**PTH-PTHrP receptor in chronic renal failure**

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Chronic renal failure (CRF) is associated with marked elevation in the concentration of plasma PTH [1] and with a significant increase in the basal levels of cytosolic calcium ([Ca\(^{2+}\)]\(i\)) of many cells including brain synaptosomes [2], pancreatic islets [3], cardiac myocytes [4], thymocytes [5], B and T lymphocytes [8,9], adipocytes [10], hepatocytes [11] and platelets [12]. This change in basal levels of [Ca\(^{2+}\)]\(i\) has been incriminated in the genesis of various components of the uraemic syndrome [13].

The elevation in the basal levels of [Ca\(^{2+}\)]\(i\) in CRF is due to both increased calcium entry into and decreased calcium exit out of the cells (Figure 1). The primary event is a PTH-induced increase in calcium influx into the cells due to activation of calcium channels inhabitable by verapamil [14]. The continued increase in PTH-induced calcium entry into cells is followed by a decrease in ATP content and in the activity of Na\(^+\)-K\(^+\) ATPase, Ca\(^{2+}\) ATPase and Na\(^+\)-Ca\(^{2+}\) exchanger of cells [2,3,4,10,11,15], Figures 1 and 2. These latter events lead to a decrease in calcium efflux out of cells.

**Fig. 1.** A schematic presentation of the sequence of events that lead to the elevation in the basal levels of [Ca\(^{2+}\)]\(i\) in cells exposed to chronic excess of PTH. Note that PTH stimulates many cellular pathways, which may vary from one cell to another, and this results in the activation of calcium channels that would permit influx of calcium into cells. Also note the various cellular events that underlie the reduction in calcium exit out of the cells. Reproduced by permission from [13].

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Fig. 3. Northern blot analysis of poly A+RNA prepared from various tissues of rats hybridized with cDNA probe of PTH-PTHrP receptor. Note that the mRNA of this receptor is present in many tissues. Reproduced by permission from [24].

Fig. 2. The chronology of the relationship between the various cellular parameters during the evolution of CRF over a period of 6 weeks. Note that [Ca^{2+}]i begins to rise as the ATP content and the activity of Ca^{2+} ATPase and Na^+–K^+ ATPase decline. Also note that [Ca^{2+}]i reaches a steady state by the 5th week of CRF despite continued rise in blood levels of PTH. Reproduced by permission from [15].

basal levels of [Ca^{2+}]i of cells. If this action of PTH continues unabated, the levels of [Ca^{2+}]i would reach values that may lead to cell death. However, the basal levels of [Ca^{2+}]i reach a steady state and do not increase further with higher plasma PTH [15], Figure 2. Thus, it appears that the cells develop a defence mechanism(s) to prevent continued increase in their [Ca^{2+}]i. One such potential mechanism is down-regulation of the PTH-PTHrP receptor so that further elevation in plasma PTH in CRF would not be able to cause additional increments in the basal levels of [Ca^{2+}]i of cells.

Indeed, Tian et al. [26] and Ureña et al. [27] showed that the amount of the mRNAs of the PTH-PTHrP receptor in the kidney is downregulated in CRF (Figure 4). This finding is not limited to the kidney but has been observed in the heart [28] (Figure 5) and liver [29] (Figure 6) of CRF rats. Thus, it appears that the downregulation of the mRNA of the PTH-PTHrP receptor in CRF is a generalized phenomenon and occurs in both the traditional and non-traditional cells for PTH action. This decrease in the mRNA of the PTH-PTHrP receptor would most likely be associated with a reduction in receptor synthesis, a decrease in receptor number and consequently a reduced action of PTH on its target cells.

The cloning of the PTH-PTHrP receptor by Abou-Samra et al. [23] permitted the examination of the presence of the PTH-PTHrP receptor in various cells. Both Tian et al. [25] and Ureña et al. [24] demonstrated that the aorta, adrenal gland, urinary bladder, brain, cerebellum, breast, bone, heart, uterus, kidney, liver, lung, skeletal muscle, ovary, placenta, skin, spleen, stomach, and testis contain the mRNA of the PTH-PTHrP receptor. Figure 3 shows the data reported from our laboratory [24]. These observations provide the molecular basis for the widespread actions of the elevated plasma PTH on many cells in CRF and on the role of the hormone in the genesis of the elevation of the basal levels of [Ca^{2+}]i of various cells in CRF.

As CRF advances, plasma PTH continues to increase; this would cause a progressive increase in the basal levels of [Ca^{2+}]i. The acute effects of PTH on the [Ca^{2+}]i of many cells is blocked by the PTH antagonists [Tyr^{34}] bPTH[7–34] NH$_2$ or [Nle$^5$, $^{18}$Tyr$^{34}$] PTH[7–34] NH$_2$ [18–22]. These observations indicate that many cells, besides those of kidney and bone, possess receptors for PTH, a phenomenon that would explain the adverse effects of excess PTH on organs function in uraemia.

