

Interactions of Growth Hormone, Insulin-Like Growth Factor-1, and Ghrelin with the Blood-Brain Barrier

Weihong Pan^{*,1}, Fred Nyberg² and Abba J. Kastin¹

¹Blood-Brain Barrier Group, Pennington Biomedical Research Center, Baton Rouge, LA, USA

²Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

Abstract: Growth hormone (GH) therapy is associated with improved neurobehavior, leading to the question whether GH crosses the blood-brain barrier (BBB) from blood after its release there from the pituitary, or whether GH exerts CNS effects by secondary mediators, particularly insulin-like growth factor (IGF)-1. GH release from the pituitary is controlled not only by hypothalamic factors, but also by ghrelin that is mainly produced in the stomach and uses its unique structure to interact with the BBB. This review summarizes studies of the permeation of GH, IGF-1, and ghrelin across the BBB, and discusses them in the context of neuroendocrine regulation. Exogenous GH has a half-life of approximately 3.8-7.6 min in mouse blood and shows a slow non-saturable permeation across the BBB, with 26.8 % remaining intact in the brain 20 min after intravenous delivery. IGF-1 crosses the BBB by saturable transport mediated by its receptors, and its interactions with the BBB are greatly affected by serum binding proteins. The interactions of ghrelin with the BBB appear to be dependent on species and show directionality. The BBB provides regulatory compartmentalization to fine-tune the CNS actions of GH and its related hormones.

Keywords: Growth hormone, IGF-1, ghrelin, blood-brain barrier, simple diffusion, receptor-mediated transport, stability, binding proteins.

1. WHY STUDY BBB PERMEATION OF GH AND RELATED HORMONES

As the use and abuse of both GH and IGF-1 are increasing, and concern about the potential misuse of ghrelin is rising [1-3], it is important to revisit how these hormones enter the brain. It is clear that they exert robust CNS effects. For example, GH supplementation can increase concentration and memory [4-6], and in children with Prader-Willi syndrome it improves sleep [7]. The blood-brain barrier (BBB) provides a regulatory interface to modulate the permeation of these small protein hormones, and mechanisms of their interactions greatly influence their dose-dependent effects. The need for replacement of GH in GH deficient children is established, but could overdosing result in excessive GH entering the CNS? In adults with declining GH concentrations in their blood, the question becomes more complicated since it is not clear how much, if any, of the physiological decrease with age requires treatment. Conversely, individuals with GH producing tumors, such as acromegaly, may have psychological problems such as mood lability, anxiety, and depression that may result from increased amounts of GH entering the brain.

Though related in their functions, GH, IGF-1, and ghrelin represent three different types of mechanisms of BBB

interactions. GH crosses the BBB by passive diffusion; this means that there would be a direct correlation between the amount of GH administered with the amount entering the CNS. IGF-1 crosses the BBB by a saturable transport system; this indicates that increasing amounts would be rate limiting for IGF-1 entry from blood to the CNS. For ghrelin, species and structural variation occurs.

2. PHARMACOKINETICS OF THE SIMPLE DIFFUSION OF GH ACROSS THE BBB

In regard to the stability of ¹²⁵I-GH (recombinant human or rat GH) in mice, high performance liquid chromatography (HPLC) showed that approximately 91% of the total radioactivity in serum in mice 20 min after intravenous (iv) injection remains intact. Acid precipitation showed similar results. In the homogenized brain from a mouse 20 min after iv injection of ¹²⁵I-GH, 86% of radioactivity (corrected for *ex vivo* degradation seen in the processing control) was acid precipitable. Gel autoradiography confirmed the presence of 22 kDa GH in all samples [8]. The serum half-life of ¹²⁵I-GH ranged from 3.8-7.6 min in different experiments in this study. The results indicate that after iv delivery, GH remains intact for a sufficiently long time to exert its biological actions and permeate the BBB. It has been established that a small protein's effect can last considerably longer than its half-life [9, 10].

