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THE DISCOVERY OF THE BLOOD-BRAIN BARRIER A PRACTICAL EXERCISE FOR PHYSIOLOGY STUDENTS

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Abstract: Brain tissue, basically irreparable after injury, is stored in a "strong box" built with several levels of protection: skull, meninges, extracellular fluids and, finally, the Blood-Brain Barrier (BBB). The BBB combines anatomical structures and physiological transport systems. The structures are the tight junctions between brain endothelial cells (CECs) that form the walls of most of the brain's approximately 400 kilometers of capillaries. Transport systems are selective processes that control substances and their rates of entry into the cerebral interstitial fluid. The formation of the BBB is influenced by paracrine signals originating from the microenvironment of CECs, involving pericytes, astrocytes and neurons. This neurovascular unit exhibits highly controlled cell-cell communication patterns, varying with factors such as age and pathologies. The concept of BHE dates back to the beginning of the 20th century, but the beginning of its history dates back to the 17th century with the work of Humphrey Ridley (1653 –1708). Ridley, by injecting mercury, or colored wax, into the cerebral veins of recently executed criminals, was the first to observe and document the complexity and low permeability of the cerebral vascular network. Two hundred years later, Paul Ehrlich resumed this research in rodents, concluding that the bright coloring he used did not stain either the brain or the cerebrospinal fluid. New research and discoveries followed, establishing the current understanding of the BBB and its role in maintaining brain homeostasis, and providing new clues for future research into possible points of pharmacological intervention.

Keywords: Blood-brain barrier; Historical description; Development; Cell-cell communication; Physiology

INTRODUCTION

The purpose of this article is to provide physiology students in a class with a "protocol" that guides and encourages them to search, discover and share scientific information through episodes in the history of physiology as a science and, ultimately, to write a research article. revision. It is also important to show them the value of each scientific achievement and the intellectual, temporal and often personal cost faced by researchers at the time, which must lead us to an attitude of humility and respect towards the pioneers, recognizing that knowledge current results are the result of your efforts and dedication.

The topic of the blood-brain barrier (BBB) – a complex system of multiple physical and physiological obstacles that selectively limit the flow of blood solutes to and from the brain – is a point of interest in the study of the central nervous system (CNS) and has the appropriate requirements for the class objectives referred to in the purpose of the article: its relatively recent history, some controversies and an example of the difficulties in chemotherapy for CNS diseases, because medications that could act on other organs may not be able to enter that system because of the BHE. The subtitles ordered in a temporal sequence correspond to the subtopics in focus. Each group of students must select a theme to develop. The process requires group work, but also intergroup dialogue so that, at the end and together, the class composes a full text of a review article. This article deals with how physiology students can be challenged to write, during a practical class of two hours (100 minutes), a review article, with "many hands", and without losing the thread that integrates the parts into a effort that must be coordinated and reinforced by the teacher.

THE FOUR INTERFACE BARRIERS

What is now understood about the blood-brain barrier (BBB) represents the culmination of a long line of investigation. The concept began to be established at the end of the 19th century, but it was only from the 1920s that the term began to be used, although its structure and underlying mechanisms were far from known.

The normal functioning of brain tissue is absolutely vital for the individual, as is the redundancy of protective structures and processes that include the skull bones, meningeal membranes, cerebrospinal fluid and various interface barriers. To provide the necessary environment for neurons, the extracellular fluids of the CNS are separated from the constantly changing environment of the blood by four main interfaces, although one of these is only present in the embryo. Of these, the two that represent the largest interface between blood and cerebral extracellular fluids are: (a) the BBB - the blood-brain barrier itself, which represents the functional interface between the central nervous system and the vascular system (in English Blood Brain Barrier, BBB;); (b) the interface that separates the cerebrospinal fluid (CSF) space from the circulating blood BCSFB (Blood/CSF). Both prevent the free paracellular diffusion of polar molecules, due to their complex morphological characteristics that include tight junctions (TJs) that interconnect endothelial and epithelial cells, respectively [1].

