MINIREVIEW

Dysregulation of Angiotensin Converting Enzyme 2 Expression and Function in Comorbid Disease Conditions Possibly Contributes to Coronavirus Infectious Disease 2019 Complication Severity

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ABSTRACT

ACE2 has emerged as a double agent in the COVID-19 ordeal, as it is both physiologically protective and virally conducive. The identification of ACE2 in as many as 72 tissues suggests that extrapulmonary invasion and damage is likely, which indeed has already been demonstrated by cardiovascular and gastrointestinal symptoms. On the other hand, identifying ACE2 dysregulation in patients with comorbidities may offer insight as to why COVID-19 symptoms are often more severe in these individuals. This may be attributed to a pre-existing proinflammatory state that is further propelled with the cytokine storm induced by SARS-CoV-2 infection or the loss of functional ACE2 expression as a result of viral internalization. Here, we aim to characterize the distribution and role of ACE2 in various organs to highlight the scope of damage that may arise upon SARS-CoV-2 invasion. Furthermore, by examining the disruption of ACE2 in several comorbid diseases, we offer insight into potential causes of increased severity of COVID-19 symptoms in certain individuals.

SIGNIFICANCE STATEMENT

Cell surface expression of ACE2 determines the tissue susceptibility for coronavirus infectious disease 2019 infection. Comorbid disease conditions altering ACE2 expression could increase the patient’s vulnerability for the disease and its complications, either directly, through modulation of viral infection, or indirectly, through alteration of inflammatory status.

Introduction

The Renin-Angiotensin System. The renin-angiotensin system (RAS) plays a key role in regulating the normal physiology and pathogenesis of cardiovascular diseases (CVDs). RAS mainly consists of a series of enzymatic reactions, with renin being the rate-limiting enzyme and angiotensin (Ang) II, generated by local RAS, being the major component acting on angiotensin receptor 1 (AT1R) and angiotensin receptor 2 (AT2R). Overstimulation of the “classic” RAS pathway involving the activation of angiotensin converting enzyme (ACE) 1 (AngII/AT1R) results in vasoconstriction, inflammation, sodium and water retention, and oxidative stress generation, thus increasing sympathetic nervous system activity, cardiovascular fibrosis, and hypertrophy. The view of RAS has changed with the identification of an ACE homolog, ACE2 (Donoghue et al., 2000). A more complex signaling network

ABBREVIATIONS: ACE, angiotensin converting enzyme; Ang, angiotensin; ARDS, acute respiratory distress syndrome; AT1R, angiotensin receptor 1; AT2R, angiotensin receptor 2; COVID-19, coronavirus infectious disease 2019; CVD, cardiovascular disease; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant-1; RAS, renin-angiotensin system; S protein, spike protein; SARS, severe acute respiratory syndrome; SARS-CoV, severe acute respiratory syndrome coronavirus; TGF-β, transforming growth factor-β; Th, T-helper; TMPRSS2, transmembrane protease serine 2; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.
mediating protective physiologic effects, along with the detrimental ones, has now been recognized. ACE2 degrades both AngI and AngII into Ang1–9 and Ang1–7, respectively. As such, another arm of RAS, ACE2/Ang1–7 and ACE2/Ang1–9, emerged and is reported to act as a physiologic antagonist to counteract RAS overactivation, although its full physiologic role is still not completely understood (Santos and Ferreira, 2007; van Twist et al., 2014).

Impact of RAS Imbalance on Various Organs. Of relevance to SARS-CoV-2 infection, lungs are rich in RAS components, including ACE1/ACE2. Imbalance in RAS pathways in favor of ACE1 exacerbatess pulmonary tissue damage, leading to acute respiratory distress syndrome (ARDS) (Marshall, 2003). AngII promotes pulmonary vasconstriction, inflammation, and fibrosis (Uhal et al., 2012). Studies have also linked both AT1/AT2R with AngII-induced lung fibrosis via a mitogen-activated protein kinase (MAPK) pathway (Marshall et al., 2000). Further, AngII stimulates transforming growth factor-β (TGF-β) and collagen expression in fibroblasts and mediates alveolar epithelial apoptosis in mice via c-Jun N-terminal kinase (JNK) phosphorylation (Uhal et al., 2007, 2011).

On the other hand, the kidney is the main source of renin, the enzyme initiating RAS cascade. Renal AT,R expression is important in regulating baseline blood pressure, and the local renal RAS activation underlies hypertension-induced renal damage (Crowley et al., 2006). AngII induces a local immunoinflammatory response, leading to glomeruloclerosis, interstitial fibrosis, albuminuria, and renal failure (Park et al., 2007). A direct link between stimulation of RAS/AT1R, NADPH oxidase, reactive oxygen species, and renal remodeling was reported (Nistala et al., 2008). Also, AngII initiates fibrosis and inflammation in kidney through stimulation of TGF-β1 signaling (Chen et al., 2012) and triggering extracellular signal-regulated kinase (Erk)/p38MAPK/Smad3 (Mothet et al., 2013) signaling—e.g., hypertension, heart failure, and diabetes—ACE2 activity confers some protection by counteracting the effect of AngII activation, possibly by reducing cellular apoptosis and oxidative stress (Shenoy et al., 2010; Chang et al., 2011; Bernardi et al., 2016). Specifically, stimulating ACE2 activity decreased the levels of TNF-α, IL-1β, and IL-6 in addition to increasing the expression—e.g., hypertension, heart failure, and diabetes—ACE2 activity confers some protection by counteracting the effect of AngII activation, possibly by reducing cellular apoptosis and oxidative stress (Shenoy et al., 2010; Chang et al., 2011; Bernardi et al., 2016). Specifially, stimulating ACE2 activity decreased the levels of TNF-α, IL-1β, and IL-6 in addition to increasing the expression of anti-inflammatory cytokine IL-10 in an animal model of autoimmune myocarditis (Sukumaran et al., 2011).

