Cardiac Renin Levels Are Not Influenced by the Amount of Resident Mast Cells

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Abstract—To investigate whether mast cells release renin in the heart, we studied renin and prorenin synthesis by such cells, using the human mast cell lines human mastocytoma 1 and LAD2, as well as fresh mast cells from mastocytosis patients. We also quantified the contribution of mast cells to cardiac renin levels in control and infarcted rat hearts. Human mastocytoma 1 cells contained and released angiotensin I–generating activity, and the inhibition of this activity by the renin inhibitor aliskiren was comparable to that of recombinant human renin. Prorenin activation with trypsin increased angiotensin I–generating activity in the medium only, suggesting release but not storage of prorenin. The adenylyl cyclase activator forskolin, the cAMP analogue 8-db-cAMP, and the degranulator compound 48/80 increased renin release without affecting prorenin. Angiotensin II blocked the forskolin-induced renin release. Angiotensin I–generating activity was undetectable in LAD2 cells and fresh mast cells. Nonperfused rat hearts contained angiotensin I–generating activity, and aliskiren blocked ≈70% of this activity. A 30-minute buffer perfusion washed away >70% of the aliskiren-inhibitable angiotensin I–generating activity. Prolonged buffer perfusion or compound 48/80 did not decrease cardiac angiotensin I–generating activity further or induce angiotensin I–generating activity release in the perfusion buffer. Results in infarcted hearts were identical, despite the increased mast cell number in such hearts. In conclusion, human mastocytoma 1 cells release renin and prorenin, and the regulation of this release resembles that of renal renin. However, this is not a uniform property of all mast cells. Mast cells appear an unlikely source of renin in the heart, both under normal and pathophysiological conditions. (Hypertension. 2009;54:315-321.)

Key Words: renin ■ prorenin ■ mast cell ■ heart ■ cAMP

The majority of cardiac angiotensin (Ang) II is produced at tissue sites by conversion of in situ synthesized Ang I.1 Yet, the renin required for this Ang generation is largely, if not completely, derived from the kidney, because after a bilateral nephrectomy, both cardiac renin and Ang II decrease to undetectable levels.2–4 Circulating, kidney-derived renin diffuses into cardiac interstitial fluid5 and/or binds to “renin receptors.” In support of this concept, the washout of renin from isolated perfused rat Langendorff hearts loaded with porcine renin followed a biphasic pattern: an initial, rapid (half-life: <0.5 minutes) phase representing a disappearance from the extracellular fluid compartment and a secondary, slower phase (half-life: ≈3 to 4 minutes), representing a disappearance from tissue sites, possibly cell-surface renin receptors.6 Two such receptors have been identified recently: the mannose 6-phosphate receptor (M6PR) and the (pro)renin receptor.7–10 M6PRs bind phosphomannosylated proteins, including renin and its inactive precursor, prorenin. Binding to such receptors is followed by internalization, intracellular proteolytic cleavage of prorenin to renin, and subsequent proteolytic/hydrolytic removal or clearance.8 M6PRs are, therefore, considered to act as clearance receptors for both renin and prorenin. (Pro)renin receptors bind renin and prorenin on the cell surface without resulting in internalization.9,10

Recently, the idea of cardiac renin being kidney derived was challenged. Silver et al11 provided evidence for the presence of renin in cardiac mast cells using both the human mastocytoma (HMC-1) cell line and frozen tissue sections of buffer-perfused Sprague-Dawley rat hearts. HMC-1 cells contained renin, as demonstrated by immunoblot and immunocytochemical analyses, and released Ang I–generating activity after their exposure to the mast cell degranulator compound 48/80 (48/80). The human renin inhibitor BILA2157 (IC50: 2.5 nmol/L),12 at a concentration of 100 nmol/L, blocked part of this Ang I–generating activity. A monoclonal mouse antirenin antibody recognized renin in mast cells in the rat heart. Moreover, in a follow-up study,13 a bolus injection of 48/80 was reported to release Ang I–generating activity from Langendorff-perfused guinea pig hearts, and a comparable release occurred after exposure of
the heart to 20 minutes of global ischemia and 30 minutes of reperfusion. BIL2157 not only blocked the Ang I–generating activity in the perfusate but also prevented the norepinephrine overflow and arrhythmias occurring after ischemia. Thus, it was concluded that ischemia promotes cardiac Ang generation by mast cell–derived renin.

