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(54) Title: NITROGEN MONOXIDE COMPOSITION FOR USE AS A DRUG

(54) Titre: COMPOSITION A BASE DE MONOXYDE D'AZOTE EN TANT QUE MEDICAMENT

(57) Abstract

The use of a gaseous composition containing nitrogen monoxide (NO) and carbon dioxide (CO_2) for preparing a drug useful for the intravascular treatment or prevention of ischaemia is disclosed.

(57) Abrégé

L'invention concerne l'utilisation d'une composition gazeuse contenant du monoxyde d'azote (NO) et du dioxyde de carbone (CO_2) pour la fabrication d'un médicament gazeux destiné au traitement ou à la prophylaxie par voie intravasculaire de l'ischémie.

COMPOUND ON A BASE OF NITROGEN MONOXIDE USED AS MEDICATION

This invention relates to the use of a stable gaseous compound on a base of nitrogen monoxide (NO) and carbon dioxide (CO_2) to produce a gaseous medication intended for the treatment or prevention, by intravascular means, of ischemia and, in particular, embolism.

Nitrogen monoxide is produced naturally in mammals by an enzyme, called NO-synthase, which is expressed in terms of its makeup by the endothelial cells, by the platelets, and by the central and peripheral nervous systems. Another form of calcium-independent NO-synthase can be induced by different stimuli (especially liposaccharides) in numerous cells, such as macrophages, the lymphocytes, the myocardial cells, the endothelial cells, and the smooth muscular cells.

Nitrogen monoxide is an important biological messenger in mammals, and this molecule plays a decisive role in the local control of hemodynamics.

It has been possible to make evident the release of NO by means of the endothelial cells in the case of variations in the blood flow. Nitrogen monoxide appears especially as a major component of the physiological adaptation of the vascular diameter to blood perfusion: reactive hyperemia is thus attenuated in a most noticeable fashion on the coronary level. Conversely, a chronic increase in the blood flow, produced by an arteriovenous fistula, increases the dependent relaxations of the endothelium.

The capacity of nitrogen monoxide, produced at the level of the vascular wall and in the neighboring tissues, to regulate precisely the vascular tonus by adaptation of the blood flow is remarkable. Likewise, it has been assumed that the NO that is released during neuronal activity could regulate the tonus of cerebral microcirculation, thus linking the activity and the cerebral blood flow. We also recall the role played by NO in the regulation of the proliferation of smooth vascular muscle which is a decisive factor in vascular compliance [sic].

Nitrogen monoxide furthermore controls the post-capillar venule permeability.

Nitrogen monoxide also participates in hormonal regulation mechanisms at the level of the kidney by inhibiting the release of renin, and on the cardiovascular level by opposing the release of the natriuretic factor (ANF).

Finally, in vivo, platelet activation is under the permanent control of endothelial nitrogen monoxide and, to a lesser degree, it is under the control of platelet NO-synthase itself. During aggregation, the platelets release nucleotides (ATP, ADP), serotonin, PAF, thromboxane A2, and vasopressin; they can also initiate the gush of coagulation by releasing

thrombin. In response to ATP, ADP, serotonin, PAF, and thrombin, the endothelial cells release NO and prostacyclin, both of which act together to prevent and counter the process of platelet aggregation.

The abnormal decline in the nitrogen monoxide rate, observed in the case of numerous diseases, seems to confirm the importance of its role in the organism. Such a decline is characteristic of hypertension, hypercholesterolemia, atherosclerosis, and diabetes.

Likewise, a very early reduction of the basal release of NO would not be the cause of problems related to the re-perfusion of ischemic areas, such as coronary thrombosis and vasospasms.

Various vasodilator agents have been developed so far on the basis of these findings. These substances, known as nitrovasodilators, produce NO in vivo and thus make up for a deficiency in the endogenous NO. By way of example, one might mention molsidomine or sodium nitroprussiate, which make it possible to prevent the phenomena of platelet adhesion and aggregation.

To make up for insufficient production of NO, there has even been a proposal for the administration of L-arginine or analogues of L-arginine, since L-arginine intervenes directly in the biosyntheses of nitrogen monoxide as a substrate of NO-synthase.

