British Journal of Pharmacology (2000) 130, 805–810

www.nature.com/bjp

Regional haemodynamic effects of recombinant murine or human leptin in conscious rats

*,[1]S.M. Gardiner, [1]P.A. Kemp, [1]J.E. March & [1]T. Bennett

[1]School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 Regional haemodynamic responses to recombinant murine or human leptin were assessed in conscious, chronically-instrumented, male, Long-Evans rats (350–450 g).

2 Human, but not murine, leptin caused a slight hindquarters vasoconstriction, but neither peptide had any effect on mean arterial blood pressure or heart rate.

3 In the presence of the β_2-adrenoceptor antagonist, ICI 118551, a hindquarters vasoconstrictor response to human leptin was not seen, and there was a tachycardia, as there was to murine leptin.

4 The nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester, (L-NAME), did not influence the cardiovascular effects of murine or human leptin.

5 The results indicate that the previously reported sympathoexcitatory effects of murine leptin in anaesthetized rats are not manifest as regional haemodynamic changes in conscious rats, and this is not due to β_2-adrenoceptor-mediated vasodilator mechanisms opposing any vasoconstrictor responses. Moreover, the ability of L-NAME to unmask a pressor effect of murine leptin in anaesthetized rats may not be apparent in the conscious state.

British Journal of Pharmacology (2000) **130,** 805–810

Keywords: Murine leptin; human leptin; nitric oxide; β_2-adrenoceptors; haemodynamics

Abbreviations: L-NAME, N^G-nitro-L-arginine methyl ester; NOS, nitric oxide synthase; OB-R_L, leptin receptor (long form); OB-R_S, leptin receptor (short form)

Introduction

Leptin is the 167 amino acid product, synthesized by adipocytes, in response to activation of the *ob* gene. The structure of leptin is highly conserved across species, with human leptin showing 84 and 83% homology with mouse and rat leptins, respectively (Zhang *et al*., 1994).

Two forms of leptin receptor have been identified, namely, OB-R_L (long form) and OB-R_S (short form (see Tartaglia, 1997, for review)). OB-R_L, which is considered to be the functional receptor, is primarily localized in the hypothalamus, and is responsible for mediating the actions of leptin on food intake and metabolic regulation (Tartaglia, 1997). The OB-R_S subtype(s) of leptin receptor are more widespread than OB-R_L receptors, but the latter are expressed at higher levels than the former (Tartaglia, 1997). It has been suggested that OB-R_S receptors may serve transport and/or clearance functions. Additionally, OB-R_S receptors may be involved in the mediation of some peripheral actions of leptin, e.g., in the kidney (Serradiel-Le Gal *et al*., 1997) and pancreas (Kieffer *et al*., 1997). It is notable that activation of ATP-sensitive K^+ channels is the final step in some hypothalamic (Spanswick *et al*., 1997) and pancreatic (Keiffer *et al*., 1997) actions of leptin.

Following the initial reports of the involvement of leptin in the regulation of energy metabolism, it has been established that this peptide may influence a variety of other processes, such as immune responses (e.g., Loffreda *et al*., 1998; Lord *et al*., 1998) and different aspects of cardiovascular regulation, including blood pressure control (Dunbar *et al*., 1997; Haynes *et al*., 1997a,b; 1999; Casto *et al*., 1998; Shek *et al*., 1998; Mark *et al*., 1999) and angiogenesis (Sierra-Honigmann *et al*., 1998; Bouloumié *et al*., 1998).

Concerning the former action, Dunbar *et al*. (1997) reported that central (i.c.v.) administration of leptin (of unspecified type) into anaesthetized male, Wistar rats caused a slow, progressive increase in mean systemic arterial blood pressure (up to about 10% above basal after 30 min). There was constriction in the iliac and superior mesenteric, but not in renal, vascular beds. The absence of renal vasoconstriction was surprising since renal, as well as lumbar, sympathetic nerve activity was increased (Dunbar *et al*., 1997).

Haynes *et al*. (1997a) reported that peripheral (i.v.) administration of recombinant murine leptin (up to 1 mg kg^{-1} over 3 h) in anaesthetized, male Sprague-Dawley rats caused an increase in sympathetic nerve activity to kidney, interscapular brown adipose tissue, hindlimb and adrenal gland, yet there were no significant changes in systolic or diastolic blood pressures or heart rate. In the light of these findings, Haynes *et al*. (1997a) raised two unresolved possibilities to explain the apparent lack of cardiovascular effects of leptin, in spite of its ability to cause widespread sympathoadrenal excitation, namely: (1) leptin-induced sympathoadrenal excitation influences metabolic functions, but not vascular tone, and/or (2) leptin has other effects which oppose the expected vasoconstrictor actions of increased sympathetic outflow. In the latter context, Haynes *et al*. (1997a) pointed out that leptin-induced diuresis (Serradeil Le-Gal *et al*., 1997; Jackson & Li, 1997), resulting in volume depletion, could offset the anticipated pressor effects of widespread vasoconstriction.

