Peptides 72 (2015) 50–56

Contents lists available at ScienceDirect

Peptides

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

Peptides at the blood brain barrier: Knowing me knowing you

Thomas P. Davis[a,*], Thomas J. Abbruscato[b], Richard D. Egleton[c]

[a] The Davis Lab, Department of Medical Pharmacology, University of Arizona, Tucson, AZ 85724-5050
[b] Texas Tech University Health Sciences Center, School of Pharmacy, Amarillo, TX 79106
[c] Joan C. Edwards School of Medicine at Marshall University, Huntington, WV 25755

ARTICLE INFO

Article history:
Received 10 April 2015
Received in revised form 20 April 2015
Accepted 21 April 2015
Available online 30 April 2015

Keywords:
Blood brain barrier
Neuropeptides
Drug delivery
Tight junction proteins
Transporter proteins
P-glycoprotein

ABSTRACT

When the Davis Lab was first asked to contribute to this special edition of Peptides to celebrate the career and influence of Abba Kastin on peptide research, it felt like a daunting task. It is difficult to really understand and appreciate the influence that Abba has had, not only on a generation of peptide researchers, but also on the field of blood brain barrier (BBB) research, unless you lived it as we did. When we look back at our careers and those of our former students, one can truly see that several of Abba's papers played an influential role in the development of our personal research programs.

During the mid to late-eighties the Davis Lab became aware of the robust discussions regarding the ability of peptides to cross the BBB [1]. As someone who researched central and peripheral neuropeptide processing and degradation throughout the 1980s [2–6], and had previously investigated a different barrier in the skin [7], the debate regarding the delivery of peptides to the brain was interesting, but we did not feel that it was applicable to the Davis Lab of the early to mid 1980s. However, the interest in this area was sparked when we observed, in collaboration with Dr. Terry Moody of the NIH, that some neuropeptides we were studying could be produced by and also regulate the function of cells associated with small cell lung cancer (autocrine growth factors) [8]. At this same time period, various groups in our medical pharmacology department at The University of Arizona were interested in the ability of synthetic opioid peptides to act as analgesics [9] with the ultimate goal to offset the negative side effects of morphine (dependence, tolerance, and constipation). This is when our interest in Abba's studies, coupled to our ongoing research program in neuropeptides, led to a change in the direction of our research focus.

In this manuscript, we will discuss some of the pivotal research and publications that contributed to this challenging and successful direction for our research program and also formed the bedrock of our early studies on the BBB that actively continues today [10–13].

"OUCH there goes MY blood brain barrier"

Perhaps the early studies for which our research group may be best known are the novel and paradigm shifting studies spearheaded by Dr.s Jason Huber, Tracy Brooks, Chris Campos and Melissa Seelbach [14–17]. Over a 10 year period these excellent young researchers produced a series of well-designed and carefully executed studies demonstrating that peripheral pain could have a profound impact on the molecular, structural and functional properties of the BBB, and that these alterations can directly affect drug delivery to the brain. However, what has been under appreciated is the role that Abba's early studies on peptide delivery across the BBB played and the influence these foundational studies had on our lab at this time.

There had been considerable interest in the development of peptide analogs of the endogenous opioids to replace morphine, as analgesics, and much of this initial work was carried out by Abba Kastin's group [18–21], in collaboration with brilliant synthetic chemistry by Dr. David Coy. However, a major stumbling block exists towards development of any analgesic peptide analog, and that is the ability of the BBB to prevent or limit the entry of peptides and their analogs into the brain. This is not however the only

* Corresponding author. Tel.: +1 502 6267643; fax: +1 502 6264053.
E-mail addresses: davistp@email.arizona.edu, davistp@u.arizona.edu (T.P. Davis).

