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RESEARCH ARTICLE

TIME-DEPENDENT EFFECT OF OBESITY ON LEPTIN-INDUCED HYDROGEN SULFIDE-MEDIATED RELAXATION OF PERIPHERAL RESISTANCE ARTERIES

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Abstract

Abnormal regulation of vascular tone plays an important role in the pathogenesis of obesity-associated hypertension. We examined the effect of leptin on vascular tone in lean and obese rats. Male Wistar rats were fed either standard diet (control group) or highly-palatable diet to induce obesity for either 1 or 3 months (obese O1 and O3 groups). Effect of leptin on vascular tone of phenylephrine-preconstricted mesenteric artery rings was examined. In addition, membrane potential of cultured endothelial cells isolated from these three groups of animals was measured by potential-sensitive fluorescent probe. The effect of leptin on hydrogen sulfide (H_2S) production by endothelial cells was measured by sulfide-sensitive microelectrode. Leptin induced concentration-dependent relaxation of mesenteric artery rings and its effect was blocked by NO synthase inhibitor, L-NAME, as well as by small- and intermediate-conductance Ca^{2+}-activated K^+ channels inhibitors, apamin and TRAM-34, indicating that vascular effect of leptin is mediated by both NO and endothelium-dependent hyperpolarization. The latter component was inhibited by H_2S-synthesizing enzyme, cystathionine γ-lyase, inhibitor, propargylglycine, as well as by H_2S scavenger, bismuth subsalicylate. The NO-dependent component of vascular effect of leptin was impaired in both obese groups whereas the EDH/H_2S-dependent component was up-regulated in the O1 and impaired in the O3 group. Leptin increased H_2S production by endothelial cells and induced cell hyperpolarization; these effects were impaired in the 3-month obesity. The results indicate that leptin induces NO- and H_2S-dependent vasorelaxation and dietary-induced obesity has a time-dependent influence on vascular effect of leptin.

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Key words: leptin, vascular tone, obesity, arterial hypertension, hydrogen sulfide, endothelium-dependent hyperpolarization

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Introduction

Obesity is a major cause of arterial hypertension worldwide (1, 2). Leptin, produced by white adipose tissue in amounts proportional to energy stores, not only inhibits food intake and increases energy expenditure, but also has many effects on peripheral tissues including the cardiovascular system (3, 4). In physiological conditions leptin has no effect on blood pressure because induces balanced stimulation of sympathetic nervous system (SNS) and endothelium-dependent vasorelaxation (5). Conditions of chronic hyperleptinemia such as obesity, metabolic syndrome or type 2 diabetes are associated with selective leptin resistance; that is pressor SNS-dependent effect of leptin is preserved whereas its vasodilating effect is impaired (6-8). Therefore, chronic hyperleptinemia contributes to the development of arterial hypertension not only in patients with obesity but also in those with chronic kidney disease, obstructive sleep apnea, polycystic ovarian syndrome and preeclampsia (9).

Previously, we (10) and others (11-14) have demonstrated that leptin in-

duces vasorelaxation by activating endothelial nitric oxide (NO) synthase as well as by inducing endothelium-dependent hyperpolarization (EDH). In addition, in a recent study we have shown that EDH-dependent component of vascular effect of leptin is mediated by hydrogen sulfide (H_2S). H_2S is the endogenously synthesized gasotransmitter produced in endothelial cells from L-cysteine by cystathionine γ-lyase (CSE) and induces hyperpolarization by activating small- and intermediate-conductance calcium-activated potassium channels (SK_{Ca} and IK_{Ca}, respectively) (15). Hyperpolarization is then transmitted to smooth muscle cells through myoendothelial gap junctions ultimately leading to vasorelaxation (16-18).

Interestingly, in rats made obese by highly palatable diet administered for 1 month NO-dependent component of vascular effect of leptin was impaired but EDH-mediated component was up-regulated. Consequently, leptin relaxed phenylephrine-preconstricted mesenteric artery rings in obese rats as potently as in lean animals (15). However, the model of obesity applied in that study was characterized by normal metabolic profile such as plasma lipids, glucose and insulin concentrations. That is, the model represents the early stage of obesity not associated with the metabolic syndrome.

