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Effects of ligustrazine on the pressure/flow relationship in isolated perfused rat lungs

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Effects of ligustrazine on the pressure/flow relationship in isolated perfused rat lungs. A. Oddoy, D. Bee, C. Emery, G. Barer.
ABSTRACT: Ligustrazine, the synthesized principle of a Chinese herbal remedy shown previously to be a pulmonary vasodilator, was tested in chronically hypoxic and normal rats. Pressure/flow, (P/$\dot{Q}$), relations were measured in isolated perfused lungs during normoxia, hypoxia and after reversal of hypoxic vasoconstriction by increasing doses of ligustrazine.

P/$\dot{Q}$ lines were linear over a wide range and extrapolation to the pressure axis gave an intercept which was the effective downstream pressure for flow. In chronically hypoxic rats the slope of the line was steeper and the intercept greater than in control rats, which we attributed to newly muscularized arterioles with tone. Hypoxia caused an increase in slope and intercept in both groups but the intercept increase was greater in chronically hypoxic rats. In both groups of rats increasing doses of ligustrazine given during continued hypoxia caused a fan of lines which moved progressively towards the control normoxic line. In chronically hypoxic rats it required only 2 mg of ligustrazine to bring the line back to the normoxic position, whereas in controls it required 4 mg. In chronically hypoxic rats the change in intercept with every dose was greater than in control rats; this suggests that ligustrazine mainly relaxes the muscle of small collapsible vessels.

The action of ligustrazine remained in both control and chronically hypoxic rats after administration of an arginine analogue which blocks synthesis of the endothelial relaxant factor nitric oxide. This and previous evidence suggest that ligustrazine is a non-endothelial-dependent pulmonary vasodilator.
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A potent, specific pulmonary vasodilator substance which could be used in severe progressive pulmonary hypertension and in post-operative pulmonary hypertensive crises is still lacking. Recently, experimental evidence in animals has shown that ligustrazine (tetramethyl pyrazine HCl), semi-synthesized principle of an ancient Chinese herbal remedy [1], is a potent and possibly relatively specific pulmonary vasodilator. The herb has been used orally with other herbs for "heart disease" for thousands of years. In isolated rat lungs it proved a powerful vasodilator and abolished hypoxic pulmonary vasoconstriction; in intact ferrets it reduced pulmonary artery pressure (Ppa) substantially more than systemic pressure [2]. Given chronically, it also partially prevented the rise in pulmonary artery pressure, right ventricular hypertrophy and muscularization of pulmonary arterioles which develop when rats are exposed chronically to hypoxia; the pressure/flow relations of the lungs came close to those of normal lungs [3].

Our first aim in this study was to look in detail at the acute haemodynamic effects of ligustrazine in normoxic control (C) and chronically hypoxic (CH) rats; we measured the effect of increasing cumulative doses of the drug on pressure/flow (P/$\dot{Q}$) relations. Previous evidence from isolated rings of pulmonary artery showed that ligustrazine acts in the absence of endothelium [4]. Our second aim was, therefore, to see if it was active in the whole isolated perfused pulmonary circulation in the absence of endothelial-derived-relaxant factor (EDRF). We blocked synthesis of nitric oxide (NO), believed to be at least one EDRF [5], with an arginine analogue, L-nitro arginine methyl ester (L-NAME). This could be an important therapeutic point since the endothelium is frequently damaged in pulmonary vascular disease. The normal pulmonary vasculature is in a state of near complete dilatation. Thus to test a vasodilator substance, preconstriction is necessary; we used hypoxic vasoconstriction.

In the chronically hypoxic rat we have a model which in some ways resembles human pulmonary hypertension; Ppa is raised, the right ventricle is hypertrophied and new smooth muscle extends into the previously

non-muscularized or only partially muscularized alveolar arterioles (50 μm or less in diameter) [6, 7]. These features are also found in human chronic bronchitis [8]. However, the rat model lacks the endothelial damage and proliferation seen in hypoxic cor pulmonale [9, 10], while in more severe pulmonary vascular disease such as "primary" pulmonary hypertension even more severe damage is found.

