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Lack of dilator effect of leptin in the hindlimb vascular bed of conscious rats

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Abstract

Leptin acts in the hypothalamus to decrease appetite and increase sympathetic nerve activity. Some in vitro studies have suggested that leptin may possess vasodilator actions that oppose sympathetically mediated vasoconstriction. We tested whether leptin is a vasodilator of the hindlimb vascular bed in vivo, and also whether it alters vasodilation to acetylcholine or sodium nitroprusside. In conscious instrumented Sprague–Dawley rats we measured iliac blood flow responses to local arterial infusion of acetylcholine (0.1 and 1 μg/min) and sodium nitroprusside (1 and 10 μg/min) before and after 2 h of intra-arterial leptin (at doses of 0.1 or 1 mg/kg, $n=10$ each) or vehicle ($n=10$). Leptin infusion over 2 h did not change mean arterial pressure, heart rate or iliac flow and conductance. In addition, leptin did not significantly alter the vasodilation (increase in the conductance ratio between infused and non-infused arteries) induced by acetylcholine, whereas pre-treatment with a nitric oxide synthesis inhibitor, N^G-nitro-L-arginine methyl ester, significantly attenuated the vasodilation to acetylcholine. Leptin also did not affect the vasodilation to sodium nitroprusside. Lack of effect of leptin on vasodilator function could not be due to the activation of the sympathetic nervous system because lumbar sympathoactivation was obtained only 4 h after the iliac infusion of 1 mg/kg/h of leptin. These findings are in accordance with our previous results and argue against a meaningful direct dilator action of leptin on resistance vessels in vivo.

Keywords: Leptin; Blood flow; Endothelium; Nitric oxide; Blood pressure

1. Introduction

Leptin, produced by adipocytes, acts in the hypothalamus to promote weight loss by decreasing appetite and increasing sympathetic nerve activity to thermogenic brown adipose tissue (Collins et al., 1996; Haynes et al., 1997). Leptin induces sympathoactivation to several other organs not usually considered thermogenic such as the kidney, hindlimb and adrenal gland (Haynes et al., 1997; Dunbar et al., 1997). This suggests that leptin contributes not only to regulation of energy homeostasis, but also to the control of cardiovascular function. However, the robust increase in sympathetic nerve activity observed after acute intravenous administration of leptin was not associated with an increase in arterial pressure (Haynes et al., 1997). This lack of acute pressor response suggests that leptin may have a peripheral vasodilator action that opposes sympathetically induced vasoconstriction, although it could also reflect the short duration of these studies.

Recently, several studies have suggested that leptin may have direct vascular effects that tend to decrease arterial pressure. Functionally competent leptin receptors are present on endothelial cells (Sierra-Honigmann et al., 1998) and leptin has been shown to enhance nitric oxide (NO) release from endothelial cells (Winters et al., 2000). Furthermore, leptin administration in rat caused a dose-dependent increase in serum NO concentrations and infusion of leptin under NO synthesis inhibition increases arterial pressure (Fruhbeck, 1999). In contrast, leptin decreases arterial pressure after suppression of sympathetic

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influence, by ganglionic blockade (Fruhbeck, 1999) or chemical sympathectomy (Lembo et al., 2000). Furthermore, in vitro studies have shown that leptin evokes an endothelium-dependent relaxation of arterial rings (Lembo et al., 2000; Kimura et al., 2000). Winters et al. (2000) studied the consequences of leptin deficiency in *ob*/*ob* mice on the aortic vascular reactivity. The vasoconstrictions induced by agonists such as norepinephrine and phenylephrine were markedly increased while the vasorelaxation induced by acetylcholine was impaired which could be corrected with leptin replacement (Winters et al., 2000). Given all these reports, it has been argued that the vasodilator effects of leptin might oppose its neurogenic pressor action.

