Comparative actions of adrenomedullin and nitroprusside: interactions with ANG II and norepinephrine

CHRISTOPHER J. CHARLES, M. GARY NICHOLLS, MIRIAM T. RADEMAKER, AND A. MARK RICHARDS
Christchurch Cardioendocrine Research Group, Christchurch School of Medicine, Christchurch, New Zealand

Received 27 April 2001; accepted in final form 30 July 2001

Comparative actions of adrenomedullin (ADM) in volume and pressure homeostasis remains undefined. Accordingly, we compared the biological responses to infusions of ADM and nitroprusside (NP; matched for reduction of arterial pressure) and assessed their effects on responses to ANG II and norepinephrine in eight conscious sheep. During matched falls in arterial pressure (8–10 mmHg, both P < 0.001) ADM and NP induced similar increases in heart rate. ADM increased cardiac output (P < 0.001), and the fall in calculated peripheral resistance was greater with ADM than NP (P = 0.013). ADM infusions raised plasma ADM levels (P < 0.001), plasma renin activity (P = 0.001), and ANG II (P < 0.001) but tended to blunt any concurrent rise in aldosterone compared with NP (P = 0.056). ADM maintained both urine flow (P < 0.001) and sodium excretion (P = 0.01) compared with falls observed with NP. ADM attenuated the vasopressor actions of exogenous ANG II (P = 0.006) but not norepinephrine. In addition, ADM antagonized the ANG II-induced rise in plasma aldosterone (P < 0.001). In conclusion, ADM induces a different spectrum of hemodynamic, renal, and endocrine actions to NP. These results clarify mechanisms by which ADM might contribute to volume and pressure homeostasis.

METHODS

The study protocol was approved by the Animal Ethics Committee of the Christchurch School of Medicine. Eight Coopworth ewes (Lincoln University Farm, Christchurch, New Zealand) were housed in an air-conditioned light-controlled room and received a diet of lucerne chaff and food pellets providing 75 mmol sodium and 150 mmol potassium per day. While the ewes were under general anesthesia (induced by 17 mg/kg thiopentone sodium and maintained by a mixture of halothane, nitrous oxide, and oxygen), a Konigsberg (P4.0) high-fidelity pressure-tip transducer was implanted in a carotid artery (Konigsberg Instruments, Pasadena, CA) for direct measurement of arterial pressure and heart rate. Polyethylene catheters were placed in the jugular veins for blood sampling and measurement of right atrial pressure (RAP), and a Swan-Ganz thermocatheter catheter (American Edwards) was placed in the pulmonary artery via the jugular vein for measurements of cardiac output. A Foley catheter was placed per urethra in the bladder to allow continuous collection of urine. The animals recovered for at least 7 days before experiments.

Each animal was studied on six occasions at least 2 days apart as follows: control + ANG II, ADM + ANG II, NE, and NP + NE. Thus, on...
two occasions, they received ADM in haemaccel, on two occasions NP, and on the other two occasions (control) the same volume of haemaccel (vehicle) alone in addition to either ANG II or NE. ADM was administered intravenously at a dose of 5.5 pmol·kg⁻¹·min⁻¹ (33 ng·kg⁻¹·min⁻¹) in a total volume of 50 ml haemaccel as a 200-min infusion commencing at 1000. NP was titrated (dose range = 2.5–20 mg/h) to achieve a fall in mean arterial pressure (MAP) matched to that induced by ADM. On 3 study days, ANG II (Hypertensin, Ciba Geigy) was coinfused (commencing 120 min after initiation of ADM, NP, or vehicle) incrementally at 2, 10, 20, and 100 ng·kg⁻¹·min⁻¹ (each for 20 min). On the other 3 study days, similarly timed coinfusions of NE (Levophed, Abbott) were given incrementally at 0.2, 0.4, 0.8, and 1.6 ng·kg⁻¹·min⁻¹ (each for 20 min). Human ADM-52 was synthesized as previously described (4). Peptides/drug study followed a balanced random design, except NP followed ADM days to ensure a matched fall in MAP.

Arterial pressure recordings using an online data-acquisition system (Dataflow, Crystal Biotech, Hopkinton, MA) commenced 40 min before infusions. Heart rate and pressures were digitally integrated in 30-s recording periods, and data from four consecutive periods were averaged and recorded at preset intervals throughout the study. MAP was monitored every 5 min during ADM and NP infusions to allow accurate titration of NP to achieve time-matched decrements in MAP. Cardiac output (thermodilution) was measured in triplicate (3 values within 10%) at 40-min intervals for the first 120 min of infusions. Calculated total peripheral resistance (CTPR) was calculated as MAP divided by cardiac output.

