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Mast Cell Degranulation Increases Mouse Mast Cell Protease 4–Dependent Vasopressor Responses to Big Endothelin-1 But Not Angiotensin I[S]

Laurence Vincent, Catherine Lapointe, Modou Lo, Hugo Gagnon, Gunnar Pejler, Shinji Takai, Robert Day, and Pedro D'Orléans-Juste

Department of Pharmacology and Physiology, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, Sherbrooke, Quebec, Canada (L.V., C.L., M.L., P.D.-J.); PhenoSwitch Bioscience Inc., Sherbrooke, Quebec, Canada (H.G.); Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden (G.P.); Department of Innovative Medicine, Osaka Medical College, Osaka, Japan (S.T.); and Department of Surgery, Division of Urology, Université de Sherbrooke, Sherbrooke, Quebec, Canada (R.D.)

ABSTRACT

Mouse mast cell protease 4 (mMCP-4), the murine functional analog to the human chymase, is a serine protease synthesized and stored in mast cell secretory granules. Our previous studies reported physiologic and pathologic roles for mMCP-4 in the maturation and synthesis of the vasoactive peptide endothelin-1 (ET-1) from its precursor, big ET-1. The aim of this study was to investigate the impact of mast cell degranulation or stabilization on mMCP-4–dependent pressor responses after the administration of big ET-1 or angiotensin I (Ang I). In anesthetized mice, mast cell degranulation induced by compound 48/80 (C48/80) or stabilization by cromolyn enhanced or repressed, respectively, the dose-dependent vasopressor responses to big ET-1 in wild-type (WT) mice but not in mMCP-4 knockout mice in a chymase inhibitor (TY-51469)–sensitive fashion. In addition, mMCP-4–dependent hydrolysis of the fluorogenic substrate Suc-Leu-Leu-Val-Tyr–7-amino-4-methylcoumarin was depleted or enhanced in peritoneal mast cells isolated from mice pretreated with C48/80 or cromolyn, respectively. Furthermore, C48/80 or cromolyn markedly increased or abolished, respectively, ET-1 (1–31) conversion from exogenous big ET-1 in WT mice peritoneal fluid–isolated mast cells, in vitro. Finally, the vasopressor responses to Ang I were unaffected by mast cell activation or stabilization, whereas those induced by the angiotensin-converting enzyme-resistant Ang I analog, [Pro[11], D-Ala[12]] Ang I, were potentiated by C48/80. Altogether, the present study shows that mast cell activation enhances the mMCP-4–dependent vasoactive properties of big ET-1 but not Ang I in the mouse model.

SIGNIFICANCE STATEMENT

The current work demonstrates a significant role for mast cell stability in the cardiovascular pharmacology of big endothelin-1 but not angiotensin I in the murine systemic circulation.

Introduction

Upon activation, mast cells undergo a biphasic secretory response involving the rapid release of prestored vasoactive mediators followed by subsequent release of de novo synthesized products, significantly modulating several physiologic and pathologic pathways (Blank, 2011). Secretion of the serine protease chymase, a major component of the mast cell secretory granules, is notably involved in the maturation of vasoactive factors such as angiotensin II (Ang II) and endothelin-1 (ET-1) (Nakano et al., 1997; Fleming, 2006), both acting in the regulation of the vascular flow and hemodynamic balance (Rossi et al., 1999).

Comparative studies in human, hamster, rat, rabbit, dog, pig, and marmoset tissues reported remarkable variations in the Ang II–forming pathways among several organs in vitro, suggesting that the chymase contribution to local Ang II generation could be species-dependent (Balcells et al., 1997; Akasu et al., 1998; Kunori et al., 2005). By adopting an in vitro mouse model, it was shown that, under physiologic conditions, angiotensin-converting enzyme (ACE) is the major Ang II–forming dipeptidyl-carboxypeptidase (Company et al., 2011). Ang II can, however, under inflammatory conditions, also be generated via mast cell activation by release of the extravascular mouse mast cell protease 4 (mMCP-4) (Company et al., 2011), the closest mouse analog to human chymase (Pejler et al., 2010). Notably, though, whether mast cell activation

ABBREVIATIONS: ACE, angiotensin-converting enzyme; AMC, 7-amino-4-methylcoumarin; Ang I, angiotensin I; Ang II, angiotensin II; BSA, bovine serum albumin; C48/80, compound 48/80; ECE, endothelin-converting enzyme; ET-1, endothelin-1; HR, heart rate; KO, knockout; MAP, mean arterial pressure; mMCP-4, mouse mast cell protease 4; NEP, neprilysin; rmMCP-4, recombinant mMCP-4; WT, wild type.

and secretion of mMCP-4 can modulate the response of the cardiovascular system to angiotensin I (Ang I) in vivo remains to be determined.

The endothelin-converting enzyme (ECE) is located on endothelial cells membrane but also on a wide variety of cell types such as vascular smooth muscle cells, macrophages, and neurons (Davenport et al., 2016). Such cells produce ET-1 through ECE-mediated conversion of big ET-1 (McMahon et al., 1991; Xu et al., 1994). An alternate pathway of ET-1 generation involves mast cell chymase, through the production of an inactive intermediate, ET-1 (1–31), as shown in human tracheal smooth muscle cells (Nakano et al., 1997). ET-1 (1–31) is subsequently cleaved by the neprilysin (NEP) to form the vasoactive peptide ET-1 (1–21), which is a prerequisite to exert its pressor properties in vivo (Fecteau et al., 2005). We have previously shown a 40% reduction in the pressor responses induced by the administration of big ET-1 in mice genetically deficient for mMCP-4 (mMCP-4 KO) (Tchougounova et al., 2003), demonstrating a dynamic role for chymase in the generation of ET-1 in vivo (Houde et al., 2013). Although mMCP-4 metabolizes big ET-1 in vivo and Ang I in vitro (Fecteau et al., 2005; Fleming, 2006), the impact of acute mast cell activation or stabilization on the hydrolytic properties of mMCP-4 in vivo remains uninvestigated. We suggest that mast cell degranulation enhances mMCP-4–dependent cardiovascular responses to big ET-1 in the mouse model in vivo.

This study therefore investigated the influence of mast cell degranulation on the chymase-dependent pressor responses to big ET-1 or Ang I in wild-type (WT) or mMCP-4 KO mice. These agonists were tested in mice treated with the mast cell degranulating agent compound 48/80 (C48/80) (Irman-Florjanc and Erjavec, 1983) or the mast cell stabilizer cromolyn (Samoszuk and Corwin, 2003) and when applicable with the chymase inhibitor TY-51469 (Koide et al., 2003). Finally, the contribution of degranulated mMCP-4 on the pressor responses to [Pro[11], D-Ala[12]] Ang I, an ACE-resistant analog (McDonald et al., 2001) was also investigated in vivo.

