Effects of Steroid Hormones on Collagen Biosynthesis in Rat Aorta and Uterus

BYUNG-MOO MIN*, WANG-KOOK KIM and DONG-KYUN CHEONG**

Departments of Oral Biochemistry and Dental Pharmacology***
Collage of Dentistry, Seoul National University, Seoul 110, Korea

[Abstract] Effects of steroid hormones on the collagen biosynthesis in aorta and uterus were studied with ovariectomized Sprague-Dawley rats. Effects of administration of hormones, such as estrogen, testosterone and prednisolone, to the ovariectomized animals were studied, comparing with the control. Each group was injected with ^{3}H-proline and sacrificed, followed by removals of aorta and uterus. Separations and quantitative analyses of proline and hydroxyproline were performed by means of thin layer chromatography; and radioactivities of the separated amino acids were assayed by liquid scintillation counter. Normally the incorporation of ^{3}H-proline into hydroxyproline was greater in uterus than in aorta, and collagen turnover rate of uterus was observed rapid as well than that of aorta. In the two tissues from ovariectomized rats, the incorporation rate of ^{3}H-proline into hydroxypoline was markedly decreased than that of the former. Changes in the turnover rate of collagen in these tissues were not observed. Decrease in ^{3}H-proline incorporation into collagen in ovariectomized rats was markedly antagonized by estrogen, but not influenced by prednisolone in the tissues tested.

Remodeling of the connective tissue components of tissues essentially involves the formation and breakdown of the collagen fibers. Of the various components, collagen is the most abundant and major protein of the connective tissues and functions as a structural protein serving principally as a prime mechanical support and elasticity of tissues.

The biosynthesis of collagen has been extensively studied in many tissues by following up the incorporation rate of ^{3}H-proline (Orlowski, 1976; Cohen *et al.*, 1977; Orlowski, 1977; Park & Cheong, 1980).

Hydroxyproline of collagen is derived from the hydroxylation of peptide-bound proline during the synthesis or the release of the nascent chains of collagen peptides (Cardinale & Udenfriend, 1974). Proline, being a precursor of collagen hydroxyproline, its conversion to hydroxyproline is a measure of the collagen synthesis (Firschein, 1967).

The growing tissue was reported to have the highest specific activity of all the tissues studied which reflects the rapid rate of the collagen synthesis in the tissues (Kao *et al.*, 1961; Smith & Allison, 1965; Orlowski, 1977; Park & Cheong, 1980).

It was reported that the incorporation of ^{3}H-proline into animal tissue was very high, but that a lesser amount was incorporated into the collagen than noncollageneous proteins (Orlowski, 1977).

Some workers found also in the study of hyper-

tension that collagen synthesis in arteries was increased but not in veins, suggesting that the vascular collagen synthesis is enhanced by a direct effect of the increased pressure on the arterial cells(Iwatsuki *et al.*, 1977).

Many other studies reported that estrogen, testosterone and cortisone can affect the collagen synthesis(Chvapil, 1959; Smith & Allison, 1965; Katz & Kappas, 1968; Cohen *et al.*, 1977; Park & Cheong, 1980).

The present investigation was performed to elucidate the rate of the collagen synthesis by means of the incorporation of ^{3}H-proline into ^{3}H-hydroxyproline as a parameter of the collagen synthesis, and to determine the effect of steroid hormones on the collagen turnover in uterus and aorta of ovariectomized rats.

MATERIALS AND METHODS

Animals

The female Sprague-Dawley rats weighing 140±20 gm which were supplied from Seoul National University animal house were used in the present experiment. After the rats were treated with sodium pentobarbital (300 mg/kg of body weight) followed by ovariectomy, they were fed for seven days prior to subjecting the animals to the present experiment.

The animals were divided into normal and ovariectomized groups. The latter was subdivided into 4 groups; control, estrogen, testosterone and prednisolone administered groups each being consisted of 12 rats.

