Histamine receptor agonist alleviates severe cardiorenal damages by eliciting anti-inflammatory programming

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Heart failure and chronic kidney disease are major causes of morbidity and mortality internationally. Although these dysfunctions are common and frequently coexist, the factors involved in their relationship in cardiorenal regulation are still largely unknown, mainly due to a lack of detailed molecular targets. Here, we found the increased plasma histamine in a preclinical mouse model of severe cardiac dysfunction, that had been cotreated with angiotensin II (Ang II), nephrectomy, and salt (ANS). The ANS mice exhibited impaired renal function accompanied with heart failure, and histamine depletion, by the genetic inactivation of histidine decarboxylase in mice, exacerbated the ANS-induced cardiac and renal abnormalities, including the reduction of left ventricular fractional shortening and renal glomerular and tubular injuries. Interestingly, while the pharmacological inhibition of the histamine receptor H3 facilitated heart failure and kidney injury in ANS mice, administration of the H3 agonist immethridine (Imm) was protective against cardiorenal damages. Transcriptome analysis of the kidney and biochemical examinations using blood samples illustrated that the increased inflammation in ANS mice was alleviated by Imm. Our results extend the pharmacological use of H3 agonists beyond the initial purposes of its drug development for neurogenerative diseases and have implications for therapeutic potential of H3 agonists that invoke the anti-inflammatory gene expression programming against cardiorenal damages.

cardiorenal damages | animal model | histamine | H3 agonist | anti-inflammation

A growing body of evidence demonstrates that the physiological communication between heart and kidney is necessary to maintain cardiovascular homeostasis (1). In recent years, many investigators have become interested in this relationship with respect to cardiorenal syndrome and in the role that chronic kidney disease plays in being a strong risk factor for heart failure (2, 3).

A number of clinical studies have examined the correlation of the renin–angiotensin system (RAS), the sympathetic nervous system, oxidative stress, and inflammation in cardiorenal syndrome (4, 5). Specifically, Ang II is a vasoconstrictive hormone in RAS that plays an essential role in the regulation of blood pressure, electrolyte, and volume homeostasis (6). Its diverse actions are mediated by several types of receptors that are expressed in a variety of target tissues including heart and kidney (7). It has been thought that Ang II is closely involved in the deterioration that occurs in cardiorenal syndrome (8–10).

The details of the underlying molecular mechanisms of cardiorenal syndrome remain unclear because of the absence of useful animal models to evaluate preclinical conditions. In the study of heart failure, isoproterenol administration and thoracic transverse aortic constriction treatment have commonly been used as a preclinical animal model (11). In the field of kidney disease, the unilateral ureteral obstruction and 5/6 nephrectomy models are frequently used (12). These cardiac and renal studies have been carried out independently, and there are few animal models which are applicable for evaluating both systems together in cardiorenal regulation. Recently, it has been reported that cotreatment with ANS results in high blood pressure-induced severe cardiac dysfunction in mice (13). In this model, Ang II induces elevating blood pressure, nephrectomy reduces kidney function, and salt causes fluid retention, together producing the adverse effects seen in cardiorenal syndrome.

Biogenic amines are produced by the decarboxylation of amino acids and are delivered to tissues via blood circulation. Neuroactive

Significance

Histamine has been known to play important roles in inflammation, and its inhibition has been expected to ameliorate the pathological statement of heart failure and chronic kidney disease. In this paper, we found that histamine is elevated in the plasma of a preclinical mouse model with severe cardiac dysfunction and showed that it acts protectively rather than harmfully on heart and kidney damages in this model. In addition, we showed that a histamine H3 agonist, Imm, prevents the cardiorenal damages in this model. Accordingly, this paper will be helpful for developing new therapeutic strategies for cardiorenal syndrome, and it will serve as a basis for repositioning the application of H3 agonists to the inflammatory heart and kidney damages.

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The authors declare no competing interest.

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amines, such as adrenaline, dopamine, and noradrenaline play central roles in regulating sympathetic action, and this suggests that they may be implicated in the pathogenesis of cardiorenal damages (14). As another member of the biogenic amines, histamine catalyzed by histidine decarboxylase (HDC) from histidine, plays a key role in the inflammatory response and is found to be elevated in patients with heart failure caused by acute myocardial infarction, by reperfusion injury, or by chronic kidney disease (15–17). Although this implies the involvement of increased histamine in the development of cardiac and renal dysfunctions, the significance and specific mechanism is entirely unknown.

In this paper, we explored the molecular basis underlying cardiorenal regulation using ANS mice as a preclinical model. We found severe renal dysfunction and heart failure and identified the protective properties of increased plasma histamine in ANS mice. Imm, an agonist of the histamine receptor H3, prevented the progression of cardiorenal damages by halting the reduction in left ventricular fractional shortening, and renal glomerular, and tubule injuries. Renal transcriptome analysis suggests that Imm has a therapeutic potential in cardiorenal syndrome through its ability to induce alteration of proinflammatory transcriptional properties.

