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Synergistic effects of apelin and leptin on isolated rat pulmonary arteries

Research Article

Irina L. Dumitriu[1*], Luminita G. Vata[1], Florin E. Zugun[1,2], Bogdan Gurzu[1]

[1] Department of Functional Sciences, Faculty of Medicine, "Gr T Popa" University of Medicine and Pharmacy, Iasi, 700115, Romania

[2] Immunopathology and Genetics Laboratory, "Sf. Spiridon" Hospital, 700111, Iasi, Romania

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Abstract: Apelin (AP) and leptin (LEP) are adipokines with vasomotor actions. Taking into account the published data on the role of obesity in the development of pulmonary hypertension, we studied the implications of apelin on leptin relaxing effects on isolated rat pulmonary arteries. LEP had vasodilatatory effects on phenylephrine-precontracted rat pulmonary arteries from normal and ovalbumin-sensitized rats, but not on rats with monocrotaline-induced pulmonary hypertension. AP13 pretreatment increased LEP effects by one-half. Our studies revealed the existence of synergistic favorable effects of these adipokines on pulmonary vessels.

Keywords: *Licorice • apelin, leptin • ovalbumin • pulmonary artery • rat*

1. Introduction

Leptin (LEP) is a well-known adipokine involved in regulation of energy homeostasis; it has a broad range of actions on cardiovascular, reproductive, neuroendocrine, immune and metabolic functions [1-4]. Previously published studies have linked LEP with inflammation and endothelial dysfunction, supporting its involvement in the pathogenesis of vascular damage either as an obesity-related or obesity-independent factor [5,6]. On the other hand, recent data published by Biasucci and colleagues [7] demonstrate that vascular function is better in severely obese (with high LEP levels) than in obese subjects and is similar to that found in normal-weight subjects. *In vivo* studies have revealed the involvement of LEP in the control of vascular tone by simultaneously producing a sympathetically mediated vasoconstriction and an endothelium-mediated vasodilation [8,9]. Depending on the experimental design or the variety of LEP used in the experiments, literature data showed that *in vivo* administration of LEP could increase [10], have no effect on [11], or decrease [12,13] vascular tone.

Taking into account all the data that came from previous investigations of LEP as a vasomotor factor involved in pathogenic mechanisms of vascular diseases, we studied the effects of LEP on vasodilation of pulmonary arteries. We compared its effects in normal rats and in two experimental model rats that combine the inflammation and endothelial dysfunction of pulmonary vessels: the ovalbumin (OVA)-induced allergic airway disease and monocrotaline (MCT)-induced pulmonary hypertension. Increased pulmonary arterial vascular tone is an important early component of pulmonary vascular disease [14]. It has been demonstrated that a specific antigen challenge causes an increase in responsiveness to contractile agonists [14] and a decrease of endothelium-dependent relaxant responses to acetylcholine [15,16]. Published data confirm that damage to the endothelium may play an important role in the development of pulmonary vascular diseases in both humans and experimental models [17,18]. MCT-induced pulmonary hypertension is a well-known experimental model of pulmonary vascular disease that has an important inflammatory component [19]. MCT causes endothelial injury and subsequent endothelial dysfunction of pulmonary arteries [20].

* E-mail: dumitriu_irinaluciana@yahoo.com

Impaired endothelium-dependent relaxation is caused by endothelial dysfunction and/or reduced vasodilator function in vascular smooth muscle. Abe and colleagues have demonstrated that both mechanisms are involved in the impaired endothelium-dependent relaxation in MCT-induced pulmonary hypertension [21].

Further, we investigated the modulation of LEP effects by another adipokine, apelin (AP13), was. Previous studies showed that AP13 could induce both endothelium-dependent and NO-mediated transient relaxations of pulmonary artery rings, and that it attenuated the response to vasoconstrictors [22,23].

