The effect of air bubbles on rabbit blood brain barrier

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Hjelde A, Bolstad G, Brubakk AO. The effect of air bubbles on rabbit blood brain barrier. Undersea Hyper Med 2002, 29(1):31-38. Several investigators have claimed that the blood brain barrier (BBB) may be broken by circulating bubbles, resulting in brain tissue edema. The aim of this study was to examine the effect of air bubbles on the permeability of BBB. Three groups of 6 rabbits were infused an isoosmotic solution of NaCl w/macrodex and 1% Tween. The solution was saturated with air bubbles and infused at rates of 50-100 ml·hr^{-1}, a total of 1.6, 3.3, or 6.6 ml in each group, respectively. Two groups, each consisting of 6 rabbits, served as controls; one was infused by a degassed isoosmotic NaCl solution and one was sham-operated. All animals were left for 30 min before they were sacrificed. Specific gravity of brain tissue samples was determined using a brombenzene/kerosene gradient column, where a decrease in specific gravity indicates local brain edema. Specific gravity was significantly lower for left ($P = 0.037$) and right ($P = 0.012$) hemisphere white matter and left ($P = 0.0015$) and right ($P = 0.002$) hemisphere gray matter for the bubble-infused animals compared to the sham-operated ones. Infusion of degassed NaCl solution alone affected white left ($P = 0.011$) and right ($P = 0.013$), but not gray matter of both hemispheres. We speculate that insufficient degassing of the fluid may cause the effect of NaCl solution on the BBB of the white matter, indicating that the vessels of the white matter are more sensitive to gas bubbles than gray matter. Increasing the number of infused bubbles had no further impact on the development of cerebral edema, indicating that a threshold value was reached already at the lowest concentration of bubbles.

air bubbles, blood brain barrier, brain edema, specific gravity

INTRODUCTION

There is a growing concern that diving can lead to permanent damage to the central nervous system even in the absence of decompression sickness (DCS). Reversible neuropsychological and neurophysiological changes have been recorded in divers during, as well as shortly after, the dives (1,2). In some of these cases clinical dysfunctions have also been demonstrated (3). Indications of neurological disorders of more permanent character, compatible with brain damage, have been reported (4). In addition to DCS cerebral arterial air embolism can also occur in other settings, for example angiography and cardiac surgery. Gas bubbles can lead to endothelial damage (5). As the number of gas bubbles increase this damage also increases (5).

Under normal conditions cerebral, unlike peripheral blood vessels, are impermeable to circulating proteins due to a layer of endothelial cells. This restriction is called the blood brain barrier (BBB) (6). The term blood brain barrier originated with the observation that trypan blue

and other dyes failed to enter brain parenchyma after intravenous injection (6). The term was used as early as 1969 (7).

Experiments involving several animal species have demonstrated that when gas bubbles are developed or introduced into the brain circulation, either through direct infusion (8-10), or as a result of lowering the environmental pressure (11-13) the BBB may be broken. Breakdown of the BBB may result in transport of proteins across the barrier and subsequent formation of edema in the brain tissue.

Breakdown of the BBB due to intravascular bubbles has been suggested as one possible initiator of neurological DCS (9,11), either through direct mechanical injury of the endothelial cells of the vessel, or through chemical substances which are released in presence of gas bubbles. Gas bubbles can cause changes in barrier permeability even in the absence of clinical manifestations of DCS (11). The most important consequence of barrier malfunction may be cerebral edema (i.e. increased water content) (6). The biphasic presentation of symptoms that often are observed in mild neurological DCS may be explained by passage of bubbles initiating the early symptoms, while the delayed symptoms may be related to a gradual development of edema in the brain tissue (9).

Based on earlier experiments where rats were decompressed from 700 kPa of air we were unable to demonstrate breakage of the BBB by measuring specific gravity of brain tissue samples (14). This observation was partly explained by a decompression rate that probably did not develop sufficient amounts of bubbles to produce such injuries. However, in another study at our laboratory we demonstrated a significant decrease in specific gravity of brain tissue in decompressed rabbits following massive bubbling 30 min *postdive* (15). Furthermore, rabbits developing fewer bubbles had no change in specific gravity (white and gray matter) 2 hrs *postdive* compared to non-exposed animals (15).

It is difficult to compare the result of one study to another due to the use of different experimental models and different ways of evaluating the BBB dysfunction. The degree of BBB permeability induced by air emboli or gas bubbles may vary with respect to dose of infused air or decompression stress. The present experiment was designed to analyze the effect of infusing increasing doses of intracarotid air bubbles on the permeability of BBB. To evaluate changes in the permeability of the blood brain barrier with respect to development of brain edema a gravimetric method, previously employed in our laboratory, was used (16).

