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Different Mechanisms of Calcitonin, Calcitonin Gene-Related Peptide, and Somatostatin Regulation by Glucocorticoids in a Cell Culture of Human Medullary Thyroid Carcinoma

Gilbert J. Cote, and Robert F. Gagel*

We have employed the TT cell line, a model for the human medullary thyroid carcinoma cell, to study the regulation of peptide hormone production by glucocorticoids. Complementary DNA probes were used to measure the calcitonin (CT), CT gene-related peptide (CGRP), and somatostatin (SRIF) mRNA levels. Dose-response experiments in serum-free medium showed that dexamethasone (six-day treatment) lowered somatostatin (to 1% of basal) and CGRP mRNA (to 50% of basal) and stimulated CT mRNA (threefold to thirteenfold) with a half-maximal effective concentration of 10^{-8} M. Time course studies for cells continuously exposed to 10^{-6} M dexamethasone showed a rapid (within hours) lowering of SRIF mRNA, whereas the effects on CT and CGRP mRNA required six to eight days. These results demonstrate the presence of two mechanisms, transcriptional (somatostatin) and posttranscriptional (the RNA splice decision to make CT or CGRP mRNA), that can be hormonally regulated. (Henry Ford Hosp Med J 1987;35:149-52)

The TT cell line is a continuous cell line originally derived from the needle biopsy of a human medullary thyroid carcinoma (1). We have employed this cell line to study the production of the peptide hormones calcitonin (CT), CT gene-related peptide (CGRP), and somatostatin (SRIF). Two precursor peptides for CT and CGRP are produced from the CT gene by alternative RNA processing, whereas the SRIF gene codes for the production of a single precursor peptide (2,3). Because the TT cell line produces all three of these peptides in significant quantities, it provides a useful model system for studying the regulation of the two genes. We have previously demonstrated an effect of the synthetic glucocorticoid dexamethasone on production of SRIF (4), as well as CT and CGRP (5). In this paper we present and compare the effects of dexamethasone treatment on CT, CGRP, and SRIF mRNA in the TT cell line. The results demonstrate the existence of two distinct mechanisms of action for dexamethasone: a rapid transcriptional effect seen on SRIF, and a more slowly developing posttranscriptional effect on the processing of primary RNA transcript derived from the CT gene.

Experimental Procedures

Cell culture methodology

The TT cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, NY), using previously described techniques (6). In experiments using serum-free medium, cells were subcultured 48 hours before treatment in serum-containing medium and then switched to serum-free medium 24 hours before the onset of treatment. Serum-free defined medium was prepared as previously described for a rat C-cell line (7). Cell counts were determined with the aid of a Coulter Counter (model ZF), as previously described (6). All steroids were added to the medium as 25 mM stocks prepared in ethanol. Control cells received an equal volume of ethanol.

Preparation of RNA

The TT cells were plated at an initial density of $\sim 3 \times 10^6$ cells/100 mm dish. The cells were allowed to attach for 24 hours before treatment. Total cellular RNA was isolated from various cell types by the guanidine isothiocyanate procedure (8), with the previously described modifications (5). Total RNA was stored dissolved in sterile distilled H_2O in the presence of vanadium-riboside complex (9) at $-70°C$.

Hybridizations

RNA dot blot hybridizations were performed by immobilization of purified total RNA onto nitrocellulose paper as described by Thomas (10). The SRIF-, CT-, and CGRP-specific probes were prepared as previously described (4,5). Prehybridization, hybridization, and washing procedures were identical to those of Thomas (10). Autoradiographs were quantitated by computer-assisted densitometric scanning with a video camera as described by Mariash et al (11).

Results

Fig 1 shows the effect of a pharmacologic dose of dexamethasone (10^{-6} M) on CT, CGRP, and SRIF mRNA levels.

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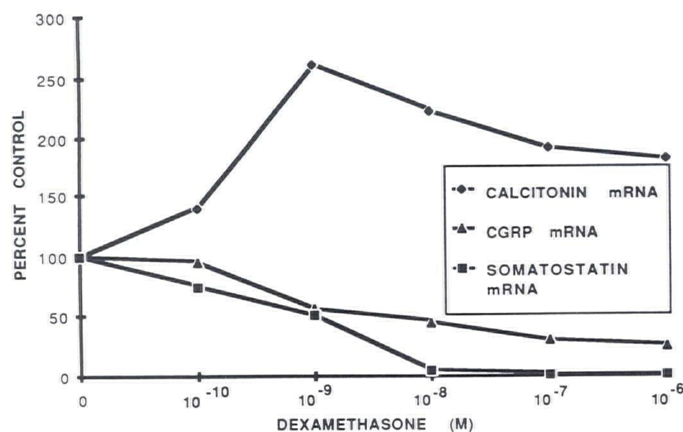

Fig 1—Time course of dexamethasone treatment on CT, CGRP, and SRIF mRNA production in the TT cells. The TT cells were plated at an initial density of ~3 × 10⁶ cells/100 mm dish and allowed to attach in growth medium (24 hours) before changing to medium containing 1 × 10⁻⁶ M dexamethasone. Medium was changed at 48-hour intervals. RNA measurements were made at indicated time points after the initiation of treatment by removal of medium and addition of guanidine isothiocyanate. The values represent the average hybridization value of two dishes as determined by dot hybridization and quantitated by a computer-assisted densitometer.

