column 5). On the other hand, PEG-SOD (300 μM) alone or combined with PEG-catalase caused a minor but insignificant decrease of MB-induced tracheal contraction ($p = 0.08$ and $p = 0.06$, respectively, $n = 5$) (Fig. 3, columns 6, 7).

**Effects of tetrodotoxin and physostigmine on MB-induced tracheal contraction**

Pretreating the tracheal preparations with tetrodotoxin (0.3 μM) had no effect on MB-induced contraction ($p = 0.07$, $n = 5$) (Fig. 3, column 8). Pretreating with physostigmine (0.1 μM) increased both the tracheal basal tension and MB-induced contraction (Fig. 4c).

**Concentration-response relationships between histamine, ACh, and MB**

MB caused a concentration-dependent tension increase of tracheal preparations; the magnitude of the maximal contractions induced by MB were 45.3% ± 3.8% compared to those by histamine (Fig. 5a), and 50.1% ± 3.0% compared to those by ACh (Fig. 5b). The combined effects of histamine and MB on increases in tracheal tension were less than those of histamine alone at higher concentrations (100-300 μM, $p < 0.05$) (Fig. 5a). Since simultaneous treatment with a full agonist and a partial agonist should have produced a lower maximal response than with a full agonist alone,[17] these results combined with the data of figure 2 suggest that MB-induced contractions partially result from the binding of histamine receptors.

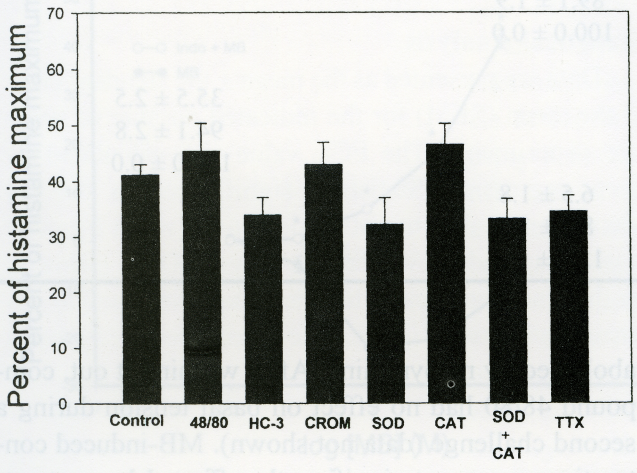


Fig. 3. Effects of preincubation of various agents on methylene blue (MB)-induced tracheal contraction. Tension changes are expressed as a percentage of histamine-induced maximum tension. The control represents 100 μM MB-induced contraction. 48/80 = compound 48/80 (2 mg/ml), HC-3 = hemicholinium-3 (100 μM), CROM = sodium cromolyn (100 μM), SOD = PEG-SOD (300 μM), CAT = PEG-catalase (300 U/ml), TTX = tetrodotoxin (0.3 μM). Each column is the mean value from at least 5 experiments, and the vertical bars represent S.E.M. Indomethacin (3 μM) was present throughout.

## DISCUSSION

Endogenous arachidonic acid metabolites such as prostaglandins have been demonstrated to be regulators of inherent muscular tone in guinea pig tracheal smooth muscle. Therefore, cyclooxygenase inhibition decreases the tracheal baseline tone.[18] In this study, we found that both indomethacin (3 μM) and MB (3-100 μM) reduced the inherent tone in guinea pig trachea (Fig. 1), whereas, in the presence of indomethacin, MB produced concentration-dependent tracheal contraction (Fig. 1). This finding led us to propose that the relaxation phase of the MB-induced tracheal basal tension change may be mediated by prostaglandin inhibition. In concert with this observation, MB has been reported to inhibit prostacyclin synthesis.[10]

Recently, Hwung et al.[9] reported that MB-induced contractions were inhibited by both the nonselective muscarinic antagonist, atropine, and the $M_3$-selective antagonist, 4-DAMP. They suggested that MB could modulate the cholinergic system in guinea pig trachea. We also observed similar results (Fig. 2). Furthermore, our data show that the nonselective muscarinic and histamine antagonist, diphenhydramine, (Table 1) completely abolished MB-induced tracheal contraction (Fig. 2). In addition, the $H_1$-selective antagonist, mepyramine, also attenuated MB-induced contraction in a dose-dependent manner (Fig. 2). On the other hand, 4-DAMP (0.1 μM) pretreatment completely abolished EFS-induced but only partially inhibited MB-induced contractions (Fig. 4b) indicating that the histamine re-