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EFFECTS OF ESTROGENS ON THE METABOLISM OF ACID MUCOPOLYSACCHARIDES IN RAT AORTA

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Abstract—The effects of estrone, 2-methoxymethyl-17α-methylestradiol-3-methyl ether (P-5780) which is an anti-atherogenic lipid-shifting steroid, and testosterone propionate on the metabolism *in vivo* of aortic acid mucopolysaccharides (AMPS) in male Wistar rats were investigated. Results were compared with the effects of these compounds on costal cartilage and eyeball tissues.

(1) The incorporation of sodium sulfate-S^{35} into AMPS in costal cartilage was found to be more than the incorporation into aorta and eyeball. The specific activities in the aorta and eyeball were higher than in the cartilage.

(2) In estrone-treated rats the specific activity of aortic AMPS was higher than those of cartilage and eyeball, while P-5780-treatment decreased the aortic AMPS level and incorporation into aortic AMPS without influencing the levels in cartilage and eyeball. Testesterone propionate had no effect on the aortic AMPS level but tended to decrease the incorporation into AMPS in the cartilage and eyeball.

(3) In hypercholesterolemic rats the AMPS levels in the aorta and eyeball tended to decrease and incorporation into all connective tissues examined tended to decrease, while there was a significant increase in the specific activity of aortic AMPS. P-5780-treatment had no significant effect on the metabolism of AMPS in the aorta and other tissues of these treated animals.

(4) On treatment with P-5780 the specific activity of perchloric acid soluble serum protein (glycoprotein) increased significantly but that of perchloric acid insoluble serum protein was unaffected. Estrone had no effect on the specific activities of either perchloric acid soluble or insoluble of serum protein.

THERE is a sexual difference in the occurrence of coronary atherosclerosis,[1–4] and estrogen has been found to prevent the disease.[5–8]

Recently, as a new approach to studies on the mechanism of this effect of estrogen, several investigators have investigated its influence upon connective tissue ground substances. These studies have a significant connection with vascular sclerosis because it has been found that the accumulation of acid mucopolysaccharides (AMPS) is an early morphological sign of atherosclerosis[9–13] and that there is progressive increase in chondroitin sulfate with age.[14] However, there is still no agreement on the effects of estrogens on connective tissue metabolism *in vitro*. Thus a steady increase in AMPS in the vascular wall on administration of estrogen has been reported by Schiff and Burn[15] and by Asboe-Hansen,[16] while others[17, 18] have reported on the inhibitory activity of estrogens.

This paper presents studies on the incorporation of sodium sulfate-S^{35} into AMPS in the aorta *in vivo*. For comparison incorporation into costal cartilage and eyeball, and serum glycoprotein of normo- and hyper-cholesterolemic rats were studied. The

effects on this incorporation of estrone, 2-methoxymethyl-17α-methylestradiol-3-methyl ether (P-5780), an anti-atherogenic lipid-shifting steroid with less feminizing activity[19, 20] and testosterone propionate were examined.

METHODS AND MATERIALS

Male Wistrar rats of 70 days of age weighing 200–235 g were used throughout the experiments. The animals were given commercial laboratory chow (CLEA's CA-1) and water *ad libitum*, and were not fasted before experiments. Animals were given a daily subcutaneous injection of 1 mg of estrone, P-5780 or testosterone propionate for 2 weeks. Hypercholesterolemic rats were obtained by feeding animals on a diet containing 1% sodium cholate, 1% cholesterol and 5% cotton seed oil for 10 weeks with or without P-5780 (2 mg/rat/day in the gabbage). For studies on the incorporation of sulfate-S^{35}, animals were sacrificed 1, 2, 4, 8 and 16 hr after intraperitoneal injection of 0·5 mc of sodium sulfate-S^{35} (specific activity 0·2 mc/mg).

