Eur Respir J
1988, 1, 706–710.

Release of thromboxane A_2 by low-dose almitrine in the hypoxic dog

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ABSTRACT: Potentiation of hypoxic pulmonary vasoconstriction by a low dose of almitrine bismesylate (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was evaluated in terms of blood chemical mediator concentrations. Time course changes in the blood concentrations of adrenaline, noradrenaline, serotonin, histamine, thromboxane TXB_2 and 6-keto-$PGF_{1\alpha}$ were monitored after administration of almitrine bismesylate for 15 min at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) 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 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ 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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Keywords: Almitrine; chemical mediators; hypoxic pulmonary vasoconstriction; prostanoids.

Received February 1988; accepted for publication April 14, 1988.

Almitrine bismesylate has been shown to improve arterial O_2 tension and therefore improve tissue oxygenation in patients with chronic obstructive lung disease (COLD) or acute respiratory failure. It has also been found to have an effect on the pulmonary circulation at low-dose levels resulting in improvement of blood gas tensions without any increase in ventilation [1, 2]. Studies conducted using radioisotopic [3] and multiple inert gas methods [4, 5] have suggested that almitrine improves blood gases by correcting ventilation/perfusion inequalities. Animal studies have led to the hypothesis that almitrine enhances hypoxic pulmonary vasoconstriction (HPVC) [6]. In particular, almitrine has been shown to enhance HPVC at low-dose (3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ or below) [7]. However, the mechanism of action still remains unknown.

Many authors have investigated the role of various chemical mediators in the mechanism of HPVC, but only a few studies are available on the relationship between almitrine and chemical mediators. Only prostacyclin has been shown to be released by almitrine [8, 9], but since prostacyclin is a vasodilator, it is not known how it may be related to the mechanism of action of almitrine. In the present study we evaluated the effect of low doses of almitrine on blood concentrations of various possible mediators for HPVC (catecholamines, serotonin, histamine and thromboxane), as well as 6-keto-$PGF_{1\alpha}$, a metabolite of prostacyclin. Changes in various haemodynamic parameters were also recorded.

Methods

Muscular paralysis was induced with 2 mg of pancuronium bromide (*i.v.*) in beagles (body weight: 10–15 kg) anaesthetized with 20 $mg \cdot kg^{-1}$ of pentobarbital (*i.v.*). A cuffed endotracheal tube was then inserted in order to ventilate the animals with room air at 15 $ml \cdot kg^{-1}$ (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 $mg \cdot kg^{-1} \cdot h^{-1}$), pancuronium bromide (0.06 $mg \cdot kg^{-1} \cdot h^{-1}$) 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$) under hypoxic conditions (n=6); 2) infusion of almitrine bismesylate (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or solvent (0.6% malic acid diluted with 10% glucose solution) was then infused at a rate of 0.5 $ml \cdot kg^{-1} \cdot 15\ min^{-1}$. 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 15 min into another five dogs under hypoxic conditions and thromboxane (TX) B_2 and 6-keto-$PGF_{1\alpha}$ 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 method. TXB_2 and 6-keto-$PGF_{1\alpha}$ which are stable metabolites of TXA_2 and prostacyclin, respectively, were isolated according to a modification of the method of POWELL [10]. Radioimmunoassay kits, (^{125}I) (NEN, Boston) were used for the determination of TXB_2 and 6-keto-$PGF_{1\alpha}$.

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 ($Paco_2$) and oxygen tension (Pao_2) when administered at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ under normoxic and hypoxic mechanical ventilation (table 1).

Table . 1. – Arterial blood gas analyses

	Period	pH	$Paco_2$ kPa	Pao_2 kPa
Hypoxia 12% O_2	Control	7.34±0.02	4.74±0.11	6.52±0.22
	Almitrine	7.36±0.01	4.64±0.15	6.72±0.20
Normoxia 21% O_2	Control	7.36±0.01	4.72±0.20	11.8±0.37
	Almitrine	7.37±0.01	4.65±0.13	12.4±0.42

$Paco_2$: arterial carbon dioxide tension; Pao_2: arterial oxygen tension. Each value represents the mean±SE of the individual maximal values before (control) and after the infusion of almitrine bismesylate (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$).