Significantly, ACE2 plays a key role in cellular entry for several coronaviruses, including SARS-CoV-2, and its wide distribution may support viral invasion and potential damage in susceptible organs (Hamming et al., 2004; Chen et al., 2020c; Coutard et al., 2020). Conversely, ACE2 activity is crucial in noncommunicable disorders, such as autoimmune, cardiovascular, metabolic, and gastrointestinal diseases, to mitigate many associated proinflammatory and pathogenic effects (Zhang and Wada, 2007; Perlot and Penninger, 2013; Simões e Silva et al., 2013; Patet el al., 2016b). Thus, inhibition of ACE2 as a therapeutic target against SARS-CoV-2 has been ruled out from the start. As such, ACE2 has emerged as the double-edged sword in the SARS-CoV-2 ordeal, as it is both physiologically protective and virally conducive (Zores and...
Rebeaud, 2020). Highlighting the distribution of ACE2 in the body will elucidate various tissue-specific symptoms that may arise upon viral infection. Moreover, identifying its dysregulation in patients with comorbidities may offer insight as to why coronavirus infectious disease 2019 (COVID-19) symptoms are often more severe in these individuals.

ACE2 Tissue Distribution

ACE2 is a zinc metalloproteinase transmembrane protein characterized by having several domains: a signal peptide, a carboxy peptidase active site, a transmembrane domain, and a cytoplasmic domain (Donoghue et al., 2000; Haga et al., 2008). Importantly, it has been detected in cell culture media, indicating that it likely also exists in an extracellular secreted form. The enzyme is abundantly found on the vascular endothelium, including capillaries, arterioles, arteries, venules, and veins, which embed every organ (Hamming et al., 2004). Besides vessels, ACE2 is also majorly detected in the tissues of the lung, heart, kidney, and testis (Donoghue et al., 2000). It is present on cells that have direct contact with the environment, particularly type 1 and 2 alveolar epithelial pneumocytes of the lungs (Hamming et al., 2004). Hamming et al. (2004) were able to further characterize the distribution of ACE2-like immunoreactive material in approximately 72 different human tissues obtained from autopsy specimens, including the brush boarder of the small intestine enterocytes and renal proximal tubular cells, cholangiocytes, the brain, the basolateral layer of the skin epithelium, and epithelial cells of the oral and nasal mucosa and nasopharynx. Interestingly, a recent investigation correlates ACE2 expression levels with SARS-CoV-2 viral loads in swabs from different tissues in infected individuals (Sungnak et al., 2020; Zou et al., 2020).

The Effect of Comorbid ACE2 Dysregulation on COVID-19–Associated Symptoms

Many diseases are associated with impaired ACE2 activity in the implicated organs. One of the major corollaries of this impairment is an elevated proinflammatory response (Kangussu et al., 2019; Patel et al., 2016b). Ultimately, tissue injury and fibrosis may result. Patients with underlying diseases such as hypertension, diabetes, and coronary heart diseases have been shown to have more severe COVID-19 symptoms (Guan et al., 2020; Huang et al., 2020). This may be attributed to the pre-existing proinflammatory state, which is further propelled by the cytokine storm induced by SARS-CoV-2. Moreover, it can also be a consequence of an interplay with RAS and thus further loss of functional ACE2 units that are shed from the cell surface or downregulated after internalization following interaction with SARS-CoV-2 (Haga et al., 2008; Services et al., 2014; Gheblawi et al., 2020).

On the other hand, extrapulmonary symptoms in infected individuals with no known underlying diseases are increasingly emerging in patients with COVID-19. Besides pneumonia, other symptoms associated with the virus span the intestinal tract, cardiovascular system, central nervous system, and others (Fang et al., 2020; Zheng et al., 2020). We propose that the emergence of such symptoms is related to the presence of ACE2 in these specific tissues. Conversely, the disruption of ACE2 in patients with underlying diseases may contribute to the severity of symptoms detected in these individuals. Therefore, it is important to highlight the distribution of ACE2 in various organs and its dysregulation in specific diseases and to monitor related symptoms in inflicted patients.

Pulmonary Disease

The Role of ACE2 in Pulmonary Diseases

Pulmonary hypertension in rats led to an enlarged right ventricle, interstitial fibrosis, and increased pulmonary wall thickness (Ferreira et al., 2009). In tandem, renin, ACE, angiotensinogen, AT1R, and proinflammatory cytokines were also elevated. These manifestations were precluded by treatment with a synthetic ACE2 activator (Ferreira et al., 2009). In a related study, rats in which pulmonary fibrosis was induced displayed reduced levels of ACE2, augmented collagen deposits, increased expression of TGF-β and other cytokines, and elevated AT1R expression (Shenoy et al., 2010). Moreover, lung injury in ACE2-knockout mice was associated with an exacerbated reduction of exercise capacity, further impairment of lung function, and increased lung fibrosis (Li et al., 2016). This phenotype was markedly attenuated by Ang1–7 or ACE2 overexpression. These findings emphasize the integral role of ACE2 in pulmonary protection against inflammation and fibrosis. Similar findings were observed upon assessing the role of ACE2 in mouse lungs in situations of inhaled endotoxins (Sodhi et al., 2018). The loss of ACE2 expression induced an activation of des-Arg9 bradykinin/bradykinin B1 receptor pathway, leading to an increase in chemokines, macrophage inflammatory protein-2, TNF-α, and neutrophil infiltration, thus exaggerating lung inflammation and injury (Sodhi et al., 2018). Even in chronic airway inflammatory conditions like asthma, Ang1–7 significantly reduced the number of autoinflammatory-related leukocytes, including macrophages, eosinophils, and neutrophils (El-Hashim et al., 2012). Ang1–7 also reduced immune cell infiltration, fibrosis, and goblet cell metaplasia, which suggests airway remodeling. As such, it becomes plausible to assume that suppression of ACE2 activity/ expression might exacerbate lung injury after viral invasion.

