Estimation of influence of high doses of cholecalciferol on thyroid parafollicular and respiratory tract neuroendocrine cells; preliminary investigations

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Abstract

The aim of this study was to compare what changes are caused by high doses of cholecalciferol (100000 UI vD_3) and $CaCl_2$ on thyroid parafollicular (C) cells and airways neuroendocrine (NE) cells in rat. Overdosage of vD_3 and $CaCl_2$ causes hypocalcaemia and strong hypercalcitoninemia in blood; C cells showed mainly signs of hypertrophy; simultaneously, the number of strong calcitoninpositive cells decreased significantly (statistically significant changes). Immunohistochemical reactions, detecting CGRP, somatostatin, synaptophysin and neuronspecific enolase did not fall under statistic analysis. Airways NE cells re-acted to hypercalcemia differently than C cells – they probably respond to different regulatory mechanisms.

Key words: Vitamin D_3, immunohistochemistry, calcitonin concentration, thyroid, lung.

Introduction

The basic hormone, synthesized in thyroid parafollicular (C) cells, is calcitonin (CT). Some airways NE cells also release CT [1, 2]. Response of thyroid C cells to chronic hypocalcaemia – after high doses of vitamin D_3 (vD_3) - has been described in detail in several publications [3, 4]; the studies did not pertain, however, to the problem of calcitonin positive airways neuroendocrine (NE) cells. The aim of this study was to compare what changes were caused by high doses of cholecalciferol [intraperitoneal (ip.) injection of 100000 UI v. D_3 (Vigantol)], and 0,5% $CaCl_2$ in drinking aqueous solution – on thyroid C cells and airways NE cells in rats.

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Material and methods

The study was performed on 40 male Wistar rats weighting c. 200g. The animals were housed under controlled, standard conditions. All the animals had a free access to drinking (0,5% aqueous solution of $CaCl_2$) and standard food. The rats were divided into 4 groups, 10 rats in each group. Three (3) experimental groups were given ip. injections of Vigantol, Merck (100000 UI v. D_3); the animals were killed under pentobarbital (Vetbutal) anaesthesia during the following intervals: Group 1D after 24h, Gr. 7D after 7 days and Group 14D after 14 days. Group 4C consisted of control rats which were given ip. injections of physiological solutions. Blood was collected for analysis: to determine blood plasma calcitonin concentration by RIA (radioimmunoassay) and the concentration levels of total and ionized calcium (Ca^{2+}). In all the applied procedures, the control reactions yielded negative results. Thyroid lobes and two specimens from the airways (the trachea, and the right lung) were fixed in Bouin's fluid. Paraffin 5-µm sections were made. Immunocytochemical reac-tions were performed, using the ABC technique. Polyclonal and monoclonal specific antibod-ies were used against: calcitonin (CT), somatostatin (SS), synaptophysin (SF), neuronspecific enolase (NSE) (everything from DakoCytomation), and CGRP (SigmaAldrich). Control reactions were also performed – in all the cases, they yielded negative results. The immunohistological preparations were subjected to a quantitative analysis, using: an Olympus Bx50 microscope, a PC computer, a morphological program for quantitative picture analysis (Lucia G, Nikon). In that way, the number of strong CT immunopositive endocrine cells of the thyroid and lung was evaluated in all the experimental groups. In statistic analyses, the results of all the calculations were compared, using an unpaired Student's t-test and non-parametric Mann-Whitney U-test. Significance was considered to be at $P < 0.05$.

Figure 1. Average concentrations of ionised Ca in blood serum in examined groups.

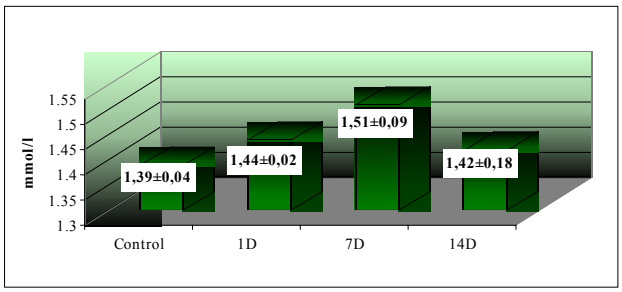


Figure 2. Average concentrations of total calcium in blood in examined groups.

