Comparison between Hippocampus-Synthesized and Circulation-Derived Sex Steroids in the Hippocampus

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Estradiol (E2) and other sex steroids play essential roles in the modulation of synaptic plasticity and neuroprotection in the hippocampus. To clarify the mechanisms for these events, it is important to determine the respective role of circulating *vs.* locally produced sex steroids in the male hippocampus. Liquid chromatography-tandem mass spectrometry in combination with novel derivatization was employed to determine the concentration of sex steroids in adult male rat hippocampus. The hippocampal levels of 17β-E2, testosterone (T), and dihydrotestosterone (DHT) were 8.4, 16.9, and 6.6 nM, respectively, and these levels were significantly higher than circulating levels. The hippocampal estrone (E1) level was, in contrast, very low around 0.015 nM. After castration to deplete circulating high level T, hippocampal levels of T and DHT decreased considerably to 18 and 3%, respectively, whereas E2 level only slightly decreased to 83%. The strong reduction in hippocampal DHT resulting from castration implies that circulating T may be a main origin of DHT. In combination with results obtained from metabolism analysis of [^{3}H]steroids, we suggest that male hippocampal E2 synthesis pathway may be androstenedione $\rightarrow$ T $\rightarrow$ E2 or dehydroepiandrosterone $\rightarrow$ androstenediol $\rightarrow$ T $\rightarrow$ E2 but not androstenedione $\rightarrow$ E1 $\rightarrow$ E2. **(*Endocrinology* 150: 5106–5112, 2009)**

Both circulation-derived sex steroids and endogenously synthesized sex steroids are present in the brain. However, comparison of these sex steroids with both origins is not well clarified about relative content or difference in function. Increasing evidence has accumulated to support the endogenous synthesis of brain-derived steroids (1–6). Synthesis of dehydroepiandrosterone (DHEA), testosterone (T), dihydrotestosterone (DHT) and 17β-estradiol (17β-E2) as well as expression of cytochromes P450(17α) and P450aromatase (P450arom) are demonstrated in the isolated hippocampus from adult rats (7–9). If the level of brain-derived steroids is comparable to or more than that of circulation-derived steroids, then brain-derived steroids may have a significant role in modulation of brain function. A considerable decrease of T has been observed in the whole brain within 1 d after castration (1). It is interesting to examine a resultant content of T and E2 after castration, with an improved methodology, to estimate the level of brain-derived T and E2.

We focus on the hippocampus, which is a good target for the neuromodulatory actions of sex hormones. Extensive studies have been performed *in vivo* to investigate the role of circulation-derived 17β-E2 for female and DHT for male in slow modulation of hippocampal synaptic plasticity (10–12). Estrogens also have specific contributions to rapid action on the synaptic plasticity as neuromodulators (13).

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

doi: 10.1210/en.2009-0305 Received March 10, 2009. Accepted July 2, 2009.
First Published Online July 9, 2009
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Abbreviations: ADione, androstenedione; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E2, estradiol; E1, estrone; 17β-HSD, 17β-hydroxysteroid dehydrogenase; LC-MS/MS, liquid chromatography with tandem mass spectrometry; m/z, mass to charge ratio; P450arom, P450aromatase; PFBz, pentafluorobenzyl; PROG, progesterone; T, testosterone.

In addition to conventional RIA (7, 14), recently, mass spectrometry has been applied to detect brain steroids such as DHEA and T in the whole brain. However, the presence of 17β-E2 or DHT has not yet been observed with mass spectrometry (15–18).

Here, we determine the accurate concentrations of hippocampus-derived estrogens and androgens by employing novel derivatization methods to improve the sensitivity of liquid chromatography with tandem mass spectrometry (LC-MS/MS). The metabolisms and participating enzymes for sex steroid production are investigated to illustrate the complete synthesis pathways in the hippocampus.

Materials and Methods

Animals, castration procedures, and chemicals used in the current study are described in supplemental material (published as supplemental data on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Young adult male or female Wistar rats were used.