In the present study, we set out to confirm the renin synthesis and release by HMC-1 cells, applying both the selective human renin inhibitor aliskiren (IC50: 0.6 mmol/L) and immunoradiometric assays (IRMAs) making use of monoclonal antibodies that recognize the active site of renin. We also studied the regulation of mast cell renin release, using known stimulators and inhibitors of renal renin release, and we investigated to what degree these cells release prorenin. Results obtained in HMC-1 cells were compared with results in the mast cell line LAD2 and in primary mast cells isolated from mastocytosis patients. Finally, we evaluated the washout of endogenous rat renin from the rat heart when perfused according to Langendorff, both with and without 48/80, to quantify the contribution of mast cells to cardiac renin levels. This was done both in control animals and in animals subjected to myocardial infarction (MI), a procedure known to significantly increase the cardiac mast cell number.

Methods

Cell Culture Studies

HMC-1 cells were a kind gift of Dr Joseph H. Butterfield (Mayo Clinic, Rochester, MN). Cells were grown in 75-cm2 culture flasks (37°C, 5% CO2) for 7 days using supplemented Iscove’s modified Dulbecco’s medium containing 10% heat-inactivated calf serum. Next, the cells were separated from the culture medium by centrifugation at 1500g at room temperature. The culture medium was collected and stored at −20°C, and the cells were resuspended in fresh medium at a concentration of ~5 million cells per milliliter. Cells (0.5 mL) were then stimulated for 4–5 hours with compound 48/80 (20 to 100 μg/mL; final concentration in medium), the β-adrenoceptor agonist isoproterenol (1 mmol/L to 1 μmol/L), the adenylyl cyclase activator forskolin (50 μmol/L), the membrane-permeable cAMP analogue 8-bromo-cAMP (1 mmol/L), Ang II (1 mmol/L to 1 μmol/L), and/or the protein kinase C inhibitor chelerythrine chloride (0.1 to 1.0 mmol/L) at 37°C. All of the drugs were obtained from Sigma and dissolved in water, except forskolin, which was dissolved in dimethyl sulfoxide. Cells incubated with vehicle served as the control. After the stimulation period, cells were collected from the stimulation medium by centrifugation at 1500g and stored at −20°C. The culture medium was collected and stored at −20°C, and the cells were resuspended in fresh medium at a concentration of ~5 million cells per milliliter. Cells (0.5 mL) were then stimulated for 4–5 hours with compound 48/80 (20 to 100 μg/mL; final concentration in medium), the β-adrenoceptor agonist isoproterenol (1 mmol/L to 1 μmol/L), the adenylyl cyclase activator forskolin (50 μmol/L), the membrane-permeable cAMP analogue 8-bromo-cAMP (1 mmol/L), Ang II (1 mmol/L to 1 μmol/L), and/or the protein kinase C inhibitor chelerythrine chloride (0.1 to 1.0 mmol/L) at 37°C. All of the drugs were obtained from Sigma and dissolved in water, except forskolin, which was dissolved in dimethyl sulfoxide. Cells incubated with vehicle served as the control. After the stimulation period, cells were collected from the stimulation medium by centrifugation at 1500g, and the medium was collected and stored at −20°C. The pellet was resuspended in lysis buffer (0.2% Triton-X in PBS) and also stored at −20°C.

The human mast cell sarcoma cell line LAD2 was obtained from the National Institutes of Health. Cells were cultured in StemPro 34 with nutrient supplement and 100-ng/mL stem cell factor for 7 days. Cells were then centrifuged, and medium was collected and stored at −20°C. The pellet was resuspended in lysis buffer (~1 million cells per milliliter) and also stored at −20°C.

Bone marrow aspirates were obtained from 2 women with systemic mastocytosis (aged 67 and 41 years). Mast cells were isolated by fluorescence-activated cell sorter (FACS Aria, BD Biosciences) on the basis of CD117-PE-Cy7 and CD33-PE expression. Cells were resuspended in lysis buffer (~0.8 and 0.2 million cells per milliliter, respectively) and stored at −20°C.

Animal Studies

Ninety-one male Sprague-Dawley rats (Harlan), weighing 300 to 400 g, were housed in groups of 2 or 3 on a 12-hour light-dark cycle. Standard rat chow and water were available ad libitum. All of the experiments were performed according to the regulations of the Erasmus MC Animal Care Committee, in accordance with the American Physiological Society Guiding Principles. Sixty rats underwent coronary artery ligation as described previously. They were then allowed to recover for 2 weeks. Of the MI animals, 36 rats died within 24 hours after coronary ligation, and 1 animal died in the subsequent 2 weeks. Three MI rats were excluded from further analysis because of insufficient infarct size.

