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REVIEW ARTICLE

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ACE2 in the second act of COVID-19 syndrome: Peptide dysregulation and possible correction with oestrogen

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Abstract

Coronavirus disease 2019 (COVID-19) has become the most critical pandemic of the 21st Century and the most severe since the 1918 influenza pandemic. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects the host by binding to angiotensin-converting enzyme 2 (ACE2). The role of ACE2 in the pathophysiology of coronavirus disease 2019 (COVID-19) is a topic of debate, with clinical and experimental evidence indicating a multifaceted relationship between ACE2 activity and disease severity. Here, we review the mechanisms by which the peptidergic substrates and products of ACE and ACE2 contribute to physiological and pathophysiological processes and hypothesise how down-regulation of ACE2 by SARS-CoV-2 cellular entry disrupts homeostasis. A better understanding of the endocrinology of the disease, in particular the neuroendocrinology of ACE2 during COVID-19, may contribute to the timely design of new therapeutic strategies, including the regulation of ACE2 itself by steroid hormones, to ameliorate the severity of COVID-19.

KEYWORDS
Ang (1-7), apelin, asthma, obesity, SARS-Cov-2

1 | INTRODUCTION

The renin–angiotensin–aldosterone system (RAAS) comprises an endocrine cascade of vasoactive peptides to mineralocorticoids that orchestrate key processes in mammalian physiology. Notably, it preserves end-organ perfusion by regulating extracellular volume, sodium and water balance, and cardiovascular activity. The RAAS is also crucially involved in the inflammatory response, epithelial cell proliferation, angiogenesis and fibrosis. Two zinc metalloproteases are pivotal players in the RAAS, namely angiotensin-converting

Zhang, Zetter and Guerra contributed equally to this work.

enzyme (ACE) and the angiotensin-converting enzyme-related carboxypeptidase (ACE2), in a complex cooperation-antagonism manner,[1] achieving homeostasis of vasomotor activity, hydroelectrolyte equilibrium, inflammatory responses and tissue growth.[2]

The severe acute respiratory syndrome coronavirus (SARS-CoV) and the current COVID-19 pathogen, SARS-CoV-2, are intimately associated with the RAAS (Figure 1 and 2), initially through the use of ACE2 as a receptor for both coronaviruses. The virus enters human cells by binding of the viral trimeric spike protein to ACE2,[3] and the spike protein is primed by the serine protease TMPRSS2, triggering the fusion of viral and cellular membranes[4] and, ultimately, virus entry into the cell. As viral infection progresses, ACE2 expression is severely diminished in the lung and other tissues.[5-8] At the advanced disease stage (usually after 14 days), the host viral burden is sharply reduced and numerous virus-negative cases are reported during the severe stages of pathophysiology.[9] Concomitantly, the host inflammatory response and hypercoagulable phase escalates and intussusceptive angiogenesis and pulmonary fibrosis formation are increased.[10,11] The above observations immediately add another layer of complexity to our understanding of the pathophysiology of COVID-19. An intrinsic question arises: could the delayed disease severity be caused in part by down-regulation of ACE2 and the corresponding accumulation of peptide substrates and depletion of peptide products?

Here, we first review the cellular physiology of ACE2 substrates and products whose generation or depletion could affect the RAAS balance and the inflammatory response. Next, we consider the epidemiological data on sex and chronic comorbidities in the pathophysiology of COVID-19 from the standpoint of these actors within the RAAS during the clinical course of the disease. Finally, we assess the rationale for potential therapeutic intervention with oestradiol, with the aim of restoring ACE2 physiological function.

2 | ACE VS ACE2 IN RAAS-RELATED EVENTS IN COVID-19

After the enzymatic cleavage of angiotensinogen by renin, ACE removes the carboxy terminal dipeptide from the decapeptide angiotensin I (Ang I) to generate angiotensin II (Ang II), a potent vasoconstrictor that maintains blood pressure and exerts pro-inflammatory, pro-fibrotic and pro-oxidative effects. Ang II also

