Review

Dual Vascular Effects of Leptin *via* Endothelium: Hypothesis and Perspective

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Abstract

Secreted by adipocytes, leptin is a hormone which regulates appetite and metabolism. Leptin secretion is proportional to the fat mass, and thus leptin concentration is raised in most obese subjects. In recent years, more and more biological effects have been attributed to leptin; one of the most well-known effects is the effect of leptin on the vascular tone. Obesity is very often associated with hypertension, and it has been known that leptin affects the blood pressure by activating the sympathetic nervous system and causing endothelial cell (EC) dysfunction. However, there has been strong evidence that leptin is able to dilate blood vessels. Such vasodilation has been shown to be EC-dependent and EC-independent. Further, both nitric oxide-dependent and nitric oxide-independent mechanisms have been reported. In this mini-review, we summarize the heterogeneous mechanisms by which leptin causes relaxation of vascular smooth muscle. We also argue that while leptin may act as a direct dilator on the vasculature in healthy subjects, hyperleptinemia in obese subjects gradually dysregulates blood pressure control by deteriorating EC functions. How these dual effects of leptin on EC might be related to EC ionic channels is also discussed.

Key Words: obesity, leptin, hypertension, endothelium

Introduction

Obesity has become pandemic in Western countries and is emerging as a serious health risk in Asian countries, including Taiwan (6). Obesity is related to insulin resistance, clinically manifested mainly as the metabolic syndrome (37). Further, obesity is very often associated with cardiovascular diseases like hypertension and atherosclerosis (37). It is of importance to understand how obesity adversely affects the cardiovascular system. The endothelium, a single layer of cells lining the inner lumen of all blood vessels, is an important tissue regulating vascular permeability, hemostasis and hemodynamics. By secreting a variety of vasodilating and vasoconstricting substances, the endothelium contributes to blood pressure adjustments (26). Endothelial dysfunction is amongst the pathologies in hypertension and atherosclerosis, and is manifested as a reduced ability of endothelial cells (EC) to relax vascular smooth muscle cells (SMC) (39). Leptin is a hormone secreted by adipocytes in proportion to the fat cell mass, and acts as a signal to the brain about the amount of stored fat (11, 12, 42). Hyperleptinemia occurs in most cases of obesity (11, 12, 42). This adipokine is believed to contribute in part to hypertension by centrally activating the sympathetic nervous system (SNS) and causing EC dysfunction (3, 35, 36). Intriguingly, leptin has also been shown to cause acute vasorelaxation in many reports, arguing for a role of this adipokine as a vasodilator (10, 20, 21, 23, 25, 28, 31). This review examines the pressor and depressor effects of leptin

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and explains their possible physiological and pathological relevance.

Obesity and Leptin

Leptin is a 167-amino acid peptide hormone secreted by adipocytes, and plasma leptin concentration rises as the adipocyte mass increases. In healthy and lean subjects, circulating leptin concentration is around 0.3 nM; in morbid obesity, the leptin concentration can rise to 6 nM (35). As the stored fat increases, the enhanced level of leptin signals the nutritional status to the hypothalamus as an anorexigenic stimulus to deter food intake and regulate energy expenditure (11, 12, 42). Yet, hyperphagia persists in obese subjects. In the vast majority of obese subjects, the level of leptin in plasma stays high, and indicates there is some malfunctioning in leptin signaling to the hypothalamus (11, 12, 42). Thus, some form of leptin resistance has developed in most obese subjects. It is now known that this leptin resistance could be due to defective mutation(s) in the leptin receptor. Hence, in mutant db/db obese mice, the db gene was found to be the leptin receptor (for a review see 16). Another cause for leptin resistance may be defective regulation of post-receptor signaling (see below). A minority of obese subjects suffers from deficiency in leptin, and their weight can be normalized by leptin injections. The rodent model of this leptin-deficiency is the ob/ob obese mouse, whose body weight and food intake can be normalized with leptin reconstitution; the ob gene was found to be leptin itself (42).

