Triiodothyronine Stimulates $^{45}$Ca-Accumulation via Synthesis of Insulin-Like Growth Factor-I in Cultures of Osteoblast-Like Cells

Osamu Kozawa, Haruhiko Tokuda, Masaichi Miwa, Jun Kotoyori, Yoshitaka Miura, Yuichi Mori and Yutaka Oiso

Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03, Japan.

First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya 466, Japan.

Abstract:
We examined the effects of triiodothyronine (T3) on $^{45}$Ca-accumulation into extracellular matrix in osteoblast-like MC3T3-E1 cells cultured for 7-week period, which was considered to be long enough to develop calcification. T3 increased $^{45}$Ca-accumulation into sodium dodecyl sulfate-insoluble, EDTA-extractable materials cultures of MC3T3-E1 cells in a dose-dependent manner in the range between 10 pM and 10 nM. Contrary to the stimulatory effect on $^{45}$Ca-accumulation, T3 inhibited DNA synthesis in MC3T3-E1 cells dose dependently between 10 pM and 10 nM. T3 stimulated the secretion of insulin-like growth factor-I (IGF-I) dose dependently between 0.1 nM and 10 nM in MC3T3-E1 cells cultured for 7 weeks. Antibodies to IGF-I suppressed the $^{45}$Ca-accumulation induced by T3 almost to the control level. These results strongly suggest that T3 stimulates $^{45}$Ca-accumulation via synthesis of IGF-I in osteoblast-like cells.

Key Words: Calcium, Triiodothyronine, Insulin-like growth factor-I, Osteoblast.

Introduction

Thyroid hormone is known as one of the major regulators of bone metabolism [1]. Bone metabolism is regulated mainly by two cell types, osteoblasts and osteoclasts [2]. The former cells are responsible for bone formation and the latter are for bone resorption. According to current understandings that the receptors of bone resorbing agents such as parathyroid hormone and 1,25-dihydroxyvitamin D₃ are present in osteoblasts, osteoblasts are considered to play important roles also in the regulation of bone resorption through local factors or cell-to-cell interaction [2]. Furthermore, triiodothyronine (T₃) receptor is recognized in osteoblast-like cells [3, 4], including MC3T3-E1 cells derived from newborn mouse calvaria [5, 6]. Thus, it is possible that effects of thyroid hormone on bone metabolism are mediated by modulation of osteoblast functions. Indeed, it has been reported that thyroid hormone increases calcium release in bone organ culture [7, 8]. It has been reported that thyroid hormone stimulates alkaline phosphatase activity [4, 9, 10], regarded as a marker of mature osteoblast phenotype [11], and inhibits proliferation [4] in osteoblast-like cells. These observations suggest that thyroid hormone modulates cellular function of osteoblasts. However, its effect on calcification promoted by osteoblasts has not yet been demonstrated.

Please address all reprint requests to: Osamu Kozawa, M.D., Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03, Japan.
It is well established that insulin-like growth factor-I (IGF-I) is a potent growth stimulator in a variety of cells including osteoblasts [12, 13]. In addition, it has recently been reported that IGF-I is produced by osteoblasts including MC3T3-E1 cells, suggesting that IGF-I acts as an autocrine or paracrine growth regulator [14, 15]. In our previous reports [16, 17], we have shown that IGF-I stimulates proliferation synergistically with a protein kinase C activator in MC3T3-E1 cells just after confluency and enhances 45Ca-accumulation without stimulating proliferation in 8-week cultures of these cells.

Osteoblast-like MC3T3-E1 cells retain many osteoblastic features such as high alkaline phosphatase activity, type I collagen synthesis and mineralization in vitro [5, 6]. Therefore, we examined the effects of T3 on 45Ca-accumulation into extracellular matrix in MC3T3-E1 cells cultured for 8-week period, which was considered to be long enough for these cells to develop calcification. In addition, we examined whether IGF-I secretion is involved in the effect of T3 on these cells. Herein, we show that T3 stimulates 45Ca-accumulation via secretion of IGF-I in cultures of osteoblast-like cells.

