

A LC-MS/MS method for the diagnostic measurement of cAMP in plasma and urine

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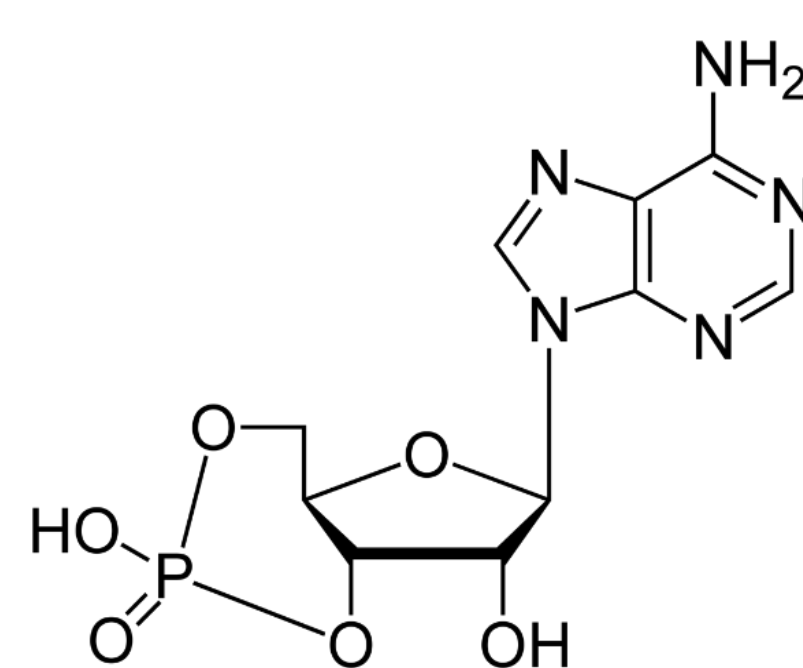
Introduction

Parathyroid hormone (PTH) plays a key role in calcium and phosphate homeostasis. Upon binding to its receptor, it can signal via a second messenger, the cyclic adenosine 3, 5' monophosphate (cAMP). Plasma and urinary cAMP concentrations are used as a diagnostic marker for pseudohypoparathyroidism (PHP).

Patients may be asymptomatic or present with skeletal phenotype (Albright's osteodystrophy) and often short stature. Subcutaneous calcification has been reported in the neonatal period¹. PHP is associated primarily with resistance to the parathyroid hormone². Patients present with low serum calcium and high phosphate, but the parathyroid hormone concentration is high. The pathogenesis of PHP has been linked to dysfunctional G Proteins (in particular, Gs alpha subunit)³. In PHP patients, cAMP fails to increase in response to PTH, including the bioactive form PTH(1-34), administration (Ellsworth-Howard Test).



