Calcium-dependent activation of phospholipase C by mechanical distension in renin-expressing As4.1 cells

MICHAEL J. RYAN,1 KENNETH W. GROSS,2 AND GEORGE HAJDUCZOK1
1Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo 14214; and 2Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, New York 14263

Received 13 December 1999; accepted in final form 22 May 2000.

Calcium-dependent activation of phospholipase C by mechanical distension in renin-expressing As4.1 cells. Am J Physiol Endocrinol Metab 279: E823–E829, 2000.—One of the major physiological regulators for the production and release of renin from the kidney is blood pressure. The juxtaglomerular (JG) cells, located primarily at the afferent arterioles leading to the glomerulus, are thought to be the baroreceptor of the kidney and adjust their ability to secrete renin in an inverse relationship to changes in pressure (mechanical force). The characteristics of JG cells that allow them to sense and respond to changes in mechanical force at the cellular level are not clear. By use of a renin-expressing clonal cell line (As4.1) as a model for JG cells, it was the purpose of this paper to identify cellular pathways that are activated by mechanical distension. Furula 2-labeled As4.1 cells were mechanically probed to observe changes of intracellular calcium concentration ([Ca2+]i). Mechanical distension of As4.1 cells resulted in an influx of Ca2+ to the cytosol, mediated by stretch-activated ion channels and dependent on the presence of extracellular Ca2+. Furthermore, cyclic mechanical distension elevated total inositol phosphates (IP) in As4.1 cells. This response was also dependent on the presence of extracellular Ca2+, and the addition of U-73122, a phospholipase C (PLC) antagonist, significantly attenuated the increase of IP. Taken together, these findings demonstrate the calcium-dependent activation of PLC and the subsequent increase of IP and [Ca2+]i, to be a potentially important pathway for the modality of pressure sensing by renin-expressing cells in response to mechanical stimulation.

THE PRODUCTION AND SECRETION of renin from juxtaglomerular (JG) cells of the kidney are crucial for the regulation of arterial blood pressure and volume homeostasis. An inverse relationship between renin and changes in arterial pressure has been well documented in vivo (3, 25). In vitro, increases of pressure (mechanical stimulation) on the renin-expressing CaLu-6 cell, as well as isolated JG cell cultures, have been shown to inhibit forskolin-stimulated renin expression (6). The mechanisms by which increased mechanical force regulates renin at the cellular and molecular level are largely unknown. There is speculation for the involvement of mechanically sensitive ion channels (MSC) and activation of second messenger pathways, including changes of intracellular calcium concentration ([Ca2+]i) (6, 25, 33).

Experimental data in mechanically stimulated endothelial cells (30) and canine cerebral arteries (37) support mobilization of intracellular Ca2+ stores and a subsequent increase in [Ca2+]i. Contrary to other secretory cells, elevated Ca2+ in isolated JG cells, individual glomeruli, and isolated perfused kidneys is recognized as an inhibitory signal to the release of renin (26). Scholz et al. (33) reported that mechanical stimulation of individual JG cells has no effect on [Ca2+]i. However, these experiments utilized isolated glomeruli to measure JG [Ca2+]i in response to stretch of the afferent arteriole, making it difficult to assess what level of stimulus was exerted on the individual JG cells. Therefore, there is little direct evidence to support or refute either the presence of MSC or the role of Ca2+ in response to changes in pressure in renin-producing cells.

The role of second messenger pathways in the cellular response to mechanical stimulation as putative mechanisms that may be involved in controlling renin has not been established. Activation by mechanical force of protein kinases A and C, adenylate cyclase, cAMP, diacylglycerol, phospholipase C (PLC), and inositol phosphate (IP) metabolites have been demonstrated in many non-renin-expressing cell types, including endothelial (4), myocardial (7), skeletal (38), and smooth muscle (22) cells. However, there has been no experimental evidence for the activation of these pathways or their role in modulating renin expression and secretion from the JG cells.

The lack of experimental evidence for the cellular regulation of renin by mechanical stress is due in part to the limited number of cellular models. As4.1 cells were developed via transgene-targeted oncogenesis in mice with a renin SV40 T antigen fusion construct and were purified from kidney neoplasm (35). This cell line
expresses both the endogenous renin gene and the transgene and has been used in our laboratory (K. W. Gross) as a model to study cellular and molecular regulation of renin (16, 28, 29). It was the purpose of this study to test the following hypotheses. 1) Mechanical distension of individual As4.1 cells elevates [Ca\(^{2+}\)]\(_i\), via MSC. 2) The influx of [Ca\(^{2+}\)]\(_i\), mediated by mechanical stimulation, triggers the activation of PLC and IP, facilitating a further increase of [Ca\(^{2+}\)]\(_i\). 3) Mechanical activation of a signal transduction pathway involving PLC, IP, and Ca\(^{2+}\) leads to downregulation of renin gene expression. The last hypothesis is the subject of our companion study (31).