Results

Kidney Impairments in ANS Mice. To verify the possibility that ANS mice are a model for cardiorenal damages, we generated and maintained these mice for 4 wk after the treatment (*SI Appendix*, Fig. S1*A*) and confirmed the cardiac dysfunction that had been shown in a previous study (13). The blood pressure of the Sham group was constant throughout for 4 wk after treatment (Fig. 1*A*), whereas it was significantly elevated from 1 wk after ANS treatment and persisted until 4 wk, associated with increased water intake and urine volume (*SI Appendix*, Fig. S1 *B* and *C*) and suppressed body weight gain (*SI Appendix*, Fig. S1*D*). We observed progressive exacerbation of decreased cardiac contractility (Fig. 1*B*), hypertrophy (*SI Appendix*, Fig. S1*E*), and up-regulated gene expression of injury markers, *Nppa* (atrial natriuretic peptide [ANP]) and *Nppb* (brain natriuretic peptide [BNP]) (*SI Appendix*, Fig. S1*F*). The Sirius-red staining revealed the right and left ventricular interstitial fibroses in the hearts of ANS mice compared with the Sham group (*SI Appendix*, Fig. S2 *A–C*).

In addition to cardiac damages, creatinine clearance (Fig. 1*C*) was significantly decreased, but urinary albumin (Fig. 1*D*), urinary neutrophil gelatinase-associated lipocalin (NGAL), and kidney NGAL mRNA expression (*SI Appendix*, Fig. S1 *G* and *H*) as markers of glomerular and tubular injuries were increased in ANS mice compared to mice that underwent a Sham procedure. In ANS mice, plasma creatinine level and blood urea nitrogen (BUN) were significantly elevated (*SI Appendix*, Fig. S1 *I* and *J*), and urinary creatinine was decreased (*SI Appendix*, Fig. S1*K*). Histological examination of kidney tissues in ANS mice using the Sirius-red staining also revealed greater interstitial fibrosis in perivascular regions than those in the Sham group (*SI Appendix*, Fig. S3 *A–C*). Additionally, electronic microscopy of glomeruli in ANS mice showed the effacement of the foot process in some podocytes (*SI Appendix*, Fig. S4*A*, black arrow) where microvilli are visible (*SI Appendix*, Fig. S4*B*, black arrowhead) and local mesangiolysis with the disappearance of the glomerular basement membrane is seen in the mesangial angle (*SI Appendix*, Fig. S4*C*, black arrow). These changes demonstrate severe kidney damages and some degree of injury in the interstitial tubules and glomeruli of ANS mice. Despite the structural changes found in the aorta from ANS mice (*SI Appendix*, Fig. S1*L*), severe edema and congestion in the lung were not observed (*SI Appendix*, Fig. S5 *A* and *B*). Collectively, we found that ANS mice present kidney dysfunction with heart failure under the high salt and elevated blood pressure conditions.

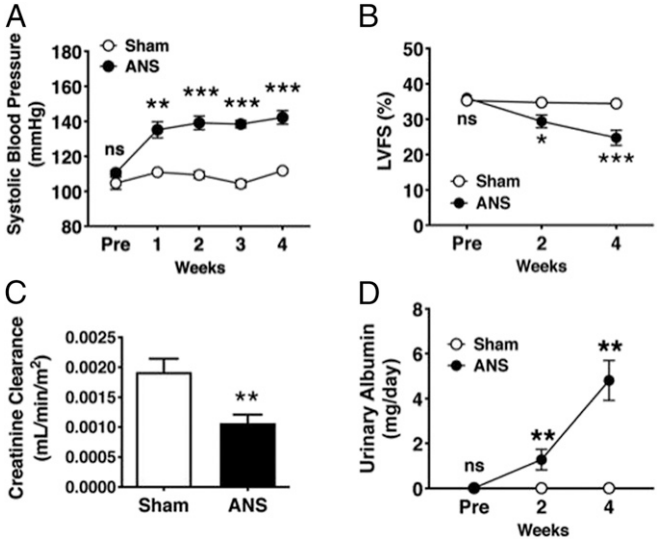

Fig. 1. Cardiorenal damages in ANS mice. Time-dependent changes in *A* the systolic blood pressure (n = 5–10) and (*B*) cardiac contractility (n = 5–12). (*C*) Creatinine clearance in Sham and ANS mice at 4 wk after ANS treatment (n = 5 to 6). (*D*) Time-dependent changes in the urinary albumin levels (n = 5 to 6). Data are shown as means ± SEM. Statistical differences were determined using Student's t test, Welch's t test, or Mann–Whitney U test (ns, not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ as compared to Sham mice).