Venous blood was drawn at 40-min intervals for the first 120 min of the infusions and at the end of each ANG II/NE dose (20-min intervals). Blood was taken into chilled EDTA tubes and centrifuged, and the plasma was stored at −80°C before assay for ADM (18), aldosterone (19), ANG II (20), cortisol (17), plasma renin activity (PRA; Ref. 7), atrial natriuretic peptide (ANP; Ref. 3), brain natriuretic peptide (BNP; Ref. 23), endothelin (2), and catecholamines (10).

Urine was collected for a 40-min baseline period immediately before infusions and then at 40-min periods for the duration of the study. Volume was measured before assay for sodium, potassium, and creatinine excretion rates by standard methods.

Statistics. Results are expressed as means ± SE. Two-way ANOVA with time as a repeated measure was used to determine time and treatment differences between ADM, NP, and control arms of the study. Statistical significance was assumed at \( P < 0.05 \). Where significant differences were identified by ANOVA, a priori Fisher’s protected least-square difference (LSD) tests were used to identify individual time points significantly different from time-matched data. Slopes obtained from linear regression of data from individual sheep were compared by Student’s \( t\)-test.

RESULTS

Experiments were completed without mishap, and data collection was complete.

ADM vs. NP (first 120 min of infusions). Compared with time-matched control and in accord with study design, MAP fell similarly in response to ADM and NP (both \( P < 0.001 \); Fig. 1). MAP fell from a baseline of 72.1 ± 3.6 to 63.9 ± 3.4 mmHg during ADM and from 69.8 ± 3.5 to 59.2 ± 3.3 mmHg during NP. There was no significant difference in the MAP response to ADM and NP. Heart rate rose similarly in response to both ADM and NP (both \( P < 0.001 \) vs. control) with no difference between the two infusions. Cardiac output increased by ~3 l/min in response to ADM (\( P < 0.001 \) vs. both control and NP) but was unaltered by NP. Compared with control, CTPR was reduced in response to both ADM and NP (both \( P < 0.001 \)), the fall being significantly greater with ADM (\( P < 0.001 \) vs. NP). Both ADM (\( P = 0.001 \)) and NP (\( P = 0.013 \)) significantly and similarly reduced hematocrit compared with control. RAP was not significantly affected by ADM compared with either control or NP (Table 1).

![Fig. 1. Hemodynamic response to intravenous infusions of adrenomedullin (ADM; \( \bullet \), at a dose of 5.5 pmol·kg⁻¹·min⁻¹), nitroprusside (NP; \( \square \), titrated at a dose to match fall in mean arterial pressure (MAP)], or vehicle control (\( \circ \)). Data are from a total of 16 studies derived from 8 sheep. Values shown are means ± SE. Individual time points significantly different from time-matched data [Fisher’s protected least-square difference (LSD) from 2-way ANOVA] are indicated as follows: \( \dagger \)ADM vs. control; \( † \)NP vs. control; \( ‡ \)ADM vs. NP. CTPR, calculated total peripheral resistance; bpm, beats/min.](http://ajpregu.physiology.org/)

AJP-Regulatory Integrative Comp Physiol • VOL 281 • DECEMBER 2001 • www.ajpregu.org
Plasma immunoreactive (IR) ADM levels remained at or below detection limit for the assay during vehicle control infusions and NP but rose as expected during ADM infusions \((P < 0.001 \text{ vs. both})\) to plateau at \(\sim 10\) pmol/l (Fig. 2). PRA was significantly increased by ADM compared with control \((P = 0.001)\). PRA tended to rise with NP but not significantly so. The rise in PRA observed with ADM was also significantly greater than during NP \((P = 0.022)\). Rises in PRA with ADM were paralleled by increased plasma ANG II \((P < 0.001 \text{ vs. control})\). NP also tended to increase plasma ANG II \((P = 0.084)\). There was no significant difference between plasma ANG II levels during ADM and NP infusions. Plasma aldosterone levels were increased in response to both ADM \((P = 0.001)\) and NP \((P < 0.001)\), marginally less with ADM compared with NP \((P = 0.056)\). Relative to control, plasma cortisol levels were increased in response to NP \((P = 0.013)\) but not ADM; however, there was no significant difference between plasma cortisol levels on the 2 days. Plasma BNP levels tended to fall with ADM \((P = 0.056 \text{ vs. control})\) but not NP (Table 1) and were significantly reduced during ADM compared with NP infusions \((P = 0.049)\). There were no significant effects of either ADM or NP on plasma ANP, NE, epinephrine, or endothelin levels (Table 1).

