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Somatostatin Effects on Cultured Human Fetal Epiphyseal Chondrocytes

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ABSTRACT. Somatostatin effects on cultured human fetal epiphyseal chondrocytes were evaluated by studying the effects of somatostatin on DNA synthesis. Cultured epiphyseal chondrocytes from human fetuses (12–40 wk old) were incubated for 48 h in Ham's F-12 serum-free medium. After this, the medium was replaced by MCDB-104 serum-free medium and the cells were incubated for an additional 48 h in the presence or absence of somatostatin 1 pM to 10 μM, with the addition of ^{3}H-thymidine (5 μCi/mL) for the last 24 h of incubation. A significant ($p < 0.02$) inhibitory effect of somatostatin (1 nM to 10 μM) on ^{3}H-thymidine DNA incorporation was observed in cultured chondrocytes from fetuses of all gestational ages studied (12–40 wk), with no significant differences among fetal ages. In conclusion, our results show that somatostatin exerts a biologic effect on cultured human fetal epiphyseal chondrocytes, as it does in its target cells. These results suggest that somatostatin could regulate human skeletal growth not only by growth hormone secretion regulation, but also by acting directly on chondrocyte metabolism. However, the physiologic significance of the latter remains to be elucidated. (*Pediatr Res* 32: 571–573, 1992)

Longitudinal bone growth results from the proliferation and differentiation of chondrocytes in epiphyseal cartilage (1). Hypothalamic somatostatin influences this process through growth hormone secretion regulation (2, 3). However, somatostatin is also secreted by other extrahypothalamic tissues (4, 5).

In humans, somatostatin has been found in hypothalamic tissue during fetal development (6), and *in vitro* studies have shown that human placental tissue is able to synthesize somatostatin (7, 8), although the biologic significance of this fact remains to be clarified.

Cultured human fetal epiphyseal chondrocytes are a suitable *in vitro* model for the study of hormone effects on cartilage growth and differentiation (9–11).

The aims of the present work were to ascertain whether somatostatin exerts biologic effects on cultured human fetal epiphyseal chondrocytes. Thus, the effects of somatostatin on ^{3}H-thymidine incorporation into DNA were evaluated in cultured chondrocytes from human fetuses 12–40 wk old.

MATERIALS AND METHODS

Materials. Plastic 75-cm^2 tissue culture flasks were from Corning Glass (Corning, NY). Ninety-six-microwell tissue culture plates were purchased from Nunc (Copenhagen, Denmark). Trypsin was from Difco Laboratories, Inc. (Detroit, MI). Collagenase was from Worthington Biochemical Corp. (Freehold, NJ). FCS and culture media were from GIBCO (Grand Island, NY). Human placental cord serum was obtained from human umbilical cord blood at delivery; a simple pool was made and used throughout the study. ^{3}H-thymidine (sp act, 22 Ci/mmol) was purchased from Amersham (Radiochemical Centre, Amersham, UK). Glass fiber filters were purchased from Whittaker (Walkersville, MD). Unisolve scintillation fluid was obtained from Koch Light Lab (Colnbrook Berks, UK) and Triton X-100 from Sigma Chemical Co. (St. Louis, MO). Synthetic cyclic somatostatin was a gift from Serono Laboratories.

Chondrocyte culture. With informed parental consent, femoral epiphyseal cartilage was obtained from human fetuses and collected within 12 h postmortem. Chondrocytes were released by enzymatic digestion and cultured as previously described (12). Briefly, after enzymatic digestion with trypsin and collagenase, a chondrocyte suspension was obtained. Approximately 500 000 cells were plated in a 75-cm^2 plastic culture flask with 12 mL of Ham's F-12 medium. The culture media were supplemented with 8% human placental cord serum, 4% FCS and 25 IU/mL penicillin. The flasks were maintained at 37°C in an atmosphere of 5% CO_2 in humid air. The cultures were fed by changing the medium every 2 d.

