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Direct cardiac and vascular actions of adrenomedullin in conscious sheep

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1 Adrenomedullin (ADM) is a recently characterized circulating hormone which affects haemodynamic, renal and pituitary function in mammals. We have shown previously that in sheep, ADM produces vasodilatation together with increases in cardiac output and contractility. However, whether these effects are direct or mediated by autonomic reflexes is unclear. The present study examined the cardiovascular actions of an intravenous infusion of ADM in conscious, chronically instrumented sheep with either sympathetic, parasympathetic or autonomic ganglion blockade, to determine the role of the autonomic nervous system in mediating these cardiovascular changes.

2 Human ADM (1–52) was infused for 60 min at 2 μg kg^{-1} h^{-1} following: (1) saline control, (2) combined α/β-adrenoceptor (sympathetic) blockade (proporanolol 0.4 mg kg^{-1} h^{-1} + phentolamine 0.15 mg kg^{-1} h^{-1} for 20 h), (3) muscarinic (parasympathetic) blockade (methscopolamine 0.05 mg kg^{-1} h^{-1} for 20 h) or (4) ganglion blockade (hexamethonium 3 mg kg^{-1} h^{-1} for 4 h). Measurements were made of mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), stroke volume (SV), total peripheral conductance (TPC), maximal aortic flow (Fmax) and maximal rate of change of aortic flow (dF/dt).

3 ADM reduced MAP by 3±1 mmHg, and increased CO (1.2±0.2 l min^{-1}), HR (14±2 beats min^{-1}), TPC (21±3 ml min^{-1} mmHg^{-1}), Fmax (2.3±0.8 l min^{-1}) and dF/dt (86±21 l min^{-1} s^{-1}) in normal sheep. In animals with α/β blockade, similar changes were observed with ADM. However, during muscarinic blockade, the increases in HR (32±4 beats min^{-1}), CO (2.1±0.4 l min^{-1}), TPC (31±4 ml min^{-1} mmHg^{-1}), Fmax (4.0±0.6 l min^{-1}), and dF/dt (150±12 l min^{-1} s^{-1}) produced by ADM were enhanced. During ganglion blockade, ADM produced a greater reduction in MAP (−10±2 mmHg) compared to controls (−3±1 mmHg). However, there was no increase in HR. The changes in CO, TPC and contractility were similar to those observed in control animals.

4 These results suggest that the vasodilator effects of ADM on the periphery and its ability to increase CO and cardiac contractility are not mediated by the autonomic nervous system, but are probably the result of direct actions of ADM on the heart and vasculature.

Keywords: Adrenomedullin; cardiac contractility; vasodilatation; autonomic nervous system

Introduction

Adrenomedullin (ADM) is a recently characterized peptide isolated from human phaeochromocytoma tissue (Kitamura *et al*., 1993a), which has potent cardiovascular actions in rats (Gardiner *et al*., 1995; He *et al*., 1995) and sheep (Parkes, 1995), and can promote natriuresis/diuresis, behavioural and hormonal changes (for review see Schell *et al*., 1996; Kitamura *et al*., 1995). Expression of ADM immunoreactivity has been shown within the adrenal medulla, lung and kidney (Kitamura *et al*., 1993b; Ichiki *et al*., 1994). Significant levels of immunoreactive ADM have also been demonstrated in the heart (Sakata *et al*., 1994; Ichiki *et al*., 1994) in both atrial and ventricular tissue, and more specifically ADM mRNA has been demonstrated within the heart ventricles (Kitamura *et al*., 1993b; Miller *et al*., 1996) suggesting that ADM may be synthesized and act locally within the heart. Binding sites for ADM have also been demonstrated in the heart with radiolabelled ADM (Owji *et al*., 1995), and the recently cloned ADM-specific receptor is expressed in heart tissue (Kapas *et al*., 1995; Miller *et al*., 1996). This suggests that the heart may be a target for locally produced or circulating ADM, where it may act in combination with its effects on peripheral vasodilatation.

Recently, Szokodi and co-workers have shown that ADM can increase the contractile force of the heart, when administered to rat isolated perfused heart preparations (Szokodi *et al*., 1996). We have shown previously that ADM can increase cardiac contractility in sheep (Parkes, 1995). However, whether this effect is direct, or mediated via autonomic reflexes remains unclear. In the present study, we compared the cardiovascular actions of 60 min infusion of human ADM (1–52) in conscious, chronically instrumented sheep during several methods of sympathetic and parasympathetic blockade, to determine the involvement of the autonomic nervous system in mediating the vasodilator and cardiac actions of this peptide.

Methods

Animals

Cross-bred adult merino ewes (40–50 kg body weight), oophorectomized and with carotid artery loops, were housed in individual metabolism cages in association with other sheep in an open laboratory. Animals were not used for at least three weeks after surgery and until they were accustomed to laboratory conditions and human contact. Sheep were fed a diet of oaten chaff (800 g day^{-1} containing 80–120 mmol kg^{-1} Na^+ and 270–380 mmol kg^{-1} K^+) and water was offered *ad libitum*. All experiments were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

Animal instrumentation and data collection

Flow probes were implanted in sheep for measurement of cardiac output and coronary blood flow as previously described (Bednarik & May, 1994a). Briefly, anaesthesia was

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induced with intravenous sodium thiopentone (15 mg kg^{-1}). Following intubation, sheep were placed on a ventilator and maintained on 2% isoflurane in air/oxygen. An electromagnetic flow (EMF) probe (InVivo Metrics, Healdsbrug CA) was implanted on the ascending aorta, and a transit-time flow probe (Transonic Systems Inc., Ithaca NY) was implanted on the left circumflex coronary artery.

