DR-3 (VDRE), this hypothesis is not strongly supported by the results from this study, as PPARγ-RXRα heterodimers bind normally to DNA in the presence of uremic plasma. Another alternative that can not be excluded is an inhibitory effect of the uremic toxin on the surface of TR and VDR DNA binding domain (DBD), disrupting its ability to binding to DNA. Patel *et al.* attributed to the formation of Schiff bases between "reactive aldehydes" and lysine residues of the DBD of the VDR to explain the inhibitory effect of the uremic ultrafiltrate on the binding of VDR to DNA [26]. Nevertheless, in another study, point mutagenesis of different lysine residues in the DBD could not confirm this idea [28]. In addition, we should consider that the uremic toxins can interact with TR and VDR, causing structural conformational changes on these receptors, consequently, impairing heterodimers formation.

We attempted to demonstrate the physiological relevance of these results by examining the effect of uremic toxins on T_3 transcriptional activation. Our results showed that uremic ultrafiltrate collected before hemodialysis inhibited T_3-induced transcriptional activation, confirming the *in vitro* findings. Conversely, in the presence of ultrafiltrate collected after hemodialysis, the transcriptional activation induced by T_3 was similar to the control group treated with ultrafiltrate collected from normal individuals. Therefore, we hypothesize that dialyzable toxins are responsible for the resistance to T_3 action documented in CRF patients.

In summary, uremic toxins circulating in the plasma of CRF patients selectively reduced the binding of TRβ1-RXRα to DNA and impaired the TRβ1 transcriptional activation mediated by T_3. Moreover, hemodialysis partially corrected this inhibitory effect, suggesting the presence of a dialyzable toxin. Since TRβ1 functions as a heterodimer with RXRα, these findings might explain some features of hypothyroidism and thyroid hormone resistance commonly found in CRF patients. Future studies are necessary to identify the toxins and further characterize the mechanisms involved in resistance to T_3 action in CRF patients.

Materials and methods

Patients and Clinical Procedures

Four patients from the chronic dialysis program of Soclimed Dialysis Clinic were enrolled in our study. All patients were men whose age ranged from 19 to 43 years with the mean age being 34 years. They appeared well nourished and clinically and laboratorial euthyroid; none had a history of thyroid disease, thyroid hormone therapy, treatment with amiodarone or clinically detectable goiter. Etiology of their chronic renal failure was as follows: chronic glomerulonephritis (2); hypertension (1); reflux nephropathy (1). Mean plasma urea level was 178 ± 44.8 mg/dL (120 to 233 mg/dL), while that of creatinine was 12.6 ± 2.7 mg/dL (9.9 to 16.3 mg/dL). Patients were on hemodialysis 3 times a week, during 4 hours using a 1.8 m^2 Fresenius® Polysulfone filter. Normal control subjects consisted of three healthy men, age ranging from 23 to 41 years, with the mean age of 32 years. The experimental protocol was approved by the Human Rights in Research Committee of the University of Brasilia and all patients and normal individuals gave their informed consent.

For the *in vitro* DNA binding assay, uremic plasma was collected immediately before and after 4 h of hemodialysis, aliquoted into 20 μL samples and stocked at -20°C. Uremic ultrafiltrate (UF) was also collected pre and post 4 h hemodialysis. Lyophilisation was used to concentrate ultrafiltrate samples. The lyophilisates were re-suspended in bidistillated water to a 10-fold concentrated solution, as effects of the UF were not detectable at lower concentrations (1 fold, 2.5 fold, 5 fold concentrated; not shown). Samples were subsequently desalted by filtration using Centricon 3 filters. Following centrifugation, the pellet was re-suspended in RPMI-1640 medium, (10% newborn bovine serum; 2 mM glutamine; 50 units/mL penicillin; 50 μg/mL streptomycin) and pH corrected to 7. Normal UF was collected from control plasma of normal individuals. The treatment solution was prepared in the same manner as the uremic solution. All experiments were performed with the uremic sample from the same patient that showed the strongest inhibitory effect.

Gel shift binding assay

Gel shift assays were used to evaluate the binding of ^{35}S-labeled TR synthesized in reticulocyte lysate on 600 fmoles of unlabeled DR-4 (5'-AGCT TC AGGTCA CAGG AGGTCA GAG -3') and inverted palindrome – F2 (5'-TTC TGACCC CATTGG AGGTCA GAG -3'); ^{35}S-labeled VDRs to unlabeled DR-3 5'- AGCT TC AGGTCA AGG AGGTCA GAG - 3') and ^{35}S-labeled PPARγ to unlabeled DR-1 (5'-AGCT TC AGGTCA G AGGTCA GAG - 3'). Sensitivity and specificity of this assay have been previously characterized [23]. Briefly, the labeled protein will migrate in the non-denaturating polyacrylamide gel only when bound to DNA. Additionally, gel shift were also performed using unlabeled synthesized TRs and ^{32}P-labeled DR-4.

In vitro receptor synthesis was performed using plasmids encoding hTRβ1 hRXRα, and hPPARγ [38] and hVDR [39] with the TNT-coupled Reticulocyte Lysate System (Promega, Madison, WI) containing a methionine-free aminoacid mixture, and either 20 μM cold methionine or ^{35}S-labeled methionine. DNA plasmid (0.2–2 μg) was added to TNT Quick Master Mix and incubated in 50 μL for 90 min at 30°C. To confirm efficiency of the translation reaction ^{35}S-labeled translated proteins were analyzed by sodium dodecyl sulfate gel electrophoresis (SDS-