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Effects of Propranolol and Indomethacin on Muscarinic Airway Reactivity in Unanesthetized Guinea Pigs[1] (42294)

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Abstract. To investigate whether endogenous β-adrenergic stimulation or cyclooxygenase products normally affect muscarinic reactivity in conscious, spontaneously breathing guinea pigs, we measured specific airway resistance (SR_{aw}) during acetylcholine (ACh) infusion before and after treatment with propranolol (10 mg/kg ip) or indomethacin (30 mg/kg ip). Airway reactivity was assessed by measuring changes in SR_{aw} upon increasing ACh infusion. We found that propranolol treatment increased reactivity to parenteral ACh, but did not change baseline SR_{aw}. Furthermore, propranolol reduced the range in muscarinic reactivity for the group, and it enhanced the reproducibility of measurements in individual animals. In contrast, indomethacin had no effect on either baseline SR_{aw} or muscarinic reactivity. Our results suggest that β-blockade of endogenous adrenergic stimulation increases the muscarinic reactivity of guinea pig airways, but does not influence resting airway tone. It appears that propranolol treatment allows a more reproducible assessment of muscarinic reactivity in the guinea pig. In contrast, cyclooxygenase products do not seem to significantly affect baseline airway resistance, reactivity, or reproducibility in the guinea pig.

A characteristic feature of asthma is the increased irritability, or hyperreactivity, of the airways to a wide variety of stimuli. Factors that may affect airway reactivity in health or disease are numerous and not well understood at present. Endogenously released catecholamines are believed to diminish reactivity of histamine in some species (1, 2), but not others (3, 4). Whether this is due to differences in species or to testing techniques, including anesthesia, is unknown. That cyclooxygenase products may affect bronchomotor tone and reactivity is suggested by the fact that indomethacin potentiates histamine- and vagal-mediated bronchoconstriction *in vitro* (3, 4). However, the effect of indomethacin on bronchial reactivity *in vivo* has not yet been fully studied.

We have previously reported that it is advantageous to test muscarinic bronchial reactivity by intravenous rather than by aerosolized challenge in that the range of reactivity in the sample population is decreased, and the reproducibility of measurements is improved (5). In this study, we have examined whether blockade of endogenous β-adrenergic stimulation or cyclooxygenase product elaboration affects basal airway resistance and muscarinic reactivity in intact, unanesthetized guinea pigs, and improves within as well as between subject testing.

Methods. *Protocol.* Male Hartley strain guinea pigs (550–750 g in weight) were used in the study. Both baseline specific airway resistance (SR_{aw} in (ml × cm H_2O)/(ml/sec)) and bronchial reactivity to intravenous acetylcholine (ACh) were determined before and after drug treatment. SR_{aw} was measured during and after a 2 min infusion of ACh (100 μg/min). Treatment consisted of propranolol (10 mg/kg ip, $N = 5$) or indomethacin (30 mg/kg ip, $N = 5$). After treatment, measurements were made $\frac{1}{2}$ hr after propranolol or 1 hr after indomethacin (4). Reactivity measurements before and after propranolol were repeated 1 week later to assess reproducibility of the testing methods.

Procedures. SR_{aw} was measured in intact, unanesthetized, spontaneously breathing guinea pigs placed in a constant volume body plethysmograph. The techniques used were

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similar to those previously described (5, 6). Briefly, each animal was positioned in a two-compartment leucite chamber designed to keep its head fixed (with mouth closed) and isolated from its body and the plethysmograph. Flow at the snout was measured using a pneumotachograph (No. 0; Fleish Instruments, Pres Lausanne, Switzerland) connected to a differential pressure transducer (Model MP45-1; Validyne, Northridge, Calif.). To prevent rebreathing of carbon dioxide from the dead-space around the nares and within the pneumotachograph, a constant flow of warm, humidified air was provided at the nares. The animal in the chamber was placed in the plethysmograph, which was equipped with another transducer for measuring changes in box pressure. The pneumotachograph was calibrated using a rotometer (Model 1355-01C1AAA; Brooks Instrument, Hatfield, Pa.) to pass known airflows through it. Changes in box pressure were calibrated (with the box tightly sealed) by rapidly delivering boluses of air from a calibrated syringe. Airflow and box pressure signals were displayed simultaneously on an x-y oscilloscope (Model 502A; Textronix, Portland, Ore.). The angle described during the rapid inspiratory phase of the animal's breathing was measured, and SR_{aw} was calculated from it (6).

