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Histological examination of the submandibular gland following experimental administration of Metizol

Metizol is a thyreostatic derived from thiourine, which inhibits the initial stages of thyroid hormone synthesis. The inhibition results from impaired formation of peroxidase--iodine complexes and thyrosine iodising in thyreoglobuline. Peripherally active Metizol blocks thyroxine conversion to triiodothyronine (3). That type of activity mechanism is used to treat hyperthyroidism.

Apart from affecting the thyroid gland, Metizol indirectly or directly acts on other organs, causing many side-effects. The most common ones include allergies such as rash, erythema (1) or changed blood picture: agranulocytosis, granulocytopenia (2). Some authors have reported that endocrine glands including the thyroid gland have a big influence on other glands, e.g. the salivary glands (1, 4, 6, 7). For example, the thyroid hormones have probably complete influence on the mitochondrial proteins synthesis in the submandibular glands, including MAO (5).

The tangential point between the thyroid gland and the submandibular gland can be the ability of the salivary gland to catch iodine from blood and transform it (13). Metizol influences excretion of the thyroid hormones and probably the submandibular gland as well.

The present experiment involved observation of the submandibular gland in white Wistar rats administered Metizol (Polfa).

MATERIAL AND METHOD

The investigations were carried out on white Wistar rats (adult males, weighing approx. 300 g each). The animals were divided into three groups (two experimental

groups and a control one); experimental group I: the animals were given Metizol for 21 days; experimental group II: the animals were given Metizol for 42 days; control group: the animals were given distilled water by means of intragastric bougie.

Metizol dissolved in distilled water was administered intragastrically at the dose of 1 mg/kg b.m. 24 hours following the last dose the animals were put to sleep by ether and the submandibular gland samples were taken for histological examination (stained with hematoxylin and eosin and Masson's method) and histochemical examination (stained by PAS's and Feulgen's methods).

The submandibular gland samples for the examination under optic microscope were fixed in Baker's fluid (1% $CaCl_2$ in 10% solution on neutralised formalin). Paraffin sections 7 μm thick were histologically and histochemically evaluated.

Following morphometric measurement have been done on the submandibular gland's samples stained with hematoxylin and eosin. Under 1000x magnified microscope, the smallest and the biggest diameter of the nucleus was taken to calculate the section's surface using πr^2 formula for a circle and πab for ellipse. Samples of the submandibular gland from each animal were taken to measure 100 nuclei from randomly chosen follicles. Then the mean surface of the section of nucleus of each group was established.

Results of examinations have been statistically modified using Student's test (8). The values of the investigated parameters and section surfaces of the cell nucleus, standard deviation, variability coefficient, differences between groups, value of test-function "t" are introduced in Table 1 and 2.

Table 1

Description	N	Middle	SD	CV	Min	Max
Salivary gland-control group	100	16.78	7.077387	42.2	7.1	45.7
Salivary gland-group I	100	14.08	6.02	42.8	3.1	37.7
Salivary gland-group II	100	12.10	5.2	41.4	3.1	28.3

t-Student test for differences between means founding unequal variations.

Table 2

Compared pairs	df	T	P	Essential
Control and group I	193	2.897869	0.004192	P<0.01
Control and group II	178	5.383944	2.28E-07	P<0.0001
Group I and group II	192	2.523217	0.01244	P<0.02

RESULTS

CONTROL GROUP

Standard staining with hematoxylin and eosine and Masson's method staining showed no deviation from normal structure of the submandibular gland. On the slides there are visible serous and mucous fragments. Serous follicles have narrow, fissured lumen. Their cells show thick arrangement. Round or oval nuclei of cells, which bounded follicles, are partly pushed to bases of cells. The final part of mucous secreting fragments – tubules, have wide lumen. The mean area of section of tubules is 1,700 μm^2. The nuclei of cells which produce mucus are always flattened, irregular and pushed to bases of cells. In mixed final sections, serous cells close ends of mucous tubules in a form of half-moons of Gianuzzi. The mean area of section of the cell nuclei of serous follicles is 16.78 μm^2.

EXPERIMENTAL GROUP I (21 DAYS OF METIZOL ADMINISTRATION)

There is an apparent increase in quantity of mucous secreting fragments – tubules. They are bigger than in the control group. The mean area of section of tubules is 2,027 μm^2. In cells which bounded tubules there is more mucus, which testifies to smaller

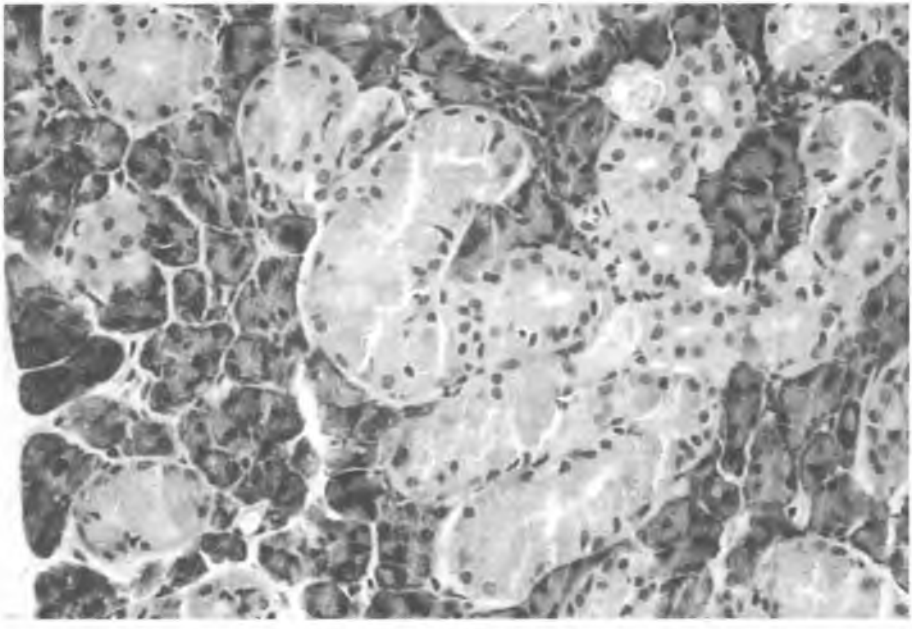

Fig. 1. The submandibular gland of the rat, experimental group I. Hematoxylin and eosine staining. Magn. 400x

amount of secretion. The serous fragments – follicles are shrinked, smaller than in the control group. They have irregular shape. In the cells which bounded follicles staining was more basophilic than in the control group. The mean area of section of the cell nuclei of serous follicles is 14.80 μm^2.

EXPERIMENTAL GROUP II (42 DAYS OF METIZOL ADMINISTRATION)

There is more mucous secreting fragments in the control group but less than in experimental group I. The mean area of section of tubules is 1,871 μm^2 – like in the control group. The appearance, number, staining of tubules and follicles also resembles the control group. The mean area of section of the cell nuclei of serous follicles is 12.10 μm^2.

