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PLATELET-ACTIVATING FACTOR STIMULATION OF PEPTIDOLEUKOTRIENE RELEASE: INHIBITION BY VASOACTIVE POLYPEPTIDE

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Leukotriene (LT) release stimulated by platelet activating factor was inhibited by vasoactive intestinal polypeptide (VIP) in an in vitro rat lung preparation. This was detected by HPLC and radioimmunoassay. LTC_4 , although the major species in the stimulatory model used, was not detected in peptide -treated preparations and LTD_4 and LTE_4 levels were considerably reduced.

Release of peptidoleukotrienes is classically associated with antigen challenge of sensitised tissue in animal models (1,2,3) and, more recently, in asthma (4). However, observations of LTC_4 and LTD_4 release stimulated by mediators such as C5a (5) and platelet-activating factor (PAF;6 and 7) have been reported.

We have examined the modulation of leukotriene release, generated by PAF stimulation of these compounds, in the rat chopped lung. Leukotrienes were identified and quantitated using a combined HPLC and radioimmunoassay procedure recently developed in this laboratory (8). Pre-incubation of the tissue with indomethacin and PGE_2 was conducted to investigate the effect of cyclooxygenase inhibitors and products on the lipoxygenase pathway.

MATERIALS AND METHODS

Rat chopped lung: Lungs from male Sprague Dawley rats (280-320 g) were perfused free of blood and chopped into approximately 1 mm fragments using a McIlwain tissue chopper, and incubated in oxygenated Tyrodes at 37°C as described previously (9). PAF (3 uM) was added to the bathing fluid and the incubation continued for 15 min. In separate experiments, indomethacin (2.8 uM), VIP (6 uM) and PGE_2 (4 uM) were added at 15 min, 2 min and 2 min respectively before addition of PAF. After removal of lung tissue by filtration, the incubation fluid was cooled on ice.

C_{18} Sep-pak extraction, HPLC and RIA were conducted as described previously (8).

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RESULTS AND DISCUSSION

The PGE_2 incubations were conducted in duplicate and the remaining experiments in triplicate, and the results are mean +/- s.e.m. (see Fig. 1).

Chopped lung preparations, incubated for 15 min, exhibited higher levels of LTC_4 and LTD_4 in indomethacin-treated samples, this increase being effectively reversed by PGE_2.

In the experiments involving addition of VIP prior to PAF stimulation, LTC_4 generation was completely blocked, as evidenced by the failure to detect immunoreactivity in the relevant fractions of the HPLC chromatogram. Concomitantly, reduced levels of LTD_4 and LTE_4 were observed.

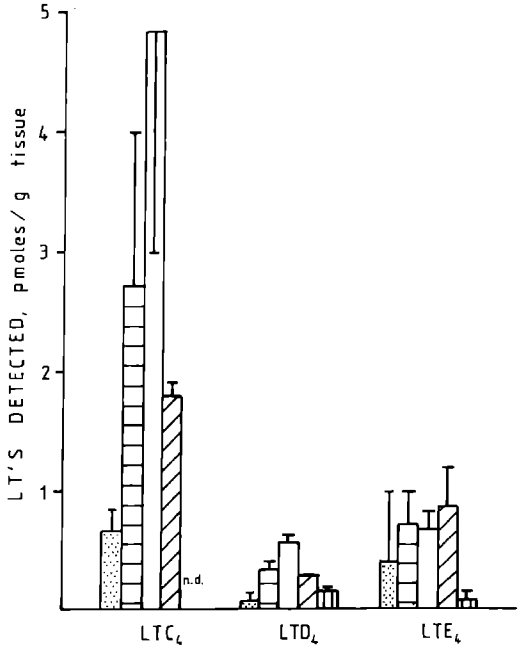

Figure 1. The levels of leukotrienes measured in PAF-stimulated chopped lung are shown. Control experiments are shown by stippled bars. The PAF-stimulated experiments are represented by horizontal hatched bars, the PAF and indomethacin experiments by open bars, the PAF, indomethacin and PGE_2 experiments by diagonally hatched bars and the PAF/VIP experiments by vertically hatched bars. No LTC_4 was detected in the PAF/VIP experiments. Results are mean +/- s.e.m. of three experiments, apart from the PAF, indomethacin and PGE_2 result, which is mean +/- s.e.m of two experiments. Only PAF and PAF/VIP results were statistically significant ($p<0.005$ for the three leukotriene species).

Whilst the results from the indomethacin and indomethacin/PGE_2 incubations cannot be regarded as statistically significant, they do however lend support to earlier pharmacological studies concerning the potentiation of SRS-A release by indomethacin (10), and the inhibition of SRS-A release by PGE_2 (11) supporting view that the arachidonate pathway is composed of a complex negative feedback system.

Importantly, the VIP inhibition of PAF-stimulated LT release appears to be highly significant since LTC_4 could not be detected in any of the three experiments involving VIP pre-incubation, despite a detection limit in the assay of the order of 3.4 fmoles.

Thus, in this model, VIP appears to act as an inhibitor of peptidoleukotriene biosynthesis or release. The mechanism by which a neuroendocrine peptide modulates arachidonate metabolite release may be associated with its role as a modifier of muscarinic receptor actions (12), and it is interesting to note that VIP has been shown to be present in rat lung (13) and mast cells (14). If the work reported here is substantiated, this would represent, to our knowledge, the first example of control of the release of a lipoxygenase product by a neuroendocrine peptide.

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