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Skull development during anuran metamorphosis

II. Role of thyroid hormone in osteogenesis

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Summary. We examined the role of thyroid hormone (TH) in mediating cranial ossification during metamorphosis in the Oriental fire-bellied toad, *Bombina orientalis*. Exogenous T_3 (3,3′,5-triiodo-L-thyronine) was administered in three treatment dosages (0.025, 0.25, and 2.5 μg) plus a control dosage via plastic micropellets implanted within the dermis of tadpoles of three Gosner developmental stages: 28/29, 30/31, 32/33. Tadpoles were recovered after 2, 4, 6, and 8 d, and scored for the presence of three bones – median parasphenoid and paired frontoparietals and exoccipitals – as seen in cleared-and-stained, whole-mount preparations. T_3 induced precocious ossification in both a stage-dependent and a dosage-dependent manner; stage dependence corresponded precisely with the degree of osteogenic differentiation at the time of hormone administration. Precocious ossification thus was due to the T_3-promoted growth and calcified matrix deposition of these centers. Differential TH sensitivity among osteogenic sites may underlie both the temporal cranial ossification sequences characteristic of metamorphosing amphibians as well as sequence differences commonly observed among taxa.

Key words: Thyroid hormone – Osteogenesis – Skull – Metamorphosis – Amphibian

Introduction

Cranial metamorphosis in anuran amphibians comprises a large-scale transformation from the cartilaginous skull of the larva, or tadpole, into the predominantly bony skull of postmetamorphic froglets and adults. The primary role of thyroid hormone (TH) in mediating similarly extensive metamorphic transformations of non-skeletal tissues in amphibians is extensively documented (reviewed by Dodd and Dodd 1976; White and Nicoll 1981), yet relatively few studies have specifically addressed the effects of TH on the skeleton, either cranial or postcranial, and our understanding of these effects is limited. For example, exogenous TH applied to amphibian larvae initiates precocious ossification (Terry 1918; Fox and Irving 1950; Kaltenbach 1953a; Dent et al. 1955; Kühn and Hammer 1956; Dundee 1957, 1961; Dent and Kirby-Smith 1963; Kemp and Hoyt 1965a, b, c, 1969a, b; Yeatman 1967), but in most cases it is not known whether the hormone acts by initiating osteogenic tissue differentiation or by stimulating matrix synthesis and calcification of pre-existing ossification centers. Furthermore, many other aspects have not been addressed, such as the degree to which TH effects are stage or dosage dependent and the role of TH in subsequent bone growth and remodeling. This dearth of detailed knowledge about the role of TH in amphibian skeletal development is especially surprising in view of the predominant role of TH in skeletal development, growth, and remodeling in amniotes (reviewed by Jowsey and Detenbeck 1969; Raisz et al. 1978; Reddi 1982; Silbermann 1983; Nijweide et al. 1986).

We earlier examined the sequence and timing of cranial ossification during metamorphosis in the Oriental fire-bellied toad, *Bombina orientalis*, a morphologically generalized, primitive anuran of the family Discoglossidae (Hanken and Hall 1984, 1988). In the present paper, we extend our focus on the early development of the osteocranium in this species by specifically addressing the role of TH in mediating the initial formation of the first cranial bones to appear – the exoccipital, the parasphenoid, and the frontoparietal. In particular, we sought to answer the following questions: (1) Does exogenous TH applied early in the larval period induce precocious cranial ossification? (2) What is the mechanism of hormone-induced osteogenesis, i.e., does TH affect tissue differentiation or proliferation of pre-existing centers? (3) Is the response to TH stage dependent? (4) Is the response to TH dosage dependent? The results reveal that exogenous TH does initiate precocious ossification that is both stage and dosage dependent. Moreover, the hormone acts specifically by accelerating growth and calcified matrix deposition within already differentiated ossification centers which typically don't proliferate until later in metamorphosis. These results, combined with others that document a role of TH in osteogenic differentiation, attest to a previously unappreciated, yet primary role of TH in mediating cranial ossification during anuran metamorphosis. They also support an endocrine model for the regulation of temporal patterns of cranial ossification in amphibians, and for the evolution of interspecific differences in such patterns.

Materials and methods

Tadpoles of *Bombina orientalis* were derived from laboratory crosses among commercially bought adults. Development was staged according to the scheme of Gosner (1960), which defines a total of 46 stages from fertilization through

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Table 1. External features of tadpoles after treatment for 8 d (all groups) and 14 d (control groups only; values are in parentheses). *SVL*, snout-vent length; *TL*, tail length; *Δ*, mean increase in Gosner developmental stage during treatment period. N equals five specimens for all values, which denote mean ± S.E.

Dosage	Implant stage	SVL	TL	Gosner stage	Δ
Control	28/29	14.7±1.0	17.9±0.8	32.4±0.8	4.0
		(15.2±0.9)	(20.0±0.8)	(35.8±0.4)	(7.4)
	30/31	15.3±0.5	20.5±1.1	35.6±0.5	4.8
		(15.8±0.8)	(21.2±0.9)	(37.0±0)	(6.2)
	32/33	16.2±0.3	21.0±0.3	36.6±0.5	4.2
		(15.8±0.8)	(21.0±2.3)	(37.4±0.5)	(5.0)
0.025 μg T_3	28/29	11.6±2.2	16.2±1.9	34.0±1.1	5.6
	30/31	14.0±0.6	18.9±1.3	35.4±0.5	5.0
	32/33	13.9±0.7	20.3±1.5	36.2±0.4	3.6
0.25 μg T_3	28/29	9.6±0.7	15.9±1.2	34.5±0.6	5.9
	30/31	9.0±0.8	14.1±2.3	36.0±0	5.4
	32/33	11.9±0.7	20.7±1.7	36.8±0.4	4.4
2.5 μg T_3	28/29	8.5±1.3	15.3±1.6	34.8±0.4	6.2
	30/31	9.8±0.6	17.7±1.4	36.6±0.5	5.6
	32/33	10.6±0.5	18.0±3.1	37.0±0	4.0