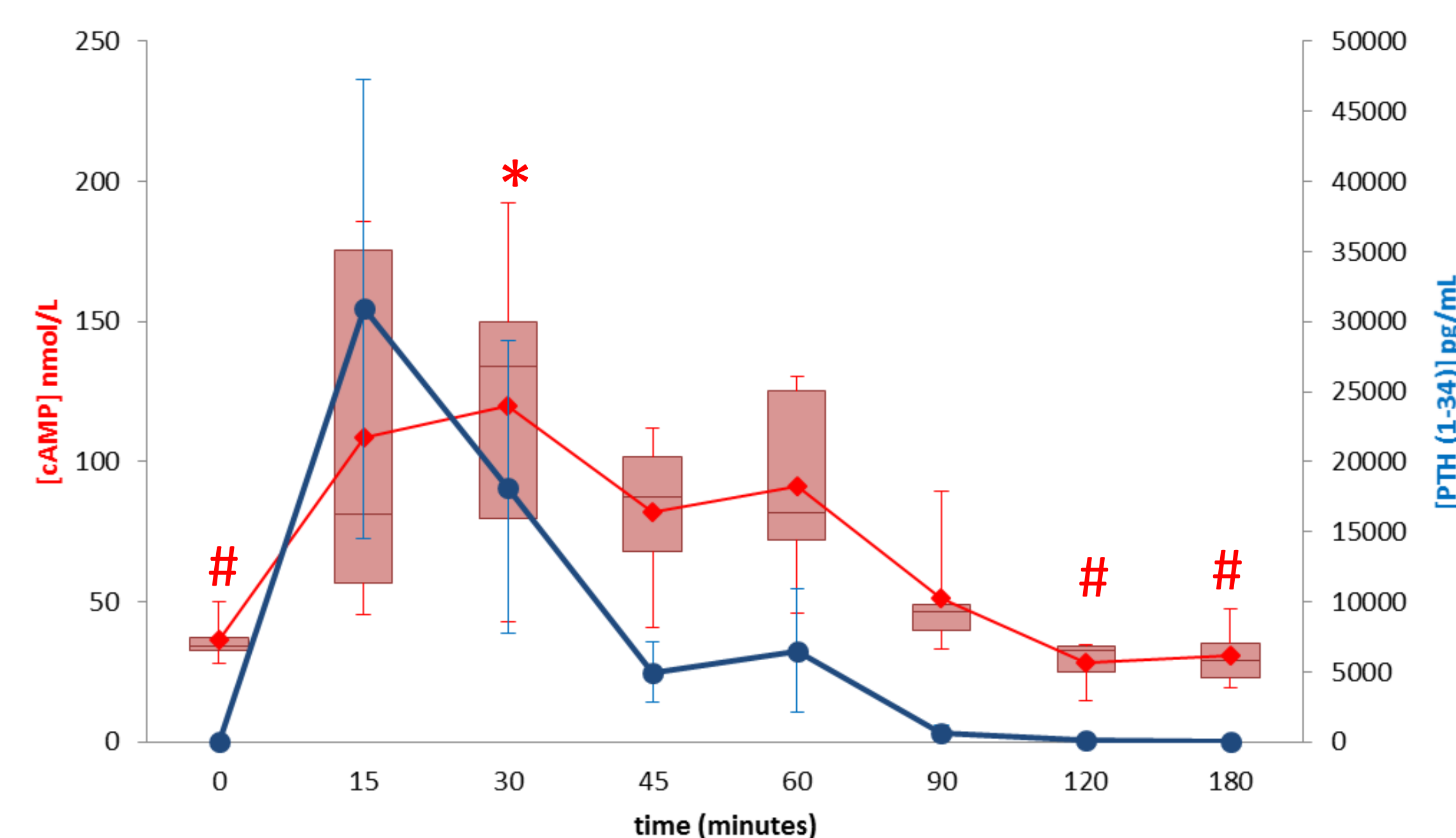
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Chemical structure of 3',5'- cAMP

Rat plasma cAMP pharmacokinetic profile

In all 5 rats, plasma PTH and cAMP increased significantly and rapidly within 15 min of dosing, reaching peak values between 15 and 30 min. PTH concentrations increased significantly by up to 6770-fold, although response to PTH is highly variable between animals. Mean plasma cAMP typically tripled, from 36.5 ± 3.7 nmol/L at baseline to a peak of 119.7 ± 26.3 nmol/L. Increase was significant at 30 min and returned to normal 120 min after ingestion of 1000mg/kg of oral PTH (1-34).



Graph showing the plasma cAMP (red) response to oral PTH (1-34) (blue) in Sprague-Dawley rats.

Objectives

- ❖ Develop and validate a high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of cAMP in plasma and urine samples.
- ❖ Investigate assay performance in pharmacokinetic studies investigating the response to an oral dose of PTH (1-34) in rats and humans.
- ❖ Test urinary samples from a patient with suspected PHP.

Methods

❖ LC-MS/MS method

- Purified cAMP (calibrators) was purchased from Sigma-Aldrich and $^{13}\text{C}_5$ -cAMP internal standard from Toronto Research Chemicals (Toronto, Canada). Analytes were extracted from EDTA plasma using a weak anion exchange solid phase extraction (MOD-SPE-CAMP-03-50, Chromatography Direct Ltd., Runcorn, UK); urine was injected without extraction. Chromatography was performed in positive electrospray ionisation mode, using a pentafluorophenyl column (MOD-LC-CAMP, Chromatography Direct Ltd) with a 10 min 2% formic acid water:acetonitrile gradient.