Our study shows that modulation of mast cell activation significantly impacts the hemodynamic responses to big ET-1 but not Ang I.

Methods

Animals. C57Bl/6 mice genitors were purchased from Charles River Canada (Montreal, QC, Canada) and housed in our facilities. Genitor mMCP-4 KO mice were provided by Dr Gunnar Pejler (Uppsala University, Sweden) and bred in our facility. All animals (male mice, 8–12 weeks old) were kept at constant room temperature (23°C) and humidity (78%) under a controlled 12-hour light/dark cycle, with standard chow and tap water ad libitum. Animal care and experimentations were approved by the Ethics Committee on Animal Research of the University of Sherbrooke following the Canadian Council on Animal Care guidelines and the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health.

Mice were anesthetized by the intramuscular administration of ketamine/xylazine (87/13 mg/kg). The intraperitoneal administration of vehicle, C48/80 (1 mg/kg) or cromolyn (50 mg/kg), 5 or 30 minutes, respectively, prior to further experimentation, was performed (Röhlich et al., 1971; Skedinger et al., 1987; Tariq et al., 2006).

Hemodynamic Monitoring in Anesthetized Mice. The method was performed as previously described (Houde et al., 2013). Anesthetized mice were cannulated with a polyethylene catheter via the left jugular vein for intravenous administration and the right carotid artery

for hemodynamic recordings using the Blood Pressure Analyzer 200A (Digi-Med, Louisville, KY). After the surgery, the mice were allowed a 15-minute stabilization period before the intravenous injection of either big ET-1 (Phoenix Pharmaceuticals Inc., Burlingame, CA), ET-1 (1–31) (Peptide Institute, Osaka, Japan), ET-1 (Enzo Life Sciences Inc. Farmingdale, NY), Ang I (TOCRIS Bioscience, Ellisville, MO), or [Pro[11], D-Ala[12]] Ang I (American Peptide Company; Sunnyvale, CA) with doses ranging from 0.001 nmol/kg to 10 nmol/kg (D'Orléans-Juste et al., 2008). Each mouse was administered a unique dose, and blood pressure parameters were continuously monitored for a maximum of 20 minutes before the mice were sacrificed by cervical dislocation. All data were analyzed using the DMSI-400 System Integrator software (System Integrator; Digi-Med). Blood pressure variations (Δ) obtained after the injection of each vasopressor were calculated as the difference between blood pressure increases postinjection and basal pressure recorded prior to administration of each agent. Recording of heart rate (HR) was initiated at the time of agonist administration. In another series of experiments, the specific chymase inhibitor TY-51469 (10 mg/kg) (Toa Eiyo limited, Osaka, Japan) was administered intravenously 20 minutes before drug administration.

Peritoneal Mast Cells Isolation. Mast cells from the peritoneal cavity were isolated from vehicle, C48/80 or cromolyn pretreated mice (as described above) and used for measurement of chymase activity as previously reported (Semaan et al., 2015). Mice anesthetized with ketamine/xylazine (87/13 mg/kg, intramuscular) were injected with 5 ml of isolation buffer (PBS pH 7.4 containing 1 mg/ml of bovine serum albumin (BSA) and 0.5 mg/ml of heparin) in the peritoneal cavity. The peritoneal fluids were collected after a 1-minute abdominal massage and centrifuged at 200g for 5 minutes. Pellets were suspended in 1 ml of RPMI-1640 medium containing 2 mM of L-glutamine, 100 U/ml of penicillin, and 1 mg/ml of BSA and subsequently incubated for 1 hour at 37°C in tissue culture dishes 35 × 10 mm to sediment and allow adherence to the plastic surface of the dish the macrophages and leukocytes, thus permitting a purification of the mast cells. RPMI-1640 medium and nonadherent cells were collected and centrifuged at 200g for 5 minutes. Pellets were suspended in 1 ml of isolation buffer, and cells were counted using a hemocytometer (zoom in to 40× magnification) according to the Moore and James method with toluidine blue (0.038%) (Moore and James, 1953). A final centrifugation was performed at 200g for 5 minutes, and pellets were suspended in PBS (pH 8) at a concentration of 1×10^4 cells/µl.

Activation of the Recombinant Enzymes. Recombinant mMCP-4 was expressed in S2 *Drosophila* cells and purified as previously described (Semaan et al., 2015). The recombinant enzymes were thawed and diluted to a concentration of 20 µg/ml in maturation buffer (50 mM 2-(N-morpholino)ethanesulfonic acid (MES), 1 mg/ml BSA, pH 5.5). Active murine cathepsin C (R&D Systems, Minneapolis, MN) was diluted to 20 µg/ml in cathepsin C buffer (50 mM MES, 50 mM NaCl, 5 mM Dithiothreitol (DTT), pH 5.5). Activation was performed by adding equal volumes of recombinant chymase and cathepsin C, adding 50 µg/ml heparin, and incubating 1 hour at room temperature. mMCP-4 activation was stopped with N-Ethylmaleimide (NEM) (3 mM) and diluted with assay buffer (20 mM Tris, 200 µM KCl, 0.02% Triton X-100, pH 9.0) to bring the recombinant enzyme concentration to 2 or 5 µg/ml, and 5 minutes was allowed to completely stop the cathepsin C–dependent reaction.

Measurement of Chymase Enzymatic Activity. Activated recombinant mMCP-4 (rmMCP-4) at concentrations of 30.85 nM (100 ng/well) and 154.32 nM (500 ng/well), in absence or presence of C48/80 (12 µM), cromolyn (600 µM) or TY-51469 (10 µM), or mast cells (1×10^5 cells), were incubated in 96 well plates with the fluorogenic substrate, Suc-Leu-Leu-Val-Tyr–7-amino-4-methylcoumarin (AMC) (10 µM) (Peptide Institute inc., Osaka, Japan). The fluorescence of the released AMC molecule, as chymotrypsin-like activity, was then measured in duplicate with an Infinite M1000 spectrophotometer (Tecan Group Ltd., Männerdorf, Swiss) with $\lambda_{ex} = 370$ nm and $\lambda_{em} = 460$ nm, at 37°C for up to 20 minutes.

Mast Cell–Specific Conversion of Big ET-1 to ET-1 (1–31) In Vitro. Big ET-1 (15 μM) was incubated with 1×10^5 mast cells. Thiorphan (1 μM), an NEP inhibitor, was added to the reaction to block conversion of ET-1 (1–31) to ET-1. C48/80 (1 μM) or cromolyn (20 μM) was added to the medium and incubated for 20 minutes at 37°C. Formic acid (4%) was added to lower the pH and to completely stop the enzymatic reaction.

Peptide samples (200 μl per tube) were analyzed via liquid chromatography–tandem mass spectrometry analysis by PhenoSwitch Bioscience Inc. Each sample was loaded on Strata-X polymeric reversed-phase columns. Peptides were eluted twice with 350 μl 75% acetonitrile, 2% formic acid. Eluates were dried, samples were then suspended in 50 μl of H_2O with 2% formic acid, and 5 μl was injected on a Halo peptide C18 50 × 0.5 mm column eluted with water, 3% DMSO, 0.2% formic acid (mobile phase A) and acetonitrile, 3% DMSO, 0.2% formic acid (mobile phase B). The flow rate was 25 μl/min for the 2.5-minute duration, and gradients started at 10% mobile phase B. ET-1, ET-1 (1–31), and big ET-1 were monitored by product ion acquisition on a TTOF5600 from AbSciex.

Statistical Analysis. All quantitative results are presented as means ± S.E.M. All graphical and statistical analysis were conducted on GraphPad Prism 8 software (GraphPad Software, La Jolla, CA). Statistical significance was determined with Student's t test for comparisons of two samples or ANOVA with Dunnett's multiple comparison tests for three or more sample sets. Statistical significance was reached when the P value was below 0.05.

Results

No Differences in Basal Hemodynamic Parameters in WT and mMCP-4 KO Mice After the Intraperitoneal Administration of C48/80 or Cromolyn. Prior to drug injections, the basal hemodynamic parameters were measured in anesthetized mice treated with either vehicle, C48/80, or cromolyn. Mean, systolic and diastolic arterial pressure, HR, and arterial pulse pressure were similar in both groups regardless of the pharmacological agent administered (Supplemental Table 1).

Interfering with Mast Cell Stability Modulates the mMCP-4–Dependent Hemodynamic Responses to Big ET-1. The dose-dependent increases in mean arterial pressure
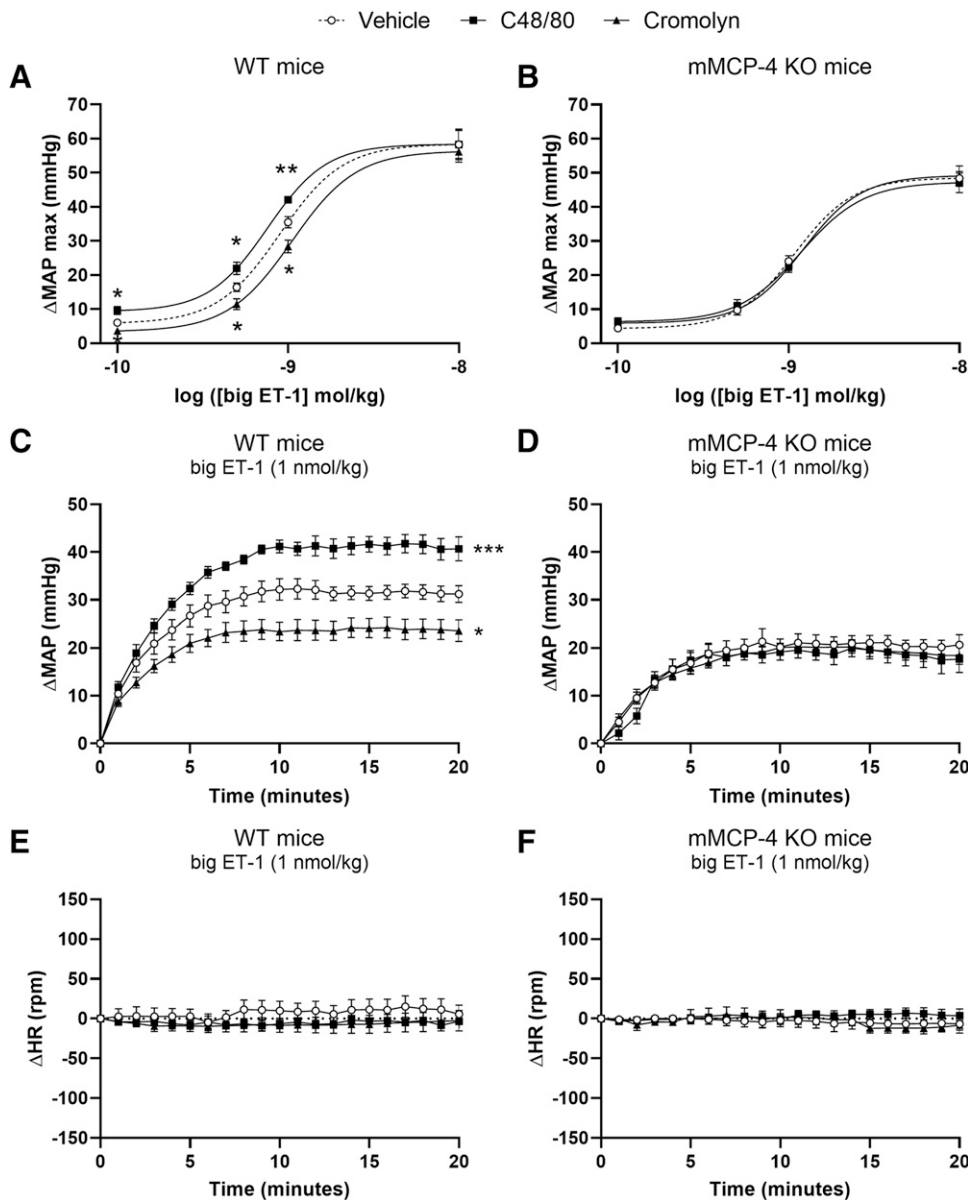
Fig. 1. Maximal variation in MAP after intravenous administration of big ET-1 in WT (A) and mMCP-4 KO (B) mice pretreated with either vehicle, C48/80 or cromolyn; time course of the ΔMAP in response to intravenous administration of a single dose of big ET-1 (1 nmol/kg) in WT (C) and mMCP-4 KO (D) mice pretreated with the above-mentioned agents, and their respective HR (E and F). Each point corresponds to the mean ± S.E.M. of the MAP increase (n = 6–10). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. WT + vehicle by one-way ANOVA with Dunnett's multiple comparison test.
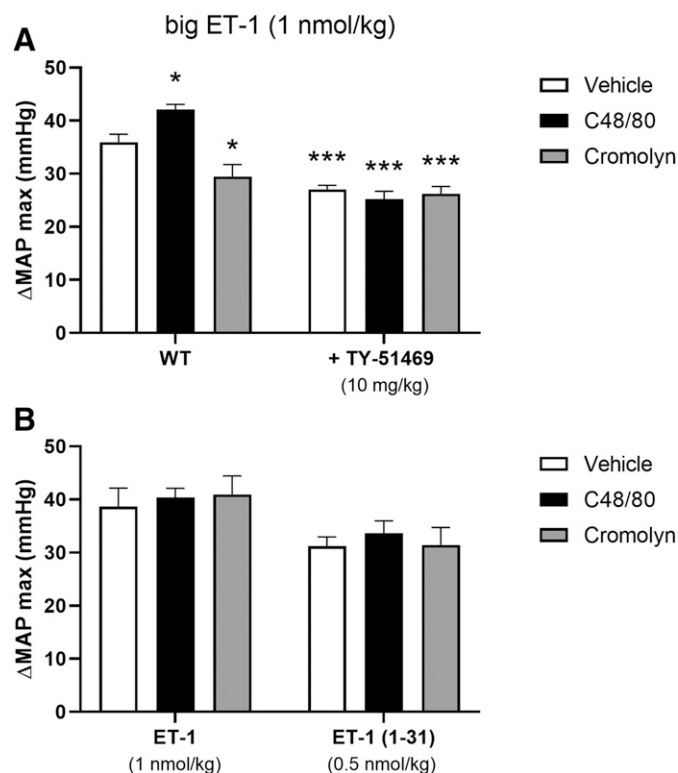
Fig. 2. (A) Maximal variation in MAP in response to big ET-1 (1 nmol/kg) in WT mice pretreated intravenously with vehicle or TY-51469 (10 mg/kg). (B) Maximal ΔMAP after intravenous administration of ET-1 (1–31) (1 nmol/kg) and ET-1 (0.5 nmol/kg) in WT mice. Each bar corresponds to the mean ± S.E.M. of the MAP increase (*n* = 5–9). *$P < 0.05$; ***$P < 0.001$ vs. WT + vehicle by one-way ANOVA with Dunnett's multiple comparison test.

(MAP) after bolus administrations (intravenous) of big ET-1 were compared in WT and mMCP-4 KO mice, treated intraperitoneally with C48/80 (1 mg/kg) or cromolyn (50 mg/kg). C48/80 potentiated the maximal increases of the MAP induced by exogenous big ET-1 at doses ranging from 0.01 to 1 nmol/kg ($P < 0.05$), with no variations of HR at all doses tested. In contrast, at the same doses, pressor responses to big ET-1 were markedly reduced in cromolyn-treated WT ($P < 0.05$) but not in mMCP-4 KO mice (Fig. 1, A and B). Figure 1, C and D, shows that the potentiating or repressing effects of C48/80 or cromolyn, respectively, occur throughout the sustained pressor responses to big ET-1 in WT mice. Finally, no effects of C48/80, cromolyn or mMCP-4 deficiency on HR in response to big ET-1 were observed (Fig. 1, E and F).

Interfering with Mast Cell Stability Does Not Influence the Hemodynamic Responses to Big ET-1 Metabolites ET-1 (1–31) or ET-1. A specific chymase inhibitor, TY-51469 (10 mg/kg, intravenous), suppressed the mMCP-4 contribution to the pressor responses to big ET-1 in WT mice ($P < 0.001$), regardless of induction of mast cell degranulation or stabilization (Fig. 2A). ET-1 (1–31) (1 nmol/kg) and ET-1 (0.5 nmol/kg) induced similar hemodynamics in WT mice treated with vehicle, C48/80, or cromolyn (Fig. 2B). Thus, mast cell stability plays no significant role in the vasoactive effects of the latter two metabolites.

C48/80 and Cromolyn Increase or Abolish, Respectively, Mast Cells Derived mMCP-4–Dependent Conversion of Big ET-1 to ET-1 (1–31). In a first series of specificity experiments, concentration-dependent chymase

activity of recombinant mMCP-4 was assessed (Supplemental Fig. 1). Furthermore, the latter serine protease activity was not modified by C48/80 or cromolyn yet abolished by TY-51469 (Fig. 3A).

The hydrolysis of the fluorogenic substrate was subsequently measured in peritoneal fluid–isolated mast cells from
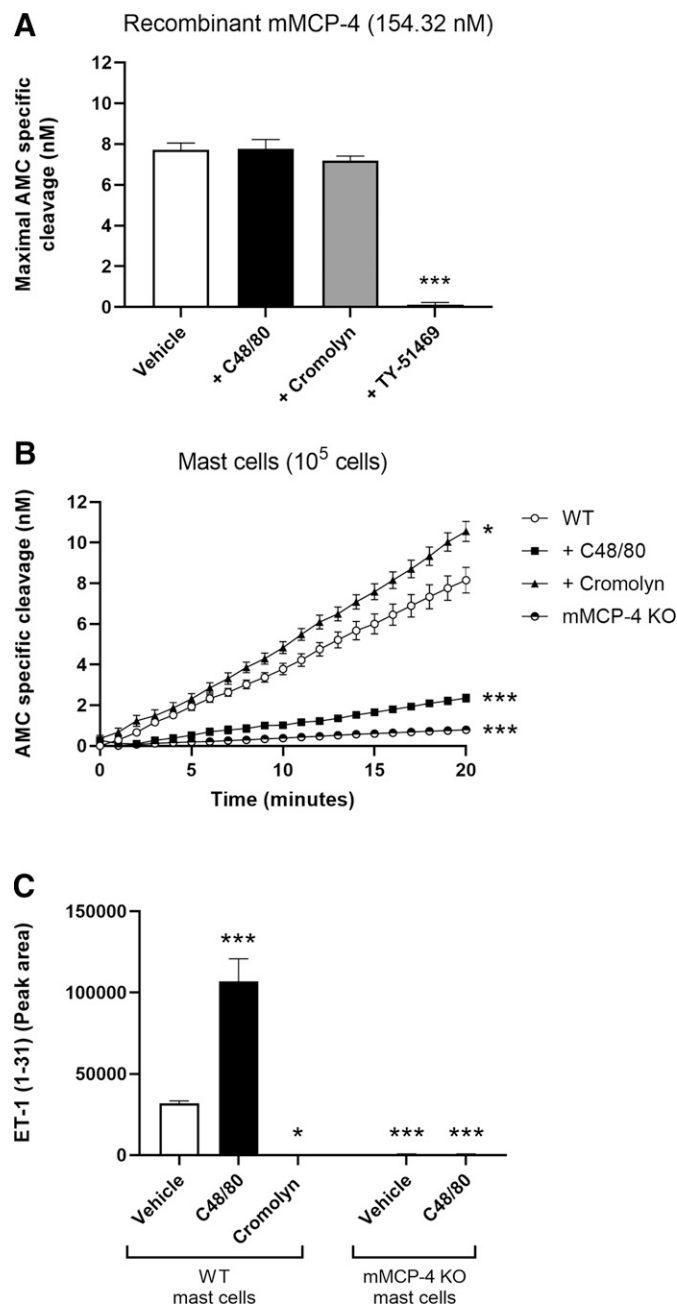

Fig. 3. (A) Quantification of the specific cleavage of the fluorogenic substrate Suc-Leu-Leu-Val-Tyr-AMC by rmMCP-4 (154.32 nM) in the presence of vehicle, C48/80 (12 μM), cromolyn (600 μM), or TY-51469 (10 μM) (*n* = 6). (B) Quantification of the specific AMC cleavage of the fluorogenic substrate measured ex vivo in peritoneal fluid-isolated mast cells derived from WT (pretreated with vehicle, C48/80 or cromolyn) or mMCP-4 KO mice (*n* = 6–10). (C) In vitro conversion of big ET-1 into ET-1 (1–31) from intact peritoneal mast cells derived from WT or mMCP-4 KO in presence of vehicle, C48/80 (1 μM) or cromolyn (20 μM) using mass spectrometry area under the curve arbitrary units (*n* = 4 to 5). Each point and bar correspond to the mean ± S.E.M. *$P < 0.05$; ***$P < 0.001$ vs. rmMCP-4 or WT + vehicle by one-way ANOVA with Dunnett's multiple comparison test.

WT and mMCP-4 KO mice pretreated with either vehicle, C48/80, or cromolyn. Figure 3B shows that WT but not mMCP-4 KO mast cells show hydrolytic activity and that C48/80 markedly reduced ($P < 0.001$) mast cells' chymase-like activity, suggesting a lower content in mMCP-4 post- degranulation. On the other hand, cromolyn produced an increase in hydrolytic activity ($P < 0.05$), suggesting a greater enzyme content in stabilized WT mast cells. We had previously reported that WT mouse peritoneal mast cells possess mMCP-4–dependent hydrolytic activity (Semaan et al., 2015; Desbiens et al., 2019). Worthy of notice, 1×10^5 isolated peritoneal mast cells were equivalent to a concentration of 150 nM of the recombinant mMCP-4 in terms of chymase-specific hydrolysis.

Finally, C48/80 and cromolyn, respectively, enhanced or abolished the production of ET-1 (1–31) from exogenous big ET-1 in mast cells isolated from WT mice peritoneal fluid ($P < 0.001$ and $P < 0.05$, respectively) (Fig. 3C). ET-1 (1–31) generation did not occur in peritoneal mast cells derived from mMCP-4 KO mice ($P < 0.001$) (Fig. 3C).

C48/80 or Cromolyn Do Not Affect Hemodynamic Responses to Ang I in Anesthetized Mice. The absence of mMCP-4–specific contributions in the generation of the potent vasopressor peptide Ang II is shown in Fig. 4. Ang I (0.001–0.5 nmol/kg) administered to either WT or mMCP-4 KO mice elicited similar vasopressor responses (Fig. 4A). Higher doses of Ang I triggered tachycardia in the anesthetized mouse model (data not shown). Figure 4, B and C, shows no effects of C48/80 or cromolyn on duration of the pressor responses to Ang I (0.01 nmol/kg), either in WT or mMCP-4 KO mice. Furthermore, no effects of C48/80, cromolyn or mMCP-4 deficiency on HR in response to Ang I were detected (Fig. 4, D and E).
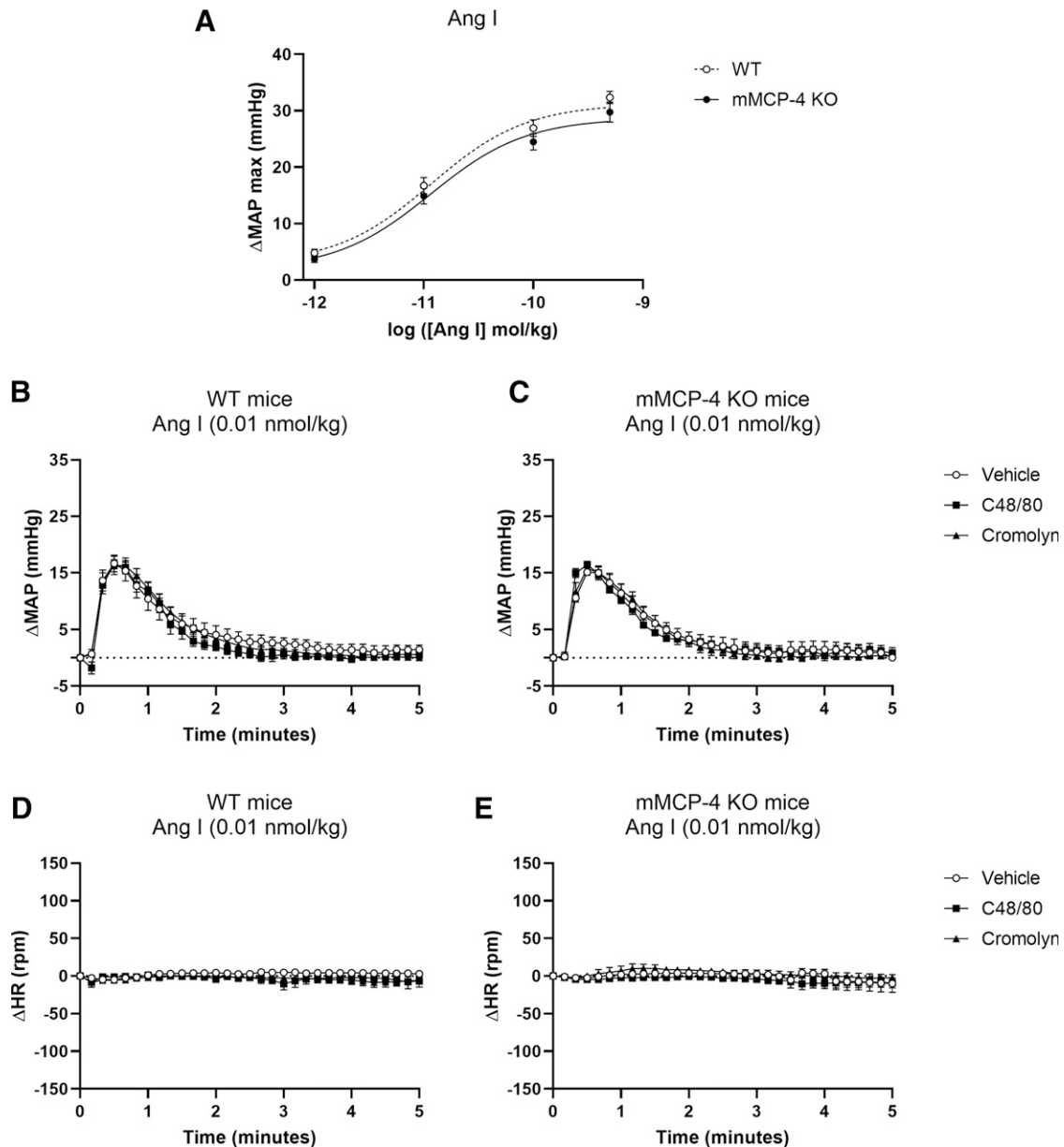
Fig. 4. (A) Maximal variation in MAP after the intravenous administration of Ang I in WT and mMCP-4 KO mice; time course of the variation of the ΔMAP in response to intravenous administration of Ang I (0.01 nmol/kg) in WT (B) and mMCP-4 KO mice (C) pretreated with vehicle, C48/80 or cromolyn, and their respective HR (D and E). Each point and bar correspond to the mean ± S.E.M. of the MAP increase of 6–8 separate experiments.

