

The impact of SARS-Cov-2 infection on the renin-angiotensin-aldosterone system and its axes. Pathogenic consequences

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Abstract

Coronaviruses were discovered and identified after a long series of researches of variants of viruses that were responsible for the common cold, with numerous characterization attempts, being considered for some time rhinoviruses. Intracellular penetration of the virus is achieved through spike glycoproteins with the two subunits - S1 and S2. The S1 subunit binds to the angiotensin-converting enzyme 2 (ACE-2) receptor on the surface of the host cell and S2 fuses with the cell membrane. We present a synthesis of the links between the viral receptor (ACE2), the place of ACE2 in the complex of the renin-angiotensin-aldosterone system, and its axes (ACE2-Ang (1-7) AngA/alamandine/RMas, the kallikrein-kinin/RMrgD system and pathogenic consequences of viral aggression.

Keywords: SARS-Cov-2 , renin-angiotensin-aldosterone system, receptors, pathophysiology.

Introduction

Coronaviruses were discovered and identified after a long series of researches of variants of viruses that were responsible for the common cold, with numerous characterization attempts, being considered for some time rhinoviruses [1].

In April 1967, Tyrell and J. Almeida identified uncharacterized respiratory viruses that were not associated with human disease. In 1968, Tyrell *et al.*

published a paper on a group of viruses that cause avian bronchitis, murine hepatitis, and upper respiratory infections in humans, registering for the first time the “coronaviruses” name [2].

According to the classification, coronaviruses that contaminate humans are, in most cases, beta-coronaviruses. The name is generated by the crown appearance that appears on the electronic microscopy images of the virus.

Intracellular penetration of the virus is achieved through spike glycoproteins with the two subunits S1 and S2. The S1 subunit binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell’s surface, and S2 fuses with the cell membrane [1].

In this process, the intervention of a transmembrane protease is essential (TMPRSS2). This protease activates the S1 spike and cleaves it, separat-

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ing it from ACE2; it also acts on S2, facilitating its fusion (in fact, the fusion of the virus) with the cell membrane, an essential phase for penetration into the cell, usually by endocytosis.

The intracellular release of the virus from the endosome is achieved by acidification or the action of an intracellular cysteine protease - cathepsin. A synthesis of the links between the viral receptor (ACE2), the place of ACE2 in the complex of the renin-angiotensin-aldosterone system and its axes (ACE2-Ang (1-7) AngA/alamandine/RMas, the kallikrein-kinin/RMrgD system and pathogenic consequences of viral aggression is presented in this paper.

The RAAS and its axes

The identification of renin by R. Tigerstedt and P.G. Bergman brought the renin-angiotensin-aldosterone system (RAAS) to light [3].

In more than 120 years, the knowledge of the multiple components and complex interrelationships has been completed, including the pharmacological arsenal that can control and modulate, to a large extent, the different axes and links of this extraordinary system.

RAAS functions are of great complexity and relevance, as shown by a brief enumeration: hydroelectric balance control, vascular resistance, blood pressure, and cardiovascular homeostasis. Its dysfunctions are associated with oxidative stress, endothelial dysfunction, inflammation, hypertension, heart failure, and kidney failure [4, 5].

In the evoked functional context, a series of peptides, which, by enzymatic cleavage, generate another succession of peptides, mostly active or inactive, according to the biological tendency of the organism have been identified.

The angiotensin-converting enzyme (ACE) is a membrane endopeptidase that converts angiotensin I (AngI (1-10)) to angiotensin II (1-8). ACE is also found in the plasma, in relatively high concentration, being derived from the endothelium. Tissue ACE, in particular, also converts other peptides belonging to the SRAA system and its axes such as Ang (1-7), bradykinin, kallikrein (Figures 1-3), alamandine, and others.

Another important enzyme in the system, also a peptidase, is the angiotensin-converting enzyme-2 (ACE2), with membrane localization, especially in endothelial cells. ACE2 is the functional receptor of the coronavirus SARS, according to Wi Li *et al.*, whose statements are still valid for SARS-Cov-2 [6].

It should be emphasized that ACE2 is the key enzyme that controls and counteracts the negative