Transitions in the multiple reaction monitoring mode were m/z 330/136 for cAMP and 335/136 for $^{13}\text{C}_5$ -cAMP. cAMP was eluted within 3.0 min. Over concentrations ranging 4.6 (lower limit of quantification) to 293.5 nmol/L, the calibration curve was linear (mean curve fits of >0.95, 5 repeats) and intra- and inter-assay precisions were <12% and <8%, respectively. Spiked recovery was $98 \pm 5\%$:

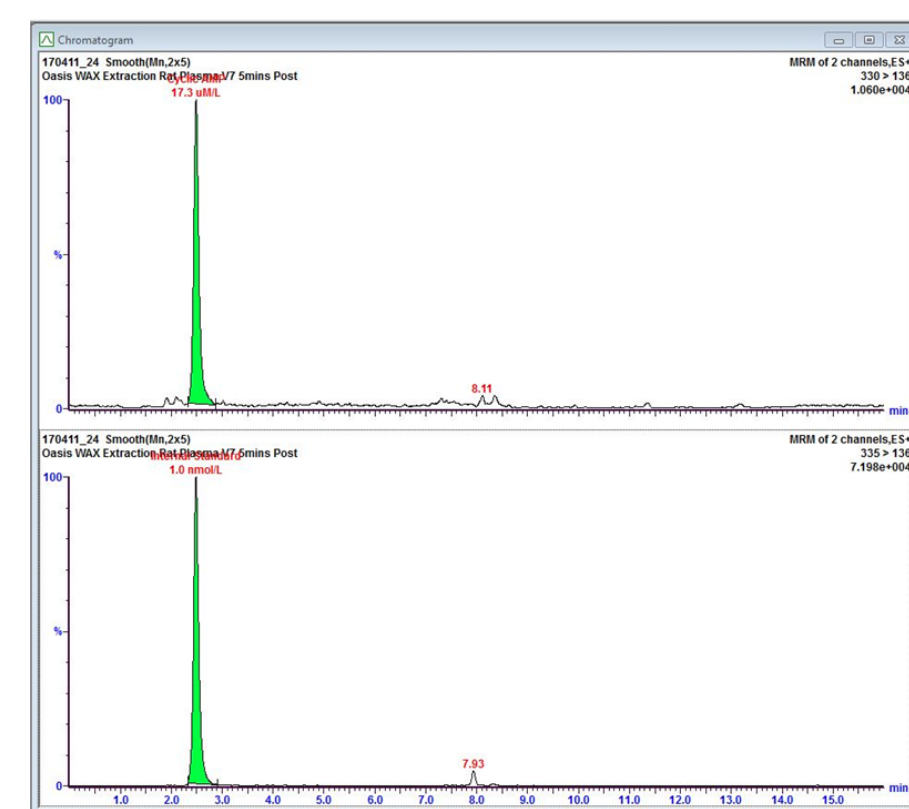
- PTH(1-34) was measured using the IDS-iSYS automated immunoassay platform (IDS Ltd., Boldon, UK).

❖ Application:

