Hypertension

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Circulating Dipeptidyl Peptidase 3 Modulates Systemic and Renal Hemodynamics Through Cleavage of Angiotensin Peptides

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BACKGROUND: High circulating DPP3 (dipeptidyl peptidase 3) has been associated with poor prognosis in critically ill patients with circulatory failure. In such situation, DPP3 could play a pathological role, putatively via an excessive angiotensin peptides cleavage. Our objective was to investigate the hemodynamics changes induced by DPP3 in mice and the relation between the observed effects and renin–angiotensin system modulation.

METHODS: Ten-week-old male C57Bl/6J mice were subjected to intravenous injection of purified human DPP3 or an anti-DPP3 antibody (procizumab). Invasive blood pressure and renal blood flow were monitored throughout the experiments. Circulating angiotensin peptides and catecholamines were measured and receptor blocking experiment performed to investigate the underlying mechanisms.

RESULTS: DPP3 administration significantly increased renal blood flow, while blood pressure was minimally affected. Conversely, procizumab led to significantly decreased renal blood flow. Angiotensin peptides measurement and an AT1R (angiotensin II receptor type 1) blockade experiment using valsartan demonstrated that the renovascular effect induced by DPP3 is due to reduced AT1R activation *via* decreased concentrations of circulating angiotensin II, III, and IV. Measurements of circulating catecholamines and an adrenergic receptor blockade by labetalol demonstrated a concomitant catecholamines release that explains blood pressure maintenance upon DPP3 administration.

CONCLUSIONS: High circulating DPP3 increases renal blood flow due to reduced AT1R activation via decreased concentrations of circulating angiotensin peptides while blood pressure is maintained by concomitant endogenous catecholamines release.
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Key Words: angiotensin II ■ blood pressure ■ catecholamines ■ circulatory failure ■ DPP3 protein, human ■ procizumab ■ renal blood flow ■ renin–angiotensin system

High circulating dipeptidyl peptidase 3 (cDPP3) concentration has been observed in the blood of critically ill patients, notably in patients with various causes of circulatory failure including septic shock, cardiogenic shock, hemorrhagic shock, or circulatory failure associated with extensive burns.[1–6] In such conditions, high cDPP3 has been associated with poor survival. Particularly relevant to the context of shock, high cDPP3 has been associated with a higher incidence of cardiac dysfunction and with the need for higher doses of catecholamine support to maintain blood pressure. Furthermore, high cDPP3 during circulatory failure has been associated with a higher incidence of acute kidney injury and a more frequent need for renal replacement therapy.

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NOVELTY AND RELEVANCE

What Is New?

In mice, DPP3 (dipeptidyl peptidase 3) administration increases renal blood flow while an anti-DPP3 antibody induces opposite effects.
The renovascular effect of DPP3 reflects decreased AT1R (angiotensin II receptor type 1) activation via angiotensin peptides cleavage.
Endogenous catecholamines release explains blood pressure maintenance upon DPP3 administration.

What Is Relevant?

DPP3 cleaves endogenous angiotensin peptides.

Clinical/Pathophysiological Implications?

In critically ill patients, high circulating DPP3 is associated with severe cardiovascular and renal failures, and poor survival.
These findings suggest that high circulating DPP3 contributes to inadequately low angiotensin II concentration and associated consequences in shock.

Nonstandard Abbreviations and Acronyms

AT1R	angiotensin II receptor type 1
cDPP3	circulating dipeptidyl peptidase 3
DPP3	dipeptidyl peptidase 3
hDPP3	human dipeptidyl peptidase 3
PCZ	procizumab
RAS	renin–angiotensin system
MAP	mean arterial pressure
RBF	renal blood flow
RVR	renovascular resistances

DPP3 is an enzyme cleaving 2 amino acids of the N-terminal extremities of small peptides, ranging from 3 to 10 amino acids. Most of its substrates have been identified in ex vivo or in vitro experiments and include angiotensin peptides and endogenous opioids such as enkephalins and endomorphins.[7–9] Low cDPP3 concentration in the bloodstream was found in healthy animals and individuals and its role in the metabolism of its known substrates remains poorly understood. Interestingly, there is a strong correlation between cDPP3 concentration and activity in critically ill patients confirming that, in acute conditions, DPP3 (dipeptidyl peptidase 3) is released in its active form.[2,10] This observation raises the possibility that, in addition to be a biomarker, high cDPP3 could contribute to circulatory failure by enhanced cleavage of vasoactive peptides. Among these peptides, angiotensin II is of particular interest, due to its effect on blood pressure maintenance, renal hemodynamics, and sodium and water homeostasis. Interestingly, defective angiotensin II-AT1R (angiotensin II receptor type 1) signaling has been reported during vasodilatory shock, with hypotension and renal dysfunction as primary consequences.[11,12] This defective signaling is classically attributed to an angiotensin-converting enzyme deficiency in the setting of a sepsis or sepsis-like endotheliopathy leading to inadequately low angiotensin II concentration.[11] However, it is likely that renin–angiotensin system (RAS) alterations in severe shock are due to additional causes, like increased degradation of angiotensin II by circulating peptidases such as cDPP3.[13]

Previous studies from our group showed that intravenous DPP3 administration to healthy mice was associated with impairment of cardiac function.[2] Inversely, administration of a monoclonal antibody inhibiting cDPP3 activity in mouse models of acute cardiac dysfunction was associated with improved left ventricular shortening fraction.[2,14] However, the precise mechanism and cDPP3 effects on other important hemodynamic variables remain largely unknown. Notably, the consequences of high cDPP3 on blood pressure are insufficiently characterized. Additionally, it has been suggested that DPP3 administration alters renal hemodynamics, either as a consequence of systemic hemodynamics impairment or due to a more specific effect on the renal vasculature.[2] Thus, the present work aimed to determine the effects of cDPP3 on systemic and renal hemodynamics and the relationship between the observed effects and the modulation of the RAS in mice.

