Effect of a short-acting NO donor on bubble formation from a saturation dive in pigs

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Møllerløkken, A., V. J. Berge, A. Jørgensen, U. Wisløff, and A. O. Brubakk. Effect of a short-acting NO donor on bubble formation from a saturation dive in pigs. J Appl Physiol 101: 1541–1545, 2006. First published July 20, 2006; doi:10.1152/japplphysiol.01191.2005.—It has previously been reported that a nitric oxide (NO) donor reduces bubble formation from an air dive and that blocking NO production increases bubble formation. The present study was initiated to see whether a short-acting NO donor (glycerol trinitrate, 5 mg/ml; Nycomed Pharma) given immediately before start of decompression would affect the amount of vascular bubbles during and after decompression from a saturation dive in pigs. A total of 14 pigs (Sus scrofa domestica of the strain Norsk landsvin) were randomly divided into an experimental (n = 7) and a control group (n = 7). The pigs were anesthetized with ketamine and α-chloralose and compressed in a hyperbaric chamber to 500 kPa (40 m of seawater) in 2 min, and they had 3-h bottom time while breathing nitrox (35 kPa O₂). The pigs were all decompressed to the surface (100 kPa) at a rate of 200 kPa/h. During decompression, the inspired Po2 of the breathing gas was kept at 100 kPa. Thirty minutes before decompression, the experimental group received a short-acting NO donor intravenously, while the control group were given equal amounts of saline. The average number of bubbles seen during the observation period decreased from 0.2 to 0.02 bubbles/cm² (P < 0.0001) in the experimental group compared with the controls. The present study gives further support to the role of NO in preventing vascular bubble formation after decompression.

PREVENTIVE MEASURES TO REDUCE the risk of decompression sickness (DCS) have traditionally involved reduction of decompression speed and breathing of O₂ (12). Previously, our laboratory has shown that both exercise and nitric oxide (NO) prevent vascular bubble formation in rats (18). This effect of exercise has also been shown in humans (4). Administration of Nω-nitro-L-arginine methyl ester, a nonselective inhibitor of NO synthase, initiated formation of vascular bubbles in sedentary but not in exercised rats (17).

Many tissues contain a nearly ever-present population of small gas bubbles, known as preexisting gas nuclei (19). These gas nuclei are believed to be the origin of the vascular bubbles that evolve during decompression caused by supersaturation of inert gas in tissues. The inner layer of blood vessels is lined by a monolayer of endothelial cells, and these are crucial in regulating vascular tone. In blood vessels, nuclei are probably attached to the blood vessel endothelium where they grow into bubbles that are dislodged into the bloodstream (9, 10). Thus any process that influences the surface properties of the endothelium may affect bubble formation in the vascular system.

Our laboratory’s previous studies have demonstrated that NO is involved in vascular bubble formation (17, 18). The present study was initiated to determine whether a short-acting NO donor given immediately before decompression from saturation would prevent vascular bubble formation during and after decompression.

MATERIALS AND METHODS

Experimental animals. A total of 14 male pigs (Sus scrofa domestica of the strain Norsk landsvin) were used. The pigs were 10–12 wk old, with body weights of 22.9 ± 2.7 kg. The experimental procedures conformed to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the protocol was approved by the Norwegian Council for Animal Research.

Pressure profile and breathing gas. The pigs were randomly assigned either to an experimental group (n = 7) or a control group (n = 7). All animals were compressed in a hyperbaric chamber to 500 kPa (40 m of seawater) in 2 min while breathing nitrox (35 kPa O₂). They remained at this pressure for 3 h (inclusive compression time). The bottom time was followed by a linear decompression to the surface (100 kPa) at a rate of 200 kPa/h, giving a total decompression time of 2 h. During decompression, the inspired Po2 of the breathing gas was kept at 100 kPa.

Surgical procedure. Before the experiments, the pigs were fasted for 16 h with free access to water. On the day of the experiment, they received premedication with 10 ml Stresnil (azaperon, Janssen-Cilag Pharma, Wien, Germany) and 2 ml Stesolid (5 mg/kg diazemepam, Dumex-Alpharma, Copenhagen, Denmark) given intramuscularly. After 20 min, atropine sulfate (1 mg iv Atropin, Nycomed Pharma) was given via an ear vein. Anesthesia was induced by thiopental sodium (5 mg/kg Pentothal Natrium, Abbott Scandinavia) and ketamine (20 mg/kg Ketalar, Pfizer). The anesthesia was maintained by a continuous intravenous infusion of ketamine in 0.9% NaCl (30 mg·kg⁻¹·h⁻¹) together with bolus doses of α-chloralose in 0.9% NaCl (10–15 mg/kg injected iv; 0.25% solution). A tracheotomy was performed to allow the pigs to breathe spontaneously through an endotracheal tube. Throughout the experiments, the pigs were in a supine position. The depth of anesthesia was maintained at an even level, as judged by clinical observation and the various measured physiological variables.