In the present study we aimed to characterize in more details vasodilating effect of leptin in lean and obese rats. In contrast to our previous study (15), we included also the group of animals fed high-calorie diet for 3 months which resulted in significant dyslipidemia and insulin resistance. The model more closely resembles the metabolic syndrome observed in most obese patients.

Materials and methods

Experimental model

The study was performed on adult male Wistar rats weighing 216 ± 4 g before the experiment. The animals were kept at a temperature of 20 ± 2°C, on a 12h light/dark cycle (lights on at 7.00 AM), and had free access to food and tap water before the experiment. The study protocol was reviewed and approved by the Bioethical Committee of the Medical University in Lublin.

After 2-week acclimation, animals were randomized to three experimental groups: (1) control, fed standard rat chow (Agropol, Motycz, Poland) ad libitum for 3 months, (2) short-term obesity (O1), fed standard chow for 2 months and then switched to highly-palatable diet for the 3rd month, (3) long-term obesity, fed highly palatable diet for 3 months. Highly palatable diet consisted of standard chow combined 1:1 (wt/wt) with a semi-liquid diet containing equal amounts of sucrose, glucose, whole milk powder and soybean powder suspended in tap water. Standard chow provided 68% calories from carbohydrates, 20% from protein and 12% from fat. The composition of highly-palatable diet was similar to standard chow (66% calories from carbohydrates, 20% from protein, and 14% from fat).

Measurement of vascular tone

Effect of leptin on vascular tone was measured *ex vivo* as described previously (15) After 3 months of feeding their respective diets, rats were anesthetized with pentobarbital (50 mg/kg ip.). Abdominal cavity was opened and blood was collected from the abdominal aorta for the measurement of leptin, insulin, glucose and lipid profile. First and second-order mesenteric artery branches (internal diameter 250–300 μm) were dissected, placed in HEPES- buffered saline solution containing 142 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 5.5 mM glucose and 10 mM HEPES (pH 7.4) saturated with 95%O_2/5% CO_2 gas mixture, cleaned of adherent connective and adipose tissue and cut into 2 mm segments. In some experiments endothelium was removed from the vessel by repeatedly passing stainless steel cannula of appropriate size through the vessel lumen. Integrity of the vessel after denudation was verified by measuring contractility induced by 60 mM KCl; only vessels with contractility comparable to those of endothelium-intact rings were used. Destruction of the endothelium was confirmed by loss of the relaxation response to acetylcholine (1 μM). Arterial segments were mounted into the multiwire myograph system DMT 610 (Danish Myo Technology, Aarhus, Denmark) and kept in HEPES-buffered saline solution bubbled with 95%O_2/5%CO_2 (pH 7.4) at 37°C throughout the experiment. Vessels were then stretched to 90% of diameter that would be obtained if the artery was subjected to 100 mmHg internal pressure, which produced maximal tension responses. Vessels were initially exposed to 60 mM KCl to measure their maximal contractility. After the 30-min washout period, concentration–response curve to the α-adrenergic agonist phenylephrine was constructed by its cumulative addition (1 nM–10 μM). Tension was expressed as the percentage of tension obtained with 60 mM KCl. After washout, segments were contracted to 75% of maximal tension with appropriate concentration of phenylephrine and then leptin was added at increasing concentrations (0.01–500 ng/ml). Relaxation in response to leptin was expressed as a percentage of sustained phenylephrine-induced contraction. If the effect of leptin was examined in the presence of inhibitors of specific pathways, respective inhibitors were administered 15 min before the first dose of leptin. The effective concentrations causing 50% of maximal response (EC_{50}) and the maximal relaxations (R_{max}) were calculated for each ring by nonlinear regres-

sion, and mean ± SD values calculated for respective experimental groups are presented in tables. Data presented on figures are relaxations induced by individual leptin concentrations (mean ± SD).

Primary culture of endothelial cells

Mesenteric artery was excised, cleaned of adherent adventitia and adipose tissue and subjected to digestion by 2 mg/ml collagenase (type II, Sigma-Aldrich) at 37°C for 45 min. Then, endothelial cells were removed by flushing with DMEM and centrifuged at 800 g for 15 min. The cells were re-suspended in RPM 1640, supplemented with 10% fetal bovine serum containing 100 U/ml penicillin and 100 U/ml streptomycin, placed in 96-well black clear bottom cell culture microplates (Thermo Scientific) at a density of 2×10^4/well and allowed to settle for 1 hour. Measurements were performed after the 1st passage to avoid changes in cell phenotype induced by culture conditions.