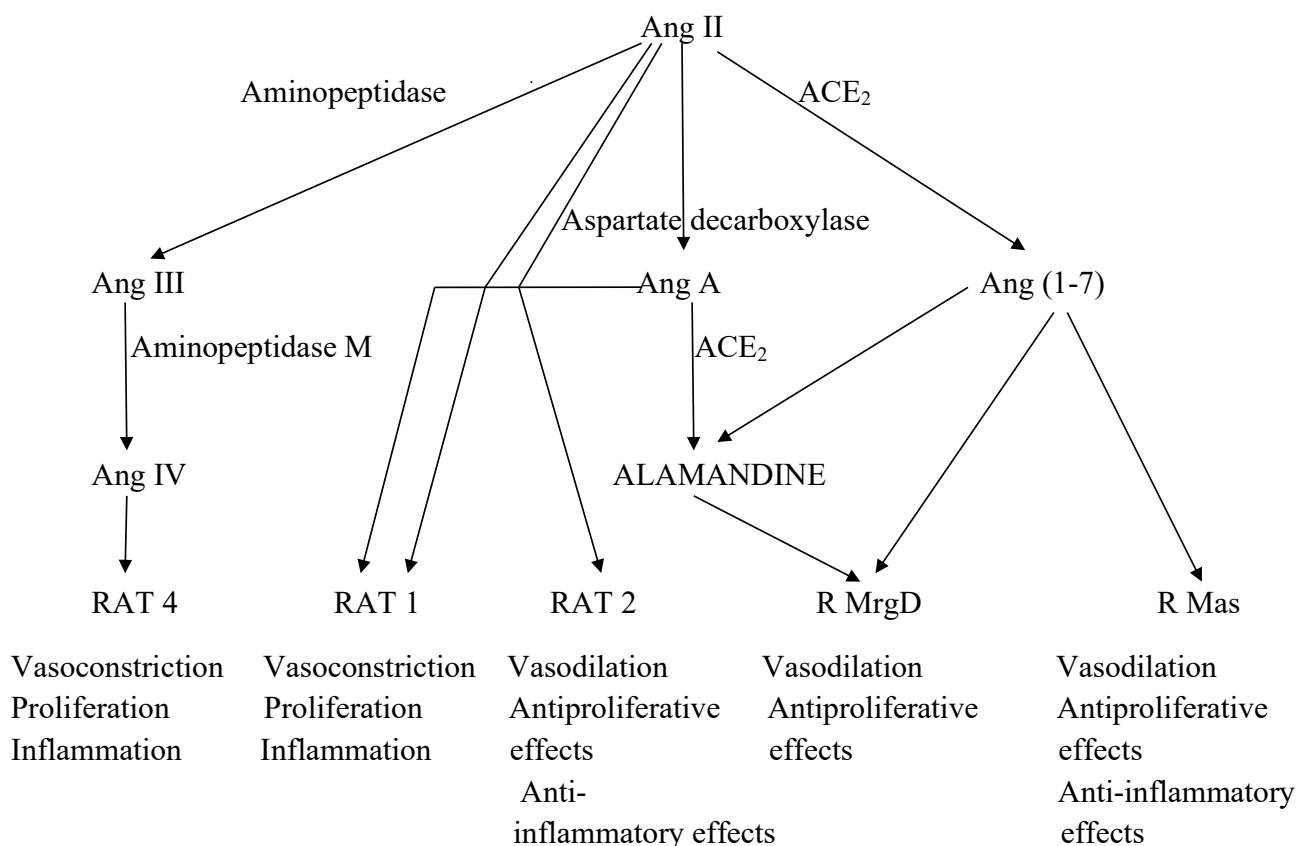


Figure 1. The relationship between Ang II and Ang (1-7)/RMas and alamandine/RMrgD and their specific receptors.

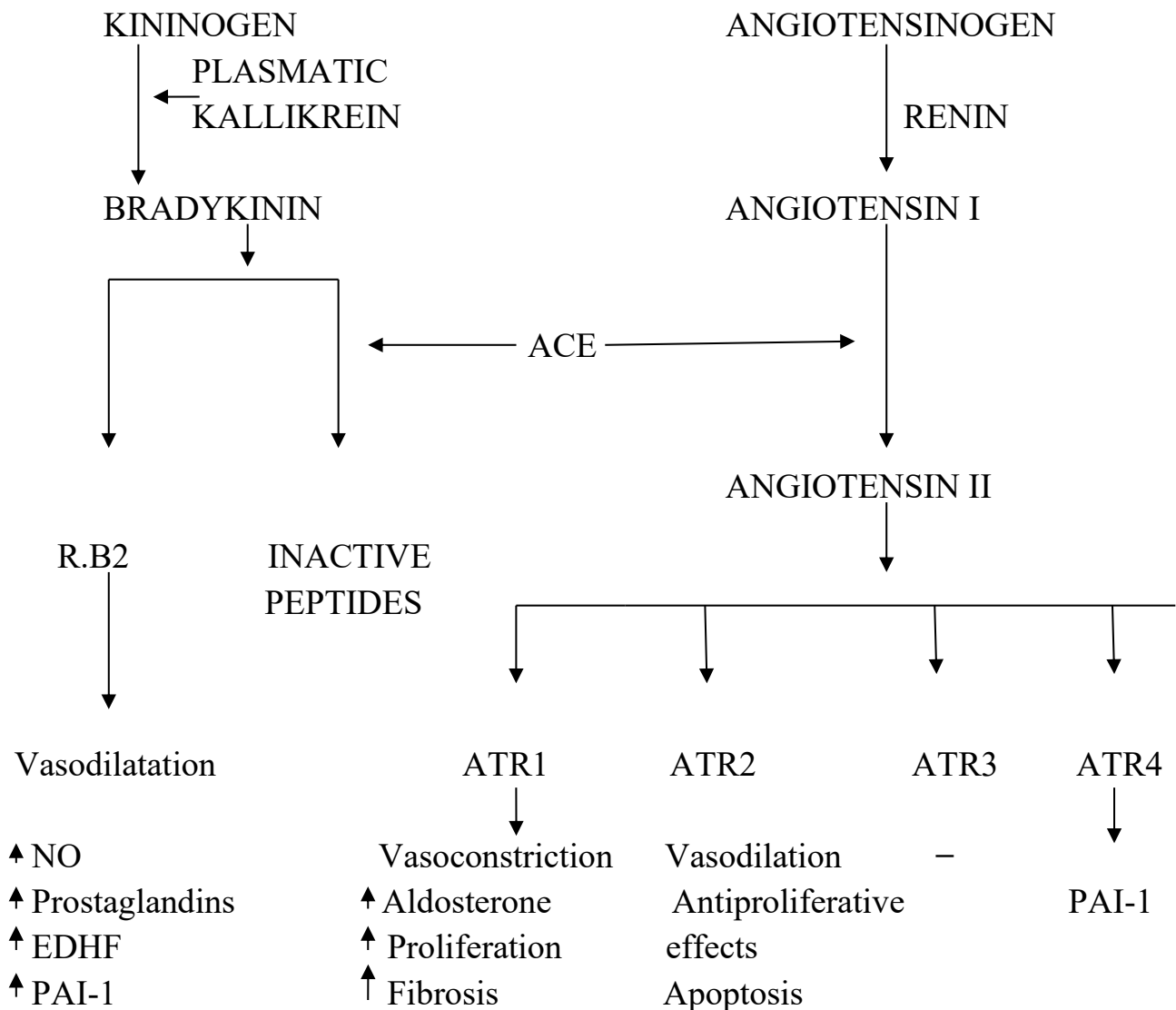


Figure 2. RAAS-Kallikrein-kinin system interrelationships. Effects of endogenous agonists on specific receptors. ACE: Angiotensin-converting enzyme; ATR: Angiotensin receptor; EDHF: Endothelium-derived hyperpolarizing factor; NO: Nitric oxide; PAI-1: Plasminogen activator inhibitor.

effects of Ang II on the cardiovascular system and lungs.