The transit-time flowprobe was connected to a Transonics T201CDS flowmeter (Transonic Systems Inc., Ithaca NY) and the EMF probe was activated by a Biotronex BL610 flowmeter (Biotronex, MD). The output voltage of the EMF meter was reset to zero with an autozero circuit, during a portion of each diastole when blood flow in the ascending aorta is assumed to be zero. The autozero circuit also incorporated a separate circuit to measure the first differential of the upstroke of systole (dF/dt) at each beat. The EMF probe was calibrated *in vivo* against thermodilution over a range of cardiac output values (Bednarik & May, 1994a). Dobutrex (dobutamine, Eli Lilly, France) was used to increase cardiac output from approximately 4 to 9 l min^{-1}. Blood pressure was measured via a tygon catheter inserted into a carotid artery loop, and connected to a pressure transducer (TDXIII, Cobe, CO) tied to the sheep's back. The pressure was corrected to compensate for the height of the transducer above heart level.

Analogue signals (mean arterial pressure, cardiac output, maximum rate of increase or aortic flow (dF/dt) and coronary flow) were collected with a PC-based data acquisition system with custom-written software. Following analogue to digital conversion, data were collected at 100 Hz for 10 s at 2 min intervals. The following cardiovascular variables were recorded: mean arterial pressure (MAP), cardiac output (CO), heart rate (HR, calculated from the aortic flow signal), dF/dt, peak aortic flow (Fmax), stroke volume (CO/HR), total peripheral conductance (TPC = CO/MAP), mean coronary blood flow (CF) and mean coronary conductance (CC = CF/MAP).

Two polyethylene cannulae were inserted in a jugular vein under local anaesthetic two days before experimentation, for intravenous administration of ADM or vehicle (normal saline), and pharmacological agents. Central venous pressure (CVP) was measured via a cathether inserted into a jugular vein to heart level. Arterial blood samples were collected via the carotid arterial tygon cathether.

Experimental protocols

Experiments were performed at least 6 days apart; 5 animals were administered the following pharmacological agents or saline as control: Hexamethonium chloride (Sigma Chemical Company, St Louis, MO) 3 mg kg^{-1} h^{-1} for 4 h before, and during infusion of ADM, produced total autonomic ganglion blockade. This was confirmed by complete abolishment of heart rate responses induced by the hypotensive actions of the vasodilator sodium nitroprusside (David Bull Labs., Mulgrave, Australia) (changes in MAP(mmHg)/HR(beats min^{-1}) in normal sheep: $-4/+12$ (0.2 μg kg^{-1}), $-9/+20$ (0.6 μg kg^{-1}), $-22/+50$ (2 μg kg^{-1}), $-24/+56$ (6 μg kg^{-1})), and the hypertensive responses produced by the α-receptor agonist phenylephrine hydrochloride (Winthrop Pharmaceuticals, New York, NY) (changes in MAP(mmHg)/HR(beats min^{-1}) in normal sheep: $+5/-5$ (0.2 μg kg^{-1}), $+7/-10$ (0.6 μg kg^{-1}), $+18/-22$ (2 μg kg^{-1}), $+46/-22$ (4 μg kg^{-1}), $+50/-35$ (6 μg kg^{-1})).

Phentolamine mesylate (Ciba-Geigy, Australia), 0.15 mg kg^{-1} h^{-1} together with propranolol hydrochloride (Sigma), 0.4 mg kg^{-1} h^{-1}, for 18 h before, and during infusion of ADM produced sympathetic α/β-adrenoceptor blockade as assessed by abolishment of blood pressure/heart rate responses induced by the β-receptor agonist isoprenaline (Sigma, 2–60 ng kg^{-1}) (range of MAP/HR changes in normal sheep: ΔMAP: -5 to -28 mmHg; ΔHR: $+4$ to $+180$ beats min^{-1}), and the α-receptor agonist phenylephrine (0.2–6.0 μg kg^{-1}).

Scopolamine methyl bromide (Sigma) 50 μg kg^{-1} for 18 h before, and during infusion of ADM produced parasympathetic muscarinic blockade as assessed by complete abolishment of blood pressure/heart rate responses induced by the muscarinic agonist carbamylcholine chloride (Sigma) (2–20 μg kg^{-1}) (range of MAP/HR changes in normal sheep: ΔMAP -9 to -20 mmHg; ΔHR: $+25$ to $+35$ beats min^{-1}) Phentolamine mesylate, 0.15 mg kg^{-1} h^{-1}, propranolol hydrochloride, 0.4 mg kg^{-1} h^{-1}, and scopolamine methyl bromide, 50 μg kg^{-1} h^{-1}, for 18 h before, and during infusion of ADM produced combined sympathetic/parasympathetic α/β/muscarinic receptor blockade as assessed by blockade of the responses to the receptor agonists described above.

ADM infusion

Following a one hour control measurement period, human ADM (1–52) (Bachem, Torrance CA) made up in normal saline, was infused intravenously via a jugular vein catheter at 2 μg kg^{-1} h^{-1} for 60 min, a sub-maximal dose based on a previous dose-response study in sheep (Parkes, 1995). Cardiovascular parameters were monitored every two minutes by the computer-based data acquisition system. Arterial blood samples for measurement of adrenocorticotropin (ACTH), corticotropin releasing factor (CRF), arginine vasopressin (AVP), cortisol and renin were collected at -10 min, $+30$ min, $+60$ min and $+120$ min (60 min after cessation of infusion). ADM was infused with saline, or during one of the four pharmacological blockade protocols in 5 sheep. Saline was also infused alone for 60 min serving as a control.