Although almitrine bismesylate significantly increased pulmonary arterial pressure (Ppa) and pulmonary vascular resistance (PVR) at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ pulmonary capillary wedge pressure (Ppcw), cardiac output ($\dot{Q}T$) 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in all groups (figs 1A, B, C and D). TXB_2, however, increased significantly in the

Table 2. – Haemodynamic variables

	Period	Ppa mmHg	Ppcw mmHg	$\dot{Q}T$ $l \cdot min^{-1}$	PVR $dyne \cdot s \cdot cm^{-5}$	SVR $dyne \cdot s \cdot cm^{-5}$
Hypoxia 12% O_2	Control	29.2±1.8	10.6±1.7	3.42±0.19	436.3±38.3	3244±253.1
	Almitrine	31.6±1.5*	11.6±2.6	3.46±0.11	507.2±39.4*	3246±230.9
Normoxia 21% O_2	Control	21.5±2.4	8.2±1.3	3.22±0.28	348.4±59.9	3082±326.6
	Almitrine	25.1±2.3*	8.6±1.9	3.23±0.26	434.1±48.1*	3198±246.8

Ppa: pulmonary artery pressure; PVR: Ppcw: pulmonary capillary wedge pressure; $\dot{Q}T$: 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$). *: $p<0.05$; significantly different from the control period.