In view of the significant contribution made by nitrogen monoxide to the maintenance of a low pulmonary circulation pressure and the importance of the resultant local vasodilator effect, it has also been suggested that NO be directly administered by way of inhalation in the treatment of acute pulmonary arterial hypertension. The extensive research done along these lines demonstrated the therapeutic effectiveness of a gaseous mixture of nitrogen monoxide and inhaled nitrogen at doses between 1 and 20 ppm of NO on patients suffering acute respiratory disorders: in fact, a reduction of pulmonary arterial hypertension, possibly accompanied by an improvement of the ventilation-perfusion ratios due to an intrapulmonary shunt, was observed.

Nevertheless, this type of administration by inhalation entails the major inconvenience of the formation of NO_2 due to the reaction of the inhaled NO with the oxygen present at the level of the pulmonary alveoli. Now, nitrogen dioxide is a substance that is highly toxic for the lungs. Another disadvantage of this method is related to the need for making a mixture consisting of nitrogen monoxide, nitrogen, and oxygen, in an extemporaneous fashion, since the contact time between NO and O_2 must be as short as possible.

Moreover, administration by way of inhalation according to all evidence restricts the action of the exogenous nitrogen monoxide to the ventilated areas of the lungs.

An intravascular injection of the mixture used, made up of nitrogen and NO, in fact, cannot be considered here since it would lead to gaseous embolism.

It is in this context that this invention was developed.

The invention thus relates to the use of a gaseous compound, containing nitrogen monoxide (NO) and carbon dioxide (CO_2), to produce a gaseous medication intended for the treatment or prevention of ischemia by the intravascular route.

The invention further relates to the use of a gaseous compound, containing nitrogen monoxide (NO) and carbon dioxide (CO_2), to produce a gaseous medication intended for the treatment or prevention of embolism by the intravascular route.

The invention may be used in the treatment of humans or animals.

In the context of this invention:

- the term ischemia refers to a total or partial stoppage of blood circulation in a localized area of the human or animal organism;

- and the term embolism as such refers to an obliteration of a blood vessel by a clot or a foreign body moved along by the blood up to the place where the caliber of the blood vessel is insufficient to permit its passage.

Embolism is thus a particular form of ischemia.

The gaseous medication involved in the invention is preferably made up of a single mixture of carbon dioxide and nitrogen monoxide; however, the addition of at least one gas to the group made up of xenon, krypton, nitrogen protoxide and their mixture to said mixture of CO_2 and NO can also be considered in the context of the invention.

The concentration of NO in the gaseous mixture of NO + CO_2 is an effective concentration, preferably between 1 and 100 ppm. A concentration of less than 1 ppm is not desirable since a minimal concentration of 2 nM of NO is detected in the blood plasma of a healthy mammal. A concentration in excess of 100 ppm leads to the progressive disappearance of the therapeutic effect. The reasons for the low activity level observed in such concentrations are as yet

unknown, but could be related to a local toxicity or possibly the saturation of the nitrogen monoxide receptors.

According to a preferred embodiment, the concentration of NO in the medication mixture is between 15 and 30 ppm.

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The stability of the gaseous medication in this invention makes it possible to store it under pressure under conventional conditions in bottles made of steel or a light alloy on a base of aluminum.

To prevent any risk of contamination, bottles made of a light alloy on a base of aluminum are preferred. The preferred storage conditions that ensure stability in excess of 2 years are a temperature between 15 and 30°C, preferably 20 and 25°C, and a pressure between 20 and 30 bar.

The gaseous medication in this invention can be administered by the intravascular route, without risk of gaseous embolism, for example, by the intra-arterial route, by the intracardial route, or by the intravenous route.

In the case of intravenous administration, a catheter that is introduced at the level of the blood vessel to be treated will be preferred. Catheters that can be used for this purpose are those that are habitually employed in this technique. The nature of the material constituting the catheter is not critical as such; nevertheless, a flexible material such as silastene is recommended. It is implanted in the blood vessel in the known manner, for example, after local anesthesia and incision. For greater ease, the catheter may be retained by ligature where upon the incision can or may not be closed up again.

During injection, the habitual necessary precautions must be taken so as to prevent platelet aggregation and coagulation at the level of the orifice.

With regard to this, a heparin-treated physiological solution could also be administered before and after injection of the gaseous medication involved in this invention.

