Molecular Pharmacology Fast Forward. Published on August 6, 2020 as DOI: 10.1124/molpharm.120.000049
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Commentary on: “Does COVID19 Infect the Brain? If So, Smokers Might Be at a Higher Risk”

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While it is of critical importance to rapidly publish the latest findings on COVID-19, recent high profile retractions of COVID-19 reports (Mehra, Desai, Kuy, Henry, & Patel, 2020; Mehra, Desai, Ruschitzka, & Patel, 2020), reminds us that it is important to be vigilant for the accuracy of this literature.

A recent paper published in Molecular Pharmacology suggests that there is an interaction of nicotinic cholinergic receptors and angiotensin-converting enzyme 2 (ACE2) in the brain that predisposes smokers to increased susceptibility to infection of the brain by SARS-CoV-2 (Kabbani & Olds, 2020), above and beyond the general damage of smoking to the airways . However, the purported mechanism for this toxicity is unsubstantiated and does not accurately reflect current knowledge of predisposing factors for COVID-19 toxicity. This commentary addresses some of these issues.

ACE2 is a protein that is considered to be part of the renin-angiotensin system as well as the primary receptor by which SARS-CoV-2 enters cells (Figure) (Hoffmann, et al., 2020). And, while there is little doubt that smoking nicotine-containing tobacco products predisposes smokers to increased disease susceptibility, this predisposition arises from adverse effects of nicotine on the cardiovascular system https://www.cdc.gov/tobacco/campaign/tips/diseases/heart-disease-stroke.html (accessed 4/25/20) as well as the inhalation of particulate and volatile substances that directly injure lung cells https://www.cdc.gov/tobacco/campaign/tips/diseases/index.html?s_cid=OSH_tips_D9389

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(accessed 4/25/20) (Oakes, Fuchs, Gardner, Lazartigues, & Yue, 2018). Specifically, it has been shown that both acute and subacute nicotine administration to female rats increased blood-brain-barrier permeability by altering tight junction proteins of the cerebral microvessel endothelial cells (Hawkins, et al., 2004), an effect independent of ACE2.

Linking increased brain susceptibility to SARS-CoV-2 infection with smoking "…based upon known functional interactions between the nicotinic receptor and ACE2." (Kabbani & Olds, 2020) is unsubstantiated, as none of the papers cited by these authors for demonstrating co-localization of NAChRs on the same brain cells as ACE2 (Changeux, 2010; Nordman, Muldoon, Clark, Damaj, & Kabbani, 2014; Tolu, et al., 2013) mention the word angiotensin or the acronym ACE2 as being associated with nicotinic receptor-containing cells. Of note, colocalization of NAChRs and components of the RAS in bronchial and alveolar epithelial cells in the lungs has been reported (Oakes, et al., 2018).

A subsequent claim for an association of NAChRs and ACE2 comes from a concurrent publication by these authors (Olds & Kabbani, 2020) "nicotine stimulation of the nAChR can increase ACE2 expression within them (Olds and Kabbani, 2020)." They cite a medRxiv paper by Cai et al., 2020 https://www.preprints.org/manuscript/202002.0051/v1 demonstrating that tobacco smoking is associated with increased ACE2 gene expression. However, a peer-reviewed manuscript from this same author noted that it has not been determined whether the 25% increase in ACE2 gene expression is due to nicotine, or the other components of inhaled tobacco smoke (Cai, Bosse, Xiao, Kheradmand, & Amos, 2020). Moreover, nicotine has been shown to decrease ACE2 expression (Oakes, et al., 2018), which contradicts the hypothesis that nicotine increases ACE2 expression (Kabbani & Olds, 2020). This implies that it is the other components of tobacco smoke, not nicotine, that increase ACE2 expression.

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Later in the manuscript the authors write “Interactions between nAChRs and ACE2 have been studied in several of these [brain] regions including the ventrolateral medulla (Deng et al., 2019)” (Kabbani & Olds, 2020). However, Deng et al., 2019 described changes in acetylcholine in this brain region in relation to ACE2 expression, and that paper does not mention NAChRs at all.