Two polyethylene catheters were introduced into the left jugular vein. One of the catheters was used for obtaining venous blood samples for blood-gas analysis; the other was used for backup. Two more catheters were positioned in the right atrium via the right jugular vein for measurements of central venous pressure and for induction of either a short-acting NO donor or equal amounts of saline. The infusion started 30 min before decompression, and it was terminated at the beginning of decompression. As our NO donor, we used the cost of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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glyceryl nitrate (glycerol trinitrate, 5 mg/ml, Nycomed Pharma). Before infusion, 20 ml of glyceryl nitrate were mixed in 250 ml 0.9% saline. The infusion rate was 0.025 ml/min, and the rate of administration was thus 0.4 μg·kg⁻¹·min⁻¹. Furthermore, two catheters were inserted into the right femoral artery and advanced into the abdominal aorta for continuous monitoring of arterial pressure and to obtain blood samples for analysis of blood-gas composition.

Deep body temperature was measured continuously throughout the experiments by a rectal thermometer, and it was adjusted through regulation of the chamber temperature. Body temperature was kept between 38 and 39°C.

Experimental setup and bubble detection. The experimental setup is shown in Fig. 1. Inside the chamber, the pig breathed from an inspired bag into an expired bag. CO₂ and O₂ in the inspired and expired gas were measured continuously (Beckman OM-11 O₂ analyzer; Beckman LB-2 medical gas analyzer) together with respiratory frequency and flow. Blood gases were measured by using a blood-gas analyzer (model ABL 330, Radiometer, Copenhagen, Denmark), and the measurements were taken 30 min into the stabilization period, 30 min into the dive, at 1.5 h into the dive, and again 2.5 h into the dive (which is the start point of nitrate infusion). Further blood gases were taken at 30 min into decompression, after 1.5 h of decompression, and after 30 min at the surface.

A transesophageal echocardiographic probe was inserted and positioned to obtain a simultaneous two-dimensional view of the pulmonary artery and the aorta (CFM 750, Vingmed Sound, Horten, Norway). Data from the echocardiographic probe were continuously transferred to a Macintosh computer that analyzed the amount of bubbles detected in the pulmonary artery and the aorta. From the images, the amount of bubbles in the right ventricular outflow tract is given as number of bubbles per square centimeter as described by Eftedal and Brubakk (5). Predive data were collected during the 1 h of stabilization.

From the onset of the 1-h stabilization period, during the dive, and throughout a 90-min positive period, the experiments were controlled by a preprogrammed computer. All measurements were recorded continuously by the computer and by the Macintosh computer used for bubble detection. A VHS recording was made from each experiment, allowing repeated measurements of the vascular bubbles if necessary.

Statistical analysis. The data were analyzed using SPSS 13.0. Data are expressed as means (SD) or as median and range. A Student’s t-test was used to compare the number of bubbles during the observation period. A Mann-Whitney U-test was used to evaluate differences in the blood gases. P < 0.05 was considered as statistically significant.

RESULTS

The formation of venous gas bubbles is presented in Fig. 2. The average number of bubbles seen during the whole observation period decreased from 0.2 to 0.02 bubbles/cm² (P < 0.0001) in the experimental group compared with the control group after injection of a short acting NO donor 30 min before start of decompression.

Table 1 shows arterial and venous O₂ and CO₂ partial pressures measured 30 min after the beginning of decompression. By using the values at 30 min into decompression, we assume that there is a steady state after the change in inspired O₂. No bubbles were detected at this stage of the decompression, and the blood-gas values reported in Table 1 are assumed to result from the change in inspired O₂.

Heart rate was significantly increased in the experimental group after infusion of the NO donor compared with the control group. Thirty minutes into decompression, heart rate

Fig. 1. Schematic figure of pressure chamber and its subsystems. TEE, transesophageal echocardiographic; PC personal computer.
was no longer significantly different (Table 2). Table 2 also gives the values for arterial and central venous blood pressure in two of the animals in the experimental group and three of the animals in the control group.

In the control group, two of the pigs died within 15 min after decompression, probably due to substantial gas bubble formation. All the animals in the experimental group survived the entire observation period (Table 3).