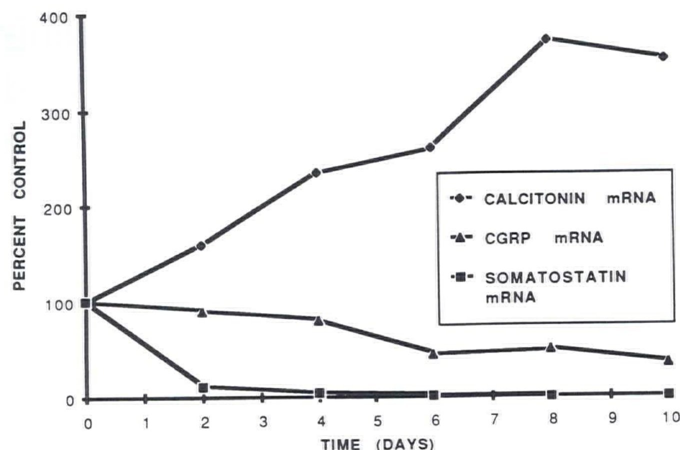

Fig 2—Dose-response curve for the effect of dexamethasone on CT, CGRP, and SRIF mRNA production. The TT cells were plated at an initial density of ~3 × 10⁶ cells/100 mm dish in serum-containing medium for 24 hours, then switched to serum-free medium for 24 hours before treatment. Cells were then grown in the presence of the indicated concentration of dexamethasone in serum-free medium for six days with medium changed at 48-hour intervals. The mRNA levels were determined as described in Fig 1.

Dexamethasone treatment affected the levels of each mRNA differently. The levels of SRIF mRNA showed a rapid decrease (levels reach 50% of control by 12 hours) and were barely detectable by 48 hours. While CGRP mRNA levels also declined as a result of dexamethasone treatment, a six-day treatment was required to achieve a 50% reduction. Unlike SRIF and CGRP, CT mRNA showed an increase over two to eight days following treatment with dexamethasone. Although this increase could be explained by an enhancement of transcription, the rate of the response and the simultaneous decrease in CGRP mRNA levels suggest a more complex explanation.

To determine whether glucocorticoids might play a physiologically significant role in the regulation of these genes, we examined the dose-dependent effects of dexamethasone on CT, CGRP, and SRIF mRNA levels (Fig 2). Again, both SRIF and CGRP mRNA levels were depressed, while CT levels were enhanced. The TT cells are typically grown in the presence of 10% fetal bovine serum, which we have found to contain approximately 10^{-9} M endogenous steroid. For this reason, the dose-response curves were generated in serum-free medium. The half-maximal doses of dexamethasone (10^{-9} M for CT, 10^{-8} M for CGRP, and 10^{-9} M for SRIF mRNA) are shifted two orders of magnitude to the left, compared to our previous findings in the presence of serum (4,5), and now approach physiologically significant levels (12).

Discussion

Differences between regulation of the CT and SRIF genes

The results presented in this and previous papers (4,5) suggest that dexamethasone acts to regulate mRNA levels by several mechanisms in the human medullary thyroid carcinoma TT cell line. Three possible points of regulation are transcription, RNA processing, and mRNA degradation. Fig 3 schematically demonstrates the production of CT, CGRP, and SRIF mRNA from their respective genes. The SRIF gene is transcribed, the transcript undergoes RNA processing in the nucleus to produce mature mRNA, and then the mRNA is typically degraded in the cytoplasm after several rounds of translation. Unlike the SRIF gene, the CT gene's primary transcript is alternatively processed to produce one of two possible mRNAs, one coding for CT and the other for CGRP. Therefore, RNA processing becomes a key regulatory step in the production of CT and CGRP mRNA.

Effect of dexamethasone on SRIF mRNA levels

We have shown that dexamethasone rapidly lowered SRIF mRNA levels. Because the ~12-hour half-life of the SRIF mRNA (data not shown) approximates the rate of decline in SRIF mRNA following dexamethasone treatment, we favor a model whereby dexamethasone acts to inhibit directly the transcription of the SRIF gene. The possibility that dexamethasone affects SRIF mRNA stability, though unlikely, has not been excluded.

