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Central insulin–angiotensin II interaction in blood pressure regulation in fructose overloaded rats

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ABSTRACT

The aim of the present study was to determine if insulin is able to modulate the pressor response to intracerebroventricularly administered angiotensin II in insulin resistant fructose overloaded rats.
Male Sprague-Dawley rats were divided into two groups: 1) Control group (C) with tap water to drink for 6 weeks (n = 36); and 2) fructose treated (F), with fructose solution (10% w/v) to drink for 6 weeks (n = 36). On the day of the experiment, anesthetized male C and F rats were intracerebroventricularly infused with insulin (12 mU/h, n = 15) or Ringer's solution as vehicle (n = 15) for 2 h. Immediately, changes in mean arterial pressure (MAP) in response to an intracerebroventricular subpressor dose of angiotensin II (5 pmol, n = 10) or vehicle (n = 5) were measured for 10 min. Then, hypothalami were removed and Akt and ERK1/2 phosphorylation levels were determined. In a subset of C (n = 10) and F (n = 20) animals, PD98059 (p44/42 MAPK inhibitor) or vehicle was administered intracerebroventricularly at a flow rate of 5 μl/min for 1 min. Ten minutes later, insulin (12 mU/h, n = 5 for each group) or vehicle (Ringer's solution, only in the F group, n = 5) was perfused for 2 h at a flow rate of 4 μl/h, and cardiovascular parameters were measured every 15 min. Immediately, changes in MAP and HR in response to a subpressor dose of Ang II (5 pmol/2 μl) were evaluated for 10 min (n = 5 for each group). In other subset of animals (n = 6 for each group), AT1 and AT2 hypothalamic receptor levels were measured by Western blotting.
Intracerebroventricular insulin pre-treatment increased the pressor response to angiotensin II in C rats. In F rats (with or without insulin pretreatment), the pressor response to angiotensin II was higher than that in vehicle pre-treated C animals, but similar to that observed in C after insulin infusion. In C rats phospho-ERK 1/2 hypothalamic levels significantly increased after angiotensin II injection in insulin pretreated animals compared to vehicle pre-treated rats, suggesting that MAPK activation might be involved in insulin potentiation of blood pressure response to angiotensin II in the brain. Phospho-ERK 1/2 hypothalamic levels were significantly increased in vehicle treated F rats compared to C, suggesting that basal MAPK activation might play a role in the enhanced response to angiotensin II observed in these animals. Finally, in F rats, either after vehicle or insulin infusion, angiotensin II injection was associated with a similar increase in phospho-ERK 1/2 hypothalamic levels, comparable to that observed after angiotensin II injection in insulin pre-treated C animals. ERK 1/2 blockade significantly reduced MAP in F rats compared to C. Moreover, ERK 1/2 inhibition completely abolished the Ang II pressor response in F rats and in insulin pre-treated C animals. All these findings suggest that insulin–angiotensin II interaction at hypothalamic level might be involved in the increase in blood pressure observed in the insulin resistant state.

1. Introduction

There is a large body of experimental evidence showing that insulin resistance and compensatory hyperinsulinemia play a role in the development of hypertension in the metabolic syndrome [1]. An animal model frequently used to study the pathophysiological pathways involved in this association is the fructose-overloaded rat [2–5]. In

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this model, it has been reported that interventions that reduce insulin levels or improve insulin sensitivity prevent blood pressure increase, supporting the notion that insulin resistance/hyperinsulinemia are causally linked to the development of hypertension in this animals [2].

Many mechanisms could be involved in the increase in blood pressure related to insulin resistance. For instance, it has been reported that insulin resistance and hyperinsulinemia are able to up regulate the number and activity of AT1 receptors at peripheral levels [6,7], as well as increase sympathetic nervous system activity at hypothalamic level [8,9], involving renin angiotensin dependent pathways [10]. On the other hand, many of the central effects of angiotensin II (Ang II) are mediated by intracellular pathways (such as the Mitogen-activated-protein-kinase — MAPK — and PI3K-akt/protein kinase B pathways) [11,12] that are also involved in the central effects of insulin [8], suggesting that insulin might influence on the central response to Ang II.

In a previous study conducted in normal rats, we reported that centrally administered insulin potentiates the pressor effects of Ang II at brain level, by means of MAPK pathway activation, suggesting a novel mechanism by which insulin influences blood pressure control at central level [13]. However, the role of this mechanism in blood pressure regulation in the insulin resistant state has not been evaluated.

