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Effect of Methysergide Pretreatment on Thrombin-induced Pulmonary Oedema in the Rat

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ABSTRACT

Microembolic pulmonary oedema was induced by injection of thrombin (500 NIH units/kg body weight) i.v. in rats in which fibrinolysis had been inhibited by pretreatment with trans-4-aminomethyl-cyclohexanoid-carboxylic acid (AMCA). To evaluate the role of serotonin in this condition the effect of pretreatment with the antiserotonin compound methysergide (2.5 mg/kg body weight) on the amount of pulmonary oedema was studied. Pretreatment with methysergide resulted in a 20 % decrease in lung weight in thrombin-treated rats. It caused a significant reduction of dilated lymph vessels, and of interstitial and alveolar oedema, as evaluated morphometrically. Methysergide pretreatment did not significantly alter the number of degranulated mast cells. Antiserotonin is thought to exert its effect by lowering the filtration pressure in the pulmonary microcirculation.

INTRODUCTION

In conditions of fibrinolysis inhibition, thrombin-induced pulmonary microembolism is followed by a profound oedema and progressive pulmonary insufficiency, both in the dog (1, 10) and in the rat. The physiological background of these events is not fully understood. In a previous presentation we have shown that this oedema is associated with an increased number of degranulated mast cells (10), as observed morphologically. Serotonin has been shown to be abundantly present in rat mast cells (8), as well as to be able to induce pulmonary oedema (4, 9, 12). It thus seemed of interest to evaluate the effect of pretreatment with the antiserotonin compound methysergide upon the development of pulmonary oedema following infusion of thrombin and inhibition of fibrinolysis in the rat.

MATERIALS AND METHODS

The experiments were performed on male Sprague-Dawley rats, from the Anticimex Farm (Stockholm, Sweden), weighing 200-250 g.

Thrombin-induced pulmonary oedema

The pulmonary dysfunction following injection of thrombin has been characterized in previous publications (10). Briefly, 500 NIH units/kg body weight (b.w.) of bovine thrombin (Topostasine®, Hoffman La Roche, Switzerland), dissolved in saline, was infused in a tail vein over a period of 5 min with a 1 ml disposable syringe graded in 0.01 ml. This infusion was given under light ether anaesthesia. Fifteen minutes earlier, the rats had been injected i.p. with 200 mg/kg b.w. of the fibrinolysis inhibitor trans-4-aminomethyl-cyclohexanoid-carboxylic acid (AMCA) (kindly supplied by Kabi-Vitrum AB, Stockholm, Sweden). All animals were killed 90 min after the thrombin infusion, and both lungs were quickly excised. The lungs were gently cleaned with gauze and weighed. They were then dried at +80°C until their weight was constant, and reweighed.

Antiserotonin

100 mg of methysergide (Sandoz AG, Basle, Switzerland) and 1 mg of methanesulphonic acid were dissolved in 5 % glucose to a final volume of 10 ml. Ten minutes before the thrombin infusion, 2.5 mg of methysergide/kg b.w. was given i.v.. This dose has been used previously in the rat to inhibit the effect of serotonin in the skin microcirculation (6) and also to attenuate the haemodynamic response to pulmonary embolism in the rabbit (19). Animals not treated with methysergide were given the solvent only.

Quantitation of fibrin deposition

The amount of fibrin deposited in the lungs was determined by a method described in detail earlier (5, 18). Human fibrinogen (grade L, AB Kabi, Sweden) was labelled with ^{125}I by the monochloride method (11) and about 500 KBq were injected i.v. on the day before the experiment. At the end of the experiment the radioactivity in the left lung and blood was recorded. The specific activity of fibrinogen, calculated from control rats, was used in the calculation.

Morphological studies

From each rat three different pieces of lung tissue, representing the lower part of the left lung, the middle right lobe and the upper right lobe were taken for examination. The pieces were immediately put into fixative containing 40 g/l of formaldehyde. Paraffin-embedded sections 5 µm thick were stained with haematoxylin and eosin, Mallory's PTAH stain and the Picro-Mallory technique (6). The slides were coded to allow a blind evaluation. In each section five fields of vision (5 times 2.54 mm^2), making 15 fields of vision per rat, were examined. For each field of vision the presence of absence of the morphological parameters listed in Table 2 was noted. In order to examine the mast-cell morphology, the left lung from separate rats was put into a fixative containing lead acetate and formaldehyde, each in a concentration of 40 g/l. After 24-48 h these lung speci-

mens were post-fixed in formaldehyde, 40 g/l, with the addition of 5 ml of glacial acetic acid per litre (14). Sections were stained in 1 % toluidine blue in 70 % ethanol. In each section the numbers of stained mast cells within five fields of vision were calculated.

Statistical evaluation

For comparison between groups, Wilcoxon-White's two sample ranks test was used.

RESULTS

Rats injected with thrombin and AMCA exhibited a dramatic increase in lung weight (Table 1). Morphologically, this corresponded to a profound interstitial and alveolar oedema (Table 2). Pretreatment with methysergide resulted in an approximately 20 % reduction in lung wet weight, compared with animals given thrombin and AMCA alone. The morphometric findings were compatible with a significantly decreased oedema both alveolarly and interstitially. Dilated lymph vessels were significantly fewer in the methysergide group. The number of stained mast cells (Table 3), which was moderately decreased in thrombin-treated animals, was not influenced by methysergide pretreatment. Neither did the antiserotonin agent interfere with the fibrinogen consumption and fibrin deposition in the lungs, as observed 5 min after termination of the thrombin infusion (Table 4).

