Involvement of Rho kinase pathway in the mechanism of renal vasoconstriction and cardiac hypertrophy in rats with experimental heart failure

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Winaver, Joseph, Elena Ovcharenko, Irit Rubinstein, Konstantin Gurbanov, Piero Pollesello, Bishara Bishara, Aaron Hoffman, and Zaid Abassi. Involvement of Rho kinase pathway in the mechanism of renal vasoconstriction and cardiac hypertrophy in rats with experimental heart failure. Am J Physiol Heart Circ Physiol 290: H2007–H2014, 2006. First published December 16, 2005; doi:10.1152/ajpheart.00600.2005.—Rho-dependent kinases serve as downstream effectors of several vasoconstrictor systems, the activities of which are upregulated in congestive heart failure (CHF). We evaluated renal and cardiac effects of Y-27632, a highly selective Rho kinase inhibitor, in an experimental model of volume-overload CHF. Effects of acute administration of Y-27632 (0.3 mg/kg) on renal hemodynamic and clearance parameters and effects of chronic treatment (10.0 mg·kg−1·day−1) for 7 days via osmotic minipumps) on cardiac contractility and cumulative Na+ excretion were studied in male Wistar rats with aortocaval fistula and control rats. The Y-27632-induced decrease in renal vascular resistance (from 40.4 ± 4.6 to 26.0 ± 3.1 resistance units, P < 0.01) in CHF rats was associated with a significant increase in total renal blood flow (+34%) and cortical and medullary blood flow (approx +37 and +27%, respectively). These values were significantly higher than those in control rats and occurred despite a decrease in mean arterial pressure (−15 mmHg). Despite the marked renal vasodilatory effect, Y-27632 did not alter glomerular filtration rate and renal Na+ excretion. Chronic administration of Y-27632 did not alter daily or cumulative renal Na+ excretion in CHF rats but was associated with a significant decrease in heart-to-body weight ratio, an index of cardiac hypertrophy: 0.32 ± 0.007, 0.46 ± 0.017, and 0.37 ± 0.006% in control, CHF, and CHF + Y-27632 rats, respectively. The findings suggest that Rho kinase-dependent pathways are involved in the mechanisms of renal vasoconstriction and cardiac hypertrophy in rats with volume-overload heart failure. Selective blockade of these signaling pathways may be considered an additional tool to improve renal perfusion and attenuate cardiac hypertrophy in heart failure.

Y-27632; congestive heart failure; renal hemodynamics; kidney

Vasoconstrictor neurohumoral systems, such as the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system, are of key importance in the pathophysiology of congestive heart failure (CHF) (10). Although they play an important compensatory role in the early stages of the disease, prolonged and excessive activation of these systems might have detrimental effects on the kidney and cardiovascular system. In particular, the kidney is highly sensitive to the action of vasoconstrictor agents, and a decrease in renal blood flow (RBF) is one of the most common pathophysiological alterations in clinical and experimental CHF (9). Increased activity of the vasoconstrictor systems may also lead to salt and water retention by the kidney and, thereby, cause a further deterioration in cardiac performance (10, 27). In addition, the RAAS, catecholamines, and other vasoconstrictor agents are involved in the induction of cardiac hypertrophy in CHF (21, 33).

Recent studies have revealed the importance of the Rho family of small G proteins and their associated kinases, Rho kinases, in the regulation of the vascular tone of various blood vessels, including the renal vasculature (6, 7, 12, 23, 28, 31). Rho kinase-activated pathways are known to promote the contraction of vascular smooth muscle cells, acting primarily by enhancement of Ca2+ sensitization through inhibition of myosin light chain phosphatase activity (29). Moreover, it has been shown that Rho kinase may act as a downstream effector in the intracellular signaling of several G protein-coupled receptors, including those of angiotensin II (ANG II), norepinephrine, and endothelin (ET)-1, the activities of which are known to be elevated in CHF (4, 13, 15, 18, 20, 22). In addition, the Rho kinase system has been implicated in the mediation of ET-1- and mechanical stress-induced hypertrophic responses in cardiac myocytes (3, 8, 20). These findings suggest that Rho kinase-dependent signaling pathways may potentially contribute to the mechanism of systemic and renal vasoconstriction and cardiac hypertrophy in CHF. Indeed, cardiac-specific overexpression of RhoA in mice resulted in a lethal form of heart failure, characterized by atrial enlargement, conduction defects, contractile failure, and generalized edema (26). Similarly, Kobayashi et al. (19) demonstrated the importance of Rho kinase pathways in the induction of cardiac dysfunction and remodeling in the failing hearts of Dahl salt-sensitive rats with CHF, and Kishi et al. (17) demonstrated that Rho kinase is involved in the increased forearm vascular resistance and impaired vasodilatation in patients with heart failure.

