ACTA STEREOL 1992; 11/2: 199-204
ORIGINAL SCIENTIFIC PAPER

MORPHOMETRICAL CHANGES IN FOLLICLES OF THE THYROID GLANDS IN CHRONIC ALCOHOLISM

Milica Matavulj [1], Milan Kecman [2], Ljiljana Somer [2]

1 University of Regensburg, Institut of Anatomy, Universitätstr. 31, D-8400 Regensburg, Germany
2 Institute of Histology and Embryology, Faculty of Medicine, Novi Sad, Yugoslavia

ABSTRACT

The effect of chronic ethanol consumption on the structure of thyroid gland follicles was the subject of our studies. Mature male Wistar rats were given 15% ethanol solution ad libitum as the only liquid offered. The animals were sacrificed in three groups: one,three and six months after the beginning of alcohol ingestion. Stereological analysis showed a significant increase in the volume density of follicular epithelium /Vve/, its thichness /t/,index of activation of the thyroid gland /Ia=Vve/Vvc/, and significant reduction in volume density of colloid /Vvc/ in all the periods examined. These results suggest the possibility that long-term alcoholism can disturb the normal structure of the thyroid gland, besides its well-known effects on the peripheral metabolism of thyroid hormones.

Keywords: thyroid gland, chronic ingestion of ethanol, stereology

INTRODUCTION

Basically, the levels are known at which ethanol influences thyroid activity. First, ethanol can directly influence both hypothalamic TRH and/or pituitary TSH content or its secretion (Prasad et al, 1984). Second, chronic ethanol ingestion results in decreased circulating thyroid hormone levels (Singh et al, 1979) and reduced activity of hepatic 5-monodeiodinases which convert thyroxine /T4/ into triiodothyronine /T3/ and reverse /T3/ into diiodothyronine /T2/ (Wu et al, 1979). Possibility that ethanol can have a direct effect on the structure of the thyroid gland has been also suggested (Prasad et al, 1984, Portolés et al, 1985), but has, unfortunately, not been supported with sufficient data. Our work was designed to investigate the effect of long-term ethanol exposure on thyroid morphology. In this connection we have studied both stereological and cytological parameters.

MATERIALS AND METHODS

A total of 153 mature male Wistar rats with 190-250 g initial body weight were used in these experiments. They were housed

in groups of 4-5 animals per cage and maintained under controlled light, temperature and humidity conditions. Animals were given regular food and 15% ethanol as the only drinking solution ad libitum. Alcoholized animals, as well as parallel control animals, were sacrificed in one, three and six months after the beginning of alcohol ingestion. Thyroid glands were fixed in Bouin's solution. Paraffin-embedded thyroids were cut serially in four-micrometer slides coloured after the method of Florantin. A number of thyroid glands were prefixed in 2,5% glutaraldehyde and fixed in 1% osmiumtetroxide. Epon embedded semithin sections were stained with methylene-blue and used for light microscopy analysis. For stereological analysis every fourth section was used from the middle of the gland to the periphery. We determined the volume density of the follicular epithelium /Vve/, and colloid /Vvc/ with grid M42 (Weibel et al, 1966), index of activation of the thyroid gland /Ia/ /Ia=Vve/Vvc/ (Kališnik, 1971) and thickness of the follicular epithelial cells /$\bar{t}$/ (Bogataj et al, 1977). Statistical analyses were estimated by Student's t-test.

RESULTS

Chronic alcohol ingestion increased significantly both the volume density of follicular epithelium /Vve/ /Fig.1/ and its thickness /$\bar{t}$ / /Fig.2/, while volume density of colloid /Vvc/ during all experimental intervals significantly decreased /Fig.3/. Index of activation of thyroid glands /Ia/, which has positive correlation with TSH level in plasma (Kališnik, 1971), was significantly increased /Fig.4/.

