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Complex Haemodynamic Changes In Vivo After Adrenomedullin Administration in Rats

L. Szekely^{1,2}, P. Vijayaraghavan¹, T. G. Sharp¹, J. W. Brown¹

Adrenomedullin (ADM) is a peptide that has potent vasodilatory effect in several vascular beds. Its effect on the haemodynamics of the systemic-pulmonary circulation is unknown. We studied the kinetics of ADM action in rats *in vivo* with pulmonary hypertension.

Sprague-Dawley rats, 6 weeks old (280–340 g), were divided into control (n = 5), MCT (n = 6) and ADM (n = 6) groups and received a single intraperitoneal dose of vehicle, monocrotaline (60 mg/kg b.wt.) and ADM_(1–50) (3 nmol/kg b.wt.) respectively. Three weeks later, systemic (P_s) and direct pulmonary arterial mean pressures (P_a, mmHg) were measured after ADM administration (6.6 pmol) every 2 min for up to 18 min.

P_s declined at 2 min in MCT and ADM groups (79.4 ± 3.5 to 76.6 ± 3.8 and 63.7 ± 5.5 to 58.7 ± 1.1 respectively), but was delayed until 4 min in the Control group (68.5 ± 1.4 to 61.8 ± 1.9). There were no significant differences in P_s 6 to 12 min following ADM administration between the groups. At 12 min, there was a 30.8 % (54.9 ± 4.0) decrease in MCT group compared to baseline (p = 0.0005). Similar kinetic patterns were observed in P_a in all three groups with a marked decrease (28.4 %) from 18.0 ± 1.0 to 12.9 ± 0.7, p = 0.0007, in MCT group after 12 min. The hypotensive effect of ADM in the P_a was shorter (8 min) in the ADM pretreated animals compared to either control or MCT groups (12 min).

The results indicate that ADM vasodilates more rapidly in MCT and ADM pretreated rats. The effect of ADM is pronounced and long lasting in MCT- and vehicle-treated rats. It may be explained by the regulated clearance of ADM and related receptors. *J Clin Basic Cardiol 2001; 4: 79–82.*

Key words: adrenomedullin, pulmonary hypertension

Adrenomedullin (ADM) is an endogenous peptide with potent hypotensive effect [1]. It was originally isolated and sequenced from human pheochromocytoma [2], but ADM and its mRNA were shown to be expressed widely in lung, heart, kidney and adrenal medulla. ADM presents in significant levels in plasma functions as a circulating hormone and regulates systemic and pulmonary pressures [3]. Plasma levels of ADM have been found to be elevated in patients with hypertension and renal failure [4]. In our previous work, we have shown that in children who underwent open heart surgery for congenital heart disease, pre-operative plasma ADM levels significantly correlated with the occurrence of postoperative pulmonary hypertension [5]. In addition, ADM has been shown to exhibit significant vasodilator activity in a number of regional vascular beds [6]. The resting pulmonary vascular tone, however, remains unaltered after ADM administration.

ADM reduces pulmonary vascular resistance only under conditions of elevated pulmonary vascular tone [7]. In spite of several *in vivo* studies, which examined the systemic hypotensive effect of ADM in anaesthetized rat and sheep models [8,9], the onset and duration of haemodynamic changes after ADM administration in the systemic and pulmonary circulations have not yet been demonstrated in rats. Injection of rats with monocrotaline (MCT, a pyrrolizide alkaloid) causes complex endothelial damage, increases vascular muscularization and capillary thrombosis. These structural changes correlate with a progressive rise in pulmonary arterial pressure and, after 3–4 weeks, with the development of pulmonary hypertension [10].

The purpose of this study was to determine the onset and duration of complex haemodynamical effects after the administration of ADM in anaesthetized rats. We compared the kinetics of ADM actions on pulmonary and systemic blood pressures in MCT and ADM pretreated rats.

Materials and Methods

The study was performed and the animals were cared for according to the guidelines of Indiana University Laboratory Animal Care and Research Committee.