Measurement of membrane potential of endothelial cells

Membrane potential of cultured endothelial cells was measured by Fluorescence Imaging Plate Reader (FLIPR) using Membrane Potential Assay Kit (Molecular Devices). The kit contains a lipophilic anionic bis-oxonol dye which can partition across the cytoplasmic membrane of live cells depending on membrane potential across the plasma membrane. Its fluorescence intensity increases when the dye is bound to cytosolic proteins. When the cells are hyperpolarized, the dye exits the cells and fluorescence signal decreases.

After reaching 80-90% confluence by cultured endothelial cells, 100 µl of appropriately diluted dye contained in the kit was added to each well and the plate was incubated for 60 min at 37°C in the 5% CO_2 atmosphere to load cells with the dye. Then the plate was transferred to FLIPR and basal fluorescence signal was read at the following settings: excitation 488 nm, laser power 0.4 W, exposure time 0.4 sec., emission filter 540-590 nm camera f-stop 4, sampling time 1 sec. Then, the appropriate tested compounds were pipetted to each well from the compound V-bottom plate by the FLIPR pipettor. Each compound was dissolved at 5× final concentration and added to a culture well in 50 µl of solvent at a rate of 40 µl/sec, and changes in fluorescence were observed for 5 min. In separate wells changes in fluorescence were measured after addition of increasing extracellular K^+ concentrations (3.6, 7.5, 15, 30, 60 and 120 mM). Membrane potential (E_m) at each K^+ concentration was calculated from the Nernst equation. The slope of the relationship between change in fluorescence and change in E_m was calculated by linear least-square regression and used to calculate test compound-induced change in E_m.

H_2S production by endothelial cells

Endothelial cells were cultured as described above in 100 µl of medium/well. H_2S production was measured in real-time in each well by the H_2S-sensitive microelectrode (ArrowH_2S™ Micro Hydrogen Sulfide Measurement System, Lazar Research Laboratories, Los Angeles, USA). Each electrode was first calibrated by measuring current in standard Na_2S solutions and then placed into the microplate well. Tested compounds were added in 10 µl of solvent and the current was recorded over the next 10 min. Maximal increase in H_2S concentration (pM/min) was calculated for each well.

Measurement of plasma insulin, leptin, glucose and lipids

Plasma insulin and leptin concentrations were assayed by EIA method using Rat Insulin EIA Kit (SPIbio, Massy, France) and Leptin Enzyme Immunoassay Kit (Cayman Chemical), respectively. Plasma triglycerides, total cholesterol, HDL-cholesterol and glucose were measured by commercially available kits (Alpha Diagnostics, Warsaw, Poland).

Reagents

Recombinant rat leptin was purchased from R&D Systems and other reagents were from from Sigma-Aldrich.

Statistical analysis

Results are expressed as mean ± SD from 8 experiments in each group. Statistical significance was evaluated by ANOVA and Tukey's post-hoc test. $P<0.05$ was considered significant.

Results

Characteristics of experimental groups

After 3 months of the experiment, body weight of rats receiving highly-palatable diet was higher than of control animals but there was no significant difference between both obese groups (Table 1). Plasma leptin concentration was about 3-fold higher in both obese groups and did not differ between groups fed high-calorie diet for 1 or 3 months. Plasma glucose and total cholesterol did not differ between groups. Plasma triglycerides and HDL-cholesterol were similar in the control group and group receiving high-calorie diet for 1 month. However, 3-month feeding with this diet resulted in significant increase in triglycerides and decrease in HDL cholesterol concentrations (Table 1).

Vasodilating effect of leptin

Both KCl and phenylephrine induced similar contractility of mesenteric artery rings in control and obese groups (not shown). Leptin induced concentration-dependent relaxation of phenyl-

Table 1. Metabolic characteristics of animals in different experimental groups.