**Mast Cell Degranulation Modulates the mMCP-4–Dependent Hemodynamic Responses to [Pro[11], D-Ala[12]] Ang I.** In contrast to Ang I, administration of the ACE-resistant analog [Pro[11], D-Ala[12]] Ang I induced pressor responses with greater amplitude and duration in WT mice when compared to their mMCP-4 KO congeners ($P < 0.01$), unmasking a chymase-dependent contribution in the vasopressor responses to this particular peptide (Fig. 5, A–C), whereas similarly to Ang I, no significant effects on HR were observed with the analog.

In addition, in WT but not mMCP-4 KO mice, C48/80 potentiated by 2.5-fold ($P = 0.001$) (in terms of amplitude and duration) the pressor responses to [Pro[11], D-Ala[12]] Ang I (Fig. 6, A–C). As positive chronotropic response to the ACE-resistant analog of Ang I was also prompted by C48/80 (Fig. 6D) in WT but not in mMCP-4 KO mice (Fig. 6, C and E). Finally, cromolyn did not modify the

cardiovascular responses to [Pro[11], D-Ala[12]] Ang I in either mouse strains.

Discussion

Our previous studies have demonstrated a contribution of mast cell–secreted mMCP-4 in the ECE-independent production of ET-1 in mice (Fecteau et al., 2005; Simard et al., 2009; Houde et al., 2013). Until the present study, however, it had not been established whether mast cell stability impacts on the mMCP-4–dependent biologic responses to big ET-1 and Ang I in vivo. This study shows that induction of mast cell degranulation by C48/80 increases mMCP-4–dependent responses to exogenous big ET-1. In contrast, the mast cell stabilizer cromolyn reduced the biologic activity and chymase-dependent hydrolysis of big ET-1. Furthermore, mMCP-4 does not significantly contribute to the vasoactive
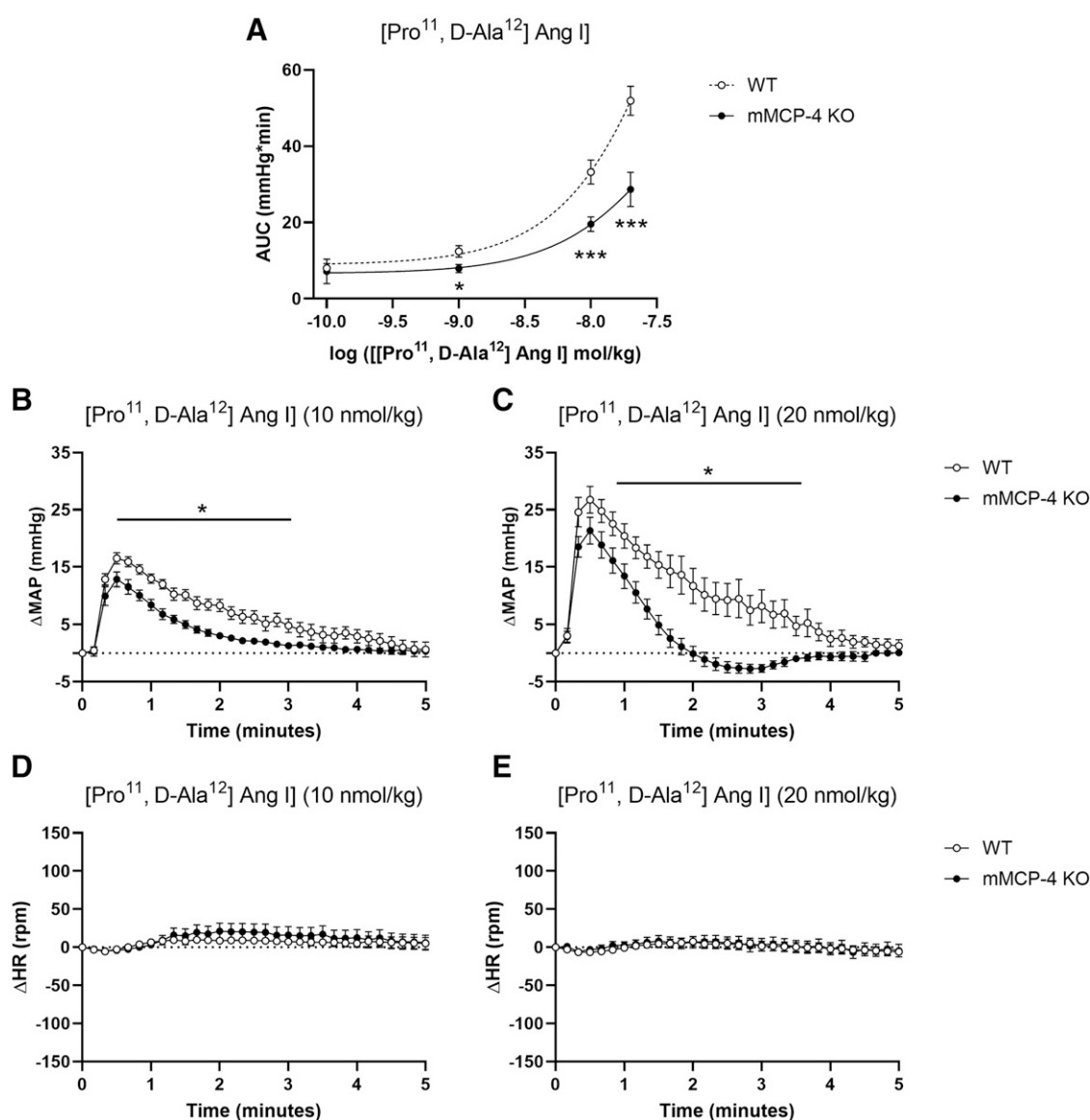
