Steroids 81 (2014) 70–73

Contents lists available at ScienceDirect

Steroids

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

Adrenal steroids in the brain: Role of the intrinsic expression of corticosteroid-binding globulin (CBG) in the stress response

Elena V. Sivukhina, Gustav F. Jirikowski *

Institute of Anatomy II, Friedrich-Schiller University Jena, Germany

ARTICLE INFO

Article history:
Available online 16 November 2013

Keywords:
Glucocorticoids
Stress response
HPA axis
Binding globulin
Immunohistochemistry

ABSTRACT

The complex interaction between hypothalamus, pituitary and adrenal glands is a key component of the neuroendocrine stress response. The major stress hormones – glucocorticoids – have both central and peripheral effects. Among the factors regulating their availability to target tissues are levels of corticosteroid-binding globulin, as the major transport protein for glucocorticoids in systemic circulation. Our recent findings demonstrated expression of corticosteroid-binding globulin in various brain regions and in different cell populations (neurons and glial cells). We showed at the cellular level the presence of corticosteroid-binding globulin in the human hypothalamus, where it was co-localized with the classical neurohypophyseal neurohormones – vasopressin and oxytocin. For the first time we demonstrated in mouse that the same gene encodes brain and liver corticosteroid-binding globulin. The full-length sequencing of hypothalamic corticosteroid-binding globulin revealed a full homology with liver corticosteroid-binding globulin cDNA. Thus, we confirmed that corticosteroid-binding globulin mRNA is produced locally within various cerebral regions and thus not transported from blood. However, the amounts of mRNA encoding corticosteroid-binding globulin are in liver about 200 times higher than in brain. The wide distribution of corticosteroid-binding globulin, distinct from the localization of glucocorticoid receptors, observed in our comparative study in rodents, led us to propose two possibilities: (1) corticosteroid-binding globulin is made in certain neurons to deliver glucocorticoids into the cell and within the cell in the absence of cytoplasmic glucocorticoid receptors or (2) is internalized into neurons specifically to deliver glucocorticoids to classical glucocorticoid receptors. Brain corticosteroid-binding globulin may be involved in the response to changing systemic glucocorticoid levels either additionally to known nuclear and membrane corticosteroid receptors or in glucocorticoid responsive brain regions devoid of these receptors. Clearly the multiple locations of corticosteroid-binding globulin within the central nervous system of humans and rodents imply multiple functional properties in normal and/or pathological conditions, which are yet to be determined. Most likely, the importance of brain corticosteroid-binding globulin exceeds the function of a mere steroid transporter.

1. Introduction

Adrenal steroids are of tremendous importance in brain. Central and systemic stress responses as well as learning and memory are likely to be controlled by glucocorticoids (GCs) and mineralocorticoids (MCs). GCs are involved in memory consolidation, while MCs seem to participate in appraisal and response to novelty [1]. Steroids are capable of crossing the blood brain barrier. It is generally accepted that GCs act via two types of receptors – MC receptors (MR) and GC receptors (GR), which are expressed by different types of cells within the brain [2–5]. However, their distribution and activity are quite different. While MRs are especially active under basal conditions with low levels of GCs (they have high affinity for GCs), GRs are mostly occupied during chronic stress, when systemic levels of GCs are increased [6]. Both receptor types have been well described and characterized in various brain regions. Several studies revealed a widespread distribution of GR mRNA and/or GR immunoreactivity within the cerebral cortex, the amygdala complex, the thalamus, the hypothalamus, the septohippocampal complex, the brainstem, the cerebellum, and the spinal cord in rats [7–10], Despite some discrepancies, owed most likely to the different antibodies used in the various studies, our recent findings on the distribution of GR immunoreactivity are

Abbreviations: CBG, corticosteroid binding globulin; CSF, cerebrospinal fluid; GC, glucocorticoid; MC, mineralocorticoid; GR, glucocorticoid receptor; MR, mineralocorticoid receptor.

* Corresponding author. Address: Inst. für Anatomie II, Teichgraben 7, 07743 Jena, Germany. Tel.: +49 3641 938553; fax: +49 3641 938552.
E-mail address: Gustav.Jirikowski@med.uni-jena.de (G.F. Jirikowski).

0039-128X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.steroids.2013.11.001

in accordance with previous observations. They confirm that GR-positive cells are highly abundant throughout numerous brain regions in intact untreated male rats [11]. Moreover, it has been demonstrated that many of the clearly GC-sensitive brain systems are devoid of GR under physiological conditions [12,13]. Among them are the magnocellular hypothalamic nuclei [11]. However, the effects of GCs on synthesis and release of both vasopressin and oxytocin in paraventricular (PVN) and supraoptic (SON) nuclei have been demonstrated [14]. Thus, the absence of GR suggests that GCs may act on these neurons in a GR-independent manner. The presence of MRs has been also reported in magnocellular hypothalamic neurons [5,15]. Brain MRs are thought to mediate effects of MCs on blood pressure and salt intake [16] rather than being involved in the regulation of the hypothalamo–pituitary–adrenal (HPA) axis [5].

According to the "classical" (genomic) concept of steroid action, the binding of GCs to GR leads to the translocation of these receptors from cytoplasm to the cell nucleus to regulate the gene transcription which may take hours (slow action of GCs) [17]. Thus, the intracellular location of GR (nuclear or cytoplasmic) is dependent on the availability of GCs to cells [18]. Among the factors regulating GCs availability to target tissues are levels of liver-derived corticosteroid-binding globulin (CBG), a well characterized 55 kDa glycoprotein [19,20] which is the major transport protein for GCs in systemic circulation [21]. It is known that more than 85% of GCs are bound to CBG [19,20].