Linear uptake of ¹²⁵I-GH from blood to brain in mice is seen 1-30 min after iv injection. The influx transfer constant of $0.23 \pm 0.07 \mu\text{l/g-min}$ and the initial volume of distribution of $15.5 \mu\text{l/g}$ in the initial study [8] are slightly higher than

*Address correspondence to this author at the Blood-Brain Barrier Group, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808, USA; Tel: (225) 763-2707; Fax: (225) 763-0261; E-mail: weihong.pan@pbrc.edu

expected for a protein of this size without a saturable transport system [11, 12]. Capillary depletion study showed that approximately 26.8% of the injected ^{125}I -GH reaching the whole brain entered brain parenchyma 10 min after iv delivery, whereas 10% remained in the cerebral vasculature. However, neither in-situ brain perfusion nor multiple-time regression analysis [13] showed the presence of a saturable transport system for GH crossing the BBB. Nonetheless, brain uptake was approximately 0.1%/gram of brain at 20 min after iv injection of ^{125}I -GH, indicating that 6.8 pg of GH was present in a gram of brain tissue. It is possible that the 150-fold excess of unlabeled GH co-administered was insufficient to inhibit the permeation of ^{125}I -GH across the BBB, and there might also be species differences (rat and human GH studied in mice since murine GH was not available at the time).

However, in-situ brain perfusion and cellular uptake assays, which should provide higher sensitivity for detection of a specific saturable transport system, also failed to identify the characteristic feature of self-inhibition by excess unlabeled GH [8]. The concentration of GH in the perfusate for in-situ brain perfusion was 5 $\mu\text{g}/\mu\text{l}$. The influx transfer constant was approximately 0.8 $\mu\text{l}/\text{g}\cdot\text{min}$ and the brain parenchyma/perfusate ratio was approximately 12.6 $\mu\text{l}/\text{g}$ at 10 min (vs a capillary update of 3.6 $\mu\text{l}/\text{g}$) [13]. Estimating that 0.03% of GH reaches the brain compartment by the infusion method (higher than that after intravenous bolus delivery), the concentration in brain capillaries at 10 min would be approximately 1.1 $\mu\text{g}/\mu\text{l}$.

Analyses of individual regions (frontal, parietal, and occipital cortices, striatum, thalamus, hypothalamus, brainstem, and cerebellum) also failed to show region-dependent transport, although transport in specific regions may exist in special cases [14, 15]. It should also be noted that there are circulating GH binding proteins [16], but their role in GH permeation across the BBB is not clear.

Although a saturable transport system for GH across the BBB has been sought but not identified, it is clear that GH receptors are present in the choroid plexus [6, 17] and in an ovine choroid plexus epithelial cell line [18]. The blood-cerebrospinal fluid (CSF) barrier may also play a role in GH transport; the high affinity GH receptor in human brain choroid plexus has a K_a of 0.63 nM^{-1} [17]. In the CSF, high GH levels were reported in acromegaly and a small increase was also found after chronic administration of hGH in subjects with GH-deficiency syndromes [19]. This suggests the presence of a transport system for GH at the blood-CSF barrier.

The highest binding of GH in the brain is seen in the choroid plexus, hypothalamus, hippocampus, pituitary, and spinal cord, whereas a lower binding density is present in the cortex [20]. The GH receptor is a single transmembrane protein belonging to the cytokine receptor family. Binding of GH leads to receptor dimerization, and phosphorylation of the receptor itself and Janus kinase (JAK)-2. The downstream signaling elements include signal transducers and activators of transcription (STAT), mitogen-activated protein kinases (MAPK), and others [21]. Since there is a

lack of sequence and domain homology of the GH receptor with that of the IGF-1 and insulin receptors, it is possible that there is no cross-inhibition of GH permeation by insulin. In the same study, the influx transfer constant of ^{125}I -insulin was $0.97 \pm 0.14 \mu\text{l}/\text{g}\cdot\text{min}$, and it was not affected by the presence of excess GH [8].

3. THE SATURABLE TRANSPORT SYSTEM AT THE BBB FOR IGF-1

GH stimulates the production of IGF-1 in mammals. IGF-1 crosses the BBB by a transport system, and this may be complementary to the direct permeation of GH to enhance the CNS effects of GH [11]. A unique feature of IGF-1 permeation across the BBB is its modulation by serum binding proteins (IGFBP and others). As a result, the half-life of IGF-1 is prolonged in the circulation, indicating an increase of biostability. Excess unlabeled IGF-1 facilitates the influx of ^{125}I -IGF-1 from blood to the CNS, explained by displacement of the tracer from the binding proteins. In the setting of in-situ brain perfusion where IGFBP is absent, excess unlabeled IGF-1 significantly inhibits the influx of ^{125}I -IGF-1. Therefore, the transport system is saturable. There is a progressive increase of IGF-1 over time in brain parenchyma rather than accumulation just in the vasculature, and intact IGF-1 is detectable in brain by HPLC after iv delivery [22]. Receptors for IGF-1 and IGF-2 are present in BBB microvessels [23, 24], and they mediate IGF-1 endocytosis *ex vivo* [24]. It is most probable that IGF-1 is transcytosed across cerebral endothelial cells through a receptor-mediated transport system.