METHODS

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animals

A total of 310 ten-week-old male C57Bl/6J mice, weighting 25±2.5 g purchased from Janvier Labs were used in all experiments. Mice were housed with a 12/12 h light-dark cycle and with ad libidum access to food and water. All experimental procedures were performed in accordance with the Directive 2010/63/eu of the European Union. The study received approval from the ethics committee Lariboisière–Villemin (S140#9385). Reporting of this work complies with ARRIVE 2.0 guidelines.

Modulation of cDPP3 Activity

Native human DPP3 (hDPP3) was purified from human red blood cell lysate as previously described and administered in all

experiments as an intravenous bolus injection of 0.55 mg/kg in 100 µL.[15] This dose of hDPP3 was chosen to produce a peak concentration ≈10× higher than observed in human pathological conditions given: (1) the rapid decrease in concentration (hDPP3 half-life 20 minutes), and (2) the basal angiotensin II concentration in mice, which is ≈10× higher than in humans.[2]

The monoclonal antibody PCZ (procizumab), an immunoglobulin G1 inhibiting DPP3 activity, was generated as previously described and administered in all experiments as an intravenous bolus injection of 10 mg/kg in 100 µL.[2]

Both hDPP3 and PCZ were provided by 4TEEN4 Pharmaceuticals GmbH (Hennigsdorf, Germany), diluted in sterile PBS buffer (hDPP3) or formulation buffer containing 10 mM L-histidine, 20 mM L-methionine, 250 mM trehalose (PCZ). PBS was used as a control injection in all experiments. Mice were randomly allocated to each condition and PBS, PCZ, or hDPP3 blindly administered.

Hemodynamic Experiments

Continuous measurements of hemodynamic variables were performed in anesthetized mice according to previously established methodology.[16] Briefly, after anesthesia by inhaled isoflurane (3% for anesthesia induction then reduced to 1% for maintenance) and analgesia with subcutaneous injection of buprenorphine 0.05 mg/kg, animals were placed on a servo-controlled table kept at 37 °C. The right femoral artery was catheterized for arterial pressure measurement, and a femoral venous catheter was used for volume replacement and administration of pharmacological agents. Sodium chloride (0.9%) was intravenously infused at a rate of 10 µL/min for fluid maintenance. Mean arterial pressure (MAP) was measured via a pressure transducer (Statham P23 DB). Renal blood flow (RBF) was measured by a flowmeter placed around the left renal artery via left lombotomy (0.5-V probe, TS420, Transonic Systems, Ithaca, NY). RBF values were controlled for zero offset, which was determined at the end of an experiment after cardiac arrest. Renovascular resistance (RVR) was estimated as the ratio of MAP/RBF. Data were recorded, stored, and analyzed using a DataTranslation analog-to-digital converter and IOX software (EMKA Technologies, Paris, France). RBF and MAP were measured before and after intravenous 100 µL bolus injections of 0.55 mg/kg hDPP3, 10 mg/kg PCZ or PBS. In some experiments, 0.8 mg/kg valsartan (Sigma Aldrich, Saint-Louis), 1.6 mg/kg naloxone (Mylan, Canonsburg) or 10 mg/kg labetalol (Aspen Pharmacare, Dublin, Ireland) was administered as a single intravenous bolus injection of 100 µL, 5 minutes before hDPP3 (0.55 mg/kg). Untreated animals received a bolus of 100 µL 0.9% sodium chloride.

In separate experiments, the systemic (MAP) and renal (RBF, RVR) vasoreactivity to 0.25 to 2 ng angiotensin II (Sigma Aldrich, Saint-Louis) or 20 ng norepinephrine (Mylan, Canonsburg) was evaluated before and after hDPP3 or PCZ administration.

Additional methods and associated references are presented in the Supplemental Material.[2,17–20]

Statistical Analyses

All statistical analyses and graphs were made using the GraphPad Prism software version 9.5.1 (GraphPad Software Inc., San Diego). The normality of distributions was assessed using the Shapiro-Wilk test and variables were expressed as means±SE or median and interquartile range as appropriate.

Due to the relatively small number of subjects per group with substantial interindividual variability in baseline values, hemodynamic changes induced by PBS, hDPP3, or PCZ administration were analyzed relative to baseline values. Absolute value traces are represented in supplemental figures for illustration, without statistical analyses. Hemodynamic data were analyzed by repeated-measures 2-way ANOVA using time and (pre)treatments as factors. In case of a significant interaction, a post hoc Dunnett's multiple comparison test was performed. Intergroup data at a specific time point were analyzed using ANOVA or the Kruskal-Wallis test as appropriate, followed by post hoc Dunnett's multiple comparison test or Dunn's test. Correlations were evaluated by the Pearson correlation coefficient (r). All tests were 2 sided and a P value of <0.05 was considered statistically significant.