metamorphosis. Breeding and rearing followed standard methods (Carlson and Ellinger 1980; Frost 1982). Eggs and fully formed tadpoles (Gosner stages 1–26) were reared in 10% Holtfreter's solution (Hamburger 1960); later stages were maintained in 20% modified Holtfreter's solution buffered with TRIZMA-7.2 (Sigma Chemical Co.). All specimens were reared at 18° ± 1° C with an alternating 12L:12D photoperiod.

Thyroid hormone (3,3′,5-triiodo-L-thyronine; T_3; Sigma Chemical Co.) was administered via dermal implants of plastic micropellets. In choosing T_3, we relied on recent evidence that it, and not T_4, has the major role in inducing metamorphosis in target tissues (Buscaglia et al. 1985). Micropellets were prepared following the procedures of Silberstein and Daniel (1982) as modified by Dr. Leland Chung, University of Texas Health Sciences Center (personal communication). Powdered hormone first was dissolved in 500 μl dimethyl sulfoxide, to which was added successively 500 μl absolute ethanol, 10 ml methylene chloride, and 1 g 95%-ethanol-washed plastic (Elvax 40P). Once the plastic dissolved (c. 30 min), the solution was poured into a plastic petri dish and quick frozen in an acetone-dry-ice bath for 20 min. The frozen block of plastic then was removed to a freezer (−20° C) for 2 d, before being vacuum-dried (−70 k-Pa) at room temperature for another 2 d.

Blocks of plastic were prepared containing 0, 0.1, 1.0, and 10 mg T_3 per g Elvax. Rectangular pellets weighing 0.25 ± 0.05 mg were cut from each block with a fine scalpel and a pair of forceps. Thus, each pellet contained one of four dosages of T_3: 0 (control), 0.025, 0.25, and 2.5 μg. Plastic blocks and cut pellets were stored desiccated at −20° C for up to 1 yr; there was no noticeable loss of potency during this time.

Pellets were implanted into tadpoles at three Gosner stages: 28/29, 30/31, and 32/33. Tadpoles first were anaesthetized in 0.03% aqueous 3-aminobenzoic acid ethyl ester buffered to pH 7.0 with sodium bicarbonate (Robinson and Scadding 1983), and then the implants placed dorsally within the dermis on the right side of the head, posteromedial to the eye and lateral to the braincase. The implant site was prepared by using a scalpel to make a longitudinal incision immediately posterior to the eye, inserting an iris scissors through the incision, and eroding a tiny pocket into which the pellet could be placed using forceps.

Tadpoles with implants were removed immediately to individual styrofoam soup cups containing 300 ml rearing medium; gentamicin sulfate, 5 mg/l, was added to prevent infection. Bleeding and other side effects of the operation were minimal; all tadpoles recovered within a few minutes of the operation. Soup cups, medium, and food were replaced every 2 d.

Tadpoles in each treatment group (dosage X implant stage) were recovered 2, 4, 6, and 8 d following implant. Control groups also were sampled after 14 d. Each animal first was anaesthetized in 30% aqueous 1,1,1-trichloro-2-methyl-2-propanol and then preserved in 10% neutral-buffered formalin. All animals were measured (snout-vent length and tail length), staged, and processed as bone-and-cartilage-stained whole mounts (Wassersug 1976; Hanken and Wassersug 1981). An ossification score was calculated for each specimen, which equaled the number of cranial bones present as revealed by red-stained, calcified matrix (paired bones were scored as one-half point for each side).

Results

External effects

Control groups

Tadpoles that received control pellets continued to develop normally but envinced little change in external cranial morphology (Table 1; Fig. 1). After 8 d, no specimen implanted at stages 28/29 or 30/31 had reached stage 37, when cranial bones are first visible in cleared-and-stained preparations of normal, i.e., untreated, specimens (Hanken and Hall 1984); three of the five specimens implanted at stages 32/33, however, had reached this stage. After 14 d, none of the implant-stage 28/29 tadpoles had reached stage 37, whereas nearly all tadpoles that received implants at stages 30/31 and 32/33 had reached this or the next stage.

T_3-treated groups

External development in T_3-treated tadpoles was decidedly different from that in controls (Table 1; Fig. 2). First, in

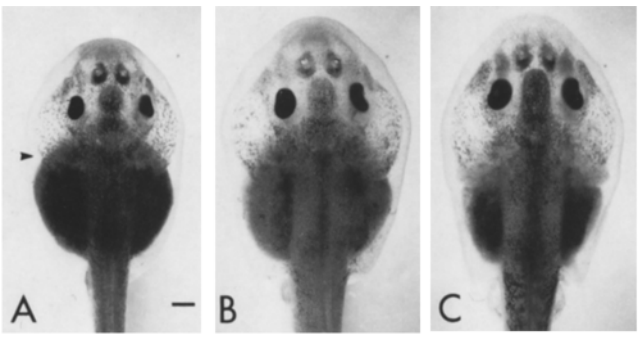

Fig. 1. External development in control tadpoles preserved after (**A**) 1, (**B**) 8, and (**C**) 14 d. Implant stage 32/33. Arrow in A indicates posterior boundary of head adjacent to rudimentary forelimbs. Scale bar, 1 mm

