Calcium sensitivity of the parathyroid in renal failure: another look with new methodology

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Abnormal calcium sensitivity in uraemia?

The parathyroid’s sensitivity to changes in ionized calcium has been a matter of controversy for more than two decades. In 1978 Brown introduced the concept of a four-parameter sigmoidal model to characterize the calcium dependency of parathyroid hormone (PTH) release from parathyroid cells in vitro. He demonstrated an increased calcium ‘set-point’, i.e. a decreased calcium sensitivity of cells derived from patients with primary and uraemic hyperparathyroidism [1]. Several clinical studies adapting the in-vitro model to the clinical setting are in line with a reduced sensing of the extracellular calcium level in patients with parathyroid adenoma [2,3]. The reduced expression of the calcium receptor on the parathyroid cell surface of patients with primary and uraemic hyperparathyroidism is also compatible with the notion of...
a calcium sensing deficiency [4]. On the other hand, many investigators failed to demonstrate a rightward shift of the calcium set-point of uraemic patients, and the role of vitamin D in the regulation of calcium sensitivity is still unclear. Calcitriol administration has been reported to lower [5], increase [6] or have no effect [7] on the calcium set-point. More confusion has arisen from the introduction of modifications of the mathematical model originally used by Brown, where the calcium set-point had been defined as the serum calcium concentration at mid-range between the maximal and minimal PTH concentration. In follow-up studies the set-point was also defined as the calcium concentration at which maximal PTH is reduced by 50% [8], or as the concentration required to suppress basal PTH levels by 50% [6]. The validity of these calculations has been discussed extensively, however a direct comparison of in-vivo and ex-vivo data has not been made.

Moreover, recent studies demonstrated that the calcium set-point is not an intrinsically stable feature, but depends on the basal calcium concentration: an increased basal ionized calcium increases the calcium set-point independently of the set-point definition used [9]. Consequently, doubts have been raised concerning the physiological and clinical relevance of the concept [10].

A new methodological approach

Further, hitherto undisputed methodological aspects are the short half-life of intact PTH, which is only 2–3 min in healthy humans, and the pulsatile mode of endogenous PTH secretion [11]. Rapid fluctuations of plasma PTH occur physiologically and may remain undetected when blood is sampled at low frequency. Moreover, the parathyroid secretory response depends not only on the absolute change, but also on the rate of change in ionized calcium: the faster calcium levels decline the more PTH is released [12,13]. Using a high-frequency (1 min) sampling protocol during acute hypo- and hypercalcaemia induced by the citrate–calcium clamp technique, we were able to demonstrate that the relationship between ionized calcium and PTH is not simply sigmoidal [11] (Fig. 1). In fact PTH secretion is maximally stimulated at a time when ionized calcium levels start to fall and steady-state hypocalcaemia is not yet achieved. This peculiar biphasic behaviour, which has escaped detection in previous studies, using a slower lowering of ionized calcium and less frequent blood sampling, is explained by a short-lasting volley of high-frequency and high-amplitude PTH pulses, settling into a more regular continuous secretion pattern on a lower, although still elevated, level at the time of maximal hypocalcaemia (Fig. 2).

Several further confounding factors have to be taken into account when comparing the secretory behaviour of the parathyroids in response to calcium changes in normal controls and patients with uraemic hyperparathyroidism. Whilst administering equal doses of citrate in a clamp infusion study, we observed a more rapid decrease in ionized calcium in uraemic patients than in healthy controls. A possible mechanism for this difference is suggested by the observed relationship between the rate of calcium change and serum albumin [14]. As albumin serves as an immediate buffer for blood ionized calcium, diminished serum albumin concentrations in uraemic patients may compromise their ability to compensate for acute changes in ionized calcium. In addition, the uraemic state is associated

Fig. 1. Plasma PTH concentrations versus serum ionized calcium levels in seven healthy adults during hypocalcaemic clamp investigations using a 1-min sampling paradigm [11]. There is no sigmoidal relationship but a brisk initial rise in PTH plasma levels, subsequently decreasing to a lower but still elevated steady state despite an ongoing decline in ionized calcium.

Fig. 2. Instantaneous PTH secretion profiles in a healthy adult as calculated by deconvolution analysis during hypocalcaemic (experiment I) and hypercalcaemic clamp studies (experiment II) performed 1 week apart. Induction of hypocalcaemia induced an initial volley of pulsatile secretory bursts, followed by a proportionate increase in the pulsatile and tonic secretion components during steady-state hypocalcaemia. Hypercalcaemia elicited a sharp, proportionate decline of pulsatile and tonic PTH secretion rates.
with skeletal demineralization and reduced sensitivity to the calcaemic action of PTH \cite{15}, which may further limit the patients’ capacity to compensate for acute alterations of ionized calcium. The more rapid rate of change in ionized calcium in uraemic patients introduces a systematic bias by providing a more powerful stimulating signal for PTH release than in healthy subjects.

