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A Novel Role of Semaphorin 3C in Modulating Systemic and Renal Hemodynamics

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Keywords

Semaphorin 3C · Mean arterial pressure · Renal blood flow

Abstract

Background: Alterations of renal hemodynamics play an essential role in renal homeostasis and kidney diseases. Recent data indicated that semaphorin 3C (SEMA3C), a secreted glycoprotein involved in vessel development, can modulate renal vascular permeability in acute kidney injury, but whether and how it might impact systemic and renal hemodynamics is unknown. **Objectives:** The objective of the study was to explore the effect of SEMA3C on systemic and renal hemodynamics. **Methods:** SEMA3C recombinant protein was administered intravenously in two-month-old wild-type mice, and the variations of mean arterial pressure, heart rate, renal blood flow, and renal vascular resistance were measured and analyzed. **Results:** Acute administration of SEMA3C induced (i) systemic hemodynamic changes, including mean arterial pressure decrease and heart rate augmentation; (ii) renal hemodynamic changes, including reduced vascular resistance and elevated renal blood flow. Continuous perfusion of SEMA3C had no significant effect on systemic or renal hemodynamics. **Conclusion:** SEMA3C is a potent vasodilator affecting both systemic and renal hemodynamics in mice.

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Introduction

Hemodynamics refers to the dynamics of the blood flow, which are essential to the body's homeostasis. One of the fundamental functions of the kidney is regulating systemic hemodynamics via the modulation of body fluid load and renal vascular resistance (RVR), mainly involving renal hemodynamic alterations, tubular regulation of fluid and ion transport, and release of kidney-derived vasoactive molecules [1]. In addition to the renin-angiotensin-aldosterone system, the kidney can also synthesize various vasoactive substances, including arachidonic acid derivatives, NO, endothelin, and kinins, to regulate blood pressure and vascular tone [2].

Semaphorin 3C (SEMA3C), an 85 kDa glycoprotein belonging to the family of semaphorins, was initially identified as an axon guidance molecule [3]. More recent studies have reported the pivotal role of SEMA3C in vascular smooth muscle cell (VSMC) migration [4] and cardiovascular system development [5], implying its possible impact on circulatory blood flow. We recently showed that in acute kidney injury (AKI), SEMA3C is de novo excreted and secreted several hours post-kidney damage and alters renal vascular permeability [6]. Considering

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that instability of systemic hemodynamics is closely related to multiorgan dysfunction and adverse outcomes in AKI [7], it is highly warranted to investigate the acute and direct effect of SEMA3C on systemic and/or renal hemodynamics, which hitherto remained largely unknown.

In our previous study, intravenous injection of SEMA3C could trigger renal endothelial hyperpermeability [6]. Because SEMA3C receptors neuropilin 1 (NRP1) and neuropilin 2 (NRP2) are expressed throughout the embryonic cardiovascular system [5] and also within the renal peritubular vasculature [6], we hypothesize that SEMA3C could elicit both systemic and renal hemodynamic changes. To confirm our hypothesis, SEMA3C recombinant protein was injected as intravenously into wild-type mice and multiple hemodynamic parameters were measured. Acute and not continuous administration of SEMA3C triggered drastic transient changes in arterial pressure, heart rate (HR), renal blood flow (RBF), and RVR. Our study provides the first evidence of a direct role for SEMA3C in modulating systemic and renal hemodynamics.

