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Angiotensin-(1-7) Influences Tryptophan Absorption in the Rat and Mouse Intestine

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Authors' contributions

This work was carried out in collaboration between all authors. Author ELB coordinated and designed the study, performed the surgical procedures and statistical analysis, interpreted the data and prepared the manuscript. Author PBL performed the data acquisition. Authors AABP and WOS performed the Western blot analysis. Author JSO performed the biochemical analyses (HPLC). Author MLO measured ACE2 activity. Author GME assisted in the surgical procedures. Author RTR provided technical support. Author AJF performed a critical review of the manuscript for intellectual content. Author RASS conceived and designed the study, interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: Components of the renin-angiotensin system are involved in absorption and regulate both fluid and electrolyte transport in the intestinal epithelium. The angiotensin-converting enzyme 2 (ACE2) is a major Angiotensin-(1-7)-forming enzyme, and a key regulator of the homeostasis of dietary tryptophan (Trp). The aim of the present study was to investigate the physiological role of the Ang-(1-7)/Mas pathway in the intestinal tryptophan absorption.

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Methodology: Two models of Mas receptor inhibition were used: treatments, in rats, with the specific Mas receptor inhibitor, the A-779, and Mas receptor knockout (KO) mice; Ang-(1-7) or Ang-(1-7) + A-779 were injected prior Trp infusion. Male Wistar rats (n = 5 in each goup), wild-type (WT) FVB/N mice and Mas (KO) FVB/N mice (n = 4-8 in each group) were anesthetized, and submitted to midline laparotomy to expose, and isolate jejunal loop. Tyrode's solution (pH 8) containing tryptophan (0. 25 mg%) was infused (0.5 mL and 0.15 min^{-1} for rats and mice, respectively) into the jejunal loop and samples were taken at 10-min intervals during the 40-min experiment.

Results: Tryptophan absorption was determined by the difference between influx and efflux. Ang-(1-7) increased tryptophan absorption in comparison to the control group (0.13 ± 0.03 vs. 0.23 ± 0.0 mg%, P = .01). ACE2 activity in the effluent of jejunal perfusion, and the expression of ACE2 in the tissue of the small intestine were higher in the group that received Ang-(1-7) compared to the control (13.6 ± 1.5 vs. 50.5 ± 4.1 and 0.14 ± 0.0 vs. 0.38 ± 0.05, (P = .001, and P =.003, respectively). Moreover, the effect of Ang-(1-7) on tryptophan absorption was blunted in Mas KO mice, in comparison to WT (9.8 ± 2.6 vs.1.7 ± 3.0).

Conclusions: The results of the present study indicate that Ang-(1-7) increases jejunal tryptophan absorption in rats and these changes are associated with increases in ACE2 activity and expression. In Mas KO mice, these actions were blunted. The findings suggest a new mechanism that is dependent on Ang-(1-7)/Mas by which ACE2 modulates tryptophan intestinal absorption.

Keywords: ACE2; Angiotensin-(1–7); tryptophan absorption; small intestine.

ABBREVIATIONS

A-779: D-Ala^7-angiotensin-(1-7) antagonist; ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme 2; Ang II: Angiotensin II; Ang-(1-7): Angiotensin-(1-7); iv: Intravenously; Mas: Selective receptor of angiotensin-(1-7); Mas-KO: Mas knockout; RAS: Renin-angiotensin system; receptor subtype 1 (AT1); 2 (AT2); Trp: Tryptophan.

1. INTRODUCTION

1. Previous studies indicate a role for angiotensin-converting enzyme 2 (ACE2) as a key regulator of the homeostasis of dietary tryptophan (Trp), which is an essential amino acid [1]. However, the underlying mechanism remains to be established.
2. Tryptophan is the biochemical precursor of a wide array of metabolites, especially serotonin (5-hidroxytryptamin), Niacin (vitamin B3) and the Kynurenine pathway, which suggest a role in regulating intestinal function. Tryptophan metabolic routes are involved in the pathogenesis of several gastrointestinal disorders, such as inflammatory bowel disease, irritable bowel disease, and Hartnup disorder [1,2,3,4,5].
3. Serotonin is a neurotransmitter derived from Trp. Serotonin is synthesized in the gut, and can diffuse into enteric nerve endings to promote the digestion and movement of food through the alimentary canal [6]. Amino acid transporter B^0AT1 is the major luminal sodium-dependent neutral amino acid transporter in the small intestine. Recent data has demonstrated that ACE2 is required for the expression of this transporter on the luminal surface of intestinal epithelial cells [3,7]. Trp absorption is mediated through B^0AT1 (SLC6A19) activity at the brush-border membrane. Both B^0AT1 and ACE2 are present in a brush-border complex, and ACE2 is required in order for B^0AT1 to be expressed normally in the membrane [2,5,8].

