Intracellular and extracellular renin have opposite effects on the regulation of heart cell volume. Implications for myocardial ischaemia

Walmor C De Mello

Abstract
The influence of intracellular renin plus angiotensinogen (Ao) as well as angiotensin (Ang) II on cell volume was investigated in myocytes isolated from the heart of four-month-old cardiomyopathic hamsters (TO-2) and normal hamsters (F1B). Measurements of cell width and cell length were performed on quiescent cells using a Px-it imaging and computer system. The cell volume was calculated assuming the cells as elliptical cylinders and taking the cell depth equal to one third of cell width. For measurements of sodium pump current, the cells were voltage clamped (holding potential -40 mV) using the whole cell configuration. Cells were exposed to Krebs solution to inhibit the pump and then to normal Krebs solution to reactivate the pump. In other experiments the cells were voltage-clamped (holding potential -40 mV) and changes in the background current elicited by renin plus Ao or by Ang II were monitored. The results indicated that: a) intracellular dialysis of renin (128 pmol Ang I/ml) plus Ao (110 pmol Ang I generated by renin by exhaustion) decreased the cell volume concurrently with the activation of the sodium pump; b) intracellular losartan (10⁻⁸ M) or extracellular ouabain (10⁻⁸ M) or extracellular renin plus Ao (10⁻⁸ M) abolished the effect of renin plus Ao on cell volume; c) intracellular Ang II (10⁻⁸ M), by itself, reduced cell volume and increased the pump current density; d) intracellular administration of renin plus Ao, at the same concentration used intracellularly, increased cell volume and inhibited the sodium pump. This increase of cell volume elicited by extracellular renin plus Ao was related to the activation of the Na-K-2Cl cotransporter; e) intracellular Ang II (10⁻⁸ M) reversed cell swelling induced by hypotonic solutions.

Conclusions. Intracellular and extracellular renin plus Ao have opposite effects on sodium pump and cell volume regulation in the failing heart. Both effects of renin plus Ao are dependent upon the formation of Ang II. Since intracellular Ang II counteracted the cell swelling induced by hypotonic solution, it is reasonable to think that the activation of the intracrine renin-angiotensin system might play a protective role during myocardial ischaemia by reducing cell volume.

Introduction
Cell volume activates stretch-sensitive ion channels and is an important contributor to metabolism, gene expression and protein synthesis. Ang II, through, hypotonic stress induced by ischaemia, for instance, leads to accumulation of metabolites intracellularly and consequent cell swelling due to water entering the cells. Although the sodium pump plays an important role on the maintenance of cell volume evidence is available that there is an interaction between Na-K-ATPase and the activation of the Na-K-2Cl cotransporter which is responsible for cell swelling in different tissues including myocytes from rabbit’s atrium. The relationship between the sodium pump and the cotransporter is complex and not totally clear. In skeletal muscle in culture, for instance, the Na-K-2Cl-mediated K uptake has been found to be insensitive to ouabain, at least during short incubations while in skeletal muscle, in situ, ouabain abolishes the cotransporter.

It is known that the circulating renin angiotensin system is an important regulator of blood volume and that intracellular and extracellular renin alters cellular functions like the inward calcium current or gap junctional conductance in the failing heart but no information is available if renin influences heart cell volume. It is the aim of this work to investigate: a) if intracellular renin plus angiotensinogen (Ao) and angiotensin (Ang) II have an effect on cell volume in normal heart; b) if the alteration in cell volume is related to activation of the sodium pump; c) if intracellular and extracellular renin have similar effects on the regulation of cell volume; d) if the effect of intracellular renin plus Ao on cell volume is greater in the failing heart when the RAS is activated.

In the present work this problem was investigated in myocytes isolated from the failing ventricle of four-month-old cardiomyopathic hamsters and the results were compared with those achieved in age-matching controls.