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and verapamil by itself has no effect on the mRNA of the receptor. These observations suggest that verapamil blocks a PTH-mediated signal that is responsible, at least in part, for the downregulation of the PTH-

PTHRP receptor in CRF. Furthermore, we found that parathyroidectomy (PTX) of normal rats was not associated with upregulation of the mRNAs of the PTH-PTHRP receptor of kidney, heart or liver [30].

Fig. 4. Northern blot analysis of mRNA of PTH-PTHRP receptor and of β-actin of kidneys from CRF rats (lanes 1–4), CRF-V animals (lanes 5–8) and normal-V rats (lanes 9–12). Mean values and 1SE of the concentration of the mRNA of the receptor relative to that of β-actin, *P < 0.01, vs. CRF-V and normal V; **P < 0.01, vs. normal-V. Reproduced by permission from [26].

Fig. 5. Upper panel: Northern blot analysis of the mRNA of the PTH-PTHRP receptor of the heart of normal rats (lanes 1–4), CRF rats (5–7), normal rats treated with verapamil (lane 8) and CRF rats treated with verapamil (lanes 9–11). Lower panel: Northern blot analysis of mRNA of PTH-PTHRP receptor of the heart from CRF rats (lanes 1–3), CRF-PTX rats (lanes 4–6), normal rats (lanes 1–3), CRF-PTX rats (lanes 4–6), normal rats (lanes 7 and 8), CRF rats treated with verapamil (lanes 9 and 10) and normal rats treated with verapamil (lanes 11–13). Reproduced by permission from [28].
Fig. 6. Northern blot analysis of mRNA of PTH-PTHrP receptor and G3PDH of liver from normal, CRF, CRF-PTX, CRF-VER (CRF-Verapamil) and normal-VER (normal-Verapamil) rats. The numbers provide the mean ±1 SE of the density of the mRNA signal of the receptor relative to that of G3PDH. The values in CRF rats are significantly \( P < 0.05 \) lower than those in normal, CRF-PTX, CRF-VER and normal-VER. The value in CRF-PTX is not different from normal. The value in CRF-VER is lower \( P < 0.05 \) than in normal-VER. Reproduced by permission from [29].

Fig. 7. Northern blot analysis of mRNA of the PTH-PTHrP receptor and of G3PDH from the kidney (upper panel), liver (middle panel) and heart (lower panel) of normal and PTX rats. There were no significant differences between the relative concentration of mRNA of the receptor and that of G3PDH in all three tissues. Reproduced by permission from [30].
receptor increased by 2- and 2.4-fold after 2 and 4 days of PTX, respectively. The reasons for this difference are not evident. Although it is unlikely, one must consider that the response of the Sprague-Dawley or Wistar rats, used by us [30] and Ureña et al. [31] to PTX is different from that of the Hebrew University strain of rats used by Kilav et al. [32]. It is more plausible, however, that the time at which the studies were done after PTX provides an explanation for the different results obtained in these three studies. It is theoretically possible that the mRNA of the PTH-PTHrP receptor of the kidney is upregulated shortly after PTX and returns to normal thereafter, suggesting that the upregulation of the mRNA of this receptor is a transitory phenomenon.

It appears, therefore that an elevation or a reduction in plasma PTH, per se, does not affect the accumulation of the mRNA of the PTH-PTHrP receptor in cells. We proposed that the PTH-mediated increase in basal levels of \([\text{Ca}^{2+}]_i\) is a major signal for the downregulation of the mRNA of PTH-PTHrP receptor in CRF. This proposal is based on the following reasoning: since PTH causes an increase in \([\text{Ca}^{2+}]_i\) of many cells in CRF and since the elevation in \([\text{Ca}^{2+}]_i\) is a major contributor to cell dysfunction in CRF, it

(Figure 7). Also Ureña et al. [31] found the amount of the mRNA of the renal PTH-PTHrP receptor in the kidney of normal PTX rats is normal. It must be mentioned, however, that our observations [30] and those of Ureña et al. [31] on the effect of PTX on PTH-PTHrP receptors of kidney are different from the results obtained by Kilav et al. [32] who found that the concentrations of the mRNA of the PTH-PTHrP receptor increased by 2- and 2.4-fold after 2 and 4 days of PTX, respectively. The reasons for this difference are not evident. Although it is unlikely, one must consider that the response of the Sprague-Dawley or Wistar rats, used by us [30] and Ureña et al. [31] to PTX is different from that of the Hebrew University strain of rats used by Kilav et al. [32]. It is more plausible, however, that the time at which the studies were done after PTX provides an explanation for the different results obtained in these three studies. It is theoretically possible that the mRNA of the PTH-PTHrP receptor of the kidney is upregulated shortly after PTX and returns to normal thereafter, suggesting that the upregulation of the mRNA of this receptor noted by Kilav et al. [32] after PTX is a transitory phenomenon.

