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Short communication

Nitric oxide synthase activity is elevated in inflammatory lung disease in humans

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Abstract

We have characterised nitric oxide (NO) synthase activity in lung samples from patients with inflammatory lung disease compared to that in normal donor lung. NO synthase activity was measured by the ability of tissue homogenates to convert L-arginine to L-citrulline. Higher levels of NO synthase activity were found in samples from patients with inflammatory lung disease (mild asthma, cystic fibrosis, obliterative bronchiolitis after lung transplantation) compared to samples from healthy donors. NO synthase activity in all samples was mainly dependent on the presence of extracellular Ca^{2+}. The increased NO synthase activity in diseased-lung samples suggests a modulatory role for nitric oxide in lung inflammation.

Keywords: Nitric oxide (NO); Asthma; Cystic fibrosis; Inflammation; Cytokine

1. Introduction

Nitric oxide (NO) is released by a variety of cells following the conversion of L-arginine to NO and L-citrulline by the enzyme NO synthase (Moncada et al., 1991). NO synthase is now known to exist in multiple isoforms including constitutive calcium-dependent isoforms present in endothelial cells (endothelial NO synthase) (Pollock et al., 1991) and some peripheral nerves (neuronal NO synthase) (Mitchell et al., 1991). In addition, an inducible isoform of NO synthase (inducible NO synthase) is expressed in both in vitro and in vivo models of inflammation. In contrast to constitutive isoforms, inducible NO synthase in macrophages (Steuher et al., 1991), vessels (Busse and Mülsch, 1990) as well as at sites of inflammation (Vane et al., 1994) is mainly Ca^{2+}-independent. Nitric oxide released by endothelial cells or peripheral nerves acts as a smooth muscle relaxant (Moncada et al., 1991) whereas the additional effects of NO released at the site of inflammation are not completely understood. However, there is evidence supporting both an inflammatory (Ialenti et al., 1993) and/or an anti-inflammatory (Whittle et al., 1990) role for NO.

In the airways of many species including guinea pig and human there is a population of NO-releasing non-adrenergic noncholinergic (inhibitory NANC; nitrergic) nerves (Barnes and Belvisi, 1993). Indeed in human airways the inhibitory NANC system represents the only neural bronchodilator mechanism. The lung also has a higher ratio of endothelial and inflammatory cells than other organs in the body. For these reasons the lung represents an organ which is likely to be susceptible to changes in NO synthase activity. Indeed, bronchial strips from patients with cystic fibrosis have a reduced functional 'nitrergic' innervation (Barnes and Belvisi, 1993). Furthermore, asthmatic patients exhale greater amounts of NO than healthy volunteers (Kharitonov et al., 1994) and the epithelial layer of biopsies taken from asthmatic patients exhibits an increased expression of inducible NO synthase suggesting that inducible NO synthase is expressed in asthma (Hamid et al., 1993). In addition, inducible NO synthase has been immunolocalised to areas of inflammation in biopsies taken from human lung (Kobzik et al.,

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1993) and to alveolar macrophages in the lung from a patient with bronchiectasis (Tracey et al., 1994). However, NO synthase activity in human lung has not been characterised.

Here we show that human lung contains Ca^{2+}-dependent NO synthase activity which is significantly increased in different types of lung inflammation (cystic fibrosis, asthma and obliterative bronchiolitis following lung transplantation).

2. Materials and methods

Lung parenchymal tissue was obtained from 5 normal (1 male, 22–36 years) and 3 mild asthmatic (1 male, 11–30 years) potential donors for heart-lung/heart transplantation. Tissues were deemed normal on the basis of previous medical histories, macroscopic histological inspection and if the donors were non-smokers with no previous history of chronic inflammatory lung disease. Tissues were also obtained from 4 heart-lung transplant recipient patients with end stage respiratory failure due to cystic fibrosis (3 male, 17–29 years) and 4 patients who were undergoing a second transplant because of chronic obliterative bronchiolitis (2 male, 17–32 years). Tissue was frozen in liquid nitrogen and stored at −80°C until NO synthase activity was measured by the ability of lung homogenates to convert [^{3}H]L-arginine to [^{3}H]L-citrulline in the presence of cofactors as described previously (Mitchell et al., 1993). Briefly, tissue was homogenised on ice in Tris (50 mM; pH 7.4) containing the protease inhibitor phenylmethylsulfonyl fluoride (1 mM) in a ratio of 1:5 (w/v). Lung homogenates were incubated at room temperature for 30 min in the presence of NADPH (1 mM); calmodulin (300 U/ml); tetrahydrobiopterin (5 μM); L-valine (50 mM); Ca^{2+} (2 mM); L-arginine (10 μM) and [^{3}H]L-arginine (0.03 μM; 180000 DPM).

In separate experiments designed to characterise the Ca^{2+} dependency of NO synthase present in human lung, activity in each sample was measured in parallel under three different conditions. (i) For total (calcium-dependent and calcium-independent) NO synthase activity, incubations were carried out in the presence of NADPH, calmodulin, tetrahydrobiopterin, L-valine, L-arginine, [^{3}H]L-arginine, Ca^{2+} (2 mM), (ii) for calcium-independent activity, calcium was replaced with EGTA (1 mM), and (iii) any NO synthase-independent conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline was determined by adding the specific NO synthase inhibitor L-N^G-nitro-L-arginine methyl ester (L-NAME; 1 mM); this conversion was subtracted from that measured under conditions (i) and (ii). This concentration of L-NAME has previously been demonstrated to completely inhibit constitutive and inducible NO synthase activity (Mitchell et al., 1993). Protein content of lung homogenates was determined using a modified Bradford assay (Mitchell et al., 1993). Data are expressed as mean ± S.E.M. Values were compared by Student's unpaired t-test.