Isolated Heart Studies

Rats were anesthetized with sodium pentobarbital (60 mg/kg, IP). Blood (0.5 mL) was collected to measure plasma renin activity. Hearts were rapidly excised, cooled in ice-cold Krebs-Henseleit buffer (composition in mmol/L: NaCl 125, KCl 4.7, NaHCO3 20, NaH2PO4 0.43, MgCl2 1.0, CaCl2 1.3, and D-glucose 9.1; pH 7.4) until contractions stopped, and then either frozen in liquid N2 or prepared for Langendorff perfusion. Continuously carbogen-gassed (95% O2/5% CO2) Krebs-Henseleit buffer at 37°C was perfused immediately after cannulation of the aorta, at a constant perfusion pressure of 80 mm Hg. A water-filled latex balloon was placed in the left ventricle via the left atrium to measure left ventricular pressure. The volume of the balloon was adjusted to achieve a stable left ventricular end-diastolic pressure of 5 mm Hg during initial equilibration, and this volume was maintained throughout the experiment. Hearts were paced at 450 bpm. Coronary flow was measured with an in-line flow probe (Transonic Systems). After 30 or 120 minutes of buffer perfusion, hearts were removed and frozen in liquid N2. In a separate series of experiments, hearts were perfused with buffer for 30 minutes and then exposed to a bolus injection of compound 48/80 (300 μg in 100 μL Krebs-Henseleit buffer). Coronary effluent was collected from 5 minutes before until 5 minutes after the exposure to compound 48/80 and stored at −20°C. Five minutes after the exposure to compound 48/80, hearts were removed and also frozen in liquid N2.

Biochemical Measurements

Renin and prorenin were measured in medium and cell lysates by enzyme-kinetic assay (EKA) and/or IRMA (Cisbio), using recombinant human renin as a control. To allow its measurement by EKA or IRMA, prorenin was converted to renin by incubating the sample for 72 hours with trypsin-Sepharose at 4°C, followed by 5-minute centrifugation at 1300 rpm to remove trypsin-Sepharose. This approach yielded the same result as prorenin activation via a 48-hour exposure at 4°C to plasmin (0.5 casemolytic U/mL; data not shown). Renin in cardiac tissue and coronary effluent were measured as described before, using rat kidney renin and rat plasma renin as controls. In short, hearts were homogenized in phosphate buffer (1:15, weight:volume; pH 7.4) and dialyzed for 48 hours at 4°C against 0.05 mol/L of glycine buffer (pH 3.3), followed by a 24-hour dialysis against phosphate buffer. This procedure effectively removes all angiotensinase activity. Because the levels in coronary effluent were expected to be low, the effluent was concentrated 10-fold by centrifugal filtration (Millipore). Finally, to verify the contribution of renin to the Ang I–generating activity detected in the EKA, all of the EKA measurements were repeated in the presence of increasing concentrations of the renin inhibitor aliskiren (1.0 pmol to 0.1 mmol/L; provided by Novartis Pharmaceuticals). Detection limits of the EKA and IRMA were 0.05 ng of Ang I per milliliter per hour and 1 pg of renin per milliliter, respectively, and 1 ng of Ang I per milliliter per hour corresponds with 2.6 pg of human renin per milliliter.

Data Analysis

Results are expressed as mean±SEM. ICS50 values were calculated as described previously. Statistical analysis was performed using 1-way ANOVA. P<0.05 was considered significant.
and medium of 48/80-stimulated cells did contain Ang I–generating activity. Comparison of these measurements with those by IRMA yielded the line of identity (Figure 1, left), suggesting that all of the Ang I–generating activity was attributable to renin. The inhibition curve obtained in the presence of increasing concentrations of aliskiren confirmed this view, because the IC$_{50}$ values for Ang I–generating activity in medium and cell lysate (0.13±0.04 and 0.18±0.07 nmol/L; Figure 1, right) were comparable to that for recombinant human renin (IC$_{50}$: 0.30 nmol/L). Full blockade of the Ang I–generating activity was observed at aliskiren concentrations of ≥0.1 μmol/L, indicating that renin is the only Ang I–generating enzyme present.

Prorenin Release by HCM-1 Cells

Trypsin treatment increased the renin level in the culture medium but not in the cell lysates or the stimulation medium of 48/80-stimulated cells (Figure 2). This indicates that only the culture medium contained prorenin. The stimulation medium of forskolin- (n=9) and 8-db-cAMP-stimulated (n=4) cells also did not contain prorenin (data not shown).