Methods
Cardiomyopathic hamsters (TO-2) four-month-old (Biobreeders, MA), at the hypertrophic stage of the disease, and normal hamsters (F1B)
controls were used. The animals were kept in the Animal House at constant temperature (24°C) and humidity following the recommendations of National Institute of Health. Animals were kept on a normal laboratory animal diet and given tap water ad libitum. The animals were anaesthetised with sodium pentobarbital (50 mg/Kg, i.p.), and the heart was removed with the animals under deep anaesthesia.

**Sodium pump current**

All experiments were performed in a small chamber mounted on the stage of an inverted phase-contrast microscope (Diaphot, Nikon). Ventricular cells were placed in a modified cultured dish in an open perfusion microincubator (Model PDMI-2, Medical System). Cells were allowed to adhere to the bottom of the chamber for 15 minutes and were superperfused with normal Krebs solution. The electrical measurements were carried out using a patch-clamp technique in a whole cell configuration with an Axon (model 200B) amplifier. Series resistance originating from the tips of the micropipettes was compensated for electronically.

To study the influence of intracellular renin plus Ao or Ang II on the sodium pump, the compounds were added to the pipette solution and dialysed into the cell. The cells were voltage clamped using the whole cell configuration. The holding potential was kept at -40 mV. The sodium pump was suppressed by superperfusing the cells with K+ -free solution for 10 minutes and then the sodium pump was reactivated by superperfusing the cells with normal Krebs solution containing 5 mM of potassium ions. In some experiments, the cells were voltage clamped at -40 mV and the changes in background current elicited by intracellular renin plus Ao or by Ang II were recorded (Ko = 5.4 mM).

**Cell isolation procedure**

Cells were obtained from the ventricles of TO-2 following the method of Powell and Twist and Tanigushi et al. The heart was removed and immediately perfused with normal Krebs solution containing: (mM): NaCl-136.5; KCl-5.4; CaCl2-1.8; MgCl2-0.53; NaH2PO4-0.3; NaHCO3-11.9; glucose 5.5; HEPES 5, pH adjusted to 7.3. After 20 minutes a Ca-free solution containing 5.4 mM of potassium ions. In some experiments, the cells were voltage clamped at -40 mV and the changes in background current elicited by intracellular renin plus Ao or by Ang II were recorded (Ko = 5.4 mM).

**Results**

The results showed that intracellular renin plus Ao and extracellular renin plus Ao both significantly increased sodium pump current compared to the control group (p<0.05). The difference between the groups was statistically significant (p<0.05).

**Measurements of cell width, length and volume**

Measurements of cell length and width were made in quiescent ventricular myocytes immersed in Krebs solution using an inverted phase contrast microscope (Nikon) and a high resolution camera (Paxcam). Images were obtained with a Pax-it imaging management system and stored on a computer. The volume of the cardiomyocytes was calculated by the following equation: V=π wdl/4 where w is the width, d the depth and l the length of the myocyte. Moreover, it was assumed that the cells were elliptical cylinders and that the depth (d) was one third of the value of width.

**Table 1**

Effect of intracellular and extracellular renin plus Ao on heart cell volume (µm³) in FIB.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After intracellular renin plus Ao</th>
<th>After extracellular renin plus Ao</th>
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<tr>
<td></td>
<td>(n=24)</td>
<td>(n=27)</td>
<td>(n=26)</td>
</tr>
<tr>
<td>20,050±998</td>
<td>17,152±970</td>
<td>24,850±1,000</td>
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<td>4 animals</td>
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**Drugs**

Ang II, ouabain, Ao and bumetanide were from Sigma Chemical Company. Losartan was from Merck Sharp and Dohme and renin, from rat kidney, was kindly provided by Dr Jan Danser, Erasmus University, The Netherlands. This sample of renin contains an amount of Ao that is 40 times lower than that of normal human blood.

**Statistical analysis**

Data are expressed as mean±SEM. Comparison between groups was done by analysis of variance. Differences were considered significant when p<0.05.