Against the background of these various findings, we hypothesized that i.v. leptin would cause concurrent constriction and dilatation in different vascular beds, resulting in no overall change in total peripheral conductance. Thus, our first objective was to test this hypothesis by measuring the effects of recombinant murine leptin on mean arterial blood pressure and renal, mesenteric and hindquarters haemodynamics in conscious rats. For comparison, we studied the effects also of recombinant human leptin in a separate group of animals, since rat leptin was not available.

*Author for correspondence.

Since Haynes *et al*. (1997a) found murine leptin caused a marked increase in adrenal sympathetic nerve activity, we speculated that adrenaline-induced, β_2-adrenoceptor-mediated, hindquarters vasodilatation (Gardiner & Bennett, 1988; Gardiner *et al*., 1991a,b; 1992) could contribute to the haemodynamic effects of leptin, particularly since very recent data indicate leptin can directly stimulate catecholamine secretion from cultured adrenal chromaffin cells (Takekoshi *et al*., 1999). Therefore, we assessed also the haemodynamic effects of murine or human leptin in the presence of the β_2-adrenoceptor antagonist, ICI 118551 (Bilski *et al*., 1983; Gardiner & Bennett, 1988; Gardiner *et al*., 1992).

While this work was in progress, Frühbeck (1999) reported that recombinant murine leptin caused an increase in mean arterial blood pressure and heart rate in anaesthetized rats, but only in the presence of the nitric oxide synthase (NOS) inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME). So, in a third experiment we compared the haemodynamic effects of recombinant murine leptin in the absence or presence of L-NAME, in the same conscious rats. Some of the results have been presented to the British Pharmacological Society (Gardiner *et al*., 1998a).

Methods

All experiments were performed on male, Long-Evans rats (350–450 g) bred in the Biomedical Services Unit in Nottingham. All surgery was performed under sodium methohexitone anaesthesia (40–60 mg kg^{-1} i.p., supplemented as required). Initially, pulsed Doppler probes were implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor changes in hindquarters flow). Seven to 14 days later, animals were anaesthetized (as above) and intravascular catheters were implanted (i.a. and i.v.). All procedures have been published in detail (Gardiner *et al*., 1990; 1991a,b; 1998b). At least 24 h after catheter implantation the following experiments were performed with the rats conscious and unrestrained.

Effects of recombinant murine leptin

On the first experimental day, rats ($n=6$) were given i.v. murine leptin in a total dose of 1 mg kg^{-1}, administered as a bolus (0.5 mg kg^{-1}) followed by a 3 h infusion (167 μg kg^{-1} h^{-1}), as used by Haynes *et al*. (1997a). On the second experimental day, the same animals were given i.v. saline (0.1 ml bolus, 0.4 ml h^{-1} infusion for 3 h). On the third experimental day, the same experimental animals were given murine leptin (as above) in the presence of ICI 118551 (0.2 mg kg^{-1}, 0.1 mg kg^{-1} h^{-1}; Gardiner *et al*., 1992), starting 30 min before leptin administration (as above).

We chose the highest dose of murine leptin used by Haynes *et al*. (1997a) and, like them, found it had no significant pressor action and little effect on heart rate (see Results). However, since we did not monitor sympathetic nerve activity, it is feasible that this dose of murine leptin is without any influence in conscious rats, possibly due to pharmacokinetic and/or pharmacodynamic differences in the conscious and anaesthetized states. Therefore, in additional animals ($n=5$) we administered murine leptin in a bolus dose of 1 mg kg^{-1} (i.e., twice the dose above as a bolus). Higher doses of murine leptin would have required prohibitively expensive amounts of peptide, and, moreover, may have resulted in grossly supraphysiological levels (see Discussion).

Effects of recombinant human leptin

On the first experimental day, rats ($n=6$) were given human leptin (dose as above for murine leptin). These animals were given saline on the second experimental day (as above). In order to control for possible effects of prior exposure to leptin, in this experiment, naive animals ($n=6$) were given human leptin (dose as above) in the presence of ICI 118551 (as above).

Effects of recombinant murine leptin in the absence or presence of L-NAME

Animals ($n=7$) were given murine leptin (i.v. bolus, 0.1 mg kg^{-1}) in the absence, or 2 h after the onset of infusion of L-NAME (3 mg kg^{-1} h^{-1}; Gardiner *et al*., 1991b; 1998b). The dose of leptin in this protocol was matched to that of Frühbeck (1999). As a time control for this experiment, rats ($n=6$) were given i.v. saline (0.1 ml bolus) 2 h after the onset of L-NAME infusion (as above).

