MORPHOMETRIC STUDY OF THE ACINAR CELL DEVELOPMENT OF MOUSE SUBMANDIBULAR GLANDS DURING THE POST NATAL PERIOD*

ESTUDO MORFOMÉTRICO DO DESENVOLVIMENTO DE CÉLULAS ACINOSAS DE GLÂNDULAS SUBMANDIBULARES DE CAMUNDONGO DURANTE O PERÍODO PÓS-NATAL.

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RESUMO

Na presente pesquisa estudamos as fases e a velocidade de crescimento da população de células acinosas de glândulas submandibulares de camundongos machos (linhagem Swiss) no período de 14 a 84 dias de vida pós-natal. Com este propósito, avaliamos através de métodos morfométricos de contagens ao microscópio de luz, a frequência e o número absoluto de células acinosas. A frequência de células, flutuou durante todo o período analisado, iniciando com um valor de 31,8% e terminando com 36,6%. A evolução do número absoluto de células acinosas mostrou duas fases distintas de significativo crescimento, uma entre 14 a 21 dias e a outra entre 28 a 84 dias do desenvolvimento; entre 21 e 28 dias não ocorreu aumento no número de células. Através da análise de regressão linear obtivemos, para representar a evolução do número de células acinosas no período de 14 a 84 dias a equação: $Y = 149,44 + 2,54 (X - 40)$. O acúmulo diário médio de células acinosas calculado com a equação foi de $2,54 . 10^5$ células/dia.

UNITERMOS

Células acinosas; Glândula submandibular; Morfometria.

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* Pesquisa desenvolvida no laboratório de Histologia, Departamento de Morfologia da Faculdade de Odontologia de Bauru-USP. Este trabalho é parte da tese apresentada à Faculdade de Odontologia de Bauru-USP, para obtenção do grau de Doutor em Diagnóstico Oral - área de Radiologia e Semiologia.

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INTRODUCTION

The submandibular glands of rats and mice exhibit two types of secretory structures: acini and convoluted granular tubules [8,11,19,21,22]. While the acini develop primarily during the first month of postpartum life, the convoluted tubules develop later, predominating during the 2nd month of development [5,6,7,10,18].

Since most of the above studies on acinar evolution mainly emphasized the changes occurring during the first month of postnatal life, in the present investigation we decided to study acinar cell development during a later period when exuberant growth of convoluted granular tubules occurs.

MATERIAL AND METHODS

General Histological Procedures

Male albino Swiss mice were obtained from the Central Animal House of the School of Dentistry of Bauru, University of São Paulo, Brazil, in groups of 5 animals per age at 14, 21, 28, 35, 42, 56 and 84 days of life.

The submandibular glands of each animal were carefully dissected and rapidly removed under ether anesthesia. Fresh mass was weighed with a precision scale (Mettler H-20) and the glands were fixed in Helly fluid (2.5 mg potassium bichromate, 5-8 mg mercury chloride in 100 ml distilled water, with 5% formalin added before use) and washed overnight in running water. The material was then processed routinely by dehydration in ethyl alcohol, clearing in xylene and paraffin embedding. Semiserial 5 µm sections were obtained and stained with Masson trichrome[14].

Determination Of Processed Gland Volume (Vp)

Gland Vp, i.e., gland volume after all the histological procedures of fixation, dehydration, clearing and embedding, was determined by the following equation:

$$Vp = m/d \cdot RF$$

where:

m = fresh gland mass

d = mouse submandibular gland density

RF = factor for the correction of the retraction provoked by histological processing.

The values used for calculation were d = 1.09 g/cm^3 and RF = 0.8576, previously obtained by PARDINI[16] and PARDINI & TAGA[17].

Morphometric Evaluation Of Absolute Acinar Cell Number

Cells counts were performed using a Zeiss microscope with a 100X immersion objective and a Zeiss Kpl 8X ocular micrometer containing a Zeiss II integration grid consisting of 10 parallel lines and 100 points symmetrically distributed over a quadrangular area.

The images of acinar cell nuclei (n) and the number of crossings (c) between these nuclear images and the parallel lines of the test system were counted in 50 histological fields selected by systematic randomization [24]. The absolute number of nuclei was calculated by the following formula:

$$N = \frac{2n}{A\,(i \cdot d + 2t)} \cdot Vp\ (AHERNE^{1})$$

where:

N = total number of nuclei

A = total area examined

i = c/n

d = distance between the lines of the test system

t = section thickness

Full details about this morphometric cell counting method are given by TAGA and SESSO [23].

