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Effects of sex hormones on congenital kyphosis in Ishibashi rats

We examined the influence of estrogen and testosterone on the development of congenital kyphosis in Ishibashi rats. Neither hormone caused a growth spurt.

Estrogen suppressed the spinal growth and the progression of kyphosis in both sexes, and accelerated narrowing of the epiphyseal plates, which became apparent at 6 weeks after birth in females and 8 weeks in males. In both sexes, maturity of the spine was accelerated and trabeculae were hypertrophied by estrogen.

Testosterone suppressed the progression of kyphosis in males, but not in females. Histologically, testosterone had no effects on growth cartilage, but did produce thinning of the bone trabeculae in males.

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In a foregoing paper, we reported the natural development of congenital kyphosis in Ishibashi rats (Moritake et al. 1983).

Differences in the sexes were noted in those groups with congenitally ventral wedge-vertebrae; no progression was detected in females but it was marked in males.

As sex hormones seem to play an important role in the adolescent growth spurt and maturation in both sexes, we studied the influence of these hormones on the development of congenital kyphosis in rats.

Material and methods

The Ishibashi rats (ISR) used had congenital kyphosis mainly in the lumbar spine, accompanied by either partial or total intervertebral bony fusion. The spines tend toward kyphosis with growth. The vertebrae adjacent to normal discs are flexible, and kyphosis is limited to the malformed spines.

Gonadectomy (ovariectomy: OX, testectomy: TX) was performed in both sexes 3 weeks after birth. Sham operations were also performed (sham OX in ten females and sham TX in nine males). Immediately after gonadectomy, subcutaneous nucheal injections were given in a dose of 20 τ per rat of estradiol benzoate (E) (OX + E in 18 females and TX + E in 14 males) or 2 mg per rat of testosterone propionate (T) (OX + T in eight females and TX + T in 12 males). These hormones were dissolved in 0.1 ml of sesame oil. Ten ovariectomized females and 12 castrated males were injected with 0.1 ml of sesame oil only for the control study. Injections were given twice weekly until age 36 weeks.

Soft radiographs were taken of the spines with the rats in extended position and under ether anesthesia, weekly between 4 and 8 weeks after birth, and biweekly thereafter. The length from the first thoracic vertebra to the sixth lumbar vertebra was measured on the radiographs, and the maximum angle of kyphosis was measured. Lines were drawn along the dorsal surface of the vertebral bodies above and below the region of maximum kyphosis, and the angle of intersection was measured with a protractor. All 93 rats in the eight groups survived at least 8 weeks after birth and those with kyphotic angles of 20 degrees or more were included.

Routine histologic examination was made in sagittal sections of the lumbar spines stained with hematoxylin and eosin. The lower epiphyseal plate of L5 vertebra was chosen as a control to examine the influence of sex hormones.

Results

The gain in body weight was suppressed after 6 weeks of age in the estrogen-treated groups, while the testosterone-treated groups showed a steady increase of weight (Figure 1).

In the estrogen-treated groups, the growth of the spine was suppressed as compared with gonadectomized groups in both sexes after 6

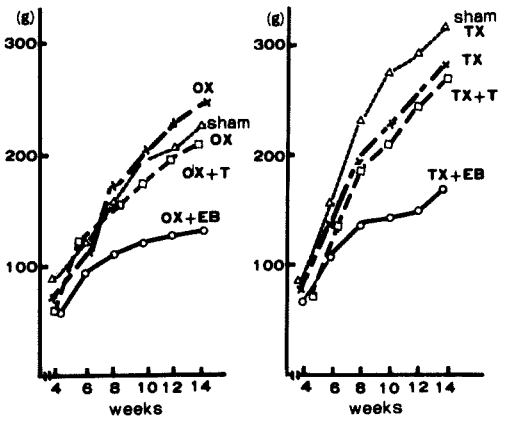

Figure 1. Increase in body weight in female rats (left) and in male rats (right).

weeks, and no growth of the spine was observed after 14 weeks (Table 1).

Ovariectomy accelerated the progress of kyphosis, which was more rapid in the OX than in the controls after 7 postnatal weeks (Table 2). Estrogen suppressed the progress which was slower in the OX + E than in the OX (Figure 2). Rapid progression from 45° at 4 weeks to 80° at 13 weeks in the OX animal contrasted with insignificant progression from 4 to 14 weeks in the OX + E animal.

Testosterone produced no effect, although in the OX + T animals, there was a slightly slower progression than in the OX animals.