**Results**

To investigate the influence of intracellular renin...
(128 pmol Ang I/ml) plus Ao (110 pmol Ang I/ml generated by renin by exhaustion) on cell volume, both compounds were added to the pipette solution and dialysed into the cell while changes on cell length and width were continuously monitored at rest, using a high resolution camera. As shown in table 1 the average cell volume for the control hamster (F1B) was 20,050±998 μm³ (n=25) and for cardiomyopathic hamster at 4 months of age, was 29,060±1,100 μm³ (n=25).

When the cells from F1B were dialysed with renin (128 pmol Ang I/ml) plus Ao (110 pmol Ang I/ml generated by renin by exhaustion) there was a decrease of cell volume within 20 minutes (see table 1) which was mainly due to a decrease of cell width. Similar experiments performed on cells of TO-2 at four months of age showed a greater decline in cell volume. Indeed, the average cell volume (29,060±1,100 μm³; n=25) was reduced to 21,266±900 μm³ (n=26) 20 minutes after the introduction of both compounds into the cell (table 2). To investigate if the decline in cell volume was related to the activation of the sodium pump, the peak pump current generated by reactivation of the Na pump previously inhibited by K-free solution was measured before and after the intracellular dialysis of renin plus Ao. Considering the different sizes of the cardiomyocytes, all values of pump current were expressed as pA/pF. The capacitance of cell membrane in these cardiomyocytes varies from 91–135 pF. Figure 1a shows the transient peak outward current elicited by reactivation of the sodium pump in control hamster. In cells dialysed with renin (128 pmol Ang I/ml) plus Ao (110 pmol Ang I/ml generated by renin by exhaustion) the peak outward current was enhanced in controls but particularly in cardiomyopathic hamsters (figure 1b).

The relationship between the sodium pump and the cell volume was confirmed by exposing the cells to Krebs solution containing ouabain (10⁻⁸ M). There was no change of cell volume or pump current when renin plus Ao were administered intracellularly in presence of ouabain in TO-2 (figure 2) as well as in controls (20,050±998 μm³ (n=25)/20,048±1,100 μm³ (n=22) (p>0.05). Moreover, losartan (10⁻⁸ M) administered to the cytosol also blocked the effect of renin on pump current and cell volume in cardiomyopathic hamsters as shown in figure 2 suggesting that Ang II is involved in the effect of renin plus Ao on cell volume. Identical results were found when cells were exposed to Krebs solution containing 1 mM of BaCl₂ to inhibit the potassium conductance (not shown).

Is the effect of intracellular renin on cell volume due to the formation of Ang II?
To investigate this possibility Ang II (10⁻⁸ M) alone was dialysed inside the cell while changes of cell length and width were monitored. As shown in figure 3, intracellular administration of Ang II in cardiomyopathic hamsters decrease the cell volume significantly, reaching a steady-state value within

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td><strong>Effect of intracellular and extracellular renin plus Ao on heart cell volume (μm³) in TO-2.</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>29,060±1,100</td>
</tr>
<tr>
<td>(n=25)</td>
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<tr>
<td>(4 animals)</td>
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<td>p&lt;0.05</td>
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**Key:** Renin (128 pmol Ang I/ml). Ao (110 pmol Ang I/ml generated by renin exhaustion); Ang = angiotensin; Ao = angiotensinogen; TO-2 = cardiomyopathic hamsters.
The effect of intracellular Ang II was abolished by intracellular administration of losartan (10^{-8} M) or by extracellular administration of ouabain (10^{-8} M) (figure 3). The change in cell volume elicited by Ang II was related to the activation of the sodium pump because the peptide increased the pump current, an effect abolished by ouabain (10^{-8} M) (figure 3). Identical results were found in normal hamsters (not shown).
Measurements of membrane potential performed on cells of TO-2 under current clamp conditions indicated a hyperpolarisation of 7±1 mV (n=25) (p<0.05) during the intracellular dialysis of Ang II which is related to the activation of an electrogenic sodium pump because it was abolished by ouabain (10^{-8} M) (not shown).