Materials and methods

Young, 33 days old, male Wistar rats (specific pathogen free, Tuck's laboratories) were exposed for 3 weeks to 10% O_2 in a normobaric hypoxic chamber [11]. After pentobarbitone anaesthesia (60 mg·kg^{-1}, *i.p.*) the isolated perfused lungs of these CH rats (n=6) were perfused by a modification of HAUGE's [12] method [13, 14] and compared with those of littermate control rats (n=5) kept in the same room in air. Lungs were ventilated with air+5% CO_2 (normoxia) or 2–3% O_2+5% CO_2, balance N_2 (hypoxia); end expiratory pressure was 2–3 mmHg. The lungs were perfused with 8–10 ml blood of normal pH (7.35–7.45, adjusted with sodium bicarbonate) at 38°C; blood for CH rats was taken from a donor since polycythaemia, present in CH rats, affects pulmonary vascular resistance (PVR) [15]. Normoxic and hypoxic ventilation was applied at 15–20 min intervals depending on the time taken to reach stable hypoxic pulmonary vasoconstriction (HPV) and a stable normoxic base line between hypoxic tests. As previously shown, this base line tends to rise slightly with time in C rats but much more so in CH rats for an unknown reason [13].

Pressure/flow line measurement

Blood flow was kept at 20 ml·min^{-1} except when measuring pressure/flow ($P/\dot{Q}$) lines. Left atrial or outflow pressure was kept at zero so that the alveolar pressure caused by positive pressure ventilation was the effective downstream pressure, or critical closing pressure, unless tone in small collapsible vessels took over this role [16–17], as it does during hypoxic vasoconstriction [18]; thus the lung was in West's zone 2. At constant flow, changes in mean Ppa represent changes in vascular resistance or in the effective downstream pressure; the nature of the changes was determined from the slope and position of the $P/\dot{Q}$ lines and is discussed in the text. Hypoxia caused a steep rise in pressure to a plateau which was usually sustained but occasionally slowly decayed. During this plateau doses of ligustrazine (20 mg·ml^{-1}, Fourth Pharmaceutical Laboratory factory, Beijing, China) were given; each caused a fall in Ppa to a new level. Figure 1 is a diagram which shows the protocol and typical responses. Sequential doses (0.5, 0.5, 1 and 2 mg given into the blood reservoir) were considered cumulative, as we had previously shown that the action lasted about 20 min; thus the final cumulative dose was 4 mg. Doses were given in 0.1–0.2 ml volumes which were too small to dilute the perfusate; similar volumes of 0.9% NaCl were given as controls. Two hypoxic tests were given first, as HPV tends to increase in the first few challenges, and ligustrazine was given during the third hypoxic challenge. $P/\dot{Q}$ lines were measured by plotting flow (Spectromed electromagnetic flow meter) against mean pressure (Druck Ltd transducers and Lectramed amplifier) on an XY recorder (Bryans model 2600 A4) at the points illustrated in figure 1; blood flow was reduced by alteration of the pump speed from 20 to 15, 10, 5 and 0 ml·min^{-1}. Lines were linear except at <5 ml·min^{-1} and were extrapolated to the pressure axis to give an intercept.

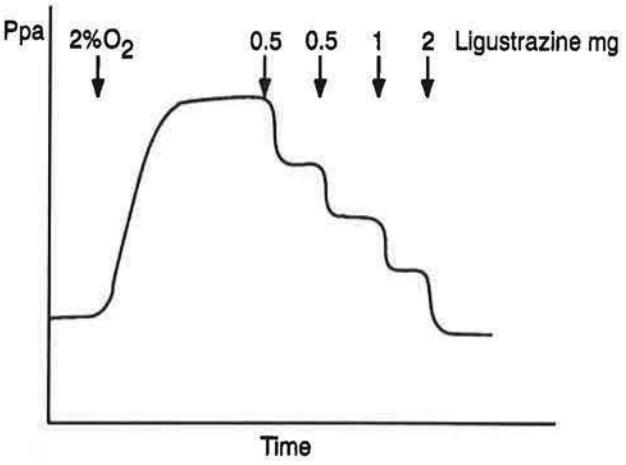

Fig. 1. – A diagram of the experimental protocol. Ventilation was changed from air to 2% O_2 which caused a rise in pressure to a stable plateau. Ligustrazine was given into the reservoir where indicated in increasing cumulative doses; actual doses are marked (↓). Pressure flow relations were taken at the hypoxic plateau and at each new lower level attained after the individual doses of ligustrazine. Ppa: pulmonary artery pressure.

