*Br. J. Pharmac.* (1982), **77**, 003–005

# ANTIGEN-INDUCED CONTRACTION OF GUINEA-PIG TRACHEA: SEARCH FOR MEDIATOR RELEASE WITH CASCADE SUPERFUSION BIOASSAY

JOHN F. BURKA

Department of Pharmacology, 9–70 Basic Medical Sciences Building, The University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Ovalbumin induces contraction of sensitized guinea-pig trachea, both in the presence and absence of indomethacin. Cascade superfusion bioassay was employed to detect mediator release. A prostaglandin E-like material was released in response to antigen in the absence of indomethacin but, no biologically active material could be detected from trachea in the presence of indomethacin. Thus the substance responsible for antigen-induced tracheal contraction, although appearing to be a lipoxygenase product, is as yet undefined.

**Introduction** Contraction of sensitized guinea-pig trachea in response to antigen has been well documented (reviewed by Chand & Eyre, 1978) but the mediators and mechanisms of this contraction are not well understood. Previous experiments have indicated that histamine only contributes in the first few minutes and does not influence the overall height and length of the contraction (Burka & Paterson, 1981). Because inhibitors of the lipoxygenase pathway of arachidonic acid metabolism inhibit the prolonged phase of the contraction, we have assumed that lipoxygenase products, possibly leukotrienes (LT) $C_4$ and $D_4$, are mediators of antigen-induced tracheal contraction. The present study was carried out to determine whether a biologically active lipoxygenase product could be detected by means of differential superfusion bioassay.

**Methods** Male English short-hair guinea-pigs (200–250 g) were sensitized with ovalbumin 100 mg subcutaneously and 100 mg intraperitoneally. The trachea was removed 3–4 weeks later, spirally cut (Constantine, 1965) and superfused in cascade (Vane, 1964) with Krebs solution (37°C, aerated with 95% $O_2$:5% $CO_2$) at a flow rate of 2.5 ml/min over a tracheal spiral from a normal guinea-pig. These tissues were superfused over either a guinea-pig ileum to assay leukotrienes or a rat stomach strip to assay prostaglandin $E_2$ ($PGE_2$). The tissues from normal guinea-pig and rat were superfused with atropine ($10^{-6}$M) and mepyramine ($10^{-6}$M), antagonists of acetylcholine and histamine respectively and indomethacin ($8.4 \times 10^{-6}$M) to inhibit the endogenous synthesis of prostaglandins and thromboxanes. In some experiments all tissues in the cascade received indomethacin. The changes in tissue tone were recorded isotonically (initial load 1 g for trachea and ileum, 2 g for rat stomach strip) using Harvard type 386 transducers connected to either a Fisher 5000 Recordall or a Harvard type 350 linear chart recorder.

Dose-response relationships of histamine and $LTD_4$ were established on the trachea and ileum and $PGE_2$ on the rat stomach strips, followed by a challenging bolus injection of ovalbumin (10 μg). Arachidonic acid (10 μg) was administered to some of the tracheae studied and the products analyzed qualitatively.

Histamine dihydrochloride, arachidonic acid, and ovalbumin were purchased from Sigma. I am grateful for the gifts of leukotriene $D_4$, and indomethacin (Merck-Frosst), mepyramine (Poulenc), and prostaglandin $E_2$ (Upjohn).

**Results** Ovalbumin (10 μg) induced a strong contraction of sensitized trachea, both in the presence and absence of indomethacin ($8.4 \times 10^{-6}$M). Neither the normal trachea nor the ileum contracted when ovalbumin was administered directly to these tissues. In the absence of indomethacin, sensitized trachea released PGE-like material in response to ovalbumin, histamine and arachidonic acid, as determined by contraction of the rat stomach strip (Figure 1a). This agreed with our previously published results (Burka & Paterson, 1980a). Administration of ovalbumin to the sensitized trachea also induced a slight contraction of the normal trachea.

Administration of ovalbumin to sensitized trachea treated with indomethacin did not result in the contraction of any tissue superfused by the effluent, including normal trachea (Figure 1b). The threshold sensitivity of normal trachea to $LTD_4$ was $10^{-11}$ mol and of the ileum $10^{-12}$ mol.

The effect of arachidonic acid ($3 \times 10^{-8}$ mol) was variable. The majority of tracheae (8/10) relaxed and the others contracted. In all cases, a PGE-like material was produced. In the presence of indomethacin, arachidonic acid induced contraction of most tracheae, although some did not respond. No. PGE-like activity was detected on the rat stomach strip and no contraction of the guinea-pig ileum was observed.

