Peptides 72 (2015) 16–19

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Peptides and the blood–brain barrier

William A. Banks*

Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System and Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA, United States

ARTICLE INFO

Article history:
Received 9 March 2015
Received in revised form 12 March 2015
Accepted 12 March 2015
Available online 21 March 2015

Keywords:
Peptides
Blood–brain barrier
AJ Kastin
Delta sleep-inducing peptide
Regulatory proteins
Cytokines

ABSTRACT

The demonstration that peptides and regulatory proteins can cross the blood–brain barrier (BBB) is one of the major contributions of Dr. Abba J. Kastin. He was the first to propose that peptides could cross the BBB, the first to show that an endogenous peptide did so, and the first to describe a saturable transport system at the BBB for peptides. His work shows that in crossing the BBB, peptides and regulatory proteins act as informational molecules, informing the brain of peripheral events. Brain-to-blood passage helps to control levels of peptides with the brain and can deliver information in the brain-to-blood direction. He showed that the transporters for peptides and proteins are not static, but respond to developmental and physiological changes and are affected by disease states. As such, the BBB is adaptive to the needs of the CNS, but when that adaption goes awry, the BBB can be a cause of disease. The mechanisms by which peptides and proteins cross the BBB offer opportunities for drug delivery of these substances or their analogs to the brain in the treatment of diseases of the central nervous system.

Published by Elsevier Inc.

How do peripherally administered peptides affect behavior? Can peptides circulating in the blood stream impact brain function independently of the vagus and other components of the afferent nervous system? Can peptides cross the blood–brain barrier (BBB)?

These and related questions were being asked by Abba Kastin when I joined his lab 35 years ago. They were tough questions without obvious approaches to answer them. And they were controversial, especially the idea that peptides could cross the BBB.

Peptides and the blood–brain barrier: the first ten years

That the newly discovered class of substances termed peptides could have profound effects on behavior was beyond question. Abba's mentor, Andrew Schally, had won the Nobel Prize just the year before for showing that the hypothalamic factors that controlled pituitary functions were peptides [48]. Abba had been instrumental in showing that the release of thyroid stimulating hormone (TSH) from the pituitary was controlled by the hypothalamic secretion of thyrotropin releasing hormone [1]. TSH, in turn, controlled the thyroid's release of thyroxine, a hormone whose excess or absence had powerful effects on behavior. But Abba's work was showing that hypothalamic and pituitary hormones had effects on behavior not likely mediated through the hypothalamic-pituitary-end organ axes. This was shown elegantly in experiments conducted in hypophysectomized animals and also with hypothalamic peptide fragments that did not induce pituitary secretions, but were nonetheless behaviorally active [32].

But if peptides had extra-pituitary effects [37], how could they mediate those effects? Abba was the first to suggest that peptides could cross the BBB [35,36,47] and the first to attempt experiments to determine whether they could or could not cross the BBB.

Why it was assumed so widely among both BBB experts and non-experts that peptides could not cross the BBB and why the suggestion that they might cross was met with severe skepticism is still puzzling to me, even after all these years. Maybe it was because it was clearly established that large proteins as typified by albumin did not cross the BBB. It may have been reasoned that since proteins are composed of amino acids and do not cross the BBB, then peptides must not be able to cross the BBB either, since they are also composed of amino acids. That such reasoning was flawed should have been evident in that it was already proven false in another case: proteins played largely structural roles (note: this era predated the discovery of "regulatory proteins"), whereas peptides had regulatory effects. It may also have been that the term "barrier" was taken too literally for a tissue that is more properly viewed as

Abbreviations: BBB, blood–brain barrier; BUI, brain uptake index; CNS, central nervous system; CSF, cerebrospinal fluid; DSIP, delta sleep-inducing peptide; IL, interleukin; MIF-1, prolyl-leucyl-glycinamide; PTS, peptide transport system; TSH, thyrotropin stimulating hormone.

* Correspondence to: rm810A/Bldg 1, 1660 S Columbian Way, Seattle, WA 98108, United States.
E-mail address: wabanks1@uw.edu

http://dx.doi.org/10.1016/j.peptides.2015.03.010
0196-9781/Published by Elsevier Inc.

a regulatory interface between the blood stream and CNS; Abba was to later highlight the dangers of reification in the discovery of the functions of peptides [33,38]. To this day, newly discovered classes of substances (e.g., cytokines and other regulatory proteins, antisense molecules) are assumed to not be able to cross "the barrier". Whatever the reasoning, for at least two decades after Abba first proposed that peptides could cross the BBB, it remained an untested assumption by many that they could not cross, with many discussion sections in papers on the behavioral effects of peptides concluding that while the mechanism by which the peptide was affecting the CNS was unknown, at least it could be assumed that they could not cross the BBB to exert the effects.