METHODS

A total of 30 rabbits (Chinchilla breed) of both genders, weighing between 2.5 and 3.5 kg, were used in this study. The rabbits were housed at the Animal Care Facilities of the University Hospital, Trondheim. The experiments were performed in accordance with the Principles of Laboratory Animal Care published by the National Institutes of Health (NIH publication no. 85-23, revised 1985). The experimental protocol was approved by the Norwegian Committee for Animal Experiments:

Experimental design. Using a randomized protocol, the rabbits were divided into 5 groups, each consisting of six animals (A: sham-operated, B: NaCl-infused, and C1, C2, C3: bubble-infused animals). They were anesthetized with intramuscular injections of midazolam (Dormicum) 0.4 ml·kg^{-1}·body weight and fluanisone/fentanyl (Hypnorm) 0.3 ml·kg^{-1}·body weight, whereafter the left common carotid artery was exposed. The anesthesia was maintained

by giving 0.1 ml Hypnorm intravenously injected when needed. Respiratory frequency was recorded approximately every 15 min during the whole experimental procedure, in order to control the depth of anesthesia.

Infusion of air bubbles. A 21 gauge hypodermic needle with a polyethylene catheter (inner diameter 0.76 mm) which was connected to a pump (Injectomat 50, Diacor A/S), was inserted into the left common carotid artery of the animals in groups B, C1, C2, and C3. Infusion with an isoosmotic solution of NaCl w/Macrodex and 1% Tween was started immediately thereafter. In groups C1, C2, and C3 the infused solution was saturated with air bubbles, as earlier described (17). The infusion rate was 50 ml·hr^{-1} for 2 min (C1), 100 ml·hr^{-1} for 2 min (C2), and 100 ml·hr^{-1} for 4 min (C3), giving a total volume of 1.6, 3.3, and 6.6 ml in the 3 groups, respectively. Two groups (A and B) served as controls. Group B was infused with a degassed isoosmotic NaCl solution at a rate of 100 ml·hr^{-1} for 2 min, giving a total injected volume of 3.3 ml, following the same procedure as for group C animals. Exposing the liquid to vacuum until no bubbling was observed performed the degassing of NaCl. Group A was sham-operated only.

Observation period. All animals were left for 30 min after start of infusion. The anesthetized rabbits were sacrificed by decapitation subsequent to an intracardial injection of KCl.

Specific gravity measurements. After decapitation, the brains were excised within 3-4 min. The brains were immediately placed in a petridish and covered with kerosene to prevent the tissue from dehydration. While immersed in kerosene, parallel samples of white and gray matter (3-5 mg) were dissected from the distal cortex of both hemispheres. The samples were taken from two 2-3 mm thick adjacent coronal sections around the middle part of the brain. Specific gravity (density, g·ml^{-1}) of these samples was determined using a translucent brombenzene/kerosene graduated column, as described elsewhere (15,18,19). The column was made by slowly adding the lighter mixture (1.030 g·ml^{-1}) of brombenzene (50 ml) and kerosene (100 ml) to the denser mixture (1.065 g·ml^{-1}) of brombenzene (57.6 ml) and kerosene (92.4 ml) into the cylinder to form a continuous gradient with densities running from 1.030 g·ml^{-1} at the top to 1.065 g·ml^{-1} at the bottom. The column was calibrated using drops of K_2SO_4 solutions with known specific gravity that ranged from 35.9 to 73.9 g·liter^{-1}, i.e. 1.0283 and 1.0572 g·ml^{-1}, respectively (20). The brain tissue samples were carefully transferred to the top of the brombenzene/kerosene column and allowed to equilibrate for 2 min before their positions in the column were recorded. Specific gravity of these samples was estimated graphically by interpolation from the position of K_2SO_4 solutions of known specific gravity.

Statistical analysis. All results are presented as mean and standard deviation. Using a one-way analysis of variance (ANOVA) the three bubble-exposed groups (C1, C2, and C3) were compared for differences in specific gravity of left and right hemisphere white and gray matter, respectively. Student's t-test was performed for comparison between two different groups. A *P* value less than 0.05 was considered significant.

RESULTS

No macroscopic signs of infarct could be detected in any of the brains. Table 1 shows the specific gravity for C1, C2, and C3 bubble-infused animals. No significant differences were found in white ($P = 0.774$) or gray ($P = 0.217$) matter between these groups. Specific gravity of left and right hemisphere white and gray matter is displayed in Figs. 1 and 2, respectively.