Urinary volume was significantly reduced by NP \((P = 0.001 \text{ vs. control})\) but was maintained by ADM (Fig. 3) and was significantly greater during ADM compared with NP \((P < 0.001)\). Similarly, urinary sodium excretion was significantly reduced during NP \((P < 0.001 \text{ vs. control})\) but not ADM. Again, urinary sodium was significantly greater during ADM compared with NP \((P = 0.01)\). Urinary potassium tended to be higher during ADM compared with both control and NP \((0.05 < P < 0.1; \text{Fig. 3})\). Urinary creatinine excretion was not significantly altered by either ADM or NP (Table 1). Creatinine clearance tended to be increased with ADM but not significantly so (Table 1). Neither plasma sodium nor creatinine levels were significantly different between any study days (data not shown). However, plasma potassium (Table 1) tended to fall with ADM \((P = 0.052)\) and was reduced \(\sim 0.4\) mmol/l by NP \((P < 0.001 \text{ vs. control}, P = 0.043 \text{ vs. ADM})\).

Responses to ANG II and NE. Plasma ANG II levels appropriately rose dose dependently during coinfu-
sions of ANG II (Fig. 4). Achieved levels of ANG II did not differ significantly between the 3 study days. Dose-dependent increments in MAP with ANG II alone were significantly and similarly attenuated by ADM ($P < 0.006$) and NP ($P < 0.001$). The ANG II-induced rise in plasma aldosterone was significantly attenuated by ADM ($P < 0.001$ vs. both control and NP) but not by NP. ANG II induced dose-dependent rises in both ANP and BNP on each day ($all P < 0.001$) with no significant difference between days.

Plasma NE levels were increased dose dependently in response to coinflusions of NE (Fig. 5). Stepped NE infusions induced dose-dependent rises in MAP on the control day, and these were not affected by ADM infusions (Fig. 5). However, NE-induced rises in MAP were significantly attenuated at all doses by NP compared with both control and ADM (both $P < 0.001$). During NE infusions, ADM induced a steady fall in plasma aldosterone levels ($P = 0.005$ vs. control, $P = 0.035$ vs. NP). Stepped NE infusions induced dose-dependent rises in plasma BNP, similar on each infusion day (all $P < 0.001$), and in plasma ANP levels on the ADM day ($P = 0.045$).

The contrasting effects of ADM and NP on NE-induced increments in MAP but similar effects on ANG II/MAP dose response curves are shown in Fig. 6. Paired comparison of mean slopes obtained from linear regression (MAP vs. log plasma NE) showed that
slopes were attenuated by NP (P < 0.001 vs. both control and ADM) but not ADM. Also shown is plasma aldosterone plotted against log plasma ANG II, where paired comparison of mean slopes obtained from linear regression showed that slopes were attenuated by ADM (P = 0.019 vs. control, P = 0.013 vs. NP) but not NP.

DISCUSSION

Previous studies have reported numerous biological actions for ADM, yet the basis for many of these effects remains unclear. In particular, it is uncertain whether the direct vasodilator action of the hormone underpins many of its other actions. Accordingly, we have compared and contrasted the hemodynamic, hormonal, and renal responses to infusions of ADM and the vasodilator NP, the action of which depends on generation of nitric oxide. We also assessed the effects of ADM on ANG II-induced aldosterone and pressor responsiveness and compared this with NE pressor responsiveness.

Overview. ADM and NP similarly reduced MAP in accord with study design, associated with similarly increased heart rate and falls in hematocrit. By contrast, cardiac output increased 50% with ADM but not NP; therefore, falls in CTPR were greater with ADM.
Pressor responsiveness to NE was attenuated by NP but not ADM. In contrast, ANG II pressor responsiveness was attenuated by both ADM and NP. ADM (but not NP) significantly increased PRA and plasma ANG II. Rises in plasma aldosterone observed with both NP and ADM tended to be less with the latter. The ANG II-induced rise in plasma aldosterone was significantly attenuated by ADM but not NP. Urinary volume and sodium excretion were markedly reduced by NP but were maintained with ADM despite a similar fall in arterial pressure. Thus ADM was relatively diuretic and natriuretic compared with pressure-matched NP data.

