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ScienceDirect
Life Sciences 81 (2007) 1411 – 1420
Life Sciences
www.elsevier.com/locate/lifescie

The cyclic AMP-dependent protein kinase A pathway is involved in progesterone effects on calcitonin secretion from TT cells

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Received 1 June 2007; accepted 28 August 2007

Abstract

It is well known that gonadal steroid hormones influence the level of plasma calcitonin (CT), but the mechanism by which progesterone affects CT secretion is not clear. Immortalized TT cells are a reliable model system for studying the endocrine function of human parafollicular cells. In the present study, the effects of progesterone on CT secretion were examined in TT cells. TT cells were incubated in medium containing vehicle (DMSO), progesterone or BSA-progesterone for 60 or 150 min, and then the levels of CT in the medium, progesterone receptors, cAMP accumulation and CT mRNA expression were measured. To study the correlation between progesterone effects and the cAMP-dependent protein kinase A (PKA) pathway, cell lysates or cells in 24-well plates were treated with either vehicle or progesterone plus RU486, SQ22536, KT5720, or 3-isobutyl-1-methylxanthine. Then, adenylyl cyclase and protein kinase A (PKA) activities were measured in the cell lysates, and the CT levels were measured in the medium from the 24-well plate. The activated cAMP response element binding protein (P-CREB) was also measured by immunofluorescence. Administration of 1 μM progesterone or 500 nM BSA-progesterone increased the secretion of CT by 381% and 100%, respectively. Progesterone receptors A and B were downregulated by progesterone treatment. The cAMP concentration, adenylyl cyclase and PKA activity, CT mRNA expression, and nuclear P-CREB concentrations all showed an increase after progesterone treatment. RU486, SQ22536 and KT5720 inhibited the progesterone-stimulated effects. These results suggest that a cAMP-dependent PKA pathway is involved in progesterone-stimulated effects on CT secretion from TT cells.

Keywords: TT cells; Calcitonin; Progesterone; cAMP-dependent pathway; Protein kinase

Introduction

It is well known that gonadal steroid hormones influence the level of plasma calcitonin (CT). For example, estrogen increases CT secretion from humans (Whitehead et al., 1981) and postmenopausal estrogen replacement therapy is effective in the prevention of rapid bone loss in clinical studies (Agnusdei et al., 1990). Both estradiol and progesterone have been shown to cause an increase of in vitro CT release from the thyroid C cells of 8-day-old rats (Greenberg et al., 1986). Treatment of ovariectomized rats with progesterone results in an increase of basal and calcium-induced secretion of plasma CT (Lu et al., 1998). Therefore, the secretion of CT from the thyroid gland might be regulated by progesterone in humans; however, the mechanism of progesterone's effects on CT secretion is not clear.

Previous studies have demonstrated that high doses of progesterone improve postnatal bone mineral accretion in preterm infants (Trotter and Pohlandt, 2000), and that preterm infants treated with estradiol and progesterone for 6 weeks postnatally show a tendency to higher bone mineral accumulation. An elevated plasma progesterone level is presented to the fetus during pregnancy (up to 1.9 μM) (Trotter and Pohlandt, 2000; Benassayag et al., 2001). Serum CT levels are also elevated in

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0024-3205/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2007.08.046

premature and newborn infants, when a period of rapid bone growth occurs (Klein et al., 1984; Body et al., 1993). There is a critical role of progesterone on enhanced CT secretion during periods of rapid bone growth; however, a cause and effect relationship between progesterone, CT and bone growth has not been established.

It is well established that progesterone influences cell functions through regulation of DNA transcription. However, progesterone might act also through a cell membrane receptor to modulate cell function (Ke and Ramirez, 1987, 1990; Dluzen and Ramirez, 1989; Peluso et al., 2001; Hanna et al., 2006). For example, progesterone can stimulate JNK activation both through the MEK/p42 MAPK pathway and MEK/p42 MAPK-independent pathways in *Xenopus* oocytes (Bagowski et al., 2001). Ni and co-workers reported that progesterone regulates CRH gene transcription via a cAMP regulatory element in the CRH promoter (Ni et al., 2004). Recently, the human membrane progesterone receptor (Jang and Yi, 2005) and receptor gene were identified (Bernauer et al., 2001). Hanna and co-workers also suggested that zebrafish membrane progestin receptor (mPR) alpha and mPR beta signal similarly upon progestin binding, resulting in rapid activation of MAPK and downregulation of adenylyl cyclase activity (Hanna et al., 2006). Thus, the mechanism of progesterone stimulation of CT secretion may occur through a pathway that is distinct from the classical effects of progesterone on gene transcription.

