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Evidence against leukotriene-mediation of propranolol-induced airway hyperreactivity to acetylcholine

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In unanaesthetized guinea-pigs, propranolol treatment (0·1 mg kg^{-1} i.v.) substantially increased reactivity to intravenous acetylcholine infusion or aerosolized histamine to a comparable degree. Neither BW755c (5 mg kg^{-1} i.v.), FPL 55712 (1 mg kg^{-1} i.v.), nor piriprost (5 mg kg^{-1} i.v.) pretreatment influenced propranolol's effect on muscarinic reactivity although BW755c abolished histaminic hyperreactivity. This suggests that propranolol-induced muscarinic hyperreactivity in the guinea-pig is not mediated by leukotrienes whereas histaminic hyperreactivity may be.

Propranolol may increase airway reactivity to different bronchoconstricting agents in a variety of species, including man (Zaid & Beall 1966; Douglas et al 1973; MacLagan & Ney 1979; Mue et al 1980). The mechanism of this effect is unclear. Several years ago, MacLagan & Ney (1979) found no correlation, and thus, no causal relationship, between the bronchospasm and the pulmonary β-adrenoceptor blockade produced by propranolol treatment. Ney (1983) found that propranolol-induced hyperreactivity to histamine or 5-hydroxytryptamine (5-HT) was inhibited by either BW755c, an antagonist of both the lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism, or FPL 55712, an antagonist of slow reacting substance of anaphylaxis. This evidence suggests that the bronchial hyperreactivity produced by propranolol to some agonists may be leukotriene-mediated. Stimulation of the airways by muscarinic agonists (Miller et al 1976) differs in several respects from that by 5-HT and histamine (Gold et al 1972). To investigate whether propranolol-induced bronchial hyperreactivity to acetylcholine (ACh) or histamine occurred in unanaesthetized guinea-pigs and whether leukotrienes were involved, we studied muscarinic or histaminic bronchomotor tone before and after propranolol treatment in guinea-pigs pretreated with BW755c. In those challenged with ACh, the effect of pretreatment with FPL 55712, or piriprost, a pyrroloprostacyclin antagonist of leukotriene C/D biosynthesis (Bach et al 1983), was also assessed.

Methods

Ten male, English short-haired guinea-pigs (550–750 g) were used. Both baseline specific airway resistance (SRaw (ml × cm H_2O/(ml s^{-1})) and bronchial reactivity to either intravenous ACh or aerosolized histamine were determined before (on 3 separate occasions) and after drug treatment. Treatment consisted of propanolol (0·5 mg kg^{-1} i.v. via an indwelling jugular venous cannula (Roum & Murlas 1984)) given 15 min before ACh or histamine. On another occasion in each animal, propranolol was followed by BW755c (5 mg kg^{-1} i.v.) administered 1 min before ACh or histamine challenges. In those animals tested with ACh, the effect of FPL 55712 (1 mg kg^{-1} i.v.) or piriprost (5 mg kg^{-1} i.v.), administered 1 min before ACh infusion, was also evaluated.

SRaw in unanaesthetized guinea-pigs was measured using a constant volume body plethysmograph. The techniques were those of Roum & Murlas (1984). Briefly, each animal was positioned in a two-compartment chamber designed to isolate the head (with mouth closed) from the body and the plethysmograph. Flow at the snout was measured using a pneumotachograph (No. 0; Fleish Instruments, Pres Laussane, Switzerland) connected to a differential pressure transducer (Model MP45-1; Validyne, Northridge, CA). Airflow and box pressure signals were displayed simultaneously on an x-y oscilloscope so that the angle described during the rapid inspiratory phase of the animal's breathing could be measured, and SRaw calculated from it.

Bronchial reactivity was assessed by measuring SRaw as a function of the rate of ACh infused or concentration of histamine inhaled. For ACh testing, if no change occurred in baseline SRaw during normal saline infusion, 1 μg kg^{-1} min^{-1} ACh was delivered, and mean SRaw for 3 consecutive breaths was recorded after steady state had been reached. At the end of 90 s, the rate of infusion was approximately doubled, and the

new steady state SRaw was measured. This process was repeated until at least doubling of the baseline SRaw occurred. Reactivity to inhaled histamine was determined using a nebulizer (model 1700, Hudson, Temecula, CA) driven by a compressed air source at 28 psi (nebulizer output: 290 ± 8 μl min^{-1}; aerosol particle size: 4·28 ± 1·62 μm aerodynamic mass median diam.). After baseline SRaw was determined, 40 breaths of 0·01% histamine were administered, and the peak SRaw over the next 4 min was recorded. The concentration of histamine was then approximately doubled, and the delivery was repeated. This process was repeated until at least a doubling of the baseline SRaw occurred.