DNA synthesis studies. These studies were carried out as previously described (10, 11). Primary confluent chondrocytes were trypsinized and plated in 96-microwell tissue culture plates at a density of 70 000 cells/well in 0.3 mL Ham's F-12 serum and antibiotic-free medium. After 48 h, this medium was aspirated and chondrocytes were incubated for an additional period of 48 h in MCDB-104 serum and antibiotic-free medium in the presence or absence of somatostatin (10 pM to 10 μM). ^{3}H-thymidine (5 μCi/mL) was added for the last 24 h of incubation. Five different wells were prepared for each somatostatin molarity evaluated, and five without somatostatin served as controls. Incubations were terminated by medium aspiration, followed by extensive cell washing with 2.5% acetic acid in distilled water. Chondrocytes were treated with 0.5% Triton X-100 (100 μL/well, 10 min) and collected in a multiple-cell harvester on glass fiber filters. The filters were dried and counted in 10 mL Unisolve in a beta-counter for 10 min. Results were expressed as cpm of radioactivity/tissue culture well.

Statistical analysis. The results are expressed as the mean ± SEM. The differences between groups were analyzed by the Mann-Whitney U test.

RESULTS

Figure 1 shows the effect of somatostatin on ^{3}H-thymidine DNA incorporation by cultured chondrocytes from a 34-wk-old human fetus after 96 h of incubation in a serum-free medium. For the last 48 h, cells were incubated in the presence or absence of somatostatin. A significant ($p < 0.02$) inhibitory effect *versus* controls was observed for 1 and 10 nM. However, no significant

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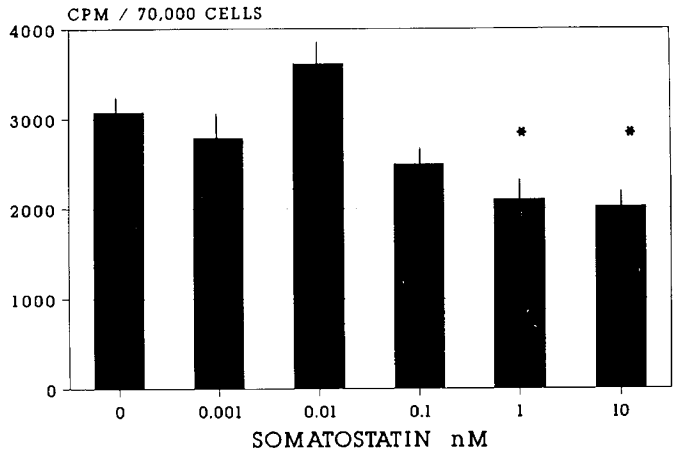

Fig. 1. Somatostatin effects on ^{3}H-thymidine DNA incorporation by cultured epiphyseal chondrocytes from a 34-wk-old human fetus. Cells were incubated in a serum-free medium for up to 96 h, the last 48 h in the presence or absence of somatostatin. *, $p < 0.02$ *vs* controls, $n = 5$.

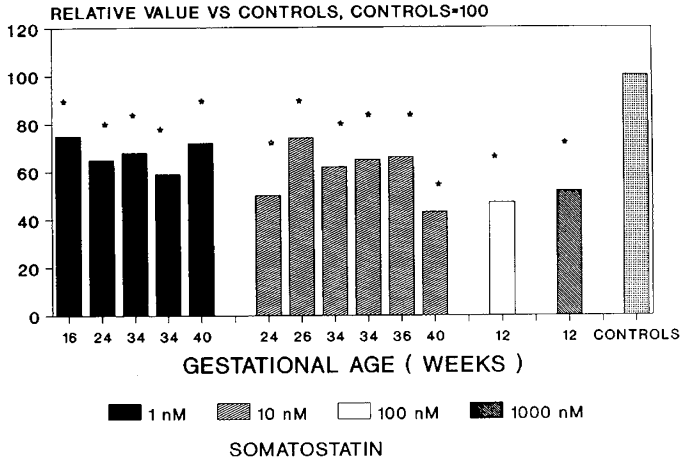

Fig. 2. Somatostatin effects on ^{3}H-thymidine DNA incorporation by cultured chondrocytes from human fetuses 12–40 wk old. Cells were incubated in a serum-free medium for up to 96 h, the last 48 h in the presence or absence of somatostatin. Each value is the mean of five points. Results are expressed as a relative value *vs* controls of the same experiment. Controls = 100. *, $p < 0.02$ *vs* controls in the same experiment.

