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MORPHOLOGICAL ALTERATIONS OF THE RAT SUBMANDIBULAR GLAND CAUSED BY LESION OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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ABSTRACT: The morphological characteristics of the rat submandibular gland were studied different of times (5, 10, 20, 40, and 90 days) after lesion of the ventromedial nucleus of the hypothalamus. The structural arrangement of the submandibular gland was the same in rats with lesion of the ventromedial nucleus and in control or sham-lesioned animals. In the first group, however, the septa were narrow, this impairing the definition of the lobular compartments. Parenchymal alterations were quite evident in lesioned animals, with acinar hypotrophy and increased numbers of granulose ducts. The remaining submandibular components, however, did not show morphological alterations when compared to those of control animals. In summary, lesion of the ventromedial nucleus of the hypothalamus produces the following changes in the rat submandibular gland: 1) decreased glandular masses, 2) acinar hypotrophy, and 3) increased numbers of granulose ducts.

KEY-WORDS: Submandibular gland; ventromedial nucleus of hypothalamus; hypothalamic lesions.

INTRODUCTION

Electrolytic lesion of the ventromedial nucleus (VMN) of the rat hypothalamus causes hyperphagia, and when food is provided in abundance the occurence of the hypothalamic obesity syndrome is observed together with metabolic and endocrine dysfunctions[2,14]. The VMN is an important area of the central nervous system which

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is related to the autonomic nervous system and to its peripheral actions[12,18]. Lesion of the VMN of dogs causes changes in the classical conditioned salivatory reflex[19].

The normal secretion of the parotid gland of rats is considered to depend on cooperation between the sympathetic and parasympathetic nerves[3]. There is a relationship between the amount of saliva and body temperature, which are both decreased after lesion of the VMN[6,7]. The electrolytic lesions of hypothalamic areas has been related with functional alterations of the salivary glands[21]. FONSECA[8] reported that rats with lesion of the median eminence of the hypothalamus show morphological impairment of the parenchyma of the submandibular gland.

The objective of the present study was to determine by histological methods the possible structural alterations occurring in the parenchyma of the rat submandibular gland after lesion of the VMN of the hypothalamus.

MATERIAL AND METHODS

Adult, male Holtzman rats weighting 250-300 g were housed in individual cages with free access to tap water and balanced food pellets (Anderson Clayton S/A).

Animals were divided into three groups. The first group was not submitted to any special treatment and was used as control. The second group (VMN lesion) was anesthetized with ether and placed in a stereotaxic apparatus. An incision was made in the skin, the periosteum was isolated and the skull was drilled with a spherical dental drill. A stainless steel monopolar electrode isolated throught except at the tip and attached to the anode was introduced into the hypotalamus according to the coordinates of the DE GROOT[5] atlas and a continuous current of 1 mA was applied for a period of 10 s. The animals in the third group (sham lesion) were submitted to the same surgical procedure, except that no current was applied.

At different times after VMN lesion or sham lesion (5, 10, 20, 40 and 90 days), each 5-animal subgroup was fasted for 12 h and then fed for 1 h[24]. The animals were anesthetized with ether and the submandibular glands were removed[4] and fixed in Bouin's fluid for 24 h at room temperature. The glands were washed in running water for 24 h, dehydrated with a growing alcohol series, cleared in xylene and embedded in paraffin by routine histological methods. Sections (6 μm) were obtained with a microtome and stained with hematoxylin-eosin and Mallory trichrome.

The brain of the lesioned animals were removed and fixed in 10% formalin for 48 h, frozen and sectioned into serial frontal 15-μm thick cuts using a freezing microtome and stained with hematoxylin-eosin.

Only the animals whose VMN had been totally destroyed by electrolytic lesion were used for analysis of the results. All histological analysis were under light microscopy.

Data of body and gland weights are reported as means ± SEM and were analyzed statistically by the Student *t*-test, with the level of significance set at 0.05.

RESULTS

Body weight and submandibular gland weight

When the body weight of VMN lesioned rats were compared with normal or sham-lesioned rats, a significant increase was observed in rats with lesion of the VMN after 40 days ($p < 0.05$, Table 1). Animals with lesion of the VMN showed also a significant decrease in submandibular gland mass in relation to control or sham-lesioned animals after 20 days of lesion ($p < 0.05$, Table 2).

TABLE 1 – Body weight (g) of rats of different experimental groups. The results are represented by mean ± SEM. *significant difference ($p < 0.05$) compared to sham group. n = 5 rats in each group

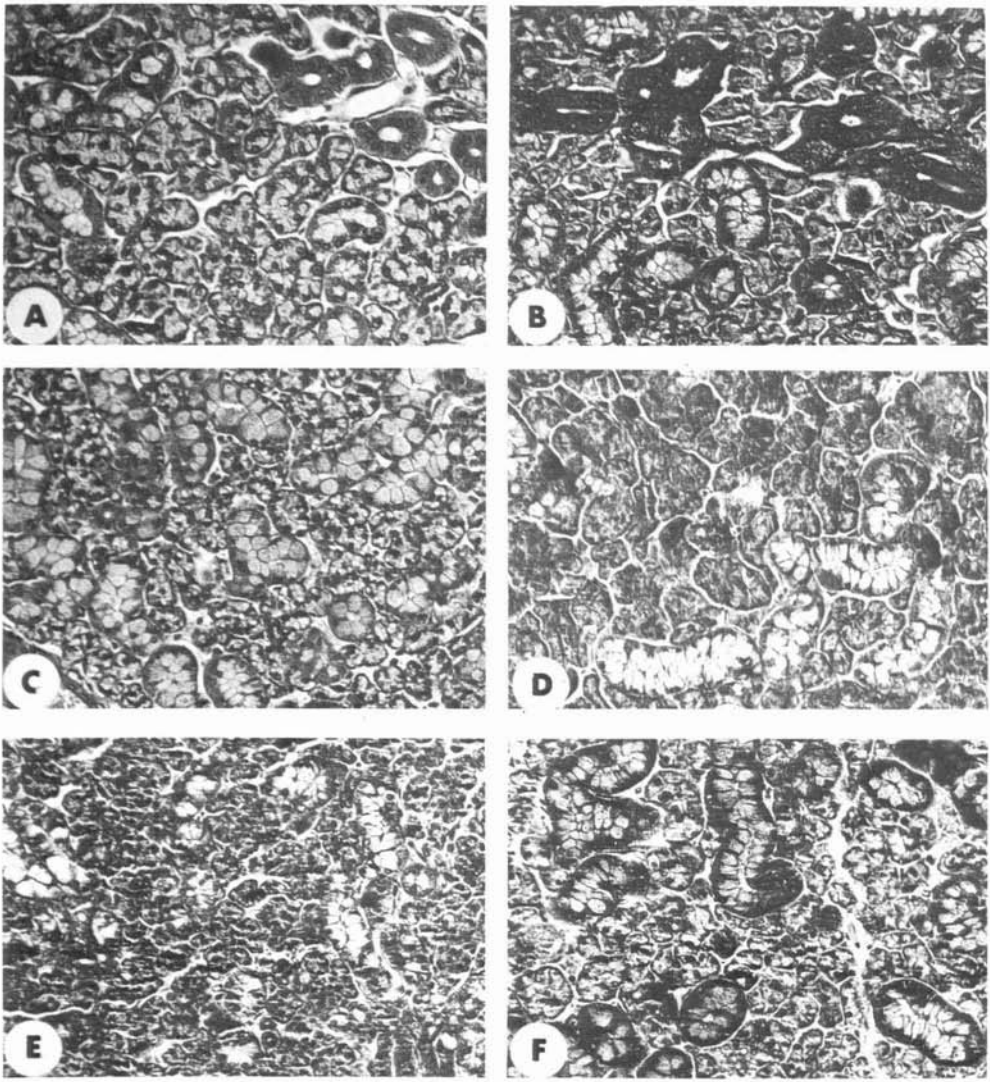
Group	Days after cerebral surgery 5	10	20	40	90
Sham	255±4	263±5	267±13	321± 5	405± 5
Lesion	260±6	268±7	302±12	373±12*	517±16*

TABLE 2 – Submandibular gland weight (mg) of sham or VMN lesioned rats at different times after surgery. The results are represented by mean ± SEM. *significant difference ($p < 0.05$) compared to sham group. n = 5 in each group

