Effects of Ketotifen, a Benzocycloheptathiophene, on Methacholine- and Acetylcholine-induced Contractions of Canine Respiratory Smooth Muscle

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Abstract: *The relaxant effects of ketotifen on isolated respiratory smooth muscle contracted by methacholine or acetylcholine were examined. Although potent dose-dependent relaxation was observed, it appears that more than 100 times the concentration is required to relax muscle contracted with cholinergic agonists than has been reported for muscle contracted by histamine. This observation offers a possible explanation for the findings of several investigators that dosages of ketotifen administered to patients protect against histamine-induced, but not methacholine- or acetylcholine-induced, bronchospasm.*

Key Words: Ketotifen; Smooth muscle; Acetylcholine; Methacholine; Asthma

INTRODUCTION

Ketotifen has been shown to provide protection against histamine-induced bronchospasm in asthmatic patients (Craps et al., 1978; Wuethrich et al., 1978; Beumer, 1979; Mattson et al., 1979a), but not against bronchospasm induced by acetylcholine (Craps et al., 1978; Wuethrich et al., 1978) or methacholine (Mattson et al., 1979b). In contrast to these clinical studies, preliminary findings on isolated canine respiratory smooth muscle in our laboratory indicated that ketotifen not only can inhibit histamine-induced, but also methacholine- and acetylcholine-induced, contractions. This report describes the relaxant effects of ketotifen on cholinergically stimulated canine tracheal smooth muscle strips.

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MATERIALS AND METHODS

Methacholine (acetyl-β-methylcholine chloride) and other chemicals were purchased from Sigma Chemical Co., St. Louis, MO. Ketotifen was kindly supplied by Sandoz Pharmaceuticals, East Hanover, NJ. Adult mongrel dogs of random sex, weighing 15–30 kg, were used in this study, each being anesthetized with intravenous pentobarbital sodium (30 mg/kg) prior to removal of the trachea. Strips of tracheal smooth muscle, approximately 2-3 mm × 15–20 mm, were suspended in 10 ml volumes of a Krebs-Ringer solution (NaCl, 117 mM; KCl, 4 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 2.4 mM; NaH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; and dextrose, 11 mM) bubbled with 95% O_2: 5% CO_2 and maintained at 37°C. Tension was measured by Grass FTO3C force-displacement transducers and recorded on a Grass polygraph. Following an equilibration period of at least 30 min at a resting tension of 1–2 g, the strips were contracted by the addition of methacholine (0.1 μM) or acetylcholine (10 μM) to the bath. In each experiment, cumulative dose-response curves for the relaxant effects of ketotifen on the contracted strips were constructed according to the method of Van Rossum (1963), and the data presented as means and standard errors of the means (SE).

RESULTS

Figure 1a shows that ketotifen produced a potent dose-dependent reversal of the contractile response to methacholine. Total relaxation (equal to 101.5 ± 2.06% of the methacholine-induced contraction) was achieved by 2 μM ketotifen and the 50% effective concentration (EC_{50}) of ketotifen against methacholine-induced contraction was about 0.44 μM. In a separate experiment (not shown), the contraction produced by methacholine was found to be blockable by atropine, confirming that the methacholine-induced contractions were mediated by muscarinic cholinergic receptors.

Figure 1b illustrates the dose-dependent reversal by ketotifen of contractions produced by 10 μM acetylcholine. This concentration of acetylcholine induced stronger average contractions (18.3 ± 2.1 g) than the concentration of methacholine (0.1 μM) used in the previously described experiment (10.8 ± 1.1 g) and the largest concentration of ketotifen tested (22 μM) produced relaxation equal to 84.6 ± 4.8% ($n=5$) of the acetylcholine-induced contraction.

DISCUSSION

Ketotifen produced a dose-dependent relaxation of canine tracheal smooth muscle placed in contraction by 0.1 μM methacholine or 10 μM acetylcholine. The EC_{50} for ketotifen on methacholine-contracted muscle was 0.44 μM, indicating that ketotifen produces a more potent relaxant effect on respiratory smooth muscle than either theophylline or 1-methyl-3-isobutylxanthine, which have EC_{50} values of 120 and 3 μM, respectively (Polson et al., 1979). Martin and Römer (1978) have reported a pD'_2 value of 8.6 for ketotifen's noncompetitive inhibition of histamine-induced contractions of guinea pig ileum. This would correspond to an EC_{50} of about 0.0025 μM, which is similar to the EC_{50} (0.0035 μM) determined in this laboratory for the relaxant effects of ketotifen on histamine-induced contractions of canine tracheal smooth muscle (unpublished observations). From these results, it appears that ketotifen is at least 100 times more potent as an inhibitor of histamine-induced than of methacholine- or acetylcholine-induced smooth muscle contraction. These findings suggest that the failure to observe any protective effect of ketotifen against cholinergically induced bronchospasm in

Abbreviations. EC_{50}: drug concentration that produces 50% of the maximum pharmacological effect.

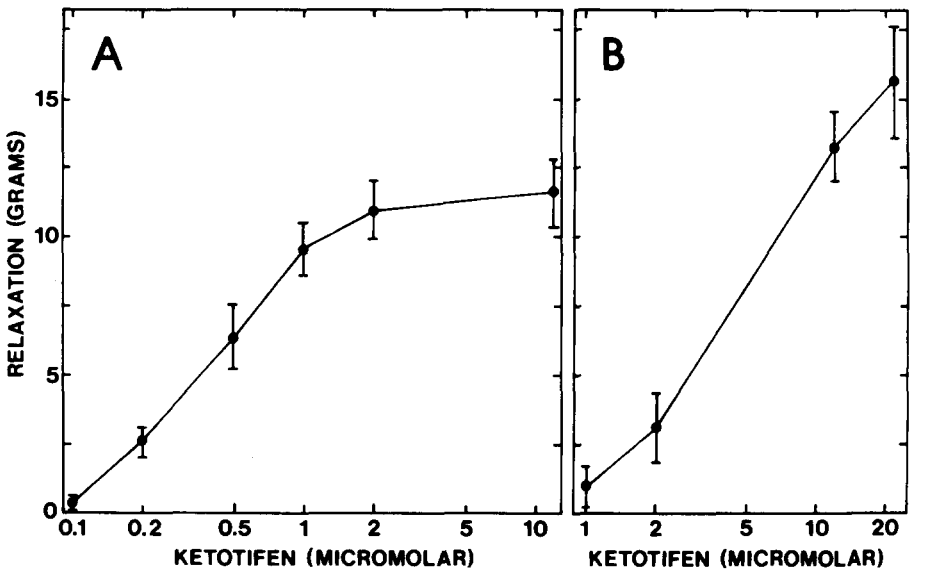

Figure 1 *Reversal by ketotifen of contractions produced by methacholine (a) or acetylcholine (b). The average contractile response of the 6 tracheal smooth muscle strips from 6 dogs in each experiment was 10.8 ± 1.1 g and 18.3 ± 2.1 g for a and b, respectively. Individual dose-response curves were generated by addition of increasing amounts of ketotifen in the continued presence of cholinergic agonist. The resulting relaxation is presented as the mean ± SE for* n=6 *at each point except at 22 μM ketotifen (panel b) where* n=5.

asthmatic patients (Craps et al., 1978; Wuethrich et al., 1978; Mattson et al., 1979b) may have been because the dosages used in the clinical studies were lower than required to produce the relaxant effects of ketotifen on cholinergically contracted respiratory smooth muscle, rather than because of any absolute inability of ketotifen to relax smooth muscle contracted by cholinergic agonists.

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