Plasma analysis

All blood samples were collected via the arterial cannula, centrifuged and plasma was frozen at -20°C until assay. Measurement of plasma ACTH was performed on unextracted plasma (assay sensitivity = 2 pg ml^{-1}; interassay variation = 11%) by use of an ACTH immunoradiometric kit (DYNOtest, Henning, GmbH, Berlin, Germany). Plasma cortisol was measured by radioimmunoassay of extracted plasma (sensitivity = 0.2 nmol l^{-1} interassay variation = 13%). For measurement of AVP, plasma was assayed by a radioimmunoassay designed for sheep plasma (sensitivity = 0.4 pg ml^{-1}; interassay variation = 13.6%) (Wintour *et al.*, 1982). Plasma renin concentration was determined by use of a modification of the antibody capture technique, by measuring the generation of angiotensin 1 (sensitivity = 0.2 pmol ml^{-1} h^{-1}; interassay variation = 9%) (Fei *et al.*, 1980). CRF was measured on extracted plasma with a specific radioimmunoassay again designed for sheep plasma (sensitivity = 2.0 pg/tube; interassay variation = 14%).

Statistics

Cardiovascular and hormonal parameters are expressed as mean $\pm$ s.e.mean, and were analysed for significant changes from control and between treatments ($P < 0.05$) by repeated measures analysis of variance, with *post-hoc* Fisher's PLSD test for individual time-point changes. Maximal percentage changes for each parameter (Figures 3 and 4) were compared between treatment groups by paired Student's *t* test (Statview IV, Abacus Resources, Berkeley, CA). Before analysis, time-course cardiovascular parameters (Figures 1 and 2) were grouped into 10 min blocks from the 2 min readings collected during the ADM infusion period. All stated changes within the Results section are significant to the $P < 0.05$ level.

Results

ADM in control sheep

Figure 1 shows the time-related cardiovascular changes of intravenous ADM infusion in five conscious sheep. ADM

(2 μg kg^{-1} h^{-1} for 60 min) produced a small reduction in MAP from 73±1 to 70±1 mmHg which was significant only after 60 min of infusion, and returned to control values within 20 min of cessation of infusion. This was associated with a rise in HR from 74±4 to 87±7 beats min^{-1}, and an increase in CO from 4.5±0.1 to 5.7±0.2 l min^{-1} after 60 min. There was no significant change in SV during the infusion period. TPC exhibited a marked increase from 61±3 to 82±2 ml min^{-1} mmHg^{-1} after 60 min infusion. All these parameters had returned to control values within 20–30 min following cessation of ADM infusion. CVP was unchanged throughout the infusion period (control: 3.5±0.5 mmHg, ADM: 4.0±0.5 mmHg). Coronary blood flow, shown in Figure 2, increased in parallel with CO from 48±1 to 59±4 ml min^{-1}, and this was associated with an increase in coronary conductance from 0.66±0.01 ml min^{-1} mmHg^{-1} to 0.84±0.06 ml min^{-1} mmHg^{-1} at 60 min of ADM infusion. Fmax exhibited an increase from 24.2±0.7 to 26.5±0.9 l min^{-1} at 60 min, and dF/dt increased from 844±21 to 930±34 l min^{-1} s^{-1} at this time point.

ADM during ganglion blockade

Infusion of hexamethonium produced a reduction in MAP, SV, Fmax and dF/dt after 4 h infusion (Table 1). These changes were associated with increases in HR, TPC and CC. Subsequent infusion of ADM (2 μg kg^{-1} h^{-1}) produced a large fall in MAP from 68±2 to 59±3 mmHg (Figure 1), when compared to the decrease seen in control (ADM alone) sheep. ADM increased CO from 5.2±0.1 to 6.3±0.2 l min^{-1}, TPC from 76±3 to 107±8 ml min^{-1} mmHg^{-1} and SV from 46±3 to 56±3 ml/beat. However, there was no change in HR. CVP remained unchanged throughout the infusion period (control: 1.5±0.5 mmHg, ADM: 2.0±0.5 mmHg). Fmax increased from 22.5±0.5 to 25.4±0.7 ml min^{-1}, and dF/dt

