Thyroid Hormones Stimulate Renal Expression of CFTR

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Key Words

CFTR • TNR-CFTR • Thyroid hormones • Hypothyroidism • Hyperthyroidism • Ion channel

Abstract

CFTR is a multifunctional protein of the ATP binding cassette family that may contribute to overall electrolyte homeostasis by acting as a chloride channel in the kidney. In renal tissues CFTR does not exist only in its full-length form, but also as a kidney-specific, truncated splice variant, TNR-CFTR. In this study we show that both forms of CFTR are regulated by thyroid hormones in rat renal tissue. Four groups of male rats were used: control, hypothyroid, hypothyroid with T_4 treatment and hyperthyroid rats. The hypothyroid rats showed a decrease of both CFTR and TNR-CFTR mRNAs (44%, and 49%, respectively, $n=5$; $p<0.05$) and proteins (30% and 37%, respectively, $n=5$, $p<0.05$) expressions, compared to control group. In hyperthyroid rats, a significant increase in both CFTR and TRN-CFTR mRNAs expressions (43% and 95%, $n=5$; $p<0.05$) and proteins (250% and 38%,

respectively, $n=5$, $p<0.05$) was observed when compared to control group. Treatment of immortalized rat proximal tubule cells (IRPTC) with T_3 (10^{-7} M) produced also an increase of CFTR mRNA expression (95%, $n=5$, $p<0.05$). Analysis of the promoter region of CFTR transfected to IRPTC showed that T_3 (10^{-7} M) stimulates the CFTR promoter (38%, $n=4$, $p<0.05$).

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Introduction

The kidneys critically regulate extra cellular fluid volume (ECFV) through the absorption and excretion of salt and water [1]. Variations in renal tubular sodium chloride (NaCl) intake or excretion mechanisms can be related to the genesis of ECFV disturbances, which can lead to systemic arterial hypertension, among other dysfunctions [1-4].

NaCl excretion is regulated by different hormones which act not only on the function of ionic transporters but also in their expression along the nephron. Atrial

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natriuretic peptide (ANP) and aldosterone exemplify two well-studied hormones that regulate ECVF [5, 6]. However, other hormones can affect ECFV. These include the thyroid hormones (THs), estrogen and progesterone [7-10]. In particular, the THs, thyroxine (T_4) and triiodothyronine (T_3), stimulate Na transport pathways by increasing either their expression or function. These pathways include the Na^+/K^+ ATPase [11] as well as coupled Na^+ transporters: the Na^+ -phosphate transporter, NaPi2 in the proximal tubule [12-14], as well as Na^+/H^+ exchangers [15]

Chloride is the main sodium co-ion in the extracellular fluid. We therefore postulated that hormones involved in the regulation of renal sodium transporters also modulate renal chloride transporters [16, 17]. The Cystic Fibrosis transmembrane conductance regulator, CFTR, is a multifunctional chloride channel [18, 19] that is mutated in the genetic disease, Cystic Fibrosis (CF) [20, 21]. CFTR is expressed in the kidney, where it is modulated by hormones related to the regulation of ECVF, such as vasopressin [9]. Interestingly, in the kidney, CFTR exists in both its full-length form and as a truncated, splicing variant, TNR-CFTR, that comprises the first half of the molecule (the first membrane spanning domain and the first nucleotide binding domain, as well as the regulatory domain; MSD1, NBD1, R). Furthermore, TNR-CFTR is unique to the kidney [22]. CF patients do not present with gross disorders of ECVF regulation, suggesting a role for TNR-CFTR. Taken together, these observations motivated us to investigate the effects of THs on the expression of CFTR and its kidney-specific splice variant, TNR-CFTR, with the goal of elucidating their physiological roles in the kidney [8, 17, 23]. Our findings suggest that THs regulate the expression of both forms of CFTR and that this operates at least partially through stimulation of the *CFTR* promoter.

Materials and Methods

Animal treatment

Male rats, Wistar, weighting of 200-250g, were housed in a room with controlled lighting (12-h light/12-h dark cycles, beginning at 8 am/8 pm) and temperature (23-26°C). Rats were rendered hypothyroid by including 0.03% methimazole (MMI, Sigma Chemical Co., St Louis, MO, USA) in the drinking water for 21 days before the sacrifice. One group, drinking tap water, served as normal controls. The MMI-treated rats also received subcutaneous (s.c.) injections of saline (hypothyroid control - Hypo) or thyroxine ($L-T_4$ - Sigma-Aldrich) at 1 μ g/100g of body weight daily for 10 days before the sacrifice. Hyperthyroidism was induced in one group of normal animals by s.c. injection

with T_4 (10 μ g /100g BW/day) for 10 days (Hyper group).