In contrast, we recently found that leptin does not have substantial direct or indirect vasodilator effects in vivo (Mitchell et al., 2001; Jalali et al., 2001). Indeed, intravenous leptin, at concentration sufficient to increase sympathetic nerve outflow, did not change arterial pressure or blood flow measured from the mesenteric, lower aortic and renal arteries (Mitchell et al., 2001). Blockade of the adrenergic system or NO synthase did not reveal any pressor effect of leptin (Mitchell et al., 2001). Furthermore, leptin did not alter the sympathetically mediated vasomotor response in hindlimb or kidney to stimulation of the splanchnic sympathetic nerve trunk (Jalali et al., 2001). However, these results did not exclude the possibility that leptin causes vasodilatation elsewhere than in the vessels we studied so far i.e. mesenteric, lower aortic and renal arteries. In addition no previous study has tested whether leptin has vasodilator effects in vivo when administered directly into a vessel, without the confounding effects of systemic dosing.

The present study was therefore designed to examine whether leptin has vasodilator actions in another resistance bed; the iliac artery. We tested whether leptin alters iliac blood flow and/or responses to acetylcholine or sodium nitroprusside (endothelium-dependent and -independent vasodilators, respectively). In addition, in the present study leptin was administered directly into the iliac artery to test whether any vasodilator effect of leptin could be revealed while minimizing systemic actions.

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley rats ($n=53$) at 14–16 weeks of age were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Rats were allowed a week to acclimatize in the animal facility. These animals had access to a standard rat chow (Teklad Premier Laboratory Diets, Madison, WI) and tap water ad libitum until the time of experimentation. All procedures were approved by the University of Iowa Animal Research Committee. At the end of each experiment rats were euthanized with an overdose of methohexital sodium (Brevital, 150 mg/kg intravenous). Recombinant murine leptin was provided by Amgen Biologicals (Thousand Oaks, CA). Acetylcholine was obtained from Novartis (Miochol-E, Duluth, GA), sodium nitroprusside from Abbott Labs (Nitropress, North Chicago, IL) and N^G-nitro-L-arginine methyl ester (L-NAME) from Sigma Chemical Co (St. Louis, MO).

2.2. Surgical procedures

2.2.1. Doppler flow probe implantation and intra-iliac arterial catheterization

On the day of the surgery, each rat was anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg, intraperitoneal (i.p.)). Under aseptic surgical conditions, a midline ventral incision was made and visceral organs retracted to expose the lower abdominal aorta and the right and left iliac arteries. Blood flow in both iliac arteries was measured by separate ultrasound Doppler probes (model ES, Iowa Doppler Products, 0.8 mm) that were placed just distal to the abdominal aorta bifurcation. When a clear Doppler echo signal was achieved on both iliac arteries, each probe was securely tied into place and wires were tunneled subcutaneously out of the nape of the animal's neck. For this isolated hindlimb preparation, the right or left iliac artery was randomly chosen to be the control bed while the other iliac artery was prepared for arterial catheterization. A snare was placed around the abdominal aorta approximately 5–10 mm above the aortic bifurcation and clamped to occlude blood flow distal to the two iliac arteries. During the brief period of occlusion, a PE-10 tubing with a 30-gauge needle was quickly inserted 3–5 mm distal to the Doppler flow probe into the lumen of the artery. The intra-iliac catheter was glued in place with Vetbond (3M, St Paul, MN). The PE-10 tubing was filled with 20% heparin solution and then plugged. The tubing was tunneled subcutaneously and then exteriorized out of the nape of the rat's neck. The other corresponding iliac artery was untouched with its Doppler probe still in place. After the completion of surgical procedures, the rats were given penicillin (1500 U, intramuscular) and were allowed to recover 4–5 days.

After recovery, rats were briefly anesthetized (methohexital sodium, Brevital, 40 mg/kg, i.p.) for implantation of carotid artery and jugular venous catheters, which were tunneled subcutaneously and exited out from the nape of the rat's neck. After complete recovery from anesthesia (4–5 h), each rat was placed in a Plexiglas restraining cage. The signal from the carotid artery catheter was split and delivered to a transducer (model P23XL, Gould Electronics, Valley View, OH) and then to an ECG/Biotach coupler (Gould Electronics) for continuous mean arterial pressure and heart rate recording. Wires from the previously implanted iliac Doppler flow probes were connected to a directional pulse Doppler flow meter (Model 545C-2, Bioengineering, University of Iowa) and the range was adjusted to achieve the maximum voltage. Signals from the right and left iliac Doppler flows as well as the blood pressure and heart rate measurements were digitized, displayed and stored on an Apple Macintosh (8500/180) computer with the use of a MacLab (model 8S, ADInstruments, Castle Hill, Australia) analog-to-digital converter with Chart v 3.6 software. Finally, the intra-iliac catheter was attached to an infusion pump (Model 355, Sage Instruments) for administration of leptin and other drugs.

