PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 120–125 (1974)

# Effects of Pulmonary Irritants on DNA, ATPase Activity, and Histamine on Rat Lung (38055)

SUSAN R. CRONIN AND SHRI N. GIRI
(Introduced by S. A. Peoples)
*Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, California 95616*

Tissue enzyme activity and biogenic amine content are conventionally expressed per unit wet weight of tissue or tissue protein. Under normal conditions the lung is no exception in this regard. The validity of conventional ways of reporting enzyme activity for the edematous lung has been subjected to criticism (1), as edematogenic agents induce a marked leakage of plasma water, protein and blood cells into pulmonary tissue, thus increasing lung weight and lung protein content several fold (2, 3). It is unlikely that alterations in enzyme activity and biogenic amine content of lung tissue resulting from edematogenic agents could be truly represented by the conventional ways. Therefore, the purpose of this investigation was two fold: (1) to compare the lung DNA content of rats subjected to a gaseous ($O_3$) and a nongaseous (thiourea) edematogenic agent with their matching control and determine if the DNA content could be employed to standardize the lung enzyme activity and biogenic amine content of edematous and nonedematous lungs; (2) to find out whether or not the adenosine triphosphatase (ATPase) activity and histamine content of lungs are altered in the pathogenesis of pulmonary edema.

ATPase, a membrane bound enzyme, was selected in the present investigation because of its key role in the active transport processes (4) that maintain the ionic homeostasis of many organ systems (5). ATPase inhibition results in an increased membrane permeability of sodium and potassium ions along with an inhibition of active transport (6). It was therefore envisioned that an interference in the ATPase activity at the pulmonary tissue level might be of primary importance in the pathogenesis of chemically induced pulmonary edema.

Lung histamine content was investigated because of its suggested involvement in the pathophysiology of pulmonary edema (7). This part of the study was extended in two age groups of rats to determine if the age-dependent susceptibility to the edematogenic effect of thiourea (8, 9) could be related to its differential effects on lung histamine content.

*Materials and Methods.* Male Sprague-Dawley rats (Simonsen Gilroy, Calif.) 75 ± 1 days old weighing approximately 360 g were used throughout the DNA and enzyme investigations. Rats 25 ± 1 days old weighing approximately 60 g were used with 70 ± 5 days old rats for the histamine determinations. Purina rat chow and $H_2O$ were provided *ad libitum*. The effects of a nongaseous edematogenic agent were determined by ip injection of 10 mg/kg thiourea (TU), 10 mg/kg phenylthiourea (PTU), or an equivalent volume of saline. The effects of a gaseous edematogenic agent were determined by exposure of rats to 4 ppm $O_3$ for 4 hr while the controls were exposed to ambient air for the same period of time. The exposure procedures were the same as followed by the pulmonary group at the University of California, Davis, California (10). Two hours after injection or ½ hr after exposure the rats were anesthetized with ether. The inferior vena cava was exposed and

severed and the animals were thus exsanguinated.

In the event of death prior to 2 hr for the injected animals, the lungs were removed immediately following death. No premature deaths occurred in the $O_3$ exposed animals. For all studies except histamine determination, the trachea was ligated and the lungs carefully excised intact. Extraneous tissue was removed and the lungs were blotted. The right and left lungs were separated from the trachea at the primary bronchi where it enters the lungs. The tissue was weighed in a tared beaker filled with ice-cold 0.25 *M* sucrose. Pulmonary edema was quantitated by use of lung weight as percent body weight (11, 12).

The lungs were then homogenized at 0–4°C in a glass–Teflon homogenizer. The homogenate was made to 20 ml and three 1.0 ml aliquots were taken for DNA determination according to Schneider (13). Lung DNA content was reported as mg DNA/lung.

For the assay of ATPase activity the homogenate was strained through 2 layers of sucrose-soaked cheese cloth before making a final volume of 20 ml. Aliquots for DNA determinations were taken as described above. For determination of ATPase activity, another aliquot was diluted 1:19 with 0.25 *M* sucrose and was used for measuring ATPase activity according to the procedure of Wahler *et al.* (14). Incubations were carried out at 37°C in a Dubnoff shaker for 20 min. In all cases, the activities of $Ca^{2+}$, $Mg^{2+}$, and $Na^+$-$K^+$ activated $Mg^{2+}$ ATPases were determined. ATPase activity was expressed in two ways: $\mu$mol inorganic phosphate ($P_i$) liberated/gm lung/hr and $\mu$mol $P_i$ liberated/mg lung DNA/hr.

