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Research Article

Glucose and Electrolyte Absorption in Sepsis: Modulation Byangiotensin-(1-7)

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Abstract

Objectives: Studies have demonstrated the involvement of the renin-angiotensin system in electrolyte homeostasis. Endotoxemia causes dose-dependent changes in jejunal glucose, water and potassium absorption. The aim of the present study was to evaluate the effect of angiotensin-(1-7) [Ang-(1-7)] on glucose and electrolytes jejunal absorption and its modulation on sepsis induced by lipopolysaccharide (LPS).

Method: Wistar rats (n=6-10) received either saline (control), Ang-(1-7) at a dose of 2.5 nmol/kg intravenously (iv) or Ang-(1-7) + antagonist of its Mas receptor (A 779). In another set of experiments (n = 7 per group), Ang-(1-7) was administrated 30 min prior to sepsis induction by LPS (3 mg/kg IV). Six hours after sepsis induction, a Tyrode solution containing twice the usual concentrations of glucose, sodium, and potassium (pH 8.0) was infused through the jejunal loop for 40 minutes to investigate the intestinal absorption of sodium and potassium.

Results: Ang-(1-7) increased glucose (51.6±3.2), and decreased sodium (32.03±1.96) and potassium (0.25±0.05) jejunal absorption when compared with the saline group (41.79±1.84, 53.01±0.94, 0.56±0.04respectively; $p<0.05$). The antagonist A-779 prevented this effect (30.54±3.34, 41.14±4.0, 0.65±0.12, respectively; $p<0.05$). LPS did not significantly affect glucose and electrolyte jejunal absorption. However, a decreased in sodium absorption was found with the combined injections of LPS+ Ang-(1-7) (21.48±6.70mM), in comparison to the control and LPS groups (55.31±4.90 and 46.86±6.86 mM, respectively; $p<0.05$).

Conclusions: These data indicate that Ang-(1-7) modulates and LPS-induced sepsis does not impair glucose and electrolyte jejunal absorption. However, Ang-(1-7) maintains its effect on sodium absorption even in sepsis.

Keywords: Lipopolysaccharide; Absorption; Jejunum; Electrolytes; Angiotensin-(1-7)

Abbreviations

A-779: Antagonist of Ang-(1-7); ACE: Angiotensin-Converting Enzyme; ACE2: Angiotensin-Converting Enzyme 2; Ang II: Angiotensin II; Ang-(1-7): Angiotensin-(1-7); ARDS: Adult Respiratory Distress Syndrome; BBM: Brush Border Membrane; IV: Intravenously; LPS: Lipopolysaccharide; Mas: Selective Receptor Of Angiotensin-(1-7); RAS: Renin-Angiotensin System; SGLT1: Sodium-Glucose Transport Proteins

Introduction

The intestinal tract acts as a selective barrier, allowing physiologic movement of important elements, such as, water, solutes, and immune modulating factors. Critically ill patients have numerous alterations in gut integrity that play a central role in the progression of sepsis and multiple organ dysfunction syndromes [1]. It is well documented that endotoxemia causes dose-dependent changes in the jejunum, namely, increased traffic and absorption of potassium and a decrease in the absorption of water and glucose [2]. Treatment with LPS inhibits the transport of fructose and this inhibition has been associated with a decrease in the amount of GLUT5 carrier protein in the brush border membrane of enterocytes [3].

The administration of LPS [4] has been used in animal models of sepsis-related adult respiratory distress syndrome (ARDS) due to its high degree of induced sepsis, which is considered a major risk factor for the development of ARDS [5]. Recently, a number of studies have demonstrated the involvement of RAS in the pathophysiology of sepsis-induced ARDS. Studies have demonstrated the involvement of the renin-angiotensin system (RAS) in electrolyte homeostasis [6]. Angiotensin-(1-7) [Ang-(1-7)] has been shown to enhance water absorption in rats and this effect was abolished by A-779, which is an antagonist of Ang-(1-7) [7].