4. Components of the renin-angiotensin system (RAS), such as angiotensin, angiotensin-converting enzyme (ACE) and receptor subtypes 1 (AT1) and 2 (AT2), are found in the brush border of the epithelial cell surface of the jejunum and ileum in rats, where these substances regulate absorption as well as both fluid and electrolyte transport [9]. RAS pathways may also be involved in the pathogenesis and treatment of inflammatory bowel disease [3,10]. The rat jejunum expresses angiotensinogen [11], which is the precursor for the local formation of angiotensin II (Ang-II) and/or other bioactive angiotensins [12]. ACE2 is a mono-carboxypeptidase that metabolizes Ang-II to Ang-(1-7), thereby maintaining a balance between the two peptides [13,14,15,16,17]. As such, it is a key modulator of the two RAS axes: ACE2/Ang II/AT1 receptor and ACE2/Ang-(1-7)/Mas receptor. Mas receptor is a functional G-protein-coupled receptor which binds Ang-(1-7).
5. We hypothesized that the Ang-(1-7)/Mas receptor pathway is also involved in the jejunal absorption of Trp. Therefore, the aim of the present study was to investigate the influence of the ACE2/Ang-(1-7)/Mas receptor pathway in the jejunal absorption of Trp, focusing on Ang-(1-7)/Mas. For such, we determined the effect of Ang-(1-7) on Trp absorption in rats and both wild-type and Mas-deficient mice. We also determined the effect of Ang-(1-7) on ACE2 expression in the gut of rats, wild-type mice and Mas knockout (Mas-KO) mice. Our results show that Ang-(1-7) stimulated Trp absorption through a mechanism involving Mas receptor. This effect was directly correlated to changes in ACE2.

2. MATERIALS AND METHODS

2.1 Animals

Male Wistar rats weighing 210 ± 20 g (n = five in each group), FVB/N wild-type (WT) mice and FVB/N Mas receptor knockout (KO) mice (source: Max Delbrück Center, Berlin, Germany) weighing 27.2 ± 3.8 g (n = four to eight in each group) were used. The animals were housed in collective cages with free access to filtered water and food. The animals were also kept under standard laboratory conditions with a 12:12-h light/dark cycle and controlled temperature (23 ± 3℃). The animals were fasted for 12 h prior to the absorption studies, but water was offered *ad libitum*.

2.2 Experimental Design

The rats were randomly divided into three groups: Group 1 (control) (n=5) received no treatment; Group 2 received Ang-(1-7) (n=5); and Group 3 received Ang-(1-7) + A-779 (specific Mas receptor inhibitor) (n=5). The control group received a corresponding volume of saline solution. In another set of experiments, the mice were randomly divided into four groups: WT group (control) (n=6) and Mas KO group (n=6); WT + Ang-(1-7) group (n =4 to 8) and Mas KO + Ang-(1-7) group (n=5 to 8). Ang-(1-7) (Millipore, CA, USA) was injected at a dose of 2.5 nmol/kg (iv) 15 min before the perfusion of Tyrode's solution. A-779 5 mg/kg (iv) (Sigma Chemical Co., St. Louis, MO, USA) was administered 10 minutes before the injection of Ang-(1-7). These drugs were dissolved in isotonic saline (0.9% NaCl) immediately prior to use.

2.3 Femoral Vein Cannulation

After anesthesia (intraperitoneal pentobarbital sodium – Cristália – 40 mg/kg) and hair removal from the inguinal region of the rats, cannulation of the inferior vena cava via the femoral vein was accomplished with a polyethylene catheter (14 cm of PE 50 tube welded by heating to 2 cm of PE 10). The cannulae were pre-filled with saline solution (NaCl 0.9%), with the distal end occluded by a metal pin. The cannulae were then externalized in the dorsal region of the rat. Cannulation was performed in all experiments immediately prior to drug administration. After anesthesia (80 mg/Kg Ketamine and 15 mg/Kg Xilazin, Syntec), the femoral vein cannulation for mice followed the same procedure as that for rats.