**Hemodynamics.** Hemodynamic effects of ADM have been reported by a number of authors. These actions include lowering of arterial pressure associated with increases in cardiac output and falls in peripheral resistance (4, 22, 16) and direct arterial dilation (1, 6). Less clear is whether baroreceptor-mediated increases in heart rate and sympathetic activity are altered by ADM compared with those observed with other vasodilators. Studies in rats have reported that ADM elicits dose-related hypotension accompanied by increases in heart rate and renal sympathetic nerve activity (SNA; Ref. 28). Studies in rabbits similarly report increases in renal SNA, but these were attenuated compared with responses induced by NP (9). Previous studies from our laboratory showed no significant rise in heart rate (although heart rate did tend to be higher during ADM infusions) and plasma ANG II levels fell in conscious sheep infused with ADM (4). The present study provides no evidence for attenuation of either heart rate or plasma catecholamine responses with ADM compared with NP. The reasons for differences in this and our previous study (4) are unclear but may relate to dose and/or duration of infusions.

The effect of ADM on cardiac output could result from alterations in cardiac preload or afterload, baroreflex-induced augmentation of efferent cardiac sympathetic activity, or a direct positive inotropic action (22, 31). The present study demonstrates a substantial increase in cardiac output with ADM quite distinct from NP, which had no observable effect. Accordingly, CTPR fell more for a given fall in arterial pressure with ADM than NP. The available evidence points to ADM being a potent vasodilator (1, 6). The consistent observation that intravenous ADM reduces peripheral resistance and that intra-arterial administration of the hormone increases local blood flow (1, 6) suggests a direct effect of ADM on arterial tone, although specific antagonism of potent constrictor hormones such as ANG II cannot be ruled out. Taken together, the rise in cardiac output with ADM could result from greater vasodilation than NP and/or a direct inotropic action but it is not possible to elucidate the exact mechanism further from our data.

The marked attenuation of the ANG II-induced rise in blood pressure observed in the present study and an earlier study from our laboratory (5) clearly demonstrates that ADM opposes the vasopressor effects of ANG II. Whether this represents direct interactions at a cell surface or intracellular level, or indicates independent directionally opposed actions that act to cancel each other out, remains to be determined. However, it is important to note that NP and ADM demonstrated similar potency at attenuating the ANG II-induced rise in pressure. Also of interest is whether ADM exerts a predilection for relaxing ANG II-induced vascular tone vs. other vasoconstrictors such as NE, as has been demonstrated, for example, with ANP (14). Indeed we demonstrate here in conscious healthy sheep that in contrast to NP, ADM did not alter the pressor response to NE. This suggests selective ability of ADM to oppose vasoconstricting agents and indicates adrenoceptor va-
soconstriction fully overwhelms the vasodilator effects of ADM.

*Hormones.* In the present study, despite similar falls in MAP, ADM significantly increased PRA and ANG II beyond trends induced by NP. Thus, in addition to reducing arterial pressure (and thereby stimulating the afferent renal arteriolar baroreceptor) and augmenting (or at least not inhibiting) delivery of chloride and sodium to the macula densa, ADM presumably acts directly on juxtaglomerular granular cells to augment renin release (12). There are conflicting reports regarding in vivo studies of the action of ADM on aldosterone. Some studies reported inhibition of endogenous (25) and ANG II-stimulated plasma aldosterone (24, 29) while other studies report little or no effect (4, 5). Despite vigorous activation of PRA and ANG II by ADM, rises in plasma aldosterone were marginally attenuated with ADM compared with responses with pressure-matched doses of NP (P = 0.056). During NE infusions, ADM induced a steady fall in plasma aldosterone levels not seen on control and NP days. Furthermore, ADM clearly attenuated plasma aldosterone responses to exogenous ANG II infusion. Thus, these results support a role for ADM in the regulation of aldosterone secretion, presumably by a direct inhibitory action on aldosterone secretion from the adrenal glomerulosa (21), especially when circulating levels of ANG II are high.