MATeRIALS AND METHODS

Cell Culture

As4.1 cells (ATCC No. CRL-2193) are a renin-expressing clonal cell line derived from the kidney neoplasm of a transgenic mouse. Cell cultures were maintained in humidified room air containing 5% CO\(_2\) at 37°C. As4.1 cells were cultured in DMEM and supplemented with 10% fetal bovine serum (FBS). Media changes were performed every 3rd day, and cell cultures were split when confluence in the flask was attained using this ratio and the standard Ca\(^{2+}\) (Flexcell International) in DMEM with 10% FBS.

Experiments were run on 6-well Collagen I-coated flexible bottom dishes before the experiment. To measure total intracellular IP from the cytosol to the membrane after activation of second messenger pathways, cells were incubated in Ca\(^{2+}\)-free Krebs solution 1 h before stretch. Preliminary studies from our laboratory (G. Hajduczok) indicated that a 1-h incubation period in Ca\(^{2+}\)-free extracellular Krebs solution was sufficient to deplete intracellular Ca\(^{2+}\) stores and reversibly inhibit agonist mobilization of [Ca\(^{2+}\)]\(_i\). For these experiments, the Ca\(^{2+}\)-free Krebs solution contained 2.5 mM EGTA in place of CaCl\(_2\). To determine the role of PLC in activation of inositol pathways in response to stretch, the PLC inhibitor U-73122 (8 μM) was added to the media for 5 min before stretch.

After stimulation of the As4.1 cells, 9% perchloric acid was added to each well to prevent the further hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)). The lysed As4.1 cell suspension was pelleted with the supernatant containing cytosolic fluid and metabolites. Pellets were saved and used in a Lowry assay to measure total protein from each well. The supernatant was run on a Dowex column and washed with several solutions, including myo-inositol (5 mM), sodium tetraborate (5 mM), and 1 M formate/0.1 M formic acid to collect the radiolabeled myo-inositol from the cytosolic fraction. myo-[\(^{3}\)H]inositol was measured with a scintillation counter and expressed as disintegrations per minute (dpm) per milligram of protein.

Stretch Protocols

Calcium measurements. Mechanical stimulation of individual As4.1 cells was accomplished using a picospritzer. The picospritzer consisted of a glass micropipette with a tip diameter of ~1 μm attached to a nitrogen pressure source (Picospritzer II, General Valve, Fairfield, NJ), and the solenoid was controlled by a pulse generator (model 1830/1831, World Precision Instruments, New Haven, CT). This system allowed control of the stimulus magnitude (as a relative pressure) as well as the duration of the stimulus applied to the cell membrane. The micropipette was filled with either normal Ringer solution or Ca\(^{2+}\)-free Ringer solution, depending on the protocol, and was positioned near (3–5 μm) the plasma membrane of As4.1 cells. Application of pressure to the micropipette caused a jet of solution to deform the membrane of the cell (cell diameter ~20 μm). In all protocols, the duration of each stimulus was 500 ms, and the pulse magnitudes ranged from pipette back-pressures of 0.6 to 2.2 atmospheres, or atm. For initial experiments, cells were superfused with normal Ringer solution and subjected to
increasing mechanical stimulation with pipettes filled with normal Ringer solution. The experiment was then repeated, superfusing the cells with 25 μM gadolinium hexachloride (Gd³⁺) in normal Ringer solution, an inhibitor of MSC as well as of voltage-activated Ca²⁺ channels (VACC) (36). We observed that As4.1 cells do not contain VACC, as evidenced by the inability of 70 mM KCl (n = 36 cells, P > 0.05) or the L-type VACC agonist BAY K 8644 (17) (10 μM, n = 17 cells, P > 0.05; 50 μM, n = 21 cells, P > 0.05) to elicit changes in [Ca²⁺]. Therefore, Gd³⁺ was sufficient to determine what role, if any, MSCs had in the cell response resulting from mechanical stimulus. To determine the source of Ca²⁺ entry into the cells after mechanical stimulation, As4.1 cells were superfused with Ca²⁺-free Ringer and picospritzed with a micropipette containing Ca²⁺-free Ringer.