Regulation of Renin Release by HCM-1 Cells

Renin release was induced by 48/80 into the medium in a concentration-dependent manner (Figure 2), without significantly lowering the renin levels in the cell lysates. The latter relates to the fact that the amount of renin released into the stimulation medium amounted to <10% of the levels in the cell lysates. Ang II and chelerythrine chloride, at concentrations ≤1 μmol/L, did not affect the 48/80-induced renin release into the medium, nor did these drugs affect renin release by themselves (Figure 3; n=4 to 5). Forskolin increased renin release into the medium to the same degree as 48/80 (Figure 3), and identical results were obtained with 8-db-cAMP (renin level in stimulation medium: 209±36% of control; n=4). Ang II and chelerythrine chloride, both at a concentration of 1 μmol/L, fully prevented the forskolin-induced renin release (Figure 3). Isoproterenol, at concentrations ≤1 μmol/L, did not affect renin release (n=5; data not shown).

LAD2 and Primary Mast Cells

Renin and prorenin levels were below the detection limit in the culture medium and cell lysates of LAD2 cells, as well as in the cell lysates of primary mast cells obtained from 2 mastocytosis patients.

Does Renin in the Rat Heart Originate in Mast Cells?

Nonperfused acidified rat heart homogenates contained Ang I–generating activity, and aliskiren blocked ~70% of this

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**Figure 1.** Left, comparison of the renin concentration in medium and lysates of HMC-1 cells measured by EKA and IRMA. For the sake of clarity, EKA data in micrograms of Ang I per milliliter per hour have been converted to nanograms of renin per milliliter. The regression line (log[renin]$_{EKA}$=0.90×log[renin]$_{IRMA}$–0.53; r=0.95) was not significantly different from the line of identity. Right, concentration-dependent inhibition of the Ang I–generating activity by aliskiren in medium and lysates of 48/80-stimulated HMC-1 cells. Data (mean±SEM of 5 experiments) have been expressed as a percentage of the Ang I–generating activity in the absence of aliskiren (control). For comparison, results obtained with recombinant human renin are also shown.

**Figure 2.** Renin in medium and lysates of HMC-1 cells before (■) and after (▲) trypsin activation of prorenin. Culture medium represents medium obtained from cells cultured for 7 days without stimulators. Stimulation medium represents medium of cells stimulated with 48/80 (0, 20, or 100 μg/mL) for 30 minutes. The lysates are derived from the 48/80-stimulated HMC-1 cells. Data are mean±SEM of 5 experiments. *P<0.05 vs before trypsin activation, #P<0.05 vs control.
activity at concentrations that fully blocked rat kidney and rat plasma renin (Figure 4). The IC₅₀ values toward the cardiac, renal, and plasma Ang I–generating activity were, respectively, 0.6±0.2, 0.2±0.1, and 0.2±0.1 μmol/L (P value not significant), ie, within the range of the previously published IC₅₀ of aliskiren for rat renin (0.1 μmol/L). Buffer perfusion greatly decreased the cardiac Ang I–generating activity, and under those conditions, the effect of aliskiren, even at high concentrations, was marginal or absent (Figure 5, top left).

Renin-attributable, Ang I–generating activity was calculated by subtracting the mean Ang I–generating activity in the presence of the 3 highest aliskiren concentrations from the Ang I–generating activity without aliskiren. Figure 5 (top right) shows that a 30-minute buffer perfusion of the heart reduced the aliskiren-inhibitable (ie, renin-attributable) Ang I–generating activity by >70%. Perfusion for longer periods (120 minutes) did not decrease this activity further, nor did exposure to 48/80 affect this activity. In the perfusion buffer, Ang I–generating activity was below the limit of detection both during 5 minutes before and during 5 minutes after exposure to 48/80. Stimulation with 48/80 reduced coronary flow from 8.7±0.6 to 4.4±0.4 mL/min (P<0.01). When performing these studies after MI (Figure 5, bottom), the results were identical, despite the increased mast cell content of the infarcted heart (Figure 6). Stimulation with 48/80 in MI hearts reduced coronary flow from 8.2±0.3 to 1.5±0.4 mL/min (P=0.02 versus control hearts). The much larger effect of 48/80 in MI hearts also supports the concept of an increased mast cell content in these hearts. However, Ang I–generating activity remained below the detection limit in the perfusion buffer of the MI hearts, both before and after 48/80 exposure.