2.4 General Procedures

Following the procedures of median xypho-pubic laparotomy, the small intestine was isolated from the duodenojejunal ligament to the end of the ileum, preserving the nerves and vascular pedicle. Two cannulae were then inserted into the extremities of the small intestinal loop – one for perfusion and the other for fluid drainage. The abdominal wall was then closed to prevent tissue dehydration. Both cannulae were exteriorized through the extremities of the abdominal suture. In a bottle connected to the catheter infusion pump, Tyrode's solution (137 mM NaCl, 2.7 mM

KCl, 1.36 mM $CaCl_2$, 0.49 mM $MgCl_2$, 11.9 mM $NaHCO_3$ and 5 mM D-glucose) was maintained at 37°C in a water bath. This solution, pH 8.0 (buffered with HCO3-), was perfused at a rate of 0.25 ml min^{-1} (rats) or 0.15 ml min^{-1} (mice) for 15 min to balance the fluids and reach a steady state within the intestinal lumen [18]. The choice of alkaline pH was due to the better solubilization of neutral amino acids.

Tyrode's solution containing Trp (0.25 mg%) was infused for 40 min under the conditions described above. For rats, effluents were collected in separate test tubes at 10-min intervals, maintained in ice, and kept in a freezer at -20℃ for subsequent biochemical analysis. For mice, samples were not collected at intervals, but for the entire 40 min. After perfusion, samples of the small intestine were collected, snap frozen in liquid nitrogen and kept frozen at- 80℃ until the analysis of ACE2 using Western blot analysis.

2.5 Biochemical Determinations

The Trp concentration was estimated using high-performance liquid chromatography, which is a well-established method for measuring amino acids [19,20]. The concentration studied was chosen as a reliable point within a Trp standard curve in diluted samples and in order not to saturate the *in vivo* absorption process. Samples were prepared from a pool consisting of equal volumes of the four effluents withdrawn at 10 min-intervals for rats or the total volume collected from mice. The protein was precipitated from the mixture using ammonium sulfate (100% saturation) with stirring. The samples were allowed to stand for 24 hours at 4℃, after which the supernatant was removed. The supernatant (500 µL) from each sample was submitted to chromatography. The chromatographic separation of Trp from 15 rats was performed on an AQUAPORE RP300 C8 column (250 mm × 4.6 mm i.d., 7 µm) using gradient elution with 0.05% trifluoracetic acid and acetonitrile at a flow rate of 1.0 ml/min. Trp was identified based on the area under the peak and UV spectrum (280 nm). The area under the peak for each rat was correlated with the area under the peak obtained from the Trp solution used in the jejunum infusion at a concentration of 0.25 mg/100 mL of Tyrode's solution.

2.6 Membrane Protein Preparation and Western Blot Analysis of ACE2

The gut was weighed and 100 mg of tissue/mL of lysis buffer (Nonidet P40 1%, sodium deoxycholate 0.5% and sodium dodecyl sulfate (SDS) 0.1%, pH 7.2) were used. During protein extraction, a tablet of a cocktail of protease inhibitors containing EDTA (Complete Mini, Roche Diagnostics, Germany), Na2VO4 1M and NaF 5M was added to each 10 mL of lysis buffer. The lysate was transferred to a 1.5-mL tube, homogenized and centrifuged at 14000 rpm for 20 min at 4℃. The supernatant was then transferred to another tube. The protein concentration was determined using the Bradford assay. Western blotting was performed with standard protocols using ACE2 [21,22]. Membrane protein lysates (60 µg) were loaded into SDS-polyacrylamide (10% gels), electrophoresed and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% powdered milk for 1 h and incubated overnight with primary antibodies against ACE2 (1:300, Santa Cruz Biotechnology, CA USA, (lot G 2312) or β-actin (1:2000, Sigma Chemical, CO, St Louis, USA). The secondary antibody was added for 1 h at room temperature. Protein band detection was performed using the Odyssey scanning system (Li-Cor, USA) and Odyssey software. The results were quantified by densitometry (Odyssey software), and normalized by the β-actin level. The ratio of the experimental to control values was then calculated.

