Histology and Histopathology

Anti-platelet agents reduce morphological changes of chronic hypoxic pulmonary hypertension

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Summary. The pathophysiologic mechanism by which chronic hypoxia causes pulmonary hypertension is unknown. If anti-platelet agents, or other pharmacologic interventions, altered the pulmonary vascular changes induced by hypoxia, information concerning the pathogenesis of the pulmonary hypertension or the potential therapeutic usefulness of the drugs might be obtained. In Study 1, rats exposed to chronic hypobaric hypoxia (Pₐ = 520 mmHg) had a pulmonary arterial medial thickness of 6.7 ± 0.6 μ compared to 4.1 ± 0.2 μ * for control, normoxic rats (*p<0.05). Administration of dipyridamole (2mg/kg/day), or sulfinpyrazone (11 mg/kg/day) in the drinking water reduced the medial thickness to 5.0 ± 0.3 μ* and 5.4 ± 0.5 μ* respectively, thus suggesting the possible involvement of platelets in the response of the media to chronic hypoxia. In Study 2, hypoxic rats treated with the calcium blocker, flunarizine, were found to have less medial hypertrophy than a control group of hypoxic rats. This observation suggests that a decrease in transmembrane calcium flux may also reduce medial hypertrophy.

Key words: Hypoxia - Rats - Platelets - Pulmonary hypertension - Calcium blockers

Introduction

Exposure to chronic hypoxia is often used as a method to induce experimental pulmonary hypertension (Herget et al., 1978; Rabinovitch et al., 1979), and thus mimic the pulmonary hypertension which occurs in chronic hypoxic lung diseases in man. This condition is manifest physiologically by increased right ventricular and pulmonary artery blood pressures and morphologically by right ventricular hypertrophy and thickening of the media of small pulmonary arteries. The pathophysiologic mechanism by which hypoxia causes pulmonary hypertension is not known. Despite earlier indications (Hauge, 1968; Hauge and Melmon, 1968), it does not appear that platelets help to mediate the acute pulmonary vascular pressor response to hypoxia in rats (McMurtry et al., 1978) or dogs (Weir et al., 1976). However, the number of circulating platelets is reduced by chronic hypobaric hypoxia in man (Gray et al., 1975), cattle (Genton et al., 1970), mice (Birks et al., 1975) and rats (DeGabriele and Pennington, 1967). Given that these platelets can be sequestered in the lungs (Gray et al., 1975), it is possible that they may contribute to the increase in pulmonary vascular resistance caused by chronic hypoxia. There is evidence that platelets are involved in the pulmonary hypertension induced by microemboli (Mlczoch et al., 1978) and by subclavian artery to pulmonary artery shunts (Van Benthuysen et al., 1981). If antiplatelet agents reduce the pulmonary vascular changes caused by chronic hypoxia, this might indicate a role for platelets in the pathogenesis of hypoxic pulmonary hypertension and the findings might be of potential therapeutic importance. Consequently pulmonary hypertension was induced in rats by exposure to chronic hypoxia. Some of these rats were treated with dipyridamole or sulfinpyrazone. Other rats exposed to chronic hypoxia were treated with the calcium channel blocker, flunarizine (Van Neuten and Janssen, 1973; Borgers et al., 1980) in an attempt to elucidate the mechanisms underlying morphologic changes in chronic hypoxic pulmonary hypertension.

Materials and methods

A total of 72 male Sprague Dawley rats were used in the experiment. Rats which were made hypoxic were housed for two weeks in a hypobaric chamber at 520 mm Hg, simulating an altitude of 3,105 meters, and were then killed while hypoxic. The chamber was returned to ambient air pressure for 30 minutes daily for feeding and
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Cleaning. All drugs were dissolved in the drinking water and fresh solutions were made each day. Drug treatment started five days before the start of hypoxia and continued throughout the experiment. Water was restricted in all groups to the amount drunk by the treated rats and commercial rat food was given ad lib.