**Inositol measurements.** As4.1 cells cultured on the 6-well Bioflex membrane dishes coated with Collagen I were used with the Flexcell Strain Unit (Flexcell International), which allowed the cells to be subjected to a mechanically active environment. The unit consists of a base plate and a computer-driven vacuum pump that allows various protocols of stretch (frequency and magnitude and duration) to be applied to the cells. The Bioflex membranes were designed such that 85% of the membrane exhibits a uniform radial strain (13). All stretch protocols for measurement of IP were performed with this system at a frequency of 1 Hz and a stretch magnitude of 24% membrane elongation. The protocol for stretch included a control plate (nonstretch) and 5, 10, 20, 30, 60, and 100 cycles of stretch.

**Statistical Analysis**

All data are presented as means ± SE. Data are considered significantly different if P < 0.05 as examined by paired t-test or repeated-measures ANOVA, depending on the experiment. The n for each experiment varied between 3 and 21, depending on the experiment and is listed with each figure. For Figs. 1–3, n refers to the number of individual cells mechanically probed. For Figs. 4–5, n refers to the number of times the experiment was repeated at each stimulus.

## RESULTS

**Calcium Response to Mechanical Stimulation**

[Ca²⁺]i was measured in individual As4.1 cells that were mechanically probed with a picospritzer. Figure 1 illustrates results from those experiments. A threshold response generating a Ca²⁺ transient could be attained with mechanical stimulation. Once threshold was reached, probing the cells with higher pressures resulted in rapid, transient increases of [Ca²⁺]i. Further increasing the pressures resulted in larger Ca²⁺ transients, thus demonstrating a pressure dependence and repeatability of this response. Stimulus intensities below the threshold were unable to elicit a response. Microscopic analysis revealed that membrane deformation was the threshold required to generate a [Ca²⁺]i transient. Thus a fluid jet increasing shear force, directed normally to the cell surface, was unable to generate a response alone unless accompanied by a membrane strain (deformation). Although these experiments demonstrate a Ca²⁺ response to a mechanical force, they do not provide any insight as to the source of Ca²⁺ that contributes to these transients.

There are two major sources that may contribute to the Ca²⁺ responses seen in Fig. 1, release from intracellular stores or extracellular influx. To determine which of these pathways was activated, resulting in the transients, the experiments were repeated while superfusing the As4.1 cells in Ca²⁺-free Ringer solution (Fig. 2). Before superfusion with Ca²⁺-free Ringer, As4.1 cells were probed for observation of the previously described transient. Upon removal of Ca²⁺ from the extracellular bath, increasing the stimulus intensity to 1.3× and 2.2× (relative to the control stimulus) was unable to elicit a rise of [Ca²⁺]i (P < 0.05). The average peak changes are illustrated in Fig. 2 (inset). These results suggest that extracellular Ca²⁺ is necessary for the As4.1 cell response to mechanical stimulation.

When we consider that a typical path of entry for ions to the cytosol may be through the opening of ion channels in the plasma membrane, it is logical to assume that the influx of Ca²⁺ occurs in the same fashion. More specifically, the potential role of MSC in this response was tested by probing As4.1 cells that were superfused with normal Ringer solution containing 25 μM Gd³⁺, a putative inhibitor of MSC. This dose of Gd³⁺ was based on preliminary studies and previous studies indicating it to be effective for blocking mechanically activated calcium currents in isolated rat nodose ganglion neurons (20, 34). The results from those experiments are illustrated in Fig. 3. Before superfusion of cells with Gd³⁺, mechanical stimulation resulted in an elevated [Ca²⁺]i. However, when Gd³⁺ was added to the preparation, increasing the stimulus intensity to over twice that of the initial stimulus (1.4× and 2.1×), it was unable to initiate the Ca²⁺ response (P < 0.05). It is therefore likely that the influx of Ca²⁺ in response to mechanical stimulation is mediated through the opening of MSC.

