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A preliminary evaluation of thyroid and respiratory tract neuroendocrine cells in the rat after experimental hypercalcaemia

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The goal of this study was to investigate the influence of experimentally induced hypercalcaemia (after 100000 UI Vigantol and $CaCl_2$) on neuroendocrine cells (NECs) in the thyroid and airways in the rat. After 24 h, 7 days and 14 days the thyroid and lungs were collected. Paraffin sections were immunocytochemically stained with specific antibodies against CGRP, calcitonin (CT) and synaptophysin (SY) in the airway NECs and thyroid C cells. The largest hypercalcaemia were observed in experimental rats after 7 days. More significant changes in the number and size of neuroendocrine cells were observed in the thyroid gland as well as in the airways. In the airways only a slight increase in the number of neuroepithelial bodies (NEBs) was observed, some of which gave evidence of hypertrophy symptoms.

Key words: hypercalcaemia, neuroendocrine cells, thyroid, airways

INTRODUCTION

The neuroendocrine cell (NECs) system is derived from neural crest ectoderm. These cells secrete similar hormones. Their origin explains the similar expression of regulatory peptide genes in the NECs of various organs, including those in the thyroid and airways epithelium [2–5]. In the thyroid they appear as C cells and in the airways as scattered single NECs and cell groups — the neuroepithelial bodies (NEBs) [1]. The basic hormone secreting from C cells is calcitonin (CT), a hormone that decreases the calcium level in serum [6]. Some lung NECs also release CT [2, 4]. The aim of our study was the evaluation of experimental hypercalcaemia on NECs localised in the airways and thyroid.

MATERIAL AND METHODS

The study was conducted on 60 male Wistar rats with an initial body weight of 200 g, which were divided into 4 identical groups. All the animals received 0.5% aqueous solution of $CaCl_2$ to drink and had free access to drink and standard food. All the experimental rats (groups 1–3) were given an intraperitoneal injection of the 100000 UI vitamin D_3 (Vigantol). The rats were killed under pentobarbital anaesthesia at the following intervals: from group 1 after 24 h, from group 2 after 7 days and from group 3 after 14 days. group 4 consisted of the control rats. At the end of the experiment blood was collected for analysis and both thyroid lobes and 2 specimens from the airways were extracted, these being the trachea and the right lung together with the lobar bronchi. The entire lungs were fixed in Bouin's fluid with intratracheal infusion and next both specimens were fixed separately for the duration. Paraffin 5 μm sections were taken. Immunocytochemical reactions were performed using ABC technique [4]. Specific antibodies (DAKO) were used against calcitonin (CT), CGRP and synaptophysin (SY). The control reactions yielded negative results. The results were compared in statistical analyses us-