Group	Control	Obese O1	Obese O3
Body weight (g)	317 ± 5	401 ± 9***	437 ± 7***
Plasma leptin (ng/mL)	4.05 ± 0.39	12.01 ± 1.04***	12.89 ± 1.07***
Plasma insulin (ng/mL)	2.11 ± 0.22	2.32 ± 0.26	3.82 ± 0.36*
Plasma glucose (mM)	6.11 ± 0.32	6.36 ± 0.39	6.36 ± 0.39
Triglycerides (mM)	0.81 ± 0.05	0.88 ± 0.08	1.28 ± 0.07*
Total cholesterol (mM)	2.08 ± 0.23	1.53 ± 0.13	1.53 ± 0.13
HDL-cholesterol (mM)	1.27 ± 0.08	1.18 ± 0.11	0.78 ± 0.07***

*p<0.05, ***p<0.001 vs. control group

Table 2. Leptin-induced relaxation of phenylephrine-preconstricted mesenteric artery rings.

	R_{max} (%)	EC_{50} (ng/ml)
Control	60.1 ± 3.7	40.3 ± 6.8
Obese O1	57.4 ± 6.4	44.8 ± 4.4
Obese O3	29.4 ± 6.3***	52.4 ± 7.0

Maximal relaxation (R_{max}) was calculated as the leptin-induced percent decrease in tension developed in response to phenylephrine. EC_{50} – leptin concentration (ng/ml) which induced a half-maximal relaxation of PE-preconstricted segments. R_{max} and EC_{50} values were calculated for each individual vascular preparation and data presented in the table are mean ± SD from 6 animals per group. ***p<0.001 vs. control group.

ephrine-preconstricted vascular preparations in all groups of rats. Whereas the effect of leptin in the O1 group was similar to control, leptin induced less vasorelaxation in the obese O3 group (Fig. 1A). High-calorie diet administered for 3 months reduced maximal leptin-induced relaxation (Table 2). Leptin concentration which produced half-maximal relaxation tended to be higher in the O3 group but the difference was not significant. In all groups of rats vasodilating effect of leptin was almost completely abolished by either endothelial denudation (not shown) or the mixture of NO synthase inhibitor, L-NAME (100 μM), SK_{Ca} channel inhibitor, apamin (5 μM), and IK_{Ca} channel inhibitor, TRAM-34 (1 μM). In contrast, cyclooxygenase inhibitor, indomethacin (10 μM) had no effect on leptin-induced vasorelaxation (Fig. 1B). These results indicate that leptin-induced vasorelaxation is mediated by NO as well as endothelium-dependent hyperpolarization but not by prostacyclin. In the subsequent studies we used leptin-induced vasorelaxation in the presence of apamin and TRAM-34 as a measure of NO-dependent component, and vasorelaxation in the presence of L-NAME as a measure of EDH-dependent component.

Leptin-induced NO-dependent vasorelaxation was almost completely abolished in both obese groups (Fig. 2A). In contrast, EDH-dependent component of leptin induced vasorelaxation was augmented in the O1 group but diminished in the O3 group (Fig. 2B). All these effects were associated with appropriate changes in maximal relaxation whereas leptin concentration which induced half-maximal relaxation remained unchanged (Table 3).

Cystathionine γ-lyase inhibitor, propargylglycine (PAG, 1 mM) as well as H_2S scavenger, bismuth (III) subsalicylate (BSS,