Experiment

Six week-old Sprague-Dawley rats (Harlan, Inc. Indianapolis, IN) weighing 280–340 g were divided into three groups: (MCT n = 6, control n = 5, ADM n = 5). MCT group rats were injected intraperitoneally with a single dose of 60 mg/kg b.wt. MCT (Sigma Chemicals Co., St. Louis, MO) dissolved in 1N HCl and neutralized with 0.5N NaOH to a dilute solution with distilled water. In the control group, the rats were given a comparable volume (0.75 ml) of the solvent used to prepare MCT solution. ADM group rats received 3 nmol/kg b.wt of rat ADM_(1–50) (Peninsula Laboratories, Belmont, CA). They were given food and tap water *ad libitum*. Weights were recorded before, on the day of injection and every fifth day thereafter.

Haemodynamic Studies

Three weeks later, the rats were anaesthetized with intramuscular injection of Ketamine (100 mg/kg; Fort Dodge Laboratories, Fort Dodge, IA) and Rompun (15 mg/kg; Miles, Shawnee, KS). After stable anaesthesia was obtained, the trachea was cannulated with a polyethylene tubing (16 G = 1.7 mm, Angiocath, Becton Dickinson, Vascular Access, Sandy, Utah), connected to a rodent ventilator (Model SAR 21), Analytical Specialities Co., St. Louis, MO) and ventilated with 21 % O₂/5 % CO₂ with a tidal volume of 2.4–2.6 ml/kg and 30–35 breaths per minute. The right carotid artery was exposed, ligated distally and cannulated with intravascular catheter (24 G = 0.7 mm) to monitor systemic arterial pressure. Another catheter was inserted into the external jugular vein for

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the systemic administration of ADM. Then the chest was opened by left lateral thoracotomy and a small catheter (24 G) was inserted into the left pulmonary artery to measure the pulmonary pressures. Mean and systolic/diastolic pressures were measured in mmHg (Model Escort Series 100 monitor, Medical Data Electronics Inc., Carlsbad, CA) and recorded before and at every two minutes after administration of 6.6 pmol ADM, up to 18 minutes. The transducers were zeroed at the right atrial level.

Statistical Analysis

All values are expressed as mean ± standard deviation. Between group data were compared using ANOVA (Statistica, Statsoft, Inc., Tulsa, OK). Values were considered to be statistically significant when p was less than 0.05.

Results

Body Weights

As illustrated in table 1, total body weights (BW) became significantly different (p ≤ 0.05) among the groups during the 3 week period. BWs were significantly elevated in the Control group (4.1 %), while the BWs of MCT animals were significantly decreased (3.7 %, p ≤ 0.05) 3 weeks after treatment when compared to their day-of-injection weights, The BWs of ADM treated rats did not show any significant change throughout the study period.

Blood Pressure Changes after ADM injection

The baseline *mean systemic pressures* (figure 1) were significantly elevated in MCT group (79.4 ± 3.5, p ≤ 0.01) when compared to ADM (63.7 ± 5.5) or Control (68.5 ± 1.4) groups. The pressures began to decline 2 minutes after a bo-

lus administration of 6.6 pmol ADM in ADM and MCT groups and after 4 minutes they became significantly lower (50.8 ± 3.3 and 66.1 ± 4.3, respectively), p ≤ 0.01 than the baseline levels. In the Control group, however, the decline was delayed until 4 minutes which was significant (61.8 ± 1.9, p ≤ 0.05). There were no significant differences in the systemic pressures 8 to 12 minutes following ADM administration between all the groups. At 12 minutes, there was 30.8 % (54.9 ± 4.0, p = 0.0002) decrease in the MCT group compared to baseline. The decreases were 17.3 % and 27.2 % in the ADM and Control groups respectively at the same time point (see insert). Recovery from the effect of ADM occurred earlier (at 12 minutes) in the ADM group, than either in MCT or Control groups.

Similar kinetic patterns were observed in *mean pulmonary artery pressures* in all three groups studied (figure 2). The baseline levels in MCT animals were significantly above (18.0 ± 1.0, p ≤ 0.01) the levels of ADM (14.6 ± 1.1) and Control (14.4 ± 1.0) rats, however the difference diminished from 10 to 18 minutes following ADM injection. Although the pressure levels tend to decline in all groups, significant decreases were seen only in MCT group from 6 to 18 minutes after ADM and at the 12 minute time point in the Control group (11.3 ± 0.5, p = 0.02). ADM was most effective in MCT group with a 28.4 % decrease (18.0 ± 1.0 to 12.9 ± 0.7, p = 0.0007) 12 minutes after administration (see insert). The hypotensive effect of ADM in the pulmonary artery was shorter (8 minutes) in the ADM pretreated rats compared to either Control or MCT pretreated rats (12 minutes).