Data analysis

Variables were monitored for a 30 min baseline period, and for up to 3 h after the onset of leptin administration. Measurements were made at appropriate time points to allow comparisons with the results reported by Haynes *et al*. (1997a) and Frühbeck (1999).

Within-group analysis was by Friedman's test, and between-group analysis by the Wilcoxon test or Mann–Whitney *U*-test, as appropriate; a P value <0.05 was taken as significant.

Peptides and drugs

The recombinant murine and human leptin used in the first two experiments were obtained from R&D Systems (Abingdon, U.K.). Both peptide preparations contained <0.1 ng endotoxin per 1 μg of peptide, and showed the appropriate functional effects on feeding behaviour and body weight (R&D Systems technical data sheet).

The recombinant murine leptin used in the experiment to assess the influence of L-NAME was obtained from Peprotech (London, U.K.), since this was the source of the murine leptin used by Frühbeck (1999) whose experimental protocol we were following.

Leptins were solubilized in sterile saline (154 mmol l^{-1} NaCl) containing 1% bovine serum albumin (BSA).

L-NAME and BSA were obtained from Sigma (U.K.); ICI 118551 was a gift from ICI Pharmaceuticals plc. L-NAME and ICI 118551 were dissolved in sterile saline (the latter with gentle warming).

Results

Haemodynamic effects of recombinant murine leptin

There were no differences in resting cardiovascular variables prior to infusion of saline or murine leptin (Table 1).

During the 3 h infusion of murine leptin, the cardiovascular changes were not different to those seen during infusion of saline (Figure 1).

In five additional animals, given murine leptin in a bolus dose of 1 mg kg^{-1} (i.e., twice that given as a bolus in the primed infusion above), there were no significant haemody-

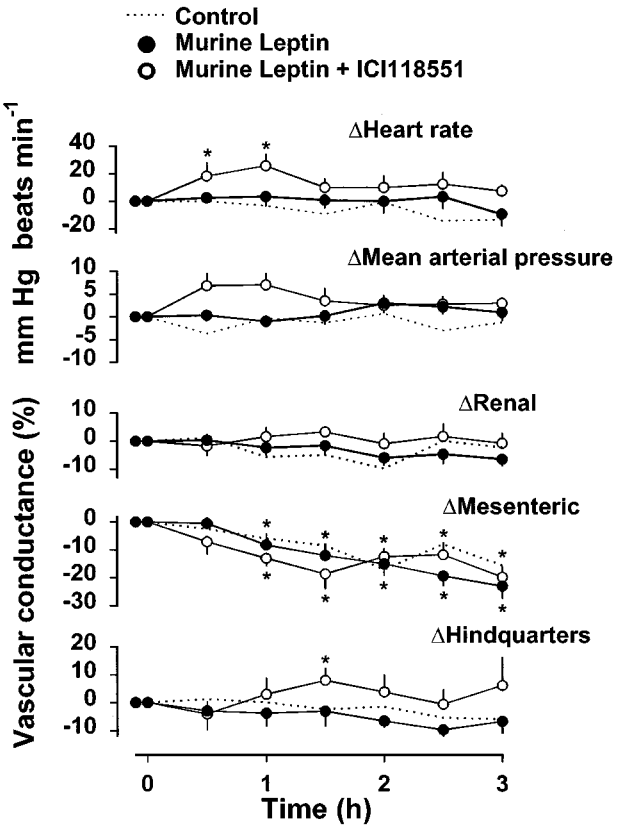

Figure 1 Cardiovascular changes during infusion of saline (control), or murine leptin, or murine leptin in the presence of ICI 118551, in the same conscious Long Evans rats ($n=6$). Values are mean and vertical bars show s.e. mean; $*P<0.05$ versus baseline for the variables indicated (Friedman's test).

namic changes other than reductions in mesenteric flow and vascular conductance (Table 2), as observed in animals receiving saline and murine leptin at the lower dose (Figure 1).

The integrated cardiovascular changes during the 3 h infusion of murine leptin were not significantly different in the absence and presence of ICI 118551 (Figure 1), although in the latter condition there was a slight and transient tachycardia and hindquarters vasodilatation. Even when values in the two conditions were compared (on a paired basis) at 1.5 and 3 h (i.e. times when the apparent separation in hindquarters vascular conductances was greatest) there was no statistically significant difference.

Haemodynamic effects of recombinant human leptin

There were no significant differences in resting cardiovascular variables prior to infusion of saline or human leptin (Table 3).

