

## **APELIN INVOLVEMENT ON ANGIOTENSIN II – INDUCED VENOCONSTRICTION IN OBESE RATS**

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### **ABSTRACT**

*Apelin (AP), the endogenous ligand of the recently de-orphanized APJ receptor is a relatively newly identified adipokine. Despite this high level of expression of both AP and APJ in the vascular wall, the vasomotor effects of AP are still under investigation. This study was undertaken to evaluate comparatively the in vitro effects of apelin on angiotensin II (Ang II) – induced constriction of various veins from normal vs. obese rats. Rings of the inferior vena cava (IVC), portal vein (PtV), femoral vein (FV), and pulmonary vein (PV) from male Sprague Dawley rats (control rats), obese prone (OP) rats and obese resistant (OR) rats were mounted between wires and isometric contraction induced by Ang II (1μM) was measured. These results showed that reactivity for Ang II was increased in IVC and FV in OR. The AP13 treatment decreased the Ang II- induced contractions by more than 30% on IVC and PtV from OR. On OP, the F13A treatment increased the contraction induced by Ang II on all studied veins. These results suggested that AP could play protective roles by antagonizing Ang II – induced venoconstriction.*

**KEYWORDS:** *apelin, angiotensin II, veins, obesity, rats*

### **1. Introduction**

The last decades has brought many data about white adipose tissue capacity to secrete hormones, named adipokines, which could be involved on the regulation of vascular reactivity and the modulation of local inflammatory responses (1). Taking into account the published studies about the obesity roles in the development of pulmonary diseases we studied the apelin implications on Ang II contractile effects on pulmonary veins from male Sprague Dawley rats

(control rats), obese prone (OP) rats and obese resistant (OR) rats.

We have recently shown that apelin (AP), the endogenous ligand of the recently de-orphanized APJ receptor and a newly identified adipokine, could have vasodilatory and NO-dependent effects (2). Even more, apelin modulated vasoconstrictor tone in rat pulmonary vessels (3) and reduced the lipopolysaccharide-increased pulmonary permeability in rats (4). This study aims to investigate the role of apelin/APJ receptors system in agonist-mediated

pulmonary venoconstriction in normal and obese rats, in order to accomplish a greater understanding of the involvement of adipokine in the regulation of pulmonary vascular tone.

## 2. Materials and methods

The experiments were conducted in Sprague Dawley rats (control rats), the OP-CD (Obese Prone) and the OR-CD (Obese Resistant) that were obtained from Charles River Laboratories (Wilmington, MA, USA). The animals were kept under conventional laboratory conditions of temperature, humidity and light, and allowed free access to water and standard rat chow (for control rats) or a high fat diet (D12266B, 32.5% fat, Research Diets, NJ, USA). Rats were given daily intraperitoneal injections of AP13 or F13A (100 nmol/kg) for 2 weeks (5). After finishing the protocol, the inferior vena cava (IVC), portal vein (PtV), femoral vein (FV), and pulmonary vein (PV) veins were quickly removed, cleaned and cut into 1-2 mm wide rings as previous described (6,7). Individual rings were then mounted in a MYO-01 MYOGRAPH SYSTEM (Experimetria LTD., Budapest, Hungary) and changes in vessel tension were recorded and analyzed by ISOSYS data acquisition system (Experimetria LTD., Budapest, Hungary). The tissue organ bath contained the Krebs-Henseleit solution containing (mM): NaCl 118, KCl 4.8, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.6, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, glucose 5.5. The Krebs-Henseleit buffer was maintained at 37°C, and bubbled continuously with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (pH=7.2–7.4). A resting tension of 0.5g for JV, FV and PtV, and 0.2g for PV was applied to each ring and then allowed to equilibrate for 45-60 minutes before starting the experiment. The bathing medium was renewed every 15 minutes. After the equilibration period, vessel rings were initially stimulated twice with 40 mM KCl as a standard stimulus. The functional integrity of the

endothelium was assessed by testing the degree of relaxation produced by adding 10 μM acetylcholine (ACh) to KCl pre-contracted rings. The rings that produced less than 70 % relaxation in response to ACh were discarded. After re-equilibration, the rings were stimulated with the Ang II (1 μM) and the peak contractile responses were measured. Results are expressed as percentage of control contraction induced by 40 mM KCl (mean ± S.E.M, n=6). The statistical significance was tested using one-way analysis of variance (ANOVA), completed by the Bonferroni method (SigmaStat software, Jandel Corporation). p<0.05 was considered statistically significant.