Bronchial reactivity was assessed by measuring SR_{aw} as a function of increasing rates of infused ACh. Agonist was delivered using a syringe pump (Model 600-910; Harvard Apparatus Co., Dover, Mass.). After baseline SR_{aw} had been measured (the highet mean SR_{aw} for three consecutive breaths), ACh in normal saline was infused via a 0.61 mm od, 0.28 mm id polyethylene cannula that had been previously placed in the external jugular vein (5). Initially, 1 μg/min ACh was delivered to the animal, and SR_{aw} for 3 consecutive breaths was again calculated after steady state had been reached. At the end of 2 min, the rate of infusion was approximately doubled, and the new steady-state SR_{aw} was measured. This process was repeated until at least a doubling of the baseline SR_{aw} occurred.

Cumulative intravenous ACh dose–response curves were constructed by plotting, on semilogarithmic paper, baseline SR_{aw} and the steady-state value of SR_{aw} for each infusion rate of ACh administered. The rate which produced a doubling of baseline SR_{aw} (ED_{200}ACh in μg/kg/min) was determined by interpolation. Changes in SR_{aw} or log-ED_{200}ACh ($\log ED_{200}$ACh pretreatment $-$ $\log ED_{200}$ACh post-treatment) were compared using the two-tailed, two-sample t test. In all cases, a level of more than 95% probability was considered significant.

Acetylcholine chloride (Sigma, St. Louis, Mo.) was diluted in heparinized saline (10 u/ml heparin in 0.9% NaCl) to 73.5 μg/ml, and propranolol (Sigma) in normal saline to 10 mg/ml. Indomethacin (Sigma) was dissolved in Tris buffer (1 *M*, pH 8.0) to 10 mg/ml.

Results. Propranolol treatment substantially increased bronchial reactivity to parenteral ACh in all five animals. Complete dose–response curves before and after propranolol are shown in Fig. 1. The mean $\log ED_{200}$ACh before propranolol was 2.05 ± 0.13, and after treatment 1.53 ± 0.09. This is demonstrated in Fig. 2, in which the change in $\log ED_{200}$ACh ($\log ED_{200}$ACh pretreatment $-$ $\log ED_{200}$ACh post-treatment) is displayed. This magnitude represents an approximately threefold shift to the left on the dose–response curve. There was no change in baseline SR_{aw} with propranolol treatment alone (Table I).

As demonstrated by dose–response measurements performed 1 week apart for each animal (Fig. 3), treatment with propranolol resulted in greater reproducibility of the determination of ED_{200}ACh. With propranolol, the ED_{200}ACh never varied more than one-half fold; without propranolol, the reactivity often varied more than onefold. The prepropranolol reactivity was generally greater 1 week after the first measurements (more than onefold in two out of the five animals studied). However, on Day 7, treatment again led to increased reactivity in four out of five of these animals. In all cases tested, however, the post-propranolol reactivity 1 week later was very similar to that found initially.

Animals that were less reactive initially showed a greater increase in reactivity with propranolol than those that were initially more reactive. This is represented in Fig. 4 where prepropranolol $\log ED_{200}$ACh is plotted against the change in $\log ED_{200}$ after treatment ($\log ED_{200}$ACh pretreatment $-$ $\log ED_{200}$ACh post-treatment). From linear regression anal-