Blockade of EDRF

In 7 normal rats (male Wistar, local stock) and a further 3 C and 4 CH littermates (as for the $P/\dot{Q}$ tests), isolated perfused lungs were set up and L-NAME (100 μg in 0.1 ml, final concentration ca. 10^{-5} M) was given into the reservoir when a consistent HPV had been established. After a 15–20 min wait to see whether L-NAME affected the normoxic pressure, ligustrazine was given into the reservoir during a further hypoxic test at peak HPV.

Statistics

Means and standard errors of the means (SEM) are quoted in text and figures. Differences between means were tested by paired or unpaired Student's t-test as appropriate; p values <0.05 were considered significant. Regression lines were calculated by the method of least squares.

Results

Although body weight of chronically hypoxic rats was less than that of controls (226±9 g *cf* 302±35, p<0.001), the same flow rate was used in both groups. We have previously shown that lung size and vascular volume are not dissimilar despite body growth retardation [6, 13]. Initial Ppa in 5 C rats was 18.1±1.2 mmHg (SEM) compared with 28.4±4.3 in 6 CH rats (p<0.05). After 1 h, when P/$\dot{Q}$ lines were measured, the values were 17.2±1.9 mmHg *cf* 37.5±3.3 (p<0.01) owing to the rise in normoxic Ppa of CH rats already mentioned. The rise in CH rats was significant (p<0.05). During the 2nd hypoxic response, volumes of 0.9% NaCl equivalent to those used for ligustrazine caused small depressions of Ppa but these were not comparable to those caused by the drug. In the 3rd hypoxic test Ppa rose to 35.1±8.5 in C rats and 56.7±12.9 in CH rats and ligustrazine reversed this in a dose dependent manner. Figure 2 shows the linear log-dose relationship in both groups (r=0.99). Baseline normoxic Ppa values were achieved after 4 mg cumulative dose in C rats and 2 mg in CH rats. In CH rats 2 mg or more brought the Ppa below the previous baseline normoxic value. On return to air ventilation there was no further fall in Ppa and no subsequent HPV could be demonstrated for 20 min after administration of ligustrazine.

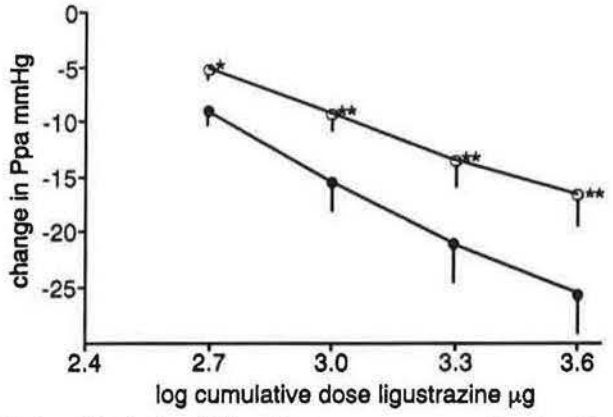

Fig. 2. – The absolute fall in pulmonary artery pressure, Ppa, from the hypoxic plateau is plotted against the log cumulative dose of ligustrazine for 5 control (C) (open circles) and 6 chronically hypoxic (CH) rats (closed circles). The reversal of hypoxic vasoconstriction was more effective in CH compared with C rats (*:p<0.01; **: p<0.001, unpaired t). —o—: control; —●—: chronic hypoxia.

Figure 3 shows mean P/$\dot{Q}$ lines for all rats, C on the left and CH on the right. Acute hypoxia caused an increase in slope and intercept in both groups but the intercept changes were greater in CH rats. Ligustrazine reduced slopes and intercepts in a dose dependent manner until the lines were similar to (C rats) or below (CH rats) those measured in normoxia. Figure 4 shows log-dose plots of the changes in slope and intercept (linear regression was 0.99 for all lines). It is apparent that, in CH rats, changes in intercept (shown above), which represent effective downstream pressure are significantly greater than in C rats (p<0.05) while changes in slope (shown below), which are a measure of vascular resistance, are similar.

