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Modulation of substance P-induced bronchoconstriction by lipoxygenase metabolites

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In anaesthetized, mechanically ventilated guinea-pigs, substance P induces a bronchoconstrictor response comprising increases in airway resistance and decreases in dynamic compliance. Eicosatetraynoic acid (ETYA, 20 mg kg^{-1} i.v.) or BW755c (20 mg kg^{-1} i.v.) potentiated the substance P-induced bronchoconstriction. Neither indomethacin (1 or 5 mg kg^{-1} i.v.) nor aspirin (20 mg kg^{-1} i.v.) significantly altered the potency of substance P on bronchomotor responses. These observations are consistent with the existence of a bronchodilator lipoxygenase metabolite(s).

Immunohistochemical identification of substance P-containing nerves in the guinea-pig respiratory tract (Nilsson et al 1977) has initiated interest in the possible role(s) of this neuropeptide in the airways. In the guinea-pig, substance P induces bronchoconstriction (Andersson & Persson 1977). Further immunohistochemical studies indicate that the substance P-containing nerves in the guinea-pig lung originate from vagal primary sensory neurons in the nodose ganglion (Terenghi et al 1983). Lundberg et al have reported that pulmonary substance P-containing sensory fibres may be stimulated antidromically to elicit bronchoconstriction and increased pulmonary vascular permeability (Lundberg & Saria 1982a, b; Lundberg et al 1983a; Saria et al 1983). In addition, exposure of the airways to irritants, such as ether or cigarette smoke, also elicits an increase in pulmonary vascular permeability (Lundberg & Saria 1983; Lundberg et al 1983b) which is prevented by a regimen of capsaicin pretreatment reported to deplete vagal substance P (Gamse et al 1981). It has recently been reported that the substance P-induced tone in the guinea-pig isolated trachea may be enhanced by metabolites of arachidonic acid (Regoli et al 1984). It is not known whether a similar effect occurs in-vivo. Since substance P may be an important regulator of bronchomotor tone, we have investigated its actions in anaesthetized guinea-pigs treated with the cyclo-oxygenase inhibitors indomethacin or aspirin, and inhibitors of both cyclo-oxygenase and lipoxygenase enzymes, eicosatetraynoic acid (ETYA) or BW755c.

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Methods

Guinea-pigs of either sex (400–650 g) were anaesthetized by an injection of a mixture of 25% w/v urethane and 0·3% w/v sodium pentobarbitone (4–6 ml kg^{-1} i.p.). The respiratory pump (Palmer) delivered 0·7 ml of air/100 g weight per stroke at a rate of 60 strokes min^{-1}. Airways resistance (R_L) and dynamic compliance (C_{dyn}) were measured according to Diamond (1972) as modified from the method of Amdur & Mead (1958). Transpulmonary pressure (TPP) was obtained by measurement of the difference in pressure between the trachea and the interior of the whole body plethysmograph using a Pye differential pressure transducer. Inspiratory and expiratory flow rates (Q) were measured across a pneumotachograph. The difference in pressure across the pneumotachograph was detected by a Statham differential pressure transducer. The preamplified (Grass Polygraph model 79D) signals representing Q and TPP were fed into a modified EAI 180 analogue hybrid computer onto which was patched a program for breath to breath on-line computation of R_L and C_{dyn} as modified from that of Mindlin (1969). The computed values of R_L and C_{dyn} were displayed and recorded on a two channel Rikadenki recorder. The carotid artery was cannulated and mean arterial blood pressure was monitored on a Grass Polygraph (Model 79D). All drugs were injected in a volume less than 0·5 ml kg^{-1} via a cannula placed in the left jugular vein

and the cannula was flushed with 0·2 ml 0·9% NaCl (saline) after each drug administration. Following surgical preparation, gallamine (4 mg kg^{-1} i.v.) was administered to prevent spontaneous respiratory movements.

