Inhibition of Pulmonary Prostaglandin Metabolism by Exposure of Animals to Oxygen or Nitrogen Dioxide

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The effects of exposure of animals to 100% O₂ and NO₂ on the rate of prostaglandin metabolism by lung and kidney were studied *in vitro*. Exposure of guinea pigs to 100% O₂ for 48 h inhibited the metabolism of prostaglandin F_{2α} by both NAD⁺- and NADP⁺-dependent prostaglandin dehydrogenase in lung, but had no effect on the metabolism in kidney. Succinate dehydrogenase, but not glucose 6-phosphate dehydrogenase, in guinea-pig lung was inhibited by exposure to 100% O₂. Exposure to 46 p.p.m. but not 16 or 29 p.p.m. NO₂ for 6 h inhibited guinea-pig lung prostaglandin dehydrogenase *in vitro*. The inhibition of pulmonary prostaglandin dehydrogenase by exposure to 100% O₂ or to 49 p.p.m. NO₂ was dependent on the duration of exposure, but returned to control values within 7 days after cessation of the exposure. The pulmonary transport system responsible for removing circulating prostaglandins from the blood was not affected by exposure to 100% O₂ as measured by using the isolated perfused lung. Kinetic analysis of the inhibition of pulmonary prostaglandin dehydrogenase activity in guinea pig exposed to 100% O₂ showed non-competitive inhibition with respect to both prostaglandin F_{2α} and NAD⁺, which suggests destruction or inactivation of the enzyme. Pulmonary prostaglandin dehydrogenase appears to be inhibited by exposure to oxidant gases, which may lead to elevated prostaglandin concentrations in the lungs or in the systemic circulation.

It is widely accepted that the lung plays an important role in the metabolism and thus, presumably, the inactivation of circulating prostaglandins. We have recently shown that before pulmonary metabolism of prostaglandins by intracellular enzymes, the prostaglandins are removed from the circulation by a transport system (Anderson & Eling, 1976). Prostaglandins are metabolized by two enzymes in lung tissue, namely, prostaglandin dehydrogenase and prostaglandin Δ^{13} -reductase (Anggard & Samuelson, 1964; Granstrom, 1971). Presumably, one function of prostaglandin dehydrogenase is to metabolize locally generated prostaglandins, whereas a second function is to degrade circulating prostaglandins. Thus, the lung appears to play a vital role in preventing high systemic circulation of prostaglandins. In addition, pulmonary tissue synthesizes and releases prostaglandins in the circulation in response to various physiological and pathological stimuli (Piper & Vane, 1969, 1971; Fanburg, 1973).

We have previously investigated the effects of exposure to 100% O₂ and SO₂ on pulmonary biosynthesis and metabolism of prostaglandins.

Exposure to 100% O₂ and SO₂ did not alter pulmonary prostaglandin biosynthesis as measured *in vitro*. Prostaglandin dehydrogenase was not inhibited by exposure to SO₂, but was inhibited after exposure to 100% O₂. We postulated that exposure of animals to other environmental oxidant gases such as NO₂ and O₃ might inhibit pulmonary prostaglandin dehydrogenase. In the present paper we have further investigated the effect of exposure to oxidant gases (O₂ and NO₂) on prostaglandin dehydrogenase and have attempted to elucidate the mechanisms involved in the inhibition of prostaglandin dehydrogenase.

Materials and Methods

[9-³H]Prostaglandin F_{2α} (sp. radioactivity 9.2 Ci/mmol) and [5,6-³H]prostaglandin E₁ (sp. radioactivity 87 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Ma, U.S.A. Prostaglandin standards were gifts from Dr. J. E. Pike, The Upjohn Company, Kalamazoo, MI, U.S.A. NAD⁺, 2,6-dichlorophenol-indophenol, phenazine methosulphate and glucose 6-phosphate (disodium salt) were purchased from Sigma Chemical Co., Pittsburgh, PA, U.S.A. Adult male guinea pigs (Hartley, 400-500 g body wt.) and rats (CD rat-

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Sprague-Dawley descendant, 250–300g body wt.) were obtained from Charles River Breeding Laboratories, Wilmington, MA, U.S.A. The 6000g pellet and the 100000g supernatant fraction of lung and kidney were prepared by the method described by Hook *et al.* (1972).

