A kinetic study of lung DNA-synthesis during simulated chronic high-altitude hypoxia

N. VÖLKEL, U. WIEGERS, V. SILL, AND J. TRAUTMANN

From I Medizinische Universitätsklinik Hamburg, Martinistrasse 52, 2000 Hamburg 20, Germany

Völkel, N., Wiegers, U., Sill, V., and Trautmann, J. (1977). Thorax, 32, 578–581. A kinetic study of lung DNA-synthesis during simulated chronic high-altitude hypoxia. Rats were exposed to chronic hypobaric hypoxia at a simulated altitude of 4250 m for 3, 6, 9, 12, 20, and 35 days. The in-vitro incorporation of ³H-thymidine into the DNA of lung tissue was measured and compared with that of normoxic controls: the obtained time course study showed a maximum increase of 345% on the ninth day of hypoxia, indicating a marked stimulation of cellular proliferation. Between the 12th and 20th day of hypoxia, the lung DNA-synthesis reached control values. No significant change in the DNA-concentration of the lungs was registered. The response to hypoxia was less impressive in rat livers used as controls.

Pulmonary hypertension is a common manifestation of altitude exposure. This has been described in different species by many authors (Alexander et al., 1960; Arias-Stella and Saldaña, 1962; Grover et al., 1963; Burton et al., 1967; Barer et al., 1970; Abrahams et al., 1971; McMurtry et al., 1973). The pulmonary hypertension is the result of a fixed increase in pulmonary vascular resistance due to structural changes in the pulmonary blood vessels that occur in the course of long-term hypoxia.

Although the histological changes are well documented by Arias-Stella and Saldaña (1962) and by Heath (1959), it is not clear whether they are hypertrophic or hyperplastic, and we are also ignorant of the time course of these changes. In order to investigate the mechanism involved and to monitor the onset and the kinetics of the expected cellular proliferation the present cyto-kinetic study of pulmonary response to chronic hypoxia was designed.

Methods

Thirty female Wistar rats weighing 220–250 g were used for the experiments. They were randomly divided into groups of three: six groups were kept in a decompression chamber for 3, 6, 9, 12, 20, and 35 days respectively and were exposed to a simulated altitude of 4250 m. Three groups were taken as controls at the beginning of the study and on day 3, 12, and 35 respectively. The animals were exposed to hypoxia for 22 hours daily and they were allowed access to food and water. After anaesthesia with 2 mg/kg pentobarbital sodium (Nembutal®) a medium thoracotomy was performed, and the middle lobe of the right lung was excised and cut into slices. Two hundred milligram portions of each lung were immediately incubated in 3 ml Krebs-Ringer medium containing 20 μCi of ³H-thymidine (¹³H-thymidine, 24-6 Ci/mmol, Amersham-Buchler, Braunschweig) at 35°C for 60 minutes. The incubation was stopped by the addition of 5 ml iced-cold 3N TCA. The slices were homogenised in 10 ml 5% TCA (w/w) and centrifuged at 3000 g for 5 minutes. The DNA of the sediment was hydrolysed in 5 ml 5% TCA at 95°C for 30 minutes. The insoluble material was removed by centrifugation. One hundred microlitre samples of the supernatant were measured in duplicate for both radioactivity and DNA (Burton, 1968). The DNA synthesis rate was expressed as specific radioactivity (cpm/μg DNA). Liver slices in portions of approximately 300 mg were run as controls.

Results

Rats that were exposed to chronic hypobaric hypoxia (440 Torr) for a variable period of time (3–35 days) increased their lung DNA synthesis considerably. Figure 1 shows a rapid and continu-
A kinetic study of lung DNA-synthesis during simulated chronic high-altitude hypoxia

![Graph](Fig. 1) Study of \(^3\)H-thymidine incorporation by lung and liver parenchyma during normoxia and hypoxia: (a) Mean values of the in vitro \(^3\)H-thymidine incorporation of rat lungs reach a maximum on the ninth day of hypobaric hypoxia (440 Torr, n=3). In spite of persisting hypoxia the cell proliferation decreases after the ninth day to control values (n=3); (b) stimulation of liver cell proliferation during hypoxia is less pronounced (n=3).

The morphological adaptation to altitude hypoxia is seen in right ventricular hypertrophy (Alexander et al., 1960; Grover et al., 1963; Barer et al., 1970; Abraham et al., 1971; Heath, 1975; Pfeiffer et al., 1976; Suggett and Mungall, 1976), medial hypertrophy of the pulmonary trunk (Heath, 1975), and medial muscularisation of the small pulmonary arterioles (Heath, 1959; Naeye and Bickerman, 1959; Meessen, 1960; Arias-Stella and Saldafia, 1962; Barer et al., 1970). These changes are encountered in rats of all age groups after a period of chronic hypoxia due to simulated high altitude (McGrath et al., 1973). Similar changes are found during long-term hypercapnia (Lodi and Viswanathan, 1974).