Table 1

Group bubble-infused	White matter left side (n=6)	White matter right side (n=6)	Grey matter left side (n=6)	Grey matter right side (n=6)
C1 (1.6 ml)	1.0399 ± 0.0021	1.0406 ± 0.0004	1.0411 ± 0.0017	1.0419 ± 0.0010
C2 (3.3 ml)	1.0398 ± 0.0009	1.0399 ± 0.0006	1.0404 ± 0.0015	1.0408 ± 0.0007
C3 (6.6 ml)	1.0402 ± 0.0017	1.0403 ± 0.0015	1.0419 ± 0.0005	1.0411 ± 0.0012

Table 1. Specific gravity of left and right hemisphere for group C1, C2, and C3. The values are presented as mean ± SD separately for gray and white matter.
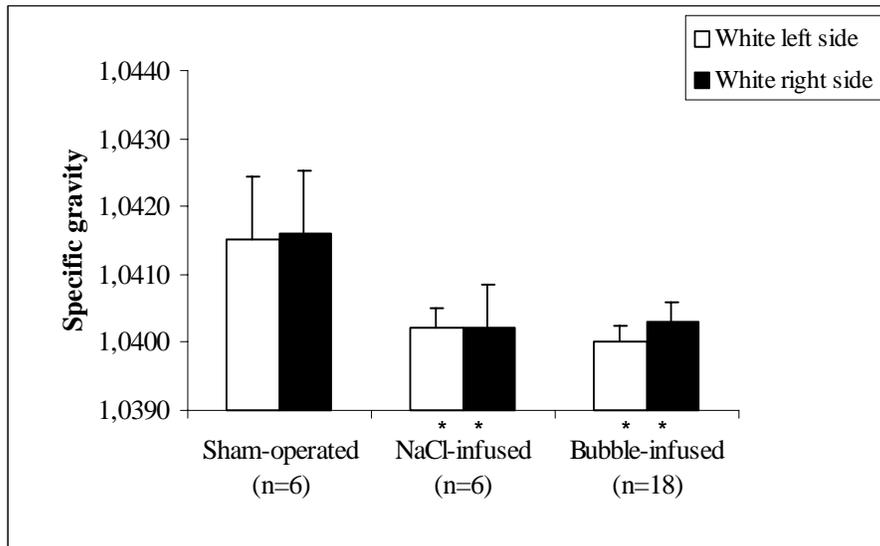
Figure 1

FIG. 1. Specific gravity of left (*open bars*) and right (*solid bars*) hemisphere white matter for sham-operated (A), NaCl-infused (B) and bubble-infused (C1-C3) animals. The values are presented as mean ± SD. Level of significance is indicated with an *asterisks* ($P< 0.05$ compared to sham-operated animals).
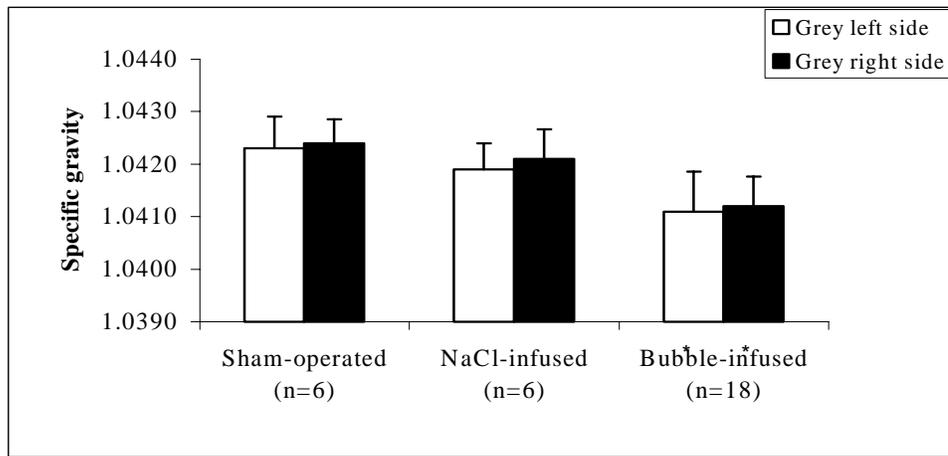
FIG. 2. Specific gravity of left (*open bars*) and right (*solid bars*) hemisphere gray matter for sham-operated (A), NaCl-infused (B) and bubble-infused (C1-C3) animals. The values are presented as mean ± SD. Level of significance is indicated with an *asterisks* ($P < 0.05$ compared to sham-operated animals).

The values are presented as mean ±SD for group A (sham-operated) and group B (NaCl-infused). For C1-C3 bubble-infused animals, values are presented pooled for the 3 groups, as no significant difference was observed between these groups. As can be seen in Figs. 1 and 2 specific gravity was significantly lower for left and right hemisphere white matter ($P = 0.037$ and $P = 0.012$) and gray matter ($P = 0.0015$ and $P = 0.002$) for the bubble-infused animals compared to the sham-operated ones. Infusion of degassed NaCl solution alone affected white, but not gray matter of both hemispheres. A significantly lower specific gravity was observed in the left ($P = 0.011$) and right ($P = 0.013$) white hemisphere of these animals (Fig. 1).