Mass-spectrometric assay of steroids

Detailed procedures are described in supplemental material.

Step 1: purification of steroids from hippocampi with normal-phase HPLC

The preparation of hippocampal homogenates from slices and the extraction of steroids were performed as described in supplemental materials and elsewhere (7). [^{3}H]Steroids were added to homogenates as internal standards. The steroid extracts were applied to a C_{18} Amprep solid-phase column (Amersham Biosciences, Arlington Heights, IL) to remove contaminating fats. Then the steroids were separated into fractions of E2, T, DHT plus DHEA, E1, androstenedione (ADione), and progesterone (PROG) using a normal-phase HPLC system (Jasco, Tokyo, Japan) with a silica gel column (Cosmosil 5SL; Nacalai Tesque, Kyoto, Japan). Plasma was prepared by centrifugation from trunk blood collected from decapitated rats (8).

Step 2: derivatization of HPLC-purified steroids before application to LC (reverse phase)-MS/MS (19, 20)

At first, 100 pg isotope-labeled steroids ([$^{13}C_4$]E2, T-d_3, DHT-d_3, [$^{13}C_4$]E1, PROG-d_4, ADione-d_4 and DHEA-d_4,) were added to steroid extracts prepared via step 1). For preparation of E2-pentafluorobenzyl (PFBz)-picolinoyl, evaporated E2 extracts from the hippocampus or evaporated total steroid extracts from plasma were reacted with 5% PFBz bromide/acetonitrile, under KOH/ethanol, for 1 h at 55 C. After evaporation, the products were reacted with 100 μl picolinoic acid solution (2% picolinoic acid, 2% 2-dimethylaminopyridine, 1% 2-methyl-6-nitrobenzoic acid in tetrahydrofuran) and 20 μl triethylamine for 0.5 h at room temperature. The reaction products dissolved in 1% acetic acid were purified using a Bond Elute C_{18} column (Varian, Palo Alto, CA). The dried sample was dissolved in elution solvent of LC. For preparation of T-17-picolinoyl-ester, DHT-17-picolinoyl-ester, E1-17-picolinoyl-ester, and DHEA-3-picolinoyl-ester, evaporated steroid extracts from the hippocampus or plasma were reacted with 100 μl picolinoic acid solution and 20 μl triethylamine for 0.5 h at room temperature. The reaction products were purified with the C_{18} column by using 80% acetonitrile. The purified T, DHT, E1, or DHEA derivative was dissolved in elution solvent of LC.

Step 3: determination of the concentration for 17β-E2, T, DHT, E1, and other steroids using LC-MS/MS

The LC-MS/MS system, which consisted of a reverse-phase LC (Agilent 1100, Agilent Technologies, Santa Clara, CA) coupled with an API 5000 triple-stage quadrupole mass spectrometer (Applied Biosystems, Foster City, CA), was operated with electrospray ionization in the positive-ion mode.

To examine specificity of LC-MS/MS analysis, samples were spiked with steroid isotopes as internal standards. Although the mass to charge ratio (m/z) transitions are different between intact steroids (*e.g.* from m/z = 558 to 339 for E2) and their isotopes (*e.g.* from m/z = 562 to 343 for [$^{13}C_4$]E2), their retention times are the same, because the affinity of intact steroids for the LC column is the same as that for their isotopes (supplemental Fig. S1).

Isotope-labeled steroid derivatives were also used for internal standards to measure recovery of steroids The recoveries of 17β-E2, T, DHT, and E1 were determined as 89 ± 8, 75 ± 4, 73 ± 5, and 76 ± 4%, respectively, after derivatization and MS/MS detection. Total recovery during all the steps was determined via ^{3}H- and isotope-labeled steroids in steps 1 and 2.

The limits of quantification for steroids were measured with blank samples, prepared alongside hippocampal samples through the whole extraction, fractionation, and purification procedures. The limits of quantification for 17β-E2, T, DHT, and E1 were 0.3, 1, 1, and 1 pg per 0.1 g hippocampal tissue or 1 ml plasma, respectively (supplemental Table S2). From the calibration curve using standard steroids dissolved in blank samples, the good linearity was observed (supplemental Fig. S2).

In situ hybridization

The hippocampal frozen slices were treated with proteinase K and postfixed. The mRNAs in the hippocampal slices were hybridized with digoxigenin-labeled sense or antisense cRNA probes. The slices were treated with ribonuclease A and stringent washes after hybridization. Then the slices were incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody. Target mRNAs were visualized by color development with reagent chemicals. Details are described in supplemental material.

Statistical analysis

Data are expressed as mean ± SEM in tables. An unpaired, two-tailed *t* test, under the assumption of unequal variances, was used to test the significance of observed differences between groups. Several numbers of independent experiments from different animals were used to determine the parameters of *t* distribution for the test. Metabolism analysis and RT-PCRs are described in supplemental material.

Results

Mass-spectrometric analysis

The concentration of sex steroids was determined for adult male rat hippocampus using a chromatogram anal-

TABLE 1. Mass-spectrometric analysis of the concentration of steroids in the hippocampus and plasma of adult male rats

	Hippocampus		Plasma	
	Intact	Castrated	Intact	Castrated
17β-E2 (ng/g wet weight or ml)	2.3 ± 0.4 (n = 6)	1.9 ± 0.2 (n = 16)	0.004 ± 0.001 (n = 5)	0.002 ± 0.000 (n = 14)
17β-E2 (nM)	8.4 ± 1.5	6.9 ± 0.8	0.014 ± 0.003	0.006 ± 0.001
T (ng/g wet weight or ml)	4.9 ± 0.7 (n = 8)	0.9 ± 0.2 (n = 16)	4.2 ± 0.5 (n = 8)	0.06 ± 0.02 (n = 16)
T (nM)	16.9 ± 2.3	3.1 ± 0.8	14.6 ± 1.7	0.20 ± 0.08
DHT (ng/g wet weight or ml)	1.9 ± 0.5 (n = 8)	0.06 ± 0.01 (n = 16)	0.18 ± 0.03 (n = 8)	0.012 ± 0.003 (n = 16)
DHT (nM)	6.6 ± 1.7	0.22 ± 0.04	0.63 ± 0.10	0.04 ± 0.01
E1 (ng/g wet weight or ml)	0.004 ± 0.001 (n = 4)	0.003 ± 0.002 (n = 4)	0.002 ± 0.001 (n = 4)	
E1 (nM)	0.015 ± 0.003	0.014 ± 0.006	0.007 ± 0.003	
ADione (ng/g wet weight or ml)	0.43 ± 0.08 (n = 4)	0.48 ± 0.00 (n = 4)	0.17 ± 0.05 (n = 4)	
ADione (nM)	1.5 ± 0.3	1.7 ± 0.0	0.61 ± 0.17	
PROG (ng/g wet weight or ml)	4.6 ± 1.1 (n = 4)	2.2 ± 1.1 (n = 4)	2.1 ± 0.6 (n = 4)	
PROG (nM)	14.6 ± 3.5	7.1 ± 3.4	6.8 ± 1.8	
DHEA (ng/g wet weight or ml)	0.08 ± 0.01 (n = 4)		0.006 ± 0.003 (n = 4)	
DHEA (nM)	0.27 ± 0.05		0.02 ± 0.01	

Intact shows the averaged values from intact and sham-operated rats, because there were no significant differences between these two groups of rats. Data are expressed as mean ± SEM. Number of animals (*i.e.* the number of hippocampi) is shown in *parentheses*. Concentration in nanomolar is calculated using the average volume of 0.14 ml for one whole hippocampus that has 0.14 ± 0.02 g wet weight (n = 86). We assumed that tissue having 1 g wet weight has an approximate volume of 1 ml, because the major part of tissue consists of water whose 1 ml weight is 1 g. The volume should be decreased by less than 10%, due to the specific volumes of proteins and lipids (0.7–0.8 ml/g) (14).

ysis of the fragmented ions of steroid derivatives (supplemental Fig. S1). Results are summarized in Table 1.