Exposure to low oxygen in a decompression chamber serves as an effective model to produce the reversible histological vascular changes resulting from chronic hypoxia (Kay et al., 1970; Abraham et al., 1971; Heath 1975; Urbanová et al., 1975) in the course of chronic bronchitis or long lasting emphysema (Naeye and Bickerman, 1959; Heath, 1970). We used this model to design a pilot study in search of a simple parameter to measure cell proliferation in the lung under hypoxic conditions.

A raised in-vitro incorporation of \(^3\)H-thymidine into lungs submitted to increasingly longer periods of hypoxia suggests a stimulation of cell proliferation. As in postnatal life, this rise of lung DNA synthesis may occur to match a new functional requirement (Crocker et al., 1970). Rats exposed to high altitude for three days show a 50% increase in pulmonary artery pressure (Moret and Duchosal, 1976), and after 10 days of simulated high altitude the mean percentage wall thickness of small pulmonary vessels is significantly raised (Reid, 1975). Our time course study provides data for increased cellular DNA synthesis activity that is upheld for several days. DNA synthesis may be

---

Discussion

The morphological adaptation to altitude hypoxia is seen in right ventricular hypertrophy (Alexander et al., 1960; Grover et al., 1963; Barer et al., 1970; Abraham et al., 1971; Heath, 1975; Pfeiffer et al., 1976; Suggett and Mungall, 1976), medial hypertrophy of the pulmonary trunk (Heath, 1975), and medial muscularisation of the small pulmonary arterioles (Heath, 1959; Naeye and Bickerman, 1959; Meessen, 1960; Arias-Stella and Saldafia, 1962; Barer et al., 1970). These changes are encountered in rats of all age groups after a period of chronic hypoxia due to simulated high altitude (McGrath et al., 1973). Similar changes are found during long-term hypercapnia (Lodi and Viswanathan, 1974).

Exposure to low oxygen in a decompression chamber serves as an effective model to produce the reversible histological vascular changes resulting from chronic hypoxia (Kay et al., 1970; Abraham et al., 1971; Heath 1975; Urbanová et al., 1975) in the course of chronic bronchitis or long lasting emphysema (Naeye and Bickerman, 1959; Heath, 1970). We used this model to design a pilot study in search of a simple parameter to measure cell proliferation in the lung under hypoxic conditions.

A raised in-vitro incorporation of \(^3\)H-thymidine into lungs submitted to increasingly longer periods of hypoxia suggests a stimulation of cell proliferation. As in postnatal life, this rise of lung DNA synthesis may occur to match a new functional requirement (Crocker et al., 1970). Rats exposed to high altitude for three days show a 50% increase in pulmonary artery pressure (Moret and Duchosal, 1976), and after 10 days of simulated high altitude the mean percentage wall thickness of small pulmonary vessels is significantly raised (Reid, 1975). Our time course study provides data for increased cellular DNA synthesis activity that is upheld for several days. DNA synthesis may be
the terminal event in a chain of metabolic processes which are preceded by changes of the DNA precursor pool resulting in a lag between the application of the stimulus and the onset of DNA synthesis (Novi, 1976). The delay varies in duration from hours to a day (Baserga, 1968). Thus a 345% increase in the DNA synthesis rate after nine days of hypoxia due to a reduction of the capacity of the DNA precursor pool seems improbable.

It is surprising that the proliferative system is turned on vigorously during an early induction phase but seems insensitive to further hypoxia. This finding may explain the results of Naeye (1962) who was unable to demonstrate a change in the mitosis index in mice lungs after chronic hypoxia. In contrast to the proliferation rate, which returns to control values and tends to reach even lower levels, the lung tissue DNA concentration remains raised, reflecting the postmitotic increase in the total cell number.

Our data suggest a transient stimulation of mitotic activity, but we cannot define the type of cell that accounts for this proliferation. Whether we deal with arteriolar wall muscle cells, intima cells, alveolar cells, fibroblasts or the mast cell hyperplasia that has been reported to occur around small vessels of hypoxic lungs (Kay and Grover, 1975; Mungall and Barer, 1975; Mungall, 1976) can only be shown by morphological studies.

A preliminary qualitative statement can be made on the basis of our first autoradiographic findings. A significantly higher index of radioactive label in the hypoxic lungs compared with the controls is found in the mesenchymal cells, in the walls of small pulmonary arteries, and in the bronchial tissue. A detailed quantitative evaluation of these results is in preparation.

References


N. Vökel, U. Wiegers, V. Sill, and J. Trautmann


A kinetic study of lung DNA-synthesis during simulated chronic high-altitude hypoxia


Requests for reprints to: Dr. N. Völkel, I Medical University Clinic, 2, Hamburg 20, Martinstrasse 52, Germany.
A kinetic study of lung DNA-synthesis during simulated chronic high altitude hypoxia.

N Völkel, U Wiegers, V Sill and J Trautmann

Thorax 1977 32: 578-581
doi: 10.1136/thx.32.5.578

Updated information and services can be found at:
http://thorax.bmj.com/content/32/5/578

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/