In the following pages, the functions of the ACE2/Ang (1-7)/RMas link are briefly outlined, emphasizing that the Ang (1-7) genesis results from Ang II and is catalyzed by ACE2.

The enzyme has cleavage functions, acting on several substrates and converts Ang II to Ang (1-7), Ang I to Ang (1-9), and as additional substrates (Ang III and Ang IV), although the preferred substrate is Ang II [7].

ACE2 is very well represented at the tissue level, predominantly at the level of the endothelium, as well as the heart, lung, kidneys, testicles, brain, and intestine.

The enzyme is not detectable in the circulation of healthy individuals but is significantly present in people with cardiovascular risk factors such as hypertension, obesity, diabetes, or hyperlipemia [8]. It

should be noted that ACE2 is elevated in patients with heart failure and can be used as a biomarker to predict all-cause mortality, including cardiovascular mortality [9].

Nepilysin, another endopeptidase, has a wide tissue expression and a large number of substrates and peptides, such as Ang I, Ang II, bradykinin, endothelin, enkephalins, and insulin.

The enzyme that hydrolyzes endothelins is the **endothelin-1** conversion enzyme; it belongs to the same family as nepilysin and is widely distributed in the cardiovascular, reproductive and endocrine systems.

Aminopeptidase A designates an enzyme/peptidase with membrane localization and is distributed in the vascular endothelium, at the intestinal and cerebral level. It acts by hydrolyzing the terminal bond, either with aspartic acid or with glutamic acid, having as substrate Ang II and cholecystokinin [7].

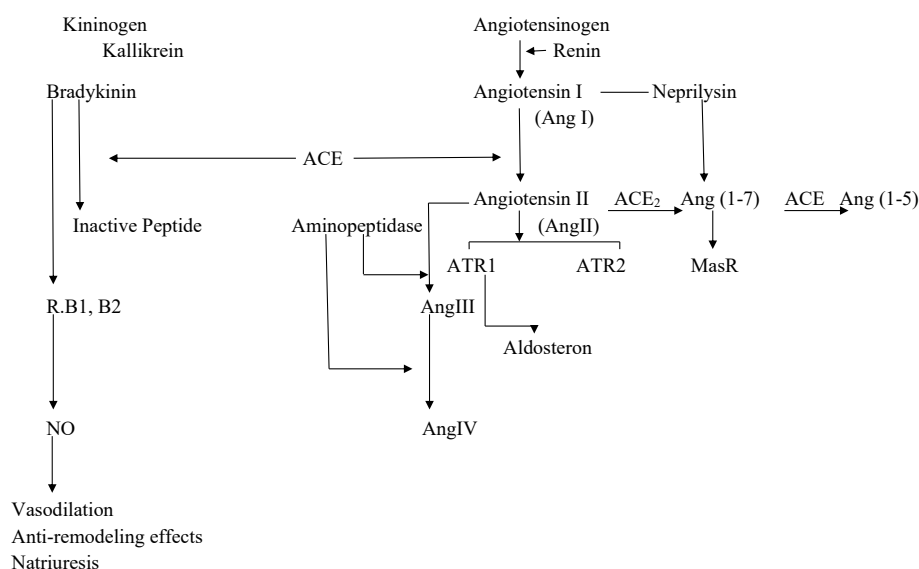


Figure 3. Renin-angiotensin-aldosterone system link with Ang II, ACE2-Ang (1-7) - RMas axis and connection with the kallikrein-bradykinin system via the angiotensin converting-enzyme (ACE).

ACE: Angiotensin-converting enzyme; ATR: Angiotensin receptor; NO: Nitric oxide.

Angiotensin II, one of the peptide compounds with very high functional significance, acts on type 1 and type 2 receptors - Angiotensin II receptor 1 (ATR1) and angiotensin II receptor 2 (ATR2), respectively; It couples with protein G10.

Table 1 shows the effects of stimulating the two receptors. ART1 stimulation generates rapid, short-term effects, but also long-term genetic effects. However, it has the common characteristic of G protein-coupled receptors. Namely, it is rapidly desensitized and internalized, thus avoiding long-term chronic activation [11].

In essence, the main axis, the first discovered and described, the renin-angiotensin-aldosterone system is connected with three other axes, namely the **ACE2-Ang (1-7) - RMas axis**, the **kallikrein-kinin-bradykinin axis**, and the **almandine-receptor axis (MrgD)**. Almandine results from the decarboxylation of aspartate or catalytic activation of AngA under the action of ACE2. It is essentially characterized by the substitution of alanine with aspartic acid from Ang (1-7). It is considered a potential mediator of RAAS (Figure 2)

ACE2 - Ang (1-7) - RMas axis

Regarding the complex functions of angiotensin (1-7), extensive experimental research has been done on Ang (1-7) functions and kinetics, highlighting extremely complex functions from which beneficial cardiovascular, metabolic, anti-thrombogenic effects, decreased cardiac remodeling, reduced area of infarction lesions, attenuation of dysfunction

post-infarction, antihypertensive effects, improvement of erectile dysfunction, muscular dystrophy, and type II diabetes should be mentioned. There is increased interest in orally-administered forms of Ang products (1-7) [11].

The effects on the central nervous system (CNS) are also described; Ang (1-7) produces opposite effects to Ang II under specific physiological conditions, highlighting neuronal regulation and complex interrelationships with RAAS. In the brain, Ang (1-7) acts as a neuromodulator, especially in the hypothalamus, the dorsomedial and ventrolateral regions of the spinal cord.