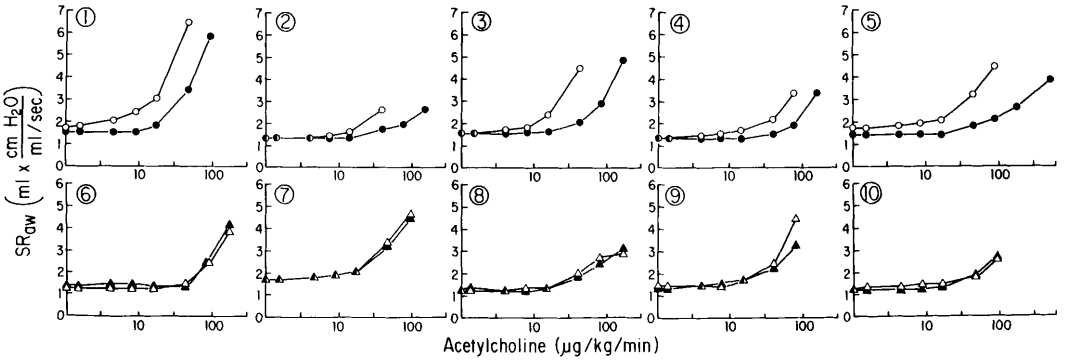

FIG. 1. Dose-response curves demonstrating effect of propranolol (No. 1-5) or indomethacin (No. 6-10) on guinea pig bronchial reactivity to intravenous ACh. Curves were obtained before (●) and after (○) propranolol (10 mg/kg); and before (▲) and after (△) indomethacin (30 mg/kg) treatment. In each case, response was measured as specific airway resistance prior to and after iv ACh infusion. Propranolol led to a shift to the left of the curves, whereas indomethacin did not.

ysis of this data, $r = 0.83$, slope = 1.09, and y intercept = 1.52. Since animals which were less sensitive initially showed a larger change after propranolol, the range of reactivity for all animals was decreased markedly with propranolol treatment (Fig. 3). The range of $ED_{200}ACh$ including measurements from both days prior to treatment was greater than 20-fold, (12.2 to 310.0 μg/kg/min) whereas that after propranolol was less than fivefold (19.5 to 60.4 μg/kg/min).

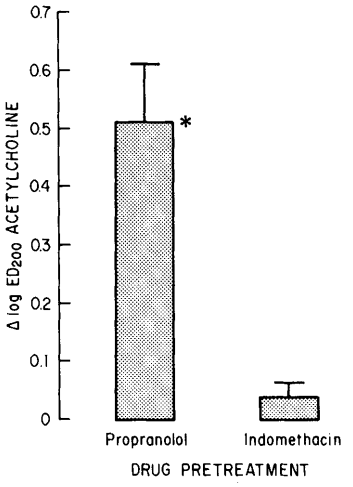

FIG. 2. Comparison of magnitude of shift in the $ED_{200}ACh$ due to propranolol or indomethacin treatment. $ED_{200}ACh$ was derived by interpolation from dose-response curves (Fig. 1). The difference (Δ log) in the log values of $ED_{200}ACh$ are calculated as ($\log ED_{200}ACh$ pretreatment − $\log ED_{200}ACh$ post-treatment). Values represent means ± SE for five guinea pigs. For propranolol, $P < 0.05$.

The time course of change in SR_{aw} during and after a 2-min infusion of ACh both pre- and postpropranolol is illustrated in Fig. 5. Without propranolol, the response was never as large as with propranolol, and was not as regular. Usually, the prepropranolol response was initially large, but recovery occurred even during the infusion; this phenomenon was seen as an "overshoot" in the response. Treatment resulted in a substantially larger response to the muscarinic agonist. In addition, treatment resulted in sustained bronchoconstriction during the infusion in those animals where a large "overshoot" occurred prior to propranolol.

TABLE I. EFFECTS OF PROPRANOLOL OR INDOMETHACIN TREATMENT ON BASELINE SR_{aw} IN THE UNANESTHETIZED GUINEA PIG

	SR_{aw} (ml × cm H_2O/(ml/sec))	
Treatment	Before	After
Propranolol (10 mg/kg, ip)	1.43 ± 0.05	1.52 ± 0.06
Indomethacin (30 mg/kg, ip)	1.37 ± 0.09	1.37 ± 0.10

Note. Values are means ± SE for five animals. There were no significant differences in SR_{aw} before and after treatment.

