Steroids 81 (2014) 13–16

Contents lists available at ScienceDirect

Steroids

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

Sex hormone binding globulin and corticosteroid binding globulin as major effectors of steroid action

Jack D. Caldwell [a,*], Gustav F. Jirikowski [b]

[a] Lake Erie College of Osteopathic Medicine, Erie, PA, USA
[b] Institute of Anatomy II, University Hospital Jena, Friedrich Schiller University, Jena, Germany

ARTICLE INFO

Article history:
Available online 20 November 2013

Keywords:
Steroid binding globulins
Membrane effects
Steroid uptake
Non genomic actions
Gonadal hormones
Glucocorticoids

ABSTRACT

Contrary to the long-held postulate of steroid-hormone binding globulin action, these protein carriers of steroids are major players in steroid actions in the body. This manuscript will focus on our work with sex hormone binding globulin (SHBG) and corticosteroid binding globulin (CBG) and demonstrate how they are actively involved in the uptake, intracellular transport, and possibly release of steroids from cells. This manuscript will also discuss our own findings that the steroid estradiol is taken up into the cell, as demonstrated by uptake of fluorescence labeled estradiol into Chinese hamster ovary (CHO) cells, and into the cytoplasm where it may have multiple actions that do not seem to involve the cell nucleus. This manuscript will focus mainly on events in two compartments of the cell, the plasma membrane and the cytoplasm.

1. Where are SHBG and CBG?

Originally, it was believed that SHBG and CBG were only made in the liver [1–3] from which they were distributed into the blood to carry steroids around to their target cells. Several laboratories have found both SHBG and CBG made in numerous peripheral organs other than the liver [4–8]. Our own laboratory followed up on the original work from David Joseph's laboratory [9] showing that androgen binding protein (ABP) was found in the hypothalamus of the brain. Seeing that ABP was localized in the magnocellular nuclei (the paraventricular and supraoptic nuclei), where we had already identified steroid-sensitive production of the neuropeptide oxytocin [10–13], we examined whether the same cells might not also contain ABP (SHBG is made from the same gene as ABP but is post-translationally glycosylated [14] and since our antibody cannot distinguish ABP from SHBG we will henceforth refer to SHBG.). It turned out that SHBG and oxytocin were extensively co-localized in both magnocellular nuclei [15–17]. These were co-localized to such an extent that they were found in the same secretory vesicles in the posterior pituitary [18] suggesting that they are co-released. We went further to suggest that these two had co-functions and to suggest a model wherein the known cell membrane receptor for oxytocin and a putative receptor for SHBG (see more below) were closely aligned in the plasma membrane [19]. Meanwhile, within the cell body, we were finding that sometimes SHBG was made in the cells in which we identified it [15] and sometimes it was found in cells that did not seem to produce it, such as in circumventricular neurons in the periventricular hypothalamus [20]. We have suggested that this indicates SHBG uptake from the cerebrospinal fluid by these cells [20], which is further explored below.

We have also seen that CBG is both made and taken up by various cells in the brain. Generally, CBG is found in the same hypothalamic nuclei as SHBG [21,22]. Interestingly, CBG is also found in neurons containing both oxytocin and vasopressin [22], which likely has importance for control of these neuropeptides in response to stress. Another part of the brain that contains CBG is the cerebellum [23]. CBG is also found in specific areas of the hippocampus, which suggests particular relationships with the intracellular glucocorticoid receptor as well as relationships with stress responses.

One value of the Rapid Responses to Steroid Hormones meetings is that attendees from different disciplines discuss the similarities of steroid actions across various organs and tissues. One very important organ that seems also to contain both SHBG and CBG is the heart. First, we identified SHBG in the heart that seemed to disappear in human hearts demonstrating dilated cardiomyopathy [6], suggesting a role for this gonadal steroid-binding protein in normal heart functioning. It might be of interest to cardiologists to explore the role of SHBG in light of the extreme gender-specificity with regard to rates of heart disease [24–26]. We have shown immunostained CBG in all cardiomyocytes [27]. However, there is as yet no evidence that CBG is produced in the heart, suggesting

* Corresponding author. Address: Lake Erie College of Osteopathic Medicine, 1858 West Grandview Blvd., Erie, PA 16509, USA. Tel.: +1 814 860 5153.
E-mail address: jcaldwell@lecom.edu (J.D. Caldwell).

0039-128X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.steroids.2013.11.010

that perhaps, particularly at times of great stress, that CBG is responsible for internalizing the corticosteroids into heart muscles.

CBG is also found in the rat olfactory system [28]. An interesting finding with regard to CBG in the olfactory system is that we identify it in axons of cells projecting out of the olfactory epithelium and to the olfactory bulb [28]. This suggests that CBG, either produced within the cell or internalized by the neurons, is transported within the cytoplasm of the cell even to the extent that it is targeted for transport along the axon. Therefore, we need to consider that these steroid-binding globulins are important in transporting steroids around within cells.