Severe Dysfunction of Heart and Kidney in ANS Mice by Lack of Histamine. It has been reported that plasma histamine levels are elevated with chronic renal disease and nephrotic syndrome or cardiac insufficiency (15–17). To evaluate the alteration of plasma histamine in this model, we quantified the levels of histamine by using liquid chromatography–tandem mass spectrometry (LC–MS/MS) (*SI Appendix*, Fig. S6). As shown in Fig. 2*A*, plasma histamine in mice with ANS treatment was significantly increased compared with that in the Sham group at 2 wk, and it was further elevated in the ANS group at 4 wk. These results prompted us to ascertain the role of histamine in cardiorenal regulation. To examine whether elevated plasma histamine in ANS mice is protective or detrimental for cardiorenal function, we used HDC knockout (KO) mice (18), those of which are unable to synthesize histamine in vivo. We generated HDC-KO/ANS mice and found that their blood pressure was similarly elevated compared with that in HDC-wild-type (WT)/ANS mice (Fig. 2*B*), but the enhanced cardiac hypertrophy and the decreased left ventricular fractional shortening (LVFS) were observed in HDC-KO/ANS mice (Fig. 2 *C* and *D*). Moreover, as parameters of kidney function, creatinine clearance (Fig. 2*E*) and urinary albumin excretion (Fig. 2*F*) in HDC-KO/ANS mice showed a greater degree of deterioration than those in WT/ANS mice, but the body weight gain, daily water intake, and urine volume were not significantly changed (*SI Appendix*, Fig. S7 *A–C*). Although the urinary NGAL level of HDC-KO/ANS mice was not different from that in HDC-WT/ANS mice (*SI Appendix*, Fig. S7*D*), the NGAL mRNA expression of the kidneys in HDC-KO/ANS mice was significantly up-regulated (*SI Appendix*, Fig. S7*E*). These results indicate that the lack of histamine in vivo accelerated both cardiac and kidney dysfunctions in the ANS model and suggest that histamine plays a protective role on the cardiorenal damages induced by ANS.

Deteriorated Heart and Kidney Damages in the ANS Model by Inhibition of H3. Four subtypes of the histamine receptor, H1, H2, H3, and H4, have been identified (19). To determine which subtype is involved in the protective effect of histamine, we administered subtype-specific receptor blockers to ANS mice. There was no difference in LVFS of ANS mice from that of the groups treated

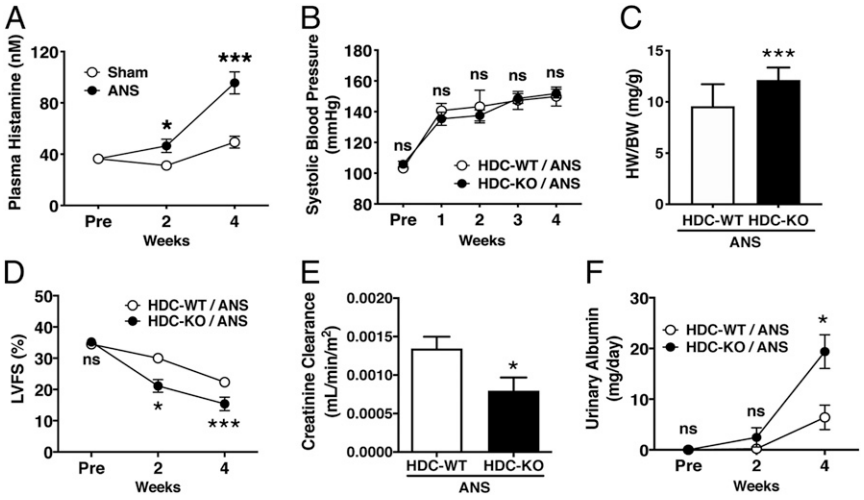

Fig. 2. Plasma histamine level in ANS mice and augmentation of heart and kidney damages in HDC-KO/ANS mice. Time-dependent changes in *A* plasma histamine levels (n = 6–9) and (*B*) systolic blood pressure (n = 6–17). Progressive exacerbation of (*C*) cardiac hypertrophy (n = 9) and (*D*) decreased cardiac contractility (n = 8–12) in HDC-WT/ANS and HDC-KO/ANS mice. (*E*) Creatinine clearance in HDC-WT/ANS and HDC-KO/ANS mice at 4 wk after ANS treatment (n = 5–7). (*F*) Time-dependent changes in the urinary albumin level (n = 5–7). Data are shown as means ± SEM. Statistical differences were determined using Student's t test, Welch's t test, or Mann–Whitney U test (ns, not significant; $^*P < 0.05$; $^{***}P < 0.001$ as compared to HDC-WT/ANS mice).

with an H1-specific blocker (H1B) cetirizine or an H2 blocker (H2B) ranitidine at 4 wk after the ANS intervention (*SI Appendix*, Fig. S8*A*, blue or green square). In contrast, ANS mice given the H3 blocker (H3B) carcinine showed significantly decreased LVFS compared to the ANS group after 2 and 4 wk (*SI Appendix*, Fig. S8*A*, red square). Among the groups treated with histamine receptor blockers, there was no significant changes in blood pressure (*SI Appendix*, Fig. S8*B*), cardiac hypertrophy (*SI Appendix*, Fig. S8*C*), or expression of the ANP and BNP genes in ANS mice after 4 wk (*SI Appendix*, Fig. S8*D*).

