

inergic contraction induced by electrical field stimulation (EFS) in a concentration-dependent fashion in rat trachea.<sup>7</sup> An endogenous NO-like factor was proposed which possibly mediates the prejunctional inhibition of cholinergic contractions through a cyclic GMP-dependent mechanism. However, Brave et al.<sup>8</sup> showed that L-N<sup>G</sup>-nitro-arginine enhanced contractions induced by EFS but had no effect on ACh release in guinea pig trachea. In addition, MB caused a concentration-dependent contraction which was inhibited by muscarinic antagonists.<sup>9</sup> However, ODQ (a potent and selective inhibitor of sGC) and the NO inhibitor, N<sup>w</sup>-nitro-L-arginine methyl ester (L-NAME), had no effect on tracheal basal tone.<sup>9</sup> Other than as a sGC inhibitor, MB has many other effects including inhibition of prostacyclin synthesis,<sup>10</sup> generation of superoxide anions,<sup>11-13</sup> inhibition of NO synthase<sup>14</sup> and cholinesterase activity,<sup>15</sup> and inhibition of muscarinic receptors.<sup>15-16</sup> We have therefore investigated the effects of MB on tracheal tension changes in the guinea pig to determine whether MB is capable of releasing endogenous ACh from cholinergic neurons or whether it is involved in other mechanisms.

## MATERIALS AND METHODS

### Tissue Preparation

Male Hartley guinea pigs (300-500 g) were sacrificed, and tracheas were rapidly isolated and immediately immersed in an oxygenated modified Krebs solution at  $37 \pm 0.5$  °C. The trachea was dissected free from the connective tissues, and then transversely cut into 4-mm-long segments of approximately 4-5 cartilaginous rings. The trachea rings were opened longitudinally along the anterior cartilaginous part and connected to stainless steel hooks. These preparations were mounted vertically in an individual water-jacketed organ bath containing 5 ml of modified Krebs solution which was continuously aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at  $37 \pm 0.5$  °C. Isometric tension was continuously measured with a force transducer (Gould, UC<sub>2</sub>) connected to a polygraph (Gould, RS 3200). Tracheal rings were placed between 2 rectangular platinum electrodes for EFS (Grass, S88 stimulator). The initial rest-

ing tension imposed on the preparation was 1 g. Experiments were performed after rinsing with a fresh buffer solution and equilibration for 60 min. EFS (10-50 V of 0.5-ms duration) was applied for 10-15 s. All contraction effects were expressed as a percentage of the maximum contraction by histamine, Ach, or MB. Negative values represent relaxation.

### Materials

The following drugs were used in this study: atropine sulphate, indomethacin, N<sup>w</sup>-nitro-L-arginine methyl ester (L-NAME), diphenhydramine hydrochloride, sodium cromolyn, methylene blue, tetrodotoxin (TTX), superoxide dismutase-polyethylene glycol (PEG-SOD), catalase-polyethylene glycol (PEG-catalase), compound 48/80 (Sigma Chem., St. Louis, MO, USA), 4-diphenylacetoxy-N-methylpiperidine methiodine (4-DAMP), hemicholinium-3 (RBI, Boston, MA, USA), ODQ, and mepyramine maleate (Tocris Cookson, Ballwin, MO, USA).

### Statistical Analysis

Data are presented as the mean  $\pm$  the standard error of the mean (SEM) for the indicated number of separate experiments. The EC<sub>50</sub> value was calculated by linear regression. Statistical significance ( $p < 0.05$ ) was determined by use of Student *t*-test when 2 groups were compared. However, one-way ANOVA followed by the least significant difference test was used to determine statistical significance when 3 or more groups were compared.

## RESULTS

### Effect of MB on tracheal basal tension in the presence or absence of indomethacin

Pretreating with indomethacin (3  $\mu$ M) relaxed the tracheal muscular tension from the initial basal tension of 1 g to about 0.5 g (time control  $92.2\% \pm 1.1\%$ ,  $n = 9$ ; indomethacin treatment  $45.6\% \pm 1.6\%$ ,  $n = 20$ ,  $p < 0.05$ ). MB alone depressed the intrinsic tone of tracheal smooth muscle (Fig. 1). In contrast, in the presence of indomethacin (3  $\mu$ M), MB induced a concentration-dependent contraction (Fig. 1). The EC<sub>50</sub> value of MB