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is plausible that the increase in $[\text{Ca}^{2+}]$ provides a negative feedback control mechanism through which the mRNA of the PTH-PTHrP receptor is downregulated, so that the increment in $[\text{Ca}^{2+}]$ of cells does not progress unabated as the blood levels of PTH continue to rise with progression of CRF, Figure 8. Support for this hypothesis is provided by the observation that the treatment of CRF rats with verapamil prevented the increase in $[\text{Ca}^{2+}]$ of many cells and either normalized or significantly increased the amount of the mRNA of PTH-PTHrP receptor in the kidney [26] (Figure 9), heart [28] (Figure 5) and liver [27] (Figure 6) despite marked elevation in plasma PTH. Also, the normalization of the $[\text{Ca}^{2+}]$ of cells of CRF animals by their prior PTX was again associated with significant improvement or normalization of the mRNA of the PTH-PTHrP receptor in these organs. It must be mentioned that Ureña et al. [31] did not find an effect of PTX on the mRNA of PTH-PTHrP receptor of the kidney of CRF animals. These authors did not measure $[\text{Ca}^{2+}]$ of the renal cells of their animals, but their data suggest that other consequences of uraemia may also affect the molecular machinery of the PTH-PTHrP receptor.

Our observations, described above, are consistent with an interaction between the PTH-mediated elevation of $[\text{Ca}^{2+}]$ and the molecular machinery of the PTH-PTHrP receptor and support the notion that such an interaction represents an adaptive and protective phenomenon against continued deleterious effects of PTH on cell function. This beneficial effect, however, is associated with an adverse trade off in that the elevated basal levels of $[\text{Ca}^{2+}]$ in CRF cause the downregulation of the mRNA of the hepatic receptors of vasopressin (V1a) and angiotensin II (AT1) [29] as well as of other proteins such as hepatic lipase [33] (Figures 10–12). It is possible that the mRNA of other hormone receptors and various proteins are similarly affected by the elevated basal levels of $[\text{Ca}^{2+}]$ in CRF. Such a phenomenon may provide an explanation for the resistance to the action of many hormones and to the multiple derangements in organs function in CRF.

It is of interest that an adverse effect of elevated basal levels of $[\text{Ca}^{2+}]$ on the molecular machinery of cells has been also observed in phosphate depletion where renal function is normal and blood levels of PTH are very low. Marcinkowski et al. [34] found that in phosphate depletion where basal levels of $[\text{Ca}^{2+}]$ of the renal proximal tubular cells are elevated, the mRNA of the PTH-PTHrP receptor of the kidney is downregulated. The normalization of the $[\text{Ca}^{2+}]$ of the renal cells resulted in reversal of the downregulation of the mRNA of this receptor.

The mechanisms through which an elevation in the basal levels of $[\text{Ca}^{2+}]$ affects the amount of mRNA are not know. The concentration of mRNA in cells is
Northern blot analysis of the mRNA of the AT1 receptor and G3PDH of liver of normal, CRF, CRF-PTX, CRF-V and normal-V rats. Each lane contains 4 μg of poly A+ RNA from different animals. The numbers provide the mean ± 1 SE of the density of the mRNA signal of the receptor relative to that of G3PDH. The values in CRF are significantly (P<0.01) lower than in normal or CRF-PTX rats. The latter value is significantly (P<0.01) lower than in normal. The values in CRF-V rats are not different from those in normal and normal-V animals. Reproduced by permission from [29].

Northern blot analysis of the mRNA of hepatic lipase and G3PDH of liver from normal CRF, CRF-V, CRF-PTX, and normal-V rats. The numbers provide the mean ± 1 SE of the density of the mRNA signal of hepatic lipase relative to that of G3PDH. The values in CRF are significantly (P<0.01) lower than those in the other four groups of animals. Reproduced by permission from [33].

determined by the balance between its synthesis and degradation. Thus, the increase in [Ca^{2+}]i may impair the transcription or processing of the mRNA and/or increase turnover of the mRNA. The data of Young and Tashjian [35] provided evidence that an increase in [Ca^{2+}]i adversely affected the rate of gene transcription of TRH receptor in the GH4C1 cells. Others have found that CRF is associated with increased degradation of albumin mRNA in liver in CRF [36,37]. Thus, either one or both mechanisms may be at work to explain the reduction in the concentration of the mRNA of PTH-PTHrP, AT1 and V1a receptors and of hepatic lipase in CRF.

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References