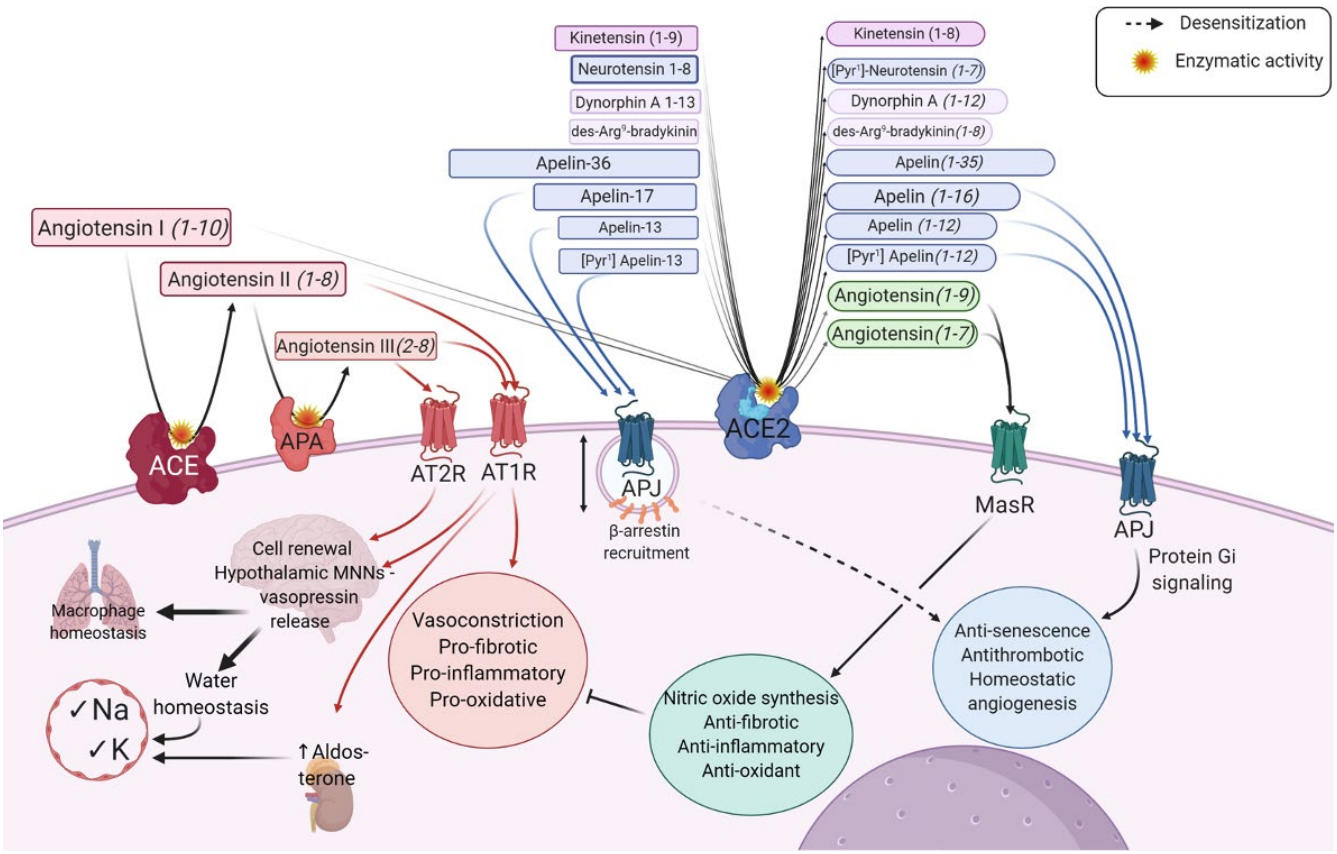

FIGURE 1 The balanced angiotensin-converting enzyme (ACE), aminopeptidase A (APA) and angiotensin-converting enzyme 2 (ACE2) physiological activities keep the corresponding peptide substrates and products in homeostasis to safeguard the physiological functions of the organism. The aggressive and protective branches of the renin-angiotensin-aldosterone system (RAAS) are represented by reddish and blueish objects, respectively. The functional interaction between the peptide ligands and their receptors, angiotensin receptor 1 (AT1R), angiotensin receptor 2 (AT2R), apelin receptor (APJ) and Mas receptor (MasR) play key roles in homeostasis with respect to blood pressure, fibrosis, inflammation, oxidation, angiogenesis and ageing. MNNs, magnocellular neurosecretory neurones

stimulates the synthesis of aldosterone, the main mineralocorticoid in the adrenal cortex, and the release of this liposoluble molecule regulates the excretion of potassium and retention of sodium by the tubular epithelium in the kidney. For the sake of simplicity, we refer to this axis as the aggressive branch of the RAAS, as symbolised by the reddish objects in Figure 1.

