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Angiotensin II-activated Ca^{2+} entry-induced release of Ca^{2+} from intracellular stores in rat portal vein myocytes

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1 The action of angiotensin II (AII) was studied in single myocytes from rat portal vein in which the cytoplasmic Ca^{2+} concentration was estimated by emission from dyes Fura-2 or Indo-1 and the Ca^{2+} channel current was measured with the whole-cell mode of the patch-clamp technique.

2 Most of the AII-evoked increases in $[Ca^{2+}]_i$ were reduced by about 60% after pretreatment with ryanodine and caffeine to deplete intracellular Ca^{2+} stores. However, in some cells the AII-induced Ca^{2+} responses were of small amplitude and resembled those obtained in the presence of ryanodine and caffeine. Both types of Ca^{2+} responses induced by AII were selectively inhibited by losartan, suggesting that the AII effects resulted from activation of the angiotensin AT_1 receptors.

3 The concentration-response curve to AII had an EC_{50} value close to 1 nM for the increase in $[Ca^{2+}]_i$ obtained after depletion of intracellular Ca^{2+} stores. This value was increased to around 18 nM in experiments where the intracellular Ca^{2+} stores were not depleted.

4 AII-evoked Ca^{2+} responses were abolished in the absence of external Ca^{2+} and in the presence of 1 μM oxodipine to block L-type Ca^{2+} channels.

5 Intracellular applications of the $InsP_3$ receptor antagonist, heparin or an anti-PdtIns antibody did not modify AII-induced Ca^{2+} responses.

6 Our results show that AII releases Ca^{2+} from intracellular stores without involving $InsP_3$ but through a Ca^{2+} release mechanism activated by Ca^{2+} influx through L-type Ca^{2+} channels.

Keywords: Angiotensin II; Ca^{2+} channels; intracellular Ca^{2+} store; anti-PdtIns antibody; smooth muscle; portal vein

Introduction

In a variety of smooth muscle cells, angiotensin II (AII) has been reported to bind to angiotensin AT_1 receptors leading to activation of a phosphatidylinositol-specific phospholipase C and generation of both inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG; for review, Lassègue *et al.*, 1994). These second messengers activate at least two major biochemical cascades. The release of $InsP_3$ mobilizes Ca^{2+} from intracellular stores, whereas DAG, in concert with cellular Ca^{2+}, activates protein kinase C (PKC). PKC may play a central role in phosphorylation of cellular proteins, including Ca^{2+} channels (Gutierrez *et al.*, 1994). Angiotensin AT_1 receptors have also been shown to regulate adenylate cyclase activity; in most systems angiotensin AT_1 receptors act via G_i protein to inhibit adenylate cyclase (Anand-Srivastava, 1983) although a stimulation of adenylate cyclase has been reported in vascular myocytes (Kubalak & Webb, 1993). Finally, in vascular and non-vascular smooth muscle cells, AII appears to stimulate voltage-dependent Ca^{2+} channels (Mironneau *et al.*, 1980; Bkaily *et al.*, 1988; Ohya & Sperelakis, 1991).

In the present study, we investigated the mechanisms underlying the action of AII on cytosolic Ca^{2+} concentration in vascular myocytes from rat portal vein. We show for the first time that AII releases Ca^{2+} from intracellular stores, without involving the $InsP_3$ receptor. The AII-induced Ca^{2+} release results from a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism, activated by the influx of Ca^{2+} through dihydropyridine-sensitive L-type Ca^{2+} channels.

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Methods

Cell preparation

Wistar rats (150-160 g) were stunned and then killed by cervical dislocation. The portal vein was cut into several pieces and incubated for 10 min in low Ca^{2+} (40 μM) physiological solution, then 0.8 mg ml^{-1} collagenase, 0.25 mg ml^{-1} pronase E, and 1 mg ml^{-1} bovine serum albumin were added at 37°C for 20 min. After this time, the solution was removed and the pieces of vein were incubated again in a fresh enzyme solution at 37°C for 20 min. Tissues were then placed in enzyme-free solution and triturated with a fire-polished Pasteur pipette to release cells. Cells were stored on glass coverslips at 4°C in physiological solution containing 0.8 mM Ca^{2+} and used on the same day, or maintained in short-term primary culture in medium M199 containing 10% foetal calf serum, 2 mM glutamine, 1 mM pyruvate, 20 units ml^{-1} penicillin, and 20 μg ml^{-1} streptomycin. In the latter case, cells were kept in an incubator gassed with 95% O_2, 5% CO_2 at 37°C and used within 36 h.

Fluorescence measurements

Cells were loaded by incubation in physiological solution containing 1 μM Fura-2-acetoxymethylester or Indo-1-acetoxy methylester for 20 min at room temperature. These cells were washed and allowed to cleave the dye to the active Fura-2 or Indo-1 compound for at least 30 min. Fura-2 or Indo-1 loading was usually uniform over the cytoplasm, and compartmentalization of the dye was never observed. Measurement of intracellular Ca^{2+} concentration with the two fluorescent dyes have been published previously and the calibration curves have been determined within cells (Pacaud *et al.*, 1993; Leprêtre *et*

al., 1994). Indo-1 or Fura-2-loaded cells were mounted in a perfusion chamber and placed on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan). Some experiments were carried out in the presence of 1 μM oxodipine (a light-stable dihydropyridine derivative) in order to inhibit voltage-dependent Ca^{2+} channels. All measurements were made at 25±1°C.

Membrane current and $[Ca^{2+}]_i$ measurements

Voltage-clamp and membrane current recordings were made with a standard patch-clamp technique using a List EPC-7 patch-clamp amplifier (Darmstadt-Eberstadt, Germany). Whole-cell membrane currents were measured with the perforated-patch method except in experiments where anti-PtdIns antibodies, heparin and Indo-1 were dialyzed into the cell with the patch pipette. In order to obtain a perforated patch, nystatin (80–100 $\mu g\ ml^{-1}$) was present in the patch pipette solution. Patch pipettes had resistances of 1–4 MΩ. Membrane potential and current records were stored and analyzed with an IBM-PC computer (P-clamp system, Axon, Foster City, CA, U.S.A.). Simultaneous measurements of intracellular calcium concentration were carried out in some experiments. Briefly, 50 μM Indo-1 was added to the pipette solution, and entered cells following establishment of the whole-cell recording mode. $[Ca^{2+}]_i$ was estimated from the 405/480 nm fluorescence ratio using a calibration determined within cells, as previously described (Pacaud *et al.*, 1993). Patch-clamp experiments were done at 30±1°C.

Antibodies

Anti-PtdIns antibodies were added to the pipette solution to allow dialysis of the cell after a break through in whole-cell recording mode. Purification and specificity of these antibodies have been reported previously (Leprêtre *et al.*, 1994).

Solutions

The normal physiological solution contained (in mM): NaCl 130, KCl 5.6, $MgCl_2$ 1, $CaCl_2$ 2, glucose 11, HEPES 10, pH 7.4 with NaOH. The basic pipette solution contained (in mM): CsCl 130, HEPES 10, pH 7.4 with CsOH. Ca^{2+}-free external solution was prepared by omitting $CaCl_2$ and by adding 0.5 mM EGTA. For the recordings of calcium channel current, 5 mM $BaCl_2$ was substituted for $CaCl_2$ in the reference solution, and CsCl was used instead of KCl in the pipette and external solutions to block outward potassium currents. In addition, 10 mM EGTA, 5 mM Na_2ATP, 1 mM $MgCl_2$ were added to the basic pipette solution. Angiotensin II was applied to the recorded cell by pressure ejection from a glass pipette for the period indicated on the records. Before each experiment a fast application of physiological solution was tested and cells with movement artefacts were excluded.