Effect of dexamethasone on CT and CGRP mRNA levels

Dexamethasone acts to enhance CT mRNA levels and decrease CGRP mRNA levels. Regulation of this event must occur at either the RNA processing step or at the level of mRNA degradation. Transcriptional regulation alone would result in parallel changes in CT and CGRP mRNA levels, such as seen after phorbol ester or cAMP analog treatment (12), and not the diver-

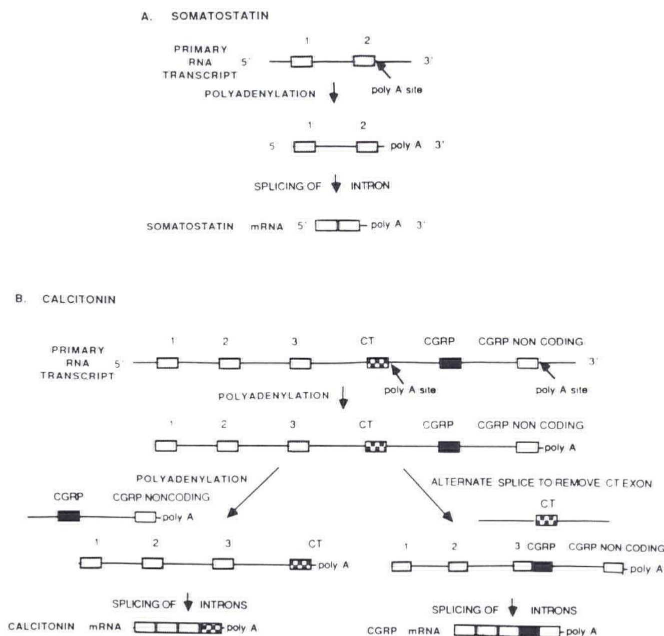

Fig 3—Schematic outline comparing RNA processing of the primary transcript from the SRIF gene and the CT gene: A) processing of the SRIF gene primary transcript to SRIF mRNA, and B) processing of the CT gene primary transcript into CT and CGRP mRNA.

gence seen here. We have previously demonstrated that dexamethasone has no effect on the half-life of either CT or CGRP mRNA (5). Therefore, we proposed that dexamethasone must be acting to affect the RNA processing of the primary transcript.

How might dexamethasone alter the RNA processing of the primary transcript of the CT gene? The first possibility to consider is a direct effect of dexamethasone on RNA processing. The dexamethasone-glucocorticoid receptor complex, or some other dexamethasone-binding protein with regulatory abilities, might affect RNA processing directly. Glucocorticoid receptors have been demonstrated to bind RNA (13), and RNA processing is brought about by protein-RNA complexes (14). However, it is unlikely that dexamethasone is acting through one of these direct mechanisms, because the time course for the effect is too slow, and a similar effect can be produced in the absence of any steroids by long-term culturing of the cells without subculturing (as described by A. deBustros et al at these proceedings). Therefore, we propose that dexamethasone is acting in an indirect fashion to alter RNA processing.

Model for the regulation of alternative RNA processing by dexamethasone

To explain the regulation of the alternative processing of the CT gene primary transcript, we favor a model where the production of CT mRNA is the unregulated pathway or "null" choice, and production of CGRP mRNA requires the presence of specific factor(s). This model is based on the available information regarding the processing of the CT gene transcript (15) and on current theories of RNA processing (14). From this model we

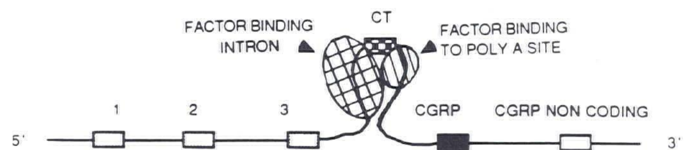

Fig 4—Proposed model for CGRP mRNA production.

predict that in the thyroid C-cell, cleavage and polyadenylation at the site immediately following the CT exon are the early steps which necessitate an exon 3 to CT exon splice (Fig 3). If our CT "null" choice is correct, then the production of CGRP mRNA would require that: 1) polyadenylation immediately after the CT exon be blocked; and 2) because the blockage of cleavage following the CT exon would be insufficient to ensure CGRP mRNA production, a mechanism must exist to prevent an exon 3 to CT exon splice. This is most easily accomplished either by preventing branch point formation within the third intron or by blocking the accessibility of the 3′ splice junction (studies have shown that spliceosome binding to the 3′ splice site is required for 5′ cleavage) (14). This blockage would then allow preferential usage of the next available 3′ splice junction (at the CGRP exon).

This model would predict the existence of protein or RNA factor(s) in CGRP-producing cell types functioning to block polyadenylation immediately after the CT exon and removal of the intron immediately before the CT exon. Whether this is accomplished by a single factor, several factors, or a complex remains to be elucidated. One such way in which the interaction could occur is diagramed in Fig 4, where separate binding sites cause the RNA to loop out while forcing CGRP mRNA production. If the production of this complex was inhibited by dexamethasone, cell cycle-dependent or cell type-dependent mechanisms, then the production of CT would be favored.

Acknowledgments

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