ACE2 was first identified as a key protein in the RAAS axis that catalyses the hydrolysis of the C-terminal residue of Ang I to Ang (1-9) and Ang II to Ang (1-7), by removing a single phenylalanine from the amino acid chain.[12,13] Ang (1-9) can be further hydrolysed by the catalytic action of ACE and conversion to Ang (1-7), which is a physiological antagonist of the Ang II axis[14,15] because it comprises a vasodilatory peptide that also inhibits inflammation and down-regulates fibrosis through Mas receptor pathway activation.[1,16] This protective branch, symbolised in blue in Figure 1A, opposes the vasoconstrictor, pro-inflammatory, pro-oxidant, pro-proliferative and pro-fibrotic actions exerted by the aggressive branch.

ACE2 hydrolyses plasma-borne and tissue-derived peptides other than those of the RAAS. A variety of biologically active peptides, in addition to Ang I and Ang II, were screened.[17] Ten peptides were found to be efficiently hydrolysed by ACE2 and, in each case, the proteolytic activity resulted in removal of the C-terminal residue only. ACE2 catalyses the efficient hydrolysis of apelin-36, apelin-17, apelin-13, [Pyr^1] apelin-13, kinetensin (1-9), dynorphin A 1-13, des-Arg^9-bradykinin and neurotensin to apelin (1-35), apelin (1-16), apelin (1-12), [Pyr^1] apelin (1-12), kinetensin (1-8), dynorphin A (1-12), des-Arg^9-bradykinin (1-8) and [Pyr^1] neurotensin (1-7), respectively (Figure 1).

Because of the host–SARS-CoV-2 viral interaction (Figure 2), ACE2 down-regulation alters the balance of the relative concentrations of its substrates and products in both plasma and tissues. First, the lack of key components of the protective branch of RAAS (ie, ACE2 > Ang1-7) leaves the aggressive branch (ie ACE > Ang II) unchecked. There is an increasing body of evidence supporting the

