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IN VITRO CHOLESTEROL BIOSYNTHESIS IN TESTICULAR TISSUE OF RAMS FED ON FLUORIDE SUPPLEMENTED RATION.

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SUMMARY

A study was carried out to investigate the effect of fluoride on cholesterol synthesis in testicular tissue of rams. Sodium fluoride was added to the rations at two levels (50 and 100 ppm) on dry weight basis. At the end of the experiment, the animals were sacrificed and the right testes of each animal was removed and incubated with 1-C^{14}- acetate in the presence and absence of HCG. Human chorionic gonadotropin stimulated cholesterol biosynthesis significantly in the control animals, but failed to do so in the fluoride treated rams. Fluoride, also, reduced cholesterol biosynthesis in the treated groups compared with the control animals. Itw was postulated that fluoride might alter cholesterol biosynthesis by influencing carbohydrate and lipid metabolic pathways.

INTRODUCTION

Fluoride ions, at high concentrations, have induced various effects on biochemical and functional aspects of the mammalian systems (1,2). The plasma membrane-bound enzyme adenylate cyclase was activated by fluoride in cardiac (3), uterine (4) and testicular (5) tissues. Increased cyclic adenosine monophosphate (cAMP), which results from activation of adenylate cyclase, has a stimulatory effect on steps involved in steroidogenesis (6,7). Luteinizing hormone activity (such as human chorionic gonadotropin, HCG) is known to induce its stimulatory effect on steroidogenesis via increasing the cAMP content(8).

The present investigation aimed at exploring the capability of testicular tissue to synthesize cholesterol, in vitro, from labeled acetate after feeding the animals rations supplemented with two levels of fluoride. The extent of stimulating the biochemical reactions, which are involved in cholesterol biosymthesis by HCG was also investigated.

MATERIALS AND METHODS

Eighteen adult Awassi rams were, at random, divided into three equal groups as follows:

1- Control (C) group: rams of this group were kept on alfalfa and concentrate ration (600g/day each) for 11 weeks.

2- T_1 group: the animals of this group were fed as in the control group, but the concentrate portion was supplemented with NaF (50 pmm, based on the dry weight of the offered ration).

3- T_2 group: the animals were treated as in T_1 group with the exception that the fluoride supplementatuion was 100pmm.

Water and green fodder were offered ad libitum to all animals during the experimental period.

On day 78, the rams were acrificed and the right testes of each animal was transfered into 1L beaker in an ice bath. The beaker contained Krebs-Ringer bicarbonate solution (PH 7.4). In a cold room (4°C), testicular mince was prepared from each testes and weighed aliquots were transfered into two sets of 25 ml inoubation flasks as follows:-

1- Five flasks each containing 4ml Krebs-Ringer bicarbonate solution plus 200mg% glucose (PH 7.4) and 100 uCi 1-C^{14}-acetate (specific activity 681 mCi/mg, Am. International Ltd, UK).

2- Five flasks each containing, in addition to the above mentioned contents, 150 I.U. human chorionic gonadotropin (gonadotrophin chorionique, I.S.H., France).

All incubation flasks were incubated in a shaking water bath at 37°C for 3 hrs. under an atmosphere of 90-95% O_2 and 5-10% CO_2. At the end of incubation time, 5ml of hexane-acetone mixture (1:1) were added to each flask and stored at -20°C.

At the time of extraction, 200ug of cold cholesterol and approximately 100,000 cpm of tritium labled cholesterol were added to each flask. After thowing and mixing, the contents of each flask were homogenized in a glass homogenizer using hexane-acetone (1:1). The homogenate was transfered into 250 ml RB flask, and rotoevaporated under negative pressure. The contents of each flask was washed three times with hexane-acetone (1:1), and transfered into 12ml conical flasks. The contents of each flask was evaporated under nitrogen gas stream in a water bath (45°C). Each sample was then thin layer chromatographed along with cholesterol standard(9). Collected cholesterol fraction was recrystallized for three times (acetone-water), to ensure purity before counting for radioactivity in weighed aliquots of dried crystals.

Organic solvents were of analytical grade and were redistilled twice before use. Purification of unlabelled (cold) cholesterol was carried out by the steps described by Tietz (1986) (10). Purified cholesterol crystals were stored in tightly stoppered glass containers at -20°C. Tritium labeled cholesterol was thin layer chromatographed before use. The developing mixture was benzene: ethylacetate (3:2), as described previously (11).

RESULTS

The values in table 1 represent the means of total counts of radioactivity per unit weight of purified

Table 1: The effect of fluoride supplementation (50 and 100 ppm) and of HCG (150 I.U.) on in vitro cholesterol biosynthesis from 1-C^{14}-acetate by the testicular tissue of Awassi rams (cpm/g).

	Control group		T_1 group		T_2 group	
	C	HCG + C	50 ppm	50 ppm + HCG	100ppm	100 ppm + HCG
First Crystallization	17916 + 922	33775 + 1647	9681 + 516	12020 + 1046	6311 + 490	7141 + 462
Second Crystallization	17659 + 901	32908 + 1722	9236 + 498	11981 + 985	6186 + 416	6990 + 471
Third Crystallization	17448 + 853[a]	32817 + 1492[A*]	8971 + 523[b]	11846 + 956[B]	6035 + 388[b]	6819 + 436[B]

- The figures represent the means of six values/group + SEM. Each value is an average of five incubtes (thirty incubates per group).

- Means with different superscripts are significantly different ($P < 0.05$).

* Significantly different ($P < 0.05$) as compared with control without the addition of HCG.

cholesterol. The values of the third crystallization was used for comparison between treatments.