ACE2 and Pulmonary Symptoms in Patients with SARS-CoV-2

ACE2 is highly expressed in the lungs (Letko et al., 2020), in which 85% of ACE2-displaying pulmonary cells were alveolar cells, implicating the alveoli as a reservoir for viral invasion (Li et al., 2020b). Recent investigation showed that although lung inflammatory disorders including asthma and chronic obstructive pulmonary disease are not associated with changes in ACE2 expression, smokers had increased pulmonary expression levels, further confirming the susceptibility of these patient groups (Grundy et al., 2020; Li et al., 2020a). Interestingly, this is thought to occur as a consequence of nicotine-mediated stimulation of α7-nicotinic acetylcholine receptors (Leung et al., 2020). Conversely, others propose that some of the SARS-CoV-2 proteins might carry amino acid sequences similar to those of known neurotoxins that block nicotinic acetylcholine receptors (Farsalinos et al., 2020b,c). This suggested a possible role for α7-nicotinic acetylcholine receptors as potential sites of interaction with SARS-CoV-2. As such, one might speculate that this interaction drives inflammatory cytokine production, whereby infected lung macrophage will be deprived of the anti-inflammatory effect.
exerted by nicotine-mediated activation of α7-nicotinic acetylcholine receptors on their surface (Kloc et al., 2020). If this competitive relationship between SARS-CoV-2 and nicotine on α7-nicotinic acetylcholine receptors is proven experimentally, it could potentially offer a molecular explanation for the clinical debate that nicotine, and hence tobacco smoking, might be protective against COVID-19 (Farsalinos et al., 2020a). On the other hand, the large surface area of the lungs favors their high susceptibility to inhaled viruses (Zhang et al., 2020a). Because of ACE2 abundance on pneumocytes and pulmonary vessels, massive SARS-CoV-2 entry and subsequent alveolar wall destruction is expected, contributing to the lung damage associated with COVID-19 (Hamming et al., 2004; Zhou et al., 2020b). Contrary to other coronaviruses, SARS-CoV-1 and SARS-CoV-2 have been found to decrease cell membrane–bound ACE2, which in turn promotes severe acute respiratory complications (Kuba et al., 2005; Haga et al., 2008; Ingraham et al., 2020), further alluding to the paradoxical nature of ACE2’s role in the course of pulmonary infections. However, other work reports a rebound in pulmonary ACE2 expression 48 hours postinfection that was correlated to inflammatory cytokine production (Li et al., 2020a), raising the question of why this induced increase in expression does not lead to the same beneficial effect associated with basal ACE2 expression/function.

Another factor that accounts for the much higher SARS-CoV-2 pulmonary infectivity is that the viral entry is mediated by cellular membrane proteases (Hoffmann et al., 2020). Transmembrane protease serine 2 (TMPRSS2) is expressed in the epithelial cells in human lungs and is involved in the regulation of airway surface liquid. Previous studies have identified TMPRSS2 as a key element in SARS-CoV pathogenesis (Matsuyama et al., 2010). TMPRSS2 enhances viral entry through cleavage of ACE2 and activation of cell membrane fusion by cleaving S protein Services et al., 2014. TMPRSS2 contributes to higher levels of cytokine and chemokine production after SARS-CoV infection. In TMPRSS2-knockout mice, the levels of monocyte chemoattractant-1 (MCP-1), IL-1α, IL-1β, and IL-12 in the lungs induced by SARS-CoV infection were much lower (Iwata-Yoshikawa et al., 2019). A new study using primary human airway epithelial cells reported that SARS-CoV-2 viral entry is highly dependent on TMPRSS2, as the inhibition of TMPRSS2 by camostat mesylate totally inhibited viral infection (Hoffmann et al., 2020). Camostat mesylate, currently used in Japan as an antiviral drug for other indications, could be useful in patients with COVID-19 (Kawase et al., 2012; Zhou et al., 2015; Yamamoto et al., 2016). After the SARS-CoV-2–induced cytokine storm, patients usually suffer from signs of microvascular dilation, increase of pleural effusions and thickening, and fibrotic streaks, which could lead to subsequent progression into acute lung injury, ARDS, and respiratory failure (Zhang et al., 2020b; Zhou et al., 2020c).

Cardiovascular Disease

ACE2 and Cardiovascular Diseases. Chronic hypertension and myocardial infarction are associated with cardiac remodeling, a pathologic process leading to heart failure and mortality (Grobe et al., 2007; Maaliki et al., 2019). The remodeling occurs after prolonged and enhanced production of AngII, and inhibition of the ACE1/AngII/AT1R system confers protection from these effects. In fact, induced overexpression of ACE2 reverses cardiac remodeling (Grobe et al., 2007) in a manner mediated by Ang1–7, which specifically reduced myocyte hypertrophy and interstitial fibrosis without affecting blood pressure. Furthermore, overexpression of ACE2 prevents cardiac hypertrophy in hypertensive rats (Sriramula et al., 2011). In a related study, ACE2 mRNA expression was markedly reduced in rat models of hypertension and was associated with defects in cardiac contractility, increased AngII levels, and upregulation of hypoxia-induced genes (Alaaeddine et al., 2019). Drugs used to treat hypertension, such as the AT1R blockers, upregulate myocardial expression of ACE2 and reduce levels of inflammatory markers like MCP-1, interleukins, and nuclear factor-κ-light-chain enhancer of activated B cells (Sukumaran et al., 2012). The ACE1/AngII/AT1R system also promotes atherosclerotic lesion formation, aneurysms, and proinflammatory cytokine secretion (Daugherty et al., 2000). ACE2 has been shown to counteract these effects. For example, knockout studies of ACE2 in mice led to plaque growth as well as an increase in several proinflammatory and atherogenic proteins, like adhesion molecules, IL-6, and MCP-1 (Thomas et al., 2010).