RESULTS

DPP3 Modulates Hemodynamics and Vascular Reactivity to angiotensin II

To assess the effect of cDPP3 on blood pressure and renal hemodynamics, cDPP3 activity was modulated in isoflurane-anaesthetized mice by intravenous injection of purified hDPP3 or the anti-DPP3 antibody PCZ that efficiently inhibits both mouse DPP3 and hDPP3 (Figure S1). Compared with PBS-injected mice, hDPP3-injected mice exhibited a transient and massive increase of RBF, whereas MAP remained constant, reflecting decreased RVR (Figure 1; Figure S2). The maximal effect of a single bolus injection of hDPP3 was reached within ~1 minute and lasted 15 to 30 minutes. Conversely, PCZ administration produced a progressive, significant reduction of RBF without change in MAP, thus, reflecting increased RVR.

As angiotensin II has been suggested to be a substrate of DPP3, we next investigated whether hDPP3 or PCZ affects systemic or renal vasoreactivity to angiotensin II. Before hDPP3 injection, repeated intravenous bolus of increasing doses of angiotensin II induced a dose-dependent increase in MAP concomitantly with a dose-dependent decrease in RBF reflecting increased RVR (ANOVA, dose-effect $P<0.05$ for all 3 variables; Figure 2; Figure S3). The maximal response was reached within ~25 s, independent of the dose. After hDPP3 administration, the systemic (MAP) and renal (RBF, RVR) vasoreactivity to angiotensin II was systematically decreased ($P<0.01$ for all 3 variables; Figure 2; Figure S3). In contrast, hDPP3 administration did not affect the vasoconstrictive effect of norepinephrine, a vasopressor which metabolism is DPP3-independent (Figure S4). Conversely, PCZ administration did not affect the systemic or renal vasoreactivity of angiotensin II (Figure 2; Figure S3).

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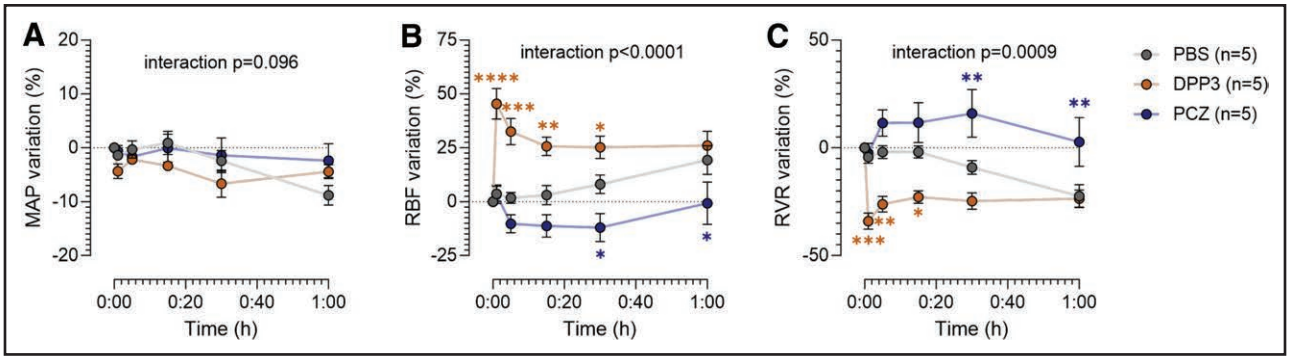

Figure 1. Effect of DPP3 (dipeptidyl peptidase 3) or PCZ (procizumab) injection on systemic and renal hemodynamics.
Mean arterial pressure (**A**), renal blood flow (**B**), and renovascular resistances variations after DPP3, PBS, or PCZ administration. Data are presented as mean±SE of relative variation compared with baseline. Comparison was made by repeated-measures 2-way ANOVA, followed by post hoc Dunnett multiple comparison test at specific time points (color stars) in case of a significant interaction. Mean arterial pressure (MAP) variation: interaction, P=0.096; time effect, P=0.0007; and treatment effect, P=0.33. Renal blood flow (RBF) variation: interaction, P<0.0001; time effect, P=0.0002; and treatment effect, P<0.0001. Renovascular resistances (RVR) variation: interaction, P=0.009; time effect, P=0.0026; treatment effect, P=0.0002. Post hoc comparisons with the PBS group. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

DPP3 Modulates the RAS In Vivo

To confirm that hDPP3 induces angiotensin peptide cleavage in vivo, circulating concentrations of angiotensin peptides were measured on stabilized plasma sampled 15 and 60 minutes after hDPP3, PBS, or PCZ injections to anesthetized mice. Compared with PBS-injected animals, hDPP3 administration was associated with decreased concentrations of angiotensin II, III, and IV, 15 minutes after injection (Figure 3). Additionally, hDPP3 administration was associated with increased angiotensin I, in relation with increased renin activity (Figure S5). Conversely, PCZ administration led to increased circulating concentrations of angiotensin II,[1–7]; Figure 3). Neither hDPP3 nor PCZ influenced aldosterone concentration (Figure S5). The effect of hDPP3 but not PCZ on circulating angiotensin peptide concentrations persisted at least for 60 min after injection (Figure S6).

