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Effect of an Acute Exposure of Guinea Pigs to NO_2 on Pulmonary Prostaglandin Dehydrogenase and Angiotensin Converting Enzyme

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Nitrogen dioxide is a ubiquitous pollutant most commonly found in industrial and automobile exhausts and tobacco smoke. This gas has been suggested by many investigators to be a pathogenic agent in that it causes pulmonary and cardiovascular damage by its oxidative action (FREEMAN & HAYDON 1964, FREEMAN et al. 1968, LUCIAK 1972, SPEIZER et al. 1980).

The biochemical mechanisms underlying NO_2-induced pathological changes in the lung have been widely investigated during the last several years and resulted into plethora of information (WITSCHI 1975, KLEINERMAN 1977, MUSTAFA & TIERNEY 1978). Thus, it has been shown that chronic exposure of guinea pigs to 2 mg/m^3 (approx. 1 ppm) of NO_2 caused a decrease of total collagen content in lung tissue and an increase of hydroxyproline in blood serum and urine (DRÓŻDŻ et al. 1977). The basic conclusion of this study is that the primary mechanism of NO_2-induced connective tissue damage is probably due to the inhibition of collagen maturation and/or activation of its catabolism. On the other hand CRESIA and coworkers (1977) reported a stimulation of DNA synthesis in the lungs of hamsters exposed intermittently to 10 ppm NO_2. Recently, CHAUDHARI et al. (1979) have shown that pulmonary prostaglandin metabolism can be altered by an acute exposure of animals to NO_2. The significance of this observed inhibition of the PGDH activity in relation to lung injury or other biochemical changes caused by NO_2 as reported by CRESIA et al. (1977) or DRÓŻDŻ et al. (1977) is not clear. It is possible that a highly reactive agent such as NO_2

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may induce non-specific injury to many different cells distributed throughout the lung and because of this non-specificity of NO_2 effect one notes so many different biochemical changes following exposure to this oxidant gas.

In an attempt to develop further insight into this matter, we have undertaken this study to determine whether NO_2, while affecting pulmonary PGDH may cause parallel effect on other vital pulmonary enzymes. With this objective in mind, we have chosen to study angiotensin converting enzyme (ACE) since this enzyme has been shown to be localized particularly in the pulmonary endothelial cell surface (SMITH & RYAN 1973) and since this cellular site, unlike that of the broncho-alveolar epithelial cell surface, is not easily exposed to the inhaled NO_2. Furthermore, in this study we have also determined the blood levels of nitrite which is formed by the inhaled NO_2 so that we may correlate the effects of NO_2 on various enzymes with its blood level. Since there is only indirect evidence of formation of nitrous and nitric acids as a result of reaction of NO_2 with water in the nasopharynx and lungs (GOLDSTEIN et al. 1977) or nitrite formation in experiments using simulated pulmonary surface (ICHIOKA 1972), in the present study the blood nitrite has been measured directly in an effort to determine the relationship between the concentrations of NO_2 in the inhaled air and the formation of nitrite in blood in relation to its effect on two pulmonary enzyme activities.

MATERIALS AND METHODS

[9-^{3}H] Prostaglandin $F_{2\alpha}$ (Specific activity, 9.2 Ci/mmol) was purchased from New England Nuclear Corp., Boston, MA. Prostaglandin $F_{2\alpha}$ THAM was a generous gift from Dr. J.E. Pike of the Upjohn Company, Kalamazoo, MI. Hippuryl-L-histidyl-L-leucine, nicotinamide adenine dinucleotide and N-1-naphthylethylenediamine dihydrochloride were obtained from Sigma Chemical Co., St. Louis, MO. Thin layer chromatography plates (plastic) coated with silica gel and liquid scintillation cocktail, Redi-Solv EP were supplied by Eastman Kodak Co., Rochester, NY and Beckman Instrument Inc., Fullerton, CA, respectively. Male Hartley guinea pigs weighing 300-400 grams were purchased from Camm Research Animals Co., New Jersey. They were maintained in a light and termperature controlled facility until used in these experiments. Food and water were available ad libitum. The NO_2 was supplied in stainless steel cylinder by Air Products and Chemicals, Inc., Tamaqua, PA.