Method of exposure

For exposure to O₂, animals were placed in air-tight polystyrene cages as described previously (Parkes & Eling, 1975). The O₂ concentration was verified by measurement of pO₂ with a blood-gas analyser.

For exposure to NO₂ animals were placed in a stainless-steel chamber of the type described by Hennes *et al.* (1968). The atmosphere of NO₂ was maintained by metering the NO₂ directly into the incoming air and was monitored by the method of Saltzman (1954). Control animals were exposed to air.

Assay of prostaglandin dehydrogenase activity

Standard assay procedure. Each incubation mixture contained 105nmol of prostaglandin F_{2x} as the tromethamine salt, 0.1 μ Ci of [³H]prostaglandin F_{2x}, 250 μ l of 8mM-NAD⁺, 100 μ l of 100000g supernatant containing 1mg of protein, 250 μ l of 0.25M-KH₂PO₄ adjusted to pH 8.0 with NaOH, and sufficient water to give a total volume of 1.0ml. Incubations were carried out for 15min at 37°C in a Dubnoff metabolic shaker. The metabolite, 15-oxoprostaglandin F_{2x} was separated and determined by the method described by Parkes & Eling (1975).

Kinetic-analysis assay procedure. The assay was optimized for enzyme and substrate concentrations and for incubation time. The prostaglandin F_{2x} (tromethamine salt), dissolved in 20 μ l of ethanol, was added to obtain final concentrations from 1 to 100 μ M. Tubes containing from 1 to 5 μ M-prostaglandin F_{2x} contained 0.25mg of protein, and those containing from 10 to 100 μ M-prostaglandin F_{2x} contained 0.50mg of protein. The NAD⁺ concentration in all tubes was 2mM, and all tubes were incubated for 10min.

The NAD⁺ concentration was varied from 2 to 1000 μ M by adding appropriate amounts of NAD⁺ dissolved in water. The prostaglandin F_{2x} concentration in all mixtures was 50 μ M, and all tubes contained 1.0mg of protein. Tubes containing less than 40 μ M-NAD⁺ were incubated for 20min, whereas those of 40 μ M-NAD⁺ or higher were incubated for 10min. The metabolite, 15-oxoprostaglandin F_{2x} was separated and determined by the method described by Parkes & Eling (1975).

Assay of glucose 6-phosphate dehydrogenase activity

The method used for analysis of glucose 6-phos-

phate dehydrogenase activity in lung tissue was the spectrophotometric procedure described by Langdon (1966).

Assay of succinate dehydrogenase

The assay of succinate dehydrogenase activity was carried out by using the method described by Singer (1974).

Isolated-perfused-lung studies

The lung-perfusion technique has been described in detail previously (Anderson & Eling, 1976). Briefly, after the animals were exposed to either 100% O₂ or air for 48h the lungs were excised, and pulmonary artery and trachea were cannulated. The lungs were perfused with Krebs-Ringer buffer containing 4.5% bovine serum albumin in a single-pass manner. After an equilibration period, the lungs were then perfused at a constant supply rate (supply rate = arterial concentration $\times$ flow rate) with media containing [³H]prostaglandin E₁. Effluent samples from the pulmonary vein were collected at various time points and analysed for total radioactivity and for metabolites. The rate of removal of prostaglandin E₁ from the circulation into the lung (transport velocity) and the rate of intracellular metabolism by prostaglandin dehydrogenase (metabolism velocity) were calculated as described by Anderson & Eling (1976). Prostaglandin E₁ was used since prostaglandin F_{2x} decreased the perfusion rates. Both prostaglandins E₁ and F_{2x} are substrates for prostaglandin dehydrogenase and the transport system responsible for removal of prostaglandins from the pulmonary vascular bed (Anderson & Eling, 1976).