It is important to point out that, in previous studies, we have evidenced many alterations in hypothalamic control of blood pressure in fructose overloaded rats [9,14,15], an animal model of insulin resistance. Interestingly, many of these alterations involve angiotensinergic pathways located in the anterior hypothalamus [14], a brain region sensitive to Ang II levels in the cerebro-spinal fluid [16].

Consequently, the aim of the present study was to determine if insulin is able to modulate the pressor response to intracerebroventricularly administered Ang II in insulin resistant fructose overloaded rats and to further explore the role of the hypothalamic renin angiotensin system in the development of hypertension in the metabolic syndrome.

2. Materials and methods

2.1. Animal preparation

A total of 72 male Sprague-Dawley rats (weighing 250–300 g at the beginning of the study) were used. Rats were kept in cages in a room with a 12-h light–dark cycle. All animals were fed standard rodent diet (Asociación Cooperativas Argentinas, Buenos Aires, Argentina) with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals and 69% starch and vitamin supplements, containing the same amount of calories. Following an acclimatization period of 7 days, rats were randomly divided into two groups, a control group (C; n = 36) that received regular diet (19% protein, 77% carbohydrate, 4% fat), and a fructose-treated group (F; n = 36) that was fed a regular diet and fructose that was administered as a 10% solution (prepared every 2 days) in drinking water for 6 weeks, as described previously [14]. Control animals were given ordinary tap water to drink throughout the entire experimental period. Animals consumed diets and fluids ad libitum.

Animal experiments were performed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals [17].

2.2. Systolic blood pressure and body weight determination

Rats were weighed previously to dietary manipulation and at the end of the study. The rats were trained to the procedure of systolic blood pressure (SBP) measurement at 1:00 PM twice/week for 2 weeks previous to the final measurement. The mean of 10 consecutive readings was used as the reported value of the SBP for each rat. Indirect SBP was measured at week 6 by means of the tail-cuff method using a blood pressure analysis system (model SC1000; Hatteras Instruments, NC, USA).

2.3. Glucose, triglycerides, and insulin measurements

All measurements were determined 5 h after food removal. Blood glucose and triglyceride determinations were test by an enzymatic colorimetric assay kit (Wiener Lab, Rosario, Argentina). Insulin levels were assessed using a rat insulin ELISA kit (Ultra Sensitive Rat Insulin ELISA Kit; Crystal Chem, IL, USA).

2.4. Acute intracerebroventricular insulin infusion followed by angiotensin II injection in C and F animals

On week 6, 30 F and 30 C rats were fasted for 5 h, weighed and anesthetized with a mixture of chloralose (50 mg kg^{-1}, i.p.) and urethane (500 mg kg^{-1}, i.p.). The left carotid artery was cannulated and connected to a Statham Gould P23ID pressure transducer (Gould Instruments, Cleveland, OH, USA) coupled to a Grass 79D polygraph (Grass Instruments, Quincy, MA, USA) for mean arterial pressure (MAP) and heart rate (HR) recording. MAP was calculated as the sum of the diastolic pressure and one-third of the pulse pressure. The HR was measured tachographically by counting the pulsatile waves of arterial pressure recording.

A 32 gage stainless-steel needle was inserted into the right lateral ventricle according to the stereotaxic coordinates A/P − 1.3 mm; L/M − 2.0 mm; and V/D − 3.5 mm; from the bregma [18].

After a 60 min stabilization period, basal MAP and HR values were determined for 30 min in C and F rats. Then, insulin (12 mU/h, n =15 for each group) or vehicle (Ringer's solution, n = 15 for each group) was perfused for 2 h at a flow rate of 4 μl/h, and cardiovascular parameters were measured every 15 min. Immediately, changes in MAP and HR in response to a subpressor dose of Ang II (5 pmol/2 μl, n = 10) or vehicle (n = 5) were evaluated for 10 min in F and C rats. The subpressor dose of Ang II (the highest dose of intracerebroventricularly-injected Ang II which failed to exert a pressor effect in C rats) was selected based on a previous work from our laboratory [13]. Finally, animals were immediately sacrificed by decapitation and hypothalami were dissected according to Palkovitz and Brownstein microdissection technique [19], removed and frozen at −70 °C until assay.

2.5. Western blotting analysis

For the evaluation of intracellular signaling response to insulin and Ang II, phospho-Akt and phospho-ERK 1/2 levels were measured in a subset of animals (n = 5 for each group) and considered as parameters of Phosphoinositide 3-kinase (PI3K) and p44/42 MAPK activation, respectively.

