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AT1 receptor autoantibodies mediate effects of metabolic syndrome on dopaminergic vulnerability

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ABSTRACT

The metabolic syndrome has been associated to chronic peripheral inflammation and related with neuroinflammation and neurodegeneration, including Parkinson's disease. However, the responsible mechanisms are unclear. Previous studies have involved the brain renin-angiotensin system in progression of Parkinson's disease and the angiotensin receptor type 1 (AT1) has been recently revealed as a major marker of dopaminergic vulnerability in humans. Dysregulation of tissue renin-angiotensin system is a key common mechanism for all major components of metabolic syndrome. Circulating AT1 agonistic autoantibodies have been observed in several inflammation-related peripheral processes, and activation of AT1 receptors of endothelial cells, dopaminergic neurons and glial cells have been observed to disrupt endothelial blood -brain barrier and induce neurodegeneration, respectively. Using a rat model, we observed that metabolic syndrome induces overactivity of nigral pro-inflammatory renin-angiotensin system axis, leading to increase in oxidative stress and neuroinflammation and enhancing dopaminergic neurodegeneration, which was inhibited by treatment with AT1 receptor blockers (ARBs). In rats, metabolic syndrome induced the increase in circulating levels of LIGHT and other major pro-inflammatory cytokines, and 27-hydroxycholesterol. Furthermore, the rats showed a significant increase in serum levels of proinflammatory AT1 and angiotensin converting enzyme 2 (ACE2) autoantibodies, which correlated with levels of several metabolic syndrome parameters. We also found AT1 and ACE2 autoantibodies in the CSF of these rats. Effects of circulating autoantibodies were confirmed by chronic infusion of AT1 autoantibodies, which induced blood–brain barrier disruption, an increase in the pro-inflammatory renin-angiotensin system activity in the *substantia nigra* and a significant enhancement in dopaminergic neuron death in two different rat models of Parkinson's disease. Observations in the rat models, were analyzed in a cohort of

Abbreviations: 27-OHC, 27-Hydroxycholesterol; 6-OHDA, 6-hydroxydopamine; AAV-9 α-syn A53T, adeno-associated viral vectors serotype 9 coding for A53T mutated human alpha-synuclein; ACE2, Angiotensin converting enzyme 2; ACE2-AA, ACE2 autoantibodies; ACEi, Angiotensin Converting Enzyme Inhibitor; ADAM17, Disintegrin And Metalloproteinase (ADAM) 17; Ang1-7, Angiotensin 1–7; Ang1-9, Angiotensin 1–9; AngI, Angiotensin I; AngII, Angiotensin II; ARBs, AT1 receptor antagonists; AT1, Angiotensin type 1; AT2, Angiotensin type 2; AT1-AA, AT1 receptor autoantibodies; BBB, Blood Brain Barrier; CAND, Candesartan; CEBEGA, Center of Experimental Biomedicine of Galicia; EBD, Evans Blue Dye; CEIm-G, Galician Drug Research Ethics Committee; CI, Confidence Interval; GPCRs, G Protein-Coupled Receptors; IL-6, Interleukin 6; IL-17, Interleukin 17; IgGs, Immunoglobulins G; IQR, interquartile range; MasR, Ang1-7 /Mas receptors; MetS, Metabolic Syndrome; PRR, prorenin receptors; RAS, Renin Angiotensin System; SD, Standard Deviation; SEM, Standard Error of the Mean; TACE, Tumor necrosis factor-alpha converting enzyme; TG2, Transglutaminase 2; TH, dopaminergic; TMPRSS, Transmembrane protease serine 2; TNFSF14, Tumor Necrosis Factor Ligand Superfamily Member 14; TNF-α, Tumor necrosis factor-alpha; W, week; ZO-1, zonula occludens-1.

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parkinsonian and non-parkinsonian patients with or without metabolic syndrome. Non-parkinsonian patients with metabolic syndrome showed significantly higher levels of AT1 autoantibodies than non-parkinsonian patients without metabolic syndrome. However, there was no significant difference between parkinsonian patients with metabolic syndrome or without metabolic syndrome, which showed higher levels of AT1 autoantibodies than non-parkinsonian controls. This is consistent with our recent studies, showing significant increase of AT1 and ACE2 autoantibodies in parkinsonian patients, which was related to dopaminergic degeneration and neuroinflammation. Altogether may lead to a vicious circle enhancing the progression of the disease that may be inhibited by strategies against production of these autoantibodies or AT1 receptor blockers (ARBs).

1. Introduction

A considerable number of studies have suggested that peripheral diseases, particularly those involving chronic inflammation, affect neuroinflammation and neurodegeneration (Labandeira et al., 2021; Pugazhenthi et al., 2017; Sandu et al., 2015). However, the mechanisms connecting peripheral and central inflammation are unclear. The metabolic syndrome (MetS) has been associated to chronic peripheral inflammation and has been related with neurodegeneration, including Parkinson's disease (Leehey et al., 2017; Nam et al., 2018; Park et al., 2021; Zhang and Tian, 2014) although there are also controversial studies (Hu, 2010; Saaksjarvi et al., 2015) and the responsible mechanisms are unclear. MetS, like neurodegeneration, is a current silent epidemic disease, and it is defined by the presence of central obesity and at least 3 of the following parameters: hypertension (HT), hypertriglyceridemia, low HDL cholesterolemia, and type-2 diabetes/hyperglycemia (Etchegoyen et al., 2018).

Previous studies from our laboratory and others have involved the brain renin-angiotensin system (RAS) in progression of Parkinson's disease and other neurodegenerative diseases by enhancing neuronal oxidative stress and neuroinflammatory processes (Campos and Pacheco, 2020; Labandeira-Garcia et al., 2013; Labandeira-Garcia et al., 2017; Wright and Harding, 2019). Interestingly (Labandeira-Garcia and Parga, 2022), a recent study has shown that the population of human dopaminergic neurons most vulnerable to degeneration in Parkinson's disease can be identified by their high expression of angiotensin type-1 (AT1) gen (Kamath et al., 2022). Dysregulation of the tissue RAS also plays a major role in several of the above-mentioned components of the MetS. Moreover, it has been suggested that dysregulation of tissue RAS is a key common mechanism for all major components of MetS (de Kloet et al., 2010; Skov et al., 2014). The tissue RAS, including brain RAS, is organized into two opposite axes that balance the system. First, a pro-oxidative and pro-inflammatory axis mainly constituted by angiotensin II (AngII) with its AT1 receptors, and prorenin with its prorenin receptors. Second, an anti-oxidative and anti-inflammatory axis formed by AngII/AT2 receptors, and Ang1-7 with its receptor Mas and Mas-related receptors (Jackson et al., 2018; Labandeira-Garcia et al., 2017; Labandeira-Garcia et al., 2021). Angiotensin converting enzyme 2 (ACE2) transforms peptides of the pro-inflammatory axis (AngI and AngII) into components of the anti-inflammatory axis (Ang1-9, and particularly Ang1-7), playing a key role for a correct balance between both axes of the system (Jackson et al., 2018; Labandeira-Garcia et al., 2017; Labandeira-Garcia et al., 2021).

It is frequently considered that MetS induces the release of circulating proinflammatory cytokines that may open blood–brain barrier (BBB) and promote neuroinflammation and progression of neurodegeneration (Schuster et al., 2018; Zhan et al., 2018). However, the mechanisms of interaction between MetS and neurodegeneration are unclear, and a possible role of RAS components in mediating this interaction has not been studied. In a recent work, we have observed that Parkinson's disease animal models and patients have a significant increase in circulating and cerebrospinal fluid (CSF) levels of autoantibodies targeting RAS components, particularly AT1 receptor autoantibodies (AT1-AA, with agonistic effect on AT1 receptors) and ACE2-AA (antagonists on ACE2), which may enhance the proinflammatory RAS axis and promote neuroinflammation and dopaminergic neurodegeneration (Labandeira et al., 2022a). AT1-AA have been observed in several peripheral processes such as preeclampsia (Campbell et al., 2018; Wallukat et al., 1999), kidney graft rejection (Dragun et al., 2005) or malign hypertension (Fu et al., 2000). In these diseases AT1-AA play a major functional role, by exacerbating the Ang II/AT1 proinflammatory RAS axis. Interestingly, activation of AT1 receptors of vascular endothelial cells plays a major role in BBB disruption (Bloch et al., 2015; Faraco et al., 2016; Fleegal-DeMotta et al., 2009; Santisteban et al., 2020; Setiadi et al., 2018). However, it is not known if AT1-AA and ACE2-AA are increased in MetS and if these autoantibodies may mediate a possible enhancing effect of MetS on dopaminergic cell death and Parkinson's disease, which has been studied in the present work.

2. Materials and methods

2.1. Experimental design

Data from animal models with the corresponding controls, and from human serum of controls and patients were used in this study. All animal experiments were carried out in accordance with the Directive 2010/63/EU, European Council Directive 86/609/EEC and the Spanish legislation (RD53/2013). Animal experiments were approved by the corresponding committee at the University of Santiago de Compostela (15005/15/002) and were carried out in the Experimental Biomedicine Centre (CEBEGA; University of Santiago de Compostela). The clinical study was approved by the Galician Drug Research Ethics Committee (CEIm-G) (protocols 2017/618, 2017/590) and was carried out in accordance with the principles of the Helsinki Declaration.

In a first series of animal models (Supplementary Fig. 1), young adult rats (2–3-month-old; males) without (controls) and with metabolic syndrome (obesity, increased blood pressure, hyperglycemia) were used to study effects of metabolic syndrome. Male rats were used because it is known that female rodents are protected against HFD-induced metabolic changes (Pettersson et al., 2012). In the *substantia nigra*, we analysed changes in RAS components, markers of oxidative stress and neuroinflammation and dopaminergic neuron vulnerability. Furthermore, we analysed changes in BBB permeability, serum levels of major pro-inflammatory cytokines and 27-hydroxycholesterol (27-OHC), and particularly changes in levels of AT1-AA and ACE2-AA in serum and CSF.