Previously, we demonstrated that rats with aortocaval fistula (ACF), an experimental model of volume-overload CHF, closely mimic the neurohumoral, renal, and cardiac manifestations of patients with severe CHF (1, 5, 32): an increase in

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activity of the vasoconstrictor RAAS and sympathetic nervous system, a decrease in RBF with Na⁺ retention by the kidney, and marked cardiac hypertrophy and dilatation. Moreover, these manifestations were dependent in part on increased activity of the RAAS and could be significantly ameliorated by treatment with angiotensin-converting enzyme (ACE) inhibitor or ANG II receptor antagonist (5, 32). The present study was undertaken to evaluate the hypothesis that Rho kinase-mediated pathways are involved in the mechanism of renal and cardiac manifestations in rats with experimental volume-overload CHF, specifically, that Rho kinase activation contributes to the generation of renal vasoconstriction and impaired salt handling by the kidney, as well as cardiac hypertrophy, in rats with ACF. Accordingly, we studied the effects of acute and chronic treatment with a highly selective Rho kinase antagonist, (R)-(+)-trans-N-(4-pyridyl)-4-(1-aminoethyl)-cyclohexancarboxamide (Y-27632), on renal hemodynamics, renal clearance parameters, and cardiac hypertrophy in rats with ACF (16).

MATERIALS AND METHODS

Male Wistar rats (Harlan Laboratories, Jerusalem, Israel; 280–350 g body wt) were maintained on standard rat chow (0.5% NaCl) and water ad libitum. The investigation was approved and conducted according to the guidelines of the Animal Use and Care Committee, Technion, and conforms with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Experimental Model

Heart failure was induced by surgical creation of an arteriovenous fistula between the abdominal aorta and the inferior vena cava (side to side, 0.9–1.2 mm OD) as originally described by Stumpe et al. (30) and adapted in our laboratory (2, 3, 32). After surgery, the rats were placed in individual metabolic cages for measurements of daily urinary Na⁺ excretion. At 7 days after surgery, the rats were subjected to one of the following experimental protocols.

Acute Studies

The rats were anesthetized with thiobutabarbital (Inactin; 100 mg/kg ip), placed on a thermoregulated (37°C) surgical table, and prepared for hemodynamic and clearance studies (5). After tracheotomy, polyethylene (PE-50) tubes were inserted into the carotid artery, jugular vein, and urinary bladder for blood pressure monitoring, infusion of various solutions, and urine collections, respectively. A solution of 0.9% saline (1.0–1.5% body wt/h) was infused throughout the experiment. After a 60-min equilibration period, the following experiments were undertaken.

Effects of incremental doses of Y-27632 on arterial pressure in control rats. Rats (n = 8) were anesthetized and placed on a temperature-controlled table as described above. Systolic, diastolic, and mean arterial pressures (SBP, DBP, and MAP, respectively) were measured in the carotid artery. Y-27632 (Toecris Cookson, Bristol, UK) was infused intravenously at 0.1, 0.3, and 1 mg/kg for 30 min at each dose. The control group of rats (n = 7) was treated with vehicle for 150 min after baseline measurements, which were obtained during infusion of saline alone.

Effects of Y-27632 on RBF, renal vascular resistance, and MAP in control and CHF rats. Total RBF was measured by ultrasonic flowmeter (model T206, Transonic, Ithaca, NY) using a flow probe (model 1RB) placed around the left renal artery, as previously described (5). MAP was continuously monitored by a pressure transducer, and renal vascular resistance (RVR) was calculated according to the following formula: RVR = MAP/RBF. After surgery and equilibration, baseline measurements were obtained for 60 min. Y-27632 (0.3 mg/kg body wt iv) was administered to control (n = 7) and CHF (n = 8) rats, and measurements were recorded for an additional 40 min.