It is regrettable that the companion paper (Olds & Kabbani, 2020) cited by Kabbani and Olds (Kabbani & Olds, 2020) also contains questionable statements, e.g., “ACE2 appears to play both protective and pathogenic roles within RAS pathways, and its direct mechanisms of function in cells remain less understood [7,8].” (Olds & Kabbani, 2020). ACE2 is an extremely well characterized protein (Feng, Xia, Santos, Speth, & Lazartigues, 2010; Raizada & Ferreira, 2007; Soler, Wysocki, & Batlle, 2008; Turner, Hiscox, & Hooper, 2004; Turner & Hooper, 2002) and has been overwhelmingly recognized as only playing a beneficial role within the renin-angiotensin system (RAS) (R. A. Santos, Ferreira, Verano-Braga, & Bader, 2013).

There is a considerable body of literature regarding the interaction of SARS-CoV-2 with the RAS, in particular ACE2 , which serves as its receptor for entry into cells (Hoffmann, et al., 2020) . Most published papers and cardiovascular medicine societies cf (Gurwitz, 2020; Reynolds, et al., 2020; Speth, 2020a, 2020b, 2020c; Sriram & Insel, 2020; Vaduganathan, et al., 2020) support the continued use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) based upon the predominant beneficial effects of reducing the ability of angiotensin II to promote inflammation acting via the AT_1 receptor (Ranjbar, et al., 2019). ACE2 by metabolizing Ang II and forming angiotensin 1-7, which has anti-inflammatory actions via its receptor Mas (R. A. S. Santos, et al., 2018) may help to

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minimize the cytokine storm (Annweiler, et al., 2020; Mahmudpour, Roozbeh, Keshavarz, Farrokhi, & Nabipour, 2020) that exacerbates lung damage associated with COVID-19.

The adverse effects of smoking on outcomes of COVID-19 infection are indisputable. However, it is necessary to accurately determine what aspect of smoking and which target tissues mediate this increase in morbidity and mortality.

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References

Annweiler, C., Cao, Z., Wu, Y., Faucon, E., Mouhat, S., Kovacic, H., & Sabatier, J. M. (2020). Counter-regulatory 'Renin-Angiotensin' System-based Candidate Drugs to Treat

COVID-19 Diseases in SARS-CoV-2-infected patients. *Infect Disord Drug Targets*. May 17. doi: 10.2174/1871526520666200518073329.

Cai, G., Bosse, Y., Xiao, F., Kheradmand, F., & Amos, C. I. (2020). Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2. *Am J Respir Crit Care Med*. Jun 15;201(12):1557-1559. doi: 10.1164/rccm.202003-0693LE.

Changeux, J. P. (2010). Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat Rev Neurosci, 11*, 389-401.

Feng, Y., Xia, H., Santos, R. A., Speth, R., & Lazartigues, E. (2010). Angiotensin-converting enzyme 2: a new target for neurogenic hypertension. *Exp.Physiol, 95*, 601-606.

Gurwitz, D. (2020). Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev Res*. Mar 4:10.1002/ddr.21656. doi: 10.1002/ddr.21656

Hawkins, B. T., Abbruscato, T. J., Egleton, R. D., Brown, R. C., Huber, J. D., Campos, C. R., & Davis, T. P. (2004). Nicotine increases in vivo blood–brain barrier permeability and alters cerebral microvascular tight junction protein distribution. *Brain Research, 1027*, 48-58.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N.-H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. Apr 16;181(2):271-280.e8. doi: 10.1016/j.cell.2020.02.052.

Kabbani, N., & Olds, J. L. (2020). Does COVID19 Infect the Brain? If So, Smokers Might Be at a Higher Risk. *Mol Pharmacol, 97*, 351-353.

Mahmudpour, M., Roozbeh, J., Keshavarz, M., Farrokhi, S., & Nabipour, I. (2020). COVID-19 cytokine storm: The anger of inflammation. *Cytokine, 133*, 155151.