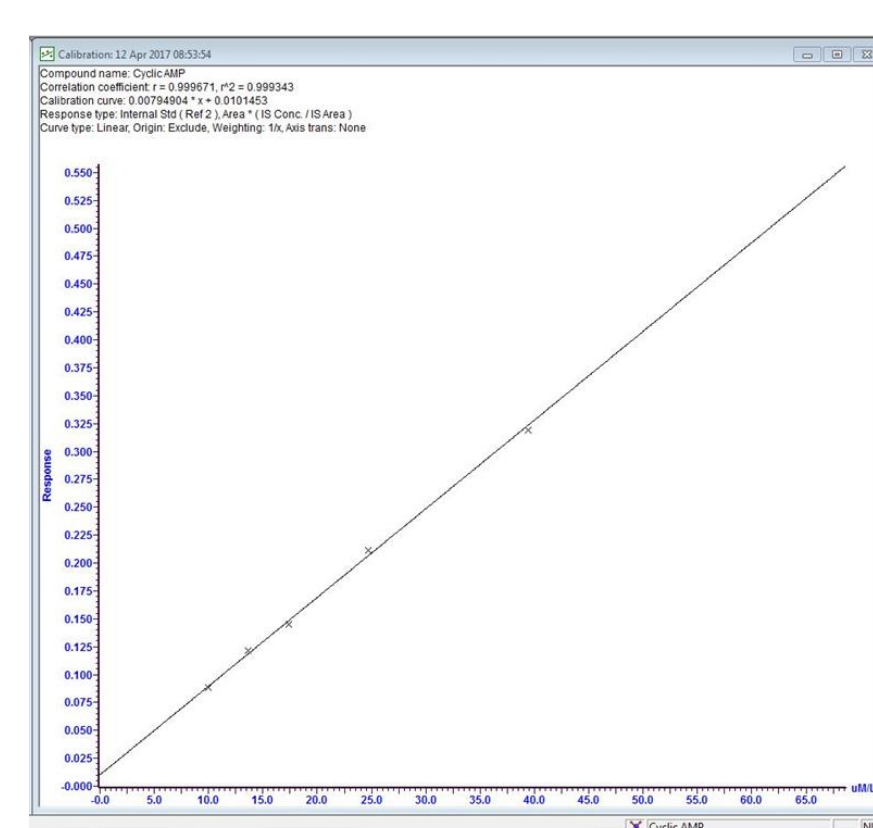
- cAMP was analysed in 5 EDTA samples obtained from Sprague-Dawley (SD) rats. A single oral dose of 1000 mg/kg PTH (1-34) (or placebo) was administered after an overnight fast. Blood samples were obtained at baseline, prior to dosing and every 15 min for 2h and then hourly for another 3h after dosing.
- A preliminary pharmacokinetic study was also performed in human (n=8) using a 1mg oral administration of PTH(1-34).
- Urinary cAMP was also analysed in a patient with suspected PHP after PTH stimulation.

❖ Statistics

- Concentrations were compared using one-way ANOVA.
- SPSS for windows version 22.0.0.1 was used and results were considered statistically significant for $p < 0.05$. * $p < 0.05$ vs 0 min and # $p < 0.05$ vs 30 min.