The understanding of the RAS 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 receptor Mas [8]. 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 the RAS. Ang-(1-7)

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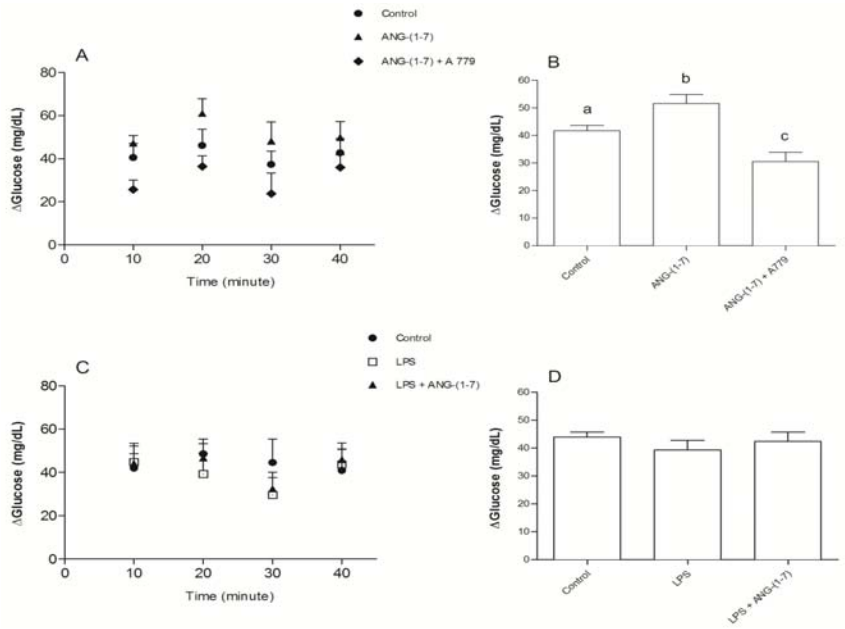

Figure 1: Effects of Ang-(1-7) (A, B) and LPS-induced sepsis (C, D) on jejunal glucose concentration. A and C represent the time course of the effect of Ang-(1-7) and LPS-induced sepsis, respectively. B and D represent mean ± SEM for all time points of each treatment. Results were expressed by the difference between influx and efflux. The glucose absorption of the group that received Ang-(1-7) (n=10) increased when compared with saline group (n=8). This effect was abolished by the selective antagonist A-779 (n=8) ($p<0.05$). There was no difference in glucose absorption in the LPS + Ang-(1-7) (n = 6) compared with control group (n = 7), either with LPS-induced sepsis group (n = 7).

is one of the main metabolites of angiotensin-converting enzyme 2 (ACE2) through its action on Ang II [9]. ACE2 less efficiently cleaves Ang I into Ang-(1-9), with the subsequent formation of Ang-(1-7) by the Angiotensin-Converting Enzyme (ACE) [10]. Thus, ACE2 counteracts the function of ACE and negatively regulates Ang II levels.

Despite considerable progress in the knowledge of RAS involvement in sepsis, no previous studies have been carried out to investigate the modulation of the jejunal absorption of electrolytes by Ang-(1-7) in the presence of this disease. The purpose of the present study was to evaluate the effects of LPS-induced sepsis on the jejunal absorption of glucose and electrolytes in rats and its modulation by Ang-(1-7).

Materials and Methods

Animals

Male Wistar rats weighing 200 to 220g were housed in collective cages with free access to filtered water and food. The animals were maintained under standard laboratory conditions of a 12:12-h light/dark cycle and controlled temperature (23±3°C). The rats were fasted for 12 h prior to the absorption studies, but water was offered ad libitum. All experiments complied with the International Principles of Animal Care and the study received approval from the local Ethics Committee on Animal Experimentation (CEUA/UFMG process Nº 35/2011).

Experimental sepsis induction

LPS from *Escherichia coli* (LPS, Sigma from E. coli serotype 055: B5) [3 mg/kg intravenously (IV)] was used for experimental sepsis induction [6, 11].

Experimental design

The animals (n=6-10) were randomly divided into four groups: control rats Group 1 (control) received no treatment; Group 2 (Ang-(1-7) received Ang-(1-7); Group 3 [(Ang-(1-7) +A-779] received Ang-(1-7) +A-779.

In another set of experiments, the animals were randomly divided into four groups (n=7 per group): Group 1 (control) received no treatment; Group 2 (LPS) was inoculated with LPS; Group 3 [LPS+Ang-(1-7)] received Ang-(1-7) and was inoculated with LPS.

Ang-(1-7) (Millipore, CA, USA) was injected at a dose of 2.5nmol/kg (IV) [12] 15 min before tyrode solution perfusion in the first set of experiments or 30 min prior to sepsis induction. A-779 5 mg/kg (IV), Sigma Chemical Co. (St. Louis, MO, USA) were administered 10 minutes before the injection of Ang-(1-7). These drugs were dissolved in isotonic saline (0.9% NaCl) immediately before use.

Femoral vein cannulation

After anesthesia (thiopentalsodium, 40 mg/kg, intraperitoneal injection) and trichotomy of the inguinal region, cannulation of the inferior vena cava via the femoral vein was accomplished with a polyethylene catheter (14 cm of polyethylene PE 50 tubing welded by heating at 2 cm PE 10). The cannulae were pre-filled with saline (NaCl 0.9%), with the distal end occluded by a metal pin. The cannulae were then externalized in the dorsal region of the mouse. This cannulation was performed in all experiments 12 hours prior to drug administration.

General procedures

Six hours after experimental inoculation, the rats were anesthetized with thiopental (40 mg/kg IV). Following the procedures of median

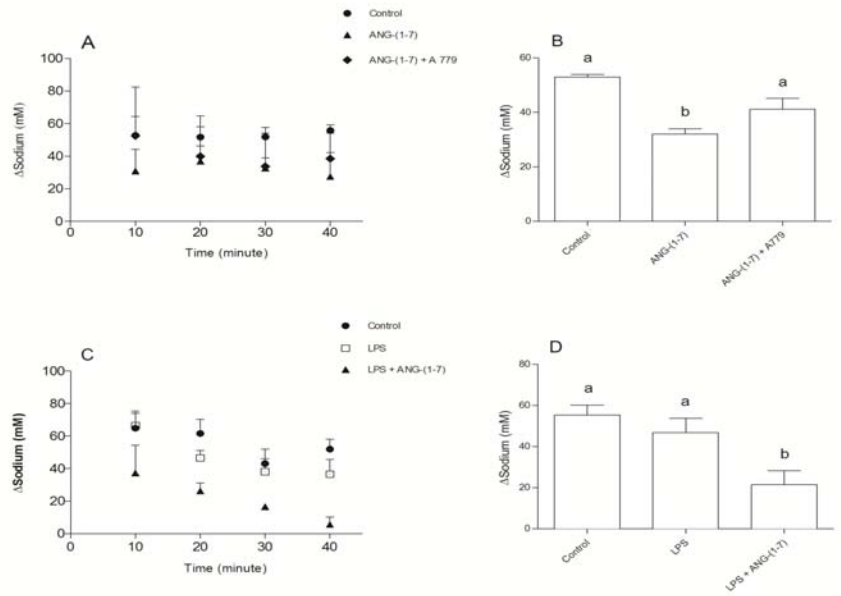

Figure 2: Effects of Ang-(1-7) (A, B) and LPS-induced sepsis (C, D) on jejunal sodium concentration. A and C represent the time course of the effect of Ang-(1-7) and LPS-induced sepsis, respectively. B and D represent mean ± SEM for all time points of each treatment. Results were expressed by the difference between influx and efflux. Ang-(1-7) (n=7) decreased sodium absorption compared with the control (n=7). This decreased was inhibited by A-779 (n=7) ($p<0.05$).In LPS-induced sepsis Angiotensin-(1-7) (n = 6) decreased sodium absorption compared with the control (n = 6), either with LPS-induced sepsis group (n = 7) ($p<0.05$).

xypho-pubic laparotomy, thesmall intestine from the duodeno jejunal ligament to the end of the ileum was isolated, 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. 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) in a bottle connected to the catheter infusion pump 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} for 15 min to equilibrate the fluids in order to reach a steady state within the intestinal lumen[13].

The animals were submitted to the Tyrode solution infusion containing twice the usual concentrations of glucose, sodium and potassium (to increase availability for absorption) for 40min under the same conditions described above. The effluents were collected separately in test tubes at 10-min intervals, maintained in ice and kept in a freezer at -20 °C for subsequent biochemical analysis.

