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CHANGES IN LUNG HYALURONIDASE ACTIVITY ASSOCIATED WITH LUNG GROWTH, INJURY AND REPAIR

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SUMMARY: We measured lung hyaluronidase activity in rats during postnatal life and during the repair of oxygen-induced lung injury. Hyaluronidase activity increased rapidly after birth and peaked at 16-fold the initial value at 8 days. The peak preceded decreased cell proliferation and the onset of differentiation; this is consistent with current concepts of the role of hyaluronidase. During the repair of lung injury, hyaluronidase activity increased to 2.5-fold the control value at 1 day post-injury, but had decreased by 3 days. This early peak is probably related to simultaneous cell proliferation and differentiation. We postulate that changes in hyaluronidase can influence lung growth and repair and that the system may be amenable to manipulation.

INTRODUCTION: The glycosaminoglycans of the extracellular matrix are thought to be important in tissue growth and repair (1-3). Studies in several organs during morphogenesis, regeneration and repair suggest that a hyaluronate-rich matrix promotes cell proliferation and migration whereas a decrease in hyaluronate combined with an increase in sulfated glycosaminoglycans favors differentiation of the proliferated cells (3-7). The enzyme hyaluronidase may thus influence growth and repair by affecting the turnover of hyaluronate (5-8); it may also favor formation of new capillaries in normal or injured tissues by causing dissolution of the pericapillary sheath (9,10).

Although increases in hyaluronidase have been reported in healing skin wounds (7) and during embryonic brain and kidney growth (6,8), changes in lung hyaluronidase during growth, injury and repair have not previously been described. In the present study, we describe the pattern of changes in rat lung hyaluronidase during postnatal growth and during the repair of oxygen-induced lung injury. We also correlate these changes with data previously reported by us and by others on the morphologic and biochemical changes occurring during these processes.

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METHODS: Pregnant female Sprague-Dawley rats (Zivic-Miller) were allowed to give birth and the new-born rats were sacrificed on the day of birth (0 days) and at intervals during the next 21 days. They were weighed and sacrificed by decapitation (0 to 4 days of age) or were anesthetized with 50 mg/kg of intraperitoneal sodium pentobarbital and sacrificed by transection of the abdominal aorta (7 to 21 days of age). The lungs were then excised and weighed, and lung hyaluronidase activity was measured.

Adult male Sprague-Dawley rats (275-300 g, Charles River Laboratories) were exposed to 100% oxygen for 60 hours in polystyrene chambers and then allowed to recover in room air as previously described (12,13). Immediately post-injury (0 days) and at 1, 2, 3, and 4 days post-injury, rats were sacrificed as above. The lungs were excised and weighed, and lung hyaluronidase activity was measured.

Lung hyaluronidase activity was measured by a modification of the method used by Belsky and Toole for chick embryo kidneys (6). Approximately 200 mg wet weight of lung tissue was homogenized in 2 volumes of water on ice using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). 0.2 ml of the homogenate was added to 0.8 ml of formate buffer (pH 3.7) containing 960 μg of hyaluronic acid (Miles Biochemical, Naperville, IL) and incubated for 20 hours at 37°C under toluene. After incubation, the toluene was aspirated and the mixture was centrifuged at 27,000 g for 30 minutes. The supernatant was then assayed for the amount of terminal N-acetylglucosamine liberated from the exogenous substrate using the method of Reissig et al (13). Hyaluronidase activity was expressed as μg of terminal N-acetylglucosamine liberated per total lung or per gram lung wet weight.

The data for the newborn lungs were analyzed by using Dunnett's test (14) to compare all time points against the value at birth. For the oxygen-exposed rats, an unpaired t test (15) was used to test the differences between post-injury lungs and controls. The 0 and 1-day post-injury lungs were compared to the pooled 0 and 1 day air controls, and the 2, 3 and 4-day post-injury lungs were compared to the pooled 2 and 3-day controls. In addition, the 1 to 4-day post-injury data were compared to the immediate post-injury value using Dunnett's test (14).