The BBB of endothelial IGF-1 receptor knockout mice functions normally in response to paracellular tracers [25]. However, it has not been determined whether these mice show a reduction of IGF-1 transport from blood to the CNS. Since the system is redundant, it is possible that IGF-1 can still reach the CNS by insulin receptor mediated transport, and that insulin and other related proteins can compensate for the somewhat reduced availability of IGF-1. As potential transporters, insulin receptors and IGF receptors might have overlapping functions dictated by their differential binding affinity to ligands, as well as by receptor density and transport kinetics. Neither endothelial overexpression of different types of receptors for side-by-side comparison of IGF-1 transport, nor the use of receptor-specific gene knockdown strategies to compare the relative contributions in BBB transport of IGF-1 and insulin have been reported. Nonetheless, results from studies with HUVEC cells suggest that IGF-1 receptors play a bigger role than insulin receptors based on their higher level of expression and greater binding of IGF-1 [26]. Although both IGF-1R and IGF-2R are present in BBB microvessels [23], IGF-1R appears to play a bigger role in IGF-1 transport.

IGF-1 is an important growth factor for the CNS, and can relieve signs of acute experimental autoimmune encephalomyelitis (EAE) [27]. It is also effective against chronic relapsing EAE after subcutaneous delivery, reduces BBB defects, and decreases the number and size of inflammatory, demyelinating, and demyelinated lesions in these SJL/J mice [28]. IGF-1 ameliorates neural damage

resulting from traumatic brain injury after intracerebroventricular (icv) injection [29], and various delivery strategies have been tested for treatment of amyotrophic lateral sclerosis (ALS), a devastating motor neuron degenerative disorder [30, 31]. This includes delivery of IGF-1 by the intrathecal route [32], stem cells [33], and viral vectors [34]. Although a clinical trial with a low-dose twice daily subcutaneous injection of IGF-1 failed to show a beneficial effect after two years [35], much remains to be determined about bioavailability based on the interactions of IGF-1 in the periphery and with the BBB [36].

4. STUDIES OF INTERACTIONS OF GHRELIN WITH THE BBB

Ghrelin is a peptide mainly produced by the gastrointestinal tract that binds to the GH-secretagogue receptor. It has a unique primary structure with an octanoyl side-chain at Ser³; this posttranslational modification appears to determine its specific bioactivity [37]. Ghrelin is a strong stimulator of GH secretion in man [38, 39]. In the mouse, ¹²⁵I-labeled human ghrelin shows bidirectional transport from either blood-to-brain or brain-to-blood. By contrast, mouse ghrelin has brain-to-blood efflux but no saturable influx from blood to brain, and its permeability coefficient is lower than that of human ghrelin. When the octanoyl side-chain is removed, des-octanoyl ghrelin crosses the BBB by nonsaturable diffusion. There is very limited transcytosis as most of the des-octanoyl ghrelin is trapped within the cerebral vasculature. After icv delivery, des-octanoyl ghrelin has a prolonged half-life in the CSF in comparison with native ghrelin of human or mouse origin [40]. The animal results are consistent with studies in RBE4 rat cerebral endothelial cells where ¹³¹I-labeled human ghrelin showed a high level of surface binding and saturable endocytosis, both at least 4-fold greater than those of obestatin, a peptide reported to be also produced by the stomach but with opposite effects from ghrelin, inhibiting food intake [41].

The influx transport of ghrelin across the BBB is modified by metabolic factors related to obesity and by starvation, and it differs with age. Transport is no longer present in aged mice maintained on a high-fat diet. In studies involving iv delivery and multiple-time regression analysis, there is an inverse correlation between body weight and ghrelin permeation. However, serum triglycerides promote the transport, and fasting also has a marginal effect of increasing the transport in studies by in-situ brain perfusion [42].

