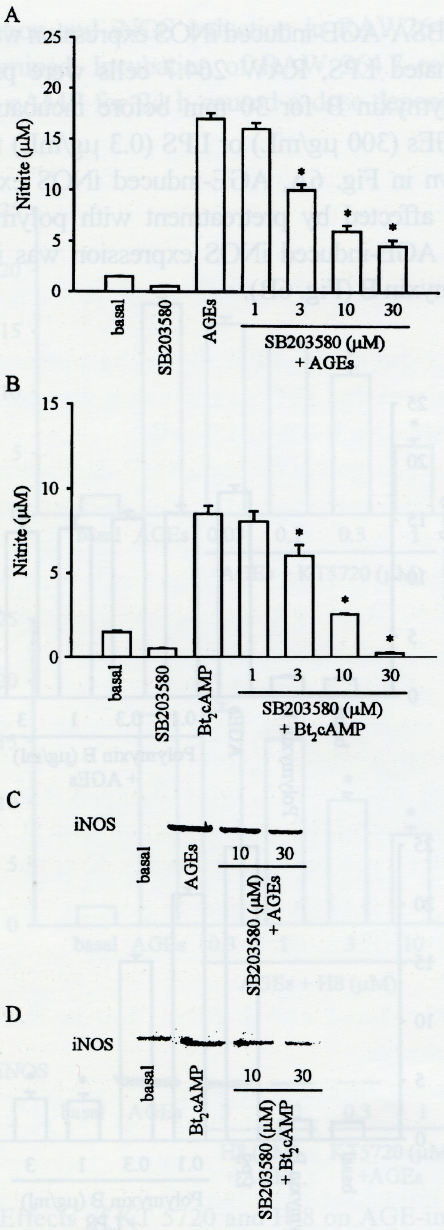

Fig. 7. Effects of SB 203580 on AGE- and dibutyryl cAMP-stimulated nitrite production and iNOS expression in RAW 264.7 cells. Cells were pretreated with different concentrations of SB 203580 (1-30 μM) for 30 min before the addition of 300 μg/mL BSA-AGEs (Panel A) or 300 μM dibutyryl cAMP (Panel B) and incubated for 24 h. Then the medium was removed and analyzed for nitrite accumulation from RAW 264.7 cells. Data represent the mean ± S.E.M. of three 3 independent experiments done in triplicate. Immuno detection of iNOS expression (Panel C and D) with an iNOS-specific antibody was performed as described in the "Methods".

Involvement of p38 MAP kinase in AGE- and dibutyryl cAMP-stimulated iNOS expression and nitrite production by RAW264.7 cells

We previously demonstrated that p38 MAPK is involved in AGE-induced iNOS expression in C6 glioma cells (Lin et al., 2001). To investigate whether the induction of iNOS in RAW 264.7 cells is mediated through this signaling pathway, a p38 MAPK inhibitor (SB 203580) was used to pretreat the cells, and then AGE- and dibutyryl cAMP-induced iNOS expression and nitrite accumulation were investigated. SB 203580 attenuated AGE- and dibutyryl cAMP-stimulated NO release (Fig. 7A, B) and iNOS induction (Fig. 7C, D) in a dose-dependent manner suggesting that cAMP-PKA acts upstream of p38 MAPK.

DISCUSSION

In the present study, we demonstrate that cAMP-dependent protein kinase plays an important role in AGE-induced iNOS expression and NO release from RAW264.7 macrophages. Furthermore, we show that pretreatment of RAW264.7 cells with the p38 MAPK inhibitior dramatically abolishes AGE- and cAMP-induced iNOS expression and NO release, suggesting that cAMP-dependent protein kinase acts upstream of P38 MAPK. Given that iNOS upregulation and peroxynitrite damage to neurons have been observed in the brain of Alzheimer disease (Smith et al., 1997), AGE-induced iNOS expression may lead to deleterious effects in the CNS.

AGEs have been shown to activate protein tyrosine kinase (Khechai et al., 1997), p21 Ras (Lander et al., 1997), protein kincase C-beta (II) (PKCβ II) (Scivittaro et al., 2000), p42/44 MAPK (Simm et al., 1997), and nuclear factor kappa B (NF-kB) (Huttunen et al., 1999, Mohamed et al., 1999). In this study, we demonstrate that the protein kinase A inhibitors, H-8 and KT 5720, and the p38 mitogen-activated protein kinase (MAPK) inhibitor, SB 203580, inhibit BSA-AGE-induced iNOS expression. Further, AGEs increase cAMP accumulation in a dose-dependent manner in RAW264.7 cells. Consistent with these findings, dibutyryl cAMP also upregulates iNOS expres-