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Induction in guinea-pigs of airway hyperreactivity and decreased lung β-adrenoceptor number by 15-hydroperoxy-arachidonic acid

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Administration to guinea-pigs of 15-hydroperoxy-arachidonic acid (0.2 mg kg^{-1} i.p.) caused a 20% reduction of lung β-adrenoceptor number. Furthermore, *in vivo*, a hyperreactivity of the airways to nebulized histamine was observed. In contrast, administration of 15-hydroxy-arachidonic acid (0.2 mg kg^{-1} i.p.) did not cause a significant change in either of these parameters.

Introduction Bronchial hyperreactivity to a variety of stimuli is one of the diagnostic features of bronchial asthma. However, the underlying pathophysiological mechanism still remains uncertain. Szentivanyi (1968) proposed that a decreased β-adrenoceptor functioning may be one of the mechanisms that contribute to the pathogenesis. RecentlyAdcock & Garland (1980) suggested that arachidonic acid metabolites formed by the lipoxygenase pathway may also play a role in airway hyperreactivity. Since corticosteroids, which are used in the therapy of bronchial asthma and inflammation, not only inhibit the liberation of arachidonic acid from the phospholipids by inhibiting phospholipase A_2 (Blackwell, Flower, Nijkamp & Vane, 1978) but also increase β-adrenoceptor function and number, these two hypotheses may be related.

In the present study we investigated the influence in guinea-pigs of 15-hydroperoxy-arachidonic acid (15–HPETE), an arachidonic acid metabolite formed by the 15-lipoxygenase pathway and of 15-hydroxy-arachidonic acid (15-HETE, a metabolite of 15-HPETE) on airway reactivity to histamine and on lung β-adrenoceptor number.

Methods Male guinea-pigs (CPB, TNO, Zeist, The Netherlands) weighing 300 to 350 g were used. The animals were anaesthetized with pentobarbitone sodium and the lungs were removed. The number of β-adrenoceptor binding sites was measured in lung membrane preparations of peripheral lung tissue (excluding the large airways) by means of a radio-ligand binding assay with [^{3}H]-dihydroalprenolol, similar to that described by Schreurs & Nijkamp (1982). In the present series of experiments propranolol 10^{-6} M was used to determine non-specific binding.

In vivo, bronchoconstriction was induced by exposure of guinea-pigs in a closed chamber to an aerosol of 0.09% solution of histamine. The time required for asphyxial collapse was used as a measure of bronchial reactivity *in vivo*. Animals undergoing collapse were removed quickly from the chamber and resuscitated if necessary. Guinea-pigs were screened by exposure on 2 separate occasions to the histamine aerosol and groups of animals were selected with matching asphyxial collapse times. The groups were injected intraperitoneally 3 h before the experiment with (a) 15-HPETE, 0.2 mg kg^{-1}; (b) 15-HETE, 0.2 mg kg^{-1}; or (c) an equivalent volume of sterile saline.

15-HPETE was prepared by incubating arachidonic acid with soybean lipoxygenase for 30 min at 0°C in 0.1 M Tris-buffer at pH 8.5. Separation from other products was achieved by thin layer chromatography (chloroform: methanol: acetic acid: water, 90:5:1:0.5) on silica gel plates. Concentrations of 15-HPETE were measured by u.v.-spectrophotometry (234 nm). 15-HETE was prepared by reduction of 15-HPETE with sodium borohydride.

Results Compared with controls given saline, pretreatment with 15-HPETE (0.2 mg kg^{-1} i.p.) 3 h earlier caused a statistically significant 20% reduction in the number of binding sites for [^{3}H]-dihydroalprenolol in peripheral lung tissue of the guinea-pig. This change was accompanied by an increased sensitivity of the respiratory tract to histamine *in vivo*, since, in a separate experiment, the asphyxial collapse time was 27% shorter than in the

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Table 1 Effect of 15-hydroperoxy-arachidonic acid (15-HPETE) and 15-hydroxy-arachidonic acid (15-HETE) on number of binding sites (B_{max}) and affinity (K_D) for [^{3}H]-dihydroalprenolol in lung homogenates of guinea-pigs and on histamine airway reactivity *in vivo*

	B_{max} (fmol mg^{-1} protein)		K_D (nM)	*Time for asphyxial collapse* (s)	
Control	878±44	(6)	1.45±0.21	157±17	(6)
15-HPETE	709±22*	(6)	1.33±0.07	115±12*	(6)
Control	928±23	(5)	1.58±0.03	174±15	(7)
15-HETE	903±19	(5)	1.48±0.08	144±19	(7)

*$P<0.05$ as compared to their respective control group (Student's t test).
Values are presented as means±s.e.mean. Number of animals are given in parentheses.
Guinea-pigs were given either 15-HPETE or 15-HETE i.p. at a dose of 0.2 mg kg^{-1}, 3 h before they were killed or before histamine challenge; control animals received saline, in an equivalent volume, similarly.

control counterparts (see Table 1).

By contrast, when 15-HETE was given in an identical manner it caused small but statistically insignificant reductions both in β-adrenoceptor binding sites and in asphyxial collapse times when compared with the saline-treated group. In neither series of experiments was there any change in the affinity of the lung β-adrenoceptors for the tritiated ligand (Table 1).

Discussion The present data show that an arachidonic acid metabolite formed by the lipoxygenase pathway may conceivably contribute to airway hyperreactivity reactions. The administration of one such metabolite, 15-HPETE, reduced the amount of β-adrenoceptor binding in guinea-pig lung tissue and caused an increased sensitivity of the airways to histamine *in vivo*. The affinity of the β-adrenoceptors for [^{3}H]-dihydroalprenolol was unaltered. It is possible that reduced functioning of lung β-adrenoceptors could be the cause of the bronchial hyperreactivity.

There are several other reports suggesting that arachidonic acid metabolities may be involved in β-adrenoceptor function. Mepacrine and tetracaine, both inhibitors of phospholipase A_2, were able to block the isoprenaline-induced decrease in β-adrenoceptor binding sites (Mallorga, Tallman, Henneberry, Hirata, Strittmatter & Axelrod, 1980). Other studies reveal an increase in β–adrenoceptor number after glucocorticoids, which also block arachidonic acid metabolism at the phospholipase A_2 level (Cheng, Goldfien, Ballard & Roberts, 1980; Foster & Harden, 1980). Our data with 15-HPETE are compatible with the previous findings. At present we cannot be sure if the phenomena described are caused by 15-HPETE itself, by one of its metabolites, or even by stimulation of other arachidonic acid metabolic pathways. However, 15-HETE caused no significant changes when given in the same dose as 15-HPETE so this metabolite does not seem to be a likely candidate.

Previously we showed that intraperitoneal injection of bacterial endotoxins and several gram-negative bacteria induce a similar reduction in β-adrenoceptor number (Scheurs, Verhoef & Nijkamp, 1983). The 15-lipoxygenase pathway is present in several cell-types related to the defence mechanism but seems to predominate in eosinophils (Turk, Maas, Brash, Roberts & Oates, 1982). Therefore, activation or infiltration of cells involved in the immune system, events which can be evoked by lipoxygenase products like leukotriene B_4 (Goetzl, 1980), may play a role in airway hypersensitivity reactions such as occur in bronchial asthma.

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