Desacyl (des-octanoyl) ghrelin can also be biologically active. It stimulates adipogenesis and inhibits glucose output in hepatocytes, and high concentrations are associated with decreased food intake. At least some of its effects may be related to its permeation from blood to brain and activation of neuronal pathways that regulate feeding [43].

5. THE CONTEXT OF BBB PERMEATION OF GH AND RELATED HORMONES ON CNS FUNCTIONS

With its peak secretion occurring during sleep, GH has long been considered the link between sleep and memory. A recent study elegantly showed the modulatory effect of GH

on hippocampal synaptic function. In rats after 3 days of sleep deprivation, NMDA-receptor mediated synaptic currents are reduced. Daily GH injection during the period of sleep deprivation prevents loss of synaptic currents and reduction of long-term potentiation and NMDA receptor 2B expression [44]. Similarly, ghrelin plays essential roles in CNS function. Ghrelin is involved in food reward and food motivation behavior by modulating the mesolimbic circuitry [45]. Ghrelin receptor (GHS-R1a) knockout mice show deficits in contextual fear conditioning, indicating impaired hippocampal emotional dependent memory, although they have normal balance, movement, coordination, and pain sensation and perform better than wildtype mice in the Morris water maze test [46]. Rat studies involving a ghrelin receptor antagonist or receptor knockout rats also confirmed a role of ghrelin signaling in the induction of locomotor sensitization to cocaine, consistent with the prominent role of ghrelin in the rewarding circuitry by crossing the BBB and acting on ventral tegmental dopamine neurons [47]. Ghrelin increases slow wave sleep, shown in elderly men though not elderly women [48], and differentially affects memory acquisition and retention [49]. During the past decade, the role of ghrelin in obstructive sleep apnea, circadian rhythm regulation, and wake promoting mechanisms has been widely investigated [50-53]. Ghrelin has positive effects on learning, memory, reward, motivation, anxiety, and depression, and shows neuroprotective and neuromodulatory effects [54]. This explains why it is implicated in cognitive function in aging [55] and Alzheimer's disease [56]. The effects of IGF-1 are also widespread and reviewed extensively elsewhere in this *Special Issue*.

Although we did not detect a saturable transport system for GH crossing the BBB, it remains possible that future studies may identify modulatory factors that influence GH permeation across the BBB. By analogy, urocortin does not show saturable transport in the basal state, but its transport system can be activated by leptin and TNF both in-vivo [57-60] and in cultured cells [61, 62]. Concentrations of the hormones as well as circadian, prandial, and nutritional influences [63] might all play a role in the physiology of BBB interactions of GH, IGF-1, and ghrelin.

6. PROSPECTIVES

The BBB is a tremendous information exchange interface, that allows peptides and proteins from the periphery to exert CNS effects. GH, IGF-1, and ghrelin have overlapping functions and they also have interactions at the BBB, not only during the course of their own transport but also in induction of BBB cellular signaling. The most pressing questions to be resolved are related to the mechanisms of their transport. For example, if there is receptor-mediated endocytosis for any of these neuroendocrine hormones, how do the transporting receptors interact at the luminal surface of the BBB? Does intracellular signaling initiated by these hormones affect their own or other transport? What are the exocytosis mechanisms? How do different cell types contributing to the BBB cooperate with each other in the course of delivering these hormones to

the CNS target cells in the parenchyma? Further, how does neuropathology (such as obesity, neurodegeneration, inflammation) regulate the transport systems?

7. SUMMARY

In this review we mainly discussed the interactions of interrelated GH, IGF-1, and ghrelin at the level of the BBB. GH does not exhibit a saturable transport across the BBB *per se*, but may stimulate the production of IGF-1 that enters the brain and spinal cord by a specific, receptor-mediated transport system. Ghrelin also shows regulated entry across the BBB, and there is a strong species difference and possible bidirectional transport. The lack of saturation of the low but meaningful permeation of GH indicates that higher concentrations of GH can be associated with greater CNS effects. However, its interaction with the blood-CSF barrier appears to have higher efficacy and is tightly regulated. The unique interactions of these three hormones with the BBB are part of their neuroendocrine regulation of memory, sleep, mood, and many other CNS functions.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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