outwardly. Furthermore, the corresponding I–V relationships and the magnitudes of currents appear similar to those of the Ang II-activated current examined in this study. The difference between currents lies in the dependence on Ca^{2+}: the Ang II-induced current requires internal Ca^{2+} for activation, while all the other above-mentioned currents do not.

Ca^{2+}-dependent Cl^- currents have been reported in rabbit ventricular myocytes (Zygmunt & Gibbons, 1991). Such currents depend strongly upon an influx of Ca^{2+} through Ca^{2+} channels and show outward rectification that is similar to that of the Ang II-induced current. The mechanism associated with the requirement for Ca^{2+} of the Ang II-induced Cl^- current is unknown. Nonetheless, it is probable either that opening of the channel depends directly on internal Ca^{2+} or that the signal-transduction pathway for activation of Ang II receptors is a Ca^{2+}-dependent process, for example a process that involves activation of protein kinase C, which in turn might phosphorylate the Cl^- channel. Recently, Walsh (1991) reported a Cl^- current that was activated by phorbol esters or other activators of protein kinase C in the ventricular myocytes of the guinea-pig. Although Ang II does not have a positive inotropic effect on the guinea-pig ventricular myocytes, this current may be related to the Cl^- current that was characterized in the present study.

The Cl^- current and positive inotropic effect

Is the Ang II-induced Cl^- current involved in the positive inotropic effect induced by the stimulation of Ang II receptors? The time required for maximum activation of the Cl^- current by Ang II was approximately 40 min from the time of application and the half-time of activation was about 20 min. By contrast, the increase in contractile force induced by Ang II reaches a maximum steady-state value within 5 min and the half-time is about 3 min (A. Ishihata & M. Endoh, unpublished observations). Thus, the time courses of the two phenomena are entirely different. Furthermore, the changes in the action potential induced by Ang II did not parallel the development of the Cl^- current: the duration of the action potential decreased transiently within the first few minutes, recovered slowly to the control level and then increased continuously for up to 7 min (the maximum time examined). The initial shortening of the duration of the action potential could be attributed to an increase in I_{K1} and the subsequent prolongation might be due to the development of the Cl^- current. Thus, it is unlikely that the Ang II-induced Cl^- current contributes to the positive inotropic effect of Ang II. The increase in amplitude of the action potential plateau, which reaches a maximum height at about 3 min, may be due to an increase in the Ca^{2+} current (Kass & Blair, 1981; Bonnardeaux & Regoli, 1974) and may contribute to the inotropic effect. However, Ikenouchi *et al.* (1992) failed to find an increase in the Ca^{2+} current or in $[Ca^{2+}]_i$ and concluded that the intracellular alkalinization induced by activation of the Na^+–H^+ exchanger is crucial for the inotropic effect in rabbit ventricular myocytes.

The functional role of the Cl^- current is unknown at present. Ang II has been shown to be involved in the development of cardiac hypertrophy (for review, see Johnston, 1992). Since a Cl^- current has been shown to be involved in the volume regulation of cells (Tseng, 1992), it is tempting to speculate that the Ang II-induced Cl^- current might be responsible, at least in part, for the chronic pathophysiological effects of Ang II, for example, cardiac hypertrophy. Although the slow development of the Ang II-induced Cl^- current supports such a possibility, further experiments are necessary to elucidate the mechanism and the role of the Ang II-induced Cl^- current in cardiac myocytes.

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