Table 1. Effect of pretreatment with the serotonin antagonist methysergide, 2.5 mg/kg b.w., on the lung weight in thrombin-induced pulmonary oedema.

Group	n	Wet weight (g)	Dry weight (g)
Untreated controls	4	1.17 ± 0.04	0.22 ± 0.01
AMCA + thrombin	10	2.22 ± 0.33	0.35 ± 0.04
Methysergide + AMCA + thrombin	11	1.80 ± 0.26**	0.33 ± 0.04

**= $p < 0.01$ compared with rats given AMCA + thrombin alone.

Table 2. Effect of pretreatment with methysergide on morphological parameters in microscopic sections from rats with thrombin-induced pulmonary oedema.

	Untreated controls	AMCA + Thrombin	AMCA + Thrombin + Methysergide
Number of animals	10	10	10
Interstitial oedema	2.10 ± 3.38	93.30 ± 6.96	84.70 ± 6.34**
Alveolar oedema	3.50 ± 4.95	82.70 ± 8.93	76.70 ± 7.26*
Dilated lymph vessels	3.50 ± 4.95	90.00 ± 7.10	80.70 ± 10.09**
Peribronchial oedema	4.20 ± 4.64	47.30 ± 19.97	31.50 ± 8.30**
Intravascular fibrin	2.10 ± 3.38	92.6 ± 6.78	90.00 ± 7.10
Extravascular fibrin	1.40 ± 2.95	72.00 ± 14.94	68.60 ± 32.48
Extravascular haemorrhage	0 ± 0	49.10 ± 12.94	61.70 ± 12.33*

*= $p < 0.05$ versus rats treated with AMCA + thrombin
**= $p < 0.01$ versus rats treated with AMCA + thrombin

Table 3. Effect of methysergide on mast cell count in the lungs in thrombin-induced pulmonary oedema in the rat.

Group	Number of animals	Number of intact mast cells per cm^2 of sectioned lung
Untreated controls	10	1532 ± 242
AMCA + thrombin	10	1274 ± 186*
Methysergide + AMCA + thrombin	10	1360 ± 210

*= $p < 0.05$ versus untreated animals.

Table 4. Effect of pretreatment with methysergide on the amount of fibrin in the lungs 5 min after induction of pulmonary embolism with AMCA + thrombin.

Groups	n	Time	Fibrinogen g/l plasma	Fibrin mg/g lung
Untreated controls	5	0	3.00 ± 0.16	0.85 ± 0.07
AMCA + thrombin	5	5'	1.24 ± 0.31	7.30 ± 1.85
Methysergide + AMCA + thrombin	5	5'	1.03 ± 0.25	7.90 ± 2.10

DISCUSSION

In this study the antiserotonin compound methysergide was found to reduce the lung weight in rats with thrombin-induced pulmonary oedema. The background of the study was the observation of a large number of degranulated mast cells in microscopic sections from rats with this condition (10). Whether this was a cause or a consequence of the pulmonary dysfunction was unclear. It therefore seemed logical to try to prevent the development of the pulmonary oedema by pretreatment with an antagonist of one of the predominant vasoactive constituents of rat mast cells, namely serotonin (8).

Also of relevance is that serotonin has profound effects on the pulmonary circulation (3, 9), causing an increase in pulmonary arterial pressure after i.v. infusion and altering the pressure gradient in the microcirculation. It has also been found to induce pulmonary oedema in the dog (12) and sheep (9). Additionally, pretreatment with methysergide has been reported to counteract the effect of acute pulmonary microembolisation (19).

Serotonin does not increase microvascular permeability in the lungs of dogs and sheep (16). In sheep, infusion of serotonin results in a pulmonary oedema with all characteristics of a haemodynamic oedema, but without signs of altered microvascular permeability (4).

The pulmonary oedema seen in the present rat model is characterized by a high albumin content, indicating a dramatically increased permeability in the pulmonary microcirculation (10).

The amount of oedema can be altered in two ways: Firstly, the Permeability Surface Area Product (PS-product), i.e. there can be an effect on the microvascular permeability (15), or secondly, the pressure gradient across endothelial cell linings of the microcirculation can be altered. It is impossible to determine which of these alternatives is responsible for the effect in the present study.

As the effect of methysergide was limited, and in view of the finding of other authors that serotonin has a profound effect on the pulmonary microcirculation by increasing the transvascular pressure (17), an alteration of the PS product does not seem to be required to explain our result.

It is not easily explained why methysergide-treated animals exhibited more extravascular haemorrhage. However, it is possible that serotonin antagonism has a negative effect on vasoconstriction in damaged tissue, or it may interfere with platelet function. To conclude, other, more dominant pathophysiological mechanisms than the liberation of serotonin from lung mast cells or trapped platelets must be involved in the pulmonary oedema observed after thrombin-induced microembolism in the rat.

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