Acinar Cell Frequency

During the acinar cell counts, the number of nuclear images of the remaining parenchymal and stromal cells was also recorded. Acinar cell frequency (%) in the gland was then calculated on the basis of the above data.

Statistical Analysis

Body mass, fresh gland mass, and frequency and absolute number of acinar cells obtained for a given age group were compared with those for the remaining groups by analysis of variance[13].

RESULTS

The results concerning the postnatal evolution of body mass, gland mass, and frequency and number of acinar cells are presented in Table 1.

TABELA I - Mean (± SEM) body mass, fresh gland mass and frequency and absolute number of acinar cells during postnatal development

age groups in day	PARAMETERS											
	body mass in g			gland mass in mg			cell frequency in %			cell number per gland		
14	6. 96	±	0.184	24.3	æ	0.74	31.8	æ	1.06	64.1	æ	1.71
21	9.28	±	0.091	39.0	æ	1.97	37.7	æ	0.69	115.3	æ	4.14
28	15.04	±	0.380	51.2	æ	1.12	34.7	æ	0.79	117.0	æ	1.76
35	22.24	±	0.532	88.7	æ	2.55	33.9	æ	0.51	147.0	æ	6.12
42	26.10	±	0.157	114.7	æ	2.78	31.2	æ	0.76	157.2	æ	5.57
56	26.38	±	0.550	139.2	æ	1.52	31.8	æ	0.81	188.4	æ	5.96
84	33.62	±	0.573	175.2	æ	4.79	36.6	æ	1.65	257.1	æ	5.22

Analysis of Table I shows that:

- Body mass gain (g) occurred during two stages, i.e., from 14 to 42 and from 56 to 84 days of postnatal life. On a percent basis, body mass (g) increased by 275% ($P<0.01$) during the first stage and by 27.4% ($P<0.01$) during the second. No significant increase in body mass occurred between the age groups of 42 and 56 days ($P>0.1$).
- Absolute gland mass (mg) increased significantly ($P<0.0l$) throughout the study period, with a variation from 24.3 to 175.2 mg between day 14 and day 84, corresponding to a 621% increase in development.
- Acinar cell frequency increased from 31.8 to 37.7% between 14 and 21 days ($P<0.01$) and decreased from 37.7 to 31.2% from 21 to 42 days ($P<0.01$), with a new increase from 31.2 to 36.6% from 42 to 84 days ($P<0.05$).
- The number of acinar cells increased from 64.1 x 10^5 on the 14th day of postnatal life to 115.3 x 10^5 on the 21st day, representing a 79.9% increase ($P<0.01$). A stabilization occurred from 21 to 28 days ($P>0.1$), followed by an increase from 117.0 x 10^5 on the 28 th day to 257.1 x 10^5 on the 84th day, corresponding to a 119.7% increase ($P<0.01$).

Regression analysis between the number of acinar cells (y) and age in days (x) yielded the following equation:

$$Y = 149.44 + 2.54\ (X - 40)$$

which is presented graphically in Figure 1.

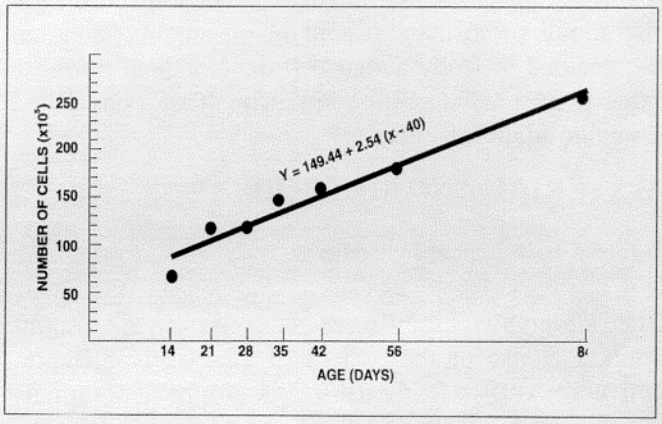

Fig. 1 Post natal evolution of the acinar cell number

The equation was used to calculate the mean daily increase in acinar cell number, which was 2.54 . 10^5 cells/day.

DISCUSSION

The acini are the terminal secretory structures of the submandibular glands of rodents and are located on the distal ends of the intercalary ducts [8,9,12]. The percent evolution of the number of acinar cells shows little variation throughout the period from 14 to 84 days of postnatal life, with small oscillations (which at times are statistically significant) of the order of approximately 1.18 times. Thus, the maximum and minimum values, respectively obtained for the 21 and 42 day age groups, were 37.7% and 31.2%.

