Transmural pressure inhibits prorenin processing in juxtaglomerular cell

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Ichihara, Atsuhito, Hiromichi Suzuki, Yutaka Miyashita, Mareo Naitoh, Matsuhiro Hayashi, and Takao Saruta. Transmural pressure inhibits prorenin processing in juxtaglomerular cell. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R220–R228, 1999.—Pressure control of renin secretion involves a complex integration of shear stress, stretch, and transmural pressure (TP). This study was designed to delineate TP control of renin secretion with minimal influence of shear stress or stretch and to determine its mechanism. Rat juxtaglomerular (JG) cells were applied to a TP-loading apparatus for 12 h. In cells conditioned with atmospheric pressure or atmospheric pressure + 40 mmHg, renin secretion rate (RSR) averaged 29.6 ± 3.7 and 14.5 ± 3.3% (P < 0.05, n = 8 cultures), respectively, and active renin content (ARC) averaged 47.3 ± 4.6 and 38.4 ± 3.4 ng of ANG I · h−1 · million cells−1 (P < 0.05, n = 10 cultures), respectively. Total renin content and renin mRNA levels were unaffected by TP. The TP-induced decrease in RSR was prevented by Ca2+-free medium and the Ca2+ channel blocker verapamil and was attenuated by thapsigargin and caffeine, which deplete intracellular Ca2+ stores. Thapsigargin and caffeine, but not Ca2+-free medium or verapamil, prevented TP-induced decreases in ARC. The adenylate cyclase activator forskolin did not modulate TP-induced decreases in RSR or ARC. These findings suggest that TP not only stimulates Ca2+ influx but also inhibits prorenin processing through an intracellular Ca2+ store-dependent mechanism and thus inhibits active renin secretion by JG cells.

mechanoreceptors; renin-angiotensin system; calcium channels; endoplasmic reticulum; adenosine 3',5'-cyclic monophosphate

An increase in renal arterial pressure interacts with the vessel wall to generate the mechanical forces acting in directions perpendicular and tangential to renal blood flow, which are called transmural pressure and shear stress, respectively. Shear stress stimulates synthesis (30, 31) and release (3, 8) of nitric oxide by endothelial cells. Acute application of nitric oxide inhibits renin secretion, but chronic exposure to nitric oxide stimulates renin secretion (39). It is therefore likely that the shear stress-nitric oxide system participates in the control of renin secretion (24, 38). In contrast, transmural pressure may modulate renin secretion through a more complex mechanism. Transmural pressure has been shown to inhibit nitric oxide release from cultured endothelial cells (17) and may modulate renin secretion through this mechanism. In addition to the nitric oxide-mediated effect, transmural pressure can directly influence renin secretion by JG cells. Transmural pressure causes tangential strain on the vessel wall and thus generates stretch. A recent study demonstrated that a 20-h load of stretch inhibited renin secretion by cultured rat JG cells (6). However, the effects of perpendicular forces without stretch on renin regulation remains undetermined because of the technical difficulty in separating pure transmural pressure from stretch.

There is evidence that perfusion pressure control of renin secretion is dependent on extracellular Ca2+ concentrations (11, 36). According to the mathematical model proposed by Fray (10), transmural pressure stretches JG cells, which depolarizes the membrane, and the subsequent activation of voltage-gated Ca2+ channels leads to Ca2+ influx. Stretch may also activate ion channels, directly resulting in Ca2+ influx without membrane depolarization. This is based on a study demonstrating that antagonists or agonists of Ca2+ channels do not influence the inverse relationship between perfusion pressure and renin secretion (37). In either case, Ca2+ influx increases cytosolic Ca2+ concentrations and inhibits renin secretion (13). Thus Ca2+ influx has been presumed to play a critical role in the control of renin secretion by transmural pressure. However, transmural pressure control of renin secretion has not been examined at the level of JG cells. A recent study provided novel evidence that intracellular Ca2+ mobilization mediates pressure-induced vasoconstriction in afferent arterioles (21). Because afferent arteriolar vascular smooth muscle cells transform metaplasticity to JG cells (1, 43), pressure control of renin secretion by JG cells may also involve intracellular Ca2+ mobilization.

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The present study was focused on transmural pressure control of renin secretion. Experiments were conducted to delineate the effect of pure transmural pressure on renin secretion by cultured JG cells and to determine the underlying intracellular mechanism. To load transmural pressure with minimal influence of stretch or shear stress, JG cells were subjected to a load transmural pressure with minimal influence of JG cells were subjected to a load transmural pressure with minimal influence of JG cells were subjected to a load transmural pressure with minimal influence of

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sure control of renin secretion. Experiments were conducted to delineate the effect of pure transmural pressure on renin secretion by cultured JG cells.