Calcium and cAMP are second messengers involved in CT secretion from thyroid C cells. In the calcium-dependent pathway, both protein kinase C (PKC) and the L-type calcium channel were used to increase the secretion of CT from C cells (McGehee et al., 1997). In the cAMP-dependent pathway, increased adenylyl cyclase and protein kinase A (PKA) activity stimulated the secretion of CT from C cells (Schultz et al., 1990; Houslay, 1992; Zink et al., 1992; Zink-Lorenz et al., 1996).

The TT cell line is a stable cell line derived from human medullary thyroid carcinoma, and it serves as a reliable model system for studying the endocrine function of human parafollicular cells (Zabel and Grzeszkowiak, 1997). TT cells produce hormones, such as human calcitonin (hCT), calcitonin gene-related peptide, somatostatin, gastrin-releasing peptide and ACTH (Gagel et al., 1986; Oosterom et al., 1986; Cote et al., 1987; Haller-Brem et al., 1988; Sunday et al., 1988).

CT gene transcription has been shown to be controlled by cAMP in TT cells (de Bustros et al., 1992). Results from numerous investigations indicate that transcription of the hCT gene is markedly increased by cAMP in these cells. The cellular response to cAMP is complex, as it requires multiple elements acting in concert. In transfection experiments, the downstream cAMP response element (CRE), combined with CT promoter sequences, generated 70% of the maximal cAMP response in TT cells. The upstream CRE and C-rich elements conferred 10 and 30% of this response, respectively. The respective TT cellular proteins were found to bind to these sequences (de Bustros et al., 1992).

A functional calcium channel has not been detected in TT cell membranes (Krautwurst et al., 1993). Calcium action is a direct effect of the secretory process in TT cells (Zabel and Grzeszkowiak, 1997). Therefore, this paper only discusses the cAMP-dependent PKA pathway of hCT secretion from TT cells.

This investigation aims to study the role of progesterone in regulating CT secretion from TT cells. To study the mechanism of progesterone effects on TT cells, inhibitors of adenylyl cyclase and PKA were used. Our hypotheses are that progesterone could increase the secretion of hCT from TT cells and that these effects occurred through increased adenylyl cyclase and PKA activity and involved regulation of DNA transcription. In the present work, we have confirmed our hypothesis that progesterone increases the release of CT in TT cells through activation of adenylyl cyclase and PKA. The cAMP-dependent PKA pathway was involved in the mechanism by which progesterone modulates CT release.

Materials and methods

Experimental design

Two treatment regimes were employed in this study. One group served as the control while the other received progesterone or 11α-Hydroxyprogesterone 11-hemisuccinate: BSA (BSA-progesterone). Time course and dose-dependency experiments were also performed. In order to explore the mechanism of progesterone effects through the cAMP-dependent PKA pathway, inhibitors of adenylyl cyclase, phosphodiesterase, PKA and progesterone were used. All experiments were repeated three times or more with similar results.

Cell culture

TT cells were purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in nutrient mixture F12 Ham Kaighn's modification (F12K) medium (Sigma, St. Louis, MO, USA), supplemented with 10% fetal calf serum (Gibco BRL, France) at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. The medium was changed twice per week.

Experiments on CT release, cAMP accumulation, and DNA transcription in TT cells

Before the experiments, TT cells (1×10^6 cells per ml) were cultured in 24-well plates in challenge medium (RPMI 1640, 10 mM HEPES, 6 mM glutamine) for 48 h. Then the cells were preincubated or primed with vehicle (DMSO), SQ22536, KT5720 or 3-isobutyl-1-methylxanthine (IBMX) in 1 ml challenge medium for 30 min. After preincubation, the cells were incubated with 1 ml challenge medium containing vehicle (DMSO) or progesterone (10^{-8}, 10^{-7}, and 10^{-6} M) for 150 min. In time course experiment, the cells were incubated with challenge medium for 30 to 180 min. In order to study the correlation between progesterone effects and the cAMP-dependent pathway, TT cells were treated with challenge medium that contained vehicle (DMSO) or progesterone with 1 μM RU486 (a progesterone antagonist), 10 μM SQ22536 (an adenylyl cyclase