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ENZYME INDUCTION BY AIR POLLUTANTS ON RESPIRATORY TISSUES OF *FUNAMBULUS PENNANTI* (WRONGH.)

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Effect of three principal air pollutants (CO, SO_2 and NO_2) on the activity of few enzymes (alkaline and acid phosphatases, 5-nucleotidase, lipase, phosphamidase and peroxidase) have been analysed histochemically in the lungs of *F. pennanti* Inhibited alkaline phosphatase reaction suggests damage to plasma membrane. Lysosomal damage is evident from depleted acid phosphatase reaction. Loss of 5-nucleotidase shows proliferation of cells, injury to endoplasmic reticulum and the plasma membrane. Inhibited lipase reaction thus observed, to reflect decreased phospholipid synthesis, essential to maintain proper surface tension. Probably these air pollutants also change the half life of enzymes.

INTRODUCTION

Air polluting gases like other toxic agents may induce or inhibit enzyme activities that further effect metabolic reactions such as oxidation and hydroxylation. Continuous and long exposure to such air pollutants namely CO, SO_2 and NO_2 in low concentrations have resulted into pathological lesions in the lungs (Freeman *et. al.*, 1964; Wolf *et al.*, 1975; Agarwal *et al.*, 1977). It, therefore, assumed that enzyme histochemical data may provide an additional information since enzyme activities are treated as markers of subcellular components and as the parameters of metabolic pathways and processes. The present investigation incorporates to analyse alkaline and acid phosphatases, 5-nucleotidase, lipase, phosphamidase and peroxidase histochemically in the lungs of *Funambulus pennanti*. Causes and significance of altered enzyme levels in exposed subjects to these pollutants have also been discussed.

MATERIAL AND METHODS

Forty common ground squirrels (*F. pennanti*) with an average starting weight of 90 g were selected from the laboratory stock. They were housed in four groups separately, and fed on laboratory chow and *ad Lib*. Each squirrel (one at a time)

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of the first group was exposed to carbon monoxide (CO) for four minutes at 500 ppm (0 05%) in an environment chamber. Squirrels of the second inhaled sulphur dioxide (SO_2) in the same way and at the same concentration and duration. Members of the third group were treated with nitrogen dioxide (NO_4) alike. Each squirrel of the fourth inspired filtered air, and served as control. Such treatment to each squirrel every alternate day continued for a fortnight. After a day of rest, the lungs were carefully removed after vivisecting the treated individuals. The chilled absolute acetone was injected then through a bronchiole, excised and immersed as such in the fixative at 4°C for 24 hrs. The frozen and paraffin sections when applicable (8—10 μ thick) from control and experimental animals were processed for alkaline and acid phosphatase, peroxidase (Gomori, 1952; Burstone, 1952), and 5-nucleotidase (Holter & Li, 1952). Suitable controls to check the validity of reactions were also run simultaneously.

RESULTS

Alkaline phosphatase

Lungs from control animals exhibited strong positive reaction for alkaline phosphatase in the walls of alveolii. Bronchiolar cells also showed a positive reaction. CO exerted adverse effects on the distribution of this enzyme. Comparatively dull reaction was observed in bronchioles and nearby alveolar cells. Alveolii exhibited a negative reaction. SO_2 also had remakable effects on this enzyme restricting its presence in the central alveolar cells. Alveolii showed no reaction. However, arterioles exhibited a positive reaction. Contrary to the above observations, NO_2 stimulated its activity. Strong positive reaction was observed in the alveolii, bronchioles and arterioles (Table I).

Acid-phosphatase

Lungs in normal squirrels showed acid phosphatase activity in the alveolar walls and in the cells around arterioles. Bronchoii exhibited a poor reaction. After carbon monoxide treatment, a diffused activity was observed in the alveolii and also in the cells around the arterioles. Dull and diffused activity was observed in alveolii, alveolar ducts, arterioles and bronchii after SO_2 and NO_2 treatments. (Table I).

5-Nucleotidase

Sections of the normal lungs from control squirrels showed strong positive reaction for 5 nucleotidase in the cells of the alveolii, arterioles and bronchii. CO treatment had no appreciable effect on the topography of this enzyme;

however, a diffused reaction could be observed. In the same region, a dull reaction was observed after SO_2 treatment also. After NO_2 exposure too, no effects on the distribution of this enzyme could be observed (Table I).

Lipase

In the lungs of normal squirrels, strong positive reaction for lipase existed in the alveolii and arterioles. Bronchii also could show a moderate reaction. After CO poisoning, a dull activity was noticed in the alveolar walls. Arterioles and bronchioles also showed a dull activity. Few cells of the alveolii exhibited a positive reaction after SO_2 treatment. After NO_2 treatment, very dull activity for lipase was localized in the alveolii and arterioles. Bronchii had no enzyme at all (Table I).

Phosphamidase

In the lung of normal squirrels, bronchioles and arterioles showed a strong positive reaction for phosphamidase. Alveolii, however, exhibited a moderate reaction. Treatment with carbon monoxide, inhibited the enzymatic activity in arterioles and the bronchii. Alveolar duct cells remained uneffected, sulphur dioxide had comparatively, negligible effects on the distribution of enzyme in arterioles. However, it was wanting from the alveolii. NO_2 had much milder effects on the lung of squirrels. Cells of the bronchii as well as alveolii exhibited a moderate but diffused reaction (Table I).

Peroxidase

Although peroxidases are widely distributed in plants, their activity is limited to relatively few sites in mammalian tissues.

In control squirrels, positive reaction was observed. No alteration in the topography of this enzyme was caused by these gases, however, a dull reaction was visualized (Table I).