Rats used for metabolic syndrome (group-A rats; n = 71) received a high fat diet with 60 % calories fat supplemented with 4 % of NaCl (D18042603; Research Diets, USA) for a period of 18 weeks. The rats were treated or not treated with the AT1 receptor blocker Candesartan (AstraZeneca). Powered candesartan was administered orally mixed with "Nocilla" hazelnut cream (Nutrexpa, Barcelona, Spain). Animals not treated with candesartan were given "Nocilla" hazelnut cream only. The dose of candesartan (candesartan cilexetil, 1 mg/Kg/day for at least 2 weeks) was based on the results of our previous studies (Rodriguez-Perez et al., 2018).

Changes in the expression of nigral RAS components were determined by RT-PCR and western blot. Proinflammatory markers particularly related with dopaminergic degeneration such as TNF-α (McCoy et al., 2011; Tansey et al., 2022), and markers of oxidative stress such as

NADPH-oxidase, were determined using enzyme-linked immunosorbent assay (ELISA) and enzymatic activity assays, respectively. Effects of MetS on dopaminergic cell vulnerability was studied by studying dopaminergic cell death after intrastriatal injection of 6-hydroxydopamine (6-OHDA). The loss of dopaminergic neurons and striatal terminals was determined by tyrosine hydroxylase (TH) immunohistochemistry, and quantifications were performed using stereological analysis with an Olympus CAST-Grid system. BBB permeability and possible disruption were studied by injection of Evans blue dye (EBD) through a femoral vein, and the subsequent analysis of cortex, striatum, and *substantia nigra* for photospectrometric detection of EBD fluorescence. Serum and CSF cytokines, 27-OHC, AT1-AA and ACE2-AA levels were quantified with specific enzyme-linked immunosorbent assay kits.

A second set of rat experiments were designed to study the effects of circulating AT1-AA infused through intraperitoneal mini-osmotic pumps (Supplementary Fig. 1). AT1-AA were obtained from serum samples of pregnant preeclamptic women, using affinity chromatography and subsequent titration with a specific enzymatic assay and quantified in a Nanodrop Spectrophotometer 252 (Thermo Fisher Scientific). AT1-AA solution was diluted to a final concentration of 0.15 μg/μL in saline solution or 0.25 μg/μL. The dose of 0.15 μg/μL was initially calculated as the lowest dose that induced a slight but significant increase in blood pressure, which showed the functionality of the infused AT1-AA. As levels of AT1-AA (human IgGs) in CSF appeared below levels of detection of our analytical procedure using 0.15 μg/μL, concentration of AT1-AA in minipumps was increased to 0.25 μg/μL in the corresponding groups. Functional effects on blood pressure were confirmed using a non-invasive pressure system meter. In a group of rats (Group-B rats; n = 24) blood and CSF were extracted one and two weeks after osmotic minipump implantation and before sacrifice. An additional group of rats (group-C rats; n = 15) were used to determine the effect of AT1-AA on BBB integrity in rats untreated and treated with the AT1 antagonist candesartan. At day 15 after osmotic mini-pump implantation, rats were killed by decapitation and the area of the *substantia nigra* in the ventral mesencephalon carefully dissected and processed for microvessel extraction followed by RT-PCR studies to determine expression of components of the tight junctions (claudin-5, occludin and zonula occludens-1). A third group of rats (group-D rats; n = 30) were used to determine the effect of circulating AT1-AA and AT1-AA plus candesartan on brain *substantia nigra*, particularly on the expression of RAS components, proinflammatory markers such as TNF-α and markers of oxidative stress such as NADPH-oxidase, determined as above. New groups of rats infused with AT1-AA were designed to study the effect of AT1-AA on dopaminergic neuron death induced by intrastriatal injection of 6-OHDA (group-E rats; n = 21) or nigral α-synuclein overexpression (group-F rats; n = 24) induced by injection of viral vectors (AAV-9 α-syn A53T), and the possible neuroprotective effect of the AT1 receptor antagonist candesartan. In these rat Parkinson's disease models, the loss of dopaminergic neurons and terminals was determined by TH immunohistochemistry and the microglial neuroinflammatory response by immunohistochemistry against OX-6 (monoclonal antiserum to major histocompatibility complex, MHC, class II). Quantifications were performed using stereological analysis with the Olympus CAST-Grid system. In addition, a control group of rats (group-G rats) were infused with AT1-AA alone (n = 6) or vehicle (n = 6), using intraperitoneally implanted mini-osmotic pumps as above, and compared with control untreated rats (n = 6) to study possible effects on dopaminergic neuron death in the absence of additional dopaminergic deleterious factors/toxins.

Observations in the rat models, particularly those related with serum levels of AT1-AA and ACE2-AA, were analyzed in a cohort of parkinsonian and non-parkinsonian patients with and without metabolic syndrome. The study was part of a retrospective observational study with a total of 106 adult non-parkinsonian controls and 117 adult Parkinson's disease patients. A total of 41 parkinsonian patients and a total of 32 non-parkinsonian subjects suffered MetS at the point of the investigation. Serum aliquots were stored at −80 °C until processed to quantify AT1-AA and ACE2-AA levels. All subjects were followed up using an electronic health information system until July 2021. AT1-AA and ACE2-AA serum levels were measured using two specific solid-phase, sandwich enzyme-linked immunosorbent assays (ELISAs) for quantitative determination of these autoantibodies.

2.2. Statistical analysis of animal experiments.

Statistical analyses were performed using SigmaPlot 11.0 (Systat Software, Inc., CA, U.S.A.). Data normality was tested with Kolmogorov–Smirnov test. When the dataset passed the normality test, parametric tests were used: Student's *t* test for two group comparisons and one-way ANOVA followed by the Student–Newman–Keuls method for multiple comparisons. For nonparametric data, multiple comparisons were carried out by Kruskal–Wallis one-way analysis of variance on ranks test followed by Student–Newman–Keuls. Pearson or Spearman's coefficients were used to study correlations between different parameters. All data were expressed as means ± SEM. Differences were considered statistically significant at $P < 0.05$. GraphPad Prism 8 software (GraphPad Inc., San Diego, CA, USA) was used to create scatter dot plot graphs.

2.3. Statistical analysis of the clinical study

Median and interquartile range (IQR) were used as central tendency and dispersion estimators, respectively. Assumptions of normality and homoscedasticity were verified using Anderson Darling test and Fligner-Killeen test, respectively. For quantitative variables, the analysis of two group comparisons were carried on using two tailed *t*-test when data followed a normal distribution. When normality assumption was rejected, Mann-Whitney *U* test was used. Welch's two-samples *t*-test was used for unequal population variances where the assumption of normality is maintained. Analysis of categorical variables was performed with Fisher's Exact Test for Count Data or Pearson's Chi-squared test. All statistical analyses were performed using R (R Core Team, 2020).

Details on the experimental procedures are included as Supplementary Methods (Appendix A).

3. Results

3.1. Characterization of MetS rat models. Proinflammatory RAS activation in the nigra of MetS rat models

Rats were followed for 18 weeks. Weight, serum levels of cholesterol, triglycerides, glucose, and blood pressure were monitored to confirm that they were significantly increased in the MetS rats used in this study relative to controls (Supplementary Fig. 2). On this basis, we studied the expression (mRNA and protein) of major RAS components in the *substantia nigra* of MetS rats as compared with control rats (Fig. 1). In the nigra of MetS rats, we observed a significant increase in expression of components of the proinflammatory axis such AT1 receptors and prorenin receptors (Fig. 1A, B), and a decrease in the expression of components of the anti-inflammatory axis including AT2 receptors, ACE2 and Mas receptors (Fig. 1C, D). These changes were inhibited by treatment of MetS rats with the AT1 receptor antagonist candesartan, which suggests that overactivation of AT1 receptors play a major role in the differences observed between MetS rats and controls in the nigral RAS. Consistent with previous observations, overactivation of the RAS proinflammatory axis was associated to significant increase in markers of inflammation, such as TNF-α (Fig. 1E), and oxidative stress, such as NADPH-oxidase activity (Fig. 1F), in the *substantia nigra*.

