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Liraglutide, a GLP-1 receptor agonist, regulates the expression of the hepatic components of RAS in rat pups subjected to perinatal caloric restriction

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Abstract:

The renin-angiotensin system (RAS) is one of the most potent regulator of physiological mechanisms related to diseases, including chronic liver disease and hepatic fibrosis. Maternal undernutrition has long term effects on metabolic dysregulation in offspring. In fact, maternal prenatal food restriction (MPFR) may alter the RAS components which results in metabolic disruption both in mothers and pups. Our aim was to examine the impact of MPFR on RAS components in the liver of male and female pups and to determine how liraglutide, administered to pregnant rats subjected to caloric restriction, can restore RAS activity in the liver. Our results identified the presence of RAS components in the liver. They were differentially expressed in male and female offsprings. Liraglutide restored the expression of RAS components (ACE, ACE2, Nln) and significantly increased the mRNA level of MasR in MPFR male pups compared to the food restrictive vehicle, whereas in female pups there was no noticeable change. Furthermore, liraglutide decreases the expression of RAS components (ACE, ACE2, Nln, AT1R, MasR,) in males from ad libitum pregnant mothers, but did not modify these factors in females. As a conclusion, liraglutide modulates the expression of different components of RAS and minimizes the harmful effects of maternal food restriction. Liraglutide restores ACE2 and MasR mRNA levels in male MPFR offspring. Activation of the protective pathway ACE2/Ang(1-7)/MasR explains that RAS has beneficial effects on liver metabolism.

Keywords:

- Liver
- Liraglutide
- Sistema-Renina-Angiotensina (RAS)
- Maternal Perinatal Food Restriction (MPFR)
- GLP1R

Introduction

In the last few decades, the discovery of functional local RAS systems, more specific receptors and new enzymes has been changed the relevance of RAS, revealing new functions for some of the intermediate products, beyond their roles as substrates along the classical route. The classical view consists of the angiotensin-converting enzyme (ACE), that convert Angiotensin I (AngI) to AngII, and AngII mediate its biological effect by angiotensin type 1 (AT1) receptor. I was established a second axis through ACE2/Ang(1-7)/Mas, whose end point is the metabolite Ang(1-7) that exerts the vasodilatory, anti-proliferative and anti-inflammatory as opposed to AngII [1] (Figure 1).

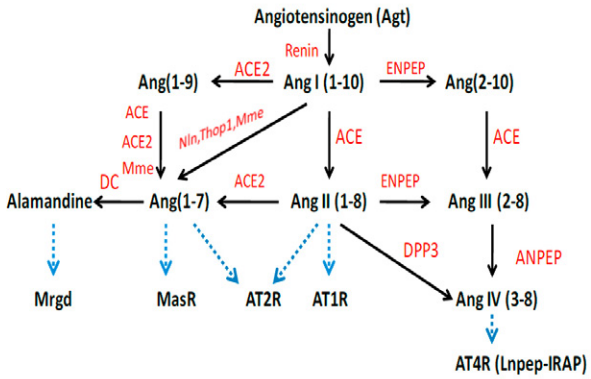

Figure 1. Non Classical RAS components. Angiotensinogen cleaved by renin and enzymatic cascade of angiotensin peptides formed that bind with different receptors and exert their effects

Liver diseases are one of the major cause of mortality worlwide. RAS is associated with pathological characteristics of chronic liver diseases including pro-fibrosis, oxidative stress and inflammatory markers [2]. Liver fibrosis is linked with increased AngII/AT1, and on the contrary antagonized by Ang(1-7)/Mas Receptor, which play a protective role [2]. In cirrhotic human liver and in rat liver injury, the activation of ACE2/Mas receptor branch promotes hepatic conversion of angiotensin II to Ang(1-7) leading to beneficial effects in liver diseases and regeneration [3].