The ovariectomized groups were injected intramuscularly steroid hormones for six days, starting from the 7th day after ovariectomy. Each group received intraperitoneally 0.7 μCi of ^{3}H-proline per gm of body weight. After 1 day, a half of rats of each group was sacrificed, the remaining half sacrificed 6 days later during which steroid hormones were injected daily, The aorta and uterus were removed and washed repeatedly in physiologic saline, and their wet weights were measured immediately.

All the rats except those of normal group were ovariectomized, and each group was injected testosterone propionate(Samil Pharm. Co., Korea, 40 mg/kg of body weight), estradiol benzoate (Samil Pharm. Co., Korea, 20 mg/kg of body weight) and prednisolone acetate(Chong Keun Dang Pharm. Co., Korea, 20 mg/kg of body weight) once a day intramuscularly, respectively. Ovariectomized rats without any streoid hormone injection were used as control.

Detection of proline and hydroxyproline by T.L.C.

The samples were collected by manual dissection and stored in a desiccator at −20°C until they were analyzed. The samples from each group were pooled, and duplicate analyses were performed and averaged.

For separations and quantitative analyses of proline and hydroxyproline samples were hydrolyzed 6N HCl for 24 hours in sealed tubes at 105°C. The hydrolysates were then evaporated to dryness and redisolved in distilled water. The two amino acids were then separated by two-dimensional thin layer chromatography and analyzed quantitatively using Thin Layer Chromatogram Scanner(Aloca Japan Radio. Co.)

Chloroform-methanol-17% ammonium hydroxide (40:40:20, by vol.) and phenl-water(75:25, V/V) were used as solvents for lst and 2nd run for T. L.C., respectively. Ninhydrin dissolved in n-butanol-acetic acid (100:3, V/V) was used to visualize amino acids.

To detect the radioactivities of proline and hydroxyproline separated by T.L.C., their spots were collected and subjected to counting by Liquid Scintillation Counter(Nuclear Enterprise Co.).

RESULTS AND DISCUSSION

We presumed that collagen synthesis and turnover rate varies not only in species, but also at different stages of development and in different tissues in the same species. The rapid rates of collagen biosynthesis and turnover may be directly related to the rate of growth of the rat tissues (Kao *et al.*, 1961; Smith & Allison, 1965; Orlowski, 1977; Park & Cheong, 1980).

Firschein(1967) using ^{14}C-proline in rats, have shown that the proximal 20% of the tibia had the highest specific activity in hydroxyproline of all the bones stuied, which reflected the rapid rate of collagen synthesis in this area of bone. The turnover rate of collagen also decreased very rapidly. Similar results have been reported by others(Bauer, 1954).

Incorporation of ^{3}H-proline into collagen hydroxyproline in the non-treated normal rats was shown that the uterus had a higher specific activity in hydroxyproline than that of the aorta. Collagen turnover rate of uterus was observed rapid as well than that of aorta (Table 1).

Table 1. *Incorporation of ^{3}H-proline into non-ovariectomized normal and ovariectomized group*

+Normal; Non-ovariectomized group. Control; Ovariectomized group. Specific Activity, cpm/mg of amino acids. ** Activity, cpm/mg wet weight of tissue. *** Total Activity, of ^{3}H/mg wet weight of tissue

Groups+	Tissues	Days after ^{3}H-pro inj.	Specific Activity*		Activity**		Total*** Activity
			Pro	Hyp	Pro	Hyp	
Normal	Aorta	1	8.66	6.96	123.9	46.0	708
		6	8.20	5.67	95.9	28.9	1037
	Uterus	1	182.49	55.06	865.0	180.6	—
		6	39.95	23.93	210.9	73.7	—
Control	Aorta	1	7.87	5.89	153.3	41.9	1003
		6	6.82	4.12	126.2	38.3	692
	Uterus	1	76.67	43.96	331.2	84.4	—
		6	37.00	11.60	249.0	65.2	—

Table 2. *Incorporation of ^{3}H-proline into estrogen administered group in ovariectomized rats*

+ Control; Ovariectomized group. Ectomy+Est.; Ovariectomized group injected with estrogen.