During the 3 h infusion of human leptin there was a progressive hindquarters vasoconstriction that was not seen during saline infusion (Figure 2), but no other changes were different in the two conditions (Figure 2).

In the presence of ICI 118551, human leptin caused a tachycardia, and this effect was significantly different from the heart rate profile in the absence of ICI 118551 (Figure 2). Although there was no hindquarters vasoconstriction to human leptin in the presence of ICI 118551, the integrated change in hindquarters vascular conductance (over 3 h) was not different in this condition compared to the vasoconstriction seen during infusion of human leptin in the absence of ICI 118551 (Figure 2). However, at the 3 h time point there was a significant difference ($P<0.05$, Mann–Whitney U-test) in the change of hindquarters vascular conductance in the two groups.

Table 1 Resting cardiovascular variables in conscious Long-Evans rats prior to infusion of murine leptin (Day 1) or saline (Day 2)

	Heart rate (beats min^{-1})	*Mean blood pressure* (mm Hg)	*Vascular conductance* ([kHz $mmHg^{-1}$]10^3) *Renal*	*Mesenteric*	*Hindquarters*
Day 1	331±10	99±2	105±13	88±8	42±4
Day 2	321±4	100±1	95±11	76±6	33±3

Values are mean±s.e.mean; $n=6$.

Table 2 Cardiovascular variables before and following bolus (1 mg kg^{-1}) injection of murine leptin in conscious, Long-Evans rats

	Heart rate (beats min^{-1})	*Mean blood pressure* (mm Hg)	*Vascular conductance* ([kHz $mmHg^{-1}$]10^3) *Renal*	*Mesenteric*	*Hindquarters*
Baseline	355±12	108±3	97±16	66±7	45±4
0.5 h	351±18	108±3	95±17	65±8	46±3
1.0 h	362±21	111±3	94±18	60±7*	44±2
1.5 h	343±11	106±2	96±17	60±6*	46±5
2.0 h	349±12	108±2	96±17	61±6*	43±3
2.5 h	352±11	111±4	92±16	60±7*	42±4
3.0 h	349±16	110±2	93±18	62±9	42±3

Values are mean±s.e.mean; $*P<0.05$ versus baseline value (Friedman's test); $n=5$

Table 3 Resting cardiovascular variables in conscious Long-Evans rats prior to infusion of human leptin (Day 1) or saline (Day 2)

	Heart rate (beats min^{-1})	*Mean blood pressure* (mm Hg)	*Vascular conductance* ([kHz $mmHg^{-1}$]10^3) *Renal*	*Mesenteric*	*Hindquarters*
Day 1	318±4	101±2	62±5	65±3	43±4
Day 2	303±6	100±2	72±7	62±4	36±5

Values are mean±s.e.mean; $n=6$

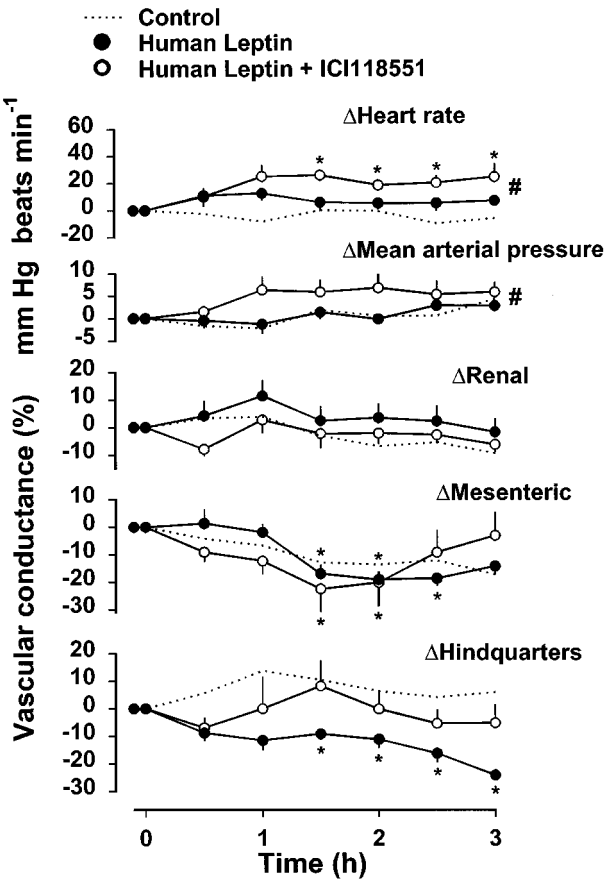

Figure 2 Cardiovascular changes during infusion of saline (control), or human leptin, or human leptin in the presence of ICI 118551. The same conscious, Long Evans rats ($n=6$) were given saline and human leptin, but human leptin in the presence of ICI 118551 ($n=6$) was administered to a group of naive rats. Values are mean and vertical bars show s.e. mean; $*P<0.05$ versus baseline for the variables indicated (Friedman's test); $\#P<0.05$ for the difference between the integrated responses (areas) in the absence and presence of ICI 118551 (Mann–Whitney *U*-test).