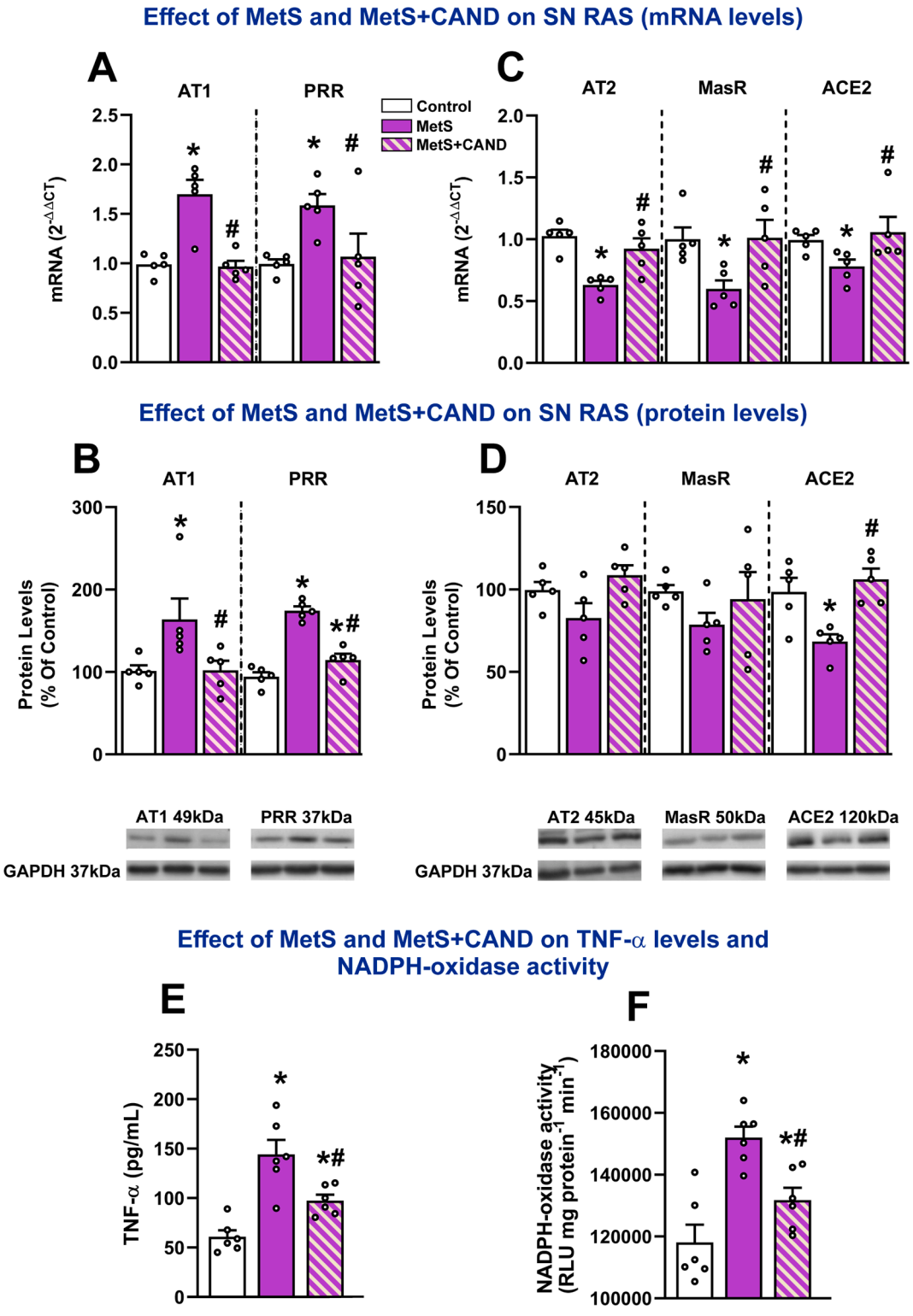

Fig. 1. Effects of metabolic syndrome (MetS) and candesartan in the *substantia nigra* of rats. mRNA (A, C) and protein (B, D) expression of pro-inflammatory (AT1 and PRR receptors; increased) and anti-inflammatory (AT2 receptors and Mas receptors, ACE2; decreased) RAS components. Markers of inflammation (TNF-α; E) and oxidative stress (NADPH-oxidase activity; F) were increased in MetS rats, which was inhibited by treatment of rats with candesartan. Data are means ± SEM. $*P < 0.05$ relative to control adult rats, $\#P < 0.05$ relative to untreated rats with MetS (one-way ANOVA with Student-Newman-Keuls Method post hoc test or Kruskal-Wallis One Way Analysis of Variance on Ranks with Student-Newman-Keuls Method post hoc test). ACE2, Angiotensin-converting enzyme 2; AT1, angiotensin type 1; AT2, angiotensin type 2; CAND, Candesartan; MasR, Mas Receptor; MetS, Metabolic Syndrome; RAS, Renin Angiotensin System; SEM, standard error of the mean; SN, *substantia nigra*.

3.2. Effect of MetS on vulnerability to dopaminergic degeneration.

A second set of MetS rats was used to know if the vulnerability to dopaminergic neurotoxin 6-OHDA was increased in the MetS model (Fig. 2). We observed that MetS rats treated with 6-OHDA showed a moderate but significant increase in dopaminergic neuron loss in the *substantia nigra* (Fig. 2A, C-E) together with a significant increase in loss of striatal dopaminergic terminals (Fig. 2B, F-H). Interestingly, the increase in vulnerability of MetS rats was inhibited by treatment of MetS rats with the AT1 antagonist candesartan, which suggests the involvement of brain RAS in the MetS-induced increase in dopaminergic vulnerability.

3.3. Increase in circulating cytokines, 27-hydroxycholesterol and RAS autoantibodies in MetS rat models

Serum levels of major pro-inflammatory cytokines, including IL-17, IL-1β, IL-6, TNF-α and LIGHT (TNFSF14) were significantly increased in MetS rats as compared with control rats (Fig. 3A). We also observed a significant increase in serum 27-OHC (Fig. 3B). MetS rats showed a significant increase in levels of circulating AT1-AA and ACE2-AA (Fig. 3C and D) and there was a significant correlation between serum levels of AT1-AA and ACE2-AA ($r = 0.670$, $P = 0.0171$) (Fig. 3E). In addition, serum levels of AT1-AA and ACE2-AA showed significant correlations with levels of major components of MetS such as cholesterol, triglycerides, and glucose, although the last was not significant for ACE2-AA (Supplementary Fig. 3). We also observed significant correlations between AT1-AA and serum levels of LIGHT, 27-OHC and interleukins IL-1β and IL-17. Serum levels ACE2-AA showed significant correlations with IL-1β and 27-OHC levels (Supplementary Figs. 4 and 5).

3.4. Increase in RAS autoantibodies in CSF of MetS rat models

We observed a significant increase in AT1-AA and ACE2-AA levels in the CSF of MetS rats as compared with controls, which suggested that circulating autoantibodies or activated B cells (intrathecal synthesis) may cross the BBB (Fig. 3F and G). This is consistent with our observations showing increase of BBB permeability in MetS rats revealed with Evans Blue dye. MetS rats injected with Evans Blue showed a significant increase dye extravasation into the studied brain regions (*substantia nigra*, striatum and cortex), revealing BBB disruption, which was inhibited by treatment of rats with the AT1 antagonist candesartan (Fig. 3H).

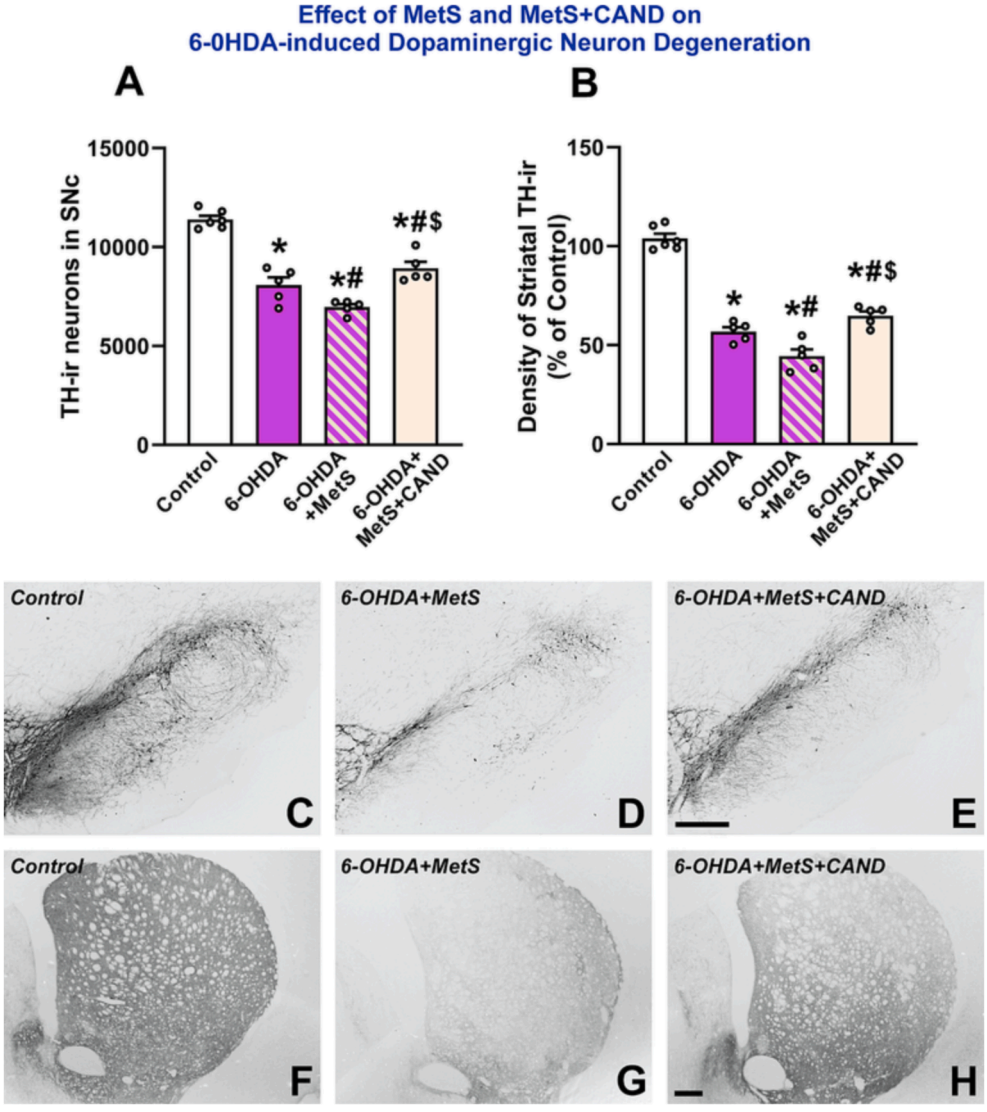

Fig. 2. Effect of MetS on vulnerability to dopaminergic degeneration. MetS enhanced the deleterious effects of intrastriatal 6-OHDA injection in rats, leading to significant increase in loss of dopaminergic (TH-ir) neurons in the nigra (A) and loss of dopaminergic terminals in the striatum (B), which was inhibited by simultaneous treatment with the AT1 receptor antagonist candesartan. Photomicrographs of rat *substantia nigra* showing dopaminergic neurons (C–E) and striatal terminals (F–H) in control rats (C, F), MetS rats treated with 6-OHDA (D, G) and MetS rats treated with 6-OHDA plus candesartan (E, H). Scale bar 200 μm. Data are given as means ± SEM. $^{*}P < 0$ 0.05 compared to the corresponding control group, $^{\#}P < 0.05$ compared to 6-OHDA group; $^{\$}P < 0.05$ compared to MetS + 6-OHDA group. One-way analysis of variance and Student–Newman–Keuls post hoc test. 6-OHDA, 6-Hydroxydopamine; CAND, Candesartan; DA, dopaminergic; MetS, Metabolic Syndrome; SEM, standard error of the mean; TH-ir, tyrosine hydroxylase immunoreactive.