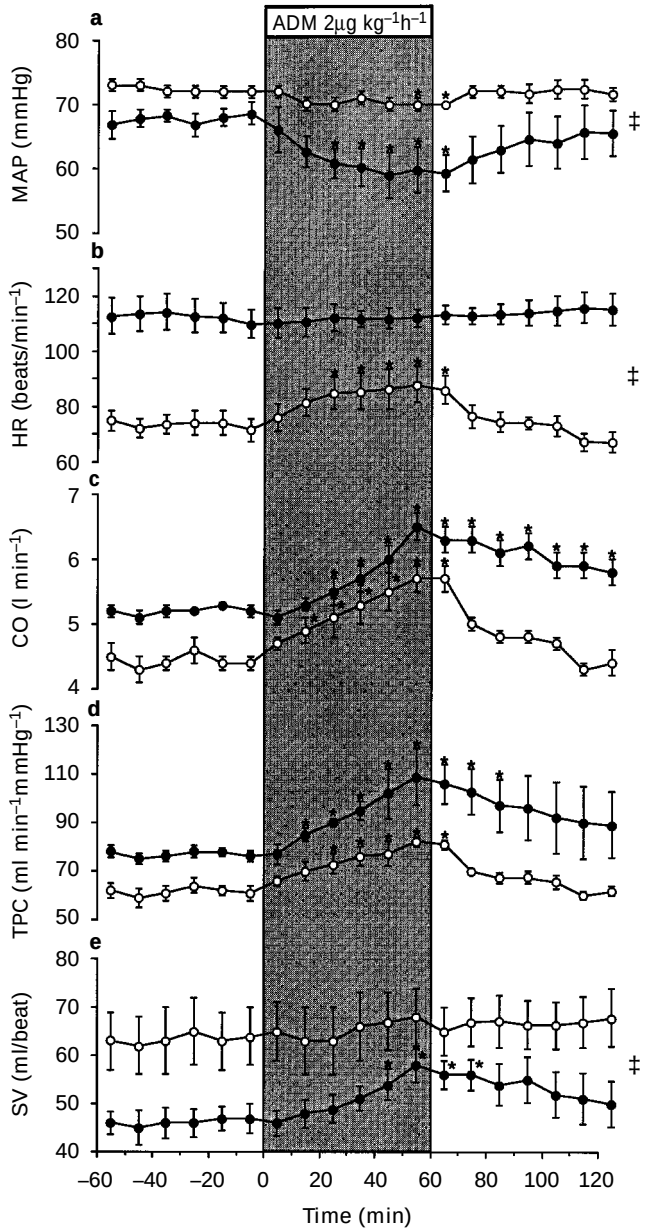

Figure 1 Haemodynamic effects of intravenous human adrenomedullin (1-52) (ADM) infused at 2 μg kg^{-1} h^{-1} in five control sheep (○) and the same five sheep with total autonomic blockade (●) (hexamethonium, 3 mg kg^{-1} h^{-1}). (a) Mean arterial pressure (MAP), (b) heart rate (HR), (c) cardiac output (CO), (d) total peripheral conductance (TPC) and (e) stroke volume (SV) were measured every 2 min by a computer-based data collection system. Results are shown as the mean of 10 min grouped readings with vertical lines showing s.e.mean. *Represents significant difference ($P<0.05$) from control period time points. ‡Represents significant difference ($P<0.05$) between ADM-induced changes in control animals versus changes in hexamethonium-treated animals.

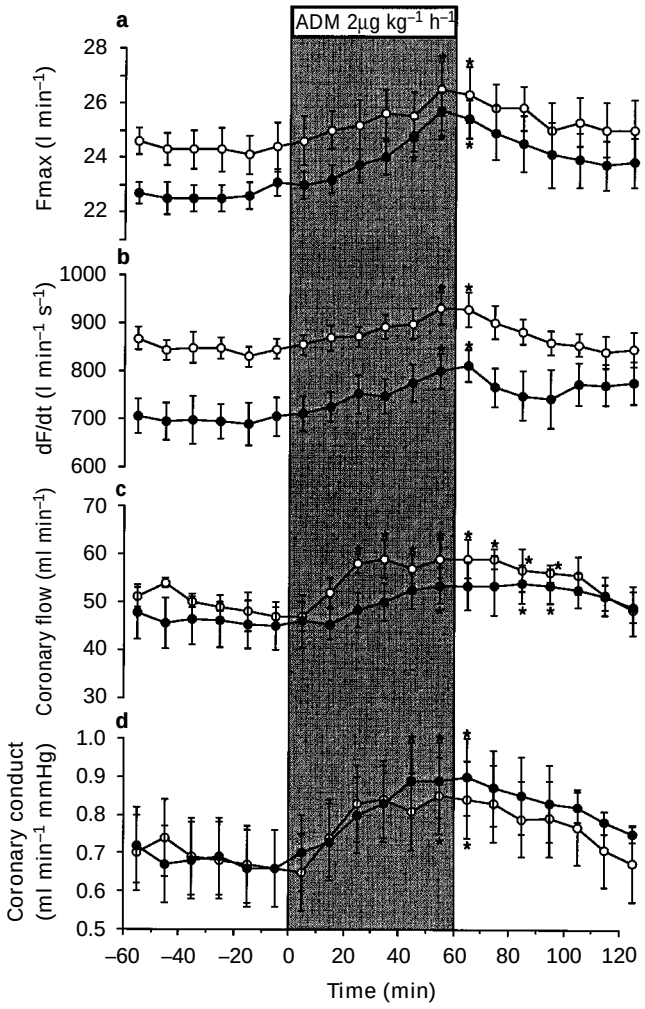

Figure 2 Haemodynamic effects of intravenous human adrenomedullin (1-52) (ADM) infused at 2 μg kg^{-1} h^{-1} in five control sheep (○) and five sheep with ganglion blockade (●) (hexamethonium, 3 mg kg^{-1} h^{-1}). (a) Peak aortic flow (Fmax), (b) aortic dF/dt, (c) coronary blood flow and (d) coronary conductance, were measured every 2 min by a computer-based data collection system. Results are shown as the mean of 10 min grouped readings with vertical lines showing s.e.mean. *Represents significant difference ($P<0.05$) from control period time points. No significant differences were observed between ADM-induced changes in control animals versus changes in hexamethonium-treated animals.