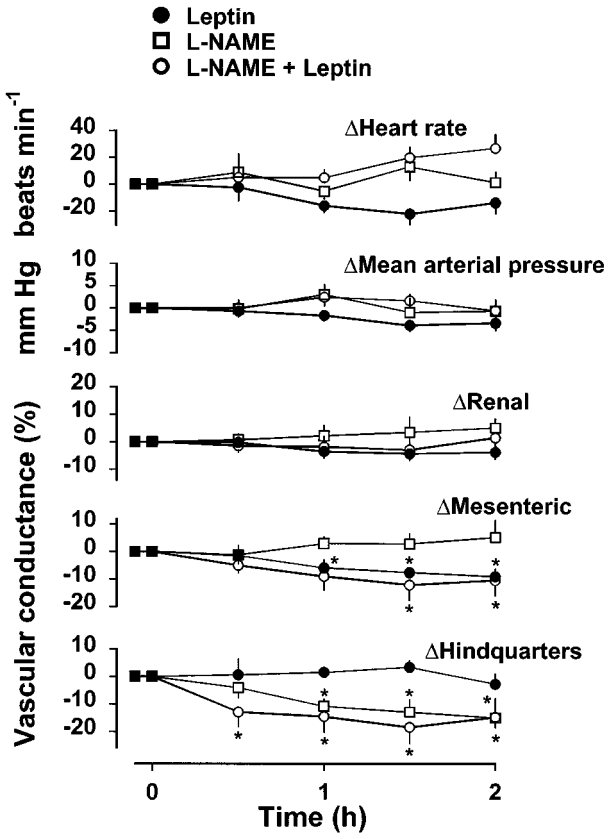

Figure 3 Cardiovascular changes following administration of murine leptin in the absence or presence of L-NAME in the same conscious Long Evans rats ($n=7$). A separate group of rats ($n=6$) was given L-NAME and saline as a time control. Values are mean and vertical bars show s.e.mean; $*P<0.05$ versus baseline for the variables indicated (Friedman's test).

Effects of recombinant murine leptin in the absence or presence of L-NAME

In the absence of L-NAME, murine leptin, as in the previous experiment (see above), had no pressor action, and no vasoconstrictor effects (Figure 3) that were different from those of saline (Figure 2). However, in the presence of L-NAME, there was a hindquarters vasoconstriction during infusion of murine leptin (Figure 3), but the change in hindquarters vascular conductance was not different to that seen in the presence of L-NAME alone (Figure 3), and there was no additional change in mean arterial blood pressure (Figure 3).

The failure of L-NAME to unmask pressor and/or vasoconstrictor effects of murine leptin was in spite of the substantial changes in cardiovascular variables caused by L-NAME itself (Figure 4).

Discussion

The experiments reported herein demonstrate that the absence of a pressor effect of murine leptin, in spite of its ability to cause widespread sympathoexcitation (Haynes *et al*., 1997a,b; 1999; Mark *et al*., 1999), is not due to concurrent vasoconstriction and vasodilatation in different vascular beds. Even in the case of recombinant human leptin, which appeared to cause a selective, but modest, vasoconstriction in the hindquarters, there was no accompanying change in systemic arterial blood pressure. Moreover, we were unable to reproduce the recent finding that a pressor action of recombinant murine leptin was unmasked in the presence of the NOS inhibitor, L-NAME (Frühbeck, 1999). However, our finding that murine leptin had no pressor effect in conscious rats is consistent with the observations made by Haynes *et al*. (1997a) and Frühbeck (1999) in anaesthetized rats.

Concern about the possibility that conscious rats may be substantially less sensitive than anaesthetized rats to the actions of leptin prompted us to assess the effects of a bolus dose (1 mg kg^{-1}) of murine leptin which was twice the highest bolus dose used by Haynes *et al*. (1997a). We saw no significant haemodynamic effects of murine leptin at this higher dose, but cost prevented us from giving even higher doses. If the explanation for our failure to observe any haemodynamic effects of murine leptin in conscious rats was due to subsensitivity to the peptide (on account of pharmacokinetic and/or pharmacodynamic differences in the conscious and anaesthetized state), then it appears the dose response relation is shifted at least 200 fold (since Haynes *et al*. (1997a) saw effects on sympathetic nerve activity at 0.1 mg kg^{-1}).

