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Several New Aspects of Bubble-Induced Central Nervous System Injury

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Several new aspects of bubble-induced central nervous system injury

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Ischaemia is a major mechanism underlying central nervous system (c.n.s.) damage in decompression sickness. Some recent experimental observations on the effect of bubble-induced ischaemia on c.n.s. tissue sharpen and extend our understanding of the pathophysiology of decompression sickness. After bubble-induced brain ischaemia, a measurable increase in ^{111}In-labelled leucocytes occurs in the injured hemisphere. By 4 h into the recovery period the cells are concentrated in zones of low blood flow, as measured by the [^{14}C]iodoantipyrine technique. The presence of these cells during the critical early hours of c.n.s. ischaemia suggests that they may contribute to the evolution of neuronal damage. Oedema is often cited as the cause of clinical deterioration after c.n.s. ischaemia or trauma. Recent evidence indicates that the presence and degree of circumscribed brain oedema is not a good predictor of the amount of nerve cell recovery (by using cortical sensory evoked response) after bubble-induced brain ischaemia. This brings into question the role of circumscribed oedema of the c.n.s. in dysfunction of post-ischemic nerve cells.

Introduction

In decompression sickness, ischaemia is a major mechanism underlying tissue damage of the central nervous system (c.n.s.) (Hallenbeck *et al.* 1975; Hallenbeck 1976). There is growing evidence that in a zone of acute ischaemic damage to c.n.s. tissue, an interaction can develop between the injured tissue and the perfusing blood (Hallenbeck 1977). The result of this interaction is a progressive rise in resistance to blood flow in the microcirculation of the injury zone. This rise in microcirculatory resistance staunches local perfusion of the tissue and may contribute to some fraction of the neuronal loss that ultimately occurs. The interaction is viewed as multifactorial and although it is not fully understood in detailed molecular terms, factor (VIII)/von Willebrand factor protein (F. (VIII)/vWF.-protein) and the prostaglandin system appear to be mediators (Hallenbeck & Furlow 1979; Hallenbeck *et al.* 1981). If, as seems very likely, injured endothelium permits increased access of contact-activated proteins (for example, Hageman factor) to subendothelial collagen, a sequence leading to production of such chemotactic factors as C_3a and C_5a could be set into motion (Stewart *et al.* 1977). Another potential stimulus for activation of chemotactic factors in decompression sickness is bubble surface activity. Surface activity proceeds from 4 nm to 10 nm zones of electrokinetic forces that exist at the interfaces between bubbles and blood and cause alteration in the secondary and tertiary configurations of native globular proteins (Lee & Hairston 1971). The globular proteins in the body ordinarily have hydrophobic groups in the interior of the protein molecule and hydrophilic groups toward the exterior of the protein molecule, which are exposed to aqueous blood. When these proteins migrate into bubble–blood interfaces, electrokinetic forces cause a molecular reorientation such that the hydrophobic groups protrude into the gas phase (bubble) and the hydrophilic groups protrude into the blood.

Enzyme activation or protein denaturation may occur, depending on factors such as time of exposure and the polarizing force of the interface relative to the strength of the intramolecular bonds of the proteins. The biological activity of some of the proteins altered at the bubble–blood surface increases. Hageman factor is converted from precursor to active form, which could lead to generation of chemotactic substances (Ghebrehiwet *et al.* 1981). We have started studying the accumulation of leucocytes in the brain damaged by bubble-induced ischaemia.

Experimental model

The model has been previously described (Hallenbeck *et al.* 1982*a*). Essentially, male mongrel dogs anaesthetized with α-chloralose (80 mg/kg) were mechanically ventilated and maintained in the physiological range with respect to blood gases, blood pH and body temperature. The femoral vessels were catheterized for measuring aortic pressure and right ventricular pressure, for sampling venous and arterial blood, and for infusing solutions as required. The right internal carotid artery was catheterized with PE-50 tubing.