Table 1 Cardiovascular changes produced by intravenous infusion of hexamethonium (Hex, 3 mg kg^{-1} h^{-1}), phentolamine (0.15 mg kg^{-1} h^{-1}), +propranolol (0.4 mg kg^{-1} h^{-1}) (PP), methscopolamine (Meth, 50 μg kg^{-1} h^{-1}) or propranolol + phentolamine + methscopolamine (PPM)

	Control	*Hex*	*Control*	*PP*	*Control*	*Meth*	*Control*	*PPM*
MAP (mmHg)	79±2	68±2*	75±2	68±3*	76±2	79±2	85±1	78±5*
HR (beats min^{-1})	83±2	113±5*	65±3	81±5*	78±8	110±10*	96±5	108±3*
CO (l min^{-1})	5.1±0.2	5.2±0.1	4.4±0.2	5.9±0.2*	5.4±0.1	5.3±0.3	4.6±0.1	4.9±0.3
TPC (l min^{-1} $mmHg^{-1}$)	65±3	76±3*	59±5	87±4*	71±3	67±4	54±4	63±4
SV (ml beat)	61±4	46±3*	68±4	73±4	69±5	48±3	48±4	45±3
Fmax (l min^{-1})	26.9±0.7	22.5±0.5*	25.3±0.6	29.9±0.6*	28.3±1	24±0.8*	24±1	27.5±5*
dF/dt (l min^{-1} s^{-1})	868±39	699±39*	807±32	830±29	993±51	802±20*	859±36	869±18
CF (ml min^{-1})	45±4	45±5	38±4	41.5±4	43±3	57±2*	52±6	38±3*
CC (ml min^{-1} $mmHg^{-1}$)	0.57±0.04	0.66±0.07*	0.51±0.06	0.61±0.04	0.57±0.08	0.72±0.05*	0.61±0.07	0.49±0.05*

Abbreviations: mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), total peripheral conductance (TPC), stroke volume (SV), maximum aortic flow (Fmax), aortic dF/dt, coronary blood flow (CF), and coronary conductance (CC) are shown as mean change±s.e.mean after 60 min infusion. *Represents significant difference ($P<0.05$) from pre-infusion (control) values.

increased from 699±39 to 812±33 l min^{-1} s^{-1} (Figure 2). CF increased from 45±4 to 53±5 ml min^{-1}, and CC increased from 0.65±0.07 to 0.89±0.08 ml min^{-1} $mmHg^{-1}$. Overall, similar percentage changes to control sheep were observed in CO, TPC, Fmax, dF/dt, CC and CF during ADM infusion, whereas there was a greater decrease in MAP and no tachycardia in the autonomically blocked sheep (Figures 3 and 4).

ADM during sympathetic blockade

Infusion of propranolol and phentolamine for 18 h promoted a small reduction in MAP, and increases in CO, HR, TPC and Fmax, with little effect on SV, dF/dt and CF (Table 1). Infusion of ADM for 60 min produced no significant change in MAP and a slightly smaller increase in TPC when compared to control animals. The changes in all other cardiovascular parameters were similar to those observed in control animals (Figures 3 and 4).

ADM during parasympathetic blockade

Infusion of scopolamine methyl bromide for 18 h did not effect MAP, CO or TPC, whereas there was a large increase in HR (Table 1). Fmax and dF/dt were significantly reduced after 18 h, and there was a rise in both CF and CC. Subsequent infusion of ADM for 60 min at 2 μg kg^{-1} h^{-1} produced similar changes in MAP, CF and CC to those seen in the control group (Table 2). However, larger increases in HR (+31±2%), CO (+42±6%), TPC (+44±5%), Fmax (+17±2%) and dF/dt (+22±2%) were observed when compared to sheep receiving ADM alone (Figures 3 and 4).

ADM during combined sympathetic/parasympathetic blockade

Combined infusion of propranolol, phentolamine and methscopolamine produced a small decrease in MAP, and this was associated with increases in HR and Fmax (Table 2). A decrease in both CF and CC was also observed, whereas there was no change in CO after 18 h infusion. Infusion of ADM for 60 min in these sheep reduced MAP by 12±3%, associated with an increase in HR of 11±2% and CO of 18±5%. The percentage increase in HR was significantly less than that observed with ADM infusion in the control group. Similar changes were observed in TPC, Fmax, dF/dt and CF to those seen with ADM alone (Figures 3 and 4).

Plasma hormonal effects

Plasma concentration of ACTH was reduced by 50% after 60 min infusion of ADM alone (Table 2) consistent with pre-