The question of peptide penetration: major technical challenges

Another factor that slowed the progress of the BBB-peptide field was a lack of established methods for examining this question. Oldendorf had recently introduced his brain uptake index (BUI) method that revolutionized the study of the BBB [40]. It allowed the brain's uptake of amino acids, glucose, and many other substances to be quantified, compared, and categorized [40–42]. With this method, Oldendorf and colleagues demonstrated that amino acids were transported across the BBB but by transporters that were specific for categories (e.g., the large neutral amino acids) and that glucose was rapidly transported by a system that also carried other hexoses. Oldendorf and colleagues also showed that morphine, methodone, codeine, and heroine all crossed the BBB in proportion to their lipid solubilities [43]. However, the BUI was not very sensitive; it was only useful for substances that had very large rates of uptake across the BBB. It could, for example, easily detect the uptake of methadone, codeine, and heroine, but not of morphine. When applied to peptides, the BUI could not reliably detect their uptake by brain [43]. Therefore, if peptides did cross the BBB, they did not do so in large amounts.

Another technical problem was that peptides have very short half-lives in the blood stream. This meant that if they entered the brain, they must do so rapidly. This presented both a conceptual dilemma (how could they affect behavior for hours if they were cleared from the blood after a few minutes?) and a technical one: any method for detection needed to be done over a short time course and to be able to distinguish intact peptide from degradation products.

A low entry rate (at best) and a short time available to enter greatly favored the idea that peptides did not cross the BBB, at least not in amounts sufficient to affect brain function. But other work countered the assumptions that these findings depended on [38]. First, it was clear that peptides were very potent and that not much peptide would need to cross the BBB to exert effects on brain. In this sense, peptides were similar to morphine in that morphine had very profound effects on the CNS, yet so little crossed that it could not be detected by the BUI method. Second, a peptide's effects could last for hours or even days after its administration, long after it was cleared from the blood stream. Peptides then challenged long-held assumptions about how substances injected into the blood stream could affect the brain [38].

The first challenge in determining whether peptides could cross the BBB was to find methods that could address the technical difficulties in studying peptides. There were only about a dozen studies on peptides and the BBB with about half of those concluding that they could cross and about half that they could not. However, all these studies were flawed in the sense that they had alternative explanations from their conclusions. For example, those studies that concluded that peptides did not cross did not use very sensitive methods. Those studies that did conclude that peptides could cross often used a sensitive approach, such as radioactively labeled peptides, but did not show that the radioactivity in the brain exceeded levels explicable by the vascular space of the brain or that the radioactive label was still attached to the peptide.

Technical challenges and delta sleep-inducing peptide

At this point in the evolution of the field, there were basically two dichotomous choices: the first, to choose to study uptake into brain tissue or uptake into cerebrospinal fluid (CSF); the second, to use radioactively labeled peptides or to follow immunoactivity with radioimmunoassays. Each of these choices had advantages and disadvantages. The main advantage of using radioactivity was its great sensitivity, but its main disadvantage was that the radioactive label could become detached from the peptide so that one was no longer assessing peptide penetration. We overcame this difficulty by using column chromatography to confirm that the radioactivity taken up into brain tissue or CSF was still attached to the peptide. The disadvantage of radioimmunoassay was that an immunoactive fragment might be crossing the BBB rather than the intact peptide. However, the advantage of our radioimmunoassay for delta sleep-inducing peptide (DSIP) was that it required eight of the nine amino acids for cross reactivity and we could easily determine with column chromatography whether it was the nonapeptide or the octapeptide that was crossing the BBB. A major advantage of CSF was that any material recovered from the CSF had clearly crossed the BBB (vascular contamination from "bloody" taps is readily assessed by a variety of methods), but the main disadvantage at that time was that some questioned how relevant CSF levels were to levels at the brain receptor. The main advantage of using brain tissue was that it was not subjected to the "relevancy" question as was CSF. The main disadvantage of using brain tissue is that its vascular space will contain peptide that has not crossed the BBB but will contaminate the tissue upon homogenization. We accounted for the vascular contribution in two ways, either by injecting a vascular marker (e.g., radioactive albumin or inulin) that allowed us to compute the contribution of vascular contamination in the brain tissue or by washing out the vascular space of the brain prior to assessment.

With these options and incorporating these solutions, we performed all possible combinations of studies: radioimmunoassay using brain tissue, radioactivity using brain tissue, radioimmunoassay using CSF, radioactivity using CSF [4,12,31,34]. In all cases, we found intact peptide entering the CNS in excess of vascular markers. This clearly demonstrated that the small peptide DSIP could cross the BBB.

We next asked the question of how DSIP could cross the BBB. We were fortunate in that we had several analogs of DSIP whose immunoactive or radioactive forms could be readily distinguished with the aid of column chromatography. We found that whatever peptide was injected into the blood was the peptide that was recovered from the CNS [12,31,34]. This, along with the results using radioactivity, ruled out the possibility that peptide appearing in the CNS originated there, having been stimulated by the increased levels in the blood. As neither the analogs nor the radioactive peptides were produced endogenously, their detection in the CSF after intravenous administration could have only occurred by passage across the BBB.

The work with DSIP and its analogs showed the surprising finding that not all the peptides crossed to the same degree. This meant that their entry could not be easily explained by residual leakiness of the BBB. We were able to show that protein binding affected the ability of the various DSIP-related peptides to enter the CSF with mostly the unbound fraction available for passage [12]. In later studies, we showed that uptake of DSIP peptides into the CSF correlated with their lipid solubility [14] and that uptake into brain was by a non-saturable mechanism [13]. We concluded that the DSIP peptides crossed the BBB by the non-saturable mechanism of transcellular diffusion.