The dogs were placed in a Kopf stereotaxic apparatus. Stainless steel screw electrodes were inserted into the skull. The recording electrode was positioned over the right sensorimotor cortex and the reference electrode was embedded in the nasal bones at their distal extreme. Electrode impedances were less than 3 kΩ. Stimulating electrodes were positioned in the left upper foreleg such that the median nerve was between them. A square-wave stimulus of 300 μs duration at a rate of 1.1 s^{-1} was led to the stimulating electrodes through a Grass Model PISU6C photoelectric stimulus isolation unit with constant current output. The strength of the stimulus was adjusted to cause a maximal cortical sensory evoked response (c.s.e.r.) over the contralateral somatosensory cortex. Potentials from the recording electrodes were initially led to a Nicolet HGA-100 preamplifier with a 0.25 Hz–10 kHz bandwidth and gain of 10^4. The output was led to a Nicolet 1073 computer of average transients and was displayed on a Tektronics 5110 oscilloscope. The output was recorded on a Hewlett-Packard 7045A *X*–*Y* plotter. A total of 64 evoked potentials (epochs) were averaged to obtain each recorded response.

After each animal was prepared, a series of not less than five c.s.e.rs was recorded as a baseline. The latency of the first positive peak, P_1, was noted, and all recorded amplitudes from P_1 to N_1, the first negative peak, were averaged to yield a control value. After these baseline measurements, 30–50 μl of room air was injected as a bolus into the catheter in the right internal carotid artery and flushed with 500 μl of saline solution. After 2 min another c.s.e.r. was recorded. If the response was suppressed to between 10% and 20% of the control amplitude, no more air was infused. If the c.s.e.r. was only partially suppressed, another 20–50 μl of air was delivered. This sequence was repeated until the c.s.e.r. amplitude was 10–20% of the control value. An animal was not eligible for inclusion in the series if it took longer than 10 min to suppress the c.s.e.r. amplitude to the desired range. Subsequently, any periodic recovery of the c.s.e.r. to 20% of control values or greater was suppressed to a level between 10% and 20% of control by periodic infusion of 20–50 μl of air. The volume of these incremental infusions of air was determined by the apparent sensitivity of the evoked response to each embolization. This cycle of alternating emergence and ischemic suppression of the evoked response was continued for 1 h. Following this 60 min period of c.s.e.r. suppression by ischaemia, the c.s.e.r. was monitored for a subsequent period of either 1 h or 4 h to assess the degree of neuronal recovery. At the conclusion of this variable recovery period, a [^{14}C]iodoantipyrine autoradiographic blood flow study was made (Sakurada *et al.* 1978).

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Leucocyte labelling

A total of 102 ml of arterial blood was drawn into two 60 ml syringes, each of which contained 9 ml of anticoagulant citrate dextrose solution USP. Fifty-one ml of blood were allocated to each syringe. The blood was processed by means of a Beckman Elutriator to isolate granulocytes. In general, 350×10^6–450×10^6 granulocytes were recovered, centrifuged at 150 *g* for 10 min and incubated with ^{111}In (1–1.5 mCi) in phosphate-buffered saline solution, pH 7.4, for 30 min. Following incubation, the cells were washed and re-suspended in 20 ml phosphate-buffered saline solution for infusion into the animal. Labelling efficiency ranged from 97–99%; only 1–3% of the tracer was in the suspension medium outside cells. The labelled granulocytes were injected intravenously into three control animals and into three other animals 10–15 min before the initiation of ischaemia by incremental air embolism. The latter three animals were monitored for 4 h after the 60 min ischaemic periods, while controls were simply observed for a similar period.

Detection of labelled granulocytes in the brain

Two techniques allowed measurement of labelled granulocytes in the brain. Sections of brain 20 μm and 40 μm thick were incubated with Kodak SB 50 X-ray film. These double-label autoradiograms permitted measurement of local blood flow, visualization of granulocyte accumulation, and correlation of local blood flow rates with focal accumulation of granulocytes. Slices of cortex of mass 50–100 mg from the sensorimotor area on the superolateral hemisphere were obtained from the injured right side and the non-injured left side, and ^{111}In activity was measured with a gamma counter. Brain–blood ratios of ^{111}In per unit mass were compared for injured and non-injured sides.

