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A Stereological Study of the Effects of Lithium on Morphology of Submandibular Gland

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Abstract: The purpose of this study was to investigate morphometrically the effects of lithium administration on the submandibular salivary gland structure in male rats. Twenty male Wistar rats randomly divided into control and experimental groups, received distilled water and 0.1% lithium carbonate solution, respectively, for a period of 8 weeks. The submandibular glands were dissected, histologically processed and sectioned. Ten to twelve sections were sampled by systematic uniform random sampling and stained with hematoxylin and eosin. The volume of the gland, volume density and absolute volume of the seromucous acini, convoluted granular tubules, striated ducts, excretory ducts and stroma of the gland were estimated by point counting. Volume weighted mean volume of the seromucous acini was also estimated by the point sampled intercepts method. The volume density and the total volume of the acini, convoluted granular tubules and striated ducts were significantly decreased in the lithium group in comparison to control group ($p<0.01$). The excretory ducts showed no statistically significant differences ($p>0.05$) and the glandular stroma increased significantly in the lithium treated group as compared with the control group ($p<0.0001$). The volume-weighted mean volume of the seromucous acini was significantly higher in the control group, than in the lithium treated group ($p<0.0001$). This study has demonstrated the occurrence of significant alternations in the submandibular gland structure of male rats following lithium administration.

Key words: Lithium, submandibular gland, rat, morphology

INTRODUCTION

Lithium is commonly used in the treatment of manic-depressive illness. It is particularly efficacious in the treatment of acute mania and in the prophylaxis of bipolar disorder (Schou, 2001; Schreiner *et al*., 2000). It is often used as adjuvant therapy in the treatment of resistant depression and as a prophylactic agent for patients with cluster headaches and as a stimulus for white blood cell production in neutropenic patients (Opresko, 1995; Gelder *et al*., 1999). Lithium can cause changes in the structure and function of salivary glands. Submandibular glands are more sensitive to metabolic and physiologic changes (Dehpour *et al*., 1995). Rat submandibular glands consist of seromucous acini, intercalated ducts, granular convoluted tubules, striated ducts and excretory ducts immersed in a highly vascularized connective tissue (Taga *et al*., 2000; Sato and Miyoshi, 1999; Garcia *et al*., 2002). Granular convoluted tubules are the specific ductal system of the submandibular gland of mice and rats. These tubules are functionally integrated into hormonal circuits; they produce regulatory peptides as well as epidermal and nerve growth factors (Danz *et al*., 1999). Most studies done on the effects of lithium on salivary glands have focused on functional and physiological aspects, but histological and quantitative study of salivary glands has received little attention. Thus, the purpose of this study was to investigate morphometrically the effects of lithium administration on the submandibular salivary gland structure in male rats, including the seromucous acini, convoluted granular tubules and striated ducts, interlobular connective tissue and excretory ducts.

MATERIALS AND METHODS

In this study 20 male Wistar rats weighing 250-300 g were selected. There was maintained a constant cycle of 12 h light and 12 h darkness and the temperature at 22±2°C, with free access to standard laboratory diet and water *ad libitum*. After 2 weeks for acclimatization, the rats were randomly divided into control and experimental groups (n = 10) and received distilled water and 0.1% lithium carbonate ($Li\ Co_3$) solution, respectively, as drinking water for a period of 8 weeks. The rats were anesthetized with Ketamine hydrochloride and their submandibular glands were dissected, removed and separated from the

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sublingual glands, weighed on an analytical scale. The glands were collected 10:00 to 12:00 am to avoid circadian variations (Assis *et al.*, 2000).

Tissue processing and morphometrical methods: One submandibular gland was randomly selected from each rat and fixed in Lillie's solution for 72 h at room temperature. The submandibular glands were dehydrated, cleared and embedded in paraplast in random orientation and then the glands exhaustively were sectioned. Ten to twelve sections of ~ 5 μm were sampled from each gland by systematic uniform random sampling (Howard and Reed, 1998). Each of these sections was stained with hematoxylin and eosin and mounted. In order to project, the whole section image on the table, a modified slide projector was used (final magnification 24). Point counting using the Cavaliers principle was employed to estimate the volume of submandibular gland using the formula:

$$V = \frac{\sum_{i=1}^{m} P.a/p.\bar{t}}{M^2}$$

Where, V is the volume of the submandibular gland, ΣP: the sum of the number of points landing within the gland profiles, a/p: the area associated with each point, t: the distance between sections and M: the magnification (Howard and Reed, 1998; Mandarim-de-Lacerda, 2003). From the sections mentioned above, five were selected by systematic random sampling. Every section was investigated using a BH2-Olympus light microscope with a projecting arm to project the image onto the table. A transparent test system was then superimposed and in five systematic random fields in each slide, points hitting the various components of the gland were counted at a final magnification of 32. Then an estimate of the volume density, Vv, of the components in the reference space was obtained using:

$$Vv = \frac{P(\text{part})}{P(\text{ref})}$$

where P(part) and P(ref) are the number of test points falling in all structure profiles and in the reference space, respectively (Howard and Reed, 1998; Gundersen and Jensen, 1985; Gundersen *et al.*, 1988). In order to estimate the absolute volume of a part, the volume density of that part was multiplied by the reference volume (volume of the submandibular gland). To determine the volume weighted mean acini volume, five systematic random fields were sampled per section. In a final magnification of 400, a grid of standard points and a set of parallel lines of random orientation were superimposed randomly onto the image and the volume weighted mean acini volume was estimated using the point sampled intercepts method (Gundersen and Jensen, 1985; Gundersen *et al.*, 1988). Measurements were performed using a 15-class ruler with a total length of 35 mm (Howard and Reed, 1998; Gundersen and Jensen, 1985; Skau *et al.*, 2001). Data were entered into a result sheet and the volume weighted mean acini volume was estimated by formula (Skau *et al.*, 2001).

$$V_v = \frac{\pi}{3} \cdot \lambda_3^3 \cdot F$$

where V_v is the volume weighted mean acini volume, λ^3_0 is the mean of the cubed measured intercepts length and F is $\left(\frac{1}{\text{Magnification}}\right)^3$

Statistical analysis: Values are reported as Mean±SD. Differences in parameters between the control and lithium groups were analyzed using the nonparametric Mann-Whitney U test. A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Mean animal weight, submandibular gland weight and volume showed no statistically significant differences between the two groups ($p>0.05$) (Table 1).