In terms of renal function, the levels of urinary albumin (*SI Appendix*, Fig. S9*A*), water intake (*SI Appendix*, Fig. S9*B*), urine volume (*SI Appendix*, Fig. S9*C*), and kidney NGAL mRNA expression (*SI Appendix*, Fig. S9*D*) were not different in ANS mice compared to the groups given the H1B, H2B, or H3B at 4 wk. On another front, in H3B-treated ANS mice, creatinine clearance (*SI Appendix*, Fig. S9*E*) was decreased, and urinary NGAL excretion (*SI Appendix*, Fig. S9*F*) was increased compared with those in ANS mice treated with saline at 4 wk. Creatinine clearance in H1B-treated ANS mice was also decreased at 4 wk compared with those in ANS mice treated with saline (*SI Appendix*, Fig. S9*E*). Our data show that only H3B deteriorated both cardiac and renal functions in ANS mice, which is similar to the observations in histamine-depleted HDC-KO/ANS mice (Fig. 2 *C*–*F*). These results prompted us to investigate the effect of H3 agonism on cardiorenal regulation.

Prevention of Heart and Kidney Dysfunctions by H3 Agonist Administration in the ANS Model. We used the histamine H3-specific agonist Imm to evaluate the effect of H3 agonism in the ANS model. The blood pressure and body weight gain in the Imm-treated ANS group were not different from those in the saline-treated ANS group (*SI Appendix*, Fig. S10 *A* and *B*). On the other hand, cardiac hypertrophy was significantly ameliorated (Fig. 3*A*), and the reduction of LVFS was significantly recovered (Fig. 3*B*) in the Imm-treated ANS group compared with that in the saline-treated ANS group. The Imm treatment attenuated the ventricular interstitial fibrosis observed in the Sirius-red staining and the increased expression of the collagen type I α 1 gene in the hearts of ANS mice (*SI Appendix*, Fig. S2 *D* and *E*).

Imm treatment did not affect water intake, urine volume, plasma BUN, and plasma creatinine, whereas increased urinary creatinine in ANS mice (*SI Appendix*, Fig. S10 *C*–*G*) resulted in the recovery of creatinine clearance (Fig. 3*C*). In addition, kidney damages indicated by the increased levels of urinary albumin, urinary NGAL excretion, kidney NGAL mRNA expression, plasma cystatin C, and urinary β2-microglobulin observed in the saline-treated ANS group were markedly attenuated in the Imm-treated ANS group (Fig. 3 *D*–*F* and *SI Appendix*, Fig. S10 *H* and *I*). Although the fibrosis in glomeruli of the saline-treated ANS group were not observed (*SI Appendix*, Fig. S3*D*), Imm treatment significantly suppressed the enhanced interstitial fibroses in perivascular regions and the increased expression of the collagen type I α 1 gene in the kidney of ANS mice (*SI Appendix*, Fig. S3 *E* and *F*). Moreover, histological examination using periodic acid–Schiff staining revealed the enlargement of glomeruli and protein casts in renal tubules of the kidneys of ANS mice (*SI Appendix*, Fig. S11*A*). The diffused mesangial growth and cell proliferation were also observed (*SI Appendix*, Fig. S11*B*), and the segmental sclerosis in the glomerulus was rarely seen in the kidney from ANS mice (*SI Appendix*, Fig. S11*B*: ANS/saline, lower panel). We found that Imm attenuated these changes in the kidneys from ANS mice (*SI Appendix*, Fig. S11 *A*–*C*), suggesting that the structural abnormalities in glomeruli and renal tubules contribute to the dysfunction of the kidneys in ANS mice. These data clearly indicate that Imm plays a protective role in the heart and kidney dysfunctions induced by ANS.

