



# Neurosteroid Transport in the Brain: Role of ABC and SLC Transporters

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Neurosteroids, comprising pregnane, androstane, and sulfated steroids can alter neuronal excitability through interaction with ligand-gated ion channels and other receptors and have therefore a therapeutic potential in several brain disorders. They can be formed in brain cells or are synthesized by an endocrine gland and reach the brain by penetrating the blood–brain barrier (BBB). Especially sulfated steroids such as pregnenolone sulfate (PregS) and dehydroepiandrosterone sulfate (DHEAS) depend on transporter proteins to cross membranes. In this review, we discuss the involvement of ATP-binding cassette (ABC)- and solute carrier (SLC)-type membrane proteins in the transport of these compounds at the BBB and in the choroid plexus (CP), but also in the secretion from neurons and glial cells. Among the ABC transporters, especially BCRP (ABCG2) and several MRP/ABCC subfamily members (MRP1, MRP4, MRP8) are expressed in the brain and known to efflux conjugated steroids. Furthermore, several SLC transporters have been shown to mediate cellular uptake of steroid sulfates. These include members of the OATP/SLCO subfamily, namely OATP1A2 and OATP2B1, as well as OAT3 (SLC22A3), which have been reported to be expressed at the BBB, in the CP and in part in neurons. Furthermore, a role of the organic solute transporter OST $\alpha$ -OST $\beta$  (SLC51A/B) in brain DHEAS/PregS homeostasis has been proposed. This transporter was reported to be localized especially in steroidogenic cells of the cerebellum and hippocampus. To date, the impact of transporters on neurosteroid homeostasis is still poorly understood. Further insights are desirable also with regard to the therapeutic potential of these compounds.

**Keywords:** ATP-binding cassette transporters, blood–brain barrier, dehydroepiandrosterone, DHEAS, neuroactive steroids, pregnenolone sulfate, solute carriers

## INTRODUCTION

Neurosteroids are cholesterol-derived compounds categorized in pregnane neurosteroids (e.g., allopregnanolone), androstane neurosteroids (e.g., androstanediol), and sulfated compounds [PregS and DHEAS] (Reddy, 2010). They can be synthesized in the central nervous system, or the compounds themselves or precursors can be taken up from the systemic circulation (Baulieu, 1997; Maninger et al., 2009). One of their main functions in the brain is to modulate

**Abbreviations:** ABC, ATP-binding cassette; BBB, blood–brain barrier; BCRP, breast cancer resistance protein; CP, choroid plexus; CSF, cerebrospinal fluid; DHEAS, dehydroepiandrosterone sulfate; E<sub>1</sub>-3-S, estrone-3-sulfate; GABA,  $\gamma$ -aminobutyric acid; MRP, multidrug resistance protein; NMDA, *N*-methyl-D-aspartate; OATP, organic anion transporting polypeptide; OST, organic solute transporter; PregS, pregnenolone sulfate; SULT, sulfotransferase; TRP, transient receptor potential.

excitability by interaction with membrane receptors and ion channels (Reddy, 2010). Accordingly, a therapeutic potential of these compounds or synthetic analogs has been discussed for a variety of brain disorders (Reddy, 2010). Local steroid biosynthesis in rodent and human brain has been studied since 1990s (Baulieu, 1997). Expression of key enzymes (e.g., P450 side-chain cleavage enzyme, P450c17) has been demonstrated in the principal neurons of several brain areas and in the microglia and astrocytes (reviewed in: Maninger et al., 2009; Hojo et al., 2011; Porcu et al., 2016). However, the relative contributions of the local synthesis and uptake from blood to CNS levels have still to be clarified and may vary between different neurosteroids. In contrast to the lipophilic unconjugated compounds, especially DHEAS and PregS do not diffuse across membranes at a sufficient rate due to their hydrophilic sulfate moiety. Therefore, this review focuses on DHEAS/PregS membrane transporters in the brain. Both compounds play important roles in age-related memory and learning. They can be formed from the non-sulfated precursors by SULTs with SULT2B1b as a major isoform in the human brain (Salman et al., 2011). The ratio of sulfated versus non-sulfated neurosteroids in the brain may be decisive, since sulfation can change the direction of the neuromodulating activity. While for example some non-sulfated neurosteroids such as allopregnanolone are potent positive modulators of GABA type A receptors (for review see Chisari et al., 2010; Reddy, 2010), DHEAS and PregS have been shown to antagonize the GABA effect on the GABA<sub>A</sub> receptor (Seljeset et al., 2015) and to be potent allosteric agonists at NMDA receptors (Wu et al., 1991; Monnet et al., 1995). Furthermore, PregS has been shown to directly activate certain TRP channels (Harteneck, 2013).