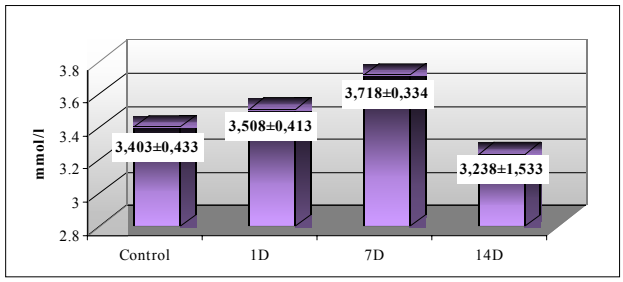


Figure 3. Average calcitonin (CT) concentrations in blood serum in examined groups.

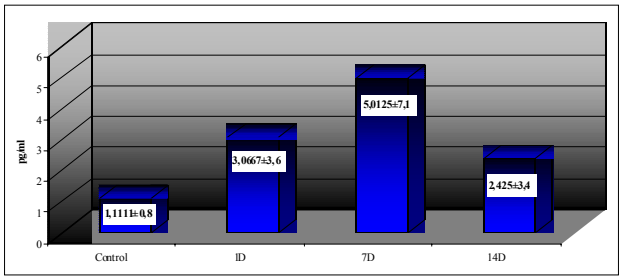


Figure 4. Comparition strong CT immunopositive NE cells in the thyroid and the lung.

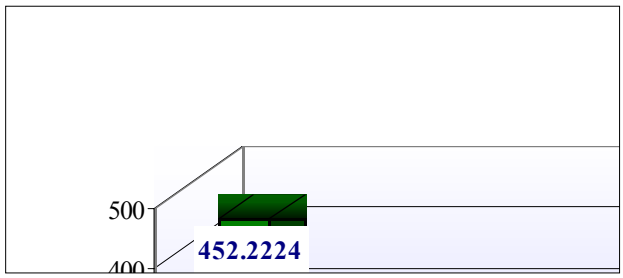


Figure 5. Control. Strong CT immunopositive reaction in the cytoplasm of thyroid C cells, x 400.

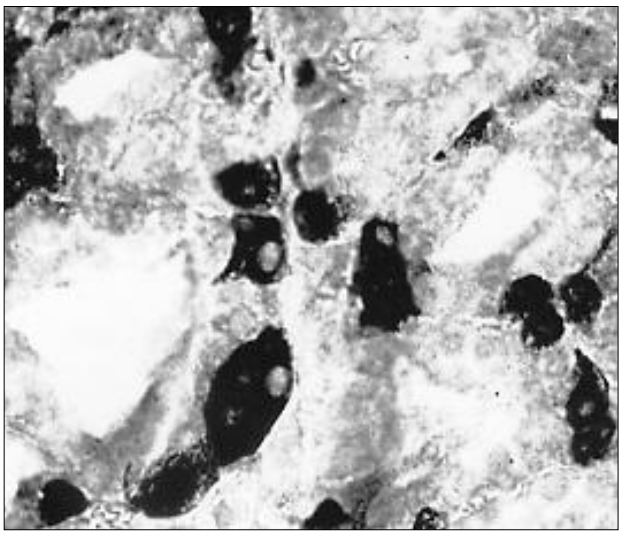
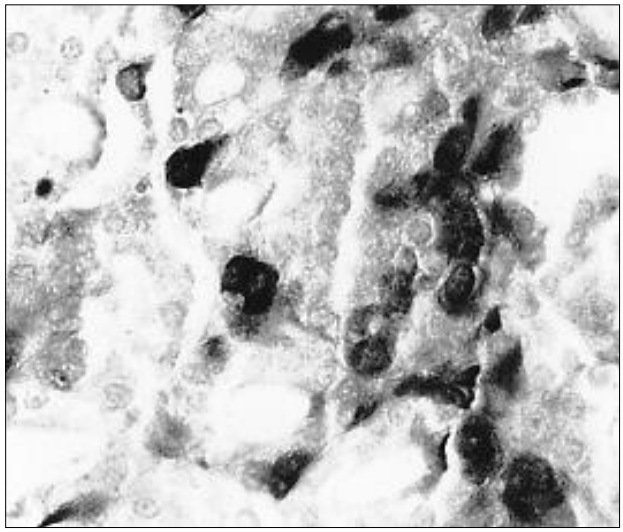


Figure 6. Group 14D. Many C cells show strong CT immunoreactivity similar to that in the control group. CT positive colouring of the environment is still visible, x 400.