Chemicals and drugs

Collagenase was obtained from Worthington (Freehold, NJ, U.S.A.); pronase (type E), bovine serum albumin, angiotensin II, and nystatin were from Sigma (St Louis, MO, U.S.A.). DuP 753 (Losartan) (2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-ethyl] imidazole, potassium salt) was from Dupont Merck (U.S.A.) and PD 123319 (S-1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4, 5, 6, 7-tetrahydro-1H-imidazo-[4,5-c]pyridine-6-carboxylic acid, difluoroacetate mono hydrate) was from Parke Davis (U.S.A.). CGP 42112A (N-α-nicotinoyl-Tyr-Lys[N-α-CBZ-Arg]-His-Pro-Ile-OH) was from Neosystem Laboratories (Strasbourg, France). M199 medium was from Flow Laboratories (Puteaux, France). Foetal bovine serum was from Flobio (Courbevoie, France). Streptomycin, penicillin, glutamine and pyruvate were from Gibco (Paisley, UK). Oxodipine was a gift from Dr Galiano (IQB, Madrid, Spain). Caffeine was from Merck (Nogent sur Marne, France). Fura-2AM, Indo-1 AM, Indo-1 and ryanodine were from Calbiochem (Meudon, France).

Data analysis

The results are expressed as means±s.e.mean. Significance was tested by means of Student's *t* test. *P* values of <0.05 were considered as significant. Inhibition and concentration-response curves were analyzed by a nonlinear least-square fitting programme, according to models involving one- or two-binding sites.

Results

Effects of angiotensin II on $[Ca^{2+}]_i$

Ejection of 10 nM AII to single myocytes induced different types of increase in $[Ca^{2+}]_i$. In 45% of the cells tested ($n=80$), AII initiated a slow and small Ca^{2+} response (Figure 1a) with a mean increase in $[Ca^{2+}]_i$ of 43±3 nM ($n=36$). The AII-induced Ca^{2+} response reached a peak 22±5 s ($n=36$) after application of AII and then returned slowly to basal Ca^{2+} value. However, in 45% of the cells tested ($n=80$), the AII-evoked Ca^{2+} response seemed to possess two components (Figure 1b,c) with a faster component superimposed on the slow Ca^{2+} response. The maximal value of the Ca^{2+} response induced by application of 10 nM AII was 119±6 nM ($n=36$). Finally, in the remaining 10% of cells tested ($n=8$) oscillatory Ca^{2+} responses (Figure 1d) were obtained in which the delay between the start of AII microejection and the peak of the first response was 21±5 s. It was noted that the initial Ca^{2+} transient (125±10 nM, $n=8$) was larger than those which followed. The amplitude of subsequent Ca^{2+} transients showed a small decline throughout the exposure to the agonist. The time course of the second and subsequent transients were similar showing little change in the rate of rise. Increasing AII concentration from 10 nM to 10 μM did not modify the pattern of activity. At 10 μM AII, the amplitude of the initial Ca^{2+} transient within the burst (128±12 nM, $n=4$) and the number of cells showing a burst of Ca^{2+} oscillations (10%, $n=40$) were similar to those obtained with 10 nM AII (Figure 1d).

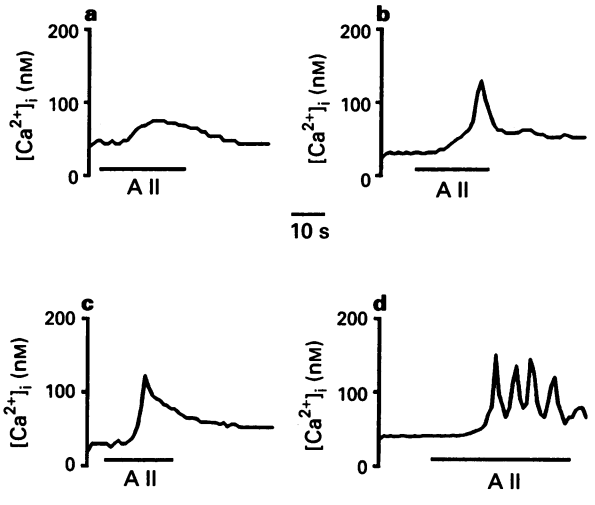

Figure 1 Effects of applications of 10 nM angiotensin II (AII) on $[Ca^{2+}]_i$ in single myocytes of rat portal vein. The cells were loaded with Fura-2AM and not patch-clamped. Four examples of AII-induced Ca^{2+} responses showing a slow and small Ca^{2+} response (a) on which a faster component can be superimposed (b, c). In some cells oscillatory Ca^{2+} responses were observed (d). In all cells, the delay between the microejection of AII and the peak of the response was greater than 10 s.