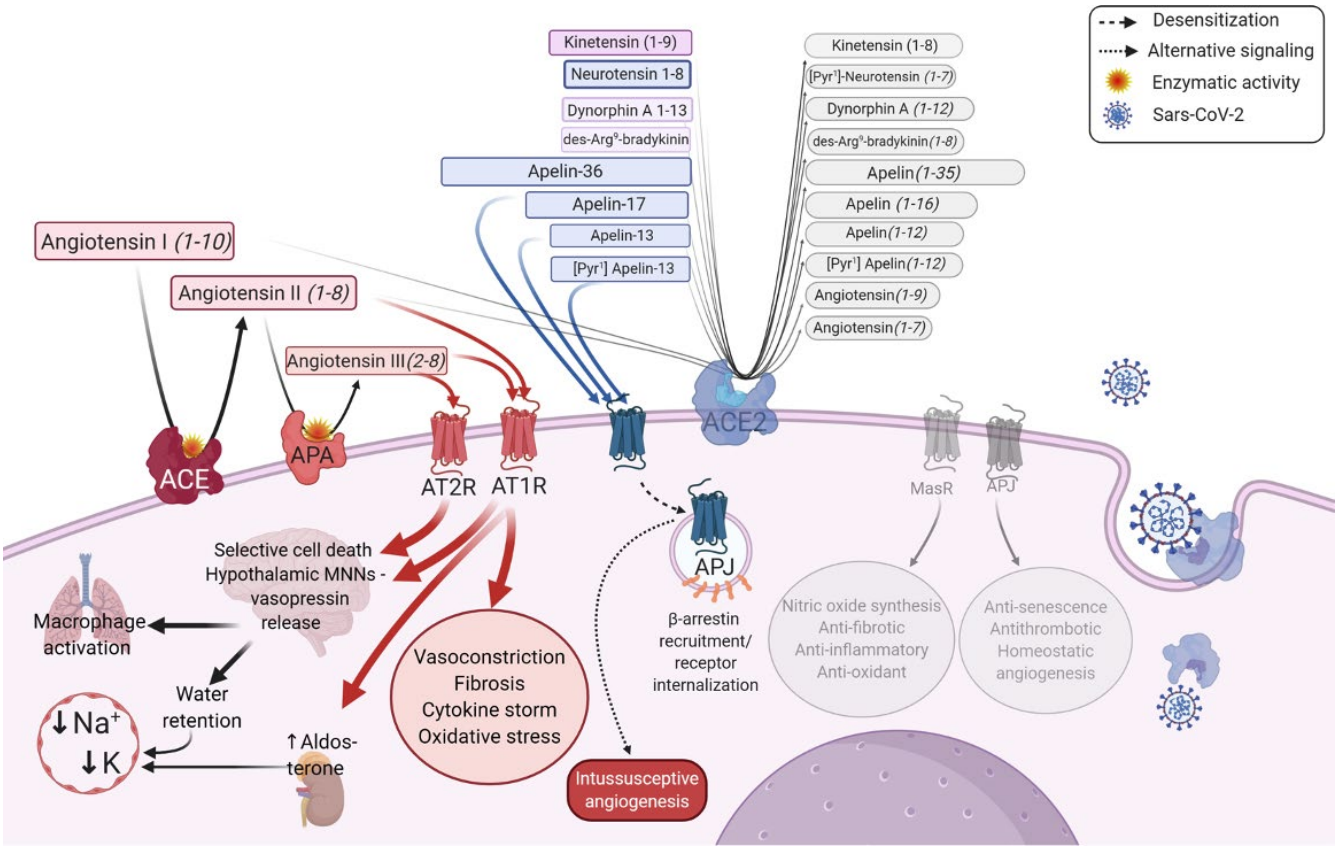

FIGURE 2 Hypothetical consequences of the marked down-regulation of angiotensin-converting enzyme 2 (ACE2) (symbolised by attenuated) as a consequence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (symbolised by) to potentiate the aggressive branch and weaken the protective branches of the renin-angiotensin-aldosterone system (RAAS), represented by reddish and blueish objects, respectively. The down-regulated metabolites and physiological functions are represented by grey objects, which drive toward increased vasoconstriction, inflammatory responses, cytokine storm, oxidative stress and intussusceptive angiogenesis, as well as the sustained activation of hypothalamic vasopressin magnocellular neurosecretory neurones (MNNs). The propposed mechanism is through potentiated angiotensin II (1-8) to angiotensin III (2-8), binding to angiotensin receptor 1 (AT1R) and angiotensin receptor 2 (AT2R) of MNN and by promoting aldosterone secretion via the AT1R pathway in the cortical adrenal gland, causing water retention, hyponatraemia and hypokalaemia by potassium diuresis. Also, the hypothetical consequences of biased protein Gi signalling by the loss of apelin metabolites, causing receptor desensitisation and internalisation of the apelin receptor (APJ) by parental apelin peptides are depicted. APA, aminopeptidase A; MasR, Mas receptor

role of an over-activated RAAS in COVID-19[1] (Figure 1B). Ang II accumulation, as a result of decreased hydrolysis by ACE2, would be predicted to lead to overstimulation of the angiotensin receptor 1 (AT1R), and the over-activation of the aggressive pathway would culminate in hypertension, thrombosis, lung edema, fibrosis and hyper-inflammatory reactions. Furthermore, Ang II stimulates steroidogenesis/aldosterone production in the adrenal cortex, which affects sodium reabsorption, water retention and potassium excretion by the kidney, leading to hypertension. It is noteworthy that hypokalaemia has recently been linked with severe forms of COVID-19.[18] Moreover, Ang II (1-8) can be hydrolysed to Ang III (2-8) under the catalytic action of another metallopeptidase, aminopeptidase A (Figure 1). Ang III is a central up-regulator of vasopressin release[19] which also exerts important effects in SARS pathogenesis (vide infra).

The apelin family of peptides comprise a group of very short half-life molecules with diverse cardiovascular, pulmonary and metabolic functions, generally opposite to those of the ACE > Ang II > AT1R axis. Apelins have been shown to demonstrate anti-senescence, antithrombotic and angiogenesis homeostasis properties.[20,21] Apelin peptides are synthesised as the 77-amino acid precursor protein preproapelin, encoded by the *APLN* gene on the long arm of chromosome X. Preproapelin is cleaved to a prohormone, apelin-55, prior to further proteolytic processing. The most studied apelin hormones are apelin-36, apelin-17, apelin-13 and a spontaneously produced N-terminal pyroglutamate form of apelin-13 designated [Pyr^1]-apelin-13. The apelin hormones are ligands of the G-protein coupled receptor APJ. Shorter apelin forms such as apelin-13 have a greater affinity for APJ than longer ones such as apelin-36.[22] ACE2 cleaves a single phenylalanine residue from the C-terminal parental forms of apelin (apelin-17, apelin-13 and [Pyr^1]-apelin-13) yielding the metabolised forms (apelin-16, apelin-12 and [Pyr^1]-apelin-13$_{(1\text{-}12)}$, respectively).[22-24] The metabolised forms have a relatively decreased affinity compared to the parental forms, albeit within the same order of magnitude.[25] Nevertheless, it has been demonstrated that the metabolised forms have less hypotensive effects.[26]