Mehra, M. R., Desai, S. S., Kuy, S., Henry, T. D., & Patel, A. N. (2020). Cardiovascular Disease, Drug Therapy, and Mortality in Covid-19. *N Engl J Med*. Jun 18;382(25):e102. doi: 10.1056/NEJMoa2007621. Retracted

Mehra, M. R., Desai, S. S., Ruschitzka, F., & Patel, A. N. (2020). RETRACTED: Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet . 2020 May 22;S0140-6736(20)31180-6. doi: 10.1016/S0140-6736(20)31180-6.

Nordman, J. C., Muldoon, P., Clark, S., Damaj, M. I., & Kabbani, N. (2014). The alpha4 nicotinic receptor promotes CD4+ T-cell proliferation and a helper T-cell immune response. *Mol Pharmacol, 85*, 50-61.

Oakes, J. M., Fuchs, R. M., Gardner, J. D., Lazartigues, E., & Yue, X. (2018). Nicotine and the renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol, 315*, R895-r906.

Olds, J. L., & Kabbani, N. (2020). Is nicotine exposure linked to cardiopulmonary vulnerability to COVID-19 in the general population? *Febs j*. Mar 18:10.1111/febs.15303. doi: 10.1111/febs.15303.

Raizada, M. K., & Ferreira, A. J. (2007). ACE2: a new target for cardiovascular disease therapeutics. *J Cardiovasc Pharmacol, 50*, 112-119.

Ranjbar, R., Shafiee, M., Hesari, A., Ferns, G. A., Ghasemi, F., & Avan, A. (2019). The potential therapeutic use of renin-angiotensin system inhibitors in the treatment of inflammatory diseases. *J Cell Physiol, 234*, 2277-2295.

Reynolds, H. R., Adhikari, S., Pulgarin, C., Troxel, A. B., Iturrate, E., Johnson, S. B., Hausvater, A., Newman, J. D., Berger, J. S., Bangalore, S., Katz, S. D., Fishman, G. I., Kunichoff, D., Chen, Y., Ogedegbe, G., & Hochman, J. S. (2020). Renin–Angiotensin–Aldosterone System Inhibitors and Risk of Covid-19. *New England Journal of Medicine*. Jun 18;382(25):2441-2448. doi: 10.1056/NEJMoa2008975.

Santos, R. A., Ferreira, A. J., Verano-Braga, T., & Bader, M. (2013). Angiotensin-converting enzyme 2, angiotensin-(1-7) and Mas: new players of the renin-angiotensin system. *J Endocrinol, 216*, R1-r17.

Santos, R. A. S., Sampaio, W. O., Alzamora, A. C., Motta-Santos, D., Alenina, N., Bader, M., & Campagnole-Santos, M. J. (2018). The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin System: Focus on Angiotensin-(1-7). *Physiol Rev, 98*, 505-553.

Soler, M. J., Wysocki, J., & Batlle, D. (2008). Angiotensin-converting enzyme 2 and the kidney. *Exp.Physiol, 93*, 549-556.

Speth, R. C. (2020a). Angiotensin II administration to COVID-19 patients is not advisable. *Crit Care, 24*, 296.

Speth, R. C. (2020b). Keep Taking Your ACE Inhibitors and ARBs During the COVID 19 Pandemic. *J Travel Med*. May 18;27(3):taaa045. doi: 10.1093/jtm/taaa045.

Speth, R. C. (2020c). Response to recent commentaries regarding the involvement of angiotensin-converting enzyme 2 (ACE2) and renin-angiotensin system blockers in SARS-CoV-2 infections. *Drug Dev Res*. Apr 17:10.1002/ddr.21672. doi: 10.1002/ddr.21672

Sriram, K., & Insel, P. A. (2020). Risks of ACE inhibitor and ARB usage in COVID-19: evaluating the evidence. *Clin Pharmacol Ther*. Aug;108(2):236-241. doi: 10.1002/cpt.1863

Tolu, S., Eddine, R., Marti, F., David, V., Graupner, M., Pons, S., Baudonnat, M., Husson, M., Besson, M., Reperant, C., Zemdegs, J., Pages, C., Hay, Y. A., Lambolez, B., Caboche, J., Gutkin, B., Gardier, A. M., Changeux, J. P., Faure, P., & Maskos, U. (2013). Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. *Mol Psychiatry, 18*, 382-393.

