ELSEVIER

European Journal of Pharmacology 285 (1995) R1–R2

ejp

Rapid communication

Leukotrienes mediate tracheal hyperresponsiveness after nitric oxide synthesis inhibition

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Received 12 September 1995; accepted 14 September 1995

Abstract

Preincubation of guinea pig tracheas with the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 120 μM) resulted in a significant upward shift of the histamine concentration-response curve with a concomitant inhibition of prostaglandin E_2 production. Preincubation of the preparations with a 5-lipoxygenase inhibitor (AA-861, 2-(12-hydroxy-5,10-dodecadiynyl)-3,5,6-trimethyl-*p*-benzoquinone) or a leukotriene C_4,D_4,E_4 receptor antagonist (FPL 55712, sodium 7-{3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy}-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate) totally blocked the L-NAME-induced tracheal hyperresponsiveness. A shift from cyclo-oxygenase to lipoxygenase products, in particular leukotrienes, is likely to be responsible for the L-NAME-induced tracheal hyperresponsiveness.

Keywords: Nitric oxide (NO); Tracheal hyperresponsiveness; Leukotriene; (Guinea pig)

Adcock and Garland (1980) demonstrated in 1980 that the guinea pig tracheal hyperresponsiveness to histamine after cyclo-oxygenase inhibition was attributable to an augmenting effect of lipoxygenase products. Now, abundant evidence has been obtained that leukotrienes are involved in airway hyperresponsiveness (O'Byrne, 1994). Recently it became clear that nitric oxide (NO) can stimulate cyclo-oxygenase, an enzyme responsible for the synthesis of prostaglandins (Salvemini et al., 1994). Indeed, in previous studies we demonstrated that the histamine-induced contractions of the guinea pig trachea were associated with the release of both nitric oxide *and* prostaglandin E_2 (Folkerts et al., 1989,1995a). Also, enhanced tracheal contractions in animal models of airway hyperresponsiveness coincide with a decreased nitric oxide *and* prostaglandin E_2 production (Nijkamp and Folkerts, 1987; Folkerts et al., 1989,1995a,b). Moreover, preincubation of tissues with the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) significantly enhances histamine responsiveness of guinea pig tracheas (Nijkamp et al., 1993). In the present study it was investigated whether the L-NAME-induced tracheal hyperresponsiveness could be explained by modulation of cyclo-oxygenase and lipoxygenase activities.

Guinea pig isolated tracheas were perfused and two hooks were inserted through opposite sides of the tracheal wall with the smooth muscle in between. Isometric tension changes were measured. The inside of the trachea was perfused independently from the outside with Krebs solution (Folkerts et al., 1995b). After 3 washes and 30 min stabilization the intraluminal side of the trachea was perfused for 30 min with the solvent solution (= Krebs buffer) or L-NAME (120 μM) and histamine concentration-response curves were made. Samples were taken from the organ bath just *before* and *after* completion of the histamine concentration-response curve and immediately frozen (−20°C). Prostaglandin E_2 and leukotriene C_4,D_4,E_4 were measured with radioimmunoassays. In a separate set of experiments histamine responsiveness in the absence and presence of L-NAME was investigated in tissues that were also incubated for 30 min with the 5-lipoxygenase inhibitor AA-861, (2-(12-hydroxy-5,10-dodecadiynyl)-3,5,6-trimethyl-*p*-benzoquinone, 10 μM), or the leukotriene C_4,D_4,E_4 receptor antagonist, FPL 55712 (sodium 7-{3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy}-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate, 10 μM). The L-NAME-induced tracheal

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SSDI 0014-2999(95)00580-3

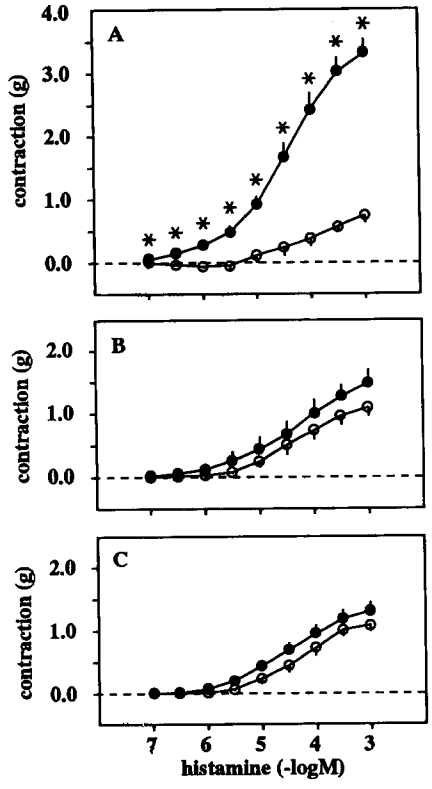

Fig. 1. Effect of L-NAME (120 μM) incubation on the responsiveness of the guinea pig tracheal tube. The (A) histamine concentration-response curve was significantly enhanced after L-NAME incubation compared to the curve for tissues incubated with the solvent solution. (B) AA-861 (10 μM, 5-lipoxygenase inhibitor) and (C) FPL 55712 (10 μM, a leukotriene C_4,D_4,E_4 receptor antagonist) significantly suppressed the L-NAME-induced tracheal hyperresponsiveness to histamine. Control = open circles, L-NAME (120 μM) = closed circles. $^*P < 0.01$, Student's unpaired t-test, $n = 6$–9.

hyperresponsiveness (Fig. 1A) was associated with an obvious decrease in prostaglandin E_2 production. After correction for the basal concentrations, the histamine-induced prostaglandin E_2 production in the control group was 31.5 ± 8.8 and in the L-NAME group 0.8 ± 3.2 pg prostaglandin E_2/100 μl organ bath fluid ($P < 0.01$, Student's unpaired t-test, $n = 3$). Under the present experimental conditions leukotriene production was below the detection limit (< 12.5 pg/100 μl). However, leukotrienes can induce airway hyperresponsiveness at very low concentrations (O'Byrne, 1994). Therefore, pharmacological experiments were performed. Incubation of control preparations with the 5-lipoxygenase inhibitor, AA-861, or the leukotriene C_4,D_4,E_4 receptor antagonist, FPL 55712, did not affect the histamine concentration-response curves. In contrast, the above-mentioned agents completely prevented the L-NAME-induced tracheal hyperresponsiveness to histamine (Fig. 1B and C).

In conclusion, inhibition of nitric oxide synthesis decreases cyclo-oxygenase activity and, maybe as a consequence, increases lipoxygenase activity. The released leukotrienes are likely to be responsible for the L-NAME-induced tracheal hyperresponsiveness.

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