J. Pharm. Pharmacol. 1996, 48: 578–580
Received June 26, 1995
Accepted January 19, 1996

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Adrenomedullin is Inactivated in the Lungs of Neonatal Piglets

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Abstract

Adrenomedullin, a novel 52 amino acid peptide normally present in adult human plasma, has been shown to induce systemic hypotension in the adult rat, pig and cat, and in the new-born piglet. Little is known about the site(s) of adrenomedullin inactivation in adults or neonates.

Groups of five 0-2-day old and 2-week old anaesthetized piglets were prepared to enable continuous monitoring of cardiac output, mean systemic arterial pressure, mean pulmonary artery pressure, mean systemic vascular resistance and mean pulmonary vascular resistance. In both age groups, injections of human adrenomedullin$_{1-52}$ into the left atrium produced significant ($P < 0.05$) reductions in mean systemic arterial pressure and mean pulmonary artery pressure. Although injections of similar doses of human adrenomedullin$_{1-52}$ into the right atrium produced significant ($P < 0.05$) decreases in mean pulmonary artery pressure, there were no appreciable alterations in mean systemic arterial pressure in either age group.

These results suggest that the systemic vasodilator properties of human adrenomedullin$_{1-52}$ are reduced upon first pass through the pulmonary circulation in 2-week old piglets, a phenomenon that is present at birth.

Adrenomedullin is a 52 amino acid peptide with potent vasoactive properties initially isolated from pheochromocytoma tissue (Kitamura et al 1993a). Studies using mRNA blot analysis indicate that adrenomedullin is expressed by porcine (Kitamura et al 1994), rat (Sakata et al 1993) and human (Kitamura et al 1993b) lung, heart, kidney, adrenal gland, duodenum, spleen and submandibular gland. The predicted sequence of porcine and human adrenomedullin is 52 amino acids in length. Porcine adrenomedullin is identical to the human isoform except for a single base replacement of Gly for Asn at position 40 (Kitamura et al 1994). Intravenous injections of both adrenomedullin isoforms have, however, been shown to dramatically reduce systemic arterial pressure in the adult rat (Kitamura et al 1993a; Perret et al 1993). Using an antibody to human adrenomedullin$_{1-12}$, Kitamura et al (1993a) have demonstrated that adrenomedullin circulates in the plasma of healthy humans at a concentration sufficiently high (19 ± 5.4 fmol mL^{-1}) to suggest a role for this peptide in blood pressure regulation. Finally, we have recently demonstrated that human adrenomedullin$_{1-52}$ also reduced mean systemic arterial pressure in new-born piglets, suggesting that adrenomedullin may as well play a role in the regulation of neonatal vascular tone (DeVito et al 1995).

Although the effects of adrenomedullin on systemic haemodynamics are now well documented, there exist few data about the effects of this peptide upon the pulmonary vascular system in adult animals, and none in the new-born age group. Lippton et al (1994) have demonstrated that direct injections of human adrenomedullin into the pulmonary arterial circuit reduced pulmonary vascular resistance in the preconstricted pulmonary vascular bed of the intact cat. These investigators also observed that systemic arterial pressure changed little during their experiments, and only at high adrenomedullin doses, suggesting that the pulmonary vascular bed may be a site of adrenomedullin inactivation. Given this background, this study was undertaken to investigate the effects of human adrenomedullin on systemic and pulmonary haemodynamics in neonatal piglets.

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Materials and Methods

All experiments were conducted under the guidelines of, and approval of, the Tulane University School of Medicine Advisory Committee for Animal Resources.

Surgical and experimental protocols

Groups ($n = 5$ per group) of 0-2-day old (1.2 ± 0.2 kg) and 2-week old (2.2 ± 0.25 kg) piglets were sedated with intramuscular ketamine hydrochloride (10–15 mg kg^{-1}) and then anaesthetized with intravenous pentobarbital sodium (30 mg kg^{-1}). Supplemental doses of pentobarbital were administered as needed to maintain a level plane of anaesthesia. Each animal underwent tracheotomy before being placed on a Harvard ventilator. Through a neck cut-down, the right internal jugular vein was cannulated and a catheter was advanced toward the right atrium to provide a port for the administration of maintenance fluid and drug injections. A brachial artery was cannulated to monitor mean systemic arterial pressure.

A left thoracotomy was then performed, and the ductus arteriosus (or its remnant) was ligated and divided. A Transonic blood flow probe was placed around the ascending aorta to monitor cardiac output (minus coronary artery blood flow). The pulmonary artery was cannulated to enable continuous measurement of mean pulmonary artery pressure. The left atrium was accessed via the left atrial appendage in order to provide an injection port. Finally, the right atrium was palpated in order to confirm proper positioning of the catheter that had previously been introduced via internal jugular venotomy. Upon completion of the surgical preparation, a 20-min time interval was allowed before instituting a given experimental protocol;

Table 1. Effects of right and left atrial bolus injections of human adrenomedullin$_{1-52}$ in 0–2–day old and 2-week old piglets on the percent change from baseline cardiac output (CO), pulmonary vascular resistance (PVR), and systemic vascular resistance (SVR).