Among the cardiovascular effects, it is worth noting the improvement of the baroreflex and chemoreflex functions, hypertension, and metabolic syndrome. Also, in heart failure, the restoration of ACE2 activity causes an increase in nitric oxide (NO) and a decrease in sympathetic activity.

ACE2 / Ang (1-7) / Mas axis exerts neuroprotective effects in ischemic or hemorrhagic stroke, anti-inflammatory effects, attenuates the effect of loss of vascular endothelial function correlated with age, increases angiogenesis in ischemic stroke, reduces the alteration of the hemato-encephalic barrier in reperfusion-related injury (Figure 1) [12].

The cardiac effects of Ang (1-7) have been extensively studied, and the following have been established: antiarrhythmic effects, coronary dilators, cardioprotective effects, anti-cardiac remodeling properties, antifibrosis and antihypertrophic effects. At the vascular level, Ang (1-7) is produced in the endothelium and has vasorelaxation effects, potentiates the vasodilating effects of bradykinin, produces antiproliferative and shows antithrombotic effects.

Table 1. The effects of stimulating the angiotensin II and bradykinin receptors.

Ang II receptors effects		Bradykinin receptors effects	
ATR ₁	ATR ₂	BR ₁	BR ₂
Vasoconstriction	Vasodilation	Vasoconstriction	Vasodilation
Hypertrophy	Apoptosis		↑NO production
Fibrosis	Cellular growth inhibition		↑Prostaglandin production
Endothelin production	Increased sodium urinary excretion		Natriuresis
Production of superoxides	Counteracts the effects of AT1 in cancer and cardio-vascular system		
Hypertension	NO release		
Sympathoadrenergic activation	Antithrombotic effects		
Vasopressin release	Antifibrotic effects		
Aldosterone and insulin release	Increase of plasma triglycerides		
Hydro-saline retention			
Cellular growth and migration			
Pro-inflammatory state			
Release of glucose from the liver			
Decreased gluconeogenesis			
Oxidative stress			

Ang II receptors (ATR₁, ATR₂); Bradykinin receptors (BR₁, BR₂).

Overall, the Ang (1-7) / Mas system is a link to counteract RAAS, counteracting, in particular, the signals generated by Ang II.

With regard to intracellular signaling, the increase in NO and cyclic GMP is considered to be a classic event in understanding the protective effect of Ang (1-7). Also, this peptide increases the release of arachidonic acid, phospholipase A, augments prostaglandin E2 activity, and prostacyclin synthesis. Counterregulation of Ang II signaling, causing phosphorylation and activation of NADPH oxidase by Ang II, and increasing the genesis of oxygen free radicals is a very relevant function [12].

Mas receptor [7, 13] was identified in 1886 as a proto-oncogene, coupled with the G protein, through which it signals the peptide Ang (1-7). The effects generated by Ang (1-7) are opposite to those generated by Ang II and mediated by RAT1 (Figure 1).

The kallikrein-kinin system is functionally coupled with the “classical” RAAS by means of the ACE enzyme, which, in context, converts the hypotensive kinins - bradykinin (1-9) and kallidin (1-10) into inactive bradykinin (1-7) and kallidin (1-8) [14].

Bradykinin and kallidin are intense agonists of the bradykinin receptors B1 and B2. ACE (Kinase II), neprilysin, and endothelin conversion enzyme cleaves and removes two amino acids from the kinin chain and inactivates the latter.

The kallikrein-kinin system and interrelationship with RAAS

Bradykinin and kallidin are generated from kininogen by kallikrein.

Kallidin can also be converted to bradykinin by a plasma aminopeptidase. Bradykinin is the key component of the kallikrein-kinin system [15]. Kinins are bradykinin B1 and B2 receptor agonists.

The conversion enzyme ACE (Kininase II), neprilysin (endopeptidase) and the endothelin conversion enzyme inactivate the above-mentioned kinins (Figure 3).

Bradykinin is inactivated by ACE and neprilysin, representing, especially ACE, the interconnection of the two systems - RAAS and bradykinin.

Bradykinin receptors, B1 (BR1) and B2 (B2R) are G-protein-coupled receptors with seven transmembrane domains.

Stimulation of the two receptors increases NO synthase activity and prostacyclin synthesis in endothelial cells and increases intracellular calcium levels. The release of NO, following the activation of the B2 receptor, is fast, in contrast to the activation of the B1 receptor, much slower (Table 1).

In essence, the effects of bradykinin, at the vascular level, consist of smooth muscle relaxation by stimulating the production of NO, prostacyclin and hyperpolarizing factor, derived from the endothelium.

Blockade of the B2 receptor with icatibant has been shown to decrease the antihypertensive effect of conversion enzyme inhibitors [15].

In fact, ACE is the link between the two systems, renin-angiotensin-aldosterone, and the kallikrein-kinin system.

The two components - Ang II and bradykinin - are connected by ACE, but in reverse: the enzyme activates the formation of Ang II from Ang I on the one hand and inactivates bradykinin on the other. The ACE inhibitor favors the presence of bradykinin and reduces the genesis of Ang II. The

result is, implicitly, a synergism generated by the conversion enzyme inhibitor, which, through the increased presence of bradykinin, amplifies the antihypertensive effect of the mentioned compound. Experimental evidence suggests that a bradykinin BR2 antagonist reduces the antihypertensive effect of the ACE inhibitor in experimental animals.

Inhibitors of bradykinin receptor activity also reduce the antihypertrophic effect of ACE inhibitors in hypertension [16, 17].

It is known that the adverse effects of ACE inhibitors, namely dry cough and angioedema, are thought to be caused by an increase in the concentration of bradykinin following the blockade of ACE, the inactivator of autacoid.

Stimulation of bradykinin receptors activates intracellular links and activates, among others, NO synthase.