The effects of $O_3$ and TU on lung histamine were determined in the following way. TU (10 mg/kg) and saline were injected ip in 70 day and 25 day old rats. A group of older rats was exposed to $O_3$ as described earlier. Following exsanguination, the lung was removed intact and put in ice-cold saline. The lung was perfused with cold isotonic saline through the trachea and the right side of the heart to remove blood from the pulmonary vascular compartment. The perfused lung was separated from the trachea, heart, and the surrounding tissue and then homogenized at 0–4°C in a glass–Teflon homogenizer with 0.25 *M* sucrose. The homogenate was made to 20 ml. Two 2.0 ml aliquots were taken for DNA determination. Three 1.0 ml aliquots were assayed for histamine by the fluorometric technique of Shore (15). Lung histamine was expressed as $\mu$g histamine/mg DNA.

Plasma histamine content was determined in 70 day old rats subjected to TU. Two hours following the injection rats were anesthetized with ether and the inferior vena cava was exposed. Blood was collected in a heparinized syringe for plasma histamine analysis. The histamine levels were expressed as $\mu$g histamine/100 ml plasma.

All data were expressed as the average $\pm$ standard error (SE). The student *t* test was applied to determine the significance of difference between the means.

*Results. Effects of $O_3$ and TU on pulmonary edema and lung DNA content.* A highly significant increase ($P < 0.001$) in lung wet weight as % body weight (index of pulmonary edema) was obtained in 75 day old rats subjected to acute $O_3$ exposure or TU administration. No difference was noticed however between the DNA content of edematous and nonedematous control lung. Figure 1 demonstrates these findings.

*Effects of $O_3$, TU and PTU on lung ATPase activity.* Table I summarizes the ATPase activities in response to varying edematogenics. The ATPase activity expressed as $\mu$mol $P_i$ liberated/gm lung/hr showed significant decreases ($P < 0.001$ to 0.01) in edematous lungs from control lungs. There was no significant difference however when this activity in both cases was expressed per mg DNA with the exception of $Na^+$-$K^+$, $Mg^{2+}$ activated ATPase in PTU-induced edema.

*Effects of $O_3$ on lung histamine content.* Histamine and DNA content were determined on the lungs of 70 day old rats ½ hr after $O_3$ exposure. Table II shows that there is no difference in the lung histamine level of $O_3$ exposed rats as compared to their controls.

*Effects of TU on histamine content of*

TABLE I. Lung ATPase Activity of 75 Day Old Rats, Expressed per g Lung and per mg DNA, in Response to TU and PTU Injection and $O_3$ Exposure.

| Expression of ATPase activity | Treatment | Average enzyme activity of 4 rat lungs ± SE: $Mg^{2+}$ ATPase | *P* value between control & edematogenics | $Na^+$-$K^+$, $Mg^{2+}$ ATPase | *P* value between control & edematogenics | $Ca^{2+}$ ATPase | *P* value between control & edematogenics |
|---|---|---|---|---|---|---|---|
| $\frac{\mu mol\ P_i\ liberated}{g\ lung/hr}$ | Control[a] | 148.71 ± 12.13 | 0.001 | 154.15 ± 13.81 | 0.01 | 158.56 ± 12.20 | 0.01 |
| | TU[b] | 52.20 ± 6.33 | | 57.14 ± 8.90 | | 61.43 ± 8.58 | |
| | Control[a] | 118.93 ± 5.06 | 0.001 | 131.34 ± 2.03 | 0.001 | 129.23 ± 3.31 | 0.001 |
| | PTU[b] | 71.05 ± 3.69 | | 78.22 ± 2.54 | | 75.71 ± 5.50 | |
| | Control[c] | 142.65 ± 5.58 | 0.001 | 165.31 ± 5.58 | 0.001 | 158.72 ± 5.95 | 0.001 |
| | $O_3$[d] | 82.22 ± 4.90 | | 84.23 ± 6.48 | | 91.07 ± 7.50 | |
| $\frac{\mu mol\ P_i\ liberated}{mg\ DNA/hr}$ | Control[a] | 17.93 ± 1.10 | NS[e] | 18.70 ± 1.72 | NS | 19.08 ± 0.89 | NS |
| | TU[b] | 19.00 ± 1.02 | | 20.46 ± 0.55 | | 22.25 ± 1.47 | |
| | Control[a] | 14.57 ± 0.79 | NS | 16.08 ± 0.56 | 0.02 | 15.85 ± 0.66 | NS |
| | PTU[b] | 16.93 ± 0.93 | | 18.62 ± 0.49 | | 17.99 ± 1.09 | |
| | Control[c] | 17.28 ± 0.34 | NS | 20.02 ± 0.18 | NS | 19.23 ± 0.42 | NS |
| | $O_3$[d] | 17.48 ± 1.24 | | 17.90 ± 1.54 | | 19.29 ± 1.39 | |

