

**NEUROSTEROID BIOSYNTHESIS**  
**IN THE BRAIN AND PITUITARY GLAND**  
**OF SALMONID FISHES**

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**Abstract**

The present study was conducted to investigate the relations between neurosteroid biosynthesis and their possible roles on migratory mechanisms of salmonid fishes, using sockeye salmon (*Oncorhynchus nerka*). First, the ability of brain to produce sex steroid hormones was examined with exogenous steroid substrates during smoltification. It was demonstrated that the brain had the ability to produce sex steroid hormones during smoltification, and the ability tended to be higher in the anterior brain than in the posterior brain. Secondly, the ability of salmon brain and pituitary to synthesize and metabolize cholesterol was examined by *in vitro* thin-layer chromatography method during sexual maturation. It was revealed that pregnenolone was identified as one of cholesterol metabolites in the brain and pituitary gland. These results suggest that neurosteroids are produced in the brain and pituitary gland of sockeye salmon, and might be involved in smoltification and sexual maturation of salmonid fishes.

**Introduction**

It is well established that peripheral sex steroid hormones regulate reproduction and migratory behaviors of salmon by acting on the central nervous system (CNS). Recently, it has been reported that steroid hormones accumulate in the

CNS of many vertebrates through mechanisms independent of peripheral sources (Robel and Baulieu, 1985, Le Goascogne et al., 1987, Tsutsui et al., 1995, 2000), and these molecules have been called “neurosteroid” or “neuroactive steroid”.

Neuroreactive steroid, 3 $\alpha$ -hydroxy-4-pregnen-20-one, which is a metabolite of progesterone, was discovered in rat pituitary and suggested that they selectively suppressed pituitary follicle stimulating hormone secretion by rapid non-genomic interaction with the Ca<sup>2+</sup>-driven cell signaling mechanism (Wiebe et al., 1997). Seasonal profiles of neurosteroid have also been investigated in *Rana nigromaculata*, and the concentrations of pregnenolone and its sulfate ester in the brain were high during the active season and low during the quiescent season, whereas plasma pregnenolone concentrations were virtually constant through the year (Takase et al., 1999).

However, little is known about the production of these steroids in the brain of salmonid fishes. In this study, we examine the ability of sockeye salmon (*Oncorhynchus nerka*) brain and pituitary gland to produce steroid hormone by biochemical analyses in order to investigate the mechanism of neurosteroid biosynthesis during smoltification and sexual maturation.

## Methods

Yearling (1+) sockeye salmon and 3-year-old (3+) lacustrine sockeye salmon were used in this study. After brain and pituitary were removed, brain was cut into small regions by dissection in ice-cold rainbow trout ringer. In 1+ fish, anterior or posterior brain was incubated with exogenous steroid substrates, 17 $\alpha$ -hydroxyprogesterone (OHP<sub>4</sub>) or testosterone (T) for 18 hrs at 15 °C. After incubation, various steroid hormone, such as T, estradiol-17 $\beta$  (E<sub>2</sub>), 11-ketotestosterone (11-KT), and 17 $\alpha$ ,20 $\beta$ -dihydroxy-4-pregnen-3-one (DHP) levels in the incubation medium were measured by time-resolved fluoroimmunoassay. In 3+ fish, each sample of brain regions (7 sections including pituitary) was incubated with <sup>14</sup>C-labeled cholesterol for 18 hrs. After incubation, steroid metabolites in the medium were separated by thin-layer chromatography (TLC) and were identified using recrystallization method.