Briefly, tissue samples were homogenized in solubilization buffer containing 1% Triton together with phosphatase and protease inhibitors as described previously [20]. Tissue extracts were centrifuged at 100,000 ×*g* for 1 h at 4 °C to eliminate insoluble material, and protein concentration in the supernatants was measured using the Bradford method as described previously [20]. In order to determine the phosphorylation levels of Akt and ERK 1/2, equal amounts of solubilized proteins (40 μg) were denatured by boiling in reducing sample buffer, resolved by SDS-PAGE, and subjected to immunoblotting with the anti-phospho-Akt (1:1000 dilution) or anti-phospho-ERK 1/2 antibodies. Akt and ERK 1/2 abundance was detected by reprobing the corresponding membranes with the anti-Akt and anti-ERK 1/2 antibodies. After extensive washing, membranes were incubated with the appropriate secondary HRP-coupled antibodies and processed for enhanced chemiluminescence using the ECL plus Western blotting detection system (Amersham Biosciences, Piscataway, NJ, USA). Bands were quantified using a Gel-Pro Analyzer 4.0 (Media Cybernetics, Inc. Bethesda, MD, USA).

2.6. AT1 and AT2 hypothalamic receptor levels in control and fructose treated rats

In order to evaluate the possible mechanisms involved in the pressor response to intracerebroventricular Ang II in F rats, AT1 and AT2 hypothalamic receptor levels were measured by Western blotting. Briefly, after 5 hour fasting, a subset of un-anesthetized animals (n = 6 for each group) was sacrificed and hypothalami were dissected as previously described and removed, frozen at −70 °C until assay and processed as mentioned above. Afterwards, to evaluate hypothalamic AT1 and AT2 receptor protein abundance, equal amount of proteins was subjected to immunoblotting with anti-AT1R or anti-AT2R receptor antibodies, respectively, as described above. Equal protein loading in the gels was confirmed by reblotting the same membranes with an anti-β-actin antibody. After being extensively washed, all membranes were incubated with the appropriate secondary HRP-coupled antibodies and processed for enhanced chemiluminescence using the ECL plus Western blotting detection system (Amersham Biosciences, Piscataway, NJ). Bands were quantified using a Gel-Pro analyzer 4.0 (Media Cybernetics, Bethesda, MD).

2.7. Protocol 2: Acute intracerebroventricular insulin infusion followed by angiotensin II injection, in the presence of MAPK inhibition in C and F rats

In a subset of C (n = 10) and F (n = 20) animals, PD98059 (p44/42 MAPK inhibitor, 1 μg/μl, F n = 10; C n = 5) or vehicle (dimethylsulphoxide, Sigma, USA, F n = 10; C n = 5) was administered intracerebroventricularly at a flow rate of 5 μl/min for 1 min. Ten minutes later, insulin (12 mU/h, n = 5 for each group) or vehicle (Ringer's solution, only in the F group, n = 5) was perfused for 2 h at a flow rate of 4 μl/h, and cardiovascular parameters were measured every 15 min.

Immediately, changes in MAP and HR in response to a subpressor dose of Ang II (5 pmol/2 μl) were evaluated for 10 min (n = 5 for each group). Finally, animals were sacrificed by decapitation and the correct location of the needle was verified by histology.

2.8. Statistical analysis

All results are expressed as mean ± SEM. Statistical analysis was performed using a GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, California, USA), by means of Student's *t* test, one-way ANOVA or two-way ANOVA followed by Bonferroni post hoc test. A p value <0.05 was considered statistically significant.

3. Results

3.1. Metabolic parameters

At the end of week 6, body weights were similar between C and F rats (Table 1). Circulating insulin, glucose and triglyceride levels were significantly higher in the F group than in the standard chow-fed group. In addition, fructose-fed rats developed a mild hypertensive state, as shown by a significant increase in SBP (Table 1).

Table 1
Metabolic characteristics of the experimental animals.

Parameters	Control	Fructose
Body weight, g	415 ± 12	420 ± 13
SBP, mmHg	113 ± 3	131 ± 2*
Glycemia, mg/ml	1.35 ± 0.3	1.53 ± 0.3*
Triglycerides, mg/ml	0.57 ± 0.07	1.03 ± 0.10*
Insulin, ng/ml	1.2 ± 0.2	2.8 ± 0.4*

* $p < 0.05$ vs C.

3.2. Acute intracerebroventricular insulin infusion followed by angiotensin II injection in C and F animals

Baseline MAP (F: 94 ± 3 mmHg vs. C: 87 ± 3 mmHg; $p < 0.05$) was significantly higher in F rats. No differences were observed in baseline HR levels between groups (F: 396 ± 8 bpm vs. C: 401 ± 10 bpm; NS).