3. Results

Homogenates of human lung from healthy donors contained significant NO synthase activity (38.2 ± 7 pmol L-citrulline mg^{-1} protein; $n = 5$; Fig. 1). NO synthase activity in diseased human lung from patients with cystic fibrosis (88.7 ± 18 pmol L-citrulline mg^{-1} protein; $n = 4$) or obliterative bronchiolitis (94 ± 21 pmol L-citrulline mg^{-1} protein; $n = 4$) was significantly ($P < 0.05$) higher than that seen in healthy donors (Fig. 1). In addition NO synthase activity in lung samples from asthmatic lung (74 ± 25 pmol L-citrulline mg^{-1} protein; $n = 3$) was higher than the activity in normal lung; however, this increase did not reach statistical significance. In all cases the conversion of arginine to citrulline by lung homogenates was greatly reduced in the presence of L-NAME (% of control remaining in the presence of L-NAME; donor, 39.7 ± 5; cystic fibrosis, 38.5 ± 10; asthma, 28.9 ± 4; obliterative bronchiolitis, 23.2 ± 8.1; $n = 3$–4). The specific NO synthase activity present in all lung homogenates was significantly reduced when Ca^{2+} was replaced with EGTA (% of control remaining when calcium was replaced with

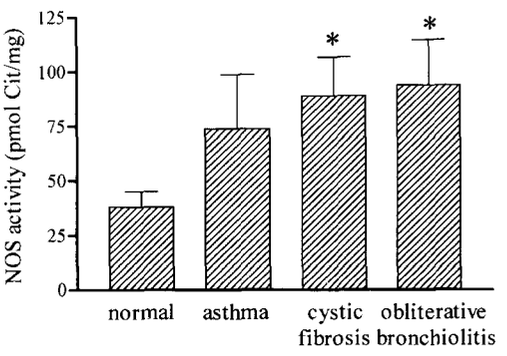

Fig. 1. Comparison of the amounts of nitric oxide (NO) synthase activity in samples of healthy human donor (normal, $n = 5$ patients), asthmatic donor (asthma, $n = 3$ patients), cystic fibrosis recipients (cystic fibrosis, $n = 4$ patients) and obliterative bronchiolitic recipients (obliterative bronchiolitis, $n = 4$ patients). NO synthase activity was measured by the ability of tissue homogenates to convert [^{3}H]L-arginine to [^{3}H]L-citrulline in the presence of co-factors (NADPH, 1 mM; tetrahydrobiopterin, 5 μM and calmodulin, 300 U/ml), calcium (2 mM) and a mixture of unlabelled and [^{3}H]L-arginine in a ratio of 1000:3 (10 μM). L-Valine (60 mM) was included in incubations to inhibit the conversion of L-arginine to L-citrulline by arginase. NO synthase activity is expressed as pmol Cit mg^{-1} protein 30 min^{-1} (mean ± S.E.M. of $n = 3$–5 patients). * $P < 0.05$ and ** $P < 0.01$ compared to the activity in donor lung samples.

EGTA; donor, 11.4 ± 0.83; cystic fibrosis, 14.4 ± 5.4; asthmatic, 29.63 ± 14.7; obliterative bronchiolitis, 17.7 ± 6.3; $n = 3–5$).

4. Discussion

Our results show that human lung tissue contains significant amounts of Ca^{2+}-dependent NO synthase activity and that inflammatory diseases such as cystic fibrosis and obliterative bronchiolitis, result in increased amounts of NO synthase activity in lung samples. Similarly NO synthase activity in asthmatic lung showed a trend to be higher than that in normal lung. The fact that the increased activity in asthmatic lung did not reach significance, when compared to normal lung, was probably due to the small sample number available to us in this group. In addition, this lung tissue was from patients with mild asthma and therefore likely to represent a more moderate inflammatory state. However, higher levels of NO synthase in asthmatic lung are consistent with previous studies showing that exhaled air from asthmatic patients contains higher amounts of NO than from healthy volunteers (Kharitonov et al., 1994). Similarly the recent observation that NO synthase activity is increased in acute and chronic inflammation (Vane et al., 1994) supports a role for NO in inflammatory diseases. The exact nature of the active isoform of NO synthase present in human lung is unknown; however, several possibilities exist. This increased NO synthase activity may be due to an up-regulation of a constitutive isoform(s) of NO synthase, such as endothelial NO synthase or neuronal NO synthase. This would be substantiated by our findings that this activity was mainly dependent on the presence of extracellular calcium. However, the calcium dependency of inducible NO synthase in human hepatocytes has recently been demonstrated (Geller et al., 1993). It is therefore possible that the high levels of NO synthase activity in human diseased lung tissue could be of the 'inducible' type. The source of this increased NO synthase activity is as yet unknown. However, positive immunostaining for inducible NO synthase has been demonstrated in epithelial cells from biopsies taken from patients with asthma (Hamid et al., 1993; Kobzik et al., 1993) and rats treated with lipopolysaccharide (Kobzik et al., 1993). In addition, inducible NO synthase immunostaining has been demonstrated in human alveolar macrophages from inflamed areas of resection tissue (Kobzik et al., 1993) and from a patient with bronchiectasis (Tracey et al., 1994). Alternatively, the increased NO synthase activity could be associated with infiltrating inflammatory cells.

The demonstration that NO synthase is elevated in pulmonary inflammation provides the impetus for continued study of the role of NO in lung disease. It is presently impossible to predict whether increased NO formation in pulmonary tissue is associated with tissue damage or protection. However, the further biochemical and molecular characterisation of NO synthase activity in diseased lung tissue will not only allow for a definition of the respective isoform present but also provide the tools necessary to identify which cells contain it.

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