Many steroids need derivatization before application to LC-MS/MS to determine their accurate concentrations in the brain where the absolute content of steroids is extremely low. We employ picolinoyl derivatization to improve limit of quantification (supplemental Table S2). In case of E2, PFBz derivatization was additionally performed simultaneously, to increase evaporation probability in electrospray ionization procedures.

Chromatographic profiles for the fragmented ions of E2-PFBz-picolinoyl showed a clear peak with the retention time of 7.01 min, which was the same as that of the standard 17β-E2 derivative (Fig. 1A). The peak corresponding to 17α-E2 was not observed around 6.79 min. In the chromatographic profiles of the fragmented ion of T-17-picolinoyl-ester, a major peak at 4.84 min and a small peak at 5.59 min were observed (Fig. 1B). In the profiles of the fragmented ion of DHT-17-picolinoyl-ester, a major peak at 5.94 min and a small peak at 6.36 min were observed for DHT-picolinoyl (Fig. 1C). In the chromatographic profiles of the fragmented ion of E1–3-picolinoyl-ester, a very small peak at 2.4 min was observed. For these steroids, the retention time of the observed steroid peak was the same as that of standard steroid-picolinoyl.

Using the average hippocampal volume of 0.14 ml (deduced from 0.14 ± 0.02 wet weight for one whole hippocampus of a 12-wk-old rat, n = 86), the average concentrations of 17β-E2, T, and DHT in the hippocampus of intact rats were calculated to be 2.3, 4.9, and 1.9 ng/g wet weight (*i.e.* 8.4, 16.9, and 6.6 nM), respectively (Table 1). In contrast, the hippocampal E1 level was extremely low, 0.004 ng/g (15 pM). The relative concentrations of 17β-E2, T, DHT, and E1 were T higher than 17β-E2 higher than DHT much higher than E1 in that order. Because no significant differences were observed in the concentrations of these steroids between intact and sham-operated rats, we pooled the data from both experimental groups as control values. The average concentration of 17β-E2, T, and DHT in plasma was 0.004, 4.2, and 0.18 ng/ml (*i.e.* 0.014, 14.6, and 0.63 nM), respectively (Table 1). Plasma E1 was approximately 0.002 ng/ml (7 pM).

The concentration of DHT was nearly 10-fold higher in the hippocampus than in plasma. The concentration of T in the hippocampus was higher than that in plasma. As shown in Fig. 1, E and F, for individual rats, a roughly linear relationship was observed between hippocampal T and plasma T and between hippocampal DHT and plasma T. No such linear relationship was observed between hippocampal E2 and plasma T.

Castration was performed to eliminate the contribution of testis-derived sex steroids (T and DHT) via the blood circulation (Table 1 and Fig. 1, D–F). After castration, the concentrations of T and DHT in plasma decreased to roughly 1/100 and 1/15 of their intact levels, respectively. Upon castration, the level of T and DHT in the hippocampus also decreased. T remained, however, at approximately 18% of the intact level (0.9 ng/g = 3.1 nM), implying that 18% of T is endogenously synthesized in the hippocampus. The sum of the castrated residual T level (3.1 nM) in the hippocampus and the intact plasma T level (14.6 nM) was roughly equal to the level of intact hippocampal T (16.9 nM) (Table 1 and Fig. 1E). A considerable decrease in DHT to 3% of the intact level (0.06 ng/g = 0.22 nM) was observed by castration (Table 1 and Fig. 1F). In contrast, castration induced only a slight de-