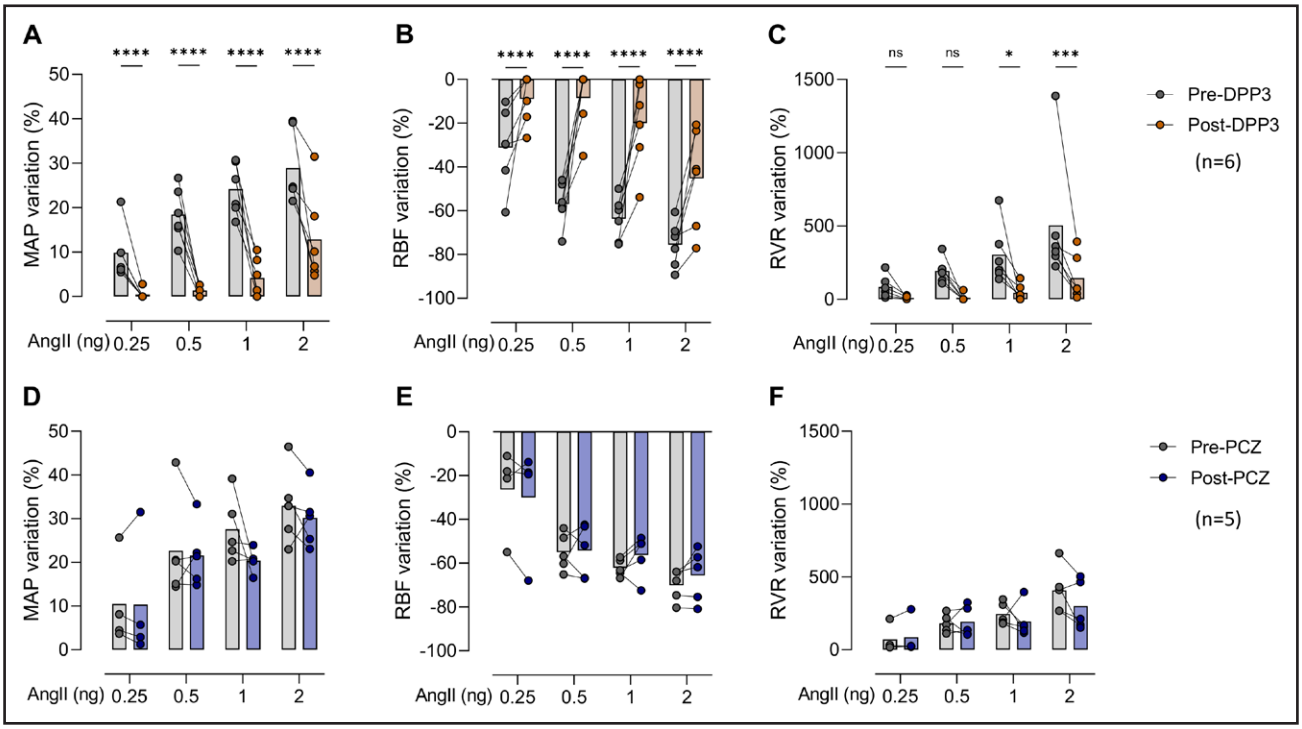

Figure 2. Effect of DPP3 (dipeptidyl peptidase 3) and PCZ (procizumab) on systemic and renal vasoreactivity to AngII (angiotensin II).
Effect of DPP3 on the peak mean arterial pressure (MAP; **A**), renal blood flow (RBF; **B**), and renovascular resistances (RVR; **C**) responses induced by increasing doses of AngII. Effect of PCZ on the peak MAP (**D**), RBF (**E**), and RVR (**F**) responses induced by increasing doses of AngII. Comparison was made by repeated-measures 2-way ANOVA followed by post hoc Šidák multiple comparison test (intergroup difference at a specific dose): *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

