*Biochemical Pharmacology*, Vol. 37, No. 4, pp. 573–576, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00


# RAPID COMMUNICATION

## POSSIBLE INVOLVEMENT OF CYTOCHROME P-450 IN THE EPITHELIUM-MODULATED RESPONSE TO METHACHOLINE IN GUINEA PIG TRACHEA

David Raeburn*, David J. Sequeira and Wayne L. Backes

Department of Pharmacology and Experimental Therapeutics,
Louisiana State University Medical Center,
New Orleans, LA 70112, U.S.A.

(Accepted 7 December 1987)

Removal of the endothelial cells lining vascular smooth muscle preparations in vitro results in the loss of an endothelium-dependent relaxant response produced by a factor released from the endothelium, the so-called endothelium-derived relaxant factor (E.D.R.F.). Damage to the endothelium with a concomitant loss of E.D.R.F. has been implicated in the pathology of several vascular diseases [1]. Similarly, mechanical removal of the epithelial cells lining the respiratory tract of experimental animals and human tissue in vitro and the loss of airway epithelium in bronchial asthma in vivo lead to an increase in the reactivity of the underlying airway smooth muscle [2-4]. An epithelium-derived relaxant factor (Ep.D.R.F.) has been proposed as the mediator in the airways.

It has been reported that E.D.R.F.-dependent relaxation of vascular preparations may be mediated by metabolites of arachidonic acid [1] and that these metabolites may be produced by neither cyclooxygenase nor lipoxygenase pathways but by the action of cytochrome P-450 present in the endothelial cells [5,6]. For this reason we decided to investigate the role of cytochrome P-450-dependent metabolism on the Ep.D.R.F.-related reduction in airway smooth muscle contractility.

## MATERIALS AND METHODS

Male, Hartley guinea pigs (250-300 g, Harlan Sprague-Dawley, Indianapolis, IN) were killed by stunning and bleeding. The trachea was removed and strips containing or lacking the epithelium were prepared for recording changes in the force of isometric contraction following stimulation with methacholine. The experimental methods and bathing solution used have been described previously [7]. Liver microsomes were prepared from rabbits treated with β-napthoflavone (an inducer of P-450 activity) as previously described [8].

Concentration-response curves to the cumulative addition of methacholine were obtained in tissues containing or lacking the epithelium under the following conditions: (a) control (no treatment), or in the presence of (b) liver microsomes (1 mg/ml) plus an NADPH-generating system, (c) NADPH-generating system only or (d) microsomes (1 mg/ml) only. The NADPH was generated by adding glucose-6-phosphate

*Author to whom correspondence should be addressed.

(5 mM), glucose-6-phosphate dehydrogenase (2 units/ml) and $NADP^+$ (1.5 mM) to organ baths (b) and (c). Each treatment was instituted 30 min prior to the addition of methacholine, and the conditions were maintained throughout the remainder of the experiment. Contractile responses to methacholine under each condition were recorded as $pD_2$ ($-\log EC_{50}$) values and the force of maximum contraction (g). Comparisons across the groups (a-d) and within the groups (+ vs -epithelium) were made by ANOVA one-way analysis of variance where $P < 0.05$ was considered significant. In the figure, the standard errors are given by the vertical bars unless enclosed by the symbol. All compounds used were purchased from Sigma Chemical Co. (St. Louis, MO).

## RESULTS

The removal of the epithelium from the control tracheal preparations resulted in an increase in sensitivity (i.e. a decreased $pD_2$ value) to methacholine without altering the maximum contractile response of the tissue (Fig. 1A, Table 1). When liver microsomes plus the NADPH-generating system were added, the increase in reactivity to methacholine seen following epithelium removal in the control tissue was abolished (Fig. 1B, Table 1). In addition, an increase in the maximum force of contraction was seen in the epithelium-containing tissue (Table 1). Addition of the NADPH-generating system alone did not abolish the increased sensitivity to methacholine following epithelium removal, although the magnitude of the shift was reduced. As before, the maximum response was increased in the epithelium-containing tissues (Table 1). The addition of the liver microsomes in the absence of the NADPH-generating system similarly did not prevent the increased tissue reactivity seen following epithelium removal (Table 1).

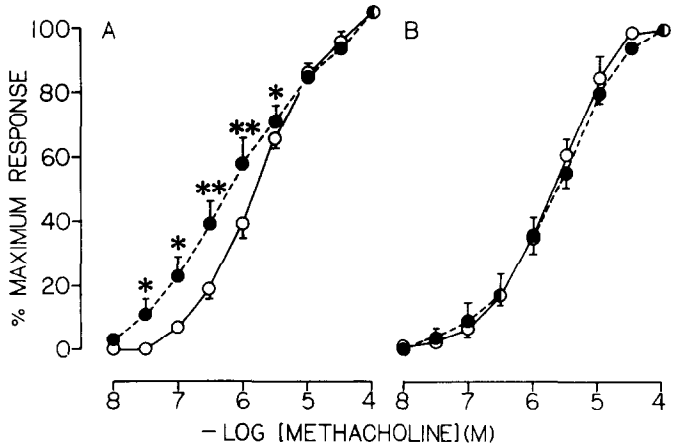


Fig. 1. Cumulative concentration-response curves to methacholine in epithelium-containing (o) or epithelium-denuded (•) strips of guinea pig trachealis plotted as a % of their individual maximum response. Panel A: control (no treatment); panel B: in the presence of microsomes and NADPH. Key: (*) $P < 0.05$, (**) $P < 0.01$. Values are means $\pm$ SE, N = 4.

