ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XLVIII, 9 SECTIO D 1993

Katedra i Zakład Histologii i Embriologii z Pracownią Cytologii Doświadczalnej. Akademia Medyczna w Lublinie
Kierownik: prof. dr hab. Irena Królikowska-Prasał

Alicja ZARĘBSKA

The Influence of Encortone on Changes of Some Parameters of Parotid Serous Cells of Female White Rats of Wistar Breed

Wpływ Encortonu na zmiany niektórych parametrów komórek surowiczych przyusznicy samic szczurów białych rasy Wistar

In the extrasubstitutional treatment of glucocorticoids the preparations showing stronger antiphlogistic action and diminished ability of check of Na^+ ions have been most frequently used. Simultaneously in the case of prolonged treatment the preparations characterized by not a very long time of reversible restraint of corticotropin should be applied. These conditions are satisfied by Encortone (Prednisone).

Since only some publications report the influence of glucocorticoids on parotid gland in a general way (6, 11) and at the same time it is known that the receptors for these hormones are almost in every tissue of the body (5) — B. W. O'Malley, U. Schrader, 1977 quoted after Polakova (10), the influence of physiological encortone doses on the changes of some parameters of this salivary gland had to be studied.

MATERIAL AND METHODS

The experiment was carried out on female rats' inbreeding Wistar race weighing 210—220 g. The animals were divided into two experimental groups and two parallel control groups considering the age of animals and stress influence. In each group there were five animals. In control group I the animals received distilled water by a stomach-tube for 7 successive mornings. In experimental group I the animals received the emulsion of Encortone (of Polfa firm) in distilled water in the dose 1 mg/kg of body mass by a stomach-tube for 7 successive mornings before feeding. In control group II the animals received distilled water in the same way as the animals of control group I for 30 successive days. In experimental group II the animals received Encortone in the same way as the animals of experimental group I for 30 successive days.

Administration of the drug in the case of both experimental groups began with full dose, namely 1 mg/kg of body mass, then decreased to half and to 1/3 in the end of the administration period. Each

animal of experimental group I received altogether 0.94 mg of Encortone and each animal of experimental group II — 3.41 mg of Encortone. The rats were fed with standard granulated fodder and had enough drinking water. They were killed by the ether anaestesia. In the experimental animals the same increase of body mass was observed as in the case of the control animals: 30 g after 1 week and 50 g after 1 month.

On the sections of parotid gland which was fixed in 4% formalin and stained with hematoxylin and eosin the following morphometric measurements were carried out: 1) measurements of cell nuclei (using magnification 1,000× the smallest and the largest diameter of nucleus was measured by projection microscope and then the sectional area was calculated using the formula Πr^2 for the circle area and Πab for the ellipse area. On the sections of gland parotid from each animal 100 nuclei in the cells of acini selected accidentally were measured. Then the mean sectional area of nuclei for each group was determined; 2) measurements of sectional area of acini (on the sections of parotid gland of each animal the area of 100 acini was measured by polar planimeter after previous projection on the technical copy paper). Taking the square magnification $1{,}000 \times 1{,}000 = 1{,}000{,}000\times$ into consideration the specific sectional area was calculated as well as 3) the mean sectional area of cell considering a number of cells in the measured acini. Investigation results were statistically calculated by the use of Student Test (12). The values of parameters: the sectional area of acini, the sectional area of cells, the number of cells in acini for each group and also standard deviation, the difference between groups and value of test function t are presented in Tables 1—4. The calculated for each parameter value of test function t for $n_1+n_2=498$ of freedom degrees was compared with the critical value of test function t for 5 and 1% risk of error $\alpha_{0.05}=1.960$ and $\alpha_{0.01}=2.576$ (12) and statistically essential differences were suitably marked.

RESULTS

In both investigated experimental groups, i.e. after 7-day and 30-day periods of Encortone treatment, essential changes of mean sectional changes of mean sectional area of nuclei were statistically observed compared with the control animals. In group I a decrease in this area but in group II — an increase (Table 1) were observed.

Similarly the mean section area of acini was increased in comparison with the control animals in experimental group I, however, it was decreased in experimen-

Table 1. Mean surface section area of secretory cell nuclei of rat parotid in μ^2

Group	Mean value	Standard deviation	Difference between means	Value of test function t
Control I	18.039	6.967	−3.543**	7.931
Experimental I Encortone 7 days	14.496	7.160	$p<0.01$	
Control II	15.410	6.333	+2.810**	6.088
Experimental II Encortone 30 days	18.220	8.149	$p<0.01$	

** Significant difference at the assumption of 1% risk of error.

tal group II — in both groups statistically essentially at 1% risk of error (Table 2).

The mean sectional area of serous cells also showed a statistically essential increase in experimental group I and statistically essential decrease in experimental group II (Table 3).