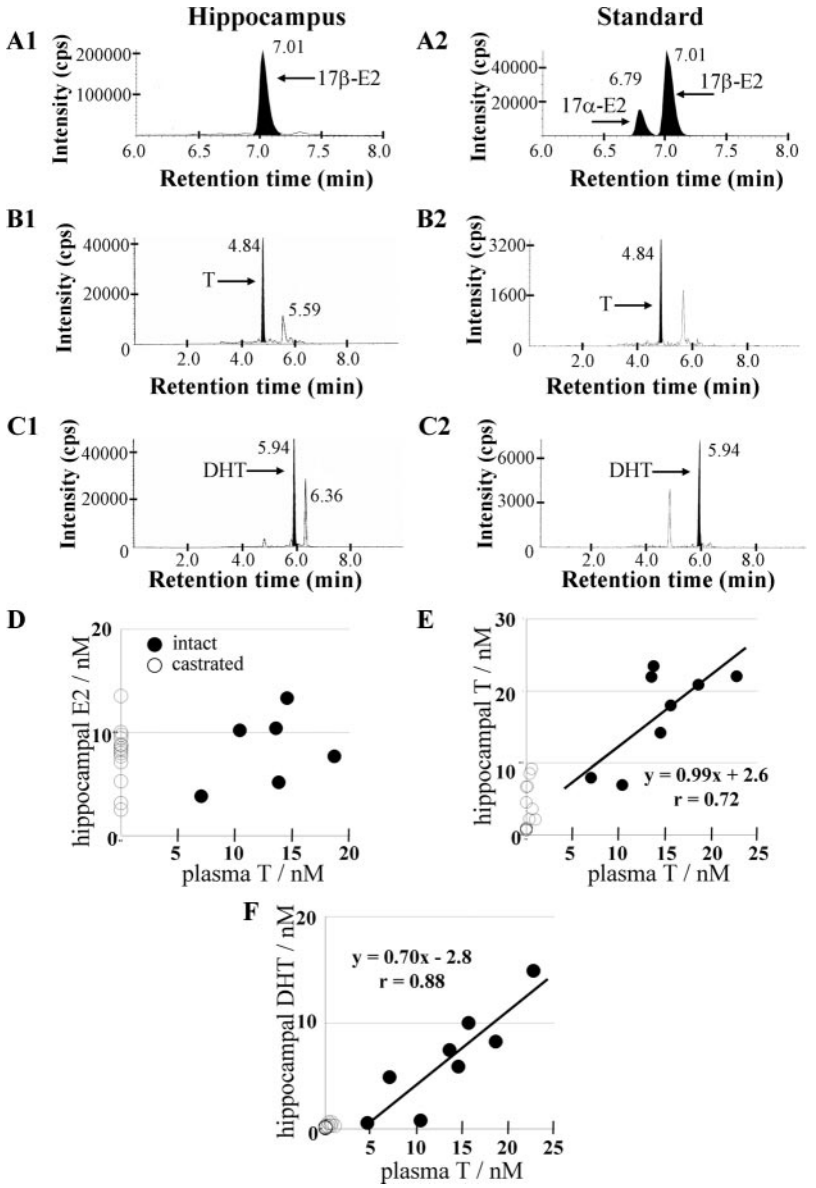

FIG. 1. Mass-spectrometric analysis of hippocampal sex steroids. A–C, LC-MS/MS ion chromatograms of E2 (A), T (B), and DHT (C). A1, B1, and C1 represent the chromatograms of the fragmented ions of each steroid derivative from the hippocampus of adult male rats (12 wk old). Shaded portions indicate the intensity of fragmented ions of E2-PFBz-picolinoyl (m/z = 339, A1), T-picolinoyl (m/z = 253, B1), and DHT-picolinoyl (m/z = 203, C1), respectively. A2, B2, and C2 represent the chromatograms of the fragmented ions of each of the standard steroid derivatives. The *vertical axis* indicates the detected intensity of the fragmented ions of each steroid derivative. The *horizontal axis* indicates the retention time of the fragmented ions; t = 7.01 min for 17β-E2 (A1), t = 4.84 min for T (B1), and t = 5.94 min for DHT (C1). The time of the injection to the LC system was defined as t = 0 min. D–F, Relationship between hippocampal sex steroids and plasma T for individual rats. *Vertical axis* is E2 (D), T (E), or DHT (F). ●, intact rats; ○, castrated rats. Note that a prepurification step using normal-phase HPLC before steroid derivatization was very important to achieve high precision and good reproducibility of LC-MS/MS determination to avoid contamination of other steroids and fats. Derivatized steroids were first separated with the LC column. In the multiple reaction monitoring mode, the instrument monitored the m/z transition (supplemental Table S2). In MS/MS procedures, the mother ion (17β-E2 derivative, m/z = 558) is selected using a first-stage mass spectrometer. This E2 derivative is broken by collision with N_2 gas, and the fragmented ion (m/z = 339) is selected using a second-stage mass spectrometer and detected (supplemental Fig. S1).