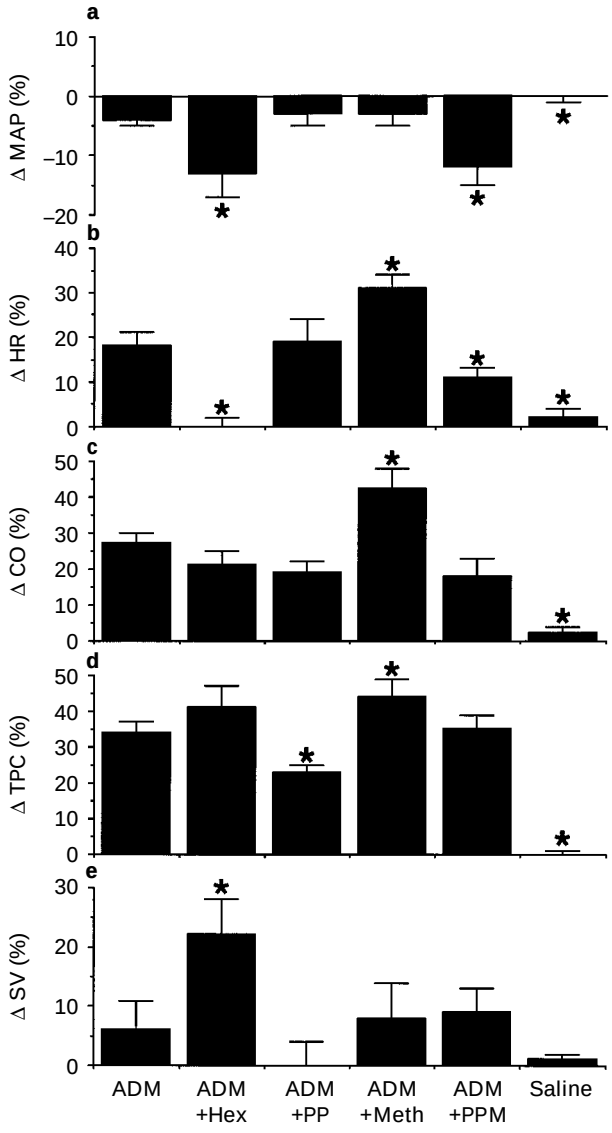

Figure 3 Comparison of systemic haemodynamic percentage changes following intravenous human adrenomedullin (ADM) infused at 2 μg kg^{-1} h^{-1} alone, or during hexamethonium (Hex), phentolamine + propranolol (PP), methscopolamine (Meth)or propranolol + phentolamine + methscopolamine (PPM). Abbreviations (a) mean arterial pressure (MAP), (b) heart rate (HR), (c) cardiac output (CO), (d) total peripheral conductance (TPC) and (e) stroke volume (SV) are shown as mean change±s.e.mean after 60 min infusion. *Represents significant difference ($P<0.05$) from animals treated with ADM alone.

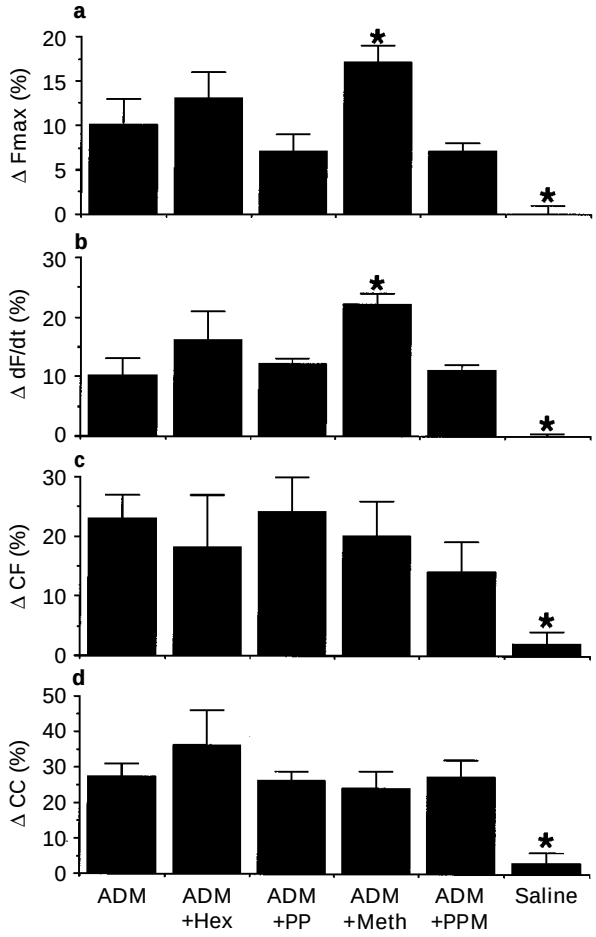

Figure 4 Comparison of cardiac haemodynamic percentage changes following intravenous human adrenomedullin (ADM) infused at 2 μg kg^{-1} h^{-1} alone, or during hexamethonium (Hex), phentolamine + propranolol (PP), methscopolamine (Meth) or propanolol + phentolamine + methscopolamine (PPM). Abbreviations: (a) maximum aortic flow (Fmax), (b) aortic dF/dt, (c) coronary blood flow (CF) and (d) coronary conductance (CC) are shown as mean change ± s.e.mean after 60 min infusion. *Represents significant difference ($P<0.05$) from animals treated with ADM alone.

vious studies in sheep (Parkes & May, 1995), and this was associated with a fall in plasma cortisol from 42 ± 10 to 20 ± 4 nmol l^{-1} after 60 min. Plasma concentration of renin increased significantly from 1.6 ± 0.2 to 3.3 ± 0.4 pmol ml^{-1} h^{-1} after 60 min ADM infusion. Plasma concentrations of AVP and CRF were not significantly changed throughout the infusion period. In the same sheep treated with hexamethonium, phentolamine/propranolol/methscopolamine or methscopolamine alone, no significant changes were observed in plasma levels of ACTH, cortisol, CRF or AVP, whereas a similar rise in renin levels was seen compared to that observed in normal sheep. However, animals with sympathetic (α/β) blockade did exhibit a slight lowering of plasma ACTH in response to ADM infusion.

Discussion

We have shown in the present study that short-term infusion of ADM in conscious sheep can produce direct effects to increase total peripheral conductance (vasodilatation), together with direct actions to increase cardiac contractility and cardiac output. These results are consistent with recent *in vitro* data, where ADM produced a dose-dependent cardiac inotropic action, as determined by increased developed tension in isolated heart preparations (Szokodi *et al*., 1996). ADM-induced increases in heart rate were abolished during treatment with hexamethonium, suggesting that this tachycardia is mediated via autonomic baroreflexes. Furthermore, the study by Szokodi (Szokodi *et al*., 1996) observed no change in cardiac rate in isolated hearts, suggesting that ADM is unlikely to produce a direct cardiac chronotropic effect *in vivo*. A recent study by Matsunaga has also demonstrated that the tachycardia associated with ADM administration in normal and spontaneously hypertensive (SHR) rats is abolished during concurrent treatment with hexamethonium (Matsunaga *et al*., 1996).