Intracerebroventricular insulin infusion for 120 min did not modify MAP compared to vehicle in C (ΔMAP: insulin: 3.4 ± 3.6 mmHg; n = 15 vs vehicle: 7.9 ± 2.6 mmHg; n = 15; NS) and F (ΔMAP: insulin: 2.6 ± 3.2 mmHg; n = 15 vs vehicle: 8.0 ± 2.2 mmHg; n = 15; NS) animals. No differences were observed in these parameters between F and C rats.

Similarly, no differences were observed in HR for 120 min of insulin or vehicle infusion in C and F rats (data not shown).

Fig. 1 shows the effects of Ang II (5 pmol) on MAP in insulin or vehicle pre-treated C and F rats. While intracerebroventricular Ang II (5 pmol) injection did not modify MAP in vehicle pre-treated C rats, it significantly increased MAP in insulin pre-treated C animals (Fig. 1A). On the other hand, F rats showed a pressor response to Ang II (5 pmol) either after vehicle or insulin pre-treatment (Fig. 1B). As expected, vehicle injection did not exert any pressor response, neither in insulin nor in vehicle pre-treated C and F rats (data not shown). There were no changes in HR after Ang II injections in any of the studied groups (data not shown).

Figs. 2 and 3 show the effects of insulin perfusion and/or Ang II intracerebroventricular injection on Akt thr 308 phosphorylation and ERK 1/2 phosphorylation in C and F rats. Meanwhile Akt thr 308 phosphorylation significantly increased in C and F rats that received insulin infusion, followed or not by Ang II intracerebroventricular injection compared to vehicle, no significantly changes were observed with Ang II injection in vehicle-pretreated C and F animals (Fig. 2A and B).

In C rats, Ang II injection increased in more than two folds of the phospho-ERK 1/2 hypothalamic levels compared to vehicle. Similarly, insulin infusion increased phospho-ERK 1-2 hypothalamic levels in more than two folds. As previously reported [13], insulin infusion followed by Ang II injection induced an increase in phospho-ERK 1/2 hypothalamic levels more than 4 times higher values than those who only received vehicle, and nearly twice than those who received Ang II without insulin pre-treatment (Fig. 3A).

In vehicle infused F rats, phospho-ERK 1/2 hypothalamic levels were two fold higher than in vehicle infused C rats. Phospho-ERK 1/2 levels after Ang II (5pmol) injection in F animals were two fold higher than those in vehicle infused F rats and four fold higher than those in the C group. Conversely, insulin infusion did not significantly increase phospho-ERK 1/2 levels compared to vehicle in F rats, meanwhile F animals treated with insulin and Ang II showed phospho-ERK 1/2 levels 2.5 fold higher compared to vehicle in F and nearly four fold higher than in vehicle treated C rats (Fig. 3B).

3.3. Hypothalamic AT1 and AT2 receptor levels in C and F animals

No significant differences were observed between C and F rats in hypothalamic AT1 (C: 1329 ± 113.5 vs. F: 1312 ± 160.6; NS) or AT2 (C: 3.04 ± 0.4 vs. F: 4.6 ± 0.6; NS) receptor levels in our experimental conditions.

3.4. Acute MAPK inhibition effects on mean arterial pressure in C and F rats

Meanwhile, as expected, vehicle injection did not modify MAP in C and F rats (data not shown), PD98059 injection excerpted a mild but significant reduction in MAP in F rats compared to C (ΔMAP: C: −3.0 ± 4.4 mmHg; n = 10 vs F: −8.8 ± 3.9 mmHg; n = 5; $p < 0.01$). No significant changes were observed in HR in any of the studied groups (data not shown).

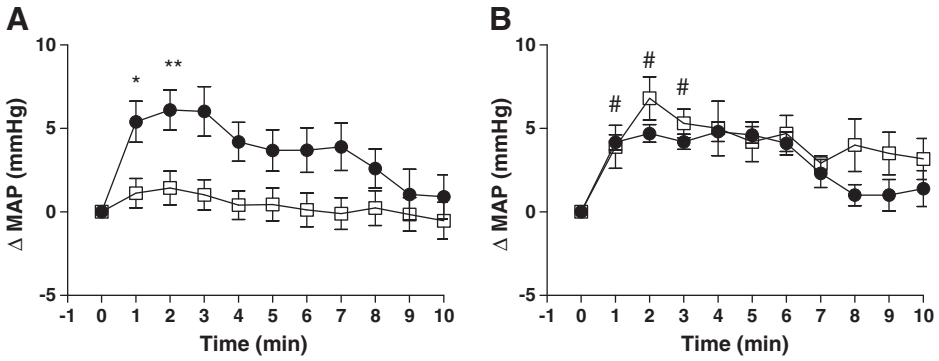

Fig. 1. Time course of effects of ICV angiotensin II injection on mean arterial pressure (ΔMAP in mmHg) in vehicle-pretreated rats (open boxes) and insulin-pretreated rats (closed circles) in C (Panel A) and F (Panel B) animals. Each point shows the mean ± SEM of ten animals. *p < 0.05 vs. the corresponding vehicle, #p < 0.05 vs vehicle in C.