Ventilation of Guinea Pigs Using a Rodent Respirator

Guinea pigs were anesthetized with nembutal (35 mg/kg, I.P.). The trachea was cannulated using polyethylene cannula. A pressure transducer for recording tracheal resistance, air pressure and respiration rate was connected to a side arm of a 3-way adaptor attached to the cannula. The other end was connected to a rodent respirator (Harvard Model 680). The respirator was set to a respiration rate of 50 strokes/min and a stroke volume of 5 ml.

The animal was allowed to breath room air during first 10-15 min through the respiratory pump. During this time the chest was opened carefully by cutting through the sternum just above the heart. A stainless steel cannula prepared from a hypodermic syringe of 18 gauge was inserted in the left ventricle of the heart. This cannula was connected to a pressure transducer through a polyethylene tube to record the ventricular pressure and to collect arterial blood. The animal was ventilated for an additional 10 min by connecting the pump to the compressed air from cylinder. At the end of 10 min of equilibration period, the control animals were allowed to breath only air while the experimental animals were exposed to air containing 96 ppm NO_2 for an additional period of 60 min. The ventricular pressure and dp/dt were monitored in both groups during the entire period of 70 min. A blood sample of about 0.8 ml was collected just before switching to NO_2 and at the end of 1 hour about 4-5 ml of blood was collected.

Processing of the Samples

In each paired experiment one animal was ventilated with air only and the other one with air containing 96 ppm NO_2. The blood samples were analyzed for nitrite level as described later. An aliquot of blood was allowed to coagulate overnight at 2-4° C and the following day serum was separated for the determination of ACE activity. At the termination of each experiment lung was quickly chilled, weighed and stored at -70° C for the determination of PGDH and ACE activities.

After overnight storage, a 10% homogenate of lungs was prepared in 0.25 M sucrose under ice-cold conditions. A suitable aliquot of homogenate was centrifuged at 5000 x g for 10 min and the resulting supernate was used for the determination of ACE activity. The remaining homogenate was centrifuged at 100,000 x g for 1 hour and the supernate thus obtained was used for the determination of PGDH activity.

Determination of Nitrite

The determination of nitrite in blood was carried out using the mehtod of SCHECHTER et al. (1972) which makes use of the SALTZMAN reaction (1954). Sodium nitrite was used as a standard and the results were calculated as µg of nitrite/ml of blood. The lower limit of the sensitivity of this method is 0.01 µg/0.1 ml blood.

Determination of Prostaglandin Dehydrogenase Activity

The method used for the assay was that of CHAUDHARI et al. (1979). $[^3H]PGF_{2\alpha}$ and 100,000 x g supernate of lung homogenate were used as a substrate and source of enzyme respectively. For the analysis of 15 keto-$[^3H]PGF_{2\alpha}$ (the metabolite) we have used silica gel coated plastic plates instead of glass plates as used in the earlier studies (CHAUDHARI et al. 1979). The chromatogram were cut into 0.5 cm pieces and counted in a Beckman Liquid

Scintillation counter using Redi-Solv EP cocktail.

Determination of Angiotensin Converting Enzyme Activity

The method employed was essentially that of LIEBERMAN (1975). This method depends upon the ability of the ACE to liberate hippuric acid from Hippuryl-L-histidyl-L-leucine. One unit of ACE activity is defined as the nanomoles hippuric acid formed per min per mg protein at 37°C under the assay conditions. The protein estimation was done by the method of SEDMAK & GROSSBERG (1977) using bovine serum albumin as a standard.

RESULTS

Table 1 summarizes the results of pulmonary PGDH activity and blood level of nitrite in five pairs of air-exposed and NO_2-exposed animals. Each pair was assessed for the NO_2-induced

Table 1

Effect of exposure of guinea pigs to 96 ppm NO_2 for 1 hour on pulmonary PGDH activity and nitrite level in blood

Paired Experiment	PGDH Activity[a] Air-exposed	PGDH Activity[a] NO_2-exposed	Percent Inhibition of PGDH Activity	Nitrite µg/ml blood Air exposed	Nitrite µg/ml blood Air exposed
1	0.606	0.333	45	ND[b]	1.82
2	0.637	0.485	24	ND	0.60
3	0.644	0.312	51	ND	1.32
4	0.364	0.204	44	ND	1.85
5	0.544	0.380	30	ND	2.10
Mean ± S.E.	0.559 ± 0.052	0.343 ± 0.046[c]	39 ± 5	ND	1.54 ± 0.27

a - Prostaglandin dehydrogenase activity is expressed as nmoles of 15-keto-$PGF_{2\alpha}$ formed/min/mg protein at 37°C.

b - Not detectable

c - Highly significant difference from control as determined by Student's 't' test, $p > 0.01$

inhibition of enzyme activity and attempts were made to correlate it with the blood level of nitrite. The PGDH activity in the NO_2-exposed animals was found to be inhibited significantly compared to the air-exposed animals. The degree of enzyme inhibition varied from 24 to 51% giving an average of 39 ± 5%. The nitrite level in the air-exposed animals was not detectable in the blood,

while there was a significant amount of nitrite in the NO_2-exposed animals ranging from 0.6 - 2.1 μg/ml of blood (mean ± S.E. = 1.54 ± 0.27). Although the least inhibition of the PGDH activity was observed at the lowest nitrite level, higher degree of the enzyme inhibition has not revealed a clear trend with respect to the blood level of nitrite.

Figure 1 shows the effect of 1 hour exposure to 96 ppm NO_2 on serum and lung ACE activities in guinea pigs. The results indicate that neither lung nor serum ACE activity is affected significantly by the exposure to NO_2 compared to their respective control values. Although it appears that the serum/lung ratio of ACE activity in the NO_2-exposed animals is lower than the controls (2.36 ± 0.39 versus 1.44 ± 0.31), the difference between these values has not been found to be statistically significant.

We have also measured left ventricular pressure and dp/dt in these experiments. It was noted that, in the air-exposed animals there was no change in these parameters after one hour of experiments compared to 0 hour, while in 2 out of 5 NO_2-exposed animals there was about 30-40 lowering of both maximum left ventricular pressure and maximum dp/dt.

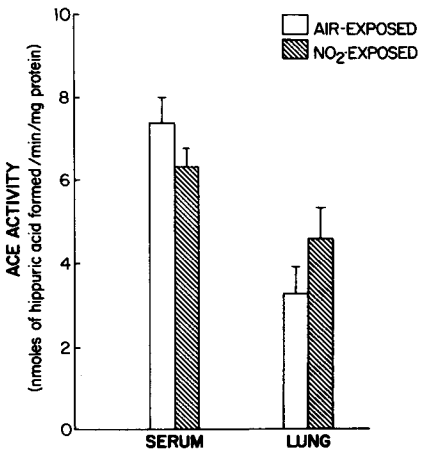

Figure 1. Effect of Exposure of Guinea Pigs to 96 ppm NO_2 for 1 hour on Serum and Lung Angiotensin Converting Enzyme Activity.

DISCUSSION

In the present study, pulmonary PGDH, an enzyme which converts biologically active prostaglandins to less active 15-keto forms, has been shown to be inhibited by an acute exposure of guinea pigs to NO_2. This enzyme inhibitory effect of NO_2 is consistent with the previous studies (CHAUDHARI et al. 1979). Furthermore, in the earlier studies a marked inhibition of PGDH has also been observed following exposure to 100% oxygen. The mechanism of

this inhibition has been suggested to be due to the destruction of the enzyme supposedly caused by free radicals produced by 100% oxygen. However, since several other pulmonary enzymes, such as prostaglandin synthetase, catechol-O-methyltransferase, glutathione S-aryltransferase (PARKES & ELING 1975) and glucose 6-phosphate dehydrogenase (CHAUDHARI et al. 1979) have not been affected by exposing the animals to pure oxygen; it appears that the oxygen exposure does not produce a generalized effect on various pulmonary enzymes. This aspect of specificity has been further investigated in the present study with respect to NO_2 in relation to an important pulmonary enzyme.