As for the injection flow rate, the expert in the field can determine it easily, since it is understood that immediate solubilization of the gaseous medication, gradually, along with its administration, is more than desirable: by doing it this way, the formation of gas bubbles can be prevented. In humans, a flow rate of less than 20 ml/mn and, preferably, less than 10 ml/mn, or even better still 8 ml/mn, will lead to optimum effectiveness. These figures, however, are not in any way intended to restrict the invention. It is quite obvious in fact that the flow rate of the injection is a function of the blood flow rate of the vessel to be treated.

The quantity of medication compound to be administered, however, depends on the age of the patient, the seriousness of the ailment from which he suffers, and the NO concentration of the injected gaseous compound.

According to the invention, it would be preferable to intervene at the level of the arterial vessel. In fact, it was found that in the arteries the concentration of oxyhemoglobin is

such that the fixation of nitrogen monoxide on the hemoglobin is definitely unfavorable. Due to this fact, the formation of methemoglobin by linkage of the nitrogen monoxide with the hemoglobin would take place only in a quantity that would be insufficient to cause a possible inhibition of the therapeutic effect of the nitrogen monoxide and especially its vasodilator and platelet anti-aggregate effect.

Recent studies show that nitrogen monoxide can also react with proteins, such as albumin, to form S-nitrosothiols, whose lifetime is longer than that of free NO. These species display biological activity comparable to that of the free NO; the effectiveness of the medication compounds involved in the invention will not be diminished.

The medication compounds of the invention are perfectly suitable for the treatment of ischemia and embolism when administered by intravascular, intra-arterial, intracardial, or intravenous injection.

Examples 1 to 3 below, illustrate the stability of the compounds involved in the invention, as well as their therapeutic usefulness, with reference to Figures 1 and 2 as enclosed.

EXAMPLE 1

Various mixtures of nitrogen monoxide and carbon dioxide were prepared and packaged in bottles of type B5, consisting of a light alloy, on a base of aluminum, sold through S.M. GERZAT, at a pressure of 24 bar. In these mixtures, the initial concentration of nitrogen monoxide was set at 20 ppm. The stability of the concentration in terms of NO was studied for 3 bottles kept at ambient temperature over a period of 16 months.

The value of the NO concentration was measured by an analyzer with chemiluminescence in the range of 0 to 100 ppm, calibrated before each measurement with the help of a standard NO/N_2 mixture at 90 ppm. The chemiluminescence analyzer employed is the TOPAZE 2020 made by COSMA.

At the end of a period of 16 months, no decomposition of the nitrogen monoxide in the NO/CO_2 mixtures, kept at ambient temperature, was detected. Indeed, the only fluctuations measured in the value of the concentration of NO remain below the precision of the analysis.

These results confirm the stability of the medication compounds involved in the invention.

EXAMPLE 2

The therapeutic usefulness of the medication compounds of the invention was demonstrated in an experimental model. More specifically, this example was aimed at evidencing the in vivo vasodilation qualities of the gaseous compounds made up of a mixture of CO_2 and NO on the coronary level, using a model of multifocal myocardial ischemia in the rat.

The test was performed on male Wistar rats with an average weight of 260/280 g, aged three months.

To administer the compounds in this invention, we installed flexible catheters emptying into the left ventricle.

The rats were made to fast for 20 hours on the evening before the experiment.

A Chloral anesthesia was performed by intraperitoneal administration of 360 mg/kg of anaesthetic. The left carotid was separated and clamped without lesion of the muscular planes. A flexible silastene catheter, filled with 2% heparin-treated physiological serum, was introduced by the retrograde path up to the left cardiac ventricle (about 5 cm of catheter). The catheter was kept in place by two ligature threads and was brought out again under the skin at the level of the dorsal portion of the head. The incision was closed up again. The animal awoke from its anesthesia within 60 to 90 minutes. The permeability of the catheter was checked and heparin-treated physiological serum remained in the catheter.

Three hours after awakening, the animals resumed their normal spontaneous activities. The end of the catheter was extended with a conventional catheter (Biotrol) with an inside diameter of 600 μ and a length of 30 cm for use in subsequent injections.

Various experiments were performed on a group of healthy rats and a group of embolized rats.

The operating record applied so as to induce multifocal myocardial ischemia is described below.

Embolization was caused by the administration, to a healthy rat, of 0.4 ml of dextrane at 20% containing 40,000 50-μ microtimed albumin microspheres, kept in suspension by agitation. The injection was administered in one minute by means of the intraventricular catheter.