In males, the influence of estrogen was more marked than in females (Table 2). Although early progress of kyphosis was found in the TX from 5 weeks, minimal progress was detected in the TX + E. Figure 3 shows a typical case with progression from 47° at 4 weeks to 69° at 13 weeks in the TX rat. On the other hand, progression was minimal in the TX + E from 4 to 14 weeks (Figure 3). Progression in the TX + T was the same as in the sham TX and slower than in the TX animals.

Table 1. Effect of estradiol (E) and testosterone (T) on spine growth in ovariectomized (OX) and testectomized (TX) Ishibashi rats. Values are spine length T1–L6. Mean (SD), mm

Group	No.	Age (wk)								
		4	6	8	10	12	14	20	30	36
Sham OX	13	5.6 (0.24)	7.1 (0.36)	8.1 (0.31)	8.7 (0.33)	9.1 (0.26)	9.2 (0.30)	9.3 (0.38)	9.6 (0.51)	9.9 (0.34)
OX	6	5.8 (0.27)	7.3 (0.26)	8.2 (0.06)	8.5 (0.38)	9.0 (0.51)	–	9.6 (0.31)	10.0 (0.37)	10.1 (0.0)
OX+E	14	5.5 (0.36)	6.7 (0.19)[a]	7.4 (0.47)[b]	7.8 (0.56)[b]	7.9 (0.35)[a]	8.1 (0.39)	8.1 (0.33)[a]	8.1 (0.28)[b]	–
Sham TX	11	5.7 (0.23)	7.9 (0.18)	9.1 (0.28)	9.7 (0.15)	10.0 (0.23)	10.1 (0.40)	10.5 (0.33)	11.2 (0.31)	11.2 (0.24)
TX	10	5.8 (0.47)	7.2 (0.20)	8.0 (0.41)	8.7 (0.29)	9.0 (0.31)	9.4 (0.22)	9.7 (0.33)	10.1 (0.12)	10.2 (0.12)
TX+E	14	5.7 (0.45)	6.9 (0.38)[b]	7.6 (0.37)[b]	8.2 (0.44)[b]	8.2 (0.30)[a]	8.5 (0.23)[a]	8.6 (0.37)[a]	8.8 (0.62)[b]	–

Statistical Analysis (Student's t-test) of difference between OX and OX + E groups, and TX and TX + EB groups respectively. [a]$p<0.01$. [b]$p<0.05$.

Table 2. Effect of estradiol (E) and testosterone (T) on kyphosis in ovariectomized (OX) and testectomized (TX) Ishibashi rats. Mean (SD) degrees

Group	No.	Age (wk)						
		5	6	7	8	10	12	14
Sham OX	10	4.4 (3.7)	4.3 (4.6)	7.2 (5.2)	8.6 (6.6)	10.1 (5.2)	10.0 (2.4)	15.4 (4.4)
OX	10	2.4 (5.3)	6.6 (4.1)	10.2 (5.4)	13.2 (10.2)	17.0 (9.6)	16.3 (11.7)	20.7 (12.0)
OX + E	18	3.0 (4.7)	2.6 (5.8)	3.7 (5.8)	4.4 (3.6)	6.0 (2.9)[a]	8.4 (5.7)	8.2 (5.5)[b]
OX + T	8	5.3 (4.3)	6.7 (4.1)	8.8 (4.6)	9.4 (4.2)	11.0 (4.7)	14.8 (7.8)	17.0 (5.8)
Sham TX	9	2.6 (3.2)	6.2 (5.2)	8.8 (8.1)	11.0 (8.0)	14.0 (7.6)	12.8 (4.6)	16.4 (7.0)
TX	12	10.6 (8.7)	16.0 (9.4)	15.7 (9.8)	21.5 (12.0)	21.8 (11.0)	23.0 (9.8)	28.0 (10.0)
TX + E	14	1.7 (3.5)	2.6 (4.2)[a]	3.2 (4.1)[a]	3.1 (7.9)[a]	5.7 (6.0)[a]	2.7 (2.3)[b]	4.8 (3.2)[a]
TX + T	12	5.6 (8.6)	7.2 (6.1)	9.6 (8.3)	10.7 (6.0)	12.5 (8.4)	14.0 (5.3)	18.3 (9.5)

Statistical analysis (Student's t-test) of difference between OX and OX + E groups and TX and TX + E groups, respectively. [a]$p<0.01$, [b]$p<0.05$.

