

Fig. 4. Concentration- and time-dependent increases in nitrite accumulation and iNOS expression caused by dibutyl cAMP in RAW 264.7 cells. Cells were incubated with various concentrations of dibutyl cAMP for 24 h, then the medium was removed and analyzed for nitrite accumulation from RAW 264.7 cells (Panel A). Data represent the mean \pm S.E.M. of three independent experiments done in triplicate. In parallel experiments, dibutyl cAMP-induced iNOS expression was analyzed. RAW 264.7 cells were incubated with various concentrations of dibutyl cAMP for 24 h (B); cells were lysed, and the cell lysate was subjected to Western blot analysis using an iNOS-specific antibody (Panel B).

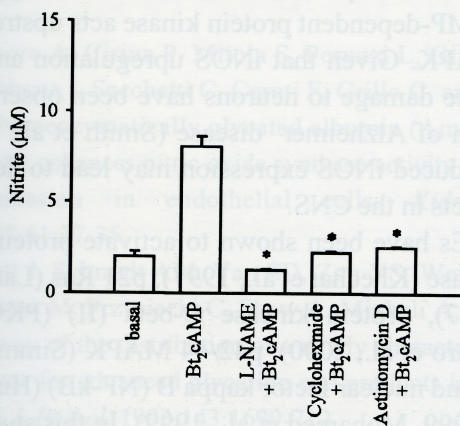


Fig. 5. Effects of *l*-NAME, cycloheximide, or actinomycin D on dibutyl cAMP-stimulated nitrite release from RAW 264.7 cells. Cells were pretreated with *l*-NAME, cycloheximide, or actinomycin D for 30 min before the addition of 300 µM dibutyl cAMP and incubated for 24 h. Then the medium was removed and analyzed for nitrite accumulation from RAW 264.7 cells. Data represent the mean \pm S.E.M. of three independent experiments done in triplicate.

whether BSA-AGE-induced iNOS expression was due to contaminated LPS, RAW 264.7 cells were pretreated with polymyxin B for 30 min before incubation with BSA-AGEs (300 µg/mL) or LPS (0.3 µg/mL) for 24 h. As shown in Fig. 6A, AGE-induced iNOS expression was not affected by pretreatment with polymyxin B, whereas AGE-induced iNOS expression was inhibited by polymyxin B (Fig. 6B).

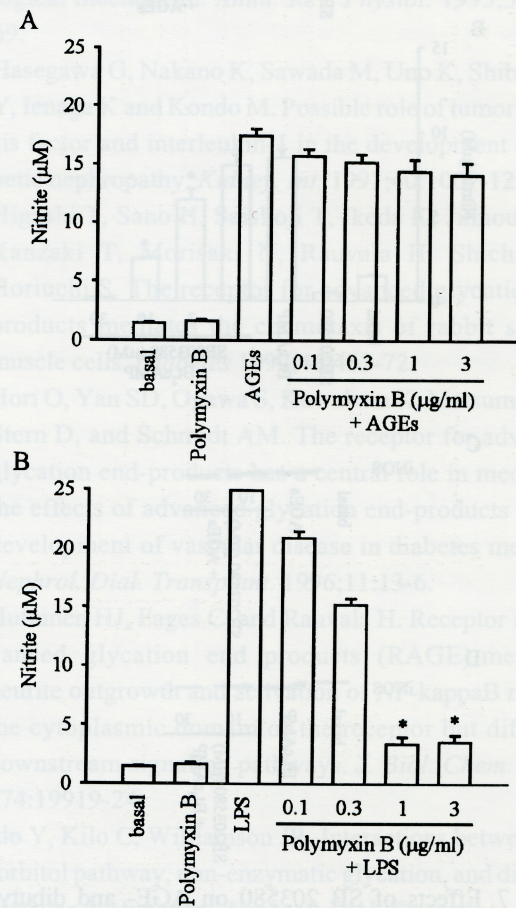


Fig. 6. Effects of polymyxin B on BSA-AGE-induced nitrite release from RAW 264.7 macrophages. In (Panel A), cells were treated with 300 µg/mL BSA alone or pretreated with different concentrations of polymyxin B for 30 min, and then incubated with 300 µg/mL of BSA-AGEs for 24 h. In Panel B, cells were pretreated with different concentrations of polymyxin B for 30 min, and then incubated with 0.3 µg/mL LPS for 24 h. Then the medium was removed and analyzed for nitrite accumulation from RAW 264.7 cells. Data represent the mean \pm S.E.M. of three independent experiments done in triplicate.