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Induction of hepatic peroxisomal β-oxidation of fatty acids by dehydroepiandrosterone (DHEA) in male F-344 rats

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Pharmacological administration of dehydroepiandrostenone (DHEA, 300 mg/kg, p.o.) for 2 weeks significantly increased ($P < 0.01$) the activity of hepatic peroxisomal β-oxidation of fatty acids in male F-344 rats. Similar effects were observed following 8 week administration of DHEA. These results are discussed in the light of oxidative stress-mediated hepatocarcinogenesis.

Dehydroepiandrosterone (DHEA), is a C_{19} steroid secreted abundantly by the mammalian adrenal cortex. Other than serving as a precursor of sex hormones in peripheral tissues, the physiological function of DHEA is not well characterized. However, in animal models, administration of DHEA inhibits cancer (Nyce et al. 1984), and moderates obesity (Yen et al. 1977), diabetes (Coleman et al. 1982) as well as atherosclerosis (Gordon et al. 1988). Despite these potential beneficial effects, DHEA exhibits the characteristics of a peroxisome proliferator and hepatocarcinogen in rats and mice (Sakuma et al. 1992).

Peroxisome proliferators represent a class of chemicals capable of producing a dramatic increase in the number and size of peroxisomes in rodent liver. These functionally diverse chemicals also cause induction of lipid metabolism enzymes and liver tumor in the rodents (Sakuma et al. 1992).

According to the oxidative stress hypothesis of peroxisome proliferator-induced hepatocarcinogenesis, an increase in free radicals results from a significant induction of peroxisomal β-oxidation of fatty acids following treatment with a peroxisome proliferator. This induction of enzyme causes an excessive production of hydrogen peroxide molecules as a reaction intermediate. Hydrogen peroxide formed, then undergo further metabolism to reactive free radicals, which cause lipid peroxidation and genomic damage to hepatocytes, ultimately resulting in hepatic tumor formation (Rao et al. 1992). Cellular defense enzymes (e.g.-catalase, glutathione peroxidase), on the other hand, are not induced proportionately to counteract this oxidative damage.

In light of these observations, the present study examined the effect of a pharmacological administration of DHEA on peroxisomal β-oxidation of fatty acids in male F-344 rats.

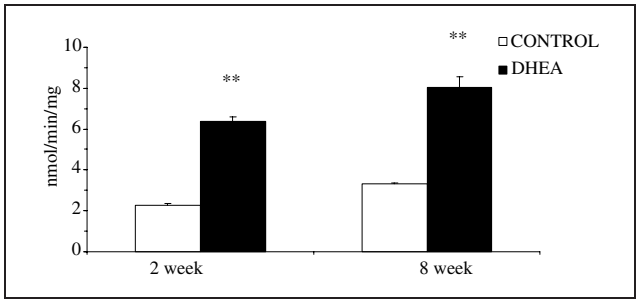

Fig.: Effect of DHEA on hepatic peroxisomal β-oxidation activity in male F-344 rats. Each bar represents mean ± S.E.M of 4–6 rats. Values represent nmoles of NADH formed/min/mg protein. **Significantly different from control animals. ($P < 0.01$)

Peroxisomal β-oxidation activity measured in rat liver homogenates increased significantly ($P < 0.01$) following DHEA treatment for 2 and 8 weeks, the increase being 2.8 and 2.4 fold respectively (Fig.). The time-point of treatment was chosen from a previous report (Khan 2005). The increase of peroxisomal β-oxidation of fatty acids in this study following 2 weeks of DHEA treatment is somewhat less than that reported by others (3.74 fold: Sakuma et al. 1992). Although the same strain of rats was used, this could be due to different age-group animals used in our study.

In the present study, enzyme activity of peroxisomal fatty acid β-oxidation was similar at both time-points. It appears that maximal induction of enzyme activity might have occurred within 2 weeks.

Previously we have shown (Khan 2005) that DHEA treatment caused a significant increase in lipid peroxidation in liver homogenate only after 8 weeks, with no change at an earlier time-point (2 weeks). The current findings, however, suggest that elevated lipid peroxide levels in DHEA-treated animals were not necessarily associated with increased peroxisomal β-oxidation of fatty acids. It is more likely that lipid peroxidation might be increased in specific cellular compartments and is not measurable in total liver homogenates. Furthermore apart from oxidative stress, other molecular mechanisms are also suggested in perturbation of cellular homeostasis by peroxisome proliferators in hepatic tissues.

Taken together, current study indicates that daily oral administration of DHEA for 2 weeks significantly increases peroximal β-oxidation in the liver and that the induction was not altered substantially even after 8 weeks of steroid treatment.

Experimental

After acclimatization, male F-344 rats initially weighing 191–224 g (Harlan Sprague Dawley, IN) maintained on normal laboratory chow and water *ad libitum* were randomly divided into 2 groups: DHEA (300 mg/kg; AKZO, Basel, Switzerland) and control. DHEA suspended in 0.5% carboxymethyl cellulose was administered at 3 ml/kg, while the control animals received the vehicle. The animals were treated once daily by gastric intubation for 2 or 8 weeks as applicable (Sakuma et al. 1992; Yamada et al. 1991; Khan 2005).

