

METHOD OF USING AMINOARGININE TO BLOCK NITRIC OXIDE FORMATION IN VITRO

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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 from arginine as a normal metabolite which is an important component of endothelium-derived relaxing factors (EDRF's). EDRF's are currently being intensively studied as participating in regulation of blood flow and vascular resistance. Incident to such study, a search has been carried out for compounds which block nitric oxide production in the body. The compound discovered for use to obtain this effect is N^G-methyl-L-arginine (Palmer, R. M. J., et al, *Nature* (London), 333, pp. 664-666, 1988). Administration of N^G-methyl-L-arginine to guinea pigs and rabbits has been shown to increase blood pressure (Aisaka, K., et al, *Biochemical and Biophysical Research Communications*, Vol. 160, No. 2, pp. 881-886, 4/28/89; Rees, D. D., et al, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 3375-3378, 5/89).

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, 9/87).

SUMMARY OF THE INVENTION

It has been discovered herein that physiologically active N^G-aminoarginine (wherein the terminology N^G indicates substitution on a guanidino nitrogen) and its pharmaceutically acceptable acid addition salts constitute superior inhibitors of nitric oxide synthesis in the body. The term physiologically active N^G-aminoarginine is used herein to mean N^G-aminoarginine selected from the group consisting of N^G-amino-L-arginine and N^G-amino-D,L-arginine. In the D,L-compound only the N^G-amino-L-arginine portion is physiologically active.

N^G-amino-L-arginine has been reported as a by-product in the reductive deprotection of N^G-nitro-L-arginine which is used in chemical synthesis of peptides. However, no report is known of the preparation and isolation of such or of N^G-amino-D,L-arginine in pharmaceutically pure form. The invention herein contemplates such preparation and isolation.

Thus composition herein which is considered to be novel is pharmaceutically pure physiologically active N^G-aminoarginine or a pharmaceutically acceptable acid addition salt thereof. The term pharmaceutically

pure is used herein to mean 99.9+ % pure (on a water-free basis).

The method of the invention herein for preparing and isolating physiologically active N^G-aminoarginine comprises the steps of:

(a) reducing N^G-nitro-L-arginine or N^G-nitro-D,L-arginine to form a mixture of physiologically active N^G-aminoarginine and arginine;

(b) treating said mixture with arginase to convert the arginine therein to ornithine thereby forming a mixture of physiologically active N^G-aminoarginine and ornithine;

(c) isolating pharmaceutically pure physiologically active N^G-aminoarginine from the mixture resulting from step (b).

The isolation of step (c) is readily carried out by chromatography or by crystallization of the N^G-aminoarginine as the flavianic acid salt.

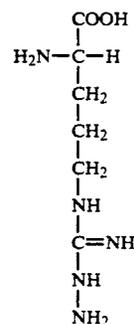
A method herein for inhibiting nitric oxide synthesis in a subject in need of such inhibition comprises administering a nitric oxide synthesis inhibiting amount of physiologically active N^G-aminoarginine or pharmaceutically acceptable acid addition salt thereof to said subject. The term "subject" is used herein to mean any mammal, including humans, where nitric oxide formation from arginine occurs. This method contemplates prophylactic as well as curative use.

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, metabolism or physiological role of nitric oxide comprises adding physiologically active N^G-aminoarginine or pharmaceutically acceptable acid addition salt thereof to a medium containing said organs, cells, or homogenates at a concentration sufficient to inhibit nitric oxide formation.

DETAILED DESCRIPTION

As previously indicated, inventive composition herein constitutes pharmaceutically pure physiologically active N^G-aminoarginine or a pharmaceutically acceptable acid addition thereof.

N^G-amino-L-arginine in free base form has the structural formula



N^G-amino-D,L-arginine in free base form consists of 50% N^G-amino-L-arginine and 50% N^G-amino-D-arginine which has the structural formula: