How Hormones Influence Composition and Physiological Function of the Brain-Blood Barrier

R. HAMPL¹, M. BIČÍKOVÁ¹, L. SOSVOROVÁ¹

¹Institute of Endocrinology, Prague, Czech Republic

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Summary
Hormones exert many actions in the brain. Their access and effects in the brain are regulated by the blood-brain barrier (BBB). Hormones as other substances may enter the brain and vice versa either by paracellular way requiring breaching tight junctions stitching the endothelial cells composing the BBB, or by passage through the cells (transcellular way). Hormones influence both ways through their receptors, both membrane and intracellular, present on/in the BBB. In the review the main examples are outlined how hormones influence the expression and function of proteins forming the tight junctions, as well as how they regulate expression and function of major protein transporters mediating transport of various substances including hormone themselves.

Key words
Blood-brain barrier • Tight junction proteins • Paracellular way • Protein transporters • Hormones

Introduction
The brain is a privileged organ coordinating and controlling main physiological functions including central nervous activity. As such it must be supplied with a sufficient energy and protected from undesired endogenous or environmental noxious substances. Indeed, the brain, though amounting only about 2% of the total body weight, it consumes as much as 18% of the whole energy. One of the tools ensuring these functions is the blood-brain barrier (BBB). It is an interface on the outer site of brain vessels, separating the brain extracellular fluid and brain parenchymal cells from the blood stream. Anatomically it is formed by capillary endothelial cells, to which, from the brain side adhere glial cells – astrocytes and pericytes (Tajes et al. 2014).

Endothelial cells are rich of a plenty of transmembrane and other proteins – ion channels, receptors, transporters and enzymes, some of which are specific for the cells forming the BBB. We distinguish the luminal (blood-facing) and abluminal (brain-facing) membranes, the composition and function of which may differ. The endothelial cells forming the BBB differ from other endothelial cells throughout the body not only by presence of more or less specific transporters and receptors, but also by tight junctions (TJ) between the cells, not allowing passage of molecules across the membrane. These junctions thus effectively prevent diffusion through the intercellular pores. They are composed of proteins, usually dimers, anchored in endothelial cell membranes, often to other proteins. Examples of such proteins are occludin, claudins and many other proteins, commonly termed junctional adhesion molecules (JAM). The characteristic feature of the tight junctions is an extremely high electric resistance (Abbott et al. 2010).

In the following paragraphs we will briefly summarize the main ways how various substances may get across the BBB, with a particular attention to hormones, with respect to their pluripotent actions in the brain. Finally we will show, how various hormones affect the function and composition of the BBB.
Transport of substance across the BBB

Generally, compounds (and in some instances even also cells) may get across the BBB by two ways: 1. By breaching tight junctions by which the cells are "stitched" (paracellular way). 2. By passing through the epithelial cells (transcellular pathway). Breaching, or more generally, impairment or even disruption of the BBB, is a typical feature of the most severe neurodegenerative diseases as Alzheimer’s or other dementias, multiple sclerosis and parkinsonism. Mechanisms of transcellular transport are more sophisticated events, with a number of various participating actors, as shown in the next text.

The main transcellular pathways include:
1. Passive diffusion in the direction of the concentration gradient. It includes carbon dioxide, water, with some exceptions due to existence of ion channels, the major electrolytes and a broad group of small lipid-soluble molecules including low-molecular hormones as unconjugated steroids. Generally, hydrophilic substances and compounds of high molecular weight do not possess the physico-chemical properties required for passive diffusion. 2. Carrier-mediated transport using transporters as transmembrane proteins on the luminal or abluminal membrane, or eventually, on both. A facilitated transport not consuming energy as well as active transport may occur here. 3. Transport based on ligand-membrane receptor interaction. A specific case of the latter represents transport of the ligands mediated by the receptor for plasma transport protein to which the ligand is bound (Hampl et al. 2014).