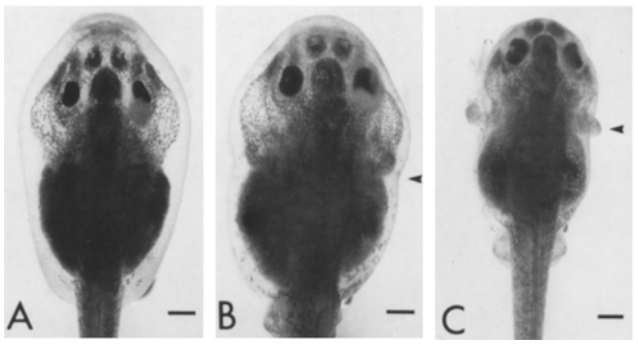

Fig. 2. External development in T_3-treated tadpoles preserved after (**A**) 1, (**B**) 4, and (**C**) 8 d. Implant stage 32/33; dosage, 2.5 μg T_3. Intact pellets are visible immediately posterior to the right eye in **A** and **B**; *arrows* in **B** and **C** indicate the transverse constriction described in the text. Scale bar, 1 mm

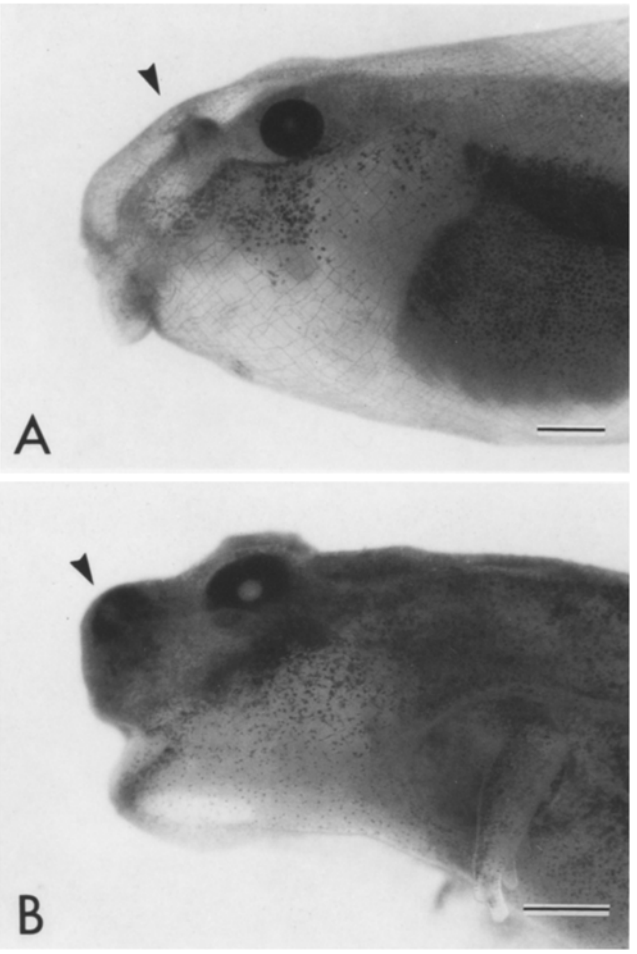

Fig. 3. Heads of (**A**) control and (**B**) T_3-treated tadpoles after 8 d. Note altered relative positions of the mouth, nostril (*arrow*), and eye in the treated specimen, which received a pellet containing 2.5 μg T_3 (highest dosage). Both specimens received implants at stage 30/31. Scale bar, 1 mm

each treatment group, snout-vent length after 8 d was smaller than in controls, reflecting the overall shortening of the body which occurs at this time. Second, development generally was accelerated, as sequential changes were "compressed" into an abnormally short interval. Third, the magnitude of these effects was proportional to dosage. And, although these effects were visible throughout the body, there was a distinct response gradient: anterior structures near the pellet, such as the mouth, typically were far advanced, whereas posterior structures, such as the hind limb and tail, remained characteristic of earlier stages. For example, over the range of stages involved here, Gosner stage reflects relative hind limb development. After 8 d, the mean stage attained by most treatment groups was not statistically different from that attained by controls ($p > 0.05$), despite obvious differences in cranial morphology. Indeed, in two of the three groups receiving the highest T_3 dosage (2.5 μg), mean increase in Gosner stage over 8 d was less than in controls. Despite the obvious anteroposterior response gradient, there was no apparent difference in response between right and left sides, cranially or postcranially, even though all pellets were implanted unilaterally.

Changes in head shape and configuration of the mouth in treated specimens can be related to the internal modifications of the cranium. Viewed dorsally, these changes included foreshortening of the snout and the appearance of a transverse constriction between the head and trunk (Fig. 2C). Viewed laterally, they included posterior migration of the mouth and jaw angle to a position ventral, and sometimes posterior, to the nostrils, loss of horny beak and resorption of circumoral papillae (Fig. 3). These effects were most pronounced in response to the higher T_3 dosages but were evoked at all implant stages.

General effects on the skull

Control groups

Aside from modest growth, skulls of control specimens were virtually unchanged after 8 d (Figs. 4A, 5A, B; Table 2). Cartilages remained in their larval configuration and no bone was visible in cleared-and-stained preparations. Larval, calcified endolymphatic sacs remained prominent in most specimens.

