Calcitonin Inhibits Prolactin Promoter Activity in Rat Pituitary GGH3 Cells

Evidence for Involvement of p42/44 Mitogen-Activated Protein Kinase in Calcitonin Action

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Previous findings from our laboratory have shown that pituitary calcitonin-like peptide (pit-CT) is synthesized and released by gonadotrophs and inhibits prolactin (PRL) release, synthesis, and lactotroph proliferation. To investigate further the regulation of PRL gene transcription by CT, we examined the effect of CT on rat PRL (rPRL) promoter activity in rat pituitary GGH3 cells. GGH3 cells were transiently transfected with rPRL promoter-luciferase and control plasmids. Thirty-six hours later, the cells were treated with CT or other agents and their effect on luciferase activity was examined. The effect of CT and/or thyrotropin-releasing hormone (TRH) on p42/44 mitogen-activated protein kinase (MAPK) activity was also investigated. CT inhibited basal rPRL promoter activity in a dose-dependent fashion, with an approximate IC₅₀ of 3 nM. The maximal inhibition occurred 1 h after the CT addition, and the peptide was equipotent in inhibiting −600 and −2500 rPRL promoter constructs. CT also inhibited TRH-, Bay K 8644-, and ionomycin-induced rPRL promoter activity. CT mimicked the actions of MEK inhibitors U0126 and PD 980089. However, CT could not inhibit rPRL promoter activity in GGH3 cells expressing constitutively active ERK1 or ERK2. CT markedly attenuated phospho-MAPK immunoreactivity in untreated as well as TRH-treated GGH3 cells. These results suggest that CT inhibits rPRL promoter activity by antagonizing Ca²⁺ and ERK1/2-mediated signaling events. They also demonstrate that CT is a potent inhibitor of early events associated with PRL gene activation and may play an important role in regulation of lactotroph function.

Key Words: Prolactin promoter; regulation; calcitonin; mitogen-activated protein kinase.

Introduction

Calcitonins (CTs) are a group of polypeptide hormones containing 32 amino acid residues (1–4). In addition to the thyroid gland, CTs are widely distributed in the central nervous system; the pituitary gland; and several other organs such as lung, uterus, and prostate (5–11). Receptors for CT have also been detected in these organs (12–15). CT exerts significant effects on the secretion and production of neurotransmitters and also alters growth and function of various target organs such as uterus, prostate, tuberoinfundibular dopaminergic neurons, as well as pituitary gland (16–19). Thus, the diversity of sites of CT production as well as its actions suggests a variety of paracrine and autocrine roles for the peptide in addition to its originally described function of regulating of serum calcium.

CT-like pituitary peptide (Pit-CT) is synthesized and released by gonadotrophs of the anterior pituitary gland (9, 20, 21). Pit-CT has been suggested as a negative regulator of lactotroph function in rat anterior pituitary gland because of its selective, potent inhibition of prolactin (PRL) biosynthesis, secretion, and lactotroph cell proliferation (22–24). Our earlier studies with rat pituitary GH3 cells showed that CT attenuates steady-state as well as thyrotropin-releasing hormone (TRH)–induced PRL mRNA levels in a dose-dependent manner by acting at the level of gene transcription (23). Since pit-CT is secreted by gonadotrophs of the anterior pituitary gland in a highly regulated fashion (25), CT can significantly influence lactotroph function in a paracrine manner. Consequently, pit-CT can affect various PRL-dependent events and may also alter the long-term development of the pituitary gland.

In the present study, we investigated the regulatory actions of CT on rat PRL (rPRL) promoter activity. We also examined the possibility that CT interferes with TRH-induced mitogen-activated protein kinase (MAPK) activation.

Results

CT Inhibits rPRL Promoter Activity, and Proximal Region of rPRL Promoter Is Sufficient for CT Action

Because CT has previously been shown to attenuate steady-state PRL mRNA abundance and PRL gene transcription in GH3 cells (23), we tested its effect on rPRL promoter
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We used two separate –2500 and –600 rPRL promoter constructs because rPRL promoter contains distal (between –1561 and –1016 relative to the transcriptional start site) and proximal (between –326 to +73 relative to the transcriptional start site) enhancer regions (26,27). The results presented in Fig. 1A show that 10 nM CT was equi-potent in inhibiting –2500 as well as –600 rPRL promoter constructs. CT caused a 67% decrease in luciferase activity of both promoters. Therefore, all subsequent experiments used only proximal (–600) promoter construct.

Inhibitory Action of CT on rPRL Promoter Is Specific

Although CT affected rPRL promoter-luciferase activity, it did not alter either RSV 400, HSV-Tet-luc, bovine α-subunit luciferase, or PRL-TK promoters (Fig. 1B).

Inhibitory Action of CT on rPRL Promoter Is Rapid

The results presented in Fig. 1C show that 10 nM CT induced a rapid decrease in –600 rPRL promoter-luciferase activity. The initial decline was seen after 10 min, reaching nadir at 60 min. Longer incubation did not cause any greater inhibition. A maximal inhibition of 63% was achieved after 1 h of incubation.

CT Inhibits rPRL Promoter Activity in Dose-Dependent Manner

In the next group of experiments, the dose dependence of CT action on –600 rPRL promoter-luciferase activity was examined. The results in Fig. 1D show the effect of various...