ACE2 and CVD Symptoms in Patients with COVID-19. Cases of acute cardiovascular anomalies have been reported in patients with COVID-19 that were previously healthy. Patients show high levels of troponin, creatine kinase, arrhythmias, and incidences of myocardial injuries, which lead to higher rates of intensive care unit admission and mortality (Guo et al., 2020; Inciardi et al., 2020; Wang et al., 2020a). Patients with high troponin levels also had high levels of C-reactive protein, suggesting an inflammatory pathway linked to the SARS-CoV-2–induced myocardial injury (Guo et al., 2020). Thus, the myocardial injury seen could be attributed to SARS-CoV-2 direct viral entry as well as an increased production of cytokines that can result in decreased coronary blood flow and oxygen supply (Oudit et al., 2009). In addition, similar to other respiratory infections (Milbrandt et al., 2009), patients with COVID-19 demonstrate elevated D-dimer levels, which were also associated with disseminated intravascular coagulation, severe symptoms, and higher risk of mortality (Guan et al., 2020; Zhou et al., 2020a). Contributory mechanisms could include a series of inflammatory-immunologic reactions, which could directly contribute to atherothrombotic plaque rupture, predisposing the patient to ischemia and thrombosis (Zhou et al., 2020a).

Significantly, ACE2-mediated viral endothelial entry could induce vascular disorders and an accelerated coagulation in patients with COVID-19 (Gallagher et al., 2008). Indeed, SARS-CoV-2 was shown to infect endothelial cells in different vascular beds in patients with COVID-19 (Varga et al., 2020). Treatment with clinical-grade human recombinant ACE2 precluded SARS-CoV-2 infection in blood vessel organoids, highlighting the importance of ACE2 in endothelial infection (Monteil et al., 2020). The endothelium plays a critical role in the regulation of the adherence of immune cells, capillary permeability, and clotting and platelet activation, all of which could be altered by viral infection (Dalrymple and Mackow, 2014). Under normal conditions, vascular endothelial growth factor (VEGF) induces repair of vascular damage. Hantavirus, a virus affecting the respiratory system, was shown to bind to VEGF and disengage the normal regulation of VEGF-induced permeability (Gavrilovskaya et al., 2012). A similar profile is
encountered in patients with COVID-19. Indeed, serum VEGF levels were elevated in patients with COVID-19 (Huang et al., 2020), possibly triggered by hypoxia and inflammatory changes and contributing to increased vascular permeability and pulmonary edema. Interestingly, increased ACE2 expression was shown to reduce VEGF production in vitro and in vivo (Cheng et al., 2016), ameliorating increased vascular permeability during lung injury (Yu et al., 2016), which raises the question of whether SARS-CoV-2–induced endothelial ACE2 downregulation could underlie this observation. These findings highlight the importance of monitoring VEGF levels in patients with COVID-19 and also suggest a possibility of introducing treatment modalities to target VEGF responses. Bevacizumab, a humanized monoclonal anti-VEGF antibody, is currently being studied for a potential beneficial effect in reducing lung injury due to increased vascular permeability (ClinicalTrials.gov: NCT04275414) (clinicaltrials.gov/ct2/show/NCT04275414, 2020).

On the other hand, accumulating evidence indicates an association between existing cardiovascular and metabolic disease with further progression of severe COVID-19 complications (Liu et al., 2020a; Rodriguez-Morales et al., 2020; Wehbe et al., 2020; Zhou et al., 2020a). Higher mortality rates and progression of ARDS were reported in patients with hypertension, diabetes, and coronary artery diseases (Wu et al., 2020; Wu and McGoogan, 2020). This could be attributed to stimulation of immunoinflammatory pathway, dysregulation of RAS, and most importantly, viral entry through upregulated ACE2, as previous work showed that patients with heart failure had higher ventricular ACE2 expression levels (Zisman et al., 2003). Indeed, a recent study reported that patients with COVID-19 and heart failure had higher cardiac ACE2 expression (Chen et al., 2020a). Specifically, higher ACE2 expression was observed in cardiac pericytes, raising the possibility that cardiac injury could be initiated by microvascular dysfunction. Moreover, ACE2 expression in vascular endothelial cells could facilitate localized vascular injury and subsequent viral spread. As such, in situations of altered vascular function, vascular inflammation and/or increase in VEGF, SARS-CoV-2 vascular viral entry, and viral spread could be aggravated.

**Inflammatory Disease: Obesity, Diabetes, and Autoimmune Disorders**

ACE2 activity has been detected in macrophages, in which its deficiency led to an increased production of AngII; proinflammatory cytokines, like TNF-α, MCP-1, IL-6, and matrix metalloproteinase-9; and endothelial adhesion molecules (Thomas et al., 2010; Thatcher et al., 2011). Importantly, overexpression of ACE2 by macrophages significantly reduced their MCP-1 production (Guo et al., 2008), and Ang1–7 treatment decreased macrophage inflammatory response (Souza and Costa-Neto, 2012). These findings further support the vital role of ACE2 in relieving the proinflammatory response; thus, both low- and high-grade inflammatory conditions could affect the pathogenesis of COVID-19. Interestingly, obesity is associated with increased RAS activity in adipose tissue (Yvan-Charvet and Quignard-Boulangé, 2011) together with increased macrophage infiltration (Weisberg et al., 2003). Adipose tissue macrophages in obesity tend to acquire macrophage (M1) polarization with increased expression of proinflammatory cytokines (Lumeng et al., 2007). Under such circumstances, adipose inflammation in obesity contributes to insulin resistance, glucose intolerance, and cardiovascular dysfunction (Jiao and Xu, 2008; Elkhatib et al., 2019). Interestingly, ACE2 deficiency enhanced M1 polarization of adipose tissue macrophages, increased adipose tissue inflammation, exacerbated the metabolic dysfunction, and worsened the associated cardiovascular function in diet-induced obesity (Thatcher et al., 2012; Patel et al., 2016a). Indeed, recent evidence implicates obesity as a risk factor for severe ARDS in patients with COVID-19 (Simonet et al., 2020). Whereas no change in adipose tissue ACE2 expression levels was observed in patients with obesity compared with lean individuals (Pinheiro et al., 2017), the increased disease severity could be attributed to the overall inflammatory state that results from an obesity-triggered systemic RAS imbalance (Engeli et al., 2005).