METHODS

Primary culture of rat JG cells. Rat JG cells were isolated from kidneys of male Sprague Dawley rats (100–150 g) in accordance with the method described previously (19, 20) and suspended at 10^6 cells/ml in culture medium consisting of RPMI 1640 with 25 mM HEPES, 0.3 g/l L-glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, 0.66 U/l insulin, and 10% fetal bovine serum. Cell number was determined with a Coulter counter (Miami, FL). The suspended cells were distributed in 1-ml aliquots into individual wells of 8-well chamber slides containing 1 ml of culture medium and incubated at 37°C. JG cells had a 48-h rest period before the beginning of the experiments. Use of immunofluorescence staining for renin confirmed that 90 ± 3% of the cells (n = 7 primary cultures) were positive for renin at 60 h after isolation (19, 20).

Pressure-loading JG cells. Pressure was loaded on JG cells with minimal contribution by shear stress or stretch as reported previously by our laboratory (16, 17) with some modification. The 8-well chamber slides were placed in a sanitary pressure vessel (model DV-5-ST; Advantec Toyo, Tokyo, Japan) that was prewarmed to 37°C. The pressure vessel was sealed tightly and then connected to tubing attached to a three-way rotary valve, a sphygmomanometer, and a pressure valve. Compressed helium was pumped in to raise the internal pressure. The sanitary pressure vessel was then put in the incubator, and the internal temperature was kept constant at 37°C. During the experiments, the loaded pressure level was monitored with a sphygmomanometer. The partial pressure of oxygen and pH of the medium averaged 155 ± 4 mmHg and 7.4 ± 0.1, respectively, and were kept constant throughout the experiments.

In addition to atmospheric pressure, transmural pressures of 0 and 40 mmHg were added to JG cells in the present study. Thus the 0-mmHg transmural pressure load means atmospheric pressure, and the values of transmural pressure added to cultured cells do not reflect in vivo absolute levels of renal perfusion pressure. Therefore, the present study investigated the effect of an increase in transmural pressure on renin synthesis and secretion in JG cells conditioned with atmospheric pressure. Because, in isolated kidneys, renin secretion decreases dramatically when renal perfusion pressure is increased by 40 mmHg from 40 to 80 mmHg and remains stable when renal perfusion pressure is increased further, we added a 40-mmHg transmural pressure to JG cells under atmospheric pressure. In addition, the chronic (12 h) effects of transmural pressure on renin secretion were assessed because our preliminary study did not identify a significant difference in renin secretion by cells exposed to atmospheric pressure and atmospheric pressure + 40 mmHg up to 3 h (7.9 ± 3.2% and 7.2 ± 3.5%, respectively, at 3 h; n = 4 cultures in each).

Measurement of renin secretion rate, active renin content, and total renin content in JG cells. After culture medium was removed, the cells were washed twice with prewarmed PBS. Each well was filled with 1 ml of Ca^2+-containing PBS and placed in the pressure-loading apparatus. Immediately before (0 h) and 12 h after the pressure loading, the cell-conditioned buffer was removed and centrifuged. The supernatants were stored at −20°C until renin activity was assayed. After being rinsed with PBS, the cells were frozen in liquid nitrogen and stored at −80°C. For assay of active and total (active + inactive) renin contents, frozen cells were homogenized in 1 ml of buffer (pH 6.0) containing (in mM) 2.6 ethylenediaminetetraacetic acid, 1.6 dimercaprol, 3.4 hydroxyquinoline sulfate, 0.2 phenylmethylsulfon fluoride, and 5 ammonium acetate. The homogenates were centrifuged at 12,000 g for 30 min, and the supernatant was removed. An aliquot of the supernatant was diluted 1:10 (PBS) for the assay of renin activity. To measure total renin content of JG cells, diluted samples (300 µl) were shaken for 18 h at 4°C with 15 µl of 0.25 g/l trypsin coupled to cyanogen bromide-activated Sepharose 4B (Amersham Pharmacia Biotech, Uppsala, Sweden) as described previously (5, 40). Thus inactive renin in the samples was converted to active renin, and then renin activity was determined. As shown in Fig. 1, the medium of rat renin cDNA-transfected Chinese hamster ovary cells (50) was diluted 1:100 in the buffer and actually had renin activities of 37 or 55 ng of ANG I·ml⁻¹·h⁻¹ after the trypsin treatment. Because the diluted medium (1:100) is estimated to contain a theoretical renin activity of 82 ng of ANG I·ml⁻¹·h⁻¹ (50), we believe that 45–62% of inactive renin in the samples was converted to active renin by the present technique. In addition, the diluted medium (1:100) containing recombinant rat prorenin was added to the cells (n = 3 cultures) and homogenized as described above, and then active and total renin contents were determined. Although active renin content of the cells with the diluted medium (1:100) was similar to that of the cells alone (47 ± 1 vs. 49 ± 3 ng of ANG I·h⁻¹·million cells⁻¹), total renin content of the cells with the diluted medium (1:100) averaged 150 ± 5 ng of ANG I·h⁻¹·million cells⁻¹ and increased by 57 ± 5 ng of ANG I·h⁻¹·million cells⁻¹ compared with that of the cells alone (93 ± 1 ng of ANG I·h⁻¹·million cells⁻¹). The increase in total renin content corresponding to the recombinant rat prorenin added to the cells was similar to the renin activity of the same dose of recombinant rat prorenin, which was determined after the trypsin treatment in the test tube. Therefore, the possibility that prorenin is destroyed, converted to renin, or changed by proteases released from cells during the present homogenization process is unlikely.

