

EJP 0300R

Rapid communication

## 13-Hydroxy-linoleic acid induces airway hyperreactivity to histamine in guinea-pigs

Paul A.J. Henricks, Ferdi Engels, Henk J. Van der Linde and Frans P. Nijkamp

Department of Pharmacology, Faculty of Pharmacy, University of Utrecht, Catharinesingel 60, 3511 GH Utrecht, The Netherlands

Received 14 March 1991, accepted 5 April 1991

Airway epithelial cells play an important role in airway smooth muscle function. Damage or removal of the epithelial lining is associated with airway smooth muscle hyperreactivity to contractile agents. Production of mediators derived from fatty acids may be one of the mechanisms by which epithelial cells modulate airway smooth muscle function. Most of the research on the production and biological activity of fatty acid metabolites has been directed towards arachidonic acid metabolism and metabolites, whereas little attention has been paid to the metabolism of linoleic acid. Linoleic acid can be converted into 9-hydroxy-linoleic acid (9-HODE) or 13-hydroxy-linoleic acid (13-HODE) by the same enzyme systems that metabolize arachidonic acid into biologically active mediators. We had previously shown that 9- and 13-HODE are formed by several cell types within the lungs, including airway epithelial cells (Oosthuizen et al., 1990) and pulmonary macrophages (Engels et al., 1986), and that HODEs interfere with the activity of reactive oxygen species released by pulmonary macrophages (Engels et al., 1989). Moreover, HODEs exert chemotactic activity on polymorphonuclear leukocytes (Henricks et al., 1991). We now investigated whether 13-HODE is of importance for the regulation of airway smooth muscle function.

The animals used were specified pathogen-free male Dunkin Hartley guinea-pigs (Harlan/Porcellus, Heatfield, U.K.) weighing 450–700 g. 13-HODE was obtained from Oxford Biomedical Research Inc. (Oxford, MI, U.S.A.). For in vitro studies, tracheas were cut into 3 rings each, suspended in organ baths and connected to isometric transducers. The experimental protocol included: (1) a 15-min phase during which 4 g tension was applied to the tracheal preparations, followed by a change to 2 g tension, (2) a 60-min equilibration period during which the medium was refreshed 3 times, (3) a

30-min incubation with 10  $\mu$ M 13-HODE or vehicle, and (4) a dose-response curve to cumulative concentrations of histamine. For in vivo studies, guinea-pigs were anaesthetized with urethane and allowed to breathe spontaneously. The pulmonary resistance and dynamic lung compliance were determined breath by breath using a computerized respiratory analyzer (Folkerts and Nijkamp; 1985). Two dose-response curves with histamine (0.4–2.0  $\mu$ g/100 g body weight intravenously) were obtained for each animal, one before and one after the administration of 13-HODE or vehicle. The solutions were applied to the airways of the animals as aerosols. 13-HODE (10  $\mu$ M) or vehicle solutions were nebulized in a one-liter box coupled to the flow head that was connected to the trachea of the animal. The animals were allowed to inhale the aerosols for 5 breaths. This procedure was repeated 5 times every 5 min.

An increased maximal contraction in response to cumulative concentrations of histamine was observed when tracheal preparations were incubated in vitro with 10  $\mu$ M 13-HODE for 30 min as compared to control incubations (maximal contractions:  $1640 \pm 80$  mgf vs.  $1090 \pm 170$  mgf;  $n = 5$ ,  $P < 0.02$ ). The  $EC_{50}$  values of the dose-response curves for histamine did not differ between 13-HODE-treated and control preparations.

The histamine-induced changes in dynamic compliance were not influenced by 13-HODE administration to guinea-pigs in vivo (data not shown). However, pulmonary resistance was significantly changed after treatment of the animals with an aerosol of 13-HODE (fig. 1). The increase in pulmonary resistance was twice as high in 13-HODE-treated animals as in control guinea-pigs after administration of 1.2, 1.6 or 2.0  $\mu$ g histamine/100 g body weight (fig. 1). No differences between the first and second curve to histamine were measured when the animals had inhaled the control aerosol (fig. 1).

The present study demonstrates for the first time that 13-HODE can induce an increased response of the airways to histamine. The increased reaction to histamine in vivo after 13-HODE administration as meas-

Correspondence to: P.A.J. Henricks, Department of Pharmacology, Faculty of Pharmacy, Catharinesingel 60, 3511 GH Utrecht, The Netherlands.

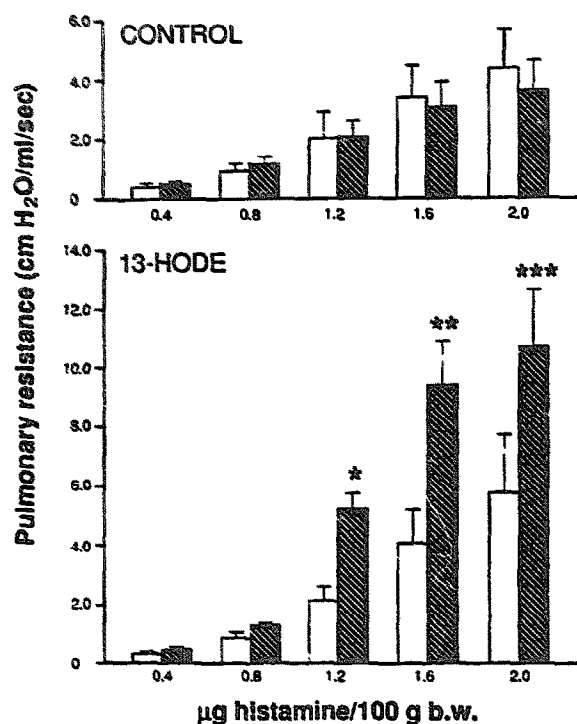


Fig. 1. Histamine-induced changes in pulmonary resistance in 13-HODE-treated (lower panel) and control (upper panel) animals before (□) and after (■) aerosol administration ( $n = 3$ ). The values represent means  $\pm$  S.E.M. \*  $P < 0.05$ , \*\*  $P < 0.005$ , \*\*\*  $P < 0.001$  as compared to values obtained before the 13-HODE administration (Student's paired t-test).

ured by the potentiated increase in pulmonary resistance points to hyperreactivity of the central respiratory airways. Bronchial hyperreactivity is a characteristic feature in asthmatic patients. Inflammation in the airways is considered to be one of the causes of bronchial hyperreactivity in asthma. Since 13-HODE also induces a chemotactic response to polymorphonuclear leukocytes (Henricks et al., 1991), products released from these cells (e.g. platelet-activating factor and leukotrienes) could be responsible for the increased re-

sponsiveness of the airways to contractile stimuli. However, a role for these inflammatory products in the induction of bronchial hyperreactivity caused by 13-HODE in our in vivo model seems unlikely because 13-HODE was able to induce hyperreactivity in tracheal preparations in vitro from which inflammatory cells were absent. Nevertheless, recruitment of inflammatory cells induced by the release of linoleic acid metabolites by epithelial cells and pulmonary macrophages can amplify bronchial hyperreactivity in vivo. 13-HODE may thus play an important role in the inflammatory component of chronic lung diseases and the induction of bronchial hyperreactivity.

### Acknowledgements

This study was supported by the Dutch Asthma Foundation (Grant 86.17).

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