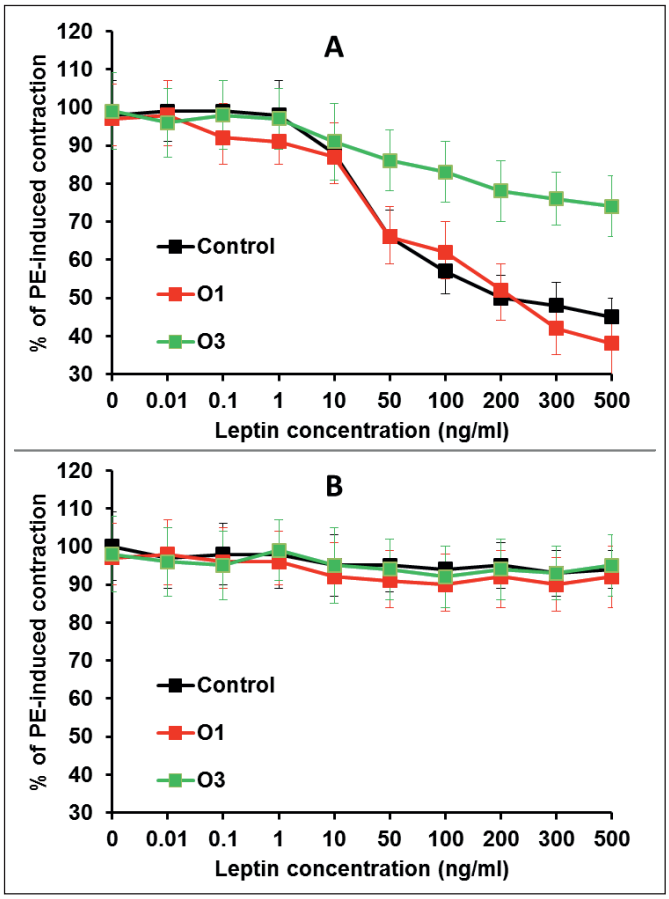

Figure 1. Effect of leptin on phenylephrine (PE)-preconstricted mesenteric artery rings of control rats (black) and rats made obese by feeding high-calorie diet for 1 month (O1, red) or 3 months (O3, green) measured in the absence of inhibitors (A) and in the presence of L-NAME, apamin and TRAM-34 (B).

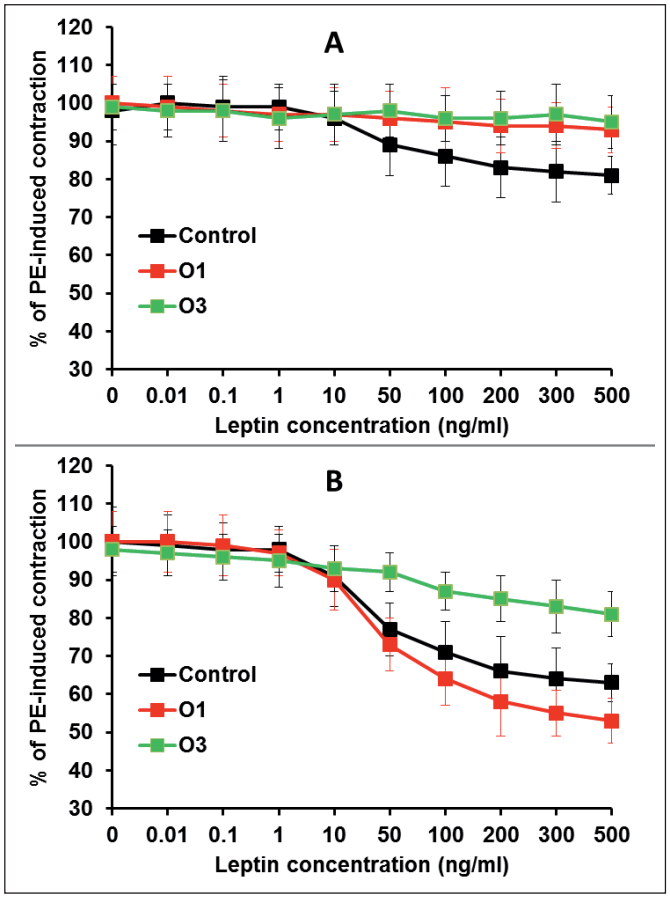

Figure 2. Effect of leptin on phenylephrine-preconstricted mesenteric artery rings in the presence of apamin and TRAM-34 (A) or L-NAME (B) in control rats (black) and rats made obese by feeding high-calorie diet for 1 month (O1, red) or 3 months (O3, green).

10 μM) almost completely abolished leptin-induced vasorelaxation measured in the presence of L-NAME in all experimental groups. PAG reduced R_{max} in control, O1 and O3 groups to 5.7 ± 2.1%, 4.8 ± 2.2% and 5.5 ± 1.8%, respectively (all $p<0.001$ vs. preparation examined without PAG), whereas BSS decreased R_{max} in these groups to 7.8%, 8.2% and 2.1%, respectively (all $p<0.001$ vs. samples without BSS).