Results

Exposure of animals to 100% O₂ for 48h inhibited the rate of prostaglandin metabolism by the lung 100000g supernatant fraction (Table 1). In guinea-pig lung supernatant both NAD⁺- and NADP⁺-dependent prostaglandin F_{2x} metabolism was inhibited by approx. 70% in animals exposed to 100% O₂. The activity of pulmonary NADP⁺-dependent prostaglandin dehydrogenase is only 5% of the NAD⁺-dependent prostaglandin dehydrogenase activity in guinea-pig lung supernatant. In rat lung supernatant the activity of the pulmonary NAD⁺-dependent prostaglandin dehydrogenase was approx. 10% of the activity observed in guinea-pig lung and was inhibited by approx. 60% on exposure to 100% O₂ for 48h. In contrast with lung tissue, the rate of prostaglandin metabolism by the guinea-pig kidney prostaglandin dehydrogenase was not significantly altered after exposure to 100% O₂ for 48h (Table 1).

The amount of 100000g supernatant protein from

Table 1. *Effect of exposure of animals to O₂ on various pulmonary and enzyme activities*

Animals were exposed to either air (control) or 100% O₂ for 48 h and killed at the end of exposure. Tissue was removed quickly and immediately put in chilled 0.25M-sucrose. The tissue was homogenized in the sucrose (1:4, w/v) and the 6000g and the 100000g supernatant fractions were obtained for enzyme analysis. For determining the prostaglandin dehydrogenase activity, prostaglandin F_{2a} was used as the substrate and either NAD⁺ or NADP⁺ as cofactor. The assay procedures were as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for duplicates determined from four separate animals for each group. 2,6-Dichlorophenol-indophenol was used as an electron acceptor for the assay of succinate dehydrogenase activity. The 100000g supernatant was used for all enzyme assays except succinate dehydrogenase, which was measured in a mitochondrial preparation (6000g). Values marked with an asterisk were significantly different from control values at $P < 0.01$.

Enzyme	Source	Enzyme activity	
		Control	Exposed
NAD ⁺ -dependent prostaglandin dehydrogenase (nmol of prostaglandin F _{2a} metabolized/min per mg of protein)	Guinea-pig lung	1.79 $\pm$ 0.09	0.59 $\pm$ 0.17*
NADP ⁺ -dependent prostaglandin dehydrogenase (nmol of prostaglandin F _{2a} metabolized/min per mg of protein)	Guinea-pig lung	0.085 $\pm$ 0.009	0.022 $\pm$ 0.004*
NAD ⁺ -dependent prostaglandin dehydrogenase (nmol of prostaglandin F _{2a} metabolized/min per mg of protein)	Rat lung	0.126 $\pm$ 0.014	0.046 $\pm$ 0.004*
NAD ⁺ -dependent prostaglandin dehydrogenase (nmol of prostaglandin F _{2a} metabolized/min per mg of protein)	Guinea-pig kidney	2.59 $\pm$ 0.05	2.40 $\pm$ 0.04
Glucose 6-phosphate dehydrogenase (nmol of NADP ⁺ reduced/min per mg of protein)	Guinea-pig lung	34.0 $\pm$ 0.9	34.0 $\pm$ 0.9
Succinate dehydrogenase (nmol of 2,6-dichlorophenol-indophenol reduced/min per mg of protein)	Guinea-pig lung	141.0 $\pm$ 3.0	99.0 $\pm$ 5.0

Fig. 1. *Prostaglandin dehydrogenase activity in lungs of O₂- and air-exposed guinea pigs*

The control group of animals was exposed to air at atmospheric pressure throughout the period of experiment. Test animals were exposed to either 100% O₂ at atmospheric pressure for various lengths of time with a maximum exposure of 48 h; or were exposed to 100% O₂ for 48 h, and thereafter to air for various lengths of time. Control and exposed animals were killed at the various times and NAD⁺-dependent prostaglandin dehydrogenase activity was measured with prostaglandin F_{2a} as substrate and NAD⁺ as cofactor. Each point represents the mean $\pm$ S.E.M. for duplicate determinations from three separate animals. The enzyme activity from control guinea pigs varied from 1.7 to 1.9 nmol of metabolite formed/min per mg of protein.

prostaglandin metabolic activity in lungs of exposed animals could be due to dilution by oedema-fluid protein. After accounting for the dilution of lung supernatant protein by oedema-fluid protein when calculating the specific activity of prostaglandin dehydrogenase in exposed animals, the prostaglandin metabolism was still decreased by 50% in exposed animals. Thus only a small fraction of the loss of prostaglandin metabolic activity is due to oedema-fluid protein.

