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# Lack of Interaction of MDL 257 and Isoproterenol: Comparison With Aminophylline

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## ABSTRACT

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MDL 257 [8-methyl-6-piperidino-s-triazolo(4,3-b)pyridazine], a potential bronchodilator, was evaluated for potential interactions with isoproterenol on isolated tracheal smooth muscle taken from either prednisone- or methocel HG-treated guinea pigs. Its effects were compared to those of aminophylline. In tracheas taken from methocel HG-injected animals, aminophylline lowered the height of isoproterenol concentrations-effect curve more than the vehicle ($P < .001$) and shifted the curve to the left ($P < .05$). MDL 257, on the other hand, had no effect. In tracheas taken from prednisone-treated animals, aminophylline and MDL 257 had no significant effect on the maximum relaxation caused by isoproterenol. However, both drugs shifted isoproterenol concentrations-effect curve to the left. Their effects were significantly different ($P < .05$) from that of the vehicle. These data suggest that, if given together, MDL 257 would not attenuate the bronchodilator effects of beta-sympathomimetic amines.

**Key words: drug interaction, tracheal smooth muscle relaxation**

## INTRODUCTION

The use of beta-sympathomimetic, aminophylline, and corticosteroid combinations in the treatment of asthma is well established. Sutherland et al. [1968] demonstrated a synergistic interaction between adrenaline and caffeine to raise cyclic AMP in rat lung slices. Subsequent studies have shown that a beta-sympathomimetic and methylxanthine combination

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interacts in a synergistic fashion to relax guinea pig and human tracheobronchial smooth muscle in vitro [Lefcoe et al., 1975]. Hanna and Roth [1979], on the other hand, reported that the combination of beta-sympathomimetics and theophylline did not act in a synergistic manner to produce relaxation of contracted tracheal smooth muscle in vitro. Moreover, some in vivo animal [Herxheimer and Stresemann, 1960; and Oskoui et al., 1970] and human studies [Taylor et al., 1985] did not support the synergistic interaction of combined beta-sympathomimetic and methylxanthines to relax precontracted tracheal smooth muscle.

MDL 257 is a potential bronchodilator [Abdallah et al., 1984; Shim et al., 1986; and Küng et al., 1986] that has no effect on airway beta-adrenergic receptors [Abdallah et al., 1984] and possesses phosphodiesterase inhibitory activity (unpublished data). The purpose of this study was to investigate the potential interaction of MDL 257 (Fig. 1) with isoproterenol on tracheas taken from vehicle- or prednisone-pretreated guinea pigs and to compare its effects to those of aminophylline.

## MATERIALS AND METHODS

### Guinea Pig Tracheal Preparation

Male guinea pigs, weighing between 400–500 g, were stunned, bled, and their tracheas were removed. Each trachea was cut into two cylindrical segments of 8 mm in length. The cylindrical segment of each tissue was placed between stainless steel hooks and suspended in a 10-ml tissue bath containing modified Burn solution at 37°C and attached to a force transducer (Gould Instruments, Cleveland) for recording isometric tension. The amount of tension was recorded through a Buxco computer system and data logger and printed out on a Texas "Silent Writer" Terminal. The preparation was allowed to equilibrate for 60 min under a resting tension of 8 g. The modified Burn · solution, which consists of NaCl (8 g/L), KCl (0.2 g/L), $CaCl_2 \cdot 2H_2O$ (0.26 g/L), $MgCl_2 \cdot 6H_2O$ (0.02 g/L), $NaHCO_3$ (1.0 g/L), $NaH_2PO_4 \cdot H_2O$ (0.05 g/L), was aerated with a mixture of 95% $O_2$ and 5% $CO_2$.