The leptin receptor belongs to the cytokine receptor superfamily. There are six isoforms of leptin receptor (Ob-Ra - Ob-Rf) arising from alternative splicing (35). Five members (Ob-Ra - Ob-Rd, Ob-Rf) have the same ligand-binding extracellular domain and differ in the length of the intracellular domain, while Ob-Re is a cytosolic protein (35). One of the major signaling pathways for leptin is the JAK/STAT (Janus kinases/signal transducers and activators of transcription) (for a review see ref 14). Different leptin receptors employ various STAT proteins; phosphorylated and dimerized STAT molecules eventually translocate into the nucleus to modify gene transcription. STAT3 is the main signaling activated by leptin in hypothalamic neurons (4). In endothelium of retina, brain and liver, leptin effects are also mediated by STAT3 (18, 30, 38), but whether the latter is the main signaling in the endothelium of other vascular beds remains to be examined. Another major pathway for leptin signaling is *via* JAK-2 stimulaton of insulin receptor substrate-2 and subsequent activation of phosphoinositol-3 kinase (PI3-K) (14, 35). PI3-K is pivotal in regulating a repertoire of downstream substrates, including Akt, protein kinase C, RhoA/Rac and ATP-sensitive K^+ (K_{ATP}) channels (14, 35). The latter plays an immediate regulatory role in cellular excitability (see below).

Remarkably, leptin is a very pleiotropic hormone. Besides regulation of food intake and energy expenditure, leptin has been found to affect female reproduction (menstruation), thermogenesis, osteogenesis, immune functions, angiogenesis and blood pressure (9). It is interesting to note that in obesity, leptin resistance is selective, as leptin is increasingly ineffective in discouraging food intake, but still able to affect blood pressure by activating SNS and impairing EC functions (3, 11, 12, 35, 36). The molecular basis for this selective leptin resistance is unclear. It has been shown that proteins of the suppressor of cytokine signaling family (SOCS) negatively regulate the JAK/STAT pathway, and leptin resistance has been proposed to be related to the elevated expression of SOCS3 protein in the arcuate nucleus of the hypothalamus (14, 29).

It is noteworthy that heightened levels of leptin are not only observed in obese subjects, but also observed in subjects under stress. Systemic stress and perceived psychological stress have been shown to raise serum leptin levels in rats and humans, respectively (22, 34).

There are multiple causal factors linking obesity and hypertension, such as raised levels of free fatty acid and endothelin-1, which are beyond the scope of this mini-review. Here we focus on discussing how leptin contributes to the regulation of blood pressure and how sustained hyperleptinemia eventually leads to hypertension. In recent years, EC has been found to be one of the major targets of leptin. A brief description of the modulation of the vascular tone by EC will facilitate understanding of how leptin-EC interaction may contribute to blood pressure regulation.

How EC Modulates the Vascular Tone

EC modulates the vascular tone by releasing an array of vasoconstricters and vasorelaxants (26). The most prominent known relaxing factors include nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF). Shear stress and agonist, by activating non-selective cation channels and the store-operated Ca^{2+} channel (SOCC, which opens upon Ca release from intracellular Ca stores), respectively, can stimulate a rise in EC cytosolic Ca^{2+} concentration, subsequently leading to enhanced NO synthesis and release (32). The vast majority of EC types lack voltage-gated Ca^{2+} channels, and therefore depolarization of EC would not cause Ca^{2+} influx (32). Indeed depolarization of EC would decrease the electrical driving force for Ca^{2+} influx. Most EC types also do not have voltage-gated K^+ channels but do have Ca^{2+}-activated K^+ (Kca) channels and K_{ATP}

channels (1, 19, 32). Kca channels are activated by a rise in cytosolic Ca^{2+} while K_{ATP} channels open upon a drop in ATP concentration in the cytosol. It has been known that Kca channels could be activated by physiological stimuli such as agonists and flow-induced shear stress, while K_{ATP} channels open in response to flow-induced sheer stress and hypoxia (1, 19, 32). The opening of Kca and K_{ATP} channels allows more K^+ efflux and thus cause hyperpolarization, which enhances Ca^{2+} influx by providing a stronger electrical driving force (1, 19, 32). Further, EC hyperpolarization can spread to SMC through EC-SMC gap junctions, providing an EDHF mechanism (1, 19, 32). Such an EDHF mechanism represents an NO-independent mechanism of SMC relaxation.