In addition to prostaglandin dehydrogenase activity, two other pulmonary dehydrogenase activities of guinea-pig lung tissue were studied *in vitro* after the animals were exposed to 100% O₂ for 48 h (Table 1). The activity of succinate dehydrogenase, a mitochondrial enzyme, was decreased by 30%. However, in contrast with prostaglandin dehydrogenase activity, glucose 6-phosphate dehydrogenase, another soluble-fraction enzyme, was not affected by the exposure.

The effect that the time of exposure to 100% O₂ had on prostaglandin dehydrogenase activity and the ability of lung tissue to recover prostaglandin dehydrogenase activity after the exposure was stopped is shown in Fig. 1. Prostaglandin dehydrogenase activity, expressed as a percentage of the control value, decreased linearly with time of exposure to 100% O₂, which is in agreement with our previous reported data (Parkes & Eling, 1975). After the exposure to 100% O₂ was stopped, prostaglandin dehydrogenase activity in lung increased with time until control values were reached in 7 days (Fig. 1).

To determine if oxidant gases other than 100% O₂

homogenates of lungs from O₂-exposed animals was in some cases slightly higher than that found in control animals and thus part of the decrease in

Table 2. *Effect of exposure to NO₂ on pulmonary prostaglandin dehydrogenase activity*

For determining prostaglandin dehydrogenase activity prostaglandin F_{2α} was the substrate and NAD⁺ was the cofactor. The assay procedure was as described in the text. Values are mean ± s.e.m. for duplicate determinations from four separate animals for each group. Control animals were exposed to air. The values for NO₂ are average concentrations as measured during the course of the exposure. The asterisk indicates that the value is significantly different from the control value at $P < 0.01$.

Species	Exposure conditions	Enzyme activity of (nmol of prostaglandin F _{2α} metabolized/min per mg of protein)		Inhibition (%)
		Control	Exposed	
Guinea pig	16 p.p.m. NO ₂ for 6h	1.26 ± 0.10	1.39 ± 0.10	0
Guinea pig	29 p.p.m. NO ₂ for 6h	1.51 ± 0.16	1.60 ± 0.10	0
Guinea pig	46 p.p.m. NO ₂ for 6h	1.26 ± 0.1	0.74 ± 0.63*	41

Fig. 2. *Effect of time of exposure of guinea pigs to NO₂ on pulmonary prostaglandin dehydrogenase activity*

Animals were exposed to either 49 p.p.m. NO₂ or air at atmospheric pressure and killed at various times. Prostaglandin dehydrogenase activity was determined in the lung by using prostaglandin F_{2α} as substrate and NAD⁺ as cofactor, as described in the text. Each point represents the mean ± s.e.m. for duplicate determinations from three separate animals. Prostaglandin dehydrogenase activity of supernatant fraction from guinea pigs varied from 1.47 to 1.73 nmol of prostaglandin F_{2α} metabolized/min per mg of protein.

will inhibit prostaglandin dehydrogenase, we exposed guinea pigs to NO₂. NO₂ was chosen since it is a known environmental pollutant, and since exposure facilities for NO₂ were available to us. We needed to examine prostaglandin dehydrogenase activity in the absence of severe tissue damage, so concentrations of 15, 30 and 50 p.p.m. were chosen on the basis of reported effects on pulmonary tissue of exposure to NO₂ for short periods of time (Hine *et al.*, 1970). With these NO₂ concentrations for 6 h exposures, no differences were observed in lung net weight or in protein content of the 100000g supernatant fraction (Table 2). Exposure to 15 and

30 p.p.m. NO₂ for 6 h failed to inhibit pulmonary prostaglandin dehydrogenase. Exposure to 50 p.p.m. NO₂ significantly decreased the prostaglandin dehydrogenase activity.