After 14 d, up to three cranial bones were visible: the paired frontoparietals and median parasphenoid, formed by intramembranous ossification, and the paired exoccipitals, formed by endochondral ossification (Fig. 4B; Table 2). Even in the most advanced specimen, however, ossification is still at an early stage (Fig. 5C, D). Each exoccipital comprises a bony cylinder investing the cartilaginous occipital arch which connects the ventromedial portion of

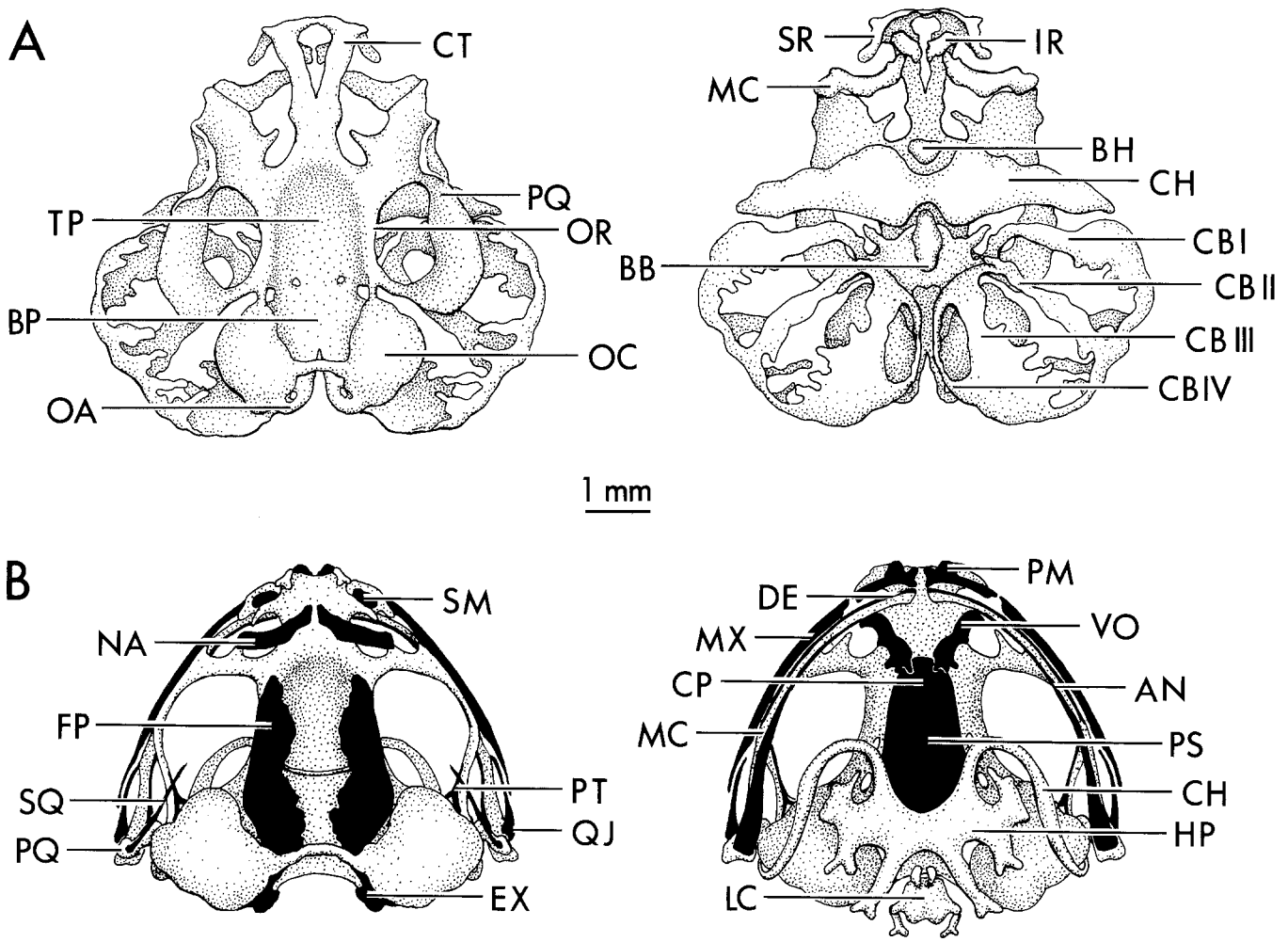

Fig. 4. Pre- and postmetamorphic skulls of *Bombina orientalis*, drawn from cleared-and-stained whole mounts: **A** Larva, Gosner stage 36. **B** Postmetamorphic froglet, stage 46. Left, dorsal views; right, ventral views. Cartilage is stippled; bone is solid black. The larval skull is very similar to that of earlier, implanted stages. The postmetamorphic specimen does not depict the full, adult complement of cranial bones, many of which are yet to form. Similarly, bones already present will become more extensive during subsequent growth and development. *Abbreviations: AN*, angulosplenial; *BB*, basibranchial cartilage; *BH*, basihyal cartilage; *BP*, basal plate; *CB I–IV*, ceratobranchial cartilages I–IV; CH, ceratohyal cartilage; *CP*, cultriform process; *CT*, cornu trabeculae; *DE*, dentary; *EX*, exoccipital; *FP*, frontoparietal; *HP*, hypobranchial plate; *IR*, infrarostral cartilage; *LC*, laryngeal cartilage; *MC*, Meckel's cartilage; *MX*, maxilla; *NA*, nasal; *OA*, occipital arch; *OC*, otic capsule; *OR*, orbital cartilage; *PM*, premaxilla; *PQ*, palatoquadrate cartilage; *PS*, parasphenoid; *PT*, pterygoid; *QJ*, quadratojugal; *SM*, septomaxilla; *SQ*, squamosal; *SR*, suprarostral cartilage; *TP*, trabecular plate; *VO*, vomer

the otic capsule with the basal plate; they both also extend onto the dorsomedial wall of their respective otic capsules. The parasphenoid is a very thin sheet of bone underlying the basal and trabecular plates. Its single, median cultriform process extends anteriorly from the level of the synotic tectum two-thirds of the way to the nasal capsules; its paired lateral wings, or alae, are rudimentary and do not yet extend very far beneath the adjacent otic capsules. Frontoparietals comprise paired, longitudinal splints of bone that run along almost the entire dorsolateral edge of the braincase, but they converge medially from the orbital cartilages no more than one third the distance to the dorsal midline. No significant structural changes in cranial cartilages were evident in any specimen (Fig. 5C, D).