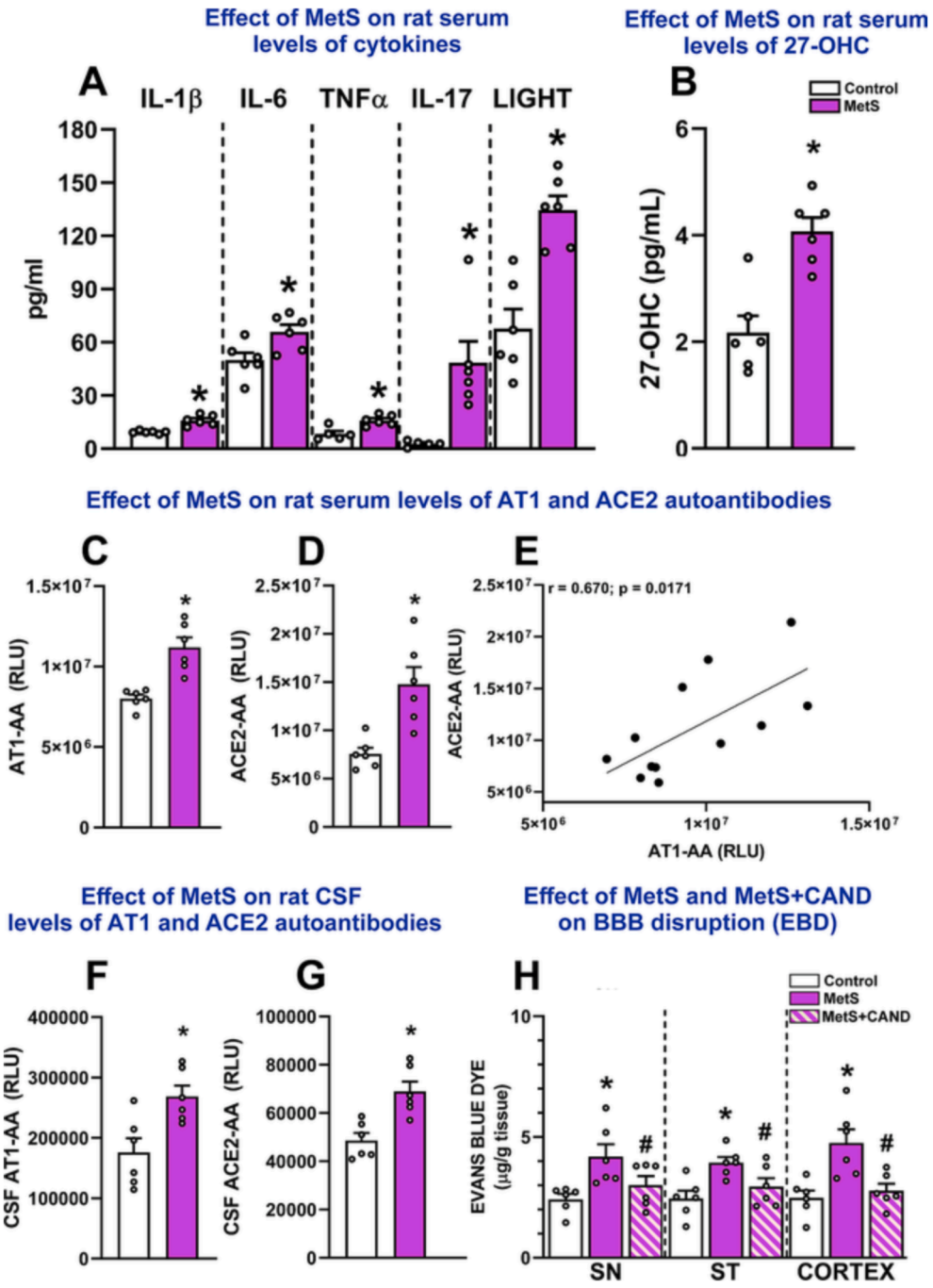

Fig. 3. Pro-inflammatory cytokines, 27-Hydroxycholesterol, AT1-AA and ACE2-AA levels in serum (A–E), and CSF (F, G), and increase in BBB permeability in MetS rats (H). Serum levels of major pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, IL-17 and LIGHT (TNFSF14) (A) and 27-OHC (B) were significantly increased in MetS rats as compared with control rats. MetS rats also showed a significant increase in levels of circulating AT1-AA and ACE2-AA (C, D) and there was a significant correlation between serum levels of AT1-AA and ACE2-AA (E, [$r = 0.670$, $P = 0.0171$]). AT1-AA and ACE2-AA levels were significantly increased in the CSF of MetS rats as compared with controls (F, G). MetS rats injected with Evans Blue showed a significant increase in dye extravasation into the *substantia nigra*, striatum and cortex, revealing BBB disruption, which was inhibited by treatment with candesartan (H). Data are given as means ± SEM. $^{*}P < 0$ 0.05 compared to the corresponding control group, $^{\#}P < 0.05$ compared to MetS group. Student-*t* test or Mann-Whitney Rank Sum Test (A–D; F–G). One-way analysis of variance and Student–Newman–Keuls post hoc test (H). BBB, blood–brain barrier; CSF, cerebrospinal fluid; EBD, Evans Blue Dye; MetS, Metabolic Syndrome; RLU, relative luminescence units; SEM, standard error of the mean; SN, *substantia nigra*; ST, striatum.

3.5. Effects of chronic intraperitoneal infusion of AT1-AA on BBB and CSF

Several previous studies have shown that levels of human immunoglobulins, including AT1-AA, administered to rodents are not significantly affected by the rodent immunological system for several weeks, and retained biological activity (Fülber et al., 2014; Zhou et al., 2008). Consistent with this, rats treated with circulating human AT1-AA, chronically released using intraperitoneally implanted mini-osmotic pumps (Alzet; 0.15 μg/μL), showed a significant increase in blood pressure as *in vivo* marker of AT1-AA activity (Fig. 4 A), as well as significant increase in levels of human IgG in serum 1 week or 2 weeks after minipump implantation (Fig. 4B). However, using this dose of AT1-AA (0.15 μg/μL), we could not detect a clear presence of human IgG in rat CSF with our detection method. Therefore, we increased concentration of AT1 (0.25 μg/μL) and checked the CSF one week after pump implantation, detecting significant levels of human IgG in rat CSF, which shows that AT1-AA can cross the BBB (Fig. 4C).

To know if circulating AT1-AA can affect the BBB, we studied the expression of major proteins involved in BBB integrity in control rats, in rats chronically treated with AT1-AA minipumps (0.15 μg/μL) and in rats treated with AT1-AA together with the AT1 receptor antagonist candesartan. Treatment with AT1-AA induced a significant decrease in levels of claudin-5, occludin and zonula occludens-1 (ZO-1) expression, which was inhibited by simultaneous treatment with candesartan (Fig. 4D). This is consistent with previous studies showing that angiotensin II can disrupt BBB by acting on endothelial AT1 receptors (see Discussion), and the present results show that serum AT1-AA can also disrupt BBB by activating AT1 receptors.

