J. Pharm. Pharmacol. 1983, 35: 755–756
Communicated March 15, 1983

Interaction of leukotriene D_4 and histamine on bronchomotor tone, in the guinea-pig

A. G. STEWART*, D. C. THOMPSON, M. R. FENNESSY, *Department of Pharmacology, University of Melbourne, Parkville, Victoria, 3052, Australia*

Leukotriene D_4 (LTD_4) has been identified as one of the major components of SRS-A (Morris et al 1980). In the guinea-pig, LTD_4 produces bronchoconstriction, increases in vascular permeability and variable cardiovascular effects dependent on the state of consciousness (Drazen et al 1980). Its release during immediate hypersensitivity reactions suggests it has a role in asthma. Since LTD_4 is released concomitantly with other bronchoconstrictor mediators (e.g. histamine, bradykinin), the possibility of mutual potentiation of bronchoconstrictor actions arises. We have investigated a possible interaction between LTD_4 and histamine in both isolated and intact airway preparations of the guinea-pig.

Methods

Guinea-pigs of either sex (Dunkin Hartley) (350–450 g), were killed by a blow to the head, and tracheae and lungs rapidly removed. Isolated tracheal and parenchymal lung strips were mounted in 25 ml organ baths in Krebs solution maintained at 37 °C and gassed with 5% CO_2 in O_2.

Tracheal strips (Emmerson & Mackay 1979) were mounted under a load of 0·5 g, equilibrated for 60 min and then exposed to a supramaximal concentration of acetylcholine (ACh 0·5 mM). Parenchymal lung strips (Drazen & Schneider 1978) were mounted under a load of 0·5 g, equilibrated for 30 min and then exposed to ACh (0·5 mM). Responses to histamine are expressed as a % of the response to ACh. Concentration-response relationships to histamine were obtained by cumulative addition of histamine to the organ bath (van Rossum 1963). To investigate the effect of LTD_4 on histamine concentration-response curves, the concentration of LTD_4 was cumulatively increased from 10 pM until a response was just produced. Histamine concentration-response curves were then constructed in both control and LTD_4-treated preparations.

Guinea-pigs of either sex (350–450 g) were anaesthetized with a mixture of 25% w/v urethane and 0·3% w/v sodium pentobarbitone (5 ml kg^{-1}). A tracheal cannula, for artificial ventilation, was then inserted. The respiration pump (Palmer) was adjusted to deliver 0·7 ml of air/100 g body weight per stroke at a stroke rate of 60 strokes min^{-1}. Intratracheal pressure (ITP) (McCulloch et al 1967) was measured by connecting a T-junction on the tracheal cannula to a Druck pressure transducer (PDCR 75). Following surgical preparation, gallamine (4 mg kg^{-1}, i.v.) was administered to prevent spontaneous respiratory movements. All drugs were injected in 0·2 ml 0·9% NaCl (saline) via a cannula inserted in the left jugular vein; and flushed in with a further 0·2 ml saline. Potentiation of histamine-induced increases in ITP were determined by reference to bracket-control histamine reponses elicited 6 min before and 6 min after the test response to histamine following LTD_4 pretreatment.

The nominal concentration of LTD_4 (as supplied by Merck Frosst, Canada), was confirmed by absorbance at 280 nm. The LTD_4 had an absorption maximum at 280 nm, with shoulders at 290 and 272 nm, closely corresponding to those previously published for LTD_4 (Lewis et al 1980a). In addition, reverse phase h.p.l.c. (Waters, C_{18} μBondapak), using 60:40 methanol–0·1% acetic acid pH 5·6 at a flow rate of 1 ml min^{-1}, showed a single major peak which eluted at 12·5 min with a minor peak at 13·75 min (approximately 8%) which may have been 11-trans LTD_4 (Lewis et al 1980b).

The drugs used were gallamine triethiodide (May & Baker); histamine diphosphate (Sigma); sodium pentobarbitone (Abbott), acetylcholine perchlorate, urethane (BDH Chemicals).

Results

The threshold constrictor concentration of LTD_4 in the parenchymal lung strips (0·19 ± 0·03 nM) was 10-fold lower than that in the isolated trachea (1·94 ± 0·48 nM). LTD_4, in threshold constrictor concentrations, had no effect on either the EC50 or maximum response to histamine ($P > 0·05$, unpaired t-test) in parenchymal or tracheal strips (Table 1).

LTD_4 (0·5 μg kg^{-1}, i.v.) administered 20 s before histamine (4 or 8 μg kg^{-1}, i.v.) significantly potentiated ($P < 0·05$, paired t-test) the responses to these doses of histamine (Table 2). There was a small bronchoconstrictor response to LTD_4 alone ($P < 0·05$, paired t-test) which, when superimposed on that to histamine alone, does not account for the increase in ITP when these bronchoconstrictors were administered 20 s apart.