The possibility that the activation of the sodium pump by Ang II be related to an influx of sodium through the surface cell membrane can be ruled out because at 0 mM Na inside the cell no change on pump current was recorded. To test the control of the intracellular Na concentration experiments were performed in cells dialysed with Npipf=0 and then superfused with ouabain (10^{-8} M). In none of these experiments (n=7) a shift of the holding current was produced by the administration of ouabain the external medium indicating a good control of ouabain the external medium.

To determine the possible degree of contamination of the effect of Ang II by different ionic currents, the K-activated current was studied at different potentials using 100 ms duration step changes in membrane potential between -120 to + 60 mV. Figure 4 shows that steady state currents elicited in presence of ouabain (10^{-8} M) were insensitive to changes in extracellular K concentration from 0–10 mM. Moreover, these currents were also insensitive to BaCl2 (2 mM) added to the bath solution (not shown) and at 5 mM Na concentration, inside the pipette, the K-dependent current was small compared to that elicited at 80 mM Na inside the cell (figure 4). These observations show the dependence on the outward current on extracellular K and intracellular Na ions.

**Intracellular and extracellular renin have opposite effects on cell volume**

The question whether extracellular renin had an effect on cell volume similar to that described above for intracellular renin was investigated. For this, measurements of cell volume were performed before and after the administration of renin (128 pmol Ang I/ml per minute) plus Ao (110 pmol Ang I generated by renin to exhaustion) to the bath. As shown in table 1 the extracellular administration of renin plus Ao increased, not decreased, the cell volume in normal hamsters. Indeed, before the administration of renin plus Ao, the average cell volume reached its peak value Ang II (10^{-8} M) and thereafter reduced cell volume and inhibited the effect of extracellular renin plus Ao on cell volume in cardiomyopathic hamsters. Similar results were found in normal hamsters. Indeed, before the administration of renin plus Ao, the cell volume was 20,001±1,300 μm^3 (n=25) and after renin plus Ao (10^{-8} M) the effects of renin plus Ao on cell volume were monitored. Figure 5 shows that

![Figure 4](https://example.com/image.png)

**Figure 4**

Current-voltage relationship in presence of ouabain (10^{-8} M) and changing the extracellular K concentration from 0–5.4 mM.

related to the formation of Ang II at heart cell membrane. Interestingly, in cells incubated with renin (128 pmol Ang I/ml per minute) plus Ao (110 pmol Ang I generated by renin to exhaustion) for 80 hours the membrane potential measured in cells under current clamp conditions, showed an appreciable increase in membrane polarisation (-100±3 mV; n=23; p<0.05) during the intracellular dialysis of Ang II (10^{-8} M) plus Ao (110 pmol Ang I/ml per minute) compared with controls (-80 ±3.2 mV; n=23; p<0.05) which is related to the activation of an electrogenic sodium pump (De Mello, unpublished).

To investigate whether the increase in cell volume caused by extracellular renin plus Ao was related to activation of the Na-K-2Cl cotransporter cells from TO-2 were treated with bumetanide (10^{-6} M) to inhibit the cotransporter. As shown in figure 5, bumetanide, by itself, reduced cell volume and inhibited the effect of extracellular renin plus Ao on cell volume in cardiomyopathic hamsters. Similar results were found in normal hamsters. Indeed, before the administration of renin plus Ao, the average cell volume was 20,001±1,300 μm^3 (n=25) and after the administration of renin plus Ao in presence of bumetanide (10^{-6} M) was 20,050±1,250 μm^3 (n=22) (four animals) (p<0.05).