Under ether anaesthesia animals were exsanguinated by cardiac puncture and blood was collected. The aorta, costal cartilage and eyeball were immediately removed, weighed and kept frozen at −20° until just before analysis. Perchloric acid soluble serum protein (glycoprotein) and perchloric acid insoluble serum protein were obtained by the method of Archord and Galambos.[21] For determination of AMPS, tissues were homogenized in acetone, denatured by heating in a water bath at 100° for 15 min and digested with activated papain at 65° for 20 hr in phosphate buffer following the method of Scott.[29] AMPS was precipitated from the digest with Rivanol reagent using the procedure described by Whitehouse and Bostrom.[23] The radioactivity of samples in toluene solution containing 0·5% 2,2-diphenyloxazole and 0·01% 2,2-*p*-phenylene-bis (5-phenyloxazole) was determined in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

Uptake of sodium sulfate-S^{35} into total AMPS *in aorta, costal cartilage and eyeball*

Rats were injected intraperitoneally with 0·5 mc of sodium sulfate-S^{35} (0·1 mc/mg) and sacrificed 1, 2, 4, 8 and 16 hr later. As shown in Fig. 1, the incorporation of radioactivity into tissue AMPS was found to reach a maximum by 1 hour after the injection. The incorporation into costal cartilage was greater than into the aorta and eyeball. However, higher specific activities and more rapid turn-over rates were found in the aorta and eyeball than in the cartilage. In the hypercholesterolemic state there was a decrease in the amount of total AMPS in the aorta and eyeball, but not in the cartilage. Moreover the incorporation of sulfate-S^{35} into these tissues decreased greatly in the order, aorta>cartilage>eyeball.

In hypercholesterolemic rats, significant increases in cholesterol levels were seen in the plasma (155%), aorta (87%) and liver (398%).

Effects of steroids on incorporation into AMPS *in the tissues*

As shown in Tables 1 and 2 after treatment with estrone for 2 weeks, a significant decrease was observed in the AMPS levels in the cartilage and eyeball, and the incorporation of sulfate-S^{35} into AMPS in the eyeball. P-5780 significantly decreased the AMPS level in the aorta and sulfate-S^{35} incorporation into aorta and eyeball. Testosterone propionate decreased the AMPS level in the cartilage and the incorporation into AMPS in the eyeball. As shown in Table 3, the specific activity of total

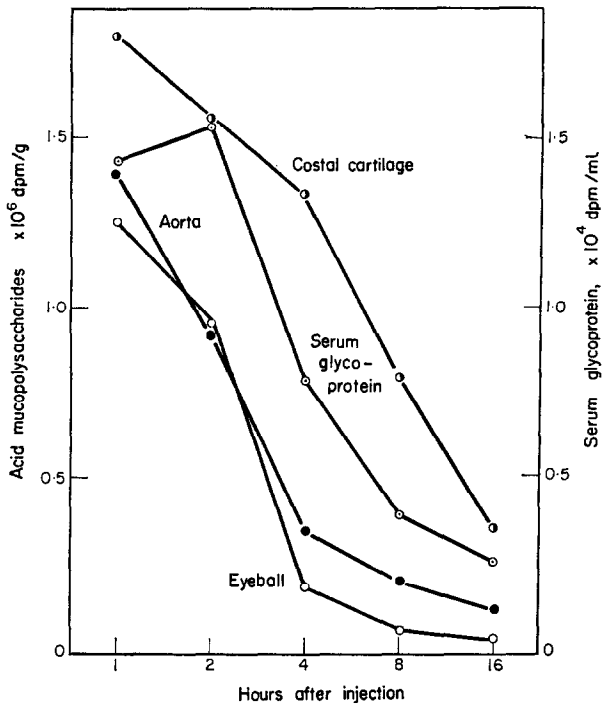

FIG. 1. Incorporation of sodium sulphate-S^{35} into tissue acid mucopolysaccharides and serum glycoprotein in rats.

AMPS derived from sulfate-S^{35} was significantly increased in the aorta by esterone and decreased in the eyeball by testosterone propionate. The specific activity of aortic AMPS was decreased in normocholesterolemic but not in hypercholesterolemic rats by treatment with P-5780. P-5780 had no significant effect on the amount of AMPS nor on the incorporation of radioactivity into AMPS in the aorta, cartilage or eyeball of hypercholesterolemic rats (Tables 1–3).