The animals were decapitated 18h after the last injection (in the case of hormone-injected animals) and blood samples were collected for hormonal evaluation [24].

All animal experiments were performed according to the NIH guide for the Care and Use of Laboratory Animals and protocols were approved by our institutional animal ethics committee.

Cell Culture

Immortalized rat proximal tubule cells (IRPTC) were kindly provided by Dr. Mello-Aires (Renal Biophysics Laboratory of the Biomedical Science Institute in University of São Paulo, Brazil). IRPTC were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, N.Y., USA), 25 mM glucose, 2 mM L-glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin (Gibco BRL, N.Y., USA). After reaching 90% confluence, cells were incubated with DMEM in the absence of fetal bovine serum for 12 h, and treated with T_3 (10^{-7} M, 10^{-8} M and 10^{-9} M) for 24h. After this procedure total RNA was extracted.

Isolation of total RNA

Total RNA was extracted from kidney or cell cultures using Trizol® reagent (Gibco BRL, Grand Island, N.Y., USA), following the manufacturer's suggested protocol. Purified, total RNA was treated with 1U/ μ l of ribonuclease (RNase)-free desoxyribonuclease (DNase) I (GIBCO-BRL, Grand Island, N.Y., USA) for 30min to eliminate any genomic DNA contamination.

Reverse-transcription polymerase Chain Reaction (RT-PCR)

RT-PCR analysis was carried out on 700 ng of total RNA using reverse transcriptase SuperScript™ (Gibco BRL, N.Y., USA).

For CFTR and TNR-CFTR PCR reactions, primers specific for both TNR-CFTR and CFTR were synthesized. These were designed by placing the sense primer before the splicing site (5' CTC AGT GGA CGA TGC CTC 3') and the antisense primer was placed after this region (5' CCT CAA CCA GAA AAA CCA G 3'). Thus, the amplified products of reverse transcribed CFTR and TNR-CFTR fragments gave fragments of two different sizes: 568 bp and 423 bp, respectively. GAPDH gene-specific primers (5' GTC TTC ACC ACC ATG GAG 3' and 5' CAT GAC AAC TTT GGC ATC 3'), that amplified a 211 bp fragment, were used for internal control reactions.

The PCR was performed under the following conditions. Briefly, an initial denaturation at 94°C for 5min was followed by 36 cycles of denaturation (94°C, 1min), annealing (54°C, 1min) and extension (72°C, 1min). The reaction was concluded with an extension for 10min at 72°C. First, the optimal PCR conditions that yielded a single band on agarose gel electrophoresis were determined for each gene originating from the RT-PCR reaction made in the same reaction tube for target gene and the internal control gene. Second, to determine whether the method was semi-quantitative, different quantities of total RNA (350; 700; 1400 e 2800 ng) were used for RT-PCR amplification for both genes in the same reaction tube (data not shown). Third,

experiments were performed to determine the optimal number of PCR cycles (26, 28, 30, 32, 34, 36, 38 or 40) that yielded PCR products in the linear range of amplification (data not shown). All PCR reactions performed included a negative control (reverse transcription without reverse transcriptase). The identity of the amplification product was confirmed by determination of the molecular size on agarose gel electrophoresis (1.5% agarose in buffer containing 40 mmol/l of Tris-acetate plus 1 mmol/l of ethylenediaminetetraacetic-acid-EDTA) and visualized by ethidium bromide staining (0.5 µg/ml) under ultraviolet light. The computer software Sigma Gel v1.1 was used (Jandel Scientific, Corte Madera, CA, USA) for densitometric analysis of the bands. Bands amplified in the same sample were analyzed by densitometry and normalized by dividing the target gene values by the corresponding GAPDH values.