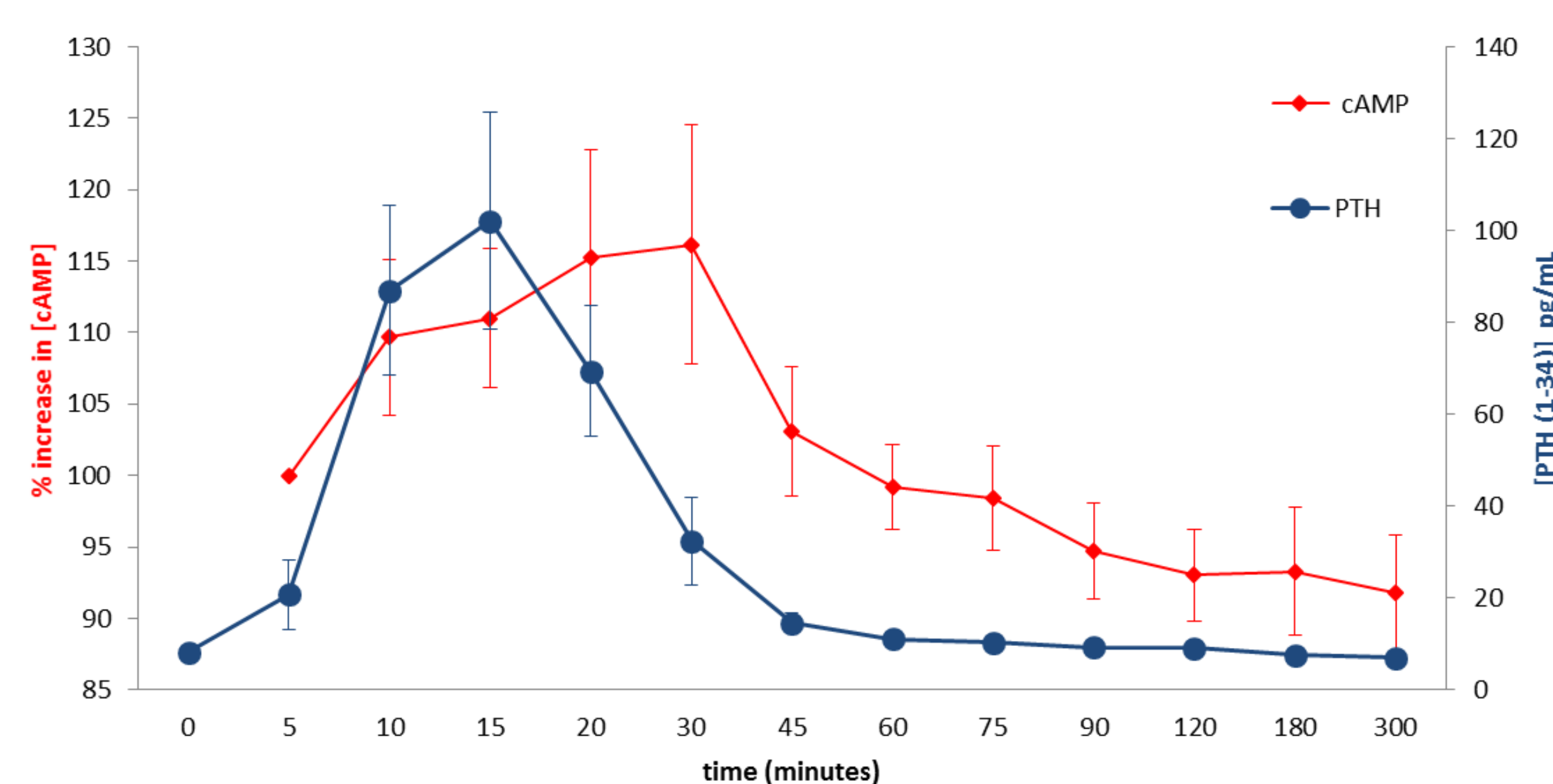
Typical cAMP peak results from rat EDTA plasma