Methods and Materials

Renal Hemodynamics Experiments

Experiments were performed on 2-month-old wild-type males weighing 28 g on average and bred into the C57BL/6J genetic background. In the mouse husbandry, temperatures of 18–23°C with 40–60% humidity are applied. Mice are subjected to a diet containing 4.5% fat with water drinking ad libitum. After analgesia (buprenorphine 0.1 mg/kg), mice were anaesthetized with 2% isoflurane and placed on a servo-controlled table at 37°C. The left femoral artery was catheterized for arterial pressure measurement and a femoral venous catheter was used for infusion of volume replacement. NaCl 0.9% was infused initially at the rate of 50 µL/min to replace surgical losses and then at 10 µL/min for maintenance. Mean arterial pressure (MAP) was measured via a pressure transducer catheter (Statham P23 DB); RBF was measured by a transonic flowmeter (0.5v probe TS420; Transonic Systems). RBF values were controlled for zero offset determined at the end of an experiment after cardiac arrest. Data were recorded, stored, and analyzed using DataTranslation analog-to-digital converter and the IOX software (EMKA Technologies). MAP, RBF, and HR were measured at basal state and during intravenous bolus injections of recombinant SEMA3C (R&D Systems) through the femoral vein (two different concentrations 2.5 and 4 µg diluted in 100 µL) or phosphate-buffered saline (PBS). Continuous perfusion was performed using a high-precision electric syringe (KD Scientific) which delivered SEMA3C given concentration at the speed of 50 µL/min through the venous catheter. The maximum changes of the transient variations in MAP, RBF, and RVR produced upon SEMA3C injection were recorded and compared with the baseline value state of each mouse. Animal experiments were conducted under animal license C75-20-01 (INSERM UMRS-1155).

Statistical Analyses

Data are expressed as mean ± SD. Statistical analyses were calculated with the Prism software (GraphPad). Comparisons between different interventions, i.e., 2.5 µg SEMA3C versus baseline (Fig. 1) and 4 µg SEMA3C versus PBS, were performed with a two-tailed *t* test. A *p* value <0.05 was considered statistically significant. The number of animals used (*n*) is given in each experiment.

Results

SEMA3C Acute Injection Induced a Significant Decrease in MAP

In a previous study, we proved that SEMA3C was de novo expressed and secreted to the blood by acutely damaged renal tubular cells [6]. In order to investigate the hemodynamic effects of this secreted glycoprotein, we injected SEMA3C intravenously and analyzed the transient systemic and renal hemodynamic changes. To this aim, we used a well-characterized SEMA3C recombinant protein, which was able to successfully induce *in vitro* endothelial-to-mesenchymal transition [5] and *in vivo* renal vascular hyperpermeability [6]. We previously observed 100% mortality (*n* = 3) a few sec following the injection of 7 µg of recombinant SEMA3C in the mouse [6]. We therefore decided to lower the injected dose and assayed the *in vivo* response following 2.5 µg and 4 µg injections of recombinant SEMA3C. We found that the intravenous injection of 2.5 µg SEMA3C elicited a rapid, robust, and transient decrease of MAP in mice (*n* = 6). Compared with the average MAP baseline of 67.9 ± 12.7 mm Hg, a peak decrease of 25% of the baseline (22.3 ± 6.3 mm Hg, *p* < 0.001, *n* = 6) was observed (Fig. 1a–b). The decrease of blood pressure was rapid, almost immediately after the injection and reached a peak decrease in approximately 15 s. This effect was transient and MAP returned to baseline value after 50 s post-injection (Fig. 1a). When stimulated with a higher dose, 4 µg of SEMA3C, the mice presented a similar MAP decrease of 25% of baseline (24.9 ± 9.5 mm Hg, *p* < 0.05, *n* = 4) (Fig. 2a–b). As the peak MAP decrease after either 2.5 µg or 4 µg SEMA3C injection was 25%, the maximum MAP decrease following SEMA3C injection should be 25% of the MAP baseline. Therefore, we concluded that intravenous bolus injection of SEMA3C recombinant protein induces a transient, rapid, and strong MAP reduction.

Increased HR after SEMA3C Acute Injection

Another change following SEMA3C injection was an increase in HR, which started 2.5 s after the drop of MAP (Fig. 1c). The average peak increase was 15% of baseline

