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In vitro 11-ketotestosterone production by the ovary of the Japanese eel, *Anguilla japonica*

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Introduction

11-Ketotestosterone (11KT) is the most potent androgen of fish. In females of some fish species, such as eels, 11KT is found at high concentrations in the serum and plays a role in lipid incorporation into the oocytes. However, the tissue responsible for the production of 11KT has remained unknown. This study aimed to identify whether the ovary possesses the ability to produce 11KT in Japanese eel. Furthermore, the putative biosynthetic pathway toward 11KT production was investigated.

Materials and methods

Eel ovaries in the late-vitellogenic or migratory nucleus stage were incubated with 1000 ng/ml of testosterone (T), androstenedione (A4),11β-hydroxyandrostenedione (11β-OHA4), adrenosterone (11KA4) or 11β-hydroxytestosterone (11β-OHT) as substrate at 20°C for 18 hours. No steroid was detected in the absence of added substrate. After incubation, steroid metabolites extracted from the media were analyzed using liquid chromatography-mass spectrometry (LC/MS). In addition, the production of 11KT was measured by time-resolved fluorescence immunoassay (TR-FIA) if 11β-OHT was used as substrate.

Results

We first investigated whether the eel ovary can produce 11KT from A4 or T. 11KT was not detected if A4 (Fig. 1a) or T (Fig. 1b) were added as substrates. Likewise, 11KA4 was not detected after addition of A4 (Fig. 1a), but 2.1% of A4 was converted to 11β-OHA4 (Fig. 1a), and 9% of A4 was converted to T (Fig. 1a), indicative of strong 17β-hydroxysteroid dehydrogenase (17β-HSD) activity. At the same time, 10% of T was converted to A4 (Fig. 1b), suggesting that eel ovaries have strong 17β-HSD activity, both as oxidase and reductase. Meanwhile, 1.8% and 1.6% of T were converted to 11β-OHA4 and 11β-OHT, respectively (Fig. 1b). We then employed 11β-OHA4 or 11β-OHT as substrates to determine whether the eel ovary could convert these steroids to 11KT. 11β-OHA4 was not converted to any detectable amount of 11KT (Fig. 1c). However, 1.4% and 0.9% of 11β-OHA4 were converted to 11KA4 and 11β-OHT, respectively (Fig. 1c, d).

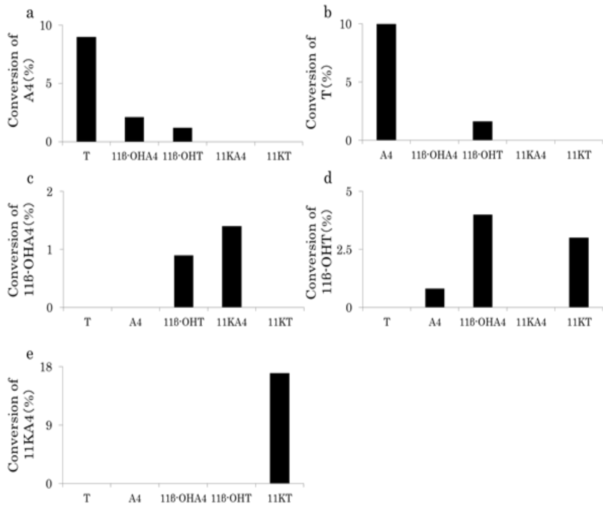

Fig. 1. Conversion ratio of A4 (a), T (b), 11β-OHA4 (c), 11β-OHT (d), 11KA4(e) into a suite of Δ4-androgens by Japanese eel ovarian fragments *in vitro*.

When 11β-OHT was added to the incubation medium, 11β-OHA4 was detected, but it was not possible to detect 11KT by LC/MS because the retention time of 11KT was very close to that of 11β-OHA4. Instead, the levels of 11KT in the media were measured using TR-FIA, identifying a 3% yield of 11KT in incubations supplemented with 11β-OHT (Fig. 1d). Simultaneously, it was estimated that 4 % of 11β-OHT was converted to 11β-OHA4, which suggests that the eel ovary has stronger 17β-oxidase than reductase activity in the presence of 11β-hydroxylated androgen substrates (Fig. 1d). Lastly, when using

11KA4 as substrate, the eel ovary converted 17% to 11KT, suggesting strong 17β-HSD activity (reductase activity) for 11KA4 (Fig. 1e).

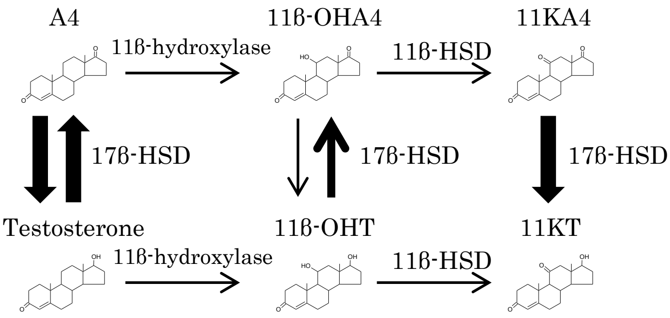

Fig. 2. Relative enzyme activity. The thicker the arrow, the stronger the substrate-supported steroidogenic activity of ovarian folliclesfrom Japanese eel in the late vitellogenic stage.

Discussion

This study did not show direct conversion from A4 or T to 11KT, but the potential for 11KT production in the eel ovary was nonetheless evident (Fig. 2). The eel ovary could produce 11β-OHT from both A4 and T, and conversion of 11β-OHT to 11KT was demonstrated. Our results did not rule out an alternative pathway *via* 11β-OHA4 and 11KA4. A third potential pathway toward 11KT production, the Δ5 (5-ene) pathway, wasnot evaluated, and therefore, conversion of 5-ene steroid substrates, such as 5-androstenediol (5-androsten-3β, 17β-diol) remains to be investigated in a future study.

References

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