![Fig. 1. Renin activity in buffer containing recombinant rat prorenin with (○) and without (●) trypsin treatment. Unpurified culture medium of recombinant rat prorenin-producing cells was diluted 1:100, 1:1,000, and 1:10,000 in buffer used in present study. After trypsin or control treatment, renin activity of diluted medium was determined as described in METHODS. Experiments were performed twice. Renin activity of culture medium itself was not detected.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00357.2004)
Renin activity was determined as previously described (19). Samples were incubated for 1 h at 37°C with plasma from bilaterally nephrectomized male Sprague-Dawley rats as the renin substrate, and renin activity was determined by the generation of ANG I from a plasma angiotensinogen substrate. ANG I levels were measured with a RIA coated-bead kit from Dinabett Radiosotope Institute (Tokyo, Japan). Renin secretion rate (RSR) was calculated as the fractional release of overall active renin (i.e., (buffer renin activity at 12 h – buffer renin activity at 0 h)/(active renin content in JG cells at 12 h + buffer renin activity at 12 h – buffer renin activity at 0 h)). Active and total renin contents of JG cells were expressed as renin activity of the sample obtained per million cells.

Renin mRNA analysis. Total RNA from frozen cells was extracted with the Total RNA Separator Kit (Clontech, Palo Alto, CA). Extracted RNA was suspended in ribonuclease-free water and quantified by measuring the absorbance at 260 nm. Renin mRNA levels in JG cells were determined by means of a semiquantitative RT-PCR as described previously (19). Total RNA from the cells was reverse transcribed with the GeneAmp RNA PCR Core Kit (PerkinElmer Cetus, Norwalk, CT). Each sample contained 0.5 µg of total RNA, 100 nmol of MgCl₂, 1,000 nmol of KCl, 200 nmol of Tris·HCl (pH 8.3), 20 nmol of each dNTP (dATP, dTTP, dGTP, and dCTP), 20 U of ribonuclease inhibitor, 50 pmol of random hexamers, and 50 U of murine leukemia virus RT in a final volume of 20 µl. After incubation for 5 min at 99°C to terminate the reaction and then stored at 4°C for 15 min, the samples were heated for 5 min at 99°C to terminate the reaction and then stored at 5°C until assayed.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard. The primers for renin and GAPDH were identical to those used in our previous studies (19, 25). The sense primers in each reaction were radiolabeled with [γ-32P]ATP (Amersham Pharmacia Biotech, Uppsala, Sweden) and phage T4 polynucleotide kinase by the Kination Kit (Toyobo, Osaka, Japan). A 5-µl sample of RT mixture was used for amplification, and 25 nmol of MgCl₂, 1,000 nmol of KCl, 200 nmol of Tris·HCl (pH 8.3), 3.75 pmol of each antisense primer, 3.75 pmol and 10³ cycles/min of each sense primer, and 0.625 U of AmpliTaq DNA polymerase were added to each sample. To minimize nonspecific amplification, we used a hot start procedure in which PCR samples were placed in a thermocycler (PerkinElmer Cetus) prewarmed to 94°C. After 2 min, PCR was performed for 25 cycles with a 30-s denaturation step at 94°C, a 60-s annealing step at 62°C, and a 75-s extension step at 72°C. We added a 5-min extension step at 72°C. The PCR products were electrophoresed on an 8% (wt/vol) polyacrylamide gel. Gels were dried on filter paper, exposed to a BAS 2000 imaging plate (Fuji Film, Tokyo, Japan) for 1 min, and quantified with a BAS 2000 Laser Image Analyzer (Fuji Film). RT yielded two clear bands with the predicted sizes of 374 bp for renin and 308 bp for GAPDH. Renin mRNA levels were assessed as renin/GAPDH ratios.

