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Release of surfactant and a myelin proteolipid apoprotein in spinal tissue by decompression

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Hills BA. Release of surfactant and a myelin proteolipid apoprotein in spinal tissue by decompression. Undersea & Hyperbaric Med 1994; 21(2):95–102.—Two experiments have been performed on sections of bovine spinal cord, the first demonstrating that surface-active phospholipid (SAPL) and myelin proteolipid protein (PLP) are released by bubbles produced by decompression. Both phospholipid and proteolipid were found to be released in amounts increasing with the extent of decompression. The immediate recruitment of surfactant to the monolayer coating the pool surface indicated that the SAPL had been "carried" at the liquid–air interface of the bubbles. In the second study, electrophoresis was used to identify a major portion of the released proteolipid as the PLP much studied in recent times for its encephalitogenic properties. These findings are offered as a possible explanation for the demyelination often found in pathologic studies of divers and for the possible role of SAPL and PLP in stabilizing microbubbles/macronuclei during recompression, especially in relation to the practice of surface decompression.

decompression sickness, myelin, proteolipid apoprotein, spinal injury, surfactant, surface decompression

In 1878, Bert (1) recorded the tendency for paraplegia to occur in preference to other CNS symptoms of DCS in both divers and caisson workers, and spinal involvement was again emphasized at the turn of the century by the clinical findings of Blick (2) and the animal studies of Boycott et al. (3). Even though the latter workers recorded spinal bubbles as "extravascular," they still endorsed the embolic mechanism originally proposed by Bert (1). This emphasis on intravascular pathophysiology has been perpetuated by Hallenbeck et al. (4) in rekindling scientific interest in this major clinical problem; they have searched beyond the arterial system for a site of occlusion that can explain the higher incidence of special involvement with respect to blood flow distribution in the CNS. Even so, the epidemiology of spinal vs. cerebral lesions of DCS continues to be a controversial issue (5, 6).

Hills and James (7) argued against any embolic mechanism for spinal DCS (SDCS) on the basis that the symptoms are not only reversed by recompression but are repeatedly reversible by pressure, recurring upon subsequent decompression in toto

with the same distribution. Since intravascular bubbles are known to be cleared when blood flow is restored by recompression (8, 9), the offending bubbles must be located in extravascular sites for their effects to be so consistently repeatable. This argument has raised the issue of why these autochthonous bubbles should form in the first instance and why they should be more prevalent in the spinal cord.

The second of these questions has been answered to some extent by the finding in this laboratory that there are lamellar bodies (LBs) in spinal tissue in essentially the same sites (10) at which other groups (6, 11, 12) have demonstrated the formation of "space-occupying lesions" upon decompression. Moreover, the distribution of LBs between spinal and cerebral tissue (3:1) corresponds to the ratio of symptoms emphasized by Hallenbeck et al. (4), although this could be fortuitous. Lamellar bodies are interesting because these are the highly surface-active "packages" in which surfactant is produced in the lung (13) where it promotes formation and stabilization of the gas–aqueous interface. The same could occur in an unwanted bubble.

The recent study from this laboratory (10) also reported a hydrophobic protein in spinal tissue which could potentiate the surface activity of the phospholipid (PL) in the same way that the hydrophobic proteins B and C, claimed to be unique to lung (14), are know to potentiate the action of pulmonary surfactant. A closer examination of the neurologic literature, however, has revealed that the hydrophobic protein is probably the "proteolipid protein" (PLP), which is a minor component of myelin relative to PL but one that is essential in myelin formation (15). Myelin contains approximately 8% protein, of which about one third is a proteolipid apoprotein (PLA) that is so hydrophobic as to be co-extracted with PL by chloroform and other typical solvents for lipids (16).

In a previous study (10) it has been shown how PLA potentiates the activity of the surface-active PL, which is the predominant component of myelin. Hence it would be highly desirable to confirm that both PLA and surface-active PL (SAPL) are actually recruited by the liquid–air interface, i.e., by the bubble surface, upon decompression. An additional reason for wishing to detect any release of PLA is that the major component has been shown to be encephalitogenic (17–20), whether the source is cerebral or spinal (21).