http://dx.doi.org/10.1016/j.peptides.2015.04.020
0196-9781/

issue with peptide drug delivery. Perhaps a more significant problem is the lack of robust metabolic stability in blood, tissue, and at the BBB, due to the presence of numerous, soluble and membrane associated, peptidases [22]. This is particularly true for the endogenous, opioid peptide Met-enkephalin, which has a plasma half-life of around five seconds [23]. However, this did not stop Abba and his team who carried out several studies on Met-enkephalin analogs showing brain entry via what was termed a Brain Uptake Index (BUI) [21]. They also demonstrated both analgesia and behavioral responses upon peripheral administration [24,25], providing evidence that synthetic peptides could be made metabolically stable, or could be given intra carotid, and could cross the BBB and elicit a biological effect. An obvious and initial challenge was to improve the enzymatic stability of peptide analogs using various chemical strategies to assure that if a peptide was shown to cross the BBB, it did so chemically intact. This point provided the initial entry of the Davis Lab into the area of peptide structure function research at the BBB, where they first demonstrated that several synthetic peptide opioid analogs could be developed that showed enhanced metabolic stability [26–28]. Many of these analogs also showed good pain relieving analgesia when given via central (icv) administration, and a few via peripheral administration in pre-clinical pain models. Biodistribution (ADME) studies also showed measurable, intact entry into the brain for several of these peptide analogs [29,30]. Though these early distribution and analgesia studies were interesting and told us that these peptides could enter the brain, the mechanism of transport through the BBB remained elusive. After re-reading several studies by Abba and Bill Banks the issue of intact peptide brain delivery soon became a focus of the Davis Lab, and this challenge was answered with very careful and detailed analytical method development and further development of BBB models. Two approaches were taken. The initial approach was to investigate peptide delivery via a modification of the classical in vitro BBB model [31]. This model was very effective at investigating chemical/biological rank order of peptide uptake as effected by the endothelium and other BBB cell types such as glia [26,28,32,33]. It also demonstrated that if BBB soluble and membrane bound peptidases were inhibited by specific protease inhibitors, then endogenous Met-enkephalin could actually have a robust brain uptake [22]. But this was not necessarily the best method for looking at endogenous, in vivo peptide transport kinetics and mechanisms, though the use of isolated cultures of brain endothelial cells did help cement the concept of an “enzymatic blood–brain barrier” [22]. This enzymatic model described a barrier that most believed was present but difficult to describe and study. The answer for us was supported in some of Abba’s earlier work in which he carried out intra carotid injections of radiolabeled peptides and measured brain uptake [21,34–36]. Further investigation by the Davis Lab revealed a number of research groups used variations of this technique to study a range of radiolabeled substance entry into the brain [37–45]. These observations of radiolabeled analogs and drug delivery resulted in the recruitment of Dr. Sarah Williams in 1994 from Dr. Malcolm Segal’s lab in St. Thomas’ Hospital, Kings College, and London. Sarah was familiar with Dr. Betza Zlokovic’s dual carotid perfusion technique in guinea-pigs [43] and also the Dr. Jane Preston adaption for rats [46]. This technique was shown to be ideal for investigating the uptake kinetics of intact radiolabeled substances that have a slow brain entry and may be enzymatically unstable to cross the BBB intact. Further the technique allowed a rigorous analysis of the tracers when coupled with state of the art HPLC procedures developed within the Davis Lab [26,47], to ensure that the brain uptake was indeed intact peptide and not enzymatically degraded products. A number of studies followed in the Davis Lab with several families of opioid peptides that demonstrated many different mechanisms, both saturable and diffusive, that could be targeted to cross the BBB [26,27,47–54]. Coupled with each of these published transport studies were various analgesia assays, which showed that not only could the peptides cross the BBB, but they were CNS and biologically active when they finally did gain entry into the brain and it was not only radiolabel that was quantified, but intact peptide. Regional CNS distribution was even analyzed in some of our early investigations and linked to brain and spinal cord sites that were known to be rich in delta- and mu-opioid receptors [49].

Hypoxia/aglycemia/stroke/neuroprotection at the BBB

Though the peptide transport studies proved to be quite fruitful in control, healthy, preclinical models, they were not fully instructive as to the mechanism of peptide drug delivery that is involved in models of disease states where drug delivery is critical. During this same time period in the Davis Lab, Abba Kastin and Bill Banks were publishing important and seminal papers indicating that BBB transport of various substances to the brain could be altered via specific disease processes [55–60]. At this same time, Dr. Tom Abbruscato had started working on a NIH fellowship in the Davis Lab studying the cerebrovascular effects of stroke on the in vitro BBB phenotype and transport characteristics. Increased cerebrovascular permeability is a critical factor in the development of vasogenic brain edema, a leading cause of death in ischemic stroke. Once again, there was a need for a disease based approach to characterize and understand ischemic brain drug delivery, especially for novel, peptide based neurotherapeutics. Early in vitro experiments suggested that astrocytes provide a protective role to the BBB endothelium during hypoxia/aglycemic conditions through the association with E-cadherin (a calcium dependent adherence protein) [61]. Additional foundational experiments deciphered the cerebrovascular effects of hypoxia and/or aglycemia. Using the membrane-impermeant marker, [^{14}C] sucrose, we found that with hypoxia alone, long exposures (48 h) were needed to result in measurable increases in BBB permeability. Hypoxia/aglycemia exposure resulted in a much shorter time (1–3 h) required for changes in paracellular permeability [62]. These in vitro, pathophysiologic experiments helped to refine in vitro conditions that provide the basis for future mechanistic, neurovascular stroke studies. Additional pharmacologic experiments verified that altered endothelial cell calcium flux was responsible for the permeability change observed after both hypoxic and hypoxic/aglycemic exposures. These pathophysiologic experiments sparked interest in understanding the contributions of the NVU to cellular and vasogenic brain edema associated with stroke, but also generated interest into the delivery of stable, peptide based, opioid receptor agonists to the ischemic brain for neuroprotection. This work continues to be developed in the Abbruscato Lab at Texas Tech University Health Sciences Center where further experiments have tested the delivery of both selective and non-selective peptide based analgesics to the ischemic brain. Biphalin, a well characterized, dimeric enkephalin based analgesic that was originally synthesized by Lipkowski et al. [63] at Arizona. Biphalin has a unique pharmacologic profile; it has high affinity to MOR and DOR and low affinity to KOR, it crosses the BBB well for a peptide, and has a good serum and brain half-life of 87 and 193 min, respectively [64]. Still to this date, biphalin has been shown to be one of the most potent, peptide based analgesics synthesized [65]. Recent experiments in Dr. Tom Abbruscato’s Lab have shown that biphalin plays a significant role in reducing cellular edema in neurons subjected to oxygen glucose deprivation by modulating the expression and function of the ion transporter Na,K,2Cl-cotransporter [66]. Further experiments also validated that these neuroprotective effects of biphalin are also seen in hippocampal slices subjected to oxygen glucose deprivation conditions [67]. Biphalin was also shown