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Intramantle pressure gradients favoring hydrocephalus development are generated in the rat brain following disruption of beta-1 integrin-matrix interactions

Introduction and Rationale

Hydrocephalus is a chronic brain disorder characterized by expansion of the ventricles and in some cases, significant neurological damage. Current treatments are only partially effective and there is an urgent need to reassess the conceptual foundation on which our understanding of this disease is based. Perhaps the greatest paradox in the hydrocephalus field is the failure of researchers to consistently measure transmantle pressure gradients in humans and in animal models of the communicating form of the disorder. Without such a gradient it is difficult to conceptualize how ventricular distention occurs.

The overall objective of our work is to investigate the mechanisms responsible for ventricular expansion in a rat model of hydrocephalus. In particular, we have been made aware of the studies performed by Dr. Reed and colleagues in Norway. His experiments demonstrated that the interstitial matrix in skin was an 'active' component in tissue pressure regulation. We surmised that if true in the brain, this matrix concept might provide an explanation for ventriculomegaly.

New Concept

Several clues exist in the literature suggesting that pressure gradients may exist in a manner and location that have not been considered seriously in the past. Based on the results of a mathematical model, one group has proposed that ventricular expansion may result from a relative reduction in interstitial pressure in the peri-ventricular area leading to the formation of an intra-mantle rather than a transmantle pressure gradient (Pena et al., Acta Neurochir 81: 59, 2002). At a theoretical level, this idea is interesting but a mechanism causing the reduction in interstitial fluid pressure was not presented. The main objective of this project is to investigate a molecular mechanism that could produce this effect.

A combination of classical physiological methods and techniques from cellular and molecular biology have provided new insights into interstitial fluid pressure regulation by the beta-1 integrins and connective tissue elements. In non-CNS tissues such as skin, the dissociation of $\alpha_1\beta_1$ integrins with the surrounding matrix fibers results in a significant reduction of interstitial fluid pressure (Wiig et al., Acta Anaesthesiol Scand 47: 111, 2003). This causes enhanced movement of water and solutes into the tissue spaces by increasing the hydrostatic pressure gradient that favours capillary fluid filtration. In the studies conduced thus far, inflammation from a variety of causes and several inflammatory mediators seem to induce this effect consistently but what is most relevant to us, is the fact that a lowering of interstitial fluid pressure can be produced by the injection of antibodies to the $\alpha_2\beta_1$ integrins which appear to be the central players in regulating this phenomenon. These results indicate that the interstitial matrix is in a dynamic state and that tension on the connective tissue fibers (mainly collagen) can be increased or decreased to modify tissue pressure as the compaction of matrix elements in

modulated.

These experiments have transformed our view of the interstitium in that the connective tissue elements and interstitial fluid pressure is now considered an active rather than a passive controller of fluid exchange. If the same is true of the brain parenchyma, we can develop a new view of hydrocephalus that incorporates perturbations in beta-1 integrin function as the defining parameter that induces ventriculomegaly. Beta-1 integrins are found throughout the brain (including the peri-ventricular area) and are believed to have an important role in the development of the brain. ***We hypothesize that perturbation of the $\alpha_2\beta_1$ integrin-matrix interaction may result in a drop in the parenchymal interstitial pressure in the peri-ventricular area leading to a pressure gradient favoring expansion of the ventricles and peri-ventricular edema.***

Recent Results

We measured periventricular pressures and ventricular pressures with servo-null micropipette system in Wistar rats after injecting a variety of anti-integrin antibodies, non-immune IgG/IgM, or ringer lactate or into a lateral ventricle. In a second group, the animals were kept for 2 weeks after similar injections and upon sacrifice, the brains were removed and assessed for hydrocephalus. In the majority of experiments in which antibodies to beta 1, alpha 2 beta 1 or alpha 2 integrin were injected (n=14), we observed a decline in periventricular pressures relative to the pre-injection values ranging from 1.10 to 4.60 cm H_2O (average reduction 2.85 ± 0.75 cm H20). Under similar circumstances, ventricular pressures either remained the same or were elevated. The ventricular to periventricular pressure gradient ranged from 4.3 to 7.0 cm H_2O (average 5.2 ± 0.85 cm H_20). Non-immune IgG/IgM and ringer lactate injection had no significant impact on any pressure (n=8). In the chronic preparations, we observed enlarged ventricles in many of the animals that received injections of anti-integrin (IgG) antibodies (14 of 18 animals, 78 %). We conclude that changes in the periventricular matrix generate pressure gradients favourable for ventricular expansion suggesting a novel mechanism for hydrocephalus development.

Mathematical Issues

1) Can one predict a ventricle size given a defined pressure gradient between the ventricles and periventricular area?

2) What is the smallest pressure gradient that would expand the ventricles?

3) What impact would elevated ICP have on the aforementioned variables? Since hydrocephalus is often associated with elevations of CSF outflow resistance and elevated CSF pressure, perhaps a 2-hit hypothesis could be generated. ***If a reduction in CSF absorption exacerbates the pressure differential caused by matrix disruption, this would support the notion a 'two-hit' hypothesis for ventriculomegaly; i.e. a disturbance in matrix integrity in the peri-ventricular area may be an initiating event in the***

development of hydrocephalus. A concurrent CSF absorption deficit may intensify the magnitude of the pressure gradient.

Significance of these studies

Apart from providing a mechanism to explain ventriculomegaly, it is of special interest to note that the reduction in tissue pressure in Reed's experiments could be reversed with selected drugs such as the anti-inflammatory agent α -trinositol and the BB isoform of platelet-derived growth factor (PDGF-BB). This provides the exciting possibility that some forms of neonatal hydrocephalus may ultimately be treatable with pharmacological agents thus reducing the dependence on problematic shunts.

References

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