## DHEAS AND PREGS LEVELS IN PLASMA, BRAIN, AND CEREBROSPINAL FLUID (CSF) IN HUMANS

While the serum concentrations of the non-sulfated DHEA and pregnenolone are in the low nM range in men and women (Labrie et al., 1997; Kancheva et al., 2011), levels of DHEAS are much higher with a slight difference between men (2.3–11.5  $\mu$ M) and women (1.6–6.2  $\mu$ M). It should be noted that here total steroid concentrations were measured. Due to the high plasma protein binding (95% for DHEAS; Wang and Bulbrook, 1969) the concentration of the unbound steroids is accordingly lower. Generally, hormone levels decrease with age, exhibiting a maximum in young men and women (~20 years; Labrie et al., 1997). Total PregS serum levels in adolescence range between 40 and 140 nM, and increase during pregnancy and at birth (de Peretti and Mappus, 1983; Kancheva et al., 2011). In contrast to DHEAS and PregS serum levels, less information is available on their tissue concentrations in the brain and in the CSF. In the human brain, region-dependent PregS concentrations between 5 and 40 nmol/kg have been reported with the highest levels in the striatum and hypothalamus. DHEAS concentrations were detected in a similar range with the highest levels in the striatum and cerebellum (Weill-Engerer et al., 2002). The CSF concentrations of DHEAS and PregS were much lower

compared to the respective serum levels [serum/CSF ratios: 584 (Preg-conjugates), 19849 (DHEA-conjugates); Kancheva et al., 2011]. These gradients are due to the fact that both compounds cannot cross the respective barriers by passive diffusion, but depend on selective uptake and efflux proteins. ABC- and solute carrier (SLC)-type transporters may not only be relevant for the transport at the BBB and the blood–CSF barrier in the CP, but also for secretion of these compounds from neurons and glial cells (Figure 1).

