RESEARCH PAPER

Possible involvement of GLP-1(9–36) in the regional haemodynamic effects of GLP-1(7–36) in conscious rats

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BACKGROUND AND PURPOSE
The incretin hormone, glucagon-like peptide (GLP)-1(7–36), is rapidly cleaved by dipeptidyl peptidase 4 (DPP-4) into GLP-1(9–36), and although it is agreed that most, if not all, of the metabolic effects are attributable to the intact peptide, the degree to which the cardiovascular effects are due to the cleavage product is unclear. The purpose of this study was to measure the regional haemodynamic effects of GLP-1(7–36), and determine the extent to which the cardiovascular effects of GLP-1(7–36) were influenced by DPP-4 inhibition and reproduced by GLP-1(9–36). Additional experiments investigated the involvement of autonomic mechanisms in the cardiovascular effects of GLP-1(7–36).

EXPERIMENTAL APPROACH
Regional haemodynamic effects of bolus doses and 4 h infusions of GLP-1(7–36) amide and GLP-1(9–36) amide were measured in conscious, chronically instrumented rats; the influence of DPP-4 inhibition and autonomic blockade on responses to GLP-1(7–36) were also assessed.

KEY RESULTS
Glucagon-like peptide-1(7–36) had clear regional haemodynamic effects comprising tachycardia, a rise in blood pressure, renal and mesenteric vasoconstriction and hindquarters vasodilatation, whereas GLP-1(9–36) was devoid of any cardiovascular actions. The effects of GLP-1(7–36) were enhanced by DPP-4 inhibition, and the tachycardia and hindquarters vasodilatation were β-adrenoceptor-mediated.

CONCLUSIONS AND IMPLICATIONS
In conscious rats, the cardiovascular effects of GLP-1(7–36) resemble those of the GLP analogue, exendin-4, and are attributable to the intact peptide rather than the cleavage product, GLP-1(9–36).

Abbreviations
DPP-4, dipeptidyl peptidase 4; GLP, glucagon-like peptide; HDAS, haemodynamics data acquisition system; VC, vascular conductance

Introduction

The insulinotropic effects of the gut hormone, glucagon-like peptide-1 (GLP-1), together with its inhibitory actions on glucagon secretion, gastric emptying, appetite and food intake, are well estab-lished and formed the basis for the development of novel therapeutic strategies for the treatment of type 2 diabetes (for review see Drucker and Nauck, 2006). GLP-1 derives from post-translational pro-cessing of proglucagon and exists in two forms, GLP-1(7–37) and GLP-1(7–36) amide, the latter

being the most abundant in the circulation. Release of GLP-1(7–36) in response to feeding is extremely rapid, but circulating levels then quickly fall (half-life 1–2 min), mainly due to enzymatic cleavage of the two N-terminal amino acids by dipeptidyl peptidase 4 (DPP-4), to form GLP-1(9–36) (Deacon *et al.*, 1995). Therefore, the therapeutic potential of native GLP-1(7–36) is limited, whereas DPP-4 inhibitors, or long-acting GLP-1 mimetics, are showing great clinical potential (Nielsen, 2005).

Glucagon-like peptide-1(7–36) binds with high affinity to the GLP-1 receptor and the beneficial glucoregulatory actions of GLP-1(7–36) are mostly, if not exclusively, GLP-1 receptor-mediated. The cleavage product of DPP-4 action, namely GLP-1(9–36), has very low affinity for the GLP-1 receptor [0.95% of the affinity of GLP-1(7–36) (Knudsen and Pridal, 1996)] and has no insulinotropic activity *per se*. Indeed there are reports of antagonist properties of GLP-1(9–36) at the GLP-1 receptor, although these may only be apparent under *in vitro* conditions (Knudsen and Pridal, 1996). However, GLP-1(9–36) may not be completely devoid of glucoregulatory actions inasmuch as some investigators (Deacon *et al.*, 2002, Meier *et al.*, 2006), but not others (Vahl *et al.*, 2003) have demonstrated a glucose-lowering effect of the peptide that was independent of insulin secretion.

In addition to the metabolic effects of GLP-1 analogues, there is increasing evidence in support of beneficial cardiovascular actions of these compounds, including vasorelaxation, protective effects in cardiac ischaemia and improved myocardial contraction in heart failure (for reviews see Grieve *et al.*, 2009; Jax, 2009). However, while it is generally agreed that GLP-1(7–36), acting at the GLP-1 receptor, is largely responsible for the effects on glucoregulation, the extent to which GLP-1(9–36) may be responsible for some, or even all, of the cardiovascular actions is less clear. For example, Nikolaidis *et al.* (2005) showed, in dogs with dilated cardiomyopathy, that even during continuous infusion of GLP-1(7–36) amide, the N-terminal dipeptide cleavage was rapid and so effective that all the measured peptide was in the truncated (i.e. 9–36) form. Furthermore, infusion of GLP-1(9–36) mimicked the effects of GLP-1(7–36) with regard to stimulating myocardial glucose uptake and improving haemodynamic status, leading the authors to conclude that in their experiments, GLP-1(9–36) was the active peptide (Nikolaidis *et al.*, 2005). In contrast, others have reported distinct cardiovascular effects of GLP-1(7–36) and GLP-1(9–36), the former, but not the latter being GLP-1 receptor-dependent (Ban *et al.*, 2008; Ossum *et al.*, 2009). Clearly, it is important to fully understand the cardiovascular actions

of GLP-1(7–36) and GLP-1(9–36) as levels of the latter may be decreased by DPP-4 inhibitors, but increased by GLP-1 analogues.

We have previously reported complex regional haemodynamic actions of the GLP-1 receptor agonist, exendin-4, in conscious rats, many of which were autonomically mediated, but some of which were resistant to GLP-1 receptor antagonism (Gardiner *et al.*, 2006a,b; 2008). Others have reported effects of GLP-1(7–36) administration on blood pressure and heart rate in rats (Barragán *et al.*, 1994; 1996; 1999), but to our knowledge, regional haemodynamic effects of GLP-1(7–36) and GLP-1(9–36) in conscious rats have not been reported. Thus, the aims of the present study were to compare the regional haemodynamic actions of GLP-1(7–36) and GLP-1(9–36), to assess the extent to which the cardiovascular effects of GLP-1(7–36) were affected by DPP-4 inhibition, and to determine the contribution of the autonomic nervous system to the cardiovascular actions of GLP-1(7–36).