DISCUSSION

The present study demonstrates that a short-acting NO donor given before decompression from a saturation dive significantly reduces the amount of venous gas bubbles in the pulmonary artery. These results further support previous findings that NO seems to be exceptionally efficient in reducing vascular gas bubble formation.

Studies have shown that almost all decompressions produce some vascular bubbles (13). Venous gas bubbles have been used as an indication of decompression stress; the occurrence of many bubbles is linked to a higher risk for DCS (13). These gas bubbles probably grow from preexistent nuclei, but the exact nature of these nuclei is not known (19). The lifetimes of such nuclei are relatively short unless they are stabilized against the surface tension, and one hypothesis is that the nuclei can only exist on hydrophobic surfaces (9). NO inhibits leukocyte as well as platelet adhesion and aggregation (3) and changes the properties of the endothelial wall (15, 16). Hence it is conceivable that these effects may reduce the number of nuclei adhering to the endothelial surface (18). This mechanism could therefore influence vascular bubble formation by reducing the stability of bubble nuclei. Another hypothesis is that changes in tissue blood flow facilitate inert-gas washout. During diving, tissues will equilibrate with the breathing gas. With an increased blood flow, this balance will be achieved more rapidly. During decompression, a more rapid change in the stability between the breathing gas and the dissolved gas pressure due to an increased blood flow in the tissues could minimize the possible gas bubble formation (11). Because of technical problems, we only have data on venous and systemic blood pressure in five animals, two of them from the experimental group. In these animals, arterial and venous blood pressure did not change during the infusion of either saline or NO, and there was no statistical difference between the two groups during the decompression or the observation period after the dive. The lower arteriovenous O2 difference in the NO-treated animals (12%) 30 min after the start of decompression indicates on one hand that the O2 extraction per unit of blood has decreased because perfusion has increased, which is also supported by the increase in heart rates seen in the experimental group. On the other hand, other studies have shown a slight decrease in cardiac output (CO) after administration of the drug, without changing the blood pressure (6). However, the experimental group showed a higher heart rate immediately after nitrate infusion compared with the control group. The effect was no longer statistically different 30 min into the decompression (Table 2). We also observed a tendency toward a higher central venous pressure in the experimental group compared with the control group. Together with the lower arteriovenous O2 difference found in the experimental group, these three observations indicate a higher CO in the experimental group than in the control group. Since the heart rates were no longer statistically different 30 min into decompression, it is tempting to speculate that a short increase in blood flow at start of decompression is enough to increase the tissue inert-gas

![Fig. 2. Amount of vascular gas bubbles during and after decompression in animals receiving nitrate and the control group. By calculating the area under the curve, we get the amount of gas that dissolves during decompression and after surfacing in the blood. Area = 4.21 bubbles cm⁻² min⁻¹ in the experimental group. Area = 39.59 bubbles cm⁻² min⁻¹ in the control group.]

Table 1. Partial pressure of blood gases for the animals given nitrate and the control animals 30 min after start of decompression

<table>
<thead>
<tr>
<th>Blood Gas and Alveolar Gas</th>
<th>Experimental Group (n = 7)</th>
<th>Control Group (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial O2</td>
<td>54.33 (6.29)</td>
<td>57.10 (6.80)</td>
</tr>
<tr>
<td>Arterial CO2</td>
<td>5.92 (1.50)</td>
<td>6.41 (1.17)</td>
</tr>
<tr>
<td>Venous O2</td>
<td>11.87 (1.64)</td>
<td>8.82 (1.62)*</td>
</tr>
<tr>
<td>Venous CO2</td>
<td>8.83 (1.06)</td>
<td>7.49 (1.33)</td>
</tr>
<tr>
<td>Alveolar O2</td>
<td>85.95 (4.88)</td>
<td>86.49 (3.33)</td>
</tr>
<tr>
<td>Alveolar CO2</td>
<td>8.45 (1.56)</td>
<td>7.75 (0.38)</td>
</tr>
</tbody>
</table>

Values are means (SD) given in kPa; n, no. of animals. *P = 0.042.
washout and hence reduce the amount of detectable gas bubbles. Based on the present study, it is not possible to state whether it is an increased inert-gas washout due to an increased blood flow following administration of nitrates or whether it is the nitrate used having an effect on the stability of the bubble nuclei or on platelet/neutrophil adhesion that caused the observed reduction in vascular gas bubble formation.