2.7 Measurement of ACE2 Activity

The supernatant samples obtained from the perfusion effluents with 100% ammonium sulfate saturation were used to measure ACE 2 activity, as described elsewhere [23,24]. The assay was conducted in the presence of captopril to eliminate any contribution of endogenous ACE, and based on the use of the Fluorogenic Peptide Substrate VI (FPSVI, 7Mca-Y-V-A-D-P-K(Dnp)-OH) (R&D systems, Minneapolis, MN, USA). ACE2 removes the C-terminal dinitrophenyl moiety of FPSVI, thereby increasing fluorescence emission at 405 nm under excitation at 320 nm. Nonspecific enzyme activity was measured by adding DX600 (1 µmol/L), which is a specific ACE2 inhibitor (Phoenix Pharmaceutical, Belmont, CA, USA). Fluorescence emission was monitored using a SpectraMax M2 Fluorescence Reader Molecular Device (Sunnyvale, CA, USA). Samples were read every minute for at least 60 minutes immediately after the addition of FPSVI at 37℃. Data (arbitrary fluorescence units) are presented as the amount of FPSVI converted into product per minute. The data obtained from the samples

were represented as the average of all perfusion effluent readings. To avoid errors, ACE2 activity was expressed per volume of perfusion effluent not normalized by protein levels, since the samples submitted to measurements were previously submitted to precipitation with 100% saturated ammonium sulfate.

2.8 Statistical Analysis

Student's *t*-test or One-Way ANOVA were used for the statistical analysis, with the level of significance set to 5% (*P < .05*).

3. RESULTS AND DISCUSSION

More than one species (Rat and mouse) were studied to test the hypothesis that Ang-(1-7) enhances Trp absorption through the activation of Mas receptor.

3.1 Effect of Ang-(1-7) on the Jejunal Tryptophan Absorption in Rat

The effect of Ang-(1-7) on Trp absorption in the jejunum of rats is displayed in Fig. 1A. Ang-(1-7) increased Trp absorption in comparison to the control, P = .01. Fig. 1B displays the expression of ACE2 in rat intestine measured by Western blotting. A striking increase in ACE2 expression was observed in rats treated with Ang-(1-7) in comparison to the control, P = .003. Fig. 1C shows the measurement of ACE2 activity in the effluent of jejunal perfusion with Trp. Ang-(1-7) treatment increased ACE2 activity, P = .001.