Methods

Animals and surgery

Adult, male, Sprague-Dawley rats, 12–15 weeks old and weighing 300–350 g, were housed in groups in a temperature-controlled (21–23°C) environment with a 12 h light–dark cycle (lights on at 0600 h) and free access to food (Teklad Global 18% Protein Rodent Diet, Bicester, Oxon, UK) and water for at least 7 days after arrival from the supplier (Charles River, Margate, Kent, UK) before any surgical intervention.

Surgery was performed in two stages. First, under general anaesthesia (fentanyl and medetomidine, 300 μg·kg^{-1} of each i.p., supplemented as required), miniature pulsed Doppler flow probes were sutured around the left renal artery, the superior mesenteric artery and the distal abdominal aorta (to monitor hindquarters haemodynamics). Following surgery, reversal of anaesthesia and provision of analgesia was achieved with atipamezole (1 mg·kg^{-1} s.c.) and buprenorphine (0.02 mg·kg^{-1} s.c.). Second, at least 10 days after the surgery for probe implantation, and following a satisfactory inspection from the Named Veterinary Surgeon, the animals were again anaesthetized, using the same regime as above, and catheters were implanted in the distal abdominal aorta via the caudal artery (for arterial blood pressure monitoring and the derivation of heart rate), and in the right jugular vein (for peptide and drug administration). Up to three separate intravenous catheters were placed in the jugular vein to enable concurrent administration of different compounds.

Experiments began 24 h after surgery for catheter implantation, with animals fully conscious and unrestrained in home cages, with free access to food and water. All procedures were carried out with approval of the University of Nottingham Local Ethical Review Committee, under Home Office Project and Personal Licence authority.

Cardiovascular recordings

Cardiovascular variables were recorded using a customized, computer-based system [haemodynamics data acquisition system (HDAS), University of Limburg, Maastricht, the Netherlands] connected to a transducer amplifier [Gould (OH, USA) model 13-4615-50] and a Doppler flowmeter [Crystal Biotech (Holliston, MA, USA) VF-1 mainframe (pulse repetition frequency 125 kHz) fitted with high velocity (HVPD-20) modules]. Raw data were sampled by HDAS every 2 ms, averaged every cardiac cycle and stored to disc at 5 s intervals.

Experimental protocols

Experiment 1. Regional haemodynamic responses to infusions or bolus doses of GLP-1(7–36) and GLP-1(9–36). One group of rats ($n = 7$) was given a 4 h i.v. infusion of either vehicle [sterile saline containing 1% bovine serum albumin (BSA), 0.4 mL·h^{-1}] or GLP-1(7–36) (6 pmol·kg^{-1}·min^{-1}) on day 1, *vice versa* on day 2, and GLP-1(7–36) (60 pmol·kg^{-1}·min^{-1}) on day 3. Cardiovascular recordings were made throughout the infusions and for 2 h after the infusions had stopped. On the last experimental day (day 4), rats were given i.v. bolus doses (0.75 and 3 nmol·kg^{-1}) of GLP-1(7–36) with at least 60 min between doses. The above protocol was followed in a separate group of rats ($n = 5$) given GLP-1(9–36) instead of GLP-1(7–36).

Experiment 2. Effects of DPP-4 inhibition on the regional haemodynamic responses to GLP-1(7–36). One group of rats was used for this experiment ($n = 10$), which ran over two experimental days. On the first day, three bolus doses of GLP-1(7–36) (0.3, 0.75 and 3 nmol·kg^{-1}) were given at 90 min intervals, 10 min following administration of either the vehicle for the DPP-4 inhibitor (0.1 mL sterile saline) ($n = 5$) or the DPP-4 inhibitor, DPPI 1c (1 mg·kg^{-1}; Wright *et al.*, 2006) ($n = 5$), with the alternative pretreatment being given on day 2.

Experiment 3. Effects of adrenoceptor antagonism on the regional haemodynamic responses to GLP-1(7–36). One group of rats was used for this experiment ($n = 7$), which ran over four experimental days. Rats were pretreated with i.v. saline (0.1 mL bolus, 0.4 mL·h^{-1} continuous infusion) on day 1, the β$_2$-adrenoceptor-selective antagonist, ICI 118551 (0.2 mg·kg^{-1}, 0.1 mg·kg^{-1}·h^{-1}) on day 2, the non-selective β-adrenoceptor antagonist, propranolol (1 mg·kg^{-1}, 0.5 mg·kg^{-1}·h^{-1}) on day 3, and propranolol plus the non-selective α-adrenoceptor antagonist, phentolamine (1 mg·kg^{-1}, 1 mg·kg^{-1}·h^{-1}) on day 4, starting 90 min before bolus administration of two doses of GLP-1(7–36) (0.75 and 3 nmol·kg^{-1}), separated by 90 min. The effectiveness of these doses of adrenoceptor antagonists has been shown elsewhere (Woolard *et al.*, 2004; Gardiner *et al.*, 2006a)

Data analysis

Data were analysed offline using software (Datview, University of Limburg, Maastricht, the Netherlands), which interfaced with HDAS. Because the data did not always fit a normal distribution, a non-parametric, two-way analysis of variance (Friedman's test; Theodorsson-Norheim, 1987) was used for within-group comparisons, and Mann–Whitney or Kruskal-Wallis (unpaired) or Wilcoxon's (paired) tests for between-group comparisons, as appropriate. Vascular conductance (VC) was calculated from the blood pressure and Doppler shift (flow) data. $P \leq 0.05$ was taken as significant.

Materials

Glucagon-like peptide-1(7–36) amide (lot number 2000763) and GLP-1(9–36) amide (lot number 2000664) were from Bachem (St Helens, UK); ICI 118551 [(+/−)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol) hydrochloride], propranolol [(RS)-1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol) hydrochloride] and DPPI 1c (1-[[[(1-hydroxymethyl)cyclopentyl]amino]acetyl]-2,5-*cis*-pyrrolidinedicarbonitrile hydrochloride) were from Tocris (Avonmouth, UK); phentolamine mesylate was from Sigma (Dorset, UK). Stock solutions of GLP-(7–36) and (9–36) were made up in sterile water for injection, and diluted in sterile saline containing 1% BSA. All drugs were dissolved in sterile water for injection. Injection volumes were 0.1 mL, and infusion rates were 0.4 mL·h^{-1}.