## TRANSPORTERS THAT MAY PLAY A ROLE IN THE TRANSPORT OF NEUROSTERIODS: ABC TRANSPORTERS

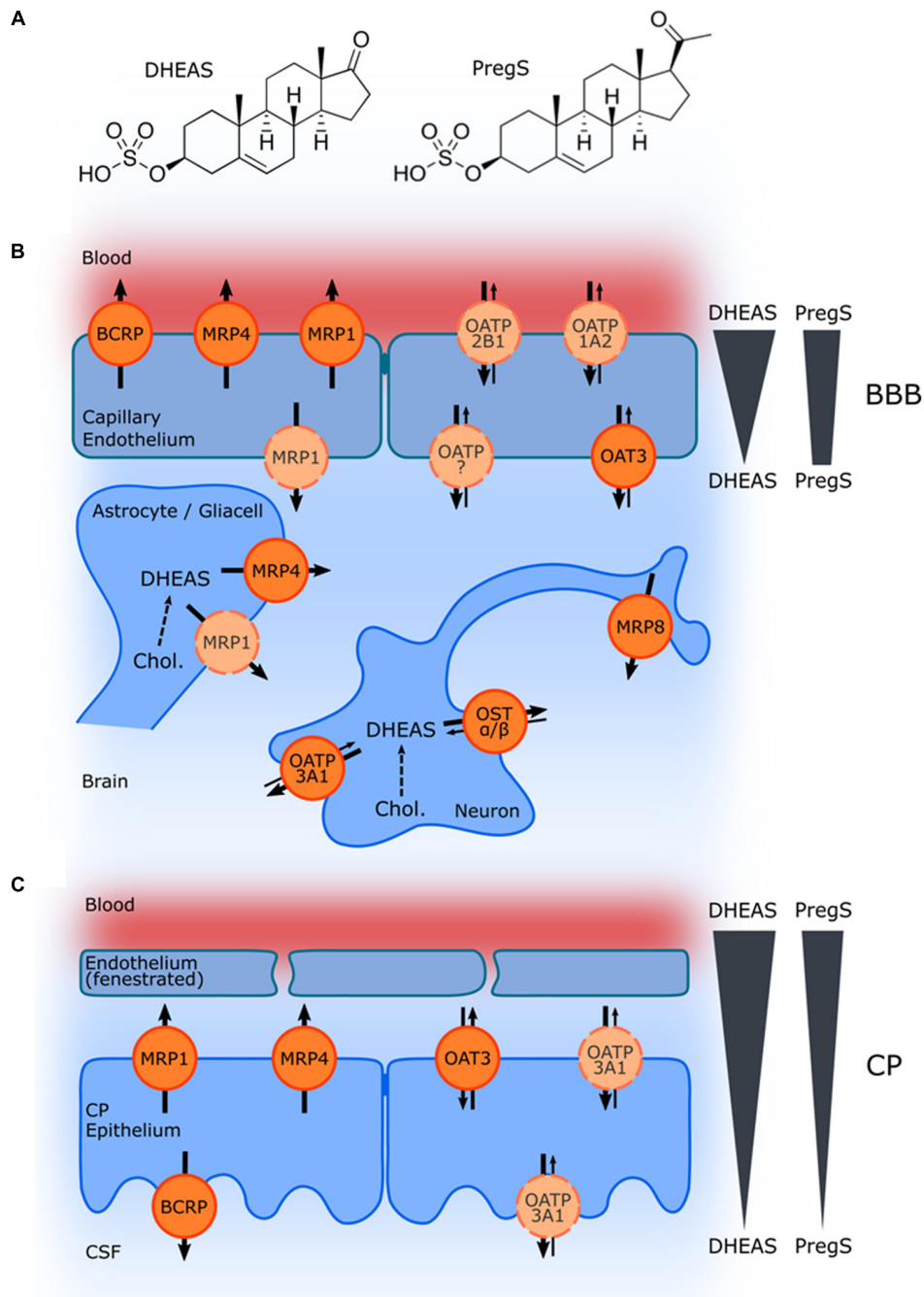
ATP-binding cassette proteins can mediate a unidirectional primary active transport of a variety of compounds across membranes. Among the ABC transporters especially ABCG2, also known as the BCRP, and several ABCC/MRP subfamily members are known efflux pumps for conjugated steroids (Suzuki et al., 2003; Haimeur et al., 2004).

### BCRP/ABCG2

BCRP/ABCG2 was initially identified as a non-P-glycoprotein and non-MRP-type resistance factor from drug-selected cell lines (Doyle et al., 1998; Miyake et al., 1999). Besides anti-cancer drugs like mitoxantrone, BCRP actively transports sulfated steroids such as E<sub>1</sub>-3-S and DHEAS, but not unconjugated or glucuronidated steroids (Imai et al., 2003; Grube et al., 2007). The  $K_m$  value for E<sub>1</sub>-3-S calculated in isolated membrane vesicles was in the low  $\mu$ M range (Table 1). In addition, androgens such as dihydrotestosterone (DHT) have been identified as BCRP substrates (Huss et al., 2005). In human brain microvessels, ABCG2/BCRP transcript (Cooray et al., 2002; Warren et al., 2009) and protein was detected as one of the most abundant ABC transporters (Shawahna et al., 2011). Immunohistochemistry showed it primarily localized at the apical (luminal) side of the endothelial cells (Cooray et al., 2002; Aronica et al., 2005; Warren et al., 2009). In addition, it was detected in the apical membrane of the CP epithelium (Roberts et al., 2008a). Hence, BCRP could be involved in limiting the penetration of peripheral DHEAS and other steroids into the brain or facilitating the elimination of brain-derived DHEAS into blood. The apical localization in the CP, on the other hand, indicate that here BCRP is able to transport neurosteroids into the CSF.