Vasodilating Mechanisms of Leptin Are Heterogeneous

Leptin-Induced Acute Vasodilation Through EC-Dependent Mechanisms

Leptin receptors are expressed in EC (3, 35, 36). It has been found that leptin could cause an acute hypotensive effect in 6-hydroxydopamine sympathectomized rats, and relax phenylephrine-precontracted rat aortic and mesenteric arterial rings at physiological concentrations (0.1-1 nM) (23). These relaxing effects were abolished by EC denudation or inhibition of NO synthase or EDHF, suggesting an EC-dependent mechanism (NO-dependent and NO-independent). However, NO synthase inhibitors were not effective in preventing the hypotensive effect of leptin, suggesting that the *in vivo* hemodynamic effect of leptin was mainly mediated by EDHF (23). Leptin-induced EC- and NO-dependent relaxation of phenylephrine-precontracted rat mesenteric artery was also reported in the same year in another laboratory (20). Such leptin-induced relaxation was substantially attenuated by lowering extracellular Cl^- concentration. The latter, however, does not affect nitroprusside-induced, EC-independent, vasorelaxation. A recent report showed that leptin (at concentrations observed in obesity) could dilate rat and dog coronary arterioles (21). Such dilation was largely prevented by EC denudation and NO synthase inhibition. There was direct evidence that leptin could induce NO release from EC (fluorimetric measurement with 4,5-diaminofluorescein) by causing phosphorylation of endothelial NO synthase at Ser 1177 (40).

Whether leptin had vasoactive effects in humans was later examined. Forearm blood flow, measured by strain-gauge plethysmography in healthy men, was shown to be significantly increased by 20% during intra-arterial infusion of leptin (31). Co-infusion of an NO synthase inhibitor did not affect leptin-induced increase in blood flow, suggesting an NO-independent mechanism in leptin-induced vasodilation. A subsequent report by the same group showed similar results in human coronary artery (25). Coronary artery diameter and blood flow (as measured by quantitative angiography and Doppler velocimetry) were found to be significantly increased by infusion of leptin into the left coronary ostium. Such leptin effects were unaffected by pre-infusion of NO synthase inhibitor, suggesting that leptin-induced coronary vasodilation may be unrelated to NO.

Leptin-Induced Vasodilation Can Be EC-Independent

Leptin appears to exert a direct, EC-independent, relaxing effect on vascular SMC. It has been shown that leptin, at pharmacological concentrations (≥ 10 nM), could inhibit angiotensin II-induced contraction in EC-denuded rat aortic rings (10). In support of this, leptin receptors have also been found in cultured vascular SMC from rat (10). In a recent report, leptin (at physiological concentration of 0.3 nM) was shown to induce relaxation in saphenous vein and internal mammary artery rings obtained from human patients with coronary artery disease (28). Such relaxation was unaffected by EC denudation or NO-synthase inhibition, suggesting an EC-independent mechanism. It is unknown whether leptin would cause such an EC-independent dilation in saphenous vein and internal mammary artery in healthy humans.

Impairment of EC Functions by Prolonged Hyperleptinemia

Although leptin has been shown to cause acute vasodilation *in vitro* and *in vivo*, such vasodilation seems to be at odds with the well known association between hyperleptinemia and hypertension in obese subjects. Therefore, it is reasonable to think that the sustained effect of raised leptin concentration is different from acute leptin effect. How sustained hyperleptinemia is casually related to hypertension is not entirely understood. Besides the known pressor effect by leptin *via* activating SNS, leptin may also raise blood pressure by causing EC dysfunction. A recent work by Knudson *et al*. (21) showed that in anesthetized, open-chest dogs, infusion of a range of leptin concentrations does not affect coronary blood flow, but high leptin concentrations significantly attenuate coronary dilation triggered by acetylcholine, but not sodium nitroprusside (direct relaxant of SMC). These results suggest that hyperleptinemia does not impair EC-independent dilation, but causes EC dysfunction. Consistent results were obtained in *in vitro* experiments in the same report (21). In dog coronary rings contracted with thromboxane A1, a

10-min pre-incubation with a high concentration (0.63 nM) but not physiological concentration (0.25 nM), of leptin significantly reduced acetylcholine-triggered relaxation. Further, the authors showed that leptin (10 nM) could cause significant vasodilation of coronary arterioles in lean Zucker rats, but not the hyperleptinemic obese Zucker rats (21).

It is not well understood how hyperleptinemia causes EC dysfunction (3, 35, 36). Generation of reactive oxygen species (ROS) by leptin in EC has been reported (5, 41). While small quantity of ROS acts as messenger molecules, chronic exposure of cells to large amounts of ROS can be harmful. In aortic EC, leptin was shown to trigger, in a protein kinase A-dependent manner, ROS formation *via* the mitochondrial electron transport chain (41). Not only are ROS damaging to lipids and proteins, superoxide anion also reacts with NO to form another toxic molecule, peroxynitrite ($ONOO^-$), and thus reduces bioavailability of NO (for a review see ref 3). ROS also contributes to EC dysfunction by up-regulating the expression of adhesion molecules and chemotactic molecules in EC, thereby promoting the adhesion and migration of monocytes to the vessel wall (7). Finally, raised level of tumor necrosis factor-α (TNF-α) as a consequence of hyperleptinemia, could also reduce the bioavailability of NO by inhibiting endothelial NO synthase (3).