Table 1. Effects of various treatments on the concentration-response curves to methacholine in tracheal preparations containing or lacking the epithelium

| Treatment* | N | $pD_2$ | Max. response (g) | % Control max. |
|---|---|---|---|---|
| Control | | | | |
| + Epi | 10 | 5.77 ± 0.07 | 0.99 ± 0.10 | 100 |
| - Epi | 10 | 6.10 ± 0.22 † | 0.88 ± 0.10 | 100 |
| Microsomes & NADPH | | | | |
| + Epi | 4 | 5.74 ± 0.17 | 1.28 ± 0.04 § | 129 ± 4 § |
| - Epi | 4 | 5.62 ± 0.10 ‖ | 0.90 ± 0.03 | 102 ± 3 |
| NADPH | | | | |
| + Epi | 4 | 5.83 ± 0.14 | 1.14 ± 0.04 ‖ | 115 ± 4 ‖ |
| - Epi | 4 | 6.14 ± 0.12 ‡ | 0.91 ± 0.12 | 103 ± 14 |
| Microsomes | | | | |
| + Epi | 2 | 5.62, 5.80 | 1.1, 1.16 | ND ¶ |
| - Epi | 2 | 6.09, 6.01** | 1.15, 1.35** | ND ¶ |

* +Epi = epithelium-containing; and -Epi = epithelium-denuded.
† $P < 0.01$ and ‡ $P < 0.05$, +Epi vs -Epi.
§ $P < 0.01$ and ‖ $P < 0.05$, treatment vs control.
**No statistical analysis was performed.
¶ Not determined.

## DISCUSSION

The results obtained indicate that an NADPH-dependent activity in liver microsomes can mimic the inhibitory effect of the epithelium on guinea pig tracheal preparations *in vitro*. The effect is seen as an epithelium-dependent shift in the concentration-response curve (Figure 1A), presumably due to the presence of a relaxing factor (Ep.D.R.F.) which mediates the response. Both NADPH and microsomes appear to be required to produce this factor, since neither component alone could affect the $pD_2$ value in the absence of the epithelium. These results indicate that the factor is not originally present in liver microsomes, but can be produced by them when NADPH is added. Due to the large amounts of cytochrome P-450 present in liver microsomes and the broad specificity for this enzyme, it is attractive to postulate a role for this heme protein in the production of this factor. In control tissues, removal of the epithelium is thought to result in the concomitant loss of a "brake" on the inherent contractility of the airway smooth muscle resulting in larger contractions for a given concentration of agonist when compared with epithelium-containing preparations. Actively metabolizing liver microsomes appear to be capable of producing a similar effect on the contractility of airway smooth muscle, suggesting that both the tracheal epithelium and liver microsomes contain common enzymes and possibly precursors necessary for the production of this relaxing factor. Another possibility, which as far as we are aware has not yet been discussed

in any detail, is that the epithelium may reduce the underlying muscle sensitivity by either preventing the generation or catalyzing the removal of an excitatory factor. In the presence of the exogenously added cytochrome P-450-activating system (liver microsomes plus NADPH), it is possible that an excitatory factor is removed by metabolism. In the intact trachea, endogenous P-450 present in the airway epithelium [9] may perform a similar function. The arachidonic acid metabolites produced by cytochrome P-450 found in the vascular endothelium are not prostaglandins but are epoxyeicosatrienoic acids [6]. These compounds have been shown to relax vascular smooth muscle in vitro and in vivo [6]. Singer and co-workers [5] have demonstrated that the cytochrome P-450 antagonist metyrapone can prevent E.D.R.F.-induced relaxant responses in vascular smooth muscle and suggest that the E.D.R.F. relaxant response may be mediated in part by the metabolism of arachidonic acid to an active product(s) which is a vasodilator. This may also be the case in Ep.D.R.F.-mediated inhibitory responses in the airways where cytochrome P-450 may be activating a relaxant factor or de-activating a contractile factor when the tissue is stimulated with bronchoconstrictor substances.

ACKNOWLEDGEMENT--We thank Parveen S. Walia for technical assistance and Thelma Evans and Demetra Castro for excellent secretarial help. This study was funded in part by grants to D.R. (N.I.H.-BRSG SO-RR-5376) and to W.L.B. (Cancer Association of Greater New Orleans and N.I.H.-B.R.S.G. SO-RR-5376).

REFERENCES

1. R.F. Furchgott and J.V. Zawadski, Nature, Lond. 288, 373 (1980).
2. N.A. Flavahan, L.L. Aarhus, T.J. Rimele and P.M. Vanhoutte, J. appl. Physiol. 58, 834 (1985).
3. D. Raeburn, D.W.P. Hay, S.G. Farmer and J.S. Fedan, Eur. J. Pharmacol. 123, 451 (1986).
4. L.A. Laitinen, M. Heino, A. Laitinen, K. Kava and T. Haahtela, Am. Rev. resp. Dis. 131, 599 (1985).
5. H.A. Singer, J.A. Saye and M.J. Peach, Blood Vessels 21, 223 (1984).
6. K.G. Proctor, J.R. Falck and J. Capdevila, Circ. Res. 60, 50 (1987).
7. D.W.P. Hay, S.G. Farmer, D. Raeburn, V.A. Robinson, W.W. Fleming and J.S. Fedan, Eur. J. Pharmacol. 129, 11 (1986).
8. W.L. Backes, I. Jansson, J.E. Mole, G.G. Gibson and J.B. Schenkman, Pharmacology 31, 155 (1985).
9. W.L. Backes and D. Raeburn, Am. Rev. resp. Dis., in press.