### Members of the MRP/ABCC Family

Anionic conjugates of lipophilic compounds are typical substrates for several members of the MRP family. As the founding member, MRP1 (ABCC1) was identified as second export pump conferring a multidrug resistance phenotype besides the MDR1/P-glycoprotein (ABCB1) (Cole et al., 1992). MRP1 was subsequently shown to preferentially transport amphiphilic anions, especially conjugates of lipophilic compounds with glutathione, glucuronate, or sulfate (Jedlitschky



**FIGURE 1** | Schematic illustration of ABC and SLC transporters putatively involved in DHEAS and PregS transport and their proposed localization. **(A)** Structures of DHEAS and PregS. **(B,C)** The ABC proteins BCRP (ABCG2), MRP1, MRP4, and MRP8 (ABCC1, ABCC4, and ABCC11), and the solute carriers OAT3 (SLC22A3), OATP1A2 (SLCO1A2), OATP2B1 (SLCO2B1), and OST $\alpha/\beta$  (SLC51A/B) may be involved in secretion of sulfated steroids from neurons and glial cells and/or in the transport across the BBB **(B)** as well as in transport at the blood–CSF barrier in the CP **(C)**. The arrows indicate the directions of substrate transport. Proteins for which there is little or controversial evidence for expression and localization in the basal or apical membrane are indicated in light orange and by a dashed line. Concentration gradients of DHEAS and PregS across the BBB and in the CP are schematically indicated at the right side.

et al., 1996; Loe et al., 1996). MRP1 transports in addition certain cationic or uncharged compounds, but only in co-transport with reduced glutathione (GSH; Loe et al., 1996). It also mediates the transport of E<sub>1</sub>-3-S and of DHEAS in a glutathione-dependent

manner (Qian et al., 2001; Zelcer et al., 2003) and is expressed in several tissues (Haimeur et al., 2004). It is also expressed in brain microvessels (Warren et al., 2009); however, the exact localization and function has not been finally clarified. It was detected to

**TABLE 1** | ABC and SLC transporters possibly involved in neurosteroid transport in the brain.

Transporter	CNS expression (mRNA and protein analysis)	CNS localization (immunohistochemistry)	Steroid substrates ( $K_m$ value)
<b>ABC Transporters</b>			
BCRP (ABCG2)	mRNA: – Total brain <sup>1</sup> – Isolated brain microvessels (BMV) <sup>2,3</sup> Protein: – BMV (H, M, Mo, R, P) <sup>4,5,6,7,8</sup> – Isolated CP <sup>9</sup>	BBB: apical (luminal) (H, R); CP: apical (H, R) <sup>2,10,11</sup>	E <sub>1</sub> -3-S (6.8 $\mu$ M) <sup>12,13</sup> DHEAS <sup>12</sup> DHT <sup>14</sup>
MRP1 (ABCC1)	mRNA: – Total brain <sup>1</sup> – BMV <sup>3</sup> – Isolated CP <sup>15</sup> Protein: – Isolated CP (H, R) <sup>9,16</sup>	BBB: apical (H, B, M), Basal (abluminal) (R, M); CP: basal (H, R) <sup>17,18,19,20</sup>	E <sub>1</sub> -3-S (+GSH) <sup>21</sup> DHEAS (+GSH) (5 $\mu$ M) <sup>22</sup> E <sub>2</sub> -17 $\beta$ G (1.5 $\mu$ M) <sup>23</sup>
MRP4 (ABCC4)	mRNA: – Total brain <sup>1</sup> – BMV <sup>3</sup> – Isolated CP <sup>15</sup> Protein: – BMV (H, M, Mo, R) <sup>4,5,6,7</sup> – Isolated CP <sup>9</sup>	BBB: apical (H, M, R), Apical + basal (B); CP: basal (H, R); Astrocytes <sup>3,11,19,20,24</sup>	DHEAS (2 $\mu$ M) <sup>22</sup> E <sub>2</sub> -17 $\beta$ G (30 $\mu$ M) <sup>22,25</sup>
MRP8 (ABCC11)	mRNA: – Total brain <sup>1</sup>	Neurons (axonal; cerebellum, cortex) <sup>26</sup>	E <sub>1</sub> -3-S (> 150 $\mu$ M) <sup>27</sup> DHEAS (13–21 $\mu$ M) <sup>26,27</sup> E <sub>2</sub> -17 $\beta$ G (63 $\mu$ M) <sup>27</sup>
<b>SLC Transporters</b>			
OATP1A2 (SLCO1A2)	mRNA: – Total brain <sup>1</sup> Protein: – BMV (M, Mo) <sup>5,6</sup>	BBB: luminal; amacrine neurons <sup>28,29,30,31</sup>	DHEAS (7 $\mu$ M) <sup>32</sup> E <sub>1</sub> -3-S (16 $\mu$ M) <sup>31</sup>
OATP2B1 (SLCO2B1)	mRNA: – Total brain <sup>1</sup> Protein: – Brain capillary cells <sup>31</sup> – BMV (M) <sup>6</sup>	BBB: luminal; CP (ependymal cells) (R); amacrine neurons <sup>11,29,30,33</sup>	E <sub>1</sub> -3-S (5–21 $\mu$ M) <sup>34,35</sup> DHEAS (9 $\mu$ M) <sup>35</sup> PregS <sup>36</sup>
OATP3A1 (SLCO3A1)	mRNA: – Total brain <sup>1</sup> Protein: – Isolated CP <sup>9</sup> – BMV (P) <sup>8</sup>	CP; neurons <sup>37</sup>	E <sub>1</sub> -3-S <sup>38</sup>
OAT3 (SLC22A3)	mRNA: – Total brain <sup>1</sup> Protein: – Isolated CP <sup>9</sup> – BMV (R) <sup>7</sup>	BBB: basal (R); CP: basal (H, R) <sup>39,40,11,41</sup>	E <sub>1</sub> -3-S (8.8 $\mu$ M) <sup>42</sup> DHEAS <sup>42</sup>
OST $\alpha$ -OST $\beta$ (SLC51A/B)		Neurons (cerebellum, Hippocampus; Purkinje cells) <sup>43</sup>	DHEAS (1.5 $\mu$ M) <sup>43</sup> PregS (6.9 $\mu$ M) <sup>43</sup>