HUNPHEY RILEY, THE 1ST DESCXRECTION

More than 300 years ago, during the Renaissance – a landmark period in the evolution of anatomy as a scientific discipline – Cambridge University physician Humphrey Ridley, MD (1653–1708) stood out as one of the first to explore the cerebral vascular network. Riley's technique, innovative at the time, involved the injection of colored wax or mercury into the cerebral vessels of recently hanged criminals, which revealed more anatomical details, due to the considerable venous engorgement in the region caused by the tourniquet on the neck. In 1695, Riley published "The Anatomy of the Brain Containing its Mechanisms and Physiology: Together with Some New Discoveries and Corrections of Ancient and Modern Authors upon that Subject", the first English-language treatise focused on neuroanatomy and illustrated by William Cooper, an important surgeon of the time. It shows the author's great fascination with the complexity of the brain, which Ridley compared to the work of a divine author, and considered that his understanding provided clarity on other issues, even if the brain itself remained mysterious. While recognizing the value of earlier books, notably Thomas Willis's "Cerebri Anatome", 1664 and Raymond Vieussens' "Neurographia universalis", 1685, Ridley's approach reflects innovative research principles. His work highlighted the importance he gave to understanding the human body through the theory of cause and effect, to the objectivity of observations through experimentation, instead of the predominant philosophical introspection, and to the analytical confrontation of discrepancies between what he observed and previous knowledge, valuing the caution in accepting information without scrutiny. In the context of neuroanatomy and neurophysiology, Ridley's most important contribution was the evidence of the extension and distribution of the cerebral vascular network and the low permeability of small cerebral vessels to a substance injected into the bloodstream compared to that observed in peripheral capillaries. Despite this, neither he fully understood the significance of his discoveries, nor did his research work achieve due recognition, remaining undervalued in

the history of neuroanatomy. [2, 3].

FROM RIDLEY TO CLAUDE BERNARD

After Ridley's work, little was known about the specificities of the brain's vascular network. It took about a century and a half until the key physiological concept of "internal milieu" was introduced by Claude Bernard (1813-1878)[4]. The physiological relevance of the constancy of the internal environment covering all body systems was described by Bernard in 1849 and later, in 1926, formulated in the concept of homeostasis by Walter Cannon (1871-1945)[4]. In turn, Joseph Barcroft (1872-1947) understood that brain function was responsible for the "controlled internal environment" of the human (or animal) body, just as this constancy had a central importance in the brain itself. [5.]

PAUL EHRLICH, 190 YEARS AFTER RIDLEY

From the mid-19th century to the beginning of the 20th century, the "new" era of chemistry, biology, physics and medicine experienced great progress thanks to the research carried out by a notable group of scientists: E. von Behring (1845 -191), M. Curie (1867- 1934), P. Curie (1859- 1906), H. E. Fischer (1859 -1919), S. Freud, (1856-1939), R. Koch (1843- 1910), I. I. Metschnikow (1854 -1916), K. Landsteiner (1868 -1943), L. Pasteur (1822 -1895), W. C. Roentgen (1845 -1923), C. von Rokitansky (1804 -1878), R. Virchow (1821- 1902), among others. Paul Ehrlich (1870-1915) was one of the most famous and influential German researchers of the time. Pioneer co-founder in the areas of hematology, immunology, pharmacology and chemotherapy (for many, the "father" of chemotherapy), great knowledge of chemical principles for the development of new biological or therapeutic concepts, Ehrlich created the concept of "magic bullet" and, in 1908 he received the Nobel Prize for Physiology or Medicine, shared with the Russian microbiologist Elie Metchnikoff (1845-1916) for their respective works that later formed the basis of humoral and cellular immunology [6].

At the same time, research carried out by German bacteriologist Paul Ehrlich, in 1885, laid the foundations for the concept of the blood-brain barrier. After intravenous administration of a variety of vital dyes (due to their low or absent toxicity, they do not cause death to the animal) in small live animals and subsequent histological examination of their tissues, Ehrlich found that the dyes diffused into the vascular network of practically all of the animal's organs, with the exception of the CNS, mainly the brain. Ehrlich's interpretation was that the brain has low or no affinity for vital dyes, with the argument that this was due to differences in binding affinity for different dyes between different tissues and not due to any special vascular properties that blocked access to the brain. dye to the brain, proposed by others. At the time, a dominant current argued that the brain had unique vascular properties, but according to Ehrlich's interpretation, the brain tissue did not absorb the dye due to a "lack of affinity" between the dye and the neural tissue and he stated: "I cannot accept that the vascular endothelium, as such, exerts different functions in different organs, so that, for example, a hepatic capillary is permeable to certain substances that will not pass through other capillaries", thus arguing against the hypothesis that the CNS barrier based on vascular properties [3,6].

