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Short communication

THE EFFECTS OF SALBUTAMOL, THEOPHYLLINE AND FPL55712 ON LEUKOTRIENE CONTRACTION OF GUINEA PIG TRACHEA

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Contraction of guinea pig tracheal smooth muscle was induced by leukotriene C_4 or leukotriene D_4. The inhibition of the smooth muscle contraction by salbutamol and theophylline was compared with the inhibition by FPL 55712. Salbutamol (5×10^{-8}-5×10^{-7} M) significantly inhibited the leukotriene-induced contraction. The degree of inhibition was greater than that observed with FPL55712 (10^{-6}-10^{-4} M). Theophylline (10^{-6}-10^{-4} M) did not affect the leukotriene-induced contraction, but enhanced the effect of salbutamol when these agents were combined.

Leukotrienes Guinea pig trachea β-Adrenoceptor agonists Theophylline

1. Introduction

Slow reacting substances (SRS) are believed to be important mediators of asthmatic bronchospasm in man. The role of SRS as potent constrictors of guinea pig (Berry and Collier, 1964) and human (Brocklehurst, 1960) airway tissue has been previously documented. A number of laboratories have demonstrated that the major biologically active constituents of SRS are leukotriene C_4 (LTC) and leukotriene D_4 (LTD) (see review; Piper and Tippins, 1980). We have demonstrated that both biosynthetic (Hanna et al., 1981) and chemically synthetized (Hanna et al., unpublished observations) LTC and LTD are potent agonists on human bronchial tissue in vitro.

β-Adrenoceptor agonists are potent bronchodilators. It has been shown that theophylline potentiates the effects of β-adrenoceptor agonists on tracheal smooth muscle tone (Lefcoe et al., 1975). A possible interaction between the β-adrenoceptor agonists and theophylline in asthmatic patients has been suggested.

In the present studies we examined the effect of salbutamol and theophylline, alone, and in combination, on the contraction induced by LTC and LTD on guinea pig trachea. The inhibitory activity of these agents was compared with that of the SRS antagonist, FPL 55712 (Augstein et al., 1973).

2. Materials and methods

Guinea pigs of either sex (300-500 g) were killed by a blow to the head. The trachea and 2 main bronchi were removed and divided into 4 segments of equal length. Each segment was then cut spirally into a strip of approximately 3 mm in width and 50 mm in length and suspended under 2 g tension in a 10 ml organ bath. Each organ bath contained Krebs-Henseleit solution maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2. Changes in tension resulting from the addition of agonists to the bath fluid were measured isometrically with Grass FTO_3 transducers and recorded on a Beckman R511A polygraph. Tissues were

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equilibrated for 1 h or until baseline tension stabilized. Cumulative concentration-response effects of LTC and LTD were examined in the presence and absence of antagonists.

The following antagonists were investigated: salbutamol, 5×10^{-8}-5×10^{-7} M, theophylline, 10^{-6}-10^{-4} M; FPL 55712, 10^{-6}-10^{-4} M. Antagonists were added 15 min before the commencement of the LTC or LTD cumulative addition. At the completion of the leukotriene concentration-response curve carbachol, 5×10^{-5} M, was added to obtain the maximum tissue response. Responses to the leukotrienes were expressed as a percentage of the maximum carbachol response. Only 1 cumulative concentration-response curve was obtained on each tissue. 1 spiral strip from each animal served as control (no added antagonist) and the 3 remaining tissues received the antagonist. The contractile responses to the leukotrienes were plotted against the molar concentrations. Results were expressed as the mean and standard error of the mean (S.E.M.) response for each concentration of agonist (LTC or LTD). Individual dose-ratios from each experiment were determined at 50% of the maximal response to LTD or LTC (ED_{50}). The log (dose ratio -1) was plotted against log antagonist concentration (M) (Arunlakshana and Schild, 1959) and a least squares regression analysis was used to determine the relationship. The individual ED_{50} values from each experiment were converted to logarithmic values (pD_2 values) and the mean and S.E.M. calculated. Results were tested for significance using Student's t-test for correlated or non-correlated data.

The drugs used were carbamycholine chloride (carbachol), propranolol hydrochloride, Sigma Chemical Co. The following drugs were generous gifts: FPL 55712, Fisons Pharmaceuticals Ltd; salbutamol sulphate, Glaxo Canada LTD; theophylline anhydride, Parke Davis Laboratories, Canada. Synthetic leukotriene C_4 and leukotriene D_4 were generously supplied by Dr. J. Rokach, Merck-Frosst Laboratories, Quebec, Canada. Solutions of LTC and LTD in distilled water were diluted with Krebs-Henseleit solution on the day of the experiment. All other drug solutions were prepared in Krebs-Henseleit solution.