Table 1. Comparison of brain–blood ratios for injured and non-injured sides of the brain

$10^{-2}\left(\frac{^{111}\text{In/mass (in brain)}}{^{111}\text{In/mass (in blood)}}\right)$	control ($n = 3$)		embolized ($n = 3$)	
	right (non-injured)	left (non-injured)	right (injured)	left (non-injured)
	1.74 ± 0.31	1.93 ± 0.43	6.37 ± 1.85	1.76 ± 0.24

The values are the mean (±s.e.) of three samples taken from the superolateral cortex.

Leucocyte accumulation

Brain–blood ratios were very similar in the two hemispheres of control animals and in the non-injured hemisphere of the embolized animals (table 1). In contrast, 4 h after a standardized ischaemic insult, brain–blood ratios increased in the injured hemisphere relative to the non-injured hemisphere and control hemispheres. Granulocytes appeared as discrete dots and were fairly evenly dispersed on autoradiograms from controls. Four hours after ischaemia the ^{111}In dots were scattered in one animal whose blood flow was relatively preserved, but in two animals with neuron-disabling flows ((15 ml/100 g) min^{-1} or less in grey matter) the isotope was concentrated in the low flow zones. The accumulation of granulocytes in the post-ischaemic brain at a time when pathophysiologic events are still in flux and recovery is possible suggests that

† 1 Ci = 3.7×10^{10} Bq.

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these cells may have a role in converting injured neurons from a non-functioning but viable state to one of irretrievable damage. It also implicates an extensive activation of blood in the injury zone and generation of chemotactic proteins.

Cerebral oedema

When an individual with air embolism affecting brain function or decompression sickness affecting cord or brain function, or cord and brain function, appears to deteriorate clinically, oedema is commonly cited as the explanation. Extensive study in a variety of experimental models has established that brain ischaemia leads to brain oedema, an increase in the volume of brain tissue due to an increase in its water content. When the oedema is massive, as in large cerebral infarctions, it can cause severe intracranial hypertension and brain herniation at several sites. Under these circumstances, the contribution of increased tissue fluid to brain damage and dysfunction would seem straightforward and unequivocal. When the oedema is circumscribed rather than massive, however, its potential for increasing neuronal injury and hindering recovery is less clear. So, this form of fluid accumulation would increase local tissue pressure, and cause a corresponding decrease in local perfusion pressure. It could also increase cell–capillary separation, thereby causing a consequent increase in oxygen diffusion distances (Bourke & Kimelberg 1977). The effect of circumscribed oedema on neuronal function and recovery nevertheless remains a subject for experimental inquiry (Katzman *et al.* 1977). Brain water content was measured in a group of 58 dogs, of which 48 were rendered ischaemic as described in the experimental model above and 10 were not (controls). The brain waters were measured as one aspect of a study designed to test the therapeutic efficacy of PGI_2, indomethacin and heparin in reversible ischaemia of the brain caused by intravascular gas (Hallenbeck *et al.* 1982*b*). The various groups are summarized in table 2.

Table 2. Experimental groups

group	number of animals	duration of recovery/min	treatment PGI_2 (75–340 ng kg^{-1} min^{-1})	treatment indomethacin (4 mg kg^{-1})	treatment heparin (300 units kg^{-1})
1†	10		0	0	0
2	11	60	0	0	0
3	11	60	+	+	+
4	6	60	+	+	0
5	5	60	+	0	0
6	5	60	0	+	0
7	5	60	+	0	+
8	5	60	0	+	+

† Group 1 was not exposed to ischaemia. All other groups were subjected to 60 min of ischaemia.

