METHOD FOR INHIBITING EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE WITH TETRACYCLINE

This application is a division of Ser. No. 08/697,815, filed Aug. 30, 1996, now U.S. Pat. No. 5,789,395.

BACKGROUND OF THE INVENTION

The invention relates to methods of reducing the generation of nitric oxide in biological systems. More specifically, the invention relates to the inhibition of inducible nitric oxide synthase in mammals.

Nitric oxide (hereinafter NO) is a recently recognized multifunctional mediator that is produced by and acts on various cells, and that participates in inflammatory and autoimmune-mediated tissue destruction. NO production is catalyzed by a family of ubiquitous enzymes called nitric oxide synthases (also nitric oxide synthetase, hereinafter NOS). NOS is a naturally expressed enzyme in mammals which catalyzes the mixed functional oxidation of L-arginine (a common amino acid) to L-citrulline and nitric oxide. The enzyme removes a guanidino nitrogen of L-arginine to form the nitric oxide. Several isoforms of the NOS enzyme have been identified, and they are generally divided into two types: constitutive NOS (hereinafter cNOS) and inducible NOS (hereinafter iNOS). Additional details concerning types and functions of some NOS enzymes are found, for example, in U.S. Pat. Nos. 5,478,946 and 5,468,630, the entire disclosures of which are incorporated herein by reference. A cDNA clone capable of expressing a human inducible NOS has been described in U.S. Pat. No. 5,468,630.

The nitric oxide product of the NOS enzymes appears to function as either a signaling or an effector molecule depending on the isoform of the NOS enzyme which is involved in its formation. The constitutive form of NOS produces small amounts of NO, which activate guanylate cyclase resulting in the formation of cyclic guanosine monophosphate (cGMP). The cGMP, in turn, mediates several specific functions, including endothelium-dependent vascular relaxation and neural transmission. By contrast, NO is produced in much larger quantities by the inducible isoforms of the enzyme, designated inducible nitric oxide synthases (iNOS). NO produced by an iNOS appears to mediate the cytotoxic activity of macrophages. Other cells which produce iNOS include endothelial cells, neutrophils, Kupffer cells and hepatocytes, and murine fibroblasts stimulated with cytokines. NO is also a chemical messenger in the brain, and appears to be produced there by a separate NOS isoform.

Several physiological activities have been ascribed to NO. Vasoactive agents such as histamine and bradykinin stimulate NO production. NO is a potent vasodilator that increases blood flow and vascular permeability. Interleukin-1 (IL-1) induces the expression of iNOS in pancreatic islets. NO appears to be a mediator of the inhibitory effects of IL-1 on islet function. Another inducer of iNOS is bacterial endotoxin, indicating that NO is involved as a mediator of endotoxic or septic shock. Other inducers of the enzyme include gamma interferon, tumor necrosis factor and other inflammatory cytokines (Collier et al. 1989). For example, tumor necrosis factor appears to be involved in the systemic hypotension associated with septic shock.

NOS is also overexpressed (expressed in increased and often abnormal amounts) in a variety of inflammatory tissues, leading some to postulate that the modulation of NO synthesis and action could represent a new approach to the treatment of inflammatory and autoimmune conditions (Vane et al. 1994, Schmidt et al. 1994). Vane and co-workers have implicated NO as an important mediator of inflammation in animal models (Vane et al. 1994). Where examined, NO formation is found to be increased in autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, Crohn's disease), and several classic inflammatory symptoms (erythema, vascular leakiness) are reversed by NOS inhibitors (Schmidt et al. 1994, Nathan et al. 1994, Marletta 1994). The most compelling evidence for NO as a mediator of tissue injury has been found in studies of arthritis, including studies carried out in animal models of that disease (McCartney-Francis et al. 1993, Stefanovic-Racic et al. 1994), as well as studies of human osteoarthritis (OA) (Amin et al. 1995a) and rheumatoid arthritis (RA) (Sakurai et al. 1995).

It has recently been observed that human OA-affected cartilage can spontaneously release NO under ex vivo conditions in quantities sufficient to cause cartilage damage (Amin et al. 1995a). An inducible NOS, designated "osteoarthritis-affected NOS" (OA-NOS), is overexpressed in OA-affected cartilage, but is not detectable in normal cartilage. The inducible OA-NOS has properties similar to neuronal NOS (based on its molecular weight and antibody cross-reactivity among α-NOS antibodies) and the 133 kD iNOS (sensitive to NF-κB and cycloheximide, upregulated by IL-1β+TNFα+LPS). NO is known to potentiate matrix degradation, which includes inhibition of proteoglycan and collagen type II synthesis (Taskiran et al. 1994, Cao et al. 1996) and upregulation of metalloproteinase activity (Murrell et al. 1995).

Several inhibitors of nitric oxide synthase have been identified. Most of these inhibitors are derivatives of L-arginine, the natural substrate of the NOS enzymes. For example, N^G-methyl-L-arginine and L-N^{ω}-nitroarginine are competitive inhibitors of NO synthesis. U.S. Pat. No. 5,358,969 describes the inhibition of NO formation in acute or chronic inflammatory diseases. The method includes administering to a mammal an NO-inhibitory amount of a methyl-, 1,1-dimethyl-, or amino-substituted guanidine compound. See also U.S. Pat. Nos. 5,246,970 and 5,246,971.

U.S. Pat. No. 5,216,025 describes the use as NO inhibitors for potentiating pressor agents in certain hypotensive patients. These inhibitors include N^G-substituted arginines in which a hydrogen on the guanidino amino group of arginine is replaced by another atomic or molecular species.

U.S. Pat. No. 5,478,946 discloses unsaturated guanidino compounds which are said to regulate nitric oxide synthase and to thereby indirectly regulate levels of cGMP. These compounds can include a variety of substituents, including C_6–C_{12} aryl groups, at various sites in the unsaturated guanidino backbone.

U.S. Pat. No. 5,480,999 discloses compounds of the structure AB, in which A is a cyclooxygenase inhibitor having an accessible acid function, and B is an arginine analog. The compounds are said to have mixed cyclooxygenase- and NOS-inhibitory activity in the same structure.

The production of nitric oxide can also be inhibited in other ways. For example, NO production can be inhibited by means of a compound which interferes with the activity of a cofactor of iNOS, such as tetrahydrobiopterin. Alternatively, net production of NO can be reduced by means of a nitric oxide scavenger. Compounds said to be suitable for use in these kinds of methods are disclosed, for