2. Material and Methods

The experiments were conducted in age-matched male Wistar rats (INCDMI "Cantacuzino", Bucharest, Romania) with body weights of 200–250 g housed under standard laboratory conditions, with free access to standard rodent chow and tap water. Three groups of animals were used: (*i*) untreated control rats (NR, n=18), (*ii*) OVA-sensitized rats (OSR, n=18), (*iii*) rats with monocrotaline-induced pulmonary hypertension (PHR, n=18). This study was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy in Iasi; it was performed according to the Helsinki convention for the use and care of animals and the European Communities Council Directive 609 of 24 November 1986.

Sensitization: The rats were sensitized against OVA by subcutaneous and intraperitoneal injection of 0.2 mL physiological saline containing 100 mg OVA and 8 mg aluminum hydroxide. The protocol was repeated 2 weeks later [24]. *In vitro challenge*: After 7 days, before starting the administration of the studied substances, vascular rings were pre-treated with OVA (100 mg/mL).

Monocrotaline (MCT)-induced pulmonary hypertension.

To induce pulmonary hypertension, rats from the PHR group received a subcutaneous injection of MCT (60 mg/kg body wt), as described previously [25].

Wire myography. Experiments were conducted on paired rings obtained from the first branches of rat pulmonary arteries (RPA) as described previously [26,27]. The RPA were removed quickly, cleaned, and cut into two rings of approximately the same width using a stereomicroscope (PZMIII, World Precision Instruments). Rings were then mounted between tungsten wires in a MYO-01 MYOGRAPH SYSTEM (Experimetria LTD., Budapest, Hungary) and changes in vessel tension were recorded and analyzed using the ISOSYS data acquisition system (Experimetria LTD., Budapest, Hungary). The tissue organ bath contained the Krebs-Henseleit solution in these concentrations (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.6; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 5.5. The Krebs-Henseleit buffer was maintained at 37°C, and bubbled continuously with a mixture of 95% O_2 and 5% CO_2 (pH=7.2–7.4). A resting tension of 0.3 g for RPA was applied to each ring and then allowed to equilibrate for 90 minutes before initiating the experimental procedure. The experiments were started simultaneously and had the same timing for both rings of the pair.

The bathing medium was renewed every 15 minutes. After the equilibration period, vessel rings were stimulated twice with 40 mM KCl. Endothelium presence was certified by existence of more then 60% relaxation of rings precontracted with KCl in response to 1 μM acetylcholine (ACh) for NR. The pairs of rings with more than 10% difference between the ACh-induced relaxations were discarded. After re-equilibration (45 min), both rings of a pair simultaneously received 1 μM phenylephrine (Phe). The Phe reached the plateau in 6–7 min. Once the plateau of the Phe-induced contractions was reached, one ring from each pair was incubated for 3 min with 10 nM AP13. After that, the dose-response curves of LEP-induced relaxation (0.01–100 ng/mL, one dose every 90 sec) [28] were constructed. To assess NO involvement, we administered 0.1 mM Nω-nitro-L-arginine-methyl ester (L-NAME) 15 min before Phe, as described previously [29].

Confirmation of OVA sensitization and pulmonary hypertension. To confirm the OVA sensitization effect on vessels, the Schultz-Dale reaction was assessed as described previously [30]. After KCl contractions and before Phe, we tested the contractile effects of OVA (10 μg/mL) on RPA rings from OSR. Experiments were continued only on rings capable of contraction to OVA by at least 90% from control contractions induced by KCl.

The RPA rings from MCT-treated rats had a very low relaxation to ACh (less than 10% of Phe-induced precontraction). After removal of RPA, the lungs were embedded in paraffin. Lung parenchyma sections were stained with haematoxylin-eosin. Pulmonary vascular remodelling was examined as described previously [31,32] in 10 (per animal) muscularized pulmonary arteries (with an external diameter of 100–150 μm). Random circular vessel profiles were selected; the external and the internal diameter were measured and reported as wall thickness (%) = (external diameter – internal diameter)/external diameter x 100. Only MCT treated rats with a calculated wall thickness higher than 60% were considered as pulmonary hypertensive rats (Figure 1).

Figure 1. Muscularized pulmonary arteries (with an external diameter less than 150 μm) from (A) NR (B) OSR and (C) PHR. Scale bars represent 100 μM. (D): The wall thickness (%) was calculated as described in the text (mean±SEM; n=60).

