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Project Summary

Ozone Exposure and Pulmonary Metabolic Effects of Mediators and Hormones

Ibert C. Wells

The effects of acute ozone exposure on smooth muscle contracting substances in lung were studied in male rats with body weights in the 180-250 g range (45-60 days of age). Ozone concentrations employed were either 0.98 or 1.96 mg/m^3 and the exposure time was four hours. The following effects were produced: (a) increases in the amounts of prostaglandin $F_2\alpha$($PGF_2\alpha$) and thromboxane A_2 (TXA_2) in the lumen fluid; (b) an increase in the angiotensin converting enzyme activity; and (c) a decrease in the rate of serotonin uptake from the blood. Histamine and slow reacting substances of anaphylaxis (SRS-A) were not released under these experimental conditions, nor was the histamine forming capacity of the lungs altered. Edema formation was variable. As indicated in the reports by previous investigators, lung succinoxidase activity was observed to be decreased by exposure to 1.96 mg/m^3 ozone for four hours, but was increased after the same exposure on each of four consecutive days.

The exposure of rats to 3.92 mg/m^3 ozone for four hours also caused increased amounts of $PGF_2\alpha$ and TXA_2 in their lungs, but this ozone dose was ineffective in this regard if 15 hours prior to it the animals had received 0.98 mg/m^3 ozone for four hours. While this low dose of ozone had a minimal effect on the lung content of $PGF_2\alpha$ and TXA_2 if measured immediately after its administration, it apparently did produce an adaptation to ozone which was evident 15 hours later.

Indomethacin is a potent inhibitor of fatty acid cyclo-oxygenase (prostaglandin synthetase), the enzyme required for the biosynthesis of all the prostaglandins, as well as the thromboxanes and the prostacyclins. The administration of this substance to rats prior to their exposure to 1.96 mg/m^3 ozone for four hours prevents the ozone caused increase of $PGF_2\alpha$ and TXA_2 in their lungs. This end result is the same as that of the apparent adaptation to ozone produced by exposure of rats to 0.98 mg/m^3 ozone for four hours. It was, therefore, anticipated that "ozone-adapted" and indomethacin-treated rats would respond similarly to chronic ozone exposure and this response would differ from that of similarly exposed, untreated controls. This possibility was studied by measuring the survival times of treated rats when continuously exposed to 8.82 mg/m^3 ozone. The average survival times of the two groups of experimental rats were significantly less than that of the control group. This result suggests that the presence of increased amounts of at least these two medi-

ators in the lungs may serve to protect the animals from the deleterious effects of ozone. Supportive of this implication is the further observation that rats treated with Clotrimazole® (Schering) survived significantly longer than control animals under such conditions. This substance is a specific inhibitor of TXA_2 synthesis, and it has been proposed that when TXA_2 synthesis is prevented, the synthesis of other prostaglandins, such as $PGF_2\alpha$, is enhanced. $PGF_2\alpha$ has the same effect on lung as TXA_2 and, though less potent, is much more stable and therefore longer acting than TXA_2.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

General Methods

Ozone exposure. Two male rats having body weights in the 180-250 g range (45-60 days of age) are placed in a closed, two compartment, glass chamber, the volume of which is six liters (0.21 CF). Air which has been cooled by passage through a coil of tubing kept at 0°C is pumped through the chamber at a rate of 12 liters per minute (C.42 CFM). Prior to its entrance into the chamber, the air is passed through a silent, controlled electric discharge to convert some of the oxygen to ozone It is introduced into the chamber below the level of the rats and is exhausted from the chamber at the level of the rats' noses Ozone concentration in the exhausted air is monitored by the spectrophotometric method of Saltzman [Anal. Chem. *31*, 1914 (1959)] and is expressed as parts per million (ppm) as officially specified [Federal Register *36* (No. 84), 8196 (1971)]. Readings are taken at intervals of 15-20 minutes and the ozone generator adjusted, if required, to maintain the ozone concentration at the desired level ± 0 10 ppm.

Preparation of lung exudate. The intact lungs are removed from the decapitated rat, blotted, placed onto ice, and weighed A small piece (50-100 mg) of the tissue is removed for the determination of the water content by heating at 110°C to constant weight. The residual fresh lung tissue is reweighed and placed in a volume (ml) of cold Tyrode's solution equal to 2.5 times its weight (grams) and cut with scissors into about 10 pieces of roughly equal size. After the mixture has remained on ice for 3-5 minutes, the lung tissue is removed and frozen. The residual Tyrode's solution is the "lung exudate" and is used immediately or is stored at -20°C until used

Quantitation of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and thromboxane A_2(TXA_2) $PGF_2\alpha$ and thromboxane B_2 (TXB_2), the mole-for-mole inactivation product of TXA_2, in the lung exudates were quantitated with the use of radioimmunoassays by Dr H H Tai at the Texas College of Osteopathic Medicine, Denton, Texas [Anal Chem *87*, 343-349 (1978)]. In these procedures, aliquots of the lung exudates are acidified and the prostaglandins and TXB_2 are extracted into ethylacetate and then separated chromatographically using columns of silica gel $PGF_2\alpha$ and TXB_2, as well as PGE_2, are then assayed by means of competitive binding measurements using specific rabbit antibodies for each constituent. In these studies, no differences in the concentrations of PGE_2 were observed