RESULTS: During postnatal growth, total lung hyaluronidase activity was relatively low on the day of birth, but increased rapidly and was more than 16 times the initial value at 8 days of age (Figure 1A). It then gradually declined, and by 14 and 21 days of age, was only about 70% and 60% respectively of the peak value. When the changes in hyaluronidase activity were expressed per g lung weight, the same overall pattern was present (Figure 1B) except that the peak value at 7 days was only 1.8 fold the value at birth and the values at 14 and 21 days were not significantly different from that at birth.

In the adult lungs undergoing oxygen injury and repair, lung hyaluronidase activity was increased to 1.4 fold the control value after 60 hours of exposure to 100% oxygen (Figure 2). During repair in air, this further increased to 2.5 fold the control value at 1 day post-injury before beginning to decline. At 2 days post-injury, hyaluronidase activity was still significant-

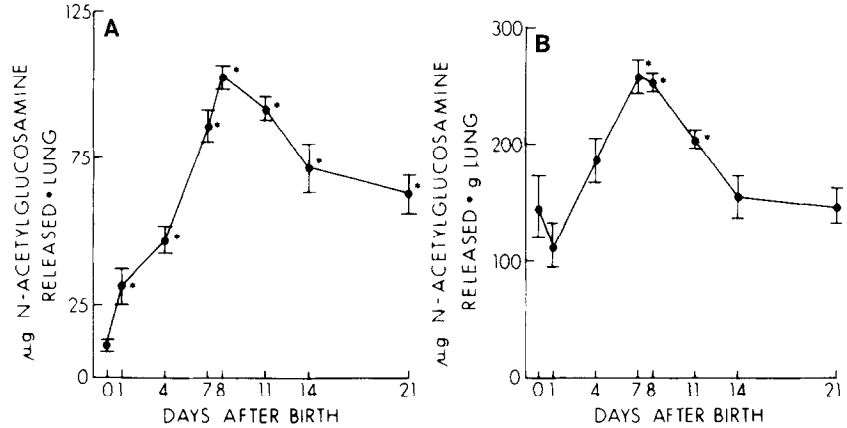

Figure 1 Changes in lung hyaluronidase activity during postnatal lung growth expressed per whole lung (A) or per g lung wet weight (B). Mean ± SEM; n = 6-8 samples at each time point. The asterisks denote p <0.05 vs. the value at birth.

ly greater than the initial post-injury value; however, by 3 and 4 days post-injury, differences were no longer present.

DISCUSSION: The peak in lung hyaluronidase at 7-8 days of age should be interpreted in the context of the morphologic and biochemical changes in the newborn rat lung. Proliferation of cells as measured by autoradiographic label-

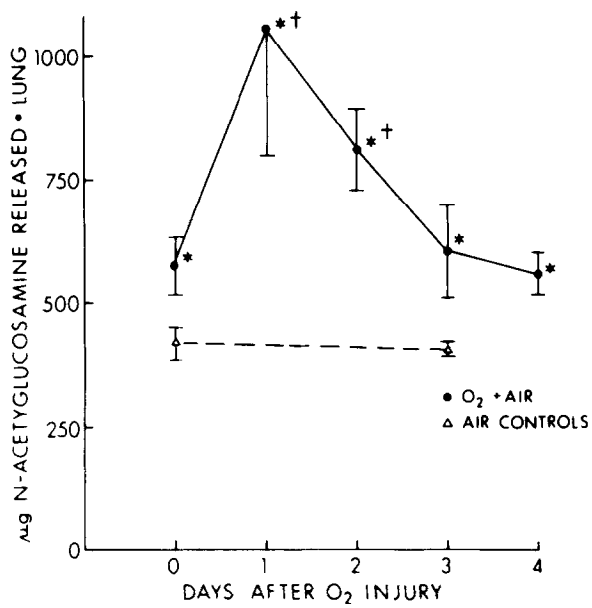

Figure 2 Changes in lung hyaluronidase activity following exposure to 100% O_2 for 60 hours. Mean ± SEM; n = 6-8 animals at each time point. The asterisks denote p <0.05 vs. controls. The crosses denote p <0.05 vs. the immediate post-injury value.

ing indices peaks at 4 days of age in fibroblasts, at 7 days in type 2 epithelial cells, and from 1 to 10 days in capillary endothelial cells (16). Lung ornithine decarboxylase activity and putrescine content, which are important in cell proliferation, also peak during this period (17). Morphologically, Burri (18) also found that between days 4 and 7 extensive restructuring of lung parenchyma with formation of secondary septae takes place. Thus, the peak in hyaluronidase activity occurs just after the period in which cell proliferation and migration are maximal and during which the hyaluronate content of the extracellular matrix is presumably relatively high. High hyaluronate content is thought to promote cell proliferation and migration by increasing the degree of hydration of the extracellular matrix and also by direct cell surface interactions which delay the onset of differentiation (4).

The period following peak hyaluronidase activity during growth and repair in other organs is characterized by decreased hyaluronate and increased sulphated glycosaminoglycan content (3,4,6); this is thought to favor cessation of cell proliferation and onset of differentiation (1,4). The changes in the newborn lung are consistent with this concept: between 7 and 13 days, labeling indices in all cell types decline sharply and differentiation of type 2 epithelial cells into type 1 epithelial cells increases as evidenced by an increase in type 1 cell number and a sharp decrease in type 2 cell number (16). We hypothesize, therefore, that the temporal pattern of hyaluronidase activity in the newborn lung can, at least partially, influence the timing and sequence of morphologic development. It remains to be seen whether factors that disrupt postnatal lung growth also affect changes in lung hyaluronidase and whether inhibition of hyaluronidase by compounds such as dextran sulphate (19) can significantly affect changes in lung morphology.

The changes in lung hyaluronidase during the repair of oxygen-induced injury bear a different relation to the morphologic and biochemical events than that described in newborns. Proliferation of cells, as well as ornithine decarboxylase activity and polyamine content are maximal between 0 and 3 days post-injury (12,20). Ornithine decarboxylase activity decreases sharply be-

tween 2 and 3 days post-injury (12). Beyond 3 days most cell proliferation ceases (12) and differentiation of type 2 epithelial cells into type 1 epithelial cells begins (21). On the other hand, lung hyaluronidase activity peaked sharply at 1 day post-injury and was above control levels throughout the first 4 days of repair.

Why does the period of peak hyaluronidase activity coincide with that of maximum cell proliferation during repair? First, if hyaluronate synthesis was increased during early repair, hyaluronate levels could be high despite the increase in hyaluronidase activity; this would allow rapid cell proliferation (and differentiation) to occur. This is seen for example in chick embryo brain development where both hyaluronidase activity and hyaluronate content are high and neuronal migrations and differentiations are occurring concurrently (8). In the lung, some cell differentiation probably occurs simultaneously with proliferation during early repair. The number of interstitial myofibroblasts, which are thought to differentiate from fibroblasts or more primitive mesenchymal cells, increases during the first 3 days of repair (22). Also, inhibition of type 2 epithelial cell proliferation and differentiation by inhibition of ornithine decarboxylase leads to an actual decrease in type 1 cell number during the first 3 days of repair, suggesting that significant replacement of type 1 cells by differentiating type 2 cells normally occurs during this period (12). A simultaneous increase in lung hyaluronidase and hyaluronate during early repair would thus be consistent with these events. In this context, it should be mentioned that the colorimetric method described by Belsky and Toole (6) for measuring hyaluronate was too insensitive, in our hands, to detect lung hyaluronate, even when multiple adult rat lungs were pooled. This can be explained by the relatively low levels of hyaluronate measured by Karlinsky in hamster lungs using 2-dimensional cellulose acetate electrophoresis and atomic absorption spectroscopy (23). These latter techniques are not presently available to us.