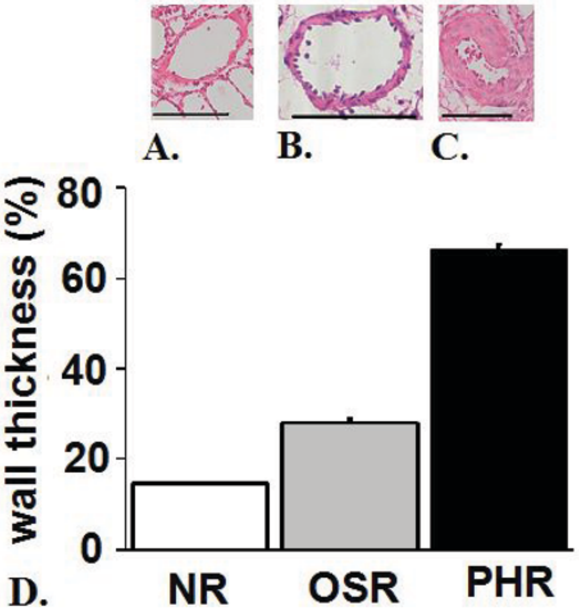
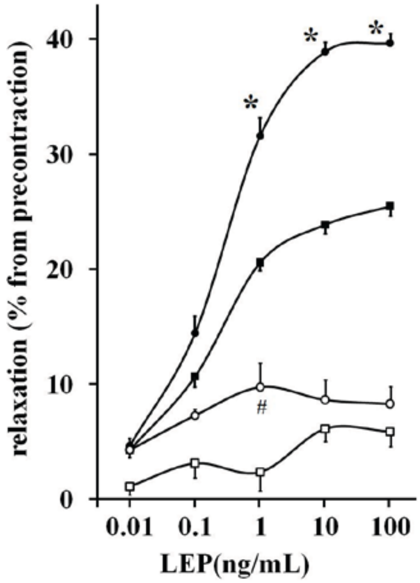


Figure 2. Vascular response of pulmonary artery rings from normal rats precontracted with phenylephrine to increasing doses of leptin (LEP), either alone (filled squares) or after preincubation with the apelin (filled circles). The vehicle effects alone or in the presence of AP13 are presented as empty squares and circles. *P<0.01 in the presence of AP13 as compared with LEP effects alone (n=6). #P<0.05 in the presence of AP13 as compared with vehicle alone (n=6). The LEP or vehicle effects are expressed as a percentage of Phe-induced precontraction (mean ± S.E.M.)



Expression of results and statistical analysis: The LEP effects were measured as a percentage of Phe-induced precontraction (mean ± S.E.M.). The statistical significance was tested for each concentration using one-way analysis of variance (ANOVA), and completed using the Bonferroni method (SigmaStat software, Jandel Corporation). P<0.05 was considered statistically significant.

KCl, Phe, OVA, aluminum hydroxide, L-NAME and MCT were all obtained from Sigma (Sigma-Aldrich Inc., St. Louis MO). AP13 was purchased from Phoenix Pharmaceuticals Inc. Leptin was purchased from Biomol GmbH (Germany). All other compounds used were of analytical grade.

3. Results

On NR (Figure 2), the 1–100 ng/mL LEP-induced relaxation of RPA were significantly higher in the presence than in the absence of 0.1 nM AP13 (31.55±1.58 *vs* 20.56±0.70, P<0.01; 38.87±0.82 *vs.* 23.85±0.80, P<0.01; 39.63±0.83 *vs.* 25.48±0.85, P<0.01).

The 0.1 mM L-NAME pretreatment (Figure 3) prevented LEP-induced vasorelaxation; in addition, in these conditions LEP had a slightly vasoconstrictor effect (Emax: 10.33±0.41% of Phe precontraction). Presence of AP13 blocked this vasoconstrictor effect of LEP, but did not re-establish the vasorelaxation.