These observations may lead to the conclusion that ACE2 down-regulation in COVID-19 increases the concentration of higher-affinity parental apelin peptides, countering the effects of the aggressive branch of the RAAS. Indeed, exogenous administration of apelin has been suggested as a treatment for COVID-19.[27] However, even though parental apelins have a higher affinity for APJ than metabolised apelins, there is an increasing volume of research suggesting that parental apelin isoforms induce receptor internalisation mediated by β-arrestin signalling, blunting the effect of high concentrations of parent apelin peptides. By contrast, metabolised apelins (ie, apelin-16, apelin-12 and [Pyr^1]-apelin-13$_{(1\text{-}12)}$) have recently been reported to display biased signalling toward the G_i pathway of the APJ receptor rather than the β-arrestin-induced internalisation pathway.[20,22,24,28] Thus, high concentrations of parental apelins and low concentrations of metabolised apelins could interfere with the beneficial anti-senescence, antithrombotic and angiogenesis homeostasis effects of the apelinergic system as a result of cell desensitisation mediated by β-arrestin internalisation of the APJ receptor (Figure 2).

Moreover, metabolised apelins may exert beneficial properties of their own. For example, [Pyr^1]-apelin-13$_{(1\text{-}12)}$, a metabolised apelin that exhibits positive inotropic and vasodilatory effects, is not detectable in individuals with excessive pulmonary vascular remodelling and abnormal angiogenesis,[25] suggesting its protective role in preventing the development of these pathological states. It is worth noting that, in the lungs of some patients who died from COVID-19, a pathological form of angiogenesis, in which capillary vessels are divided by partition (intussusceptive angiogenesis), was associated with hypoxia and microthrombosis.[29] Parental apelins are known to induce angiogenesis and capillary sprout formation in different tissues under physiological and pathological conditions.[30,31]

ACE2 plays a significant role in the regulation of other peptides involved in the inflammatory response, such as des-Arg(9)-bradykinin, neurotensin, dynorphin and kinetensin.[12,17] The opioid peptide des-Arg(9)-bradykinin has been shown to promote neutrophil infiltration and inflammation and increase fluid permeability into tissues causing oedema.[32] Its accumulation, after ACE2 down-regulation, has been regarded as an important pathophysiological component of COVID-19.[33] Moreover, bradykinin, the precursor of des-Arg(9)-bradykinin, has been shown to modulate the chemoreceptor sensitivity to hypoxia and hypercapnia in carotid bodies[34,35] where ACE and ACE2 expression is actively modulated under hypoxia.[35,36] SARS-CoV-2 induced changes in the activity of these proteolytic enzymes might alter the delicate peptidergic environment and provoke a failure of the carotid bodies to detect abnormal oxygen levels. Kinetensin is known to induce mast cell degranulation releasing histamine and other inflammatory mediators and increase vascular permeability resulting in oedema;[37,38] however, it has not been investigated in COVID-19 pathophysiology. Neurotensin is a peptide produced mainly in the gut and brain. Among its gastrointestinal functions, it promotes chloride secretion, intestinal cell growth, and intestinal inflammatory and stress responses via the activation of its NTR1 receptor.[39] Increased neurotensin concentrations in the gut after down-regulation of ACE2 in colonic mucosa might be involved in the commonly observed gastrointestinal symptoms of COVID-19.

3 | RAAS IMBALANCE AND IMMUNE FUNCTION

Alveolar macrophages, which reside near type I and II pneumocytes, are the first line of defence against respiratory pathogens and one of the first cells to be infected by SARS-CoV-2 in the lungs.[40] They express the SARS-CoV-2 receptor, ACE2, in basal and activated states[41] and prominently participate in viral clearance by orchestrating antiviral interferon-mediated responses.[42] However, during COVID-19, macrophages spread SARS-CoV-2 to lymphatic nodes[43] and trigger pathological inflammation, tissue damage and cytokine

storms.[40,44] These phenomena could be well explained by overstimulation of the aggressive branch of RAAS via the loss of effects of the protective ACE2 branch.