Ang II (rat), KCl, ACh, were all obtained from Sigma-Aldrich Inc. (Germany). Apelin and the antagonist Apelin-13 (Ala13, F13A) were purchased from Phoenix Europe GmbH (Germany). All the other compounds used were of analytical grade.

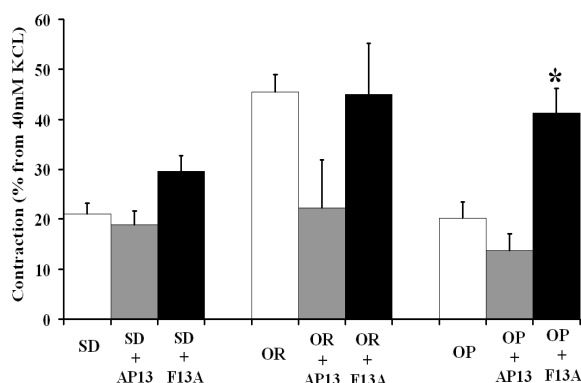
## 3. Results

The F13A treatment amplified the Ang II – induced contractions on IVC from control rats (88.78±5.71 vs. 62.97±7.91) and OP rats (96.18±7.16 vs. 68.72±6.02). On OR rats Ang II contractile effects were higher (90.84±7.25 vs. 62.97±7.91) as compared with control rats. IVC from AP13 treated OR rats had a lower contractile response to Ang II as compared with untreated OR rats (48.51±10.34 vs. 90.84±7.25).

Both the AP13 treatment and F13A treatment significantly decreased (by an average of 30%) and increased (by an average of 20%), respectively, the Ang II – induced contractions on portal vein from SD, OR and OP rats.

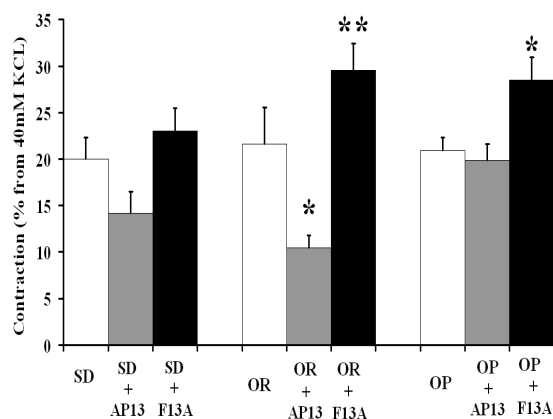
On FV (fig. 1), there are significant differences between Ang II – induced contractions on OR vs. SD rats (45.42±3.61 vs. 21.11±2.18) and on OP vs. OR rats (20.28±3.14 vs. 45.42±3.61). The

F13A treatment increased the Ang II effects on OP rats ( $41.21 \pm 5.11$  vs.  $20.28 \pm 3.14$ ).



**Figure 1.** Contractions induced by Ang II on FV from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). \*:  $p < 0.05$  as compared with SD from the same protocol.

Neither AP13 nor F13A modified the Ang II – induced contractions on PV (fig. 2) of SD rats. On the contrary, on OR rats the AP13 treatment and F13A treatment significantly decreased (by more than 50%) and increased (by than 30%), respectively.



**Figure 2.** Contractions induced by Ang II on PV from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). \*:  $p < 0.05$  and \*\*:  $p < 0.01$  as compared with SD from the same protocol.

On OP rats only the F13A treatment significantly modified the Ang II – induced

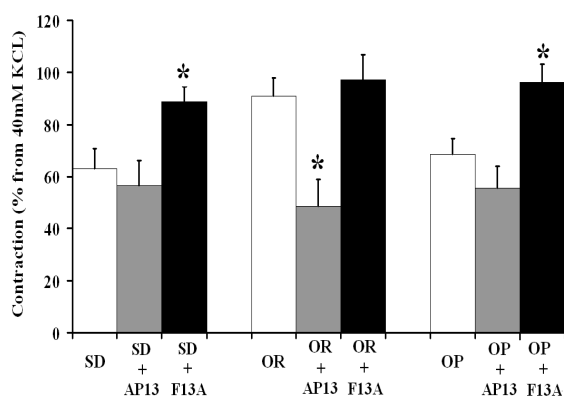
contractions ( $28.55 \pm 2.41$  vs.  $20.90 \pm 1.38$ ). On the other hand, even if there was no difference between Ang II – induced contractions on OP and OR rats, after the AP13 treatment, the responses of PV to Ang II was significantly lower on OR rats as compared with OP rats ( $10.50 \pm 1.24$  vs.  $19.82 \pm 1.79$ ).

