TITLE

Agents for Blocking Endothelin Derived Relaxing Factor

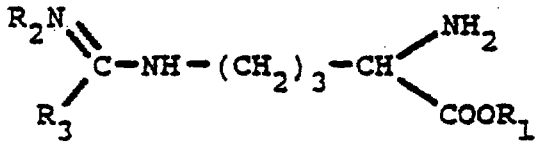
DESCRIPTION

The invention relates to agents for blocking the effect or the production of endothelin derived relaxing factor (EDRF). Such blocking agents are useful for the treatment of various shocks, for example, stresses, septic shocks or traumatic shocks.

Sepsis and endotoxemia are still the major causes of death in surgical intensive care units despite the use of large amounts and specific antibiotics, careful monitoring and operative interventions. Non-surviving patients tend to have a lower peripheral vascular resistance described as "unrelenting hypotension". Indeed, patients present a deep vasodilatation especially in the preterminal phase and die of peripheral vascular failure more than of cardiac failure. Moreover, the persistant vasodilatation in these patients is only temporarily responsive to infused catecholamines (or other vasoconstrictor agents) and cannot usually be restored due to a "vascular hyporesponsiveness" which is the major factor contributing to mortality.

The present invention relates to the treatment of vascular hyporesponsiveness in various shocks states such as sepsis, endotoxemia and other diseases leading to persistant and deep systemic vasodilatation. The treatment includes the administration of an effective amount of a blocking agent of the effect or the production of endothelin derived relaxing factor (EDRF) or nitric oxide like factor.

In particular, the blocking agents with which the invention is concerned are L-aminoacids of the general formula I



I

wherein R_1 represents a hydrogen atom or a methyl or ethyl group, R_2 represents a hydrogen atom or a nitro group and R_3 represents an amino, methylamino, ethylamino, methyl or ethyl group, with the proviso that if R_2 represents a hydrogen atom then R_3 does not represent an amino group. These L-aminoacids are known compounds, having been disclosed in EP 230037 and other publications. However, their known use is as cytoprotective agents. We have found that these L-aminoacids are able to restore depressed response to catecholamines and to effectively inhibit vascular hyporeactivity.

Accordingly the invention provides use of an L-aminoacid as above defined for the preparation of a medicament for the treatment by perfusion of shock states.

The preferred L-aminoacids for this use are L-2-amino-5-(1-methylamino-1-imino-methylamino)-pentanoic acid (I : $R_1 = R_2 = H, R_3 = NHCH_3$) which is also known as L-N-monomethyl-arginine and is hereinafter referred to as "L-NMMA";

L-2-amino-5-(1-imino-ethylamino)-pentanoic acid (I : $R_1 = R_2 = H$, $R_3 = CH_3$) which is also known as L-iminoethyl--ornithine and is hereinafter referred to as "L-N10"; and

methyl L-2-amino-5-(1-nitroimino-1-amino-methylamino)--pentanoate (I : $R_1 = CH_3$, $R_2 = NO_2$, $R_3 = NH_2$) which is also known as L-nitroarginine methyl ester and is hereinafter referred to as "L-NAME".

We have also found that a highly significant synergistic effect is achieved by administering the L-aminoacids I in admixture with a cyclooxygenase blocker, such as indomethacin or aspirin. Accordingly the invention further provides a pharmaceutical composition comprising an L-aminoacid I as above defined in admixture with a cyclooxygenase blocker.

For the experimental demonstration, much previous evidence has shown that animal models of shock in vivo and in vitro well mimick the human vascular hyporesponsiveness to pressor neurotransmitters or hormones (Wichterman K.A., Baue A.E., Chaudry T.H. Sepsis and septic shock. A review of laboratory models and a proposal. J. of Surgical Res. 29, 189-201 (1980), Parrat J.R. Alteration in vascular reactivity in sepsis and endotoxemia. In : Vincent J.L. (Ed.) Update in intensive care and emergency medicine. Springer vol. 8, 299-308, 1989). This abnormal vascular responsiveness and the effect of blocking agents of EDRF can be well demonstrated in vascular tissues removed from animals in shock.