3.5. Acute intracerebroventricular insulin infusion followed by angiotensin II injection in C and F animals after MAPK inhibition

There were no differences in MAP responses after 2 h of insulin infusion between PD98059 and vehicle-pretreated C (ΔMAP: PD98059: 5.3 ± 2.9 mmHg; n = 5 vs vehicle: 4.8 ± 3.4 mmHg; n = 5; NS) or F (ΔMAP: PD98059: 7.8 ± 3.5 mmHg; n = 5 vs vehicle: 5.7 ± 2.9 mmHg; n = 5; NS) rats. Similarly, no differences were observed in MAP responses after 2 h of vehicle infusion between PD98059 and vehicle-pretreated F rats (data not shown).

As previously reported [13], intracerebroventricular preadministration of the MAPK inhibitor PD98059 significantly abolished pressor response to Ang II (5 pmol) in insulin pre-treated C rats (ΔMAP: PD98059: 0.8 ± 0.5 mmHg; n = 5 vs vehicle: 6.1 ± 1.9 mmHg; n = 5; p < 0.05). Similarly, PD98059 significantly abolished pressor response to Ang II (5 pmol) in insulin (ΔMAP: PD98059: 0.01 ± 0.6 mmHg; n = 5 vs vehicle: 4.9 ± 0.6 mmHg; n = 5; p < 0.05) or vehicle pre-treated (ΔMAP: PD98059: 0.5 ± 0.5 mmHg; n = 5 vs vehicle: 6.7 ± 1.9 mmHg; n = 5; p < 0.05) F animals. No changes were found in HR after Ang II injections in any of the studied groups (data not shown).

4. Discussion

The present study adds new evidence regarding insulin–angiotensin crosstalk on the central regulation of blood pressure. Firstly, confirming previous findings from our laboratory, intracerebroventricular insulin pre-treatment increased the pressor response to Ang II in C rats. Secondly, in F rats (with or without insulin-pretreatment), the pressor response to Ang II was higher than that in vehicle pre-treated C animals, but similar to that observed in C after insulin infusion. Thirdly, as previously reported, in C rats phospho-ERK 1/2 hypothalamic levels significantly increased after Ang II injection in insulin pre-treated animals compared to vehicle pre-treated rats, suggesting that MAPK activation might be involved in insulin potentiation of blood pressure response to Ang II in the brain. Fourthly, phospho-ERK 1/2 hypothalamic levels were significantly increased in vehicle treated F rats compared to those in C, suggesting that basal MAPK activation might play a role in the enhanced response to Ang II observed in these animals. Fifthly, in F rats, either after vehicle or insulin infusion, Ang II injection was associated with a similar increase in phospho-ERK 1/2 hypothalamic levels, comparable to that observed after Ang II injection in insulin pre-treated C animals. Finally, ERK 1/2 blockade significantly reduced MAP in F rats compared to C, suggesting that central MAPK activity contributes to blood pressure maintenance in insulin resistant animals. Moreover, ERK 1/2 inhibition completely abolished the Ang II pressor response in F rats and in insulin pre-treated C animals, supporting the notion that central MAPK pathway activation is involved in the pressor response to Ang II in the brain. All these findings suggest that insulin–Ang II interaction at hypothalamic level might be involved in the increase in blood pressure observed in the insulin resistant state.

Fructose overloaded rats have been widely used for the study of the pathophysiology of hypertension associated with insulin resistance [21–23]. In this model, we and other authors have reported changes in the renin angiotensin system, evidenced by increases in plasma levels of angiotensin II [5,7] and in the expression of AT1 receptor mRNA in adipose tissue [7], increases in AT1 receptor density in cardiac left ventricle [22] and in locus coeruleus [24], and an enhancement in the pressor response to angiotensin II at different levels [25], including the central nervous system [14].