Fig. 5. (A) Dose-response curves [area under the curve (AUC)] of the ACE-resistant analog ([Pro[11], D-Ala[12]] Ang I) in WT and mMCP-4 KO mice ($n = 4$–9); time course of maximal variation in MAP in response to intravenous administration of [Pro[11], D-Ala[12]] Ang I [(B) 10 nmol/kg ($n = 8$–9); (C) 20 nmol/kg ($n = 9$)] in WT and mMCP-4 KO mice, and their respective HR (D and E). Each point corresponds to the mean ± S.E.M. of the MAP increase. *$P < 0.05$; ***$P < 0.001$ vs. WT + vehicle by Student's t test.
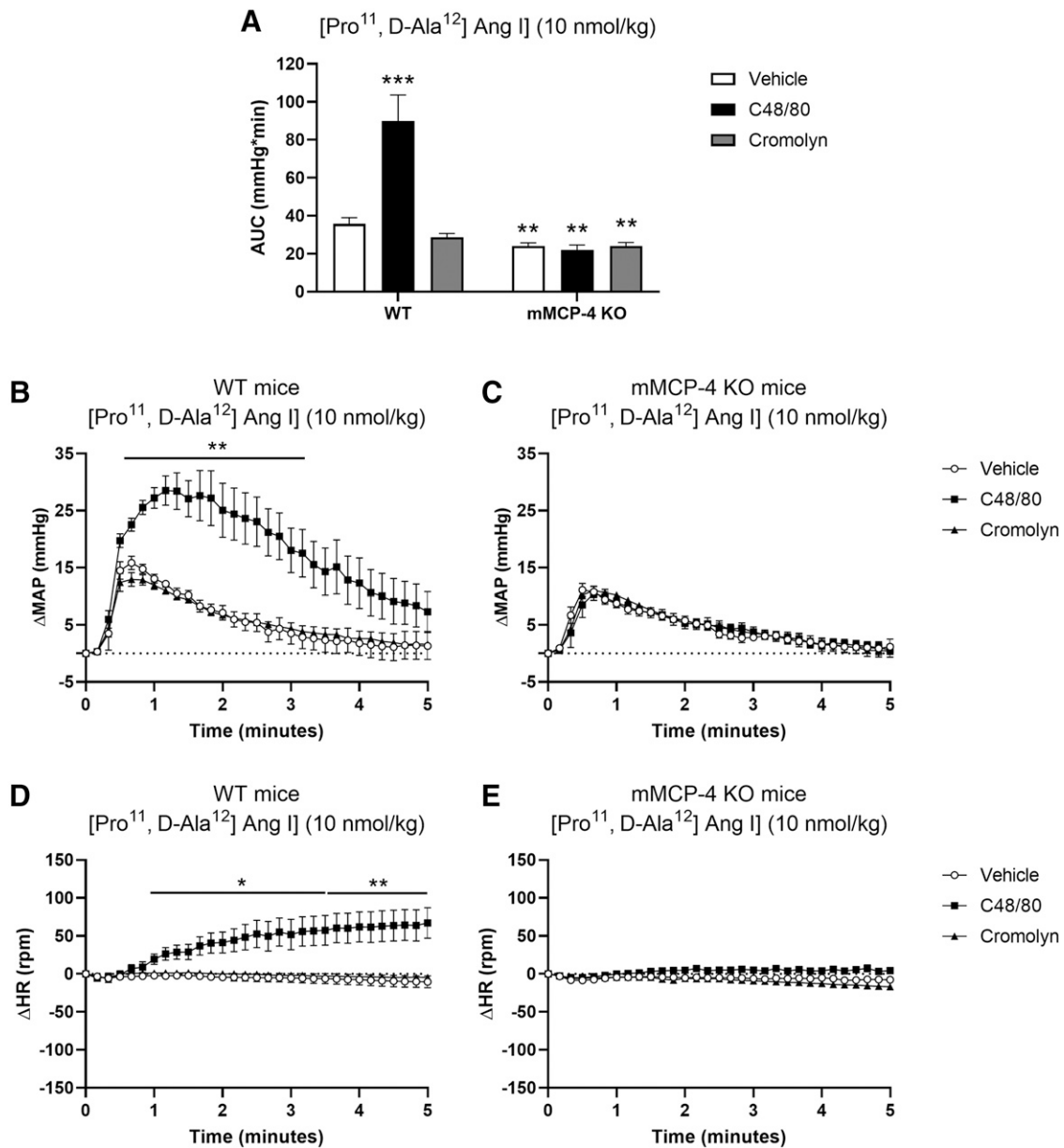
Fig. 6. (A) Time course [area under the curve (AUC)] of [Pro^{11}, D-Ala^{12}] Ang I (10 nmol/kg) in WT or mMCP-4 KO mice pretreated with either vehicle, C48/80 or cromolyn; maximal variation in MAP in response to intravenous administration of [Pro^{11}, D-Ala^{12}] Ang I in (B) WT and (C) mMCP-4 KO mice, and their respective HR (D and E). Each point and bar correspond to the mean ± S.E.M. of 7 to 8 separate experiments. *$P < 0.05$; **$P < 0.01$ vs. WT + vehicle by one-way ANOVA with Dunnett's multiple comparison test (A) and Student's t test (B and D).

properties of Ang I, suggesting an important role of mast cell stability in the ECE-independent cardiovascular properties of big ET-1 but not in the ACE-independent pressor responses to Ang I in vivo.

NEP is required to produce ET-1 from chymase-generated ET-1 (1–31) in vivo (Fecteau et al., 2005). Data reported prior to the present study suggests that repression of mMCP-4 in mice does not affect endothelin receptors or NEP expression in the mouse cardiovascular system (Houde et al., 2013). We herein show that ET-1 (1–31) and ET-1 administration induce similar hemodynamic responses regardless of whether mast cells are activated or stabilized, thus illustrating that secreted mMCP-4 plays no significant role in the cardiovascular response to these two endothelin metabolites in the mouse model.

