Angiotensin Converting Enzyme and Chymase Activity in the Feline Heart and Serum

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(Received 24 February 2003/Accepted 16 June 2003)

ABSTRACT. The feline cardiac and serum angiotensin converting enzyme (ACE) and chymase activities were determined and compared in dogs, and hamsters. In all three species, cardiac chymase activity exceeded ACE activity; however, there were some differences. In cats, left ventricular ACE and chymase activities (0.15 ± 0.01 and 0.59 ± 0.1 mlU/mg-protein, respectively) were lower than in dogs (0.42 ± 0.05; p<0.01 and 2.0 ± 0.4 mlU/mg-protein; p<0.01) and hamsters (0.93 ± 0.06; p<0.001 and 2.1 ± 0.2 mlU/mg-protein; p<0.01); in contrast, serum ACE activities was higher in cats (12.7 ± 1.0 mlU/ml) than in dogs (5.9 ± 0.6 mlU/ml; p<0.001). The relative contribution of chymase (cats: 84.0 ± 5.1%, dogs: 81.4 ± 3.4%, and hamsters: 72.6 ± 5.6%) to ANG-II formation in the heart was greater than that of ACE in these animals (cats: 10.9 ± 4.1%, dogs: 11.5 ± 3.6%, and hamsters: 17.2 ± 0.8%). These species-specific differences suggest that the efficacy of renin-angiotensin system modulating agents may differ among species.

KEY WORDS: angiotensin converting enzyme inhibitor, angiotensin II type-1 receptor blocker, canine.


Angiotensin converting enzyme (ACE) inhibitors are used to treat severe heart failure in dogs and cats [18, 26]. Since there are multiple angiotensin II (ANG II) forming pathways in cardiac tissue, ACE inhibitors fail to inhibit completely the conversion of angiotensin I (ANG I) to ANG II in the heart of dogs [30]. In particular, chymostatin-sensitive angiotensin-generating enzyme, or chymase, a serine protease with extremely high affinity for ANG I, was recently identified in the heart and vascular tissues of humans [32], dogs [9], baboons [13], and hamsters [22], but not in rats [2]. ACE is bound to the membranes of endothelial cells [17], whereas chymase is stored in vesicles in the intracellular compartments of mast cells and other interstitial cells in the heart [32]. It has been reported that chymase-induced ANG-II formation in the heart is dominant in the dog, hamster, baboon, and human [1]. However, there are no reports on the predominance of ACE- versus chymase-induced ANG-II formation in the heart tissues of cats.

The objective of this study was to clarify the ANG II-forming capacity of ACE and chymase in the cat heart, and to compare it with that of dogs and hamsters. Although ACE inhibitors are considered essential for treatment of heart failure, they fail to inhibit chymase. Angiotensin II type-1 receptor blockers (ARB) antagonize angiotensin II generated by ACE and chymase, which might result in greater restrain of the renin-angiotensin system. Therefore, it is important to understand the renin-angiotensin system in the heart to evaluate the efficacy of those agents in heart failure.

The Institutional Laboratory Animal Care and Use Committee of The School of Veterinary Medicine and Animal Science of Kitasato University approved this study.

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Six mature mongrel dogs (7.5–11 kg) and seven mature mongrel cats (2.5–3.3 kg) of either sex were used in these experiments. Routine laboratory tests, including blood tests and urinalysis, confirmed that the animals were clinically healthy. They were housed in individual cages and fed commercial dry food (p/d, Hill’s Colgate Japan, Tokyo, Japan) twice a day. Water was given ad libitum. Seven twelve-week-old male Syrian hamsters weighting 90–110 g were also used in this study. The hamsters were housed in a cage in a climate-controlled room (24°C) in the animal care unit, on a 12-hr light-dark cycle, and fed pellets. They were also given water ad libitum. After being euthanatized with pentobarbital, samples of the free left ventricular (LV) wall were obtained from the animals. The tissues were cut into small blocks, immediately frozen in liquid nitrogen, and stored at –80°C until the biochemical assays.

