Premature babies have altered sensory responses in later life

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Premature infants who need intensive care or surgery are less sensitive to thermal (hot and cold) sensations later in life, according to research conducted at UCL (University College London). The study, published in the journal *Pain*, suggests that pain and injury related to major medical interventions in early development may alter how children respond to painful stimuli much later in life.

Researchers have made several, subtle changes in the structure of a key protein, dramatically increasing its ability to drive blood clotting, according to a study published in a December edition of the Journal of Thrombosis and Haemostasis. The findings have profound implications for the treatment of hemophilia, the inherited blood disorder that causes easy or excessive bleeding in 30,000 Americans.

In most cases, hemophilia is caused by a lack of a protein, factor VIII, one of several proteins in a chain reaction that enables blood to solidify, or clot, to plug wounds after injury. Current preventive treatment consists of genetically engineered factor VIII administered by injection, but one quarter of those born with no factor VIII suffer severe immune reactions that render the treatment inactive. In addition, current treatment costs as much as $200,000 per patient per year. Researchers at the University of Rochester Medical Center have been studying the structure and function of factor VIII for decades, and are now making subtle changes in the protein with the goal of offering more effective, less burdensome treatment.

In past research and in the current study, a team led by Philip Fay, Ph.D., professor in the Department of Biochemistry and Biophysics at the University of Rochester Medical Center, has identified key amino acids (out of the more than 2,300 building blocks making up factor VIII) with the potential, if replaced, to change the performance of entire protein. Fay, along with partner, Hironao Wakabayashi, M.D., research assistant professor, has filed patent applications for the factor VIII redesigns.

"Our goal is to improve upon nature by developing gain-of-function factor VIII proteins that are superior to the version of the protein found in healthy individuals," Fay said. "These more potent forms are not likely to occur naturally since they would theoretically result in excessive clotting, blocked arteries and heart attacks in otherwise healthy people. In patients with hemophilia, however, enhanced clotting is desirable. On the other hand, we have sought to change the protein's structure as little as possible, because the immune system is more likely to react the more we change it."

More Effective Without Drawing Notice

Blood clotting involves more than a dozen proteins known to take part in a cascade of chemical reactions inside blood vessels following injury, with each factor, or complex of factors, activating the next in the chain. Named for roman numerals, these clotting factors include factor VIII, which is modified slightly to form an active cofactor, VIIIa, and partners with factor IX to activate factor X. Factor X creates a burst of thrombin, which in turn generates fibrin, the sticky protein strands that form a web-like clot over damaged tissue.

As Fay's team gained a deeper understanding of Factor VIII's structure over 20 years, it gradually became clear how they could change it to make the protein more likely to drive clotting. In 2005, he and Wakabayashi published work showing that swapping out a naturally

occurring glutamic acid with an alanine at a specific spot in the calcium binding site on factor VIII doubled its ability to bind with factor IX and increased clotting. Factor VIII has on its surface pocket-like chains of amino acids shaped to hold calcium, a metallic element that makes factor VIII better able to bind to factor IX.

While the past work increased the protein's activity (binding with factor IX), the current work dramatically increased the stability of factor VIII. In this context, stability is the ability of the protein to withstand the rigors of manufacturing and of exposure to the human blood stream. It must resist forces that would unravel it, or tear it apart, before it can have its therapeutic effect. Standard stability tests include exposing a protein to increased temperature (thermal stability) or detergents (chemical stability). In the case of factor VIII, stability measures the strength with which the other factor VIII domains hold on to the A2 domain, the piece most likely to break away. Inactive Factor VIII is converted into active Factor VIIIa by protein when clotting begins, but this cannot happen though if the A2 domain of factor VIII has already fallen off.

As pharmaceutical companies manufacture the current hemophilia treatment, recombinant factor VIII, the protein is grown in a cell culture, purified in several steps, freeze-dried to convert it into powder and added to saline before it can be injected into the body. A small but significant portion of the protein is unraveled or broken up during this bruising process, but a more durable version could mean the delivery of more active protein by the time it hits the body, researchers said. Furthermore, when more stable factor VIII is converted into factor VIIIa, the VIIIa cofactor better holds onto the A2 domain, which is the reason that the current study found that more stable mutants increase clotting.

In a recent paper in the journal Blood, the team used molecular biology

techniques to swap out those three charged residues with residues that carried no charge, removing barrier to the closing attraction between factor VIII's domains. The fact that oil separates from water has been harnessed by life. Cells have oily barriers that keep out the watery world, and the insides of blood proteins like factor VIII "prefer" non-charged, oily interactions to carry out their functions. Of course, nature is not perfect, so some charged residues are buried within proteins, including within factor VIII domain interfaces.

In the current paper, the team compared the chemical and thermal stability of mutated forms of factor VIII containing one of the single mutations proved earlier to increase stability against mutant forms with combinations of the same mutations. In all cases buried charged amino acids were replaced by non-charged substitutes. While they found several of the single mutations doubled the stability of factor VIII, and of factor VIIIa, certain combinations of mutations increased stability by up to ten times, suggesting synergy between the mutations. Another benefit of this approach is that any changes to the structure are buried within the protein, so they would not attract notice by the blood-based immune system that might react to them.

Specifically, Fay and Wakabayashi found three amino acids (Asp519, Glu665, and Glu1984) located in the interfaces between the A2 domain with domains A1 and A3, were reducing the strength of the attraction between the domains because of their charges. Their removal via mutations increased factor VIII thermal stability by 200 percent and its chemical stability by 30 percent increase. It reduced the ability of A2 to break away from factor VIIIa by 800 percent, and brought about an increase in thrombin generation capacity of 220 percent.

"Moving forward, we are excited about this line of work because we have identified further changes that promise the engineering of an even more stable factor VIII, and we now have designed a version of the

protein that combines increased stability with better calcium binding for increased activity, a version that pharmaceutical companies are starting to show interest in," Fay said. "Our goal from these redesigns remains to prolong the treatment effect, make it less expensive and lessen the chances of rejection by the immune system."

Source: University College London