Effect of leptin and Na_2S on endothelial cell membrane potential

Both leptin (100 ng/ml) and H_2S donor, Na_2S (100 μM) induced membrane hyperpolarization in cultured endothelial cells (Fig. 3A). The effect of leptin was abolished by Jak2 kinase inhibitor, AG-490 (10 μM), CSE inhibitor, PAG (1 mM) or a mixture of apamin (5 μM) and TRAM-34 (1 μM). In contrast, hyperpo-

Table 3. Leptin-induced relaxation of phenylephrine-preconstricted mesenteric artery rings in different experimental groups.

	NO-dependent		EDH-dependent	
	R_{max}	EC_{50}	R_{max}	EC_{50}
Control	20.8 ± 4.2	49.8 ± 10.9.	40.4 ± 2.3	38.6 ± 6.5
Obese O1	6.2 ± 1.1***	42.8 ± 12.7	52.1 ± 6.5*	45.7 ± 10.1
Obese O3	5.7 ± 2.3***	57.2 ± 11.9	20.2 ± 2.0***	49.2 ± 8.5

*p<0.05, ***p<0.001 vs. control group.

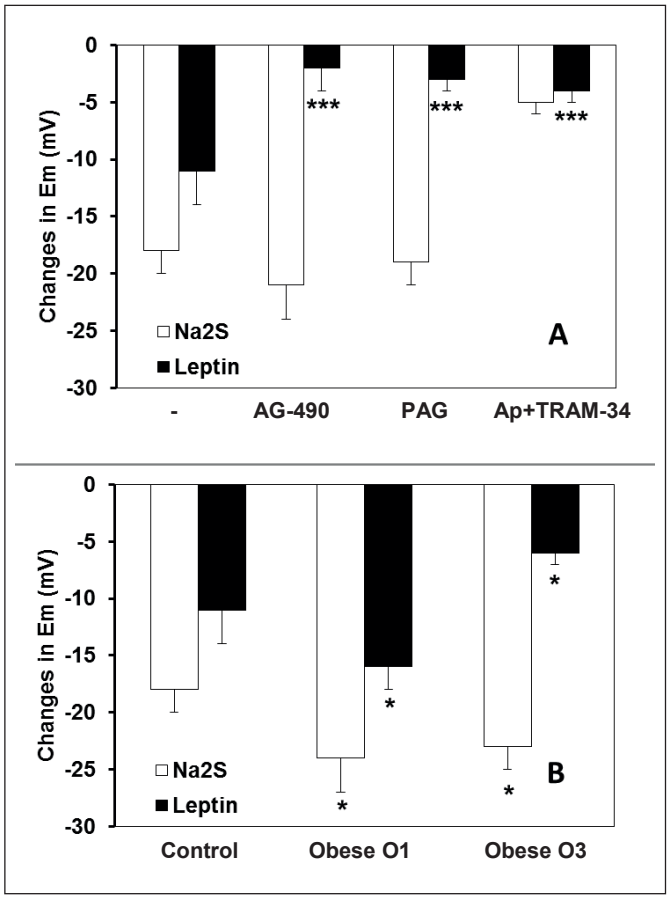

Figure 3. Effect of Na_2S (100 μM) and leptin (100 ng/ml) on membrane potential (E_m) of mesenteric artery endothelial cells. A: Effects of Na_2S and leptin were measured in the absence of any inhibitors as well as in the presence of AG-490 (10 μM), propargylglycine (PAG, 1 mM) or apamin and TRAM-34. B: Effect of leptin in control, obese O1 and obese O3 rats. *p<0.05 vs. change in E_m induced by the respective agonist in the control group, ***p<0.001 vs. change in E_m induced by the respective agonist in the absence of any inhibitors.

larizing effect of Na_2S was markedly attenuated by apamin and TRAM-34 but was not altered by either AG-490 or PAG.

Hyperpolarizing effect of Na_2S was greater in both obese groups than in control rats (Fig. 3B). In contrast, leptin induced more marked hyperpolarization of endothelial cells from the O1 group than from control rats, but less marked hyperpolarization in the O3 group.

Effect of leptin on H_2S production by endothelial cells

Leptin (100 ng/ml) increased H_2S production by endothelial cells. This effect was inhibited by AG-490 or propargylglycine but not by apamin and TRAM-34 (Fig. 4A). The effect of leptin on H_2S production in the O1 group did not differ from control.