Angiotensin converting enzyme activity. Lung tissue samples remaining from the preparation of lung exudates are frozen and thawed twice and homogenized in 0 05 M phosphate buffer, pH 7 0, 2 0 ml per gram of tissue The homogenates are strained through gauze, frozen and thawed twice, and centrifuged for two hours at 2,000 x g and 4°C. The supernatant solutions are diluted with 40 volumes of buffer [0 5 M NaCl, 0.05 M sodium phosphate (pH 8 0), 0.05 percent Brij 35] to establish optimal assay conditions. The angiotensin converting enzyme activity radioassay system obtained from Ventrex Labs, Inc (217 Read Street, Portland, ME 04103), is used to determine the enzyme activities in these diluted solutions This assay system uses [^{3}H]-hippuryl-glycyl-glycine for enzyme substrate and the enzyme activity is expressed as nanomoles of [^{3}H]-hippuric acid generated per minute per gram of lung.

Uptake of blood serotonin by lung Rats which have been fasted overnight are used. Both control and exposed animals are anesthetized with Nembutal (5 mg/100g body weight, I.P) The abdominal aorta is cannulated and the inferior vena cava is exposed A tourniquet is applied to the neck of the animal and immediately thereafter a saline solution of serotonin [0 10 ml/200 g body weight, containing 10 μg (^{14}C) serotonin (3-5 μCi/μmole)] is injected into the vena cava Blood flowing from the aortic cannula is then collected for the next minute in a heparin-containing tube. Each blood sample is hemolyzed by freezing and thawing, is diluted to 8 0 ml with distilled water and the total protein is precipitated by the addition of 2 0 ml of 40 percent (w/v) trichloroacetic acid One ml of the supernate is added to 15 ml of scintillation cocktail and the total dpm are determined with a liquid scintillation spectrometer (Beckman LS-150) A 0.1 ml aliquot of the serotonin solution used for injection is treated similarly and the percentage of the injected dose not taken up by the lung is calculated for each animal

Experimental Results

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and thromboxane A_2(TXA_2) Male rats were exposed to 1 96 mg/m^3 ozone for four hours and the lung exudates were prepared as described above The results of $PGF_2\alpha$ and TXB_2 assays are reported in Table 1

Conclusions and Recommendations

These studies have revealed three effects on the lung of acute ozone exposure Each of these effects involves a smooth muscle contracting substance(s): (a) the increased content of $PGF_2\alpha$ and TXB_2 in lung, (b) the increased activity of angiotensin converting enzyme, and (c) the decreased ability of the lung to remove serotonin from the blood The first effect would seem to be concerned only with the lung itself, whereas the other two effects would seem to be concerned with the interaction of the lungs and other systems of the organism

The effort in these studies was largely directed at identifying the effects produced by acute ozone exposure and ascertaining the minimum dose of ozone required to produce each effect These objectives were essentially achieved in the case of the increases of $PGF_2\alpha$ and TXB_2 in the lung. The minimal doses of ozone necessary to produce the two remaining observed effects remain to be determined However, because of the potential importance of these two effects, the investigations of them should be completed

Table 1. *Effect of Ozone Exposure on Lung Content of Prostaglandins and Thromboxane*

Rats	Body Weight	Lung Weight	Ozone Concentration	Lung Exudates		
				TXB_2[a]	PGE_2[a]	$PGF_{2\alpha}$[a]
	(g)	(g)	(mg/m^3)	(ng/g)	(ng/g)	(ng/g)
Control	282 ± 20 (8)[b]	0.92 ± 079 (8)	0	15.3 ± 2 11 (6)	15.4 ± 2 63 (8)	8.4 ± 1.67 (8)
Exposed[c]	249 ± 3 (20)	1 23 ± 029 (18)	1 96	25 0 ± 2 82 (8)	14 7 ± 1.06 (8)	13.0 ± 1 17 (9)
Difference[d]	NS	S		S	NS	S

[a] TXB_2 thromboxane B_2, PGE_2 prostaglandin E_2, $PGF_{2\alpha}$ prostaglandin $F_{2\alpha}$ Determined by Dr H H Tai (Texas College of Osteopathic Medicine, Denton, Texas) using radioimmunoassay

[b] Mean ± S E Number of animals is in parentheses

[c] Time four hours

[d] Significant difference, P 0 02, student's "t" test

From a consideration of the overall project, one is intrigued by the recurring question of "adaptation to ozone" or "ozone tolerance," its mechanism or mechanisms and its significance relative to health effects on the exposed animal It would appear that this basic question must be answered before a decision can be reached as to which of the acute effects of ozone are significant from the standpoint of injury Further, the mechanism of the phenomenon must be understood in order to make effects of chronic ozone exposure

Ibert C Wells is with Creighton University, Omaha, NE 68178
***Donald E. Gardner** is the EPA Project Officer (see below)*
The complete report, entitled "Ozone Exposure and Pulmonary Metabolic Effects of Mediators and Hormones," (Order No PB 81-222 408; Cost. $5 00, subject to change) will be available only from
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone 703-487-4650
The EPA Project Officer can be contacted at
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