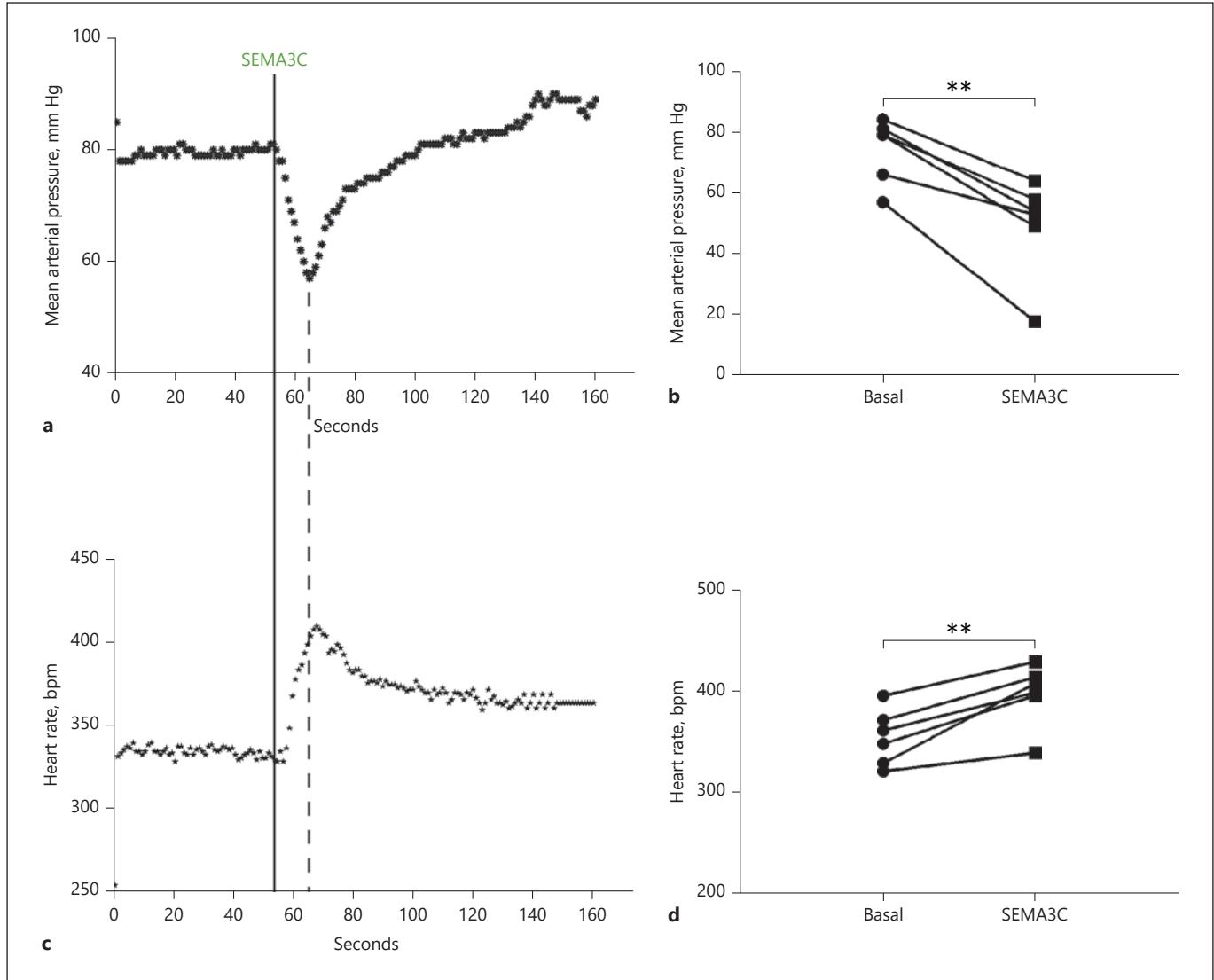


Fig. 1. Intravenous SEMA3C injection induced mean arterial pressure (MAP) decrease and heart rate (HR) elevation. **a, c** MAP and HR changes in wild-type mice post-intravenous injection of 2.5 μ g SEMA3C recombinant protein. Black solid line indicates the time of SEMA3C injection. Both solid and dashed line indicate that changes in MAP precede the ones observed for HR. **b, d** Mouse MAP and HR before (basal) and after SEMA3C injection ($n = 6$). ** $p \leq 0.01$.

(42.5 ± 16.8 bpm, $p < 0.001$, $n = 9$) and occurred 2.5 s after the maximum decrease of MAP (Fig. 1c). A similar effect was observed with the higher dose of SEMA3C (data not shown). Considering that HR elevation followed the decrease of MAP and the % change was lower compared to MAP, the transient and acute HR increase is likely due to a compensatory reaction caused by the abrupt decrease of MAP and aiming to maintain organ perfusion.

Dynamic Changes in RBF and RVR Post-SEMA3C Acute Injection

We next evaluated renal hemodynamic parameters after intravenous injection of SEMA3C. Several sec after 100 μ L injection of PBS, RBF presented a mild elevation, probably due to increased circulatory volume (Fig. 2c). Intravenous SEMA3C injection showed a biphasic response regarding RBF: first, RBF was reduced from about 0.7 mL/min to 0.05 mL/min in 8 s (Fig. 2c). Subsequently,

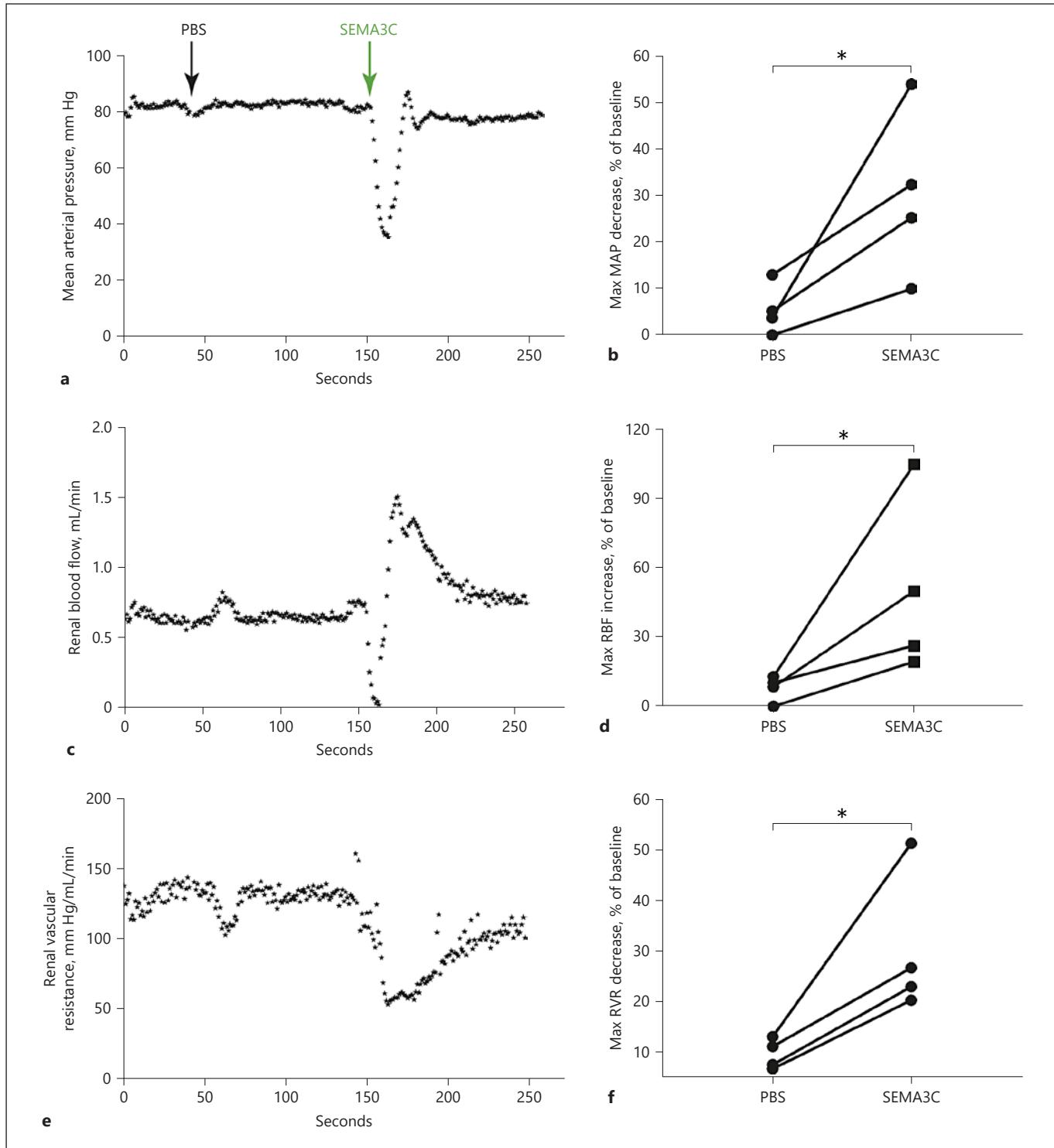


Fig. 2. Acute intravenous injection of SEMA3C induced MAP decrease, RBF elevation, and RVR reduction. MAP (**a**), RBF (**c**), and RVR (**e**) changes in wild-type mice post-intravenous injection of 100 μ L PBS, then 4 μ g/100 μ L SEMA3C recombinant protein and percentage of maximum MAP reduction (**b**), RBF increase (**d**), and RVR decrease (**f**) after the injection of PBS and SEMA3C ($n = 4$). * $p < 0.05$.

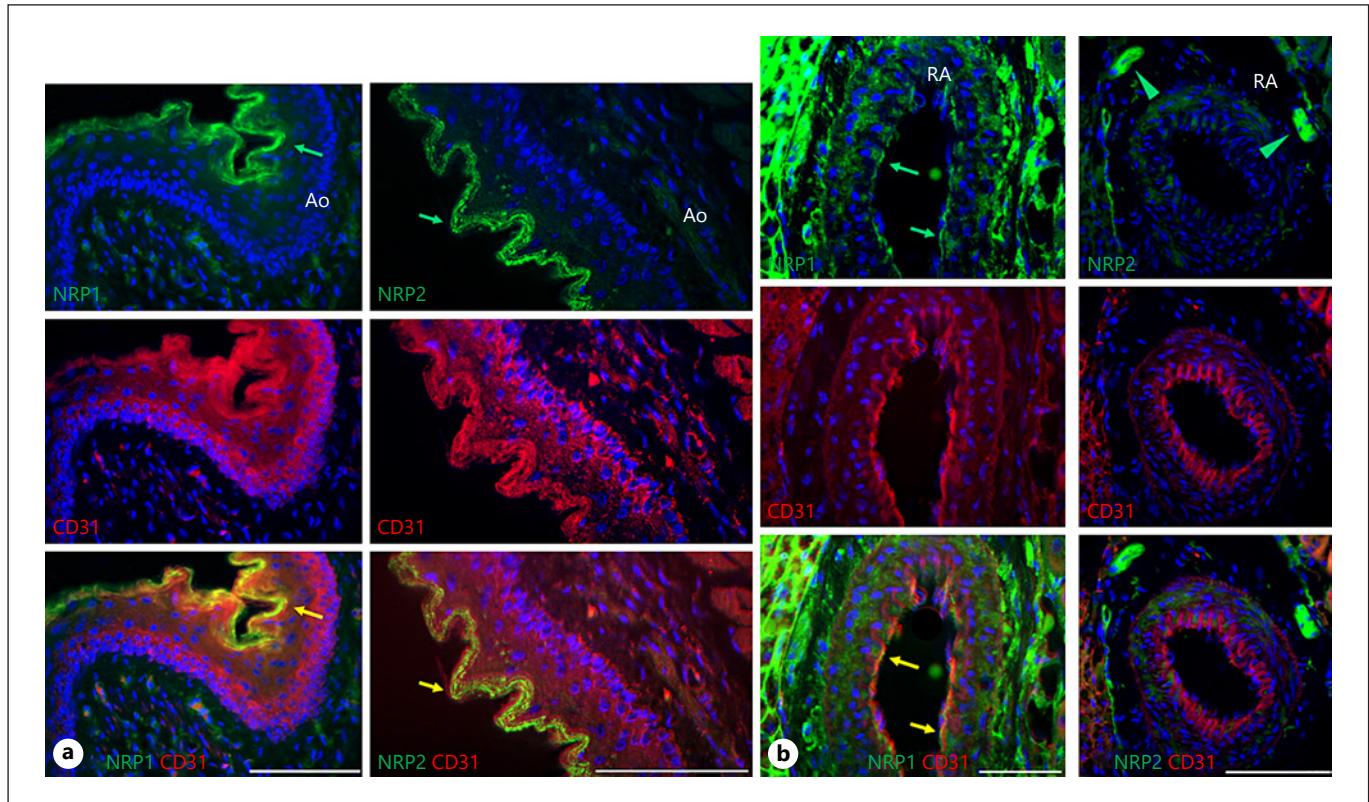


Fig. 3. SEMA3C receptors neuropilin 1 (NRP1) and neuropilin 2 (NRP2) are differentially expressed in adult mouse aorta and renal artery. Wild-type aorta (**a**) and renal artery (**b**) were immunolabeled for neuropilin 1 (NRP1) or neuropilin 2 (NRP2) and endothelial marker CD31. While both NRP1 and NRP2 are expressed in CD31-positive endothelial cells of the aorta, the endothelium of the renal artery is only positive for NRP1 (arrows). Conversely, NRP2 marks specific structures resembling nerve fibers (arrowheads).

in parallel with the restoration of MAP, RBF rapidly recovered within 10 s while reaching a peak value approximately twice as high as the baseline value, about 1.5 mL/min, and then fell back to baseline in about 50 s (Fig. 2c). The average augmentation of RBF after SEMA3C injection was 40% of baseline ($50.2 \pm 19.4\%$), while that of PBS injection was 10% of the baseline ($7.9 \pm 2.8\%$), and the difference was significant ($42.2 \pm 19.6\%, p < 0.05, n = 6$) (Fig. 2d). The elevation of RBF was caused by the alteration of RVR (Fig. 2e). It decreased mildly at a few sec post-PBS injection and then reached its peak value at about 8 s post-SEMA3C injection, followed by a reduction to about half the baseline value at around 18 s post-SEMA3C injection, and then slowly recovered to a stable value lower than baseline (105 mm Hg/mL/min, respectively, Fig. 2e). The overall average RVR decrease triggered by SEMA3C injection was of 30% of baseline ($30.4 \pm 7.1\%$), while that of PBS injection was 10% of the baseline ($9.7 \pm$