In the first study, 42 rats (150-200g) were divided into four groups: 1) normoxia without drug \((n = 10)\), 2) hypoxia without drug \((n = 10)\), 3) hypoxia with dipyridamole, 2 mg/kg/day. \((n = 11)\) (Persantine, Boehringer Ingelheim), 4) hypoxia with sulfinpyrazone, 11 mg/kg/day. \((n = 11)\) (Anturane, Ciba-Geigy). In the second study 30 rats (325-350g) were divided into three groups \((n = 10\) for each\): 1) normoxia without drug, 2) hypoxia without drug, 3) hypoxia with flunarizine, 4 mg/kg/day (Sibelium, Janssen Pharmaceutica). In all the studies the rats were killed by the intraperitoneal injection of sodium pentobarbital. The dorsal aorta was cut to remove blood from the heart and lungs. The lungs were instilled in the closed chest with Bouins fixative through a tracheal cannula at a pressure of 12-15 cm water (Wagenvoort and Wagenvoort, 1977). Sagittal paraffin sections were stained with hematoxylin-eosin for morphology and Miller's elastic stain for morphometry (Miller, 1971). The hearts were removed, opened to evacuate trapped blood and fixed in 10% formalin. The right ventricle (RV) was separated from the left ventricle and interventricular septum (LV + S) (Fulton et al., 1952) and both components were weighed separately. Right ventricular hypertrophy was indicated by decreasing \((LV + S)/RV\) weight ratios. To determine pulmonary arterial changes, the distance between the external and internal elastic laminae was measured at four equidistant sites in cross-sectioned small arteries, and the mean was calculated to determine the average medial thickness (Keith and Will, 1981). Ten arteries per animal were measured, ranging in outer diameter from 50-100 microns. Analysis of variance and Duncan's multiple range test were used to assess differences between groups. The tissues were coded so that all measurements were made without knowledge of the experimental group from which the tissue was derived.

Results

Hypoxic control rats had significantly increased medial thickness of the small pulmonary arteries and significantly smaller \((LV + S)/RV\) ratios than had normoxic controls (Table 1). The decreased \((LV + S)/RV\) ratios were caused by an increase in the weight of the right ventricle alone. The hypoxia-induced medial thickening was not prevented, but was significantly reduced by dipyridamole, sulfinpyrazone and flunarizine (Table 1). None of these drugs had any effect on \((LV + S)/RV\) ratios.

The hypoxic rats in the first study did not gain body weight as readily as the normoxic controls, whereas the older rats in the second study gained little weight and showed no difference in body weight between any groups (Table 1). There was no effect on body weight, as all hypoxic groups with or without drug had similar weights within both experiments.

Discussion

Chronic hypoxia produced an increase in medial

Table 1. The effects of dipyridamole, sulfinpyrazone, and flunarizine on hypoxia induced cardiopulmonary changes in rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of rats</th>
<th>Body weight (grams)</th>
<th>Lung arterial medial thickness (microns)</th>
<th>((LV + S)/RV) weight ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia only</td>
<td>10</td>
<td>303 ± 6</td>
<td>4.15 ± 0.23</td>
<td>3.83 ± 0.13</td>
</tr>
<tr>
<td>Hypoxia only</td>
<td>10</td>
<td>270 ± 3</td>
<td>6.74 ± 0.59*</td>
<td>3.04 ± 0.10*</td>
</tr>
<tr>
<td>Hypoxia with dipyridamole</td>
<td>11</td>
<td>265 ± 5*</td>
<td>5.03 ± 0.29†</td>
<td>2.94 ± 0.12*</td>
</tr>
<tr>
<td>Hypoxia with sulfinpyrazone</td>
<td>11</td>
<td>265 ± 4*</td>
<td>5.42 ± 0.47†</td>
<td>2.98 ± 0.14*</td>
</tr>
<tr>
<td><strong>Study II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia only</td>
<td>10</td>
<td>375 ± 2</td>
<td>2.91 ± 0.15*</td>
<td>3.46 ± 0.12</td>
</tr>
<tr>
<td>Hypoxia only</td>
<td>10</td>
<td>364 ± 6</td>
<td>6.23 ± 0.18</td>
<td>2.90 ± 0.15</td>
</tr>
<tr>
<td>Hypoxia with flunarizine</td>
<td>10</td>
<td>372 ± 7</td>
<td>5.25 ± 0.34†</td>
<td>2.73 ± 0.09*</td>
</tr>
</tbody>
</table>

