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MICE PARAFOLLICULAR AND INTRATHYROID MAST CELLS UNDER THYROTROPIN INFLUENCE

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ABSTRACT

The influence of sodium perchlorate and thyroxine on the parafollicular cells and mast cells of the mice thyroid gland was studied.

It was found out that perchlorate caused a significant hyperplasia and hypertrophy of the parafollicular cells as well as intrathyroid mast cells. The concentration of calcitonin in the serum of the perchlorate group from the 8th day onward was higher than in the control group.

The application of thyroxine caused a significantly increased number of the parafollicular cells during the first 32 days and a significantly decreased number of these cells after 64 days. The number of mast cells significantly decreased after 2 days. The number of mast cells significantly decreased after 2 days, but they disappeared totally from the gland from the 8th day on.

The concentration of calcitonin was lower in the thyroxine group than in the control group during the first 16 days, but it increased abruptly after 32 days and after 64 days was five times higher than in the control group.

Key words: perchlorate, thyroxine, thyrotropin, calcitonin, parafollicular cells, mast cells, mice

INTRODUCTION

There are three lines of endocrine cells in the thyroid gland. The follicular cells secreting the hormones thyroxine (T_4) and triiodothyronine (T_3) are the most numerous, but there are only a few parafollicular cells (about 1% in mice), which secrete in addition to calcitonin also somatostatin, katakalcin, calcitonin-gene related peptide (CGRP) (Ali-Rachedi et al. 1983, Zabel, 1984), calbindin (Zabel et al., 1988), serotonin and other peptides. The third line of endocrine cells are intrathyroid mast cells secreting other biological substances besides histamin and heparin and in rodents serotonin too. The number of intrathyroid mast cells varies in various animal species, but in mice they are scarce (Melander et al. 1971; Ericson et al., 1972).

The aim of our study was to check whether the parafollicular cells and intrathyroid mast cells also react to thyrotropine blood concentration, this being a specific stimulus for follicular cells. In order to verify this crosswise reactivity we have produced experimentaly the state of hyperthyrotropinemia or hypothyrotropinemia and studied qualitative and quantitative microscopical changes in these cells as well as the blood concentration of several hormones biochemically.

MATERIAL AND METHODS

We performed three experiments. In the first one 72 male mice Balb/c strain, 6 weeks old, 18-25 g in weight were studied. The mice were divided into 6 experimental groups of 6 animals each. Group 1 was given 1,2% sodium perchlorate ($NaClO_4$) (Irenat, Troponwerke, Koeln) in drinking water for 2 days, group 2 for 4, group 3 for 8, group 4 for 16, group 5 for 32 and group 6 for 64 days. Each of these groups had corresponding controls with 6 animals in each group, drinking tap water without perchlorate. The animals were fed with pellets for laboratory animals.

In the second experiment there were 60 male mice, of the same strain, age and weight. They were divided into the same groups as in the first experiment, 5 animals in each group. The experimental groups drank 0.02% L-thyroxine (Sodium salt of L-Thyroxin $C_{15}H_{10}J_4NO_4 \times H_2O$ Merck). The experimental animals also had corresponding control groups as described in the first experiment, 5 animals in each group.

In addition the blood from the vena femoralis was taken from five or six animals in each group of the first and second experiment to determine the concentrations of the hormones T_4, T_3 and calcitonin by the radioimmunoassay method. As there was not enough blood from each animal to determine all 3 hormones, the blood of the animals in a group was pooled.

In the third experiment on 36 male mice hold in the same conditions as in the first and second experiment, the accumulation of radioiodine in the thyroid gland was determined. Two animals were taken from the perchlorate, control and thyroxine group after 2, 4, 8, 16, 32 and 64 days respectively and were injected with 2.5 μg ^{131}I. Two days later the percentage of accumulated iodine in the thyroid gland was measured by the use of a scintillation counter.