Materials and Methods

**Materials**

4CaCl2 (37.05 mCi/mg) and 4Cr (394.19 mCi/mg) were purchased from Du Pont/NEN (Boston, MA). [methyl-3H] thymidine (83 Ci/mmol), IGF-I radioimmunoassay kit and NCS tissue solubilizer were purchased from Amersham Japan, Inc. (Tokyo, Japan). T3 was purchased from Sigma Chemical Co. (St. Louis, MO). Antibodies to human somatomedin C were provided by Eiken Chemical Co. (Tokyo, Japan). Source for the antibodies was Nichols Institute Diagnostics (San Juan Capistrano, CA) [18]. Other materials and chemicals were obtained from commercial sources.

**Cell Culture**

Cloned osteoblast-like cells, MC3T3-E1, were generously provided by Dr. M. Kumegawa (Meikai University, Sakado, Japan) and maintained in α-minimum essential medium (α-MEM) containing 10 % fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5 % CO2/95 % air. The cells (5 × 10^4) were seeded into 35-mm diameter dishes in 2 ml of α-MEM containing 10 % FBS, and cultured up to 7 weeks being fed every 3 days. The medium was replaced with 2 ml of α-MEM containing 0.3 % bovine serum albumin (BSA) 48 h prior to experiments.

4Ca-Accumulation Study

The cultured cells for 7 weeks were incubated with 1 ml of α-MEM containing 0.3 % BSA, T3 and 4CaCl2 (5 μCi/dish) for various periods. 4Ca-accumulation experiments were performed as described previously [17]. Briefly, at the end of the incubation, the cells were washed with 1 ml of phosphate-buffered saline (PBS) four times, and solubilized with 1 ml of 0.1 % sodium dodecyl sulfate (SDS). Since the extraction of Ca with 4.13 % EDTA has been a widely applied procedure for the decalcification of biological materials [19], the remaining SDS-insoluble materials were washed with 1 ml of 0.1 % SDS four times and then extracted by overnight incubation with 1 ml of PBS containing 4.13 % EDTA. The radioactivity in the extracts was determined. When indicated, the cells were incubated T3 and antibodies to somatomedin C which is identical with IGF-I [20], simultaneously. The dose of the antibodies to IGF-I was determined taking account of our previous study [21].

4Cr-Release Study

4Cr-release was examined as described [22] with minor modifications. In brief, the cultured cells for 8 weeks were incubated with 1 ml of α-MEM containing 0.3 % BSA and 2.5 μCi of 4Cr for 1 h. After washing with 1 ml of PBS four times, the cells were incubated with 1 ml of α-MEM containing 0.5 % BSA and various doses of T3 for 120 h. The radioactivity in the medium was determined. The maximum release of 4Cr was determined by addition of 0.2 % Triton X-100.

DNA Synthesis Study

The cultured cells for 7 weeks were incubated with 1 ml of α-MEM containing 0.3 % BSA and T3 for 28 h. [methyl-3H] thymidine (2 μCi/dish) was added 6 h before the end of the incubation. The incubation was terminated by adding 1 ml of 10 % trichloroacetic acid, and the radioactivity in the acid-insoluble materials was determined [23].

Assay for IGF-I

The cultured cells were incubated with T3 in 1 ml of α-MEM containing 0.3 % BSA for various periods. The medium was aspirated and stored at −20 °C. IGF-I in the medium was extracted from specific binding proteins by the acid-ethanol treatment [24] and measured with a radioimmunoassay kit.

**Determination**

The radioactivity of 4Ca- and 3H-samples was determined with a LKB RackBeta 1219 liquid scintillation spectrometer.

**Statistical Analysis**

The data were analyzed by Student’s t-test.