2.2.2. Nerve recordings

Sympathetic nerve activity to hindlimb was measured by multi-fiber recording as described previously (Haynes et al., 1997; Mitchell et al., 2001). Briefly, rats were anesthetized with an anesthetic cocktail consisting of ketamine and xylazine (95 mg ketamine/9.5 mg xylazine per kg, i.p.). With an abdominal incision, a chain of the lumbar sympathetic nerve was isolated and placed on a bipolar platinum–iridium electrode and then covered with silicone gel (Kwik-Cast, World Precision Instruments, Inc). In addition, catheterization of the iliac and carotid arteries and the jugular vein were also performed in the same animal as above. Rats were allowed to recover completely from anesthesia (4–5 h) before starting recording SNA in the conscious animal. To ensure that electrical noise was excluded in the assessment of sympathetic outflow, sympathetic nerve activity was corrected for post-mortem background activity.

2.3. Experimental procedure

2.3.1. Baseline effects of acetylcholine and sodium nitroprusside on isolated iliac blood flow

In some rats ($n=40$), after a stable 10 min baseline was obtained, two doses of acetylcholine (0.1 and 1.0 μg/min) and two doses of sodium nitroprusside (1.0 and 10 μg/min) were infused, at a rate of 10 μl/min, into one iliac artery for 10 min each, until a steady dose-dependant increase in blood flow was achieved. Time was then allowed (20 min) between different doses of acetylcholine and sodium nitroprusside for all hemodynamic measurements to return to baseline values.

2.3.2. Effects of saline, leptin or L-NAME on isolated iliac blood flow

After baseline responses to acetylcholine and sodium nitroprusside were established, rats were infused into the iliac artery for 2 h with either saline (10 μl/min, $n=10$), leptin at doses of 0.1 and 1.0 mg/kg/h ($n=10$ each) or L-NAME (30 μg/kg/h, $n=10$) and then acetylcholine and sodium nitroprusside were re-administered using the same protocol as baseline studies above. The infusion of saline, leptin or L-NAME was maintained during the administration of acetylcholine and sodium nitroprusside.

2.3.3. Effects of saline, leptin on lumbar sympathetic nerve activity

The baseline lumbar sympathetic nerve activity was recorded for 10–15 min. Saline ($n=5$) or leptin at a dose of 0.1 ($n=4$) or 1 mg/kg/h ($n=4$) was then infused in the iliac artery at a rate of 10 μl/min for 2 h. The lumbar SNA was recorded continuously during 4 h after starting the infusion.

2.4. Data analysis

The right and left iliac blood flow measurements were recorded in megahertz and corrected for background noise by subtracting post-mortem measurements from measurements taken during the study. To convert flow measurements to conductance, the corrected values were divided by contemporaneous mean arterial pressure and expressed as MHz/mm Hg. Baseline hemodynamic values were obtained by averaging three separate 60 s time periods that were stable during the control period. Likewise, three 60 s periods were averaged during the stable peak response to each dose of acetylcholine and sodium nitroprusside, before and after the co-infusion of, either, saline or leptin (0.1 or 1.0 mg/kg/h) or L-NAME (30 μg/kg/h). To minimize variability caused by systemic hemodynamic changes, iliac conductance data were expressed as a ratio between infused and non-infused arteries. Data for flow, conductance and lumbar sympathetic nerve activity are expressed as percentage change from baseline with 0% as baseline. Results are expressed as mean±S.E.M. Statistical comparisons were made with the use of Student t test or repeated-measures factorial analysis of variance (ANOVA) with Newmann–Keuhls test for post hoc comparison.