TABLE 1. ACID MUCOPOLYSACCHARIDES LEVELS

Treatment	No. of rats	Aorta		Costal cartilage		Eyeball	
		μg/g tissues	%	mg/g tissue	%	μg/g tissue	%
Normocholesterolemic							
None	7	56·9 ± 12·5*	0	4·27 ± 0·92	0	24·8 ± 3·3	0
Esterone	5	52·4 ± 17·3	− 8	2·70 ± 0·12†	−37	11·9 ± 0·9‡	−52
P-5780	5	31·8 ± 2·4†	−44	5·61 ± 0·75	+31	18·1 ± 3·5	−27
Testosterone propionate	5	53·2 ± 25·4	− 7	1·81 ± 0·29‡	−57	33·8 ± 9·6	+36
Hypercholesterolemic§							
None	8	41·4	−27	4·01	− 6	15·2	−39
P-5780	5	40·2	−29	4·38	+ 3	16·0	−35

* Standard error of mean.
† Significant difference from control, *P*-value less than 0·05.
‡ *P*-value less than 0·01.
§ Pooled sample.

Incorporation of sodium sulfate-S^{35} into serum perchloric acid soluble (glycoprotein) and insoluble serum protein fractions

As shown in Fig. 1, maximum incorporation into serum glycoprotein occurred about 2 hours after injection of sulfate-S^{35}. More of the injected sulfate-S^{35} was incorporated into the perchloric acid insoluble fraction than into glycoprotein. The two fractions were found to have almost the same specific activities, though the average concentration in perchloric acid insoluble protein was twice that in glycoprotein.

TABLE 2. INCORPORATION OF SODIUM SULFATE-S^{35} INTO ACID MUCOPOLYSACCHARIDES

Treatment	No. of rats	Aorta $10^5 \times$ dis/min/g	%	Costal cartilage $10^6 \times$ dis/min/g	%	Eyeball $10^5 \times$ dis/min/g	%
Normocholesterolemic							
None	7	9·07 ± 0·32*	0	1·30 ± 0·61	0	7·64 ± 0·77	0
Esterone	5	12·17 ± 2·75	+34	0·32 ± 0·04	−76	3·29 ± 0·47‡	−57
P-5780	5	4·90 ± 0·02†	−46	1·23 ± 0·25	− 6	5·20 ± 0·68†	−32
Testosterone propionate	5	8·42 ± 0·92	− 7	0·50 ± 0·08	−61	5·79 ± 0·31†	−24
Hypercholesterolemic§							
None	8	2·61	−71	0·365	−72	3·19	−58
P-5780	5	2·52	−72	0·299	−77	2·87	−62

* Standard error of mean.
† Significant difference from control, *P*-value less than 0·05.
‡ *P*-value less than 0·01.
§ Pooled sample.

TABLE 3. SPECIFIC ACTIVITY OF ACID MUCOPOLYSACCHARIDES FROM SODIUM SULFATE-S^{35} INCORPORATED *in vivo*

Treatment	No. of rats	Aorta $10^7 \times$ dis/min/mg	%	Costal cartilage $10 \times$ dis/min/mg	%	Eyeball $10^7 \times$ dis/min/mg	%
Normocholesterolemic							
None	8	2·12 ± 0·62*	0	2·60 ± 0·76	0	3·64 ± 0·76	0
Esterone	5	5·51 ± 1·34‡	+161	1·25 ± 0·12	−52	2·87 ± 0·58	−21
P-5780	5	1·58 ± 0·08	−25	2·47 ± 0·39	− 5	3·43 ± 0·98	− 5
Testosterone propionate	5	1·87 ± 0·50	−11	2·43 ± 0·54	− 7	1·37 ± 0·29†	−62
Hypercholesterolemic§							
None	8	6·31	+198	0·909	−65	2·10	−42
P-5780	5	6·28	+197	0·682	−74	1·12	−69

* Standard error of mean.
† Significant difference, from control *P*-value less than 0·05.
‡ *P*-value less than 0·01.
§ Pooled sample.