Chymase activity in the homogenate of the LV was determined according to Dell’Italia et al. [9] and Baleells et al. [2]. After the LV samples had been thawed and weighed, they were minced and homogenized using a Polytron homogenizer (T25 basic, IKA-WERKE GMBH, Staufen, Germany) in 10 vol (w/v) of 20 mM sodium phosphate buffer, pH 8.3 at 4°C. The homogenate was centrifuged at 42,000 g for 30 min at 4°C, and this procedure was repeated twice. The final tissue pellets were resuspended in 100 mM phosphate buffer, pH 8.3, containing 300 mM NaCl, 500 mM KCl, and 0.1% TritonX-100. The protein concentration was determined according to Lowry et al. [19], using bovine serum albumin as a standard. All chemical materials were purchased from Sigma Chemical, St. Louis, U.S.A.

The chymase activity was measured using ANG I as the substrate. For the ANG-II-forming chymase activity assays, the samples were pre-incubated for 30 min at room temperature with an inhibitor solution contained 5 mM EDTA, 100 µM captopril, 1 mM o-phenanthrolone, and 20 µM aprotinin with or without 100 µM chymostatin. The samples were
then incubated for 60 min at 37°C with 600 µM ANG II. The reactions were terminated by the addition of ice-cold ethanol in a 1:3 vol/vol sample-to-ethanol ratio. After centrifuging the reaction mixture at 12,000 g for 10 min, the supernatant was transferred to a new tube and evaporated to dryness. The residue was resuspended in 100 µl distilled water.

The ANG II generated was quantified using high-performance liquid chromatography with a C18 silica reversed phase column (YMC-Pack Polymer C18, YMC, Kyoto, Japan). The peak area corresponding to a synthetic ANG II standard was integrated to calculate absolute ANG-II formation. Chymase activity was defined as chymostatin-inhibitable ANG-II formation, and 1 unit resulted in the formation of 1 µmol of ANG II per minute. This assay inhibits other known ANG-II-forming enzymes, including ACE, cathepsin G, kallikrein, chymotrypsin, trypsin, and carboxypeptidases, by >93% [9, 17].

ACE activity in the LV and serum was determined according to Danser et al. [8], and measured in duplicate with a commercial kit (ACE color, Fujirebio, Tokyo, Japan) containing p-hydroxybenzoyl-glycyl-L-histidyl-L-leucine as a synthetic substrate. Multiple dilutions of the homogenate were incubated for 1 hr with 10 mM substrate at 37°C and pH 8.3. As controls, the homogenate was incubated with substrate in the presence of 3 mM disodium-EDTA. After one hour, the reaction of ACE with the synthetic substrate was stopped by the addition of stopper/developer solution containing 3 mM disodium-EDTA. The tubes were mixed, incubated at 37°C for 10 min to allow formation of the index color from the converted substrate, and centrifuged at 3,000 g for 5 min at room temperature. The absorbance at 505 nm (UV-1200, Shimazu, Kyoto, Japan) was then read.

All the data are given as the mean ± SE. Analysis by one-way ANOVA was used, followed by post hoc testing (Tukey’s test), to compare the chymase and ACE activities in the same organ from the same species. A value of $p<0.05$ was considered statistically significant.

The total ANG-II-forming activity in cats (0.7 mU/mg, n=7) was lower than in dogs (2.4 mU/mg, n=6, $p<0.001$) or hamsters (2.6 mU/mg, n=7, $p<0.001$). The ACE activity in the LV of cats was 0.15 ± 0.01 mU/mg-protein (n=7), which was lower than in dogs (0.42 ± 0.05 mU/mg-protein, n=6, $p<0.01$) and hamsters (0.93 ± 0.06 mU/mg-protein, n=7, $p<0.001$) (Fig. 1, left). The ACE activity in the LV in these animals was lower than that of the serum. The serum ACE activity of cats (12.7 ± 1.0 mU/ml, n=7) was lower than in hamsters (16.8 ± 0.7 mU/ml, n=7, $p<0.001$), but it was higher than in dogs (5.9 ± 0.6 mU/ml, n=6, $p<0.001$) (Fig. 1, right). The chymase activity in the LV of cats (0.59 ± 0.1 mU/mg-protein, n=7) was lower than in dogs (2.0 ± 0.4 mU/mg-protein, n=6, $p<0.01$) and hamsters (2.1 ± 0.2 mU/mg-protein, n=7, $p<0.01$) (Fig. 2, hatched columns). The relative contribution of chymase to ANG-II formation in the heart was greater than that of ACE in dogs (81.4 ± 3.4 and 11.5 ± 3.6%, respectively), hamsters (72.6 ± 5.6 and 17.2 ± 0.8%, respectively), and cats (84.0 ± 5.1 and 10.9 ± 4.1%, respectively).