## Results and Discussion

T, E<sub>2</sub>, 11-KT, and DHP productions in the anterior and posterior brain in the presence of OHP<sub>4</sub> or T was higher than in the absence of these substrates in both sexes. DHP production in the female brain was higher in May, but T, E<sub>2</sub>, and 11-KT productions increased in June (Fig. 1). On the other hand, T and DHP

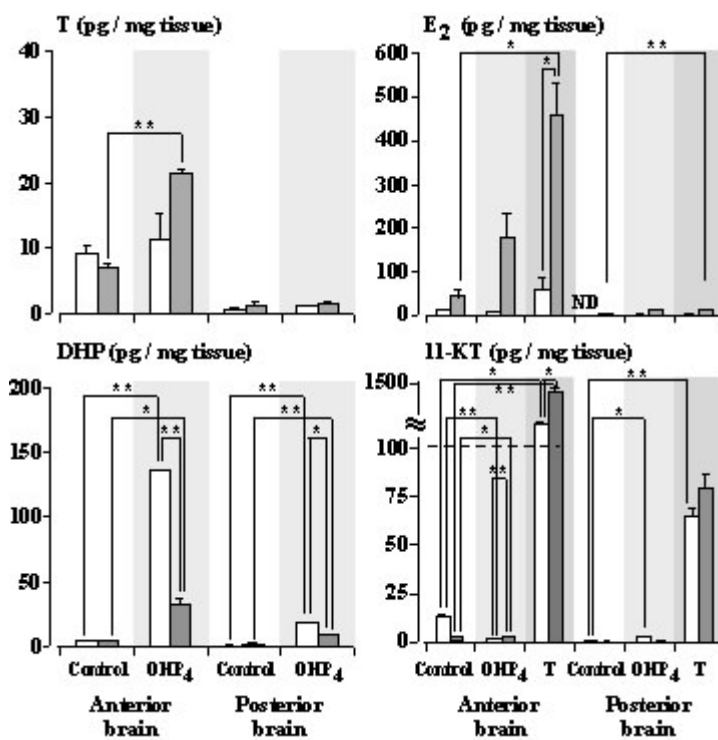


Figure 1. Changes in T, E<sub>2</sub>, 11-KT, and DHP production in the anterior and posterior brain of female sockeye salmon during smoltification in May (open column) and June (closed column) 2001. Each tissue was incubated with 0.1 μg/ml OHP<sub>4</sub> (light shadow phase), T (dark shadow phase), or Ringer only (control). Each values represent the means±SEM of three replicates from three fishes. Significant differences at 5% (\*) and 1% (\*\*) levels are indicated.

production in the male brain was higher in May than in June, but E<sub>2</sub> production increased in June (Fig. 2). Moreover, the ability to produce sex steroids tended to be higher in the anterior brain compared with the posterior brain of both sexes. These data reveal the evidence of the ability of sex steroid hormone production in the brain suggesting the existence of steroid hormone converting enzymes in the brain of sockeye salmon.

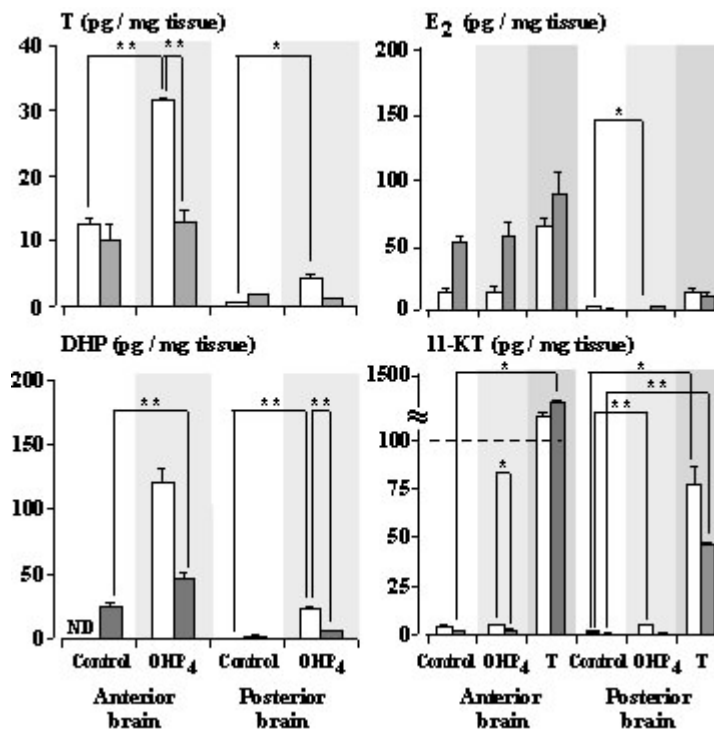


Figure 2. Changes in T, E<sub>2</sub>, 11-KT, and DHP production in the anterior and posterior brain of male sockeye salmon during smoltification from May (open column) to June (closed column) 2001. Each tissue were incubated with 0.1 μg/ml OHP<sub>4</sub> (light shadow phase), T (dark shadow phase), or Ringer only (control). Each values represent the means±SEM of three replicates from three fishes. Significant differences at 5% (\*) and 1% (\*\*) levels are indicated.