0007-1188/82/090003-03 $01.00

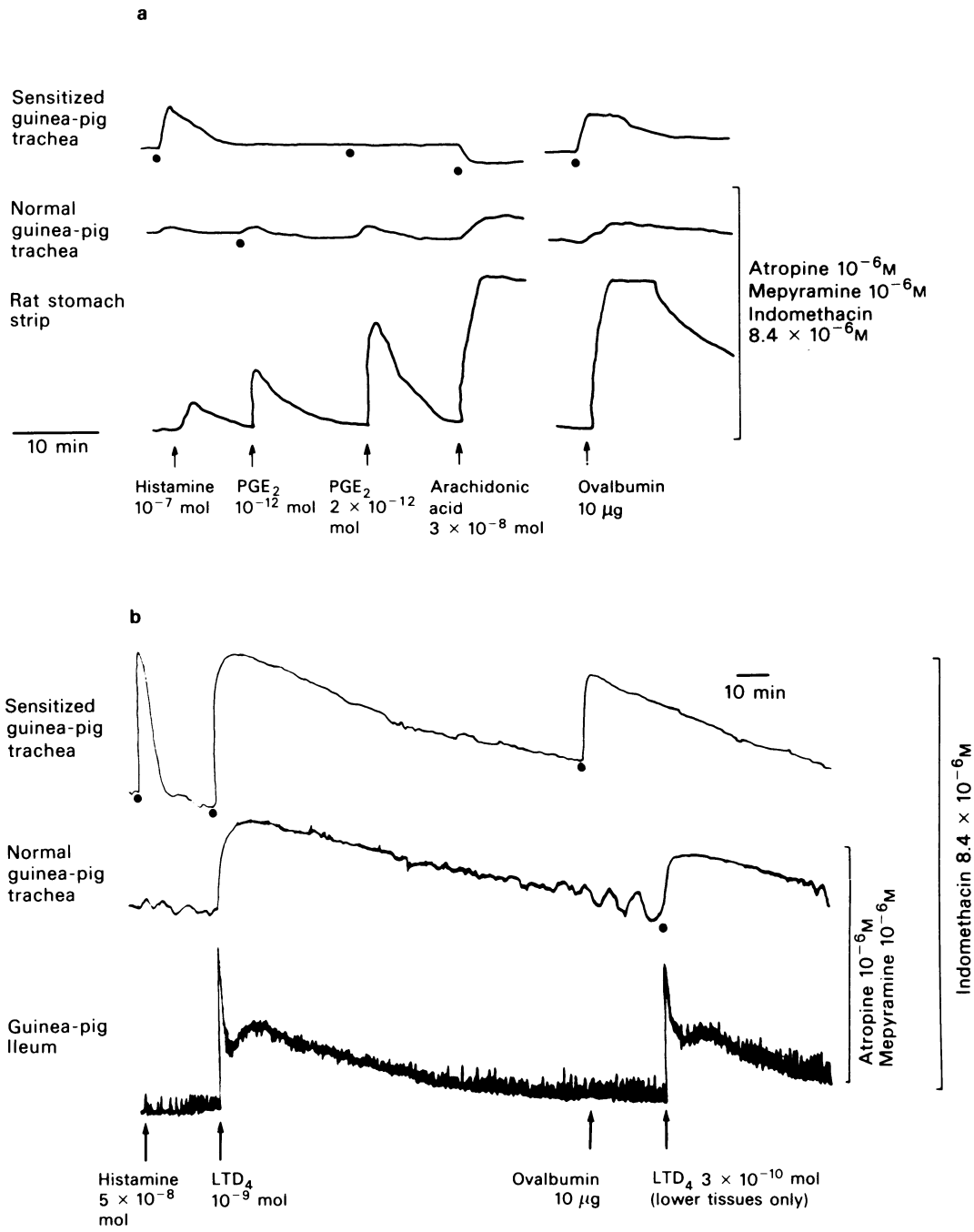


**Figure 1** Cascade superfusion of sensitized guinea-pig trachea over selected bioassay tissues in the absence (a) or presence (b) of indomethacin. The dots (●) indicate the time and point of addition in the cascade of exogenous mediators or antigen.

**Discussion** Previous studies had shown that $PGE_2$ was the major cyclo-oxygenase product produced by the trachea (Burka, Ali, McDonald & Paterson, 1981) and that inhibition of cyclo-oxygenase by indomethacin enhanced the magnitude of tracheal contraction induced by antigen (Burka & Paterson, 1980b). Indeed, contraction of sensitized trachea on ovalbumin challenge resulted in contraction of normal trachea and rat stomach strip. In contrast, if the sensitized trachea was treated with indomethacin to block the synthesis of prostaglandins and thromboxanes, these contractions were not observed, although the sensitized trachea still contracted to ovalbumin.