A second possible explanation for the early peak in lung hyaluronidase during repair is Bok's hypothesis (9) that hyaluronidase plays a crucial role

in capillary growth by causing dissolution of the pericapillary sheath. This would be consistent with the rapid increase in capillary endothelial cell number and luminal surface are during the first 3 days of repair (12,20). However, the experimental data supporting this concept is at best scanty (10).

What further studies on lung growth and repair are suggested by our findings? First, the changes in lung hyaluronidase should be correlated with changes in hyaluronate and other glycosaminoglycans during growth and repair. Also, the temporal pattern of changes in hyaluronidase and hyaluronate during abnormal or modified growth and repair should be determined and correlated with the morphologic and biochemical changes. Perhaps most importantly, the effects on lung growth and repair of agents which inhibit hyaluronidase (19) or alter glycosaminoglycan metabolism (24) should be investigated since these may provide a means of manipulating the system.

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REFERENCES:

1. Toole, B.P. (1981) Cell Biology of Extracellular Matrix, pp. 259-294, Plenum Press, New York.
2. Bernfield, M., and Banerjee, S.D. (1982) Dev. Biol. 90, 291-305.
3. Reid, T., and Flint, M.H. (1974) J. Embryol. Exp. Morph. 31, 489-495.
4. Toole, B.P. (1982) Tissue Res. 10, 93-100.
5. Maurer, P.H., and Hudack, S.S. (1952) Arch. Biochem. Biophys. 38, 49-53.
6. Belsky, E., and Toole, B.P. (1983) Cell Differ. 12, 61-66.
7. Bertolami, C.N., and Donoff, R.B. (1978) J. Surg. Res. 25, 256-259.
8. Polansky, J.R., Toole, B.P., and Gross, J. (1974) Science 183, 862-864.
9. Bok, S.W. (1979) Med. Hypotheses 5, 1183-1200.
10. Zweifach, B.W., and Chambers, R. (1950) Ann. N. Y. Acad. Sci. 52, 1047-1051.
11. Thet, L.A., Wrobel, D.J., Crapo, J.D., and Shelburne, J.D. (1983) Lab. Invest. 4, 448-457.
12. Thet, L.A., Parra, S.C., Shelburne, J.D., and McCann, P.P. (1983) Amer. Rev. Respir. Dis., in press.
13. Reissig, J.L., Strominger, J.L., and Leloir, L.F. (1955) J. Biol. Chem. 217, 959-966.
14. Dunnett, C.W. (1974) Biometrics 20, 482-491.
15. Snedecor, G.W., and Cochran, W.G. (1980) Statistical Methods 6th edition, pp. 83-106, Iowa State University Press, Ames, Iowa.
16. Kauffman, S.L., Burri, P.H., and Weibel, E.R. (1974) Anat. Rec. 180, 63-76.

17. Thet, L.A., Parra, S.C., Diekema, K.A., and McCann, P.P. (1983) Amer. Rev. Respir. Dis. 127(4 Suppl), 209.
18. Burri, P.H. (1974) Anat. Rec. 180, 77-98.
19. Zimmerman, K., Preinl, G., Ludwig, H., and Greulich, K.O. (1983) J. Cancer Res. Clin. Oncol. 105, 189-190.
20. Thet, L.A., Parra, S.C., Shelburne, J.D., and McCann, P.P. (1983) Clin. Res. 31, 422A.
21. Adamson, I.Y., and Bowden, D.H. (1974) Lab. Invest. 30, 35-42.
22. Thet, L.A., Parra, S.C., and Shelburne, J.D. (1983) Fed. Proc. 42(4), 1025.
23. Karlinsky, J.B. (1982) Am. Rev. Respir. Dis. 125, 85-88.
24. Thompson, H.A., and Spooner, B.S. (1982) Dev. Biol. 89, 417-424.