Caloric restriction during the gestational period is also related to alteration in RAS components leading to metabolic dysregulation and diseases, besides other complications in mothers and offspring [4]. In undernourished individuals AGT and ACE level are increased in adipose tissue which cause lipid accumulation [5]. Low protein intake in early life enhances RAS activity, associated with metabolic changes, cardiovascular impairment and high blood pressure during adulthood [6].

Aim

This study aimed to elucidate the effects of maternal food restriction (MPFR) in the liver expression of several components of RAS in male and female pups, and to determine if liraglutide administration (an analogue of GLP-1 receptor) during the gestational period may modulate the offspring RAS system.

Material and Methods

Pregnant Sprague-Dawley rats were randomly assigned to 50% food restriction (MPFR) or ad libitum control (CT) groups at day of pregnancy 12 (GD12). From GD14 to parturition, pregnant FR and CT rats were treated with liraglutide (100µg / kg / 12 hours, sc.) or vehicle (saline). At postnatal day 21 and before weaning 16 males and females (CT and FR) were euthanized. mRNA expression levels of angiotensinogen (AGT), interleukin-6 (IL-6), ACE, ACE2, neprilysin (Mme), neurolysin (Nln), thimet oligopeptidase (Thop1), AT1R, MasR, Leucyl and Cystinyl Aminopeptidase (Lnpep) and Mas-related G-protein coupled receptor member D (MRGPRD), were assessed in the liver by quantitative real-time polymerase chain reaction. Data were presented as mean ± SEM. Statistical significance was determined by Analysis of Variance. Differences were statistically significant following conventions at $p \leq 0.05$.

Results

This study aimed to elucidate the effects of maternal food restriction (MPFR) in the liver expression of several components of RAS in male and female pups, and to determine if liraglutide administration (an analogue of GLP-1 receptor) during the gestational period may modulate the offspring RAS system.

1. Effect of liraglutide on Angiotensinogen in MPFR male and female rat pups:

The mRNA expression of the AGT was evaluated in both male and female pups. Perinatal caloric restriction reduces angiotensinogen levels only in male rat liver. Liraglutide did not have any effect in AGT mRNA expression. (Figure 2).

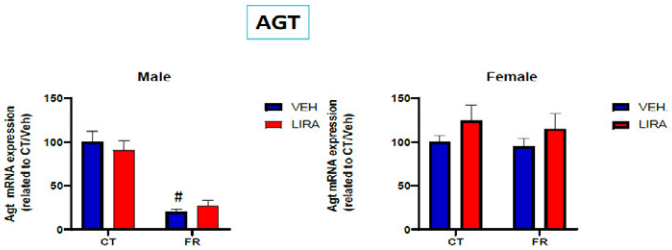

Figure 2. AGT mRNA levels in the liver of male and female rat pups. Data are mean + SEM, # $p \leq 0.05$, CT vs FR; Tukey's multiple comparison test

2. Effect of liraglutide on RAS enzymes in MPFR male and female rat pups:

The mRNA expression of the ACE, ACE-2, Nln, Mme, Thop1 was studied in the liver of male and female pups. MPFR decreased mRNA levels of ACE, ACE2 and, Nln in male pups. In females, MPFR decreased Nln mRNA expression without having any effect in ACE or ACE2 mRNA levels. Liraglutide Thop1 mRNA in control females and ACE in MPFR females. Any effect by MPFR or liraglutide were observed in Mme mRNA levels (Figure 3).

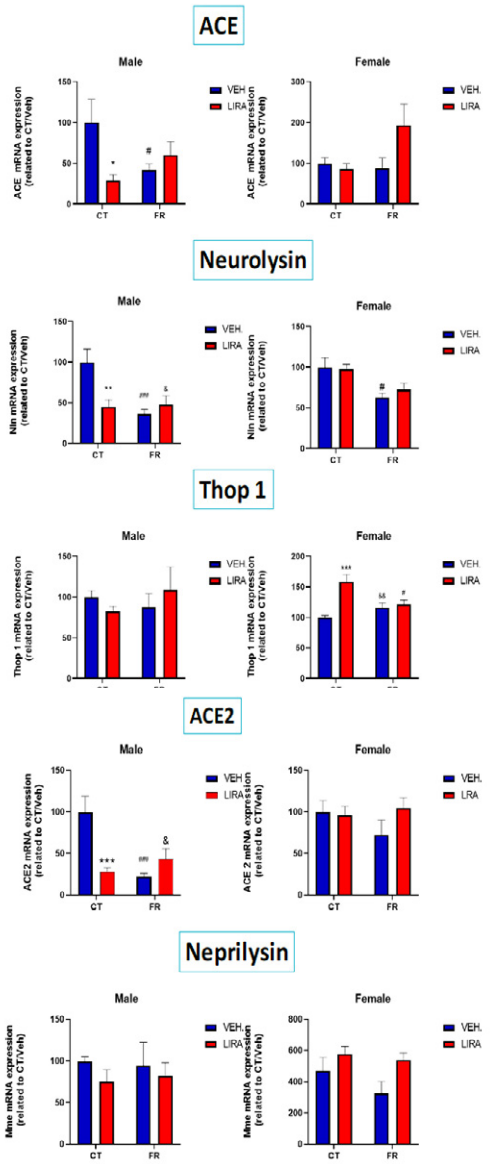

*Figure 3. RAS enzymes mRNA levels in the liver of male and female rat pups. Angiotensin converting enzyme (ACE) and Angiotensin converting enzyme 2 (ACE2), Neurolysin (Nln), Neprilysin (Mme), Thimet oligopeptidase (Thope 1) were studied. Data are mean + SEM, * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 veh vs lir; # p ≤ 0.05, ## p ≤ 0.01, CT vs FR; Tukey's multiple comparison test*

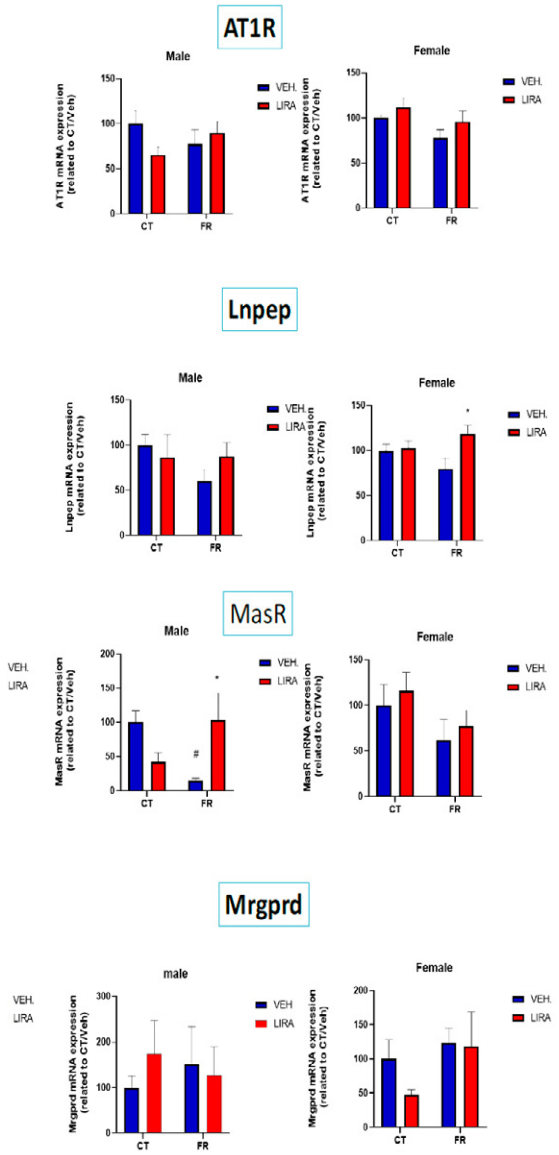

*Figure 4. RAS receptors mRNA levels in the liver of male and female rat pups. Angiotensin I receptor (AT1R), Mas receptor, Leucyl And Cystinyl Aminopeptidase (Lnpep), Mas-related G-protein coupled receptor member D (Mrgprd), were studied. Data are mean + SEM, * p ≤ 0.05, veh vs lir; # p ≤ 0.05, CT vs FR; Tukey's multiple comparison test*