The transport of major nutrients – glucose, lipids and amino acids, depends on their molecular weight and physico-chemical properties. Many of them have their own more or less specific transporters. Glucose supply to the brain is of crucial importance, and the BBB is abundant in membrane glucose transporters, the number of which, in contrast to other cells is mostly independent on insulin (Barros et al. 2007).

Most (but not all) lipoproteins are transported to and out of the BBB by using plasma lipoprotein carriers and their membrane receptors. In contrast to transport from circulation to other tissues, only high-density lipoproteins (HDL) appear to traverse the BBB (Wang and Ecke 2014). Among the lipids, brain cholesterol, amounting as much as almost 20 % of the entire body’s cholesterol is of particular importance because it is an essential constituent of myelin forming neuronal sheaths.

The blood-borne cholesterol, however, can only with difficulty traverse the BBB; in fact, only its minor portion uses plasma lipoproteins, as it is common for cholesterol transport from blood to other tissues. Therefore most of the brain cholesterol must be formed there de novo (Jeitner et al. 2011). Cholesterol in the brain undergoes intensive metabolism, including formation of neurosteroids. Regarding their transport across the BBB, of importance are cholesterol hydroxyderivatives (oxysterols), which can freely cross the BBB. While the major metabolite for efflux is 24S-hydroxycholesterol, in the opposite direction it is 27-hydroxycholesterol. This remarkable mechanism helps to maintain the brain cholesterol homeostasis (Björkhem 2006).

Different transporters were described for neutral as well as charged amino acids (Smith 2000, Hawkins et al. 2006). A large transport protein superfamily transporting also anionic amino acids and thyroid hormones are known as organic anion transporting polypeptides, abbreviated OATPs (Wirth et al. 2014). These transmembrane proteins mediate the translocation of a broad spectrum of amphipathic substrates as anionic polypeptides and also many drugs, toxins and xenobiotics and, last but not least, also steroid conjugates. They are expressed in many tissues, not only in endothelial cells forming the BBB (Hagenbuch and Meier 2003).

Generally, peptides and proteins from blood stream, including a large number of peptide hormones, can overcome the BBB only with difficulty by simple diffusion. In most instances their transport is ensured by selective transporters, which, in concert with enzymes on both sides of the endothelial membrane, can modify the permeability for these substances (Banks 2008a). An important superfamily of transmembrane transporters, with a very broad substrate specificity, are proteins of the so called ATP-binding cassette (ABC). They occur not only on the BBB, but on the most of endothelial cells. Permeability glycoprotein or P-glycoprotein (pgP) is of particular importance there. Its role consists in prevention of flux of some lipophilic drugs into the brain and, at the same time, it facilitates the removal of many substances from the brain, both in an ATP-dependent manner. Proteins of the ATP-binding cassette were tested with a view to support the clearance of neurotoxic peptides accumulating in the brain such as amyloid-β (Aβ) peptides in Alzheimer’s disease (Löscher and Potschka 2005, Shen and Zhang 2010).

As mentioned already, glucose transport to the brain does not depend on insulin, but insulin acts there as
an important regulator of central glucose metabolism. Via
the insulin receptor substrate present in the brain cells, it
initiates signaling pathways leading to the regulation of
not only peripheral metabolism, but also feeding behavior
and memory and thus contributes to maintenance of many
neural functions, such as neuronal growth, differentiation,
neuromodulation and neuroprotection (Ramalingam and
Kim 2014). Circulating insulin crosses the BBB using its
own saturable transporter. The physico-chemical
properties of the insulin transporter may be affected by
pathological factors such as hyperglycemia and diabetes.
Insulin resistance within central nervous systems is
typical for Alzheimer’s dementia (Banks et al. 2012).