Suppression of ANS-Induced Kidney Inflammation by an H3 Agonist. To understand the molecular basis of the renal damage induced by the ANS model and the protective effects of Imm, RNA-sequencing (RNA-seq) analysis of the kidney was performed. Principle component analysis (PCA) showed a clear differentiation between the Sham (blue dots) and the ANS (green dots) groups, and the Imm-treated ANS group (red dots) was also separated from the ANS group (Fig. 4 *A* and *B*). Filtering characteristics of fold change > 2 (false discovery rate $P < 0.05$) were used to identify the differentially expressed genes (DEGs). Volcano plot analysis indicated that the gene expression profile in the ANS group markedly differed from that of the Sham group and that the Imm-treated ANS group showed gene expression profiles that are distinct from those in the ANS group and from the Sham group (*SI Appendix*, Fig. S12 *A*–*C*). Unsupervised clustering of reads per kilobase per million values showed identically

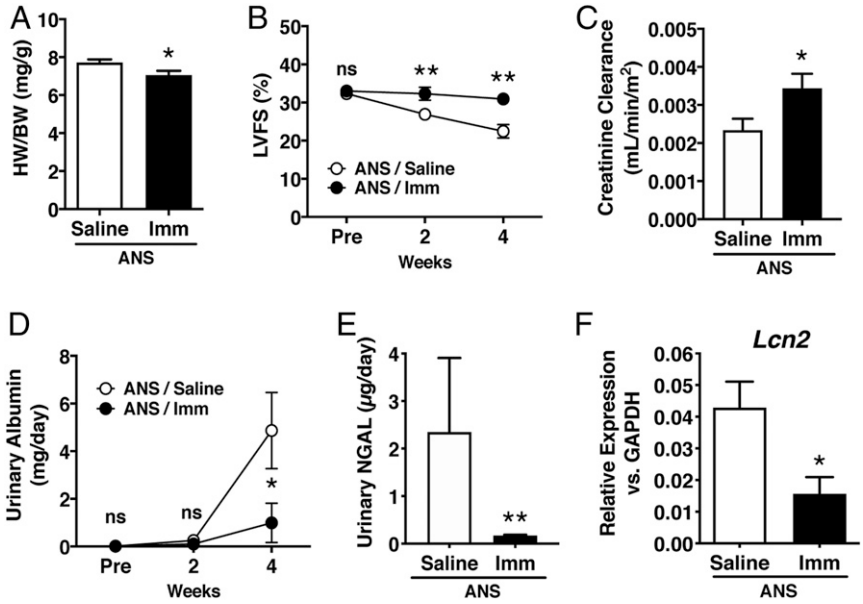

Fig. 3. Prevention of ANS-induced heart and kidney dysfunctions by the H3 agonist. Effect of H3 agonist treatment on *A* cardiac hypertrophy ($n = 9$), (*B*) contractility ($n = 8$–10), (*C*) creatinine clearance ($n = 6$), (*D*) urinary albumin ($n = 5$), (*E*) urinary NGAL ($n = 5$), and (*F*) mRNA expression level of the NGAL (Lcn2) gene ($n = 6$) in Imm-treated ANS mice. Data are shown as means ± SEM. Statistical differences were determined using Student's *t* test or Mann–Whitney *U* test (ns, not significant; $*P < 0.05$; $**P < 0.01$ as compared to ANS/saline mice).

separated clustering of expression profiles between the Sham and the ANS groups, suggesting that each group displayed a unique gene expression signature. The Imm-treated ANS group also exhibited a different signature from that of both the Sham and the ANS groups (*SI Appendix*, Fig. S12*D*).

In comparison with the Sham group, 1,283 (1,010 up- and 273 down-regulated) unique genes were significantly changed in the ANS group (Dataset S1). Meanwhile, the dataset of ANS kidneys treated with Imm showed significant changes in 234 (65 up- and 169 down-regulated) genes compared with the ANS group. Using these results, we compared the DEGs in the ANS- and Imm-treated groups to identify the genes on which the H3 agonist exerted renal protective effects. We focused on the genes in which the expression levels were up-regulated in the ANS group and down-regulated in the Imm group. Among transcripts, 169 DEGs in the Imm group were down-regulated relative to the ANS group (Fig. 4*C*, blue circle), and of these, 150 transcripts were overlapped with the transcripts that were up-regulated in the ANS group (Fig. 4*C*, gray circle).

Gene ontology (GO) enrichment analysis in biological processes and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using the ToppGene Suite (20) analyses of these differentially expressed 150 transcripts revealed gene sets with significantly down-regulated responses to proinflammatory signals in the Imm-treated ANS group versus the ANS group. The proinflammatory signals included inflammatory response (GO:0002526 and GO:0006954), immune response (GO:0006955), and the tumor necrosis factor (TNF) signaling pathway (KEGG:812256) (Fig. 4*D*). In the kidney, Imm treatment led to a reduction in the ANS-induced elevation of mRNA expression in the *Il6*, *Tnf*, *C3*, and chemokine *Cxcl2* genes (*SI Appendix*, Fig. S13*A*), the major regulators of the acute phase response in various proinflammatory cascades (21–23). Furthermore, Imm attenuated the increased mRNA expression of *Il6*, *C3*, and *Cxcl2* by ANS treatment in the heart and liver (*SI Appendix*, Fig. S13 *B* and *C*). These analyses allowed us to validate that the levels of serum amyloid A (SAA) and C-reactive protein (CRP), the systemic acute inflammatory markers (24, 25) in ANS- and Imm-treated ANS mice. As shown in Fig. 4 *E* and *F*, plasma SAA and CRP levels were significantly elevated in ANS mice and were lowered by the administration of Imm. These data implicate the H3 agonist plays a role in transcriptional regulation of proinflammatory genes in the kidneys of ANS mice.