This implies that a cyclo-oxygenase product released by the sensitized trachea in response to antigen was responsible for the contraction of normal trachea. The product is probably $PGE_2$ since it is the major cyclo-oxygenase product released by trachea (Burka *et al.*, 1981) and $PGE_2$ contracts trachea in the presence of indomethacin (Burka & Paterson, 1980b; Gardiner & Collier, 1980).

The enhancement of antigen-induced tracheal contraction by indomethacin was assumed to be caused by a combination of decreased negative feedback and increased synthesis of lipoxygenase products, possibly leukotrienes (Burka & Paterson, 1980b). The results in trachea are in contrast to the small airways where leukotriene-induced constriction is inhibited by cyclo-oxygenase inhibitors and is attributed to thromboxane $A_2$ (Piper, Samhoun, Tippins, Williams, Palmer & Peck, 1981). To optimize the synthesis of biologically active lipoxygenase products, sensitized trachea was treated with indomethacin. Surprisingly, neither the normal trachea nor the ileum contracted in response to antigen although they were highly sensitive to $LTD_4$. The results suggest that the substance responsible for contracting sensitized trachea following antigen challenge is either released in close proximity to the contractile tissue (i.e. smooth muscle cells) and then diluted so that measurement by bioassay is not possible, or is rapidly metabolized to a biologically inactive product near the site of action. The latter explanation is less likely because $LTD_4$ is equiactive on the normal trachea and ileum whether added to the sensitized trachea or lower in the cascade. In contrast, $PGE_2$ is synthesized and released from trachea in sufficient quantities for bioassay (Burka & Paterson, 1980a; this study), as is slow reacting substance (i.e., $LTD_4$ and $LTC_4$) from lung parenchyma (Fleisch, Haisch, & Spaethe, 1981). Thus, the substance(s) responsible for antigen-induced tracheal contraction, although appearing to be a lipoxygenase product, is as yet undefined.

This work was supported by a grant from the Medical Research Council of Canada (MA6646) and an establishment grant from the Alberta Heritage Foundation for Medical Research (AHFMR). The author is an AHFMR Scholar.

**References**

BURKA, J.F., ALI, M., McDONALD, J.W.D. & PATERSON, N.A.M. (1981). Immunological and non-immunological synthesis and release of prostaglandins and thromboxanes from isolated guinea-pig trachea. *Prostaglandins*, **22,** 683–691.

BURKA, J.F. & PATERSON, N.A.M. (1980a). Inhibition of antigen-induced release of prostaglandin-like material from guinea-pig trachea by antihistamines, FPL55712 and atropine. *J. Pharm. Pharmac.*, **32,** 869–870.

BURKA, J.F. & PATERSON, N.A.M. (1980b). Evidence for lipoxygenase pathway involvement in allergic tracheal contraction. *Prostaglandins*, **19,** 499–515.

BURKA, J.F. & PATERSON, N.A.M. (1981). The effects of SRS-A and histamine antagonists on antigen-induced contraction of guinea pig trachea. *Eur. J. Pharmac.*, **70,** 489–499.

CHAND, N. & EYRE, P. (1978). The Schultz-Dale reaction: a review. *Agents & Actions*, **8,** 171–184.

CONSTANTINE, J.W. (1965). The spirally cut tracheal strip preparation. *J. Pharm. Pharmac.*, **17,** 384.

FLEISCH, J.H., HAISCH, K.D. & SPAETHE, S.M. (1982). Antigen or ionophore releases slow reacting substance (SRS) during contraction of guinea pig lung parenchyma. *J. Allergy Clin. Immunol.*, **69,** 107 (abstract).

GARDINER, P.J. & COLLIER, H.O.J. (1980). Specific receptors for prostaglandins in airways. *Prostaglandins*, **19,** 819–841.

PIPER, P.J., SAMHOUN, M.N., TIPPINS, J.R., WILLIAMS, T.J., PALMER, M.A., & PECK, M.J. (1981). Pharmacological studies on pure SRS-A, and synthetic leukotrienes $C_4$ and $D_4$. In *SRS-A and Leukotrienes.* ed. Piper, P.J. pp. 81–99. Chichester: Research and Studies Press.

VANE, J.R. (1964). The use of isolated organs for detecting active substances in the circulatory blood. *Br. J. Pharmac., Chemother.*, **23,** 360–373.

(*Received April 20, 1982.*)