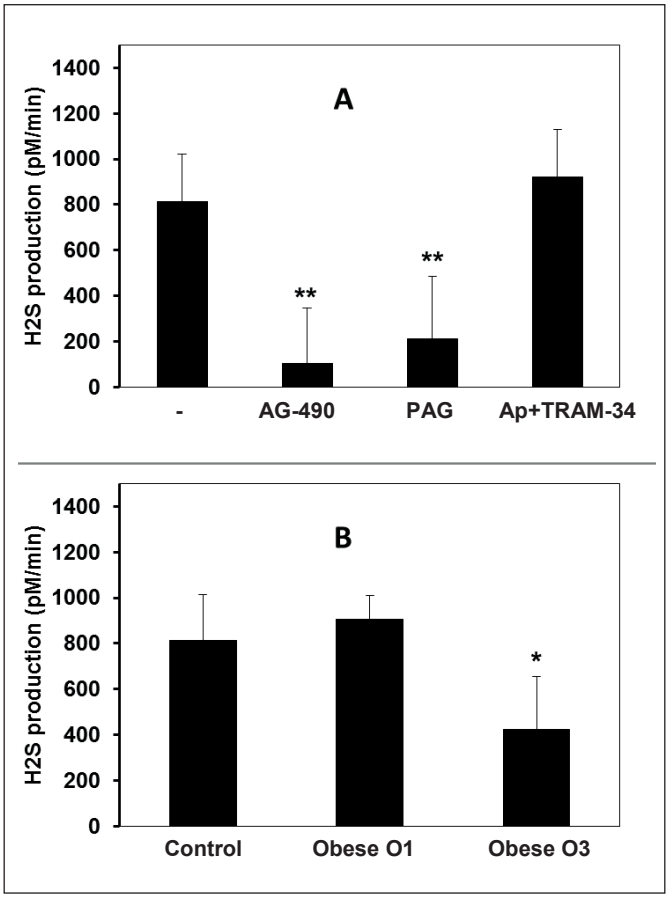

Figure 4. Effect of leptin (100 ng/ml) on H_2S production by cultured endothelial cells. A: Effect of leptin on endothelial cells of control rats in the presence of AG-490 (10 μM), propargylglycine (PAG, 1 mM) or apamin and TRAM-34. B: Effect of leptin on H_2S production by endothelial cells obtained from control, obese O1 and obese O3 groups. *p<0.05 vs. control group, **p<0.01 vs. H_2S production by endothelial cells treated with leptin without any inhibitors.

However, the effect of leptin on H_2S production by endothelial cells obtained from rats made obese by feeding high-calorie diet for 3 month was by 47.1% lower than in control group (Fig. 4B).

Discussion

Obesity is associated with abnormal endothelium-dependent vasorelaxation in response to different stimuli such as acetylcholine, insulin and fluid shear stress (19-21). Several previous studies have demonstrated that leptin stimulates endothelial NO synthase and induces NO-mediated vasorelaxation (22-27). However, although NO is a main endothelium-derived vasodilator in large conduit arteries, its contribution progressively decreases while moving to peripheral small resistance vessels which play a more important role in the regulation of total peripheral resistance and blood pressure and where endothelium-dependent hyperpolarization becomes the predominant mechanism (28, 29). In the present study we demonstrated that leptin induced endothelium-dependent relaxation of peripheral resistance arteries. The effect of leptin was mediated by both NO and endothelium-dependent hyperpolarization; the latter mechanism being the main contributor. In addition, we observed that leptin-induced endothelium-dependent hyperpolarization was mediated by H_2S which is supported by the following observations: (i) leptin stimulated H_2S production by cultured endothelial cells, (ii) leptin induced hyperpolarization of endothelial cells and this effect was sensitive to CSE inhibitor, (iii) Na_2S mimicked the hyperpolarizing effect of leptin, and both leptin- and Na_2S-induced hyperpolarization were abolished by apamin and TRAM-34, (iv) the L-NAME-resistant component of leptin-induced vasorelaxation was inhibited by CSE inhibitor as well as by H_2S scavenger. Taken together, these results indicate that leptin stimulates CSE-dependent H_2S production in endothelial cells and H_2S then induces hyperpolarization by activating small- and intermediate-conductance Ca^{2+}-activated potassium channels.