Experimental protocols. Primary cultures of JG cells were divided into two groups: wells loaded with atmospheric pressure and wells loaded with atmospheric pressure + 40 mmHg for 12 h. Each group included at least 2 wells, and mean values per a primary culture were determined in each group. In the first series of experiments, the cells were conditioned in Ca²⁺-containing PBS (control buffer) during the pressure load, and RSR, active renin content, total (inactive + active) renin content, and renin mRNA levels were determined. In the second series of experiments, the effects of extracellular Ca²⁺ on transmural pressure control of RSR and active renin content were assessed. The cells were conditioned in control buffer (1.5 mM Ca²⁺) or Ca²⁺-free buffer containing 0.5 mM ethylenediaminetetraacetic acid (0 mM Ca²⁺) during the pressure load. In the third series of experiments, we investigated effects of the L-type Ca²⁺ channel blocker verapamil (Sigma, St. Louis, MO), thapsigargin (Sigma), which depletes inositol 1,4,5-tris-phosphate (IP₃)-sensitive intracellular Ca²⁺ stores (26, 45), and caffeine (Sigma), which depletes ryanodine-sensitive intracellular Ca²⁺ stores (47) on transmural pressure control of RSR and active renin content. The cells were conditioned in control buffer or buffer including 50 µM verapamil, 1 µM thapsigargin, and 10 mM caffeine during the pressure load. In the final series of experiments that examined the contribution of cAMP-dependent pathways to transmural control of renin regulation, the cells were conditioned in control buffer or buffer including the adenylyl cyclase activator forskolin (3 µM; WAKO, Osaka, Japan) during the pressure load.

Statistical analysis. Data were analyzed by paired t-test. Differences between treatments were assessed by one-way factorial ANOVA with Scheffe’s F test. Statistical significance was defined as P < 0.05, and the results are shown as means ± SE.

RESULTS

Effects of transmural pressure on RSR, active renin content, and renin mRNA levels in JG cells. Figure 2 illustrates the inhibitory effect of 12-h exposure to increased transmural pressure on renin secretion by JG cells. In control cells treated for 12 h with atmospheric pressure, 29.6 ± 3.7% of the active renin generated by JG cells was secreted into the medium over 12 h. In contrast, in the cells exposed for 12 h to atmospheric pressure + 40 mmHg, RSR averaged 14.5 ± 3.3% and was significantly lower than that observed in control cells.

Addition of 40 mmHg of transmural pressure decreased active renin content but did not influence total renin content. As shown in Fig. 3, active renin content averaged 47.3 ± 4.6 ng of ANG I·h⁻¹·million cells⁻¹ in the cells treated with a 12-h atmospheric pressure. In the cells exposed to atmospheric pressure + 40 mmHg for 12 h, active renin content averaged 38.4 ± 3.4 ng of ANG I·h⁻¹·million cells⁻¹ and was significantly lower than the active renin contents of cells treated for 0 h (46.9 ± 5.0 ng of ANG I·h⁻¹·million cells⁻¹; n = 10

![Fig. 2. Renin secretion rate (RSR) in juxtaglomerular (JG) cells treated with 12-h loads of atmospheric pressure (AP) and AP + 40 mmHg transmural pressure. Experiment was performed with 8 primary cultures. *P < 0.05 vs. AP.](http://ajpregu.physiology.org/)
12 h with atmospheric pressure. The total renin contents of cells treated for 12 h with atmospheric pressure and atmospheric pressure + 40 mmHg averaged 110.3 ± 19.7 and 110.5 ± 20.3 ng of ANG I·h⁻¹·million cells⁻¹, respectively; these results were similar to each other and did not differ from those observed at 0 h (110.7 ± 22.9 ng of ANG I·h⁻¹·million cells⁻¹; n = 6 cultures). Total renin contents were approximately twofold greater than active renin values. This relationship was not different from those of previous studies (6, 18).

![Fig. 3](http://ajpregu.physiology.org/)

**Fig. 3.** Active (A) and total renin content (B) in JG cells treated with 12-h load of AP and AP + 40 mmHg transmural pressure. Active renin content was determined with 10 primary cultures. In 6 primary cultures, total renin content was also determined. *P < 0.05 vs. AP.