A Hypothesis: When Obesity Progresses, Leptin Loses Its Hypotensive Effect as It Causes EC Dysfunction

It can be seen that the vasodilating effects of leptin can be very heterogeneous in nature: EC-independent and EC-dependent. In the latter case it can be either NO-dependent or independent. Difference in species and blood vessels tested may in part explain this heterogeneity. It is possible that in healthy non-obese subjects, the physiological concentrations of leptin may cause vasodilation, but the pressor effect of leptin through SNS activation may counteract such effect. This is strongly supported by the evidence obtained by Fruhbeck (13). In this report, leptin treatment to rats under NO synthase inhibition (L-NAME) led to a rise in blood pressure. Leptin treatment to rats under ganglionic blockade (chlorisondamine) resulted in a drop in blood pressure compared with the control (chlorisondamine treatment alone). Then, leptin-induced hypotension under ganglionic blockade was abrogated by NO synthase inhibition.

Indeed, a number of studies using either transgenic or genetically defective animals have provided insights that the SNS-activating effect of leptin may override its effect on EC. Blood pressure in transgenic mice over-expressing leptin is higher than the control littermates, and could be normalized after α-adrenergic or ganglionic blockade (2). By contrast, the profoundly obese, leptin-deficient ob/ob mice have blood pressure lower than the wild type controls (2). Although body weight is reduced, blood pressure is elevated in these ob/ob mice after leptin treatment. Besides, leptin-resistant agouti obese mice exhibited hyperleptinemia and heightened blood pressure (8, 24).

Therefore, it is possible that in lean subjects, there is a dynamic equilibrium between leptin SNS effects and EC effects in contributing partially to blood pressure regulation. In overweight or mildly obese subjects, the hypotensive effect of leptin mediated by EC-dependent vasodilation may still partially offset the pressor effect of leptin *via* SNS activation. However, as obesity progresses, chronic and severe hyperleptinemia gradually damages EC. Consequently, EC dysfunction, together with SNS activation, contribute to obesity-associated hypertension.

Future Perspectives

Leptin can induce NO formation by directly activating endothelial NO synthase, which may account for its vasodilation and hypotensive effects. It remains to be explored if leptin would affect other machineries which are essential to NO synthesis and release. Since the vasodilating effects of leptin is acute (seconds to minutes), one of the possibilities would be direct modulation of EC ionic channels. Leptin has been shown to activate K_{ATP} channels in rat insulin-secreting CR1-G1 cells and arcuate nucleus neurons (17, 27). Leptin has also been shown to activate Kca channels in hippocampal neurons (33). If leptin also activates Kca and K_{ATP} channels in EC, it would provide a mechanism for promoting NO release by hyperpolarizing EC and thus facilitating Ca^{2+} influx. Such EC hyperpolarization, which may spread to SMC *via* gap junction, can also partly explain the reported NO-independent mechanism of vasorelaxation. Electrophysiological characterization of EC upon acute leptin challenge would certainly be warranted in the future.

It remains to be established how exactly leptin causes EC dysfunction. Excess ROS generated in EC upon hyperleptinemia would reduce NO bioavailability, and it is possible that ROS may also adversely affect EC ionic channels essential to NO synthesis and release. Damaging of ionic channels by oxidative stress has been well documented (15). Hence, it would be of interest to examine which EC ion channels (such as SOCC, K_{ATP}, Kca channels or other non-selective cation channels) would be affected by sustained hyperleptinemia.

It may be of pharmacological and therapeutic interests to develop selective leptin receptor agonists

and antagonists. However, there is currently no commercially available probes for leptin receptors. It is even doubtful about the feasibility of developing tissue-selective leptin antagonist as all the five plasma membrane-bound leptin receptor isoforms have the same extracellular domain. Designs of pharmacological intervention may be targeted towards the variable intracellular domains of the leptin receptor isoforms, or the associated diverse signaling pathways.

Conclusion

Leptin is a hormone excessively secreted by adipocytes in obesity, and has been a culprit for hypertension. Leptin-triggered acute vasodilation may counteract the simultaneous activation of the SNS. As obesity progresses, EC-dependent vasodilation is likely to be less and less effective in relaxing SMC, as sustained hyperleptinemia may eventually lead to EC dysfunction. Further investigation of acute and sustained effects of leptin on EC signaling is needed.

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