L-NAME either caused no rise or only an occasional trivial rise in normoxic Ppa in the 7 C rats tested, as previously shown [19]; however, in the former study it systematically caused a rise in CH rats. A subsequent hypoxic challenge in the present C rats showed a greatly enhanced response as it did in both C and CH rats in the earlier study. Figure 5 shows that during this test, 4 mg ligustrazine greatly reduced Ppa as before but not to baseline pressure. Subsequent hypoxic tests gave reduced rises in Ppa but could be obtained within 5–10 min, unlike those in the above tests without L-NAME. In the further 3 C and 4 CH littermate rats L-NAME was given as just described but during the subsequent hypoxic test a series of doses (0.5, 0.5, 1, 2, total cumulative dose 4 mg) gave results similar to those seen in the P/$\dot{Q}$ experiments. The numbers were too few to compare C with CH rats but in 2 CH rats (and no C rat) the total dose brought the Ppa below the previous normoxic level during continued hypoxia.

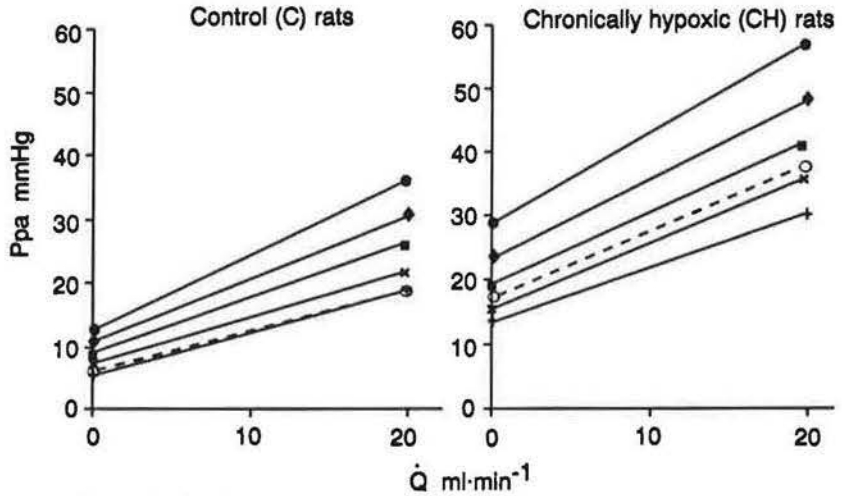

Fig. 3. – The mean pressure flow, Ppa/$\dot{Q}$, lines are drawn for control rats on the left and chronically hypoxic rats on the right. Open circles (dashed lines) inducate the normoxic lines, the closed circles are the hypoxic lines which show raised intercept and slope. Ligustrazine was given during hypoxia and reversed both intercept and slope; the different cumulative doses are shown in the key. LIG: ligustrazine. —o : air; —●: 2% O_2; —♦: 0.5 mg LIG; —■ : 1.0 mg LIG; —×: 2.0 mg LIG; —+ : 4.0 mg LIG.

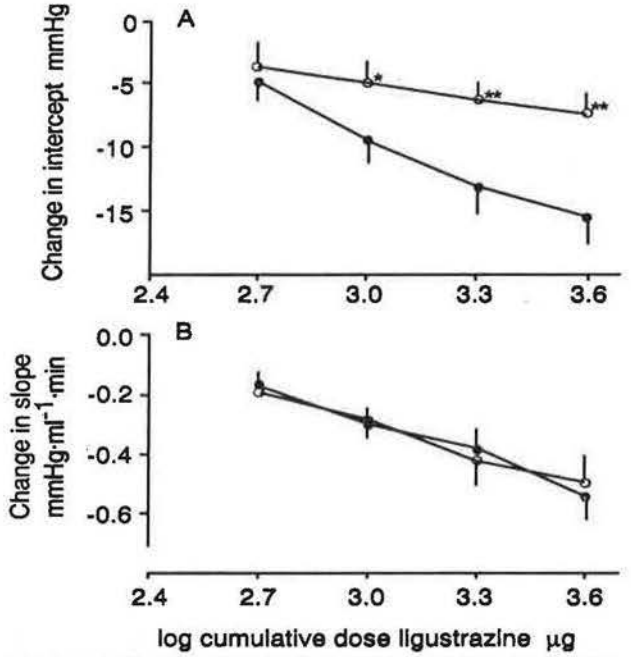

Fig. 4. – The fall in intercept (A) and slope (B) from the pressure/flow relationships at hypoxic plateau (see fig. 3) are plotted against the log cumulative dose of ligustrazine for control (C), (open circles) and chronically hypoxic(CH) rats (closed circles). Changes in slope (pulmonary vascular resistance) are similar in both groups but the fall in intercept value is significantly greater in CH than C rats (*:p<0.01; **: p<0.001, unpaired t).—⊖—: control; —●—: chronic hypoxia.

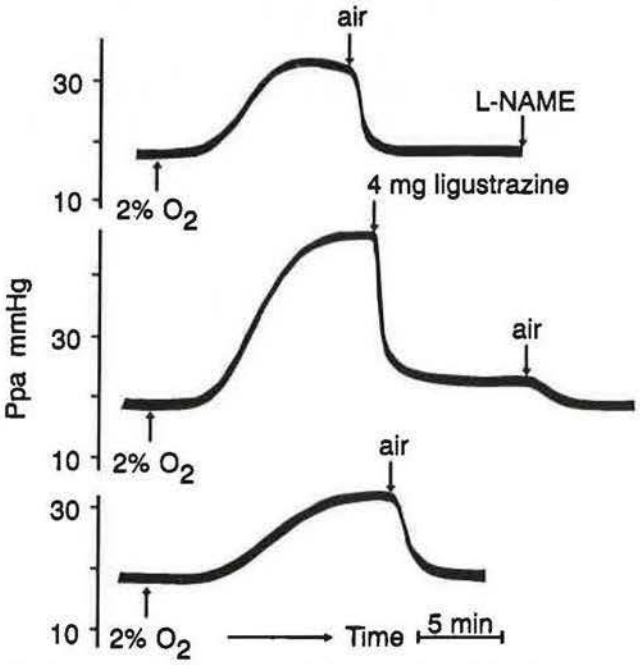

Fig. 5. – A tracing from a record of a typical isolated perfused lung preparation illustrating the effect of L-nitro arginine methyl ester (L-NAME) on hypoxic vasoconstriction and its reversal by ligustrazine in a control rat. The basal normoxic pressure was constant throughout (17.9–18.2mmHg). The top line shows the rise in pulmonary artery pressure, Ppa, caused by ventilation with 2% O_2 and the fall with air. L-NAME (100 μg) was given into the reservoir after this test and the next hypoxic test (20 min later) shown on the 2nd line is enhanced. 4 mg ligustrazine also given into the reservoir was still able to reverse hypoxic vasoconstriction. There is no time interval between the 2nd and 3rd tracings yet another reduced hypoxic vasoconstriction is demonstrated.

Discussion

Ligustrazine caused a dose-dependent reduction in hypoxic pulmonary vasoconstriction in both control and chronically hypoxic rats; it appeared more effective in the latter. Moreover, it reduced the baseline normoxic Ppa in chronically hypoxic rats such that the increase in this pressure with time was abolished; thus, this rise, which can be extreme, is not caused by oedema but by vasoconstriction. It is not likely to be due to release of potassium ions through greater haemolysis in CH rats since we used normal blood for the perfusate in both groups.

The greater decrease in intercept after ligustrazine in CH compared with C rats is of interest. These rats have newly developed muscle in very small arterioles (50 μm or less in diameter) which normally have little or no muscle. We have shown elsewhere that the main site of hypoxic vasoconstriction may move distally to these vessels in chronic hypoxia [18]. These small vessels may constrict and act as Starling resistors during hypoxia to form the effective downstream pressure or critical closing pressure; a lesser or similar effect of hypoxia on resistance may perhaps be attributed to distension of larger vessels due to the rise in Ppa caused by the intensely constricted small vessels. The present work suggests that ligustrazine acts on these small vessels, an important point for possible therapy because vasodilators must affect small intrapulmonary vessels as well as larger vessels. Differential effects between large and small vessels are well documented and it was recently shown that ligustrazine had a similar or possibly greater action on small compared with large intrapulmonary vessels [4, 20].