* different from the normoxic control group; \(p < .05\)
† different from the hypoxic untreated group; \(p < .05\)

Means and standard errors are shown.
thickness of the pulmonary arteries and a decrease in the 
(LV + S)/RV ratio, as anticipated. The administration, 
of dipyridamole, sulfinpyrazone or flunarizine reduced 
the medial thickness of the arteries toward normoxic 
levels but the ratio (LV + S)/RV was unchanged. The 
reason for this dissociation between the medial thickness 
changes and the right ventricular hypertrophy is not 
clear. It may be there was enough hypoxic pulmonary 
vasoconstriction to cause pulmonary hypertension and 
stimulate right ventricular hypertrophy, but that the 
incriminate platelets as a factor in the stimulation of 
medial hypertension. The action of sulfinpyrazone is 
more likely to be directly related to its effect on platelet 
behavior, through the inhibition of thromboxane 
synthesis (Patrono et al., 1980). The similar results of 
both "anti-platelet" agents makes it possible that 
platelets are involved in the pulmonary vascular 
response to chronic hypoxia. This suggestion is 
strengthened by a study which demonstrated increased 
platelet activation in patients with chronic obstructive 
airways disease (Nenci et al., 1982). Platelet production 
of malondialdehyde was reduced, which occurs in 
"overstimulated" platelets, and plasma levels of β - 
thromboglobulin, an indicator of platelet activation, 
were increased. After administration of dipyridamole for 
ten days these markers of platelet activation returned 
close to normal levels. Other experiments suggest that 
platelets have a role in the increased pulmonary vascular 
resistance occurring in subclavian artery to pulmonary 
artery shunts (Van Benthuysen et al., 1981).

Calcium channel blockers, such as verapamil, have 
been shown to reduce the acute pulmonary pressor 
response to hypoxia in the isolated perfused rat lung 
(McMurtry et al., 1976) and the anesthetized dog 
(Tucker et al., 1976; Archer et al., 1985). The 
administration of verapamil (4 mg i.p. twice daily) to rats 
exposed for 20 days to chronic hypoxia reduced the 
severity of right ventricular hypertrophy in another 
experiment (Davidson et al., 1978), but medial thickness 
as not examined. In the present study the calcium 
channel blocker, flunarizine, was found to reduce the 
degree of pulmonary arteriolar medial hypertrophy 
stimulated by hypoxia, but did not prevent the increase 
in right ventricular weight. The variation in the results 
could secondary to the differences in the methods of 
drug administration, the dose used, the length of the 
studies, or the pharmacology of the two calcium 
blockers. It seems possible however, that the normal 
response of the pulmonary vasculature to chronic 
hypoxia, like that to acute hypoxia, requires transmembrane calcium flux. This could involve the reactivity of the smooth muscle itself, the release of a 
constrictor mediator, or the aggregation of platelets. 
Calcium channel blockers have been reported to inhibit 
platelet aggregation (Ono and Kimura, 1981), and in this 
respect flunarizine might mimic the actions of 
dipyridamole and sulfinpyrazone.

The results observed in these hypoxic rats suggest that 
platelets may be involved in the development of 
pulmonary arterial medial hypertrophy. The calcium 
channel blocker, flunarizine, produced a modest 
reduction in medial hypertrophy, in keeping with the 
effects of verapamil on the acute pulmonary pressor 
response to hypoxia (McMurtry et al., 1976; Tucker et al., 
1976). These observations, together with the finding 
that nifedipine will inhibit experimentally induced 
bronchoconstriction in the guinea pig (Fanta et al., 
1982), and exercise-induced asthma in man (Cerrini et al., 
1981), support the use of calcium channel blockers in 
some patients with pulmonary hypertension secondary 
to chronic hypoxic lung disease (Kennedy et al., 1984; 
Muramoto et al., 1985).

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