Biochemical determinations

Effluent potassium and sodium ion concentrations were determined by flame photometry (FC 280, Celm). The glucose concentration was determined by an enzymatic method based on the use of glucose oxidase (Glucose PAP Liquiform, Lab test, Brasil). The results were expressed by the difference between influx and efflux.

Histological and morphometric analysis of the lung

The left lung was stored in 10% PBS buffered formalin and embedded in paraffin. Sections of 5 µm were prepared and stained with hematoxylin and eosin. The lung parenchyma of each animal was visualized using a 40X objective for scanning 30 random images using a microcamera(JVC TK-1270/RGB, Tokyo, Japan). Allcells contained in each image were quantified using the KS300 software program from the Carl Zeiss Image Analyzer(Oberkochen, Germany). The nuclei of leukocytes and all cell types present in lung tissue were counted based on the selection of nuclear pixels from the real image, which were transformed into a binary image for subsequent analysis [14].The count obtained from normal lung tissue was considered the normal pattern of cellularity (without inflammatory infiltrate) A histopathological examination of the lung was performed to confirm the presence of lung injury induced by sepsis.

Statistical Analysis

All values are presented as mean ± SE. Data were submitted to the Kolmogorov-Smirnov test. Split-plot analysis of variance (ANOVA) followed by the Student-Newman-Keuls method were used for the statistical analysis, with the level of significance set to5% ($p< 0.05$). All analysis and graphics were performed with the Graph Pad Prism Software (version 5.0, Graph Pad Software, Inc., La Jolla, CA, USA).

Results and Discussion

(Figure 1) displays the effect of Ang-(1-7) (A, B) and LPS-induced sepsis (C, D) on glucose absorption in the jejunum. The glucose absorption of the group that received Ang-(1-7) (n=10) increased when compared with saline group (n=8). This effect was abolished by the selective antagonist A-779 (n=8). No difference was found among groups in LPS-induced sepsis (n=6-7).

The effect of Ang-(1-7) (A, B) and LPS-induced sepsis(C, D) on sodium absorption in the jejunum is shown in (Figure 2). Ang-(1-7) (n=7) decreased sodium absorption compared with the control (n=7). This decreased was inhibited by A-779 (n=7) ($p<0.05$). No difference was found between the LPS and control group. However, a decrease in absorption was found with the combined injections of LPS+ Ang-(1-7) compared with the control and LPS groups (n=5-7).