A fructose-enriched diet induced metabolic changes characterized by increased basal plasma triglyceride, glucose and insulin concentrations and elevated systolic blood pressure without changes in resting HR and body weight. These results are in agreement with metabolic and hemodynamic profiles previously reported in this model [5,26].

As used in a previous study [13], we selected an insulin dose of 12 mU/h, taking into account that this rate of intracerebroventricular insulin infusion raised sympathetic activity and produced central nervous system levels of insulin at least as high as those found under pathophysiological conditions or in severe obesity, but without increasing plasma levels of insulin or modifying plasma glucose levels [8,27] nor modifying blood pressure or HR [27]. In accordance with these previous studies, central insulin infusion did not modify blood pressure or HR in any of the studied groups. Consequently, the absence of a direct acute effect of centrally administered insulin on blood pressure, even in the insulin resistant state, leads us to evaluate the role of the interaction of this hormone with other systems, such as the renin angiotensin system, as a possible link between hyperinsulinemia and hypertension.

It has been reported that the activation of the PI3K-akt/protein kinase B and Ras/Raf MAPK intracellular pathways is involved in the stimulation of the sympathetic nervous system by insulin at the central level [8,28]. Taking into account that part of the central effects of Ang II regarding sympathetic nervous system stimulation and blood pressure control is also mediated by the activation of MAPK [12], and, in some pathological conditions, PI3K pathways [29–31], in a previous study [13] we explored if central insulin–Ang II interaction might potentiate the pressor effects of Ang II at brain level. Briefly, we evidenced that insulin administration at brain level potentiated the pressor response to intracerebroventricularly administered Ang II and significantly increased hypothalamic phospho-ERK 1/2 levels in response to this hormone. Interestingly, ERK 1/2 inhibition by means of PD98059 (a p44/42 MAPK inhibitor) abolished the blood pressure response induced by Ang II in this experimental conditions. These findings lead us to investigate if insulin is able to modulate the pressor response to intracerebroventricularly administered Ang II in insulin resistant F rats and to further explore the role of the hypothalamic renin angiotensin system in the development of hypertension in the metabolic syndrome.

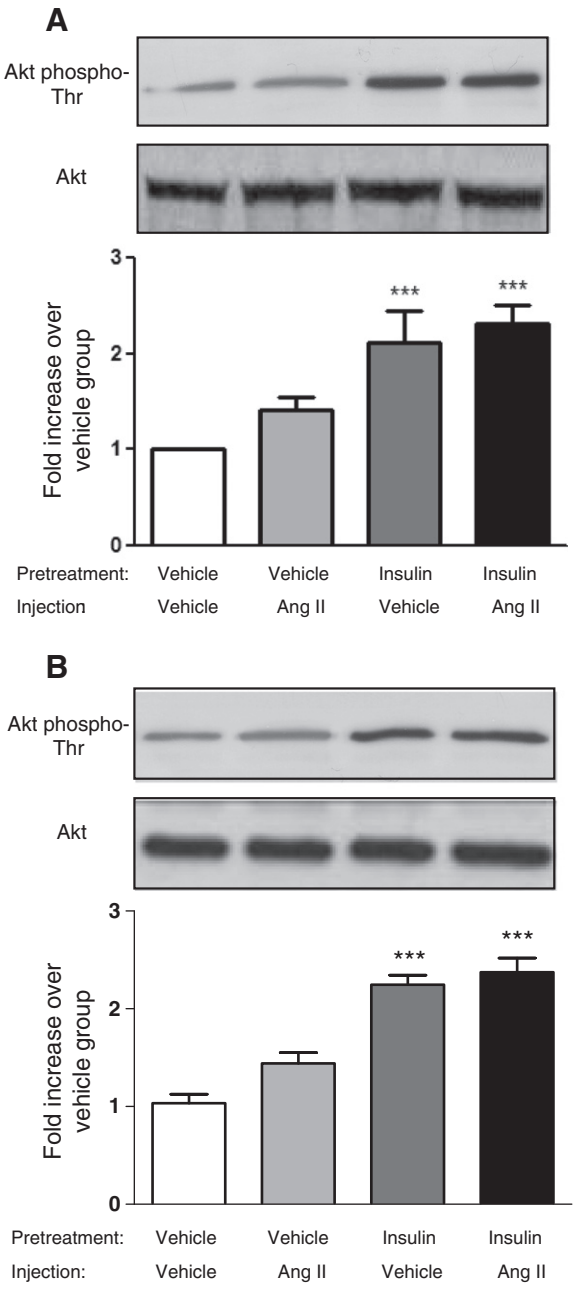

Fig. 2. Effects of angiotensin II and insulin on Akt Thr308 phosphorylation in control (Panel A) and fructose treated (Panel B) rats. To determine protein abundance, membranes were reprobed with anti-Akt antibodies. Data are expressed as mean ± SEM of five animals. ***p < 0.001 vs. the corresponding vehicle.