Discussion

The molecular mechanisms and factors involved in the progression or slowing of cardiorenal damages are not yet well understood. Greater clarification would be beneficial for developing new therapeutic approaches for cardiorenal syndrome. To this end, various rodent models of cardiorenal syndrome have been used including myocardial infarction with unilateral nephrectomy (26) or with a 5/6 nephrectomy (27), and doxorubicin-induced cardiorenal toxicity (28). The ANS model has recently been developed as a promising heart failure model with the focus on the cardiac injury in these mice (13). In the present paper, we also found reduced renal function, including severe albuminuria and tubular damage, along with the expected cardiac dysfunction (Fig. 1 and *SI Appendix*, Figs. S1–S4). These findings indicate that ANS mice exhibit symptoms of both heart and kidney damages, similar to those seen in cardiorenal syndrome and, therefore, can be considered as potential candidates for a simple preclinical animal model of this syndrome. We also identified histamine as a factor involved in cardiorenal homeostasis using HDC-KO and ANS mice and illustrated that an H3 histamine agonist, Imm, has a protective effect against both the heart and kidney dysfunctions induced by ANS treatment.

Histamine plays a central role in the body's allergic response, particularly, in the skin, nose, throat, and lung. These responses are crucially important for the inflammatory reaction, which is pivotal for the overall Imm response (29). One of our key findings was that plasma histamine was increased by ANS treatment (Fig. 2*A*). Regarding the association of histamine with renal injury, it has been previously demonstrated that a bolus injection of the platelet activating factor causes histological damages in the proximal tubule and significant histamine release in isolated perfused rat kidneys (30). Enhanced histamine synthesis in the kidneys of rats with streptozotocin-induced diabetes has also been previously reported (31). While the functional relevance of