For the compounds of the invention, this was evidenced by the following experiments :

Sprague Dawley rats (220-330 g) have received a 10 mg/kg ip injection of Escherichia Coli endotoxin (0114B4 Sigma). After 3 hours, rats were sacrificed by cervical dislocation and the thoracic aorta removed and cleaned of the surrounding tissue. Rings 2 mm wide were suspended under a tension of 2 g at 37°C in organ bath containing 10 ml of Krebs Henseleit physiological solution and gassed with 95 % O_2/5 % CO_2. Contractile responses were measured using force displacement transducers (Auguet M., Delaflotte S., P.E. Chabrier, P. Braquet Comparative effects of endotelin and phorbol 12-13 dibutyrate in rat aorta. Life Sci. 45, 21, 2051-2059, 1989).

In some experiments, the endothelium was gently disrupted (-E). Phenylephrine (PE) induced contraction was stable over the time in control rings of animals receiving saline solution (0.9 % NaCl) with (E+) or without (E-) endothelium. The arginine derivative (10, 30 or 100 μM) had no significant effect per se.

Adversely, rings from animals treated with endotoxin showed, despite a similar contractile effect to PE, a loss of tonicity within the time referred as vascular hyporeactivity. This phenomenon was accentuated with intact endothelium (E+). The compounds of the invention (at 10, 30 or 100 μM) were able to reverse the loss of tonicity indicating that these compounds could inhibit the vascular hyporesponsiveness in preparations with or without endothelium.

The effect of the compounds of the invention was specific to the inhibition of EDRF generation whereas L-arginine, the natural precursor of nitric oxide, enhanced the loss of tonicity in endotoxin treated preparation.

In some experiments, the compounds of the invention were introduced in the bath 105 mn after PE when the tissue has completely its tonicity. In these conditions, the compounds of the invention, alone, were able to curatively and totally restore the contraction and therefore contribute extensively to vascular hyporesponsiveness to vasoconstrictor agents in shock. It has been also found that the action of the compounds of the invention might be strongly increased when associated to blockers of cyclooxygenase such as aspirin and indomethacin for instance. This was evidenced by the following in vivo experimentation.

Male Sprague Dawley rats (280-320 g) were pithed and perfused continuously with endotoxin (EDTX, Escherichia Coli lipopolysaccharide OIII: B4; 300 µg/kg/h) for 60 min. This resulted in a systemic hypotension (decrease of DBP (diastolic blood pressure) of 40%, a vascular hyporeactivity to stimulation of pressor agents accompanied by hemoconcentration and leukocytopenia. The vascular reactivity was measured by constructing dose-response curves to methoxamine (a α_1-agonist) in a cumulative fashion and by calculating the ED_{50} (Effective dose 50%). The ED_{50} values for methoxamine were 79 ± 9 µg/kg and 278 ± 34 µg/kg for control and EDTX-treated rats repectively (n = 24 animals). Animals were perfused with the drugs for 60 min. The number of rats in each group is 5 or 6. Results are presented in the following table. A 60 min perfusion of endotoxin lipopolysaccharide (300 µg/kg/h) to pithed rats led to hypotension and impaired the vascular reactivity to pressor agents as observed in septic and endotoxinic shock in human. These vascular hyporeactivity can be inhibited in a dose dependent manner by blockers of EDRF such as L-NMMA, L-NAME or L-NIO confirming the in vitro results. Their effects on blood pressure are however less marked. Association of blockers of cyclooxygenase (Aspirin, Indomethacin for instance...) and blockers of EDRF results on a highly significative synergestic protective effect in both vascular hyperactivity and decrease of blood pressure induced by shock.

It should be noticed that when associating both kinds of compounds the resulting activity is far more important that the one corresponding to a mere addition of the activity of both components.

TOXICITY

An acute toxicity study of the compounds of this invention has been conducted on rats and mice but no death was noticed at the maximum administrable dosis.

POSOLOGY

For the treatment of shock the usual posology comprises the administration by perfusion of 10 to 500 mg/hour, dissolved or suspended in a serum, of the selected compound of the invention, when used alone. The duration of treatment has to be determined in each case in relationship with a sufficient recovery of the patient. In case of co-administration of one of the compounds according to the invention with a blocker of cycloogenase, the dose for one hour of perfusion contains 10 to 100 mg of the selected compound according to the invention, associated with, 0.1 to 1 mg, if indomethacin is used, or 2 to 200 mg, if aspirin is used, or the corresponding amounts of other blockers of cycloogenase.

	Dose in mg/kg/h	Vascular reactivity (methoxamine) ED_{50} (µg/kg)
Control		79 ± 9
EDTX treated animals		278 ± 34
L-NMMA	12.5	246 ± 31
L-NMMA	50	189 ± 15
L-NMMA	100	130 ± 8
L-NAME	10	238 ± 26
L-NAME	30	121 ± 11
L-NAME	100	105 ± 7
L-NIO	50	200 ± 10
L-NIO	400	138 ± 12
ASPIRIN	3.75	248 ± 24
ASPIRIN	150	136 ± 29
ASPIRIN	300	107 ± 10
INDOMETHACIN	0.5	254 ± 22
INDOMETHACIN	20	128 ± 16
ASPIRIN + L-NMMA	3.75 50	76 ± 15
ASPIRIN + L-NAME	3.75 30	79 ± 12
ASPIRIN + L-NMMA	150 50	58 ± 5
ASPIRIN + L-NAME	150 30	62 ± 4
INDOMETHACIN + L-NMMA	0.5 50	80 ± 8
INDOMETHACIN + L-NAME	0.5 30	74 ± 7

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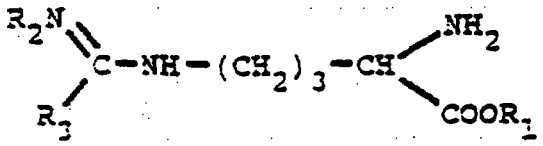
CLAIMS

1. Use of an L-aminoacid of the general formula



wherein R_1 represents a hydrogen atom or a methyl or ethyl group, R_2 represents a hydrogen atom or a nitro group and R_3 represents an amino, methylamino, ethylamino, methyl or ethyl group with the proviso that if R_2 represents a hydrogen atom then R_3 does not represent an amino group for the preparation of a medicament for the treatment by perfusion of shock states.

2. Use of L-2-amino-5-(1-methylamino)-1-imino-methylamino)--pentanoic acid for the preparation of a medicament for the treatment by perfusion of shock states.

3. Use of L-2-amino-5-(1-imino-ethylamino)-pentanoic acid for the preparation of a medicament for the treatment by perfusion of shock states.

4. Use of methyl L-2-amino-5-(1-nitroimino-1-amino--methylamino)-pentanoate for the preparation of a medicament for the treatment by perfusion of shock states.

5. A pharmaceutical composition comprising an L-aminoacid as defined in claim 1 in admixture with a cyclooxygenase blocker and with a pharmaceutically acceptable diluent or carrier.

6. A pharmaceutical composition comprising L-2-amino-5--(1-methylamino-1-imino-methylamino)-pentanoic acid) in admixture with a cyclooxygenase blocker and with a pharmaceutically acceptable diluent or carrier.

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7. A pharmaceutical composition comprising L-2-amino-5--(1-imino-ethylamino)-pentanoic acid in admixture with a cyclooxygenase blocker and with a pharmaceutically acceptable diluent or carrier.

8. A pharmaceutical composition comprising methyl L-2--amino-5-(1-nitroimino-1-amino-methylamino)-pentanoate in admixture with a cyclooxygenase blocker and with a pharmaceutically acceptable diluent or carrier.

9. A pharmaceutical composition according to any of claims 5 to 8 in which the cyclooxygenase blocker is indomethacin.

10. A pharmaceutical composition according to claim 9 and comprising from 10 to 100 mg of the L-aminoacid and from 0.1 to 1 mg of indomethacin.

11. A pharmaceutical composition according to any of claims 5 to 8 in which the cycloxygenase blocker is aspirin.

12. A pharmaceutical composition according to claim 11 and comprising from 10 to 100 mg of the L-aminoacid and from 2 to 200 mg of aspirin.