Inositol (1,4,5)-trisphosphate (IP₃) and ryanodine receptors (RYRs) represent two principal intracellular Ca²⁺ channels responsible for mobilization of Ca²⁺ from intracellular stores (2). A regenerative process of
CALCULAR PATHWAYS ACTIVATED BY STRETCH

Fig. 2. Dependence of the cell response to mechanical stimulation on the presence of extracellular calcium. An individual fura 2-loaded As4.1 cell was probed with a picospritzer (500-ms pulses) and during superfusion in Ca²⁺-free Ringer solution (bar). Mean [Ca²⁺], of mechanically stimulated cells is shown (inset) in the presence and absence of calcium. Results were significantly different (*P < 0.01) from control as assessed by repeated-measures ANOVA (n = 16 cells). Stimulus intensities ranged from 1.0 to 2.2 atm.

calcium-induced calcium release (CICR) can occur when a small extracellular influx of Ca²⁺ triggers a large release from intracellular stores, resulting in large calcium transients and repetitive spikes (2). This process of Ca²⁺ release from intracellular stores can occur via either a RYR or an IP₃ channel. To test for a RYR-sensitive pathway, As4.1 cells were superfused in Ca²⁺-free Ringer solution (bar). Mean [Ca²⁺], of mechanically stimulated cells is shown (inset) in the presence and absence of Gomez. Results were significantly different (*P < 0.01) from control as assessed by repeated-measures ANOVA (n = 16 cells). Stimulus intensities ranged from 1.0 to 2.2 atm.

Second Messenger Activation by Mechanical Distension

Although Ca²⁺ is a second messenger and its regulation is important for normal cell function, there are other potential pathways that may be activated by changes in mechanical force. The activation of PLC and total cytosolic inositol by mechanical stimulation and its dependence on extracellular Ca²⁺ were investigated. After mechanically stimulating myo-[³H]inositol-labeled As4.1 cells that were incubated in either Krebs-Ringer or Ca²⁺-free Krebs solution, total cytosolic IP was measured. Data from those experiments are illustrated in Fig. 4. In cells that were bathed in normal Krebs (control curve), total cytosolic IP was elevated significantly after 20 cycles of stretch and remained elevated for the duration of 100 cycles. These data were compared with mechanically stimulated cells that were bathed with Ca²⁺-free Krebs (0 Ca²⁺ trace). Over the course of 100 cycles of stretch, no significant change in IP was observed, thus indicating the dependence of this pathway on the presence of extracellular Ca²⁺.

Evidence for Ca²⁺ directly activating PLC (40) and speculation that mechanical forces can directly hydrolyze PI₃ in the plasma membrane (1) led to the investigation of the potential activation of PLC by Ca²⁺ in the As4.1 cellular response to mechanical distension. Cells were incubated with the PLC inhibitor U-73122 for 5 min with 10 or 100 μM ryanodine to determine whether the calcium transients elicited by repetitive mechanical stimulation would be decreased (2, 11). During 10 μM ryanodine superfusion, the second [Ca²⁺], transient elicited by mechanical stimulation did not significantly differ from the initial transient (n = 13 cells, initial peak [Ca²⁺], = 328 ± 22 nM vs. second peak [Ca²⁺], = 309 ± 33 nM, P > 0.05). Simi-

Fig. 3. Effect of gadolinium hexachloride (Gd³⁺) on As4.1 cell calcium response to mechanical stimulation. Individual fura 2-loaded cells were mechanically probed with a picospritzer (500-ms pulses) in the presence and absence of Gd³⁺. Mean [Ca²⁺], of mechanically stimulated cells is shown (inset) in the presence and absence of Gd³⁺. Results were significantly different from control (‡P < 0.01) as assessed by repeated-measures ANOVA (n = 17 cells). Stimulus intensities ranged from 1.0 to 2.2 atm.
(8 µM) and subjected to the same stretch protocols described above. Because JG cells are thought to be modified smooth muscle cells (5), this dose was chosen on the basis of a study (14) indicating that the EC\textsubscript{50} for U-73122 in vascular smooth muscle cells was 1.25 µM, with maximal inhibition occurring at 10 µM. Therefore, the submaximal dose of 8 µM was used in preliminary experiments and found to be effective. In the presence of U-73122, the increase from baseline (unstimulated) IP was only 18% compared with a 60% (P < 0.01) increase in IP in cells stretched in the absence of U-73122 (Fig. 5). Thus activation of PLC was necessary for elevation of cytosolic IP to occur.