3. Results

To demonstrate the endothelium-dependent and endothelium-independent response of the iliac conductance to acetylcholine and sodium nitroprusside, respectively, we assessed the changes in the infused/non-infused conductance ratio induced by acetylcholine and sodium nitroprusside in presence or absence of L-NAME. In absence of L-NAME, administration of either acetylcholine or sodium nitroprusside caused a significant ($P=0.008$ and $P=0.009$, respectively) and dose-dependent increase in conductance ratio (Fig. 1). A significant decrease in mean arterial pressure (from 139±4 to 121±6 mm Hg, $P<0.05$) and increase in heart rate (from 441±19 to 497±18 bpm, $P<0.05$) was observed with the highest dose of sodium nitroprusside only. L-NAME caused a significant increase in mean arterial pressure (from 139±4 to 155±4 mm Hg, $P=0.009$) and a decrease in heart rate (from 441±19 to 369±13 bpm, $P=0.004$). As expected, infusion of L-NAME significantly attenuated the increase in the conductance ratio induced by 0.1 ($P=0.006$) and 1 μg/min ($P=0.002$) acetylcholine. However, L-NAME did not alter vasodilation to sodium nitroprusside (Fig. 1).

To examine whether leptin-induced hindlimb vasodilatation we tested the effects of 2 h leptin infusion. As shown in Table 1, infusion of leptin in the iliac artery, at a dose of 0.1 and 1 mg/kg/h, or vehicle did not change significantly mean arterial pressure, heart rate, and right and left iliac flow or conductance. In addition, leptin infusion did not affect the increase in conductance ratio in response to acetylcholine (Fig. 2). Similarly, and as depicted in Fig. 3, leptin did not alter vasodilation to sodium nitroprusside.

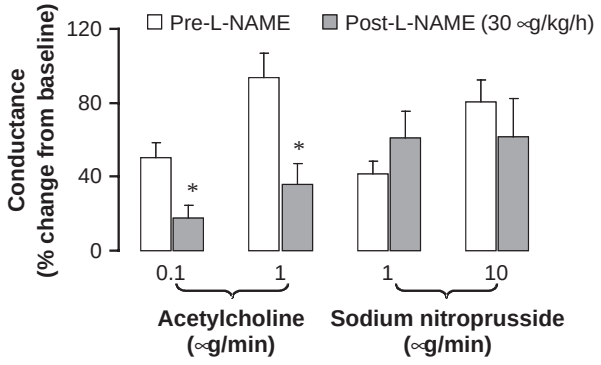

Fig. 1. Change in the conductance ratio induced by different doses of acetylcholine and sodium nitroprusside before (□) and after (■) L-NAME treatment (30 μg/kg/h). L-NAME attenuates the vasodilation induced by acetylcholine. Data expressed as mean±S.E.M. represent the percentage change from baseline of the ratio between infused and non-infused arteries. *$P<0.05$ vs. pre-L-NAME.

Table 1
Arterial pressure, heart rate, and flow and conductance (of infused and non-infused iliac arteries) obtained from Sprague–Dawley rats at baseline and 2 h after infusion of vehicle or leptin (0.1 and 1 mg/k/h) in the iliac artery

Treatment	Mean arterial pressure (mm Hg)		Heart rate (bpm)		Flow (mHz)				Conductance (Hz/mm Hg)			
	Basal	2 h	Basal	2 h	Infused artery		Non-infused artery		Infused artery		Non-infused artery	
					Basal	2 h	Basal	2 h	Basal	2 h	Basal	2 h
Vehicle	126±6	119±5	439±24	455±29	1.09±0.17	1.18±0.16	0.89±0.09	0.93±0.12	8.8±1.5	10.0±1.3	7.0±0.7	7.8±0.9
Leptin (0.1 mg/kg/h)	123±2	118±2	457±17	470±19	1.76±0.26	1.85±0.27	0.86±0.09	0.84±0.09	14.4±2.2	15.7±2.4	7.0±0.8	7.2±0.8
Leptin (1 mg/kg/h)	128±2	122±2	406±18	417±21	1.31±0.16	1.29±0.17	1.01±0.08	0.96±0.12	10.4±1.4	10.7±1.4	7.9±0.8	8.0±1.1

Data are expressed as mean±S.E.M.

In order to assess whether administration of leptin could induce sympathoactivation that may mask its vasodilatory effects we examined, in the same conditions, the effect of infusion of leptin in the iliac artery on lumbar sympathetic nerve activity in conscious rats. As shown in Fig. 4, infusion of vehicle ($n=5$) or leptin at a dose of 0.1 mg/kg/h ($n=4$) had no significant effect on lumbar sympathetic nerve activity either 2 or 4 h after starting the infusion. While lumbar sympathetic nerve activity was slightly but not significantly increased (54±12%, $P=0.08$ vs. vehicle) after 2 h of leptin infusion at a dose of 1 mg/kg/h ($n=4$) after 4 h clear and significant lumbar sympathoactivation was observed (129±18%, $P<0.001$ vs. vehicle).

4. Discussion

In the present study, we investigated the effect of leptin on the hindlimb resistance bed. Our results suggest that, in

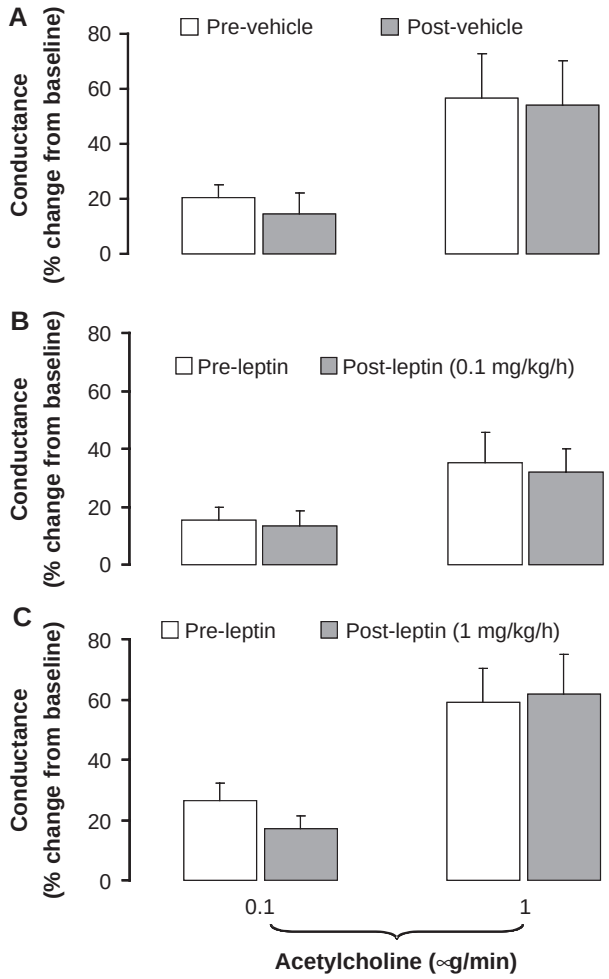

Fig. 2. Change in the conductance ratio in response to acetylcholine (at doses of 0.1 and 1 μg/min) before (□) and after 2 h of treatment (■) with vehicle (A) or leptin at doses of 0.1 (B) and 1 mg/kg/h (C). Infusion of leptin has no effect on the vasodilation to acetylcholine. Data expressed as mean±S.E.M. represent the percentage change from baseline of the ratio between infused and non-infused arteries.

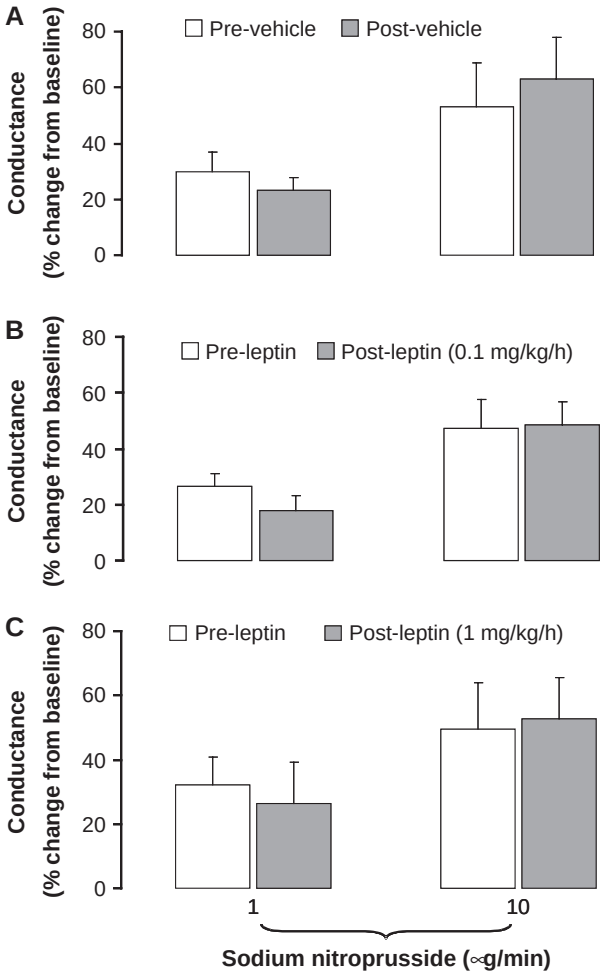

Fig. 3. Change in the conductance ratio in response to sodium nitroprusside (at doses of 1 and 10 μg/min) before (□) and after 2 h of treatment (■) with vehicle (A) or leptin at doses of 0.1 (B) and 1 mg/kg/h (C). Infusion of leptin has no effect on the vasodilation to sodium nitroprusside. Data expressed as mean ± S.E.M. represent the percentage change from baseline of the ratio between infused and non-infused arteries.

vivo, intra-arterial leptin does not have a substantial vasodilator action in the hindlimb. Indeed, leptin did not affect the increase in hindlimb blood flow induced by acetylcholine or sodium nitroprusside. These data are in accordance with our previous reports where leptin did not alter mesenteric, lower aortic or renal conductance (Mitchell et al., 2001; Jalali et al., 2001). Our failure to elicit any hemodynamic effects of leptin cannot be explained by an ineffective dose because we have previously shown that the dose of leptin used in this study caused a significant increase in sympathetic outflow in rats, when administered intravenously (Haynes et al., 1997; Mitchell et al., 2001). We have also shown here that intra-iliac artery administration of leptin caused significant increase in lumbar sympathetic outflow after 4 h of infusion.

Leptin has been demonstrated to activate NO synthesis in endothelial cells and to promote arterial relaxation in vitro (Sierra-Honigmann et al., 1998; Winters et al., 2000). Furthermore, blockade of NO synthase has been shown to reveal a pro-hypertensive effect of leptin (Fruhbeck, 1999) while inhibition of the sympathetic nervous system revealed its pro-hypotensive action (Fruhbeck, 1999; Lembo et al., 2000). Absence of leptin in *ob*/*ob* mice causes abnormalities in vascular endothelial and contractile function in vessels that were rescued by leptin replacement suggesting an important role for leptin in vasodilator function (Winters et al., 2000). In contrast to these reports, Gardiner et al. (2000) found no evidence for a vasodilator action of leptin in conscious rats. These authors showed that leptin do not change blood flow in different beds including renal, mesenteric and abdominal arteries, and presence of NO synthase inhibitor, L-NAME, failed to unmask any pressor effect of leptin (Gardiner et al., 2000). Similarly, we have shown that leptin did not change regional blood flows (Mitchell et al., 2001; Jalali et al., 2001). Furthermore, we found that blockade of NO synthesis or sympathetic nervous

