

N⁶-(HYDRAZINOIMINOMETHYL)LYSINE AND METHOD OF INHIBITING NITRIC OXIDE FORMATION IN BODY

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TECHNICAL FIELD

This invention is directed to novel inhibitors of biological nitric oxide formation.

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 endothelium-derived 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 (Iyengar, R., et al, Proc. Natl. Acad. Sci, USA, Vol. 84, pp. 6369-6373, September, 1987).

More recently it has been established that the enzyme forming nitric oxide from arginine, i.e., nitric oxide synthase, occurs in two distinct isoforms, namely the constitutive isoform and the inducible isoform. The constitutive isoform is present in normal endothelial cells, neurons and some other tissues. Formation of nitric oxide by the constitutive isoform in endothelial cells is thought to play a role in normal blood pressure regulation. The inducible isoform of nitric oxide synthase has been isolated from activated macrophages and is induced by various cytokines or combinations of cytokines in endothelial cells and vascular smooth muscle cells. It is thought that in sepsis or cytokine-induced shock that the observed life-threatening hypotension is due mainly or wholly to overproduction of nitric oxide by the inducible isoform of nitric oxide synthase. The specific tissue(s) responsible are not yet known but are likely to include endothelium and vascular smooth muscle.

Because of the physiological importance of the above, a search has been carried out for compounds which will block nitric oxide production in the body. The first compound discovered for use to obtain this effect is N^G-methyl-L-arginine (referred to herein as NMMA) (Palmer, R. M. J., et al, Nature (London), 333, pp. 664-666, 1988; Palmer, R. M. J., et al, Biochem. Biophys. Res. Commun. 153, pp. 1251-1256, 1988). Administration of NMMA to guinea pigs and rabbits has been shown to increase blood pressure (Aisaka, K., et al, Biochem. Biophys. Res. Commun. 160, No. 2, pp. 881-886, Apr. 28, 1989; Rees, D. D., et al, Proc. Natl. Acad. Sci. USA, Vol. 86, pp. 3375-3378, May, 1989). A second compound that has been discovered for use to obtain this effect is N^G-nitro-L-arginine (referred to herein as NNA). This compound, often used as its more soluble methyl ester, is a good inhibitor of the constitu-

tive form of nitric oxide synthase but is less effective than NMMA as an inhibitor of the inducible isoform (S. S. Gross et al, Biochem. Biophys. Res. Commun. 170, pp. 96-103 (1990)). Still another inhibitor is N^G-amino-L-arginine (referred to as NAA). This compound and its use as an inhibitor are described in Griffith, U.S. patent application Ser. No. 07/406,897, now U.S. Pat. No. 5,059,712. NAA is an excellent inhibitor of both the constitutive and inducible isoforms of nitric oxide synthase and has been shown to reverse the severe hypotension observed in endotoxic shock and cytokine-induced shock in dogs. NAA, however, can show some toxicity when administered for the length of time necessary to fully control the hypotension of these conditions.

SUMMARY OF THE INVENTION

In a broad aspect, the instant invention is directed to inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering agent which preferentially inhibits inducible isoform of nitric oxide synthase over constitutive isoform of nitric oxide synthase, in a nitric oxide synthesis inhibiting amount, to said subject.

It has been discovered herein that physiologically active N⁶-(hydrazinoiminomethyl)lysine and its pharmaceutically acceptable acid addition salts constitute superior inhibitors of nitric oxide synthesis in the body and are more selective than NMMA, NNA, and NAA in inhibiting the inducible isoform of nitric oxide synthase over the constitutive isoform of nitric oxide synthase and are substantially less toxic than NAA and its acid addition salts. The term N⁶-(hydrazinoiminomethyl)lysine means N⁶-(hydrazinoiminomethyl)lysine in free base form. The term physiologically active N⁶-(hydrazinoiminomethyl)lysine means N⁶-(hydrazinoiminomethyl)lysine containing L-enantiomer thereof; in other words, the L-enantiomer portion is physiologically active. Preferably, the physiologically active N⁶-(hydrazinoiminomethyl)lysine is constituted of from 50% to 100% L-enantiomer with any remainder being D-enantiomer, e.g., of 50% L-enantiomer and 50% D-enantiomer, very preferably of 100% L-enantiomer. The term pharmaceutically acceptable acid addition salts means those acid addition salts that are acceptable for pharmaceutical purposes.

For the uses set forth hereinafter, compositions containing more than 99% by weight (on a water-free basis) of agent selected from the group consisting of physiologically active N⁶-(hydrazinoiminomethyl)lysine and pharmaceutically acceptable acid addition salts thereof, are preferred.

A method herein for inhibiting nitric oxide synthesis in a subject in need of such inhibition comprises administering a nitric oxide synthesis inhibiting amount of agent selected from the group consisting of physiologically active N⁶-(hydrazinoiminomethyl)lysine and pharmaceutically acceptable acid addition salts thereof, to said subject. In an application of this method, said agent is administered to a subject who has or is at risk to have increased synthesis of nitric oxide by inducible isoform of nitric oxide synthase, and is administered to preferentially inhibit inducible isoform of nitric oxide synthase over constitutive isoform of nitric oxide synthase.

A method herein for blocking nitric oxide formation from arginine in in vitro studies including studies with isolated organs, intact cells, cell homogenates and tissue homogenates to elucidate or control the biosynthesis,