0.2 ml of physiological serum was then administered by the same route to push the assembly of microspheres into the ventricle. These microspheres were distributed in the organism as a function of fractions of cardiac flow and, in particular, in the myocardium. Each microsphere clogged one arteriole and led to one microembolization. After the administration of the microspheres, there was an interval of 30 minutes for the development of the ischemia prior to the appearance of edema. The rats in the other half of the lot were not embolized.

The catheter was then connected to a gaseous flow rate regulator, connected to a source for the supply of gas to be injected.

Indocyanine green (10 μl) was placed in the catheter to display the movement of the physiological serum visually. The gas flow regulator is opened and the gas is pushed at constant flow rate into the catheter for one minute.

After this interval of one minute, the catheter is disconnected from the regulator and the rest of the gas located in the catheter is pushed by 0.2 ml of physiological serum.

Immediately (10 seconds) after a solution of isopropyl iodo amphetamine (IAMP) marked with 125 iodine in a volume of 0.2 ml was administered by the same route and pushed by 0.2 ml of physiological serum. Five minutes after this later administration, the rats were sacrificed by decapitation. Blood was sampled on heparin and was rapidly centrifuged to isolate the plasma and the blood corpuscles. Around 200 mg of liver were sampled. The heart was taken out and separated into endocardium and epicardium by dissection.

The radioactivity of the four tissues, plasma, liver, epicardium and endocardium was then counted by means of gama spectrometry (Appareil Intertechnique) adjusting the A channel on the tip of the 125 iodine.

The results obtained are expressed in terms of numbers of beats per minute and in terms of grams of tissues.

The radioactivity of the plasma enabled us to calculate a blood flow rate in each tissue, considering the average cardiac flow rate, which is 180 ml/mn in rats.

The tissue flow rates are expressed in terms of ml/mn/100 g of tissue.

EXAMPLE 2A

A first series of experiments was performed in accordance with the general procedure described above, using 12 rats, including 6 healthy and 6 embolized, without gas injection.

The corresponding control figures for the blood flow rates were given in Table A below.

TABLE A

	Blood Flow Rate (ml/mn/100 g)	
	Control rats	Embolized rats
Endocardium	28.7 +/- 1.4	20.5 +/- 1.1**
Epicardium	24.6 +/- 1.1	23.3 +/- 0.8
R = Endo/Epi	1.16 +/- 0.15	0.87 +/- 0.02**
Liver	73 +/- 3	68 +/- 3

n = 6, m +/- ESM, ** p = 0.01 (t test of student) comparison between embolized or not embolized.

The basal flow rate of the endocardium is greater than that of the epicardium, hence a ratio of endocardium over epicardium amounting to more than 1. Embolization has more of an effect on the endocardium than on the epicardium, hence there is a significant inversion of the ratio. Embolization leads to a significant decline in the cardiac flow rates, whereas, at the level of the liver, the organ taken as reference, the observed decline is very minor.

EXAMPLE 2B

A second series of experiments were performed according to the general method described in connection with Example 2, using a group of 6 healthy rats to whom mixtures of NO and CO_2 were administered.

The NO was perfused for one minute at doses of 20 ppm with a flow rate of 0.4; 0.6; respectively, 0.8 ml/mn and 200 ppm with a flow rate of 0.6 ml/mn.

Table B below shows all of the results obtained:

TABLE B

Blood Flow Rate (ml/mn/100 g)				
	C1=20 ppm d2=0.4 ml/mn	C1=20 ppm d2=0.6 ml/mn	C1=20 ppm d2=0.8 ml/mn	C1=200 ppm d2=0.6 ml/mn
Endocardium	44.8 +/- 1.0	51.2 +/- 1.2	55.8 +/- 1.4	34.5 +/- 1.4
Epicardium	48.2 +/- 1.5	57.8 +/- 1.7	64.2 +/- 1.2	36.7 +/- 1.6
R=Endo/Epi	0.93 +/- 0.02	0.99 +/- 0.01	0.87 +/- 0.01	0.94 +/- 0.01
Liver	79 +/- 4	78 +/- 4	74 +/- 2	66 +/- 6

n = 6, m+/- ESM
1: C represents the concentration of NO in the administered gas.
2: d represents the flow rate of the gas during the injection.

The flows at the level of the endocardium and the epicardium are significantly augmented by the low doses of NO.