Turner, A. J., Hiscox, J. A., & Hooper, N. M. (2004). ACE2: from vasopeptidase to SARS virus receptor. *Trends Pharmacol.Sci., 25*, 291-294.

Turner, A. J., & Hooper, N. M. (2002). The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends Pharmacol.Sci., 23*, 177-183.

Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J. J. V., Pfeffer, M. A., & Solomon, S. D. (2020). Renin–Angiotensin–Aldosterone System Inhibitors in Patients with Covid-19. *New England Journal of Medicine*. Apr 23;382(17):1653-1659. doi: 10.1056/NEJMsr2005760

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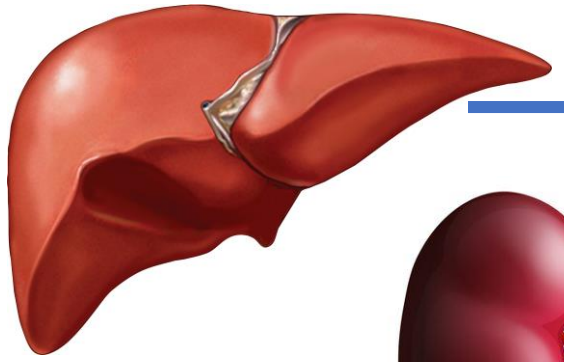
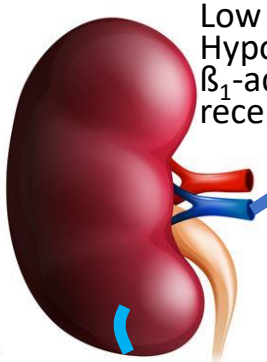
Legend for Figure: Simplified diagram of renin-angiotensin system. SARS-CoV-2 spike protein S1 domain binds to the extracellular domain of ACE2 leading to internalization of the SARS-CoV-2-ACE2 complex which decreases ACE2 expression on cell membranes. ACE2, and AT_1 and Mas receptors are present in lung and kidney, two of the tissues most adversely affected by SARS-CoV-2 infection. Angiotensin 1-5 has no established function. BP is blood pressure. Liver image from https://www.pcosnutrition.com/fattyliver/ Kidney image is from https://www.alportsyndrome.org/stages-of-kidney-disease/ Lung image is from https://www.dreamstime.com/stock-images-pulmonary-hypertension-image27276044 SARS-CoV-2 image from https://www.cdc.gov/media/subtopic/images.htm



Angiotensinogen

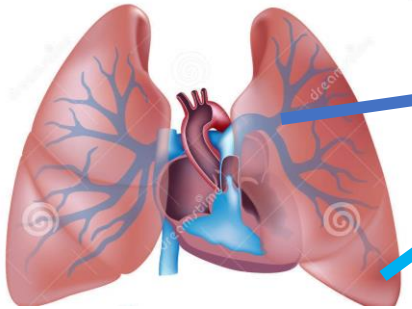
Low BP
Hyponatremia
ß$_1$-adrenergic
receptor



Renin

Angiotensin I

ACE

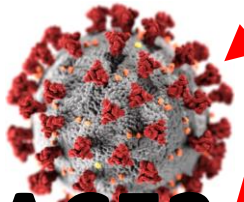


Angiotensin II

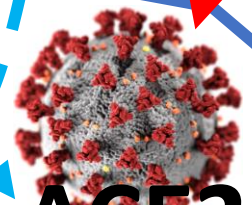
AT_1R

Vasoconstriction
Fibrosis
Inflammation

SARS-CoV-2



ACE2



ACE2
PrCP

Neprilysin
Prolylendopeptidase
Neurolysin
Thimet oligopeptidase

Angiotensin 1-9

ACE

Angiotensin 1-7

AT_2R

Mas

Vasodilation
Anti-fibrosis
Anti-inflammatory

ACE

Angiotensin 1-5

ACE is angiotensin-converting enzyme. Also known as ACE1
ACE2 is angiotensin-converting enzyme 2
PrCP is prolylcarboxypeptidase previously known as angiotensinase A