The extent of inhibition of prostaglandin dehydrogenase activity was dependent on the duration of exposure to NO₂. As seen in Fig. 2, a biphasic response was observed: an increase in activity was observed after exposure for 1 h, followed by a time-dependent inhibition. This biphasic response was not observed with O₂ exposure. At 50 p.p.m., NO₂ appeared to be significantly more potent than 100% O₂ in producing inhibition of prostaglandin dehydrogenase.

To examine the kinetics of inhibition of prostaglandin dehydrogenase by O₂ exposure in guinea-pig lung, we determined the enzymic rates *in vitro* at various substrate and cofactor concentrations by using an optimized assay system. As seen in Fig. 3(a), the metabolic activity obtained by using enzyme fractions from exposed and control animals at a fixed NAD⁺ concentration indicates non-competitive inhibition with respect to prostaglandin F_{2α}. Double-reciprocal plots also indicate non-competitive inhibition with respect to NAD⁺ when the prostaglandin F_{2α} concentration is fixed (Fig. 3b). These results are consistent with the destruction of prostaglandin dehydrogenase by exposure to 100% O₂.

The pulmonary system that inactivates prostaglandins consists of a transport mechanism that removes the prostaglandins from the circulation into the lung cells and intracellular enzymes, i.e. prostaglandin dehydrogenase and others, which metabolize the prostaglandins (Anderson & Eling, 1976). We investigated the effect that 100% O₂ exposure had on the ability of the lung to inactivate circulating prostaglandins by using the isolated perfused lung system as a model (Table 3). There was little or no effect on the rate of removal of prostaglandin from the circulation by either the rat or guinea-pig lung for the range of supply rates examined. With the isolated perfused rat lung, inhibition of prostaglandin

dehydrogenase activity of the intact organ was observed regardless of the supply rate, but the inhibition increased with increasing concentration. This is consistent with the uncompetitive inhibition observed *in vitro*. The degree of inhibition observed in guinea-pig perfused lung was also dependent on the amount of prostaglandin supplied and only at supply rates exceeding 2.0nmol/s was significant inhibition of prostaglandin E₁ metabolism observed.

Discussion

We have shown that prostaglandin dehydrogenase activity of rat and guinea-pig lung was inhibited after animals were exposed to 100% O₂ or to NO₂. In addition to the inhibition of prostaglandin dehydrogenase activity as measured under assay conditions *in vitro*, inhibition was also observed by using an isolated perfused system as an estimate of prostaglandin dehydrogenase activity *in vivo*.

Fig. 3. Effect of 100% O₂ exposure on prostaglandin dehydrogenase activity (a) with respect to prostaglandin F_{2α} at a fixed NAD⁺ concentration of 2mM and (b) with respect to NAD⁺ at a fixed prostaglandin F_{2α} concentration of 50μM

Prostaglandin F_{2α} was used as substrate and NAD⁺ as cofactor for the determination of pulmonary prostaglandin dehydrogenase activity in guinea pigs as described in the text. The lines were obtained by linear regression analysis. *v* represents nmol of prostaglandin F_{2α} metabolized/min per mg of protein.

Table 3. Effect of exposure of guinea pig and rat to 100% O₂ on transport and metabolism of prostaglandin measured in isolated perfused lung

The percentage inhibition of transport velocity and metabolism velocity in isolated perfused lung obtained from O₂-exposed animals was calculated by comparison with the control by using the same supply rate. Each value represents the percentage inhibition calculated from one control and one exposed animal. [³H]Prostaglandin E₁ was used as substrate for the transport and enzyme system.

		Inhibition (%)	
		Transport velocity	Metabolism velocity
Guinea pig	Supply rate (nmol/s)		
	0.0012	4	4
	0.1129	0	0
	0.8560	4	10
	2.0770	1	30
	9.6464	2	41
Rat	Supply rate (nmol/s)		
	0.0543	0	57
	0.0594	0	60
	0.9681	7	80
	1.0022	10	84

This inhibition of the inactivation of circulating prostaglandin by 100% O₂ exposure was due to inhibition of prostaglandin dehydrogenase rather than inhibition of the carrier system, which is the initial step in the pulmonary inactivation of prostaglandins (Anderson & Eling, 1976).