Figure 1, attached, was plotted on the basis of the results obtained from Examples 2A and 2B; it shows the variations in the blood flow at the level of the endocardium and the epicardium as a function of the perfusion flow in healthy rats. It follows clearly from this curve that the tissue blood flow increases in proportion to the perfusion flow, that is to say, to the total quantity of injected NO.

Nevertheless, we do find a definite decline in the blood flow with a major increase (by a factor of 10) of the NO concentration in the administered gas.

EXAMPLE 2C

A third series of experiments was performed along the general method described in Example 2, using a group of 6 embolized rats to which mixtures of NO and CO_2 were administered.

The NO was perfused for one minute at doses of 20 ppm with a flow of 0.4; 0.6; respectively, 0.8 ml/mn and 200 ppm with a flow rate of 0.6 ml/mn.

TABLE C

Blood Flow Rate (ml/mn/100 g)				
	C1=20 ppm d2=0.4 ml/mn	C1=20 ppm d2=0.6 ml/mn	C1=20 ppm d2=0.8 ml/mn	C1=200 ppm d2=0.6 ml/mn
Endocardium	42.2 +/- 1.3	46.2 +/- 1.5	51.8 +/- 1.5	38.0 +/- 1.8
Epicardium	41.2 +/- 1.4	43.2 +/- 1.0	44.2 +/- 1.3	38.8 +/- 2.0
R=Endo/Epi	1.03 +/- 0.03	1.07 +/- 0.02	1.17 +/- 0.03	0.98 +/- 0.01
Liver	69 +/- 3	68 +/- 3	67 +/- 3	68 +/- 3

n = 6, m+/- ESM

1: C represents the concentration of NO in the administered gas.

2: d represents the flow rate of the gas during the injection.

As a function of the administered dose, NO brings about an increase in the flow at the level of the endocardium. To a much lesser degree, an identical effect at the level of the epicardium can be observed. This is expressed by a restoration of the R ratio as in the controls.

Figure 2 combines the results obtained with this example and those of Example 2A, involving the use of embolized animals to which no gas was administered. More specifically, the curve in Figure 2 traces the changes in the blood flow at the level of the epicardium and the endocardium as a function of the injected gas flow.

Compared to the nonembolized animals, in fact, NO, in small doses, brings about a redistribution of the flow toward the endocardium, which is the most ischemized region. This effect is associated with the overall increase in the flow.

NO thus redistributes the blood toward the most embolized regions, hence the usefulness of the compounds of the invention in the treatment of acute infarct.

This experience furthermore evidences the absence of activity on the part of the NO that was administered at the level of the liver, thus confirming the local action of nitrogen monoxide. Moreover, we find once again that the therapeutic effect is diminished in case of an excessively high concentration (200 ppm) of NO in the injected gas.

Blood was taken from each rat after each administration of gas (Examples 2B and 2C). The red blood corpuscles that were separated from the plasma were hemolyzed and the percentage of methemoglobin, by comparison to the hemoglobin, was determined by means

of ultraviolet spectrometry, using a UNICAM instrument. It was impossible to evidence any change in the methemoglobin rate when compared to the blood sample taken from the control rat.

EXAMPLE 3

This example is a study of the possible toxicity of gaseous NO administered intracardially. Using the same administration procedure as stated in connection with Example 2A, we proceeded to the following three experiments:

EXAMPLE 3A

Two rats received a perfusion of NO + CO_2 at a concentration of 200 ppm and a flow rate of 0.8 ml/mn for 15 minutes. Transitory disorders in behavior were observed during and at the end of the profusion; then the rats resumed normal behavior (there was no mortality after eight days).

EXAMPLE 3B

Four rats received a perfusion of NO + CO_2 at a concentration of 200 ppm and a flow rate of 1.2 ml/mn for 15 minutes. After convulsions, the animals developed paralysis of the hindquarters. Two of the four animals died during the hour following the end of perfusion. The other two survived, but remained partially paralyzed.

EXAMPLE 3C

Two rats received a perfusion of CO_2 at a flow rate of 1.2 ml/mn for 15 minutes. Paralysis of the hindquarters was observed without mortality.

The perfusion of NO + CO_2 at a concentration of 200 ppm and a flow rate of 1.2 ml/mn for 15 minutes led to a 50% mortality of the animals, whereas a flow rate of 0.8 [illegible] ml/mn did not show any mortality. It seemed however, that the speed of perfusion is decisive, since CO_2 turns out to be almost toxic as well.