Bradykinin is recognized as a stimulator of prostaglandin biosynthesis in several ways, and one was already mentioned - increasing the intracellular concentration of calcium, promoting phosphorylation of phospholipase A2, which translocates from the cytosol into the cell membrane, stimulating the activity of phospholipase A2 (calcium-dependent, or calcium-independent) and releases arachidonic acid from membrane phospholipids, a source for prostaglandin biosynthesis [14].

As a result, the bradykinin system is characterized by experimental effects such as pain, inflammation, increased vascular permeability [14].

Interestingly, these effects are not entirely antagonized by non-steroidal anti-inflammatory drugs such as cyclooxygenase inhibitors because other second messengers are involved in this type of effect as well. Bradykinin is involved in the control of oxidative stress following the stimulation of the B2 receptor. Interestingly, the B2 receptor and NO synthase blockers decrease the protective effects of ACE inhibitors, suggesting that, in certain contexts, inactivating the effects of bradykinin is perhaps more beneficial than blocking the formation of AngII itself [14].

In essence, ACE inhibitors also increase the kallikrein-kinin system's activity, probably independently of the blood-pressure-lowering effects.

On the other hand, of great therapeutic relevance are the specific inhibitors of the bradykinin system as well as its agonists for the treatment of hyperinflammatory syndromes, angioedema, septic shock, and others.

The contribution of bradykinin is also relevant to its involvement in the fibrinolytic system, potentiating the effects of the ACE inhibitor.

The latter improves endothelial function by increasing the vasodilator response to various agonists. ACE inhibitors, but not ATR1 blockers, potentiate the effects of endogenous bradykinin, the release of tissue plasminogen activator in peripheral and coronary vessels [15], thus improving fibrinolyt-

ic balance and endothelial function, processes potentiated by conversion enzyme inhibitors.

The angiotensin A/alamandine/RMrg D axis, a component of the renin-angiotensin-aldosterone system, consists of two active components, alamandine, the metabolite of angiotensin A, but with opposite effects. That is, angiotensin A interacting directly with ATR1 produces vasoconstriction and proliferation and, indirectly, alamandine - its active metabolite (via RMrgD), produces protective effects (vasodilation, antiproliferative effects).

Alamandine results from angiotensin A, under the catalytic action of ACE2 or from Ang (1-7), by decarboxylation. Alamandine is releasing NO following the interaction with the MrgD receptor [18].

The alamandine receptor is RMrgD, i.e., the G-protein-coupled receptor D, related to the Mas receptor.

RMrgD is a MAS proto-oncogene whose functions are correlated with neuronal excitability and neuropathic pain, having a wide tissue distribution, being associated with various inflammatory pathologies identified in atherosclerosis and lung cancer. It was found that it can also be activated by Ang (1-7), its activation involving the adenylate cyclase cascade, cyclic AMP, and protein kinase A.

It is admitted that RMrgD has a central role and involvement in cardiovascular pathology, implicitly having a significant relevance, given its activation by both alamandine and Ang (1-7) [18].

Angiotensin A is derived from Ang II under the action of mononuclear leukocyte-derived aspartate decarboxylase (MLDAD) from mononuclear leukocytes, implicitly differing from the source compound by an amino acid alanine instead of aspartic acid [19].

Angiotensin A interacts with both types of Ang II receptors, ATR1 and ATR2, with the hypothesis that the affinity for AT1 would be higher than that of AT2.

The vasoconstrictor effects of Ang A result in a blood pressure increase in a dose-dependent manner, these effects being antagonized by sartans such as losartan or candesartan. In essence, it is accepted that the hypertensive effects of Ang A are mediated by ATR1 activation.

Ang A also has an affinity for the ATR2 receptor, but effects such as natriuresis, neuroprotection, and fibrosis inhibition become relevant in the presence of the ATR1 blockade [18].

SARS-CoV 2: pathogenic mechanisms following dysfunction of RAAS and its axes

The classic and extended renin-angiotensin-aldosterone system and its interconnected axes is a com-

plex of great homeostatic significance in normal physiology and implicitly in the pathology of the cardiovascular system.

Its functional connections with the sympathetic-adrenergic system further provide the integrative picture of the major cardiovascular regulatory and control functions of the evoked systems.

The impact of the SARS-Cov-2 virus on the ACE2 enzyme that controls several links of the renin-angiotensin-aldosterone system but also of the three axes, functionally connected with the classical SRAA system, results in complex pathogenic consequences. The main impact involves the upper respiratory tract and the oral cavity, as presented in the subchapter on the ACE2 distribution in systems, organs and tissues.

From the upper respiratory tract, the infection with the virus in question descends and finally affects the lung. It may spread to other organs and systems in medium, severe, or critical cases.

RAAS and the mentioned axes control the homeostasis of the cardiovascular system and the respiratory system. The dysfunction of RAAS and connected axes generates complex disorders such as hypertension, endothelial dysfunction, inflammation, fibrosis, atherosclerosis, vascular rigidity, diabetes, and others.

The functional imbalance between the classical renin-angiotensin-aldosterone system and related axes is frequently invoked in the literature for the understanding and pharmacological control of cardiovascular and pulmonary diseases related to this dysfunction, including the context of Covid-19 [20–22].

The two effects controlled by ACE2 with consequences on pulmonary and cardiovascular homeostasis aim at the degradation of Ang II (as the main effect of classical RAAS which produces vasoconstriction, oxidative stress, inflammation, fibrosis, and hydrosaline retention) and the net increase of Ang (1-7) which, through its Mas receptor, produces effects to counteract those generated by Ang II via ATR1. The alamandine axis and its RMrgD receptor potentiate the effects of the Ang (1-7) / Rmas axis [23].