The present study also argues in favor of a significant impact of mast cell stability in the production of ET-1 (1–31) in vitro. The loss of big ET-1 conversion capacity in mast cells derived from mMCP-4 KO mice is correlated with the absence of chymase-like hydrolysis in the in vitro series of experiments using the fluorogenic substrate Suc-Leu-Leu-Val-Tyr-AMC. These results show that induction of mast cell degranulation by C48/80 leads to the release of chymase, whereas cromolyn stabilizes the mMCP-4 content within secretory granules.

Borland et al. (1998) reported that, in the human saphenous vein, chymase is located within the adventitia (outer layer of the vessel) and is colocated with mast cell populations, whereas ACE is mainly found in the vascular endothelium. Borland et al. (1998) also suggested that circulating Ang I would be more accessible to ACE than to chymase. In addition,

circulating chymase is rapidly and irreversibly inhibited by plasma proteins such as alpha-macroglobulins and alpha-1-antichymotrypsin, which limits the contribution of the mast cell serine protease in the production of vascular Ang II (Walter et al., 1996). Tissue chymase thus is likely to be of greater importance in the functional effects of Ang II on the smooth muscle cells of blood vessel walls than in the systemic circulation (Borland et al., 1998). Moreover, we previously reported no differences in the pressor responses to Ang I in nonanesthetized WT and mMCP-4 KO mice (Semaan et al., 2015). In concordance with those results, we show in the present study that modulation of mast cell degranulation does not impact the vasopressor responses to exogenous Ang I in vivo, suggesting that chymase may not be as biologically relevant for the Ang II precursor as for big ET-1 hydrolysis in the murine systemic circulation. Given the abundance of angiotensin-converting enzyme in the mouse in vivo (Balcells et al., 1997), it is therefore unlikely that chymase is involved in the production of vascular Ang II under physiologic or pathologic conditions in mice.

In contrast, the present study suggests that chymase modulates the cardiovascular responses to the ACE-resistant analog, [Pro^{11}, D-Ala^{12}] Ang I (Li et al., 2004), in the mouse model. Using ACE inhibitors (captopril and enalapril) as well as a general inhibitor of chymotrypsin-like enzymes (chymostatin), Borland et al. (1998) reported a partial inhibition of the contractile properties of Ang I in human saphenous veins, suggesting the existence of both ACE- and chymase-independent pathways in the generation of Ang II. However, since several additional enzymes such as trypsin, chymotrypsin, tonin, cathepsin G, and kallikrein can also generate Ang II from Ang I in vitro (Becari et al., 2011), the contribution of these enzymes in the cardiovascular responses to Ang I remains to be investigated in vivo. Interestingly, the present study also reports the sensitivity of the ACE-resistant Ang I analog ([Pro^{11}, D-Ala^{12}] Ang I) to mast cell degranulation. Specifically, [Pro^{11}, D-Ala^{12}] Ang I requires mMCP-4 for its optimal vasoactive responses and is susceptible to mast cell degranulation in the mouse model in vivo. Notably, the finding that cromolyn did not reduce the response to the same analog in contrast to that of big ET-1 suggests that [Pro^{11}, D-Ala^{12}] Ang I is cleaved by interstitially located active mMCP-4 (Takai et al., 2010) rather than by the same enzyme stored in mast cells.

Baroreceptors are activated by changes in blood pressure, resulting in variations in cardiac chronotropism/inotropism and systemic vascular resistance ((Guo et al., 1982); Knape and van Zwieten, 1988). In the present study, no effects on the heart rate were observed even at the highest doses tested of the two endogenous vasopressors big ET-1 or Ang I. In contrast, a submaximal dose (10^{-8} mol/kg) of [Pro^{11}, D-Ala^{12}] Ang I induced significant increases in HR only in C48/80 pretreated mice, caused perhaps by higher plasma levels of chymase-produced Ang II and subsequent activation of the baroreceptor function through the Ang II type 1 receptor (Wong et al., 1993; (Matsumura et al., 1999)).

A significant role for mMCP-4 in a mouse model of experimental autoimmune encephalomyelitis was also recently reported by our laboratory (Desbiens et al., 2016, 2019). Those studies showed an increase in the mMCP-4–dependent production of ET-1 in the periphery and the central nervous system. More recently, Pinke et al. (2020)

reported that mast cell stabilization with ketotifen lowers disease prevalence and severity as well as the expression of chymase and carboxypeptidase A in mice, thus supporting a potentially therapeutic indication for mast cell stabilizers in multiple sclerosis (Pinke et al., 2020). Deleterious roles for mast cell–derived chymase have also been shown in experimental models of cardiac infarct, ischemia/reperfusion, and atherosclerosis (Jin et al., 2003; Tejada et al., 2016; Houde et al., 2018). Finally, patients with myocardial infarction show increased levels of plasmatic and cardiac ET-1 (1–31) and chymase activity in the same organ (Oka et al., 2014). Whether increased chymase production prompted by mast cells activation under inflammatory conditions is correlated with enhanced production of ET-1 levels in cardiovascular diseases, however, remains to be investigated.

Vascular or autoimmune diseases in which inflammatory processes associated with mast cell activation have been documented (Hermans et al., 2019; Kempuraj et al., 2020) and, interestingly, also recently reported in coronavirus disease 2019 patients with hyperinflammation (Afrin et al., 2020). Furthermore, Ahmad et al. (2019) suggested that stabilizing mast cells could prevent cardiac remodeling since chymase has a high affinity for the human Ang I peptide, in vitro. Agents enhancing mast cell stability may thus have beneficiary effects by reducing the deleterious contribution of chymase, Ang II, and as shown in this study, ET-1, in proinflammatory cardiac and vascular disorders.

Authorship Contributions

Participated in research design: Vincent, Lapointe, D'Orléans-Juste.

Conducted experiments: Vincent, Lo, Gagnon.

Performed data analysis: Vincent, Gagnon.

Contributed new reagents or analytic tools: Pejler, Takai, Day, D'Orléans-Juste.

Wrote or contributed to the writing of the manuscript: Vincent, Lapointe, Pejler, D'Orléans-Juste.

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Address correspondence to: Pedro D'Orléans-Juste, Department of Pharmacology and Physiology, Université de Sherbrooke, 3001, 12e Ave. Nord, Sherbrooke, Quebec, Canada J1H 5N4. E-mail: labpdj@usherbrooke.ca