The thyroid glands were fixed in Bouin's solution, cut into 6 μm step serial sections, the interval of the steps being 60 μm. One series was stained with toluidine blue at pH 4 and PAS, the second series according to Fernandez-Pasquale (1976) and with toluidine blue. The first series was used for counting the mast cells, the second for the parafollicular cells, taking into account all the slices. Using Weibel's multipurpose test system (Weibel, 1979) at an objective magnification of x63 we determined several stereological variables, such as: the volume density of the epithelium and the colloid, the activation index of the gland (Kališnik, 1972), the volume and numerical volume densities (according to Pajer and Kališnik, 1984) of the parafollicular cells and mast cells. The absolute (total) volume of the thyroid gland was stereologically determined. The absolute (total) volume and number of these cells were estimated. The statistical evaluation of the data was performed using the Student's t-test. Regarding the stereological methodology unless indicated otherwise the standard methods were used (cf. Weibel, 1979).

RESULTS

Biochemical

The accumulation of radioiodine was determined with the aim of confirming that the application of antithyroid drug and thyroxine was efficient. It was found that the percentage of radioiodine accumulated in the thyroid gland decreases steeply in the perchlorate group due to partial blockade of the iodine pump.

After 64 days it was 21 times lower than in control group, though it was 9 times lower after the application of thyroxin (Fig. 1).

The concentration of the hormones T_4 and T_3 in the perchlorate as well as in the thyroxine group showed expected results (Fig. 2). The concentration of calcitonin in the perchlorate groups were significantly higher compared with the controls, except after 4 days. In the thyroxin group lower concentrations of calcitonin were obtained after the first 16 days compared with the controls, but a sharp rise after 32 and 64 days was observed, showing a considerable release of calcitonin from the parafollicular cells.

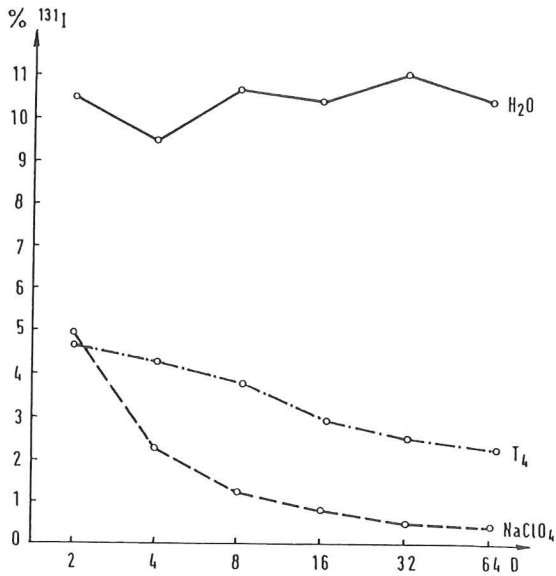

Fig. 1. Accumulation percentage of the radioiodine ^{131}I in the perchlorate ($NaClO_4$), thyroxine (T_4) and control (H_2O) groups during 64 days (D) of the experiment. Data were obtained on blood samples pooled from 2 animals at each term.

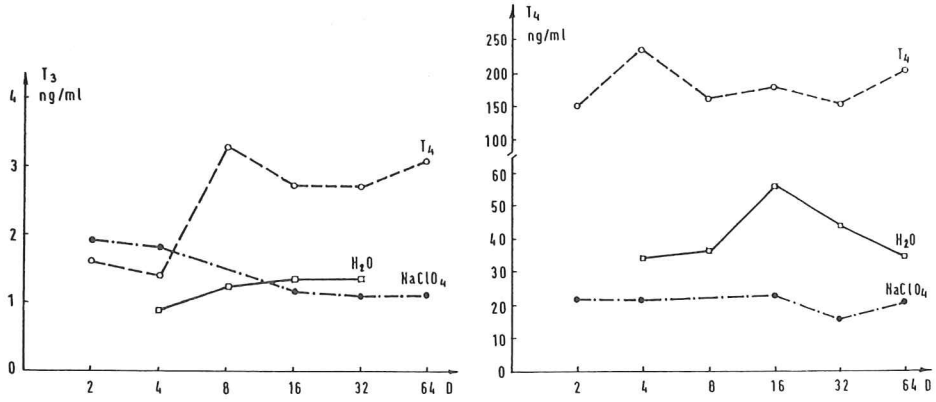

Fig. 2. Concentrations of triiodothyronine (T_3) and thyroxine (T_4) in the pooled blood samples from 5 or 6 animals of the perchlorate ($NaClO_4$), thyroxine (T_4) and control (H_2O) groups of animals during 64 days (D) of the experiment.