As monocytes mature into macrophages, they express ACE and metabolise Ang I to Ang II.[45] Ang-II up-regulates the synthesis of the pro-inflammatory cytokines tumor necrosis factor α, interleukin (IL)-6 and IL-1β and promotes the generation of toxic reactive oxygen species through AT1R.[46] Additionally, Ang III (Figure 1) increases migratory activity of monocytes by inducing the expression of monocyte chemoattractant protein 1 and proinflammatory transcription factors.[47] Ang III and dynorphin stimulate the secretion of vasopressin, which appears to be affected in severe COVID-19 patients, in whom a pronounced euvolemic hyponatraemia has been observed.[48,49] Vasopressin has been recently identified as an immunomodulator that drives inflammatory responses in the lung by triggering macrophage activation and polarisation to a reparative phenotype, increasing lung fibrosis and reducing the effectiveness for pathogen clearance.[50]

Antigenic processing and adaptive immune response are also altered in SARS-CoV-2 infected individuals with poor clinical outcomes.[51] In particular, Ang II promotes dendritic cell maturation and proinflammatory cytokine synthesis.[52] Moreover, Ang II drives antigenic presentation (through MHC II) to naïve T cells inducing vigorous clonal expansion of T $CD4^+$ cells,[53] up-regulates proinflammatory cytokine synthesis by activated T lymphocytes, and stimulates selectin and chemokine expression, leading to increased leukocyte adhesion in tissues.[54,55] Thus, despite the fact that the adaptive immune response is crucial for containing SARS-CoV-2 infection itself, an unbalanced RAAS system without the protective/regulatory ACE2 branch could lead to unbalanced proliferation of T $CD4^+$ lymphocytes with reduced efficiency for viral clearance and a high capacity of intense inflammatory response orchestration.

4 | OBESITY AND ASTHMA AS EXAMPLES OF CONDITIONS ASSOCIATED WITH ALTERED ACE2 EXPRESSION AND THEIR RELATIONSHIP WITH COVID-19

All of the above suggest that ACE2 is a pivotal element in COVID-19 pathophysiology. In otherwise uncompromised patients, down-regulation in later stages of the disease could account for many of the severe complications and lethality. Additionally, epidemiological studies spanning various populations indicate that comorbidities such as obesity, hypertension and diabetes carry a risk for increased disease severity and mortality.[56-62] This is partly explained by the fact that these individuals are less capable of coping with the disease. Nevertheless it has been demonstrated that comorbidities affect the expression of ACE2 and this could be a gateway to explain the increased rates of severe COVID-19 and mortality associated with these conditions.[63] A delineation of the role of ACE2 in obesity and asthma, and its relationship with COVID-19 severity and mortality, serves to further illustrate this point.

Obesity is one of the most common chronic comorbidities associated with negative outcomes of COVID-19.[56,62] It is also associated with overactivity of the RAAS and this may help to explain why the prevalence of hypertension is higher among obese patients.[64] Hypertension in obesity is hypothesised to be secondary to increased synthesis of angiotensinogen and local production of Ang II by adipocytes.[65] Consequentially, ACE2 expression is up-regulated as a compensatory response, albeit insufficient to counter the aggressive arm of the RAAS.[66,67] In COVID-19, increased levels of ACE2 in adipose tissue may provide a viral reservoir in obese patients.[68] Perhaps more importantly, abrupt down-regulation of ACE2 in later stages of the disease may lead to a state of unopposed activity of the already hyperactive aggressive branch of the RAAS which, as previously noted, leads to tissue damage.[69] Although ACE2 regulation varies in different pathological states,[70] the salient point regarding obese patients is that, although disequilibrium favours the aggressive over the protective branches of the RAAS, both systems are in a steady state prior to infection. In COVID-19, a sudden down-regulation of ACE2 can lead to rapid overactivation of the aggressive branch of the RAAS coupled with an acute decrease in the protective branch.

Asthma is a comorbidity that was assumed to be associated with worse outcomes in COVID-19. However, recently, it has been reported that, overall, it may not be associated with severe disease or mortality.[71] A possible explanation is that, although asthma predisposes to respiratory distress, ACE2 is down-regulated in asthma and other respiratory allergies, and therefore patients are less susceptible to infection by SARS-CoV-2.[72] Given the fact that ACE2 down-regulation is considered to be an important factor attributed to complications in later course of the disease, it could be assumed that asthmatic patients should be at increased risk for a poor prognosis. Although this may be partly explained by other factors (eg, type 2 immune response and treatments predominantly used in this population), we suggest that asthmatic patients may tolerate better the decrease of ACE2 associated with SARS-CoV-2 infection because of chronic adaptation to relatively lower ACE2 expression.[73] Consistent with this hypothesis, and considering that ACE2 has an inverse relationship with age, asthma does appear to be a comorbidity associated with severe COVID-19 in younger populations, as corroborated by the fact that hospitalised young patients have a higher prevalence of asthma than adults;[71] possibly, younger patients could be more susceptible to the sudden decrease in activity of ACE2 because they are not as habituated as adults to low levels of activity of this enzyme.