Although Ehrlich's experiments unintentionally marked a critical step towards one of the most crucial concepts in neurobiology and pharmacology, given the importance of the BBB in drug delivery, neurological diseases, and general CNS

homeostasis, he himself argued against the hypothesis that existence of such a vascular barrier and, therefore, Ehrlich is named "contrary discoverer of the phenomenon of the blood-brain barrier"[7].

SPECIAL PROPERTIES OF BRAIN ENDOTHELIAL CELLS

It is important to clarify here that, according to Saunders and collaborators[8], contrary to what is sometimes pointed out, neither the BBB was described for the first time by Ehrlich (1885), nor Lewandowsky (1900) seems to have used in his texts for the first time, the term "Blut-Hirnschranke" barrier (in German, blood-brain barrier), which is justified by a possible error of deficient translations that was perpetuated [8].

Paul Ehrlich's work, although misinterpreted by himself, provided empirical evidence that paved the way for the discovery of the specialized vascular structure in the CNS and for the concept of the blood-brain barrier. The idea of special properties of the endothelial cells of cerebral blood vessels that limit the entry of substances into the brain was proposed, among others, by Biedl and Kraus in 1898[9], Roux and Borrel in 1898[10] and Lewandowsky in 1900[11] based on the comparison of intrathecal and parenteral injections of materials with neurotoxic effects, such as the bile salts, after injection by the first route.

While the understanding of brain barriers was progressing in Germany, in England, Charles Roy (1854-1897) and Charles Sherrington (1857-1952), suggested that the brain had an intrinsic mechanism that separated the blood supply from the neuronal tissue and that this could vary locally according to the various functional states of the brain. They also discovered that fat-soluble molecules, such as morphine and caffeine, were able to pass into neural tissue, while others, which were fat-insoluble, did not pass through. [12], despite not having realized the scope of the results of their research in understanding the functioning of the BHE.

FIRST AND SECOND EXPERIENCES OF EDWIN GOLDMANN

During the period from 1909 to 1913, Edwin Goldmann (1862–1913), a student of Ehrlich, conducted the so-called "first and second Goldmann experiments". Through them, he proved his teacher wrong and contributed decisively to the formulation of the initial concept of "brain barriers". In the first, he replicated the previous studies of Ehrlich, his mentor, by systemically administering trypan blue dye intravenously. He then confirmed that the entire body blushed blue, except for the brain and spinal cord. In the second, he chose to inject the dye directly into the cerebral ventricles of dogs and rabbits, which allowed the dye to reach the cerebrospinal fluid (CSF) and demonstrated that the dye acted throughout the brain, but remained confined to the cerebral vascular network, without stain any peripheral organ. Not only did Goldmann refute Ehrlich's affinity theory, as he had shown that the lack of staining in the CNS was not due to incompatibility of the dye with brain tissue, but he demonstrated, on the one hand, that the brain was physiologically separate from the rest of the body. and, on the other hand, that there was no relevant barrier between the CSF and the brain parenchyma (a CSF-brain barrier). Goldman had produced convincing evidence of the existence of some type of barrier that "physiologically separated" the brain and the rest of the body through an interface that worked bidirectionally and blocked the brain from systemic circulation. Based on the comparison he established between the placenta (the injection of trypan blue into the maternal circulation stained the placenta, but exempted the fetus from color)

and the choroid plexuses, he further suggested that the CSF, with access to the brain tissue through the choroid plexuses, was the means of transporting substances to the brain, giving the epithelial cells of the choroid plexus the role of "physiological limiting membrane" in the CNS, with the brain's blood vessels being impermeable to most substrates [13, 14].