Recently the biochemical determination of calcitonin has been reproduced in another experiment after 64 days of perchlorate or thyroxine application the results were essentially very similar to the previous results.

Morphological

Microscopical analyses showed great differences between the experimental and control groups.

Control group

The thyroid follicles were more or less spherical, smaller in the centre and bigger in the periphery. The follicular epithelial cells were mostly cuboidal, depending on the functional state of the cells, the borders between the follicles were clear, the lumen of the follicles was filled with the colloid. The parafollicular cells were concentrated chiefly in the central region of the middle third of the lobes, less frequently subcapsulary. They were usually located in the basal region of the follicles, wedged between follicular cells away from the colloid. Intrathyroid mast cells were usually very rare and were located in the interstitium between follicles. In general their shape depended on the available space.

Perchlorate group

The follicular structure of the gland was completely lost after 64 days' application of $NaClO_4$. The follicles were irregularly shaped, the epithelial cells were higher than normal. The cells protruded into the follicular lumen, the colloid disappeared almost completely. The borders between the follicles disappeared, the follicles merged with neighbouring ones and new ones budded.
The thyroid gland showed all the signs of the hyperplasia and hypertrophy of the follicular cells. The parafollicular cells were distributed chiefly in the central sections of the thyroid gland, but some of them were placed at the periphery. Usually they were single, seldom in small groups. The shape of these cells were irregular and many of them had cytoplasmatic processes, interpolated between the follicular cells. The number of intrathyroid mast cells was significantly increased during the experiment and many of them were near the blood vessels. Some of them were partly degranulated with scarce granules. It may be assumed that mast cells after total degranulation after having used the described histological techniques could not be identified. But the cytoplasm of the other were packed with metachromatic granules so that the nucleus was covered. They were also noticed in the immediate vicinity of the parafollicular cells.

Thyroxine group

64 days' administration of L-thyroxine caused the follicular cells to become lowered and flattened. The follicles were large not only in the periphery but also in the central part of the lobes. The distribution of the parafollicular cells was similar to that in the control group, that is, they were in the centre of the central sections of the lobes. But the number of the central sections where the parafollicular cells were noticed was smaller than in the controls. Usually they were heaped up between the follicles. Individual parafollicular cells were noticed between the follicular cells in the follicle wall, and it seemed that they stuck out in the follicular lumen. The size of these cells differed, some of them were large if we take into account those with cytoplasmatic processes. Intrathyroid mast cells, normally very few in the mice thyroid gland, totally disappeared from the gland from the 8^{th} day on. After 32 days they were not found even subcapsularly.

Stereological

Quantitative results obtained by stereological analysis from the first experiment are showing that in the perchlorate groups in comparison to the control groups from 8th day of the experiment the activation index was significantly higher (Fig. 3). Significantly higher total numbers and total volumes of the parafollicular cells were observed in perchlorate groups in comparison to the controls from 16th day of the experiment (Fig. 4). The same is true also for total number and volume of the intrathyroid mast cells (Fig. 5).

Quantitative results obtained by stereological analysis from the second experiment are showing that the activation index in the thyroxine groups was significantly reduced in comparison to the control groups from 4th day of the experiment (Fig. 6). The total number and total volume of the parafollicular cells were significantly higher in the thyroxine groups in comparison to the control groups from 4th or 2nd day to 32nd or 16th day respectively, thereafter an abrupt and highly significant decrease of these values was observed (Fig. 7). Total number of the intrathyroid mast cells was significantly smaller in the thyroxine groups in comparison to the control ones during the whole duration of the experiment (Fig. 8). The total volume of these cells was negligibly small from the 8th day of the experiment and is not presented grafically.