Plasma BNP levels were reduced by ADM compared with NP. The mechanism is unclear, but in vitro data indicate suppression of ANP mRNA expression by ADM in neonatal rat cardiocytes (30). This study is the first to demonstrate stepped NE infusions increase plasma BNP levels, presumably reflecting increased cardiac transmural stress and/or direct actions of NE on BNP secretion. Although Thibault et al. (32) note that both ANP and BNP can be stored in the same atrial granules and hence assume that the regulation of their secretion is the same, to our knowledge there have been no previous reports of NE- or ANG II-induced activation of BNP secretion. Despite previous reports that NE promotes secretion of ANP (27, 32), effects of NE on plasma ANP levels were less dramatic in the present study. Both plasma ANP and BNP were increased by stepped infusions of ANG II consistent with the accepted role for ANG II in secretion of ANP (27, 32).

Inasmuch as the amino acid sequence of ovine ADM is yet to be determined, we infused the human form in the present studies. There is a high degree of homology for ADM between species sequences thus far identified (26). On the other hand, it appears that bioactivity may be species specific (15), hence it will ultimately be important to determine the biological actions of the hormone in the species of origin. Using an assay developed for measuring ADM in human plasma (18), we could not confirm detectable IR-ADM in sheep plasma, endogenous levels were at or below detection limit of the assay. Nevertheless, at the dose employed in the present study, achieved plasma levels of ~10 pmol/l are at the upper physiological limit for humans (normal range in our laboratory with this assay is 2.7–10.1 pmol/l). Therefore biological activity observed at these circulating levels may have physiological relevance. Doses of ANG II employed in the present study covered a broad spectrum of the dose-response curve, with achieved plasma levels during the lowest three doses within the physiological-pathophysiologichal range and the highest dose being supraphysiologichal. NE doses employed covered a similar range from physiological to supraphysiologichal and were chosen to induce similar increments in arterial pressure to those observed with ANG II.

*Renal.* It remains unclear whether ADM induces diuresis and natriuresis in normal animals at physiologically relevant circulating levels. Intrarenal administration of ADM has been reported to be natriuretic and diuretic in anesthetized rats (11) and dogs (8). These authors also showed that ADM significantly decreased renal vascular resistance and increased renal blood flow via pre- and postglomerular arteriolar vasodilation. ADM is natriuretic in an ovine pacing model of heart failure (25) but not in normal conscious sheep (4). In the present study, ADM demonstrated greater urinary volume and sodium excretion compared with pressure-matched NP data. Thus, despite significant falls in arterial pressure (and presumably renal perfusion pressure) with subtle, physiologically relevant rises in plasma ADM levels, renal excretion of fluid and sodium was maintained, indicating a significant shift in the pressure-natriuresis curve.

In conclusion, low-dose infusions of ADM administered to conscious sheep induced significant hemodynamic actions, including reduced MAP and increased cardiac output. Concurrently, ADM activated PRA and ANG II and yet resulted in greater urinary volume and sodium excretion than with pressure-matched NP. ADM clearly antagonized the vasopressor and aldosterone responses to administered ANG II but not NE. These results clarify mechanisms by which ADM might contribute to volume and pressure homeostasis.

**Perspectives**

Plasma concentrations of ADM in some conditions are sufficient to suggest a role for the circulating peptide in the regulation of arterial pressure. Furthermore, biological actions previously reported for ADM are consistent with a potential role for ADM in volume and pressure homeostasis. Nonetheless, the precise role of ADM in normal mammalian homeostasis is far from settled despite a growing body of literature. Many early studies employed pharmacological doses of ADM given for short periods, making interpretation of the physiological actions of ADM difficult. Moreover, many studies focused on a single system and failed to view the actions of ADM as integrative (cardiovascular, renal, and hormonal) functions. Proof that ADM is a physiologically relevant hormone requires (among other things) the demonstration of end organ responses to changes in plasma peptide levels encompassing those observed in normal health. We reported here
significant hemodynamic, hormonal, and renal preserving actions (compared with pressure-matched NP infusions) of ADM infused at a dose that raises circulating levels within the physiological range observed in humans. Furthermore, ADM clearly antagonized the vasopressor and aldosterone responses to administered ANG II but not NE. Thus these results clearly point to a physiological or pathophysiological role for ADM in pressure and volume homeostasis. Future studies employing transgenic animals (under and overexpression of ADM) and ADM antagonists will further elucidate such a role.

We are grateful to staff of the Christchurch School of Medicine Animal Laboratory for assistance with animal studies and Christchurch Cardiorenocrine Laboratory staff for hormone assays. Support was provided through grants from the National Heart Foundation of New Zealand and Health Research Council of New Zealand.

REFERENCES