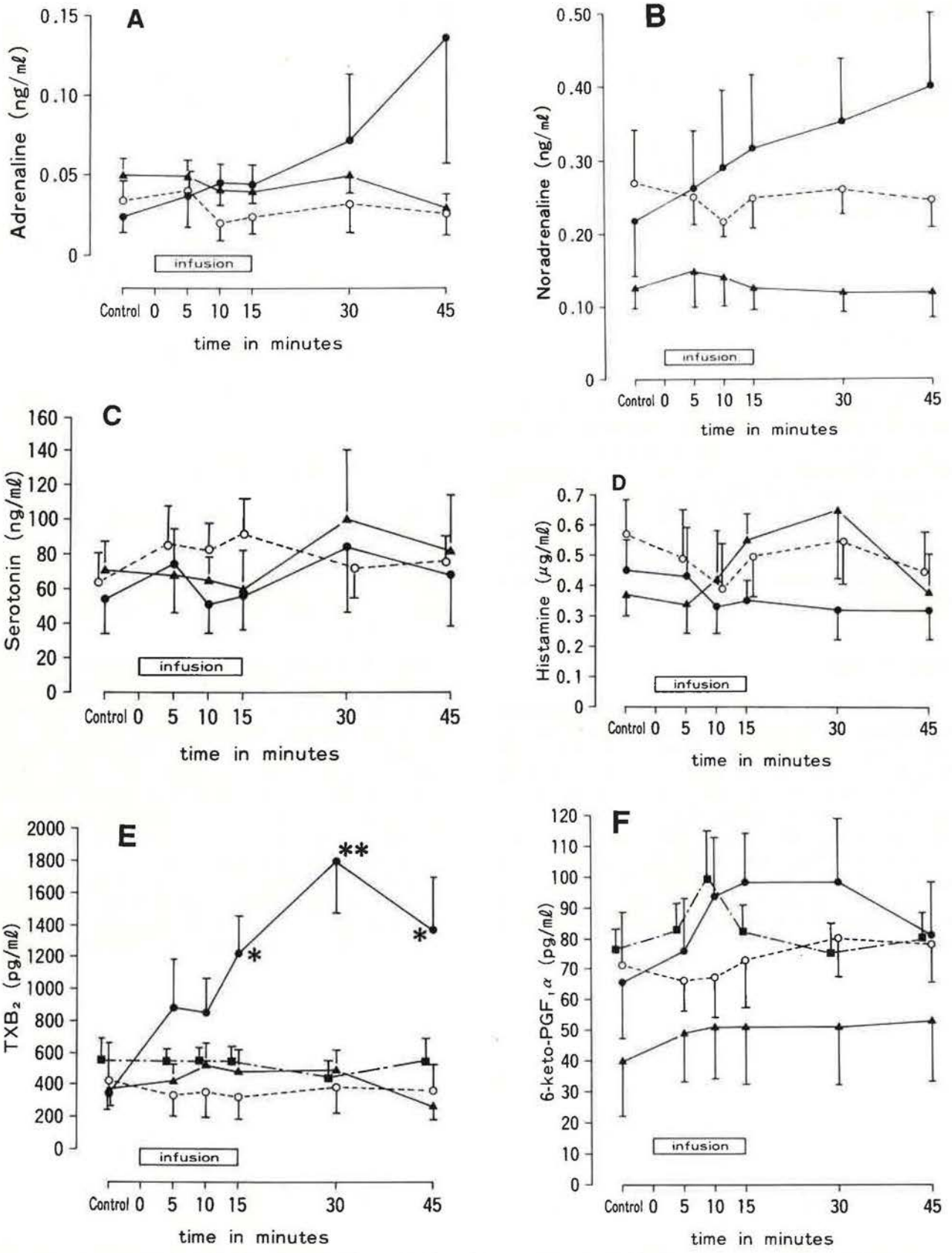

Fig. 1. – Concentrations of chemical mediators during and after almitrine or solvent infusion. A: adrenaline; B: noradrenaline; C: serotonin; D: histamine; E: thromboxane B_2; F: 6-keto-$PGF_1\alpha$ ●——● : hypoxia+almitrine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$); —— : normoxia+almitrine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$); ○----○ : hypoxia+solvent; □–·–□ : hypoxia+almitrine (5 $\mu g \cdot kg^{-1} \cdot min^{-1}$); *: $p<0.05$; **: $p<0.01$ significantly different from the control period.

almitrine bismesylate (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) group under 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or solvent was infused in hypoxic conditions or almitrine bismesylate (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused in normoxic conditions (fig. 1E). 6-keto-$PGF_1\alpha$ showed a tendency to increase in the almitrine bismesylate (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) groups under hypoxic conditions, but the increase was not significant. There was no significant change in the almitrine bismesylate (1 $\mu g \cdot kg \cdot min^{-1}$) 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 vasoconstriction (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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ significantly increased TXB_2 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-PGF_1 in the present study. This increase, however, α did not reach statistical significance. Both prostacyclin and TXA_2 are prostaglandin endoperoxide products in the cyclooxygenase pathway. Prostacyclin is a potent vasodilator, while TXA_2 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 Pao_2 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 preconstricted.

Nakanishi *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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ group, whilst no change was observed in the 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ group. In the present study, almitrine bismesylate significantly increased TXB_2 under hypoxic conditions when it was administered at a low-dose (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$), but this change was not seen under normoxic conditions or in the 5 $\mu g \cdot kg \cdot min^{-1}$ group. These findings suggest that almitrine, when administered at a low-dose (1 $\mu g \cdot kg \cdot min^{-1}$), 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ almitrine bismesylate group treated under normoxic conditions, although neither TXB_2 nor 6-keto-$PGF_{1\alpha}$ 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.

Acknowledgements: We are grateful to Nihon Servier Co. Ltd (Tokyo) for the supply of almitrine bismesylate

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RÉSUMÉ: La potentialisation de la vasoconstriction pulmonaire hypoxique, par une faible dose de bismesylate d'almitrine (1 µg·kg·min) a été évaluée par les dosages des concentrations sanguines des médiateurs chimiques. Nous avons évalué les modifications dans la temps des concentrations sanguines d'adrénaline, de noradrénaline, de sérotonique, d'histamine, de thromboxane (TX) B_2 et de 6-keto-$PGF_{1\alpha}$, après administration de bismeylate d'almitrine pendant 15 minutes à raison de 1 µg·kg·min chez des chiens "beagles" hypoxiques et normoxiques. La faible dose d'almitrine a augmenté significativement les niveaux de TXB_2 chez les beagles hypoxiques, mais les niveaux sont resté virtuellement inchangés chez les animaux normoxiques traités par le bismesylate d'almitrine, et chez les animaux hypoxiques traités par le solvant. Les niveaux de TXB_2 n'ont pas augmenté lorsque l'infusion d'almitrine a été donnée à la dose de 5 µg·kg·min pendant 15 minutes dans les conditions hypoxiques. Ces observations suggèrent que l'almitrine est impliqué dans le métabolisme de l'acide arachidonique lors d'infusions à faible taux, et que la libération de thromboxane en provenance des zones pulmonaires hypoxiques pourrait provoquer une vasoconstriction locale.