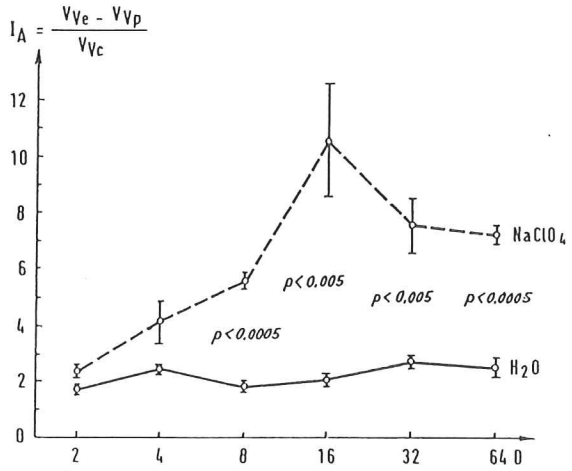

Fig. 3. Activation index (AI ± 1SE) of the treated (------$NaClO_4$) and control (------H_2O) animals with regard to the duration of the experiment.

V_{Ve} volume density of the epithelial cells
V_{Vp} volume density of the parafollicular cells
V_{Vc} volume density of the colloid

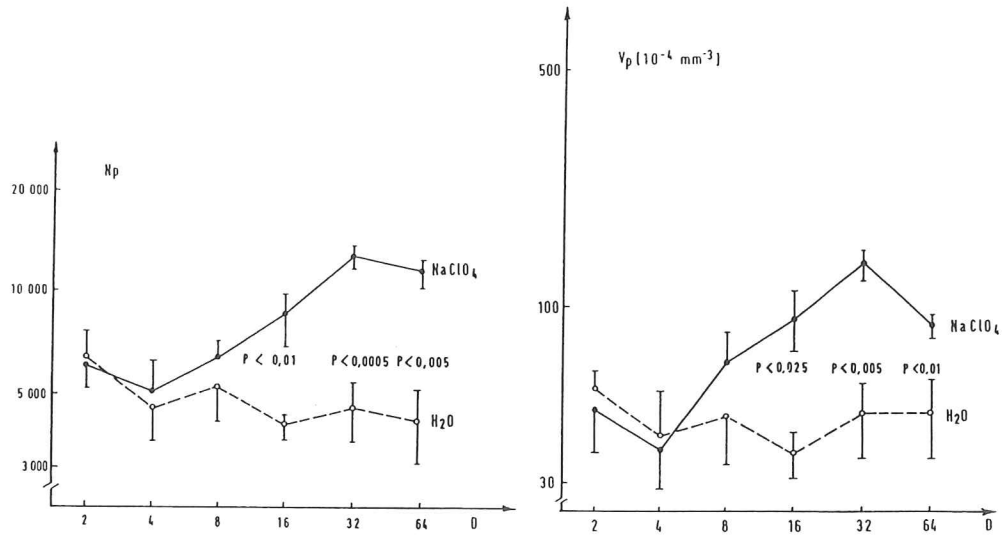

Fig. 4. Absolute (total) number ($N_p \pm 1SE$) and absolute (total) volume ($V_p \pm 1SE$) of the parafollicular cells in perchlorate ($NaClO_4$) and control (H_2O) groups of animals.