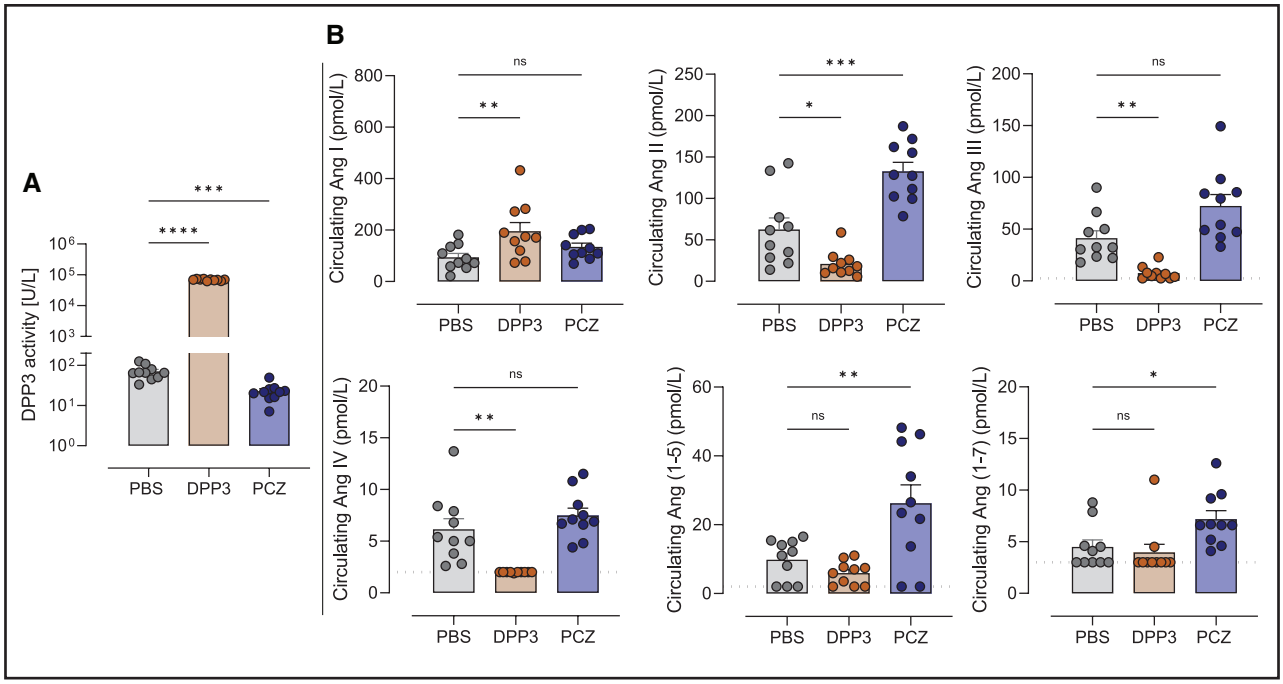

Figure 3. DPP3 (dipeptidyl peptidase 3) activity and circulating angiotensin peptides 15 minutes after PBS, DPP3, or PCZ (procizumab) administration.

Comparison was made by ANOVA or the Kruskal-Wallis test as appropriate (n=10/group). Ang indicates angiotensin. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

Renovascular Effect of hDPP3 Implicates Decreased Angiotensin Type 1 Receptor Agonism

We next investigated whether the renovascular effect observed after hDPP3 administration is due to angiotensin cleavage. Thus, mice were pretreated with valsartan, an AT1R antagonist. As expected, pretreatment with valsartan decreased MAP (−12%, from 89±5 to 78±5 mm Hg; P=0.001), increased RBF (+71%, from 0.70±0.12 to 1.2±0.34 mL/min; P=0.0215), reflecting decreased RVR (−41%, from 121±26 to 72±24 mm Hg mL^{-1} min; P=0.0125). Because these hemodynamic effects were transient, only the maximum response to subsequent hDPP3 administration, measured at 1 minute after injection, was analyzed. Compared with untreated mice, valsartan-pretreated mice exhibited decreased responses to hDPP3 (Figure 4; Figures S7 and S8). Notably, the renovascular hemodynamic response to valsartan, as well as to the subsequent injection of hDPP3, displayed important interindividual variability suggesting that AT1R blockade was not complete in all animals. Nevertheless, we observed a strong negative correlation between the effect induced by valsartan on RBF and the added effect of subsequent hDPP3 injection, suggesting that the more efficient AT1R blockade is, the less additive hDPP3 effect is observed (RBF, r=−0.959; P=0.0025 and RVR, r=−0.923; P=0.0085). Administration of a higher dose of valsartan to ensure complete AT1R blockade was not possible due to poor tolerance. In contrast, a pretreatment by the pan-opioid receptor antagonist naloxone produced a marginal hemodynamic effect (MAP, −4%, from 93±9 to 89±6 mm Hg; P=0.19; RBF, +11%, from 0.61±0.068 to 0.68±0.081 mL/min; P=0.0442; RVR, −14%, from 155±18 to 134±14 mm Hg.mL^{-1} min; P=0.10) and did not alter the response to subsequent hDPP3 administration (Figures S9 and S10). Taken together, these data suggest that hDPP3 mediates its effect on renal hemodynamics through decreased AT1R signaling.

hDPP3 Injection Do Not Alter Renal Function in Mice

To investigate the effect of hDPP3 or PCZ injection on glomerular function, water, and sodium homeostasis, we measured creatinine, urea, sodium, potassium, osmolality, and total plasma proteins on samples taken 60 minutes after injection. Compared with PBS-injected mice, neither hDPP3 nor PCZ produced significant changes on any of these variables (Table S1).

To further investigate a potential transient effect of hDPP3 or PCZ on renal function, we estimated the glomerular filtration rate using transcutaneous measurement of fluorescein isothiocyanate-sinistrin clearance. The estimated GFR measured following PBS, hDPP3 or PCZ injection did not differ significantly between the groups (Figures S11 and S12).

DPP3-Induced Catecholamines Release Maintains Blood Pressure

To explain the maintenance of a stable blood pressure upon hDPP3 administration, we tested whether an

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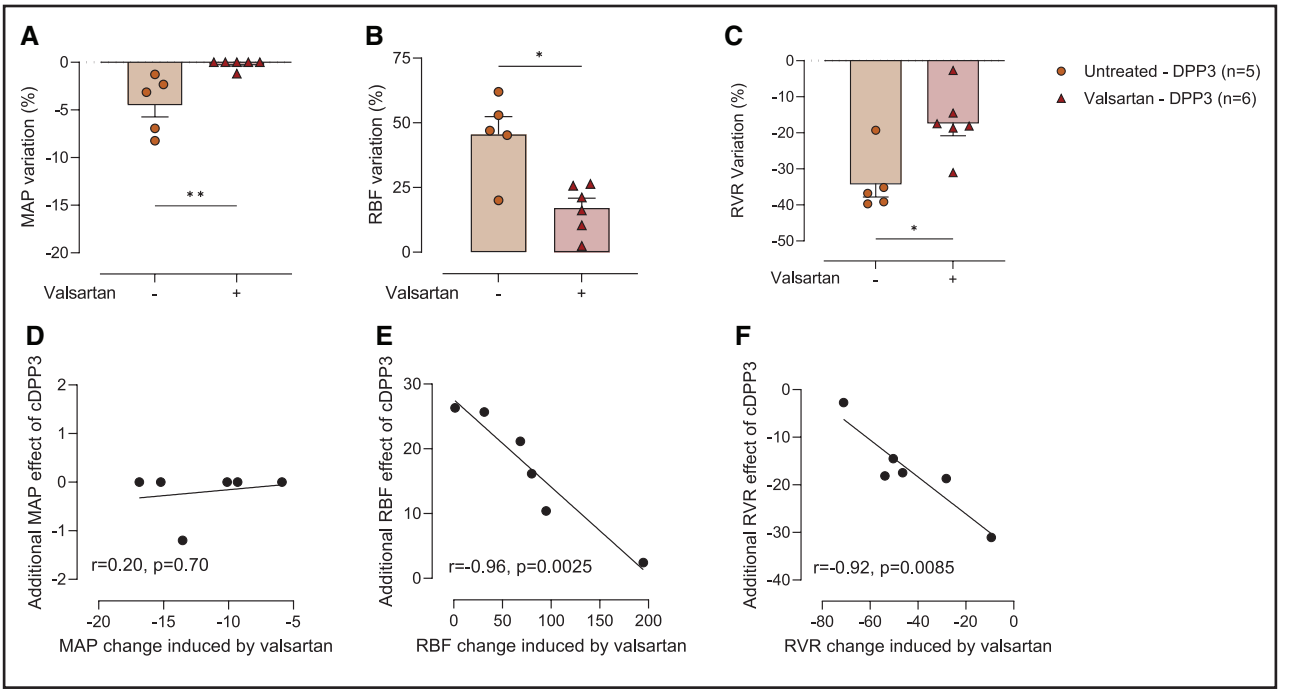

Figure 4. Effects of a pretreatment by valsartan on the systemic and renal hemodynamic effects induced by DPP3 (dipeptidyl peptidase 3). Maximal effect (T+1min) on mean arterial pressure (MAP; A), renal blood flow (B), and renovascular resistances (C) variations after DPP3 in mice pretreated (n=6) or not (n=5) with the AT1R antagonist valsartan.

Correlation between the effect induced by valsartan and the additional effect produced by DPP3 administration on mean arterial pressure (**D**), renal blood flow (**E**), and renovascular resistances (**F**). Data are presented as mean±SE. RBF indicates renal blood flow; and RVR, renovascular resistances. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

endogenous phenomenon was involved to counteract the loss of systemic vasoconstrictive effect associated to excessive angiotensin II cleavage. To address this question, we measured the concentrations of circulating endogenous catecholamines, namely epinephrine, norepinephrine, and dopamine at different time points (5, 15 and 60 min) after PBS, hDPP3 or PCZ administration. Compared with the PBS-injected group, mice injected with hDPP3 exhibited a progressive release of catecholamines, reaching maximal concentrations at 60 min while the PCZ group showed a tendency for the opposite effect (Table). To confirm that catecholamines release prevents decreased blood pressure after hDPP3 injection, we pretreated mice with an α- and β-adrenergic receptor blocker, labetalol, before hDPP3 administration. As expected, labetalol pretreatment induced a sustained decrease of MAP (−13%, from 84±6 to 73±3 mm Hg; P=0.022), without significant changes of RBF (−7%, from 0.71±0.064 to 0.66±0.13 mL/min; P=0.36) nor RVR (−2%, from 121±9 to 118±26 mm Hg mL^{-1} min; P=0.75). Upon hDPP3 administration, an abrupt fall of blood pressure was observed in labetalol-pretreated mice, contrasting with untreated mice (Figure 5; Figure S13). Additionally, pretreatment by labetalol was associated with enhanced hDPP3 effects on renal hemodynamics. Notably, pretreatment by valsartan was associated with decreased norepinephrine after PBS injection and abrogated catecholamines release upon hDPP3 administration, suggesting that catecholamines are not solely a consequence of the circulatory stress induced by decreased AT1R agonism (Figure S14). Taken together, these findings confirm that a concomitant endogenous catecholamine release accounts for blood pressure maintenance despite the DPP3-mediated cleavage of AT1R agonists.

DISCUSSION

In the present work, we show that hDPP3 administration to healthy mice decreases renovascular resistance leading to increased renal blood flow, while blood pressure is minimally affected. Additionally, hDPP3 administration is associated with decreased systemic and renal vasoreactivity to angiotensin II. We demonstrate that the renovascular effect induced by hDPP3 is due to reduced AT1R activation via decreased concentrations of circulating angiotensin II, III, and IV. An additional novel finding is that hDPP3 administration is associated with catecholamines release which maintains blood pressure despite decreased concentration of vasoconstrictive angiotensin peptides.