Previous studies in sheep have shown that ADM produces a very modest reduction in blood pressure (Parkes & May, 1995), which is associated with large increases in cardiac output and heart rate. Therefore when compared to rats, sheep are very sensitive to the cardiac/vasodilator actions of ADM, but can maintain blood pressure via reflex tachycardia. Similar conservative hypotensive actions have been observed following injection of ADM in man (Lewis *et al*., 1995), suggesting that

Table 2 Plasma hormonal changes following intravenous human adrenomedullin (ADM) infused at 2 μg kg^{-1} h^{-1} alone, or during hexamethonium (Hex), phentolamine + propranolol (PP), methscopolamine (Meth) or propranolol + phentolamine + methscopolamine (PPM)

		ADM	*Hex*	*PP*	*Meth*	*PPM*
ACTH (pg ml^{-1})	C	44 ± 12	48 ± 12	32 ± 3	23 ± 7	21 ± 2
	30 min	22 ± 7*	58 ± 9	24 ± 3*	25 ± 2	19 ± 2
	60 min	19 ± 5*	62 ± 8$^{\#}$	17 ± 3*	26 ± 3$^{\#}$	26 ± 7$^{\#}$
	post	13 ± 4*	57 ± 8	27 ± 3	22 ± 3	27 ± 3
Renin (pmol ml^{-1} h^{-1})	C	1.6 ± 0.2	1.6 ± 0.5	1.2 ± 0.1	1.7 ± 0.5	0.9 ± 0.2
	30 min	2.9 ± 0.3*	3.8 ± 0.4*	1.6 ± 0.2	3.4 ± 0.4*	2.6 ± 0.5*
	60 min	3.3 ± 0.4*	3.7 ± 0.3*	2.2 ± 0.3*	4.6 ± 0.7*	4.1 ± 0.7*
	post	1.8 ± 0.3*	3.2 ± 0.8*	1.1 ± 0.1	1.9 ± 0.4	1.8 ± 0.5
Cortisol (nmol l^{-1})	C	42 ± 10	66 ± 22	22 ± 3	41 ± 12	57 ± 12
	30 min	20 ± 6*	49 ± 16	20 ± 3	30 ± 11	70 ± 23
	60 min	20 ± 4*	61 ± 18$^{\#}$	19 ± 3	33 ± 9$^{\#}$	63 ± 29$^{\#}$
	post	35 ± 7	43 ± 14	23 ± 2	22 ± 4	66 ± 27
CRF (pg ml^{-1})	C	20 ± 2	17 ± 2	23 ± 2	17 ± 2	17 ± 2
	30 min	20 ± 3	19 ± 3	22 ± 3	21 ± 2	20 ± 4
	60 min	20 ± 4	21 ± 2	20 ± 2	20 ± 3	19 ± 4
	post	0.7 ± 0.2	25 ± 9	20 ± 2	20 ± 4	20 ± 6
AVP (pg ml^{-1})	C	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.1	0.8 ± 0.2	NM
	30 min	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.1	0.9 ± 0.2	NM
	60 min	0.6 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	NM
	post	0.6 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	0.9 ± 0.2	NM

*Represents significant difference ($P<0.05$) from pre-ADM values. $^{\#}$Represents significant difference ($P<0.05$) from changes observed with ADM alone. NM = not measured.

the hypotensive response to ADM may be more marked in rats, than in sheep or man. During treatment with hexamethonium, the hypotensive action of ADM is enhanced by 2–3 fold, and this is likely to be due to the absence of any reflex tachycardia, together with a slightly larger increase in peripheral conductance and a smaller rise in cardiac output. However, during ganglion blockade, ADM increased stroke volume. Again, this may be a consequence of the lack of ADM-induced tachycardia in these sheep, as cardiac filling time would remain constant, so that the increase in contractility would cause a rise in stroke volume. Central venous pressure was unchanged during infusion of ADM in control and hexamethonium-treated sheep, suggesting that the ADM-induced increases in cardiac contractility and cardiac output are not merely a consequence of elevated cardiac filling pressure. Furthermore, in the hexamethonium-treated animals, ADM could increase cardiac output, cardiac contractility and total peripheral conductance to the same extent as in normal animals, suggesting that these effects of ADM are not mediated via the autonomic nervous system and are probably direct actions.

The increase in cardiac output observed during ADM infusion is potentially mediated via a combination of factors which result in improved cardiac function. Firstly, the direct increase in cardiac contractility produced by ADM will stimulate the heart to elevate output. Secondly, the decrease in afterload will enable easier blood flow from the heart and increase cardiac output. Thirdly, ADM may produce direct dilatation of the coronary arteries as evidenced by the observed increase in coronary flow and conductance. This will provide a greater supply of blood to the heart, but may also be a secondary response to increased metabolic demand on the heart due to an increase in cardiac work.

The cardiovascular actions of ADM observed in conscious sheep are consistent with data from two recent studies in which the haemodynamic and regional blood flow effects of ADM in conscious rats were examined (Gardiner *et al*., 1995; He *et al*., 1995). In the study by He and coworkers, ADM produced a decrease in MAP, associated with increases in HR, CO and increased blood flow to the heart, kidney, adrenal, lung and spleen, when measured with radioactive microspheres. They suggested that because ADM increased blood flow to organs which exhibit high levels of ADM immunoreactivity, the peptide may act more as a local vasodilator. The increase in plasma renin concentration observed during ADM infusion in all protocols within the present study may be a consequence of the renal vasodilator action of ADM, and may also contribute to the maintenance of blood pressure in the face of total peripheral vasodilatation. Furthermore, because ADM is known to increase the levels of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) *in vitro* (Eguchi *et al*., 1994), this may directly stimulate release of renin from the juxtaglomerular cells within the kidney. It is unlikely that the renin elevation is a consequence of renal sympathetic nerve stimulation, as the effect was still present in the animals with ganglion blockade.