Group	Days after cerebral surgery 5	10	20	40	90
Sham	519±27	529±13	574±17	550± 1	523± 6
Lesion	559±20	503±27	431±14*	491±12*	410±16*

Morphology of the submandibular gland in normal and sham-lesioned rats

Morphologically, the submandibular gland of normal and sham-lesioned rats showed a complex and orderly arrangement of the structural components. The parenchyma consisted of specialized epithelial cells, with acini, intercalary ducts, striated ducts, granulose ducts, and excretory ducts. The acini had seromucosal characteristics and were small and formed of 3 to 4 cells of high pyramidal shape facing a small lumen. The cytoplasma showed fine granulations and discrete basophilia at the apical and basal poles, respectively. The spheroid nucleus was located in the basal third and showed weak chromatin and a well visible nucleolus (Fig. 1).



Figures – Photomicrograph of histological sections of the submandibular gland at different times after VMN lesion. Hematoxylin-eosin, 200 X.

1. Control group: panoramic aspect of the seromucosal acini, striated ducts and septa.
2. Control group: morphological characteristics of acini, granulose ducts and striated ducts.
3. Lesioned group (5 days): the acini appear hypotrophic and light cells predominate over dark cells in the granulose ducts.
4. Lesioned group (10 days): generalized acinar hypotrophy and increased acidophilia of granulose ducts.
5. Lesioned group (20 and 40 days): granulose ducts with numerous secretory granules. The acini are atrophic and lightly granules. The acini are atrophic and lightly stained.
6. Lesioned group (90 days): increased numbers of granule ducts.

The intercalary duct was directly attached to the acinus and was branched, short and small in diameter, and lined with a floor epithelium whose cells sometimes contained secretory granules. The striated and granulose ducts were the longest and more twisting ducts. They lined with a single layer of cylindric epithelial cells containing an ovoid nucleus in the middle third and basal striated nucleus in the infranuclear region. In addition, the granulose ducts were enriched with secretory granules in the apical cytoplasma, which were well visualized with Mallory trichrome (Fig. 2).

Morphological changes of the submandibular gland in rats with lesion of the VMN

After 5 days of VMN lesion, the acini were hypotrophic with cells showing altered staining characteristics. The nucleus, although spheroidal, showed dense chromatin. In the granulose ducts light cells predominated in relation to dark cells. The apical secretory granules were few and the nucleus remained vesiculose (Fig. 3).

After 10 days of lesion, the glandular changes were more intense. The acini were atrophic and lightly stained, with low cells having a dense nucleus close to the cell membrane. Cytoplasmatic basophilia was also reduced (Fig. 3). The cells of the granulose ducts continued to be high, with the cytoplasm showing slightly increased acidophilia when compared with the parenchyma of the submandibular gland of control rats (Fig. 3).

In the groups examined after 20 and 40 days of lesion, the acini were generally atrophic and lightly stained. Some cells showed signs of cytoplasm vacuolization (Fig. 4 and 5) and the granulose ducts were slightly increased in number. The apical cytoplasm of these cells also became enriched with acidophilic granules.

Finally, in the group examined 90 days of lesion, the acini continued to be atrophic and lightly stained, with pynnotic nuclei (Fig. 6). Granulose ducts sharply predominated over acini and enriched with secretory granules.

DISCUSSION

The present data concerning the variation on body weight coincide with those reported by ANAND & BROBECK[2], HETHERINGTON & RANSON[14] and ROZKOWSKA & FONBERG[20], who demonstrated that animals with bilateral VMN lesion in the hypothalamus show hyperphagia and increased body weight.

The observed decrease in submandibular gland weight after VMN lesion could be compared with the results reported by LACASSAGNE & CHAMORRO[16] and SHAFER & MUHLER[22], who detected decreased glandular weight after hypophysectomy. FONSECA[8] also observed decreased glandular weight in rats after electrolytic lesion of the median eminence.