Control responses to doses of substance P in the range 0·5–4·0 μg kg^{-1} i.v. were obtained such that one response elicited an increase in R_L of less than 100% and a higher dose elicited an increase in R_L of greater than 100%. These doses of substance P elicited decreases in C_{dyn} of less than, and greater than 50%, respectively. The doses of substance P required to elicit 100% increases in R_L and 50% decreases in C_{dyn} were determined by linear regression. The use of linear regression to obtain the logs of the doses eliciting 100% increases in R_L and 50% decreases in C_{dyn} is justified since, in the present study, a linear relation between the log dose and the response was found for R_L ($r = 0·383$, $n = 92$, $P < 0·001$) and for C_{dyn} ($r = -0·484$, $n = 92$, $P < 0·001$). The relatively low correlations probably reflect a large degree of inter-animal variability in sensitivity to substance P. In all experiments a 20 min period followed, after which responsiveness to substance P was again determined. In separate experiments, one of the vehicles (0·5 ml saline kg^{-1} or 0·5 ml 0·1 M Na_2CO_3 kg^{-1}), indomethacin (1 or 5 mg kg^{-1} i.v.), aspirin (20 mg kg^{-1} i.v.), BW755c (20 mg kg^{-1} i.v.) or ETYA (20 mg kg^{-1} i.v.) was administered at the commencement of the 20 min treatment following initial determinations of responsiveness to substance P. Data are presented as the changes in the log ED100 R_L or log ED50 C_{dyn} values for substance P in the second period compared with that in the first period, together with the antilogs of these values, i.e., the potency ratios. Statistical significance was determined by comparison of the log shifts obtained in animals treated with enzyme inhibitors to those obtained in the vehicle-treated animals using the unpaired Student's *t*-test.

For clarity, in the Table, post-treatment sensitivity to the bronchoconstrictor actions of substance P is presented in the first four results columns with statistically significant changes only in the last two columns.

Drugs were: aspirin (Nicholas, Australia); BW755c [(3-amino-1-(*m*-trifluoromethyl)phenyl) pyrazoline HCl] (Wellcome Research Laboratories), eicosatetraynoic acid (Roche); gallamine triethiodide (May & Baker); indomethacin (Merck, Sharp & Dohme); sodium pentobarbitone (Abbott); substance P (Sigma); urethane (BDH Chemicals).

Results

Substance P (0·5–4·0 μg kg^{-1} i.v.) elicited bronchoconstrictor responses in anaesthetized guinea-pigs comprising increases in R_L and decreases in C_{dyn} with no degree of selectivity for either of these parameters (Table 1). Substance P (0·5–4·0 μg kg^{-1} i.v.) also elicited decreases in mean arterial blood pressure. However, the bronchoconstrictor action of substance P peaked after 15–20 s whereas the hypotensive action was maximal after 30–60 s and more prolonged (3–6 min) than the bronchoconstriction (<60 s). Before the treatments there were no significant differences in the sensitivity of the different treatment groups to substance P-induced bronchoconstriction ($P > 0·05$, unpaired Student's *t*-test) with the exception of the BW755c-treated group which was significantly less sensitive to substance P

Table 1. Responsiveness of central (R_L) and peripheral (C_{dyn}) airways to the bronchoconstrictor actions of substance P in the first period (before treatments) and the second period (after treatments). Also included are the effects of modulators of arachidonic acid metabolism on bronchoconstrictor responses to substance P (0·5–4·0 μg kg^{-1} i.v.) in anaesthetized guinea-pigs.