Groups+	Tissues	Days after ^{3}H-pro inj.	Specific Activity*		Activity**		Total*** Activity
			Pro	Hyp	Pro	Hyp	
Control	Aotra	1	7.87	5.89	153.3	41.9	1003
		1	6.82	4.12	126.2	38.3	692
	Uterus	1	76.67	43.96	331.2	84.4	—
		6	37.00	11.60	249.0	65.2	—
Ectomy + Est.	Aorta	1	6.26	4.95	166.0	50.0	527
		6	9.65	6.51	158.2	38.4	301
	Uterus	1	181.13	41.63	488.1	72.4	—
		6	134.19	146.15	319.4	82.9	—

In ovariectomized rats, the incorporation of ^{3}H-proline into collagen hydroxyproline was markedly decreased than that of the non-treated normal rats in aorta and uterus. Collagen turnover rate was almost equal in these tissues(Table 1).

In the two tissues from ovariectomized rats injected with estradiol benzoate, the specific activity in hydroxyproline was considerably increased than that of the control group at the 6th day, particulary in the uterus(Table 2).

It was reported that estrogens tended to decrease bone absorption(Karz & Kappas, 1968), and the amounts of estrogen secretion in the patients with osteoporosis was found to be decreased (Smith, 1967). In the studies of changes in collagen content with the uterus, skin and vagina of gonadectomized young rats, Morgan (1963) found a reduced collagen concentration, but an increase in total hydroxyproline(a measure of collagen) when estradiol benzoate was given either subcutaneously or topically.

In present investigations, the decreased rate of ^{3}H-proline incorporation into collagen by ovariectomy was markedly and moderately antagonized by estrogen in the uterus and aorta(Table 2)

Smith & Allison(1965) reported that the response of dermal collagen to testosterone propionate was found to vary with age and sex.

Table 3. *Incorporation of ^{3}H-proline into testosterone administered group in ovariectomized rats*

+ Control; Ovariectomized group. Ectom+Testo.; Ovariectomized group injected with testosterone.

Groups	Tissues	Days after. ^{3}H-pro inj.	Specific Activity*		Activity**		Tatal*** Activity
			Pro	Hyp	Pro	Hyp	
Control	Aorta	1	7.87	5.89	153.3	41.9	1003
		6	6.82	4.12	126.2	38.3	692
	Utetus	1	76.67	43.96	331.2	84.4	—
		6	37.00	11.60	249.0	65.2	—
Ectomy + Testo.	Aorta	1	6.74	4.02	120.0	49.4	422
		6	2.55	2.39	80.2	38.9	895
	Uterus	1	—	—	—	—	—
		6	—	—	—	—	—

Table 4. *Incorporation of ^{3}H-proline into prednisolone administered group in ovariectomized rats*

+ Control; Ovariectomized group. Ectomy+Predni.; Ovariectomized group injected with prednisolone.

Groups+	Tissues	Days after ^{3}H-Pro inj.	Specific Activity*		Activity**		Tatal*** Activity
			Pro	Hyp	Pro	Hyp	
Control	Aorta	1	7.87	5.89	153.3	41.9	1003
		6	6.82	4.12	126.2	38.3	692
	Uterus	1	76.67	43.96	331.2	84.4	—
		6	37.00	11.60	249.0	65.2	—
Ectomy + Predni.	Aorta	1	4.14	4.85	54.3	28.6	320
		6	4.48	3.89	175.6	69.3	460
	Uterus	1	73.43	21.42	368.6	72.0	—
		6	68.78	23.95	325.3	58.9	—

