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Complex Haemodynamic Changes In Vivo After Adrenomedullin Administration in Rats

L. Szekely1,2, 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 (Ps) and direct pulmonary arterial mean pressures (Pa, mmHg) were measured after ADM administration (6.6 pmol) every 2 min for up to 18 min.

Ps 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 Ps 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 Pa in all three groups with a marked decrease following ADM administration between the groups. At 12 min, there was a 28.4 % (54.9 ± 4.0) decrease in MCT group after 12 min. The hypotensive effect of ADM in the Ps was 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

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 ADM 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 anesthesia 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
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. Be-
tween group data were compared using ANOVA (Statistica,
Statsoft, Inc., Tulsa, OK). Values were considered to be statisti-
cally significant when p was less than 0.05.

Results

Body Weights
As illustrated in table 1, total body weights (BW) became sig-
nificantly 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 signifi-
cantly 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 signifi-
cantly 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 admin-
istration between all the groups. At 12 minutes, there was 30.8 %
(54.9 ± 4.0, p = 0.0002) decrease in the MCT group com-
pared 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 min-
utes after ADM and at the 12 minute time point in the Con-
trol 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
</tr>
<tr>
<td>Control</td>
<td>296.0 ± 1.8</td>
</tr>
<tr>
<td>MCT</td>
<td>296.7 ± 5.2</td>
</tr>
<tr>
<td>ADM</td>
<td>300.0 ± 6.1</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation and expressed as gram
* p ≤ 0.05 versus day 0 after injection, * 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 ad-
ministration.

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 ad-
ministration.
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 seem 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 a 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 vasodilation [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(1-53) 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.

**References:**

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