Determination of wet tissue mass and dry tissue mass and calculation of water content

From a 2 mm thick section of coronal tissue in the sensorimotor cortex of the right hemisphere, two grey-matter samples were excised from the superolateral cerebral cortex and two samples of underlying white matter were also obtained. The samples were individually

placed in tared, stoppered weighing vials and immediately weighed on a five-place Mettler HL52 balance. The weighing vials had previously been baked to remove adsorbed water and had returned to room temperature in a desiccator over Drierite (calcium sulphate). The tissue samples were dried to a constant mass (as determined in preliminary experiments) in a 110 °C oven for 48 h and subsequently brought to room temperature in a desiccator over Drierite. The tared vials were then re-weighed to yield the dry mass. The percentage of solids for each sample was calculated:

$$\text{solids } (\%) = \frac{\text{dry mass of tissue/g}}{\text{wet mass of tissue/g}} \times 100.$$

The percentage of water was determined as:

$$\text{water } (\%) = 100 - \text{solids } (\%) = \frac{\text{wet mass} - \text{dry mass}}{\text{wet mass}} \times 100.$$

The duplicate determinations for the percentage of water in grey matter were averaged for each animal and the same procedure was followed for white matter.

TABLE 3. PERCENTAGE WATER (MEAN ± S.E.)

group	grey matter	white matter	percentage recovery c.s.e.r.
1	81.1 ± 0.2	66.6 ± 0.3	—
2	83.1 ± 0.4	67.7 ± 0.4	22 ± 3
3	82.4 ± 0.3	67.3 ± 0.8	50 ± 4
4	83.6 ± 0.6	67.5 ± 0.7	27 ± 8
5	81.8 ± 0.6	65.4 ± 0.7	21 ± 4
6	81.7 ± 0.8	66.6 ± 0.4	22 ± 8
7	83.2 ± 0.2	67.3 ± 1.0	18 ± 6
8	82.7 ± 0.5	67.3 ± 0.4	15 ± 4

RESULTS

The percentage of water (mean ± s.e.) and percentage of c.s.e.r. recovery (mean ± s.e.) are shown for each group in table 3. In grey matter the mean percentage of brain water increased in all embolized groups so that 1 h of ischaemia in this model produced oedema regardless of the type of infusate administered subsequently. One-way analysis of variance (ANOVA) revealed no difference in the percentage of grey matter among the embolized groups (groups 2–8) monitored for a 1 h recovery period ($p > 0.1$). A three-way comparison of the percentage of grey matter water was made between control animals not subjected to embolization (group 1, 81.1 ± 0.2), animals receiving effective drug therapy (group 3, 82.4 ± 0.3), and animals receiving an infusate without therapeutic benefit (groups 2 and 4–8, 82.8 ± 0.2). The differences between these groups were signficant at $p < 0.001$ by ANOVA. Both the effective therapy group and the ineffectual infusate group differed significantly from controls ($p < 0.01$), but did not differ from each other ($p > 0.1$) by Bonferroni testing.

The mean percentage of brain water in white matter of embolized groups was not significantly different from that of controls (table 3). Therefore, in correlations of brain oedema with other variables, only associations with grey matter water were analyzed. When the

percentage of grey matter water in embolized Groups 2–8 was correlated with the percentage of c.s.e.r. recovery, no association was found; $R = -0.01$, $p > 0.10$. Moreover, sorting and recombining embolized animals into an effective therapy group (group 3) and an ineffectual infusate group (groups 2, 4–8), and analysing each of these two groupings separately failed to reveal any correlation between amount of brain oedema as indicated by the percentage of grey matter water and the percentage of c.s.e.r. recovery; $R = -0.09$, $p > 0.10$ and $R = 0.15$, $p > 0.10$, respectively.