The amplification effects of AP13 on LEP-induced vasorelaxation of RPA were preserved on OSR (Figure 4). Pretreatment with 10 nM AP13 significantly increased the effects of 1–100 ng/mL LEP on OSR (18.16±0.67 vs. 11.84±0.75, P<0.01; 30.59±1.61 vs. 18.49±1.12, P<0.01; 30.71±1.25 vs. 21.70±1.29, P<0.05).

In contrast, the LEP vasodilator effects were smaller on OSR (Figure 4) as compared with those of NR (Figure 2), either in the absence (3.49±0.30 *vs.* 10.64±0.94, 11.84±0.75 *vs.* 20.56±0.70, 18.49±1.12 *vs.* 23.85±0.80 for 0.1–10 ng/mL LEP) or in the presence of 10 nM AP13 (5.11±0.15 vs. 14.41±1.48, 18.16±0.67 vs. 31.55±1.58, 30.59±1.61 vs. 38.87±0.82, 30.71±1.25 vs. 39.63±0.83 for 0.1–100 ng/mL LEP).

There were no observed vasodilatory effects of LEP on PHR; further, the 10 and 100 ng/mL LEP induced only slight vasoconstrictor effects (Figure 5).

4. Discussion

Our data showed that LEP has vasodilatory effects on Phe-precontracted RPA (Figure 2). Incubation in 0.1 mM L-NAME prevented and could even reverse LEP-induced vasodilatation, supporting the NO dependence on LEP actions (Figure 2). These results agree with

Figure 3. Effects of Nω-nitro-L-arginine-methyl ester (0.1mM) on leptin (LEP)-induced vasodilatation on phenylephrine-precontracted pulmonary artery rings in the absence (empty bars) or in the presence of apelin (filled bars) (n=6). The gray bars accompanying the empty or filled bars represent the effects of vehicle (alone or in the presence of apelin). *: $P<0.05$ as compared with vehicle. The LEP or vehicle effects are expressed as a percentage of Phe-induced precontraction (mean ± S.E.M.)

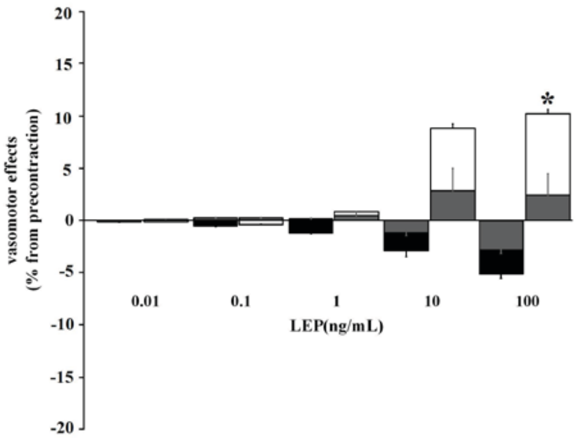


Figure 5. Vascular response of pulmonary artery rings from rats with pulmonary hypertension, precontracted with phenylephrine to increasing doses of leptin (LEP), either alone (squares) or after preincubation with apelin (circles). The vehicle effects alone or in the presence of apelin are presented as empty squares and circles. *$P<0.05$ as compared with vehicle alone (n=6). The LEP or vehicle effects are expressed as a percentage of Phe-induced precontraction (mean ± S.E.M.)