NO is produced by endothelial cells as a response to an increase in shear stress caused by increased blood flow (7), and it is the most important vasodilator released by endothelial cells (1). Once released from the endothelium, NO diffuses through the vascular wall and into the smooth muscle cells, where it activates the cytosolic enzyme. This enzyme activation increases levels of cellular cGMP, which causes relaxation of the vessel wall. In a previous study in rats, we demonstrated that NO given immediately before a 45-min air dive effectively reduced bubble formation (18). Because of the fact that NO reduces venous tone through a reduction of the preload and hence reduces CO (6, 8), part of the effect on bubble formation could have been caused by a reduction in gas uptake. This is less likely in the present study since we are close to saturation and since our NO donor was given at the end of the bottom phase of the dive. This would strengthen our hypothesis that the reduction in bubble formation is caused by other mechanisms.

Previously, Wislöff et al. (17) have shown that inhibition of basal synthesis of NO will induce bubble formation in rats that normally do not produce bubbles. They suggested that the exercise-induced protection against bubble formation is mediated via the NO pathway. However, they also discovered that NO blocking increased bubble formation in sedentary but not in exercised rats (17), which indicates that the exercise effect might be mediated by other factors than NO. Increased blood flow during exercise could also “wash away” the nuclei, but since there was no effect of the exercise closer to the dive than 20 h, they concluded that this was unlikely. Recent work by Blatteau et al. (2), which found that aerobic exercise 2 h before a dive to 30 m of seawater decreases bubble formation after decompression, has again raised this possibility.

The NO donor used in this study was glyceryl nitrate at an infusion rate of 0.4 μg·kg⁻¹·min⁻¹. This dosage is twice the dosage recommended in humans. During pilot studies, it became evident that a lower dosage had no effect on bubble formation. Glyceryl nitrate is a drug with significant secondary effects, with hypotension being one of them. The preventive use of drugs in commercial diving is forbidden by the international labor laws. But in the search for the basic mechanisms behind serious decompression sickness, pharmacological means can increase our understanding of these mechanisms. And also the potential of pharmacological means to increase safety in emergency situations, such as in submarine escapes, must be explored. The saturation conditions and decompression used in the present study were identical to those used in a previous study by Reinertsen et al. (14). This procedure achieves 97% saturation as judged from the N₂ level of the venous blood. All in all, we would expect the drug to have no effect on gas uptake in this long dive (3 h), which is close to saturation, and if anything, the drug could lead to a reduction in blood flow and hence gas elimination during the initial part of decompression due to decrease in preload and hence a reduced CO.

The present study does not allow us to determine if an increase in blood flow leading to an improved washout of N₂ or changes in endothelial properties that remove bubble nuclei are responsible for the reduction in bubble formation. Both effects may be of importance. Regardless of mechanism, NO seems to be exceptionally efficient in reducing bubble formation both in the previous study and in the one performed here.

**ACKNOWLEDGMENTS**

The assistance of Anne-Lise Ustad, Nina Lundset, and Stig Koteng in performing the experiments is highly appreciated. Arnfinn Sira is acknowledged for excellent technical assistance, and the personnel at the Centre for Experimental Animals at St Olavs Hospital in Trondheim for professional handling of the animals. Finally, Olav S. Ettedal is acknowledged for thorough and patient proofreading.

**GRANTS**

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**Table 2. Heart rate, mean arterial pressure, and central venous pressure in the experimental and the control group after infusion of nitrate, 30 min into decompression, and at the surface**

<table>
<thead>
<tr>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR, beats/min</td>
<td>MAP, mmHg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental group</td>
<td>139.2 (31.1)</td>
<td>76.3 (4.3)</td>
</tr>
<tr>
<td>Control group</td>
<td>103.7 (27.2)</td>
<td>81.1 (21.3)</td>
</tr>
</tbody>
</table>

Values are means (SD). HR, heart rate; MAP, mean arterial pressure; CVP, central venous pressure. Time 1 is immediately after nitrate-0.9% NaCl infusion; time 2 is 30 min into decompression; time 3 is at surface after decompression. For all HR measurements, n = 7 animals in both groups. For the measurements of MAP and CVP, data are from 2 animals in the experimental group and 3 animals in the control group. *P = 0.048. †P = 0.051. ‡P = 0.138.

**Table 3. Effects of nitrate on bubble formation**

<table>
<thead>
<tr>
<th></th>
<th>Experimental Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of bubbles, bubbles/cm²</td>
<td>0.02 (0.02)</td>
<td>0.2 (0.5)*</td>
</tr>
<tr>
<td>Survival, no. of animals</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

Values for no. of bubbles are means (SD) for 7 animals in both groups. *P < 0.0001.
REFERENCES