[a] Ip injection of a volume of saline equal to that of TU or PTU.
[b] 10 mg/kg was injected ip. The lungs were removed 2 hr after injection except in the case of premature death.
[c] Exposed to ambient air for 4 hr.
[d] Exposed to ozone 4 ppm for 4 hr.
[e] Not significant.

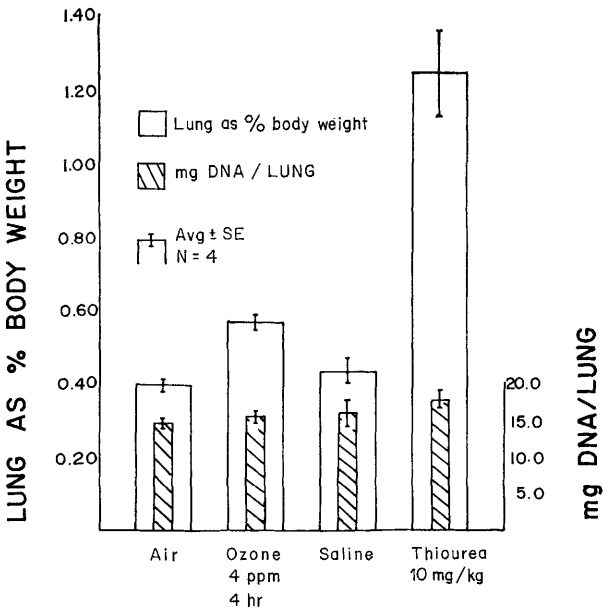


FIG. 1. The effects of $O_3$ and TU on rat lung as percent body weight and lung DNA content. The lung as percent body weight is an indication of pulmonary edema. Note the constancy of lung DNA content in control and edematous lungs.

*lung and plasma in rats of 2 age groups.* Two hours after ip administration of 10 mg/kg TU, rats were sacrificed and histamine and DNA were determined in the lungs of both 25 and 70 day old rats. Plasma histamine was determined in the 70 day old rats only and expressed as $\mu$g histamine/100 ml plasma. The data summarized in Table III shows a significantly higher ($P < 0.02$) amount of histamine in the lung of the 70 day old rats than in those of the 25 day old rats. The lung histamine content of the TU-treated 70 day old rats was significantly less ($P < 0.05$) than the controls, while no such difference was seen with the TU-treatment in the 25 day old rats. Also noted was a significant ($P < 0.05$) elevation in the plasma histamine content of the former group of rats following TU administration.

TABLE II. Effect of $O_3$ Exposure (4 ppm for 4 hr) on Lung Histamine Content of 75 Day Old Rats.

| Lung histamine in $\mu$g/mg lung DNA Avg ± SE | |
|---|---|
| Ambient air (control) | Ozone |
| 1.650 ± 0.324 (6) [a] | 1.239[b] ± 0.113 (6) |

[a] Figures in parenthesis are the numbers of animals used.

[b] $t$ value between control and $O_3$ is not significant.

*Discussion.* The genesis of pulmonary edema is unequivocally associated with the leakage of plasma water, protein, and blood cells from the vascular compartment into lung tissue. The result is an increase in lung weight and protein content. Previously, this has hindered the realistic assessment of the level of enzyme activity and biogenic amines supposedly altered in response to edematogenic agents. For instance, Skillen (1) has attributed decreased monoamine oxidase activity in edematous lung as compared to control to dilution caused by increased lung weight. The present report has shown a similar decrease in ATPase activity of edematous lung when expressed per g lung. However, no significant change in enzyme activity was noticed when the activity for

TABLE III. Effects of Thiourea on Histamine Content of Lung and Plasma in Rats.