5 | SEX DIFFERENCES AND CONSIDERATION FOR OESTROGEN THERAPEUTIC INTERVENTION

Women are less susceptible to COVID-19. Epidemiological studies have shown that males, although only slightly more likely to be infected than females, account for most COVID-19 severe cases and

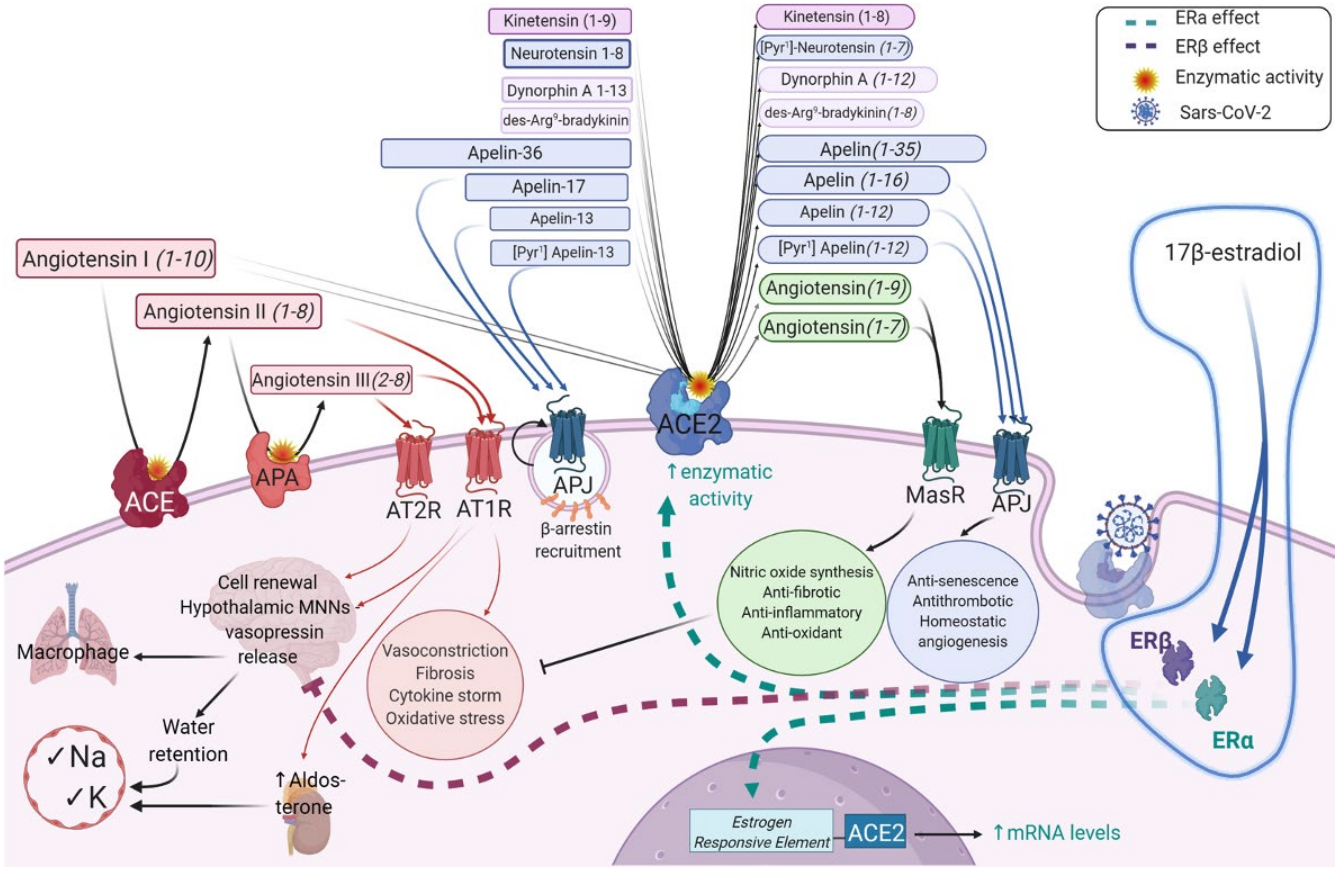

FIGURE 3 The possible improvement of the down-regulated pathways via the effects of oestrogen therapy through both α and β oestrogen receptors in COVID-19 patients as a result of increasing the enzymatic activity and expression of angiotensin-converting enzyme 2 (ACE2), restoring peptide metabolism, and balancing water metabolism and the inflammatory response through regulation of vasopressin release. AT1R/AT2R, angiotensin receptor 1 and 2, respectively; APJ, apelin receptor; MMNs, magnocellular neurosecretory neurones. APA, aminopeptidase A; ER, oestrogen receptor; MasR, Mas receptor

