Positive effects of pollution?

SIR — It took more than a decade to realize that the toxic gas nitric oxide (NO), a common air pollutant, is a signalling molecule in the body¹. As most of the actions of NO in the cardiovascular and nervous systems are mediated by soluble guanylyl cyclase², this enzyme represents the main intracellular receptor for NO. High-affinity binding of NO to the prosthetic haem group of soluble guanylyl cyclase results in a marked increase in cyclic (c)GMP formation³. Here, we demonstrate that soluble guanylyl cyclase purified from bovine lung is highly sensitive to small amounts of atmospheric NO generated by traffic and industrial combustion.

We measured the activity of this enzyme in close proximity to the Berlin inner city circular highway every half hour (see figure), and found that it paralleled nearby atmospheric NO concentrations (correlation coefficient 0.99), yielding as much as sixfold activation at an NO concentration of 550 parts per billion (p.p.b.).

We found further evidence for the stimulatory role of atmospheric NO on soluble guanylyl cyclase by showing that enzyme activity is not affected by atmospheric NO concentration in the presence of the NO scavenger oxyhaemoglobin (see figure). Maximally stimulated guanylyl cyclase activity (10 μ M diethylamine-NO complex) is constant over the indicated time and is independent of the NO concentration in the atmosphere. A plot of atmospheric NO concentration versus guanylyl cyclase activity in three independent experiments on different days revealed a linear relationship (y -intercept: 44, slope: 0.2) with a correlation coefficient of 0.94. Other air pollutants such as NO₂, CO, SO₂ and O₃ have correlation coefficients of 0.42, 0.87, 0.80 and -0.54, respectively.

Of these gases, only CO has been reported to stimulate soluble guanylyl cyclase⁴; but in contrast to the 400-fold stimulation by NO^{5,6}, CO stimulates the enzyme by a factor of only 4–6. Because of its higher concentration and much higher efficiency, we conclude that indeed NO,

Effect of atmospheric NO concentrations on the activity of purified bovine soluble guanylyl cyclase. Guanylyl cyclase activity was measured every half hour 30 feet from the Berlin city circular highway during the indicated time range in the absence of oxyhaemoglobin (filled circles) or in the presence of oxyhaemoglobin (65 μ M; filled triangles) or the NO-liberating diethylamine-NO complex (10 μ M; filled squares). The NO concentration in the atmosphere (open diamonds) was measured by chemiluminescence by the Department for City Development and Environment of Berlin at the same site. NO values are means of two consecutive 3-min measurements (18 averaged 10-s measurements) before and after the full or half hour. The marked decrease in atmospheric NO with concomitant reduction of cGMP production around 7:30 a.m. was due to sudden onset of wind.

rather than CO, is the primary stimulant of guanylyl cyclase in polluted air.

As soluble guanylyl cyclase is highly abundant in lung tissue, these results suggest a physiological effect of atmospheric NO in the lung. Indeed, beneficial consequences of inhaled NO (especially at concentrations of 100 to 1,000 p.p.b.^{7,8}) have been demonstrated in patients suffering from pulmonary hypertension and severe acute respiratory failure⁹. These atmospheric NO concentrations lead to 4–10 fold activation of guanylyl cyclase purified from lung. Thus, the data presented here argue in favour of using inhalation of low

concentrations of NO to produce a cGMP-mediated vasodilatory effect in treating lung disorders. There seem to be no effects on blood haemoglobin of inhaled NO^{7,8}, although there is recent evidence¹⁰ that haemoglobin is a transport molecule for NO.

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Oxidative damage in Alzheimer's

SIR — Oxidative stress results in the formation of free carbonyls derived from lipids, proteins, carbohydrates and nucleic acids¹. For example, metal-catalysed 'site-specific' oxidation of several amino-acid

side-chains produces aldehydes or ketones², and peroxidation of lipids generates reactive aldehydes such as malondialdehyde and hydroxynonenal. Here, we describe a highly sensitive, specific immunocytochemical technique, by using *in situ* DNP labelling linked to an antibody system, specifically to detect biomacromolecule-bound carbonyl-reactivity. This technique allows exact determination of the subcellular location of oxidative damage. We applied this method to tissues from cases of Alzheimer's disease, because oxidative events including lipoperoxidative, glycoxidative and other oxidative protein modifications have been reported and implicated in the pathogenesis of this disease³.

We studied non-aldehyde fixed (methacarn) hippocampal brain tissue from six Alzheimer's cases (age 76–90), three age-matched controls (age 70–78) and three younger controls (age 32–64). We sequentially rehydrated tissue sections, incubated them for 20 min in 3% H₂O₂ to inhibit endogenous peroxidase activity, and exposed them for 1 hour to 0.01% 2,4-dinitrophenylhydrazine (DNP). We detected bound DNP immunocytochemically with a rat monoclonal antibody against DNP followed by peroxidase-linked goat

Cases of Alzheimer's disease showing free carbonyls in neurofibrillary tangles (arrows) and in cytoplasm of neurons (large arrowheads) and nuclei of both glia and neurons (small arrowheads). By contrast, in age-matched and young controls, as well as for Alzheimer's samples after reduction of carbonyls by sodium borohydride (NaBH₄), none of these structures is labelled. Scale bar, 50 μ m. Further details of methods are available directly from M.A.S. and will be published in full elsewhere.

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