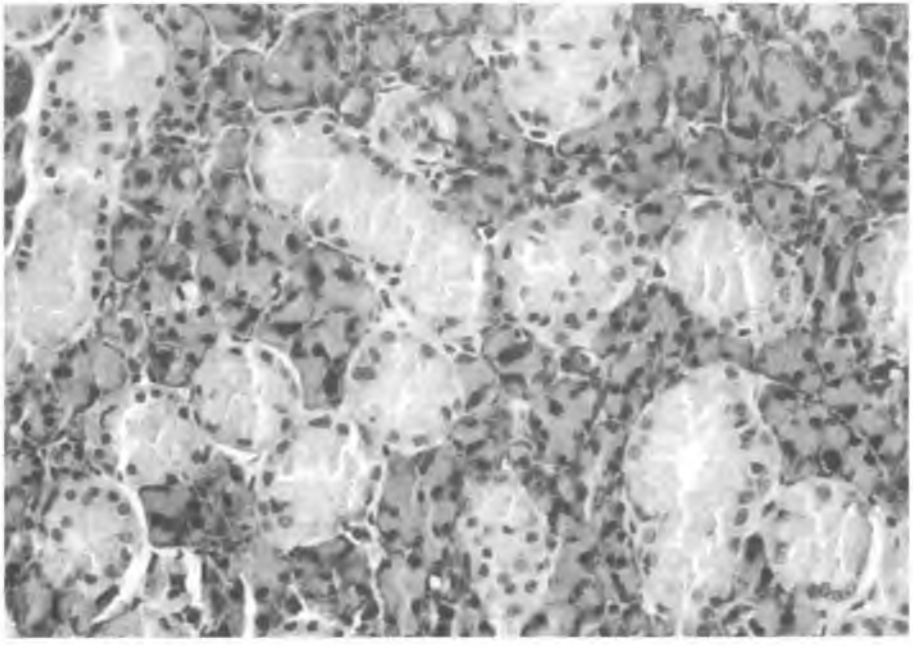

Fig. 2. The submandibular gland of the rat, experimental group II. Hematoxylin and eosin staining. Magn. 400x

DISCUSSION

Metizol – the thiourine derivative is a drug used in hyperthyroidism, where it decreases the thyroid hormones levels (3). It is known that thyroid as endocrine gland has connections with other glands: pituitary gland, suprarenal glands, salivary glands (4, 5, 10). Metizol, as it was expected, influences physiology and morphology of the submandibular gland.

21 days' administration of Metizol at the dose of 1 mg/kg b.m./24h resulted in the changed appearance and number of serous and mucous secreting fragments. The number of mucous secreting fragments – tubules increased.

The mean area of their section increases either. The cells which bounded tubules contain more secretion. The mean area of section of the follicles decreases. The follicles are shrinked and they have an irregular shape. Morphometric investigations show that 21 days' Metizol administration causes statistically important increase of the mean area of section of the cell nuclei (about 1.98 μm^2). After 42 days' Metizol administration the picture of the submandibular gland became similar to the control group. The mean area of section of the cell nuclei, however, decreases (about 4.68 μm^2 in comparison with control group). We can conclude that Metizol by decreasing the thyroid hormones level inhibits cellular divisions.

CONCLUSIONS

1. Three-week administration of Metizol initiates reactions, which result in increased secretory function of tubules, but also indecreased secretory function of follicles. The mean area of section of the cell nuclei decreases.

2. Six-week administration of Metizol results in comeback to the initial stage in microscopic picture of the submandibular gland, which may be the evidence of adaptative mechanisms in the body.

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SUMMARY

The submandibular gland of the white Wistar rats was examined. The animals were given Metizol for 21 days and 42 days at the dose of 1 mg/kg b.m./24 h. The submandibular gland samples were taken for histological and histochemical examination. Then they were stained with hematoxylin and eosine as well as by Masson's, PAS's and Feulgen's method. The mean area of section of cell nuclei was measured. The results of examination were counted statistically. The following changes were noticed: After 21 days of administration of Metizol in the submandibular gland the mean area of the tubules was increased. The quantity of tubules increased as well. In the tubules cells some more secretion was noticed. The follicles shrank. After 42 days of administration of Metizol the appearance, number and stainability of the tubules and follicles were similar to control group.

Badania histologiczne ślinianki podżuchwowej po doświadczalnym podawaniu Metizolu

Badano śliniankę podżuchwową szczurów rasy Wistar, którym podawano Metizol przez 21 dni i 42 dni w dawce 1 mg/kgm.c./24 godz. Stosowano barwienia H+E, met. PAS, met. Feulgena, met. Massona. Mierzono powierzchnię przekroju jąder komórkowych, powierzchnię przekroju cewek wydzielniczych i do uzyskanych danych stosowano

obliczenia statystyczne. Zaobserwowano następujące zmiany. Po 21 dniach podawania Metizolu w śliniance podżuchwowej zwiększyła się liczba cewek i zwiększyła się powierzchnia ich przekroju. W komórkach budujących cewki zaobserwowano większą ilość wydzieliny. Natomiast pęcherzyki uległy obkurczeniu. Po 42 dniach podawania Metizolu wygląd, liczba i barwliwość cewek i pęcherzyków upodobniły się do grupy kontrolnej.