It is important to note that other experiments had already demonstrated that some structure or process interrupts the movement of dyes to the CNS, however, they are rarely named in the context of the BBB. As early as 1898, A. Biedl and R. Kraus had discovered that bile acids caused convulsions and coma when injected directly into the brain, although they were not neurotoxic when administered intravenously. M. Lewandowsky, in 1900, reported similar results with the administration of sodium ferrocyanide. The interpretation of the three researchers was based on the consideration of special permeability properties in cerebral blood capillaries. [15]. Still according to Saunders and collaborators [7], the absence of staining of the brain and spinal cord had already been previously described in adult animals injected with trypan red in 1905 by Franke [16] and with methylene blue in 1906 by Bouffard [17].

LINA STERN, 1921: "BARRIÈRE HÉMATO ENCÉPHALIQUE"

The idea that cerebral capillaries hold the anatomical basis of the barrier proposed by Goldmann had not yet been confirmed, as at the beginning of the 20th century there was no method capable of examining the fine ultrastructure of blood vessels. Hence the hypothesis continues to be deduced indirectly through lateral research. In the 1920s, Lina Stern (1878-1968) and her collaborators carried out experiments that consisted of injecting various substances into specific anatomical compartments related to the CNS and the circulatory system – in the subarachnoid space, in the cerebral ventricles and in the blood. The substances were injected after the guinea pigs' nephrectomy to minimize urinary loss of the substance and ensure the reliability of measurements. Subsequently, the CSF was extracted to analyze the presence, or not, of the substance. Substances such as bromide, thiocyanate, strychnine, morphine, atropine and bile salts were capable of penetrating the CNS, but iodine, ferrocyanide, salicylate, curare, adrenaline, bile pigments, eosin and fluorescein maintained are systematically absent from the CSF.

The results obtained led Stern and Gautier, in 1921,[18], thinking about an obstacle that prevents the passage of substances from blood plasma to the brain as a protective barrier for the nervous system, following what was proposed in 1920 by von Monakow [14]. On April 21, 1921, during a communication at the Geneva Medical Society, Lina Stern clearly applied the term "barrière hématoencéphalique" (blood-brain barrier). Later that year, Stern published his conceptual article on the topic in the ``Schweizer Archiv für Neurologie und Psychiatrie`` (Swiss Archive of Neurology and Psychiatry)[19]. However, the concept they developed envisaged a practically absolute barrier between blood and nervous tissue, without considering the nutritional role of the highly efficient vascular circulation of the brain parenchyma, leaving this function limited to the small vascular regions of the choroid plexuses, whose blood flow represents just a small fraction of the total.

The most relevant contribution of this research was the better definition of the selective permeability of this interface, which concluded that it was little or none for the entry of many substances into the brain space and more permissive for the exit of compounds administered in the brain into the bloodstream.[18, 20]. This research, however, was practically ignored.

THE LIPID SOLUBILITY OF THE SUBSTANCE: A DETERMINING FACTOR

The progress that was being recorded in analytical techniques, which began to dispense with the use of basic dyes (such as those used by Ehrlich, Goldmann and Lewandowsky, etc.), as well as the increasing number of compounds available for experiments, such as radioisotopes that made it possible the quantification of markers made it possible to clarify many aspects of the barrier's permeability. However, until the 1950s, there was still a lot of controversy surrounding the true existence of brain barriers. Among others, works such as those by Tschirgi21 and Davson and his collaborators14 were limited: the first of these, to demonstrating that the reason why certain dyes did not cross the brain was due to their binding to plasma proteins, predominantly to albumin which, thus, it made it impossible for them to cross the barrier; the second, starting from the analysis of the behavior of small molecules, electrolytes and non-electrolytes, confirming that the lipid solubility of the substance was one of the main determinants for its penetration into the brain.

THE BLOOD BRAIN BARRIER CONTROL EVIDENCE

Research on the BBB was decisively marked by the introduction of electron microscopy in the 1930s. The technique, with an unprecedented resolution capacity, made it possible to identify the structural basis of the barrier and made it possible to distinguish between the BBB (brain-blood tissue) and the blood-cerebrospinal fluid (CSF) barrier. In 1955, two teams of researchers, Dempsey and Wislocki22 and Breemen and Clemente23, identified the brain capillaries as the locus of the BBB.