The volume density and the total volume of the acini, convoluted granular tubules and striated ducts were significantly decreased in lithium group in comparison to the control group ($p<0.01$) (Table 2).

The excretory ducts showed no significant differences ($p>0.05$) and the glandular stroma increased significantly in the lithium group as compared with the control group ($p<0.0001$).

The volume weighted mean of the seromucous acini were significantly higher in the control group, than in the lithium group ($p<0.0001$) (Table 2).

This study has demonstrated the occurrence of significant alternations in the submandibular gland structure of male rats following the administration of lithium. The most important findings were the decrease in volume density and absolute volume of seromucous acini,

Table 1: Body weight, submandibular gland weight and volume in control and lithium treated groups

Parameter	Control group	Lithium treated group
Body mass (g)	269.90±4.9	279.30±5.4
Submandibular gland mass (mg)	373.90±5.79	378.55±9.28
Gland volume (mm^3)	253.97±1.79	257.34±1.83

Values are means±SD

Table 2: Stereological dimensions of the different submandibular gland structures for control and lithium treated groups

	Volume density (%)		Absolute Volume (mm^3)		Volume weighted mean volume (μm^3)	
Parameter	Control group	Lithium treated group	Control group	Lithium treated group	Control group	Lithium treated group
Seromucous acini	53.04±1.30	46.40±1.63[b]	129.32±1.64	121.88±1.36[b]	273453.4±7715.37	219048.9±5617.86[c]
Convoluted granular tubules	26.54±0.51	23.26±0.79[b]	61.41±1.22	55.35±1.57[a]		
Striated ducts	2.65±0.07	2.36±0.09[b]	6.56±0.08	5.07±0.15[a]		
Excretory ducts	2.12±0.12	2.06±0.06	4.87±0.12	5.02±0.09		
Stroma	15.62±1.46	25.92±1.31[c]	52.40±0.85	69.2±1.48[c]		

Values are mean±SD, [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.0001$

convoluted granular tubules and striated ducts and increase in volume density and absolute volume of glandular stroma in the lithium treated group in comparison to the control group.

The seromucous acini secrete both protein and a considerable amount of polysaccharides (Gartner and Hiatt, 2001). Granular convoluted tubules are the specific ductal system of the submandibular gland of mice and rats. These tubules are functionally integrated into hormonal circuits; they produce regulatory peptides as well as epidermal and nerve growth factors (Danz *et al*., 1999). The striated ducts have Na^+-ATPase that pumps sodium out of the cell into the connective tissue, thus conserving sodium (Gartner and Hiatt, 2001). Thus it seems that the decrease in volume density and total volume of these structures can cause a reduction in concentrations of proteins, enzymes and electrolytes in the saliva.

In this respect it is reported that acute and chronic lithium treatment in rats decrease in the concentrations of electrolytes including Na^+, K^+, Ca^+ and total protein of the submandibular gland saliva. These changes attributed to inhibition of the cAMP-signaling pathway in the salivary glands (Dehpour *et al*., 1995). Neither histological nor stereological studies were conducted; the authors suggested that the functional changes may have been caused by the reduction of acinar, granular and striated duct volume. Abdollahi *et al*. (1999) reported that chronic lithium treatment in rats promotes a significant decrease in the submandibular gland's dry weight and total protein concentration. It seems likely that the properties of lithium to alter intracellular cAMP and its ability to suppress DNA and protein synthesis in the acini and convoluted granular tubules could be related to the stereological changes.

It should be pointed out that lithium therapy is also known to impair the synthesis and release of thyroid hormones, subsequently increasing the pituitary secretion of TSH by its inhibitory action on ATP activity and cAMP (Iqbal *et al*., 2001). In rodents a hypothyroidism picture occurs in the course of lithium therapy and studies on human lithium treated patients also showed that as many as a quarter to a third of patients on long-term lithium therapy develop hypothyroidism (Valle *et al*., 1993; Salata and Klein, 1987; Lazarus, 1998; Kusalic and Engelsmann, 1999). On the other hand, Oncu *et al*. (2004) reported histological changes on rats sublingual glands after thyroidectomy including enlargement of mucous acini and tubules, dilatation of ducts and increase in tissue mass and adipocytes in the stroma. Noorafshan (Noorafshan, 2001) showed that the volume density, the total volume of convoluted granular tubules and the volume weighted mean seromucous acini volume in rats submandibular gland with hypothyroidism significantly decreased in comparison to controls. Concluding that thyroid hormones have an influence on the submandibular gland and that the lack of thyroid hormones can produce significant quantitative changes in the structure of that gland. Based on the above data we speculate that the changes in the stereological parameters of the salivary gland observed in here may have been caused by lithium- induced hypothyroidism. Future studies will be helpful in further understanding the effects of lithium on the salivary gland structure.

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