Effects of Y-27632 on intrarenal blood flow in control and CHF rats. The left kidney was exposed in control (n = 7) and CHF (n = 8) rats through a midabdominal incision. Cortical and medullary blood flow (CBF and MBF, respectively) were measured simultaneously by a dual-channel laser-Doppler flowmeter (model 4001, Master Perimed) using two needle probes, as previously described (5, 14). Y-27632 (0.3 mg/kg) was administered, and CBF and MBF were measured at 5-min intervals for 30 min.

Effects of Y-27632 on renal clearance parameters in control and CHF rats. In additional groups of control (n = 9) and CHF (n = 9) rats, the acute effects of the drug on glomerular filtration rate (GFR) and absolute and fractional Na⁺ excretion rates (UNaV and FENa, respectively) were studied. Rats were prepared as described in previous protocols, except the abdominal cavity was not opened. A solution of 2% inulin in 0.9% saline was infused throughout the experiment. After surgery and equilibration, two 30-min baseline clearance periods were obtained. Y-27632 was administered first as a bolus injection (0.3 mg/kg) and then as a sustained infusion of the same dose per hour. Three or four additional clearance periods were obtained under the influence of the drug. Urine volume was determined gravimetrically. Blood samples were obtained in the middle of every second clearance period.

Chronic Studies

Effects of chronic administration of Y-27632 on Na⁺ excretion and cardiac hypertrophy. Y-27632 was dissolved in saline and loaded into osmotic minipumps (model 2001, Alzet Pharmaceuticals) at a final concentration sufficient to deliver 10 mg·kg⁻¹·day⁻¹ for 7 days, similar to the protocol reported previously by Kobayashi et al. (19) in Dahl salt-sensitive rats with CHF. Sham-operated control (n = 6) and CHF (n = 12) rats were studied. After 4 days in individual metabolic cages (baseline period), sham operation or ACF was performed. In rats with ACF, an osmotic minipump containing Y-27632 (n = 6) or vehicle (n = 6) was inserted into the peritoneal cavity during the operation. The rats were allowed to recover and returned to their metabolic cages for daily measurements of Na⁺ excretion. At 7 days after surgery, the rats were anesthetized by Inactin, and MAP was measured by a polyethylene catheter inserted into the carotid artery. The heart was removed and weighed, and the heart-to-body weight ratio was calculated.

Effects of Y-27632 on renal hemodynamics in CHF rats chronically treated with the ACE inhibitor enalapril. The last set of experiments was designed to evaluate whether Y-27632 exerts a residual vasodilatory effect in the presence of blockade of the renin-angiotensin system. In rats with ACF (n = 8), the ACE inhibitor enalapril (100 mg/l of drinking water) was administered for 5 days, starting on the day of operation. Previously, we found that this protocol results in a near-complete blockade of the renin-angiotensin system (assessed by measurement of the vasoconstrictor response to angiotensin I). At 6 days after surgery, the rats were anesthetized by Inactin and subjected to the experimental protocol described in Acute Studies.

Chemical Analysis

Na⁺ concentration in plasma and urine was determined by flame photometry (model IL 943, Instrumentation Laboratories). Inulin concentration was determined by the colorimetric anthrone method (11). GFR was equated with the clearance of inulin.

Statistical Analysis

Statistical significance was assessed by one-way analysis of variance (ANOVA), ANOVA for repeated measures, or two-way ANOVA, as

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appropriate. Dunnett’s test and Tukey’s multiple comparisons test were used for data point comparisons within each group. \( P < 0.05 \) was considered statistically significant. Values are means ± SE.

RESULTS

Effects of Incremental Doses of Y-27632 on Arterial Pressure in Control Rats

In preliminary experiments, we found that Y-27632 was stable (92–95%) in saline buffer and plasma at 37°C for up to 500 min. The effects of incremental doses of Y-27632 (0.1, 0.3, and 1.0 mg·kg\(^{-1}\)·h\(^{-1}\)) on SBP, DBP, and MAP in normal rats are shown in Fig. 1. All doses produced a significant decrease in these parameters. Although 0.1 mg·kg\(^{-1}\)·h\(^{-1}\) caused a mild decrease in MAP, 1.0 mg·kg\(^{-1}\)·h\(^{-1}\) produced severe hypotension. In comparison, 0.3 mg·kg\(^{-1}\)·h\(^{-1}\) produced moderate and significant hemodynamic effects; therefore, it was used in the subsequent acute protocols throughout the present study. Additionally, in other sets of experiments, we found that Y-27632 at 0.3 mg·kg\(^{-1}\)·h\(^{-1}\) partially blocked the pressor effects of ET-1, ANG II, and epinephrine in anesthetized rats (data not shown).