DISCUSSION

Bubbles that enter the brain circulation may pass through the arterioles and capillary beds without obstructing blood flow. Nevertheless, these bubbles might still disrupt brain function. Breakage of the BBB has been demonstrated in rabbits and rats as a result of exposure to compression and decompression (21,22) as well as in guinea pigs in response to microbubbles injected into the carotid artery (8,9).

The present study has demonstrated significant reduction in specific gravity of white and gray brain matter from rabbits infused with air bubbles into the left common carotid artery. However, it is questionable whether this reduction is due to air bubbles alone or other factors related to the infusion procedure. In white matter, edema was developed both after infusion of NaCl solution only, and when air bubbles with surfactant were added to the medium. In contrast,

control rats injected with 5 ml plasma only, introduced slowly over a 2 min period into the right carotid artery, exhibit no damage to the BBB permeability (9).

The mechanism behind the development of edema in response to infusion of NaCl solution only is not clear. Blood pressure was not monitored during these experiments. Thus, an increase in local blood pressure due to the infusion, which again would tend to induce edema (6), could be a possible explanation for the observed effects. However, at the slow infusion rates used in this study, a pressure increase is rather unlikely. Furthermore, pressure induced increase in cerebrovascular permeability is reversed within 10-20 min after the pressure has normalized (23). Uptake of albumin tracers, used as an indicator of permeability changes in the vessel wall, can be observed as late as 24 hrs after injection of gas into a carotid artery (24). We suggest that a more likely explanation is that the NaCl solution was not properly degassed before infusion and thus still contained some air bubbles. Furthermore, it is possible that simply transferring the liquid to the syringe may have caused air bubble formation.

The fact that BBB change following NaCl solution infusion only happens in white and not in gray matter, may possibly be explained by the observation that subcortical white matter have been reported to be more susceptible to air emboli induced BBB leakage and edema than gray matter (6,10). Damage to cortical vessels followed by preferential spread of protein and water throughout the white matter is seen in a variety of edema models as reviewed in (6).

No significant difference in specific gravity was found between infusion of 1.6, 3.3 or 6.6 ml solution saturated with air bubbles. This is in contrast to earlier observations demonstrating a correlation between the amount of intravascular bubbles and increased BBB permeability to trypan blue (11). In addition, injection of air (50, 100, or 150 μl/kg) caused a dose-dependent increase in neurological impairment in rabbits (25). Previous results from our laboratory indicate that the number of bubbles in the pulmonary artery after decompression is related to the degree of endothelial damage (5).

Increased permeability of cerebral vasculature could be due to permeability changes induced by chemical factors released or activated by microbubbles or direct mechanical injury of blood vessels (26). Mechanical effects can be caused by gas bubbles obstructing the blood flow or by disrupting the endothelial layer lining the vessels and the surrounding tissue (27). Bubble-endothelium interactions can induce endothelial leak and extravasation of fluid. In the brain vasculature these effects may cause a decreased function of the BBB (28). In a previous study we suggested that massive bubbling following decompression in a rabbit model caused a mechanical damage in BBB with subsequent increase in cerebral edema within 30 minutes *postdive* (15). The exact biochemical mechanism for the observed endothelial damage after air embolism is not known, but is probably related to leukocyte activation (29) leading to release of toxic oxygen metabolites (30). In a canine model, labeled granulocytes have been shown to accumulate in the brain after air embolism (31). Schoettle et al thus found a significant correlation between neutrophil granulocytes accumulation and cerebral edema (32). Oxygen metabolites, when infused intracerebrally into rats, induced changes in the permeability of the BBB, causing cellular injury and edema (33).

The edema fluid accumulated within the tissue was estimated by measuring the specific gravity of selected brain regions on a brombenzene-kerosene density column. Bothe and coworkers have shown that the main determinant of specific gravity of edematous brain tissue is water content (34). The use of specific gravity in the measurement of cerebral edema is well documented in the literature (18,19,and 34). A significant decrease in specific gravity in response to increased water content of the brain tissue was demonstrated in these studies. Obviously, a

gradual uptake of water leads to a decreased density. In studies on brain edema with microgravimetric techniques it is of utmost importance to minimize shifts of water between the sample and the medium. By using a brombenzene-kerosene density gradient as we did, tissue water is not lost into the hydrophobic medium. The individual density values obtained in this study and in previous studies from our laboratory were well reproducible and showed good repeatability (14,15).

In conclusion, this study indicates that infusion of both NaCl solution and the introduction of air bubbles into the common carotid artery caused increased vascular permeability in the white matter. It is suggested that insufficient degassing of the NaCl solution is the reason for the observed cerebral edema when no visible bubbles were infused. However, increasing the number of bubbles infused had no further impact on the development of cerebral edema, indicating that a threshold value was reached already at the lowest concentration of bubbles. Only infused bubbles led to edema in the gray matter, indicating a higher threshold for BBB damage.

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