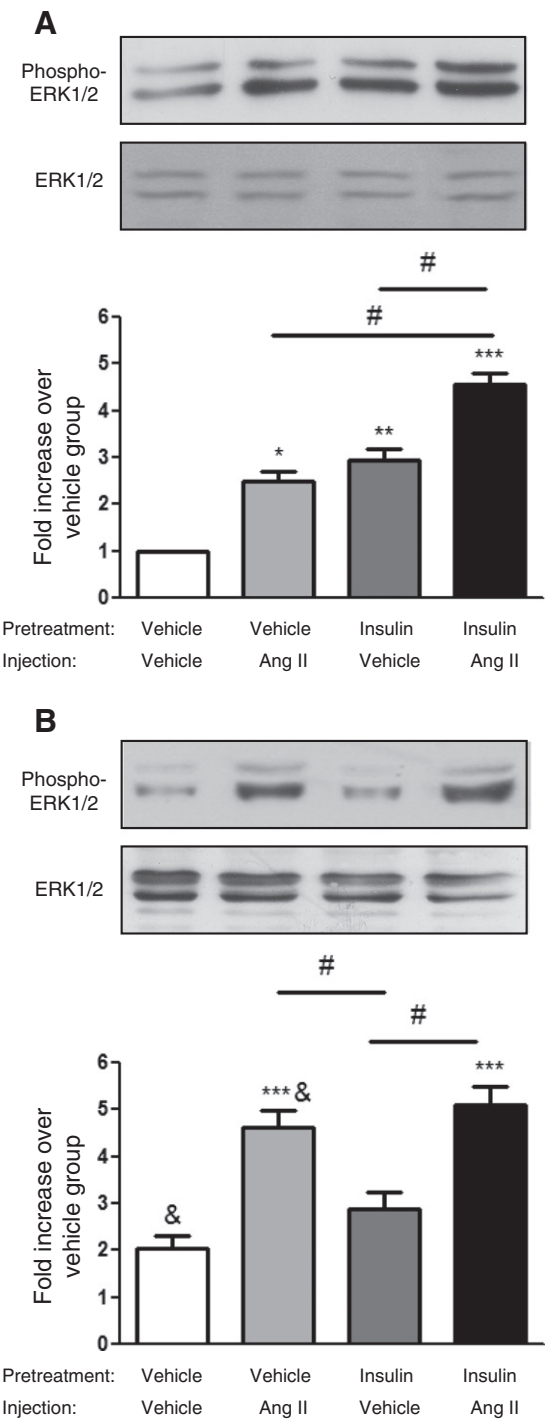

Fig. 3. Effects of angiotensin II and insulin on ERK 1/2 phosphorylation in control (Panel A) and fructose treated (Panel B) animals. To determine protein abundance, membranes were reprobed with anti-ERK 1/2 antibodies. Data are expressed as mean ± SEM of five animals. *p < 0.05 vs. the corresponding vehicle; **p < 0.01 vs. the corresponding vehicle; ***p < 0.001 vs. the corresponding vehicle; #p < 0.05 vs other group; &p < 0.01 vs the same group in C.

Confirming our previous findings, we observed that meanwhile Ang II 5 pmol did not excerpt any appreciable effect on MAP in vehicle-pretreated rats, the same dose significantly increased MAP in insulin-pretreated C rats. On the other hand, even in vehicle pretreated or in insulin pretreated F rats, Ang II 5 pmol was associated with a significant increase in MAP in these insulin resistant animals. Taking into account that, contrary to that observed in C rats, insulin pre-treatment did not modify the blood pressure response to Ang II in F, it is possible to postulate that this response might be already potentiated in F animals, as a consequence of the chronic hyperinsulinemic state present in this animal model. Moreover, the existence of higher hypothalamic basal phospho-ERK 1/2 levels in F rats further supports the notion that this pathway might be tonically activated in fructose overloaded rats.

Interestingly, this enhanced pressor response to centrally administered Ang II in F rats is in accordance with a previous study from our laboratory [14]. Briefly, we found an increase in the pressor response to Ang II injected into the anterior hypothalamic area of F rats, as well as a higher AT1 receptor's tone at this level in insulin resistant rats compared to control animals, suggesting that the overactivation of hypothalamic angiotensinergic pathways may be involved in the maintenance of high blood pressure values in F rats.