1.5%), and the difference was also significant ($20.6 \pm 7.3\%, p < 0.05, n = 6$) (Fig. 2f). We propose that the initial increase of RVR and decrease of RBF were due to the strong and abrupt decrease of MAP, which resulted in a peripheral vascular constriction to maintain MAP, while the decrease of RVR (and the corresponding increase of RBF) that followed was due to a renal vasodilator effect of SEMA3C when reaching the kidney.

In the pathophysiological state such as AKI, SEMA3C is continuously expressed, even 48 h post-injury [6], raising the possibility that systemic diffusion of the protein aggravates hemodynamic instability caused by disrupted kidney function. However, when we systemically delivered SEMA3C over a period of 21 min, we did not observe any hemodynamic changes at the given dose of 4 µg (online suppl. Fig. 1A, B; for all online suppl. material, see www.karger.com/doi/10.1159/000528259). Furthermore, while we found that acute injection of 7 µg SEMA3C was lethal

for the mice ($n = 3$), no hemodynamic changes could be detected when 8 µg SEMA3C was delivered over the course of 21 min (online suppl. Fig. 1C). We conclude that continuous diffusion of SEMA3C at the given concentrations had no effect on systemic and renal hemodynamics.

SEMA3C Receptors Neuropilin 1 and Neuropilin 2 Are Expressed in the Endothelial Wall Surrounding the Aorta and the Renal Artery

With the aim of understanding which vessels could respond to SEMA3C systemic bolus injection, we examined SEMA3C receptor expression within the arterial wall of the aorta and of the afferent renal artery. We performed double immunostaining of NRP1, NRP2, and the endothelial-specific marker CD31 on sections of the aorta and of the kidney artery obtained from wild-type adult mice. We found that both NRP1 and NRP2 are expressed in CD31-positive endothelial cells of the aorta (Fig. 3a). In the kidney, solely NRP1 is expressed in the endothelium of the renal artery (Fig. 3b).

Discussion

In this study, a comprehensive analysis of multiple hemodynamic parameters was performed to explore the vasoactive role of SEMA3C. Our previous work demonstrated that SEMA3C acted as a vascular permeability factor that was de novo produced by renal tubular cells and secreted to the blood in AKI [6]. Here, we expanded the analysis and investigated the effects of SEMA3C on both systemic and renal hemodynamics. We found that acute injection of SEMA3C decreases MAP, a major vascular effect that could be associated with the clinical features of AKI, i.e., hypotension and organ hypoperfusion. This systemic effect was followed by an increased HR, probably due to a compensatory response to maintain organ perfusion. However, we cannot exclude the possibility that SEMA3C regulates HR directly. Indeed, another molecule in the class 3 semaphorin family, SEMA3A, participates in heart rhythm regulation [8, 9]. Furthermore, blocking of class 3 semaphorin receptor NRP1 signal could lead to sinus bradycardia in adult mice [10]. Therefore, SEMA3C could modulate HR activity through its receptor NRP1 on the sympathetic nervous system [10].

Another key finding is that SEMA3C can reduce RVR and elevate RBF to strengthen renal reperfusion. As a result, hemodynamic alterations caused by SEMA3C could deteriorate ischemia-reperfusion injury of the kidney, suggesting a potential pathogenic mechanism of aggra-

vated kidney structural and functional impairment in AKI [7, 11]. However, this effect is observed solely when SEMA3C administration is delivered acutely, suggesting that a threshold in the signaling cascade should be reached to trigger the hemodynamic changes. As is known, the contraction/dilation of VSMCs, whether calcium-dependent or -independent, requires upstream signals of appropriate strength [12]. And the chronic injection of SEMA3C might keep its blood concentration under the threshold, thus unable to elicit hemodynamic changes. One limitation of our study might therefore be the given dose of SEMA3C during the continuous perfusion.

The mechanism by which SEMA3C could trigger systemic and renal hemodynamic changes is likely to involve cellular crosstalk between endothelial cells and VSMC in the main conductive arteries [13]. VSMCs maintain vessel tone and regulate arterial pressure and vessel resistance [14]. Physiological communications between these two cell types are essential in the homeostasis of mature vessels. They consist in direct cell contact and also in indirect interactions via the extracellular matrix or through soluble secreted molecules and extracellular vesicles [14]. We found that the endothelial cells of the aorta and of the renal artery express SEMA3C receptor NRP1, with the aorta expressing both receptors NRP1 and NRP2 and the renal artery expressing mainly NRP1. Our data suggest that the recombinant SEMA3C protein binds to its receptors on the arterial wall and can potentially elicit a VSMC response by cellular crosstalk. A good candidate for this regulation is the endothelial-specific coreceptor plexinD1 which forms a complex with SEMA3C receptor NRP1 to sense shear stress imparted by blood flow variation [15].

Relaxation of VSMC could lead to reduced vascular tone [10, 16]. For systemic hemodynamics, this effect decreased MAP and caused renal hypoperfusion. For renal hemodynamics, this effect decreased vascular resistance and therefore augmented RBF after MAP change but also aggravated renal tissue edema in AKI [6]. Nevertheless, further investigations utilizing tissue-specific *Nrp1* knock-out animals should determine the endothelial versus SMC contribution to the SEMA3C response.

There are some limitations of our current study. We only studied the impact of SEMA3C on a group of male mice. Assuming that gender could influence renal hemodynamics in both physiological [17] and pathological conditions [18], a complementary study should consider including both genders in the tested groups. In addition, systemic and renal hemodynamics could be affected by aging, mainly through arterial stiffness and autonomic dysfunction [19]. We only included 8-week-old animals

in this study, and an aging component should be added in future studies.

In summary, our data demonstrate that SEMA3C can reduce systemic arterial pressure and cause hemodynamic changes in the kidney. Our study reveals a novel effect of SEMA3C on modulating systemic and renal hemodynamics, as well as contributing to a deeper understanding of its role in the pathophysiological context.

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Statement of Ethics

Experimental procedure was approved by the French Ethics Committee APAFIS #11991.

Conflict of Interest Statement

The authors have declared that no conflict of interest exists.

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Author Contributions

Sandrine Placier, Anxiang Cai, Liliane Louedec, Perrine Frère, Souhila Ouchelouche, and Amelie Calmont conducted the experiments and acquired data. Amelie Calmont designed the research study. Christos Chatziantoniou and Amelie Calmont provided the funding. Anaxiang Cai, Sandrine Placier, Amelie Calmont, and Christos Chatziantoniou wrote the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.