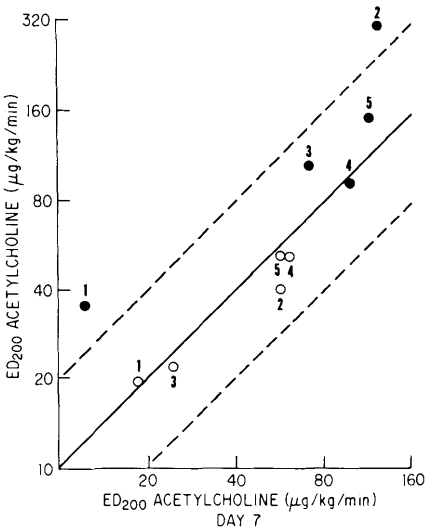

FIG. 3. Correlation of effective rate of ACh infusion that doubled baseline specific airway resistance ($ED_{200}ACh$) on 2 separate days 1 week apart. Solid circles represent measurements made prior to propranolol treatment, and open circles represent those made after. Integers next to symbols represent subject numbers from Fig. 1. Note striking improvement in reproducibility with propranolol treatment. Solid lines represent lines of identity; hatched indicate onefold changes in $ED_{200}ACh$.

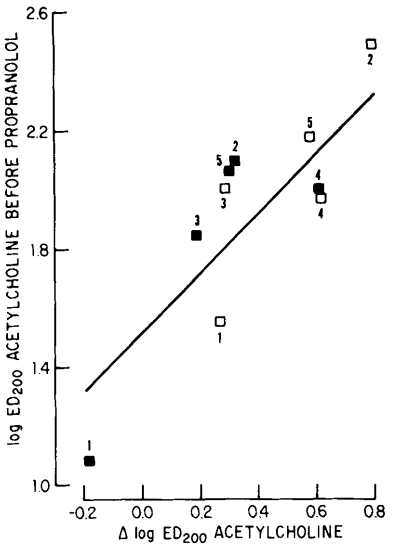

FIG. 4. Correlation between prepropranolol $logED_{200}ACh$ and the difference in $logED_{200}ACh$ after treatment ($logED_{200}ACh$ pretreatment − $logED_{200}ACh$ post-treatment) for the five animals tested on Day 1 (□) and on Day 7 (■). Integers next to symbols represent subject numbers from Fig. 1. Note that guinea pigs with the highest prepropranolol $ED_{200}ACh$ showed the largest changes after treatment; and the most reactive animals showed the least.

In contrast to the effects seen with propranolol, indomethacin treatment did not change bronchial reactivity to parenteral ACh in any of the animals. Complete dose-response curves before and after indomethacin are shown in Fig. 1. The change in $logED_{200}ACh$ was 0.07 ± 0.05 (Fig. 2) and was not significantly different from zero. Hence, no shift in the dose-response curve was observed with indomethacin treatment. In addition, there was no change in baseline SR_{aw} due to indomethacin treatment (Table I).

Discussion. Previously published work concerning the effect of propranolol on lung mechanics and reactivity in this species has focused on changes in dynamic compliance to aerosolized or infused histamine (1, 2). The *in vivo* effect of this bronchoconstrictor is probably more complex than that of a muscarinic agonist in that it has both direct influences on airway muscle (mediated by different receptors) and indirect ones via cholinergic pathways (7). In our study, we found that β-adrenergic blockade increased airway sensitivity to parenteral ACh. It did not affect specific airway resistance, however. We also found that propranolol treatment reduced the range in reac-

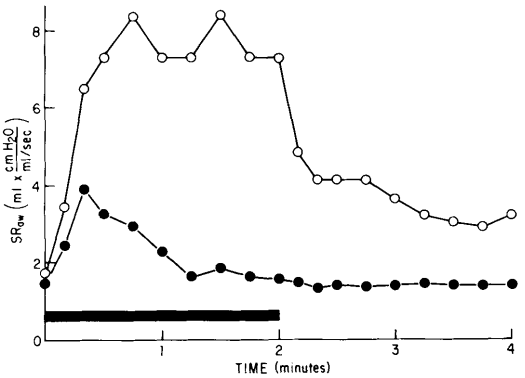

FIG. 5. Time course of change in SR_{aw} during and following intravenous administration of ACh (100 μg/min) before (●) and after (○) propranolol treatment. ACh (solid bar) was delivered for 2 min. Note that there was substantial recovery in SR_{aw} during ACh infusion when the guinea pig was not propranolol-treated, but not when the animal was.

tivity among the group of animals studied and enhanced the reproducibility of serial parenteral testings in individual animals. In contrast, indomethacin treatment (in doses reported by others to decrease baseline resistance (4) and to affect reactivity to exogenously administered arachidonic acid metabolites (8)) caused no change in SR_{aw} or muscarinic reactivity in unanesthetized guinea pigs.