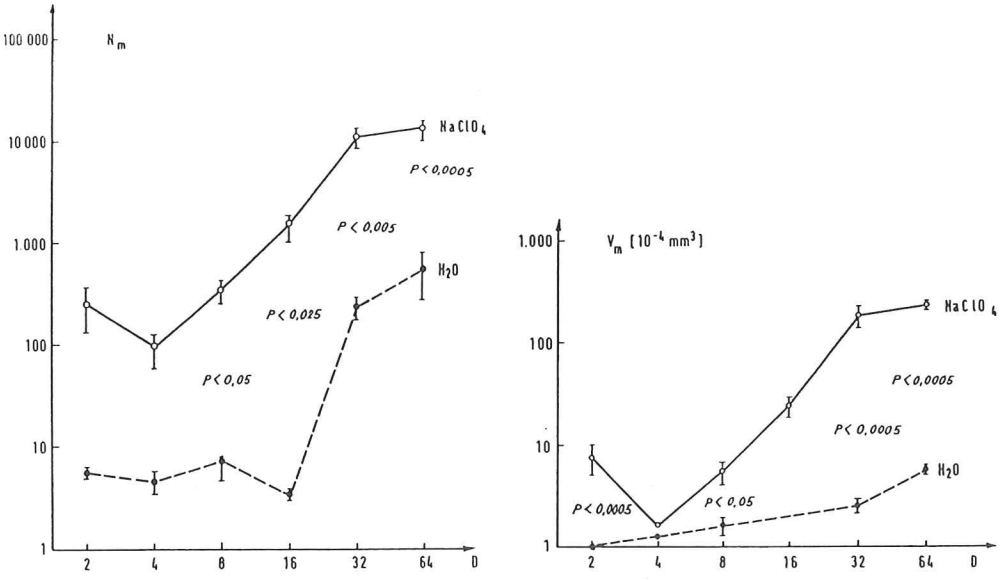

Fig. 5. Absolute (total) number ($N_m \pm 1SE$) and absolute (total) volume ($V_m \pm 1SE$) of the intrathyroid mast cells in perchlorate ($NaClO_4$) and control (H_2O) group of animals from 2nd to 64th day of experiment.

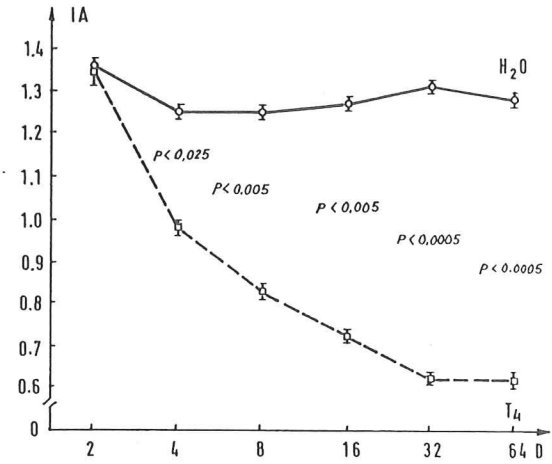

Fig. 6. Activation index (AI ± 1SE) of the thyroxine (T_4) and control (H_2O) groups of animals.

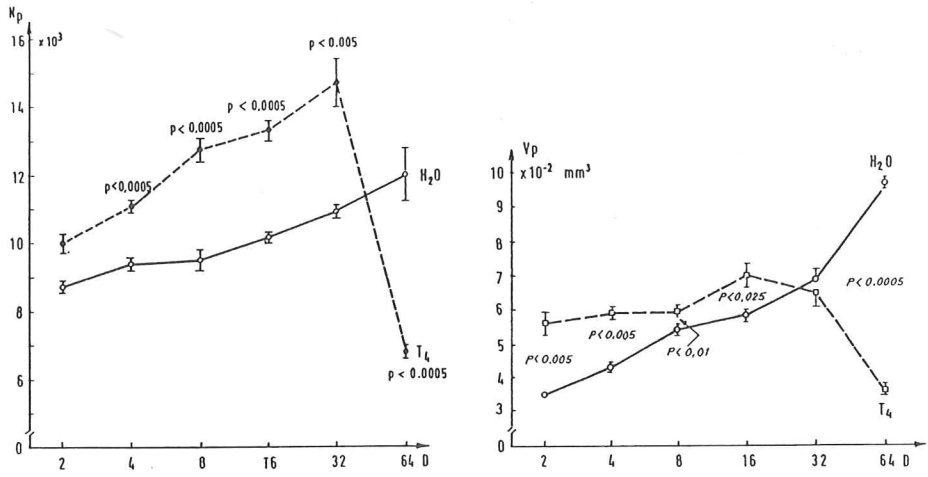

Fig. 7. Absolute (total) number (N_p ± 1SE) and absolute (total) volume (V_p ± 1SE) of the parafollicular cells in the thyroxine (T_4) and control animals (H_2O).