In the experiments of Haynes *et al*. (1997a), it was suggested that a murine leptin-induced diuresis (causing volume depletion) might offset the anticipated pressor effects of widespread vasoconstriction. However, it would be surprising if these two effects were so finely matched that no change in systemic arterial blood pressure was apparent. Furthermore, if murine leptin was

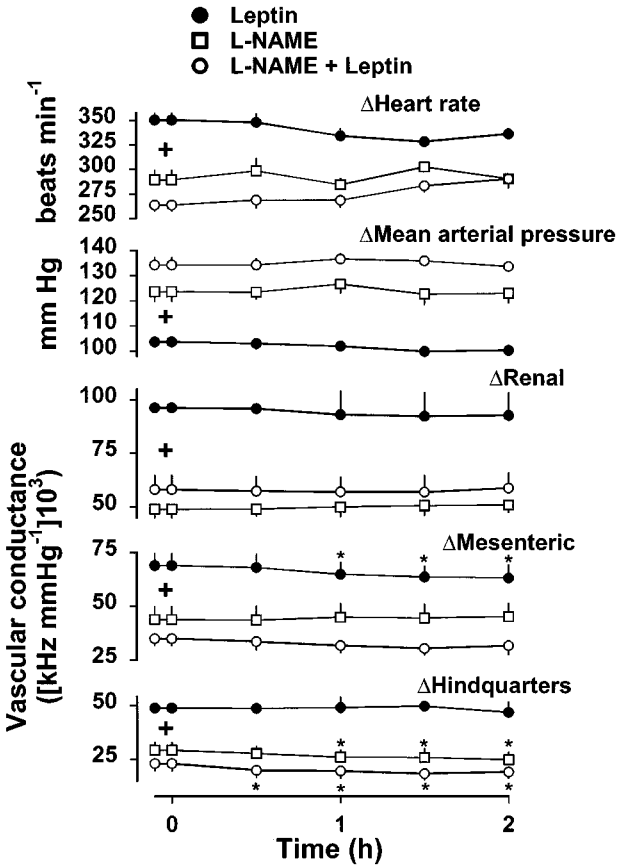

Figure 4 Absolute values for cardiovascular variables in the experiment for which normalised values are shown in Figure 3. L-NAME had substantial pressor and bradycardiac effects, accompanied by reductions in renal, mesenteric and hindquarters vascular conductances. Values are mean; $+P<0.05$ for resting values in the absence and presence of L-NAME; $*P<0.05$ versus resting value for the variables indicated (Friedman's test).

acting to cause volume depletion and sympathoexcitation concurrently, both these effects should have resulted in reduced peripheral blood flow, yet in renal, mesenteric and hindquarters vascular beds no such changes were seen. In this context it is notable that human leptin has been found to have greater natriuretic/diuretic effects than either mouse or rat leptin in the anaesthetized rat (Jackson & Li, 1997; Jackson & Herzer, 1999), although such findings may depend on the experimental protocol (Serradeil Le-Gal *et al*., 1997; Villareal *et al*., 1998). These disparities indicate a dissociation between any putative cardiovascular and renal actions of murine, human and rat leptin. However, we did observe a vasoconstrictor (hindquarters) response to human leptin which, it could be argued, is consistent with this peptide causing a greater volume depletion. But, in previous studies we have shown that volume depletion causes substantial constriction in renal, mesenteric and hindquarters vascular beds (Gardiner *et al*., 1989), so the apparently selective hindquarters vasoconstrictor effects of human leptin is not the expected response to volume depletion. Whatever the explanation of this observation, the finding of a significant hindquarters vasoconstrictor response to human leptin, in the absence of any pressor action, indicates that the dose of the peptide used was exerting a functional effect.

Haynes *et al*. (1997a) also suggested that the sympathoexcitation caused by murine leptin might be directed at metabolic, rather than vascular, targets. The actions of leptin to increase core temperature, metabolic rate (Pelleymounter *et al*., 1995) and energy expenditure (Halaas *et al*., 1995) would be expected to influence vascular tone, most likely causing vasodilatation. Thus, it could be argued that the tendency of leptin to cause vasodilatation, through its metabolic actions, balanced its expected vasoconstrictor action (through its sympathoexcitatory influence). As mentioned above, however, it would be surprising if such opposing actions resulted in no measurable change in any variable.