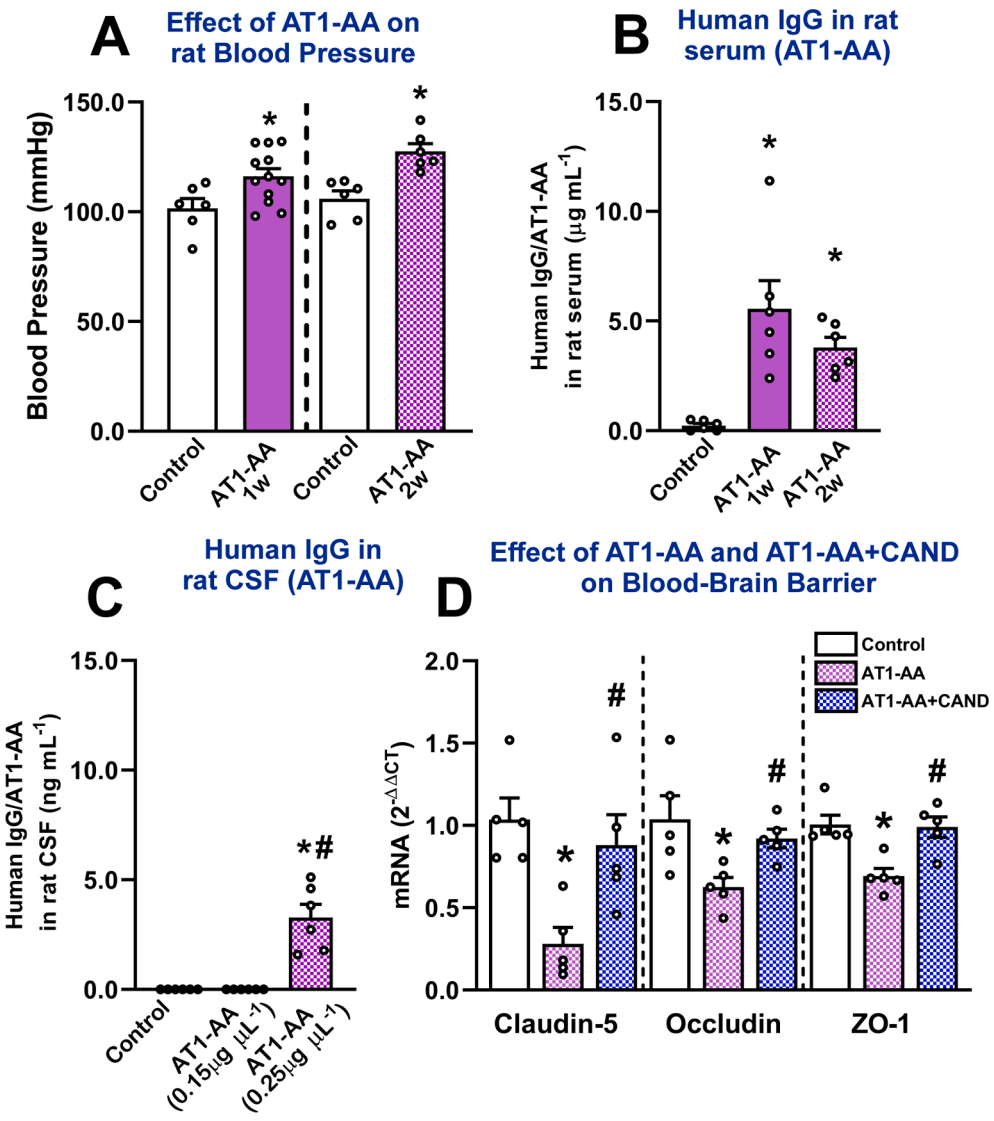

Fig. 4. AT1-AA administration using intraperitoneal minipumps. A significant increase in blood pressure revealed *in vivo* activity of AT1-AA (A). The presence of human IgGs was confirmed in serum both 1 week and 2 weeks after pump implantation (B). Detection of human IgGs in rat CSF with the present method required an increase in AT1-AA concentration in pumps (0.25 μg μL^{-1}) (C). Postmortem analysis of microvessels of the nigral region using RT-PCR revealed a significant decrease in the expression of components of the tight junctions (claudin-5, occludin and zonula occludens-1) 2 weeks after pump implantation (0.15 μg μL^{-1}), which was inhibited by simultaneous treatment with candesartan (D). Data are given as means ± SEM. $*P < 0.05$ compared to the corresponding control group, $\#P < 0.05$ relative to AT1-AA. Student's *t*-test (A), Kruskal-Wallis One Way Analysis of Variance on Ranks with Student-Newman-Keuls Method post hoc test (B, C) and One-way analysis of variance and Student–Newman–Keuls post hoc test (D). AT1-AA, AT1 receptor autoantibodies; CAND, Candesartan; CSF, cerebrospinal fluid; IgGs, Immunoglobulins G; SEM, standard error of the mean; w, week; ZO-1: zonula occludens-1.

3.6. Effects of chronic intraperitoneal infusion of AT1-AA on nigral RAS components, proinflammatory markers, and dopaminergic vulnerability

As observed in MetS rats, administration of AT1-AA led to an increase in expression of the nigral pro-inflammatory RAS components (AT1 and prorenin receptors) (Fig. 5A, C) and a decrease in expression of the anti-inflammatory RAS components (AT2 and Mas receptors) (Fig. 5 B, D). Consistent with this, administration of AT1-AA also induced a significant increase in nigral markers of oxidative stress, such as NADPH-oxidase activity (Fig. 5E), and markers of inflammation, such as TNF-α, a key mediator of dopaminergic degeneration (Fig. 5F), which was inhibited by the AT1 receptor antagonist candesartan.

Effects of AT1-AA infusion on dopaminergic vulnerability were studied using two different rat Parkinson's disease models. In a first model (intrastriatal injection of 6-OHDA) the 6-OHDA-induced loss of dopaminergic neurons in the *substantia nigra* (Fig. 6 A, C-E), and loss of dopaminergic terminals in the striatum (Fig. 6 B, F-H), was significantly increased in rats simultaneously treated with AT1-AA using intraperitoneally implanted mini-osmotic pumps. The increase in dopaminergic neuron death was inhibited by treatment with the AT1 receptor antagonist candesartan, confirming the role of AT1 receptor overactivation in the AT1-AA-induced deleterious effects. Effects of AT1-AA infusion on dopaminergic vulnerability were confirmed in a second Parkinson's disease rat model, in which we induced dopaminergic cell death using neurospecific adeno-associated viral vectors serotype 9 coding for human alpha-synuclein, under the control of a synapsin promoter, to induce dopaminergic neuron degeneration. We have described the characteristics of this model in detail elsewhere (Rodriguez-Perez et al., 2018). The loss of dopaminergic neurons in the *substantia nigra* (Fig. 6 A′, C′–E′) and the loss of dopaminergic striatal terminals (Fig. 6 B′, F′–H′) were significantly increased by peripheral AT1-AA administration and blocked by simultaneous treatment with the AT1 antagonist candesartan. As observed in our previous studies, the loss of dopaminergic neurons was accompanied by a significant increase in the microglial neuroinflammatory response, which was markedly increased by administration of AT1-AA in the present experiments and inhibited by treatment of rats with the AT1 antagonist candesartan (Supplementary Fig. 6). In addition, a group of control rats were treated with vehicle or AT1-AA alone (without any simultaneous dopaminergic deleterious factor/toxin) or vehicle, which did not induce any significant loss of dopaminergic neurons, at least with the doses used in the present study (Fig. 6 I-L).

3.7. Data from parkinsonian and non-parkinsonian patients with and without MetS. Patient characteristics

A total of 106 non-parkinsonian controls (mean age: 64.8 years old ± 9.02 Standard Deviation (SD); of which 45 were men and 51 women)

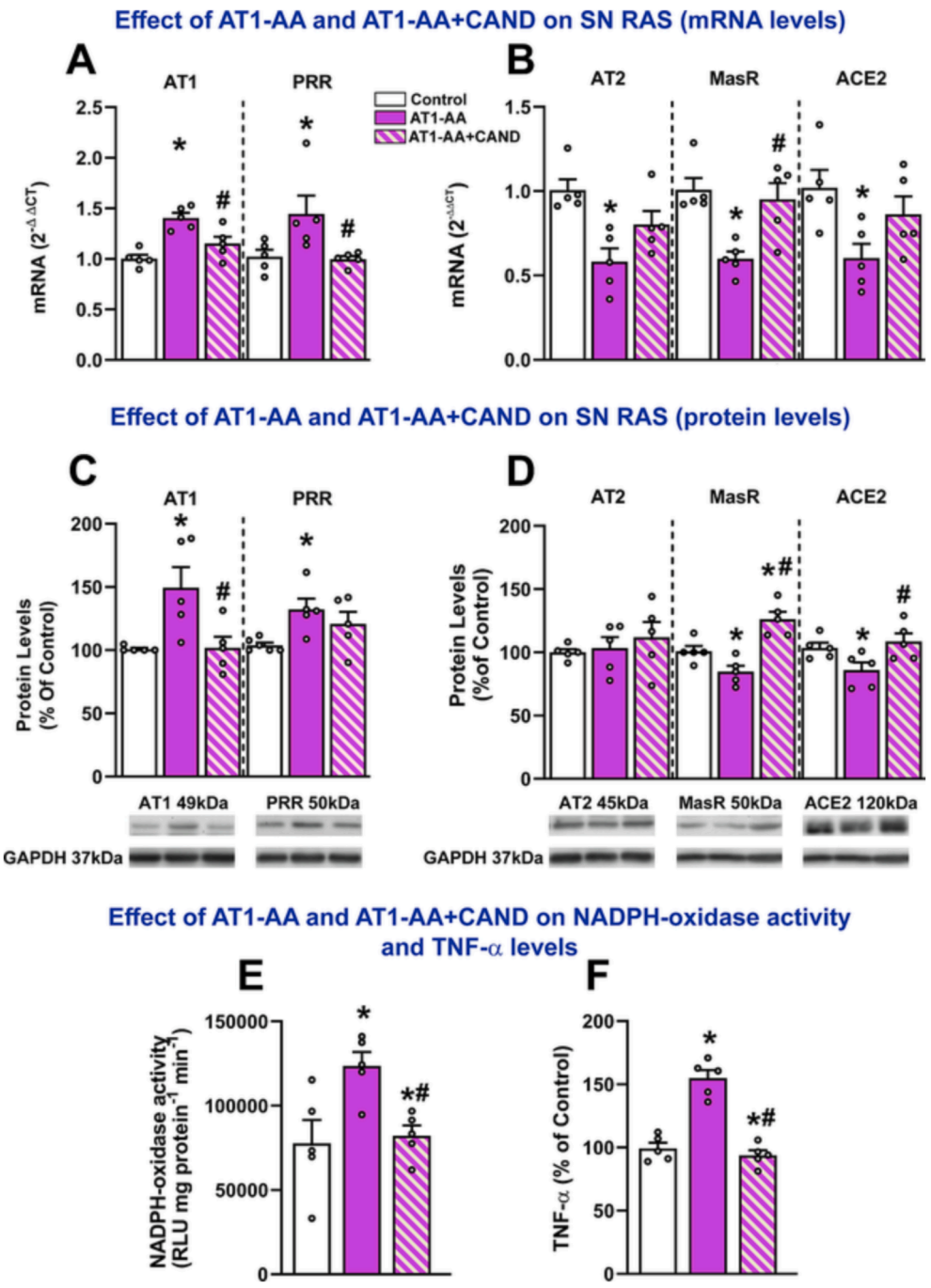

Fig. 5. Effects of AT1-AA administration and candesartan in the *substantia nigra* of rats. mRNA (A, B) and protein (C, D) expression of pro-inflammatory (AT1 and pro-renin receptors; A, C) and anti-inflammatory (AT2 and Mas receptors, ACE2; B, D) RAS components. Markers of oxidative stress (NADPH-oxidase activity; E) and inflammation (TNF-α; F) were increased by AT1-AA infusion, which was inhibited by treatment of rats with AT1 receptor antagonist candesartan. Data are means ± SEM. *$P < 0.05$ relative to control adult rats, # $P < 0.05$ relative to rats treated with AT1-AA (one-way ANOVA with Student-Newman-Keuls Method post hoc test or Kruskal-Wallis One Way Analysis of Variance on Ranks with Student-Newman-Keuls Method post hoc test). ACE2, Angiotensin-converting enzyme 2; AT1, angiotensin type 1; AT2, angiotensin type 2; CAND, Candesartan; MasR, Mas Receptor; MetS, Metabolic Syndrome; RAS, Renin Angiotensin System; SEM, standard error of the mean; SN, *substantia nigra*.

