perturbations of the hypothalamic-pituitary-thyroid hormone endocrine axis. The peripheral thyroid hormone metabolism is also altered in patients with CRF [5-9].

In uremia various thyroid hormone physiological characteristics are altered. Total and free tyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) levels in the serum are frequently reduced in patients with CRF [10,11]. Reduced T_3 levels might be explained by decreased peripheral tissue conversion of T_4 to T_3 [12]. Most CRF patients, however, are considered to be euthyroid as evidenced by normal thyroid-stimulating hormone (TSH) levels [11,13]. In addition, the prevalence of thyroid diseases, including goiter and hypothyroidism, are also higher in CRF patients than in the general population [10].

Thyroid hormones control numerous aspects of mammalian development and metabolism, of which most of these actions are mediated by specific thyroid hormone receptors (TRs). An important metabolic activity of thyroid hormones is to increase oxygen consumption of target tissues [14,15]. In fact, in experimental renal failure and in uremic patients the expected increase in basal oxygen consumption following the administration of T_3 is not observed, suggesting that CRF is associated with resistance to thyroid hormone action [16-18]. However, it is currently not known whether thyroid hormone receptors play a role in the thyroid dysfunction observed in uremic patients.

TRs are ligand-regulated transcription factors of the nuclear receptor superfamily which includes steroid hormones and vitamin D receptors and also PPARγ [15,19,20]. TRs modulate gene expression by binding specific DNA sequences, known as thyroid response elements (TREs), found in the promoters of TR-regulated genes. TREs are composed of repeats of the consensus half-site AGGTCA in a variety of different orientations, including direct repeats spaced by four nucleotides (DR-4), inverted palindromes (F2) and palindromes [21,22]. In the presence of T_3, TRs preferentially form heterodimers with the retinoid X receptors (RXRs) although unliganded TR binds to DNA as either homodimers or monomers [20,23].

In recent years, it has become apparent that uremic toxins can impair the function of some nuclear receptors, such as the vitamin D receptor (VDR). Previous studies suggest that uremic toxins inhibit the binding of VDR to DNA and can contribute to the vitamin D resistance observed in CRF patients [24-28]. It is, therefore, conceivable that uremia also induces modifications in thyroid hormone receptors and consequently plays a role in the thyroid hormone dysfunction observed in uremic patients.

We investigated the effects of uremia on TRβ1 function by studying the ability of TRs to bind to DNA sequences in the presence or absence of uremic plasma collected from CRF patients. Our results showed that uremic plasma significantly reduced the binding of TR heterodimers (TRβ1-RXRα), but not of homodimers (TRβ1-TRβ1) to DR-4. Furthermore, uremic plasma also inhibited the binding of a VDR heterodimer (VDR-RXR) to DR-3, while the binding of PPARγ to DR-1 remained unaltered. Moreover, hemodialysis (HD) diminished the inhibitory effect of CRF patients' plasma on the binding of both TR and VDR heterodimers to DNA. When human promonocyte cells were incubated with ultrafiltrate collected Pre-HD the transcriptional activation induced by T_3 was inhibited. This inhibition was lost when the cells were treated with ultrafiltrate collected after-HD. Thus, we suggest that dialyzable uremic toxins selectively block the binding of TRβ1-RXRα and VDR-RXR heterodimers to DNA and reduce the transcriptional activities regulated by these receptors. These results indicate that the thyroid hormone dysfunction observed in uremia may be partially explained by T_3 resistance induced by impaired TRβ1 function.

Results

Uremic plasma inhibits the binding of hTRβ1-hRXRα on DR-4

To study the effects of uremic plasma on the ability of TRβ1 to bind to a specific TRE we analyzed the binding of hRXRα-hTRβ1 heterodimers to DR-4. In this assay, the protein-DNA complex was visualized by labeling the TRβ1 with ^{35}S. In the presence of T_3, the addition of increasing amounts (Figure 1; lanes 2–4) of plasma from normal individuals improved the binding of hRXRα-hTRβ1 to DNA. Conversely, TR incubation with uremic plasma (lanes 5–7) collected prior to hemodialysis significantly reduced the binding of heterodimers (RXR-TR) to DR-4. Band densitometry analysis of 5 independent experiments showed that uremic plasma reduced hRXRα-hTRβ1-DR-4 complex formation by 77 ± 15%, compared to plasma from normal subject (not shown). Similar results were observed for the thyroid response element F2 (inverted palindrome) in which uremic plasma also inhibited the RXR-TR binding to DNA (not shown). Pretreatment of hTRβ1 with T_3 failed to improve the ability of the dimer hRXRα-hTRβ1 to bind to DNA.

We used uremic plasma from four different patients to determine whether the observed inhibition of RXRα-TRβ1 binding to DNA (DR-4) was patient specific. Uremic plasma samples from all four patients inhibited RXRα-TRβ1 binding to DR-4 to various degrees (not shown). However, we could not detect any correlation between TR binding impairment and abnormalities in plasma levels of urea, creatinine or thyroid hormone.