SRINIVASAN & CHANG[21] obtained similar results, with a maximum value of 37.7% at 21 days of age and a minimum value of 28.0% at 84 days of pospartum life. In a study of the submandibular gland of the rat, these

same investigators [20] detected stabilization in the percentages of acinar cells from the 4th to the 12th week of development.

In absolute terms, the number of cells obtained in the present study increased substantially from 64.1 x 10^5 to 257.1 x 10^5 cells from the 14th to the 84th day of postnatal life, corresponding to a quadruplication during the study period. An interesting fact was the lack of increase in absolute number of acinar cells observed between the 21st and 28th day, with a predominance in volume gain. This may have been linked to the change in feeding habits of the animals. It should be pointed out that it was during this period that the secretory cells of the convoluted granular tubules started to differentiate [2,5,7,21].

SRINIVASAN & CHANG[21] obtained an increase of approximately 6.3 times in absolute acinar cell number from 14 to 84 days, i.e., 39.6 x 10^5 cells at the beginning of the study and 250.0 x 10^5 cells at the end. An interesting fact concerning the number of acinar cells obtained in the two studies is that, even through our animals initially presented approximately 61.9% more cells than the animals studied by SRINIVASAN & CHANG[21], the results obtained at the end of the study were practically of the same magnitude in both investigations. This indicates that the differences between the two studies within the period analyzed were probably due to a lack of concordance of proliferative activity peaks, i.e., the periods of occurrence of highest or lowest cell production differed between the two studies. Thus, whereas in the study by SRINIVASAN & CHANG [21] the increase in absolute acinar cell number was low at the beginning of the period and markedly increased between 28 and 42 days of age, practically stabilizing at 42 days of postnatal age, in our study the increase in acinar cell number was high at the beginning, absent between 21 and 28 days and significant for the remainder of the study period.

In a study of the subamandibular gland of the rat, SRINIVASAN & CHANG [20] obtained an 85.7% increase in acinar cell number between the 4th and 8th week of development.

The growth rate of acinar cells was calculated by the regression equation which was best associated and the mean daily increase obtained was 2.54 x 10^5 cell/day.

It should be pointed out that the period of acinar cell growth, defined by JACOBY & LEESON[9] as acinar phase, starts at birth and continues until the 8th week of life in the rat[20] and until the 6th week in the mouse[21]. Even though this is quite an extended period, the major events for acinar development occur during the first two weeks of postnatal life. Thus, within this 2-week interval, the acini develop from transitory structures designated as terminal tubules [9]. Initially, the acinar cells are formed from proacinar cells which are present in small amounts in the terminal tubules[2,4]. After depletion of the proacinar cell population, the secretory cells characteristic of the terminal tubules start to transform into acinar cells[2,4]. The cells thus formed proliferate and new acinar cells are formed until the end of the acinar phase.

In our mouse strain, the acinar cell population expanded until the 12th week of development, when the number of cells reached the value obtained by PARDINI[15] for adult animals.

Running title: Development of acinar cells of mouse submandibular glands

ABSTRACT

We studied the phases and rate of growth of the acinar cell population of the submandibular glands of male albino Swiss mice from 14 to 84 days of postnatal life. The frequency and absolute number of acinar cells were evaluated by morphometric counting methods using the light microscope. Cell frequency fluctuated throughout the study period from an initial value of 31.8% to a final value of 36.6%. The absolute number of acinar cells showed two distinct phases of significant growth, i.e., from 14 to 21 and from 28 to 84 days of development. Cell number did not increase between 21 and 28 days. Linear regression analysis yielded the following equation for the evolution of number of acinar cells from 14 to 84 days: $Y = 149.44 + 2.54 (x - 40)$. Mean daily acinar cell accumulation calculated by the equation was 2.54. 10^5 cells/day.