Unless otherwise specified, the data refer to human tissue and transporter protein. B, bovine; BBB, blood–brain barrier (capillary endothelial cells); BMV, isolated brain microvessels; CP, choroid plexus; DHEAS, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; E<sub>1</sub>-3-S, estrone-3-sulfate; E<sub>2</sub>-17 $\beta$ G, estradiol-17 $\beta$ -glucuronide; H, human; M, mouse; Mo, monkey; P, pig; R, rat; PregS, pregnenolone sulfate. <sup>1</sup>Nishimura and Naito, 2005; <sup>2</sup>Cooray et al., 2002; <sup>3</sup>Warren et al., 2009; <sup>4</sup>Shawahna et al., 2011; <sup>5</sup>Ito et al., 2011; <sup>6</sup>Uchida et al., 2011; <sup>7</sup>Hoshi et al., 2013; <sup>8</sup>Kubo et al., 2015; <sup>9</sup>Uchida et al., 2015; <sup>10</sup>Aronica et al., 2005; <sup>11</sup>Roberts et al., 2008a; <sup>12</sup>Grube et al., 2007; <sup>13</sup>Imai et al., 2003; <sup>14</sup>Huss et al., 2005; <sup>15</sup>Niehof and Borlak, 2009; <sup>16</sup>Gazzin et al., 2008; <sup>17</sup>Rao et al., 1999; <sup>18</sup>Wijnholds et al., 2000; <sup>19</sup>Nies et al., 2004; <sup>20</sup>Zhang et al., 2004; <sup>21</sup>Qian et al., 2001; <sup>22</sup>Zelcer et al., 2003; <sup>23</sup>Jedlitschky et al., 1996; <sup>24</sup>Leggas et al., 2004; <sup>25</sup>Chen et al., 2001; <sup>26</sup>Bortfeld et al., 2006; <sup>27</sup>Chen et al., 2005; <sup>28</sup>Gao et al., 2000; <sup>29</sup>Bronger et al., 2005; <sup>30</sup>Gao et al., 2015; <sup>31</sup>Lee et al., 2005; <sup>32</sup>Kullak-Ublick et al., 1998; <sup>33</sup>Ji et al., 2012; <sup>34</sup>Pizzagalli et al., 2003; <sup>35</sup>Hagenbuch and Stieger, 2013; <sup>36</sup>Grube et al., 2006; <sup>37</sup>Huber et al., 2007; <sup>38</sup>Tamai et al., 2000; <sup>39</sup>Alebouyeh et al., 2003; <sup>40</sup>Kikuchi et al., 2003; <sup>41</sup>Mori et al., 2003; <sup>42</sup>Burckhardt, 2012; <sup>43</sup>Fang et al., 2010.