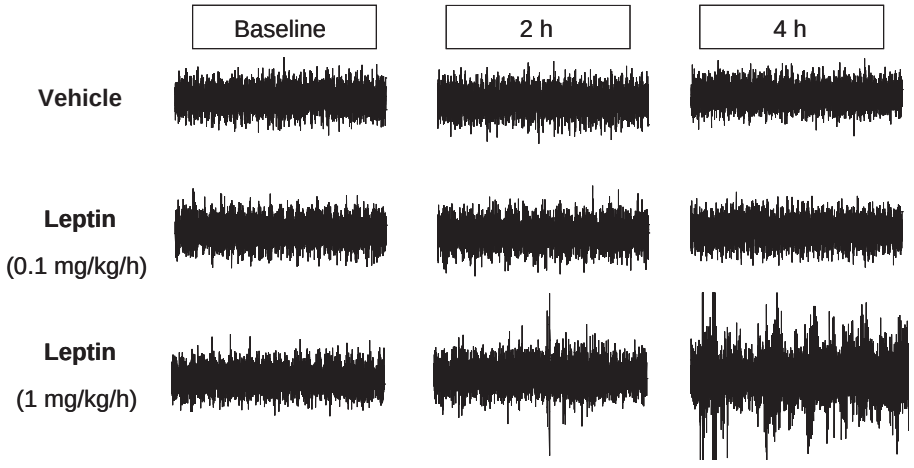

Fig. 4. Effects of leptin infusion in the iliac artery on the lumbar sympathetic nerve activity in conscious rat. Examples of the neurograms are shown before (baseline), 2 and 4 h after starting the infusion of the vehicle or leptin at a dose of 0.1 and 1 mg/kg/h. Only the higher dose of leptin caused significant sympathoactivation at 4 h, but not at 2 h.

system did not reveal any pressor or depressor effects of leptin (Mitchell et al., 2001). In addition, leptin did not alter the sympathetically mediated vasomotor response to nerve stimulation (Jalali et al., 2001). Our present results of no direct vasodilator actions of leptin are consistent with these previous reports (Mitchell et al., 2001; Jalali et al., 2001; Gardiner et al., 2000).

Because leptin clearly causes widespread sympathoactivation it would be expected that, in the absence of opposing vasodilator actions, leptin would increase arterial pressure. It could be that leptin causes sympathoactivation to metabolically active tissues, but not to the vasculature to increase vasomotor tone. Alternatively, sustained and longer duration of sympathoexcitation might be required for leptin to increase arterial pressure, for example, through its effect on renal sodium reabsorption. This is supported by the significant increase in arterial pressure observed after 6 h of leptin infusion (Correia et al., 2001a) as well as after long-term administration of leptin in rats (Shek et al., 1998; Correia et al., 2001b). Furthermore, hyperleptinemia in agouti obese mice chronically elevates arterial pressure (Mark et al., 1999). Transgenic mice with hepatic overexpression of leptin also have higher arterial pressure than their littermates control and sympathetic blockade normalizes arterial pressure in these transgenic mice (Aizawa-Abe et al., 2000). Finally, absence of leptin in the *ob*/*ob* mice is associated with lower arterial pressure (Mark et al., 1999). Thus, leptin-induced sympathoactivation appears to be physiologically relevant for the control of arterial pressure with probable adverse effects of high circulating levels of this hormone.

Some limitations of the present study need to be addressed. First, we have tested the vascular effects of only 2 doses of acetylcholine and sodium nitroprusside, which might be not sufficient to reveal any vasodilator action of leptin. However, if leptin caused a shift in the acetylcholine and/or sodium nitroprusside dose–response curves this would be revealed at the lower doses used in our study. Second, the vasodilator effect of leptin in vivo might not be powerful enough to be detected in the presence of vasoconstriction from leptin's action to increase sympathetic nerve activity. This is unlikely because we performed these studies in a time range that precede the increase in sympathetic nerve activity induced by leptin. Indeed, iliac artery administration of leptin caused no significant sympathoactivation after 2 h, which correspond to the time range in which our experiments were performed. We have also shown previously that blockade of sympathetic nervous system using α-adrenergic blockers did not reveal any vasodilator action of leptin in vivo (Mitchell et al., 2001). Finally, the experiments were performed 4–5 h after anesthesia. It is possible that such short-term recovery period may have interfered with the responses of the hemodynamic parameters to leptin and other stimuli.

In summary, direct administration of leptin in the iliac artery caused no significant change in blood pressure and hindlimb resistance bed. Leptin also did not affect the increase in iliac blood flow induced by acetylcholine or sodium nitroprusside. All together, our data suggest that leptin does not have direct vasodilator effects in vivo. These findings support the concept that the chronic hemodynamic actions of leptin are likely to be predominantly due to sympathetic activation.

Acknowledgements

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