#### 4. Discussion

Apelin is the endogenous ligand of recently de-orphanised APJ receptors. In rat tissues, the APJ receptor and apelin mRNAs were found in the lung, heart, skeletal muscle, kidney, brain and liver (8). Even more, *in situ* hybridisation histochemistry studies revealed intense APJ receptor gene expression in the parenchyma of the lung (9). In rats, the highest expression of APJ mRNA was detected in the lung, suggesting that APJ and its ligand play an important role in the pulmonary system (10). Until now there are no references about functional importance of AP – APJ pulmonary system. Considering our data about NO-dependent inhibitory effects of AP13 on Ang II-induced venoconstriction, we previously showed that AP13 could stimulate NO synthesis in both airways and pulmonary vessels (11). More recently, apelin has been described as an adipocyte-secreted factor (adipokine), markedly up-regulated in obesity. By acting as circulating hormone or paracrine factor, adipokines are involved in physiological regulations (fat depot development, energy storage, metabolism or eating behavior) or in the promotion of obesity-associated disorders as vascular dysfunctions (12).

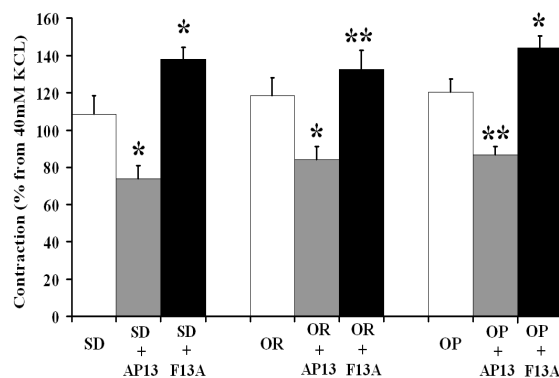
Taking into account (I) the increased of AP level in obesity (12) (II) the functional importance of tissue synthesized AP (3) and (III) the impact of veins dysfunction on vascular beds circulation (13,14) we comparatively assessed the isolated veins reactivity to Ang II on control rats, OR rats and OP rats untreated or treated with either AP13 or F13A (the blocker of APJ receptors).

The Ang II – induced contractions were significantly increased on OP and OR rats, as compared with SD rats, only for FV (fig. 3). Either APJ receptors stimulating or APJ receptors blocking prevent differences between OP/OR rats and SD rats, emphasizing importance of AP/APJ receptors system in modulation of obesity – induced modulation of FV reactivity. Administration of AP13 decreased Ang II induced contraction of FV and PV (on OR rats) and PtV (on all experimental models). Blocking of APJ receptors by ip administration of F13A increased the venous reactivity for all studied veins but only on OP rats. On control (SD) rats the F13A effects were significantly only for IVC (fig. 3) and PtV (fig. 4).



**Figure 1.** Contractions induced by Ang II on IVC from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). \*:  $p < 0.05$  as compared with SD from the same protocol.

On OR rats the F13A effects were significantly only for PtV (fig. 4) and PV (fig. 2). These data suggested that on OP rats were the AP levels are increased (12), the AP/APJ receptors system could have a vasorelaxant role. For PtV, the AP13 or F13A effects are significantly on all studied experimental models sustaining the importance of the AP/APJ receptors system in regulation of portal circulation.



**Figure 4.** Contractions induced by Ang II on PtV from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). \*:  $p < 0.05$  and \*\*:  $p < 0.01$  as compared with SD from the same protocol.

Taking into account the well known contribution of obesity in the progression of various venous diseases (15, 16) our data could suggest the involvement of apelin/APJ receptor system in dysregulation of venous tone and reactivity as a new component of obesity related induced vascular diseases.

## 5. Conclusions

These results suggested that AP/APJ system could play protective roles by antagonizing Ang II – induced venoconstriction mainly on obese rats. The functional importance of AP/APJ system on venous tone regulation is determined by vascular beds studied.

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