Thyrotropin hormone (TSH) participates in mitotic division and interferes with cell metabolism and its presence in plasma is important for maintaining the growth and development of various tissues and organs. The growth of the submandibular glands is delayed in thyroid deficiency[11,13]. A significant decrease in TSH-producing cells occurs in the hypophysis of young rats with bilateral VMN

lesions. The production of tyrotropin hormone releasing factor (TRF) is interrupted, and hypophyseal, adrenal and gonodal changes are observed[15].

In the present study, 5 days after surgery, the acini of lesioned rats was hypotrophic and became atrophic after 10, 20, 40 and 90 days. It was also observed an increase in granulose ducts in the lesioned groups. The acinar hypotrophy and the increase in granulose ducts observed in VMN lesioned rats can also be compared to those reported by FONSECA[8] after lesion of the median eminence.

Sympathetic and parasympathetic nervous system innervate the salivary gland[10,17,23]. These authors demonstrated that pre-ganglionary denervation of the parasympathetic system causes atrophy of the granulose ducts of the salivary glands. The adrenergic system could be important for the acinar enlargement of the rat submandibular gland as was showed by ALVES & MACHADO[1] during Chagas' disease.

Electrical stimulation of the medial hypothalamic area (sympathetic) accelerates the synthesis of secretory material, and stimulation of the parasympathetic lateral hypothalamic zone increases the discharge of this material[9]. Electrolytic lesion of the VMN results in changes in the reflexes of cranial nerves to appetitive stimuli such as insulin and gastric acid secretion[18] and reduces the salivary secretion[7]. Electrolytic lesion of the lateral hypothalamus of dogs also reduces salivary secretion[19]. Lesion of hypothalamic area as VMN could change the discharge of the autonomic nervous system and by this may to produce secretory and morphological alterations in salivary glands as described in this work.

In conclusion, lesion of the VMN caused a decrease in glandular mass, acinar hypotrophy and increased granulose ducts. These changes may have induced by changes in endocrine and/or autonomic nervous mechanism.

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RESUMO: As características morfológicas da glândula submandibular de ratos foram estudadas em diferentes períodos de tempo (5, 10, 20, 40 e 90 dias) após a lesão do núcleo ventromedial do hipotálamo. O arranjo estrutural da glândula submandibular foi o mesmo em ratos com lesão do núcleo ventromedial e nos ratos controle ou com lesão fictícia. No primeiro grupo, entretanto, o septo estava estreitado, dificultando a definição dos compartimentos lobulares. Alterações do parênquima foram bastante evidentes nos ratos lesados, com hipotrofia acinar e aumento no número de ductos granulosos. Os componentes submandibulares restantes, entretanto, não apresentaram alterações quando comparados com aqueles dos animais controles. Em resumo, a lesão do núcleo ventromedial do hipotálamo produz as seguintes mudanças na glândula submandibular do rato: 1) diminuição da massa glandular; 2) hipotrofia acinar; e 3) aumento no número de ductos granulosos.

UNITERMOS: Glândula submandibular; núcleo ventromedial do hipotálamo; lesão hipotalâmica.

REFERENCES

1. ALVES, J. B. & MACHADO, C. R. S. – Effect of adrenoceptor blockers on the acinar enlargement induced by Chagas' disease in the rat submandibular glands. *Braz. J. med. biol. Res., 19:* 69-73, 1986.
2. ANAND, B. K. & BROBECK; J. R. – Hypothalamic control of food intake in rats and cats. *Yale J. biol. Med., 24:* 123-40, 1951.
3. ASKING, B. & GJÖRSTRUP, P. – Amylase secretion in response to activation of different autonomic receptors. *Acta physiol. scand., 109:* 407-13, 1980.
4. CHEYNE, V. D. – A description of the salivary glands of the rat and a procedure for their extirpation. *J. dent. Res., 18:* 457-67, 1939.
5. DE GROOT, J. – The rat hypothalamus in stereotaxic coordinates. *J. comp. Neurol., 113:* 389, 1959.
6. FLYNN, F. W.; SCHRER, D. L. & MITCHELL, J. C. – Reduced salivation in rat following ventromedial hypothalamic lesion. *Physiol. & Behav., 24:* 451-5, 1980.
7. FLYNN, F. W.; EVEY, L. A. & MITCHELL, J. C. – Heat-induced saliva secretion and thermoregulation in female rats with ventromedial hypothalamic lesion. *Physiol. & Behav., 26:* 779-82, 1981.
8. FONSECA, E. C. *Contribuição ao estudo das glândulas submandibulares após lesão da eminência média do hipotálamo em ratos.* Araçatuba, Fac. Odont. Araçatuba, UNESP, 1984 (Tese de Mestrado).
9. FUJIMOTO, K.; SHIRASUNA, K.; MIYAZAKI, T.; SHIOTANI, Y.; SAKAGAMI, M. & BAN, T. – Ultrastructural changes in rabbit parotid gland induced by electrical stimulation of the hypothalamus. *Med. J. Osaka Univ., 22:* 215-43, 1972.