Tissues were contracted with 38.6 mM KCl, a concentration previously determined from concentration-contraction curves to produce 80% of the maximal response. Isoproterenol (from Sigma Chemical Co., St. Louis) was added to the tissues in a cumulative manner ($1 \times 10^{-9}$ to $1 \times 10^{-5}$ M) until maximal relaxation responses were obtained. Mean data points ± S.E. are presented in figures as percent relaxation *vs.* −Log [isoproterenol] M. The relaxant effect of each concentration of isoproterenol is expressed as a percentage of the maximal relaxation produced by isoproterenol. After thorough washing and equilibration of the tissues to reestablish a baseline, the tissues were again contracted with 38.6 mM KCl. Concentration ($ED_{10}^*$) of aminophylline (theophylline ethylenediamine) Lot No. 22-UV-118, from Gane's Chemical Works (Carlstadt, NJ) or MDL 257 [8-methyl-6-piperidino-s-triazolo(4,3-

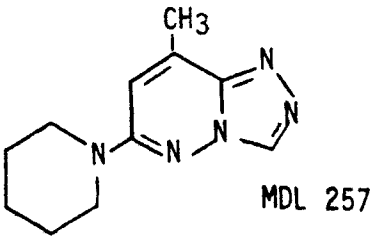


Fig. 1. Chemical structure of MDL 257.

*$ED_{10}$ is the concentration that produced 10% of the maximal relaxation produced by either MDL 257 or aminophylline, which were previously determined from concentration-effect curves.

*b*)pyridazine], from Merrell Dow Research Institute (Indianapolis, IN) or their vehicle were then added. When the effect of MDL 257 and aminophylline reached a plateau, a concentration-response curve to isoproterenol was obtained and expressed as a percentage of the maximal relaxation produced by isoproterenol prior to the addition of aminophylline or MDL 257. All test compounds were dissolved in water and prepared freshly each day.

This study was performed using tracheas obtained from guinea pigs that had been injected subcutaneously (SC) with 0.5% methocel HG solution (hydroxypropyl methylcellulose, The Dow Chemical Company, Midland, MI) for 5 days. It was then repeated using tracheas obtained from guinea pigs that had been injected SC with prednisone (5 mg/kg, suspended in 0.5% methocel HG solution) for 5 consecutive days before sacrifice.

All isoproterenol dose-response curves, both pre- and post treatment, were fitted with the following three-parameter logistic function [DeLean et al., 1978]: $C = C_{max}\,(1 - 1/[1 + (dose/Ed_{50})]p)$, where C = measured tissue relaxation, $C_{max}$ = maximum relaxation, dose = treatment application dose, $ED_{50}$ = dose at 50% of $C_{max}$, and p = shape parameter.

The nonlinear regression modeling procedure NLIN in the statistical analysis system (SAS) commercial package was applied in obtaining estimates of the parameters $C_{max}$, $ED_{50}$, and p [SAS User's Guide, 1982]. The parameters $C_{max}$ and $ED_{50}$ for the pretreatment dose-response curves were analyzed to test for consistency over the experimental conditions, which included animal types—prednisone-naive vs. prednisone-exposed; 3 experiment days; 3 treatment modalities—vehicle, aminophylline, and MDL 257.

Deviations in the treatment response curves from pretreatment were quantified as follows: $C_{max}$ expressed as relative percent of the pretreatment value, and $ED_{50}$ converted to a difference from the pretreatment value.

These parameters adjusted for pretreatment were compared in a factorial analysis of variance with the experimental conditions mentioned above constituting factors. Where warranted by significance probabilities below .10 in the analysis of variance (ANOVA), specific comparisons were made by means of t-tests based on the error mean square from ANOVA table.

## RESULTS

In tracheas taken from methocel HG-treated animals, aminophylline shifted isoproterenol concentration-effect curve to the left ($P < .05$), while MDL 257 and the vehicle had no significant effect when the $ED_{50}$s of isoproterenol before treatments were compared to its $ED_{50}$s after treatments. The $ED_{50}$s of isoproterenol in the three sets of tracheas were 3.9, 3.7, and $3.0 \times 10^{-8}$ M before and 4.9, 2.7, and $2.9 \times 10^{-8}$ M after the addition of vehicle, aminophylline, and MDL 257, respectively (Table 1). The effect of aminophylline was significantly different ($P < .05$) from that of the vehicle. All treatments reduced the maximum relaxation caused by isoproterenol in comparison to its pretreatment level. The reduction caused by aminophylline, MDL 257, and vehicle was 34, 20, and 12%, respectively (Table 2). The lowering effect of aminophylline was significantly ($P < .001$) different from vehicle.