Moreover, functional and clinical evidence supports the role of vascular inflammation induced by metabolic diseases in vascular impairment and CVD (Assar et al., 2016; Ormazabal et al., 2018). Patients with type 2 diabetes mellitus have reduced levels of ACE2 expression in various organs, which correlates with inflammatory changes especially in the kidney (Bindom and Lazartigues, 2009). Along the same lines, high levels of VEGF were observed that were correlated with high fasting blood glucose and glycosylated hemoglobin (HbA1c) levels (Zhang et al., 2018). Increases in VEGF reflected the severity of endothelial dysfunction in patients with diabetes and may lead to microvascular complications. In addition, higher levels of VEGF are associated with diabetes-induced central and peripheral neuropathy (Atif et al., 2017; Jerić et al., 2017; Tang et al., 2018). Collectively, vascular inflammation in patients with CVD and higher expression of VEGF in patients with diabetes could be additional factors to enhance viral entry and viral spread, which consequently increase COVID-19 severity in those patients. It is noteworthy that increased usage of VEGF inhibitors could induce cardiovascular and nephrological consequences; as such, extreme care should be taken in treatment with such patients (Kikuchi et al., 2019). Moreover, it is noteworthy that patients who previously recovered from SARS-CoV-1 infection reported high blood pressure as well as altered metabolism 12 years later (Wu et al., 2017). Knowing the similarities between SARS-CoV-1 and -2, a similar profile could also occur in patients with COVID-19.

ACE2 is also implicated in the autoimmune disease of rheumatoid arthritis. Mouse models of antigen-induced arthritis portrayed elevated levels of neutrophils in the knee joints, and treatments with Ang1–7 or its analog, AVE0991, were able to reduce neutrophil migration in joints and periarticular tissue (da Silveira et al., 2010). Likewise, the elevated levels of proinflammatory cytokines TNF-α, chemokine (C-X-C motif) ligand, and IL-1β were reduced by Ang1–7 treatment together with hypernociception, the pain index of arthritis (da Silveira et al., 2010). Interestingly, this effect was not mediated by hypotensive or vasodilatory actions. Thus, Ang1–7 is able to mediate these effects by inhibiting local production of cytokines and, importantly, by hindering leukocyte-endothelium adhesion and rolling on the microvasculature of the knee joint. Importantly, Ang1–7 receptors have been detected on microvessels as well as leukocytes (Nie et al., 2009). These findings suggest that loss of ACE2 activity in
patients with arthritis may inflame symptoms and cytokine production. Significantly, a recent meta-analysis showed some indication that autoimmune diseases might be associated with an increased risk of COVID-19 severity and mortality (Liu et al., 2020b); on the other hand, emerging reports indicate that SARS-CoV-2 infection precedes the development of various autoimmune disorders (Galeotti and Bayry, 2020). In either case, the role of ACE2 expression or activity alterations has not been examined.

Renal Disease

ACE2 in Renal Disease. ACE2 is abundantly expressed on the vascular endothelium supplying the kidneys and on the tubular and glomerular epithelium (Lely et al., 2004). Mice lacking ACE2 develop late-onset nephrotic glomerulosclerosis (Oudit et al., 2006) and more profound diabetic renal injury (Wong et al., 2007), where ACE2 activity was shown to be protective by reducing local AngII levels (Batlle et al., 2012). Indeed, in a related study of diabetic mice, RAS imbalance involving ACE2 downregulation was found to be involved in renal damage mediated via elevated reactive oxygen species (Chang et al., 2011). Importantly, the renoprotective effects of ACE2 are not only restricted to chronic disorders. While renal ischemia and reperfusion trigger acute kidney injury via activation of inflammatory cascades involving increased migration of leukocytes and release of cytokines, treatment with the Ang1–7 analog AVE0991 reduced renal injury and inhibited leukocyte migration in mice post–ischemia reperfusion (Barroso et al., 2012). These findings broadly categorize the ACE2 axis once again as anti-inflammatory. However, the role of ACE2/Ang1–7 appears to be rather complex and should be approached with caution, as other studies have conversely demonstrated that this pathway may contribute to certain forms of kidney injury in diabetes, including fibrogenesis and renal hypertrophy (Tikellis et al., 2008), possibly affected by differences in disease stage, locally activated signaling pathways, and dosages of the activating or inhibiting agents used (Zimmerman and Burns, 2012).

ACE2 and Renal Symptoms in Patients with SARS-CoV-2. Acute kidney injury was identified in up to 25% of patients admitted with COVID-19 (Fanelli et al., 2020), representing a higher mortality risk in these patients (Wilson and Calfee, 2020). A similar profile was seen in two other studies in which patients showing progressive increases in blood urea nitrogen and creatinine had higher mortality rates (Chen et al., 2020b; Wang et al., 2020a). The mechanism behind this renal injury could be multifactorial. Although ischemic injury and inflammatory cytokines are likely to contribute to renal damage, direct SARS-CoV-2 entry through ACE2 to the renal cells could still be a major factor. SARS-CoV-2 entry in a human kidney cell line required priming by both TMPRSS2 and the endosomal cysteine proteases cathepsin B and L (Hoffmann et al., 2020). However, viral entry in ACE2-expressing HEK293 cells was reported to be mediated through endocytosis in which other proteins, such as PIKfyve, (Phosphoinositide kinase, FYVE-type zinc finger containing phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase type III) two pore segment channel 2, and cathepsin L, were found essential for virus entry (Ou et al., 2020). Significantly, a recent study showed cellular damage and direct ultrastructural evidence of viral infection in proximal tubular epithelial cells in postmortem examination of a patient with COVID-19 and acute kidney injury (Farkash et al., 2020). Yet other studies argue against a causal relationship between COVID-19 infection and acute kidney injury (Wang et al., 2020b). Indeed, the same factors that contribute to SARS-CoV-2 infection severity and involve alteration of ACE2 activity/expression, including age, obesity, and diabetes, contribute to increased risk of acute renal dysfunction (Fanelli et al., 2020), making the establishment of causal relationships and the examination of a role for ACE2 difficult in this context.