Several studies have shown that the *in vitro* vasodilator actions of ADM can be blocked by the CGRP antagonist CGRP 8-37 (Nakamura *et al*., 1995; Kitamura *et al*., 1995). However, a detailed study in conscious rats by Gardiner and co-workers (Gardiner *et al*., 1995) has demonstrated that renal, mesenteric and hindquarter vasodilator actions of ADM cannot be blocked by CGRP 8-37. In addition, CGRP produces a different haemodynamic effect to ADM in rats (Gardiner *et al*., 1995). In the sheep, infusion of CGRP at equimolar doses to that used for ADM in this study, has no effect on cardiac output (Braslis *et al*., 1988) or cardiac contractility, although there was a small increase in coronary blood flow. This lends further support to the fact that ADM can bind and activate ADM-specific receptors, independent of actions mediated via binding to CGRP receptors, localized in similar tissues. Hence, the cardivascular profiles of ADM and CGRP appear different in conscious animals, even though they may both produce their vasodilator action via elevating levels of cyclic AMP within the vascular wall.

The similar cardiovascular responses to ADM observed in control sheep and sheep with α/β adrenoceptor blockade, suggest that the sympathetic nervous system may not play a role in mediating the cardio-stimulant actions of ADM. Interestingly, ADM was still able to produce an increase in heart rate during adrenoceptor blockade, suggesting that the tachycardia is not a consequence of activation of the sympathetic arm of the baroreflex. *In vitro* studies have shown that there is no direct chronotropic activity of ADM in isolated heart preparations (Szokodi *et al*., 1996), hence this tachycardic activity may be mediated via non-sympathetic pathways which are inhibited by ganglion blockade with hexamethonium. ADM has recently been shown to produce direct activation of area postrema neurones within the brainstem *in vitro* (Allen & Ferguson, 1996). Hence, it is possible that circulating ADM may reach this brain nucleus to mediate vagal withdrawal, and subsequent heart rate elevation, as the area postrema lacks a blood brain barrier.

In the animals treated with the muscarinic inhibitor, methscopolamine, we observed an enhanced response to ADM with regard to effects on heart rate, cardiac output and cardiac contractility. In these animals, we observed a greater vasodilator action of ADM, which may stimulate sympathetic reflexes to further increase cardiac rate, and subsequently potentiate the increase in cardiac output. This hypothesis is supported by the fact that during combined sympathetic/parasympathetic blockade, we could significantly attenuate the change in heart rate seen with ADM. It is interesting to note that in sheep with combined α/β/muscarinic receptor blockade, ADM could still produce a significant, but attenuated increase in heart rate, but had no effect on stroke volume. In the hexamethonium-treated animals, the ADM-induced tachycardia was absent, whereas stroke volume increased significantly. The difference in the effect of ADM on stroke volume between the two treatments may be explained by the partially sustained tachycardia with ADM in the combined α/β/muscarinic blockade group, as the reduction in cardiac filling time will prevent any large increase in stroke volume from occurring. It is also feasible that ganglion blockade with hexamethonium may suppress cardiovascular reflexes which cannot be totally inhibited by antagonism of α/β/muscarinic receptors alone.

A recent study in conscious rabbits has shown that infusion of ADM increases both renal sympathetic nerve activity and plasma levels of noradrenaline (Fukuhara *et al*., 1995). This would suggest that ADM may produce a stimulation of the sympathetic nervous system to modify cardiovascular function. However, this same study suggested that ADM may attenuate baroreflex-mediated sympathetic activation of heart rate, as a smaller tachycardic response was seen to ADM, when compared to the response to a dose of sodium nitroprusside (SNP) producing an equivalent hypotensive change. In sheep, the tachycardic responses ($+12$ to $+13$ beats min^{-1}) to an equivalent hypotensive dose (-3 to -4 mmHg) of SNP and ADM were not significantly different. Until the effect of ADM on sympathetic activity is measured directly, the effect of this peptide on sympathetic control of haemodynamics remains unclear. Takahashi and co-workers have shown that an intracerebraventicular injection of ADM in anaesthetized rats gradually increased blood pressure and sympathetic nervous discharge (Takahashi *et al*., 1994). In addition, the study by Allen (Allen & Ferguson, 1996) has shown that ADM can specifically stimulate neurones within the area postrema of the brainstem, a nucleus known to be involved in sympathetic control of the cardiovascular system. Hence, ADM appears to have contrasting effects on the regulation of blood pressure within the brain and periphery.

Consistent with previous studies in sheep (Parkes & May, 1995), we observed a 50% reduction in plasma ACTH and cortisol levels during ADM infusion. It is unlikely that the fall in circulating ACTH levels would contribute to any cardio-

vascular changes during ADM infusion in normal sheep, as the earliest haemodynamic changes seen with an infusion of ACTH in sheep occur after 4 h of infusion (Bednarik & May, 1994b). Interestingly, we saw no significant reduction in either plasma ACTH or cortisol levels in animals pretreated with hexamethonium, phentolamine/propranolol/methscopolamine or methscopolamine alone, suggesting that there may be a cholinergic-dependent mechanism which can mediate this action of ADM on pituitary/adrenal secretion.

In conclusion, we have shown for the first time in conscious animals, that ADM may have direct actions on the heart to increase cardiac output and cardiac contractility, as these changes are not mediated by the autonomic nervous system. Furthermore, the peripheral vasodilator action of ADM is not dependent on autonomic-mediated changes to reduce afterload, and may be due to a direct effect on vascular smooth muscle cells, consistent with results from *in vitro* studies. Subsequent studies are necessary to evaluate the specific effect of this novel hormone on the activity of the autonomic nervous system. However, ADM is now emerging as an important modulator of mammalian cardiovascular homeostasis.

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