3. Effect of liraglutide on RAS receptors in MPFR male and female rat pups:

The reduced MasR mRNA expression induced by MPFR was restored by the liraglutide treatment in the liver of male pups. No effects were observed in females, neither by MPFR nor the treatment with Liraglutide. Moreover, no MPFR nor liraglutide effect was observed in the AT1R, Lnpep, Mrgprd mRNA levels in the liver of both male and female pups (Figure 4).

In MPFR male pups, the reduction of angiotensinogen, ACE and ACE2, together with the marked reduction of MasR, unbalances the activity of RAS facilitating the action of AngII and abolishing that of Ang(1-7) in the liver. This imbalance increases the susceptibility to inflammation and liver fibrosis in males. Liraglutide may contribute to improving the response to Ang(1-7) by increasing the expression of MasR.

Discussion

Dietary restriction during pregnancy can lead to non-alcoholic fatty liver disease/steatohepatitis [5]. The pathological characteristics of chronic liver diseases include enhanced fibrosis, oxidative stress and inflammatory markers. The RAS is associated with all these processes [2]. RAS is commonly activated in chronic liver diseases and AII is a critical factor to promote fibrosis [7]. Previous studies have demonstrated that RAS is related to NAFLD and also indicate that ACE/AngII/AT1R axis inhibition or ACE2/Ang(1-7)/Mas axis activation may represent effective targets for NAFLD treatment [3]. The GLP-1 analogue liraglutide modulate both axis of RAS, especially augmenting the expression levels of ACE-2, which in turn will drive to the reduction of circulating AngII and the marked increase of Ang(1-7) [3]. GLP-1 receptor agonist also minimizes the harmful effects of maternal food restriction [7,8]. There is evidence of complete local RAS in mice liver of both cancerous and normal cells [2, 3, 9]. In our study we have demonstrated the mRNA expression of key components and enzymes of RAS in the liver of pups from maternal food restricted mothers. In addition, MPFR alters the gene mRNA expression of some of this molecules, reducing angiotesogen, ACE, ACE2 and neurolisyn in male pups and increasing Thop1 and reducing neurolisyn in females. Furthermore, MPFR reduces MasR expression levels in males without having any effect in females. Liraglutide, is able to modulate the RAS expression levels in lung, especially stimulating the expression of ACE-2 [8,10], that leads to increase production of Ang(1–7), activating the MasR [8,9]. Ang(1–7) reduces lung fibrosis and pulmonary arterial hypertension and activates pathways that promote to lung homeostasis [10]. GLP-1 receptor agonist also minimizes the harmful effects of maternal food restriction [8]. Other reports have shown that GLP-1R activation might play a role in regulation of RAS components in different organs. In this regard, the treatment with liraglutide during pregnancy is able of restoring MasR in the liver of male pups. In males from control mothers treated with liraglutide reduced ACE, ACE2 and neurolysin mRNA expression were reduced.

Based upon findings we can be concluded, liraglutide restore the ACE2/MasR mRNA level in prenatal food restricted male pups and modulate the activities of the RAS components. The elevation in protective component of RAS, ACE2/An(1-7)/MasR, shows male pups are more prone to beneficial effects. Liraglutide modulate the expression level of different components of RAS and minimizes the harmful effects of maternal food restriction, showing a clear sexual dimorphism. The RAS regulation with liraglutide may be a target strategy serving as therapeutic regimen to prevent and treat chronic liver disease as well as acute liver injury.

Acknowledgments

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