MATERIALS AND METHODS

Principles

The concept that SAPL and myelin proteolipid apoprotein (MPA) could be released from spinal tissue by bubble formation was tested by decompressing sections of excised bovine spinal cord suspended in saline and then analyzing the surrounding fluid for PL and proteolipid. Co-extraction of SAPL and PLA from the surrounding saline with chloroform left behind any organic phosphate or water-soluble protein which would otherwise invalidate the subsequent analysis using the standard method of Rouser et al. (22) for PL and the standard method of Böhlen et al. (23) for proteolipid. The Böhlen method was selected as insensitive to the PL content of the sample. The PLA released was then examined by electrophoresis according to a special method designed for myelin proteolipids to determine whether it contains

the encephalitogenic component that comprises about one third of bovine myelin protein (15).

Materials

Bovine spinal cord was used because the MPA component has been confirmed as encephalitogenic (17, 18, 21) and its identification by electrophoresis has been described in detail. Five cords from adult steers were excised at the abattoir and equilibrated for 30 min in saline saturated with air at 25°C. The cords were then cut into sections approximately 3 cm in length and the pia dissected from each, after which they were rinsed in saline to remove any blood or other extracellular material.

Decompression

Each section of fresh spinal cord was weighed and suspended horizontally in 100 ml of saline in a beaker at 25°C. Each beaker was then placed in a vacuum desiccator and a different vacuum applied to each for 60 min, absolute decompression ranging from zero (for controls) up to 635 mmHg. Barometric pressure ranged between 694 and 703 mmHg, our laboratory being located at altitude. Bubbles were seen to form in all decompressed tissues and, when large enough, would burst out of the tissue and rise to the surface of the saline. After the hour, the samples were returned to ambient pressure and each section of cord was lifted out of its saline by means of the sutures, and the saline analyzed for any SAPL and MPA released by the bubbles. This procedure was repeated for five different cords.

Analysis

Any SAPL or PLA released into the saline by bubble formation, or otherwise, was extracted using the standard solvent (chloroform:methanol 3:1) following the Folch and Stoffyn procedure as applied to myelin (16). The solvent extract was evaporated to dryness under N_2 and a known weight of the residue converted to phosphate by use of perchlorate, following the standard method of Rouser et al. (22). The phosphate generated was then mixed with ammonium phosphomolybdate and compared with known phosphate standards for color intensity using a spectrophotometer. This analysis enabled the released SAPL to be determined as elemental phosphorus and was repeated 3 times on each sample.

Protein analysis

Another portion of the residue from the solvent extraction was dispersed in 0.05 *M* sodium phosphate buffered to a pH of 8, and fluorescamine solution in acetone 30 mg/100 ml) was added according to the standard method of Böhlen et al. (23). The fluorescence developed at $\lambda = 475$ nm was then measured on a spectrofluorometer with excitation set at $\lambda = 390$ nm and compared with standard solutions of bovine serum albumin (BSA). Assays are then quoted in terms of equivalent milligrams of BSA per milliliter. The insensitivity to PL was confirmed in ancillary tests in which phosphatidylcholine was added to the test material with no effect.

Electrophoresis

Electrophoresis was also performed on the residue to identify the spinal proteolipid(s) released by decompression. This technique employed a slab gel with an acrylamide gradient of 75–26% in buffers containing sodium dodecyl sulfate in accordance with the standard method of Maizel (24). Test samples were run against Novex standard solutions of known proteins.

Surface activity

In an ancillary experiment, a platinum flag suspended from a force transducer was dipped into the same pool of saline surrounding the tissue before and after a decompression to 515 mmHg of vacuum. This enabled the surface tension to be measured by the Wilhelmy method used in an earlier study (10).

The saline was then poured into a Langmuir trough and 2 h allowed for any surfactant to relocate as a monolayer at the surface of the new pool. Surface area was cycled between 100 and 20% of its initial value as a further test of surface activity.