Fentanyl citrate was from Janssen-Cilag (High-Wycombe, UK); medetomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were from Pfizer (Sandwich, Kent, UK); buprenorphine (Vetergesic) was from Alstoe Animal Health (York, UK).

Drug/molecular target nomenclature conforms with the British Journal of Pharmacology Guide to Receptors and Channels (Alexander *et al.*, 2009).
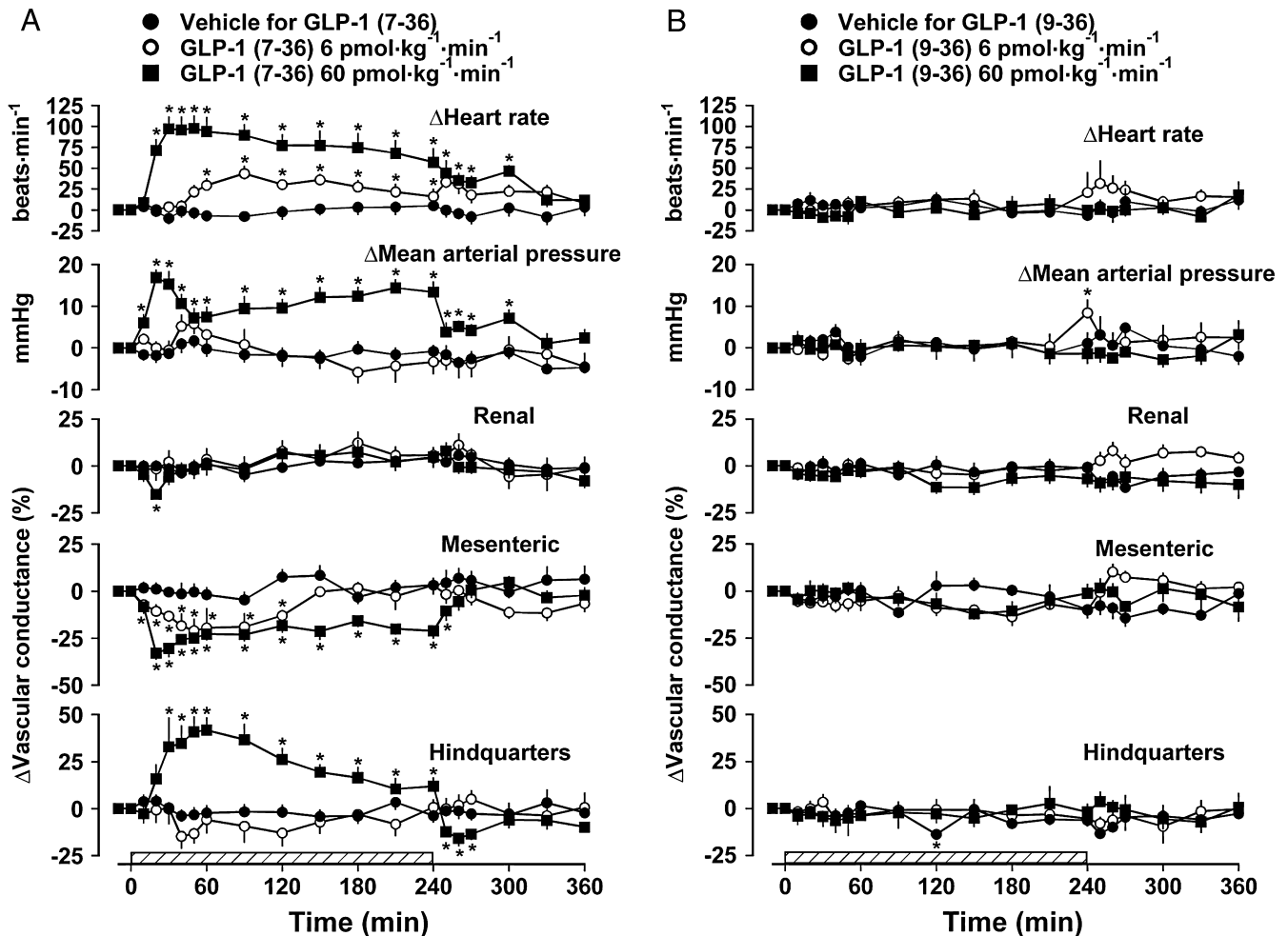
Figure 1

Cardiovascular responses during and after 4 h i.v. infusions of (A) glucagon-like peptide (GLP)-1(7–36) ($n = 7$) or (B) GLP-1(9–36) ($n = 5$) in conscious Sprague-Dawley rats. The vehicle was 1% BSA infused at 0.4 mL·h^{-1}, and the period of infusion is indicated by the hatched bar. Values are mean and vertical bars show SEM. *$P < 0.05$ versus baseline (Friedman's test).

Results

Experiment 1. Regional haemodynamic responses to infusions or bolus doses of GLP-1(7–36) and GLP-1(9–36)

Infusions. There were no differences between resting cardiovascular variables on the three occasions prior to infusion of GLP-1(7–36) 6 pmol·kg^{-1}·min^{-1} [heart rate 364 ± 5 beats·min^{-1}, mean BP 109 ± 2 mmHg, renal VC 76 ± 8 (kHz·mmHg^{-1})10^3, mesenteric VC 72 ± 9 (kHz·mmHg^{-1})10^3, hindquarters VC 41 ± 7 (kHz·mmHg^{-1})10^3], vehicle [heart rate 348 ± 8 beats·min^{-1}, mean BP 112 ± 2 mmHg, renal VC 75 ± 7 (kHz·mmHg^{-1})10^3, mesenteric VC 62 ± 8 (kHz·mmHg^{-1})10^3, hindquarters VC 37 ± 3 (kHz·mmHg^{-1})10^3] or GLP-1(7–36) 60 pmol·kg^{-1}·min^{-1} [heart rate 326 ± 8 beats·min^{-1}, mean BP 108