T_3-treated groups

In general, exogenous T_3 initiated precocious cranial ossification (Fig. 6C, D; Table 2). As many as three bones were visible after 8 d; as in the control groups, these were the parasphenoid, the frontoparietal, and the exoccipital. Effects on the osteocranium typically were not visible until day 6; one specimen implanted with the highest dosage at Gosner 30/31 had a visible parasphenoid after 4 d. Cranial ossification in the most advanced treated specimen was comparable to that in the most advanced control specimen recovered after 14 d (see above). Many treated specimens had no bone, however, even after 8 d.

T_3 treatment also initiated the metamorphosis of larval cartilages (Fig. 6A–D). This included reorientation and remodeling of palatoquadrate and Meckel's cartilages, resorption of cornu trabeculae, suprarostral and infrarostral cartilages, and repatterning of the hyobranchial skeleton. While independent of implant stage, this response was clearly dosage dependent (Hanken and Summers 1988). Effects on cartilage, unlike those on bone, were visible as early as 2 d and were well advanced by 4 d.

As with external effects, there was no apparent right-left asymmetry in the response of the developing cranial skeleton to the unilateral hormone administration.

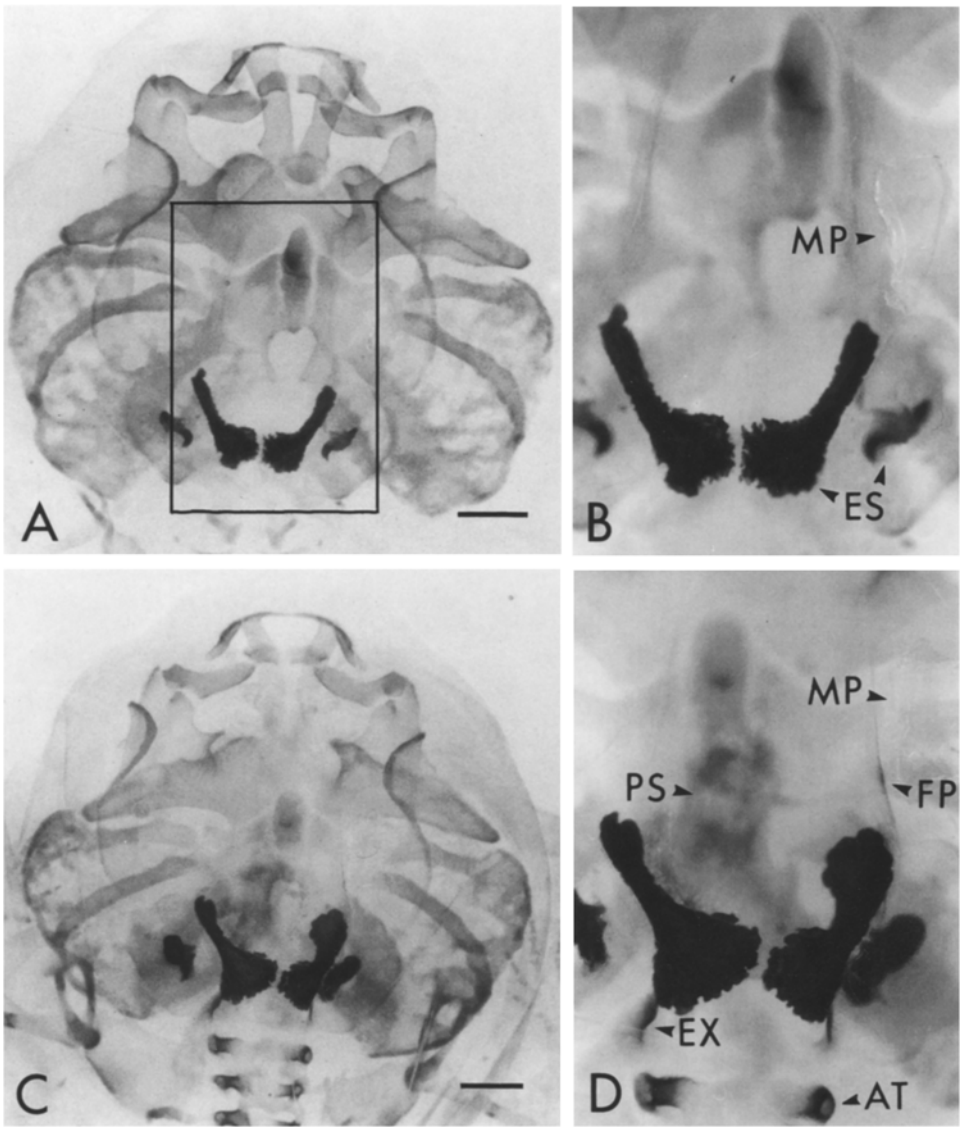

Fig. 5. Skulls of control tadpoles that received implants at stage 32/33 and were recovered after 8 d (**A, B**) and 14 d (**C, D**). Both skulls are shown in dorsal view; **B** and **D** are close-ups of the braincase region (*inset* in **A**). Alizarin-red-stained calcified tissues, including the prominent endolymphatic sacs (*ES*), appear dark against the lighter, alcian-blue-stained cartilage in **D**; the transparent, plastic micropellet (*MP*) is also visible in both skulls. *AT*, atlas vertebra. Scale bar, 1 mm