UNITERMOS

Acinar cells; Submandibular gland; Morphometry

AGRADECIMENTOS

Os autores agradecem ao Sr. Giovanni Quagliato pela colaboração na parte técnica e a Sra. Beonildes Teresinha Ruiz Correia pelos serviços de digitação do texto.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Aherne, W. Methods of counting discrete tissue components in microscopical sections. *J. Roy. Micr. Soc., v.87,* p.493-508, 1967.
2. ALVARES, E.P.; SESSO, A. Cell proliferation, differentation and transformation in the rat submandibular gland during early postnatal growth. A quantitative and morphological study. *Arch. Histol. Jap., v.38*(3), p.177-208, 1975.
3. ARANCIBIA, S.; ASSENMANCHER, I. Les glandes sous-maxillaires dans le contexte endocrinien. *J. Biol. Buccale, v.13*, p.185-203, 1985.
4. CHANG, W.W.L. Cell population changes during acinus formation in the postnatal rat submandibular gland. *Anat. Rec., v.178, n.*2, p.187-201, 1974.
5. CUTLER, L. S. ; CHAUDHRY, A.P. Cytodifferentiation of the acinar cells of the rat submandibular gland. *Develop. Biol., v.41, p.*31-41, 1974.
6. GRESIK, E. W. Postnatal development changes in submandibular glands of rat and mice. *J. Histochem. Cytochem., v.28*, n.8, p. 860-870, 1980.
7. GRESIK, E.W.; Mac RAE, E.W. The postnatal development of the sexually dimorphic duct system and of amylase activity in the submandibular glands of mice. *Cell. Tiss. Res., v. 157*, p. 411-422, 1975.
8. JACOBY, F. Observations on the postnatal development of the mouse submaxillary gland. *J. Anat. v.93*, p.579, 1959. Abstract .
9. JACOBY, F. ; LEESON, C. R. The postnatal development of the rat submaxillary gland. *J. Anat., v. 93*, p.201-216, 1959.
10. JUNQUEIRA, L.C.U.; RABINOVITCH, M. ;FAYER, A.B. Études sur le dimorphism sexual de la glande sous-maxillaire chez la souris. I. Époque de l' apparition et évolution des grains de secretion des tubules secréteurs. *Rev. Brasil. Biol., v.7*, p.435-438, 1947.
11. KAIHO, M.; NAKAMURA, T.; KUMEGAWA, M. Morphological studies on the syntesis of secretory granules in convoluted tubules of mouse submandibular gland. *Anat. Rec., v.183*, n.3, p.405-420, 1975.
12. LEESON, C.R. ; JACOBY, F. An electron microscopic study of the rat submaxillary gland during its postnatal development and in the adult. *J. Anat., v.93*, p.287-295, 1959.
13. LISON, L. S*tatisque appliqueé a la biologie expérimentale*. Paris, Gouthiers - Villars, 1958, p. 304.
14. MARTOJA, R. & MARTOJA-PERSON, M. *Técnicas de histologia animal*. Barcelona, Toray-Masson, 1970.
15. PARDINI, L. C. *Estudo morfométrico da glândula submandibular do camundongo. Comparação entre sexos*. Tese de Mestrado - Faculdade de Odontologia de Bauru, Universidade de São Paulo, Brasil/.
16. PARDINI, L.C. *Estudo morfométrico da evolução da população de células do ducto granuloso da glândula submandibular do camundongo durante a vida pós-natal,* 1987/ Tese de Doutorado - Faculdade de Odontologia de Bauru, Universidade de São Paulo, Brasil/.
17. PARDINI, L.C. ; TAGA, R. (1991): "Em publicação".
18. RAYNAUD, J. Variation avec l'age, de la structure histologique de la glande sous-maxillaire des souris femelles. *C. R. Soc. Biol. Fil., v.137*, p. 304-305, 1943.
19. RAYNAUD, J. Controle hormonal de la glande sous-maxillaire de la souris. *Bull. Biol. Fr. Belg., v.94,* n.4, p. 399-523, 1960.
20. SRINIVASAN, R. ; CHANG, W.W.L. The development of the granular convoluted duct in the rat submandibular gland. *Anat. Rec., v. 182*, n.1, p. 29-40, 1975.
21. SRINIVASAN, R. ; CHANG, W.W.L. The postnatal development of the submandibular glands of the mouse. *Cell. Tiss. Res., 198*: 363-371, 1979.
22. SRIVASTAVA, H. C. Development of acinar cell in the rat submandibular gland. *J. Anat., v. 123*, n. 2, p. 459-465, 1977.
23. TAGA, R.; SESSO, A. Avaliação do número de células de órgãos pela dosagem bioquímica de DNA em homogeinizados e por contagem direta através de métodos morfométricos. *Ciênc. Cult.,* v.30, n.10, p. 1232-1236, 1978.
24. WEIBEL, E. R. Stereological principles for morphometry in electron microscopic cytology. Int. *Rev. Cytol., v. 26*, p. 235-302, 1969.

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