± 2 mmHg, renal VC 75 ± 8 (kHz·mmHg^{-1})10^3, mesenteric VC 75 ± 10 (kHz·mmHg^{-1})10^3, hindquarters VC 40 ± 3 (kHz·mmHg^{-1})10^3]. Infusion of vehicle had no consistent cardiovascular effects, and GLP-1(7–36) (6 pmol·kg^{-1}·min^{-1}) caused only modest tachycardia ($P < 0.05$ from 60 min onwards) and transient mesenteric vasoconstriction ($P < 0.05$ between 20 and 120 min) (Figure 1A). In contrast, at a dose of 60 pmol·kg^{-1}·min^{-1}, GLP-1(7–36) caused marked tachycardia ($P < 0.05$ from 20 min onwards), a rise in BP ($P < 0.05$ from 10 min onwards), transient renal vasoconstriction ($P < 0.05$ at 20 min only), pronounced and persistent mesenteric vasoconstriction ($P < 0.05$ from 10 min onwards) and hindquarters vasodilatation that was delayed in onset ($P < 0.05$ from 30 min onwards) and tended to wane during the infusion (Figure 1A). When the infusion was switched off, heart rate and
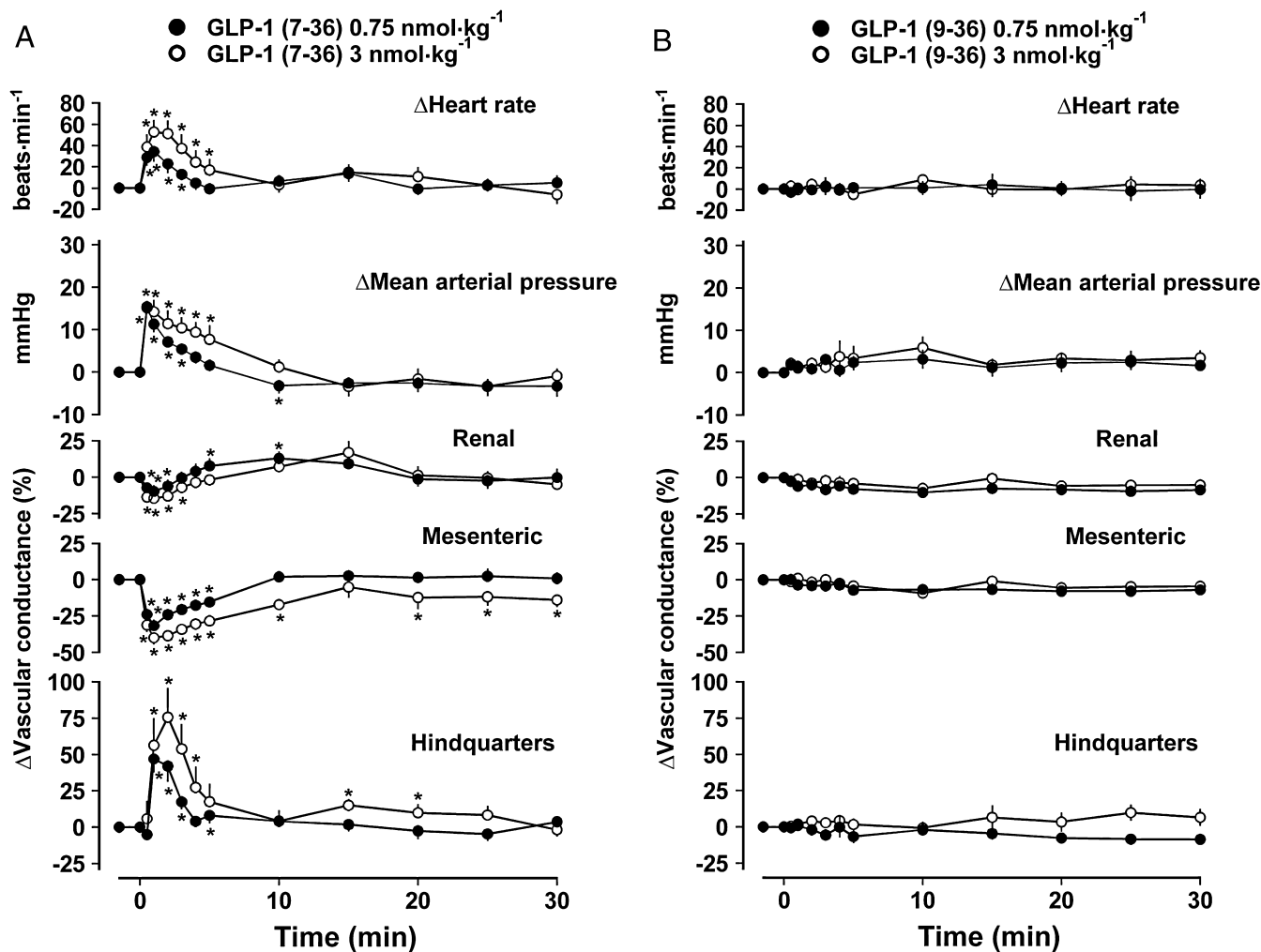
Figure 2

Cardiovascular effects of bolus doses (0.1 mL) of (A) glucagon-like peptide (GLP)-1(7–36) ($n = 7$) and (B) GLP-1(9–36) ($n = 4$) in conscious Sprague-Dawley rats. Values are mean and vertical bars show SEM. *$P < 0.05$ versus baseline (Friedman's test).

blood pressure remained above baseline for 60 min, whereas mesenteric VC returned to baseline within 20 min and in the hindquarters, the vasodilatation promptly (within 10 min) changed to modest vasoconstriction (Figure 1A).

Cardiovascular variables prior to infusion of GLP-1(9–36) 6 pmol·kg^{-1}·min^{-1}, 60 pmol·kg^{-1}·min^{-1} or vehicle were not different [heart rate 350 ± 20, 342 ± 5, 350 ± 15 beats·min^{-1}, mean BP 106 ± 4, 104 ± 3, 106 ± 3 mmHg, renal VC 76 ± 12, 74 ± 10, 77 ± 11 (kHz·mmHg^{-1})10^3, mesenteric VC 87 ± 10, 81 ± 16, 89 ± 6 (kHz·mmHg^{-1})10^3, hindquarters VC 42 ± 5, 42 ± 7, 41 ± 8 (kHz·mmHg^{-1})10^3 respectively]. There were no consistent cardiovascular effects of vehicle or GLP-1(9–36) infusion at either dose (Figure 1B).

Bolus doses. Bolus doses of GLP-1(7–36) caused tachycardia, an increase in blood pressure, renal and

mesenteric vasoconstriction and hindquarters vasodilatation, the duration of which were greater with the higher dose, such that the integrated (0–10 min) changes were significantly different between doses ($P < 0.05$, Wilcoxon's test) (Figure 2A). In contrast, equimolar bolus doses of GLP-1(9–36) had no significant cardiovascular effects (Figure 2B). Indeed, even a dose of 90 nmol·kg^{-1} GLP-1(9–36), which was given to one animal, was devoid of any cardiovascular actions (data not shown).

