# BLOCKING INDUCTION OF TETRAHYDROBIOPTERIN TO BLOCK INDUCTION OF NITRIC OXIDE SYNTHESIS

## CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of U.S. Ser. No. 08/063,067, filed May 20, 1993 which is a continuation of U.S. Ser. No. 07/813,507, filed Dec. 26, 1991 now abandoned.

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## TECHNICAL FIELD

This invention is directed to a novel method of inhibiting the induction of nitric oxide formation in biological systems by bacterial endotoxins and cytokines.

## BACKGROUND OF THE INVENTION

For several decades nitroglycerin has been administered to humans as a vasodilating agent in the treatment of cardiovascular disease. Recently, it has been shown that nitroglycerin so administered is converted in the body to nitric oxide which is the pharmacologically active metabolite. Still more recently, nitric oxide has been shown to be formed enzymatically from arginine as a normal metabolite which is an important component of endotheliumderived relaxing factors (EDRFs). EDRFs are currently being intensively studied as participating in regulation of blood flow and vascular resistance. In addition to vascular endothelium, macrophages have also been shown to produce nitric oxide in the body which is a component of their cell killing and/or cytostatic function.

It has been established that the enzyme forming nitric oxide from arginine, i.e., nitric oxide synthase, occurs in two distinct types, namely the constitutive forms and an inducible form. Constitutive forms are present in normal endothelial cells, certain neurons and some other tissues. Formation of nitric oxide by the constitutive form in endothelial cells is thought to play a role in normal blood pressure regulation. The inducible form of nitric oxide synthase has been found to be present in activated macrophages and is induced in endothelial cells and vascular cells, for example, by various cytokines and/or microbial products. It is thought that in sepsis or cytokine-induced shock, overproduction of nitric oxide by the inducible form of nitric oxide synthase plays an important role in the observed life-threatening hypotension. Furthermore, it is thought that overproduction of nitric oxide by the inducible form of nitric oxide synthase is a basis for insensitivity to pressor agents such as $\alpha_1$-adrenergic agonists, used in the treatment of septic or cytokine-induced shock in patients. Moreover, it is thought that overproduction of nitric oxide by the inducible form of nitric oxide synthase is involved in inflammation incident to an immune response.

Considerable research effort has been expended to discover inhibitors of nitric oxide synthase activity. Before the work described herein, said research effort has been directed at uncovering arginine antagonists which inhibit nitric oxide synthase activity. A problem with use of the arginine antagonists for this purpose is that the ones uncovered thus far block not only inducible nitric oxide synthase activity but also constitutive nitric oxide synthase activity; and any specificity of inhibition of any particular arginine antagonist for inducible nitric oxide synthase activity is not so high that it is possible to block hypotension-causing, pathological overproduction of nitric oxide (an inducible enzyme-mediated process) to a therapeutically adequate extent (i.e., so that clinically serious hypotension that would normally occur in sepsis or cytokine-induced shock is avoided or so that pressor agent sensitivity is restored), and, at the same time, not block the physiological nitric oxide synthesis which is thought to play a role in neural function and normal blood pressure regulation (constitutive enzyme-mediated processes) and thereby avoid the toxicity (e.g. neuronal toxicity and hypertension) associated with interfering with physiological nitric oxide synthesis.



## SUMMARY OF THE INVENTIONS

The inventions herein do not rely primarily on arginine antagonists but rather use a novel approach to selectively block the induction of nitric oxide synthesis by cytokines and/or microbial products (e.g., bacterial endotoxins) with a reduced inhibitory effect on physiological (constitutive enzyme-mediated) nitric oxide production.

The inventions herein draw on the recent discovery that tetrahydrobiopterin is a cofactor in the induction of nitric oxide synthesis (Kwon, N. C., et al, J. Biol. Chem. 264:20496–20501, 1989, and Tayeh, M. A., et al J. Biol Chem. 264:19654–19658, 1989).

The inventions herein also draw on the discovery that cytokines, e.g., interferons including interferon-gamma, tumor necrosis factor, interleukin-1 and interleukin-2, have been found to markedly increase tetrahydrobiopterin levels in various cells (Werner, E., et al, Biochem. J 262:861–866, 1989; Kerler, F., et al, Experimental Cell Research 189, 151–156, 1990; Ziegler, I., et al, The Journal of Biological Chemistry, 265, No. 28, 17026–17030, Oct. 5, 1990), the discovery in the course of the inventions herein that tetrahydrobiopterin synthesis is induced by bacterial endotoxins, the discoveries that tetrahydrobiopterin synthesis occurs via a guanosine triphosphate pathway and via a pterin salvage pathway (Nichol, C., et al, Ann. Rev. Biochem. 54, 729–764, 1985; Milstien, S., et al, Biochem. and Biophys. Res. Comm., 128, No. 3, 1099–1107, 1985; Kaufman, S., et al, J. Biol. Chem., 234, 2683–2688, 10/59; Kaufman, S., J. Biol. Chem., 242, 3934–3943, Sep. 10, 1967), and the discovery in the course of the inventions herein that the continuous production of tetrahydrobiopterin, via a guanosine triphosphate pathway or a pterin salvage pathway, is not required for maintaining constitutive nitric oxide synthase activity over at least a period of hours.

It has been discovered herein that inhibiting the synthesis of tetrahydrobiopterin in vascular cells via the guanosine triphosphate pathway and/or the pterin salvage pathway selectively inhibits the induction of nitric oxide synthesis in said cells by bacterial endotoxins and cytokines, i.e., performs such inhibiting without affecting physiological constitutive enzyme-mediated nitric oxide synthesis. The inhibition of nitric oxide synthesis in smooth muscle cells in accordance with the invention is an unexpected result since it has been shown that macrophages which are "normal"i.e., are not induced for nitric oxide synthesis, already contain enough tetrahydrobiopterin for a maximal rate of nitric oxide synthesis (see Kwon, N. C., et al, J. Biol. Chem. 264:20496–2501, 1989).

In a first embodiment the invention herein is directed at a method of inhibiting induced nitric oxide synthesis from arginine in vascular cells in a subject in need of said inhibition (e.g,. for prophylaxis or treatment of systemic hypotension or to restore vascular contractile sensitivity to