**Intracellular Ang II reverses the increase in cell volume elicited by hypotonic solution**

To investigate if Ang II is able to revert the cell swelling induced by hypotonic solution, cells were exposed to hypotonic Krebs solution prepared by diluting the normal solution to one-third. Measurements of cell volume of TO-2 were made before and during the effect of hypotonic solution. As shown in figure 6 the cell volume was appreciably increased in presence of hypotonic solution. As soon as the increment in volume reached its peak value Ang II (10^{-8} M) was dialysed into the cell and changes in cell volume were monitored. Figure 6 shows that...
intracellular Ang II reverses the effect of hypotonic solution on cell volume. In other experiments intracellular Ang II prevented the increase in cell volume produced by hypotonic solutions (not shown). Similar results were found in FIB (not shown).

Discussion

The present results indicate that intracellular administration of renin plus Ao activates the sodium pump and reduces the cell volume in normal as well as in the failing heart. This result contrasts with that found when renin plus Ao were added to the extracellular fluid because, in this case, the sodium pump was inhibited and the cell volume was increased. The role of the sodium pump on the decrease of cell volume caused by renin plus Ao was supported by the finding that ouabain, administered extracellularly, suppressed the effect of intracellular renin plus Ao on cell volume and pump current. Since intracellular Ang II, by itself, reduced the cell volume, it is reasonable to think that the effect of renin plus Ao was related to the formation of Ang II inside the cell. This view is supported by the observation that losartan added to the cytosol, abolished the effect of intracellular renin plus Ao on cell volume.

Although the effect of intracellular renin plus Ao on cell volume is related to the activation of the sodium pump, the question remains whether the inhibition of the Na-K-2Cl cotransporter is also involved in the decline of cell volume. Indeed, evidence is available that during hyperosmotic conditions, cell shrinkage is mediated by Na-K-ATPase and Na-K-2Cl cotransporter.11 Because during heart failure the Na-K-2Cl cotransporter is constantly activated12 and overexpressed,13 one cannot rule out the possibility that the inhibition of the cotransporter be responsible for the decline of cell volume. The other alternative is that intracellular Ang II reduces the cell volume by activating the Na-K-ATPase independently of the cotransporter. Evidence is available that in renal epithelia the peptide stimulates the Na-K-ATPase through angiotensin type 1 receptor activation and recruitment of Na-K-ATPase to the cell membrane.
cell membrane. Furthermore, the stimulation of Na-K-ATPase caused by the peptide is dependent on the phosphorylation of alpha-subunit at Ser-11 and Ser-18 residues.  

The effect of extracellular renin plus Ao on cell volume was due to activation of the Na-K-2Cl cotransporter because bumetanide, an inhibitor of the Na-K-2Cl cotransporter, suppressed the effect of extracellular renin plus Ao on cell volume. The relationship between Na-K-ATPase and Na-K-2Cl cotransporter, however, is complex and far from clear and further studies are needed to clarify this relationship, particularly during heart failure.

The present results have important implications particularly for myocardial ischemia because the activation of the circulating RAS might be harmful to the ischaemic heart due to the activation of the cotransporter and consequent increase in heart cell volume. Inhibition of the circulating RAS or activation of the intracrine renin-angiotensin system counteracts the harmful effects of Na-K-2Cl cotransporter activation with consequent decrease of cell volume. Indeed, the finding that intracellular Ang II reverses the cell swelling induced by hypotonic solution supports this view. Moreover, it is known that myocardial ischemia causes an increase of cell volume through the activation of Na-K-2Cl cotransporter and that swelling is an essential component of ischemia/reperfusion in myocardial cells. In addition, Ca^{2+} transporters and channels are probably involved in the regulation of heart cell volume. Although a normal rat cardiac renin seems to come from plasma, the presence of a transcript of renin, which remains inside the cell and is up-regulated in heart cells during myocardial infarction as well as renin internalization, raises the possibility that intracellular renin plays an important role on the regulation of heart cell volume.

In conclusion, the present results indicate that the activation of the intracrine RAS plays an important role on the regulation of cell volume in the failing heart. On the other hand, it counteracts the effect of the circulating RAS on sodium pump and cell volume regulation.

**Acknowledgement**

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**References**