Release of thromboxane A\(_2\) by low-dose almitrine in the hypoxic dog

R. Chuma, O. Tanaka, Y. Hoshino, H. Obara, S. Iwai

ABSTRACT: Potentiation of hypoxic pulmonary vasoconstriction by a low dose of almitrine bismesylate (1 µg·kg\(^{-1}\)·min\(^{-1}\)) was evaluated in terms of blood concentrations of adrenaline, noradrenaline, serotonin, histamine, thromboxane TXB\(_2\) and 6-keto-PGF\(_{1\alpha}\) monitored after administration of almitrine bismesylate for 15 min at 1 µg·kg\(^{-1}\)·min\(^{-1}\) in hypoxic and normoxic beagles. The low almitrine dose significantly increased TXB\(_2\) levels in hypoxic beagles, but the levels remained virtually unchanged in the normoxic animals with almitrine bismesylate and in the hypoxic animals with solvent. TXB\(_2\) levels did not increase when the almitrine infusion was increased to 3 µg·kg\(^{-1}\)·min\(^{-1}\) for 15 min in hypoxic conditions. These findings suggest that almitrine is involved in arachidonic acid metabolism at a low rate of infusion and that thromboxane release from hypoxic areas of the lung may cause local vasoconstriction.

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Methods

Muscular paralysis was induced with 2 mg of pancuronium bromide (i.v.) in beagles (body weight: 10–15 kg) anaesthetized with 20 mg·kg\(^{-1}\) of pentobarbital (i.v.). A cuffed endotracheal tube was then inserted in order to ventilate the animals with room air at 15 ml·kg\(^{-1}\)·min\(^{-1}\) (20 times per min) using an animal respirator (R-60, Aika Co., Tokyo). The animals were kept in a supine position and a polyethylene catheter was inserted into the femoral artery in order to determine arterial pressure and facilitate blood collection. In addition, the anterior cubital veins were catheterized in order to infuse pentobarbital (5 mg·kg\(^{-1}\)·h\(^{-1}\)), pancuronium bromide (0.06 mg·kg\(^{-1}\)·h\(^{-1}\)) and almitrine or solvent. A Swan-Ganz catheter was inserted into the pulmonary artery through the right jugular vein. Pressures were determined using Statham P23 pressure transducers and continuously recorded using a physiological recorder (Polygraph Type 361, San-Ei instrument Co., Tokyo). Arterial blood samples were immediately analysed using a blood gas analyser (ABL 3, Radiometer, Copenhagen). Cardiac output was measured by thermodilution. Pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) were calculated by dividing [mean pulmonary arterial pressure (Ppa)-mean pulmonary capillary wedge pressure (Ppcw)] and [mean systemic arterial pressure (BP)-right atrial pressure (Pra)], respectively, by cardiac output.
Experimental design

The animals were divided into three groups: 1) infusion of almitrine bismesylate (1 μg·kg⁻¹·min⁻¹) under hypoxic conditions (n=6); 2) infusion of almitrine bismesylate (1 μg·kg⁻¹·min⁻¹) under normoxic conditions (n=6) and; 3) infusion of the solvent under hypoxic conditions (n=5). After preparation, all of the animals were ventilated with room air for approximately 30 min. The experiment was started when the haemodynamic parameters were stable. Animals in the hypoxic and normoxic groups were continuously ventilated for 60 min using 12% oxygen and room air, respectively. Haemodynamic parameters were determined and blood samples were obtained at the same time for measurement of chemical mediators and for blood gas analysis (control period).

Almitrine bismesylate (1 μg·kg⁻¹·min⁻¹) or solvent (0.6% malic acid diluted with 10% glucose solution) was then infused at a rate of 0.5 μg·kg⁻¹·15 min⁻¹. Measurement of haemodynamic parameters and blood sampling was conducted 5, 10, 15, 30 and 45 min after the start of infusion. All blood samples for assay were immediately cooled and centrifuged. The plasma was stored at -70°C until used for chemical assay of the six mediators.

Almitrine bismesylate was infused at 5 μg·kg⁻¹·min⁻¹ for 15 min into another five dogs under hypoxic conditions and thromboxane (TX) B₂ and 6-keto-PGF₁α were determined.

Chemical assay

Adrenaline and noradrenaline levels were determined by fluorometry following separation using high performance liquid chromatography (HPLC). Serotonin was measured by HPLC using an electrochemical detector, whilst histamine was determined by fluorometry using a spectrophotofluorometer and the ortho-phthalaldehyde reagent [10]. Radioimmunoassay kits, (125I) (NEN, Boston) were used for the determination of TXB₂ and 6-keto-PGF₁α.

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Adrenaline and noradrenaline levels were determined by fluorometry following separation using high performance liquid chromatography (HPLC). Serotonin was measured by HPLC using an electrochemical detector, whilst histamine was determined by fluorometry using a spectrophotofluorometer and the ortho-phthalaldehyde method. TXB₂ and 6-keto-PGF₁α are stable metabolites of TXA₂ and prostacyclin, respectively, were isolated according to a modification of the method of Powell [10]. Radioimmunoassay kits, (125I) (NEN, Boston) were used for the determination of TXB₂ and 6-keto-PGF₁α.

