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POLYAMINE METABOLISM IN RAT LUNGS WITH OXYGEN TOXICITY

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SUMMARY: Ornithine decarboxylase activity increases 2-fold above control after 1 day and 25-fold after 3 days of exposure to 0.85 atm oxygen. Putrescine content nearly doubled by 72 hours which may reflect increased activity of ornithine decarboxylase. Spermidine and spermine content did not increase until after 3 days of exposure which was consistent with the delayed increase of S-adenosylmethionine decarboxylase activity. The results suggest that antimetabolites of polyamine metabolism may be useful to suppress excessive cellular proliferation in the lung after acute lung injury.

INTRODUCTION: Aliphatic polyamines promote cellular proliferation and differentiation in many tissues (1, 2). The diamine putrescine and the polyamines spermidine and spermine have been the focus of polyamine research; their metabolism is closely intertwined and there is sequential formation of putrescine, spermidine and spermine. The first step, putrescine formation, requires ODC, which catalyzes the formation of putrescine from ornithine. Subsequent addition of propylamine groups yields first spermidine and then spermine. If the normal accumulation of these substances is prevented, DNA synthesis may be suppressed.

Recognizing that polyamines promote DNA, RNA and protein synthesis (2) in many settings, we postulated that they may play an important role in recovery from lung injury and the subsequent cell proliferation (interstitial pneumonitis) that can follow. Recent advances in polyamine metabolism have introduced concepts and metabolic inhibitors that may eventually permit control of these processes. There is also evidence that hyperoxia suppresses cardiac ODC

Abbreviations: ODC - ornithine decarboxylase; SAMDC - S-adenosylmethionine decarboxylase

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activity by oxidizing essential sulfhydryl groups (3) and, therefore, the reported suppression of cell proliferation by hyperoxia (4) might be related to suppression of polyamine metabolism. In this initial study we did not find such an effect of hyperoxia upon lung ODC; lung polyamine metabolism increased during O_2 toxicity.

METHOD: Male Sprague-Dawley rats (60 days old weighing 250-300g) from Hilltop Laboratories were divided into control and exposure groups; all were placed in stainless steel cages and provided with food and water ad lib. The control group was kept under a laminar flow hood, whereas the exposure group was placed in an 11 ft^3 stainless steel chamber (5). Oxygen and compressed air were mixed to provide 0.85 atm O_2. At the appropriate time, animals were deeply anesthetized with sodium pentabarbital (60 mg/kg) and, after removing the lungs, we perfused them with 0.9% saline and then trimmed them of hilar tissue. They were then homogenized for 30 seconds with a polytron homogenizer (Brinkman) and the volume was adjusted to 5.0 ml with a solution consisting of 48 mM sodium phosphate buffer (pH 7.2), 0.1 mM pyridoxal 5'phosphate, 5 mM dithiothreitol, and 0.1 mM EDTA. Aliquots were taken for protein, DNA and polyamine determinations. The remainder of the homogenate was centrifuged at 30,000g for 30 minutes at 4°C. Aliquots of the supernatant were taken for protein, ODC and SAMDC determinations. ODC was determined by the method of Lowe et al (6) with the following modifications: 20 mM sodium phosphate buffer (pH 7.2), 0.5 mM EDTA, 0.9 mM L-ornithine, 0.5 µCi L-[1-^{14}C] ornithine and 0.2 ml NCS (Amersham) coated filter paper in plastic center wells. SAMDC was determined by a modification of the method of Prakash et al (7). We used 0.1 µCi of S-adenosyl-L-[carboxyl-^{14}C] methionine and omitted aminoguanidine and pargyline.

The polyamines were extracted by a method similar to that of Seiler (8). After homogenization in 0.2N $HClO_4$ the supernatant was collected following centrifugation and an aliquot was dansylated for 18 hours. Proline was added to terminate the reaction and the polyamines were extracted into ethyl acetate. The ethyl acetate was concentrated and injected into a high pressure liquid chromatograph (Varian 5020, Fluorochrom Detector, excitation at 360 nm and emission at 440 nm). The polyamines were separated using a reverse phase column and eluted with a gradient of water: acetonitrile beginning with 50:50 and ending with 5:95. Values were corrected for recovery using 1,6 diamino-hexane as an internal standard. Details of this procedure will be published separately.