ACE inhibitors are now widely used as first-line drugs in the treatment of congestive heart failure in humans [23], dogs [7, 14], and cats [26]. In humans, ACE inhibitor therapy favorably affects cardiocyte remodeling, and reduces

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**Fig. 1.** Angiotensin converting enzyme (ACE) activity in the left ventricular (LV) and serum of dogs, hamsters, and cats. Each bar represents the mean ± SE. * is $p<0.01$. ** is $p<0.001$.

**Fig. 2.** Chymase activities in the left ventricle (LV) of dogs, hamsters, and cats. Each bar represents the mean ± SE. * is $p<0.01$. ** is $p<0.001$. 

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mortality in patients with severe chronic heart failure [6, 11, 29]. ACE inhibitors mainly inhibit circulatory and endothelial cell membrane-bound ACE [16]; however, they may fail to completely suppress the renin-angiotensin system in heart failure, due to the so-called ‘escape phenomenon’ [3, 4]. It has been suggested that ANG II formed by chymase, which is insensitive to ACE inhibitors, is one limitation of ACE inhibitor treatment [32]. Since circulatory chymase activity has not been detected [20], LV tissue chymase may play a role in cardiac cell remodeling [27, 28]. Recently, specific suppressors of the renin-angiotensin system, such as renin inhibitors and ARB, have been developed [5, 25]. However, there are no significant differences in the mortality, sudden death, or resuscitated arrest rates in ACE inhibitor and ARB treatment groups [24]. By contrast, Dell’Italia et al. [10] showed increased chymase activity in the LV with volume overload in dogs, but not with pressure overload in a two-kidney-one-clip model in baboons [13] and hamsters [15]. These reports suggest that the regulation of LV tissue and the circulatory renin-angiotensin system differ with cardiac loading conditions. Therefore, the efficacy of renin-angiotensin system modulating agents, such as ACE inhibitors, ARB, etc., might also differ with cardiac loading conditions, which affect the renin-angiotensin system.

In cats, chymase (84%) dominated cardiac ANG-II generation compared to ACE (11%). Chymase was also dominant in dogs (81%) and hamsters (73%). However, total ANG-II formation in the LV was lower in cats than in dogs or hamsters. The serum ACE activity was higher in cats than in dogs. These results suggest that the role of the renin-angiotensin system in the circulation and LV tissues, which affects blood pressure and cell remodeling, differs in cats and dogs. If the circulatory renin-angiotensin system were more important in cats, perhaps ACE-inhibitor treatment would be more beneficial in cats than in dogs. However, since the acute hypotensive effect of ACE inhibitors is equivalent in dogs [12, 21] and cats [31], a comparative study of the chronic effects of ACE inhibitors is required.

In conclusion, ANG-II formation by chymase in the LV predominates in cats, dogs, and hamsters, although less total ANG-II formation in the LV was observed in cats. These results suggest that the role of the renin-angiotensin system in circulation and tissues differs in these animals, therefore that the efficacies of ACE inhibitors and ARB may also differ with species.

ACKNOWLEDGMENTS. This study was supported in part by a Grant-in-Aid for General Scientific Research (C-11836011 and C-12833009) from the Japanese Ministry of Education, Science and Culture. Grant for Scientific Research on Priority Area from Kitasato University School of Veterinary Medicine and Animal Sciences. Dr. Kutushige Nakano is much appreciated for supporting this study.

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