The essential factor in the genesis of such disturbances is generated by the imbalance and reset (allostasis) of the RAA system and its axes, especially ACE2-Ang (1-7) - Rmas, Ang A/alamandina/RMrgD, and last but not least, the kallikrein/kinin axis. The homeostatic balance of positive and deteriorating effects is largely maintained by the activity of ACE2, which converts AngII to Ang (1-7) with pulmonary protective effects - similar to some extent to ATR2 stimulation (Figure 1).

Some authors systematize the two-mentioned categories of effects in correlation, on the one hand with the Ang II/ATR1 pathway and on the other hand, with the ACE2/Ang (1-7) Rmas antagonist pathway [24].

The down-regulation of the ACE2 concentration following the interaction with SARS-Cov-2 generates the increase of the Ang II concentration, which unbalances the RAA system and the connected axes, implicitly amplifying the deteriorating effects.

Binding of the virus to ACE2 also causes a detachment of the enzyme from the membrane and its transition to a soluble state [24].

Moreover, the predominance of Ang II versus Ang (1-7) implies the increase of ATR1 activity with its described effects and with a consequent further decrease of ACE2. These processes tilt the balance even more in favor of the Ang II activity, resulting in a system reset with pathological consequences of different amplitudes.

Ang II is markedly increased - a linear increase correlated with the viral load and lung damage in Covid-19 [25].

At the same time, various cytokines (IL6, IL-10, TNF alpha) increase without being able to specify whether this increase is produced by Ang II, or the increase of the latter is a result of the cytokine storm.

Ang II receptor blockade or ACE inhibition does not influence mortality in Covid-19 infection associated with cardiovascular disease [26]. The problem of treatment interference, probably with Covid-19 disease, with conversion enzyme inhibitors and Ang II receptor blockers, has generated relevant scientific opinions [22, 27].

In essence, most scientific societies do not recommend stopping treatment with the two types of RAAS blockers [23].

Moreover, analyzing the consequences of the imbalance of RAAS links on the genesis of lung lesions in SARS-Cov-2 infection, it is suggested that the pharmacological inhibitors may restore the balance between classical RAAS and related axes.

In essence, the ACE inhibitor decreases the genesis of Ang II and its effects on ATR1, increases the Ang I substrate for ACE2, and decreases the conversion of Ag (1-9) to Ag (1-7), implicitly increasing the activity of Ag (1-9) on ATR1. Because ACE inactivates Ang (1-7) by converting it to inactive Ag (1-5), the ACE inhibitor increases the presence of Ang (1-7).

In addition, ATR1 blockers prevent the effects of Ang II at their level, with the consecutive increase (positive feedback) of Ang II, a substrate for ACE2 [23]. ACE2 also generates alamandine, and its effects have been described - it acts on RMrgD with similar effects to Ang (1-7).

The above-cited authors summarize the experimental and clinical data that argue for the benefits of Ang II conversion enzyme inhibitors and ATR1 blockers.

Such direct benefits on the lung are:

- Reduction of cellular and capillary damage in the lungs and preservation of alveolar-capillary function, including gas exchange;

- Decrease of capillary permeability, reduction of pulmonary interstitial fluid transudate, and cell and protein exudate;
- Reducing the genesis of pre-inflammatory cytokines and decreasing inflammation;
- Reducing the production of interstitial mesenchymal cells and preventing (reducing) pulmonary fibrosis;
- Preservation of lung compliance;
- Reduction of pulmonary vascular thrombosis;
- Reduction of oxidative stress, vasodilation, decrease in pulmonary arterial pressure.

Below are the most known pathogenic processes which produce damage to systems, organs and tissues that shape the severity of Covid-19 disease. The effects of SARS-Cov-2 or the consequences generated by the infection are characterized by vasoconstriction, prothrombotic and proinflammatory status. These are produced inevitably by the activation or blockage of some complex physiological links. A suggestive expression used recently by an author is that "Covid-19 usurps host regulatory networks" [28]. Knowing the links that control these mechanisms allow the therapeutic or prophylactic approach to these dysfunctions generated by SARS-Cov-2, as outlined in this manuscript.

The impact of SARS-Cov-2 on the human body, targeting the ACE2 enzyme, with a wide tissue distribution, generates a complex pathology with more or less specific characteristics, generated by the specific anatomical-clinical system or organ, or the analyzed tissue:

- Pulmonary involvement with acute respiratory failure - severe acute respiratory syndrome (SARS) with characteristic anatomopathological characteristics [29];
- Damage to the cardiovascular system;
- Vascular damage, especially at the endothelial level, generating a systemic endothelitis;
- Acute respiratory distress syndrome (ARDS);
- Damage to the central nervous system, evident from the onset of the disease by symptoms such as anosmia and ageusia;
- Genesis of multiple organ damage, leading to multiple organ failure;
- Hyperinflammatory condition, often uncontrollable, hypercoagulability;
- Cytokine storm;
- Bradykinin storm;

Next, a synthesis of these dysfunctions illustrates the complex clinical picture of the Covid-19 disease. Penetration of the virus into the host cell generally causes damage to the upper respiratory tract and lungs in most people with symptoms of Covid-19. It generates the syndrome of these organs with fever, respiratory disorders that often precede respiratory failure. Penetration of the virus into the cell triggers an intense immune response, with the secretion of inflammatory cytokines and chemokines that can generate respiratory distress syndrome and multiple organ failure [30]. The cited authors describe lesions

(presented in various published studies), in the respiratory tract, especially in the lung, vascular, urinary system, gastrointestinal tract, cardiovascular system, nervous system, reproductive system, and skin.

In essence, it results that the SARS-Cov-2 virus spreads widely in the epithelial layer of the respiratory and digestive tract, in the distal convoluted tubules and kidneys, in the sweat glands, and in the testicular epithelium.