Besides insulin, there is an array of peptide
hormones controlling energy homeostasis such as the
white adipose tissue-produced leptin, adipokines and
gastric ghrelin. They control appetite and thermogenesis
in the brain, resulting in a decrease in obesity. Leptin
mediates a negative feedback loop between the adipose
tissue and brain (Kalra and Kalra 2010). Leptin and
peptides of this group use their own receptor-transporters
to cross the BBB (Pan and Kastin 2007). A failure of the
leptin transporter in the BBB may result in leptin
resistance in obese subjects (Banks 2008b).

When referring to peptides passing across the
BBB, we must not leave out a multifarious array of
cytokines. It should be pointed out that they not only pass
across the BBB from the circulation, but that they are
synthesized in the brain as well, and even that they are
expressed in the BBB endothelial cells. Through their
receptors on both sides of the BBB they affect directly or
indirectly composition and function of the BBB, as will
be discussed in the next paragraphs (Banks 2005, Banks
et al. 1995). Impaired BBB permeability accompanied by
altered cytokine composition can be one of the factors
leading to normal pressure hydrocephalus (Sosvorova et
al. 2015).

Effects of hormones on the composition and
function of the BBB: paracellular way

The BBB is rich in hormonal receptors of all
kinds, both on its membrane and inside the cells.
Therefore hormones may affect the properties and
function of the barrier by non-genomic as well as
genomic mechanism. Generally, hormones and other
biologically active substances may act on the BBB in two
ways: 1. to influence tight junction integrity and thus
change the BBB permeability (paracellular way) and,
2. to affect properties or the expression of their
transporters (transcellular way).

Most reports published today on the effects of
hormones on the BBB permeability concerned steroids,
especially glucocorticoids (GCs) and, to a less extent,
estrogens.

The effects of GC on the BBB are an example of
their complex antiinflammatory actions. It has been
repeatedly demonstrated that GCs increase the tightness of
TJ by upregulating expression of TJ proteins such as
claudin, occludin and vascular endothelial adherin
through their binding to glucocorticoid receptors (GR),
rich in the BBB forming endothelial cells (Dietrich 2004,
evidence on genomic action of GCs on TJ protein
occludin via their receptors in murine capillary
endothelial cell was provided by Förster et al. (2005).
The authors used immortalized BBB endothelial cells
retaining their binding end proteosynthetic as well as
bioelectric abilities, enabling to study occludin
expression. GCs enhanced it and this effect was
completely abolished by GR antagonist mifepristone
(RU 486). The experiments have brought another
interesting finding that occludin expression by GCs was
potentiated by insulin by increasing stabilization of
GR protein via insulin signaling cascade.

The positive effect of GCs on tightness of the
BBB may be further strengthened by up-regulation of
especial tissue inhibitors of the BBB-destroying
metalloproteinases. Matrix metalloproteinases (MMPs),
out of which the most important is MMP-9 called also
gelatinase-B, possess their own tissue inhibitors
(TIMMP(s), the expression of which is increased by GCs.
Both direct effect as well as via expression of selective
cytokines may cause matrix metalloproteases inhibition
(Forster et al. 2007). This mechanism resembles another
one, responsible for reduction of inflammatory mediators
formation (leukotrienes and others) by GC, namely
inhibition of phospholipase A2 enzyme, catalyzing
hydrolysis of phosphoglycerol esters of arachidonic acid,
by up-regulation of its inhibitors, lipocortins (Magrioti
and Kokotos 2013).

Glucocorticoids, as mentioned already, exert
many of their actions via cytokines, but cytokines
themselves influence permeability of the BBB as well
through their signaling, especially at neuroinflammation.
The BBB is rich of cytokine receptors forming
endothelial membrane microdomains. These receptors
may- but need not be identical with their transporters. In
addition, endothelial cells and adjacent glial cell of the BBB are the sites of cytokine biosynthesis. For review of cytokine modulatory effects on the BBB properties and function see e.g. Pan et al. (2011).

Finally, let us mention well known antiinflammatory effect of GCs, taking part also at the BBB, and resulting in reducing of leukocyte infiltration across the disrupted barrier. It consists in countering the upregulation of intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 (ICAM-1 and VCAM-1, respectively) (Salvador et al. 2014).