RESULTS

Surface activity

In the ancillary experiment, the release of bubbles from the cord by decompression reduced the surface tension of saline from 68.4 dyn/cm (mN/m)—with cord immersed—to 26.9 dyn/cm after 1 h of decompression. When that liquid was transferred to the Langmuir trough and any surfactant allowed to relocate at the new interface, the recruited monolayer proved particularly surface active, as demonstrated by the relationship between the surface tension and surface areas shown in Fig. 1.

Analysis of saline

Simple immersion of a section of spinal cord in 100 ml of saline for 1 h released very little PL or PLP, even with the pia removed. Upon decompression, however,

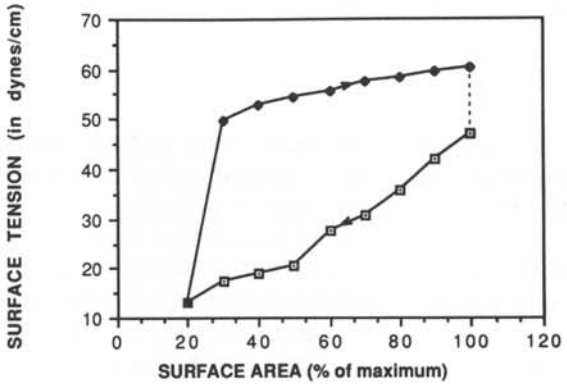

FIG. 1—Plot of surface tension vs. surface area for the first cycle of surfactant recruited to the pool surface 2 h after the saline surrounding the spinal cord during decompression had been transferred to a Langmuir trough. Note the very low surface tension reached upon surface compression of the monolayer and the large hysteresis loop—both typical of SAPL recruited to the liquid–air interface.

the amounts of both PL and PLP increased substantially, as seen in Fig. 2, and show a substantial increase with the extent of decompression, especially for PLP. The increases are highly significant statistically with $P < 0.01$ according to the paired t test.

Electrophoresis

When run against solutions of standard proteins under electrophoresis, the developed gel for the solvent extract is shown in Fig. 3. In both test runs it can be seen that there is a dense band corresponding to a molecular weight of 18,000–20,000.

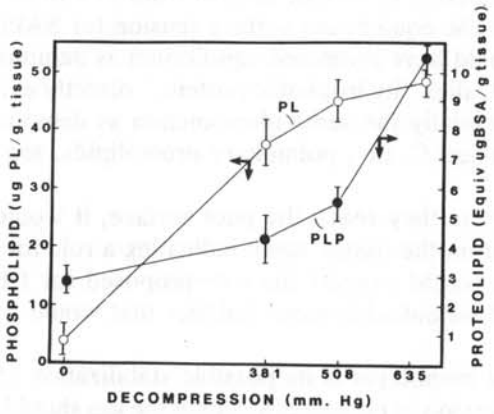

FIG. 2—Plots of PL and PLA both show a large increase in quantity released into the saline surrounding the cord sections as decompression is increased.

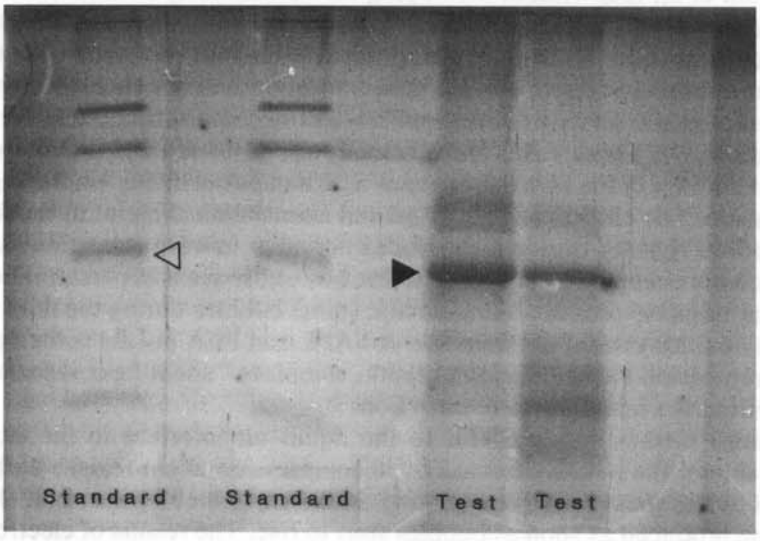

FIG. 3—Use of electrophoresis to demonstrate proteins in the chloroform extract from saline surrounding the decompressed sections of spinal cord. *Open arrow* shows a known protein of molecular weight 21,400 in the Novex controls run concurrently; the known protein places the dense band, indicated by a *solid arrow* in the test lanes, within a molecular weight range of 18,000–20,000 and close to the encephalitogenic myelin PLA of 18,750 (15).

This encompasses the value of 18,750 attributed to the encephalitogenic proteolipid found in appreciable quantities in bovine myelin (15) and is probably the MPA DM-20 known to cross-react with PLP-specific T cell clones (21).

DISCUSSION

Bubble formation in spinal tissue facilitates the release of both PL and PLP from spinal tissue. The SAPL is probably carried at the air–aqueous interface of the bubbles as they rise to the surface of the pool of saline in which the cord is immersed. They eventually burst at the surface, presumably contributing their surfactant to the pool surface as witnessed by the reduction in surface tension from 68.4 to 26.9 dyn/cm. The latter value coincides with the equilibrium surface tension for SAPL (25), in which the released proteolipid could have promoted equilibrium as demonstrated in our earlier study using proteolipid, alias "hydrophobic protein," directly extracted from spinal tissue (10). This is essentially the same phenomenon as demonstrated when lung "surfactant proteins" B and C, i.e., pulmonary proteolipids, are added to a surfactant monolayer (15).

If surfactant is lining the bubbles as they reach the pool surface, it would have been doing so before their release from the tissue, again indicating a role for SAPL (and LBs) in their formation. This would support the role proposed for LBs and surfactant in general in initiating those autochthonous bubbles that would seem to be responsible for SDCS.

Another implication of surfactant monolayer is its possible stabilization of small bubbles/macronuclei upon recompression to pressures at which the gas should return to solution. The physics of such a mechanism was discussed in detail some years ago by Yount et al. (26) without identifying the surfactant system that SAPL could provide. A very practical manifestation could occur during the common practice of surface decompression. Some time ago it was demonstrated how any upward excursion to a supersaturation depth during a dive could induce a remarkably high incidence of CNS symptoms in goats when the subsequent decompression was "titrated" to a bends point (27). Those cases were actually spinal bends and are supported by subsequent findings of the high incidence of SDCS in ocean diving employing surface decompression. The clinical and experimental animal data all point to the formation of some bubble "ghost" during the surface interval or upward excursion, which can survive recompression and yet be reactivated by subsequent supersaturation of the surrounding tissue to produce symptomatic spinal bubbles during the final stages of decompression. Maybe the combination of SAPL and PLA in LBs is the agent that, upon recompression to the "bottom" depth, stabilizes "silent" gas separating from solution during the initial upward excursion.

In addition to transporting SAPL to the liquid–air interface in the experiment described above, the bubbles formed by decompression might release both PL and proteolipid by the shear action of rupturing cells, as indicated by the slight cloudiness of the saline produced as soon as bubbles start to rise. The results of electrophoresis (Fig. 3) have major clinical implications insofar as the proteolipid released by the bubbles clearly includes the encephalitogenic PLP well characterized in bovine spinal tissue (15). This apoprotein is currently generating much interest in the neurologic literature for its ability to induce experimental animal encephalomyelitis in rabbits,

guinea pigs, rats, and all strains of mice, because this model shares many clinical and histopathologic features with multiple sclerosis (MS) (17). Hence the release of PLP by bubbles could provide a mechanism supporting the association of decompression with MS (28) or, at least, with myelin degeneration found in the spinal cords of commercial divers—even in those with no recorded incidence of neurologic DCS (29) or in those receiving an otherwise successful treatment (30).

Clearly the findings of this study require further investigation, including repetition of these experiments on cerebral tissue in animals and the search for any PLP released into the circulation in divers with neurologic DCS.

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