Treatment	n	ED100 R_L (μg kg^{-1}) (95% confidence interval) Before treatment	ED100 R_L (μg kg^{-1}) (95% confidence interval) After treatment	ED50 C_{dyn} (μg kg^{-1}) (95% confidence interval) Before treatment	ED50 C_{dyn} (μg kg^{-1}) (95% confidence interval) After treatment	Change in log ED100 R_L (Potency ratio)	Change in log ED50 C_{dyn} (Potency ratio)
0·1 M Na_2CO_3 0·5 ml kg^{-1}	7	0·85 (0·45–1·58)	0·68 (0·45–1·01)	0·88 (0·50–1·55)	0·77 (0·49–1·22)	−0·098 ± 0·085 (0·80)	−0·058 ± 0·072 (0·88)
Indomethacin 1 mg kg^{-1}	6	1·23 (0·78–1·94)	0·81 (0·54–1·21)	1·24 (0·80–1·94)	0·76 (0·45–1·29)	−0·184 ± 0·056 (0·65)	−0·213 ± 0·081 (0·61)
Indomethacin 5 mg kg^{-1}	5	1·70 (0·74–3·88)	1·14 (0·50–2·56)	1·56 (0·75–3·26)	1·17 (0·54–2·50)	−0·174 ± 0·039 (0·67)	−0·126 ± 0·058 (0·75)
ETYA 20 mg kg^{-1}	7	0·98 (0·52–1·85)	0·46 (0·29–0·70)	1·01 (0·68–1·49)	0·53 (0·34–0·84)	−0·333 ± 0·073 (0·46)	−0·277 ± 0·030* (0·53)
Aspirin 20 mg kg^{-1}	4	0·66 (0·29–1·51)	0·60 (0·24–1·51)	0·84 (0·30–2·26)	0·72 (0·30–1·71)	−0·037 ± 0·043 (0·92)	−0·083 ± 0·023 (0·83)
Saline 0·5 ml kg^{-1}	8	1·43 (0·99–2·06)	1·14 (0·80–1·61)	1·48 (0·88–2·47)	1·23 (0·38–1·37)	−0·100 ± 0·043 (0·79)	−0·052 ± 0·062 (0·89)
BW755c 20 mg kg^{-1}	5	4·51 (1·73–11·78)	1·06 (0·37–3·01)	4·01 (1·56–10·33)	1·31 (0·59–2·92)	−0·629 ± 0·096†‡ (0·24)	−0·485 ± 0·090†‡ (0·33)

*$P < 0·05$, †$P < 0·001$, unpaired Student's *t*-test compared to the log shift obtained in vehicle-treated animals.
‡ The shift in BW755c-treated animals is compared with that in saline-treated animals since BW755c was dissolved in saline whereas the vehicle for the other pretreatments was 0·1 M Na_2CO_3.

($P < 0.05$, unpaired Student's t-test). Nevertheless, the ability of BW755c to potentiate substance P did not correlate with the initial sensitivity to substance P ($r = 0.172$, $P > 0.1$). Treatment with either aspirin (20 mg kg^{-1} i.v.) or indomethacin (1 or 5 mg kg^{-1} i.v.) did not significantly alter ($P > 0.10$, unpaired Student's t-test) the potency of substance P on R_L or C_{dyn} (Table 1). In contrast, treatment with ETYA (20 mg kg^{-1} i.v.) or BW755c (20 mg kg^{-1} i.v.) increased the potency of substance P on C_{dyn} ($P < 0.02$, unpaired Student's t-test). The potency of substance P on increases in R_L was significantly increased by BW755c ($P < 0.001$, unpaired Student's t-test) whereas ETYA treatment did not have a significant effect ($0.1 > P > 0.05$, unpaired Student's t-test).

Discussion

Substance P-induced bronchoconstrictor responses in both central (R_L) and peripheral (C_{dyn}) airways were enhanced by the combined cyclo-oxygenase, lipoxygenase inhibitor, BW755c, whereas ETYA enhanced responses only in peripheral airways. On the other hand, inhibition of cyclo-oxygenase by indomethacin or aspirin did not significantly alter the potency of substance P. These observations are in contrast to those of Regoli et al (1984), who showed that indomethacin increased the contractile response of the guinea-pig isolated trachea to a single concentration of substance P. They also reported that inhibition of lipoxygenase and cyclo-oxygenase enzymes using mepacrine, BW755c or ETYA did not result in enhancement. Earlier studies by Orehek et al (1975) indicated that indomethacin, but not ETYA, inhibited the contractile response of the isolated trachea to low concentrations of histamine and acetylcholine. Thus, an examination of the effect of indomethacin on the contractile effects of low concentrations of substance P may reconcile the apparent discrepancy between in-vivo and in-vitro data.

To explain the present findings, it is necessary to postulate the existence of a lipoxygenase metabolite formed in-vivo or in airways distal to the trachea which activates a bronchodilator mechanism. Since indomethacin treatment results in both a reduction in cyclo-oxygenase metabolites and, as reported by Walker et al (1980), an increase in lipoxygenase metabolites, the anticipated enhancement by indomethacin in the present study may have been masked by an increased generation of the postulated bronchodilator lipoxygenase product. Enhancement of the bronchoconstrictor effect of substance P by ETYA or BW755c suggests that the predominant influence of products of arachidonic acid in-vivo is inhibitory and that the inhibitory product may be formed by a lipoxygenase enzyme.

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