Effect of Hypophysectomy on Sex Transformation in Frog Tadpoles*

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Synopsis

This work aims to clarify whether the pars distalis of the pituitary gland plays an indispensable role in heat-induced sex transformation from ovaries to testes. Intact and hypophysectomized tadpoles of the sex semidifferentiated race of Rana catesbeiana were reared in water at 20°C as controls and at 30°C as experimentals. The results showed that ovaries of the hypophysectomized tadpoles were sex transformed to testes completely or partially both at 20°C and 30°C, demonstrating independence of spontaneous and heat-induced sex transformation on the pars distalis. The findings were in agreement with our previous work that sex transformation of ovaries was independent of thyroid function, and also with reports from other laboratories that sex transformation induced with sex hormones likewise occurred in hypophysectomized as well as in intact tadpoles. Therefore, it is believed that the pars distalis is not essential for sex transformation and that heat delivers, without mediation through endocrine glands, a direct and destructive assault on the cortex of the gonad which elicits the antagonistic activity of the medulla, resulting in sex transformation.

Heat-induced sex transformation from ovaries to testes in frog tadpoles has been an old problem since the first report by Witschi in 1929. However, the mechanism still remains obscure. There exists the possibility that sex transformation may be due to a direct and detrimental assault of heat on the cortex of the gonad, without any mediation through endocrine glands. Contrast to this concept is the well established fact of an intimate developmental and functional relationship between the pars distalis of the pituitary and gonad. The pituitary may play a role in sex transformation. In order to clarify this seemingly conflict, the present experiment was designed to investigate sex differentiation of hypophysectomized tadpoles after heat treatment. The results will elucidate the role of the pituitary gland played in heat-induced sex transformation.

Materials and Methods

Intact and surgically hypophysectomized tadpoles (Rugh, 1962) of the sex semidifferentiated race of Rana catesbeiana from a single mating were used in these experiments. At the age of 3 months after fertilization, the intact tadpoles reared in water at 20°C were at the foot paddle stage and were about 3 gm in body weight while the hypophysectomized ones were still at the hindlimb bud stage, silvery in color and weighing about 2.5 gm. Both groups were then subjected to heat treatment for 2 or 5 months in the aged tap water at 20 ± 1°C as controls and 30 ± 1°C as experimentals. The tadpoles were reared under standard conditions as described previously (Hsü et al., 1971; Yü et al., 1972).

After heat treatment, the tadpoles were killed.
Differentiation of the gonads was examined histologically with hematoxylin and eosin stained serial paraffin sections.

**Results**

The results of sex differentiation and relevant data of the tadpoles are summarized in Table 1.

**I. Sex transformation at 20°C**

At the age of 8 months, 57% of the gonads of intact tadpoles were normal ovaries while the rest consisted of typical testes and partially transformed ovaries in various degrees toward male characteristics (Table 1). This spontaneous sex transformation showed that the tadpoles used belonged to the sex semi-differented race (Hsu and Liang, 1970; Yü et al., 1972).

Sex transformation occurred likewise in hypophysectomized tadpoles (Table 1, HYPX), indicating the pars distalis was not essential for spontaneous sex transformation.

The advancement of sex transformation of 8-month-old tadpoles over that of 5-month-old ones (Table 1) made it clear that transformation progressed with age. However, there was an off limit of this natural sex conversion which would be reached when a balanced 1:1 ratio appeared.

**II. Sex transformation at 30°C**

After heat treatment, ovaries of both intact and hypophysectomized tadpoles were invariably transformed toward the male side (Table 1), demonstrating that heat-induced sex transformation was also independent of the pars distalis. The Table further indicated that a longer period of heat treatment induced more sex converts so that 5-month-treatment for hypophysectomized tadpoles resulted in absence of normal ovaries while 2-month-treatment produced 38% of the gonads as

<table>
<thead>
<tr>
<th>Tadpole</th>
<th>Temperature treatment (°C)</th>
<th>Final age in month</th>
<th>Typical ovary</th>
<th>Intersex</th>
<th>Typical testis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Intermediate</td>
<td>Final</td>
</tr>
<tr>
<td>Intact</td>
<td>20</td>
<td>8M</td>
<td>17 (57%)</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>HYPX</td>
<td>20</td>
<td>5M</td>
<td>4 (80%)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HYPX</td>
<td>20</td>
<td>8M</td>
<td>5 (50%)</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Intact</td>
<td>30, 2M</td>
<td>8M</td>
<td>4 (20%)</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>HYPX</td>
<td>30, 2M</td>
<td>5M</td>
<td>3 (38%)</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HYPX</td>
<td>30, 5M</td>
<td>8M</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td>11</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate % of gonads in respective groups

The photomicrographs are transverse sections through gonads of hypophysectomized tadpoles; all tissues were fixed in Bouin's solution and stained with haematoxylin and eosin.