Heart Rates

There were no significant differences in the heart rate between the groups throughout the measurements (data not shown).

Table 1. Weights of Rats

Groups	Days after injection					
	-2	0	5	10	15	21
Control	296.0 ± 1.8	296.0 ± 2.3	302.0 ± 1.7	304.0 ± 1.1	310.0 ± 2.0 ^o *	308.0 ± 1.70 ^o *
MCT	296.7 ± 5.2	290.0 ± 4.5	287.3 ± 4.1	286.7 ± 3.0	283.3 ± 2.6	279.3 ± 4.10 ^o *
ADM	300.0 ± 6.1	302.0 ± 4.2	298.0 ± 5.5	302.0 ± 4.2	300.0 ± 7.1	295.0 ± 4.20 ^o

Values are mean ± standard deviation and expressed as gram
 * p ≤ 0.05 versus day 0 after injection, ^o p ≤ 0.05 versus other groups.

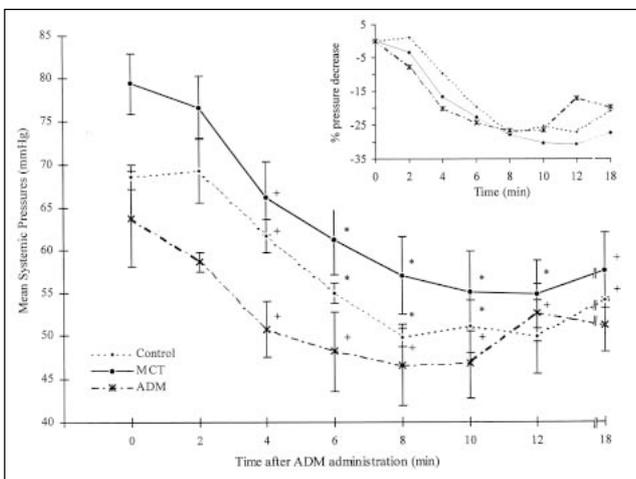


Figure 1. Changes in mean systemic (carotid) artery pressures after ADM injection up to 18 minutes. The values expressed in mmHg. (+ p ≤ 0.05, * p: ≤ 0.001 compared to levels on ADM administration.)
 Insert: Percent decrease of systemic blood pressure after ADM administration.

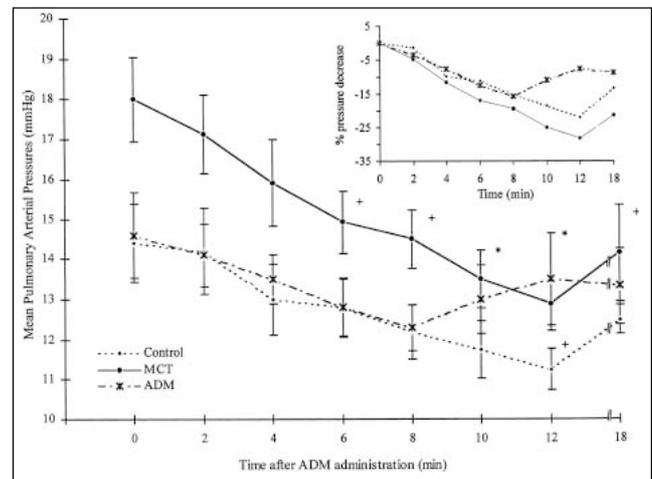


Figure 2. Changes in mean pulmonary artery pressures after ADM injection up to 18 minutes. The values expressed in mmHg. (+ p: ≤ 0.05, * p: ≤ 0.001 compared to levels on ADM administration.)
 Insert: Percent decrease of pulmonary blood pressure after ADM administration.

After 2 minutes following 6.6 pmol ADM injection, the heart rates increased significantly from baseline and remained elevated during the 18 minute period.

Discussion

In this study we evaluated the onset and duration of haemodynamic changes due to ADM administration in anaesthetized rats, that were pretreated either with MCT to cause elevated pulmonary pressures or with ADM.