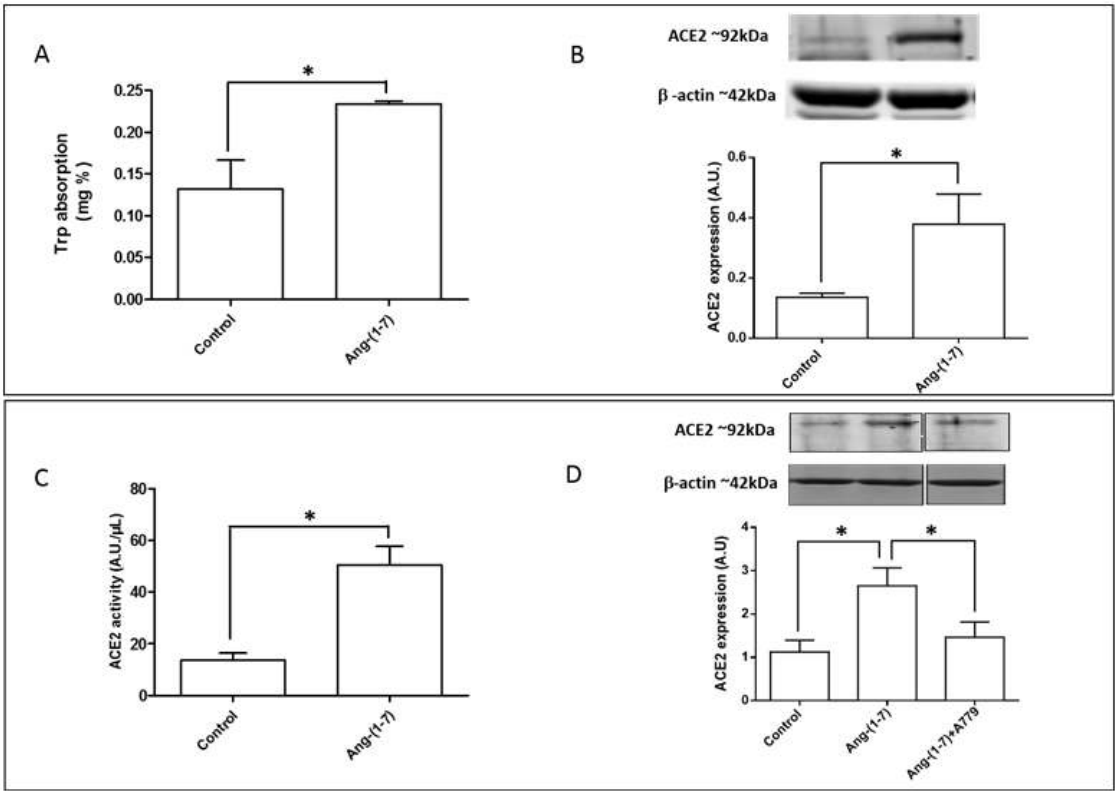

Fig. 1A. Effect of Ang-(1-7) on jejunal tryptophan absorption. B. Western blot analysis showing levels of ACE2 expression in small intestine. C. Measurement of ACE2 activity in jejuna typtophan perfusion. D. Western blot analysis showing levels of ACE2 expression in small intestine perfused with Tyrode's solution after inhibition of Mas receptor by A-779, without tryptophan

*Data expressed as Mean ± S.E.M = Mean values ± Standard error of means of all time points of each rat (n = 5). Ang-(1-7) increased jejunal Trp absorption in rats * P = .01. This effect was associated with a marked increase in ACE2 expression, and ACE2 activity in the gut of animals treated with Ang-(1-7) * P = .003 and * P = .001, respectively. The Ang-(1-7)-induced increase of ACE2 expression in the gut was blunted in presence of the Mas-receptor inhibitor, A-779 *P <. 05*

Fig. 1 D displays the ACE2 Western blot of the intestine in rats perfused with Tyrode's solution. The Mas inhibitor (A-779) abolished the increase in ACE2 expression induced by Ang-(1-7), $P < .05$. These findings are confirmed in Fig. 1B, which displays ACE2 expression in the presence of Ang-(1-7).

3.2 Effect of Ang-(1-7) on the Jejunal Tryptophan Absorption in Mice

Tryptophan absorption and ACE2 expression in the intestine of mice under basal conditions are displayed in Figs. 2A and B, respectively. No difference was found between wild-type and Mas KO mice under basal conditions. Ang-(1-7) did not alter Trp absorption in Mas knockout (KO) mice in comparison to the wild-type mice used as control (Fig. 2C), or in comparison with the baseline (Fig. 2C vs Fig. 2A). This result is accompanied by the absence of an Ang-(1-7) effect on ACE2 in the intestinal wall, in Mas KO mice (Fig. 2D).

The novel findings of the present study are that Ang-(1-7) increases Trp absorption in mice and rats and that this effect is impaired in Mas KO mice. Forty to sixty minutes after treatment with Ang-(1-7), ACE2 activity was increased in the effluent of the jejunal perfusion, and ACE2 expression was increased in the small intestine tissue in rats. However, the Ang-(1-7)-induced increase in ACE2 protein in the intestinal wall of mice was less pronounced than that found in rats and was completely absent in Mas-KO mice.

A possible mechanism for the increase in ACE2 expression would be that enhanced Ang-(1-7) levels could lead to inhibition of the "disintegrin and metalloprotease (ADAM)17 family" with consequent down- regulation of intestinal ACE2 shedding. Indeed, previous studies showed the involvement of metalloprotease in the shedding of trans-membrane ACE2 [25,26].