2. Evidence for internalization of SHBG and CBG

As is suggested by our evidence from the brain, there may be some mechanism for the internalization of SHBG and CBG into cells. We found that periventricular neurons contained, but did not produce SHBG [15,15,20], suggesting that they take SHBG up from the cerebrospinal fluid to which they sent processes. More recently, we found that Alexa 455-labeled SHBG was taken up by these same periventricular cells upon intracerebroventricular injection [20]. There were specific neurons surrounding the third cerebroventricle that internalized SHBG [20]. SHBG was also taken up by neurons in the paraventricular nucleus, where we had previously demonstrated it was made [15], in the medial forebrain bundle, and in the lateral hypothalamus [20]. SHBG was also internalized by a portion of the cells in the choroid plexus, an important circumventricular area. In that same paper we examined the uptake of radiolabeled SHBG into cells *in vitro*. In mouse hippocampal HT22 cells, we found that only cells stably transfected with ER-β (but not with ER-α) internalized SHBG and also that pre-binding with the steroid dihydrotestosterone (DHT) blocked SHBG's uptake [20]. From this first finding we suggested that there might be a special relationship between ER-β and SHBG such that the first one was necessary for either binding of SHBG to the plasma membrane or that ER-β stimulation of gene transcription was necessary for the expression of proteins that were involved in the internalization of SHBG. The second finding suggested that whatever mechanism, which could include a cell-surface receptor for SHBG, that internalized SHBG, it was blocked by the presence of DHT. Rosner's laboratory had previously postulated the presence of an SHBG receptor in the plasma membrane of prostate cells [29–31]. In their model, only an unliganded (with no bound steroid) SHBG would bind to the receptor, while prior binding of DHT by SHBG blocked its binding by the membrane-associated receptor. Our evidence that DHT also blocked the uptake of SHBG in brain suggested that a receptor similar to the one postulated by Rosner [20] was responsible for, or at least involved in, the uptake of SHBG in the brain.

3. Uptake and accumulation of fluorescent estradiol

It appears that one critical function for SHBG and CBG, at least in some cells, is to internalize steroids. It must be noted that all that has been tested of this postulate so far is the internalization of either SHBG or CBG themselves. That is, we have not yet demonstrated that their associated steroids are bound by them when they are internalized. One way to analyze uptake of steroids directly is to utilize a labeled steroid such as estradiol. So far *in vivo* accumulation of steroids has been studied on a cellular level with radiolabeled steroids and subsequent autoradiography in histological specimens. We recently developed a low molecular weight fluorescent estrogen agonist [47]. With this compound we treated Chinese hamster ovary (CHO) cells and monitored uptake with high resolution video microscopy. With this method we observed a rapid buildup of fluorescent steroid on the outside of the cell membrane. Within a very short time after introduction of estradiol, cells extended long microvilli from their outer surfaces, suggesting that specific membrane-associated binding sites accumulated estradiol. This observation contradicts the concept of immediate passive diffusion of steroids across the plasma membrane. Only after reaching a maximum concentration at the cell membrane did fluorescent estradiol enter into the cytoplasm and fill a distinct portion of the perinuclear space suggesting that steroid internalization is driven by some concentration-dependent process. It took several minutes for estradiol to concentrate on the nuclear envelope, while internalization of fluorescent estradiol into the nucleus was only occasionally observed. Membrane accumulations and internalization of fluorescent estradiol could be prevented by pretreatment of cultures with an antibody to SHBG, indicating that the presence of the intact binding globulin was essential for uptake and internalization of the labeled steroid.

This agrees with the speculation that steroids do many things within the cytoplasm before even entering the nucleus, or that they do not always enter the nucleus to have their actions. In some recordings, however, it was apparent that fluorescent estradiol accumulated on the outer edge of the nucleus, indicating another mechanism of steroid concentration, perhaps sequestering the steroid for subsequent translocation into the nuclear core. As yet no one has proposed a mechanism whereby steroids would be concentrated on the nuclear envelope.

The presence of estradiol accumulation on the outer surface of the cell may indicate the presence of SHBG and/or its receptor, or may indicate the presence of any of the multiple estradiol receptors that have been suggested to exist in the plasma membrane [32–38]. Another, as yet unexplored, postulate is that microvilli extend out from the cell to accumulate steroids. Clara Szego, who was a pioneer of the modern study of rapid steroid effects, had fascinating data that no one has been able to explain. In one study she found very rapid (within 30 s) induction of microvilli on the outer surface of uterine cells by estradiol treatment [39]. Many studies have shown that estradiol treatments over a longer period of time increase the number of dendritic spines [40–43] in neurons. To our knowledge, no one has ever examined the appearance of such growths in less than a matter of days. It is possible that estradiol induces the appearance of dendritic spines within a much shorter period of time. In a very interesting, but much ignored study, Kipp and Ramirez [44] found that estradiol binds to one end of tubulin while testosterone binds to the other end. Perhaps due to this site-specificity in binding, they have opposite effects on tubulin growth. Whereas estradiol only affected tubulin polymerization, and thus growth, testosterone acted only via depolymerization and thus limiting growth [44]. Perhaps estradiol, by some non-genomic mechanism acting directly on the tubulin itself, stimulates formation of the scaffolding of microvilli by almost immediately inducing extension of microtubules. With this capacity to distort cell shape, estradiol and perhaps other steroids, could have extensive physiological effects on diverse target cells.

4. Novel models of steroid and steroid-binding globulin actions

In the introduction to this volume, we suggested that, in spite of the apparent complexity that currently exists for non-genomic effects of steroids, there may be key parts of the system that will prove to be critical sites of action. We also suggested that determining how the steroids work at those sites will lead us into a better understanding of how steroids actually affect physiological functions. Obviously, this is trading a larger black box of obscurity for a smaller one. We have already suggested a putative membrane steroid receptor that has not been identified that interacts at the plasma membrane level with transmitter receptors, such as the