Effects of Ca²⁺-free medium on transmural pressure controls of RSR and active renin content. Figure 5 shows the effects of 12-h exposure to transmural pressure on renin secretion and active renin content in JG cells incubated in control buffer and Ca²⁺-free buffer. In the cells incubated in control buffer, addition of 40 mmHg of transmural pressure significantly decreased RSR from 27.1 ± 4.4 to 13.4 ± 4.4%. Removal of Ca²⁺

![Fig. 5](http://ajpregu.physiology.org/)

**Fig. 5.** Effects of 12-h exposure to TP(−) or TP(+) on RSR (A) and active renin content (B) in JG cells incubated with control buffer or Ca²⁺-free buffer. Experiment was performed with 6 primary cultures.

*P < 0.05 for TP(+) vs. TP(−); †P < 0.05 for Ca²⁺-free vs. control buffer at TP(−).
from the extracellular fluid significantly increased RSR in atmospheric pressure-loaded cells to 70.0 ± 12.3% and prevented the pressure-induced decrease in RSR. In the cells treated with Ca²⁺-free buffer and atmospheric pressure + 40 mmHg, RSR averaged 66.7 ± 12.1% and was similar to the RSR observed in cells treated with Ca²⁺-free buffer and atmospheric pressure. In contrast, Ca²⁺-free medium did not influence the active renin content under atmospheric pressure or the transmural pressure-induced decrease in active renin content. Addition of 40 mmHg of transmural pressure significantly decreased active renin content in control cells (51.0 ± 6.8 and 41.2 ± 4.9 ng of ANG I·h⁻¹·million cells⁻¹ for atmospheric pressure and atmospheric pressure + 40 mmHg, respectively) and cells incubated with Ca²⁺-free buffer (49.8 ± 5.9 and 41.8 ± 6.7 ng of ANG I·h⁻¹·million cells⁻¹ for atmospheric pressure and atmospheric pressure + 40 mmHg, respectively). The magnitudes of decrease in active renin content were similar (9.8 ± 3.8 and 8.0 ± 2.9 ng of ANG I·h⁻¹·million cells⁻¹ for cells incubated with control and Ca²⁺-free buffer, respectively). In cells incubated with Ca²⁺-free buffer, trypan blue exclusion staining showed cell viabilities of 96.8 ± 1.4% and 96.7 ± 1.1% (n = 6 primary cultures each) after 12-h loads of atmospheric pressure and atmospheric pressure + 40 mmHg, respectively.

Effects of verapamil, thapsigargin, and caffeine on transmural pressure controls of RSR and active renin content. Table 1 summarizes the effects of 12-h exposure to transmural pressure on renin secretion in untreated JG cells and cells treated with verapamil, thapsigargin, or caffeine. In untreated cells, addition of 40 mmHg of transmural pressure significantly decreased RSR. Administration of verapamil significantly increased RSR in the atmospheric pressure-loaded cells and eliminated the pressure-induced decrease in RSR. Treatment with thapsigargin or caffeine also significantly increased RSR in the atmospheric pressure-loaded cells and attenuated, but did not completely prevent, the pressure-induced decrease in RSR.

Table 2 shows the effects of 12-h exposure to transmural pressure on active renin content in untreated cells and cells treated with these agents. Active renin content in untreated cells was reduced significantly by addition of 40 mmHg of transmural pressure. Verapamil did not affect either the active renin content of atmospheric pressure-loaded cells or the pressure-induced decrease in active renin content. In contrast, thapsigargin and caffeine did not influence active renin content in the atmospheric pressure-loaded cells but completely prevented the pressure-induced decrease in active renin content. Trypan blue exclusion staining indicated respective cell viabilities of 98.8 ± 0.5%, 98.0 ± 0.5%, and 98.7 ± 0.4% (n = 6 primary cultures each) in the atmospheric pressure-loaded cells treated with verapamil, thapsigargin, or caffeine, and respective cell viabilities of 98.5 ± 0.4%, 97.3 ± 0.6%, and 98.3 ± 0.3% (n = 6 primary cultures each) in the atmospheric pressure + 40 mmHg-loaded cells treated with verapamil, thapsigargin, or caffeine.