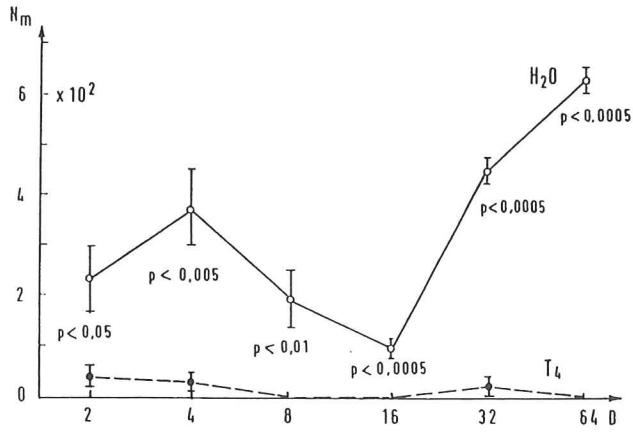

Fig. 8. Absolute (total) number of the mast cells ($N_m \pm$ 1SE) in the thyroid gland in the thyroxine (T_4) and control (H_2O) animals.

DISCUSSION

It is known that perchlorate ions partly suppress the iodine pump in follicular cells, decreasing the secretion of the thyroid gland hormones. The effect of this is reduced feed back inhibition of the hypothalamic centres and adenohypophysial thyrotropic cells, which stimulate the thyroid gland, with the resulting increase of thyroliberin (TRH) and thyrotropin (TSH) secretion (Pajer, 1988). The effect of increased thyrotropin secretion is not only stimulation of the follicular cells but also of the intrathyroid mast cells as has been shown earlier (Wynford-Thomas and Stringer, 1982). We found that the reaction of the intrathyroid mast cells is quicker, stronger and more protracted than the reaction of the follicular cells. The paracrine secretion of intrathyroid mast cells is described in the literature (Melander et al., 1973). Ericson et al. (1970) and Maayan et al. (1971) supossed that the serotonin is a possible secondary thyroid messenger stimulating the follicular cell secretion. But the experiment "in vitro" with FRTL-5 cell line did not support this hypothesis (Gabrščček, 1991). Zabel (1985) in an "in vitro" experiment proved that serotonin (also contained in the mast cells of rodents) accelerates the release of the calcitonin from the parafollicular cells. The higher calcitonin concentrations together with lower T_4 and T_3 concentrations could act synergistically, reducing so osteoclastic activity (Auverx and Bouillon, 1986; Hoffman et al., 1986; Raisz, 1988).

The opposite effects have been produced by the administration of exogenous T_4. One of the effects is reduced TRH and TSH secretion, resulting in diminished follicular cell activity. Moreover, the decreased TSH concentration resulted in the definite disappearance of the intrathyroid mast cells, resulting in the stop of exocytosis from the parafollicular cells, visible in the first month of the experiment. The appearance of the parafollicular cells filled up with granules accompanied with lower blood calcitonin concentrations has been named by us "pseudohyperplasia". The combined effect of the increased T_4 and T_3 blood concentrations on the one hand and decreased blood calcitonin concentration on the other at the beginning of the experiment was supposed to result in an increased osteoclastic activity, mobilising high quantities of calcium from bone tissue. We have observed at the end of the first and especially of the second

month an intensive degranulation of the parafollicular cells, obvious also as a decrease of their number; it was accompanied by an abrupt increase of blood calcitonin concentration.

In the interpretation of our experiment we have taken into account only some factors, which are under control or an object of observation. A direct effect of sodium perchlorate or thyroxine on the thyroid cells has not been excluded although it does not seem possible. In further research we should include the examination of other factors important for calcium homeostasis e.g. bone tissue with osteoclasts, osteoblasts and the parathyroid glands. Thus the assumption about change in osteoclastic activity with resulting blood calcium concentration will be verified.

It seems that some contradictory data in the literature regarding the reactivity of the parafollicular cells on the TSH could be resolved by taking into account the role of the intrathyroid mast cells and their influence on the exocitosis from the parafollicular cells. Further "in vitro" studies are necessary to elucidate relations between the various endocrine cells of the thyroid gland (Kališnik et al., 1988).

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