Results and discussion

After intraperitoneal injection of 100000 UI vD_3 and constant loading of $CaCl_2$, a statistically significant increase of the concentration of ionised calcium (Ca^{2+}) occurred in serum in the following groups: 1D (after one day) and even more significant in Group 7D (Fig. 1). In Group 14D, the increase was statistically insignificant; there were big individual differences. Changes in total Ca concentration in blood were less obvious in the experimental groups, in comparison to Ca^{2+} (Fig. 2). The most obvious, statistically significant changes of the experiment were observed in CT concentrations in serum (Fig. 3). The biggest increase of CT concentration in serum was in Group 2D. In the control group, the majority of thyroid C cells displayed a strong CT immunopositive reaction in cytoplasm (Fig. 5). In Group 1D, a statistically significant and very strong decrease in the number of strong CT immunopositive C cells was found; in their surroundings, strong diffusive CT immunopositive colouring to the background was observed. Weakly CT immunopositive C cells were difficult to distinguish from the environment (Fig. 6). Weaker, but also statistically significant decrease in the number of strong CT immunopositive thyroid C cells was observed in Group 7D. The comparison of specimens from CT immunopositive reactions with other immunohistochemically stained specimens (SF, NSE and CGRP) indicated that probably not real decrease of thyroid C cells occurred in the experimental animals. The morphological changes indicated hypertrophy of thyroid C cells and a more intense secretion of

Figure 7. 14D group. Weakly CT immunopositive reaction in single and in group airways NE cells at bronchiolar bifurcation epithelium, x 400.

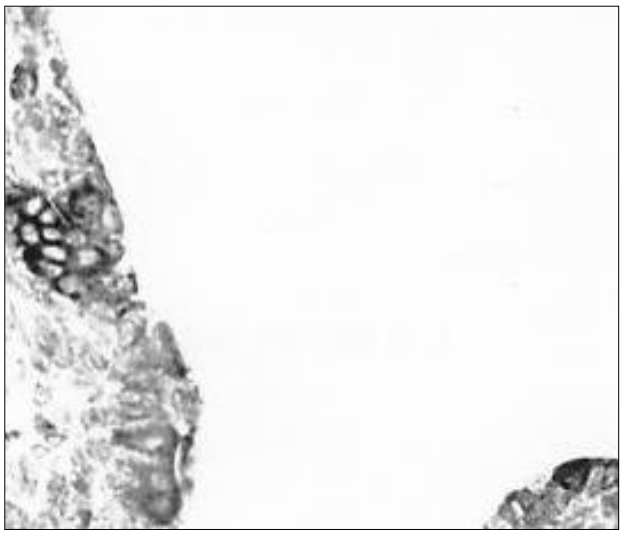
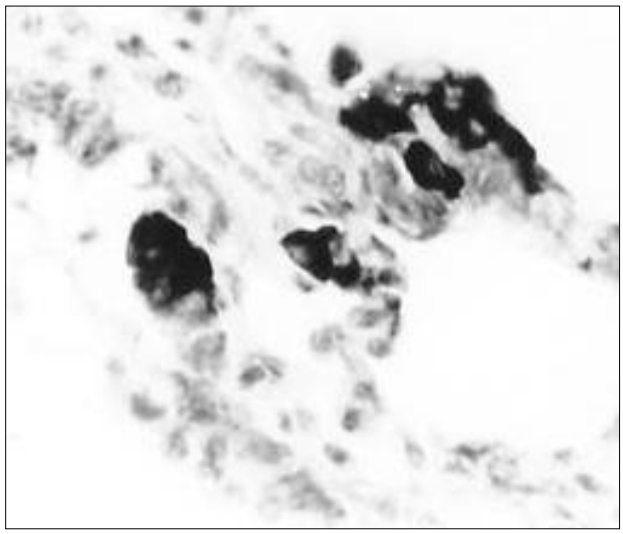


Figure 8. 14D group. Strong synaptophysin positive reaction in NE cells in the lung , x 400.



calcitonin. Moreover, the majority of researchers observed a significant decrease of CT immunoreactivity in C cells [3], some also found a relatively not high proliferative activity of thyroid C cells in rats with hypocalcaemia [4]. A few C cells demonstrated the presence of somatostatin in and all the experimental groups – here SS positive reaction was stronger than that in the control. A distinctly positive correlation was found between serum concentrations of calcitonin and Ca^{2+}. A negative correlation was observed between the number of strong CT immunopositive C cells and serum concentrations of CT, as well as Ca^{2+} (Fig. 1, 2, 3, 4). However, no correlation was found between those concentrations and the number of strongly CT immunopositive airways NE cells (Fig. 6, 7). Airways NE cells reacted to hypocalcaemia differently than C cells (Fig. 7, 8) - they probably respond to different regulatory mechanisms.

References

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