Since murine leptin clearly influences sympathetic outlow to the adrenal gland (Haynes *et al*., 1997a) and causes the release of adrenaline and noradrenaline from cultured porcine adrenal medullary chromaffin cells (Takekoshi *et al*., 1999), we anticipated that β_2-adrenoceptor-mediated vasodilatation in the hindquarters (Gardiner *et al*., 1991a,b; 1992) might be masking any pressor effect of constriction caused by leptin in other vascular beds. In other experiments (Bennett *et al*., 1989) we have shown that the β_2-adrenoceptor antagonist, ICI 118551, is capable of revealing pressor and vasoconstrictor effects of interventions (such as insulin-induced hypoglycaemia), which otherwise appear to have little cardiovascular influence. Notwithstanding our expectations, in the presence of ICI 118551, neither murine nor human leptin had any greater vasoconstrictor or pressor effect than in its absence. Hence, we could find no evidence for β_2-adrenoceptor-mediated vasodilator events being recruited by leptin. On the contrary, in the presence of ICI 118551, murine leptin caused a slight tachycardia and hindquarters vasodilatation, while human leptin caused a clear tachycardia, and no hindquarters vasoconstriction (which was seen in the absence of ICI 118551). These apparent effects are not straightforward to explain, since they are directionally opposite to those predicted on the basis of an involvement of β_2-adrenoceptor-mediated events in the responses to murine or human leptin. However, the fact that some significant responses to murine leptin occurred under these conditions tends to argue against the possibility that we failed to use sufficient peptide to exert any action (see above).

We had performed the experiments, and obtained the results described above, before the publication of the paper by Frühbeck (1999), in which it was reported that release of NO opposes the pressor effect of sympathoexcitation caused by murine leptin. Clearly, it was important to attempt to reproduce the findings of Frühbeck (1999) in our experimental model. In spite of using recombinant murine leptin from the same source as that used by Frühbeck (1999), and using L-NAME treatment which we have demonstrated to have substantial effects on haemodynamic status and responses to vasodilators that modulate NO release (Gardiner *et al*., 1990; 1991a,b; 1992; 1998b), we were unable to show that L-NAME unmasked vasoconstrictor or pressor actions of murine leptin.

In conclusion, in conscious, unrestrained, male, Long Evans rats, recombinant murine or human leptin, given systemically, are without pressor actions, even when β_2-adrenoceptors are antagonized, or NO production is inhibited. Our findings extend previous observations by showing that the lack of an acute pressor action of murine or human leptin, is not due to concurrent activation of opposing vasodilator and vasoconstrictor mechanisms.

References

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1989). Regional haemodynamic effects of hypoglycaemia in conscious, Long-Evans rats. *J. Physiol.,* **416,** 57P.

BILSKI, A.J., HALLIDAY, S.E., FITZGERALD, J.D. & WALE, J.L. (1983). The pharmacology of a β_2-selective adrenoceptor antagonist (ICI 118551). *J. Cardiovasc. Pharmacol.,* **5,** 430–437.

BOULOUMIÉ, A., DREXLER, H.C.A., LAFONTAN, M. & BUSSE, R. (1998). Leptin, the product of Ob gene, promotes angiogenesis. *Circ. Res.,* **83,** 1059–1066.

CASTO, R.M., VANNESS, J.M. & OVERTON, J.M. (1998). Effects of central leptin administration on blood pressure in normotensive rats. *Neurosci. Lett.,* **246,** 29–32.

DUNBAR, J.C., HU, Y. & LU, H. (1997). Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes,* **46,** 2040–2043.

FRÜHBECK, G. (1999). Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes,* **48,** 903–908.

GARDINER, S.M. & BENNETT, T. (1988). Regional hemodynamic responses to adrenoceptor antagonism in conscious rats. *Am. J. Physiol.,* **255,** H813–H824.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989). Regional hemodynamic changes following hypovolemia in conscious rats. *Am. J. Physiol.,* **256,** R1076–R1083.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990). Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats; effects of N^G-nitro-L-arginine methyl ester. *Br. J. Pharmacol.,* **101,** 632–639.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991a). Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to acetylcholine, 5′-N-ethylcarboxamidoadenosine or salbutamol in conscious rats. *Br. J. Pharmacol.,* **103,** 1725–1732.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991b). Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to adrenaline or BRL 38227 in conscious rats. *Br. J. Pharmacol.,* **104,** 731–737.

GARDINER, S.M., KEMP, P.A., BENNETT, T., BOSE, C., FOULKES, R. & HUGHES, B. (1992). Involvement of β_2-adrenoceptors in the regional haemodynamic responses to bradykinin in conscious rats. *Br. J. Pharmacol.,* **105,** 839–848.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1998a). Recombinant human, but not murine, leptin causes hindquarters vasoconstriction in conscious rats. *Br. J. Pharmacol.,* **125,** 91P.

GARDINER, S.M., MARCH, J.E., KEMP, P.A. & BENNETT, T. (1998b). The contribution of nitric oxide to cardiovascular status and responses to vasodilators in conscious, hypertensive, transgenic ((mRen-2)27) rats. *Br. J. Pharmacol.,* **124,** 299–306.

