Effect of peripherally derived steroid hormones on the expression of steroidogenic enzymes in the rat choroid plexus

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Abstract: Peripherally derived steroids affect steroid production in the brain via the blood–brain barrier. However, steroid concentrations are lower in the cerebrospinal fluid than those in the blood, indicating restricted influx of steroids because of their metabolism by choroid plexus (CP) epithelial cells. Here, we analyzed the gene expression of steroidogenic enzymes [cholesterol side-chain cleavage enzyme (P450scs), 17α-hydroxylase/C17-C20 lyase (P450c17), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), aromatase (Cyp19α1), and 5α-reductase type 1 (5α-R1)]. These genes were expressed to a lesser extent in the CP than in the testis and to a similar extent in the cerebral cortex. However, P450scs levels were higher in the CP than in the cerebral cortex, whereas Cyp19α1 levels showed the opposite trend. We also evaluated the effects of orchietomy and testosterone on the expression of these genes. P450c17 and 5α-R1 levels were unaffected by orchietomy, whereas P450scs and 3β-HSD levels were increased and decreased, respectively. Cyp19α1 expression increased upon testosterone treatment, whereas that of 17β-HSD decreased upon orchietomy or administration of testosterone. Immunohistochemistry analysis revealed that 17β-HSD was expressed in the cytoplasm of CP epithelial cells. These results indicate that CP epithelial cells synthesize and convert the certain types of steroids to contribute to the homeostasis of steroids in the brain. J. Med. Invest. 68: 238-243, August, 2021

Keywords: steroidogenic enzyme, choroid plexus, orchietomy, testosterone, immunohistochemistry

INTRODUCTION

Steroid hormones are produced in the adrenal glands, testis, and ovaries and circulate in the blood to act throughout the body. Neurons in the central nervous system have also been reported to produce steroid hormones de novo (1, 2). Estrogen protects estrogen-producing neurons from oxidative stress (3, 4). Steroid hormones are lipid-soluble molecules that cross membranes and affect the central nervous system. Evidence suggests that the levels of steroid hormones in the cerebrospinal fluid are lower compared to those in the blood. The blood–brain and blood–cerebrospinal fluid barriers block the entry of substances into the brain (5) and may control the inflow of steroid hormones. We predicted that the metabolism of steroid hormones in choroid plexus (CP) epithelial cells is one among the mechanisms responsible for this barrier function.

The CP plays an important role in the nutritional homeostasis of the whole brain via the cerebrospinal fluid (6). Accordingly, CP epithelial cells have been shown to constitute the blood–cerebrospinal fluid barrier, with their distal sides facing the cerebrospinal fluid and proximal ends facing the fenestrated capillary, thereby creating an interface between the cerebrospinal fluid and peripheral blood (7). The levels of D-amino acid oxidase, an enzyme involved in the metabolism of D-amino acids, are lower in the cerebrospinal fluid than those in the blood, similar to steroid hormones (8).

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EXPERIMENTAL PROCEDURES

Animals

Eight-week-old male Wistar rats were subjected to orchietomy or sham operation. One week after the operation, orchietomized animals were divided into 2 groups (n = 4). Rats in one group were subcutaneously administered testosterone (2 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), whereas those in the other group were administered sesame oil (as a solvent) at 10:00 a.m. daily for 7 d. Sham-operated animals were treated in the same manner. All experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals adopted by the Committee on Animal Research in Tokushima University (No, 11031), and were accredited by the Japanese Ministry of