and 117 Parkinson's disease patients (mean age: 69.43 years old ± 10.09 SD; of which 57 were men and 40 women) were enrolled in this study. The parkinsonian patient group included 41 subjects with Metabolic Syndrome (35 % of the parkinsonian cohort) and the non-parkinsonian group included 32 subjects with Metabolic Syndrome (33.3 % of non-parkinsonian cohort). Main clinical features are presented in Supplementary Tables 1 and 2.

3.8. AT1-AA and ACE2-AA in parkinsonian and non-parkinsonian patients with or without metabolic syndrome

In the non-parkinsonian/MetS group, median of AT1-AA concentrations were 9.076 [Interquartile range (IQR) 6.271–11.667] U/mL, and in the non-parkinsonian/non-MetS group were 7.384 [IQR 4.575–8.856] U/m. Median of ACE2-AA concentrations were 7.887 [IQR 4.907–12.469] U/mL in the non-parkinsonian/MetS group and 5.957 [IQR 2.727–14.302] U/mL for subjects in the non-parkinsonian/non-MetS group. Wilcoxon-Mann-Whitney test analysis revealed significantly higher serum levels for AT1-AA ($P = 0.037$; $W = 961$) in non-parkinsonian/MetS group than in the non-parkinsonian/non-MetS group. (Fig. 7A). However, ACE2-AA serum levels in non-parkinsonian/MetS subjects were not significantly different to the non-parkinsonian/non-MetS group (p = 0.16; $W = 841$) (Fig. 7B).

In the parkinsonian cohort of patients, no differences were found in AT1-AA (p = 0.579; $W = 1655.5$) and ACE2-AA (p = 0.305; $W = 1378$) serum levels between individuals with or without MetS (Fig. 7C, D), and parkinsonian patients (with or without MetS) showed significantly higher levels of autoantibodies for AT1-AA; U/mL ($P < 0.001$; $W =$

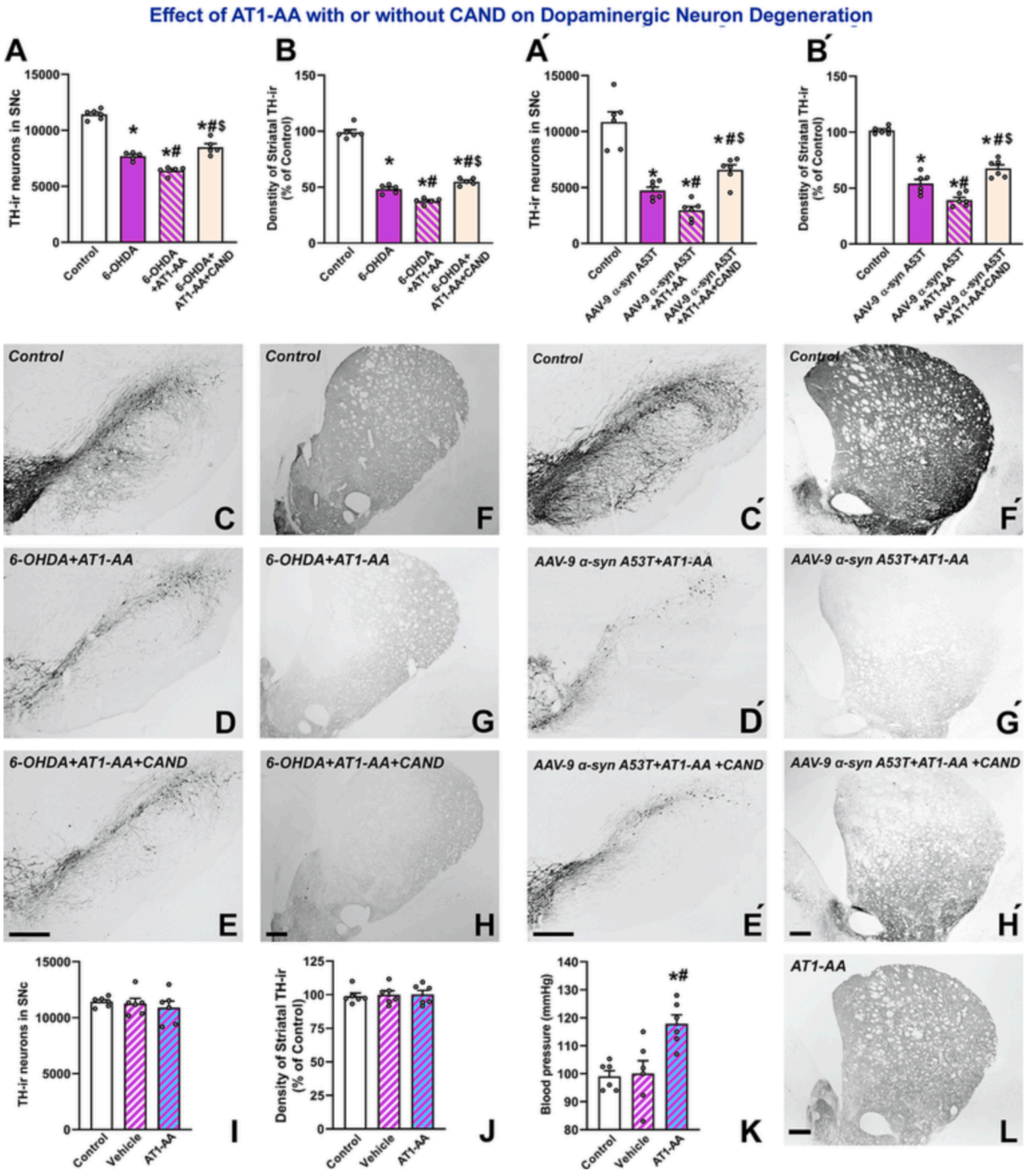

Fig. 6. AT1-AA enhanced dopaminergic neuron death. In rats, AT1-AA administration using intraperitoneal minipumps enhanced the deleterious effects of injection of the dopaminergic neurotoxin 6-OHDA (A–H), or the injection of neurospecific adeno-associated viral vectors serotype 9 (AAV-9) coding for mutated human alpha-synuclein A53T (α-syn A53T) (A′–H′). AT1-AA induced significant increase in loss of dopaminergic (TH-ir) neurons in the nigra (A, A′) and loss of dopaminergic terminals in the striatum (B, B′), which was inhibited by simultaneous treatment with the AT1 receptor antagonist candesartan. Photomicrographs of rat *substantia nigra* showing dopaminergic neurons (C–E and C′–E′) and striatal terminals (F–H and F′–H′) in control rats (C, F and C′, F′), rats treated with 6-OHDA and AT1-AA (D, G), rats treated with AAV-9 α-syn A53T and AT1-AA (D,′ G′), and rats treated with 6-OHDA and AT1-AA plus candesartan (E, H) or AAV-9 α-synA53T and AT1-AA plus candesartan (E′, H′). Scale bar 200 μm. Data are given as means ± SEM. $^{*}P < 0$ 0.05 compared to the corresponding control group, $^{\#}P < 0.05$ compared to 6-OHDA group; $^{\$}P < 0.05$ compared to 6-OHDA + AT1-AA group. One-way analysis of variance and Student–Newman–Keuls post hoc test. 6-OHDA = 6-Hydroxydopamine; AAV-9 α-syn A53T, adeno-associated viral vectors serotype 9 (AAV-9) coding for mutated human alpha-synuclein A53T; AT1-AA, AT1 receptor autoantibodies; CAND, Candesartan; SEM, standard error of the mean; TH-ir, tyrosine hydroxylase immunoreactivity.

1732) and for ACE2-AA ($P < 0.001$; $W = 1577$) than the non-parkinsonian/non-MetS subjects. This suggests that other factors, different from MetS, may induce increased levels of AT1-AA and ACE2-AA in the parkinsonian cohort. This is consistent with our recent study showing that the dopaminergic lesion and the accompanying neuroinflammatory response can also induce an increase in circulating AT1-AA (Labandeira et al., 2022a).