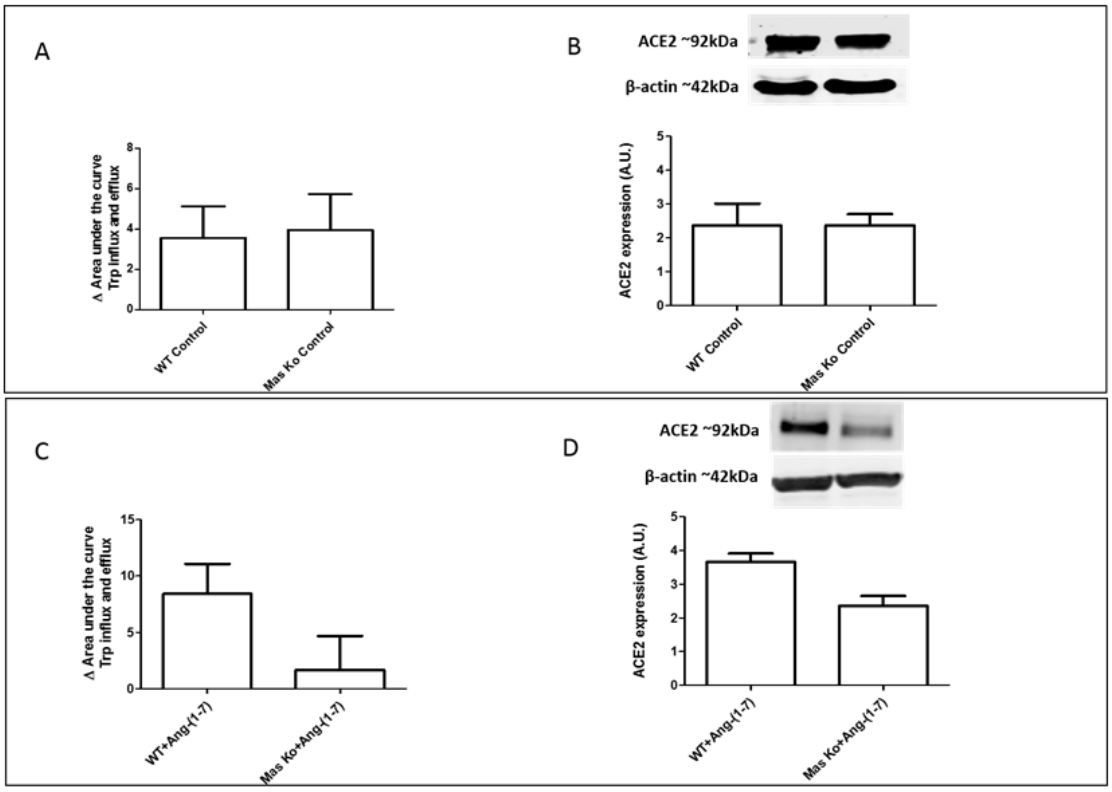

Fig. 2A. Tryptophan absorption in mice under basal conditions. B. Western blot analysis showing levels of ACE2 expression in small intestine of Mas knockout and wild-type mice under basal conditions. C and D. After treatment with Ang-(1-7)

Data expressed as Mean ± S.E.M. Ang-(1-7) did not induce Trp absorption in Mas KO mice (n = 8) (Fig. 2C vs Fig. 2A). ACE2 protein expression was strongly reduced in Mas KO (n=5) group of mice in comparison to wild-type group (n=4), after treatment with Ang-(1-7). No difference was found between wild-type and Mas KO mice under basal conditions (n=6)

The understanding of the renin-angiotensin system has advanced with the characterization of Ang-(1-7) as an important regulator of cardiovascular function and the identification of this peptide as an endogenous ligand for the G protein-coupled Mas receptor [21,27]. A number of studies have shown that many of the biological actions of Ang-(1-7) are opposed to those of angiotensin II (Ang II), suggesting a counter-regulatory function of this peptide in RAS pathways. Ang-(1-7) is one of the main metabolites of ACE2 through its action on Ang II [16]. Thus, ACE2 counteracts the function of ACE and negatively regulates Ang II levels. In this study, we provide evidence for a novel role of Ang-(1-7): tryptophan absorption.