Twenty-four hours following the last treatment, the animals were euthanized by CO_2 (100%). Immediately, prior to death, the livers of the rats were perfused *in situ* with chilled buffered saline (pH 7.4) (Borges et al. 1993). Liver homogenates were prepared in 20 mM Tris HCl containing 0.25 M sucrose (pH 7.4) and aliquots were frozen at −70 °C until further analyses were performed. All other chemicals and reagents used were of the highest grade commercially available.

Peroxisomal β-oxidation was determined spectrophotometrically in the liver homogenates by monitoring changes in absorbance at 340 nm (Lazarow 1981). Protein concentration of the liver sample was measured according to Bradford (1976) using bovine gamma globulin as standard. Data are reported

as mean ±S.E.M. (standard error of the mean). Student's T-test was used for statistical analysis using the statistical package GB STAT (version 5.0).

References

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

Borges T, Glauert HP, Robertson LW (1993) Perfluorodecanoic acid noncompetitively inhibits the peroxisomal enzymes enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase. Toxicol Appl Pharmacol 118: 8–15.

Coleman DL, Leiter EH, Schwizer RW (1988) Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. Diabetes 31: 830–833.

Gordon GB, Bush DE, Weisman HF (1988) Reduction of atherosclerosis by administration of dehydroepiandrosterone. A study in the hypercholesterolemic New Zealand white rabbit with aortic intimal injury. J Clin Invest 82: 712–720.

Lazarow PB (1981) Assay of peroxisomal β-oxidation of fatty acids. Methods Enzymol 72: 315–319.

Khan SA (2005) Effects of dehydroepiandrosterone (DHEA) on ubiquinone and catalase in the livers of male F-344 rats. Biol Pharm Bull 28: 1301–1303.

Nyce JW, Magee PN, Hard GC, Schwartz AG (1984) Inhibition of 1,2-dimethylhydrazine-induced colon tumorigenesis in Balb/c mice by dehydroepiandrosterone. Carcinogenesis 5: 57–62.

Rao MS, Subbarao V, Yeldani AV, Reddy JK (1992) Hepatocarcinogenicity of dehydroepiandrosterone in the rat. Cancer Res 52: 2977–2979.

Sakuma M, Yamada J, Suga T (1992) Comparison of inducing effect of dehydroepiandrosterone on hepatic peroxisome proliferation-associated enzymes in several rodent species. Biochem Pharmacol 43: 1269–1273.

Yamada J, Sakuma M, Ikeda T, Fukuda K, Suga T (1991) Characteristics of dehydroepiandrosterone as a peroxisome proliferator. Biochim Biophys Acta 1092: 233–243.

Yen TT, Allan JA, Pearson DV, Acton JM, Greenberg MM (1977) Prevention of obesity in Avy/a mice by dehydroepiandrosterone. Lipids 12: 409–413.

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Walter Krösche (1882–1957) and the Mannich Reaction

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Walter Krösche was the Ph.D. student of Carl Mannich, with whom the latter published the school forming paper where the first 'Mannich reaction' has been described. For the first time biographic details and photos of Krösche are published here in a short biographic sketch.

Every chemistry student has to learn the Mannich reaction, and many who are working in that field of organic synthesis refer to the first paper entitled "Ueber ein Kondensationsprodukt aus Formaldehyd, Ammoniak und Antipyrin" ("About a condensation product of formaldehyde, ammonia, and antipyrine") published in 1912 in *Archiv der Pharmazie* (Mannich and Krösche 1912). This was the Ph. D. thesis of Walter Krösche (Krösche 1912). Whereas one can easily find information about Carl Mannich (Friedrich 1991; Böhme 1955), no information is available about Walter Krösche so far. Some lucky circumstances allowed me to discover a number of interesting details about the life of that remarkable man, which are published here.

Walter Krösche was born in Berlin October 8th, 1882 as son of the merchant Hermann Krösche. The family came from Schöttmar (now part of Bad Salzufflen) in Westphalia, Germany. He attended the Lessing Gymnasium (Berlin), a well known school, until the so-called *Obersecunda*, i.e., until year 11, which is two years before the German *Abitur*, the school-leaving examination preparing for University. After that he started an apprenticeship as pharmacist (*Apothekerlehre*) and served in pharmacies in Berlin, Lörrach near Basel (Switzerland), and Geneva (Switzerland) until Easter 1906. Then he started to study Pharmacy at Berlin University. In October 1906 he took leave for half a year to serve in the army (first half as so-called 'one year volunteer', *Einjährig-Freiwilliger*). September 29th, 1909 he graduated as a pharmacist and thereafter, Easter 1910, he started again university studies with the aim of getting a Ph.D. In parallel to these studies, at Easter 1911, he passed the *Abitur* at the *Siemens-Oberrealschule* (Charlottenburg, Berlin). In Prussia the *Abitur* was a necessary prerequisite for obtaining a Ph.D., but not for becoming a pharmacist (Friedrich and Müller-Jahncke 2005). July 24th, 1912 he was awarded a Ph.D., and in the doctorate certificate, issued in Latin, Walter Nernst is given as his promoter. Indeed, Walther Hermann Nernst