In order to test the possible involvement of a Ca^{2+}-induced Ca^{2+} release mechanism in the AII-induced rise in $[Ca^{2+}]_i$, the AII-induced Ca^{2+} responses were evoked in cells treated with caffeine and ryanodine. Caffeine is known to increase the open probability of the Ca^{2+}-activated Ca^{2+} release channel (Hermann-Frank *et al.*, 1991). When added to the extracellular solution, caffeine (10 mM) caused a large transient increase in $[Ca^{2+}]_i$ that reached 312±22 nM ($n=8$), as shown in Figure 2a. When $[Ca^{2+}]_i$ returned to basal level application of 10 or 100 nM AII produced small Ca^{2+} responses (59±13 nM, $n=14$) in cell batches in which the AII-induced Ca^{2+} responses measured under control conditions were of large amplitude (112±11 nM, $n=14$).

Ryanodine is thought to inhibit the CICR mechanism by binding to Ca^{2+}-release channels and stabilizing an open subconductance state (Meissner, 1986). When the cells were preincubated in the presence of 10 μM ryanodine for 60 min (Figure 2b), the first caffeine application induced a reduced Ca^{2+} response (200±25 nM, $n=35$). After this response, the basal $[Ca^{2+}]_i$ level was slightly increased to 85±4 nM ($n=35$). A second application of caffeine failed to induce any response. Under these conditions, the AII-induced Ca^{2+} responses were of small amplitude (45±7 nM, $n=35$) in comparison to those measured in control conditions (110±10 nM, $n=35$). These results show that in the presence of caffeine and ryanodine which prevent activation of the CICR mechanism, AII evoked a single type of Ca^{2+} response of similar amplitude (around 50 nM) in all the cells tested ($n=35$). Concentration-response curves for AII were obtained from control cells and from cells treated with ryanodine and caffeine. As illustrated in Figure 3, the maximal Ca^{2+} response evoked by AII was obtained at 1 μM. The concentrations producing half-maximal response (EC_{50}) were estimated to be 18.5±3.5 nM in control conditions ($n=3$) and 1.5±0.2 nM after caffeine and ryanodine treatment ($n=3$).

Effect of AII antagonists on the AII-evoked Ca^{2+} response

The pharmacological profile of the AII-evoked Ca^{2+} response was examined with compounds selective for the angiotensin AT_1 and AT_2 receptors. Two non-peptide antagonists of the angiotensin AT_1 receptors, losartan and DuP532 inhibited in a concentration-dependent manner the [^{3}H]-AII binding in rat portal vein strips with inhibition constants in the nanomolar range for the angiotensin AT_1 receptors (Pelet *et al.*, 1995). As illustrated in Figure 4a, 100 nM losartan completely abolished the AII responses ($n=15$) as expected from the radioligand experiments. The selective non-peptide antagonist of angiotensin AT_2 receptors, PD123319 was not used because this compound inhibited in a concentration-dependent manner the Ca^{2+} channel current ($n=5$). Therefore, we used CGP42112A, a peptide antagonist which shows a high affinity for angiotensin AT_2 receptors (around 2.5 nM) and a low affinity for angiotensin AT_1 receptors (around 3.5 μM) on [^{3}H]-AII bind-

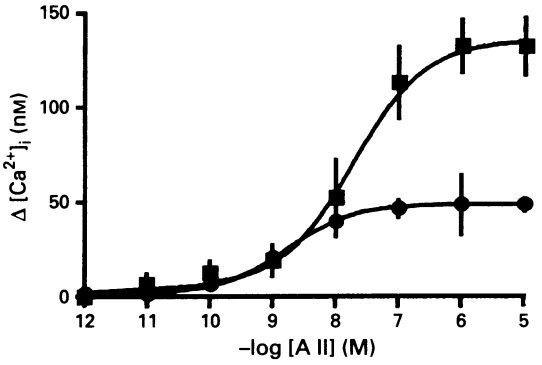

Figure 3 Concentration-response curves to angiotensin II (AII). $[Ca^{2+}]_i$ values are expressed as a percentage of the maximal response induced by AII in control conditions (■) and after depletion of the intracellular Ca^{2+} store by pretreatment with 10 μM ryanodine for 60 min and 10 mM caffeine for 1 min (●). The cells were loaded with Indo-1AM and not patch-clamped. Each point represents the mean±s.e.mean for 5–15 cells. The curves were fitted to the data by means of a non-linear least-square fitting programme, according to a single binding site model.