It has been demonstrated that the deficiency of ACE2 (ACE2 knockout animals) causes critical impairment of local Trp absorption in the small intestine [1]. Bröer et al. [8] analyzed mice lacking the apical broad-spectrum neutral (0) amino acid transporter B^0AT1 (Slc6a19) and demonstrated that the Na^+-dependent uptake of neutral amino acids into intestine brush-border membrane vesicles was abolished. In Slc6a19 nullizygous mice, the major neutral amino acid transporter is an active regulator of amino acid signaling and whole body homeostasis. The B^0AT1 transporter appears to be the major mechanism for neutral amino acid uptake in the intestine [2]. The present findings broaden this knowledge, demonstrating a stimulatory effect of Ang-(1-7) on jejunal Trp absorption and that this effect is associated with a local increase in ACE2 expression and activity. Moreover, pre-treatment with a Mas antagonist, or its genetic deletion, abolished the Ang-(1-7)-induced increase in local ACE2 expression. The absence of the effect of Ang-(1-7) on Trp absorption in Mas-KO mice indicates that this is a Mas-mediated effect. It is worth mentioning that there are many questions to be addressed in future studies such as: What is the mechanism linking higher ACE2 protein and Trp transport? Is there a correlation between the expressions of ACE2 and tryptophan transporter in basal or after stimulation with Ang-(1-7)? Despite these questions, this study describe a significant effect of one of the main ACE2 products, Ang-1(1-7), on the tryptophan absorption in rats and mice.

4. CONCLUSION

The results of the present study suggest a new mechanism that is dependent on Ang-(1-7)/Mas by which ACE2 modulates tryptophan intestinal absorption in rats. In mice, this absorption seems to be mediated by Mas receptors and less dependent on ACE2 activation. Trp is an essential amino acid and a precursor of serotonin, which is a neurotransmitter that promotes the digestion and movement of food through the alimentary canal. The results confirm our hypotheses and expand our knowledge on gastrointestinal system absorption. Furthermore, our data suggest that Ang-(1-7) and the G protein-coupled receptor (Mas) could be of great interest as a targets for pharmacological interventions in disorders associated with the diet, such as the absorption of amino acids (tryptophan).

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments complied with international principles of animal care, and this study received approval from the local ethics committee on animal experimentation (CEUA/UFMG process N^o 49 /2012).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature. 2012;487:477-483. DOI: 10.1038/nature11228
2. Bröer A, Klingel K, Kowalczuk S, Rasko JEJ, Cavanaugh J, Bröer S. Molecular cloning of mouse amino acid transporter system B^0, a neutral amino acid transporter related to hartnup disorder. J Biol Chem. 2004;279(23):24467-76. DOI: 10.1074/jbc.M400904200
3. Camargo SMR, Singer D, Makrides V, Huggel K, Pos KM, Wagner CA, et al.

Tissue-Specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations. Gastroenterology. 2009;136:872-882.
DOI: 10.1053/j.gastro.2008.10.055

4. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, Tryptophan metabolism and the brain-gut-microbiome axis. Behav Brain Res. 2015;277:32-48.
DOI: 10.1016/j.bbr.2014.07.027
5. Seow HF, Bröer S, Bröer A, Bailey CG, Potter SJ, Cavanaugh JA, Rasko JEJ. Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19. Nat Genet. 2004;36(9):1003-7.
DOI: 10.1038/ng1406
6. Rosen CJ. Serotonin's secrets. Nature Medicine. 2009;15(2):145–6.
7. Kowalczuck S, Bröer A, Tietze N, Vanslambrouck JM, Rasko JEJ, Bröer S. A protein complex in the brush-border membrane explains a hartnup disorder allele. FASEB J. 2008;22:2880-7.
DOI: 10.1096/fj.08-107300
8. Bröer A, Juelich T, Vanslambrouch JM, Tietze N, Solomon PS, Holst J, Bailey CG, Rasko JE, Bröer S. Impaired nutrient signaling and body weight control in Na^+ neutral amino acid cotransporter (SLC6A19)-deficient mouse. J Biol Chem. 2011;286(30):26638-51.
DOI: 10.1074/jbc.M111.241323
9. Wong TP, Debnam ES, Leung PS. Involvement of an enterocyte renin-angiotensin system in the local control of SGLT1-dependent glucose uptake across the rat small intestine brush border membrane. J Physiol. 2007;584(2):613-23.
DOI: 10.1113/jphysiol.2007.138578
10. Fändriks L. The renin–angiotensin system and the gastrointestinal mucosa. Acta Physiologica. 2011;201:157–67.
DOI: 10.1111/j.1748-1716.2010.02165.x
11. Fändriks L. The angiotensin II type 2 receptor and the gastrointestinal tract. J Renin Angiotensin Aldosterone Syst. 2010;11(1):43–8.
DOI: 10.1177/1470320309347788
12. Nagata S, Kato J, Kuwasako K, Kitamura K. Plasma and tissue levels of proangiotensin-12 and components of the renin-angiotensin system (RAS) following low or high-salt feeding in rats. Peptides. 2010;31(5):889–92.
DOI: 10.1016/j.peptides.2010.02.008
13. Ferreira AJ, Santos RAS, Bradford CN, Mecca AP, Sumners C, Katovich MJ, Raizada MK. Therapeutic implications of the vasoprotective axis of the renin-angiotensin system in cardiovascular diseases. Hypertension. 2010;55:207–13.
DOI:10.1161/HYPERTENSIONAHA.109.140145
14. Raizada MK, Ferreira AJ. ACE2: A new target for cardiovascular disease therapeutics. J Cardiovas Pharmacol. 2007;50(2):112-19.
15. Santos RAS, Campagnole-Santos MJ, Andrade SP. Angiotensin-(1-7): An update. Regul Pept. 2000;91:45–62.
16. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxy-peptidase. J Biol Chem. 2002;277(17): 14838–43.
DOI: 10.1074/jbc.M200581200
17. Zisman LS, Meixell GE, Bristow MR, Canver CC. Angiotensin-(1-7) formation in the intact human heart: *In vivo* dependence on angiotensin II as substrate. Circulation. 2003;108:1679–81.
DOI:10.1161/01.CIR.0000094733.61689.D4
18. Borges EL, Viana MP. Does pH of tyrode solution modify glucose and electrolyte jejunal absorption in rats? J. Biophysical Chemistry. 2012;3:127-31.
DOI: 10.4236/jbpc.2012.32014
19. Delhaye S, Landry J. High-performance liquid chromatography and ultraviolet spectrophotometry for quantitation of tryptophan in barytic hydrolysates. Analytical Biochemistry. 1986;159:175-8.
20. Pinhati RR, Polonini HC, Brandão AF, Raposo NRB, Corchs F, Gattaz WF. Quantification of tryptophan in plasma by high-performance liquid chromatography. Quim Nova. 2012;35(3):623-26.
21. Santos RAS, Ferreira AJ, Verano-Braga T, Bader M. Angiotensin-converting enzyme 2, angiotensin-(1–7) and Mas: New players of the renin–angiotensin system. J Endocrinol. 2013;216:R1–R17.
22. Savergnini SQ, Ianzer D, Carvalho MBL, Ferreira AJ, Silva GAB, Marques FD, et al. The novel mas agonist, CGEN-856S, attenuates isoproterenol-induced cardiac

remodeling and myocardial infarction injury in rats. PLOS One. 2013;8(3): e57757.
DOI: 10.1371/journal.pone.0057757

23. Huang L, Sexton DJ, Skogerson K, Devlin M, Smith R, Sanyal I, et al. Novel peptide inhibitors of angiotensin-converting enzyme 2. J. Biol Chem. 2003;278(18): 15532-0.
DOI: 10.1074/jbc.M212934200
24. Huentelman MJ, Zubcevic J, Katovich MJ, Raizada MK. Cloning and characterization of a secreted form of angiotensin-converting enzyme 2. Regul Pept. 2004;122:61-7.
DOI: 10.1016/j.regpep.2004.05.003
25. Salem ESB, Grobe N, Elased KM. Insulin treatment attenuates renal ADAM 17 and ACE2 shedding in diabetic Akita Mice. Am J Renal Physiol. 2014;306:F629-F639.
DOI: 10.1152/ajrenal.00516.2013
26. Xia H, Sriramula S, Chhabra KH, Lazartigues F. Brain ACE2 shedding contributes to the development of neurogenic hypertension. Circ Res. 2013;113:1087-96.
DOI: 10.1161/CIRCESAHA.113.301811
27. Santos RAS, Simões e Silva AC, Maric C, Silva DMR, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SVB, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schultheiss HP, Speth R, Walther T. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci. 2003;100:8258-3.

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