was 56.7 ± 1.7 μM.

Effects of various antagonists on MB-induced tracheal contraction

The mechanism involved in MB-induced contraction was investigated by using the nonselective muscarinic and histamine antagonist, diphenhydramine (0.01-10 μM); the nonselective muscarinic antagonist, atropine (0.01-10 μM); the M_3-selective antagonist, 4-DAMP (0.01-10 μM); and the H_1-selective antagonist, mepyramine (0.01-10 μM). These antagonists were added to tracheas which had attained maximum tension induced by 100 μM MB. All of these antagonists caused a concentration-dependent relaxation of MB-induced tension. Especially, diphenhydramine completely abolished MB-induced contraction at a concentration of 1 μM (n = 6) (Fig. 2). The maximum inhibitions by atropine, 4-DAMP, and mepyramine were 88.4% ± 3.9% (n =6), 80.3% ± 4.3% (n =6) and 72.3% ± 3.3% (n = 6), respectively (Fig. 2).

Effects of various antagonists on ACh- or histamine-induced tracheal contractions

Both ACh and histamine produced concentration-dependent tracheal contractions (data not shown). The 3 μM ACh-induced or 6 μM histamine-induced tensions with magnitudes similar to 100 μM MB-induced tension were used in this study. ACh- and histamine-induced tracheal contractions were completely abolished by 0.1 μM and 3 μM diphenhydramine, respectively (n = 6). Both atropine and 4-DAMP completely abolished ACh-induced contraction (0.1 μM, n = 6), whereas only partial inhibition was observed for histamine-induced contraction (58.1% ± 3.4% and 67.5% ± 2.2% respectively, n = 6). Mepyramine (0.03 μM) abolished histamine-induced contraction completely (n = 6), whereas ACh-induced contraction was not significantly affected by mepyramine (Table 1).

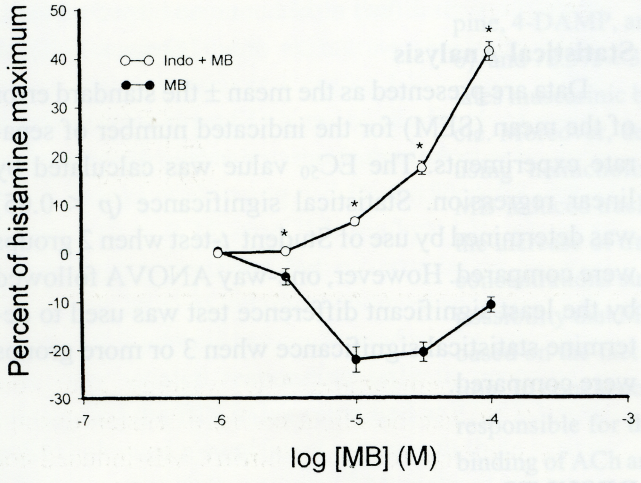

Fig. 1. Cumulative concentration-response curves for muscular tension changes of guinea pig tracheal preparations caused by methylene blue (MB). MB-induced contractions are expressed as a percentage of histamine-induced maximum tension. MB (●) alone depresses spontaneous muscular tension. In contrast, when pretreating with 3 μM indomethacin (○), MB increases tracheal muscular tension in a dose-dependent manner. Positive and negative values represent contraction and relaxation, respectively. Each point is the mean value from at least 6 experiments, and the vertical bars represent S.E.M. * $p < 0.05$ as compared with the MB value.

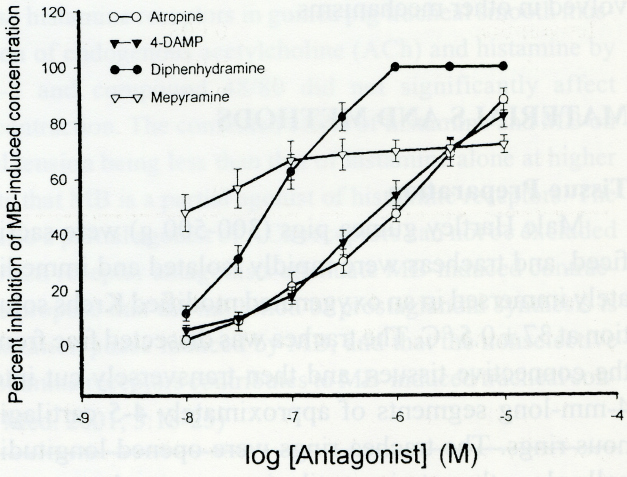

Fig. 2. Effects of various antagonists in methylene blue-induced tracheal contractions. Tracheal relaxations are expressed as a percentage of MB-induced maximum tension. All tests were performed in the presence of 3 μM indomethacin. Atropine (○), 4-DAMP (▼), diphenhydramine (●), and mepyramine (▽) depress MB-induced tracheal contraction in a concentration-dependent manner. Each point is the mean value from at least 6 experiments, and the vertical bars represent S.E.M.