The results demonstrate that human chorionic gonadotropine (HCG) exerted its stimulatory effect on cholesterol biosynthesis by almost doubling the incorporation of 1-C^{14}-acetate into cholesterol by the in vitro incubated testicular tissue of the control group (table 1). The increment, 88%, at the third crystallization was statisticall significant ($P < 0.05$). The incorporation of 1-C^{14}-acetate into cholesterol was significantly reduced in the testicular tissue samples of both T_1 and T_2 groups of rams, compared with the control group. Furthermore, HCG failed to induce a significant increase in the incorporation of labeled acetate into cholesterol in the treated animals, compared with that observed in the control group (table 1). It can be seen that HCG caused a 32% increment in cholesterol biosynthesis in T_1, and only 13% in T_2 at the third crystallization results.

DISCUSSION

The results showed that ram testicular tissue was capable of synthesizing cholesterol from 1-C^{14}-acetate as a precursor. Furthermore, HCG stimulated the synthesis rate in testicular tissue of the control group. Luteinizing hormone activity has been reported to stimulate the biosynthesis of testicular cholesterol from acetate (12).

With regard to incorporation of radioactivity into cholesterol, both levels of fluoride (50 and 100 ppm) resulted in reduction of biosynthesis rate. The effect was fluoride level dependent when reduction percentage was considered. The testicular tissue of the treated animals did not respond normally to the addition of HCG in respect to cholesterol biosynthesis. Although, fluoride as well as HCG activate adenylate cyclase enzyme and increase endogenous cAMP, cholesterol synthesis was reduced. This could be attributed to a

possible impairment of specific biochemical steps that are involved in glucose and fatty acids metabolism, which are necessary for the formation of acetyl-CoA (the building unit for cholesterol). It is reported that the activiy of lactate dehydrogenase (13), succinate dehydrogenase (14), glucose-6-phosphate dehydrogenase (15), and isocitric dehydrogenase (16), were reduced or inhibited by fluoride. These enzymes regulate important steps on glucose metabolic pathways. Fluoride has also been reported to induce structural changes, and depress the energy producing ability of the mitochondria (16, 17). On the other hand, fluoride inhibits the enzyme acyl-CoA synthetase involved in fatty acid oxidation (18). These cholesterol synthesis might be reduced by fluoride, and hence cholesterol synthesis. This might explain the observed reduction in cholesterol biosynthesis in the fluoride treated animals.

REFERENCES

1. Zhi-Zhong, G.; Pei-Si, Y. and Zong-Jie, Z. (1989). An experimental study of blood biochemical diagnostic indices for chronic fluorosis. Fluoride, 22:112-118.

2. Lee, J. (1990). Fluoride and osteoporosis. Fluoride, 23:51-54.

3. Chatelain, P.: Robbercht, P.; DeNeef, P.; Camus, J. and Christophe, J. (1983). Thermodependence of basal and stimulated cardiac adenylate cyclase activity in normotensive and spontaneously hypertensive rat. J. Mol. Cell. Cardiol., 15:811-820.

4. Krimnsland, S. (1980). Adenylate cyclase activity in the uterine cervix of neontal and immature mice: influence of estradiol-17 beta. J. Endocrinol., 84:255-260.

5. Al-Jeboory, A.; Hussain, S.; Al-Ani, W.; Al-Jiboori, N. and Mehdi, A.W.R. (1993). Cyclic adenosine monophosohate in serum and testicular tissue of rams fed on high fluoride containing ration. Iraqi J. Vet. Med., 17:159-164.

6. Haynes, R.; Sutherland, E. and Rall, T. (1960). The role of cyclic adenylic acid in hormone action. Rec. Prog. Horm. Res., 16:121-129.

7. Dempsy, M.E. (1974). Regulation of steroid biosynthesis. Am. Rev. Biochem., 43:967-990.

8. Nugent, C.L.; Lopata, A. and Gould, M.K. (1975). The effects of exogenous gonadotropins on ovarian adenylate cyclase activity. Endocrinology, 97(3): 581-587.

9. Neher, R. (1964). Steroid Chromatography. Elsevier Publishing Co., New York.

10. Tietz, W.N. (1986). Textbook of clinical chemistry. W.B. Saunders Co. philadelphia.

11. Van Lier, J.E. and Smith, L.L. (1969). Sterol metabolism: V. A chromatographic examination of human brain sterols. Texas Reports of Biology and Medicine. 27(1): 167-176.

12. Hall, P.F. (1963). The effect of interstitial-cell-stimulating hormone on the biosynthesis of testicular cholesterol from acetate-1-c^{14}. Biochemistry, 2(6): 1232-1237.

13. Chitra, T.; Reddy, M.M. and Ramana Rao, J.V. (1983). Levels of muscle and liver tissue enzymes in channa punctatus bloch exposed to NaF. Fluoride, 16(1): 48-51.

14. Chinoy, N.J. and Sequeira, E. (1989). Fluoride induced biochemichal changes in reproductive organs of male mice. Fluoride, 22(2): 78-85.

15. Carlson, J.R. and Suttie, J.W. (1966). Pentose phosphate pathway enzymes and glucose oxidation in fluoride fed rats. Am. J. Physiol. 210:79-83.

16. Singh, S.J.P. and Thapar, S.P. (1989). Effect of fluoride in excess on lipid constituents of respiratory organs in albino rabbits. Fluoride, 22(1):33-39.

17. Chongwan, Z. and Daijei, H. (1988). Ultrastructural findings in liver, kidenys, thyroid gland and cardiac muscle of rabbits following sodium fluoride administration. Fluoride, 21(1): 32-38.

18. Dousset, J.C.; Rioufol, C.; Philibert, C. and Bourbon, P. (1987). Effects of inhaled HF on cholesterol, carbohydrate and tricarboxylic acid metabolism in guinea pigs. Fluoride, 20(3): 137-141.