HALAAS, J.L., GAJIWALLA, K.S., MAFFEI, M., COHEN, S.L., CHAIT, B.T., RABINOWITZ, D., LALLONE, R.L., BURLEY, S.K. & FRIEDMAN, J.M. (1995). Weight reducing effects of the plasma protein encoded by the obese gene. *Science,* **269,** 543–546.

HAYNES, W.G., MORGAN, D.A., DJALALI, A., SIVITZ, W.I. & MARK, A.L. (1999). Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. *Hypertension,* **33,** 542–547.

HAYNES, W.G., MORGAN, D.A., WALSH, S.A., MARK, A.L. & SIVITZ, W.I. (1997a). Receptor-mediated regional sympathetic nerve activation by leptin. *J. Clin. Invest.,* **100,** 270–278.

HAYNES, W.G., SIVITZ, W.I., MORGAN, D.A., WALSH, A. & MARK, A.L. (1997b). Sympathetic and cardiorenal actions of leptin. *Hypertension,* **30,** 619–623.

JACKSON, E.K. & HERZER, W.A. (1999). A comparison of the natriuretic/diuretic effects of rat vs. human leptin in the rat. *Am. J. Physiol.,* **277,** F761–F765.

JACKSON, E.K. & LI, P. (1997). Human leptin has natriuretic activity in the rat. *Am. J. Physiol.,* **272,** F333–F338.

KIEFFER, T.J., HELLER, R.S., LEECH, C.A., HOLTZ, G.G. & HABENER, J.F. (1997). Leptin suppression of insulin secretion by the activation of ATP-sensitive K^+ channels in pancreatic β-cells. *Diabetes,* **46,** 1087–1093.

LOFFREDA, S., YANG, S.Q., LIN, H.Z., KARP, C.L., BRENGMAN, M.L., WANG, D.J., DLEIN, A.S., BULKLEY, G.B., BAO, C., NOBLE, P.W., LANE, M.D. & DIEHL, A.M. (1998). Leptin regulates proinflammatory immune responses. *FASEB J.,* **12,** 57–65.

LORD, G.M., MATARESE, G., HOWARD, J.K., BAKER, R.J., BLOOM, S.R. & LECHLER, R.I. (1998). Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature,* **394,** 897–901.

MARK, A.L., CORREIA, M., MORGAN, D.A., SHAFFER, R.A. & HAYNES, W.G. (1999). Obesity-induced hypertension: new concepts from the emerging biology of obesity. *Hypertension,* **33,** 537–541.

PELLEYMOUNTER, M.A., CULLEN, M.J., BAKER, M.B., HECHT, R., WINTERS, D., BOONE, T. & COLLINS, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science,* **269,** 540–543.

SERRADEIL LE-GAL, C., RAUFASTE, D., BROSSARD, G., POUZET, B., MARTY, E., MAFFRAND, J.-P. & LE FUR, G. (1997). Characterization and localization of leptin receptors in the rat kidney. *FEBS Lett.,* **404,** 185–191.

SHEK, E.W., BRANDS, M.W. & HALL, J.E. (1998). Chronic leptin infusion increases arterial pressure. *Hypertension,* **31,** 409–414.

SIERRA-HONIGMANN, M.R., NATH, A.K., MURAKAMI, C., GARCÍA-CARDEÑA, G., PAPAPETROPOULOS, A., SESSA, W.C., MADGE, L.A., SCHECHNER, J.S., SCHWABB, M.B., POLVERINI, P.J. & FLORES-RIVEROS, J.R. (1998). Biological action of leptin as an angiogenic factor. *Science,* **281,** 1683–1686.

SPANSWICK, D., SMITH, M.A., GROPPI, V.E., LOGAN, S.D. & ASHFORD, M.L.J. (1997). Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature,* **390,** 521–525.

TAKEKOSHI, K., MOTOOKA, M., ISOBE, K., NOMURA, F., MANMOKU, T., ISHII, K. & NAKAI, T. (1999). Lepin directly stimulates catecholamine secretion and synthesis in cultured porcine adrenal medullary chromaffin cells. *Biochem. Biophys. Res. Commun.,* **261,** 426–431.

TARTAGLIA, L.A. (1997). The leptin receptor. *J. Biol. Chem.,* **272,** 6093–6096.

VILLARREAL, D., REAMS, G., FREEMAN, R.H. & TARABEN, A. (1998). Renal effects of leptin in normotensive, hypertensive, and obese rats. *Am. J. Physiol.,* **275,** F2056–F2060.

ZHANG, Y., PROENCA, R., MAFFEI, M., BARONE, M., LEOPOLD, L. & FRIEDMAN, J.M. (1994). Positional cloning of the mouse *obese* gene and its human homologue. *Nature,* **372,** 425–432.

(Received February 16, 2000
Accepted March 22, 2000)