

Chien-Ming Chen,<sup>a</sup> MS  
Chih-Hsiung Wu,<sup>b</sup> MD  
Chien-Huang Lin,<sup>a</sup> PhD  
Chien-Shi Chang,<sup>a</sup> MS  
Hornng-Mo Lee,<sup>a</sup> PhD

<sup>a</sup>Graduate Institute of Biomedical  
Technology and Faculty of Medical  
Technology

<sup>b</sup>School of Medicine, Taipei Medical  
University

**Key Words**

Advanced glycosylation end products  
cAMP  
iNOS  
RAW 264.7 cells

## Involvement of cAMP-dependent Protein Kinase in BSA-AGE-induced iNOS Expression and Nitrite Production in RAW 264.7 Macrophages

**ABSTRACT**

**Background.** Advanced glycosylation end products (AGEs) have been implicated in the structural and functional alterations of proteins that occur during aging and long-term diabetes. Previously, we demonstrated that p38 MAPK is involved in the AGE-induced iNOS expression in C6 glioma cells.

**Aim.** To investigate the roles of cAMP-dependent protein kinase in AGE-induced iNOS expression.

**Methods.** RAW 264.7 cells were incubated with BSA-AGEs or dibutyryl cAMP, and the AGE-induced iNOS expression, nitrite release, and cAMP accumulation were analyzed.

**Results.** AGEs caused dose- and time-dependent increases in nitrite accumulation and iNOS expression in murine RAW 264.7 macrophages. The AGE-stimulated NO production and iNOS expression were dose-dependently inhibited by the PKA inhibitors, KT 5720 and H-8. AGE-stimulated cAMP production began at 1 h, reaching a maximum at 24 h. Treatment of RAW 264.7 macrophages with dibutyryl cAMP resulted in dose-dependent nitrite release and iNOS induction. The iNOS expression was not affected by polymyxin B, a lipopolysaccharide (LPS) inhibitor, suggesting that the iNOS induction is not due to LPS contamination in the BSA-AGEs preparation. Pretreatment of cells with the p38 MAPK inhibitor (SB 203580) inhibited AGE- and dibutyryl cAMP-stimulated nitrite release and iNOS induction.

**Conclusion.** Our results suggest that AGEs may increase intracellular cAMP, which in turn activates PKA and results in p38 MAPK activation, iNOS induction, and NO production in RAW 264.7 macrophages.

(N. Taipei J. Med. 2002; 4:108-116)

**Abbreviation used:**

AGEs = Advanced glycosylation end products;  
BSA = bovine serum albumin;  
iNOS = inducible nitric oxide;

ELISA = enzyme linked immunosorbent assay;  
PBS = phosphate buffered saline;  
ATF-2 = activation transcription factor-2;  
TBST = Tris-buffered saline with Tween 20.

Received: March 27, 2002  
Accepted: April 26, 2002

Correspondence: Dr. Hornng-Mo Lee, Graduate Institute of Biomedical  
Technology, Taipei Medical University, 250 Wu-Hsing Street, Taipei  
110-31, Taiwan, R.O.C.  
Tel: 886-2-2736-1661 ext. 3310; Fax: 886-2-2732-4510;  
E-mail: leehornng@tmu.edu.tw