Effects of forskolin on transmural pressure controls of RSR and active renin content. Figure 6 illustrates the effects of 12-h exposure to transmural pressure on renin secretion and active renin content in untreated JG cells and cells treated with forskolin. In untreated control cells, addition of 40 mmHg of transmural pressure significantly reduced RSR from 23.0 ± 2.2% to 9.3 ± 1.9%. In the presence of forskolin, RSR of the atmospheric pressure-loaded cells increased significantly to 51.1 ± 6.7%, and addition of 40 mmHg of transmural pressure also decreased RSR significantly to 33.9 ± 5.7%. This decrease in RSR (17.2 ± 4.9%) was similar to that observed in the absence of forskolin (13.7 ± 3.6%). Active renin content in untreated control cells averaged 48.2 ± 7.5 and 37.8 ± 6.1 ng of ANG I·h⁻¹·million cells⁻¹ for atmospheric pressure and atmospheric pressure + 40 mmHg, respectively, and thus transmural pressure decreased active renin content by 10.4 ± 2.9 ng of ANG I·h⁻¹·million cells⁻¹. Addition of forskolin significantly increased active renin content of the atmospheric pressure-loaded cells (65.8 ± 7.5 ng of ANG I·h⁻¹·million cells⁻¹) but did not influence the pressure-induced decrease in active renin content. In the presence of forskolin, active renin content of the atmospheric pressure + 40 mmHg-loaded cells averaged 51.4 ± 7.3 ng of ANG I·h⁻¹·million cells⁻¹, and thus the pressure-induced decrease in active renin content (14.4 ± 4.8 ng of ANG I·h⁻¹·million cells⁻¹) was not different from that obtained in the absence of atmospheric pressure.

Table 1. Transmural pressure control of RSR in untreated JG cells and cells treated with verapamil, thapsigargin, and caffeine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RSR at AP</th>
<th>RSR at AP + 40 mmHg</th>
<th>ΔRSR</th>
</tr>
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<tbody>
<tr>
<td>Ununtreated</td>
<td>27.9± 4.4</td>
<td>11.7± 2.5*</td>
<td>−16.1± 3.1</td>
</tr>
<tr>
<td>Verapamil, 50 μM</td>
<td>44.7± 6.3†</td>
<td>42.6± 9.0</td>
<td>−2.2± 2.0†</td>
</tr>
<tr>
<td>Thapsigargin, 1 μM</td>
<td>37.4± 6.0†</td>
<td>32.2± 5.1*</td>
<td>−5.2± 1.8†</td>
</tr>
<tr>
<td>Caffeine, 10 mM</td>
<td>38.6± 4.4†</td>
<td>32.0± 3.5*</td>
<td>−6.6± 2.5†</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6 primary cultures. Renin secretion rate (RSR, %) was measured after 12-h exposure to atmospheric pressure (AP) and AP + 40 mmHg. Individual values of ΔRSR (%) were determined by subtracting RSR of AP from that obtained at AP + 40 mmHg. *P < 0.05 vs. AP; †P < 0.05 vs. untreated cells. JG, juxtaglomerular.

Data are means ± SE; n = 6 primary cultures. Active renin content (ng of ANG I·h⁻¹·million cells⁻¹) was measured after 12-h exposure to AP and AP + 40 mmHg. Individual values of Δactive renin content (ng of ANG I·h⁻¹·million cells⁻¹) were determined by subtracting value of active renin content at AP from that obtained at AP + 40 mmHg. *P < 0.05 vs. AP; †P < 0.05 vs. untreated cells.
forskolin. In the forskolin-treated cells, trypan blue exclusion staining indicated cell viabilities of 99.2 ± 0.4% and 98.8 ± 0.2% (n = 5 primary cultures each) after 12-h loads of atmospheric pressure and atmospheric pressure + 40 mmHg, respectively.

**DISCUSSION**

Chronic elevation of arterial pressure causes vascular changes that lead to left ventricular hypertrophy (7). High arterial pressure also inhibits renin secretion from the kidney and thus inactivates the renin-angiotensin system (13). Because the renin-angiotensin system is a chronic determinant of vascular resistance, hypertrophy, and remodeling (7), its inactivation causes a reduction of vascular resistance and an attenuation of the pressure-induced vascular structural changes. Therefore, pressure control of renin secretion is considered to be one of the in vivo mechanisms against chronic sustained high perfusion pressure. Based on this idea, the present study addressed the chronic effects of pure transmural pressure on renin secretion by JG cells. The results of the present study demonstrated that a 12-h addition of 40 mmHg of transmural pressure on cultured JG cells caused a significant decrease in RSR. In addition to the inhibitory effect on RSR, the same pressure load significantly decreased the active renin content of JG cells. Because the total renin content of JG cells was not influenced by identical pressure loads, these results indicate that the transmural pressure loads in the present study inhibit prorenin processing and thus decrease active renin content. The decrease in active renin content may account in part for the inhibitory effect of transmural pressure on renin secretion. The same pressure load did not influence renin mRNA levels in JG cells, whereas a chronic load of stretch decreases renin mRNA levels in JG cells (6). Therefore, the possibility that the transmural pressures loaded in the present study caused stretch is unlikely, although we cannot exclude the possibility that minimal shear stress, which might exist at the cell level, might influence renin mRNA levels significantly.