Abbreviations

P450scs: cholesterol side-chain cleavage enzyme, P450c17: 17α-hydroxylase/C17-C20 lyase, 3β-HSD: 3β-hydroxysteroid dehydrogenase, 17β-HSD1: 17β-hydroxysteroid dehydrogenase type 1, Cyp19α1: aromatase, 5α-R1: 5α-reductase type 1, RT-PCR: reverse transcription-polymerase chain reaction, Tes: testis, Ad: adrenal gland, Cx: cerebral cortex, CP: choroid plexus
Rats administered testosterone or sesame oil were decapitated under anesthesia [0.3 mg/kg medetomidine hydrochloride (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), 4 mg/kg midazolam (Astellas Pharma Inc. Tokyo, Japan), and 5 mg/kg butorphanol tartrate (Meiji Seika Pharma Co., Ltd., Tokyo, Japan)] at 3 h after treatment. Subsequently, the brains, testes (Tes), and adrenal glands (Ad) were removed. The cerebral cortices (Cx) of the anterior brain and CP of the fourth ventricle were used for the experiments, whereas the olfactory bulb, hypothalamus, and thalamus were discarded (Alves et al., 2009). Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA). The absorption spectrum (220-350 nm) was measured with a NanoDrop Spectrophotometer (NanoDrop Technologies, USA). RNA quantity and quality were calculated using the NanoDrop spectrometer. The PCR mixture contained 200 ng cDNA, 0.5 μM of each primer, and 1× Go Taq Green Master Mix (Promega, Madison, WI, USA) in a total volume of 25 μL. Amplification was performed using the Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The PCR cycle parameters were as follows: initial denaturation for 5 min at 95°C, denaturation for 30 s at 95°C, primer annealing for 45 s at 51-66°C, extension at 74°C for 45 s, and final extension at 74°C for 7 min. The primer sequences, annealing temperature, PCR cycles, and predicted product size are listed in Table 1. The concentration of the purified product was measured using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). For fluorescence microscopy, the sections were viewed under a microscope (Eclipse E800; Nikon, Tokyo, Japan) after treatment with the primary antibody and ABC kit (Vector Laboratories, Reading, UK). The sections were then stained with uranyl acetate and embedded in Epon epoxy resin (TAAB Laboratories, Reading, UK). Statistical analysis was performed using Student's t-test. p < 0.05 was considered to indicate statistically significant results.

### Table 1. Primers used for semiquantitative reverse transcription-polymerase chain reaction (PCR)

| Enzyme name | Accession No. | Primer sequence | PCR Ta cycles Product (bp) |
|-------------|---------------|-----------------|---------------------------|
| P450scc     | NM_017286     | forward 5'-AGAATTGGTCTCAAACCAAGAGG-3′ reverse 5'-GTGTGGCATTTCATAAAGGTTTC-3′ | 59 33 213 60 35 579 |
| 3β-HSD      | NM_001007719  | forward 5'-GCAGCAGCAGTGTGACATGACAGAGC-3′ reverse 5'-AAGGTTCACAGTGGAATCAAGG-3′ | 62 32 491 |
| P450c17     | NM_012755     | forward 5'-TTGCCACGGTGAGGAGACATC-3′ reverse 5'-ACTGATCTGGCTGGTCCCATT-3′ | 66 35 357 |
| 17β-HSD     | NM_054007     | forward 5'-GCTGGCCGACAGATGGAACTA-3′ reverse 5'-AGTTGGGAGGACATTCCAACA-3′ | 59 33 213 |
| Aromatase   | NM_017085     | forward 5'-CCATCAACGACAGATGGAAC-3′ reverse 5'-TCCACGTCTCTTCAAGGAA-3′ | 53 28 368 |
| 5α-reductase| NM_017070     | forward 5'-CGGCTGACAGACAGATGGAAC-3′ reverse 5'-ACTGATCTGGCTGACT-3′ | 55 33 213 |
| GAPDH       | NM_017008     | forward 5'-GTGAAAGTACCTTGTTGACAG-3′ reverse 5'-GTTGAAGCAGCCAGTGAACCT-3′ | 55 21 300 |

Ta: Annealing temperature.
RESULTS

We performed RT-PCR to evaluate the gene expression of P450scc, P450c17, 3β-HSD, 17β-HSD1, Cyp19a1, and 5α-R1 in the rat CP. The Tes expressed all tested genes, whereas the Ad expressed P450scc, P450c17, 3β-HSD, and 5α-R1 genes but not the 17β-HSD1 and Cyp19a1 genes (Fig. 1). P450scc, P450c17, 3β-HSD, 17β-HSD1, Cyp19a1, and 5α-R1 were found to be expressed in the CP (Fig. 1).

We also examined the influence of peripherally derived testosterone on the expression of steroidogenic enzyme genes by semi-quantitative RT-PCR. The levels of P450scc decreased slightly in rats subjected to orchiectomy but were not significantly different from those in the control group (Fig. 2A). The expression levels of P450c17 and 3β-HSD were unaffected by orchiectomy or testosterone replenishment (Fig. 2B, C). Furthermore, the gene expression of 17β-HSD1 was significantly decreased in the oil-treated group compared to in the testosterone-treated group in sham-operated mice (sham (oil); mean ± SD = 1.33 ± 0.19, sham (Tes); mean ± SD = 0.86 ± 0.08, p = 0.037, Fig. 2D). In contrast, the gene expression level of Cyp19a1 was significantly increased in the testosterone-treated group compared to in the oil-treated group in sham-operated mice (sham (oil); mean ± SD = 0.17 ± 0.24, sham (Tes); mean ± SD = 0.96 ± 0.32, p = 0.028, Fig. 2E). Finally, 5α-R1 levels remained unchanged upon orchiectomy or testosterone replenishment (Fig. 2F).

To investigate whether any of the steroidogenic enzymes is also present in the CP and to histologically confirm the results of gene expression analysis, we performed immunohistochemistry evaluation. We used an anti-17β-HSD1 antibody, which has been reported to be specific (9). Immunostaining revealed immunopositive signals in antibody-reacted tissues compared

Fig. 1. The mRNA levels of steroidogenic enzymes in the rat testis (Tes), adrenal gland (Ad), cerebral cortex (Cx), and choroid plexus (CP). (A) RT-PCR products, and histograms of (B) P450scc, (C) P450c17, (D) 3β-HSD, (E) 17β-HSD1, (F) Cyp19a1, and (G) 5α-R1 comparing expression levels (all n = 4). Histograms represent the results of semiquantitative analysis of the expression of steroidogenic enzymes.
to in preimmune serum-reacted tissues. At the optical microscopy level, these signals were observed in the cytoplasm of CP epithelial cells and not in the connective tissue or blood vessels that surrounded by CP cells (Fig. 3). In addition, to observe the subcellular localization of 17β-HSD1 in the CP by ultrastructure analysis, we applied immunohistochemistry in electron microscopy. Transmission electron microscopy confirmed that 17β-HSD1 was localized to the cytoplasm (Fig. 4).

Fig. 3. Immunohistochemical analysis of rat CP in the fourth ventricle using rabbit anti-17β-HSD1 antibody and rabbit serum (preimmune). The immunoreactivity of 17β-HSD1 was detected in CP epithelial cells. Scale bar = 50 μm.

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### Fig. 2.

mRNA levels of steroidogenic enzymes detected in the rat CP by RT-PCR with (A) P450scc, (B) P450c17, (C) 3β-HSD, (D) 17β-HSD1, (E) Cyp19a1, (F) 5α-R1, and GAPDH-specific primers. Lane 1: sham operation + sesame oil; lane 2: orchiectomy + sesame oil; lane 3: sham operation + testosterone; lane 4: orchiectomy + testosterone (all n = 4). Histograms represent the results of semiquantitative analysis of the expression of steroidogenic enzymes in CP. (A)–(C) and (F) Not significantly different from that of the control group. (D) Significantly decreased in the oil-treated group compared to the testosterone-treated group in sham-operated mice (Sham (oil); mean ± SD = 1.33 ± 0.19, sham (Tes); mean ± SD = 0.86 ± 0.08, p = 0.037). (E) Significantly increased in the testosterone-treated group compared to the oil-treated group in sham-operated mice (Sham (oil); mean ± SD = 0.17 ± 0.24, sham (Tes); mean ± SD = 0.96 ± 0.32, p = 0.026).
DISCUSSION