Experiment 2. Effects of DPP-4 inhibition on the regional haemodynamic responses to GLP-1(7–36)

There were no cardiovascular effects associated with administration of DPPI 1c, hence cardiovascular variables prior to administration of GLP-1(7–36) in

Table 1

Resting cardiovascular variables immediately prior to administration of different doses of glucagon-like peptide (GLP)-1(7–36) following pretreatment with either (a) vehicle or (b) DPPI 1c

	0.3 nmol·kg⁻¹	0.75 nmol·kg⁻¹	3 nmol·kg⁻¹
(a) vehicle			
Heart rate (beats·min⁻¹)	346 ± 8	350 ± 8	360 ± 12
Blood pressure (mmHg)	109 ± 3	111 ± 3	106 ± 3
Renal VC (units)	93 ± 7	93 ± 7	97 ± 7
Mesenteric VC (units)	80 ± 11	68 ± 10	83 ± 11
Hindquarters VC (units)	47 ± 4	46 ± 4	45 ± 3
(b) DPPI 1c			
Heart rate (beats·min⁻¹)	348 ± 9	358 ± 9	347 ± 9
Blood pressure (mmHg)	108 ± 3	112 ± 4	110 ± 4
Renal VC (units)	96 ± 5	95 ± 6	90 ± 5
Mesenteric VC (units)	89 ± 16	78 ± 7	80 ± 6
Hindquarters VC (units)	51 ± 6	44 ± 4	43 ± 4

Values are mean ± SEM, $n = 10$. Units for vascular conductance (VC) are $(kHz·mmHg^{-1})10^3$.
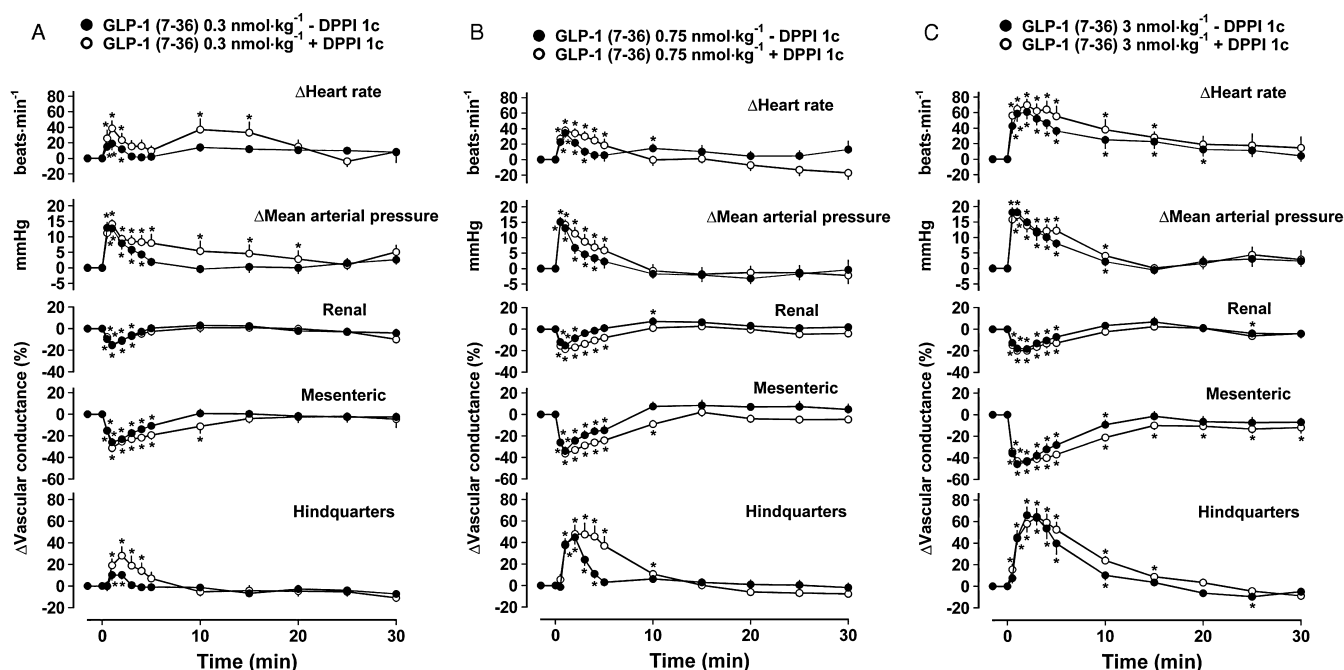
Figure 3

Effects of dipeptidyl peptidase 4 (DPP-4) inhibition with DPPI 1c on cardiovascular responses to glucagon-like peptide (GLP)-1(7–36) at (A) 0.3 nmol·kg⁻¹, (B) 0.75 nmol·kg⁻¹ and (C) 3 nmol·kg⁻¹ in conscious Sprague-Dawley rats ($n = 10$). Values are mean and vertical bars show SEM. *$P < 0.05$ versus baseline (Friedman's test). Statistical comparisons of integrated responses are given in the text.

the presence of either vehicle or DPPI 1c were not different (Table 1). In the control condition, GLP-1(7–36) caused increases in blood pressure and heart rate associated with renal and mesenteric vasoconstriction and hindquarters vasodilatation (Figure 3). Following DPPI 1c administration, the cardiovascular effects of the lower doses of GLP-1(7–36) were generally more prolonged (Figure 3A,B), although only the integrated (0–10 min) changes in heart rate (0.3 nmol·kg⁻¹ dose), blood pressure (0.3 nmol·g⁻¹ dose) and hindquarters VC (0.3 and 0.75 nmol·kg⁻¹ doses) were significantly different ($P < 0.05$,

Table 2

Resting cardiovascular variables immediately prior to administration of glucagon-like peptide (GLP)-1(7–36) 0.75 nmol·kg^{-1} (a) or 3 nmol·kg^{-1} (b) following treatment with saline, ICI 118551, propranolol or propranolol plus phentolamine