3. Results

The physiological antagonist, salbutamol, induced an initial decrease in tension when added to

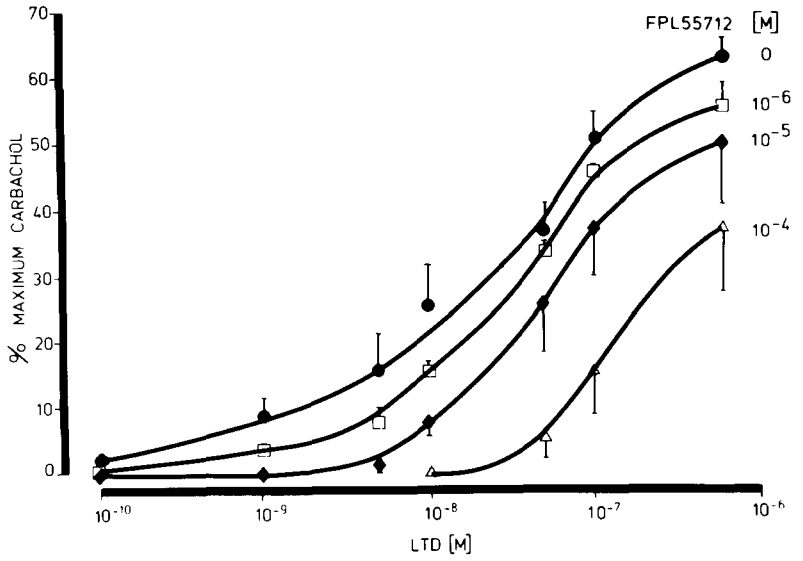

Fig. 1. Effects of salbutamol and theophylline on the contraction induced by leukotriene D (LTD) on guinea pig trachea (n = 5). Results are expressed as percentages of the maximum response to carbachol (5×10^{-5} M). Vertical bars denote S.E.M.

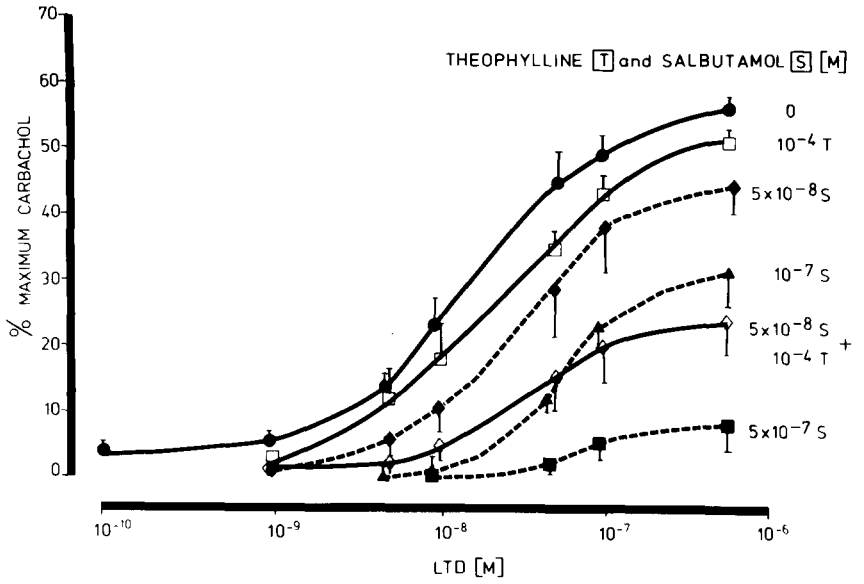

Fig. 2. The effect of FPL 55712 on the contraction induced by leukotriene D (LTD) on guinea pig trachea. Results are expressed as percentages of the maximum response to carbachol (5×10^{-5} M). Vertical bars denote S.E.M.

the organ bath fluid. The concentration-response curves to LTD and LTC were commenced from this lowered baseline. The pD_2 value for LTD was 8.07 ± 0.17 and 8.24 ± 0.41 for LTC.