Recently, it has been shown, correlated with the distribution of ACE2, that the virus can be transmitted through the fecal-oral route, through urine, and through the skin. Histopathological data allow identifying a specific sequence of pathological changes, quite early, in the lung parenchyma, correlated with the images obtained before the death of some patients [31]. The correlation of these data highlights that microvascular damage has a major role in the progression of the disease. Thus, these data suggest that the pulmonary microcirculation is damaged early, at the beginning of the disease, being a progressive pathogenic mechanism to a severe or critical form [32].

Based on recently published data, we are facing a systemic endothelitis with complex consequences, both on the transport of oxygen to the alveolar level and coagulation, thrombogenesis and coagulopathy [29]. Endothelial dysfunction, secondary to viral invasion and interaction with ACE2, alters the vascular functional balance in favor of vasoconstriction, secondary ischemia, inflammation, tissue edema and procoagulant activity [33].

In a synthetic presentation, the clinical consequences of endothelial dysfunction and microvascular thrombosis in Covid-19 infection are revealed [34]. In essence, the authors present clinical manifestations such as:

- Pulmonary microvascular thrombosis;
- Pulmonary embolism secondary to venous thromboembolism;
- Deep vein thrombosis;
- Cerebral venous thrombosis;
- Cerebrovascular damage: occlusions of the large vessels, intracerebral hemorrhage, cerebral vasculitis, neurological and psychological complications, encephalopathy, psychotic symptoms, status epilepticus, anosmia and ageusia, as direct effects of the viral invasion of the brain;
- Cardiovascular damage: cardiomyopathy, acute myocardial injury, increased troponin, and peripheral ischemia.

Ang II conversion enzyme - Receptor for the SARS-Cov-2 coronavirus

Beta coronaviruses include SARS-Cov (severe acute respiratory syndrome) and MERS-Cov (Middle East-

ern respiratory coronavirus syndrome), which are highly pathogenic to humans. SARS-Cov produced in 2002 and 2003 an epidemic with a lethality rate of about 10% while the Middle East epidemic pandemic produced by MERS-Cov in 2012 had a fatality rate of about 36% [35].

Recognition of the receptor by the virus is the first essential step, sine qua non for the infection to occur and, at the same time, a determinant of the number of hosts, interspecies, at which the infection can occur.

The SARS-Cov-2 virus has a strong structural resemblance to SARS-Cov.

There are four structural proteins: spike surface glycoprotein (S), membrane protein M, nucleocapsid protein (N), envelope (E), and accessory proteins. The glycosylated ectodomain and protein M comprise three trans-membrane (TM) domains. The M and E proteins are required for morphogenesis and assembly, while S glycoproteins comprising two S1 and S2 subunits are viral fusion proteins [36].

Protein S is involved in the interaction with tissue ACE2. There are two forms of the ACE2 protein: cellular, bound to the cell membrane, and a circulating, soluble form [37].

Cellular ACE2 is expressed in abundance in tissues, in pneumocytes, enterocytes, in the vascular endothelium, in the heart, kidneys, and brain. It should be noted that ACE2 is absent in the spleen, thymus, lymph nodes, bone marrow, and cells of the immune system - T, B lymphocytes and macrophages [37].

The circulating enzyme is cleaved from the membrane bond by a metalloproteinase, in competition with a transmembrane serine-protease - TMPRSS2. The latter enzyme cleaves the membrane bond of SARS-Cov-2 and allows the virus to penetrate the host cell, either by endocytosis or by fusion of the viral membrane with the host cell membrane. The latter mechanism is 100 times more active than endocytosis for viral replication [38].

Intracellular penetration of SARS-Cov-2 depends on cellular ACE2 and TMPRSS2, which detach it from the cell membrane, both enzymes being co-expressed in various tissues. The virus can also bind to circulating ACE2, but the virus cannot replicate extracellularly. Circulating ACE2 is thought to protect the lung from viral infection.

What are the functions of circulating ACE2, and what potential and real implications does it have for the SARS-Cov-2 infection?

Considering the two enzymes with crucial functions in the RAA system and the Ang (1-7) / Rmas axis - ACE and ACE2, it can be admitted, in enzymatic

terms, that the latter behaves functionally as an opponent of ACE [37].

The cellular form of ACE2 produces effects through Ang (1-7), inducing vasorelaxation, cardioprotection, antioxidation, and anti-inflammation. Last but not least, it inhibits Ang II-induced signaling. ACE2 is also present in a circulating form, in much lower concentrations. Instead, it was found that circulating ACE2 is increased in hypertension, heart failure, diabetes, and chronic renal failure [37]. This increased concentration of the circulating form is interpreted as a compensatory defense reaction versus the adverse effects of Ang II. That is, we are dealing with an adaptive increase in the context of potential functional imbalances of RAAS. ACE2 is cleaved from membrane connections by ADAM17, and the active form of the enzyme is released into the extracellular environment, where it acts on Ang I by converting it to Ang (1-9) and on Ang II, which generates Ang (1-7) with opposite effects. As a result of two relatively independent processes, the “shedding” of ACE2 into the extracellular environment and the “binding” by SARS-Cov-2 of ACE2, a phenomenon of decreasing the presence of ACE2 in the functional landscape of the ACE2/Ang(1-7) / Rmas axis occurs.

The disturbance of the balance between the RAA system and the mentioned axis explains the viral pathogenesis, with the pulmonary and vascular target (systemic endothelitis), the generalized, uncontrollable inflammation, the cytokine storm, ARDS [39–41].

What role can we identify for ACE2 in the pathogenic context of SARS-Cov-2?

From the brief data presented, it results that ACE2 is the viral receptor that conditions the penetration into the cell and implicitly the invasion of the organism and the defining pathogenesis of Covid-19 disease.

Since 2004, correlated with the definition of ACE2 receptor for SARS coronaviruses, it was stated that we must decipher the distribution of the ACE2 receptor throughout body tissues (localization by immunochemical methods) in order to understand the pathogenesis of this virus [44].

The authors emphasize the marked presence of ACE2 in the endothelial cells of large and small vessels, arterial and venous, as well as in the vascular smooth muscle.