crease in the 17β-E2 to 1.9 ng/g (6.9 nM) in the hippocampus (Fig. 1D).

The observed high level of E2 as well as only slight decrease by castration in the male hippocampus are not an artifact of the determination. To prove this, we did determine the hippocampal level of E2 in 12-wk-old Wistar female rats. We could follow the change of the E2 level of the female rat hippocampus dependent on the estrous cycle such as 1.7 ± 0.4 nM at proestrus, 1.0 ± 0.3 nM at estrus, 0.5 ± 0.1 nM at diestrus-1, and 0.7 ± 0.2 nM at diestrus-2. We also observed estrous cycle-dependent plasma E2 level such as 0.120 ± 0.015 nM at proestrus, 0.020 ± 0.015 nM at estrus, 0.008 ± 0.005 nM at diestrus-1, and 0.026 ± 0.005 nM at diestrus-2. Three animals (hippocampi) were used for each condition. These results demonstrate that a nanomolar level of male hippocampal E2 truly exists.

The hippocampal levels of DHEA, PROG, and ADione were determined to be 0.08, 4.6, and 0.43 ng/g, respectively (*i.e.* 0.27, 14.6, and 1.5 nM) (Table 1). The plasma concentrations of these steroids were significantly lower than their hippocampal concentrations (Table 1).

To confirm the assay accuracy, the hippocampal homogenate spiked with known amounts of the steroids was prepared, and its concentration of steroid was determined (supplemental Table S1). Satisfactory accuracy was obtained, supporting the accuracy of determined hippocampal steroid content in Table 1. The limits of quantification were defined in supplemental Table S2 as the lowest value with an acceptable accuracy (90–110%) and precision [*i.e.* relative standard deviation (RSD) <10%]. The results of intra- and interassay were shown in supplemental Table S2. The RSD for intra- and interassay was less than 7.2 and 8.9%, respectively.

Male hippocampal sex steroid metabolism

We needed to analyze the pathway of steroid metabolism (supplemental Fig. S3) because mass-spectromet-