Salbutamol shifted the concentration-response curve to LTD to the right and depressed the maximum response (fig. 1). The maximum contraction to LTD was $54.6 \pm 3.1\%$ ($n = 5$) of the carbachol response. This compares with a value of $47.8 \pm 19\%$ for LTC ($n = 5$; not shown). A concentration of 5×10^{-8} M salbutamol changed the pD_2 from a value of 8.07 ± 0.17 to 7.68 ± 0.17 for LTD. Higher concentrations of salbutamol, 10^{-7} M, 5×10^{-7} M gave values of 7.20 ± 0.03 and 7.09 ± 0.10 respectively ($P < 0.05$; $n = 5$). Similar results were obtained with LTC and salbutamol. The effects of 5×10^{-8} M salbutamol on both the LTC and LTD responses were abolished when the tissues were pre-incubated with 10^{-6} M propranolol.

Theophylline, at the concentrations investigated, (10^{-6}, 10^{-5}, 10^{-4} M) induced an initial fall in baseline tissue tone. The LTD-induced contraction was not modified by theophylline (fig. 1). However, when a concentration of 10^{-4} M theophylline and 5×10^{-8} M salbutamol were combined, the shift of the LTD concentration-response curve to the right and depression of the maximum response were greater than that observed with either drug alone. The pD_2 value was 7.30 ± 0.16 (control, 8.07 ± 0.17) and the maximum contraction to LTD was $23.2 \pm 6.4\%$ in the presence of salbutamol, 5×10^{-8} M and theophylline, 10^{-4} M. This concentration of theophylline produced a similar effect when used in combination with higher concentrations of salbutamol.

The effects of FPL 55712 on the LTD-induced contraction are shown in fig. 2. There was a progressive shift to the right of the LTD concentration-response curve with increasing concentrations of FPL 55712. A concentration of 10^{-4} M FPL 55712 shifted the pD_2 value from 7.65 ± 0.29 to 6.82 ± 0.21 (not significant) and depressed the maximum response to $37.1 \pm 13.0\%$ (control, $63.2 \pm 2.7\%$, $n = 3$; not significant). Analysis of the shift of the concentration-response curves (Arunlakshana and Schild, 1959) indicated that FPL 55712 was not a competitive antagonist of LTD on the guinea-pig trachea. The slope of the plot of log (dose ratio -1) against log antagonist concentra-

tion was 0.61, which was significantly different from unity.

4. Discussion

Both LTC and LTD produced a concentration-dependent contraction of guinea pig trachea. Although the pD_2 values of 8.24 ± 0.41 (LTC) and 8.07 ± 0.17 (LTD) suggested the two leukotrienes differed in potency, this difference was not significant. The concentration-response curves were similar and there was no significant difference in the maximum contraciton. These data differ from studies on guinea pig trachea by Drazen et al. (1980), in which it was suggested LTD was significantly more potent than LTC. The data agree with Hedqvist et al. (1980), using guinea pig trachea, and previous studies from this laboratory using human airway smooth muscle (Hanna et al., 1981). The present results agree with previous reports that LTC and LTD are less efficacious agonists than carbachol (Drazen et al., 1980; Hanna et al., 1981). The β-adrenoceptor agonist, salbutamol was a potent physiological antagonist of leukotriene-induced contractions. This antagonism is consistent with previous reports regarding inhibition of other smooth muscle contractile stimuli by salbutamol. Although individual concentrations of theophylline, up to 10^{-4} M, did not modify the LTD-induced contraction, combination of theophylline with salbutamol increased the effect of each salbutamol concentration investigated. The observed potentiation is consistent with previous in vitro data examining the activity of these agonists (Lefcoe et al., 1975).

The two physiological antagonists, as well as salbutamol alone, reduced the leukotriene-induced airway smooth muscle contraction to a greater degree than the SRS antagonist, FPL 55712. This suggests that the activity of FPL 55712 on airway tissue may be less than that observed previously on the guinea pig ileum. Our studies also show that unlike its activity on guinea pig ileum, FPL 55712 fails to show competitive antagonism of leukotrienes on guinea pig trachea.

The leukotrienes may play a major role in the pathogenesis of asthma. Inhibition of the smooth muscle contraction induced by the leukotrienes is, therefore, an important property of pharmacologic agents used in the treatment of this disease. Our data is compatible with in vivo studies showing that administration of FPL 55712 to asthmatic patients was less efficacious than salbutamol in improving pulmonary function (Lee et al., 1981). We suggest that the lack of effect of FPL 55712 in asthmatics cannot be construed as evidence against a role for leukotrienes in the causation of asthma. These results suggest that the β-adrenoceptor agonists are more effective than the specific antagonists available at present. The present study also provides support for the use of theophylline in combination with β-adrenoceptor agonists to enhance the therapeutic effect.

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