After confirming that MetS contributes to the increase in serum levels of AT1-AA in the non-parkinsonian cohort, we decided to study whether each individual parameter that defines MetS contributes to the increase

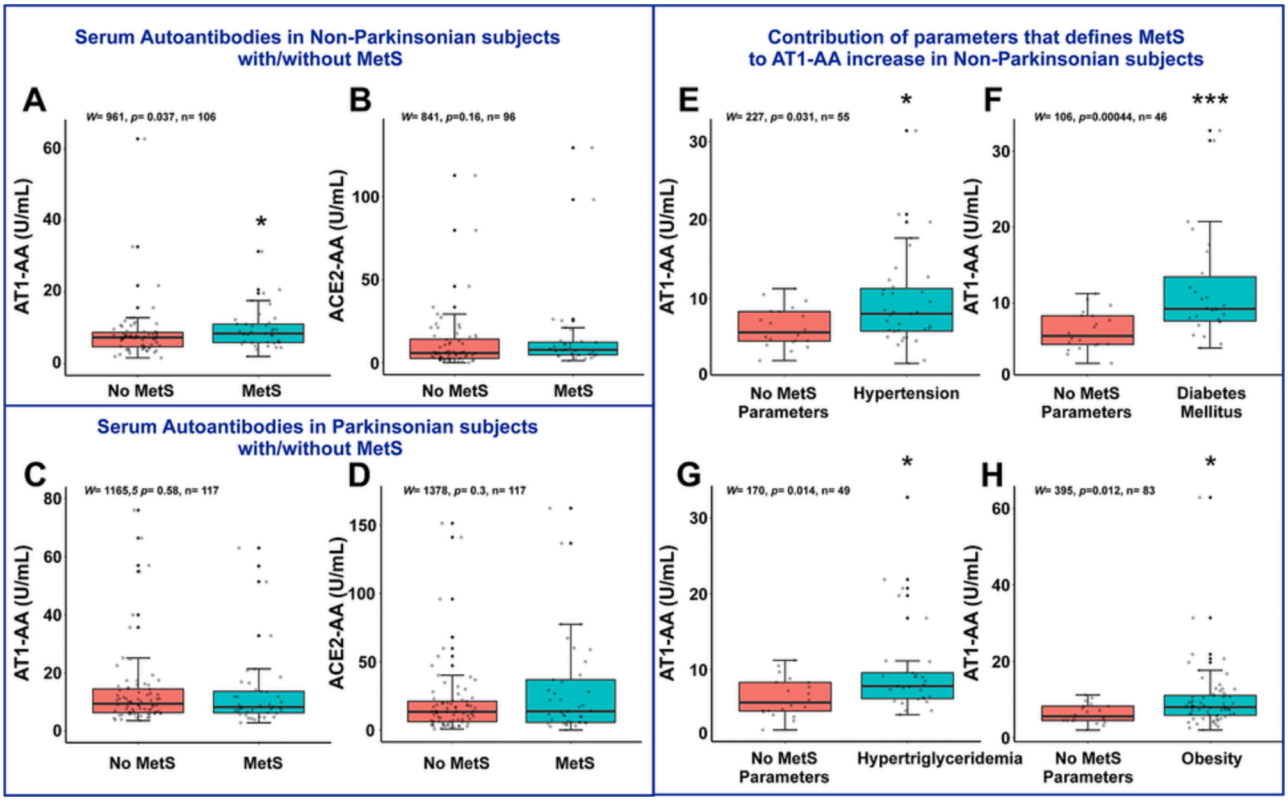

Fig. 7. Autoantibody serum levels in non-parkinsonian (A, B, E–H) and parkinsonian (C, D) subjects without or with Metabolic syndrome (MetS). In non-parkinsonian patients, MetS patients had significantly higher serum levels of AT1-AA (A; Wilcoxon test, $W = 961$, $P = 0.037$) than subjects without MetS. However, the ACE2-AA serum levels were not significantly different in subjects with MetS relative to subjects without MetS (B; *$W = 841$; $P = 0.16$*). In parkinsonian patients, no significant differences in serum levels of AT1-AA (C) or ACE2-AA (D) were observed between subjects with MetS and subjects without MetS. In non-parkinsonian subjects, each individual parameter that defines MetS contributed to the increase observed in AT1-AA serum levels relative to a group of subjects that did not present any of the MetS parameters: Hypertension (E; *$W = 227$, $P = 0.031$*), Diabetes (F; $W = 106$, $P = 0.00044$), Hypertriglyceridemia (G; $W = 170$, $P = 0.014$) and obesity (H; $W = 395$, $P = 0.012$) induced significant increase in AT1-AA levels. Data distribution is shown using a box plot with boxes representing the IQR and the median (black line) and whiskers representing ± 1.5 IQR. *$P < 0.05$; ***$P < 0.001$. AT1-AA, AT1 receptor autoantibodies; ACE2-AA, ACE2 Autoantibodies; IQR, Interquartile range; MetS, Metabolic Syndrome.

observed in autoantibody serum levels. To this end, a group of subjects that did not present any of the MetS parameters was defined in the non-parkinsonian cohort and possible differences were tested using Wilcoxon-Mann-Whitney test analysis. In the non-parkinsonian group, significantly higher serum levels for AT1-AA were observed in hypertension ($P < 0.05$; $W = 227$; Fig. 7E), diabetic patients ($P < 0.001$; $W = 106$; Fig. 7F), patients with hypertriglyceridemia ($P < 0.05$; $W = 170$; Fig. 7G), and obese patients ($P < 0.05$; $W = 395$; Fig. 7H) but no differences were found for Hipo-HDL ($P = 0.087$, $W = 430$). However, ACE2-AA levels were not increased with any of the studied parameters when comparing non-parkinsonian subjects that did not present any of the MetS parameters vs non-parkinsonian subjects suffering one MetS parameter (Supplementary Table 3).

In the parkinsonian cohort, no differences in AT1-AA and ACE2-AA serum levels were found between subjects without parameters of MetS and patients suffering one of the parameters of MetS (Hypertension, diabetes, hypertriglyceridemia, obesity, and Hypo-HDL) (Supplementary Table 4).

Recent studies have suggested that incidence and risk of Parkinson's disease gradually increases with the number of MetS components present in patients (Nam et al., 2018). In the non-parkinsonian cohort, we studied the existence of possible differences in serum levels for AT1-AA and for ACE2-AA between subjects suffering two parameters of MetS and subjects without any of the parameters of MetS, and we observed significant differences in AT1-AA levels and no significant differences in ACE2-AA levels as detailed in Supplementary Tables 5 and 6. In parkinsonian patients, no differences in AA-AT1 or AA-ACE2 serum levels were found between subjects without parameters of MetS and patients suffering two of the parameters of MetS (Supplementary Table 7).

4. Discussion

Using a MetS rat model, our results show that MetS significantly increases vulnerability of dopaminergic neurons to degeneration. MetS induces overactivity of local RAS pro-inflammatory axis that increases oxidative stress and neuroinflammation and enhances neurodegeneration, which can be inhibited by treatment with AT1 receptor antagonists. In addition, other mechanisms closely related to oxidative stress and neuroinflammation may contribute to the increase in dopaminergic vulnerability induced by AT1 receptor overactivity. Several studies have shown that RAS modulation affects α-synuclein aggregation, although controversial results have been published, possibly related to the use of different cell types and experimental conditions (Grammatopoulos et al., 2007; Sato et al., 2014; Kim et al., 2021). In a series of recent *in vitro* and *in vivo* studies, we have observed that both Ang II and AT1-AA increase α-synuclein aggregation in dopaminergic neurons (Dominguez-Meijide and Labandeira-Garcia, unpublished observations). Furthermore, it has been observed that the signal transduction pathways of insulin and Ang II/AT1 share a number of downstream effectors and cross-talk at multiple levels resulting in inhibition of insulin signaling and insulin resistance (Ramalingam et al., 2017; Zhou et al., 2012), and several experimental and clinical studies showed that pharmacological blockade of AT1 improves insulin

sensitivity in peripheral tissues (Huang et al., 2020; Yao et al., 2020). Insulin resistance has been related to increased dopaminergic vulnerability in both PD patients and an PD animal models, including those based on α-synA53T over-expression used in the present study, where dopaminergic neurons were insulin resistant. (Bassil et al., 2022; Labandeira et al., 2021; Labandeira et al., 2022b).

Interestingly (Labandeira-Garcia and Parga, 2022), AT1 gene expression has been recently revealed as a major marker of dopaminergic neuron vulnerability in humans (Kamath et al., 2022). In rats, MetS also induced the increase in circulating levels of LIGHT and other major pro-inflammatory cytokines, together with an increase in 27-OHC levels. Furthermore, MetS rats showed a significant increase in serum levels of proinflammatory AT1-AA and ACE2-AA, which correlated with levels of several MetS parameters such as cholesterol, glucose and triglycerides, and with serum levels of several major pro-inflammatory cytokines and 27-OHC. We also found AT1-AA and ACE2-AA in the CSF of MetS rats, suggesting an increase in BBB permeability to autoantibodies or activated B cells (intrathecal synthesis), which was confirmed by observing an increase in permeability to Evans Blue, and it is consistent with previous studies showing the activation of AT1 receptors (in this case by AT1-AA) as an initial and key step for BBB disruption (see below). Possible effects of circulating autoantibodies on dopaminergic degeneration were confirmed by chronic intraperitoneal infusion of AT1-AA, which induced BBB disruption, an increase in the pro-inflammatory RAS activity in the *substantia nigra* and a significant enhancement in dopaminergic neuron death in two different Parkinson's disease rat models. At the doses used in the present study, administration of AT1-AA alone (i.e. without any simultaneous dopaminergic deleterious factor/toxin) did not induce significant dopaminergic neuron death, which is consistent with that observed in previous experiments administrating low doses of angiotensin II alone (Rodriguez-Pallares et al., 2008) or AT1-AA alone to dopaminergic neuron cultures (Labandeira et al., 2022a).