10. FUJIWARA, M.; TANAKA, C.; HATTORI, K. & HONJO, T. – Uptake of noradrenaline by the adrenergic fibers of the submaxillary and sublingual glands of the rats. *Biochem. Pharmacol., 15:* 2113-7, 1966.

11. GRAD, B. & LEBLOND, C. P. – The necessity of testis and thyroid hormones for the maintenance of the serous tubules of the submaxillary glands in the male rats. *Endocrinology, 45:* 250-6, 1949.

12. GROSSMAN, S. P. – The VMN: a center for affection reactions, satiety or both? *Physiol. & Behav., 1:* 1-10, 1966.

13. HAMMETT, F. S. – Studies of the thyroid apparatus. The growth of the heart, lungs, liver, kidneys, spleen, submaxillary glands and eyeballs in male and female albino rat thyroparathyroidectomized and parathyroidectomized when 100 days age. *Am. J. Physiol., 32:* 75-94, 1923.

14. HETHERINGTON, A. W. & RANSON, S. W. – The spontaneous activity and food intake of rats with hypothalamic lesions. *Am. J. Physiol., 136:* 609-17, 1942.

15. HILL, D. L.; ALMLI, C. R.; FISHER, R. S. & WILLIAMS, D. – Damage to the ventromedial hypothalamic nucleus of newborn rats: growth, ingestion, and neuroendocrine dysfunction. *Exp. Neurol., 71:* 191-202, 1981.

16. LACASSAGNE, A. & CHAMORRO, A. – Reaction à la testosterona de la gland sous maxillaire atrophie consecutivement à l'hypophysectomie chez la souris. *C. R. Mém. Soc. Biol., 134:* 232, 1940.

17. NORBERG, K. A. & OLSON, L. – Adrenergic innervation of the salivatory glands in the rat. *Z. Zellforsch. Mikrosk. Anat., 68:* 183-9, 1965.

18. POWLEY, T. L. – The ventromedial hypothalamic sydrome, satiety, and a cephalic phase hypothesis. *Psychol. Rev., 84:* 126, 1977.

19. ROZKOWSKA, E. & FONBERG, E. – Impairment of salivary reflexes after lateral hypothalamic lesions in dogs. *Acta Neurobiol. Exp., 32:* 711-20, 1972.

20. ROZKOWSKA, E. & FONBERG, E. – Salivary reactions after ventromedial hypothalamic lesions in dogs. *Acta Neurobiol. Exp., 33:* 553-62, 1973.

21. SAAD, W. A.; UTRILLA, L. S.; SABBAG, Y. & CAMARGO, L. A. A. – Efeitos de lesões hipotalâmicas sobre as glândulas salivares. Estudo histoquímico. *In:* REUNIÃO ANUAL DA SBPC, 28., Brasília. *Apud: Ciênc. Cult., 28*(7): 387, 1976 (Abstr. 145)

22. SHAFER, W. G. & MUHLER, J. C. – Experimental dental caries. VI. The effect of hyphysectomy on dental caries and the salivatory glands of the rat. *J. dent. Res., 34:* 531-6, 1955.

23. SNELL, R. S. – The histochemical appearances of cholinesterase in the parasympathetic nerves supplying the submandibular and sublingual salivary glands of the rat. *J. Anat., 92:* 535-43, 1958.

24. TAMARIN, A. & SREEBNY, L. M. – Effects of total inanition on the submaxillary gland of the rat. *Arch. oral Biol., 7:* 469-80, 1962.

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