In separate studies DNA synthesis was estimated by the incorporation of radioactivity into DNA 90 minutes after injecting 2 µCi/g bodyweight of [methyl-^{3}H] thymidine (Amersham) intraperitoneally. DNA was extracted by the method of Baird et al (9).

DNA content was determined by the method of Schneider (10). Statistical analysis was performed using Student's T-test.

RESULTS: ODC activity increased 2-fold above control after 1 day and 25-fold after 3 days of O_2 exposure (fig. 1). It remained elevated throughout 9 days of O_2 exposure (7 days 0.85 atm O_2 plus 2 days in 1.0 atm O_2), but after an additional 8 days in 1.0 atm O_2 it had decreased (fig. 1). We have expressed all values per whole lung because lung injury is associated with vascular

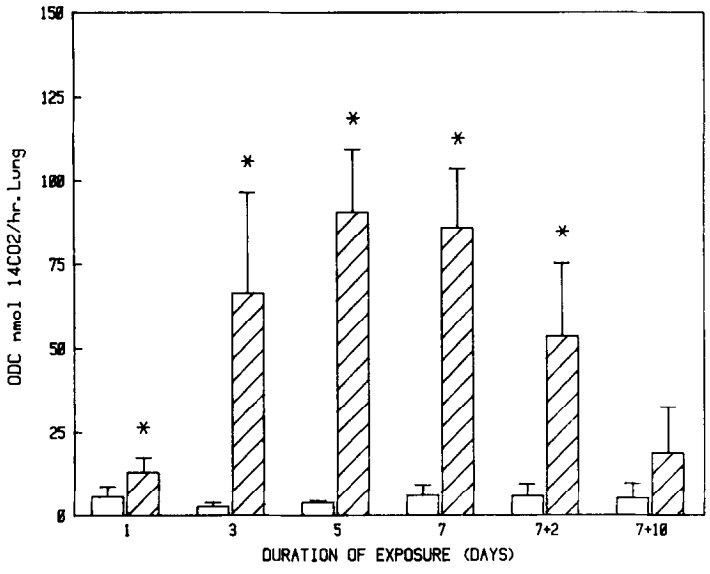

Figure 1. ODC activity in rat lung during O_2 exposure (0.85 atm O_2 for 7 days followed by 1.0 atm O_2 for 10 days).
Air-exposed Oxygen-exposed
* Statistically significant ($p<0.05$) N=5

congestion and edema with increases of protein. DNA content was 15% increased ($p<0.05$) only at 5 days. Putrescine increased within 2 days and was 5-fold greater than control by 7 days (fig. 2A). It remained elevated throughout the 17 days of O_2 exposure. In contrast with the rapid increase of ODC, SAMDC activity did not increase significantly until the 5th day. (fig. 2B).

Consistent with the later increase of SAMDC activity the contents of spermidine and spermine did not increase until the 5th day of O_2 exposure (fig. 2C and 2D).

^3H thymidine incorporation into DNA increased abruptly on the 5th day of exposure (table 1).

DISCUSSION: Hyperoxia damages the lung's epithelium and endothelium within 2 or 3 days of exposure (11) and their repair requires cell growth. However, accompanying interstitial cell proliferation may eventually dominate and produce severe interstitial pneumonitis.

Although rat lung cells proliferate by 5 days in 0.85 atm O_2, hyperoxia initially suppresses cell proliferation. Oxygen between 0.3 and 1.0 atm inhibits DNA synthesis and cell division in cultured cells for up to 4 days

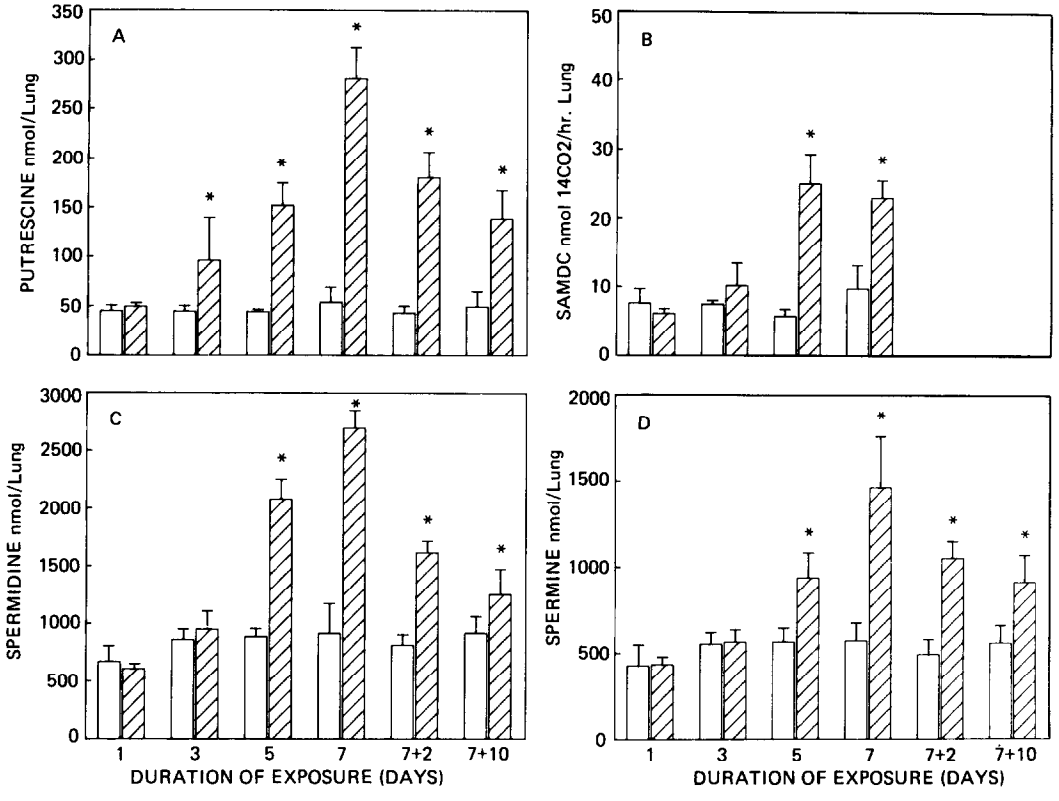

Figure 2. Effects of O_2 exposure upon: (A) putrescine, (B) SAMDC activity, (C) spermidine and (D) spermine in rat lung, (0.85 atm O_2 for 7 days followed by 1.0 atm for 10 days).
□ Air-exposed ▨ Oxygen-exposed
* Statistically significant ($p<0.05$) N=5

(4) and 1.0 atm O_2 suppresses ^3H thymidine labelling of mouse lung cells below control values for at least 48 hours (12). We observed that ODC activity increased an additional 2-fold 2 days after removing rats from 1.0 atm O_2 (unpublished observation) which is consistent with a sudden escape from suppression. The mechanism by which O_2 transiently suppresses cell growth is not known but it might alter polyamine metabolism. Prostatic and hepatic ODC have SH groups which are apparently required for activity (3) but may be

Table 1: DNA Synthesis in Lungs Exposed to 0.85 atm O_2 (DPM/μg DNA)

	1 Day	3 Days	5 Days
Control	70 ± 69	72 ± 20	81 ± 60
Exposed	48 ± 10	82 ± 32	1450 ± 271*

*Statistically significant ($p<0.05$) N=5
Each value represents mean ± S.D.

attacked by oxidants; cardiac ODC activity can be suppressed by hyperoxia (3). Oxygen toxicity associated with increased superoxide production might decrease putrescine production by attacking ODC. Such an effect could limit cell growth in the injured lung because putrescine appears to be essential for liver growth following partial hepatectomy (13). Although we found major increases of ODC activity with 0.85 atm (fig. 1) and 1.0 atm (14), it is possible that even greater increases might have occurred if SH groups were not attacked. Nevertheless, polyamines are probably present in sufficient quantity to enable cells to proliferate at a rate greater than normal, particulary after several days when marked increases of polyamine content become apparent. DNA synthesis, as judged by ^{3}H thymidine incorporation into lung DNA, did not change significantly by 3 days of exposure but it increased more than 15-fold by the 5th day (table 1). Therefore, increased DNA synthesis occurred during the same interval as the increase of spermidine and spermine content. The increase of polyamine metabolism may be delayed after the beginning of O_2 exposure because 0.85 atm O_2 does not produce immediate injury and cell growth may not start for 2 or 3 days. In contrast with 0.85 atm O_2, ozone causes an almost immediate injury to the lung and we have found that it causes a major increase of polyamine metabolism within 2 days (unpublished observation).

It is likely that different cell types within the lung proliferate at different times (15). After injury by butylated hydroxytoluene, the type II epithelial cells in the mouse lung proliferate primarily between 2 and 5 days, the interstitial cells begin proliferating after 3 days and continue through the 9th day, whereas the endothelial cells proliferate between the 5th and 9th days (15). A marked increase of type II cells can be detected within 72 hours of exposure to 0.85 atm O_2 and cell growth is present shortly afterward (16). By 7 days there are nearly twice as many type II cells present in these rat lungs as in controls but the number of interstitial cells has also increased 2-fold (17).

Cell proliferation in the endothelium and epithelium may be necessary to maintain their integrity, but interstitial cell proliferation has little known

benefit and excessive interstitial cell proliferation may be lethal. Although polyamine content of the whole lung increases between days 3 and 7, we cannot ascribe the changes to any one cell type.

However, these increases are consistent with the marked cellular proliferation that occurs in O_2 toxic lungs and it may be possible to alter these responses by using drugs which alter polyamine metabolism (18). We found no evidence that hyperoxia suppresses ODC actiivy after 48-72 hours. Few biochemical changes are detectable within 24 hours of exposure to 0.85 atm O_2 (19) but the marked changes of polyamine metabolsim that occur suggest that this may be a sensitive method to determine lung injury.

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REFERENCES

1. Pegg, A.E. and Williams-Ashman, H.G. (1981) Polyamines in Biology and Medicine, pp. 3-42, Marcel Dekker, Inc., New York.
2. Heby, O. and Janne, J. (1981) Polyamines in Biology and Medicine, pp. 243-310, Marcel Dekker, Inc., New York.
3. Guarnieri, C., Lugaresi, A., Flamigni, F., Muscari, C., and Caldarera, C.M. (1982) Biochim. Biophys. Acta. 718 (2), 157-164.
4. Haugaard, N. (1968) Physiol. Rev. 48, 311-373.
5. Hinners, R.G., Burkeart, J.K., and Punte, C.L. (1968) Arch. Environ. Hlth. 16, 194-206.
6. Lowe, N., Verma, A.K. and Boutwell, R.K. (1978) J. Invest. Derma. 71, 417-418.
7. Prakash, N.J., Schechter, P.J., Grove, J. and Koch-Weser, J. (1978) Cancer Res. 38, 3059-3062.
8. Seiler, N. and Wiechmann, M. (1970) Progress in Thin Layer Chromatography and Related Methods, pp. 94-144, Ann Arbor-Humphrey Science, London.
9. Baird, W.M., Sedgewick, J.A. and Boutwell, R.K. (1971) Cancer Res. 31, 1434-1439.
10. Schneider, W.C. (1945) J. Biol. Chem. 161, 293-303.
11. Kapanci, Y., Weibel, E., Kaplan, H. and Robinson, F. (1969) Lab. Invest. 20, 101-118.
12. Evans, M., Hackney, J., and Bils, R. (1969) Aerospace Med. 40, 1365-1368.
13. Pösö, H. and Pegg, A. (1982) Biochim. Biophys. Acta. 696, 179-186.
14. Hacker, A. and Tierney, D.F. Am. Rev. Respir. Dis. (1981) 123, 227 (abstract).
15. Adamson, I.Y.R., Bowden, D.H., Cote, M.C. and Witschi, H.P. (1977) Lab. Invest. 36, 26-32.
16. Crapo, J.D., Marsh-Salin, J., Ingram, P. and Pratt, P.C. (1978) J. Appl. Physiol., Respirat. Environ. Exercise Physiol. 44, 370-379.
17. Young, S., Crapo, J., Kremers, S. and Brumley, G. (1982) Lab. Invest. 46, 570-576.
18. Pegg, A.E. and McCann, P.P. Am. J. Physiol. (1982) 243 (Cell Physiol. 12), C212-C221.
19. Mustafa, M.G. and Tierney, D.F. (1978) Am. Rev. Respir. Dis. 118, 1061-1090.