The Reproductive System

ACE2 in Reproductive Diseases. Although ACE1, AngII, AT1R, AT2R, ACE2, Ang1–7, and Ang1–7 receptors are all involved in the reproductive physiology in both males and females, ACE2 was found to play an important role in the testis, with expression specifically enriched in Leydig cells and cells in the seminiferous tubules (Donoghue et al., 2000; Pan et al., 2013). In males, ACE1/AngII and ACE2/Ang1–7 balance drives optimal male fertility, including steroidogenesis, epididymal contractility, and sperm cell function. Males with impaired spermatogenesis do not have detected levels of Ang1–7 or Ang1–7 receptors in seminiferous tubules compared with healthy males (Reis et al., 2010). Also, lower levels of ACE2, Ang1–7, and Ang1–7 receptors in the testis are correlated to severe impairment in spermatogenesis and lower testicular weight (Reis et al., 2010). ACE2 is specifically implicated in spermatogenesis because of its presence in Leydig cells, which produce sex hormones. Moreover, ACE2 is being increasingly considered as a therapeutic mechanism to improve male fertility (Pan et al., 2013). In addition, the expression of ACE2, Ang1–7, and Ang1–7 receptors in human ovaries is directly affected by gonadotropin, suggesting an important role in ovarian physiology, follicular development, and ovulation (Pereira et al., 2009; Reis et al., 2011). Collectively, disruption in ACE2 in male and female reproductive cells may contribute to reproductive impairments and polycystic ovarian syndrome, respectively. Furthermore, its presence within the testes makes them vulnerable to possible damage by SARS-CoV-2.

ACE2 and Reproductive Impairment in Patients with SARS-CoV-2. SARS-CoV-2-induced viral testicular tissue damage has been reported. SARS-CoV leads to germ destruction, loss of spermatozoan in seminiferous tubules, and leukocyte infiltration (Xu et al., 2006). The possibility of viral orchitis after SARS-CoV-2 infection potentially leading to testicular damage and infertility remains high (Cardona Maya et al., 2020). As such, it is recommended that proper care is given to male patients with COVID-19 to guard against possible orchitis, and follow-up in males recovering from COVID-19 is equally important. Furthermore, exacerbation of polycystic ovarian syndrome symptoms in females may also need proper follow-up.

The Digestive System

ACE2 and the Digestive System. Although ACE2 is a key component of the RAS system, it has an independent role in the gut. ACE2 is necessary for the expression of transporters of neutral amino acids in the small intestine (Hashimoto et al., 2012; Perlot and Penninger, 2013). Furthermore, ACE2 has been shown to confer protection against
colitis. This was also directly correlated with a reduced uptake of tryptophan, one of the main functions of ACE2 in the gut. A reduction in tryptophan results in a reduction in the mammalian target of rapamycin pathway in the small intestine. This impairs the expression of antimicrobial peptides, leading to an altered intestinal microbiome and thus an increased susceptibility to colitis (Perlot and Penninger, 2013). Hence, ACE2 has a key role in maintaining a protective healthy biome in the gut, and its function involves a mammalian target of rapamycin pathway.

**ACE2 and Digestive Impairment in Patients with SARS-CoV-2.** Reports from Wuhan highlighted the enteric involvement of COVID-19: 2%–10% of patients with COVID-19 initially presented to hospitals with gastrointestinal symptoms, such as diarrhea, abdominal pain, and vomiting (Chen et al., 2020b; Yang et al., 2020). Patients expressed symptoms 1 to 2 days prior to the development of respiratory symptoms and fever. Moreover, patients admitted to intensive care units were more likely to report abdominal pain and anorexia (Wang et al., 2020a). The intestine represents one of the biggest immune system compartments and is highly vascularized and innervated. Previous studies reported that the gut impacts the viral immune and neuronal systems and also affects pulmonary function (Mowat and Agace, 2014; Shenoy et al., 2014). In fact, the progression of intestinal infection, inflammation, and subsequent live virus emergence in the lungs suggests the development of sequential respiratory infection. Intestinal cells are highly permissive to coronaviruses regardless of the stage of cell differentiation, suggesting a high sensitivity of the intestinal epithelial cells to these viruses (Cinatl et al., 2004; Zhou et al., 2017). Furthermore, ACE2 is expressed in the oral cavity, particularly in epithelial cells of the tongue. Also, lymphocytes in the oral cavity express ACE2 receptors in a similar density as those in the lungs (Xu et al., 2020). The stomach and the esophagus have not yet displayed infected epithelial cells. SARS-CoV-2 infection could have been initiated by eating raw food from the Wuhan market, the center of coronavirus outbreak. Yet, ACE2 being located in the basolateral membrane of the oral mucosa makes the viral uptake via this route less likely to be efficient. However, its presence on the apical surface of the enterocytes and the detection of the virus in stool samples provides a possible explanation and highlights the possibility of fecal-to-oral transmission, as stated by previous studies (Yeo et al., 2020).

### The Bile Duct

**ACE2 and Biliary Impairment in Patients with SARS-CoV-2.** The possible involvement of hepatotoxicity in patients with COVID-19 is questionable. Liver injury is found to be more prevalent in severe than in mild cases of COVID-19, and significant increases in liver enzymes and bilirubin were reported in 20%–37% of cases (Chen et al., 2020b; Huang et al., 2020). Taking this into account, it is not clear whether liver injury in patients with COVID-19 is mainly due to direct entry of the virus into liver cells or induced by certain drugs after treatment. ACE2 has not been detected on hepatocytes but rather on cholangiocytes of the bile ducts (Hamming et al., 2004). However, the role of ACE2 in liver disease is of great importance, as it participates in the regulation of liver inflammation, tissue remodeling, and liver fibrosis (Rajapaksha et al., 2019). The protective pathway of ACE2 is thought to be mediated through a significant reduction in AngII-induced fibrosis, TGF-β, and NADPH oxidase (Rajapaksha et al., 2019). A study by Chai et al. identified cholangiocyte-specific expression of ACE2, which could have been a possible route of SARS-CoV-2 entry and hence could lead to profound liver toxicity in patients with COVID-19 (X. Chai et al., preprint, DOI: https://doi.org/10.1101/2020.02.03.931766). Moreover, ACE2 expression and activity were shown to be induced as a response to chronic liver injury (Osterreicher et al., 2009). As such, this begs the question of whether patients with chronic hepatic inflammatory conditions might be at a higher risk of SARS-CoV-2–induced liver damage. Subsequently proper monitoring of liver function should be undertaken to guard against liver injury in patients with COVID-19.