deaths.[4] Moreover, male patients with comorbidities have a higher risk of developing a critically-ill status compared to men without comorbidities, whereas there is no such association in women.[74] Interestingly, the female reproductive hormones, oestrogen and progesterone, down-regulate ACE. Some evidence also supports the notion that oestrogens and progesterone might exert a protective effect on females through direct antiviral activity and immune-mediated mechanisms, thus explaining the higher COVID-19 severity in post-menopausal women.[75] From a peptide biology point of view, 17β-oestradiol (E2) promotes ACE2 mRNA abundance through effects at the oestrogen receptor-α (ERα) via ERα-mediated binding at the ACE2 promoter (Figure 3), as well as increased ACE2 enzymatic activity[76-78] (Figure 3).

As previously noted, COVID-19 pathophysiology is not only characterised by an exacerbated inflammatory response, but also by a hypercoagulable state. In animal models, it has been demonstrated that oestrogen reduces platelet aggregation and thrombus formation, whereas androgens enhance them. Under conditions of normal levels of oestrogen exposure, this also appears to apply in humans because the risk of thromboembolism is higher in men throughout the life cycle whereas women's risk is lowest in their fertile years, gradually increasing during menopause.[79] However, it is worth noting that conditions of supraphysiological oestrogen levels such as pregnancy and the use of oral oestrogen-containing contraceptives increase the production of procoagulant factors (eg, factor X, factor XII) at the same time as decreasing the production of anticoagulant factors (eg, protein S and antithrombin III). Although this may warrant caution with respect to the use of oestrogen in COVID-19, in hormone replacement therapy, the risk of thromboembolism appears to increase significantly only beyond the fourth month of treatment.[80]

Further investigations are needed to assess the effects of hormone therapy and hormone deprivation in male and female patients, given their potential implications in modulating the severity and mortality of COVID-19.

6 | CONCLUSIONS

Through the analysis presented in this review, we hypothesise that rapid down-regulation of ACE2 by SARS-CoV-2 infection could result in a depletion of both Ang (1-7) and metabolised apelins such

as [Pyr^{1}] apelin-13$_{(1\text{-}12)}$, (the protective branch of the RAAS system) and concomitantly increase other molecules, including Ang II, parent apelins and other pro-inflammatory peptides (the aggressive branch of the RAAS) that require inactivation by ACE2 such as bradykinin, neurotensin, kinetensin and dynorphin. This would result in several pathophysiological consequences depending on the affected tissue. In the lungs, it may exacerbate non-resolutive inflammation and vasoconstriction of lung vasculature. At the endothelium, it would accelerate endothelial senescence via the accumulation of free oxygen radicals, as well as induction of vessel growth by intussusceptive angiogenesis resulting in abnormal capillary beds, leading to blood stasis that, together with inflammation and endothelial damage, results in the formation of multifocal thrombi, pulmonary hypertension, oedema and, ultimately, organ failure.

Given the urgency to find alternative therapeutic strategies, the exploration of oestrogen as a therapeutic option for ameliorating the severity of COVID-19, based on its effects on the up-regulation of the expression of ACE2, appears eminently worthwhile. E2 is a US Food and Drug Administration-approved therapeutic agent for hormone replacement therapy in females (menopause, etc.) and males (for prostate cancer treatment). A regimen of E2 that could restore the physiological distribution and functions of ACE2 and catalyse the hydrolysis of key peptides critical for re-establishing equilibrium of the RAAS could be crucial in blunting the severity and mortality of COVID-19.

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AUTHOR CONTRIBUTIONS

Limei Zhang: Conceptualisation; funding acquisition; investigation; project administration; writing – original draft; writing – review & editing. **Mario A. Zetter:** Conceptualisation; investigation; visualisation; writing – original draft; writing – review & editing. **Enrique C. Guerra:** Conceptualisation; investigation; writing – original draft; writing – review & editing. **Vito S. Hernández:** Conceptualisation; investigation; writing – original draft; writing – review & editing. **Sushil K. Mahata:** Conceptualisation; funding acquisition; investigation; project administration; writing – original draft; writing – review & editing. **Lee E. Eiden:** Conceptualisation; investigation; writing – original draft; writing – review & editing.

PEER REVIEW

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DATA AVAILABILITY

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

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