Table I. Distribution of enzymes in lungs of *F. pennanti* after exposure to three air polluting gases.

Object	Treatment	Alveolar walls	Alveolar duct	Bronchiole	Arteriole
Alkaline phosphatase	Control	++	+	++	+
	CO	±	±	±	—
	SO_2	+	+	+	+
	NO_2	+++	+	++	++
Acid phosphatase	Control	++	+	±	++
	CO	+	±	±	+
	SO_2	±	±	±	
	NO_2	+	±	±	±
5-nucleotidase	Control	++	+	+	++
	CO	+	±	±	+
	SO_2	±		±	±
	NO_2	±	±	+	±
Lipase	Control	+	+	+	+
	CO	±	+	±	±
	SO_2	±	±	—	—
Phospha-midase	Control	+	+	++	++
	CO	±	±	—	—
	SO_2	—	—	—	+
	NO_2	±	—	+	+
Peroxidase	Control	—	+	—	+
	CO	—	+	—	+
	SO_2	—	+	—	+
	NO_2	—	+	—	+

+++ Indicates intense, ++ strong, + moderate, ± dull, — no activity.

DISCUSSION

Functional significance of alkaline phosphatase was first studied by Moog (1946). Its localization in the plasma-membrane perhaps played a role in the transport of phosphate through cellular membranes. This transport was facilitated by a phosphatase or phospho-transferase action (Hardonk & Koudstaal, 1976). After exposure to air pollutants, this function specially in the pulmonary system was

impaired as supported by present observations. Jorgeson & Barry (1973) studied the distribution of few enzymes in the respiratory tract of Syrian hamsters after exposing them to tobacco smoke. On the basis of relationship that exists between plasma-membrane and alkaline phosphatase, damage to plasma membrane assumably the responsible factor for inhibited alkaline phosphatase reaction as evident by exposure to these three air pollutants in the present study.

The *pH* of tissues may be another factor that enables the enzyme to work on substrate. Since alkaline phosphatase is a key enzyme, a change in its activity should cause profound changes in cellular metabolic pathways.

Another enzyme, acid phosphatase pre-eminently regarded, the marker enzyme has been found in golgi cisternae, and not to lysosomal fraction (Farquhar *et al.*, 1974). Furthermore, Ide & Ischman (1969) have suggested that the lysosomal enzymes undergo metabolic transformations *in vivo* resulting in change of substrate specificity. Effects of these irritating gases, it seems, are not merely confined to surface (s) but they also interfered with cellular organelles as evident by present observations. A precise correlation between the activity of lysosomal enzymes and tissue function (s) is difficult to establish. It may be assigned that lysosomal enzymes diffuse through cell-membrane into the cell sap and attach the lysosomal membrane from outside or by endocytosis. They probably disrupt the lysosomal membrane from inside after fusion of the lysosome with the endocytic vacuole. In the absence of experimental evidences these hypotheses appear pure speculations, though functional role of lysosomes in cell damage has been reviewed by Weismann (1967), Allison (1968), and Chayen & Bitensky (1971). Mechanism of their damage and role after exposure to these air pollutants in the present study warrants further study.

The enzyme 5 nucleotidase used as a marker enzyme (Evans & Gurd, 1973) is located in the plasma-membrane of most of the cells. Biochemical and cytochemical data suggest that this enzyme is also associated with the membranes of endoplasmic reticulum (Hardonk & Koudstaal, 1976). Goldberg (1973), Klaushofer & Bock (1974) suggested that a reverse correlation between proliferation and 5 nucleotidase activity occurs. Thus loss of its activity from lungs after exposure to these gases showed proliferation of the cells. Moreover, Wallach & Knufermann (1973) added that this enzyme is involved in the transfer of nucleotides across the plasma-membrane. Present observations using air pollutants indicate a blockade in such transfer. However, the response of endoplasmic reticulum and plasma membrane to these poisonous gases is ultimately reflected by the observations on 5-nucleotidase.

Isoenzyme studies have shown that numerous forms of esterases taki g part in various metabolic processes are present in several tissues (Hermes *et al*, 1975). In general these processes are related to phospholipid metabolism. Inhibited lipase reaction observed in the lungs of *F. pennanti* after exposure to CO, SO_2 and NO_2 reflected decreased phospholipid synthesis, necessary to maintain proper surface tension.

Enzyme phosphamidase though known to hydrolyse P-N bond of the amides of phosphoric acid (Holzer & Duntze, 1971), but loss of its activity and poor reaction from the lungs signifies its susceptibility to irritating gases.

Oxidases are usually ferrous proteins or ferroproporphyrin proteins. Baker *et al* (1973) believed that the mixed function oxidase activity in the chick embryo and adult mouse was not impaired by CO inhalation. No statistics on the effect of these pollutants directly on peroxidase is available. Depleted reaction thus observed signify inhibited catalysis of the reactions. A decrease in enzyme activity or loss may be due to changes in the association of enzyme and lipid molecules. Further more polar and a-polar parts of the lipid molecule may influence the enzymatic confirmation. Thus it can be concluded that regulation of membrane bound enzyme activities may be altered by changes in the micro-environment of the cell. This change is brought about to some extent by CO SO_2 and NO_2 gases through their interference with membranous lipids and proteins.

Presumably, observed change in enzyme activity is the result of variation in the level of enzyme protein with the consequent consideration of cellular orgarelles as highly dynamic structures. These enzymes may have different half lives under varying physiological or pathological conditions. Thus the air pollutants like, CO, SO_2 and NO_2 may change half life of these enzymes, consequently altering condi tional state of cellular organelles and modifying the protein and lipid metabolism.

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