### The Nervous System

**ACE2 and the Brain.** ACE2 is also found in the brain (Xu et al., 2011). The highest activity of ACE2 in the central nervous system is detected in the hypothalamus (Xu et al., 2011). AngII has been shown to reduce levels of ACE2 expression in cerebral and medullary astrocytes, although the exact role is still elusive. Conversely, ACE2 overexpression in the paraventricular nucleus has been shown to directly impede AngII-induced hypertension (Sriramula et al., 2011). As such, aside from the local neurogenic effects, even within the brain, ACE2 can impart cardioprotective roles.

**ACE2 and Central Nervous System Symptoms in Patients with SARS-CoV-2.** Of the most common atypical symptoms in patients with COVID-19 are the neurologic signs, which were present in 45.5% of patients with severe cases (L. Mao et al., preprint, DOI: https://doi.org/10.1101/2020.02.22.20026500). In this study, headache (13.1%), dizziness (16.8%), and impaired consciousness (14.8%) were the most common signs of viral neuroinvasion. Other studies also reported the prevalence of neurologic symptoms as well (Baig et al., 2020; Chen et al., 2020b; Wang et al., 2020a). Moreover, a new COVID-19 case was reported to have altered mental status that developed into hemorrhagic necrotizing encephalopathy (Poyiadji et al., 2020). Moreover, a sudden loss of smell and/or taste is common in patients infected with SARS-CoV-2, possibly occurring without any other typical symptoms, which highlights central nervous system involvement as a COVID-19 manifestation (Gautier and Ravussin, 2020). Previous studies detected SARS-CoV in the brain. The virus was administered via the intranasal route, where it enters through the olfactory neurons and heavily infects several areas of the brain, including piriform and infralimbic cortices, basal ganglia, and midbrain with first- or second-order connections with the olfactory bulb (Netland et al., 2008). Other sites, including those in the brainstem, were shown to have been possibly infected via the oral route. In this study, SARS-CoV infection induced neuronal death in absence of encephalitis in mice. Moreover, SARS-CoV is thought to invade the peripheral nerve terminals and then get access to the central nervous system via the synaptic-connected route (Li et al., 2012, 2013). More studies have documented the neuroinvasion of other coronaviruses, in which it was shown to spread through peripheral nerves into the medullary neurons involved in the peristaltic movement of the digestive system, leading to vomiting (Li et al., 2020c). The transfer between nerves could...
be through clathrin-mediated endocytosis/exocytosis pathway (Li et al., 2013). As for SARS-CoV-2, the latency period of the virus could be sufficient for neuroinvasion. Besides, severe development of COVID-19 could be linked to neuroinvasion and subsequent dysfunction in the cardiorespiratory center in the brainstem, as reported elsewhere (Netland et al., 2008). On the other hand, in addition to viral entry to neurons via ACE2 receptors, brain ischemia could also take place as a result of downregulation of ACE2 expression (Simões E Silva et al., 2017). As such, care should be taken to guide toward proper treatment and prevention of possible neuroinvasion of SARS-CoV-2–induced cardiorespiratory failure (Li et al., 2020c). Altered consciousness could be as devastating as respiratory distress syndrome.

**ACE2 Protein Interactions and Associated Signaling Pathways**

**ACE2 Interacts with Various Proteins.** It is well known now that SARS-CoV-2 and SARS-CoV share 76% amino acid identity and use ACE2 as a receptor for entry (Lu et al., 2020). The spike proteins (S proteins) of coronaviruses are responsible for attachment to target cells and cellular invasion (Fehr and Perlman, 2015). Priming of SARS-CoV S proteins by host cell proteases is fundamental for proper viral uptake (Simmons et al., 2004, 2005; Matsuyama and Taguchi, 2009). Several proteases have been previously reported to activate SARS-CoV S protein. Yet in the absence of proteases on the cell surface, SARS-CoV entry occurs through the endosomal pathway, in which S protein is activated by endosomal proteases. However, when proteases are expressed on the cell surface, viral replication is shown to be 100 times higher (Matsuyama et al., 2005). In the latter case, S proteins will be activated and cleaved at S1/S2 and S2’ site. The cleavage at S2’ site exposes S2 domains and enables the partition of membrane by fusion peptide and internal fusion peptide and facilitates viral entry (Lu et al., 2015).

Although ACE2 is the receptor for SARS-CoV-1 and SARS-CoV-2, there are major differences between infectivity, onset of symptoms, severity of symptoms, and mortality rate. SARS-CoV-2 poses an immense challenge compared with the SARS pandemic of 2003. Whereas a total of 8098 patients worldwide got infected with the first SARS-CoV, out of whom 774 had died (https://www.cdc.gov/sars/about/fs-sars.html), up to the time this manuscript was written, SARS-CoV-2 has resulted in over 56 million infections and over 1 million deaths. Whereas 100% of patients with SARS-CoV-1 had fever, only around 44% of individuals with SARS-CoV-2 displayed elevated temperatures. Additionally, 3.8% of patients with SARS-CoV-2 have displayed diarrhea compared with the 22% of individuals infected with SARS-CoV-1 (Huang et al., 2020). One explanation for this difference is that the S protein of SARS-CoV-2 binds with greater affinity to ACE2 than SARS-CoV-1. The disparity in the furin-like S2’ cleavage site between SARS-CoV-1 and -2 may impart unique ACE2-protein interactions and signaling cascades that ultimately play a role in the differences noted between these two coronaviruses (Coutard et al., 2020). Because of these reasons, it is necessary to characterize established and potential interactions with the receptor.