	Saline	ICI 118551	Propranolol	Propranolol + phentolamine
(a) 0.75 nmol·kg^{-1}				
Heart rate (beats·min^{-1})	368 ± 12	351 ± 7	343 ± 9	357 ± 4
Blood pressure (mmHg)	112 ± 3	111 ± 3	107 ± 2	100 ± 3*
Renal VC (units)	71 ± 8	59 ± 5	70 ± 6	61 ± 8
Mesenteric VC (units)	75 ± 10	71 ± 8	72 ± 7	73 ± 11
Hindquarters VC (units)	45 ± 7	36 ± 5	42 ± 5	60 ± 10
(b) 3 nmol·kg^{-1}				
Heart rate (beats·min^{-1})	371 ± 12	358 ± 7	339 ± 3*	347 ± 11
Blood pressure (mmHg)	110 ± 3	113 ± 3	104 ± 2	104 ± 3
Renal VC (units)	69 ± 8	62 ± 5	67 ± 5	61 ± 5
Mesenteric VC (units)	72 ± 9	66 ± 5	74 ± 8	69 ± 8
Hindquarters VC (units)	42 ± 7	36 ± 5	43 ± 6	52 ± 5

Values are mean ± SEM, $n = 7$. Units for vascular conductance (VC) are (kHz·mmHg^{-1})10^3.
*$P < 0.05$ versus saline (Friedman's test).

Wilcoxon's test). The mesenteric vasoconstrictor effects of the 3 nmol·kg^{-1} dose of GLP were more prolonged following treatment with DPPI 1c, but none of the integrated changes at that dose were significantly different (Figure 3C).

Experiment 3. Effects of adrenoceptor antagonism on the regional haemodynamic responses to GLP-1(7–36)

Resting cardiovascular variables prior to administration of GLP-1(7–36) are shown in Table 2. Following saline administration, the cardiovascular effects of bolus doses of GLP-1(7–36) (0.75 and 3 nmol·kg^{-1}) were as described above, namely tachycardia, a rise in blood pressure, renal and mesenteric vasoconstriction and hindquarters vasodilatation (Figures 4A and 5A).

Pretreatment with ICI 118551 ablated the hindquarters vasodilator effects and enhanced the pressor actions [$P < 0.05$ for integrated (0–10 min) changes] of both doses of GLP-1(7–36); it also caused some enhancement of the renal vasoconstrictor effect [$P < 0.05$ for integrated (0–10 min) change at 3 nmol·kg^{-1} dose only], and while the magnitude of the initial tachycardic effect of both doses of GLP-1(7–36) was unaffected by ICI 118551, the duration was reduced, and there was a second phase of tachycardia (Figures 4A and 5A).

In the presence of propranolol, the integrated (0–10 min) GLP-1(7–36)-induced increases in heart rate and hindquarters VC were significantly ($P <$

0.05) reduced or abolished, whereas the rises in blood pressure and renal and mesenteric vasoconstrictions ($P < 0.05$ for 3 nmol·kg^{-1} dose only) were enhanced (Figures 4B and 5B).

Pretreatment with propranolol together with phentolamine affected the GLP-1(7–36)-induced changes in heart rate and hindquarters haemodynamics in the same way as propranolol alone, except that under those conditions, the pressor and vasoconstrictor effects of GLP were not enhanced (Figures 4C and 5C).
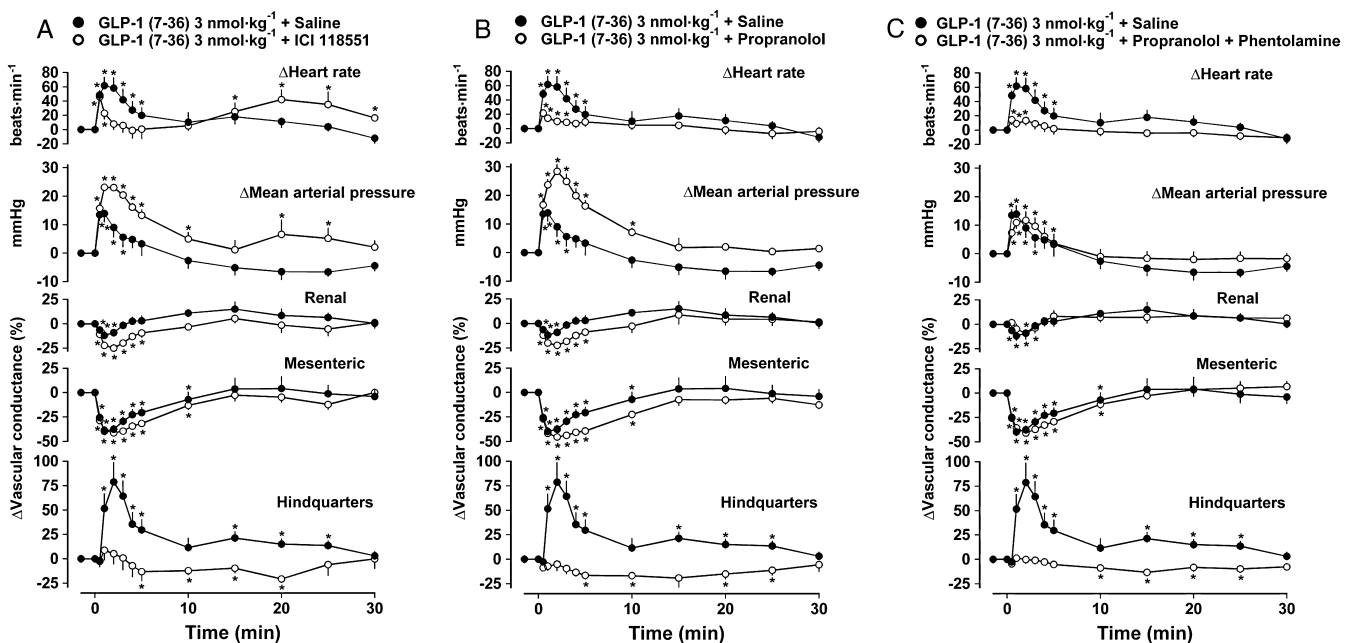
Discussion and conclusions

The objectives of this study were, in conscious freely moving rats, to compare the regional haemodynamic actions of GLP-1(7–36) and GLP-1(9–36), to assess the extent to which the cardiovascular effects of GLP-1(7–36) were affected by DPP-4 inhibition and to determine the contribution of the autonomic nervous system to the cardiovascular actions of GLP-1(7–36). The main findings were that GLP-1(7–36) had clear, dose-dependent, regionally selective haemodynamic effects that were enhanced in the presence of a DPP-4 inhibitor, and were, in part, mediated by the autonomic nervous system, whereas GLP-1(9–36) was devoid of any haemodynamic actions. Collectively, these findings indicate that in normal conscious rats, GLP-1(7–36), rather than its cleavage product, is the active peptide responsible for the haemodynamic effects.
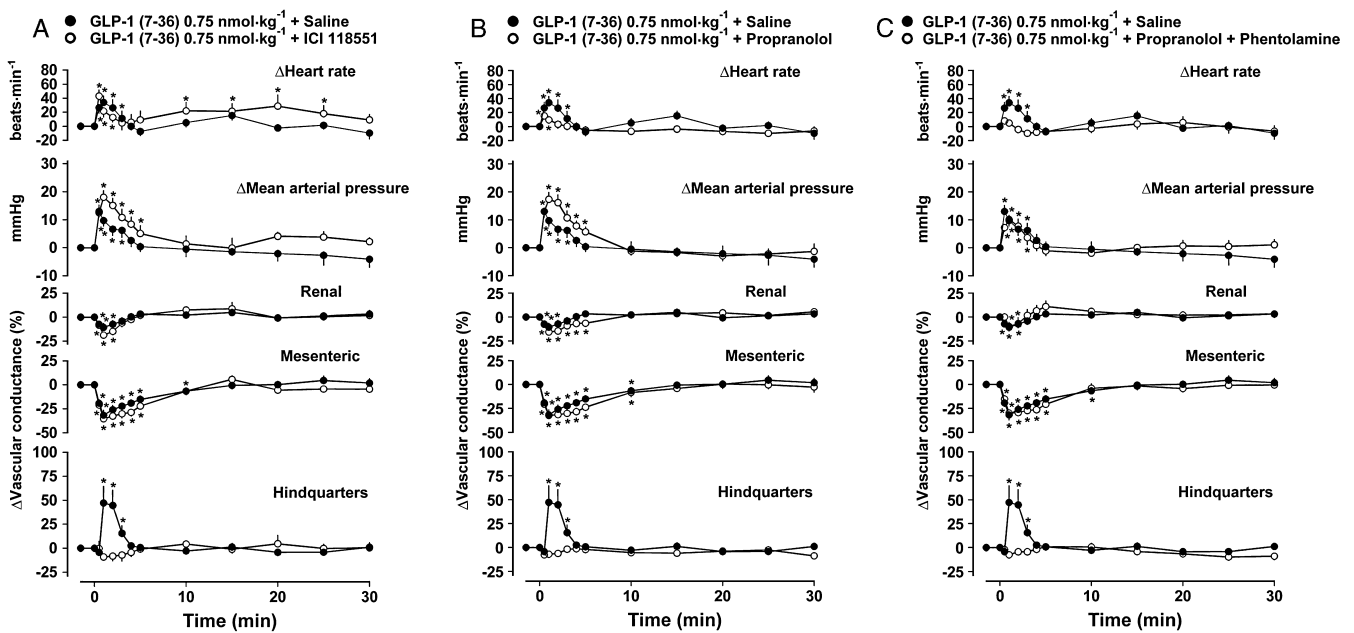
Figure 4

Cardiovascular effects of glucagon-like peptide (GLP)-1(7–36) (0.75 nmol·kg^{-1}) in the presence of saline or (A) ICI 118551 (0.2 mg·kg^{-1}, 0.1 mg·kg^{-1}·h^{-1}), (B) propranolol (1 mg·kg^{-1}, 0.5 mg·kg^{-1}·h^{-1}) and (C) propranolol (1 mg·kg^{-1}, 0.5 mg·kg^{-1} h^{-1}) together with phentolamine (1 mg·kg^{-1}, 1 mg·kg^{-1}·h^{-1}) in conscious Sprague-Dawley rats (n = 8). Values are mean and vertical bars show SEM. *P < 0.05 versus baseline (Friedman's test). Statistical comparisons of integrated responses are given in the text.

Figure 5

Cardiovascular effects of glucagon-like peptide (GLP)-1(7–36) (3 nmol·kg^{-1}) in the presence of saline or (A) ICI 118551 (0.2 mg·kg^{-1}, 0.1 mg·kg^{-1}·h^{-1}), (B) propranolol (1 mg·kg^{-1}, 0.5 mg·kg^{-1}·h^{-1}) and (C) propranolol (1 mg·kg^{-1}, 0.5 mg·kg^{-1}·h^{-1}) together with phentolamine (1 mg·kg^{-1}, 1 mg·kg^{-1}·h^{-1}) in conscious Sprague-Dawley rats (n = 8). Values are mean and vertical bars show SEM. *P < 0.05 versus baseline (Friedman's test). Statistical comparisons of integrated responses are given in the text.

The possibility that GLP-1(9–36) may have cardiovascular actions is of importance, as the use of either long-acting GLP-1(7–36) analogues or DPP-4 inhibitors to prolong the actions of GLP-1(7–36) is of great clinical interest in the treatment of type 2 diabetes, and if the cleavage product, GLP-1(9–36), is responsible for some or all of the recognized beneficial cardiovascular effects, then inhibition of DPP-4 may not be the best approach. Evidence that GLP-1(9–36) can exert cardiovascular actions comes, directly or indirectly, from several different studies, albeit none in normal conscious rats. Thus, in dogs with dilated cardiomyopathy, Nikolaidis *et al.* (2005) showed that infusion of GLP-1(9–36) improved left ventricular pressures and contractility and increased myocardial glucose uptake in an analogous manner to infusion of GLP-1(7–36), and as the latter was rapidly cleaved despite continuous infusion, they concluded that GLP-1(9–36) was the active peptide. More recently, cardioprotective effects of GLP-1(9–36) in ischaemia–reperfusion injury have been reported in rats (Sonne *et al.*, 2008) and mice (Ban *et al.*, 2008), although in a very recent study GLP-1(9–36) was found not to be cardioprotective and to exert a modest negative inotropic effect (Ossum *et al.*, 2009). In addition, *in vitro* studies have shown vasorelaxant effects of GLP-1(9–36) that were of an equivalent magnitude to those of GLP-1(7–36) (Ban *et al.*, 2008; Green *et al.*, 2008). Furthermore, in anaesthetized rats (Barragán *et al.*, 1994; Bojanowska and Stempniak, 2002), bolus doses of GLP-1(7–36) caused an initial rise followed by modest fall in blood pressure, tempting the speculation that the secondary fall might be due to a vasodilator action of the cleavage product (Grieve *et al.*, 2009). Against this background, we were surprised that GLP-1(9–36) in our study had no measurable cardiovascular actions, either when given as a continuous infusion for 4 h or when given as bolus injections at equimolar doses to GLP-1(7–36) that had clear-cut effects. However, our findings that the cardiovascular effects of low doses of GLP-1(7–36) were prolonged by inhibition of DPP-4 are consistent with the suggestion that the DPP-4 cleavage product was not the active peptide in our experiments. It is possible therefore that *in vivo*, the cardiovascular actions of GLP-1(9–36) are restricted to cardiac actions in diseased states. Alternatively, or additionally, species differences could explain the apparent disparity between our findings in rats, and those of Nikolaidis *et al.* (2005) in dogs. It was interesting to note that the DPP-4 inhibitor alone had no effect on baseline haemodynamic status. This is consistent with a very recent publication using both genetic and pharmacological inhibition of DPP-4 in mice, and showing no effect on cardiovascular structure or function under normal conditions (Sauvé *et al.*, 2010). This presumably indicates that the circulating levels of endogenous peptide are too low to exert cardiovascular effects, even when breakdown is inhibited.

This is not the first study to describe pressor and tachycardic effects of systemic administration of bolus doses of GLP-1(7–36) in rats (Barragán *et al.*, 1994; 1996; 1999; Bojanowska and Stempniak, 2002; Yamamoto *et al.*, 2002), but to our knowledge it is the first to describe the associated regional vascular changes. Here we show a regional haemodynamic profile for GLP-1(7–36), which closely resembles that of the GLP-1 receptor agonist, exendin-4, that is, pronounced hindquarters vasodilatation and mesenteric vasoconstriction, with a more modest degree of vasoconstriction in the renal vascular bed (Gardiner *et al.*, 2006a). We previously showed that the hindquarters vasodilator effect of exendin-4 was largely β_2-adrenoceptor-mediated, secondary to GLP-1 receptor-induced adrenaline release from the adrenal medulla (Gardiner *et al.*, 2006a). The present results showing inhibition of the hindquarters vasodilator effects of GLP-1(7–36) by ICI 118551 indicate a β_2-adrenoceptor-mediated event, most likely via the same mechanisms as exendin-4. In our previous studies, ICI 118551 also, somewhat unexpectedly, markedly reduced the tachycardic effect of exendin-4 whereas in the present study, the effect of ICI 118551 on the heart rate response to GLP-1(7–36) was less obvious. We suggested that some of the inhibition of the tachycardic effect of exendin-4 in ICI 118551-treated animals was due to activation of vagally mediated baroreflex bradycardia, triggered by the enhanced pressor effect that was secondary to the reduced vasodilatation (Gardiner *et al.*, 2006a). Although the pressor effect of GLP-1(7–36) was enhanced by ICI 118551, the degree of enhancement was less than that seen with exendin-4, and hence the degree of baroreflex buffering would be less, thus explaining the lesser inhibition of the tachycardia. Treatment with propranolol, however, had a clear inhibitory effect on the heart rate response to GLP-1(7–36), as previously reported for exendin-4 (Gardiner *et al.*, 2006a), consistent with sympathetic activation being the main mechanism. This contrasts with the conclusions of Barragán *et al.* (1994) who reported no significant effect of propranolol on the heart rate response to GLP-1(7–36) or exendin-4 in anaesthetized rats although there was enhancement of the pressor effect. We have no explanation for this difference in outcomes, although their data are shown as % change in heart rate and if propranolol had reduced resting heart rate in their anaesthetized animals, then in absolute terms, the tachycardia

may have been reduced. In this study, as previously with exendin-4, the enhanced pressor effect of GLP-1(7–36) seen with propranolol treatment did not occur when the animals were treated with phentolamine together with propranolol. Because this could not be explained by a marked diminution by phentolamine of the vasoconstrictor effects, we suggest it is due to inhibition of a cardiac α-adrenoceptor-mediated positive inotropic effect (Broadley *et al.*, 1999). It was interesting that there was no evidence for a major contribution from α-adrenoceptors to the vasoconstrictor effects of GLP-1(7–36). We previously reported vasoconstrictor effects of exendin-4 that were not sympathetically mediated and resistant to inhibition of endogenous angiotensin, vasopressin, endothelin, neuropeptide Y and prostanoids (Gardiner *et al.*, 2008). The extent to which the vasoconstrictor effects of GLP-1(7–36) are independent of those mechanisms has not been explored here, but given the similarity in cardiovascular profiles of the agonists, it seems likely that similar underlying mechanisms are responsible.

In the experiments where GLP-1(7–36) was infused, the regional haemodynamic profile of the high dose (60 pmol·kg^{-1}·min^{-1}) was consistent with that described above for the bolus doses although the hindquarters vasodilatation tended to wane, and the lower dose (6 pmol·kg^{-1}·min^{-1}) had only transient effects on mesenteric VC and relatively late-onset and modest effects on heart rate. This could suggest that during the infusion, the peptide was being degraded to some extent, although Parkes *et al.* (2001), using an assay that was specific for GLP-1(7–36), showed that there was a sustained plasma level of peptide during continuous 3 h i.v. infusions of doses between 2 and 2000 pmol·kg^{-1}·min^{-1}. Thus, the waning effect could represent desensitization of the GLP-1 receptor and/or activation of reflex counter-regulatory mechanisms; indeed even for the stable GLP-1 analogue, exendin-4, the hindquarters vasodilator effect during infusion tended to wane (Gardiner *et al.*, 2008).

In summary, the present data suggest that in normal conscious rats, GLP-1(7–36), rather than the DPP-4 cleavage product, GLP-1(9–36), is the active peptide with regard to haemodynamic actions. GLP-1 receptors are widely distributed peripherally (pancreas, heart, aorta, stomach, lung and kidney) and centrally in cardiovascular regulatory regions (brainstem, hypothalamus), and signal through the adenylate cyclase-cAMP transduction pathway (for review see Kieffer and Habener, 1999). In previous studies, use of exendin-(9–39) as a GLP-1 receptor antagonist showed some, but not all, the cardiovascular effects of exendin-4 were GLP-1 receptor-mediated (Gardiner *et al.*, 2006a). The extent to which the cardiovascular responses to GLP-1(7–36) observed here were mediated either centrally or peripherally by GLP-1 receptors was not investigated in this study.

Conflicts of interest

None.

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