effects *versus* controls were observed for the other molarities evaluated. Similar results were observed when somatostatin 0.001 to 10 nM effects were evaluated in cultured chondrocytes from human fetuses 16, 24, 26, 34, 36, and 40 wk old (data not shown). In addition, somatostatin 100 and 1000 nM significantly ($p < 0.02$) inhibited DNA-^{3}H-thymidine incorporation by cultured chondrocytes from a 12-wk-old human fetus (data not shown).

Figure 2 summarizes the effects of somatostatin on ^{3}H-thymidine DNA incorporation by cultured chondrocytes from human fetuses 12–40 wk old. Cells were incubated for 96 h in a serum-free medium, the last 48 h in the presence or absence of somatostatin. Results are expressed as a relative value *versus* controls, with controls being 100. A significant ($p < 0.02$ or more *versus* the absolute value of controls in the same experiment) inhibitory effect was observed for somatostatin 1 nM to 10 μM for all gestational ages evaluated (12–40 wk).

DISCUSSION

Cultured human fetal epiphyseal chondrocytes incubated in a serum-free medium for periods of time up to 96 h are able to incorporate ^{3}H-thymidine into DNA, and this suggests that these cells may synthesize growth factors, which, acting in a paracrine/autocrine way, might regulate their own growth and differentiation as has also been described in other chondrocyte culture models (13–15). Our earlier unpublished results concur with this fact, inasmuch as we found that cultured human fetal epiphyseal chondrocytes synthesize IGF-I, IGF-II, and epidermal growth factor and that these growth factors significantly stimulate ^{3}H-thymidine-DNA incorporation by these cultured cells.

Somatostatin at molarity concentrations similar to those at which it exerts biologic effects on its target cells (16–20) significantly inhibits DNA synthesis in cultured human fetal epiphyseal chondrocytes. These results are in agreement with the mechanism of action of somatostatin, which inhibits secretory (21) and proliferative (16–20) processes in a large variety of cells.

The mechanism of action of somatostatin inhibiting DNA synthesis in cultured human fetal epiphyseal chondrocytes remains to be elucidated. Recently, it has been published that, in cultured FRTL5 thyroid follicular cells of the rat, somatostatin inhibits the stimulatory effect of IFG-I on DNA synthesis (20). From these data, it may be speculated that in our human fetal chondrocyte culture model somatostatin might inhibit the stimulatory effect of one or more of the several growth factors synthesized by these cells (see above), but at present this is speculative and remains to be proved.

Long-term somatostatin analog treatment of children with excessive adult height prediction has shown a significant reduction in growth velocity during therapy, and this has been attributed to the somatostatin-induced decrease in growth hormone secretion (22, 23). However, according to several experimental data that show that somatostatin binds to and exerts biologic effects on neonatal rat calvaria (17) and neonatal rat long bones (24), that somatostatin inhibits proliferation and differentiation of cartilage and bone cells (25), and that the administration of somatostatin to mice inhibits longitudinal bone growth (26), it has been suggested that the somatostatin effect on the reduction of growth velocity in these children might in part be due to a direct effect of somatostatin on skeletal growth (27). Epiphyseal cartilage plays a central role in skeletal growth (1), and our results clearly show that somatostatin inhibits DNA synthesis in cultured human fetal epiphyseal chondrocytes and support the idea that these cells might be a target for somatostatin action. Our results also suggest that somatostatin might regulate skeletal growth, not only by regulating growth hormone secretion but also by acting locally on epiphyseal chondrocyte metabolism. However, the significance of the latter in physiologic skeletal growth regulation remains to be clarified.

In conclusion, our data show that cultured human fetal epiphyseal chondrocytes are able to elicit a biologic response to somatostatin, as occurs in somatostatin target cells. Our results also suggest that somatostatin could regulate human skeletal growth not only through growth hormone secretion regulation but also by exerting a local effect on epiphyseal cartilage regulating chondrocyte metabolism.

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