Although the importance of Ca²⁺ in the mechanism of pressure control of renin secretion has been demonstrated in isolated perfused kidney (11, 13, 36), the differential mechanisms of the control of renin secretion by shear stress, stretch, and transmural pressure remain poorly understood. Therefore, we addressed the mechanism responsible for the effect of transmural pressure on renin secretion, using Ca²⁺-free medium and agents that reduce cytosolic Ca²⁺ concentrations in different manners. Although cytosolic Ca²⁺ is known to inhibit exocytosis of renin-secretory granules (23), the Ca²⁺-related treatment and agents significantly increased RSR in JG cells treated for 12 h with atmospheric pressure. These results indicate that these treatments and agents efficiently modulate the Ca²⁺ environment and decrease cytosolic Ca²⁺ concentrations in JG cells.

Extracellular Ca²⁺ concentrations play a key role in perfusion pressure control of renin secretion in the isolated kidney (11, 36). Calcium-free medium abolishes the shear stress-induced production of nitric oxide (3) and the subsequent vasodilation (8). Cell stretch theoretically activates a voltage-operated Ca²⁺ channel (10) and/or Ca²⁺-permeable ion channels (37). Therefore, control of renin secretion by shear stress and stretch appears to depend on extracellular Ca²⁺ concentrations. The present study demonstrated that Ca²⁺-free buffer also prevents the inhibitory influences of transmural pressure on renin secretion. Furthermore, transmural pressure-mediated inhibition of renin secretion was also blocked by verapamil. This was consistent with reports that Ca²⁺ channels mediate perfusion pressure control of renin release from isolated kidneys (13). These results suggest that, at the level of JG cells, transmural pressure control of renin secretion is dependent on extracellular Ca²⁺ and L-type Ca²⁺ channels.

A recent study suggested that an intracellular Ca²⁺ store contributes to the pressure-mediated vasoconstriction of afferent arterioles (21). Because afferent arteriolar smooth muscle cells transform metaplastically to JG cells (1, 43), the nature of the pressure-induced intracellular Ca²⁺ mobilization may be maintained in JG cells. Therefore, we investigated the contribution of intracellular Ca²⁺ stores on transmural pressure control of renin secretion. Thapsigargin inhibits the endoplasmic reticulum Ca²⁺-ATPase and prevents Ca²⁺ uptake into intracellular stores (26, 45). Because of an
endogenous leak, inhibition of Ca\(^{2+}\) uptake by thapsigargin leads to depletion of intracellular stores (27). Caffeine induces Ca\(^{2+}\) release from sarcoplasmic reticulum via the ryanodine receptor, and thus long-term application depletes intracellular Ca\(^{2+}\) stores (47). In the present study, transmural pressure-mediated inhibition of renin secretion was also attenuated by thapsigargin and caffeine. This suggests that transmural pressure inhibits renin secretion in part by stimulating Ca\(^{2+}\) release from intracellular stores. Transmural pressure-induced decrease in JG cell active renin content was inhibited completely by thapsigargin and caffeine but was not influenced by Ca\(^{2+}\)-free medium or verapamil. Therefore, transmural pressure inhibits processing of prorenin to active renin through release of Ca\(^{2+}\) from intracellular stores. Although inhibition of prorenin processing has an inhibitory effect on renin secretion, the present study does not address to what degree the inhibition of prorenin processing by transmural pressure contributes to the decrease in renin secretion.

Caffeine can increase intracellular levels of cyclic nucleotides by inhibiting phosphodiesterase activities (14). Transmural pressure may inhibit prorenin processing by reducing intracellular levels of cAMP and/or guanosine 3',5'-cyclic monophosphate (cGMP), and caffeine may prevent inhibition of prorenin processing by increasing levels of cyclic nucleotides. However, previous studies demonstrated that administration of membrane-permeable cGMP does not influence active renin content (19) or renin mRNA levels (9) in JG cells. It is therefore unlikely that cGMP mediates transmural pressure-induced decreases in active renin content and RSR. In contrast, cAMP promotes prorenin processing to active renin by contributing a favorable acidic environment for renin-secretory granules (23). In our study, forskolin increased RSR and active renin content of JG cells treated for 12 h with atmospheric pressure. However, the transmural pressure load inhibited RSR and active renin content both in the absence and presence of forskolin, suggesting that transmural pressure control of renin secretion by JG cells does not involve the cAMP pathway.