be luminal in humans, bovine, and murine brain (Nies et al., 2004; Zhang et al., 2004), but also localization at the basolateral (abluminal) membrane was described in mice and rats (Kilic et al., 2008; Roberts et al., 2008a). Some groups did not detect MRP1 protein in human brain capillaries at all (Rao et al., 1999; Aronica et al., 2005). Because of the inconsistent and low-level

expression, the relevance of this transporter at the BBB is so far unclear. It may play a more important role in the CP. Here, MRP1 mRNA was detected in rat and human tissue (Gazzin et al., 2008; Niehof and Borlak, 2009) and the protein was localized at the basolateral membrane (Rao et al., 1999; Wijnholds et al., 2000; Gazzin et al., 2008). Thus, it may be involved in the efflux

of conjugated steroids from the CSF into blood. In addition, MRP1 mRNA was detected in cultured rat and human astrocytes (Hirrlinger et al., 2001; Spiegl-Kreinecker et al., 2002). However, the protein could not be detected in human glial cells or neurons in immunohistochemical studies (Nies et al., 2004; Aronica et al., 2005). MRP1 and MRP2 (ABCC2) share a similar substrate spectrum, but MRP2 is mainly expressed in polarized epithelial cells (Keppler, 2011). Immunostaining at the apical membrane in brain capillaries was described in rats (Miller et al., 2000), but was not observed in human and bovine brain (Nies et al., 2004; Zhang et al., 2004). Similarly, MRP3 (ABCC3) protein was not detected in human brain (Nies et al., 2004).

A more relevant efflux transporter for conjugated steroids in the brain may be MRP4/ABCC4, which exhibits a unique broad substrate specificity. MRP4 shows the remarkable capacity to transport cyclic nucleotides and MRP4 has been established as an independent regulator of intracellular cAMP levels in several cell types (Ritter et al., 2005; Jedlitschky et al., 2012; Belleville-Rolland et al., 2016). Furthermore, MRP4 transports lipid mediators such as prostanoids and conjugated steroids. DHEAS is transported by MRP4 in a glutathione-independent manner and with high affinity ( $K_m$  of 2  $\mu\text{M}$ ; Zelcer et al., 2003). It is expressed in several tissues especially in the prostate, kidney, blood cells, and brain (Kool et al., 1997; Haimeur et al., 2004; Nishimura and Naito, 2005; Niehof and Borlak, 2009; Warren et al., 2009; Shawahna et al., 2011). Here, it was localized apically in human, rodent and bovine capillaries (Nies et al., 2004; Leggas et al., 2004; Zhang et al., 2004; Roberts et al., 2008a). An additional detection at the basolateral membrane was only described in bovine brain (Zhang et al., 2004). MRP4 expression was also detected in human CP (Niehof and Borlak, 2009; Uchida et al., 2015) and it was localized to the basolateral membrane in human and murine tissue (Leggas et al., 2004). Moreover, it is expressed in glial cells. Immunofluorescence studies in the human brain revealed staining mainly in astrocytes of the subcortical white matter (Nies et al., 2004). Since glial cells are able to synthesize neurosteroids, MRP4 may account for the efflux of DHEAS and other neurosteroids from these cells for a paracrine action. Astrocytes play a critical role for the development and function of neurons and these cells in turn are regulated by steroid hormones as progesterone and DHEA (Acaz-Fonseca et al., 2016; Arbo et al., 2016). At the BBB and the CP, MRP4 may contribute to the transport of sulfated steroids from brain and CSF into blood.