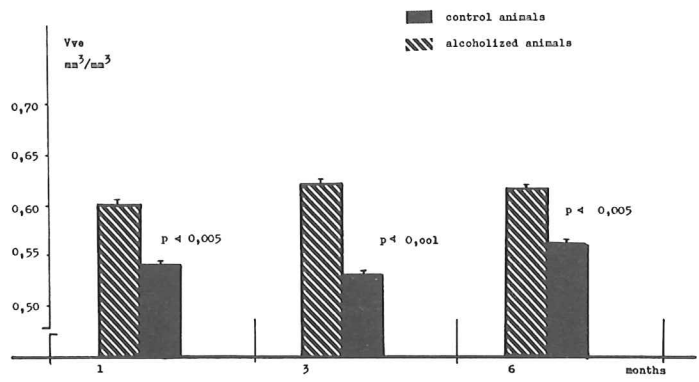

Fig.1. The mean volume density of the thyroid epithelium /Ve/ ± standard errors /vertiacl bar/ in control and alcoholized animals are given.

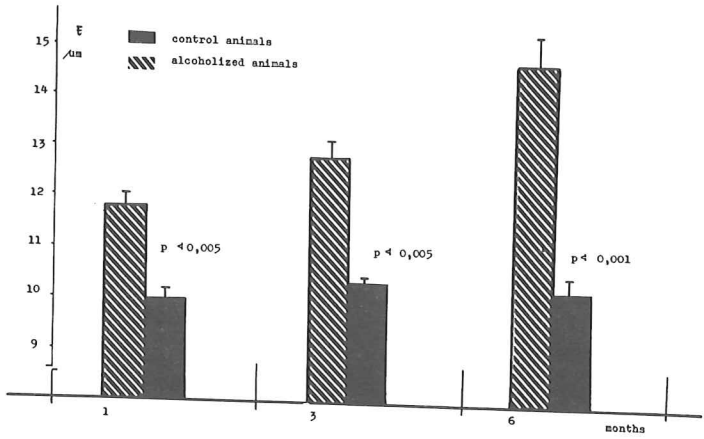

Fig. 2. Thickness of epithelium of thyroid follicles /$\bar{t}$/ in control and alcoholized animals. The mean ± standard errors /vertical bar/ are given.

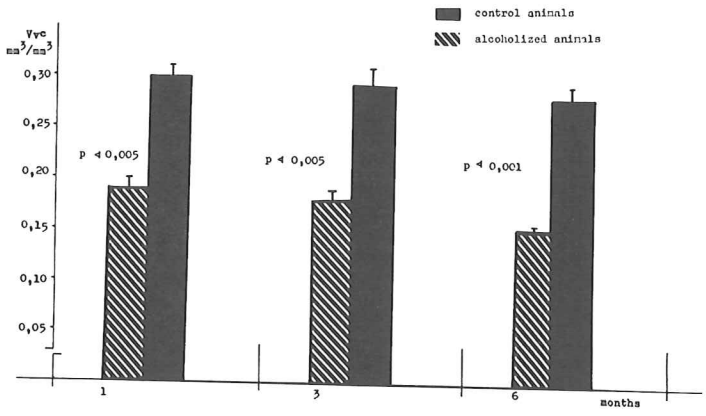

Fig. 3. Volume density of colloid /Vvc/ in control and alcoholized animals. The mean ± standard errors /vrtical bar/ are given.

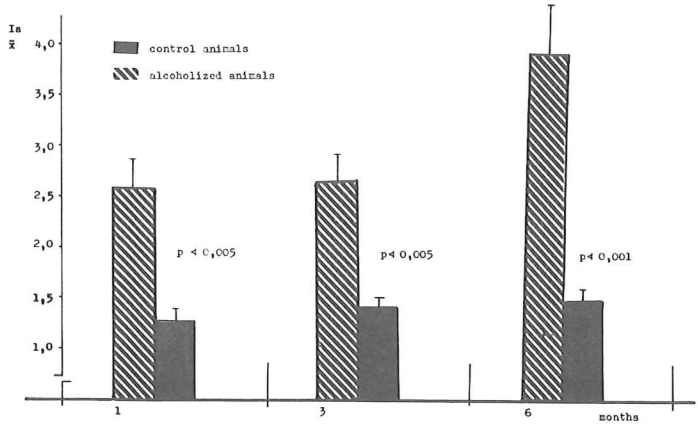

Fig.4. Activation index /Ia/ of thyroid gland in control and alcoholized animals. The mean ± standard errors /vertical bar/ are given.