Table 4 summarizes the effects of steroid treatment on serum proteins. A significant increase in the total undialyzable S^{35} in the serum resulted from treatment of estrone and a lesser but still significant increase from treatment with P-5780. Estrone increased the amount of glycoprotein and decreased the amount of perchloric acid insoluble

serum protein, while P-5780 significantly decreased the amount of both serum fractions. The only significant change in the specific activities of these fractions was the increase in specific activity of glycoprotein caused by P-5780. Estrone tended to increase the specific activity of the perchloric acid insoluble serum protein but the increase was not statistically significant.

TABLE 4. INCORPORATION OF SODIUM SULFATE-S^{35} INTO SERUM GLYCOPROTEIN AND PERCHLORIC ACID INSOLUBLE

Treatment	No. of rats	Serum dis/min	Perchloric acid soluble (serum glycoprotein)		Perchloric acid insoluble	
		non-dialyzable dis/min to total dis/min	mg/ml	sp. act. dis/min/mg	mg/ml	sp. act. dis/min/mg
None	8	0·00276	130·5	140·7 ± 4·5*	238·3	147·2 ± 3·9
Estrone	5	0·00708†	194·1†	140·3 ± 25·0	107·0‡	200·3 ± 48·9
P-5780	5	0·00464	83·3†	231·2 ± 16·8‡	158·8‡	128·8 ± 19·3

* Standard error of mean.
† Significant difference from control, *P*-value less than 0·05.
‡ *P*-value less than 0·01

DISCUSSION

Daily injection of estrone into rats for 2 weeks had no significant influence upon the aortic AMPS level but caused a significant decrease in the AMPS levels in costal cartilage and eyeball. A similar decrease in the chondroitin sulfate B of male rat skin caused by estrogen has been reported,[17] and also an increase in mucopolysaccharides of rat skin[24] and AMPS in all connective tissues.[16] These conflicting reports have been explained by differences in the methods used, in whether experiments were conducted *in vitro* or *in vivo*, the dose of estrogen administered and in the sex of the animals used.

Few reports have been published on the dynamic effects of estrogen upon the incorporation of labelled compounds into tissue AMPS. Priest and Koplitz[17] have demonstrated the inhibitory effect of estradiol on the incorporation sulfate-S^{35} into the AMPS of rat aorta and cartilage *in vitro*. In contrast, the present *in vivo* experiments show that estrone treatment increases incorporation into AMPS in the aorta and tends to decrease incorporation into cartilage and eyeball. P-5780 greatly decreased the aortic AMPS level and incorporation of sulfate-S^{35} into aortic AMPS thus causing a reduction in the specific activity in the aorta. The anti-atherosclerotic action of P-5780 in rabbits[19] might be explained as due to a reduction in the incorporation into AMPS in the aorta, cartilage and eyeball, and an increased specific activity in the aorta. Treatment of these animals with P-5780 did not affect the elevated specific activity of aortic AMPS. Thus, the turn-over rate of AMPS in the rat aorta was accelerated by estrone and in the hypercholesterolemic state.

The serum glycoprotein level has been shown to decrease after acute hepatocellular injury[21, 25] and this is associated with an elevation in the specific activity of glycoprotein following sodium sulfate-S^{35} administration. There is also an increase in rapid or abnormal connective tissue metabolism in various conditions such as collagen disease, infections, cancer and during childhood. The effect of P-5780 resembles that

of acute parenchymal liver cell damage. As demonstrated previously,[20] no pathological changes or significant influence on serum GOT, GTP or alkaline phosphatase were observed in the liver of rats after prolonged treatment with P-5780. The hepatic response to P-5780 of a change in serum glycoprotein may be related to the antiatherosclerotic and hypocholesterolemic properties of this compound, because it induced elevation of acetate-1-C^{14} incorporation into rat liver cholesterol without causing any change in the liver and plasma cholesterol levels.

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