A further member of the MRP family, the MRP8 (ABCC11) may be relevant with respect to neurosteroid transport. MRP8 was shown to transport DHEAS in isolated membrane vesicles with a  $K_m$  value of 13–21  $\mu\text{M}$ , whereas the  $K_m$  was above 150  $\mu\text{M}$  for MRP8-mediated transport of  $E_1$ -3-S (Chen et al., 2005; Bortfeld et al., 2006). In immunofluorescence studies, it was detected preferentially in the white matter of the cortex and cerebellum and co-localized with neurofilaments indicating localization in neuronal axons (Bortfeld et al., 2006). In addition, weak immunostaining was detected in the gray matter and also in the axons of peripheral neurons. The axonal localization implies that MRP8 can mediate presynaptic efflux of neurosteroids from neurons and thus could directly participate in modulating postsynaptic neurotransmitter receptors (Bortfeld et al., 2006).

## Uptake (SLC) Transporters

Besides ABC-type efflux transporters, neurosteroid concentrations in the brain may also be modulated by uptake transporters. Since several members of the SLC superfamily have been identified as uptake transporters for steroid conjugates in general, these transporters are interesting candidates for the transport of neurosteroids. In fact, several SLCs have been shown to mediate cellular uptake of DHEAS and PregS.

## Members of the OATP/SLCO Family

Among the SLC transporters, the OATP (SLCO) family is probably the most interesting one in this context. In humans, 11 SLCO transporters exist, organized in six families (Hagenbuch and Stieger, 2013). The physiological substrate profile of the OATP transporters comprises a wide variety of endogenous organic anions including bile acids, bilirubin, thyroid hormones, and prostaglandins (Hagenbuch and Stieger, 2013). In addition, OATPs transport steroid hormone conjugates like  $E_1$ -3-S (nearly all OATPs) or estradiol-17 $\beta$ -glucuronide (OATP1A2, OATP1B1, OATP1B3, OATP1C1, and OATP4A1) and the neurosteroid DHEAS (OATP1A2, OATP1B1, OATP1B3, and OATP2B1) (Hagenbuch and Stieger, 2013). The affinity of these transporters toward DHEAS was slightly above (OATP1B1 and OATP1B3) or in the range (OATP2B1 and OATP1A2) (**Table 1**) of the physiological plasma concentration (1.6–11.5  $\mu\text{M}$ ; Labrie et al., 1997) indicating an *in vivo* relevance of these findings. OATP1B1 and OATP1B3 are almost exclusively expressed in the human liver. Here, they are responsible for cellular uptake as a prerequisite for hepatic metabolism and elimination. In turn, the expression and function of these transporters affect systemic DHEAS levels as shown by enhanced DHEAS levels in monkeys and rats after treatment with the unspecific OATP inhibitor rifampicin (Watanabe et al., 2015; Nishizawa et al., 2017). Assuming a DHEAS transport/uptake from the blood into the brain/CSF (Kancheva et al., 2010), changes in DHEAS plasma concentration might indirectly influence the concentration in these compartments. Therefore, OATPs localized in the BBB and/or the CP are of special interest. Indeed, OATP1A2, OATP1C1, OATP2B1, and OATP3A1 have been detected in these structures in humans (Kullak-Ublick et al., 1998; Pizzagalli et al., 2002; Huber et al., 2007; Roberts et al., 2008b; Ji et al., 2012). Since OATP1C1 is a thyroid hormone transporter and the function of OATP3A4 is only poorly understood, at present OATP1A2 and OATP2B1 are probably the most interesting members concerning neurosteroid transport in the brain. Both proteins are expressed in the endothelial cells of the BBB presumably in the luminal membrane predisposing them as transporters for uptake into the brain (Gao et al., 2000; Bronger et al., 2005; Lee et al., 2005). In addition, OATP1A2 and OATP2B1 are also expressed in other CNS cell types. While both transporters have been identified in amacrine neurons of the retina, OATP1A2 was additionally found in hippocampal pyramidal and granule cells (Gao et al., 2015). Besides DHEAS, PregS levels in the brain may also be influenced by uptake transporters in the BBB, even though plasma concentrations of PregS were up to two orders of magnitude below the