Effects of Y-27632 on MAP and Renal and Intrarenal Hemodynamics in Control and CHF Rats

Baseline MAP and RBF were significantly lower and RVR was higher in CHF than in control rats (Fig. 2). Bolus injection of Y-27632 caused a significant, yet transient, decrease in MAP in control rats. This notion is further supported by measurements of intrarenal blood flow in response to the drug (Fig. 3). Administration of Y-27632 to control rats caused a gradual decrease in these parameters. Although 0.1 mg·kg\(^{-1}\)·h\(^{-1}\) caused a mild decrease in MAP, 1.0 mg·kg\(^{-1}\)·h\(^{-1}\) produced severe hypotension. In comparison, 0.3 mg·kg\(^{-1}\)·h\(^{-1}\) produced moderate and significant hemodynamic effects; therefore, it was used in the subsequent acute protocols throughout the present study. Additionally, in other sets of experiments, we found that Y-27632 at 0.3 mg·kg\(^{-1}\)·h\(^{-1}\) partially blocked the pressor effects of ET-1, ANG II, and epinephrine in anesthetized rats (data not shown).

Acute Effects of Y-27632 on GFR and Na\(^+\) Excretion in Control and CHF Rats

Figure 4 summarizes the results of the acute administration of the drug on GFR, U\(_{\text{Na}}\)V, and FENa in control and CHF rats. Baseline GFR and U\(_{\text{Na}}\)V tended to be lower in CHF than in control animals. Acute administration of the drug was not associated with an increase in GFR or Na\(^+\) excretion in control or CHF rats. In CHF rats, there was a significant decrease in U\(_{\text{Na}}\)V (from 0.37 ± 0.14 to 0.15 ± 0.04 μeq/min, \( P < 0.05 \)) and FENa (from 0.22 ± 0.1 to 0.06 ± 0.01%, \( P < 0.05 \)) from baseline to the final clearance period (C3). Thus, despite the prominent renal vasodilatory effect of Y-27632 in CHF rats, it did not cause a parallel increase in GFR or Na\(^+\) excretion.

Effects of Chronic Administration of Y-27632 on Daily and Cumulative Na\(^+\) Excretion

Figure 5 summarizes the effects of chronic administration of Y-27632 (via osmotic minipumps, for 7 days) on daily and
cumulative Na⁺ excretion in control and CHF rats. In agreement with our previous reports, absolute and cumulative Na⁺ excretions were lower in CHF than in sham-operated control rats (5, 32). Moreover, similar to the findings in the acute protocols, chronic administration of the drug to rats with CHF did not increase urinary Na⁺ excretion. Daily Na⁺ excretion as well as cumulative Na⁺ excretion tended to be lower (although not statistically significantly) in the CHF group infused with Y-27632 than in the CHF group infused with the vehicle only (Fig. 5). This may be attributed in part to the significant decrease (P < 0.01) in MAP observed on day 7 of the experiment in Y-27632-treated CHF rats (84 ± 6 mmHg) compared with CHF rats not treated with the drug (116 ± 5 mmHg). Taken together with the results of the acute studies, these findings indicate that the drug does not affect urinary Na⁺ excretion.

**Effects of Chronic Treatment With Y-27632 on Heart-to-Body Weight Ratio**

Figure 6 summarizes the effects of chronic treatment with Y-27632 on heart-to-body weight ratio, an index of cardiac hypertrophy. Heart-to-body weight ratio was significantly higher in CHF than in sham-operated control rats and was further reduced by chronic treatment with Y-27632: 0.32 ±...
0.007, 0.46 ± 0.017, and 0.37 ± 0.006% for control, CHF, and CHF + Y-27632, respectively. Thus administration of the drug for 7 days after surgery was associated with a significant reduction in cardiac hypertrophy in rats with experimental CHF.