Table 2. Ossification scores (±S.E.). Each bone present in cleared and stained preparations counted as one point; paired bones were scored as one-half point for each side. *N* equals six for each score

Dosage (μg T_3)	Implant stage	Days after implant				
		2	4	6	8	14[a]
Control	28/29	–	–	–	–	0.16±0.16
	30/31	–	–	–	–	1.0 ±0.45
	32/33	–	–	–	–	2.0 ±0.63
0.025	28/29	–	–	–	–	
	30/31	–	–	–	–	
	32/33	–	–	–	–	
0.25	28/29	–	–	–	–	
	30/31	–	–	–	0.16±0.16	
	32/33	–	–	0.33±0.21	0.33±0.21	
2.5	28/29	–	–	–	–	
	30/31	–	0.16±0.16	0.33±0.21	0.7 ±0.33	
	32/33	–	–	1.3 ±0.21	1.7 ±0.33	

[a] Only control groups were sampled after 14 d

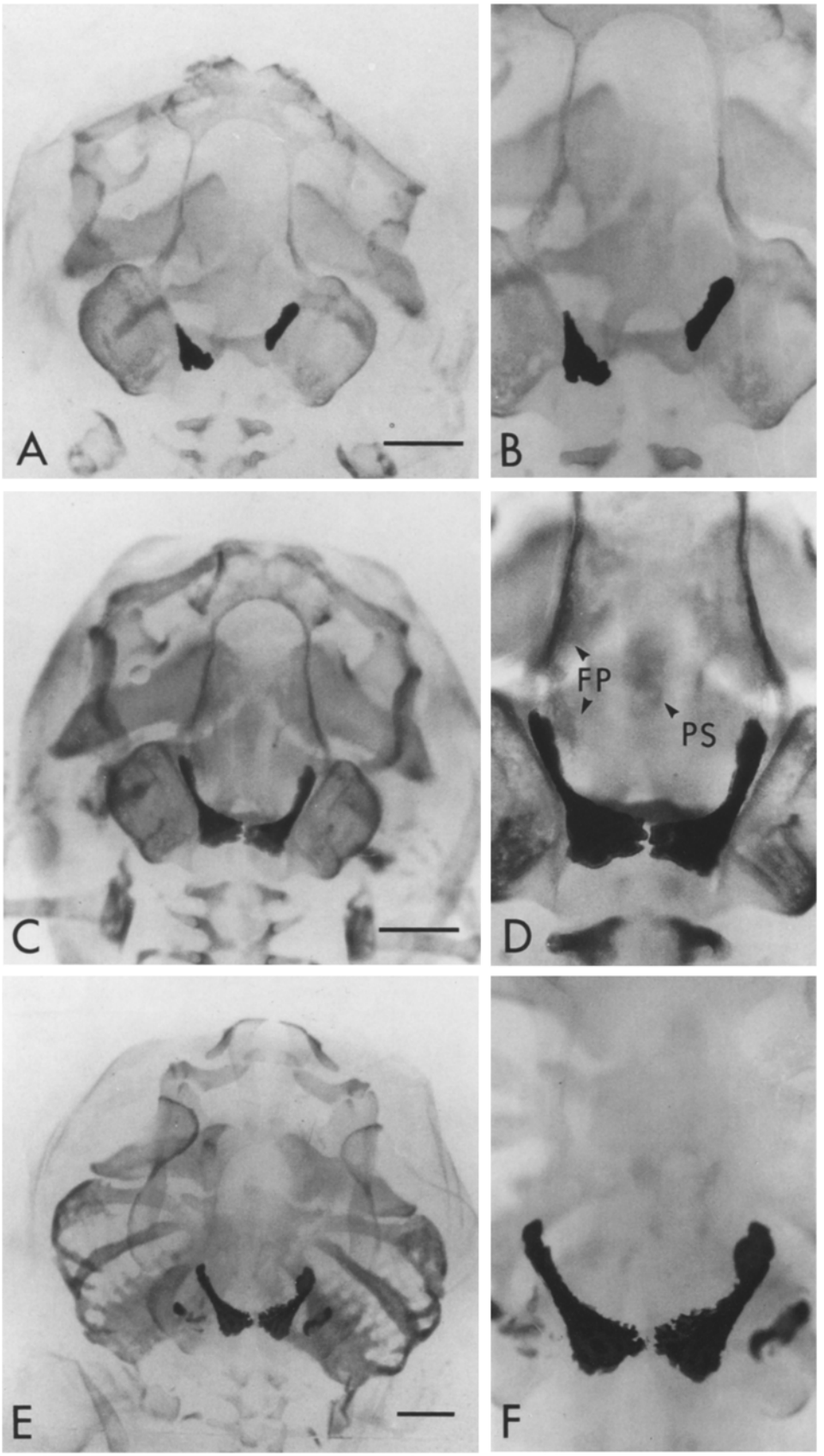

Fig. 6. Skulls of T_3-treated tadpoles recovered after 8 d. **A, B** Implant stage 28/29, 2.5 μg T_3. **C, D** Implant stage 32/33, 2.5 μg T_3. **E, F** Implant stage 32/33, 0.025 μg T_3. All skulls are shown in dorsal view; **B, D,** and **F** are closeups of the braincase region (see inset, Fig. 5A). Bones are visible only in the specimen implanted at the latest stage and with the highest dosage (**C, D**); the ventral parasphenoid, which is not in focus in this dorsal view, appears as a dark oval in the midline. Extensive transformation of larval cartilages has occurred in both specimens receiving the highest dosage (**A–D**); the specimen receiving the lowest dosage retains the larval configuration (**E, F**). Scale bar, 1 mm

