INTRODUCTION

Aging or prolonged elevation of glucose levels in diabetic patients results in a number of complications including nephropathy, atherosclerosis, retinopathy, neuropathy, and cataracts. These complications have been related to advanced glycosylation end products (AGEs). AGEs are fluorescent substances formed by the non-enzymatic Maillard reaction, and have been considered to be an important factor in mediating diabetic sequelae (Brownlee, 1991).

AGEs can be recognized by specific AGE receptors (Brett et al., 1993) and exert various biological effects. The selective presence of AGE receptors has recently been demonstrated in endothelium (Wautier et al., 1996), mononuclear phagocytes (Schmidt et al., 1993), smooth muscle cells, mesangial cells, and certain neurons (Hori et al., 1996). An AGE-receptor complex can trigger signal transduction resulting in the production of tumor necrosis factor (TNF) and interleukin-1 (IL-1) (Hasegawa et al., 1991). AGEs regulate many cell functions through AGE-specific receptors, or RAGEs (Li et al., 1996; Yamagishi et al., 1996; Higashi et al., 1997; Smedsrod et al., 1997). Activation of RAGEs may trigger a p21 (ras)-dependent mitogen-activated protein kinase pathway in many cell types (Lander et al., 1997; Satoh et al., 1997; Simm et al., 1997). There are 3 important groups of MAPKs, including p42/44 MAPK, also known as an extracellular signal-regulated kinase 1/2 (ERK 1/2), stress-activated protein kinase (SAPK)/c-jun N-terminal kinase (JNK), and p38 MAPK. The p42/44 MAPK pathway is preferentially activated by growth factors and mitogens, whereas the SAPK/JNK and p38 MAPK pathways are preferentially activated by inflammatory cytokines and various forms of stress (Denhardt, 1996).

Nitric oxide is a diffusible gas generated enzymatically from L-arginine and molecular oxygen by NO synthase. To date, at least 3 different types of nitric oxide synthases have been characterized. The endothelial type (eNOS) and the neuronal type (nNOS) are constitutively expressed, whereas the inducible type (iNOS) is induced by a variety of signals in many cell lines (Knowles and Moncada, 1994). Nitric oxide plays important roles in both physiological and pathological conditions. At low concentrations, nitric oxide has been shown to serve as a neurotransmitter and a vasodilator, while at high concentrations it is toxic and may be important in several neurodegenerative diseases (Gross and Wolin, 1995). AGEs have been shown to induce iNOS expression in a variety of cell lines (Rojas et al., 1996; Amore et al., 1997; Lin et al., 2001). We previously demonstrated that p38 MAPK is involved in AGE-induced iNOS expression in C6 glioma cells (Lin et al., 2001).

In the present study, the roles of cAMP-dependent protein kinase in the induction of iNOS in response to AGEs were studied. Our data reveal that AGEs might stimulate cAMP production resulting in PKA activation, iNOS induction, and ultimately, NO release from RAW 264.7 cells.

MATERIALS AND METHODS

Materials

Affinity-purified rabbit polyclonal antibody to iNOS was obtained from Transduction Laboratory (Lexington, KY). Dulbecco Modified Eagle Medium (D-MEM), fetal bovine serum (FBS), glutamine, gentamycin, penicillin, and streptomycin were purchased from Life Technologies (Gaithersburg, MD). Rabbit monoclonal antibodies to iNOS were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). KT 5720, H-8, SB203580, and other inhibitors were from Calbiochem (San Diego, CA). All other chemicals were from Sigma (St. Louis, MO). HRP-conjugated anti-rabbit IgG antibody and HRP-conjugated anti-biotin antibody (1:1000) were from Santa Cruz Biotechnology.

Methods

Preparation of albumin-derived advanced glycosylation end products

Bovine serum albumin (BSA)-derived AGEs were prepared by incubating 1 M glucose with 50 mg/mL BSA in phosphate-buffered saline (PBS, pH 7.4) for at