**Effects of Y-27632 on Renal Hemodynamics in CHF Rats in the Presence of Chronic ACE Blockade**

In the final set of experiments, we evaluated the effects of acute administration of Y-27632 on MAP, RBF, and RVR in CHF rats chronically treated with high doses of enalapril to block the renin-angiotensin system. The results of this set of experiments are shown in Fig. 7. In CHF rats chronically treated with enalapril, basal RVR was lower and RBF was higher than in CHF rats with an intact renin-angiotensin system. Baseline MAP did not differ between the two groups. Administration of Y-27632, in the face of ACE blockade, was associated with residual hypotensive and renal vasodilatory effects. Although these actions were mild, they were still significant in enalapril-treated CHF rats. This might indicate...
that Y-27632 can exert a mild vasodilator effect independent of the presence of ANG II. The mechanism of that residual effect was not evaluated in the present study. Nevertheless, it is noteworthy that other systems acting via G protein-coupled receptors, such as ET and norepinephrine, are also activated in this experimental model of CHF.

DISCUSSION

The findings of the present study provide novel information on the involvement of Rho kinase signaling pathways in the mediation of renal vasoconstriction and cardiac hypertrophy in rats with experimental CHF. Our data clearly demonstrate that acute administration of Y-27632, a highly selective and potent Rho kinase inhibitor, produced a renal vasodilatory action with a longer duration and a greater magnitude in rats with experimental CHF than in controls. This renal vasodilatation was associated with a significant increase in total RBF in CHF rats due to enhanced perfusion of the renal cortex and medulla. Moreover, despite the improvement in renal hemodynamics, acute administration of Y-27632 did not cause a concomitant increase in GFR or urinary Na⁺ excretion. Similarly, Na⁺ excretion was not increased by chronic (7 days) administration of the drug. Finally, chronic treatment with Y-27632 was associated with a significant decrease in heart-to-body weight ratio, an index of cardiac hypertrophy, in rats with experimental CHF. Thus our findings provide additional support for studies that established the involvement of Rho kinase-mediated pathways in the pathophysiology of cardiovascular disorders (28, 31).

The most prominent effects of Y-27632 revealed in the present study were the renal and systemic vasodilatory properties of the drug. Sensitivity to the renal vasodilatory action of the drug was greater in rats with experimental CHF than in control animals, suggesting a greater dependence of renal vasoconstriction on Rho kinase-mediated pathways in CHF. The latter is compatible with the notion that Rho kinase may act in the downstream signaling of several vasoconstrictor systems, such as RAAS, ET-1, and the sympathetic nervous system, the activities of which are elevated in CHF. Indeed, it is well accepted that increased activity of these neurohumoral systems plays a major role in the induction of renal vasoconstriction in CHF (9, 10). Thus it is possible that, beyond their action to increase cytosolic Ca²⁺, these vasoconstrictor systems also act to induce Ca²⁺ sensitization through Rho kinase-mediated signaling (12, 29). The latter is thought to occur by modulation of the level of phosphorylation of the myosin light chain in vascular smooth muscle cells mainly through the inhibition of myosin phosphatase. Thus Rho kinase phosphorylates the myosin-binding subunit of myosin phosphatase, rendering the myosin phosphatase inactive and, thereby, increasing the levels of the phosphorylated myosin light chain (12, 29, 31). Y-27632, by interfering with the Rho kinase-mediated Ca²⁺ sensitization, may lead to enhanced renal vasodilatation.

In addition to its action as a renal vasodilator, Y-27632 also caused a transient hypotensive effect, possibly because the drug may also act as a systemic vasodilator. In contrast to the enhanced renal vasodilatory effect of Y-27632 in rats with CHF compared with control rats, the systemic hypotensive effect of the drug appeared to be blunted in CHF rats. Although the decrease in MAP and the increase in RBF may reflect the vasodilatory properties of Y-27632, other factors, such as impaired myocardial contractility, could contribute to the blunted hypotensive response in heart failure. In addition, inherent differences in the involvement of Rho kinase-mediated pathways may exist between the renal and other vascular beds and, thereby, explain the different sensitivity to the drug.