Table 2. Mean surface section area of serous acini of rat parotid in μ^2

Group	Mean value	Standard deviation	Difference between means	Value of test function t
Control I	525.240	224.061	+79.580**	5.335
Experimental I Encortone 7 days	604.820	247.102	$p<0.01$	
Control II	503.200	375.940	−62.378**	3.288
Experimental II Encortone 30 days	440.822	196.632	$p<0.01$	

** See Table 1.

Table 3. Mean surface sectional area of secretory cells in rat parotid in μ^2

Group	Mean value	Standard deviation	Difference between means	Value of test function t
Control I	83.998	30.222	+13.935**	6.721
Experimental I Encortone 7 days	97.933	35.155	$p<0.01$	
Control II	88.310	34.587	−5.982**	2.614
Experimental II Encortone 30 days	82.328	37.711	$p<0.01$	

** See Table 1.

Slightly marked changes referred to a number of serous cells forming acini. In experimental group I there was also observed an increase but not essential statistically. However, in experimental group II the decrease of cell number was statistically essential only at 5% risk of error (Table 4).

DISCUSSION

Investigations of changes of some parameters of parotid serous cells can be interesting from the clinical point of view. Parotid gland is of great importance in physiological function of a digestive tract and in the first period of life it is

Table 4. Mean number of serous cells in secretory acini of rat parotid

Group	Mean value	Standard deviation	Difference between means	Value of test function t
Control I	6.414	2.352	+0.258	1.587
Experimental I Encortone 7 days	6.672	2.771	$p>0.05$	
Control II	6.228	2.755	−0.41*	2.452
Experimental II Encortone 30 days	5.818	2.528	$p<0.05$ $p>0.01$	

* Significant difference at the assumption of 5% risk of error.

necessary for normal growth and development by parotin secretion (13). Due to its chemical structure glucocorticoid hormones penetrate into all cells of the body and on majority of them exert their different influence depending on the character of tissue and organ (3, 6, 8). The influence of glucocorticoids on pancreas is known (9, 10); it is the gland which shows many common features with parotid.

The application of a physiological dose of Encortone in my experiment and the detection of statistically essential changes in the investigated parameters point to high sensitivity of this salivary gland to the influence of glucocorticoids.

After 1 week of Encortone treatment the increase of sectional area of acini in the parotid gland, serous cells as well as cell number in acini was observed. These changes are probably due to the increased mobilization of cells for secretion. The simultaneous decrease of section nuclei area of serous cells can be a result of the increased exchange (mRNA) between nucleus and cytoplasm and also a result of the division of some nuclei.

Hypertrophy and hyperplasia of pancreas serous cells as well as epithelium of lead ducts after hydrocortisone treatment were observed by Morriset et al. (8) and Bourry et al. (2) who report the secreting activity increase of pancreas serous cells after hydrocortisone treatment with a simultaneous decrease in trypsin and lipase concentrations. Deschodt et al. (4) also observed the increase of amylase and chymotrypsin activity after administration of great doses of hydrocortisone (2.5—5.0 mg/100 g b.m.).

The decrease in sectional area of cell nuclei and increase in acini area as well as a number of cells in parotid gland acini of female rats were observed after 5, 10, 15 days of testosterone treatment (15, 16). Similar changes in the parotid in the case of androgens and glucocorticoids influences can be associated with a similar biochemical character of molecules of these hormones. In the case of lack of androgens in males glucocorticoids bind with the androgen receptors (7), and

also modulate receptors capacity and concentration for EGF (1). It can be supposed that in the female parotid gland there is a number of receptors for androgens (14). It is also possible that in females glucocorticoids bind with the estrogen receptors.

After 30 days of Encortone treatment the reverse changes to those observed after 7 days of the drug administration were observed. The sectional area of nuclei increased statistically to a considerable degree in comparison with the control animals. The sectional area of acini and cells and also the number of cells in acini decreased statistically to a great extent. The increase in sectional area of cell nuclei can be a result of both mRNA and protein accumulation in nucleus and smaller exchange between nucleus and cytoplasm. Both phenomenona can occur simultaneously. The decrease in other parameters points to a smaller activity of cells which can be evidence of "metabolic tiredness" observed in glands after intensive secretion. Ricciardi et al. (11) report that dexamethasone administered in the dose 2 mg/kg of b.m. for 14 days caused atrophy of acini in the rat submaxillary gland and changes of cell shape and pyknosis of nuclei. Such pyknosis was not observed in my experiment. The decrease in sectional area of cells and acini observed in the light microscope can point to hyperplasia of cells and formation of new acini with a smaller number of cells. It cannot be excluded that distinct atrophic changes developed with prolonged time of hormone treatment are followed by the decrease in these parameters.