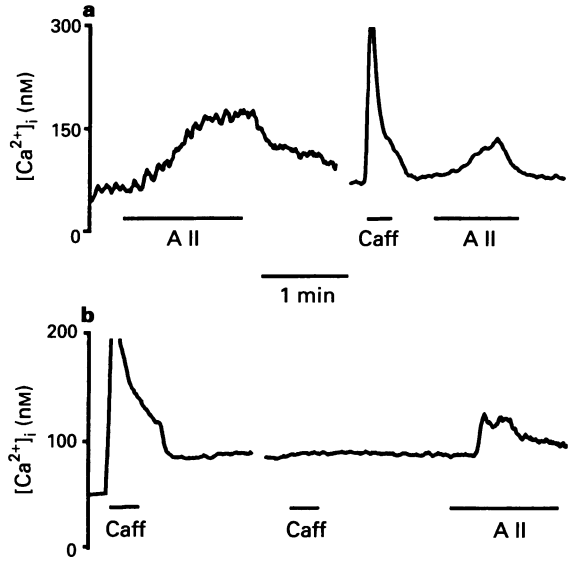

Figure 2 Effects of ryanodine and caffeine on the increase in $[Ca^{2+}]_i$ induced by 10 nM angiotensin II (AII). (a) After depletion of the intracellular Ca^{2+} stores by application of 10 mM caffeine (Caff), the amplitude of the AII-induced Ca^{2+} response was reduced by about 50%. (b) When the cells were preincubated in the presence of 10 μM ryanodine for 60 min, the first 10 mM caffeine application induced a Ca^{2+} response, but the second one was ineffective. Under these conditions, the AII-induced Ca^{2+} responses were small with amplitudes ranging from 40 to 55 nM. The cells were loaded with Indo-1AM and not patch-clamped.

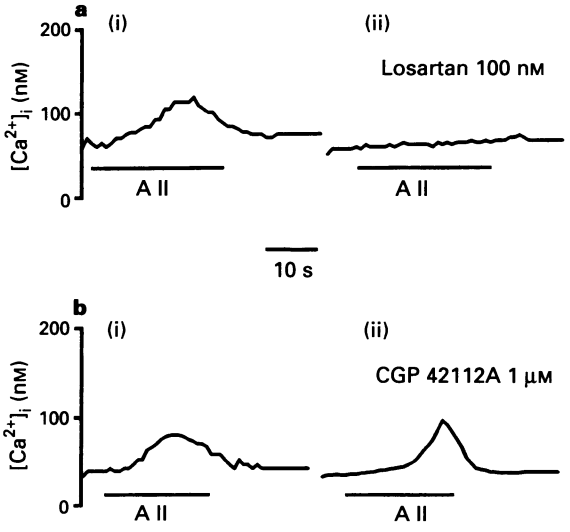

Figure 4 Effects of angiotensin II (AII) antagonists on the AII induced Ca^{2+} responses obtained after pretreatment with 10 μM ryanodine for 60 min and 10 mM caffeine for 1 min. (a) The increase in $[Ca^{2+}]_i$ induced by 10 nM AII (i) was completely blocked after addition of 100 nM losartan (ii) for 5 min. (b) The AII-induced increase in $[Ca^{2+}]_i$ (i) was unaffected in the presence of 100 nM CGP42112A (ii) for 5 min. The cells were loaded with Fura-2AM and not patch-clamped.

ing to portal vein strips (Pelet *et al.*, 1995). At a concentration which completely blocked the angiotensin AT_2 receptors (100 nM) the AII-evoked Ca^{2+} response was unaffected (control: 54 ± 10 nM; in the presence of CGP42112A: 61 ± 11 nM; $n=8$, Figure 4b). These results show that the AII-induced Ca^{2+} response is mediated mainly through activation of angiotensin AT_1 receptors.