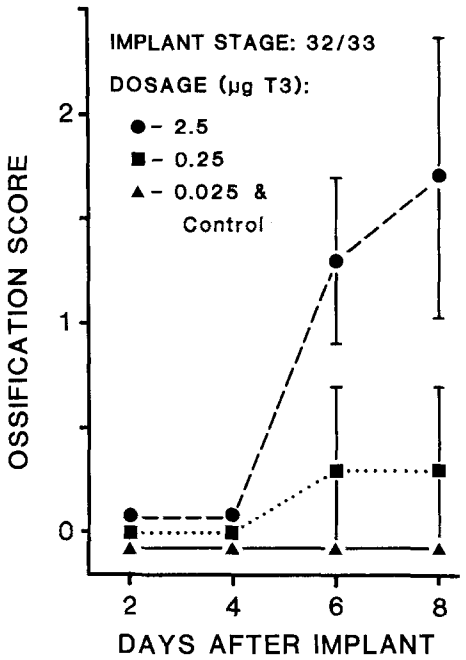

Fig. 7. Dosage-dependent response to T_3 following micropellet implantation at stage 32/33. Ossification score equals the mean number of skull bones present in each specimen ($N=6$). Vertical bar, $\pm$2S.E.

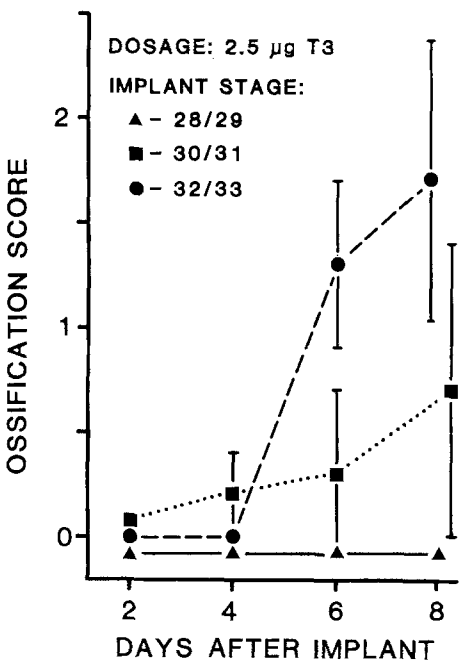

Fig. 8. Stage-dependent response following implantation of micropellets containing 2.5 µg T_3. Ossification score equals the mean number of skull bones present in each specimen ($N=6$). Vertical bar, $\pm$2S.E.

Dosage dependence

Extent of cranial ossification after 8 d was proportional to T_3 dosage (Table 2). No bone was visible at the lowest dosage, 0.025 µg, regardless of implant stage. At the intermediate dosage, 0.25 µg, a few specimens had at most the parasphenoid or both frontoparietals (ossification score equals 1); mean ossification score did not exceed 0.33. At the highest dosage, 2.5 µg, the exoccipital also was present in some specimens; mean ossification score ranged as high as 1.7. Considering only the latest implant stage, 32/33, differences in mean ossification score among the three implant dosages are statistically significant ($p<0.05$) (Fig. 7).

Stage dependence

Two of the three T_3 dosages – 0.25 and 2.5 µg – evoked precocious ossification. At each dosage, responses after 6 d and 8 d were directly proportional to implant stage (Table 2). For example, after 8 d, animals that received 2.5 µg T_3 at stage 32/33 had, on average, one more bone than those receiving implants at stage 30/31; animals receiving implants at stage 28/29 had no visible ossification (Fig. 8). Animals receiving pellets at stage 32/33 were the only ones to show as many as three bones after 8 d.

Discussion

Thyroid hormone and cranial metamorphosis

In our experiment, exogenous T_3 induced precocious cranial ossification in tadpoles within 6 to 8 d. Moreover, the response was both stage and dosage dependent. Dosage dependence is common in studies of TH and skeletal development in amniotes (e.g., Orbai and Gozariu 1982); it may represent increased saturation of TH receptors on the target tissues at higher dosages. Stage-dependent response to hormones (including TH) and teratogenic agents is also a common feature of skeletal development in a wide variety of embryonic structures in amniotes, including chick limbs (Fell and Mellanby 1955, 1956; Hall 1985), mouse secondary palates (Turley et al. 1985), and rat facial processes (Takakubo et al. 1986). It can be considered a prime example of the more pervasive, general phenomenon of critical periods during growth and development (Scott 1986). To our knowledge, however, the present work is the first demonstration of stage-dependent cranial ossification in response to TH administration during amphibian metamorphosis.