It thus appears that the toxic doses are definitely higher than the active doses; this permits therapeutic use of the compounds of the invention without any major risk.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Use of a gaseous compound including nitrogen monoxide (NO) and carbon dioxide (CO_2) for the production of a gaseous medication intended for the treatment or prevention of ischemia by the intravascular route.

2. Use of a gaseous compound including nitrogen monoxide (NO) and carbon dioxide (CO_2) for the production of a gaseous medication intended for the treatment or prevention of embolism by the intravascular route.

3. Utilization according to any one of claims 1 or 2, wherein said gaseous medication furthermore includes at least one gas chosen from among the group made up of nitrogen protoxide, xenon, krypton, and their mixtures.

4. Utilazation according to any one of claims 1 to 3, wherein said gaseous medication furthermore includes an effective concentration of NO.

5. Utilization according to any one of claims 1 to 4, wherein said gaseous medication contains a NO concentration between 1 and 100 ppm.

6. Utilization according to any one of claims 1 to 5, wherein said gaseous medication contains a NO concentration between 15 and 30 ppm.

7. Utilization according to any one of claims 1 to 6, wherein the intravascular route is the intra-arterial route.

8. Utilization according to any one of claims 1 to 6, wherein the intravascular route is the intracardial route.

9. Utilization according to any one of claims 1 to 6, wherein the intravascular route is the intraveneous route.

10. The use according to claim 1, substantially as herein described with reference to any one of the Examples.

11. The use according to claim 1, substantially as herein described with reference to the accompanying drawings.

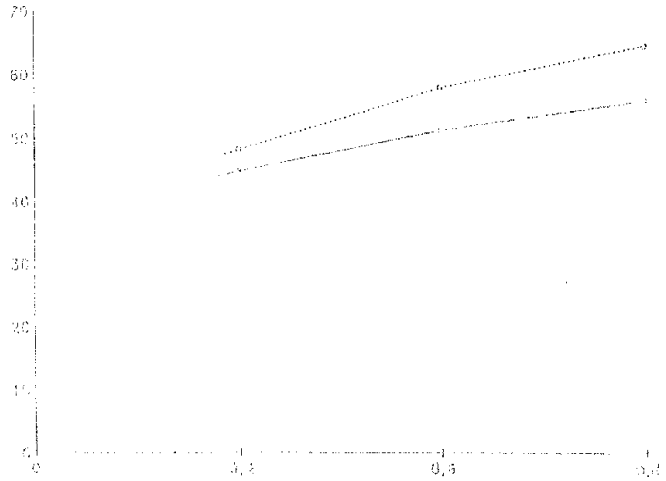
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DOSE-RESPONSE CURVE IN CONTROL RAT
Perfusion of NO at 250 P.P.M

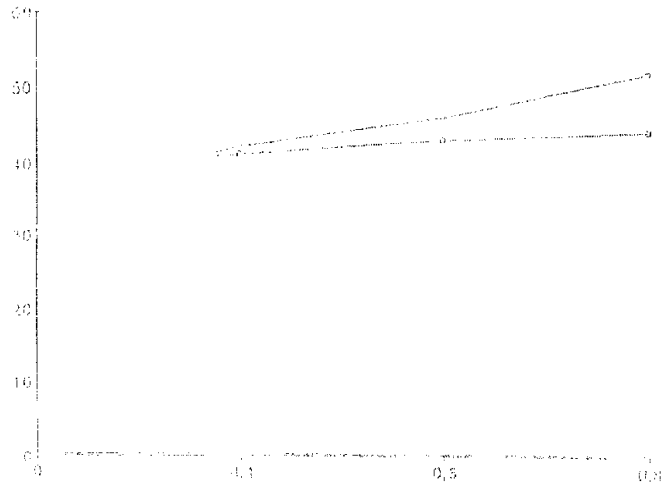


FLOW RATE
ml/min/100 g

PERFUSION FLOW RATE ml/min
Endocardium
Epicardium

FIGURE 1

DOSE RESPONSE CURVE IN EMBOLIZED RAT
Perfusion of NO at 200 PPM



FLOW RATE
ml/min/100 g

PERFUSION FLOW RATE ml/min
Endocardium
Epicardium

FIGURE 2