We have shown that a bolus administration of synthetic rat ADM₍₁₋₅₀₎ was capable of rapidly reducing systemic and pulmonary arterial pressures for at least 18 minutes in anaesthetized rats. Changes in these pressures due to the presence of ADM, however, were complicated by a number of its effects such as increased total peripheral conductance [11], cardiac output, and heart rate. The tachycardia is mediated, as other studies have shown, via autonomic baroreflexes, which were abolished during ganglion blockade [9]. The increased cardiac output is due to higher heart frequency and the positive inotropic action, as determined by the increased development of tension in isolated heart preparations [12].

Our results indicate that under conditions of elevated vascular tone, ADM has a more potent hypotensive effect, both in systemic and pulmonary circulation. The significantly elevated baseline pulmonary and carotid arterial pressures diminished considerably more in MCT group than in the other two groups. As a result of this pronounced decline in MCT treated rats, the systemic and pulmonary arterial pressures reached a similar range in 8–18 minutes and 10–18 minutes after ADM administration respectively. It corresponds with the results of other studies that ADM has vasodilatory activity in the pulmonary vascular bed only under conditions of high vascular resistance [7].

This clinically relevant observation can be explained by a wide variety of intracellular effects of ADM. It has been shown that there are at least two mechanisms of action through which ADM elicits its hypotensive effect, and the mechanisms of action seems to depend on the target cell types. First of all, ADM has a direct vascular smooth muscle relaxing effect [13], mediated by an increase in the intracellular cAMP levels, leading to decrease in intracellular Ca²⁺ concentration as well as the Ca²⁺ sensitivity of contractile apparatus [14] and activating myosin light chain kinase. Secondly, in endothelial cells ADM has an indirect vasodilator activity, where it activates phospholipase C and stimulates NO synthase to increase the levels of NO causing vasodilatation [15]. Interestingly, ADM can induce NO synthesis and release from vascular smooth muscle cells in the presence of interleukin-1 or under conditions of vascular injury [16]. This additional effect can augment the marked vascular dilatation in pulmonary hypertension.

Our data also indicates that ADM vasodilates more rapidly in rats with elevated pulmonary pressures (MCT) and with ADM pretreatment than in controls, while the duration of ADM effect is longer in MCT treated and control rats than in ADM pretreated rats. Studies have shown that plasma and right ventricular ADM levels increased after MCT treatment and might be involved in the auto-compensatory mechanisms against pulmonary hypertension [17]. Taken together, our findings may be explained by an altered clearance of ADM and its related receptors. This has led to the speculation that possibly there is an up-regulation of ADM receptors after MCT pretreatment that may result in a faster onset of action, which after ADM pretreatment somehow result in a fast but short lasting vasodilation. In our study, the duration of the vasodilator responses to rat ADM₍₁₋₅₀₎ was shorter than

the 30–40 minutes for the decrease in systemic arterial and in mesenteric perfusion pressures as reported by other studies [6,8,18]. The explanation for the shorter duration of ADM action is uncertain but may involve differences in species of animals studied or even differences in the ADM fragments used, like human ADM₍₁₋₅₂₎ or ADM₍₁₅₋₅₂₎.

Studies in rat models have shown that pulmonary hypertension can be developed 3 weeks after the administration of a single subcutaneous dose of MCT [10]. In our model, the pulmonary pressures became elevated when compared with the control, but remained below the desired level of pulmonary hypertension. As a result of incomplete pulmonary hypertension, the right ventricular compensation might have maintained the appropriate filling pressure for the left heart. This can explain the elevated systemic blood pressure seen after MCT treatment, since MCT not only causes endothelial cell damage in the pulmonary but also in the systemic circulation. In our study, when compared with the published results, the lower pulmonary pressures obtained in MCT treated rats could be due to the older age of the rats used and the different route of MCT administration (intraperitoneal) that might have influenced the development pulmonary hypertension.

In conclusion, the results of the present investigation demonstrate that the effects of ADM vary in intensity, onset and duration in different haemodynamic conditions such as resting or mild pulmonary hypertension and even after ADM pretreatment. These features may suggest the potential capacity of ADM in clinical usage for the treatment of severe as well as mild pulmonary hypertensive events. Studies for elucidating the underlying mechanisms involved in these processes, the changes in ADM and its related receptors and the role of different intracellular messengers are underway in our laboratory.

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