In addition to documenting a stage-specific response to exogenous T_3, we directly ascribe this response to the state of osteogenic differentiation at the time of hormone administration. In *B. orientalis*, ossification centers corresponding to the frontoparietal, parasphenoid, and exoccipital bones differentiate between stages 28 and 33, when they comprise distinct periostea and extracellular matrix: the first appearance of these bones in cleared-and-stained preparations at or after stage 37 represents subsequent cell proliferation and calcified matrix deposition (Hanken and Hall 1988). Thus, the implant stages used completely straddled the period during metamorphosis when these bones are differentiating. Specimens receiving T_3 after differentiation (stage 32/33) showed the most extreme response, and those receiving the hormone before differentiation (stage 28/29) showed the least response. We interpret the effect of T_3 as to promote the growth of preexisting ossification centers. (Specimens receiving hormone during the intermediate stage – 30/31 – gave a predictably intermediate response.) In effect, the pellets prematurely provided a surge in T_3 that characteristically occurs later, during prometamorphosis and metamorphic climax (sensu Etkin 1968; White and Nicoll 1981), and which presumably underlies the growth and enhanced visibility of ossification centers at that time.

Does TH also promote differentiation of ossification centers during amphibian metamorphosis? During mammalian embryogenesis, TH promotes the proliferation, and

possibly differentiation, of bone (Silberberg and Silberberg 1940; Kan and Cruess 1987); it also promotes the maturation of cartilage that precedes endochondral ossification in mammals and birds (Silberberg and Silberberg 1938; Burch and Lebovitz 1982a, b). Our results provide no evidence of similar effects in anurans, but because of our experimental design we would not have expected to produce such evidence. Similarly, many of the earlier studies of TH mediation of cranial ossification during amphibian metamorphosis did not specifically address this question (e.g., Kühn and Hammer 1956; Kemp and Hoyt 1965a, b, 1969a). A few studies, however, suggest that TH indeed plays such a role.

Two studies represent attempts to use TH to induce metamorphosis in species of "neotenic" salamanders that typically fail to metamorphose. Consequently, adults lack all or most cranial bones usually present in urodeles. In one study, induced metamorphosis in the cave salamander, *Gyrinophilus palleucus*, included the formation of paired maxillae (Dent and Kirby-Smith 1963); in the other, partial metamorphosis induced in another cave salamander, *Typhlomolge rathbuni*, was accompanied by the formation of paired maxillae and a small, median ossification presumed homologous to the normally paired septomaxilla (Dundee 1957). In neither case would ossification centers corresponding to these bones be expected in normal, i.e., non-metamorphosed, animals.

Two other studies examined the developmental basis of TH-induced precocious ossification of the anuran hindlimb skeleton. In *Rana pipiens*, Kemp and Hoyt (1965c, 1969b) interpreted TH as inducing the differentiation of osteoblasts from perichondrial cells in the femur, whereas Fox and Irving (1950) demonstrated in *Xenopus laevis* that TH can promote the maturative events in cartilage that precede endochondral ossification and the deposition of periosteal bone.

These results are especially provocative because of the questions they raise concerning the relation between TH receptivity of osteogenic tissues and the inductive interactions that typically underlie skeletal differentiation (Hall 1982). In the development of other integumental derivatives – for example, mouse mammary gland – requisite tissue interactions precede, and in fact confer, hormone receptivity (Heuberger et al. 1982).

Finally this study revealed no differential response of the cranial skeleton to exogenous T_3 between implanted and non-implanted sides, despite a conspicuous anteroposterior response gradient involving external features. Local, i.e., asymmetric, response to unilateral hormone implants characterized previous studies of TH effects on non-skeletal cranial tissues during anuran metamorphosis (Kaltenbach 1953a, b, c). Numerous additional experimental studies also document a direct effect of TH on amniote skeletal development in vitro (Burch and Lebovitz 1982a, b). We do not believe that the lack of a local response in our study challenges the prevailing view of direct TH action on skeletal tissues. Instead, we interpret our results to indicate that exogenous T_3 diffused throughout the head, likely via the circulatory system (cranial vessels often were cut while implanting pellets), thereby causing a systemic response. We cannot exclude the possibility that bone formation was due at least in part to endogenous hormone, although clearly endogenous TH alone was insufficient to evoke a positive response in controls.

Hormonal model for ontogenetic trajectories and diversity

Theoretical models incorporating differential TH sensitivity among target tissues have been proposed to explain the sequential, integrated changes characteristic of anuran metamorphosis (e.g., Chou and Kollros 1974; Kollros 1981). Previously we suggested a model for temporal integration of differentiation events that applied specifically to the skull: the cranial ossification sequence is the manifestation of intracranial variation in TH sensitivity among osteogenic sites combined with temporally varying levels of circulating hormone (Hanken and Hall 1984). Our experimental results, including other studies cited earlier, are consistent with this model, although they alone do not offer unequivocal proof. Ossification centers corresponding to the parasphenoid, exoccipital, and frontoparietal bones are sensitive to T_3, and they respond to administration of exogenous hormone by precocious growth and calcified matrix deposition. We predict that other cranial bones, which subsequently differentiate and grow when circulating levels of T_3 are much higher, correspondingly have higher thresholds of sensitivity, or at least need more prolonged exposure to a given level of hormone to develop. Alternatively, they simply may acquire sensitivity to T_3 later than the earlier forming bones.

A corollary of this model is the proposal that interspecific variation in ossification sequence is due to differential TH sensitivity of one or more ossification sites in different taxa, and/or differences in the TH profile during metamorphosis (Kemp and Hoyt 1969a). Additional comparative studies, however, are needed to establish whether such a mechanism actually underlies differences in cranial ossification among metamorphosing species. Similarly, only future research can identify how mechanisms of hormonal control have been modified in the evolution of specialized life history modes such as direct development, where the adult osteocranium forms during embryogenesis in the absence of a discrete metamorphosis (Lynn 1942).

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