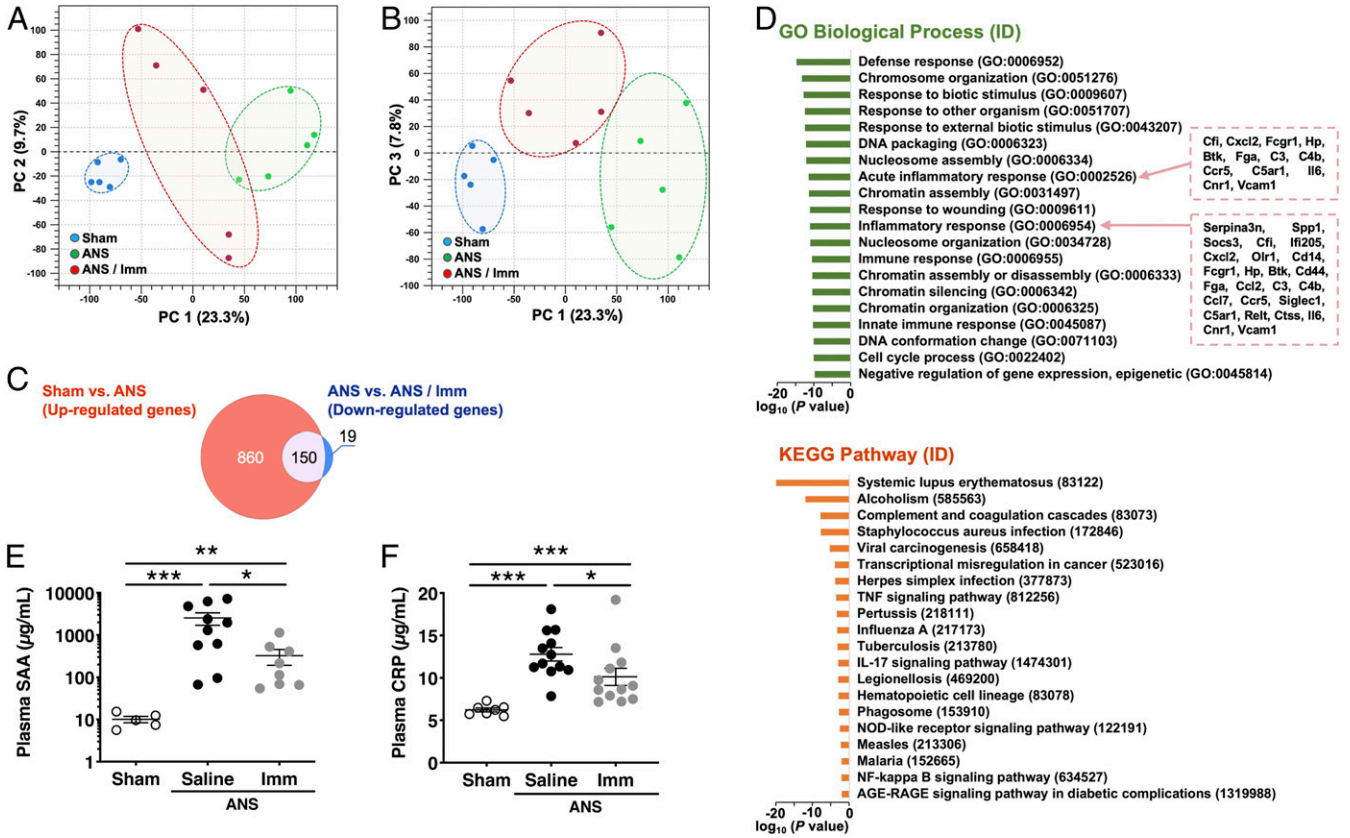

Fig. 4. H3 agonist-induced expression of anti-inflammatory programming genes in the kidney from ANS mice. (*A* and *B*) PCA of renal transcripts from Sham, ANS, and Imm-treated ANS mice. (*C*) Venn diagram, the intersection of genes between the up-regulated genes in Sham versus ANS and down-regulated genes in ANS versus ANS/Imm was identified. (*D*) Distribution of the GO biological process (*Top*) and KEGG pathway (*Bottom*) mapped from 150 genes in which the expression levels are up-regulated in the ANS group and down-regulated in the ANS group treated with Imm. Effect of H3 agonist treatment on the (*E*) SAA level ($n = 5$–10) and (*F*) plasma CRP level ($n = 7$–12) in Sham, ANS, and Imm-treated ANS mice. Data are shown as means ± SEM. Statistical differences were determined using Student's *t* test, Welch's *t* test, or Mann–Whitney *U* test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ as compared to Sham).

histamine and renal damage was not fully understood through this demonstration, the elevated histamine was suggestive of its involvement in the progression of renal lesions. Unexpectedly, however, as shown in Fig. 2, ANS/HDC-KO mice exhibited severe kidney dysfunction in addition to heart failure, indicating that histamine is a novel factor participating in cardiorenal regulation and has a protective quality against the development of the heart and kidney damages induced by ANS treatment.

Histamine exerts its biological activity via the interaction with four distinct G protein-coupled histamine receptors. Among these histamine receptor subtypes, H1 and H2 are expressed in the hearts and kidneys, and H3 is expressed in the ending of sympathetic nerves in these tissues (32, 33). With regard to the relationship between histamine receptor blockers and cardiovascular disease, it has been shown that H2B can attenuate the ischemia and reperfusion induced in the myocardia of mice (34) and has improved the clinical condition of heart failure in humans (35). It should be noted that cardiac dysfunction is enhanced only by H3B administration at both 2 and 4 wk after ANS treatment (*SI Appendix*, Fig. S8*A*), suggesting the protective effect of histamine on ANS-induced cardiac damage. Previous studies have shown that H3B increased norepinephrine overflow in Langendorff-perfused guinea pig hearts subjected to ischemia reperfusion injury, leading to arrhythmias (36). These findings suggest that histamine H3B has a detrimental effect on a progressive condition of heart failure.

Concerning the association between histamine blockade and renal injury, the protective role of H1B on streptozotocin-induced diabetic nephropathy and vascular dysfunction has been described (31). According to this view, H1B preconditioning alleviated the ischemia reperfusion injury of the kidneys in mice (37). In these studies, antioxidative stress and anti-inflammatory effects were suggested as the protective actions of H1B. Moreover, H2B reduced histamine-induced monocyte migration across the glomerular endothelial cells in vitro (38). In Fig. 2, we identified the enhanced heart and kidney dysfunctions in ANS/HDC-KO mice, suggesting the protective effect of histamine. The reduction of creatinine clearance was aggravated at 4 wk in ANS mice when they were treated with H1B or H3B (*SI Appendix*, Fig. S9*E*). From these results, we ascertained that H3B, not H1B nor H2B, complicated both the cardiac and the renal failures in ANS mice.

In a contrasting situation of H3 antagonism, it is increasingly recognized that H3 agonism is involved in the regulation of cardiac and renal damages. For instance, in a rat model of isoproterenol-induced myocardial infarction, imetit, an H3 agonist, blunted the elevation of antioxidant markers and histopathological alterations in the heart (39). It has also been documented that (R)α-methylhistamine, another H3 agonist, inhibited kidney noradrenergic neurotransmission in anesthetized dogs (40). These examples imply that H3 agonists have a protective quality in cardiac and renal dysfunctions.