Since it was observed that access to the perivascular space was interrupted by the cytoplasmic projections of astrocytes, these neuroglial cells have become a central point in the study of the BBB. What was visible indicated that the feet of astrocytes interrupted access to the perivascular space, leading to the assumption that this was the barrier or that astrocytes played a crucial role in its formation 24,25,26.

The most concrete empirical understanding of the BHE ended up emerging from the works of Reese and Karnovsky27, Brightman and Reese28 and Joó29. Through them, scientists were able to identify an electron-dense zone in the cerebral endothelial cells (CECs) of the brain capillaries of mice, the presence of specialized tight junctions (TJs) in the apical region of these cells and the polar nature of cerebral capillary CECs with a reduced number of pinocytic vesicles in their apical and basolateral poles. The interpretation of these facts led them to argue, with evidence, that the structural basis of the BBB does not reside in the feet of astrocytes, but rather in the endothelial cells of the brain capillaries themselves.

THE BLOOD BRAIN BARRIER

The study of cerebral vascular networks and the highly specialized phenotype of the BBB continued, as a result of technological advances, in particular through the development of in vitro models of the BBB that mimic the microcirculation of the BBB and allow the study of the mechanisms of specialized transport of molecules in cerebral capillaries. isolated from several mammal species. In this field, the work of the Cancilla and DeBault30,31,32 group stands out, which, in the early 1980s, isolated CECs from mouse brain capillaries and demonstrated the essential function of cell-cell interactions, in particular CECs - cells surrounding glial cells, in maintaining the properties of the BBB, the

expression of the enzyme (γ-GT) (gamma-glutamyltranspeptidase), responsible for the proteolytic cleavage of peptides into amino acids, is significant in cerebral CECs.

Knowledge about the BBB, at the end of the 1980s, included the physical properties observable by TEM (transmission electron microscopy), its transport and polarity selectivity and the action of the γ-GT enzyme in restricting/distributing the diffusion of compounds circulating in the blood or of brain origin, but the main molecules responsible for these properties, such as the proteins that form tight junctions or efflux pumps, had not yet been identified, just as the central role of cerebral pericytes in the construction of the BBB was unknown.

In the following decades, the profusion of new analytical tools in molecular biology and biochemistry allowed the separation, identification and study of the key proteins of the junctional complexes in CECs responsible for the drastic restriction of the paracellular passage of circulating compounds, microvesicles and cells such as lymphocytes or macrophages (which only cross the BBB in response to brain inflammation) present in the blood. It has been defined that the selectivity of the BBB for brain compounds or metabolites is associated with the polarized expression of specific transporters and efflux pumps that act on the surfaces of endothelial cells.

Knowledge about the BBB continues to progress, but it is known that it is a specific structure of the neurovascular unit with the function of maintaining CNS homeostasis, strictly regulating the transfer of ions, molecules and cells between blood and neural tissue, protecting the CNS of harmful molecules, including neurotoxic elements, blood cells and pathogens present in the blood. The blood-brain barrier is composed of sealed cerebral endothelial cells (CECs), tight junctions (TJs), astrocytes, pericytes, neurons and microglia. Neighboring astrocytes, pericytes and basement membranes surround CECs, providing structural support and membrane stability, together forming the exceptionally low permeability of the BBB, where only certain fat-soluble molecules, essential nutrients and specific ions pass through specialized transport mechanisms. The basement membrane of CECs contains enzymes capable of destroying certain substances, such as epinephrine and norepinephrine, preventing neuronal overstimulation. Astrocytes and pericytes also signal CECs to form tight junctions. In certain regions of the brain, the BBB is more permeable (newborns and premature infants) or completely absent (e.g., vomiting center in the brainstem and hypothalamus), allowing specialized functions such as monitoring blood composition.

The neurovascular unit is a concept that describes all the cells and molecules that interact at the blood-CNS interface, induce and regulate the series of different structural, transport, metabolic and adhesion properties that make CECs different from endothelial cells in non-neural tissues, that is, the expression of the BBB phenotype in CECs. In this scenario, the spatio-temporal organization of the BBB results from a sophisticated intercellular dialogue between endothelial cells and nearby cells, such as astrocytes and neurons, being an essential phenotypic induction process for the BBB during brain development and to maintain brain homeostasis throughout of life.

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