As angiotensin II is the main circulating AT1R agonist due to its relative abundance and its maximal receptor affinity, it is likely that the hemodynamic changes observed in our experiments primarily reflect DPP3-mediated angiotensin II cleavage. Nevertheless, as angiotensin III and IV also have some affinity for the

Table. Circulating Catecholamines Concentrations at 5, 15, and 60 min After PBS, DPP3, or PCZ Administration

Timing	PBS	DPP3	PCZ	*P* value
Norepinephrine (pmol/L)				
5 min	10 321±2402	12 342±2331	8734±2392	0.008
15 min	10 800±2221	10 378±2863	7327±2953*	0.0201
60 min	9411±1352	17 744±3993***	7500±1094**	<0.0001
Epinephrine (pmol/L)				
5 min	1503±485.2	1961±494.9	1195±282.4	0.0035
15 min	1349±575.7	2436±824.1	873.2±223.0	0.0002
60 min	1282±174.8	3325±1337*	765.4±152.3*	<0.0001
Dopamine (pmol/L)				
5 min	2484±519.3	2718±683,4	2471±586.1	0,5916
15 min	1961±723.3	2624±698.8	1780±352.8	0.0122
60 min	1720±505.1	2834±596.5****	1234±250.8	<0.0001

Comparisons were made using ANOVA or the Kruskal-Wallis test. Post hoc comparisons with PBS group: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 in comparison with the PBS group at the same time point. n=10/group at each time point. DPP3 indicates dipeptidyl peptidase 3; and PCZ, procizumab.

AT1R receptor, decreased concentrations of these peptides upon hDPP3 administration could also play a minor role.[21] Our data show clearly that DPP3 can cleave angiotensin II in vivo while most previous works have identified potential DPP3 substrates based on in vitro or ex vivo experiments. To our knowledge, only 1 study investigated angiotensin II cleavage in vivo in an experimental setting where exogenous angiotensin II was administered to artificially induce hypertension.[22] Interestingly, in the latter study, DPP3 administration reduced blood pressure in angiotensin II-infused hypertensive mice while it did not affect hemodynamics in norepinephrine-induced hypertensive mice nor normotensive mice. These results as well as ours establish that the action of DPP3 on blood pressure is mediated via the cleavage of angiotensin II and becomes apparent when systemic hemodynamics are in a state of dependence toward angiotensin II.

Moreover, we show that DPP3 effect on renal hemodynamics, caused by decreased stimulation of AT1R, was consistent with the known vasoactive properties of angiotensin II. In the absence of angiotensin II-AT1R signaling, as observed during treatment with the AT1R antagonist valsartan, the decrease in total RVR induces an increase of RBF. As angiotensin II tends to exert a preferential, but not exclusive, vasoconstrictor effect on the efferent glomerular arteriole, loss of AT1R-dependent tone tends to decrease glomerular filtration pressure, and thus glomerular filtration rate.[23–25] However, isolated loss of AT1R tone such as induced by short-term pharmacological blockade in young, healthy, sodium-repleted animals or humans seems insufficient to induce significant changes of glomerular filtration rate.[26,27] Thus, the lack of short-term alteration of GFR in our experiments could be related to the brevity of the hemodynamic modifications induced by a single bolus of hDPP3 or the integrity of endogenous mechanisms compensating for the isolated loss of AT1R tone.

Our data demonstrate that inhibition of physiological cDPP3 activity by PCZ in mice is associated with significant biochemical (increased angiotensin II concentration) and hemodynamic (decreased RBF) changes, thus, proving that cDPP3 activity is of significance concerning angiotensin metabolism, even in physiological conditions. The small amplitude of PCZ-induced hemodynamic changes could be related to the overall low sensitivity of mice to angiotensin II, to the incomplete inhibition of cDPP3 activity by PCZ or because of the low baseline cDPP3 activity in healthy mice. Increased angiotensin,[1–7] a vasodilatory peptide could also have partially counteracted the effect of increased concentration of AT1R agonists after PCZ administration.[28] A more pronounced effect of PCZ could be observed in a