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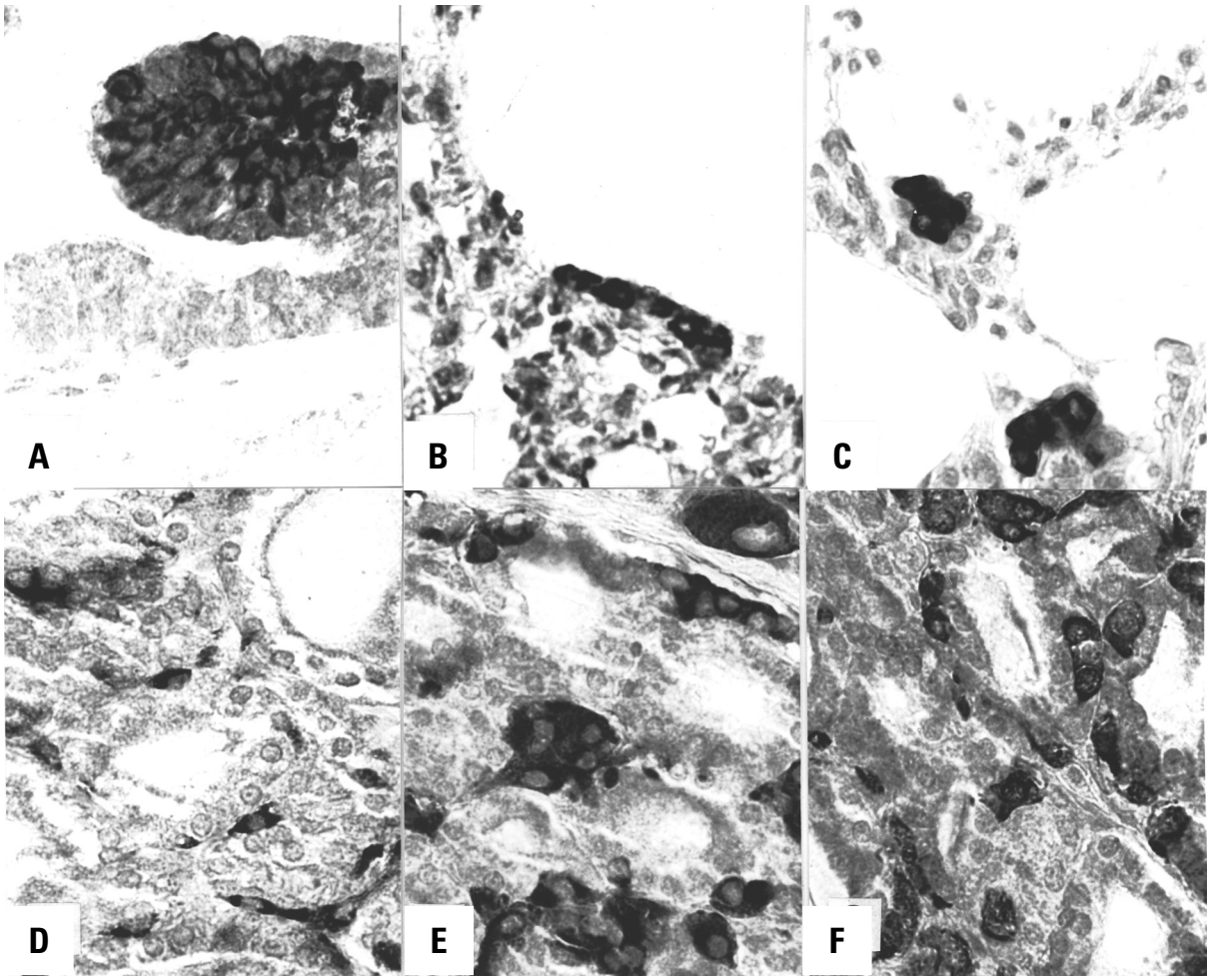

Figure 1 A. Rat 7 days (7d) after Vigantol and calcium administration. NEB immunostained for calcitonin (CT) at lobar bronchus — region of bronchus bifurcation (× 200); **B.** Control rat. Strong CT immunopositive reaction in the endocrine cells of NEB at bronchiolar bifurcation epithelium (× 400); **C.** Rat 14 days after Vigantol and calcium administration. CGRP immunopositive reaction in 2 NEBs in lung alveolar epithelium (× 400); **D.** Control rat. Thyroid gland. Synaptophysin SY immunopositive reaction in the C cells (× 400); **E.** Rat 7 days (7d). SY immunopositive reaction in the C cells is marked less obviously in many thyroid C cells than in the control rat. Some C cells are of very large size and have large light nuclei (× 400); **F.** Rat 7 days (7d). CT immunopositive reaction in the cytoplasm of thyroid C cells; in numerous cells weakly marked. The majority of C cells have large round nucle. Some cells are of large size (× 400);

ing an unpaired Student's *t*-test. Significance was taken as $p < 0.05$.

RESULTS AND DISCUSSION

The blood investigation revealed hypercalcaemia (a significant increase of Ca^{2+} concentration in blood serum, $p < 0.05$) in the experimental rats which were killed after 7 days from Vigantol administration (group 2). In group 3 (after 14 days) a significant decrease of Ca^{2+} concentration in the blood was visible in the majority of the animals investigated. The experimental hypercalcaemia caused a statistically significant increase in the number of thyroid C cells (compare, Fig. 1D, F) and symptoms of hypertrophy in certain C cells (Fig. 1E). These cells demonstrated characteristics of augmentation of secretory activity (enlargement, light nuclei, a small number of secretory granules in the cytoplasm, enlargement cells). Hypercalcaemia does not influence the number of single airway neuroendocrine cells. However, it does increase the number of NEBs (Fig. 1B, C), and some of these undergo hypertrophy (Fig. 1A). The above experiment will be extended with an examination of calcitonin concentration in the blood serum.

We know that NEB neuroendocrine cells can also participate in the hormonal regulation of Ca^{2+} concentration in the blood serum of the rat, although to a lesser degree than thyroid C cells.

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