| Age (days) | Lung histamine in μg/mg lung DNA Avg ± SE | | P value between control and TU | Plasma histamine in μg/100 ml plasma Avg ± SE | | P value between control and TU |
|---|---|---|---|---|---|---|
| | Control[a] | Thiourea[c] | | Control[a] | Thiourea[c] | |
| 25 | 0.618 ± 0.080 (4) [b] | 0.570 ± 0.065 (4) | NS | — | — | |
| 70 | 1.566 ± 0.218 (4) | 0.724 ± 0.083 (4) | <0.05 | 2.08 ± 0.33 (7) | 4.38 ± 0.94 (11) | <0.05 |
| P value between 25 & 70 day old rats | <0.02 | NS | | | | |

[a] Saline injected ip in a volume equivalent to the drug solution.
[b] Figures in parentheses are the numbers of animals used.
[c] Administered 10 mg/kg ip. Lungs removed 2 hr later and perfused before processing for histamine determination. See the Method Section for details.

both edematous and control lung was expressed per mg lung DNA.

This discrepancy in enzyme activity for the edematous lung is easily understood if one considers the insignificant change in lung DNA content concomitant with the striking increase in lung weight. Rats subjected to $O_3$ or TU exhibited pulmonary edema. Although TU exceeded $O_3$ in the magnitude of edema produced, no difference in the lung DNA content of these rats was found (Fig. 1). This strongly suggests that the DNA content remains constant and further, that it remains unaffected by the magnitude of edema induced under the edematogenic conditions herein specified. The constancy of lung DNA in normal and edematous lungs makes it a better choice for standardizing enzyme activity and biogenic amine content, thus allowing a realistic comparison of the changes involved in edematous and nonedematous (control) lungs. This standardization would obviate the dilution caused by edema fluid and thereby reflect the actual changes occurring at the lung cellular level in response to edematogenic agents.

Lungs from rats exposed to edematogenic agents and their respective controls must be perfused prior to determination of lung histamine or serotonin. The importance of this perfusion cannot be minimized because of the leakage of serotonin and histamine containing white blood cells in the edematous lungs. If these cells are not removed from the pulmonary vascular compartment and the airspace then there is a great liklihood of masking the true response to a given edematogenic agent. We believe that the lack of perfusion coupled with varying degrees of edema has most likely been a major factor in contributing to the data conflict (1, 16, 17, 18) regarding the effects of edematogenic agents on histamine and serotonin content of the lungs.

Utilization of lung DNA coupled with lung perfusion enabled us to overcome some of the difficulties previously encountered in rats subjected to edematogenic agents. The data summarized in Table III demonstrates that the lung histamine content of 70 day old rats was 2.5 times that of 25 day old

rats. These results are in agreement with and support of Jaques' (16) earlier finding that the lung histamine content of older rats is much higher than that of younger rats even when considered on a wet weight basis.

A remarkable decrease in the lung histamine content with a concomitant increase in the plasma histamine was found in 70 day old rats subjected to an acute edematogenic dose of TU. There was, however, no histamine depletion in rats of the same age group when exposed to an acute edematogenic concentration of $O_3$. This suggests the possibility of histamine involvement in TU-induced but not in $O_3$-induced pulmonary edema. The above hypothesis is further strengthened by our finding that the dose of TU producing acute pulmonary edema and depleting lung histamine in 70 day old rats failed to produce any of these changes in 25 day old rats.

The age dependent susceptibility to the edematogenic effect of TU compounds (8, 9) has been known, but unexplained, for a long time. It is conceivable that the presence of a higher amount of lung histamine in older rats might play a key role in the increased susceptibility to the edematogenic effect of TU. Whether the depletion of histamine from the lung of older rats in response to TU is a cause or an effect of pulmonary edema is not known at present.

*Summary.* The amount of lung DNA is unchanged in pulmonary edema induced by TU or $O_3$ while lung weight increases significantly. The constancy of lung DNA enabled us to make a valid comparison of ATPase activity and histamine content between normal and edematous lungs. There was no difference in ATPase activity between the 2 groups when expressed per mg DNA in contrast to a striking decrease in the edematous lung when expressed per g wet lung weight. TU but not $O_3$ caused the depletion of histamine from the lungs of 70 day old rats with a concomitant increase in plasma histamine. TU had no effect on lung histamine of 25 day old rats. The possible involvement of histamine in the pathophysiology of TU-induced pulmonary edema is discussed.

This research was supported in part by a grant from the Tuberculosis and Respiratory Disease Association of California and the University of California, Davis, Chancellor's Patent Fund. The authors are indebted to Mr. L. Richard Karrt and Mr. Timothy E. Cronin for technical assistance.

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Received Dec. 3, 1973. P.S.E.B.M., 1974, Vol. 146.