**Discussion**

This study confirms the observation by Silver et al that HMC-1 cells store and release Ang I–generating activity. Although mast cells have been reported earlier to synthesize the angiotensinogen-cleaving enzyme cathepsin D, our study, making use of the selective human renin inhibitor aliskiren (IC₅₀ for human cathepsin D: 5 μmol/L) and monoclonal antibodies that recognize the active site of renin, now demonstrates that renin is the sole contributor to the Ang I–generating activity in the lysates and medium of HMC-1 cells. The current findings also indicate that activation of the adenylyl cyclase-cAMP pathway stimulates renin release from HMC-1 cells, whereas interference with the Ca²⁺-protein kinase C pathway blocks this release. In addition, HCM-1 cells release prorenin in a constitutive manner without storing the inactive precursor of renin intracellularly. Thus, the regulation of (pro)renin release in HMC-1 cells is identical to that in other renin-producing cells, including renal juxtaglomerular cells and adrenal glomerulosa cells.

However, activation of the adenylyl cyclase-cAMP pathway with the nonselective β-adrenoceptor agonist isoproterenol did not affect renin release from HMC-1 cells. This most likely relates to the mast cell–stabilizing action of β₂-adrenoceptors, which are the predominant β-adrenoceptors on mast cells. Such an effect would oppose the well-known β₁-adrenoceptor–mediated stimulation of renin release. Indeed, mast cell stabilizers (eg, cromolyn and lodoxamide) suppress the release of Ang I–generating activity from mast cells.

Furthermore, the mast cell degranulator compound 48/80 enhanced renin release from HMC-1 cells, and this effect could not be blocked by activation of the Ca²⁺-protein kinase C pathway. This is probably attributable to the nonspecific action of basic secretagogues like 48/80, involving multiple receptors and/or direct intracellular actions. Whether mast cell degranulators and stabilizers also interfere with renin
release by non–HMC-1 cell renin–producing cells is currently unknown.

Unexpectedly, fresh mast cells obtained from mastocytosis patients and the mast cell line LAD2 did not contain or release Ang I–generating activity. Apparently, therefore, HMC-1 cells are not representative of all mast cells, and, thus, the proposal that mast cells are a source of renin in the heart needs to be viewed with care. This proposal also challenges the current concept that cardiac renin is strictly kidney derived. This concept is based on the inability to detect renin mRNA at cardiac tissue sites, the disappearance of renin from the heart after bilateral nephrectomy, and the lack of renin synthesis by cardiac fibroblasts and myocytes. In fact, the majority of cardiac renin disappears within minutes when perfusing the heart with a renin-free buffer, in full agreement with studies showing that cardiac renin is largely confined to the interstitial fluid compartment. Obviously, some retention may occur at sites that do not readily exchange with the extracellular fluid compartment. For instance, M6PR-internalized (pro)renin is cleared intracellularly (with a half-life of several hours) without being released to the extracellular fluid. In addition, renin stored in mast cell granules would only be released after exposure to a mast cell degranulator.

To investigate these possibilities, in view of the discrepant findings in HMC-1 cells, LAD2 cells, and fresh mast cells, we quantified cardiac renin after perfusing the rat heart with a renin-free buffer for several hours or after exposure of the heart to the mast cell degranulator 48/80. When expressed per gram of wet weight, nonbuffer-perfused rat hearts contained 

Figure 5. Concentration-dependent inhibition of the Ang I–generating activity by aliskiren in heart homogenates (left) and the cardiac renin levels calculated from these curves (right). Top, control rats; bottom, MI rats. Data represent nonbuffer-perfused rat hearts (0'), and hearts that had been buffer perfused for 30 minutes (30'), 120 minutes (120'), or 30 minutes followed by exposure to compound 48/80 (30' + 48/80). Data are mean ± SEM of 4 to 8 experiments. *P<0.05 vs 0', #P<0.10 vs 0'.

Figure 6. Mast cells (*) stained with toluidine blue in transversal sections of the cardiac left ventricle obtained from a control rat (left) and a rat that underwent an MI 14 days before the removal of the heart (right). Hearts were perfused with Krebs-Henseleit buffer for 30 minutes and fixed in 3.5% to 4.0% formaldehyde solution. Scale bar: 25 μm.
renin in quantities that were of the same order of magnitude as the levels per milliliter of blood plasma. This is identical to the situation in porcine and human hearts. In our experiments, Ang I–generating activity was measured after acidification of the cardiac homogenates. This procedure effectively removes angiotensinases but also activates prorenin. We did not attempt to measure Ang I–generating activity without the acidification step, and, thus, the cardiac renin in the present study represents the sum of renin and prorenin. However, previous studies have already indicated that the increase in Ang I–generating activity of cardiac homogenates after acidification is marginal or absent, thereby implying that the heart contains predominantly renin and virtually no prorenin. Approximately 25% to 30% of the Ang I–generating activity in the cardiac homogenates could not be inhibited by aliskiren. Thus, nonrenin enzymes like cathepsin D also contributed to the Ang I–generating activity in the homogenized rat heart. This has been noted before, but does not necessarily imply that such nonrenin enzymes generate Ang I under in vivo circumstances.