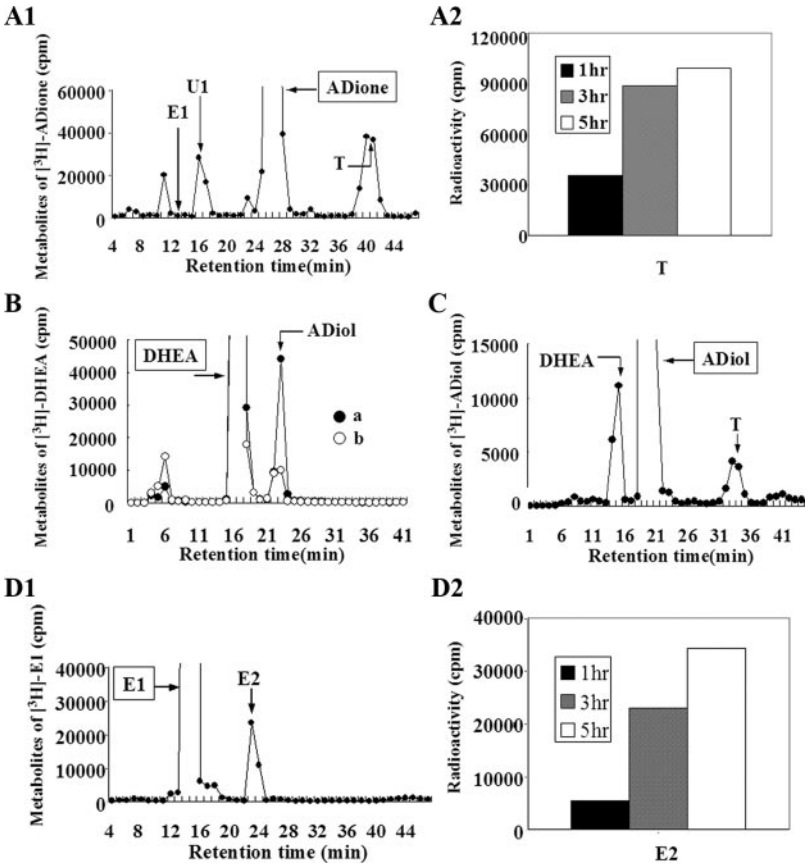

FIG. 2. Normal-phase HPLC analysis of steroid metabolism in hippocampal slices from adult male rat. Hippocampal slices from the adult male rats were incubated with 5×10^6 cpm ^{3}H-labeled steroid substrate for 1, 3, and 5 h. A portion of the purified radioactive metabolites (total of 10^6 cpm) was analyzed using an HPLC system. The rate of steroid production was normalized as products (cpm) per gram wet weight per 5 h. A1, HPLC profiles of [^{3}H]ADione metabolites. U1 indicates unknown product. As judged from the retention time, U1 (17 min) was not E1 (13 min). A2, Time dependence of the production for T from ADione. The production is the average of three experiments. B, HPLC profiles of [^{3}H]DHEA metabolites. Hippocampal slices were incubated in the absence of an inhibitor (line a) or in the presence of 40 μM RM352–26 (28), a specific inhibitor of 17β-HSD type 3 (line b). C, HPLC profiles of [^{3}H]androstenediol (ADiol) metabolites. D1, HPLC profiles of [^{3}H]E1 metabolites. D2, Time dependence of E2 production from E1. E2 production is the average of three experiments. The *arrows* designate the elution peak positions calibrated with standard [^{14}C]steroids. The retention time of the (same) standard [^{14}C]steroid differed between each *panel*, due to the different elution experiments using the different silica gel columns. The *vertical axis* indicates ^{3}H radioactivity (cpm). More than three independent experiments were performed for each of these analyses.

ric determination shows only the contents of individual steroids. The metabolism of radioactive steroids in hippocampal slices was investigated using normal-phase HPLC. Typical results of HPLC analysis are illustrated in Fig. 2, and the production rates are summarized in supplemental Table S3. Detailed results and discussion are described in supplemental material (Table S3 and Figs. S4 and S5).

Molecular biological analysis

Cellular localization and expression of each subtypes of 5α-reductase, 17β-hydroxysteroid dehydrogenase (17β-HSD), 3β-HSD, and 3α-HSD, responsible for sex steroidogenesis, were examined, because this important information had not been fully clarified. The expression level of mRNA for 17β-HSD (types 1 and 3), 3β-HSD type1, 5α-reductase, and P450arom was not changed within 5% by castration, suggesting that the activity of local sex steroid synthesis may not be changed by castration. Typical RT-PCR patterns of mRNA transcripts are shown in supplemental Fig. S6. To identify the cellular localization of 5α-reductase and 17β-HSD (type 1), *in situ* hybridization was performed. Significant expression of 5α-reductase (types 1 and 2) as well as 17β-HSD was observed in pyramidal neurons and granule neurons (supplemental Fig. S7).