Ligustrazine did not reduce Ppa in CH rats to normoxic levels seen in C rats. It is evident that part of the raised Ppa in these rats, as in human pulmonary hypertension, cannot be resolved by reduction of tone because it is due to structural remodelling [7]; the small vessels are of normal external diameter but the lumen is narrowed by the new muscle bounded internally by a new elastic lamina [21]. Many attempts have been made to prevent these changes caused in animals by hypoxic exposure with vasodilator substances [22]. Several drugs, including ligustrazine [3], have attenuated the changes when given simultaneously with hypoxic exposure but only calcium channel inhibitors have brought about some attenuation after the changes have developed [23]. Our results in ferrets suggested that ligustrazine is a more potent dilator of pulmonary than systemic vessels [2]; this is an important point which needs substantiation in other circumstances. Many pulmonary vasodilator substances have failed clinically because they caused systemic hypotension.

Our findings with L-NAME and the previous findings of Liu *et al.* [4] that ligustrazine continues to dilate preconstricted lung vessels after removal of endothelium, indicate that it is a non-endothelial-dependent vasodilator. This is an important therapeutic consideration, because both pharmacological and pathological evidence suggest that the endothelium is often

severely damaged in human pulmonary hypertension [9, 10]. This is especially true in severe progressive cases, such as "primary pulmonary hypertension" where cardiac output is impaired and a reduction in pressure is imperative. Ligustrazine should be further investigated because it is effective orally, has a prolonged action and clinical tests in China have already been undertaken.

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Les effets de la ligustrazine, principe chimique d'une herbe médicinale chinoise ancienne, sur les relations pression/débit dans les poumons de rats isolés et perfusés. A. Oddoy, D. Bee, C. Emery, G. Barer.

RÉSUMÉ: La ligustrazine, principe synthétisé d'une herbe médicinale chinoise dont l'effet vasodilatateur pulmonaire avait été démontré précédemment, a été testée chez des rats chroniquement hypoxiques et normaux. Les relations pression/débit et P/$\dot{Q}$ ont été mesurées dans les poumons isolés et perfusés au cours de la normoxie, de l'hypoxie et après correction de la vasoconstriction hypoxique par des doses croissantes de ligustrazine.

Les lignes P/$\dot{Q}$ s'avèrent linéaires sur une large zone, et l'extrapolation sur l'axe des pressions donne un intercept qui est la pression effective en aval pour le débit. Chez les rats chroniquement hypoxiques, la forme de la ligne est plus verticale et l'intercept plus grand que chez les rats contrôle, ce que nous avons attribué à des artérioles nouvellement musculairisées et toniques. L'hypoxie a provoqué une augmentation de la pente et de l'intercept dans les deux groupes, mais l'augmentation d'intercept est plus marquée chez les rats chroniquement hypoxiques. Dans les deux groupes de rats, des doses croissantes de ligustrazine, données pendant l'hypoxie continue, provoquent un éventail de lignes qui se déplacent progressivement vers la ligne normoxique contrôle. Chez les rats chroniquement hypoxiques, il suffisait de 2 mg de ligustrazine pour ramener la ligne à la position normoxique, alors que chez les contrôles il en fallait 4. Chez les rats chroniquement hypoxiques, la modification d'intercept avec chaque dose est plus importante que chez les rats contrôle. Ceci suggère que la ligustrazine provoque principalement une relaxation des muscles des petits vaisseaux collabables.

L'action de la ligustrazine persiste à la fois chez les rats contrôle et chroniquement hypoxiques après administration d'un analogue de l'arginine, qui bloque la synthèse de l'oxyde nitrique, facteur relaxant endothélial. Ceci, ainsi que des observations antérieures, suggère que la ligustrazine est un vasodilatateur pulmonaire non dépendant de l'endothélium.
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