A novel approach to characterize inherent functional properties of an endocrine gland delivering pulsatile hormone signals is to look at the true secretory kinetics and not merely at the hormone plasma levels. A major breakthrough in the study of hormone signalling was the advent of the multiparameter deconvolution technique, a sophisticated mathematical algorithm that permits the calculation of instantaneous glandular secretion rates underlying a plasma concentration pattern by separating the secretory processes from the continuously acting elimination of hormone from the bloodstream (Fig. 3) \cite{16}. The hormone secretion rate can be further differentiated into the pulsatile, e.g. episodic, secretory events and a basal, non-pulsatile (tonic) secretion component. Further analysis of the frequency, duration, amplitude, mass and orderliness of the pulsatile events gives a detailed insight into the secretory behaviour of endocrine glands.

The normal secretory pattern of PTH

In the first application of this methodology to plasma PTH concentration profiles in healthy adults, we observed a pulsatile secretion mode consisting of seven pulses per hour, accounting for around 30% of total PTH secretion \cite{11}. The remaining 70% were attributable to continuous (tonic) PTH release. In patients with uraemic hyperparathyroidism, total PTH secretion rate was increased by 8-fold, resulting from a proportionate increase in pulsatile and tonic secretion rates \cite{14}. The prolonged PTH half-life in uraemic patients accounted only for a 2–3-fold increase in basal plasma PTH levels.

The PTH secretory pattern in uraemia

Modulation of serum ionized calcium by infusion of sodium citrate elicited an immediate, frequency- and amplitude-mediated, selective increase in the pulsatile secretion component, that was diminished by more than 60% in uraemic patients vs controls \cite{14}. With the more rapid decline in ionized calcium levels in the patients, one can speculate that the observed differences might have been even more pronounced if an equally strong hypocalcaemic stimulus had been provided in patients and controls. Interestingly, we observed that the frequency of the PTH bursts, while elevated at baseline, was less up-regulated in the patients as compared to controls.

The complementary experiment (the induction of hypercalcaemia) suppressed total PTH secretion in patients and controls, however, the relative change was again much weaker in the patient group. PTH pulse frequency remained unchanged in the patients in contrast to a 30% decrease in controls \cite{14}.

The reduced capability of the uraemic parathyroid glands to adapt to changes in ionized calcium by modulation of pulse frequency and pulse amplitude was even more pronounced in patients on haemodialysis compared to subjects with pre-terminal renal failure. These profound alterations provide in-vivo evidence for a reduced sensitivity of the parathyroid glands to ionized calcium, and may represent a functional correlate to the observed reduction in calcium receptor density of the parathyroid cell surface in uraemia \cite{4}.

Issues in interpretation

The remarkable advances in the description of minute-to-minute PTH secretion achieved by the deconvolution approach adds a new level of complexity to the regulation of parathyroid function, and introduces new questions to be addressed by further research. First of all, what is the morphological substrate of the dual,
e.g. pulsatile and tonic mode of PTH release? Do two types of secretory granules, one constitutive and one responsive to calcium signals coexist within the parathyroidal cells? Is there differential recruitment of specific cell subpopulations in response to endocrine, neuronal and metabolic signals? Secondly, what are the intracellular signalling pathways linking the binding of calcium to its membrane receptor directly to the exocytosis of preformed PTH granules, permitting an instantaneous secretory response to minute changes in ambient calcium concentrations? Thirdly, what mechanisms underlie the parathyroid quadruplets ability to secrete PTH in a synchronous, pulsatile fashion? Is neuronal input a prerequisite for PTH pulsatility? Alternatively, are PTH pulses part of a spontaneous non-linear feedback system including reciprocal oscillations of ionized calcium? The study of patients with denervated single parathyroid glands after parathyroidectomy and autotransplantation should be useful to differentiate these possibilities. Finally, what is the biological function of the endogenous oscillations of plasma PTH? Do target tissues respond differently to tonic, pulsatile and combined PTH signals? Recent evidence in animals and humans suggests that bone metabolism is indeed affected by the temporal pattern of PTH administration [17,18]. Cell studies suggest that different second messenger pathways may be involved depending on the duration of exposure to PTH [19]. Extensive further in-vitro research will be required to reveal whether specific information is transmitted to the target cells via modulations of the temporal pattern of plasma PTH concentrations. The full understanding of the mechanism and biological relevance of PTH pulsatility may eventually lead to new therapeutic strategies in the treatment of various disorders of PTH action or secretion.

**References**


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