Before we consider the effect of NO_2 it is essential to realize that since NO_2 is relatively less water soluble gas, it does not diffuse through the conducting airways. Instead, NO_2 distributes over the distal lung epitheliuem and gets entry into the blood stream via interstitium and endothelial cell-layers. Indeed, the study of GOLDSTEIN et al. (1977) has shown that the dissemination of NO_2 is mediated by blood system. Thus, it would appear that NO_2 because of its oxidative interaction with cell types of the distal lung can affect indiscriminately various macromolecules and cause generalized lung injury. In a way the inhibitory effect of NO_2 on the PGDH activity as observed in this study as well as in the previous study (CHAUDHARI et al. 1979) appears to indicate such a possibility since PGDH is supposedly present in various types of lung cells. However, our finding of no effect on the ACE activity while there was marked inhibition of the PGDH activity following exposure to 96 ppm NO_2 argues against this possibility. The present study clearly indicates that the inhibition of PGDH seems to show a specific effect of NO_2 on this pulmonary enzyme, by the concentration of NO_2 used in this study.

This study also documents for the first time, the amount of nitrite found in the blood as determined by the use of the Saltzman reaction (SALTZMAN 1954) at the time when PGDH activity is being affected due to NO_2 exposure. The presence of a significant blood level of nitrite as observed under in vivo conditions of our experiments is a confirmation of the previous study (ICHIOKA 1972) where wetted wall tubes were used to simulate pulmonary surfaces and shown to produce nitrite following absorption of NO_2.

Since nitrite has been shown to react with hemoglobin of the blood to form methemoglobin, in various studies attempts have been made to correlate blood methemoglobin levels with NO_2 exposure in laboratory animals (MACQUIDDY et al. 1941; HENSCHLER et al. 1964). During an investigation of human subjects, HENSCHLER & LÜDTKE (1963) observed an increase in methemoglobin formation in the blood by 1% of the total hemoglobin following an exposure to 20 ppm NO_2 for 2 hours. In these types of studies, however, one does not get a true reflection of nitrite formed in the blood since methemoglobin is constantly being converted to hemoglobin by the methemoglobin reductase present in the blood. For this reason the measurement of nitrite in the blood may be a better and direct indicator than the methemoglobin level for the assessment of

systemic abosrption of NO_2 from the inhaled air. The direct measurement of blood nitrite is also quite sensitive in that one can measure an amount as low as 0.1 µg/ml. In terms of NO_2 exposure this would mean that one should be able to detect the blood nitrite formation due to 10 ppm (or even lower) of NO_2 exposure since in all probability there exists a linear relationship between the inhaled NO_2 concentration and nitrite formation.

Since pulmonary PGDH was selectively inhibited by NO_2 inhalation and produced a significant level of nitrite in the blood, it is our contention that inhibition of PGDH may be related to the level of nitrite in pulmonary tissue cytosol. Therefore, we suggest that this type of correlation needs to be established by using a larger number of animals in a study of concentration-response relationship.

Although in our experiments we did not observe any changes in serum and lung ACE in the NO_2-exposed animals, it has been shown by MOLTENI et al. (1974) that serum and lung ACE activities rise in response to chronic alveolar hypoxia in the experimental animals. Recently, it was reported that the human alveolar macrophages of cigarette smokers showed a higher level of ACE activity compared to non-smokers (HINMAN et al. 1979). Our results suggest that this enhancement in the ACE activity may not be related to NO_2 found in the cigarette smoke. Using thiourea as the pulmonary edemagenic agent HOLLINGER et al. (1980) have demonstrated that the acute lung injury is associated with an increase in ACE activity of serum, and a decrease in lung ACE activity. It has been suggested by these workers that the observed effects are related to the damage of vascular endothelial cells. Since in the present study 96 ppm NO_2 has not affected ACE, we may speculate that the short exposures to tolerated levels of NO_2 may not affect the integrity of endothelial cells in a manner similar to that of thiourea. However, at the concentration and conditions under which the NO_2 exposure shows pulmonary edema (HINE et al. 1970), the ACE activity is expected to be affected due to possible loss of integrity of the vascular endothelial structure.

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