Cumulative ACh or histamine dose-response curves were constructed by plotting, on semilogarithmic paper, baseline SRaw and the peak values of SRaw for each infusion rate (ACh) or aerosolized dose (histamine) administered. The rate which produced a doubling of baseline SRaw (ED200 ACh in μg kg^{-1} min^{-1}) was determined by interpolation. Animals showing a greater than 50% decrease in the ED200 ACh or ED200 histamine post-propranolol, were considered hyperreactive. Changes in SRaw or log ED200 (ACh or histamine) (log ED200 pre-propranol–log ED200 post-propranolol) in relation to the initial values for the animal groups were compared using the two-tailed, two-sample *t*-test. In all cases, differences were considered significant for $P < 0.05$.

Drugs. Acetylcholine chloride (Sigma) was diluted in 0·9% NaCl (saline) to 73·5 μg ml^{-1}; histamine dihydrochloride (Sigma) was diluted in phosphate-buffered saline (composition in mM: NaCl, 144; KCl, 3·2; Na_2HPO_4, 6·5; KH_2PO_4, 1·5; $CaCl_2 \cdot 6H_2O$, 0·5; pH 7·4 at 37 °C); propranolol (Sigma) in saline to 1 mg ml^{-1}; BW755c (3-amino-1-[(*m*-trifluoromethyl)phenyl]-2-pyrazoline, Wellcome) in saline to 5 mg ml^{-1}; FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl[4*H*]-chromene-2-carboxylate, Merck Frost) in saline to 1 mg ml^{-1}; and piriprost (U-60,257 Upjohn) in Tris buffer (pH 8·0) to 10 mg ml^{-1}. All drug dilutions were made up immediately before use.

Results

In all animals before drug treatment, neither SRaw nor bronchial reactivity to i.v. ACh infusion or aerosolized histamine was significantly different on three separate occasions. Propranolol treatment substantially increased reactivity in all animals tested with either ACh or histamine (Fig. 1). However, no significant change in baseline SRaw occurred in either group. The change in log ED200 ACh after propranolol for one group was similar to the change in log ED200 histamine for the other (Table 1).

Treatment with BW755c, FPL 55712 or piriprost after propranolol, did not affect SRaw in any animal tested, whereas BW755c abolished propranolol-induced histaminic hyperreactivity in all animals studied (Fig. 1); no significant change in muscarinic reactivity post-propranolol occurred in animals treated with BW755c, FPL 55712 or piriprost (Table 1).

Table 1. Effects of drug treatment on specific airway resistance (SRaw) and muscarinic or histaminic reactivity in unanaesthetized guinea-pigs.

	Change in SRaw after treatment (ml × cm H_2O/ml s^{-1})		Change in log ED200 (ACh or histamine) after treatment	
Treatment	ACh group	Histamine group	ACh group	Histamine group
Non (NS)	0·04 ± 0·02	0·03 ± 0·02	0·03 ± 0·01	0·04 ± 0·02
Propranolol alone	0·09 ± 0·03	0·07 ± 0·03	0·48 ± 0·10	0·46 ± 0·10
Propranolol plus:				
BW755c	0·04 ± 0·02	0·05 ± 0·02	0·45 ± 0·08	0·07 ± 0·04*
FPL 55712	0·08 ± 0·03	—	0·41 ± 0·09	—
piripost	0·05 ± 0·02	—	0·50 ± 0·09	—

Values represent means ± s.e. for 5 animals in each group.
* Denotes significant difference from propranolol alone treatment ($P < 0.01$).