H3 and H4 are highly sensitive to histamine in comparison with H1 and H2 (41). Although it has recently been reported that serum histamine levels in patients with heart failure and a mouse model of myocardial infarction were increased (17), this suggested a preventive role of H1 and H2 for the development of heart failure. On the other hand, as H3 has the 1,000-fold higher

affinity for histamine than H1 and H2 (41), one might consider that the change in local and systemic concentrations of histamine and the presence of its receptor subtypes in tissues add differential specificity to histamine responses in vivo. In this regard, a 2.63-fold increase in plasma histamine concentration in ANS mice at 4 wk after treatment with that in pretreatment (Fig. 2*A*) may be able to act on H3 prior to H1 and H2. To further evaluate the selective role of H3 agonists on ANS-induced cardiorenal damages, we chose Imm because it exhibits a 300-fold selectivity at H3 over the related H4 (42). We verified that Imm significantly prevented the development of the ANS-induced dysfunction of the heart and kidney (Fig. 3), suggesting an alternative potential of Imm to alleviate the progression of cardiac and renal damages.

Among the differentially expressed kidney genes in the Sham-, ANS-, and Imm-treated ANS groups, we showed that the proinflammatory signal related gene sets up-regulating in the ANS group were down-regulated in the Imm-treated group (Fig. 4*D*) and that systemic inflammatory markers and proinflammatory responses were reduced by Imm treatment (Fig. 4 *E* and *F*). The regulatory function of H3 on the inflammatory response has been previously described in the context of the central nervous system (CNS). H3 negatively regulates susceptibility to autoimmune inflammatory disease of the CNS (43, 44) and attenuates peripheral inflammatory signals involved in the neurogenic control of immune responses (45). Furthermore, the significance of IL-6 and TNF on the acute phase inflammatory reaction has been established (21, 46). This knowledge may corroborate our findings regarding that inflammatory responses have an effect upon ANS-induced tissue damages and support our data suggesting that Imm-induced programming of anti-inflammatory gene expression plays a crucial role on the prevention of the tissue damages in ANS mice.

In addition to the kidney, the gene expression profiles of the heart from ANS mice were examined using RNA seq (*SI Appendix*, Fig. S14 *A–E*). Although, in comparison with the Sham group, 85 (60 up- and 151 down-regulated) unique genes were significantly changed in the ANS group (Dataset S2), there were no functionally identified genes among the DEGs found in the ANS- and Imm-treated groups in which the H3 agonist works for cardioprotection. In contrast, of 60 up-regulated genes, gene sets with significantly up-regulated responses to fibrotic processes included the extracellular matrix organization (GO:0030198) and extracellular matrix (ECM) receptor interaction (KEGG:83068) were identified by GO enrichment analysis and the KEGG pathway analysis (*SI Appendix*, Fig. S14*F*). In the hearts of ANS mice, *SI Appendix*, Fig. S2 showed obvious fibrotic changes at 4 wk after ANS treatment, consistent with the characteristics of ANS mice in which the severe fibrotic damages were evident (13), and *SI Appendix*, Fig. S3 *A* and *B* indicated that the H3 agonist attenuated fibrotic response and recovered contractility and hypertrophy in the hearts of ANS mice. Interestingly, the agonist suppressed inflammatory genes including IL-6 and C3 in the hearts from ANS mice (*SI Appendix*, Fig. S13*B*), suggesting that it has a cardiac anti-inflammatory property.

In conclusion, our paper reveals that plasma histamine is elevated in mice with cardiorenal damages induced by ANS treatment and that H3 agonism plays an important role in the protective actions on both heart and kidney damages through the alteration of gene expression in the anti-inflammatory programming of both tissues. Therapeutic targeting of the regulation of anti-inflammatory programming using the selective H3 agonist could, therefore, be beneficial in the treatment or prevention of cardiorenal dysfunction.

Materials and Methods

Details of the methods used in this paper, such as the animal model, ANS heart failure model, sampling materials, physiological analysis, urine collection, kidney function analysis, histological analysis, transmission electron microscopy, sample processing for LC-MS/MS analysis, LC-MS/MS analysis of plasma histamine, RNA analysis, transcriptome analysis, measurement of SAA, CRP, BUN, cystatin-C, and statistical analysis are described in the *SI Appendix*. All animal experiments in this study were carried out humanely after approval from the Institutional Animal Experiment Committee of the University of Tsukuba. Experiments were performed in accordance with the Regulation of Animal Experiments of the University of Tsukuba, and the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Data Availability. All data needed to evaluate the conclusions in the paper are present in this paper. RNA-seq data were deposited in the NCBI's Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE100635).

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