As previously reported [13], Akt thr 308 phosphorylation (an index of PI3K pathway activation) increased in a similar manner in C rats that received insulin infusion (followed or not by Ang II intracerebroventricular injection) compared to vehicle. Similar results were observed in F animals, suggesting that this pathway response to insulin treatment at hypothalamic level is not altered in the insulin resistant state.

It is important to point out that other authors report changes in hypothalamic PI3K pathway activation in response to insulin in other animal models of insulin resistance, such as the fat fed rat [32] or obese Zucker rats [33]. However, as far as we know, this is the first study that evaluated the effects of the central administration of insulin in fructose overloaded rats. Consequently, we cannot rule out the possibility that the absence of differences in Akt thr 308 phosphorylation levels between F and C rats observed in the present study is a consequence of our experimental conditions. Furthermore, to further explore this issue, it would be useful to repeat the present study in other experimental conditions (such as fructose overloaded rats treated for a longer period of time) and to evaluate the effects of PI3K pathway blockade on blood pressure response to Ang II in F animals. However, as in the present study we decided to focalize on MAPK activation role in blood pressure control in insulin resistant rats, we decided to leave this line of research for future studies.

Confirming previous findings, hypothalamic phospho-ERK 1/2 levels significantly increased after insulin infusion or Ang II injection compared to vehicle. Moreover, these levels were significantly increased in insulin pre-treated C rats after Ang II administration compared to those that only received insulin or Ang II alone, suggesting that the activation of the same intracellular pathway by insulin and Ang II might be responsible for the higher hypothalamic phospho-ERK 1/2 levels observed after the combined treatment. On the other hand, in F animals (that, as mentioned above, presented basal phospho-ERK 1/2 levels significantly higher than C rats), insulin infusion alone did not modify phospho-ERK 1/2 levels compared to vehicle. Conversely, Ang II injection, either after vehicle or insulin infusion, significantly increased phospho-ERK 1/2 levels in F rats. Interestingly, phospho-ERK 1/2 levels after Ang II injection in F rats were significantly higher than those observed in C animals after receiving the same treatment, but similar to those found in C rats after receiving insulin and Ang II combined treatment.

As previously mentioned, ERK1/2 activation is involved in many central actions of Ang II. For instance, Wei et al. have demonstrated that this intracellular pathway plays a key role in mediating the effects of brain renin angiotensin system on sympathetic nerve activity and control of blood pressure [12]. However, although this evidence is in accordance with other studies that confirm a stimulatory effect of Ang II on intrahypothalamic MAPK activation [34,35], Cheng et al. have reported that Ang II can also inhibit ERK 1/2 phosphorylation at other central levels, such as the nucleus tractus solitarii [36]. Nevertheless, in the present study, Ang II induced a significant increase in phospho-ERK 1/2 hypothalamic level in both in C and F rats.

To further clarify the differences observed in blood pressure response to intracerebroventricularly administered Ang II between F and C rats, we decided to evaluate AT1 and AT2 receptor densities at hypothalamic level. In accordance with Iyer et al. [37], hypothalamic AT1 receptor density showed similar levels between experimental groups. Similarly, no differences were observed in AT2 receptors, suggesting that the mechanisms involved in the enhanced response to Ang II in F rats occur at post-receptor level, supporting the hypothesis that MAPK activation at hypothalamic level plays a role in the maintenance of high blood pressure values observed in insulin resistant rats.

In accordance with Wei et al. [12], in the present study central ERK 1/2 blockade by means of PD98059 administration had no acute effects on MAP in C Sprague Dawley rats. Conversely, PD98059 injection significantly reduced MAP in F rats, supporting the notion that MAPK activity has a role in the maintenance of high blood pressure levels in the insulin resistant state. Furthermore, PD98059 completely abolished the blood pressure response to Ang II in insulin pretreated C rats (as previously reported), as well as in F overloaded rats, confirming that this intracellular pathway plays a key role in the central pressor effects of Ang II in these experimental conditions.

In conclusion, meanwhile centrally administered insulin seems to potentiate the pressor effects of Ang II at hypothalamic level by means of MAPK pathway activation in C animals, insulin pre-treatment does not modify blood pressure response or ERK 1/2 phosphorylation after Ang II injection in F animals. Taking into account that blood pressure response to Ang II as well as basal ERK 1/2 phosphorylation levels are already enhanced in insulin resistant rats, we hypothesize that the chronic hyperinsulinemia present in F rats might be already sensitizing to Ang II at hypothalamic level in this experimental model.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.regpep.2013.06.001.

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