In tracheas taken from prednisone-treated guinea pigs, both aminophylline and MDL 257 shifted isoproterenol concentration-effect curve to the left, while the vehicle caused a slight shift to the right as evaluated by their $ED_{50}$ values. However, only the effect of MDL 257 was significantly different ($P < .05$) from its pretreatment value. The $ED_{50}$s of isoproterenol in the three sets of tracheas were 4.2, 4.0, and $4.7 \times 10^{-8}$ M before and 4.5, 2.7, and $3.2 \times 10^{-8}$ M after the addition of vehicle, aminophylline and MDL 257, respectively (Table 1). The $ED_{50}$s of isoproterenol after MDL 257 and aminophylline were significantly different ($P < .05$) from its $ED_{50}$ after the addition of vehicle. Aminophylline caused 20% reduction of isoproterenol maximum relaxation in comparison to its pretreatment level (Table 2). On the other hand, MDL 257 and vehicle caused only 5% and 3% reduction, respectively. The effect of aminophylline and MDL 257 were not significantly different from that of the vehicle.

**TABLE 1. Interaction of MDL 257 Aminophylline and Isoproterenol on Tracheal Cylindrical Segments Taken From Methocel HG-or Prednisone-treated Guinea Pigs[a]**

| Groups | Treatment | $ED_{50S}$ Means ± SEM × $10^{-8}$ (M) Pretreatment[a] | Posttreatment |
|---|---|---|---|
| Methocel HG-treated guinea pigs | Vehicle | 3.9 ± 0.73 | 4.9 ± 0.89 |
| | Aminophylline | 3.7 ± 0.93 | 2.7 ± 0.77[c] |
| | MDL 257 | 3.0 ± 0.40 | 2.9 ± 0.36 |
| Prednisone-treated guinea pigs | Vehicle | 4.2 ± 0.85 | 4.5 ± 0.81 |
| | Aminophylline | 4.0 ± 0.53 | 2.7 ± 0.49[b] |
| | MDL 257 | 4.7 ± 0.93 | 3.2 ± 0.71[c] |

[a]Tissues were preincubated with an $ED_{10}$ concentration of MDL 257, aminophylline, or an equal volume of vehicle before the cumulative addition of isoproterenol. Five to six tracheas from different guinea pigs were used for each treatment.
[b]Significantly different ($P < .05$) from vehicle-treated group.
[c]Significantly different ($P < .05$) from vehicle-treated group and from its own pretreatment value.

**TABLE 2. Percent Inhibition of Isoproterenol-Induced Maximal Relaxation of Guinea Pig Tracheal Smooth Muscle by Aminophylline, MDL 257, and Vehicle†**

| Groups | Percent Inhibition Vehicle | Aminophylline | MDL 257 |
|---|---|---|---|
| Tracheas from methocel HG-treated animals | 12 | 34* | 20 |
| Tracheas from prednisone-treated animals | 3 | 20 | 5 |

†Five to six tracheas from different guinea pigs were used for each treatment.
*Significantly different ($P < .001$) from vehicle group.

## DISCUSSION

Our data show that MDL 257 had no effect on the bronchodilator activity of isoproterenol in tracheas taken from vehicle-treated animals. This finding is not surprising since it is known that the bronchodilator effect of MDL 257 is not mediated through the beta-adrenergic system [Abdallah et al., 1984]. Aminophylline, on the other hand, potentiated the action of medium concentrations of isoproterenol and physiologically antagonized the activities of high concentrations. It is possible that aminophylline pretreatment directly or indirectly desensitized some of the beta-adrenergic receptors in the trachea. The consequence of this partial desensitization became apparent at the maximum concentrations of isoproterenol. It is known, for example, that aminophylline caused the release of epinephrine from adrenal medulla, and its bronchodilator activity in guinea pigs [Ward and Tomlinson, 1984] and man [Mackay, et al., 1983] is blocked by propranolol. It is possible, therefore, that aminophylline causes the release of catecholamine from guinea pig trachea, which is known to possess catecholamine uptake processes [Foster, 1968,1969], and thus enhances the desensitization of the adrenergic receptors, which occurred owing to their prior exposure to high concentrations of isoproterenol. This explanation became plausible since it is known that terbutaline [Salonen et al., 1985] and salbutamol [Ward and Tomlinson, 1984] antagonized the bronchodilator activity of aminophylline. It is conceivable, therefore, that the opposite may be true: namely, aminophylline pretreatment may antagonize the tracheal smooth muscle relaxant effect of isoproterenol.