Fig. 1. Normal immature ovary at 20°C. (×150)
Fig. 2. Sex transformed ovary of intermediate stage, heat-treated for 5 months; the large dark cell was a degenerating auxocyte. (×300)
Fig. 3. Sex transformed ovary of advanced stage; heat-treated for 5 months; arrows show rete cells embracing polymorphonuclear gonia and the vas deferens. (×480)
Fig. 4. Normal immature testis at 20°C. (×480)
normal ovaries.

In contrast to gonad differentiation at 20°C, no off limit of sex transformation was observed at 30°C. The result was concordant with our previous finding and was due to additive effect of heat and gene(s) (Hsu and Liang, 1970; Yü et al., 1972).

Histologically, the process of either spontaneous or heat induced sex transformation in hypophysectomized tadpoles was exactly the same as that observed in intact and thyroidectomized ones (Hsü et al., 1971; Yü et al., 1972). Figure 1 shows a normal ovary of hypophysectomized tadpole consisting of a few peripherally situated oogonia and synaptenes and numerous centrally located auxocytes. The ovary was not different structurally from that of an intact tadpole at the same age. After heat treatment, the transformed ovaries at the initial stage showed proliferation of secondary oogonia, some entering into synaptene stage, and beginning of degeneration of auxocytes. At an intermediate stage, degeneration of auxocytes went on further, resulting in empty spaces in the ovary forming the secondary ovarial sac, and the synaptenes were increased in number (Fig. 2). At the final stage of ovarian transformation, all auxocytes had disappeared; however, some synaptenes were still there; the characteristic rete cells appeared aligning mostly along the rim of the secondary ovarial sac and embracing the now interiorly placed gonia (Fig. 3). At the hilar region of this gonad, a section of the vas deferens was found (Fig. 3). In a section of the posterior part of this gonad, a few degenerated auxocytes were observed among the small germ cells. The presence of degenerated auxocytes added an evidence that this male-like gonad was once an ovary. Finally, a completely transformed ovary was not different from a testis, which was a solid structure composed of rete cells intermingled with spermatogonia (Fig. 4).

At the end of the experiment, the hypophysectomized tadpoles remained at the hindlimb bud stage and albino in color, and no pituitaries were found in all the albino tadpoles after dissection. Thus it is concluded that sex transformation from ovaries to testes does not depend on the presence of the pars distalis.

Discussion

The classical experiment of Smith (1916) as well as later works (Atwell, 1933; Humphrey, 1933; Smith, 1939; Chang, 1955; Chang and Witschi, 1955; Yoshikura, 1959a) all indicated that early hypophysectomy in amphibian larvae did not impair normal differentiation of gonads until the completion of metamorphosis in the controls. The present study not only offered additional evidence for this concept but also demonstrated that the pars distalis played no part either in spontaneous or in heat-induced sex transformation.

The present results agreed with our previous work that sex transformation of ovaries into testes was independent of thyroid function (Yü et al., 1972). The finding also collaborated with those from other laboratories that sex transformation due to sex hormone likewise occurred in hypophysectomized tadpoles as well as in intact ones. Mintz and Foote (1947) observed sex reversal in female tadpoles of *R. catesbeiana* by testosterone injection after hypophysectomy. Similar result with *Pleurodeles* (Mintz and Gallien, 1954) was also reported. Chang and Witschi (1955) found that estradiol in high dose or methyltestosterone modified sex differentiation toward male side in hypophysectomized exactly as in intact tadpoles of *R. pipiens*. In *vitro* ovaries were also liable to sex inversion by the addition of testosterone in the culture medium (Foote and Foote, 1959).

However, our results were at variance with the finding of Yoshikura (1959b, 1961). He took a different view namely that the pituitary gland played an indispensable role in sex transformation. The belief was based on his
results that ovaries of hyposectomized tadpoles were not transformed into testes at 30°C, whereas the same heat treatment produced masculinization of ovaries with pituitary grafts in hypophysectomized tadpoles. The controversy could be due to difference in sex race: differentiated race in Yoshikura’s experiment and semidifferentiated race in our materials. However, we had discussed this point in our previous paper (Yü et al., 1972) and concluded that there should be no differential response by these two races to heat treatment.

When Yoshikura’s data and plates were scrutinized carefully, it appeared that his heat-treated hypophysectomized tadpoles did exhibit some masculinized ovaries. He admitted there were three intersexes in hypophysectomized tadpoles after heat treatment (Re Table 2; Yoshikura, 1959b); there should be more in our view. Yoshikura classified ovaries into 6 types according to decreasing normality; types 4 and 5 were regarded as masculinized ovaries. However, there listed three of type 4 ovaries in his heat-treated hypophysectomized tadpoles (Re Table 4; Yoshikura, 1961).

Therefore, we believe that the difference of opinion between his and ours rested on recognition of the transformed or masculinized ovaries. We then suggest that Yoshikura might have misinterpreted his results and that the concept of dependence of sex transformation on the presence of the pituitary gland could be erroneous. We propose, therefore, that heat delivers, without mediation through endocrine glands, a direct and destructive effect on the cortex of the gonad which elicits the antagonistic activity of the medulla, resulting in sex transformation.

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References