Western blot analysis

Expression of CFTR and TNR-CFTR protein in whole kidneys of the different groups was assessed and compared by western blot. Kidneys were homogenized in a solution containing 250 mmol/l sucrose, 1 mmol/l EDTA, 20 mmol/l imidazole, pH 7.2, and the following protease inhibitors: 1 mmol/l 4-(2-aminoethyl)-benzenesulfonyl fluoride, 1 mmol/l benzamide, 10 mg/l leupeptin, 1 mg/l pepstatin A, 1 mg/l aprotinin, and 1 mg/l chymostatin. Homogenization was carried out on ice using a Potter apparatus. The homogenate was centrifuged at 1000xg for 10min. The supernatant was saved, the pellet was suspended in three volumes of the same medium, and the centrifugation was repeated. Both supernatants were mixed and centrifuged at 10,000xg for 20min to separate mitochondria. The ensuing supernatant was centrifuged at 100,000xg for 1h, the pellet from this centrifugation contained cell membranes that were suspended in ice-cold homogenization buffer. Protein concentration was quantified by the Bradford assay using bovine serum albumin (BSA) as the standard [25].

All the extracts were solubilized by heating at 95°C for 2min in buffer solution (15 g/l SDS, 10 mmol/l Tris-Cl pH 6.8, 6 g/l DTT, and 60 ml/l glycerol). The membrane proteins (200 µg/lane) were subjected to 7.5% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene difluoride membrane (PVDF, Bio-Rad, Hercules, CA). The membranes were then blocked with 5% nonfat milk in a Tris-buffered saline with Tween 20 0.05% (T-TBS 0.05%).

Immunodetection of CFTR and TNR-CFTR was performed using a mouse monoclonal antibody (1:1000 dilution) directed at the MSD1 region (amino acids 386-412; Upstate Biotechnology, Lake Placid NY, USA), that specifically recognizes both CFTR and TNR-CFTR proteins [26]. A mouse monoclonal antibody (1:3000 dilution) generated against the N-terminus of β -actin (Novus Biologicals, Littleton, CO 80160, USA) was used as an internal control. Sequential incubation was performed with an alkaline phosphatase-coupled secondary antibody goat anti-rabbit IgG (1:1000). Protein bands were detected using an alkaline solution (100mM NaCl, 5mM MgCl₂, 100mM Tris, pH 9.5), 75 mg/ml nitroblue tetrazolium chloride (NBT) and 50 mg/ml 5-bromo-4-chloro-3-indolylphosphate

p-toluidine (BCIP) (all from Life Technologies, Rockville, IL, USA) for 5min.

The obtained TNR-CFTR and β -actin bands from a given sample were analyzed by densitometry and normalized by dividing the TNR-CFTR values in each lane by the corresponding β -actin values in the same lane.

Construction of CFTR-Promoter Reporter Vector

PCR was used to amplify the region from the Xba I restriction site at -2247 to position +52 of the CFTR promoter by using a fragment of the promoter. Promoter locations are relative to the major transcription start site in T84 cells [27]. The PCR fragment, containing a BamHI restriction site at the 3' end, was ligated with an oligonucleotide SacI/Xba I adapter into the inh-luc construct [28] digested with Sac I and Bgl II to construct CFTR-luc.

Transient Transfections

Transient transfections were performed using subconfluent IRPTC cells grown on 12-well plates; 1.5 µg of CFTR-Luc promoter construct (kindly provided by Dr. G. Stanley McKnight, Department of Pharmacology, University of Washington, Seattle, WA, USA) and 0.5 µg of pSV- β -galactosidase plasmid (kindly provide by Dr. Peter Kopp, Division of Endocrinology, Northwestern University, Chicago, Illinois, USA) were added to each well using the Lipofectamine 2000 kit (Invitrogen, San Diego, CA, USA). After transfection, cells were washed and treated with T₃ at 10⁻⁷M, 10⁻⁸M and 10⁻⁹M, and after 24h, cultures were harvested and assayed for luciferase activity.

Luciferase activity was quantified using 200 µl of cell lysate, 100 µl of D-luciferin (Molecular Probes Inc., Eugene, OR, USA) and 100 µl of luciferase assay buffer (25 mM gly-gly, 15 mM MgSO₄, 4 mM EGTA, 15 mM KH₂PO₄, 6 mM ATP and 3 mM dithiothreitol) per sample. Light emission was detected using a TD-20/20 Luminometer (Turner Designs, Ann Arbor, MI, USA) for 30s at room temperature. To normalize the results, β -galactosidase activity was quantified by reaction of cell lysates (30 µl) with 2-nitrophenyl-bD-galactopyranoside (ONPG) reagent (60 mM Na₂HPO₄, 40 mM NaH₂PO₄ and 2 mg/ml of ONPG; 160 µl) and buffer β -gal (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgCl₂ and 50 mM β -mercaptoethanol; 810 µl). The reactions were quantified by reading the absorbance at 420 nm.

Radioimmunoassay

Plasma T₄ was measured by specific radioimmunoassay using specific antibodies (Kit Coat A Count, Diagnostic Products Corporation, Los Angeles, USA), according to the manufacturer's suggested protocol.

Statistical analysis

Data are reported as mean $\pm$ SEM. One-way ANOVA followed by Newman-Keuls multiple comparisons test was employed for assessment of significance. Differences were considered to be significant at $p < 0.05$.

	Control	Hypothyroid	Hypothyroid animals with T4 replacement	Hyperthyroid
T4 plasma level (µg/dl)	3.047±0.45 (n=7)	0.01±0.00* (n=5)	2.56±0.20 (n=3)	0.430±0.95* (n=6)

Table 1. T₄ plasma level was measured by radioimmunoassay in the different experimental groups. Results are represented as mean ± SE. * p < 0.05 vs. control.

Fig. 1. Modulation of CFTR mRNA and protein by thyroid hormones. A Expression of CFTR mRNA in kidney of control, hypo- and hyperthyroid rats according to semi-quantitative RT-PCR analysis. Graphs represent mean ± SEM for the ratio between the densitometric values of CFTR and GAPDH bands obtained in RT-PCR experiments. B Expression of CFTR protein in kidney of control, hypo- and hyperthyroid rats according to western blot analysis. Graphs represent mean ± SEM for the ratio between the densitometric values of CFTR and β-actin bands obtained in western blot experiments. Hypothyroid rats were treated with saline (HYPO) or 1 µg/100g of BW of thyroxine (Hypo + T4): for 10 days before they were killed. One group of normal rats received T4 in the dose of 10 µg/100g of BW for 10 days (HYPER) * p < 0.05 vs. control (n = 5). C CFTR chloride channel mRNA expression in IRPT cells. Representative agarose gel of semi-quantitative RT-PCR of CFTR and GAPDH genes, and graphic representation of the mean ± SE of the ratio between densitometric values of CFTR and GAPDH. IRPT cells were not treated with T3 (control) or treated with 10⁻⁷; 10⁻⁸ and 10⁻⁹ M of T3. * P < 0.01 vs. control (n = 3).

Fig. 2. Modulation of TNR-CFTR mRNA and protein by thyroid hormones. **A** Expression of TNR-CFTR mRNA in kidney of control, hypo- and hyperthyroid rats according to semi-quantitative RT-PCR analysis. Graphs represent mean $\pm$ SEM for the ratio between the densitometric values of CFTR and GAPDH bands obtained in RT-PCR experiments. **B** Expression of TNR-CFTR protein in kidney of control, hypo- and hyperthyroid rats according to western blot analysis. Graphs represent mean $\pm$ SEM for the ratio between the densitometric values of CFTR and β -actin bands obtained in western blot experiments. Hypothyroid rats were treated with saline (HYPO) or 1 μ g/100g of BW of thyroxine (Hypo + T₄); for 10 days before they were killed. One group of normal rats received T₄ in the dose of 10 μ g/100g of BW for 10 days (HYPER) * $p < 0.05$ vs. control (n = 5).

Results

T₄ plasma levels

Hypothyroid animals showed nearly undetectable levels of T₄ (0.01 ± 0.00 , n = 5, $p < 0.05$). Treatment of hypothyroid rats with a physiological dose of thyroxine (1 μ g/100g BW/day; 10 days) normalized T₄ levels. On the other hand, animals treated with ten-fold higher doses of T₄ developed hyperthyroidism (6.43 ± 0.95 μ g/dl, n = 6, $p < 0.05$; Table 1).

Chloride channel expression

CFTR. Semi-quantitative RT-PCR showed that CFTR mRNA expression varied between euthyroid, hypo and hypothyroid animals. Densitometric analysis of bands corresponding to CFTR (Fig. 1A), previously standardized by GAPDH mRNA, demonstrated that hypothyroid animals presented a CFTR expression decrease (44%) compared to control levels (n = 6, $p < 0.05$, Fig. 1A). Treatment of hypothyroid rats with physiological dose of thyroxine (1 μ g/100g of BW/day) for 10 days normalized

CFTR mRNA expression (n = 5, $p > 0.05$, Fig. 1A), whereas the treatment of normal rats with a supraphysiological dose of T₄ (10 μ g/100g of BW/day) for 10 days induced an increase of 43% when compared to normal rats (n = 5; $p < 0.05$, Fig. 1A). As shown in Figure 1B, CFTR protein had a similar pattern of expression modulation as compared to that observed on the mRNA level. Lack of TH caused a 30% decrease in CFTR protein expression compared to control animals (n = 5; $p < 0.05$, Fig. 1B). CFTR protein levels were normalized with the physiological dose of T₄ and, importantly, were increased after the supraphysiological dose of thyroxine (250%, n = 5; $p < 0.05$, Fig. 1B).