Steroid hormones selectively permeate the blood–brain barrier (10). Aldosterone and cortisol exhibit low levels of permeability, whereas progesterone, estradiol, and testosterone exhibit high potential for permeability. Hormones with high permeability are produced in the gonads and pass through the blood–brain barrier, and thus may act on neurons or glial cells. However, the correlation between steroid hormones and the CP, which is responsible for maintaining central nervous system homeostasis via the cerebrospinal fluid, remains poorly understood. We found that genes encoding enzymes involved in the metabolism of steroid hormones were expressed in the CP (Fig. 1 and 2). The levels of steroidogenic enzymes in the CP were much lower than those in the Tes (Fig. 1). Thus, we predicted that limited amounts of metabolized steroids flow from the blood to the cerebrospinal fluid. However, the expression of P450ccc was higher than that in the Cx (Fig. 1B). Notably, P450ccc, which is known to result in the production of pregnenolone from cholesterol, was present in the CP, suggesting that CP epithelial cells not only metabolize steroid hormones, but also possess the ability to de novo synthesize steroid hormones from cholesterol. Immunostaining (Fig. 3) and immunoelectron microscopy (Fig. 4) also revealed that 17β-HSD1 was expressed in the cytoplasm in CP epithelial cells, in accordance with previous biochemical results (11).

Taken together, we propose 3 hypotheses for the significance of steroid hormone-metabolizing enzymes in the CP. 1) Enzymes present in CP epithelial cells may act as a cerebrospinal fluid barrier, regulating the influx of steroid hormones from the blood vessels to the cerebrospinal fluid. 2) Steroid hormones may be directly synthesized by CP epithelial cells and secreted into the cerebrospinal fluid. 3) Steroid hormones discharged from the cerebrospinal fluid into the blood may be metabolized in the CP. As these hypotheses could not be verified by functional analysis, we measured the expression of steroid hormone synthesizing enzymes in primary cultured CP epithelial cells isolated from the CP of the lateral ventricle to determine whether steroid hormones were synthesized in these cells.

Androgen receptors are expressed in the brain and the expression levels were altered by steroid hormone levels (12, 13). As such, changes in the expression of steroid hormone-metabolizing enzymes in CP epithelial cells in response to administration of testosterone in this study may have contributed to the variations observed in the levels of steroid hormones in the peripheral blood. This phenomenon indicated that CP epithelial cells perceive peripheral steroids via their respective receptors and metabolize the hormones, thereby providing a potential mechanism for homeostasis of steroid hormones in the cerebrospinal fluid (Fig. 5).

Fig. 4. Transmission electron microscopy of rat CP. Immunoelectron microscopy revealed the cytoplasmic localization of 17β-HSD1. Bar = 5 μm.

Fig. 5. Graphic abstract of steroidogenic enzymes in the rat choroid plexus. The upper half of the image shows a sagittal section of a rat. The pink structures in each ventricle represent the choroid plexus. We analyzed the expression of steroidogenic enzymes in the choroid plexus in the fourth ventricle. Enzymes involved in the steroid synthesis pathway shown in the lower half of the figure were present.
As the expression of steroidogenic enzymes in the CP was much lower than that in Tes, we considered that the metabolism of steroids in the CP was limited upon entry of the hormones into the cerebrospinal fluid from the blood. However, gene expression in the CP changed in the presence of peripheral steroid hormones. Although the gene expression profiles in the CP and Cx were similar for most genes, the expression of P450scc and Cyp19a1 in the CP differed from that in the Cx. Thus, among the steroids required in the brain, some are synthesized as precursors in the CP and may eventually be converted in the brain.

DECLARATION OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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