Dose	1 μg		3 μg		10μg	
Injection site	Left atrium	Right atrium	Left atrium	Right atrium	Left atrium	Right atrium
0–2-Day old						
CO	6·3 ± 5·6	21·8 ± 9·9	12·6 ± 3·1*	3·9 ± 0·6*	24·5 ± 12·0	8·3 ± 3·6
PVR	− 5·7 ± 3·6	− 36·6 ± 7·1*	− 31·2 ± 6·4*	− 20·4 ± 4·3*	− 23·7 ± 4·4*	− 26·0 ± 4·7*
SVR	− 19·9 ± 2·6*	− 21·7 ± 10·2	− 20·5 ± 2·3*	− 9·1 ± 2·9*	− 18·1 ± 4·5*	− 16·4 ± 5·1
2-Week Old						
CO	8·6 ± 6·7	1·1 ± 3·2	1·1 ± 1·3	13·7 ± 6·5	25·1 ± 12·2	8·7 ± 3·8
PVR	− 15·4 ± 9·3	− 12·6 ± 3.6*	− 16·6 ± 3·5*	− 22·1 ± 4·1*	− 37·8 ± 14·3*	− 35·0 ± 4·9*
SVR	− 23·1 ± 3·2*	− 3·2 ± 4·0	− 13·1 ± 2·9*	− 12·5 ± 6·8	− 33·1 ± 4·9*	− 15·8 ± 6·3

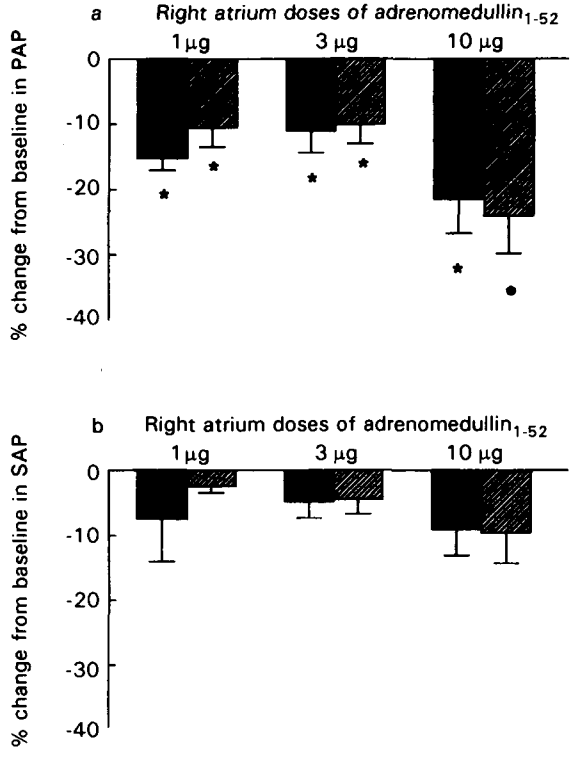

FIG. 1. Effects of right atrial bolus injections of human adrenomedullin$_{1-52}$ in 0-2-day old (■) and 2-week old (▨) piglets on the percent change from baseline (a) mean pulmonary arterial pressure (PAP) and (b) mean systemic arterial pressure (SAP). *$P < 0.05$ vs baseline.

during this time we ensured that the mean systemic and pulmonary artery pressures remained at steady, baseline levels.

After obtaining stable baseline haemodynamic measurements, 1-, 3- and 10-μg doses of human adrenomedullin$_{1-52}$ were administered by bolus injection, in random sequence, into the right and left atrial ports. Cardiac output (mL min^{-1}), mean systemic arterial pressure (mmHg) and mean pulmonary artery pressure (mmHg) were recorded just before, and 0·5, 1, 3, and 5 min after a given peptide injection. Between each injection, sufficient time was allotted in order to ensure that mean systemic and pulmonary arterial pressures had returned to their respective baseline values. Mean systemic (pulmonary) vascular resistance (mmHg mL^{-1} min^{-1}) was calculated by dividing mean systemic (pulmonary) artery pressure by cardiac output.

Drugs

Synthetic human adrenomedullin$_{1-52}$ (Peptides International, Belmont, CA, USA) was dissolved in 0·9% NaCl to give a concentration of 0·1 mg mL^{-1}.

Data expression and statistical analysis

All data are expressed as mean (± s.e.) percent peak changes from baseline values. Statistical analysis was performed using Student's paired *t*-test, with a $P < 0.05$ accepted as a significant difference.

Results

In all experimental protocols, the effects of bolus infusions of adrenomedullin$_{1-52}$ in 0-2-day old piglets were identical to those observed in the 2-week old animals. Right atrial injection of human adrenomedullin$_{1-52}$ produced an immediate dramatic reduction in mean pulmonary artery pressure, reaching a nadir after 1 min with each administered dose (Fig. 1a); pulmonary artery pressure typically returned to baseline within 3 min. Pulmonary vascular resistance likewise declined significantly following each dose of adrenomedullin (Table 1). It should be noted that these same animals experienced no significant changes in mean systemic arterial pressure (Fig. 1b). Although mild increases in cardiac output and decreases in mean systemic vascular resistance were observed (Table 1), these changes were, with a single exception, not statistically significant.

In contrast, left atrial injections of all doses of human adrenomedullin$_{1-52}$ dramatically reduced mean systemic arterial pressure, again reaching a maximum decline after 1 min (Fig. 2a). Likewise, there was a decline in mean systemic vascular resistance (Table 1), concomitant with a slight, statistically insignificant increase in cardiac output (Table 1). These animals also experienced a significant decline in mean pulmonary artery pressure — which reached a nadir about 1 min after the decrease in mean systemic arterial pressure — after virtually all administered doses (Fig. 2b). Pulmonary vascular resistance was also observed to decrease in concert with the decline in pulmonary artery pressure (Table 1).

Discussion

The observation that adrenomedullin$_{1-52}$ produced marked, albeit short-lived, reductions in mean systemic arterial pressure and systemic vascular resistance in new-born piglets — while

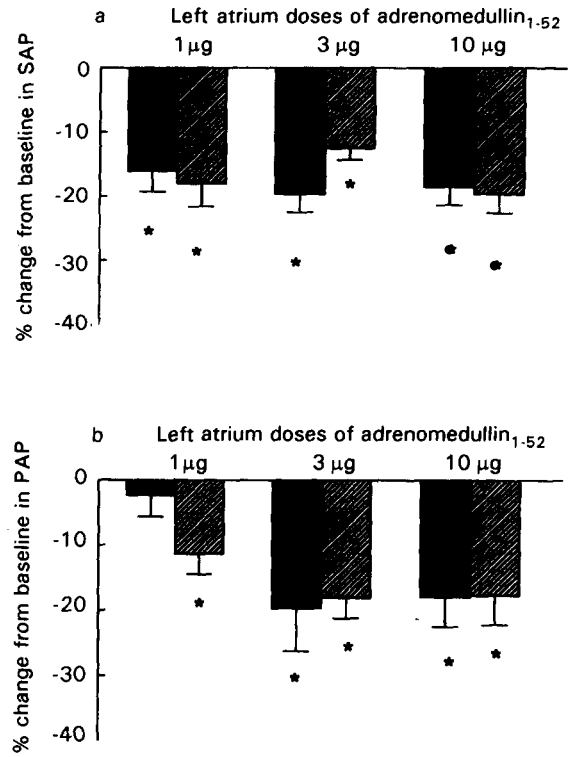

FIG. 2. Effects of left atrial bolus injections of human adrenomedullin$_{1-52}$ in 0-2-day old (■) and 2-week old (▨) piglets on the percent change from baseline (a) mean systemic arterial pressure (SAP) and (b) mean pulmonary arterial pressure (PAP). *$P < 0.05$ vs baseline.

maintaining cardiac output — is consistent with previous reports of its effects when administered to adult animals (Kitamura et al 1993a; Hao et al 1994). That left heart injections of adrenomedullin$_{1-52}$ also produced reductions in mean pulmonary artery pressure (and pulmonary vascular resistance) that were virtually identical in magnitude (though, as expected, delayed in onset relative to the changes in mean systemic arterial pressure) to those seen following right heart injections of this peptide strongly suggests, however, that adrenomedullin$_{1-52}$ is not inactivated to an appreciable extent by tissues or organs within the systemic arterial circuitry.

In contrast, right heart injections of adrenomedullin$_{1-52}$ in doses identical to those given via the left atrial infusion port, produced insignificant reductions in mean systemic arterial pressure and systemic vascular resistance, implying that adrenomedullin$_{1-52}$ not only exerts vasodilator effects within the pulmonary vascular system, as has been previously reported to occur in the adult cat (Lippton et al 1994), but also is probably inactivated somewhere within the pulmonary circulation.

It is recognized that these observations regarding the lack of an appreciable systemic effect of adrenomedullin$_{1-52}$ when given by the peripheral intravenous route to neonatal piglets may appear at odds with our previously reported finding that intravenous adrenomedullin$_{1-52}$ did cause a reduction in mean systemic arterial pressure and systemic vascular resistance in new-born piglets (DeVito et al 1995). Several differences in experimental design could, however, account for these discrepancies, including: the doses of adrenomedullin$_{1-52}$ used herein (1–10 μg) were considerably less than those used in the previous experiments (30 μg); we made no attempt to ligate the ductus arteriosus in the previously reported experiments.

Irrespective of these differences, these data strongly suggest that adrenomedullin$_{1-52}$ possesses potent systemic and pulmonary vasodilator properties that are functional at birth. That adrenomedullin$_{1-52}$ appears to be inactivated primarily within the pulmonary circuitry implies that this agent may play a physiological role in mediating pulmonary vasodilation.

Acknowledgements

This work was supported by the Louisiana Education Quality Support Fund. Dr Ferrara is a recipient of the Society for Surgery of the Alimentary Tract Career Development Award.

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