There are reports that thapsigargin influences Ca\(^{2+}\) channels and thus modulates Ca\(^{2+}\) influx (4, 34, 41, 49). In vascular smooth muscle cells, thapsigargin increases Ca\(^{2+}\) influx by activating L-type Ca\(^{2+}\) channel blocker-sensitive (41, 49) and insensitive pathways (49). In the present study, thapsigargin stimulated renin secretion by the cells treated for 12 h with atmospheric pressure, which indicates that thapsigargin decreases cytosolic Ca\(^{2+}\) concentrations. Therefore, Ca\(^{2+}\) influx, even in the presence of thapsigargin, should be negligible or overcome by the specific effect of thapsigargin on intracellular Ca\(^{2+}\) stores. Other studies have shown that thapsigargin inhibits voltage-operated Ca\(^{2+}\) channels in adrenal glomerulosa cells (34) and in a smooth muscle cell line, A7r5 (4). In the present study, the transmural pressure-induced decrease in active renin content was eliminated completely by thapsigargin but was not influenced by extracellular Ca\(^{2+}\) or verapamil. In addition, recent studies have demonstrated that 1 µM thapsigargin does not inhibit afferent arteriolar vasoconstriction caused by membrane depolarization (22, 42). Therefore, the possibility that such nonspecific effects confounded our findings is unlikely.

Although the present study demonstrated that transmural pressure inhibits prorenin processing by stimulating Ca\(^{2+}\) release from intracellular stores, two processes in this mechanism remain unclear. One is how Ca\(^{2+}\) release from intracellular stores is stimulated by transmural pressure. In the present study, this process was inhibited by thapsigargin and caffeine, which deplete IP\(_3\)-sensitive (26, 45) and ryanodine-sensitive intracellular Ca\(^{2+}\) stores (47), respectively, suggesting that transmural pressure may influence intracellular Ca\(^{2+}\) stores without involvement of a specific messenger molecule. However, caffeine can increase intracellular cGMP levels (14), and elevated cGMP levels can inhibit phosphodiesterase activity in renin-secretory granules (2, 29), these enzymes may mediate intracellular Ca\(^{2+}\) store-dependent inhibition of prorenin processing. However, PC1 and PC5 have not been detected in human JG cells (29, 33). Cathepsin B may also contribute to the inhibition of prorenin processing by transmural pressure. Studies have shown that cathepsin B is a prorenin processing enzyme (18, 48) that is present in renin-secretory granules of JG cells (44). Because addition of Ca\(^{2+}\) reduces the enzyme activity of cathepsin B in test tubes (35), transmural pressure may influence cathepsin B activity in renin-secretory granules by modulating the intracellular Ca\(^{2+}\) environment.

Although alterations in pressure-dependent renin secretion occur within several minutes in vivo, our preliminary study showed that pure transmural pressure load without shear stress or stretch has no acute influence on renin secretion. Therefore, pure transmural pressure may not significantly contribute to acute inhibition of renin secretion by renal perfusion pressure, and shear stress and stretch may play an important role in the acute phase of the pressure control of renin secretion. Alternatively, acute signals of transmu-
rual pressure may be somehow attenuated in the present experimental preparation.

In conclusion, at the level of JG cells, transmural pressure, with minimal if any shear stress or stretch, inhibited renin secretion and reduced active renin content without influencing total renin content or renin mRNA levels. As in a mathematical stretch receptor model proposed previously (10), transmural pressure-mediated inhibition of renin secretion is dependent on extracellular Ca\(^{2+}\) levels and Ca\(^{2+}\) channels. Distinct from the stretch receptor model, transmural pressure decreased the active renin content of JG cells by stimulating Ca\(^{2+}\) release from intracellular stores. Thus pure transmural pressure inhibits prorenin processing to active renin through an intracellular Ca\(^{2+}\) store-dependent mechanism. The inhibition of prorenin processing may contribute in part to the inhibitory control of renin secretion by transmural pressure.

Perspectives

Although acute elevation in renal arterial pressure is well known to elicit a prompt inhibition of renin secretion from JG cells, pressure control of renin regulation during sustained high arterial pressure has received less attention. The present study provides first evidence that, at the level of JG cells, chronic elevation in transmural pressure inhibits prorenin processing through intracellular Ca\(^{2+}\) store-dependent mechanisms. Therefore, in chronic conditions such as arterial hypertension, high arterial pressure may decrease intrarenal renin activity through inhibiting prorenin processing in JG cells. Future studies will be necessary to determine in vivo effects of sustained high arterial pressure on renin regulation and to clarify the intracellular Ca\(^{2+}\) store-dependent mechanism responsible for pressure-mediated inhibition of prorenin processing.

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