119. Effect of Neurohypophyseal Hormones on Thyroxine-Induced Resorption of Tadpole Tail *in vitro*

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According to Platt and his coworkers, injections of oxytocin (OT), lysine-vasopressin (LVP) or arginine vasotocin (AVT) into *Ambystoma tigrinum* larvae,^{11),12)} invariably caused an acceleration of gill resorption, whereas tail fin resorption was promoted only by injections of OT, the other hormones being without effects. Since it is well established that amphibian neurohypophyseal hormones are mesotocin (MT) and AVT,^{1),3),9)} the present study was undertaken to see whether either of these two hormones has any effect on thyroxin (T_4)-induced shrinkage of tail tissue of toad tadpoles *in vitro*.

Materials and methods. Premetamorphic tadpoles of *Bufo japonicus formosus* were used. A 4 mm-long segment excised from tail of each tadpole at the region about 10 mm apart from its base was cultured at 22°C in a well of the culture plate (16 mm in diameter, Corning) containing 2 ml of Gey's medium diluted to adjust to amphibians⁵⁾ (pH 7.2) in a sterile condition. The medium was supplemented with T_4 (2.5×10^{-7} M; Sigma) and/or either MT (20–200 ng/ml; Bachem) or AVT (2–200 ng/ml; Cambridge Research Biochemical). Throughout the experimental period, daily profiles of each tail piece were drawn on drawing paper using a microprojector (Bellco Glass Inc., JPV-194) and the areas were measured with an automatic area meter (Hayashi Denko). The extent of tail resorption was estimated as the percentage change from the initial area.

Results. Segments of tail cultured in the medium containing T_4 began to shrink on the day following the initiation of culture and after 3 days, they decreased to about 40% of the initial size (Figs. 1 and 2). Acceleration by MT (20–200 ng/ml) of T_4 -induced shrinkage was clear-cut 2 days after the start of culture (Fig. 1). In the absence of T_4 , MT did not call forth any visible change in segments. By contrast, AVT (2–200 ng/ml) was without effect on tail segments, regardless of the presence or absence of T_4 (Fig. 2). In the latter case, only representative data (culture in 20 ng/ml AVT) are shown.

Discussion. Platt and LiClause²⁾ have reported that OT administration brings about an acceleration of T_4 -induced resorption of tail fin in larval *Ambystoma tigrinum* and abolishes the anti-metamorphic action of prolactin on the fin. According to these workers, OT has a dehydrating effect on the explanted tail tissue.¹²⁾ Since T_4 induces a loss of water and sodium from larval tiger salamander tissue and prolactin promotes their retention,¹⁰⁾ they concluded that OT accelerates T_4 -induced metamorphosis and block anti-metamorphic action of prolactin through its diuretic effect on the tissue.

Fig. 1

Fig. 2

Figs. 1 and 2. 1: Effect of MT on T_4 -induced resorption of tail segment of toad tadpoles. Tail segments were maintained in medium containing 2.5×10^{-7} M T_4 (—●—), T_4 +20 ng/ml MT (—■—), T_4 +200 ng/ml MT (—▲—), 20 ng/ml MT (—□—), 200 ng/ml MT (—△—), or in control medium (—○—). Each point and vertical line represent the mean of 6–8 determinations and standard error of the mean, respectively. Means with the same superscript do not differ significantly from each other at $P < 0.05$ (Student's *t*-test). 2: Effect of AVT on T_4 -induced resorption of tail segments of toad tadpoles. Tail segments were cultured in medium containing 2.5×10^{-7} T_4 (—●—), T_4 +2 ng/ml AVT (—▼—), T_4 +20 ng/ml AVT (—■—), T_4 +200 ng/ml AVT (—▲—), 20 ng/AVT (—□—), or in control medium (—○—). Each point and vertical line represent the mean of 6 determinations and standard error of the mean, respectively. Means with the same superscript do not differ significantly from each other at $P < 0.05$ (Student's *t*-test).

The present experiment revealed that MT, one of the two amphibian neurohypophyseal hormones, was effective in accelerating T_4 -induced shrinkage of tail segments of toad tadpoles by acting directly on the tissue. At present, however, the mechanism of action of MT on the tail is not clear, although it is known that MT has diuretic effect on many amphibians.^{4),8)}

AVT, another neurohypophyseal hormone of amphibians, was found to be ineffective in accelerating T_4 -induced shrinkage of tail segments. Platt and Hill have also reported that administration of AVT to tiger salamander larvae had no effect on tail fin, although AVT interfered with the inhibition of tail fin regression by prolactin.¹¹⁾

Recently, evidence has accumulated for the involvement of hormones other than thyroid hormone, i.e. prolactin and adrenal steroids in the process of amphibian metamorphosis.^{2),6),13)} The findings reported here indicate that MT accelerates T_4 -induced shrinkage of tadpole tail *in vitro*. However, concentrations of MT in the frog circulation as measured by radioimmunoassay are reported to be 2–20 pg/ml, which are much lower than those used in the present experiment.⁷⁾ Further studies are needed to determine whether the neurohypophyseal hormone plays a physiological role in regulating amphibian metamorphosis.

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