Conclusions

1. 7-day administration of Encortone in the physiological dose (1 mg/kg of b.m.) caused in parotid gland of female rats the statistically essential increase in sectional area of acini, serous cells and increase in a number of cells in acini as well as the decrease in sectional area of cell nuclei. These changes are probably due to the increased mobilization of cells for secretion.

2. 30-day Encortone administration in an identical manner caused the statistically essential decrease in sectional area of acini, serous cells, and the decrease in a number of cells in acini, as well as the increase in sectional area of cell nuclei. The observed changes can be a symptom of "metabolic tiredness" of serous cells after the intensive secretion.

REFERENCES

1. Baker J. B. et al.: Dexamethasone modulates binding and action of epidermal growth factor in serum-free cell culture. Proc. Natl. Acad. Sci. USA **75**, 1882, 1978.
2. Bourry J., Sarles H.: Influence de la corticothérapie prolongée sur la sécrétion pancréatique externe de l'homme. Gastroentérol. Clin. Biol. **2**, 139, 1978.

3. Chao J., Margolius H. S.: Differential effects of testosterone, thyroxine and cortisol on rat submandibular gland versus renal kallikrein. Endocrinology **113**, 2221, 1983.
4. Deschodt-Lanckman M., et al.: Hormonal and dietary adaptation of rat pancreatic hydrolases before and after weaning. Am. J. Physiol. **226**, 39, 1974.
5. Hartwig W., Kasperlik-Załuska A.: Kortykotropina, hormony kory nadnerczy i adrenostatyki [in:] Pawlikowski M.: Leczenie hormonami i pochodnymi hormonów. PZWL, Warszawa 1988.
6. Kostulak A.: The effect of ACTH and cortisone on the behaviour of arylsulphatases in the salivary glands of white rat. Acta Histochem. **58**, 63, 1977.
7. Maruyama S., Sato S.: Binding of glucocorticoids to the androgen receptor of mouse submandibular glands. J. Endocrinol. **106**, 329, 1985.
8. Morisset J., Jolicoeur L.: Effect of hydrocortisone on pancreatic growth in rats. Am. J. Physiol. **239**, G95, 1980.
9. Otsuki M. et al.: Hydrocortisone treatment increases the sensitivity and responsiveness to cholecystokinine in rat pancreas. Am. J. Physiol. **251**, G364, 1989.
10. Polyakova T. J.: Synthesis and excretion of the pancreatic secrete in white rats under conditions of abundant hydrocortisone. Arch. Anat. **1**, 65, 1986.
11. Ricciardi M. P., Paparelli A., Pellegrini A.: Morphofunctional research on the effects of steroid-stimulating and -inhibiting drugs on the major salivary glands of rats. Z. Mikrosk. Anat. Forsch. (Leipzig) **103**, 257, 1989.
12. Sawicki F.: Elementy statystyki dla lekarzy. PZWL, Warszawa 1982.
13. Szymczyk T.: Procesy biochemiczne w jamie ustnej [in:] Grosfeldowa O.: Fizjologia narządu żucia. PZWL, Warszawa 1981.
14. Verchoeven C., Wilson J. D.: Cytosol androgen binding in submandibular gland and kidney of the normal mouse with testicular feminization. Endocrinology **99**, 79, 1976.
15. Zarębska A.: Effect of testosterone on secretory segments of rat parotid gland. Folia Morphol. (Warszawa) **40**, 97, 1981.
16. Zaręska A.: Wpływ testosteronu na morfologię jąder komórkowych i ilość kwasów nukleinowych w komórkach gruczołowych przyusznicy szczura. Endokr. Pol. **33**, 151, 1982.

Otrzymano 1993.06.23.

STRESZCZENIE

Badano wpływ Encortonu w dawce 1 mg/kg m.c. na odcinki wydzielnicze przyuszcznicy samic szczurów. Zmiany powierzchni przekroju jąder komórkowych, komórek wydzielniczych, pęcherzyków i zmiany liczby komórek w pęcherzykach były analizowane statystycznie. Pomiary wykonano na skrawkach gruczołu barwionych H+E, stosując mikroskop projekcyjny i planimetr biegunowy.

Po 1 tygodniu podawania Encortonu stwierdzono statystycznie istotny wzrost powierzchni przekroju pęcherzyków i komórek, wzrost liczby komórek w pęcherzykach oraz zmniejszenie powierzchni przekroju jąder komórkowych. Takie zmiany wskazują na zwiększoną aktywność komórek wydzielniczych ślinianki.

Po 30 dniach podawania Encortonu stwierdzono zwiększenie powierzchni przekroju jąder komórkowych, natomiast zmniejszenie powierzchni przekroju pęcherzyków, komórek i liczby komórek w pęcherzykach. Zmiany te mogą być wyrazem „metabolicznego zmęczenia" gruczołu.