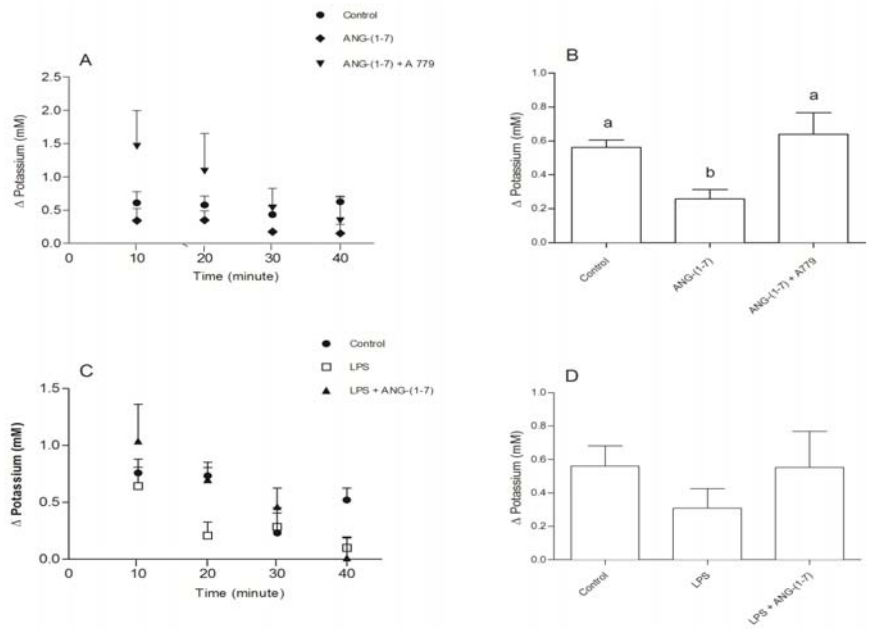

Figure 3: Effects of Ang-(1-7) (A, B) and LPS-induced sepsis (C, D) on jejunal potassium concentration. A and C represent the time course of the effect of Ang-(1-7) and LPS-induced sepsis, respectively. B and D represent mean ± SEM for all time points of each treatment. Results were expressed by the difference between influx and efflux. Ang-(1-7) (n = 8) decreased potassium absorption in comparison to the control (n = 7). This decreased was inhibited by A-779 (n = 6) ($p<0.05$). There was a decreased in potassium absorption in the LPS + Ang-(1-7) (n = 4) compared with control group (n =6), either with LPS-induced sepsis group (n = 6) ($p<0.05$).

Figure 3 shows the effect of Ang-(1-7) (A, B) and LPS-induced sepsis (C, D) on jejunal potassium concentration. Ang-(1-7) (n=6) decreased potassium absorption in comparison to the control (n=7). This decreased was inhibited by A-779 (n=7). No difference was found among groups in LPS-induced sepsis (n=4-6 per group).

Images selected as representative of the histological findings of rat parenchyma in the control and LPS-induced sepsis groups are displayed in (Figure 4). No major histological abnormalities were found in the lung parenchyma of the control group (Figure 4 A, B). The sepsis group (Figure 4 C, D), however, shows severe lung injury with edema, massive inflammatory cell infiltration, capillary congestion, and exudation. These changes contributed to the thickening of the alveolar-capillary membrane and loss of the normal architecture of the lung parenchyma. No difference was found in the experimental groups that received LPS associated with Ang-(1-7) and compared with the group that received LPS alone. The quantitative analysis of lung parenchyma demonstrated greater inflammatory infiltrate in the lungs of LPS-induced sepsis groups compared with the control group (n=7 per group) (Fig 4E).

The novel finding of the present study was that angiotensin-(1-7) administration increases glucose and reduces the jejunal absorption of sodium and potassium. LPS treatment alone did not interfere with intestinal glucose and electrolytes absorption. However, sepsis attenuated the effect of Ang-(1-7) on glucose and potassium absorption. The effect of Ang-(1-7) on sodium absorption was not influenced by sepsis.

The intestinal mucosa is responsible for the absorption of nutrients and for the separation of the potentially toxic luminal content from the host. Experimental animal studies have shown that gut wall integrity loss is involved in the development of various inflammatory syndromes, including sepsis [1]. Sepsis is the main cause of death in critically ill patients and is associated with severe problems such as impaired gastrointestinal function [15]. Patients with acute sepsis exhibit increased gastrointestinal permeability and decreased gastrointestinal functional absorptive capacity in comparison with healthy control subjects [16]. In addition, these patients are often in tolerant of enteral feedings due to a combination of motility disturbances and impaired absorptive function [2]. The mechanism involved in impaired absorptive function is not clearly established up to now and data of Ang-(1-7) effects on jejunal absorption in sepsis conditions are not available.

The present results confirm that LPS induces ARDS (positive control), as demonstrated by severe lung injury with edema and massive inflammatory infiltrate, capillary congestion and exudation. These changes lead to the thickening of the alveolar capillary membrane and loss of the normal architecture of the lung parenchyma in the experimental groups.

A previous controlled study found that doses of 1or 5 mg/kg of LPS led to a proportional decrease in the transport of glucose [2]. In contrast, no difference in glucose absorption was found among the groups in the present investigation with an LPS dose of 3 mg/kg. This discrepancy may be due to the period of sepsis induction (90 minutes versus 6 hours) as well as the different strains of rats employed.

The results of the present study suggest that the administration of exogenous Ang-(1-7) modulates jejunal glucose and electrolyte absorption by a Mas-mediated mechanism since these effects were blocked by A-779 a selective antagonist of Mas receptor. These