DISCUSSION

The experiments in the current work describe for the first time a potentially important cellular pathway that is activated by changes in mechanical force in a renin-expressing cell line, As4.1. In many studies, the inverse relationship between renin release, or expression, and pressure has been demonstrated. Evidence for this relationship has been provided by experiments utilizing experimentally induced hypertension in rats (24), isolated perfused rat kidneys (12, 25), and isolated perfused glomeruli (3). Although these studies have provided valuable information about the physiological regulation of renin by pressure, the baroreceptor properties of the JG cell have not been addressed. Cyclic stretch of both primary JG cells and the renin-expressing CaLu-6 cells (pulmonary clonal cell line) resulted in a reduced renin expression (6). However, very little has been addressed regarding potential cellular pathways that are activated by stretch leading to reduced expression or secretion of renin. Using As4.1 cells provides advantages over past experimental cellular models. As4.1 is the first kidney-derived renin to express and secrete a clonal cell line. Although the origin within the kidney is not clear, these cells contain both the renin transgene (renin SV40 T antigen) and the endogenous renin gene, suggesting that they are derived from bona fide JG cells. As4.1 cells are morphologically similar to JG cells in vivo, because electron microscopy reveals the presence of large, electron-dense, renin-containing granules (35). Finally, As4.1 provides a pure cell line that stably expresses renin over long periods in culture, making it much simpler for investigation of the control of renin at the cellular and molecular levels (35).

Using As4.1, then, as a model for the JG cell, we mechanically probed individual cells. It has been speculated by others that the JG response to increased pressure potentially involves an elevated [Ca\textsuperscript{2+}]i (32). Ca\textsuperscript{2+}, paradoxically, has been shown to be an inhibitory signal to the release of renin (9, 27). The role of elevated Ca\textsuperscript{2+} in regulating renin gene expression is not clear, although binding of calmodulin and subsequent activation of phosphorylation cascades could certainly provide some molecular control. As yet, the latter has not been demonstrated. The experiments shown in our study describe the ability of As4.1 cells to respond to mechanical stimulation with an increased [Ca\textsuperscript{2+}]i. Furthermore, the response was dependent on the presence of extracellular Ca\textsuperscript{2+}, and the influx of Ca\textsuperscript{2+} was mediated by MSC, as evidenced by the abolition of the response in the presence of Gd\textsuperscript{3+}. Gd\textsuperscript{3+} is a nonspecific MSC blocker and can also inhibit VACCs.

There are, however, conflicting reports as to the presence and role of VACCs in JG cells. Experiments by Jones-Dombi and Churchill (18) provide evidence in isolated perfused rat kidneys that the Ca\textsuperscript{2+} channel agonist BAY K 8644 inhibits renin secretion and that VACC blockers abolish the inhibitory effect of increased perfusion pressure on renin release, thus supporting the role of VACC in the control of renin (17). Scholz et al. (32) demonstrated that the presence of Ca\textsuperscript{2+} was necessary for the pressure-dependent inhibition of renin release in isolated rat kidneys. These studies demonstrate the importance of Ca\textsuperscript{2+} in response to changing pressure in the whole kidney, but they do not address the role of VACC at the level of the JG cell. Electrophysiological measurements on isolated JG cells did not reveal the presence of VACC or changes in [Ca\textsuperscript{2+}]i by membrane depolarization (21). Stimulation of As4.1 cells with either 70 mM KCl or BAY K 8644 containing Ringer did not alter [Ca\textsuperscript{2+}]i, demonstrating the absence of VACC in our study. This suggests that Gd\textsuperscript{3+} is acting through the inhibition of MSC, rather than nonspecific VACC antagonism.

The presence of MSCs has been shown in numerous cell types, but these channels may not necessarily be ubiquitous. Mechanical stimulation of individual isolated rat nodose ganglion neurons results in a rise of [Ca\textsuperscript{2+}]i that was inhibited by gadolinium (20, 34). Furthermore, in the study by Kraske et al. (20), mechanosensitive channels could not be identified in a cell type thought not to have any mechanosensitive properties (BC3H1 cell line). Thus this mechanically triggered response may not be present in all cell types.

External influx of Ca\textsuperscript{2+} cannot alone account for the changes of [Ca\textsuperscript{2+}]i in response to mechanical stimulation. Calculations reveal that changes of [Ca\textsuperscript{2+}]i due to membrane-permeant pathways would be minimal.