Typical cAMP standard curve

Human plasma cAMP pharmacokinetic profile

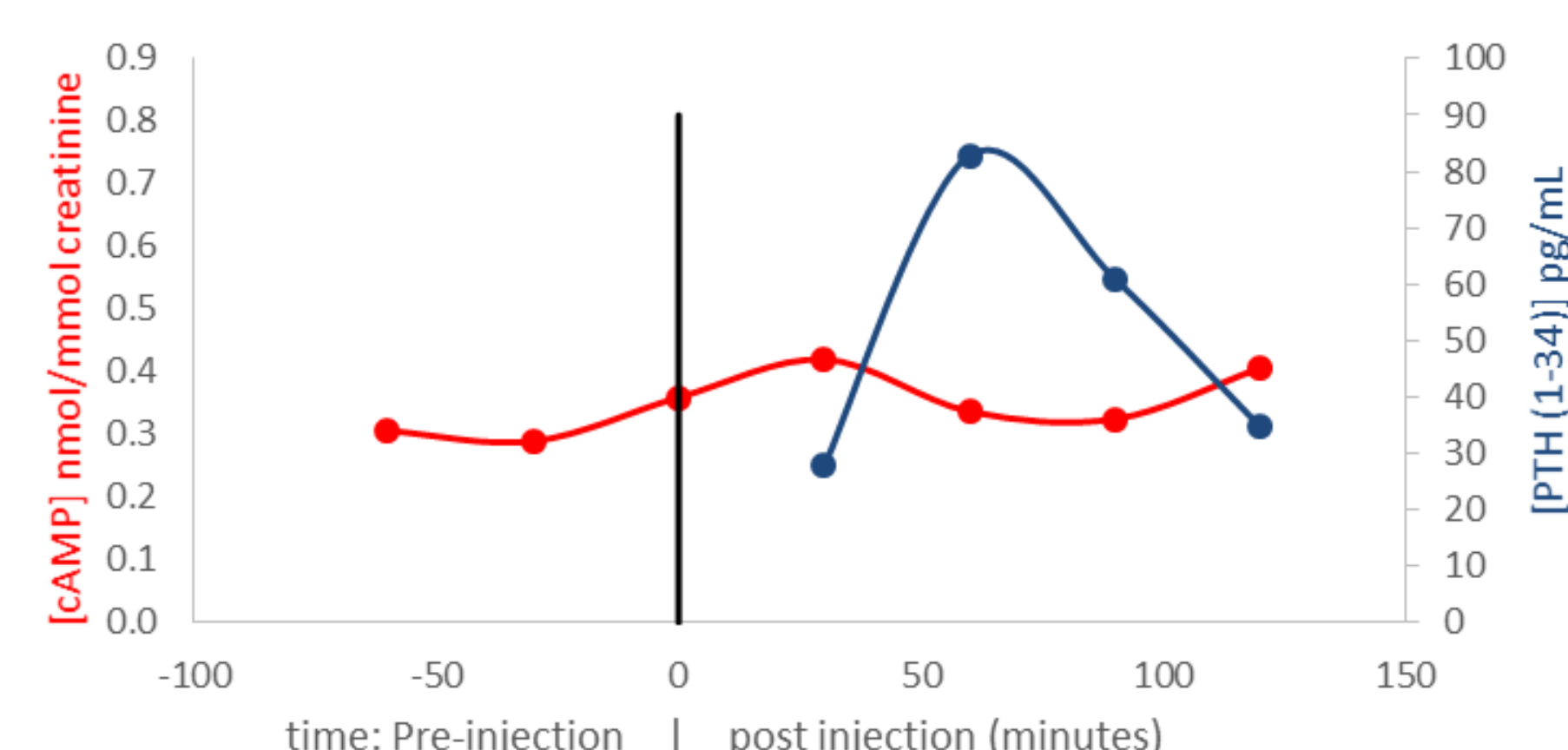
Eight human samples were analysed after oral administration of PTH(1-34) (1mg). Mean baseline levels of PTH were 8pg/mL (range 8-10pg/mL). PTH Tmax was 20-30 min and Cmax was 102 ± 24 pg/mL. The increase in plasma PTH was accompanied by a modest increase in plasma cAMP (up to 16% by 30min relative to baseline), variable between individuals.



Increase in plasma cAMP in human (n=8) in response to oral PTH (1-34)

cAMP in pseudohypoparathyroidism

PTH was measured every 30 min, following a standard 20µg sc injection of teriparatide (Forsteo). Plasma concentration of PTH increased from 28 to 83 pg/mL. Urinary cAMP concentrations were measured by LCMS every 30 min, from 1hr before to 2hrs after injection and were standardized against creatinine concentration. No significant changes in urine cAMP clearance was observed following the injection of teriparatide.



Plasma PTH concentration and urine cAMP excretion after subcutaneous injection of Forsteo in a patient with suspected PHP.

Conclusions

We developed a robust and selective method for quantifying cAMP in both urine and plasma.

- ❖ We also showed the utility in determining cAMP in biological systems. In SD rats, we were able to demonstrate the pharmacokinetic profile of PTH and cAMP after an oral administration of PTH(1-34). The cyclic AMP response to an oral administration of PTH (1-34) at 1mg (PTH Tmax = 20-30min) was modest in the human and increased by up to 16 % 30min after ingestion.
- ❖ The diagnosis of PHP was confirmed in a patient as shown by the lack of change in the clearance ratio urine cAMP : creatinine after an sc administration of Forsteo which induced a 3-fold increase in plasma PTH (1-34).

References:

1- Adachi M *et al.*, (2009). "Ectopic calcification as discernible manifestation in neonates with pseudohypoparathyroidism type 1a" Int J Endocrinol. 2009;2009:931057. 2- T.Bastepe M (2008). "The GNAS locus and pseudohypoparathyroidism". Adv. Exp. Med. Biol. 626: 27-40. 3-Spiegel AM (1999). "Hormone resistance caused by mutations in G proteins and G protein-coupled receptors". J Pediatr Endocrinol Metab. 1999 Apr;12 Suppl 1:303-9.