The most prominent cytological changes in the thyroidal follicular cells of the chronic alcoholized rats were the appearance of variously shaped apical protrusions and intracellular large colloid droplets, so-colled "colloid balls" /Fig.5/. These structure were seen after all experimental periods.

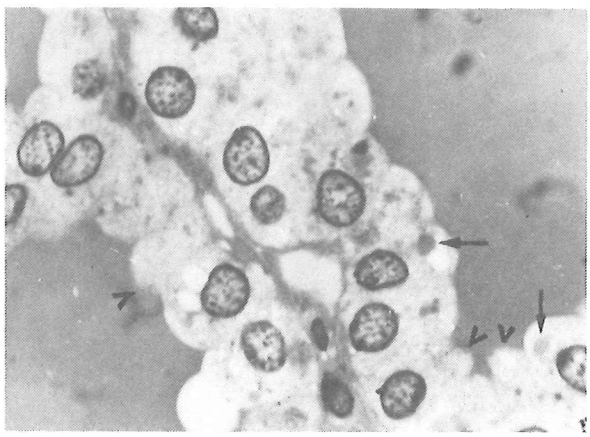

Fig. 5. Thyroid gland, follicular epithelium, after three months' ingestion of 15% ethanol. Protrusions of the apical cell surface /arrowheads/ and large colloid droplets /arrow/ are present. Semithin section, methylene blue. x 1000.

DISCUSSION

The results of the stereological analysis of follicles clearly show that chronic ethanol ingestion results in the activation of the thyroid gland. This is substantiated by the significant increase in the thyroidal activation index. This change reflects the significant increase in volume density of epithelium /Vve/ and the decrease in volume density of colloid /Vvc/. In contrast, in experiments similar to ours the administration of 20% ethanol resulted in the decrease of T4, T3 and rT3 levels (Singh et al, 1979). Some other authors also reported that chronic alcohol ingestion decrease pituitary-thyroid measures in rats (Prasad et al, 1984, Mason et al, 1984, Portolès et al, 1984). These findings suggest that increase in thyroidal activation index need not automatically mean true activity increase. Here, however, it may be a sign of slowdown in the synthesis and/or secretion of thyroid hormones. Thus, it is possible that appearence of large colloid droplets /colloid balls/ noticed in numerous follicular cells after several months alcoholization, can be considered a result of disturbed balance between exo- and endocytic processes (Pantić, 1974). In our conditions this hypothetic asynchrony between secretion and absorption could also explain the decrease in volume density of colloid. Several mechanisms could be postulated to account for the development of the hypothyroidism resulting from chronic ethanol consumption. A number of authors have proposed that alcohol liver disease itself (Green et al, 1974, Pamanter et Boyden, 1984), together with other factors such as nutrition (Shank et al, 1974), may cause decrease of thyroid hormone levels. These alterations are freqently associated with heavy alcohol intake in man but chronic ethanol traetmant with relatively low concentration /20%/ may not have been so severe as to induce pathophysiological changes in the livers of rats (Singh et al, 1979, Barak et al, 1985).In chronic alcoholized rats reduction of plasma T4 level was not due to altered plasma binding proteins because at the same time, T3 resin uptake values were not changed (Prasad et al, 1984). This reflects that liver functions were normal. It seems clear, therefore, that there exist factors other than liver disease which may help explain the reductions in circulating T4 and T3. Malnutrition observed in chronic alcoholized rats was also mentioned as a probable cause of these abnormalities in thyroid hormone serum levels (Teschke et al, 1983). Recent findings, however, have indicated that hypothalamic-pituitary-thyroid axis /HPT/ measures of long-term ethanol-treated animals, when compared to those of a control group fed a nutritionally complete isocaloric diet, show a significant decrease in T4, T3, rT3 and basal TSH (Mason et al, 1988). The latter authors concluded that "these differences did not appear to result from caloric deprivation alone". In connection with this, a certain number of investigators support the concept that ethanol per se aside from liver disease and nutritional factors, has a direct effect on thyroid physiology and creates a hypothyroid state (Prasad et al, 1984, Portolès et al, 1985). Studies in vitro demonstrated that consumption of 7,5% ethanol for 6 weeks in rats leads to alterations of all three HPT axis levels and induces: 1/ thyroidal resistance to TSH action, 2/ an exaggerated TSH-response to TRH, and 3/ possible defects in hypothalamic TRH secretion (Prasad et al, 1984). These results justify the supposed direct effect of