As expected, within 30 minutes after the start of buffer perfusion, >70% of cardiac renin (ie, aliskiren-inhibitable, Ang I–generating activity) disappeared. Longer perfusion did not decrease renin further, and exposure to 48/80, at quantities that were shown previously to release Ang I–generating activity in the coronary effluent of the isolated perfused guinea pig heart, neither decreased the cardiac renin content nor resulted in the appearance of detectable renin quantities in the coronary effluent. The cardiac renin content after 30 minutes of buffer perfusion amounted to 2 ng of Ang I per gram of wet weight per hour. At an estimated rat heart weight of 1.5 g, a coronary flow of 5 mL/min, and provided that the effluent in our assay was concentrated 10 times, this should have resulted in a coronary effluent renin level of 10×(2.0 × 1.5)/(5.0 × 0.5) = 1.2 ng of Ang I per milliliter per hour, had all of the cardiac renin been released within 5 minutes after the exposure of the heart to 48/80. Even if only 10% of this cardiac renin had been released, the renin level in the coronary perfusate would still have been above the detection limit of our assay. Because this was not the case, we conclude that the remaining cardiac renin was neither located in a compartment accessible to 48/80 (ie, storage granules) nor in a compartment that allowed further washout. It might have been present in cardiac cells, eg, after binding to and internalization by M6PRs. This finding, in combination with the rapid washout of angiotensinogen from cardiac tissue sites (half-time: <1 minute), strongly argues against the possibility of mast cell renin contributing to Ang I generation in the isolated rat heart. Such local Ang generation has been proposed in the isolated, perfused guinea pig and mouse hearts after ischemia and reperfusion (another condition resulting in mast cell degranulation), although actual measurements of Ang I and II under these conditions were not performed.

Importantly, 2 weeks after an MI, when mast cells have accumulated at cardiac tissue sites (Figure 6), the findings on cardiac renin were identical to those in control hearts. If anything, renin tended to be lower in MI hearts, and, again, 48/80 did not induce renin release or lower cardiac renin. Nevertheless, the 48/80-induced coronary flow reduction was much stronger in infarcted hearts, suggesting that a factor other than renin was massively released from mast cells in infarcted hearts, eg, serotonin. However, the identity of this factor was not investigated in the present study.

Perspectives

HMC-1 cells synthesize renin and prorenin, but this is not a uniform property of all human mast cells. Evidence to support the idea that mast cell–derived renin contributes to cardiac Ang production in humans is currently lacking but seems unlikely in view of the current study and previous measurements in human heart tissue. The present study also does not support such contribution in the infarcted rat heart, although such hearts display an enhanced mast cell content. This finding opposes observations on mast cell–derived, renin-synthesizing Ang I in buffer-perfused guinea pig and mouse hearts after ischemia and reperfusion. A further complicating factor in the isolated perfused rat heart to allow such production would have been the low or undetectable levels of angiotensinogen at cardiac tissue sites after buffer perfusion (≈0.4 pmol/mL in interstitial fluid after 30 minutes of buffer perfusion, ie, 3 orders of magnitude below the levels in blood plasma). Using the following equation:

\[ V = V_{\text{max}} \times \frac{[S]}{K_m + [S]}, \]

where \( V_{\text{max}} \) = a Michaelis constant of 2400 pmol/mL, and \([S] = 0.4 \text{ pmol/mL}, it can be calculated that the maximum degree of Ang I generation (under the unlikely condition that all of the cardiac renin had access to angiotensinogen) after 30 minutes is 0.3 pg of Ang I per gram per hour. This is far below the range required to result in physiologically relevant Ang concentrations. Clearly, therefore, the role of mast cell–derived renin is unique for the guinea pig and mouse hearts and cannot be translated to the rat heart. In addition, the importance of mast cell–derived renin, if any, in humans needs to be reconsidered.

Disclosures

None.

References

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