Discussion

LTD_4 potentiates the bronchoconstrictor action of histamine in-vivo. However, neither the potency of histamine nor its maximal response was altered by

* Correspondence.

Table 1. The effect of threshold constrictor concentrations of leukotriene D_4 (LTD_4) on histamine concentration-response curves in isolated airways smooth muscle preparations of the guinea-pig. Responses are expressed as % of the response to acetylcholine (0·5 mM).

Tissue	Concentration of LTD_4 (μM)	EC50 for histamine (μM)	Maximum response to histamine
Trachea	—	3·6 ± 0·7	131·9 ± 10·3
	1·94 ± 0·48	3·5 ± 0·3	126·4 ± 5·5
Parenchyma	—	0·9 ± 0·1	223·3 ± 22·5
	0·19 ± 0·03	1·2 ± 0·4	226·4 ± 27·7

Values are mean ± s.e. of 7 guinea-pigs.
EC50 is the concentration which produced half the maximal response.

Table 2. The effect of LTD_4 (0·5 μg kg^{-1}, i.v.) on the increase in intratracheal pressure (ITP) produced by histamine (4 or 8 μg kg^{-1}) in anaesthetized guinea-pigs. LTD_4 or saline was administered 20 s before the histamine. ITP was measured when the responses were maximal.

Pretreatment	Treatment	ITP (cm H_2O)
—	—	8·03 ± 0·40 (resting ITP)
LTD_4 0·5 μg kg^{-1}	—	9·00 ± 0·37*
Saline 0·2 ml	histamine 4 μg kg^{-1}	11·17 ± 2·66*
LTD_4 0·5 μg kg^{-1}	histamine 4 μg kg^{-1}	19·83 ± 4·69*,**
Saline 0·2 ml	histamine 8 μg kg^{-1}	18·75 ± 3·27*
LTD_4 0·5 μg kg^{-1}	histamine 8 μg kg^{-1}	28·71 ± 2·21*,**

Values are mean ± s.e. of 6–9 guinea-pigs.
* $P < 0·05$, paired t-test compared to resting ITP.
** $P < 0·05$, paired t-test, compared to saline pretreatment for the same dose of histamine.

LTD_4 in either tracheal strips (central airways) or parenchymal strips (peripheral airways). Thus there appears to be no direct interaction between LTD_4 and histamine on the smooth muscle cell. The potency of LTD_4, both in-vitro and in-vivo, observed in this study was less than that obtained by Drazen et al (1980). In view of this, spectral data and the elution profile of LTD_4 on reverse phase h.p.l.c. were obtained and confirmed both the concentration and chemical nature of the material used as LTD_4 (see Methods). Possible reasons for this difference in observed potencies include genetic and dietary differences with respect to guinea-pigs and sensitivity of measurements of bronchomotor tone.

Both the importance and mechanism of the LTD_4-induced potentiation of the bronchoconstrictor action of histamine are yet to be established. However, LTD_4 has been proposed as a primary mediator of asthma (Dahlen et al 1980) and, furthermore, bronchial hyper-reactivity appears to be invariably associated with this disease (Nadel 1980). As such, the data reported here appear consistent with the working hypothesis that LTD_4 may be a mediator of bronchial hyper-reactivity.

We would like to thank Dr J. Rokach (Merck Frosst, Canada, Inc.) for generous gifts of leukotriene D_4.

REFERENCES

Dahlen, S., Hedqvist, P., Hammarström, S., Samuelsson, B. (1980) Nature (London) 288: 484–486

Drazen, J. M., Austen, K. F., Lewis, R. A., Clark, D. A., Goto, G., Marfat, A., Corey, E. J. (1980) Proc. Natl. Acad. Sci. 77: 4354–4358

Drazen, J. M., Schneider, M. W. (1978) J. Clin. Invest. 61: 1441–1447

Emmerson, J., Mackay, D. (1979) J. Pharm. Pharmacol. 31: 798

Lewis, R. A., Austen, K. F., Drazen, J. M., Clark, D. A., Marfat, A., Corey, E. J. (1980a) Proc. Natl. Acad. Sci. 77: 3710–3714

Lewis, R. A., Drazen, J. M., Austen, K. F., Clark, D. A., Corey, E. J. (1980b) Biochem. Biophys. Res. Commun. 96: 271–277

McCulloch, M. W., Proctor, C., Rand, M. J. (1967) Eur. J. Pharmacol. 2: 214–223

Morris, H. R., Taylor, C. W., Piper, P. J., Tippins, J. R. (1980) Nature (London) 285: 104–106

Nadel, J. A. (1980) Prog. Respir. Dis. 14: 1–8

van Rossum, J. M. (1963) Arch. Int. Pharmacodyn. Thér. 143: 299-330