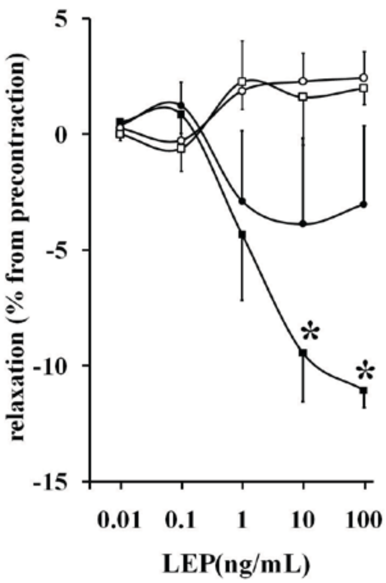
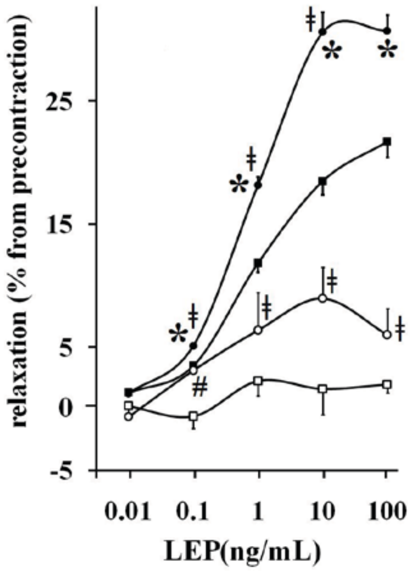


Figure 4. Vascular response of pulmonary artery rings from ovalbumin-sensitized rats precontracted with phenylephrine to increasing doses of leptin (LEP), either alone (squares) or after preincubation with apelin (circles). The vehicle effects alone or in the presence of apelin are presented as empty squares and circles. *$P<0.05$ as compared with LEP effects alone (n=6). #$P<0.01$ in the presence of apelin as compared with vehicle alone (n=6). ‡$P<0.05$ compared to normal rats (see text for details). The LEP or vehicle effects are expressed as a percentage of Phe-induced precontraction (mean ± S.E.M.)



published data that strongly indicate that leptin has both *in vivo* and *in vitro* endothelium-dependent vasodilatory actions. Leptin induced concentration-dependent dilation in rat aorta and coronary arterioles [28,33,34]. In rat mesenteric artery rings, leptin might produce vasodilatation by nitric oxide-dependent mechanisms [35] or by smooth muscle hyperpolarization [33]. In human and rat endothelium, leptin activates NO formation through a Ca^{2+}-independent mechanism that involves Akt phosphorylation and, consequently, the activation of endothelial NO synthase [28]. The hypotensive effect of leptin may be dependent on a mechanism involving both conduit and resistance arteries, in which leptin exercises its vasorelaxant effect through nitric oxide or through EDHF mechanisms [33]. The localization of leptin receptors mainly on the endothelium of the vessels reinforces the concept that the direct leptin vasorelaxation is entirely dependent on endothelial mechanisms [34]. Therefore, our data agreed with published results. In addition, the LEP vasodilatatory effects are significantly lower in OSR (Figure 3) and almost ceased in PHR (Figure 4), a possible result of vascular distress produced by bronchial allergic disease [36] or by hypertension [23].

Another interesting finding is the benefic effects of AP13 on LEP vasodilatatory effects. AP stimulates NO production in the isolated rat aorta [37] and causes NO-dependent arterial vasodilatation *in vivo* in humans [38]. Further, our previous experiments showed that at high doses AP had endothelium-dependent and partially NO-independent vasodilatory effects on Phe-precontracted rat pulmonary arteries [22]. For this study, 10 nM AP13 was added to the organ bath, on basal tone, with 15 minutes before Phe. At this concentration, AP13 did not significantly modify the vasoconstrictor agents on rat pulmonary arteries [23,36] but, as we demonstrated, could increase the LEP-induced vasodilatation on NR by an average of more than 40%. The dramatic decrease of AP13 effects in the presence of 0.1 mM L-NAME (Figure 1) suggests a large dependence on NO synthesis. The AP13 effect was maintained on pulmonary vessels from OSR, but not from PHR. These results are in agreement with those obtained by Andersen and colleagues [23] and could be the result of reduced AP13 effects on vessels with endothelial malfunction.

5. Conclusion

Adipokines could have a regulatory role in pathological situations. In agreement with published data, our results show that LEP could have vasodilatory effects on Phe-precontracted rat pulmonary arteries. Further, LEP-induced vasodilatation increased and could be preserved (during allergic lung disease) in presence of AP13. These results suggest the existence of synergistic favorable effects of these two adipokines, AP and LEP, at least on pulmonary vessels either in normal, or in particular, in a disease state.

Acknowledgements

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