Past studies concerning the effect of propranolol on airway mechanics in other species have produced differing results. In monkeys, Pare and colleagues (9) found no potentiation of the airway response to inhaled histamine after inhaled propranolol. In dogs, Snapper *et al.* (10) were unable to alter the airway reactivity to inhaled histamine after intravenous propranolol, although they did see a significant effect on dynamic lung compliance (C_{dyn}). Studies by Mue *et al.* (11) point to the complexity of effects that may be present here. In monkeys, they found parenteral propranolol increased reactivity to intravenous methacholine. However, it did not potentiate the response to intravenous histamine, or to either methacholine or histamine. Thus, β-adrenergic modulation of airway reactivity may be dependent on the technique employed (5), or the bronchoconstrictor administered, as well as on the species investigated.

In the guinea pig, we found that propranolol treatment invariably potentiated the response to a given dose of ACh, although propranolol had no effect on baseline SR_{aw} at 10 mg/kg ip. This later point is in agreement with the findings of Douglas *et al.* (1) and Drazen (2). Furthermore, in untreated animals, we often saw recovery in SR_{aw} from the initial response to ACh while it was being infused. This phenomenon was not reported by Drazen (2), who was also interested in the mechanism of propranolol-induced potentiation of the response to bronchoconstriction administration. It should be noted that he measured a different parameter (C_{dyn}) and used a different bronchoconstrictor (histamine). Because recovery during ACh infusion was minimized with propranolol, we suspect that, in the normal state, β-adrenergic-receptor-mediated bronchodilation occurs in response to bronchoconstriction.

It has been suggested that variation in the range of responsiveness to a bronchoconstrictor within an animal group may be due to variation in β-adrenergic responsiveness of their airways (1). Clearly, we found that the range of reactivity to intravenous ACh was decreased with propranolol treatment. In addition, there was a correlation between the initial reactivity and the magnitude of the change in reactivity after propranolol: the initially less reactive animals showed a greater increase in reactivity than the more reactive ones. Douglas and colleagues (1) have made similar observations in the guinea pig. These findings suggest that the responsiveness of the airways to β-adrenergic stimulation may be an important determinant of bronchial reactivity to a bronchoconstrictor.

The possibility that cyclooxygenase products may affect airway resistance or reactivity in the guinea pig is suggested by various *in vitro* studies in the literature. Adcock and Garland (12) found that indomethacin substantially augmented histamine-induced tracheal muscle contraction. In contrast, compounds which inhibited either the lipoxygenase pathway or both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism did not augment contractions induced by histamine. Jones *et al.* (13) found that the responsiveness of guinea pig trachea muscle to both cholinergic nerve and exogenous ACh stimulation was augmented by indomethacin treatment. Taken together, these studies suggest that the increased responsiveness of airway muscle after indomethacin-induced cyclooxygenase inhibition may be due to diversion of arachidonic acid metabolism toward lipoxygenase products.

In contrast to these known *in vitro* effects, knowledge as to the *in vivo* effects of indomethacin on airway reactivity is very limited. What is available in the literature suggests that indomethacin does not affect bronchial reactivity. We found no change in either baseline SR_{aw} or muscarinic reactivity in guinea pigs treated with indomethacin in doses comparable to those used by others who have reported changes in lung mechanics in the guinea pig. Using the same dose we used in this study, Leitch *et al.* (8) found that the bronchoconstricting effects of administered leukotriene C_4 or D_4 were substantially altered. Brink *et al.* (4) found the same dose of indomethacin did not affect histamine reactivity, although they did observe a decrease in basal

lung resistance after treatment. Hopefully, additional research will clarify the discrepancies between the known *in vitro* effects of indomethacin on airway muscle responsiveness and the uncertain *in vivo* ones.

Factors that may affect airway reactivity in lung disease are numerous and not well understood at present. In the future, it will be important to fully investigate whether endogenous adrenergic and cyclooxygenase product responsiveness of the airways are important determinants of bronchial reactivity in experimental or naturally occurring diseases such as asthma.

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