In the intact-organ system, the rate-limiting step for the metabolism can either be the transport of the prostaglandin into the lung cells or the intracellular metabolism by prostaglandin dehydrogenase. At the lower supply rates, the rate-limiting step is the transport, whereas at higher supply rates it is the intracellular metabolism (Anderson & Eling, 1976). Since the specific activity of prostaglandin dehydrogenase is higher in the guinea pig than in the rat, the intracellular metabolism will become the rate-limiting step at a higher supply rate in the guinea pig than in the rat. This may explain why inhibition of metabolism of circulating prostaglandin was not observed in the guinea-pig isolated perfused lung until higher supply rates were used.

The activity of guinea-pig pulmonary NAD⁺-dependent prostaglandin dehydrogenase returned to control values 7 days after cessation of the O₂ exposure. One obvious explanation for the recovery of enzymic activity is synthesis of new enzyme. Rapid synthesis *de novo* of prostaglandin dehydrogenase would be predicted from the reported turnover rate of prostaglandin dehydrogenase (Blackwell *et al.*, 1975). The lack of rapid recovery of prostaglandin dehydrogenase activity after O₂ exposure suggests that animals or human subjects on 100% O₂ may have high prostaglandin concentrations in the blood and tissue for a considerable time after the cessation of O₂ exposure.

Kinetic studies showed that inhibition of prostaglandin dehydrogenase after exposure to 100% O₂ was non-competitive with respect to either prostaglandin F_{2x} or NAD⁺. One possible explanation for the result could be the destruction of prostaglandin dehydrogenase by the oxidant gas. This hypothesis is consistent with the observed susceptibility to inactivation of prostaglandin dehydrogenase during purification (Marrazzi & Anderson, 1974).

Our results show that exposure to O₂ inhibits pulmonary prostaglandin dehydrogenase, but has no effect on renal prostaglandin dehydrogenase activity. These results suggest that there is a tissue specificity with respect to inhibition of this enzyme activity. No major differences have been reported for pulmonary and renal prostaglandin dehydrogenase (Marrazzi & Anderson, 1974). Thus the tissue selectivity of inhibition of prostaglandin dehydrogenase activity after O₂ exposure is probably due to the high concentration of O₂ in lung tissue during O₂ exposure. Since the lung is a major site for the inactivation of circulating prostaglandins, inhi-

bition of pulmonary prostaglandin dehydrogenase by exposure to oxidant gases may increase circulating prostaglandins. However, since renal prostaglandin dehydrogenase is not affected by O₂ exposure, one cannot rule out significant inactivation of circulating prostaglandins by the kidney. The effect of inhibition of pulmonary prostaglandin dehydrogenase on circulating prostaglandin concentrations could therefore only be determined by monitoring these concentrations *in vivo*.

We examined the effect of animal exposure to O₂ on the activities of other pulmonary enzyme systems. The enzymic activities of catechol *O*-methyltransferase, glucose 6-phosphate dehydrogenase and glutathione *S*-aryltransferase (Parkes & Eling, 1975), enzymes found in the 100000g supernatant fraction and microsomal prostaglandin synthetase were not inhibited by O₂ exposure. Thus O₂ exposure does not produce a general inhibition of soluble enzymes. In contrast with glucose 6-phosphate dehydrogenase, a mitochondrial dehydrogenase, succinate dehydrogenase, was inhibited by 100% O₂ exposure. Thus O₂ exposure may affect some of the lung's dehydrogenases in a similar manner, but general inhibition of dehydrogenases does not occur as a result of exposure to O₂.

The lung has been shown to release prostaglandins, thromboxanes, prostacyclin and metabolites into the circulation. During anaphylaxis, significantly large quantities of these vasoactive substances are released (Piper & Vane, 1971; Dawson *et al.*, 1976). It has been shown that exposure of guinea pigs to NO₂ and O₃, but not to SO₂, increased the symptoms of anaphylaxis (Matsumura, 1970; Matsumura *et al.*, 1972). Since NO₂ and O₂ inhibit pulmonary prostaglandin dehydrogenase, the increase in anaphylaxis may be in part the result of increased prostaglandin concentrations resulting from the inhibition of their metabolism. This would suggest a potentially important role of prostaglandin dehydrogenase in deactivating prostaglandins formed within the lung.

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