Effects of Ca^{2+}-free solution and Ca^{2+} channel antagonists

In myocytes maintained at a holding potential of -50 mV, ejection of 10 nM AII in 2 mM Ca^{2+} solution did not induce a noticeable inward current ($n=15$), in contrast to application of 10 μM noradrenaline (Leprêtre *et al.*, 1994). As shown in Figure 5a, the AII-evoked Ca^{2+} response was suppressed in Ca^{2+}-free 0.5 mM EGTA external solution for 30 s ($n=11$). A similar suppression of the Ca^{2+} response was also obtained in the presence of 1 μM oxodipine (a light-stable dihydropyridine) for 5 min (Figure 5b; $n=16$). These results suggest that the AII-evoked Ca^{2+} response is dependent on Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels.

Effects of heparin and anti-PdtIns antibody

In an attempt to test the possible involvement of $InsP_3$-induced Ca^{2+} release in the AII-evoked rise in $[Ca^{2+}]_i$, heparin (5 mg ml^{-1}), a competitive antagonist of the $InsP_3$ binding to its receptors (Guillemette *et al.*, 1989), was added to the pipette solution. The effect of AII was studied with heparin in the pipette solution in cells clamped at -50 mV (Figure 6a,b). In the presence of heparin 5 mg ml^{-1} for 5 min, the basal $[Ca^{2+}]_i$ (62 ± 9 nM, $n=7$) was not different from that obtained under control conditions (64 ± 8 nM, $n=6$). In the presence of heparin, AII induced a rise in $[Ca^{2+}]_i$ in control cells (Figure 6a) as well as in cells treated with ryanodine and caffeine (Figure 6b). The peak Ca^{2+} responses reached 121 ± 12 nM ($n=5$) in untreated cells and 57 ± 8 nM ($n=5$) in ryanodine-treated cells, respectively and thus, were not significantly different from those obtained in the absence of heparin (114 ± 7 nM and 50 ± 11 nM, $n=15$, respectively).

When the anti-PdtIns antibody (12.5 μg ml^{-1}) was added to the pipette solution for 3 min, the noradrenaline-induced transient Ca^{2+} response was completely inhibited (Leprêtre *et al.*, 1994). With concentrations of 12.5 and 25 μg ml^{-1} anti-PdtIns antibody, the two types of AII-evoked Ca^{2+} responses were not affected (control: 56 ± 12 nM, Figure 6c, and 140 ± 22 nM, $n=6$, respectively; in the presence of anti-PdtIns antibody: 47 ± 11 nM, Figure 6c, and 134 ± 43 nM, $n=6$, respectively). These results indicate that the $InsP_3$-induced Ca^{2+} release does not participate in the AII-evoked Ca^{2+} response.

Discussion

In this study, we have shown that activation of angiotensin AT_1 receptors in myocytes of rat portal vein produces an increase in $[Ca^{2+}]_i$ that is dependent on both Ca^{2+} influx through L-type Ca^{2+} channels and Ca^{2+} release from intracellular stores. The AII-induced Ca^{2+} release results from a ryanodine-sensitive Ca^{2+}-induced Ca^{2+} release mechanism and does not involve the $InsP_3$ receptor.

The AII-induced activation of L-type Ca^{2+} channels is supported by the observation that AII-evoked Ca^{2+} responses are suppressed in Ca^{2+}-free solution and in the presence of 1 μM oxodipine which selectively inhibits voltage-dependent Ca^{2+} channels (Baron *et al.*, 1994). Thus, AII-evoked increase in $[Ca^{2+}]_i$ appears to be dependent on Ca^{2+} influx in a manner similar to that induced by activation of α_{2A}-adrenoceptors (Leprêtre & Mironneau, 1994). However, the rise in $[Ca^{2+}]_i$ induced by α_{2A}-adrenoceptor activation (around 30 nM) was not affected by pretreatment with ryanodine and caffeine. This is in contrast to the present data which show that the AII-induced $[Ca^{2+}]_i$ rise was reduced by about 60% when it was