Observations in the rat models, particularly those related to AT1-AA and ACE2-AA, were analyzed in a cohort of parkinsonian and non-parkinsonian patients with and without MetS. As observed in the animal model, non-parkinsonian/MetS patients showed significantly higher levels of AT1-AA than non-parkinsonian/non-MetS controls. Regarding circulating ACE2-AA, non-parkinsonian/MetS patients were not significantly different to those of non-parkinsonian/non-MetS controls, possibly because of the reduced size of our sample. Different parameters of MetS such as hypertension, diabetes, hypertriglyceridemia and obesity and combinations of 2 of these parameters were also associated to increased levels of AT1-AA. Interestingly, there was no significant difference between parkinsonian patients with MetS or without MetS, and parkinsonian patients showed levels significantly higher than non-parkinsonian subjects without MetS, which suggests that other factors are inducing autoantibodies in the parkinsonian population in addition to those produced in MetS. This is consistent with a recent study, showing significant increase of AT1-AA and ACE2-AA levels in Parkinson's disease patients, which was related to the dopaminergic degeneration and neurodegeneration-related neuroinflammation (Labandeira et al., 2022a).

An important number of experimental and clinical studies have related MetS (Leehey et al., 2017; Nam et al., 2018; Park et al., 2021; Zhang and Tian, 2014) and several of the MetS components such as obesity (Chen et al., 2014; Hu et al., 2006), diabetes (Labandeira et al., 2021), hypercholesterolemia (Hu et al., 2008) and hypertension (Chen et al., 2019) with higher risk of Parkinson's disease, and risk of Parkinson's disease may gradually increase with the number of MetS components involved (Nam et al., 2018). Controversial findings have also been reported (Hu, 2010; Saaksjarvi et al., 2015). However, the present results in animal models confirm an increase in dopaminergic vulnerability, and that brain RAS and, particularly, circulating autoantibodies against AT1 receptors are involved. An increase in dopaminergic vulnerability by overactivation of the RAS pro-inflammatory axis, particularly AT1 receptor overactivation, has been shown in several previous studies in different Parkinson's disease models (Joglar et al., 2009; Rodriguez-Pallares et al., 2008; Rodriguez-Perez et al., 2018), and a recent study has shown that the human dopaminergic neurons expressing highest levels of AT1 receptor gene are the most vulnerable to degeneration in Parkinson's disease (Kamath et al., 2022). Consistent with this, a decrease in risk of Parkinson's disease in patients treated with AT1 antagonists has also been observed in recent studies (Jo et al., 2022; Lin et al., 2022). Overactivation of AT1 receptors by agonistic AT1-AA may explain the increase in dopaminergic vulnerability in MetS, as observed in the rat model. It has been observed that AT1-AA, in addition to their agonistic effect on AT1 receptors, also stabilize in a permanent activation and upregulate AT1 receptor expression by blocking AT1 internalization (Labandeira-Garcia et al., 2021; Villar-Cheda et al., 2017), which further increases RAS pro-inflammatory axis activation (Liu et al., 2015; Liu et al., 2019; Liu et al., 2014). AT1-AA have previously been related to several diseases, being particularly relevant in pathophysiology of preeclampsia (Campbell et al., 2018; Wallukat et al., 1999), kidney graft rejection (Dragun et al., 2005), but they are also involved in several inflammation-related peripheral diseases, including malign hypertension (Fu et al., 2000), primary aldosteronism (Meyer et al., 2020) and others (Gunther et al., 2014).

The mechanisms responsible for generation of AT1-AA are still unclear. Infusion of proinflammatory cytokines such as IL-6 or TNF-α induced an increase in levels of AT1-AA in animal models, suggesting that some inflammation-related cytokines may be involved in this process (Irani et al., 2010; Lamarca et al., 2011). In MetS patients, an increase in several pro-inflammatory cytokines has been previously observed various studies (Bassols et al., 2010; Saunders et al., 2018; Tan et al., 2021). In the rat MetS model, we observed an increase in levels of serum IL-17, IL-1β, IL-6, TNF-α and LIGHT (TNFSF14), which may be involved in the increase in levels of circulating AT1-AA in these rats. The increase in levels of TNFSF14 (LIGHT) is particularly interesting. LIGHT, acting via tissue transglutaminase 2 (TG2), has been involved in production AT1-AA (Liu et al., 2015), as TG2 can modify AT1 receptors (Liu et al., 2017; Liu et al., 2014), and the TG2-modified AT1 receptors act as neoantigens leading to AT1-AA generation (Liu et al., 2017). In addition, TG2-induced modification of AT1 receptors stabilizes AT1 in permanent activation and interferes AT1 internalization for intracellular destruction, which results in increased AT1 expression and AT1 receptor sensitization (Liu et al., 2019). Consistent with this, we observed a positive correlation between LIGHT and AT1-AA levels in MetS rats. In addition, it is known that LIGHT induces pro-inflammatory responses and promotes production of several of the above-mentioned cytokines (Herro and Croft, 2016). It has been also suggested that AT1-AA production is enhanced in processes that increase death of cells generating debris with abundant modified AT1 receptors, which can act as autoantigens for B cells (Cabral-Marques et al., 2019; Ludwig et al., 2017), and this may also occur with cells in MetS (Eguchi and Feldstein, 2014; Gautheron et al., 2020).

Our data using osmotic minipump infusions suggest that circulating AT1-AA can disrupt BBB, enter CSF and affect brain. Several previous studies have suggested that an increase in levels of pro-inflammatory cytokines in MetS patients (Bassols et al., 2010; Saunders et al., 2018; Tan et al., 2021), increases BBB permeability (Argaw et al., 2006; Rochfort and Cummins, 2015). However, AT1-AA, may also play a major role in this process. AT1-AA acting on AT1 endothelial receptors appear as a major mechanism for BBB disruption. In hypertension, several previous studies have shown that stimulation of endothelial AT1 receptors and perivascular macrophages by circulating AngII, and not the hypertension itself, plays a major role in hypertension-induced BBB disruption, which is inhibited by AT1 antagonists and not by other antihypertensive drugs (Bloch et al., 2015; Faraco et al., 2016; Fleegal-DeMotta et al., 2009; Santisteban et al., 2020; Setiadi et al., 2018).

We also found an increase in levels of circulating ACE2-AA in MetS

rats. However, the increase did not reach statistical significance in our cohort of MetS patients, which may be related to the small size of our sample or a weaker induction of ACE2-AA than AT1-AA in MetS. The mechanisms responsible for ACE2-AA production are less known than those for AT1-AA. In fact, only a few studies have reported upregulation of serum ACE2-AA up to now (Miziolek et al., 2021; Takahashi et al., 2010). It has been suggested that and increase in circulating ACE2 may potentiate generation of neoantigens for production of ACE2-AA (McMillan et al., 2021; Rodriguez-Perez et al., 2021; Townsend, 2020). As in the case of AT1-AA and other G protein-coupled receptors (GPCR), neoantigens can be formed from cell debris containing ACE2 (Negi and Das, 2020; Sun et al., 2020). However, increasing levels of circulating ACE2 can be due to shedding of ACE2 into a soluble form from the cell surface ACE2 (i. e. transmembrane ACE2). ACE2 shedding is produced by proteases such ADAM17 (TACE, TNF-α-converting enzyme) and TMPRSS (transmembrane protease serine 2), which have been intensely studied as SARS-COV-2-related mechanisms in COVID-19 (Hoffmann et al., 2020; Lambert et al., 2005). Interestingly, it is known that AT1 receptor stimulation increases ADAM17 and TMPRS activity leading to ACE2 shedding (Deshotels et al., 2014; Pedrosa et al., 2021; Valenzuela et al., 2021; Xu et al., 2017). Therefore, increased stimulation of AT1 receptors by AT1-AA may contribute to ACE2 shedding and generation of ACE2-AA. The correlation between levels of AT1-AA and ACE2-AA observed in MetS rats ($r = 0.670$, $P = 0.0171$) is consistent with this hypothesis. However, more specific studies on mechanisms responsible for ACE2-AA are necessary. In any case, AT1-AA by increasing AT1 receptor activity and ACE2 shedding, and ACE2-AA by antagonistic effect on transmembrane ACE2 would shift the RAS balance towards the pro-inflammatory axis enhancing neuroinflammation and dopaminergic degeneration.

In the MetS model we observed a significant correlation between AT1-AA and ACE2-AA levels with circulating cholesterol but also with its metabolite and 27OH cholesterol. This appears particularly relevant in the context of MetS and neurodegeneration. It has been suggested that the deleterious effects of hypercholesterolemia on cognitive dysfunction and neurodegeneration may be mediated by increased flux of 27OH cholesterol across the BBB (Bjorkhem et al., 2009; Loera-Valencia et al., 2019). However, recent studies concluded that circulating 27OH cholesterol does not have a direct effect on BBB permeability, and that an unknown circulatory factor with a level correlated to level of 27OH cholesterol may increase BBB permeability (Parrado-Fernandez et al., 2021). The present study suggests that circulating AT1-AA may be this unknown factor increasing permeability of BBB, as strong correlation between AT1-AA and 27OH cholesterol was observed in our model. Consistent with this, previous studies have suggested that brain RAS may mediate deleterious effects of 27-OHC (Mateos et al., 2011).

In conclusion, the present results show that peripheral chronic inflammatory processes such as those observed in the MetS may generate circulating and CSF AT1-AA and ACE2-AA that may disrupt BBB and increase progression of neuroinflammation and dopaminergic neurodegeneration. In a previous study we observed that neuroinflammation and dopaminergic degeneration can also produce circulating and CSF AT1-AA and ACE2 autoantibodies. Altogether may lead to a vicious circle enhancing the progression of the disease that may be inhibited by strategies against production of these autoantibodies or AT1 receptor antagonists (ARBs).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Data statement

The data that support the findings of this study are available as Supplementary material (Appendix B) and from the corresponding author, upon reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2022.12.009.

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