In Vitro Effects of Estradiol on the Aorta of Chickens

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Experiments reported elsewhere, suggesting that estrogenic hormones, besides modifying blood lipid levels, may prevent atherosclerosis through a direct action on the vessels themselves, received an apparent support when injected estradiol, 7 H3 was found within the arterial walls of normal and cholesterol-fed rabbits. Nevertheless, a direct action of the hormone on the arteries has not been reported, with the notable exception of the studies of Werthessen. In the present experiment, it is demonstrated that 2 isomers of estradiol may enhance in vitro the activity of an unspecific alkaline phosphomonoesterase in the aorta of chickens.

Methods

The aorta of 10 white leghorn cockerels, weighing between 500 and 1,000 Gm., was removed under sterile conditions and cut into 10 to 12 rings of approximate 2 mm. in length. These were incubated during 72 hours in modified roller tubes (fig. 1) with a balanced saline medium into which 30 per cent of bovine serum, 0.05 per cent of ethanol, as well as streptomycin and penicillin were incorporated. Aortic rings were also incubated with the same medium except that 2 μg. of 17α- or of 17β-estradiol* were added per ml. Throughout the experiment, pH was maintained by changing the medium as required. At the end of the experimental period, the tissues were blotted with filter paper and weighed; they were then frozen and cut in 10 μ sections with a microtome, homogenized and the alkaline phosphatase activity in approximately 20 mg. of aorta determined by the method described by Kaplan and Narahara for blood serum. Incubation was carried out at 37 C. for 30 minutes with disodium phenylphosphate as substrate and with sodium borate buffer at pH 9.4, as already described. Determinations were in duplicate and expressed as μM of phenol liberated per milligram of wet tissue, and the results were compared with Student’s “t” test for paired elements according with Fisher’s tables.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>With added steroid 2 μg./ml.</th>
<th>Paired controls</th>
<th>Difference</th>
<th>“t”</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>17α-estradiol</td>
<td>9</td>
<td>0.145±0.022</td>
<td>0.048±0.014</td>
<td>0.097</td>
<td>6.500</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>10</td>
<td>0.136±0.022</td>
<td>0.045±0.014</td>
<td>0.091</td>
<td>6.500</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Determinations performed by duplicate. Average ± standard error.

Results

The cells and fibers of control and experimental tissues maintained good staining properties at the end of the incubation periods. There was a relative increase in the interstitial fluid, so that comparisons of the enzymatic ac-
tivity before and after incubation was meaningless. Nevertheless, as it can be seen in table 1, both estradiol isomers increased the alkaline phosphatase activity in the chicken aorta when compared with corresponding paired controls.

Discussion

The present experiments demonstrate that both isomers of estradiol may enhance in vitro the alkaline phosphatase activity in the aorta of chicks. This action of the hormone is not restricted to the cardiovascular tissues, since it is apparently similar to the one obtained in the female genital tract of mammals, in the plasma of birds, as well as in the aorta of chickens and of rats after injection of the hormone. Furthermore, in vitro studies have also shown that estrogenic hormones may influence the oxygen consumption of isolated systems. Consequently, these results, as well as those demonstrated in the present paper, lend further support to the hypothesis that estrogenic hormones may influence the metabolic activity of the arteries through a direct local action. Estrogens in such a respect could then possibly have a similar action as in the uterus, vagina, mammary glands and skin, where they also are able to induce structural modifications when locally applied.

The significance of our findings in connection with enhancement of aortic atherosclerosis, as seen in birds after diethylstilbestrol administration, or with prevention and regression of coronary atherosclerosis in cholesterol-fed cockerels after estrogenic therapy, remains to be clarified. Before placing undue emphasis on results obtained in isolated systems, it seems appropriate to quote the cautious words of Hechter: "... in vitro reactions with hormones may be completely unrelated... to the nature of essential underlying cellular mechanisms involved in hormone response." Nevertheless, the fact that injection of estradiol increases alkaline phosphatase activity in the aorta of chickens and of rats and that similar results are seen in vitro, apparently demonstrates that estrogens may influence arterial metabolism by modifying local enzymatic mechanisms. Much more work on this field is clearly needed before such modified local mechanisms could be linked with atherogenesis.
Summary
Aortas of chickens were incubated during 72 hours with 17α- or with 17β-estradiol and the alkaline phosphomonoesterase activity determined. Paired experiments demonstrated that both isomers are able to enhance the activity of the enzyme in vitro. The possible relation of these results with atherogenesis is discussed.

Summary in Interlingua
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References
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