Elucidation of these mechanisms is important for treatment of many neurodegenerative inflammatory diseases such a multiple sclerosis and others by GC (Salvador et al. 2014).

Though not so efficient as glucocorticoids, estrogens are other steroids influencing the permeability of the BBB: estradiol through its receptor in the BBB-forming endothelial cell up-regulates expression of occludin in a similar way as GCs (Sandoval and Witt 2011). Either directly via their receptors or indirectly through cytokine signaling estrogens reduce activation of adhesion molecules and consequently penetration of leukocytes across the barrier (Witt and Sandoval 2014).

Increased expression of tight junction proteins was reported even by natural plant products with mild estrogenic properties as is flavonoid baicalin (Zhu et al. 2012). Since many phytoestrogens and also estrogen-like compounds from the environment, known as endocrine disruptors, may influence the properties and function of the BBB, new studies addressing this topics may be expected.

**Effects of hormones on the composition and function of the BBB: transcellular way**

Unlike paracellular effects, so far there are not many reports on the effect of hormones on transport of various substances across the BBB. Most of them were performed with experimental animals or in cell cultures.

Of interest may be the effect of estradiol on the expression of glucose transporter-1 by estradiol in rats (Shi and Simpkins 1997). Both types of estradiol receptors (α and β) are present in the endothelial cells of the BBB and through them estradiol regulates expression of several proteins of the ATP-binding cassette (ABC). Among them the most important is breast cancer resistance protein (BCRP). This protein is responsible for extrusion of a variety of therapeutic drugs, including cytostatics, and thus diminishes their pharmacological efficacy in the brain. Estradiol induces a down-regulation of BCRP on transcriptional and translational levels via the activation of ERβ in rat brain capillaries (Mahringer and Fricker 2010). The function of transporters of the ATP-binding cassette may be also modulated indirectly by cytokines (see above) (Pan et al. 2011).

Glucocorticoids, besides their plethora effects on the BBB permeability are also involved in regulation of key transporters of various substrates and drugs. Out of them already mentioned permeability phosphoglycoprotein or pgP, the product of multidrug resistance gene, is of particular importance, with respect to its neuroprotective role as it actively pumps substrates back into the capillary lumen. Experiments with developing murine fetuses indicated that pgP expression is upregulated by synthetic GCs (Petropoulos et al. 2010).

Expression of polyfunctional transporters operating in (on) the BBB could be also regulated by androgens: Organic anion transporter 3 (OAT3) mRNA in the cultured rat BBB cells was induced by treatment with dihydrotestosterone (DHT) by genomic way through androgen receptors since the effect was completely abolished by antiandrogen flutamide. This protein among others mediates transport of thyroid hormones and steroid conjugates (Ohtsuki et al. 2005).

**Conclusion**

There are many reports on effects of hormones in the brain, as well as on their transport across the BBB. Surprisingly, only scarce information is available how they affect BBB composition and their own transport. The endothelial cells composing the BBB are rich in hormonal receptors, both membrane and intracellular.

In this review we tried to outline the main ways how hormones regulate expression of tight junctions and adhesion proteins and thus the BBB permeability, as well as their effects on expression and function of various transporters including those transporting hormones themselves. It concerns first of all glucocorticoids, used for many years as powerful antiinflammatory agents. In general they increase the tightness of the BBB. Their pluripotent effect consists in up-regulation of TJ proteins expression and, at the same time in down-regulation of destructive enzymes by increasing expression of their specific inhibitors. In addition GCs regulate expression of important protein transporters responsible for removal of detrimental substances from the brain. Of interest are also
beneficial effects of estrogens and even also androgens, acting in similar way as GCs.

The effects of hormones on the BBB is an open lively topics and new reports addressing it may be expected.

Conflict of Interest
There is no conflict of interest.

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