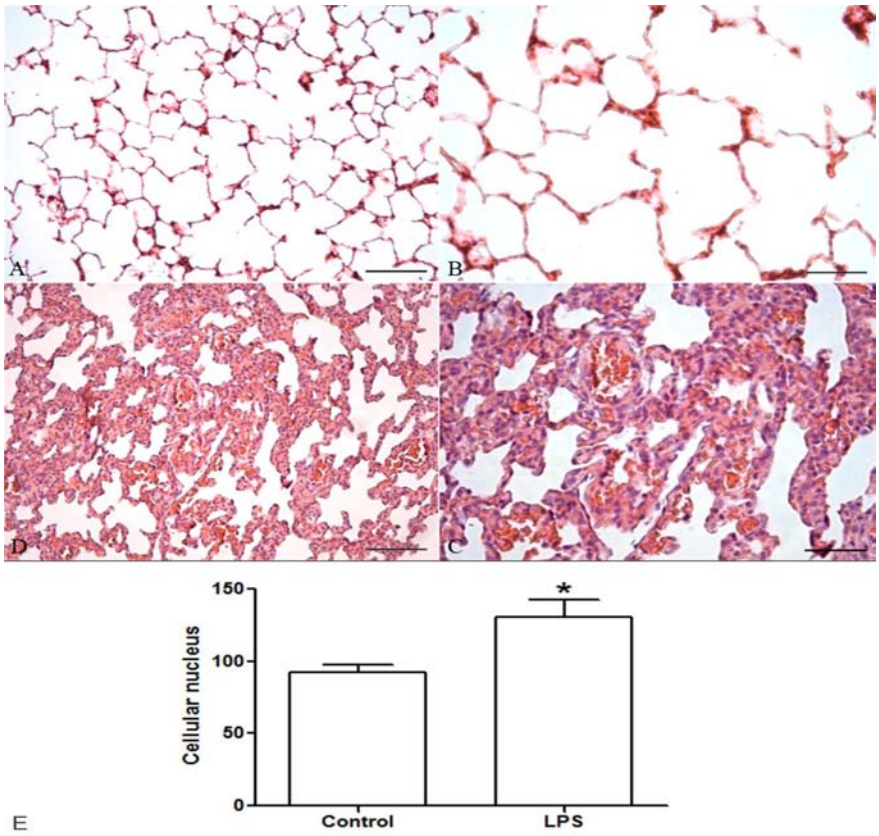

Figure 4: Photomicrography of rat lung parenchyma; pulmonary histological sections stained with hematoxylin-eosin in control group (Figure 4A, B), and LPS-induced sepsis group (Figure 4C, D). No major histological abnormalities were observed in control group. Lung injury is characterized by large amount of inflammatory cells, edema, and congestion of the alveolar capillaries leading a diffuse thickening of the alveolar septum. The morphometric assessment demonstrated greater inflammatory infiltrate in the lungs of LPS-induced sepsis group in comparison to the control group (n = 7 per group) (Figure 4E). Bar 100 µm, B and D; 50 µm: A and C.

findings show, for the first time, that Ang-(1-7) influences intestinal absorption in baseline conditions.

A recent study on type 1 diabetes mellitus have shown the expression of the components of the ACE2-Ang-(1-7)-Mas receptor system in jejunal enterocytes and the involvement of Ang-(1-7) in the control of sodium-glucose transport proteins (SGLT1)-mediated glucose transport across the intestinal brush border membrane [17]. Moreover, a higher expression of ACE2 have been demonstrated in the ileum of rats [18] suggesting the presence of Ang-(1–7), main metabolic product of ACE2. These data corroborate with the hypotheses that Ang-(1-7) is involved in the modulation of the intestinal absorptive process.

Ang-(1-7) infusion generally opposes or attenuates the effects of Ang II. It has been reported that in the kidney, in contrast to Ang II, Ang-(1-7) produces diuretic/natriuretic effects [19]. This could be partly due to the inhibition of sodium and water reabsorption along the nephron segments [19,20,21,22]. Regarding the present study, Ang-(1-7) effect in sodium absorption seems to be the same as observed in nephron segments. Interestingly in the presence of LPS-induced sepsis, this effect was maintained. Further studies are necessary to clarify the mechanism of the effects of Ang-(1-7) on sodium and glucose absorption.

Conclusion

In the present study, we demonstrate for the first time, that Ang-(1-7) modulates jejunal glucose and electrolytes absorption. In addition, within the period studied acute sepsis does not interfere with the absorption of glucose and potassium in a murine model. On the other hand, sepsis blunted the effects of Ang-(1-7) on glucose absorption without interfering with its effect on sodium absorption.

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