Despite the improvement in renal perfusion, acute administration of Y-27632 to rats with CHF did not cause a concomitant increase in GFR; therefore, it might be suggested that the vasodilatory action of the drug was restricted primarily to the
efferent arteriole. This, however, would seem to contradict the recent findings of Nakamura et al. (23) that Rho kinase played a dominant role in mediating basal and ANG II-induced tone of the afferent, but not efferent, arteriole in the isolated perfused rat hydrenephrotic kidney. In a similar preparation, Cavarape et al. (6) demonstrated that topical application of Y-27632 caused a preferential dilatation of preglomerular vessels that was greater than that of the efferent arteriole. Indeed, it is possible that Y-27632 relaxed post- and preglomerular vessels, but the concomitant decrease in MAP induced by the drug resulted in a decline in glomerular hydrostatic pressure. Further studies using direct measurements of glomerular hemodynamic parameters are required to elucidate the exact action of the drug in our rats.

Also of interest is the finding that renal Na\(^+\) excretion was not affected by short-term or chronic (7 days) administration of Y-27632 in rats with CHF. During chronic treatment with the drug, there was a clear tendency to a decrease in daily and cumulative Na\(^+\) excretion in the treated vs. untreated rats with CHF (Fig. 5). This may also be attributed to the marked decrease in MAP caused by Y-27632 in the present study or, alternatively, to the exaggerated activation of the RAAS in CHF, as we reported previously (1). It should be emphasized that ANG II has direct stimulatory effects on Na\(^+\)/H\(^+\) exchange in normal and hypertensive rats by reducing the activity of the Na\(^+\)/H\(^+\) exchanger in the proximal nephron. They used a continuous infusion of a nonhypotensive dose of the drug, and because they carefully monitored MAP, they were able to discard experiments in which MAP decreased by >5% of the basal level. Under these conditions, an effect of Y-27632 on renal tubular Na\(^+\) transport could be exposed (24).

Previously, we demonstrated that ACE inhibition and ANG II receptor blockade by eprosartan increased renal perfusion and, at the same time, significantly increased urinary Na\(^+\) excretion when administered chronically in the same experimental model (5, 32). In that respect, the findings of the present study differ considerably by showing an antinatriuretic tendency in response to chronic administration of Y-27632. This could suggest that Rho kinase inhibition may be less effective than ACE inhibitors or ANG II receptor antagonists as a treatment modality in CHF. It is possible that the significant reduction in MAP observed after chronic treatment with Y-27632 in rats with CHF masked any direct action of the drug on renal tubular Na\(^+\) transport.

Finally, our findings demonstrate that chronic treatment with Y-27632, starting on the day of the surgical creation of the ACF, significantly attenuated the development of cardiac hypertrophy in rats with experimental CHF. Kobayashi et al. (19) originally demonstrated that chronic treatment (for 7 wk) with the same dose of Y-27632 ameliorated cardiac hypertrophy and improved cardiac contractility in Dahl salt-sensitive rats developing CHF. In their study, treatment was administered from the left ventricular hypertrophy stage (week 11) to the CHF stage (week 18). Our study confirms their findings on the antihypertrophic properties of the drug and, further, suggests that earlier treatment might be beneficial as well. However, because in the clinical setting the treatment is utilized usually after development of the disease, our approach does not mimic such a setting.

Previous results reported by Ruzicka et al. (25) and from our laboratory (5) demonstrated that ANG II receptor blockade largely prevented cardiac hypertrophy in rats with ACF. These findings may underscore the dominant role of the renin-angiotensin system in the development of myocardial enlargement and remodeling in this model of volume overload-induced cardiac hypertrophy. Our present data further suggest that this growth effect may be mediated in part through Rho kinase-dependent signaling. Previous in vitro studies also demonstrated that the ET-1-induced hypertrophic response in cultured rat cardiomyocytes can be ameliorated by treatment with Y-27632 (3, 20). Whether this potential mechanism contributed to the beneficial effect of the drug on cardiac enlargement in the present study remains to be established.

In summary, the findings of the present study suggest that Rho kinase-mediated pathways may be involved in the mechanism of renal vasoconstriction and cardiac hypertrophy in rats with volume-overload CHF. Further studies are required to evaluate whether selective Rho kinase antagonists may be useful as an additional treatment modality to improve renal perfusion and attenuate cardiac hypertrophy in CHF.

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