Our finding that aminophylline potentiated the action of medium concentrations of isoproterenol agrees with the findings of Bertelli et al. [1973] and Pihlajamaki et al. [1972] who reported that beta-sympathomimetic and methylxanthine interact in a synergistic manner. Hanna and Roth [1979], on the other hand, reported that fixed combinations of salbutamol and

theophylline interacted in an additive rather than a synergistic manner. The discrepancy between our results and those of Hanna and Roth could be due to difference in the experimental design and spasmogens used. Jenne and Shaughnessy [1985] state that the bronchodilator activity of isoproterenol in dogs varies with both severity and etiology of bronchospasm. We studied the effect of a single low concentration of aminophylline on the concentration-response curve of isoproterenol. Moreover, we contracted the trachea with potassium chloride, while Hanna and Roth employed histamine, which is known to release other mediators such as thromboxane $A_2$ and leukotrienes [Dahlen et al., 1983]. Leukotrienes, unlike potassium chloride and histamine, which mobilize extracellular calcium [Hudgins and Weiss, 1968; Creese and Denborough, 1981], mobilize intracellular calcium [Tucker and Weichman, 1983; Weichman et al., 1983]. Low concentrations of leukotrienes, however, might increase calcium influx [Sasaki et al., 1984]. This difference in spasmogens and concentrations used and the initial contractile state may influence the relaxant effect of isoproterenol. This explanation is indirectly supported by the findings of Torphy [1984] that an increasing concentration of methacholine shifted the isoproterenol dose-response curve to the right. This shift by methacholine was greater than that caused by equieffective concentration of leukotriene $D_4$.

Tracheas taken from prednisone-treated animals tended to respond with a greater maximal relaxation to isoproterenol when compared to tracheas taken from vehicle-injected animals. This was true independent of drug or diluent treatment. These data agree with those of Geddes et al. [1974] who reported that in vitro, hydrocortisone and methyl prednisolone potentiated the responses to isoproterenol and other beta agonists on the guinea pig tracheal and human bronchial smooth muscles.

A number of possibilities can be considered in order to explain the potentiation of the relaxant effect of isoproterenol by prednisone. Previous studies have shown that hydrocortisone stimulates adenylcylase activity of leukocytes from normal and asthmatic patients [Logsdon et al., 1972]. It was suggested that corticosteroids can alter the beta-adrenoceptors in vascular smooth muscle [Besse and Bass, 1966] and increase their number and function in human astrocytoma cells [Foster and Harden, 1980]. Lee and Reed [1977] stated that glucorticosteroids increased the cyclic AMP level response of human lymphocytes to isoproterenol. This response does not require the biosynthesis of macromolecules, and it may depend partially on the inhibitory effect of steroids on cyclic nucleotide phosphodiesterase. Moreover, it is possible that some corticosteroids potentiate the effect of isoproterenol by inhibiting its extraneural uptake [Salt, 1972]. This explanation becomes plausible since Foster [1968, 1969] had demonstrated that catecholamine uptake processes could be operating in the tracheobronchial smooth muscle.

If prednisone, indeed, inhibits cyclic AMP phosphodiesterase activity in guinea pig trachea, then it would have an additive effect to that of MDL 257, which is known to inhibit cyclic AMP phosphodiesterase in guinea pig airways (unpublished data). This suggestion may explain why MDL 257 increased smooth muscle relaxant activity of isoproterenol only in prednisone-treated animals.

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