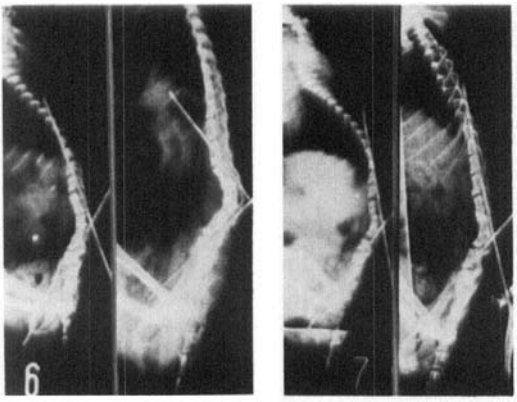

Figure 2. An ovariectomized rat with marked progress of kyphosis with growth (left), and an ovariectomized and estrogen-treated rat with practically no progress of kyphosis (right).

Testosterone suppressed the progress of kyphosis in males but had no effect in females. In both sexes, the progression of congenital curvature was more obviously suppressed in those groups under the influence of estrogen (sham OX, OX + E and TX + E) than in those influenced by testosterone (OX + T, sham TX and TX + T). In castrated groups (OX and TX), the progression rate in the early stages (4–6 weeks) was faster in males than in females.

Histologically, in females, no differences in the growth plates and trabeculae were observed in the OX and the OX + E animals at 4 weeks. The epiphyseal cartilage showed sound development at 6 weeks in the OX animals. In

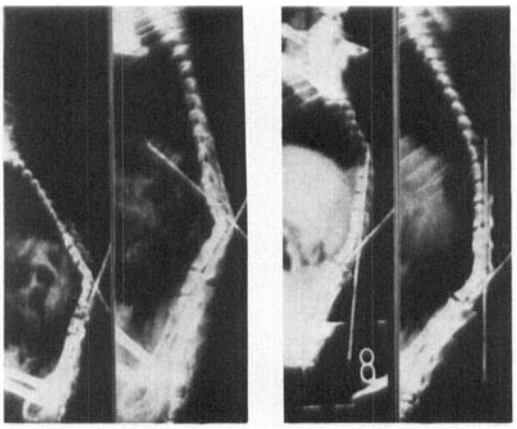

Figure 3. A testectomized rat with progress of kyphosis with growth (left) and a testectomized and estrogen-treated rat with practically no progression (right).

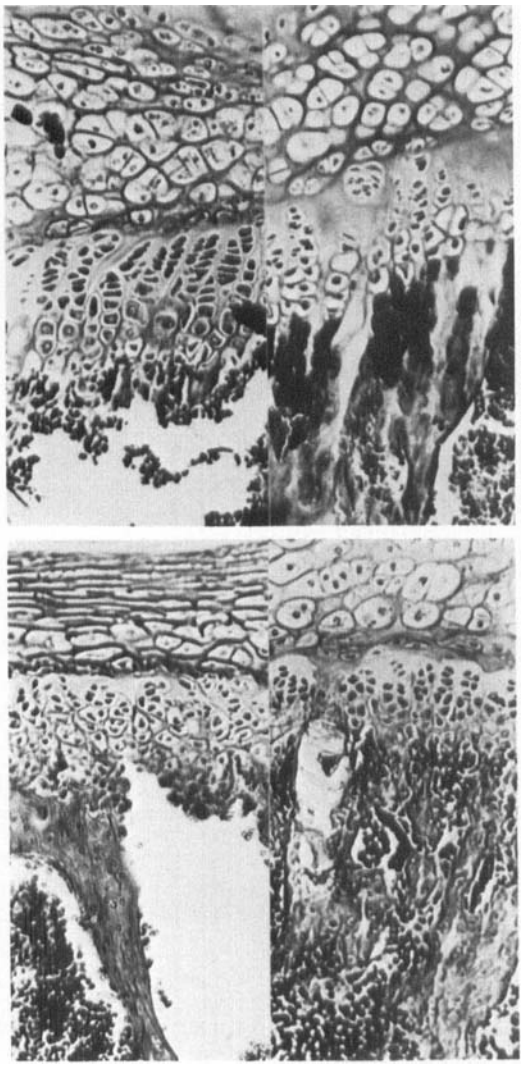

Figure 4. Photomicrographs of lower growth plate of the L5 vertebrae at 6, upper, and 12 weeks, lower, in an ovariectomized rat (left), and in an ovariectomized and estrogen-treated rat (right). Left, almost normal development. Right, maturing cartilage almost absent, zone of proliferating cartilage narrow with shrinkage of nuclei, accelerated calcification with hypertrophic and condensed bone trabeculae. (Hematoxylin and eosin, ×20).