To examine further whether TH action on CFTR gene expression in the kidney was direct, IRPT cells were used as an isolated system. CFTR mRNA expression showed an increase with the inclusion of T₃ at 10^{-8} M (35%, n = 5; $p > 0.05$) and 10^{-7} M (95%, n = 5, $p < 0.05$, Fig. 1C). Treatment with T₃ at 10^{-9} M did not give even an indication of an effect.

Fig. 3. Modulation of CFTR chloride channel gene promoter region in IRPT cells by T₃. Graph shows luciferase activity in transfected IRPT cells without T₃ (control); or treated with 10⁻⁷ and 10⁻⁸ M T₃. Luciferase activity was normalized by co-transfection with pSV-β-galactosidase plasmid. (n = 4). * p < 0.05 vs. control.

TNR-CFTR

TNR-CFTR mRNA expression analysis showed a pattern very similar to the one observed for CFTR mRNA, but the modulation turned out to be more sensitive. Hypothyroid animals had their renal expression of TNR-CFTR mRNA reduced in 49% (n = 5; p < 0.05, Fig. 2A). In hyperthyroid animals, we observed an increase of 95% in mRNA expression (n = 5; p < 0.05), whereas animals receiving hormone replacement presented TNR-CFTR mRNA levels similar to control (n = 5, p > 0.05, Fig. 2A).

Western blot analysis of TNR-CFTR protein showed modulation similar to its mRNA. Hypothyroid group presented decreased levels of protein expression of 37%, (n = 5; p < 0.05, Fig 2B) whereas the hyperthyroid group increased TNR-CFTR protein expression in 38% (n = 4; p < 0.05, Fig. 2B). Hypo+T₄ had their TNR-CFTR protein levels returned to control levels (Fig. 2B).

CFTR promoter region analysis

Luciferase reporter gene assay suggests that T₃ is capable of modulating CFTR gene promoter region. Cells transfected with the plasmid containing the CFTR promoter region associated to luciferase gene presented enzyme activity, indicating a successful transient transfection. Transfected cells treated with 10⁻⁸M T₃ showed no significant difference from non-treated transfected cells (control), whereas treatment with 10⁻⁷M T₃ presented a significant increase in luciferase activity of 38% (n = 4, p < 0.05, Fig. 3).

Discussion

Renal sodium chloride (NaCl) excretion is regulated by paracrine and endocrine factors, which modulate the activity and/or expression ion transporters [29]. Hormones, such as atrial natriuretic peptide (ANP) and aldosterone, can modulate sodium and chloride renal excretion and, consequently, the amount of water in the urine [22, 30-32]. Although THs generally are not regarded as classical ECFV regulators, specific receptors for T₃ are present in kidney epithelium, suggesting the potential for a direct hormonal action of this hormone [33]. Indeed, the thyroid hormones, thyroxine (T₄) and triiodothyronine (T₃) affect renal morphology as well as the activity of ion transporters via alteration of their function and/or expression along the nephron. Thus, depending on their plasma levels, THs can regulate renal NaCl excretion [34]. In hypothyroidism, both decreases in glomerular filtration rate and disturbances in the urine concentrating capacity are observed [30]. Also, THs stimulate expression of the Na⁺/K⁺ ATPase [11], as well as the activity of the Na⁺/H⁺ exchanger in the proximal tubules [15, 35]. Specifically, THs stimulate, two important sodium transporters in proximal tubules: the type 3 Na⁺/H⁺ exchanger - NHE3 and type 2 Na⁺-phosphate cotransporter - NaPi2 [12-14].

In addition to the profound changes in sodium handling, THs also affect the transport of chloride. Our previous work demonstrated that THs increase the ubiquitously expressed chloride channel, ClC-2, in rat renal proximal straight and convoluted tubules [17]. In the present study, we undertook an analysis of the effect of THs on CFTR, another important chloride channel. CFTR is expressed in different epithelia, where it transports Cl⁻ and also functions as a modulator of other conductances such as Na⁺, K⁺ and Cl⁻ [18, 19]. Its dysfunction causes CF, a genetic disease characterized by impairment of transepithelial ionic transport and hence airway, pancreatic and intestinal disease [20, 21]. ECFV-related hormones, such as vasopressin modulate both CFTR and ClC-2, and these two channels also co-localize in the kidney [9]. Thus, of the many different chloride transporters expressed along the nephron [22, 36, 37], we chose to investigate the effects of THs on the expression of CFTR and its kidney-specific splice variant, TNR-CFTR.

TNR-CFTR is generated by alternative splicing of the CFTR gene. This protein was first described in 1996 by Morales et al [22]. TNR-CFTR comprises the first half of the CFTR molecule, including the first membrane-

spanning domain (MSD1), a nucleotide-binding domain (NBD1) and the R (regulatory) domain [22]. TNR-CFTR is capable of transporting Cl⁻, and its electrophysiological characteristics resemble those of the full-length CFTR channel. Also like full-length CFTR, TNR-CFTR modulates other ionic conductances [22]. To date, very little is known about its physiological role, although its subcellular distribution pattern provides some clues. TNR-CFTR is abundantly expressed in the inner and outer kidney medulla of human and mice, suggesting that this CFTR variant chloride channel participates in renal concentrating mechanisms [22].

The present work demonstrates, for the first time, that renal expression levels of both CFTR and TNR-CFTR transcript and protein are modulated by THs. The decrease observed for expression of both the CFTR mRNA (44%) and TNR-CFTR mRNA (49%) in the hypothyroidism model is comparable to the reduction observed for renal expression of sodium transporters in the same model [38]. The greater sodium retention that is associated with hyperthyroidism is caused by an increase in ion reabsorption that can be explained by the stimulation of nephronal sodium transporter gene expression [17, 39]. We now report concomitant increases in the mRNA encoding the chloride transporters, CFTR and TNR-CFTR mRNA, of 43% and 95%, respectively, in hyperthyroid rats. Moreover, compared to control groups, hyperthyroid animals showed an increase in TNR-CFTR protein expression of 38%, whereas full-length CFTR protein demonstrated a more marked augmentation to 250% of control levels. The modulation by THs of both TNR-CFTR and full-length CFTR expression may underlie the changes in tubular chloride transport observed by others using the same model [38]. These results therefore suggest a physiological role for CFTR and TNR-CFTR in kidney ionic transport. Importantly, this includes not only CFTR-mediated chloride transport but also extends to CFTR's function as a modulator of other conductances, including other

renal chloride channels. Changes on CFTR and TNR-CFTR expression could have far-reaching consequences in the ionic transport dynamic throughout the nephronal segments where these channels normally reside.

It is important to highlight that both mRNA and protein expression of CFTR and TNR-CFTR were restored to control values when hypothyroid animals were subjected to T₄ administration, a fact that points out the importance of THs in the maintenance of the basal expression of these channels. Our own previous studies show that renal expression of another important chloride channel, CIC-2, is modulated by THs [17]. It is tempting to speculate that THs exert tandem regulation of both of these chloride channels.

In hyperthyroid animals, the increase in TNR-CFTR mRNA abundance was more outstanding. This allows us to speculate that the presence and excess of TH might stimulate alternative splicing. This in turn would deviate the processing of CFTR pre-mRNA for the formation of TNR-CFTR mRNA.

Our *in vitro* experiments using immortalized rat proximal tubule cells (IRPTC), suggest a steep dose-dependence of CFTR mRNA expression by THs. These results showed that the TH effect on CFTR mRNA expression occur, at least in part, via its promoter region stimulation [22, 35, 40].

Modulation of mRNA levels occurs generally by post-transcriptional regulation or changes on gene transcription rate. Luciferase activity studies in IRPT cells demonstrated a transcriptional activation profile that mirrors the expression of CFTR mRNA observed in T₃-treated IRPT cells. However, the post-transcriptional regulation effects of THs remain to be evaluated in a future study.

In conclusion, we have demonstrated the modulation of CFTR and TNR-CFTR expression by THs. These findings suggest the importance of these channels in transepithelial transport and, hence, in the renal regulation of extracellular fluid volume.

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