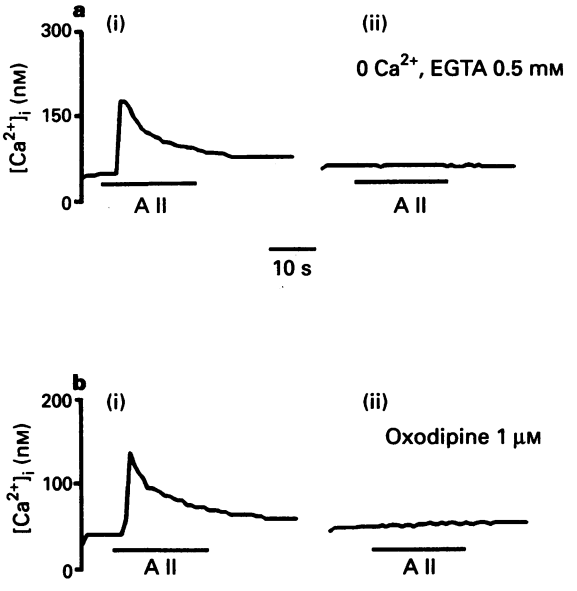

Figure 5 Effects of Ca^{2+}-free solution and oxodipine on the angiotensin II (AII)-induced increase in $[Ca^{2+}]_i$. (a) The Ca^{2+} response induced by 10 nM AII (i) was removed after incubation in Ca^{2+}-free 0.5 mM EGTA-containing solution for 3 min (ii). (b) The Ca^{2+} response induced by 10 nM AII (i) was blocked after addition of 1 μM oxodipine for 5 min (ii). The cells were loaded with Fura-2AM and not patch-clamped.

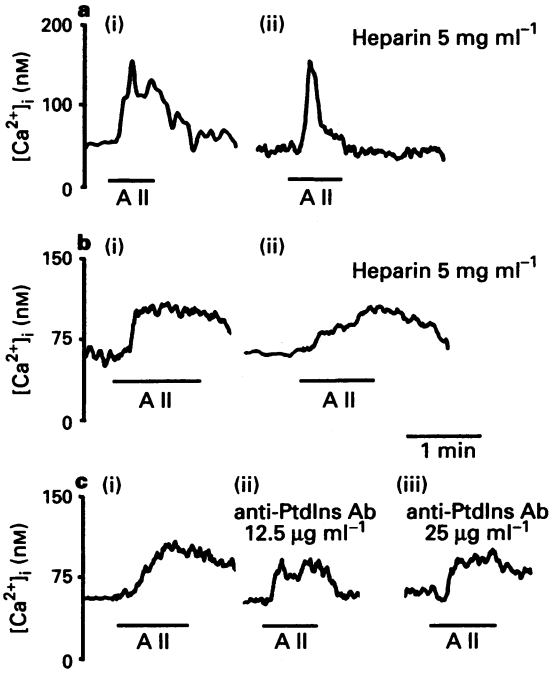

Figure 6 Effects of heparin and anti-PtdIns antibody on the increase in $[Ca^{2+}]_i$ induced by 10 nM angiotensin II (AII) at holding potential of -50 mV. (a, b) AII was applied 1.5 min (i) and 5 min (ii) after break through into the whole-cell recording mode in external control solution (a) or after pretreatment with 10 μM ryanodine for 60 min and 10 mM caffeine for 1 min (b). The pipette solution contained 5 mg ml^{-1} heparin. In (c), the cells were dialyzed with a normal pipette solution (i) and with a pipette solution containing 12.5 μg ml^{-1} (ii) or 25 μg ml^{-1} (iii) anti-PtdIns antibody for 3 min. The cells were loaded with Indo-1 and patch-clamped.

elicited immediately after caffeine application or after pretreatment with ryanodine and caffeine. This indicates that AII-induced Ca^{2+} influx triggers Ca^{2+} release from caffeine- and ryanodine-sensitive Ca^{2+} stores. As the AII-induced Ca^{2+} responses are around 50 nM in myocytes in which the intracellular Ca^{2+} stores have been largely depleted, this suggests that the Ca^{2+} threshold for activation of the CICR mechanism may range between 30–50 nM in venous myocytes. Involvement of the CICR mechanism in AII-induced Ca^{2+} responses is also supported by the fact that the concentration-response curve to AII established in myocytes pretreated with ryanodine and caffeine is shifted to the left with an EC_{50} value decreasing from 18 nM in the presence of the CICR mechanism to 1.5 nM in the absence of the amplification mechanism. The EC_{50} value obtained in the absence of the CICR mechanism is similar to that obtained on stimulation of the Ca^{2+} channel current by AII which is in the nanomolar range (N. Macrez-Leprêtre, J.L. Morel & J. Mironneau, unpublished data).

In several vascular and non-vascular smooth muscles, AII-induced mobilization of the intracellular Ca^{2+} stores has been ascribed to AII-induced stimulation of $InsP_3$ formation, with the generated $InsP_3$ mediating the release of stored Ca^{2+} (Varol *et al.*, 1989; Pfeilschifter *et al.*, 1989; Sachinidis *et al.*, 1993). However, in our experiments, the AII-induced rise in $[Ca^{2+}]_i$ was not affected in the presence of heparin or anti-PdtIns antibody in the pipette solution suggesting that the Ca^{2+} releasing action of AII could not be due to $InsP_3$ generation or to a positive regulation of $InsP_3$-induced Ca^{2+} release by the Ca^{2+} that enters the cell via L-type Ca^{2+} channels. Thus, it is proposed, for the first time, that the rise in $[Ca^{2+}]_i$ elicited by AII in venous myocytes is due to both Ca^{2+} influx and Ca^{2+} store release, via activation of the CICR mechanism. In venous myocytes, a CICR mechanism that could be initiated by Ca^{2+} influx through voltage-dependent Ca^{2+} channels has been previously identified in our laboratory (Grégoire *et al.*, 1993).

In arterial smooth muscle cells, AII-induced oscillations in $[Ca^{2+}]_i$ have been reported previously, which strongly depend on external Ca^{2+} concentration (Johnson *et al.*, 1991). Two categories of mechanism have been postulated to explain Ca^{2+} oscillations. The first model proposes a Ca^{2+}-mediated positive feedback regulation of $InsP_3$ production by phospholipase C, which results in a burst of $InsP_3$ production (Petersen & Wakui, 1990). Another mechanism is that the Ca^{2+} released from the stores will further enhance Ca^{2+} release. In this case, a rise in $[Ca^{2+}]_i$ from whatever source, sensitizes the $InsP_3$ receptor/channel or the ryanodine receptor/channel to Ca^{2+} and then induces a Ca^{2+}-induced Ca^{2+} release mechanism (Berridge, 1993; Dupont & Goldbeter, 1994). Our results are consistent with the proposal that Ca^{2+} oscillations induced by AII are generated by the caffeine- and ryanodine-sensitive CICR mechanism. They also suggest that activation of the ryanodine-receptor/channel may require a much smaller $[Ca^{2+}]_i$ (around 50 nM) than that needed to activate the $InsP_3$ receptor/channel (160 nM; Iino *et al.*, 1993).

Inhibition of AII-evoked Ca^{2+} responses by losartan but not by CGP42112A indicates that AII binds essentially to angiotensin AT_1 receptors. This result is supported by binding data obtained in rat portal vein smooth muscle which have identified two subpopulations of angiotensin II receptors (Pelet *et al.*, 1995). The angiotensin AT_1 receptor subpopulation represents 75% of the total binding sites and shows high affinity for losartan and low affinity for CGP42112A. The angiotensin AT_2 subpopulation represents 25% of the total binding sites and shows low affinity for losartan and high affinity for CGP42112A. Both angiotensin AT_1 and AT_2 receptor subtypes have been proposed as mediators of the contractile response of rat portal vein smooth muscle to AII (Pelet *et al.*, 1995). Our results suggest that activation of angiotensin AT_2 receptors is not involved in the modulation of $[Ca^{2+}]_i$ in venous myocytes. Further experiments are required to identify the mechanism by which angiotensin AT_2 receptors may modulate the contractile activity of smooth muscle without affecting Ca^{2+} homeostasis.

In conclusion, the present study demonstrated that in rat portal vein myocytes activation of angiotensin AT_1 receptors promotes an increase in $[Ca^{2+}]_i$ which depends on both Ca^{2+} influx through L-type Ca^{2+} channels and Ca^{2+}-induced Ca^{2+} release from the ryanodine-sensitive Ca^{2+} store, without involving the $InsP_3$ receptor.

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