the OX + E animals, on the other hand, the zone of maturing cartilage was all but absent, the zone of proliferating cartilage showed narrowing, and the nuclei of the cartilage cells showed shrinkage. Calcification was accelerated and the trabeculae of bone hypertrophied in the zone of calcifying cartilage. The hypertrophy of the trabeculae was more obvious in the OX + E, and less obvious in the OX ani-

mals. Estrogen thus induced an acceleration of bone maturity and condensation of bone trabeculae after 6 weeks. At 12 weeks, the epiphyseal cartilage had also narrowed in the OX and the OX + E, and differences were not apparent. Trabeculae showed a noticeable condensation in the OX + E (Figure 4). Unlike estrogen, testosterone produced no clear effects in female rats. The growth cartilage in the OX + T animals showed similar findings to those in the OX animals.

In males, the effects of estrogen on growth of epiphyseal cartilage were detectable at 4 weeks. Calcification was found in the zone of calcifying cartilage. At 8 weeks, slight narrowing of the zone of proliferating cartilage and shrinkage of nuclei were detected. The array of cartilage cells became more obvious and the trabeculae became hypertrophied in the TX + E animals.

In males given testosterone, the changes in the epiphyseal cartilage were similar to those in the sham TX animals, where the trabeculae showed less condensation in the TX + T than in the TX + E animals.

Discussion

Deterioration of congenital spinal malformation occurs during the first 3 years of life and again during the adolescent growth spurt, as first reported by Bampfield (1845) and re-confirmed by numerous investigators. In boys, testosterone seems to play an important role in the adolescent growth spurt; the serum level increases while the level of growth hormone remains constant (Hibi 1980). In girls, the mechanisms involved in the growth spurt are poorly understood. The serum level of growth hormone appears to be unchanged. An androgen of adrenal origin has been implicated (Nordwall & Willner 1975), and Hibi (1980) has recently suggested that estrogen might have a physiological action.

Thus estrogen and testosterone are thought to play an important role in eliciting the adolescent growth spurt. The action of these hormones on organs is not proportional to hormone levels, but is biphasic and differs with species, age, sex, etc. Estrogen and testosterone also affect bone growth and its maturity.

It is now widely accepted that estrogen exerts three major effects on the structure of the skeleton: inhibition of linear growth of long and flat bone, acceleration of skeletal development, and condensation of bone. Proliferation of epiphyseal and articular cartilage is inhibited, while maturation of chondrocytes is accelerated. Testosterone promotes all phases of enchondral ossification, but stimulation of proliferation of chondrocytes is usually less conspicuous than the intensification of hypertrophy, calcification and ossification (Silberg & Silberg 1971).

Sanada et al. (1978) reported studies on the enzyme activity in connective tissue and concluded that estrogen stimulates the lysyl oxidase activity and accelerates the maturation of collagen and elastin in the extracellular space.

Longitudinal growth of the spine takes place in epiphyseal plates, just as in the long bones. Simpson et al. (1950) reported that the velocity of growth slowed down at 100 days after birth in the tibiae of rats. In Ishibashi rats, the growth of the spine continues until 14 weeks of life, and after then little growth is seen.

The influence of sex hormones on the spine has not been examined thoroughly. Mizuno (1961) examined the effect in D.D. strain mice of 0.5 mg of estradiol benzoate or 2.5 mg of testosterone propionate per week. He stated that on administration of estrogen, there were changes in vertebrae similar to those mentioned above. The changes in the spine were slight in comparison with those in the femur, sex differences were not marked, and the changes were greatest in the thoracolumbar junction. On administration of testosterone, changes were detected from the sixth week, and were slight.

Our dose of 20 τ of estradiol benzoate twice weekly was the minimal effective dose required to induce estrus and the increase of the elastin content, the fiber diameter and the ratio of the central elastin to the peripheral microfibril in the hip joint capsule of rats (Shikata et al. 1979). In our cases, neither estrogen nor testosterone elicited a growth spurt. On the other hand, 20 τ of estrogen accelerated bone maturity, elicited early closure of the epiphyseal plates and suppressed the growth of the

spine. Histologically the growth plates were narrowed at 6 weeks after birth in both sexes; the narrowing of the growth plates coincided with the suppression of kyphotic progression which was influenced more by estrogen than by testosterone.

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