Embolized animals were sorted into two groups by the presence ($n = 9$) or absence ($n = 38$) of 'neuron-disabling' flows defined as (15 ml/100 g) min^{-1}, or less for grey matter, and (6 ml/100 g) min^{-1}, or less for white matter (Hallenbeck *et al.* 1982*a*). The percentages of c.s.e.r. recoveries in these two groups were significantly different by the student *t*-test: 'neuron-disabling' flow present gave $13.9 \pm 2.9\%$ c.s.e.r. recovery; 'neuron-disabling' flow absent gave $30.9 \pm 2.9\%$ c.s.e.r. recovery (mean $\pm$ s.e.); $p < 0.01$. The two groups did not differ with regard to percentage of grey matter water: 'neuron-disabling' flow present gave 83.1 ± 0.5; 'neuron-disabling' flow absent gave 82.6 ± 0.2 (mean $\pm$ s.e.); $p > 0.10$. They did, however, both differ from controls: $p < 0.05$ and $p < 0.01$, respectively, by one-way ANOVA and Bonferroni testing.

The relationship between circumscribed oedema and post-ischaemic neuronal function

If the local increase in tissue fluid that constitutes circumscribed oedema causes neuronal damage or exacerbates existing neuronal damage, one would expect a strong negative correlation between brain water content and a quantifiable index of neuronal function, such as the percentage of c.s.e.r. recovery. Furthermore, the presence of brain oedema should contribute to poor recovery regardless of whether therapy was given, and effective therapy should produce a concomitant decrease in cerebral oedema. These consequences of the hypothesis 'circumscribed oedema *per se* causes neuronal damage or exacerbates existing damage' were not observed as true in the present study. It would appear then, that regarding reversible focal ischaemia, a degree of cerebral oedema such as that observed in this study, is not a good predictor of the degree of post-ischaemic neuronal recovery. This does not, of course, preclude the possibility that oedema exceeding some higher threshold of severity would correlate better with neuronal recovery.

An alternative possibility is that brain ischaemia engenders two parallel processes that are not necessarily tightly coupled. Ischaemia creates the metabolic conditions that provoke an increased cellular imbibition of water (Leaf 1970; Chan & Fishman 1980; Bourke *et al.* 1970) and increases vascular leakiness through stimulation of pinocytosis (Westergaard *et al.* 1976; Simionescu & Palade 1976) and also, perhaps, through disruption of the interendothelial junctions (Hekmatpanah 1978). These perturbations lead to an increase in brain tissue water. Concomitantly, metabolic derangements of brain cells (some of which cause accumulation of tissue fluid) and a multifactorial interaction at the blood–endothelial interface (of which some components cause accumulation of tissue fluid) develop, which have a direct influence on neuronal function and recovery.

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Conclusions

The experimental model by which these studies were performed produces ischaemic c.n.s. damage as a consequence of intravascular gas bubbles. While not decompression sickness *per se*, the model produces a relatively controlled and standardized ischaemic insult and therein lies its value. Since circulatory impairment resulting from intravascular gas bubbles is common to both decompression sickness and this model, it is assumed that principles derived from the model have some relevance to decompression sickness in addition to cerebral air embolism.

The findings in the very preliminary work on granulocyte labelling suggest that bubble-induced ischaemia activates chemotactic proteins at an early stage and that granulocytes may be involved in the evolution of c.n.s. tissue damage. The cerebral oedema studies integrate nicely with these findings. The observation that neither the presence nor the amount of circumscribed oedema of grey matter is a good predictor of post-ischaemic neuronal recovery indicates that, up to a point, accumulation of fluid itself does not determine functional outcome after ischaemia. The changes in vessel permeability that accompany ischaemic oedema would, however, permit increased access of contact-activated proteins, such as Hageman factor, to subendothelial collagen (Stewart *et al.* 1977). The wide-ranging consequences of this protein leakage and activation would theoretically include activation of thrombosis, coagulation, fibrinolysis, kinins and the complement system (Hallenbeck & Andersen 1982). Several derivatives of these reaction sequences are chemotactic and should lead to granulocyte accumulation in the injury zone. The results of these interactions between blood and damaged tissue may well be major determinants of the extent of neuronal recovery following focal ischaemia of the c.n.s.

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