2. The role of brain CBG

CBG is a member of serine proteinase inhibitor (SERPIN) It is mainly produced by hepatocytes [22,23]. The extrahepatic expression of CBG (e.g., in Fallopian tube, ovaries, placenta, normal endometrium, kidney, white adipose tissue, anterior pituitary lobe, lung and testis) has been also demonstrated [23–28].

The intrinsic expression of CBG in rat brain has been reported before by various authors [28–31]. Recently we extended the data obtained previously by the use of a unique mouse model in which expression of the CBG gene is totally abolished [32]. For the first time we described the presence of CBG in the human hypothalamus at the cellular level. So far CBG had only been detected in the human central nervous system with immunoassays of cerebrospinal fluid (CSF) [33,34]. We found CBG predominantly in neurons of the SON, magnocellular and parvocellular portions of the PVN, perifornical region, sexually dimorphic nucleus, the bed nucleus of the stria terminalis, lateral hypothalamic and medial preoptic area [35]. Individual CBG-positive perikarya were also seen in the ventromedial hypothalamic nucleus. Although we only examined the human hypothalamus, it cannot be assumed that immunostaining for CBG is confined to this portion of the brain. We observed specific CBG immunoreactivity in functionally diverse brain regions outside the hypothalamus including the nucleus accumbens, the thalamus, the septum, the hippocampus, the globus pallidus, the amygdala, the diagonal band of Broca, the cerebral cortex and the cerebellum within the rat and mouse brain [11,36].

In human hypothalamus CBG immunoreactivity was co-localized with the classical neurohypophyseal nonapeptides – vasopressin and oxytocin [35]. While only a small portion of vasopressinergic neurons were CBG-positive, oxytocinergic perikarya overlapped with CBG to a larger extent, which is similar to the situation observed in rats [29]. Moreover, we located CBG in axonal varicosities throughout the lateral hypothalamus, the periventricular nucleus, in the internal zone of the median eminence, the infundibulum and in Herring's bodies of the neurohypophysis [11,35,36]. Thus, it can be concluded that CBG in magnocellular neurons is subject to axoplasmic transport and terminal release into the portal or systemic circulation in the respective neurohemal organs, along with two important stress peptides vasopressin and oxytocin, which suggests CBG is involved in the HPA axis reactions during stress. In previous immunoelectron microscopical study we observed a similar morphology for sex hormone-binding globulin (SHBG) and oxytocin in the rat hypothalamo-neurohypophyseal system, where they were colocalized in identical secretory vesicles in the median eminence and neurohypophysis [37]. Tissue preservation of human brain material does not allow for such ultrastructural studies. However, it is likely that there may be a similar situation for CBG in humans. CBG from such neurons could also access the CSF. CBG has been detected in human CSF but in lower concentrations than in plasma. Due to its size, CBG is unlikely to cross the blood brain barrier. CSF levels of CBG increase as systemic GC levels drop [33,34].

Results of our colocalization study with neuronal marker protein (NeuN) demonstrated CBG immunoreactivity in many neurons within the amygdala, the prefrontal cortex and the hippocampus [11,36], regions known to be connected with central stress responses [38,39]. Interestingly, phenotypic analysis of CBG knock-out mice demonstrated brain-specific deficits in animal's stress responses [32]. In particular, despair-like behavior and altered memory responses during stress were observed in these mice. These brain-specific alterations have been linked to the lack of plasma CBG although a possible contribution of brain CBG cannot be excluded at present [40]. Thus, the specific involvement of brain CBG compared to plasma CBG has yet to be defined.

CBG positive staining was found in granule- and Purkinje-cells in the mouse cerebellum [36]. Little is known about the effects of steroids on cerebellar structures. However, postnatal exposure to clinically routine doses of hydrocortisone or dexamethasone was associated with impaired cerebellar growth [41]. Another study revealed that GCs increase cerebellar cell death and induce apoptosis in immature granule neurons via a non-genomic mechanism [42].

3. The relationship between brain CBG and GR

Results of our colocalization study showed that the co-expression of CBG with nuclear GR seems to be differentially localized within the rat brain [11]. While in the cerebral cortex both proteins seem to be localized in identical neurons, CBG and GR localizations are distinct in the different hippocampal regions. This is particularly true for areas known to be affected by changing GC levels like the hippocampus and the amygdala complex [39]. Cells in the hippocampal CA3 region are known to be MR-positive [5]. In a recent *in vitro* study CBG expression and GC dependent CBG secretion was demonstrated in glial cells that were devoid of GR [43], suggesting that GCs immediately release CBG in a process that does not involve GRs. Results of our immunohistochemical study showed that the magnocellular hypothalamic nuclei contained CBG immunoreactivity rather than GR [11]. Although the effect of systemic GCs on these cells has been demonstrated [14], the involvement of both vasopressin and oxytocin in the central control of stress response is without doubt [38]. Thus, the absence of GR suggests that GCs may act on these systems in a GR-independent manner. The presence of MR has also been reported in magnocellular hypothalamic neurons [5,15]. Here MR are thought to mediate effects of MCs on blood pressure and salt intake [16] rather than being involved in the regulation of the HPA axis [5].

4. Localization of CBG in non-neuronal cells in brain

Our recent study provided for the first time a detailed analysis of the cellular distribution of CBG throughout the brain. Immunostaining for CBG and for the glial markers – glial fibrillary acidic