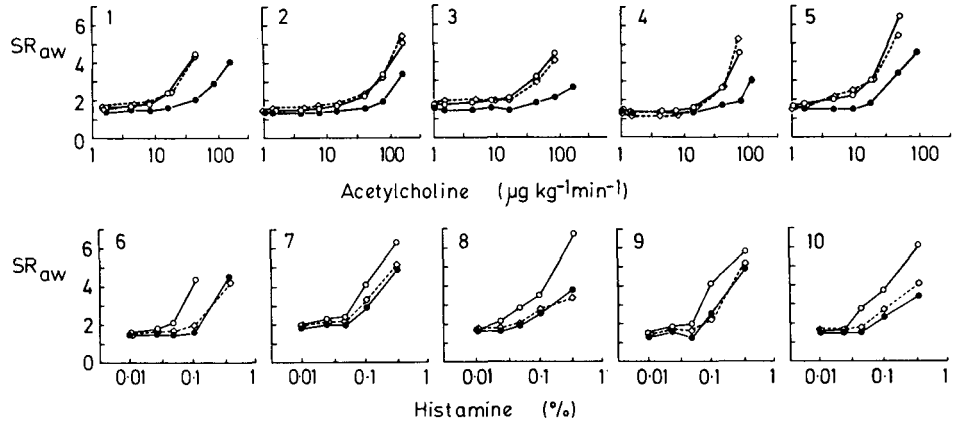

Fig. 1. Dose-response curves demonstrating effect of propranolol on guinea-pig bronchial reactivity to i.v. ACh (cases 1–5) or inhaled histamine (cases 6–10). Each panel illustrates data from one animal. For each case, curves were obtained before propranolol (●), and after propranolol (0·5 mg kg^{-1} i.v.) treatment either with (◇ and broken lines) or without (○) BW755c pretreatment.

Discussion

Treatment with either BW755c or FPL 77512 failed to inhibit propranolol-induced muscarinic bronchial hyperreactivity in unanaesthetized guinea-pigs. This is in distinct contrast to the effect of BW755c on propranolol-induced hyperreactivity to histamine that we found in awake animals, or that others have reported in anaesthetized guinea-pigs (Ney 1983). Thus, the differences in results do not appear to relate to the effects of anaesthesia on bronchomotor tone. Furthermore, it seems clear that the differential effect of lipoxygenase product antagonists on histaminic versus muscarinic hyperreactivity is not a result of differences in baseline SRaw or the degree of hyperresponsiveness induced by propranolol in that these were no different for the histamine- and acetylcholine-tested groups in our study. Moreover, piriprost (Bach et al 1983), a leukotriene antagonist which is a more potent blocker of ozone-induced muscarinic hyperreactivity than the other two in our experience (Murlas & Lee 1985), also had no effect on propranolol-induced muscarinic responsiveness. In previous studies, we have found that all three of these compounds administered intravenously, either by constant infusion or by bolus, are effective in preventing ozone-induced bronchial hyperreactivity to intravenous ACh in the guinea-pig (Lee & Murlas 1985; Murlas & Lee 1985).

There is considerable uncertainty at present regarding the mechanism(s) involved in propranolol-induced airway hyperreactivity. In guinea-pigs, MacLagan & Ney (1973) demonstrated that (+)-propranolol, the isomer with weak β-adrenergic blocking activity, was as effective as (±)-propranolol in producing histamine hyperreactivity. Furthermore, this effect was shorter-lasting than were the signs of true β-blockade. These results indicate that the increased bronchomotor tone produced by propranolol may be unrelated to its β-adrenoceptor blocking properties.

The mechanism(s) by which leukotrienes may be involved in propranolol potentiation of airway reactivity to certain bronchoconstrictors like histamine and 5-HT, but not to others like ACh, remains to be determined. The available data, including those of the present study, suggest certain possibilities. ACh-induced bronchoconstriction in-vivo probably occurs by direct stimulation of the muscarinic receptors of airway smooth muscle cells (Miller et al 1976; Murlas et al 1982). It is unaffected by hexamethonium pretreatment but can be abolished by atropine pretreatment. Leukotriene D_4 does not appear to augment airway muscle responsiveness to ACh in-vitro (Weiss & Bellino 1985). In contrast, both histamine (Shore et al 1983) and 5-HT (Sheller et al 1982) appear to cause bronchoconstriction, in part, by stimulating the pre-synaptic release of ACh from post-ganglionic fibres which innervate the airways. In-vitro, leukotriene D_4 appears to potentiate airway muscle responsiveness to histamine (Weiss & Bellino 1985). Thus, it is possible that leukotrienes participate in propranolol-induced airway hyperreactivity to some bronchoconstrictors like histamine by augmenting the pre-synaptic ACh release stimulated by such agonists. This merits further investigation.

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