The most important finding of the present study is that obesity had a time-dependent effect on leptin-induced vasorelaxation. Vascular effect of leptin was preserved in short-term obesity induced by high-calorie diet administered for 1 month but was impaired in the 3-month obese group. More specifically, we found that NO-dependent component of leptin-induced vasorelaxation was similarly impaired in both obese groups whereas EDH-dependent component was augmented in the 1-month obese group and diminished in the 3-month obese group. Recently, we have demonstrated that diminished effect of leptin on NO in short-term obesity results from chronic hyperleptinemia. Indeed, administration of leptin receptor antagonist normalized the effect of leptin on NO in 1-month obese rats al-

though tended to aggravate high calorie diet-induced increase in body weight (15). If the same mechanism is responsible for the impairment of leptin-induced NO in the long-term obesity remains to be established.

Interestingly, in the 1-month obese group the EDH-dependent component of vascular effect of leptin was up-regulated thus compensating for deficiency of NO and resulting in normal overall leptin-induced vasorelaxation. Recently, we have demonstrated that endothelium-dependent vasodilating effect of H_2S donor, GYY4137, is enhanced in short-term obesity suggesting increased sensitivity to H_2S (15). Herein we demonstrate that both Na_2S and leptin induce greater hyperpolarization of endothelial cells in 1-month obesity. On the other hand, leptin-induced H_2S production was similar in control and O1 obese groups. These results indicate that sensitivity of endothelial cells to hyperpolarizing effect of H_2S was enhanced in these animals. The mechanism of this phenomenon is unclear at present. It has been demonstrated that high fat diet-induced obesity in the rat is associated with up-regulation of intermediate-conductance Ca^{2+}-activated K^+ channels and greater sensitivity to vasodilating effect of its activator, 1-EBIO (30). Alternatively, stimulating effect of H_2S on potassium channels results from sulfhydration of critical thiol groups; that is conversion of thiol (-SH) to persulfide (-SSH) groups in protein cysteine residues. As oxidized cysteine sulfenic (-SOH) groups are more susceptible to sulfhydration than reduced thiols (31), one may speculate that obesity-induced oxidative stress results in augmented effect of H_2S.

Interestingly, greater sensitivity to hyperpolarizing effect of Na_2S was also observed in endothelial cells obtained from the 3-month obese group. However, leptin stimulated less H_2S production, lower hyperpolarization and less EDH-dependent vasorelaxation in this group. These data indicate that despite greater sensitivity to H_2S, the amount of gasotransmitter produced in response to leptin was insufficient to support efficient vasorelaxation. The reason why effect of leptin on H_2S production was impaired in long-term obesity is unclear at present. This group was characterized by hyperinsulinemia, hypertriglyceridemia and low HDL-cholesterol which are all features of insulin resistance. Leptin and insulin share some common signaling mechanisms in endothelial cells. For example, both hormones stimulate phosphoinositide 3-kinase (PI3K) by increasing tyrosine phosphorylation of insulin receptor substrate IRS-1, although leptin increases IRS-1-phosphorylation by Jak2 kinase and insulin by insulin receptor β-subunit kinase. The IRS-1/PI3K-mediated effect of insulin on endothelial NO synthase is impaired in the metabolic syndrome (21). The mechanism through which leptin stimulates CSE in endothelial cells is unclear at present (except that Jak2 is involved, see Fig. 4A), but it may be speculated that insulin resistance impairs effect of leptin on endothelial cells as well.

In conclusion, the results of this study indicate that: (i) leptin induces endothelium-dependent relaxation of peripheral resistance arteries mediated by both NO and endothelium-dependent hyperpolarization, (ii) leptin stimulates CSE-dependent H_2S production by endothelial cells and H_2S hyperpolarizes these cells by activating small- and/or intermediate conductance calcium-activated potassium channels, (iii) obesity has a time-dependent effect on leptin-induced vasorelaxation; short-term obesity impairs NO and augments EDH/H_2S component whereas long-term obesity impairs both mechanisms, (iv) obesity is associated with increased sensitivity of endothelial cells to hyperpolarizing effect of H_2S, (5) stimulatory effect of leptin on endothelial CSE is impaired in long-term obesity associated with insulin resistance and hyperlipidemia.

Conflict of interest

The authors declare no conflict of interest.

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