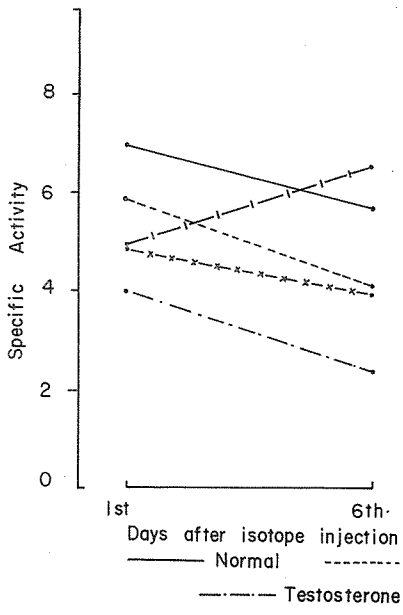

Fig. 1 *Specific activity in hydroxybroline of the aorta*

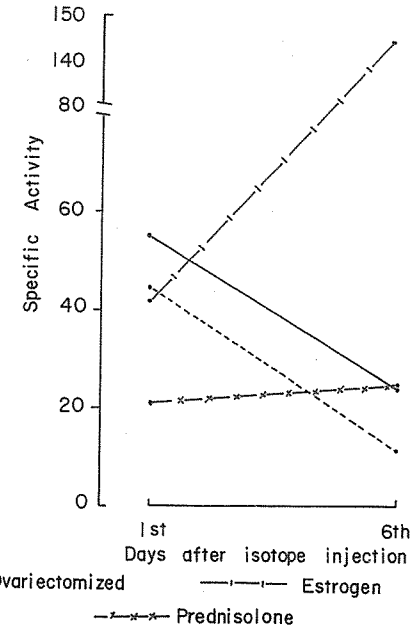

Fig. 2 *Specific activity in hydroxyproline of the uterus*

There was no significant increase in the contents of collagen in testosterone treated male rats. Jorgenson & Schmidt (1962) also reported that testosterone did not alter hydroxyproline content of granulation tissue in the rats. In testosterone treated female rats, it was also reported that no significant decrease or trend toward lower collagen were observed(Smith & Allison, 1965). An anabolic effect of androgen on collagen was observed in bone tissue by Kowalewski(1962), Landau & Kappas(1965).

Park & Cheong(1980) reported that the decreased rate of ^{3}H-proline incorporation into collagen by ovariectomy was slightly antagonized by testosterone in the teeth and gingiva. Other workers reported that testosterone has been shown to increase collagen formation in the skin of capons (Katz & Kappas, 1968).

In this report, the aorta from testosterone propionate treated rats had a lower incorporation rate than control group (Table 3). The results of the present investigation are different from others(Katz & Kappas, 1968; Park & Cheong, 1980). So this point deserves further study.

The incorporation of glycine-2-^{14}C into skin and femur was decreased in cortisone acetate treated rats(Smith & Allison, 1965) and Chvapil (1959) noted diminished hydroxyproline formation in systemically cortisone treated rats and chick embryos when measuring ^{14}C-proline incorporation. Furthermore, some workers reported that steroids enhanced collagenase activity(Houck, 1968; Brown *et al.*, 1970; Dougherty *et al.*, 1973; Hook *et al.*, 1973). In the study of the effect of corticosteroids on collagen synthesis, Cohen *et al.* (1977) showed that collagen synthesis was suppressed by long-term administration of massive systemic dose of methylprednisolone acetate and Park & Cheong(1980)recently reported that specific activity in hydroxyproline was markedly decreased in gingiva of prednisolone acetate treated female rats.

In present investigation, the aorta and uterus of ovariectomized rats injected with prednisolone acetate had a lower initial specific activity in hydroxyproline than that of control and turnover of collagen was shown no change during whole

experiments(Table 4).

In the view of the results, we presumably demonstrated that the effect of prednisolone acetate administration in ovariectomized rats was not influenced on collagen turnover in the aorta and uterus of rats.

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