ethanol on the thyroid gland in adult rats. A corresponding effect has also been seen in other endocrine glands e.g. testes (Van Thiel et al, 1975). In the light of this, morphometrical and morphological thyroid gland changes under our experimental conditions may be the result of direct effect of ethanol on one of the HPT axis levels leading to primary, secondary or tertiary hypothyroidism. However, which mechanism is really involved remains obscure. The present study gives evidence that chronic alcoholization can disturb normal thyroidal structure and we hope that our further investigations will reveal more details.

REFERENCES

Barak AJ,Tuma DJ, Beckenhauer C.Ethanol, the choline requirement methylation and liver injury.Life Sci 1985; 37: 789-791.

Bogataj M,Kališnik M,Poredos P.A simplified indirect method of determination of the average shell thickness of the hollow spheres from samples in sections.Folia Anat Iugosl 1977; 6: 45-49 (in Serbocroatian).

Green JRG,Movat NG,Fisher RA,Anderson DC.Thyroid function and thyroid regulation in euthyroid men with chronic liver disease: evidence of multiple abnormalites.Gut 1977; 17: 426- 430.

Kališnik M.Histometric thyroid gland activation index.J Micr /Oxf/ 1971; 95: 345-348.

Mason GA, Stanley DA,Walker CH,Hashimoto H, Noonam LR,Prange AJ. Chronic alcohol ingestion decrease pituitary-thyroid axis measures in Fisher-344 rats.Alcohol Clin Exp Res 1988; 12: 731-734.

Pamenter WR,Boyden TW.Interactions of ethanol with hypothalamic-pituitary-thyroid axis.Life Sci 1984; 34: 707-713.

Pantić V.The cytophysiology of thyroid cells.Int Rev Citol 1974; 38: 153-243.

Portolès M,Sachis R,Guerri C.Effect of chronic and acute administration on plasma thyroid hormone levels in the rat. IRCS Med Sci 1985; 13: 616-618.

Prasad C,Mori H,Wilber JH,Edwards RM.Chronic alcohol consumption alters hypothalamic-pituitary-thyroid axis regulation in rats.Neuroendocrinol Lett 1984; 6: 169-175.

Shank ML,Singh SP, Blivaiss B,Kabir MA,Williams K,Premachandra BN. Ethanol inhibition of pituitary-thyroid axis: an effect secondary to nutritional deficiency.Endocrinol Res 1984; 10: 139-150.

Singh SP,Petal GO,Kabir M,Premachandra BN.Serum T4, T3 and reverse T3 in ethanol fed rats.Life Sci 1979; 25: 889-894.

Teschke R,Moreno F,Heinen E, Herrmann J,Krüskemper L,Strohmeyer G. Hepatic thyroid hormone levels following chronic alcohol consumption:direct experimental evidence in rats against the existence of a hyperthyroid hepatic state.Hepatology 1983; 3/4: 469-474.

Van Thiel DH,Gavarel JS,Laster R,Goodman MD.Alcohol-induced testicular atrophy.Gastroenterology 1975; 69: 326-332.

Weibel ER,Kistler DS, Scherle XF.Practical stereological methods for morphometric cytology.J Cell Biol 1966; 30: 2-29.

Wu YS,Wiechmann A,Chopra IJ,Linos KP.Effects of ethanol on peripheral thyroid hormone metabolism in rats.Clin Res 1979; 27: 25A.

Received: 1990-06-26
Accepted: 1992-07-21