Table 2. - Haemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>Period</th>
<th>Ppa mmHg</th>
<th>Ppcw mmHg</th>
<th>Qt l·min⁻¹</th>
<th>PVR dyne·s·cm⁻³</th>
<th>SVR dyne·s·cm⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>Control</td>
<td>29.2±1.8</td>
<td>10.6±1.7</td>
<td>3.42±0.19</td>
<td>436.3±38.3</td>
<td>3244±253.1</td>
</tr>
<tr>
<td></td>
<td>Almitrine</td>
<td>31.6±1.5*</td>
<td>11.6±2.6</td>
<td>3.46±0.11</td>
<td>507.2±39.4*</td>
<td>3246±230.9</td>
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<tr>
<td>Normoxia</td>
<td>Control</td>
<td>21.5±2.4</td>
<td>8.2±1.3</td>
<td>3.22±0.28</td>
<td>348.4±59.9</td>
<td>3082±326.6</td>
</tr>
<tr>
<td></td>
<td>Almitrine</td>
<td>25.1±3.2*</td>
<td>8.6±1.9</td>
<td>3.23±0.26</td>
<td>434.1±48.1*</td>
<td>3198±246.8</td>
</tr>
</tbody>
</table>

Ppa: pulmonary artery pressure; Ppcw: pulmonary capillary wedge pressure; Qt: cardiac output; PVR: pulmonary vascular resistance; SVR: systemic vascular resistance. Each value represents the mean±sE of the individual maximum values before (control) and after the infusion of almitrine bismesylate (1 μg·kg⁻¹·min⁻¹). *: p<0.05; significantly different from the control period.

Statistics

All data are presented as mean±sE. Statistical analysis was performed using two-way analysis of variance for repeated measurements, followed by Scheffe’s test and Student’s paired t-test.

Results

Almitrine bismesylate induced no significant change in arterial blood pH, carbon dioxide tension (Paco₂) and oxygen tension (Pao₂) when administered at 1 μg·kg⁻¹·min⁻¹ under normoxic and hypoxic mechanical ventilation (table 1).

Table 1. - Arterial blood gas analyses

<table>
<thead>
<tr>
<th></th>
<th>Period</th>
<th>pH</th>
<th>Paco₂ kPa</th>
<th>Pao₂ kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>Control</td>
<td>7.36±0.01</td>
<td>4.64±0.15</td>
<td>6.72±0.20</td>
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<tr>
<td></td>
<td>Almitrine</td>
<td>7.36±0.01</td>
<td>4.64±0.15</td>
<td>6.72±0.20</td>
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<tr>
<td>Normoxia</td>
<td>Control</td>
<td>7.36±0.01</td>
<td>4.72±0.20</td>
<td>11.8±0.37</td>
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<tr>
<td></td>
<td>Almitrine</td>
<td>7.37±0.01</td>
<td>4.65±0.13</td>
<td>12.4±0.42</td>
</tr>
</tbody>
</table>

Paco₂: arterial carbon dioxide tension; Pao₂: arterial oxygen tension. Each value represents the mean±sE of the individual maximal values before (control) and after the infusion of almitrine bismesylate (1 μg·kg⁻¹·min⁻¹).

Although almitrine bismesylate significantly increased pulmonary arterial pressure (Ppa) and pulmonary vascular resistance (PVR) at 1 μg·kg⁻¹·min⁻¹ pulmonary capillary wedge pressure (Ppcw), cardiac output (Qt) and systemic vascular resistance (SVR) remained unchanged (table 2). Almitrine infusion caused a sustained slight increase in the Ppa value under hypoxic conditions but the increase was transient under normoxic conditions. In the solvent group under hypoxic conditions there were no significant haemodynamic changes.

Blood catecholamine, serotonin and histamine levels remained unchanged after the administration of almitrine bismesylate at 1 μg·kg⁻¹·min⁻¹ in all groups (figs 1A, B, C and D). TXB₂, however, increased significantly in the...
Fig. 1. - Concentrations of chemical mediators during and after almitrine or solvent infusion. A: adrenaline; B: noradrenaline; C: serotonin; D: histamine; E: thromboxane B₂; F: 6-keto-PGF₁α. • – •: hypoxia+almitrine (1 μg·kg⁻¹·min⁻¹); —: normoxia+almitrine (1 μg·kg⁻¹·min⁻¹); oo–oo: hypoxia+solvent; oo–oo: hypoxia+almitrine (5 μg·kg⁻¹·min⁻¹); *: p<0.05; **: p<0.01 significantly different from the control period.
shown to potentiate hypoxic conditions, reaching a level about 6 times higher than the control 30 min after the start of infusion. This change was not seen when almitrine bismesylate (5 μg·kg⁻¹·min⁻¹) or solvent was infused in hypoxic conditions or almitrine bismesylate (1 μg·kg⁻¹·min⁻¹) was infused in normoxic conditions (fig. 1E). 6-keto-PGF₁α showed a tendency to increase in the almitrine bismesylate (1 μg·kg⁻¹·min⁻¹) and 5 μg·kg⁻¹·min⁻¹) groups under hypoxic conditions, but the increase was not significant. There was no significant change in the almitrine bismesylate (1 μg·kg⁻¹·min⁻¹) normoxic group or in the solvent hypoxic group (fig. 1F).