In 3+ fish, pregnenolone was identified as one of cholesterol metabolites in the brain and pituitary on August. The ability of olfactory bulb to convert cholesterol is shown in Fig. 4. However, major metabolite band was not identified in this study, and further studies are in progress to examine the detail pathway of neurosteroid biosyntheses from pregnenolone.

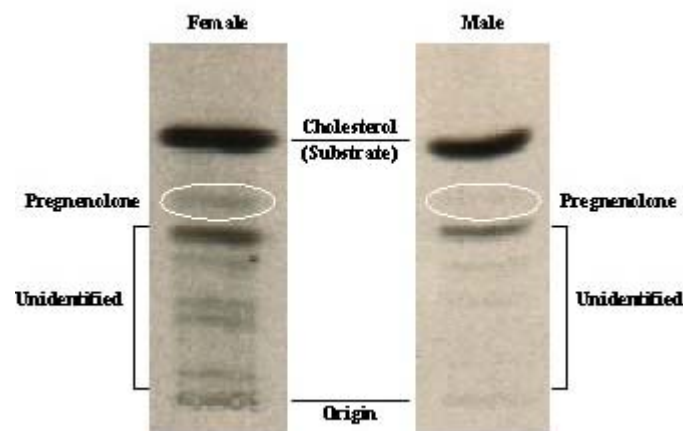


Figure 4. Autoradiographs of TLC plates showing the metabolites by incubating with  $^{14}\text{C}$ -labeled cholesterol in the olfactory bulb of lacustrine sockeye salmon in August.

From these results, it is clearly demonstrated that neurosteroids are produced in the brain and pituitary gland of salmonid fishes. Since smoltification and sexual maturation are believed to correspond with imprinting and homing phenomena of salmonid fishes, roles of neurosteroids are highly interesting to investigate during these critical period of salmon migration. Several intensive biochemical analyses are under investigation to clarify the neurosteroids biosynthesis and their important roles on migratory behavior of salmonid fishes.

## References

- Robel, P. and E.E. Baulieu. 1985. Neuro-steroids, 3 $\alpha$ -hydroxy- $\Delta^5$ -derivatives in the rodent brain. *Neurochem. Int.* 7: 953-958
- Le Goascogne, C., P. Robel, M. Gouézou, N. Sananès, E.E. Baulieu, and M. Waterman. 1987. Neurosteroids: Cytochrome P-450<sub>SCC</sub> in rat brain. *Science*. 237: 1212-1214
- Takase, M., K. Ukene, T. Yamazaki, S. Kominami, and K. Tsutsui. 1999. Pregnenolone, pregnenolone sulfate and cytochrome P450 side-chain cleavage enzyme in amphibian brain and their seasonal changes. *Endocrinology*. 140: 1936-1944
- Tsutsui, K. and K. Yamazaki. 1995. Avian neurosteroids. I. Pregnenolone biosynthesis in the quail brain. *Brain Res.* 678: 1-9
- Tsutsui, K., K. Ukene, M. Usui, H. Sakamoto, and M. Takase. 2000. Novel brain function: biosynthesis and actions of neurosteroids in neurons. *Neurosci. Res.* 36: 261-273
- Wiebe, J. P., D. Boushy, and M. Wolfe. 1997. Synthesis, metabolism and levels of the neuroreactive steroids, 3 $\alpha$ -hydroxy-4-pregnen-20-one (3 $\alpha$ HP), in rat pituitary. *Brain Res.* 764: 158-166