Discussion

Higher level of sex steroids in hippocampus than in circulation

Hippocampal levels of these sex steroids were higher than circulating levels (Table 1). Because the volume of hippocampus is very small (nearly 0.1 ml for one whole hippocampus), the calculated concentrations were relatively high in the nanomolar range except for E1. The absolute content of E2 was only around 0.23 ng in one hippocampus with 0.14 g (Table 1). Although steroid production capacity is strong in the gonads (except for E2 in male), circulation levels of steroids are also in the nanomolar range due to dilution in approximately 20 ml blood (200-fold of the hippocampal volume). Although the hippocampal expression levels of enzymes (such as P450arom) are approximately 1/200 of those in gonads (7, 13), they need to fill only a small hippocampal volume (1/200 of the blood volume). Taken together, sex steroid concentrations could be higher in the hippocampus than in the blood. Although 17α-E2 exists in the mouse hippocampus and its level elevates upon castration (21), 17α-E2 was not detected in the hippocampus of intact and castrated male rats in the current study (Fig. 1A). This discrepancy may be due to the difference between mouse and rat.

Modulation of T, E2, and DHT levels by castration

Circulating T has been considered as a major source of male brain E2. Therefore, evaluation of the relative

amount of hippocampus-derived and testis-derived T in the male hippocampus was performed. Circulating T level (14.6 nM = 4.2 ng/ml) is very high in male animals as compared with other circulating sex steroids such as E1 and E2 (around 10 pM = 3 pg/ml) (22, 23). The sum of the residual hippocampal T level after castration and the intact plasma T level was roughly equal to the level of the intact hippocampal T (Table 1). The roughly linear relationship was also observed between plasma T and hippocampal T (Fig. 1E). Collectively, nearly all the circulating T may penetrate into the hippocampus.

The male hippocampal E2 level was only slightly decreased by castration, which significantly decreased hippocampal T (a substrate for E2 production) (Fig. 1, D and E). The high hippocampal E2 level (6.9 nM = 1.9 ng/g) after depletion of circulating T may be maintained by an efficient conversion of hippocampus-derived T (3.1 nM) to E2. Another reason for the high level of hippocampal E2 may be the high stability of E2 in the hippocampus. The conversion of E2 to other metabolites (such as E1) was extremely slow, and nearly 95% of [^{3}H]E2 remained stable even after 5 h (7). Because the levels of hippocampal E1 (current study) and circulating E1 (23) are extremely low in male rats, the T → E2, rather than the E1 → E2, pathway is deduced to be a main pathway in the male hippocampus.

Previous determination of brain sex steroids

The concentrations of 17β-E2, T, and DHEA have been measured using RIA in the hippocampus (4, 7, 8, 29). By using mass spectrometry (15–18), the presence of various steroids such as DHEA, PROG, and T has been demonstrated for the whole-brain extracts of adult rats. However, 17β-E2, E1, and DHT have not yet been detected in those studies. The reported concentrations in whole male brain have been 0.3–2.5 ng/g for T, 0.08–0.27 ng/g for DHEA, and 0.7–1.9 ng/g for PROG (15, 17, 18). These concentrations are qualitatively similar to those observed in the current study. Concerning circulating sex steroids of male rats, the currently measured levels were in reasonable agreement with the reported levels (15, 22, 23).

Functional significance of hippocampus-derived sex steroids

Most of the observed hippocampus-derived sex steroids are probably bioavailable and not just stored in cells. Hippocampus-derived steroids may act locally because they can bind to drive estrogen or androgen receptors within the hippocampal neurons (13). They are not necessary to be transported to outside of the hippocampus, different from the situation of circulating steroids.

Concerning hippocampal function, hippocampus-derived E2 regulates the density of spines and enhances long-term depression as a modulator (13, 24, 25). In pathological aspect, hippocampus-derived E2 may protect neurons from damages such as kainic acid administration (26, 27). We observed sex differences in the steroidogenic pathway (concerning E1, for example) in the hippocampus.

Acknowledgments

We thank Prof. J. A. Rose (Ritsumeikan University) for critical reading of the manuscript. K. Nakajima, M. Fujiwara, M. Watanabe (University of Tokyo), and M. Okuyama (Aska Pharma Medical) are acknowledged for technical assistance. Aska Pharma Medical is a new name of the previous Teikoku Hormone Research Center.

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S. Higo was supported by the Japan Society for the Promotion of Science research fellowships for young scientists (JSPS Research Fellow).

Disclosure Summary: The authors have nothing to disclose.

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