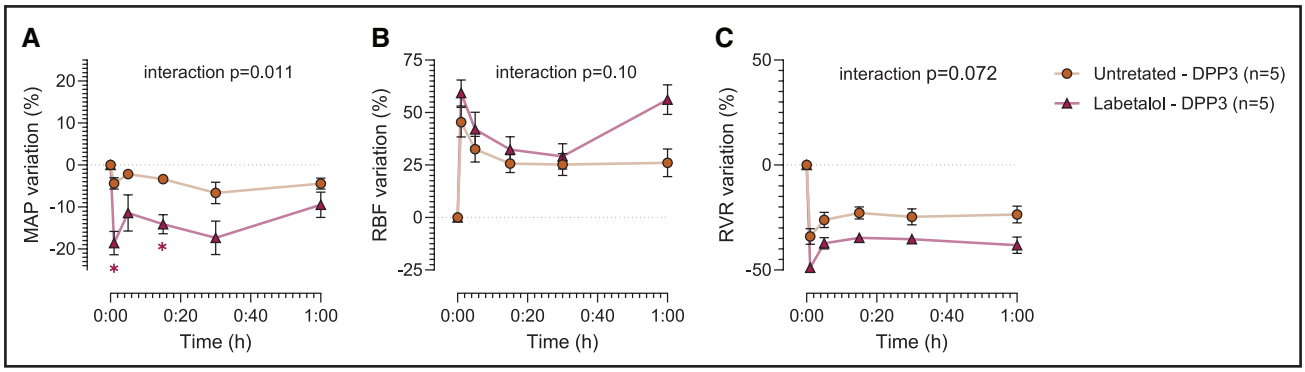

Figure 5. Effects of a pretreatment by labetalol on the systemic and renal hemodynamic effects induced by DPP3 (dipeptidyl peptidase 3). Mean arterial pressure (MAP; A), renal blood flow (RBF; B), and renovascular resistances (RVR) variations after DPP3 in mice pretreated (n=5) or not (n=5) with the α- and β-adrenergic receptors antagonist labetalol.
Data are presented as mean±SE. Comparison was made by repeated-measures 2-way ANOVA, followed by post hoc Dunnett multiple comparison test at a specific time points (color stars) in case of a significant interaction. MAP variation: interaction P=0.011, time effect P=0.0003, and treatment effect P=0.0064. RBF variation: interaction, P=0.10; time effect, P<0.0001; and treatment effect, P=0.045. RVR variation: interaction, P=0.071; time effect, P<0.0001; and treatment effect, P=0.0018. Post hoc comparisons: *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

pathological condition where cDPP3 is elevated and the classical RAS activated. From a therapeutic perspective, it is interesting to note that cDPP3 inhibition by PCZ is not associated with major hemodynamic changes when cDPP3 is low.

Our results could have multiple implications:

We have demonstrated that DPP3 cleaves angiotensin II in vivo, providing arguments to the view that high cDPP3 can contribute to the recently reported RAS alterations during shock, reproducing the typical RAS pattern observed in patients: High renin, decreased angiotensin II, and increased angiotensin I leading to a high angiotensin I/angiotensin II ratio.[11,13,29] Indeed, as angiotensin II decreases, insufficient stimulation of AT1R triggers renin release by juxtaglomerular cells, due to a known biofeedback mechanism.[30] Furthermore, the finding that DPP3 is associated with decreased systemic and renal vasoreactivity to angiotensin II questions the relevance of administering angiotensin II in situations where cDPP3 is high. Indeed, high cDPP3 could explain why some patients have a poor hemodynamic response to angiotensin II.[31,32]

Furthermore, the administration of hDPP3 to mice had a minimal effect on blood pressure, due to a concomitant release of endogenous catecholamines. In a pathological condition, where sympathetic stimulation is often already maximal, DPP3 release could be associated with further decrease of blood pressure. Alternatively, blood pressure could be maintained further stimulating catecholamines release and thus at a cost of potentially undesirable effects.[33–36] Inversely, inhibition of cDPP3 activity could be an interesting strategy aiming at an effective decatecholaminisation during circulatory failure.[37] As the mechanism linking DPP3 to catecholamines release is still unclear, further work is needed to elucidate the precise mechanisms at play.

Our study also has some limitations.

First, our experiments were limited to exploring the role of cDPP3 in otherwise healthy animals. The current design allows for a clean investigation of the mode of action of DPP3 and PCZ without the complex multifactorial effects of acute circulatory failure. Future studies should focus on more complex pathological models.

Second, only mice models were used in our study. Future studies on large animal models could allow a more detailed characterization of the macrocirculatory and microcirculatory effects of cDPP3 and its inhibition.

Third, a progressive increase of RBF in the PBS-injected (control) group, putatively ascribed to a progressive vasodilation due to prolonged anesthesia limits the interpretation of late hemodynamic changes.

Finally, if our work excludes a role of endogenous opioids in the hemodynamic changes observed, a potential role of known (angiotensin, enkephalins, or endomorphins peptides) or still unknown DPP3 substrates on other end points is not completely excluded. Further work may focus on identifying these substrates and their specific effects.

In conclusion, our study demonstrates that hDPP3 administration to mice induces a transient drop in RVR caused by enhanced degradation of angiotensin II leading to increased RBF whereas blood pressure is maintained by means of increased circulating endogenous catecholamines. These findings strengthen the fact that cDPP3 is an actor of circulatory failure and contributes to RAS alterations and associated consequences in shock.

PERSPECTIVES

Our work highlights the importance of circulating DPP3 in angiotensin peptides metabolism and suggests a mechanism explaining the poor prognosis associated with high cDPP3 in critically ill patients. Additionally, relevant biochemical (circulating angiotensin peptides, renin, and catecholamines) and clinical (blood pressure, renal hemodynamics, and function) end points could be used in future experimental and clinical studies dedicated to the role of circulating dipeptidyl peptidase 3 in pathological conditions. In such conditions, further work could focus on the mechanisms of DPP3 release into the circulation. Finally, this study suggests that excessive angiotensin peptides degradation by high circulating DPP3 during circulatory failure could be targeted by PCZ.

ARTICLE INFORMATION

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A. Picod, A. Mebazaa, and F. Azibani designed the study. All authors contributed to data acquisition and interpretation. A. Picod, A. Mebazaa, and F. Azibani drafted the article, and all other authors critically reviewed the work and made substantial contributions. All authors approved the final version of the article and agree to be accountable for all aspects of the work.

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