![Graph](Image)
(10^{-15}-10^{-10} \text{ M range}) when an estimated number of MSC, permeability and conductance of these channels, electrochemical driving force for Ca\textsuperscript{2+}, and channel open time are taken into consideration. Our data show that the Ca\textsuperscript{2+} response to mechanical stimulation occurs in the range of 100–500 nM. These discrepancies suggest that intracellular stores of Ca\textsuperscript{2+} also must contribute to A4.1 cell response to mechanical distension. Ca\textsuperscript{2+} activation of PLC, IP\textsubscript{3}, and the subsequent mobilization of intracellular Ca\textsuperscript{2+} stores can explain this phenomenon if we consider the inhibition of the response in the absence of extracellular Ca\textsuperscript{2+} (Fig. 2). Activation of PLC by Ca\textsuperscript{2+} has been demonstrated in human platelet cells (39), rat cerebellar granule cells (8), and canine smooth muscle cells (40). Furthermore, Ca\textsuperscript{2+} influx through Gd\textsuperscript{3+}-sensitive MSC and its subsequent activation of PLC have been shown in rabbit aorta (22). Interestingly, JG cells are thought to be related to smooth muscle (5). The experimental evidence described herein illustrates a cyclic stretch-induced increase in IP that is dependent on the presence of extracellular Ca\textsuperscript{2+} as well as a functional PLC. Taken together with the measurements of [Ca\textsuperscript{2+}]i, in response to mechanical stimulation, our data are consistent with such a CICR mechanism. Furthermore, it appears that the intracellular release pathway for Ca\textsuperscript{2+} contributing to CICR is mediated via the IP\textsubscript{3} receptor Ca\textsuperscript{2+} release channel, because the mechanically induced peak responses were unaltered in the presence of ryanodine. Thus the activation of the Ca\textsuperscript{2+}/IP\textsubscript{3} pathway in response to stimulation may provide the basis for communicating a mechanical signal from the plasma membrane to the nucleus (2), ultimately leading to the control of renin gene expression.

Regulation of this second messenger pathway by mechanical force, by itself, is interesting. However, it is important to question whether or not this pathway is physiologically relevant to the control of renin. Preliminary data from our laboratories indicate that renin expression is inhibited by >50% of control in the presence of endothelin-1 (ET-1). ET-1 is produced and secreted by endothelial cells and has extremely potent vasoconstricting actions. It is well known to function through activation of G proteins, PLC, IP\textsubscript{3}, and mobilization of intracellular Ca\textsuperscript{2+} stores (10). There is also growing evidence that ET-1 exerts an inhibitory influence on the secretion of renin from the kidney in a Ca\textsuperscript{2+}-dependent manner (19). The humoral activation of a second messenger pathway that paralyses one that is activated by mechanical distension may be sufficient to exert an appropriate physiological response in the renin-expressing A4.1 cell.

The current body of work demonstrates the activation of a second messenger pathway by changes in mechanical force in a renin-expressing cell line. This sensory mechanism appears to begin with the influx of Ca\textsuperscript{2+} across the plasma membrane via the opening of gadolinium-sensitive MSC. Activation of PLC by this Ca\textsuperscript{2+} trigger ensues, leading to IP\textsubscript{2} hydrolysis and increased IP in the cytosol. IP in turn can bind to receptors on the endoplasmic reticulum to mobilize intracellular stores of Ca\textsuperscript{2+}. The significance of such a pathway in the regulation of renin gene expression or secretion has yet to be determined. In addition, although similar signal transduction pathways may exist in other cell types, it is not clear whether the potential pathways activated by mechanical forces in the present study are unique to A4.1 cells, other renin-expressing cells, or smooth muscle cells. However, the use of humoral agents to activate phospholipid pathways similar to those activated by mechanical force may prove useful for better understanding their physiological significance. Continued investigation into these mechanisms is important for understanding the proposed baroreceptor properties of renin-expressing cells and their control. The effect of mechanical activation of a signal transduction pathway involving PLC, IP\textsubscript{3}, and Ca\textsuperscript{2+} leading to a down-regulation of renin gene expression is the subject of our companion study (31).

We extend our sincere gratitude to Maureen Adol for technical expertise. In addition, we thank the laboratory of Dr. Fred Sachs for assistance with the Flexcell apparatus.

This work was supported in part by National Institutes of Health Grants HL-49405 (G. Hajduczok), HL-48459 (K. W. Gross), and CA-16056 (K. W. Gross), and American Heart Association Grant 92-310G (G. Hajduczok). M. J. Ryan was partially supported by a Mark Diamond predoctoral grant.

Current address for M. J. Ryan: 2000 Medical Laboratory, Department of Internal Medicine, University of Iowa, Iowa City, IA 52242.

REFERENCES


39. Watson SP, Poole A, and Asselin J. Ethylene glycol bis(beta-aminoethyl ether)-1,1′-tetraacetic acid (EGTA) and the tyrosphostin ST271 inhibit phospholipase C in human platelets by preventing Ca2+ entry. Mol Pharmacol 47: 823–830, 1995.