Discussion

Several chemical mediators, such as catecholamines, serotonin, histamine and prostanoids, have been linked with hypoxic pulmonary vasconstriction (HPVC). Currently, however, these mediators are not thought to be highly involved in the onset of HPVC [11, 12]. In fact, the present study showed no significant change in any chemical mediator in the solvent group under hypoxic conditions. However, almitrine bismesylate, when infused at 1 μg·kg⁻¹·min⁻¹ significantly increased TXB₂, but none of other chemical mediators measured in the present study. Prostacyclin, which had already been shown to be released by almitrine [8, 9], also demonstrated a tendency to increase as judged by 6-keto-PGF₁α in the present study. This increase, however, did not reach statistical significance. Both prostacyclin and TXA₂ are prostaglandin endoperoxide products in the cyclooxygenase pathway. Prostacyclin is a potent vasodilator, while TXA₂ is a potent vasoconstrictor. The fact that almitrine influences these two different prostanoids as judged by the stable metabolites suggests that it affects the arachidonic acid metabolic pathway by causing phospholipid breakdown.

Exogenous administration of arachidonic acid, a precursor of prostanoids, may result in the formation of both vasodilating and vasoconstricting prostanoids in the lungs [13, 14]. The resulting responses to the prostaglandin precursors are dependent on the substrate concentration, rate and method of administration, activity of biosynthetic enzymes and basal pulmonary vascular tone. There may also be species differences. For this reason, pulmonary blood vessels showed different reactions to the administration of arachidonic acid depending on the experimental conditions [13]. In particular, arachidonic acid was associated with a depressor response when pulmonary vascular resistance was initially high [13, 14]. Almitrine has been shown to improve Pao₂ in patients with COLD [15-17] or acute respiratory failure [18]. This is sometimes associated with an increase in pulmonary arterial pressure, especially after a single dose [15, 16, 18, 19], whilst long-term administration for one year did not show any increase [17]. In animal experiments the effect of almitrine has been variable. It has been shown to potentiate HPVC [6], to have little effect [20, 21] or in some cases to dilate pulmonary blood vessels which were markedly constricted by hypoxia [22, 23].

These inconsistent findings may be explained by differences in doses of almitrine, the rate and mode of administration, basal pulmonary vascular tone, individual variability or the animal species used. In fact, in the present study, almitrine caused an increase in pulmonary arterial pressure in the hypoxic group, however, the magnitude of increase was smaller than in the normoxic group, in which the pulmonary blood vessels were pre-constricted.

Nakashima et al. [7] found that almitrine affected HPVC in different ways depending on its rate of infusion. They subjected the left lower lobe of dogs to hypoxic challenge using a separate ventilation technique and monitored changes in blood flow in the left lower lobe after administration of almitrine bismesylate. Blood flow in hypoxic areas decreased significantly in the 1 μg·kg⁻¹·min⁻¹ group, whilst no change was observed in the 5 μg·kg⁻¹·min⁻¹ group. In the present study, almitrine bismesylate significantly increased TXB₂ under hypoxic conditions when it was administered at a low-dose (1 μg·kg⁻¹·min⁻¹), but this change was not seen under normoxic conditions or in the 5 μg·kg⁻¹·min⁻¹ group. These findings suggest that almitrine, when administered at a low-dose (1 μg·kg⁻¹·min⁻¹), releases thromboxane selectively in hypoxic areas and causes local vasoconstriction, returning the overall pulmonary ventilation/blood flow ratio towards normal.

The lungs, however, are able to produce many vasoconstricting or vasodilating arachidonic acid metabolites in addition to thromboxane and prostacyclin. In addition to products of the cyclooxygenase pathway, leukotrienes and lipoxygenase intermediates also strongly constrict blood vessels [24, 25]. In the present study, pulmonary arterial pressure showed transient increases in the 1 μg·kg⁻¹·min⁻¹ almitrine bismesylate group treated under normoxic conditions, although neither TXB₂ nor 6-keto-PGF₁α showed any significant changes. Increases in pulmonary arterial pressure under normoxic conditions with almitrine has also been reported by other authors [22, 23]. Other arachidonic acid metabolites which were not determined in the present study may be involved in this increase in pulmonary arterial pressure. Further studies are needed to clarify the relationship between almitrine and arachidonic acid metabolism.

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References

3. Rigaud D, Dubois F, Ansquer JC, Brambilla C, Godart I, Parmelle B. - Modifications des rapports ventilation-perfu-