On the apical membrane of intestinal enterocytes, ACE2 has been shown to colocalize with the broad neutral amino acid transporter B^AT1 (SLC6A19, Sodium-dependent neutral amino acid transporter) and CD13 (Jando et al., 2017). CD13 is a type II transmembrane zinc metalloprotease that catalyzes the removal of amino terminal amino acids from peptides. The expression of B^AT1 directly depends on the coexpression of ACE2. B^AT1 is also detected in the kidney proximal tubule in association with collectrin (TMEM27, transmembrane protein 27), which interestingly shares the same membrane-anchoring domain of ACE2. In this context, ACE2 is considered a dimer, and the interaction with B^AT1 suggests that S protein is able to bind to two sites. The collectrin-like membrane-anchoring domain in ACE2 has been shown to enable the interaction with B^AT1. Collectrin is considered a homolog of ACE2 (Zhang and Wada, 2007). Not only is it involved in amino acid uptake, but it has roles in the development of renal epithelial cilia and insulin secretion. Importantly, it can interact with the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) complex via snapin. SNARE is indispensable for enabling the fusion of vesicles to their target intracellular compartments and directing vesicles to the extracellular membrane, as in the case of secreting insulin (Martens and McMahon, 2008). It is worth examining the role of ACE2 in similar processes, particularly in the context of viral uptake. Although B^AT1 is not necessarily prominent in the lungs, the homology between ACE2 and collectrin may highlight similar roles for this receptor, making it prudent to delineate it in the context of viral infection.

TMPRSS2 is a type II membrane serine protease that has been shown to cleave the S protein to allow efficient entry (Matsuyama et al., 2010). It should be noted that HAT (histone acetyl transferase) is a similar protein that was shown to activate SARS-COV-1 but, to our knowledge, has not been confirmed in the context of SARS-COV-2. Importantly, TMPRSS2 can also cleave the ACE2 receptor, and this event is implicated in increased viral invasion. However, the processing of ACE2 does not have any effect on cleavage of the viral S protein itself. Increased expression of TMPRSS2 is directly associated with increased viral entry. As such, the association of TMPRSS2 with ACE2 spurs increased viral entry by cleavage of ACE2 and enhances S protein membrane fusion by direct cleavage of the S protein (Services et al., 2014).

Cathepsin L is also a well studied protease in SARS-COV infections. Contrary to TMPRSS2, cathepsin L is an endosomal and lysosomal pH-dependent protease that processes S protein after uptake. However, initial cleavage by TMPRSS2 is independent of cathepsin L and is in fact the main way of entry (Services et al., 2014).

Combination of the S protein with ACE2 also triggers processing of ACE2 by disintegrin and metalloproteidase domain 16/tumor necrosis factor-α converting enzyme. This leads to shedding of a functional ACE2 into extracellular space and promotes uptake of SARS-CoV-1. It is likely that a similar mechanism occurs upon S protein binding of SARS-CoV-2 (Services et al., 2014).

Another protease that is worth examining is furin and its related proteins. The unique cleavage site on S2’ is postulated to interact with furin or furin-like proteases, which are associated with more potent virulence. Hence, upon conducting a protein blast, we detected proteins with considerable sequence homology that may also potentially interact with the S protein (Table 1).
TABLE 1
Potential proteins that may be involved in S protein processing and ACE2 interaction

<table>
<thead>
<tr>
<th>Protein Implicated in SARS-CoV-2 Entry</th>
<th>BLASTp Results in Human Protein Data Base</th>
<th>Sequence Identity (Filter: min ~50%)</th>
<th>Relevant (UniProtData Base)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furin pro tease</td>
<td>Caderhin-related family member 4</td>
<td>55.6%</td>
<td>Expressed in lung, uterine tube, testis (similar pattern to ACE2 prominent distribution) single-pass transmembrane protein.</td>
</tr>
<tr>
<td>Isoform 7 of tumor necrosis factor receptor superfamily member 25</td>
<td>Interacts with receptor adapter protein TRADD and activates NF-kB, which are involved in inflammatory-induced apoptosis. May play a role in regulating lymphocyte homeostasis.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BLASTp, protein-protein basic local alignment research tool; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TRADD, tumor necrosis factor type 1-associated DEATH domain protein.

Signal Pathways Associated with SARS-CoV-2 Entry. Not only is ACE2 the mode of entry of SARS-CoV-1 and -2, but it is also involved in its major symptom, the untreatable ARDS. MAPK pathways involving ERK, p38, and JNK are significantly activated and associated with the process of ARDS. Lipopolysaccharide-induced lung damage is diminished upon inhibition of p38 and JNK. The activity of p38 in the lung is linked to pulmonary edema and fibrosis. In the context of the heart, it was shown that ACE2 can directly inhibit ERK, thereby reducing cardiac fibrosis. We have previously highlighted the involvement of MAPK pathways in the manifestation of cardiovascular diseases like hypertrophy and atherosclerosis, as well as the possible hijack of these pathways to assist SARS-CoV-2 host internalization and replication (Webhe et al., 2020).

ACE2 present on microvascular endothelial cells has been shown to inhibit local ERK and JNK pathways, thereby avoiding apoptosis and inflammation under lipopolysaccharide-induced inflammatory conditions (Li et al., 2016). It is worth examining whether inhibition of ACE2 to block viral entry, while infusion with Ang1–7, is sufficient to maintain the protective effect of ACE2 on various organs involved.

Concluding Remarks
SARS-CoV-2 invasion is proving to be a complex and multifaceted issue, making it difficult to quell. Much of the complexity arises from the wide physiologic distribution of its favored receptor, ACE2. Adding further insult to injury is the dual role of ACE2 in this viral dilemma. As we have demonstrated, the protective role of this receptor is important in mitigating several diseases and offering proper homeostasis. Potential therapeutic mechanisms should include ways to preserve its regulatory role while shunting downstream events that may be hijacked for viral entry. For example, further investigation in protein-protein interactions and signaling pathways associated with ACE2 may offer various perspectives on how to subvert viral-induced uptake and organ injury.

Authorship Contributions
Wrote or contributed to the writing of the manuscript: Hammond, Wehbe, Abdelhady, Kobeissy, Eid, El-Yazbi.

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Along with the previous bibliography, a few more recent studies that are relevant to the discussion:


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