ACE2 has also been shown to be significantly present in the alveolar epithelial cells of the normal lungs, nasal mucosa and nasopharynx, smooth muscles, gastric, small intestine and colon, and vascular endothelium at these levels. Remarkably, ACE2 is

abundantly present in enterocytes in the small intestine, in its entirety, but not in the colon.

The presence of ACE2 in the epidermis and hair follicles was described.

A consistent presence has been shown in the brain, especially in endothelial cells and vascular smooth muscles. The presence of ACE2 in the spleen, thymus, lymph nodes, bone marrow, and immune system cells was not shown. The result is a remarkable presence of ACE2 in the lung alveolar epithelial cells and enterocytes. The authors comment that these are the tissues that come into contact with the external environment. In addition, ACE2 is clearly present in arterial and venous endothelial cells and the smooth muscles of all studied organs, i.e., in the lung, gastrointestinal, cardiovascular and renal tissues.

The ACE2 distribution suggests an explanation of the extensive and rapid lung pathology produced by SARS-Cov, starting with cytopathological changes at the alveolar-capillary interface, viral expansion, destruction of the alveolar wall with progressive and rapid alveolar diffuse damage.

In recent works, a meta-analysis that correlated the data already presented showed that ACE2 is highly expressed in the lungs in the context of the existence of comorbidities [43].

A recent paper showed a high expression of ACE2 receptors for SARS-Cov-2 in the epithelial cells of the oral mucosa and the tongue [44].

The expression of ACE in the tissues throughout the body highlights potential pathways of contamination. Thus, the mentioned authors highlighted a high ACE2 expression in alveolar type II cells in the lung, upper esophagus, ileum and colon enterocytes, cholangiocytes, myocardial cells, kidneys, and urothelial cells in the bladder. All these tissues may present a potential risk of Covid-19 infection.

Kallikrein-Kinin-Bradykinin system. Is there a bradykinin storm identified in Covid-19 infection?

The interaction of SARS-Cov-2 with ACE2 through host cell membrane fusion, endocytosis and viral multiplication, and involvement of the host cell genome are principle processes that condition the Covid-19 disease. The interaction with ACE2, a widely distributed enzyme in the tissues of the human body, triggers, in addition to severe lung damage, a systemic endothelitis with complex consequences, chaotic systemic inflammation, multiple organ failure, ARDS and often death in severe and critical forms.

Comorbidities favor viral invasion and involve an unfavorable prognosis. One of the most relevant systemic manifestations is the cytokine storm with

its consecutive dysfunctional release and a systemic hyperinflammatory process with serious consequences, multiple organ failure, and respiratory distress syndrome. It was found that both consensually pathogenic and as a component of the cytokine storm syndrome, the kallikrein-kinin-bradykinin system has an essential role in the pathogenesis of Covid-19. It is known that an adverse effect of converting enzyme inhibitors is angioedema. The genesis is due to the blockade of the conversion enzyme that also inactivates bradykinin. One of the common side effects of converting enzyme inhibitors is a dry, often rebellious cough. The authors, referring to the involvement of this system in the pathogenesis of Covid-19, mention that it has not been investigated under the mentioned aspect [45]. A similar paper shows the disorder of the bradykinin signal in the respiratory complications of Covid-19 [46].

In essence, it becomes clear the hypothesis of a loop, a vicious circle, respectively des-arg (9) → bradykinin and bradykinin → mediation of inflammation → injury → inflammation that probably generates critical respiratory complications in Covid-19 infection. The authors suggest that icatibant, a B2 bradykinin receptor blocker that blocks angioedema, may be useful in the treatment of respiratory distress syndrome produced in Covid-19. The authors cite that, strangely, in Covid-19, we also encounter the dry cough syndrome, similar to that produced after conversion enzyme inhibitors administration.

In an exploratory off-label study with icatibant in patients with Covid-19 administered in the early stages of the disease, this medication significantly improved oxygenation, reducing the need for additional oxygen [45]. It is suggested to extend the clinical research in a large randomized study in the pathology of Covid-19. In essence, synergistically with the anti-SARS-Cov-2 antiviral treatment, the pharmacokinetic-kinin system that generates angioedema is pharmacologically controlled with icatibant (Firazyr), a selective blocker of bradykinin B2 receptors. There is no bradykinin B1 receptor blocker yet, but there is a Lanadelumab-type plasma kallikrein inhibitor. The excessive, dysfunctional inflammatory process is controlled by controlling interleukins (IL-6, IL-1 β , TNF), C-reactive protein, with anti-inflammatory agents (anticytokines) such as anakinra, tocilizumab, eculizumab.

The hypercoagulation state is assessed and treated with heparin, along with fibrosis prevention, corticosteroid therapy and assessment of the ventilatory strategy [45].

Polak *et al.* approached the pathophysiology data from a dynamic, evolutionary aspect, and defined three stages correlated with opportune and adequate pharmacotherapy [47]: 1) the first stage consists of the viral invasion and the associated pathogenic process; the therapeutic strategy targets the virus with antiviral medication; 2) the vascu-

lar stage, consequent to the endothelial aggression and the consequent inflammatory process; in this context, anti-inflammatory, anticoagulant and anti-complement medication represent adequate therapeutic indications; 3) the third stage concerns the pulmonary fibrotic sequelae that require specific antifibrotic treatment. The authors mention that the hallmarks for determining these stages cannot be obtained by tissue biopsy but successively, by viral RNA detection (RT-PCR), assessment of cytokines, inflammatory markers and lung imaging evaluation.

Pharmacological manipulation of RAAS remains an open problem regarding the SARS-Cov-2 infection, both in terms of prevention of Ang I genesis, ACE inhibition, ATR1 blockade, administration of aldosterone antagonists and modulation of ACE2 - the viral receptor [32,48].

Conflict of Interest

The author confirms that there are no conflicts of interest.

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