DHEAS levels (Sanchez-Guijo et al., 2015). Like for DHEAS, OATP-transporters are interesting candidates in this context. While PregS significantly inhibits OATP2B1 function (St Pierre et al., 2002; Grube et al., 2006) first reports indicated no direct transport of PregS by this transporter (Pizzagalli et al., 2003; Grube et al., 2006). Interestingly, OATP2B1-mediated transport of E<sub>1</sub>-3-S and DHEAS was stimulated by steroid hormones like progesterone (Grube et al., 2006; Koenen et al., 2012). Under these conditions, PregS was also transported by OATP2B1 (Grube et al., 2006). PregS transport by other OATPs has not been studied so far. With regard to the CP only limited information is available about OATP expression and function in humans. In a recent LC-MS/MS-based study examining transporter protein expression in this structure, only OATP3A1 was detected, while OATP1A2 and OATP1C1 were below the detection limit and OATP2B1 was not analyzed (Ji et al., 2012). This finding was quite surprising, since in animal models several OATPs have been detected in the CP and shown to be involved in the neurosteroid transport into the liquor (Asaba et al., 2000; Choudhuri et al., 2003; Ji et al., 2012). Due to the limited information available, the significance of OATP3A1 in this context cannot be conclusively assessed. However, a transport of E<sub>1</sub>-3-S has also been shown for the OATP3A1 and two splice variants of the transporter are selectively expressed in the apical and basal membrane of the ependymal cells of the CP (Tamai et al., 2000; Huber et al., 2007).

### Other Organic Anion Transporters (OST $\alpha$ -OST $\beta$ , OATs)

Besides OATPs, an interaction of neurosteroids (mainly DHEAS and PregS) with several further organic anion transporters has been reported. For example, both sulfated neurosteroids have been shown to be transported with high affinity by the organic solute transporter OST $\alpha$ -OST $\beta$  [ $K_m$ : 1.5  $\mu$ M (DHEAS) and 6.9  $\mu$ M (PregS)] (Fang et al., 2010). The heterodimer OST $\alpha$ -OST $\beta$  is a relatively new member of the SLC family (SLC51) and encoded by two genes (SLC51A and SLC51B). Like OATPs, the transport mechanism is facilitated diffusion; therefore OST $\alpha$ -OST $\beta$ -mediated transport is dependent on the electrochemical gradient of its substrates (Ballatori et al., 2013). In the human brain, the transporter is expressed in Purkinje cells and hippocampal neurons (Fang et al., 2010). Both regions are well known for their function in the process of learning and memory, and hippocampal neurons have been suggested as a target site for PregS action (Akwa et al., 2001).

A third group of SLC transporters involved in the CNS distribution of sulfated neurosteroids are the organic anion transporters (OATs), which are part of the SLC22A branch (Burckhardt, 2012). The pivotal role of these transporters is the

excretion of water-soluble organic anions in the kidney. However, selected members are also present in other organs including the brain (Burckhardt, 2012). In the brain OAT3, is probably the most interesting member of this family. The transporter is expressed in the BBB as well as the CP (Alebouyeh et al., 2003; Kikuchi et al., 2003; Uchida et al., 2015). In a mouse model, OAT3 was characterized as the DHEAS transporter in part responsible for the DHEAS efflux across the BBB (Miyajima et al., 2011). Besides OAT3, mRNA expression of OAT1 and OAT2 has also been shown for the human brain (Lopez-Nieto et al., 1997; Alebouyeh et al., 2003; Cropp et al., 2008); however, protein data for these transporters are limited.

## CONCLUSION AND PERSPECTIVES

The detailed functions of the described ABC and SLC transporters in brain are still poorly understood. Even data on the expression and localization, e.g., at the BBB are often controversial. Knock-out mice of the ABC and some SLC transporters are available, but have been mainly used to study the role of these transporters for brain penetration of certain drugs (Dallas et al., 2006; Chaves et al., 2014). These studies are in part hampered by overlapping substrate specificities of several transporters and with respect to neurosteroids by the fact of negligible levels of sulfated steroids in rodent brain (Liu et al., 2003; Liere et al., 2009). Furthermore, a number of functional genetic variants in several transporter genes are known (Bruhn and Cascorbi, 2014); however, their impact on neurosteroid transport is so far largely unknown. Variations in transporter function may affect concentrations and action of several neurosteroids in brain. Therefore, a better understanding of these processes is an important aspect also in the context of a possible therapeutic use of these compounds.

## AUTHOR CONTRIBUTIONS

MG and GJ conceived and wrote the manuscript. PH designed **Figure 1** and revised the manuscript. All authors read and approved the manuscript for publication.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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