HIGH TEMPERATURE CAUSES MASCULINIZATION OF GENETICALLY FEMALE MEDAKA BY ELEVATION OF CORTISOL LEVEL

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Introduction:
In poikilothermic vertebrates, sex determination is sometimes influenced by temperature. Many studies of teleost fish show that environmental factors such as pH, density, and social factors also can change the sex ratio. However, little is known about the molecular mechanisms underlying environmental sex determination in these species. More recently, in the Japanese flounder (Paralichthys olivaceus), which exhibits temperature-dependent sex determination (TSD), cortisol caused female-to-male sex reversal [1]. These observations suggest that cortisol may be involved in TSD of various species.

Medaka (Oryzias latipes) is a small laboratory fish with an XX/XY sex determination system. Recently, it was reported that XX medaka can be sex-reversed into phenotypic males by high water temperature (HT) treatment during the sex differentiation period [2]. To elucidate molecular mechanisms underlying TSD in medaka, we investigated the effects of cortisol, HT and metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone), an inhibitor for 11β-hydroxylase which is involved in cortisol synthesis, on sex differentiation in medaka.

Methods:
The FLFII medaka stock was used [3]. This stock allows identification of genotypic sex by the appearance of leucophores before the onset of sex differentiation. Medaka embryos and larvae were reared in ERM (17 mM NaCl, 0.4 mM KCl, 0.27 mM CaCl₂, 2H₂O, 0.66 mM MgSO₄, pH 7) mixed with or without cortisol (Sigma-Aldrich, Gillingham, UK) at 26°C from 0 days post-fertilization (dpf) to 5 days post-hatching (dph). On the other hand, HT experiment was carried out by rearing them in ERM mixed with or without metyrapone (Sigma-Aldrich) at 33°C from 0 dpf to 5 dph. After treatments, these fishes were maintained up to adults at 26°C under a 14 h light and 10 h dark cycle. Steroid hormones in five pooled fishes were extracted in diethyl ether as described previously [4]. Cortisol levels were measured using a cortisol EIA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. The phenotypic sex of each adult fish was determined by histological observation of gonads [5].

Results and Discussion:
HT causes elevation of whole-body levels of cortisol, while metyrapone inhibits the elevation by HT treatment during sexual differentiation of medaka
Whole-body levels of cortisol were measured by enzyme-immunoassay at 0 dpf in the FLFII medaka larvae reared at 26°C and 33°C during gonadal sex differentiation. At 0 dpf, cortisol levels in the cortisol-treated larvae reared at 26°C or untreated fishes reared at 33°C were significantly higher than those in the untreated larvae reared at 26°C or metyrapone-treated fishes reared at 33°C. This indicates that HT causes elevation of cortisol level, whereas metyrapone inhibits the elevation by HT treatment in the medaka larvae during sex differentiation.

Cortisol causes female-to-male sex reversal and metyrapone inhibits HT-induced masculinization of XX medaka
To investigate whether cortisol induces masculinization of XX medaka in a similar way to HT treatment, we treated the FLFII medaka with or without cortisol at 26°C, or with or without metyrapone at 33°C from 0 dpf to 5 dph. Histological observations at the adult stage showed that both cortisol and 33°C treatments caused female-to-male sex reversal and that the sex-reversed XX fishes had typical testes. Moreover, masculinization by 33°C treatment was completely counteracted by administration of metyrapone. Therefore, we suggest that masculinization of XX medaka by HT treatment may be attributable to an elevation of cortisol level during gonadal sex differentiation.

Conclusion:
This study has presented the first evidence of the involvement of cortisol in TSD of the medaka. We have demonstrated that cortisol causes female-to-male sex reversal and that metyrapone inhibits HT-induced masculinization of XX medaka. HT treatment caused elevation of whole-body levels of cortisol, while metyrapone suppressed the elevation by HT treatment during sexual differentiation. Therefore, these findings strongly suggest that the masculinization of XX medaka induced by HT is mediated by the elevation of cortisol level.
References: