Plasma Cyclic Nucleotide Determination in the Investigation of Hypocalcemia

ERIC MALLET, JEAN-PIERRE BASUYAU, PHILIPPE BRUNELLE, AND CLAUDE H. DE MENIBUS

Unité d'explorations fonctionnelle, Pavillon de Pédiatrie Hôpital Charles Nicolle, Centre Hospitalo-Universitaire de Rouen (E.M., C.H.D.M.) and Laboratoire de Biochimie, Centre Henri Becquerel, 76038 Rouen Cédex (France)
(J.P.B., P.B.)

Summary

Changes in plasma adenosine 3'5' (cAMP) and guanosine (cGMP) monophosphate, measured by specific radioimmunoassay, after 150 USP/M2 of bovine parathyroid hormone (bPTH) iv administered were studied in children with pseudohypoparathyroidism, with idiopathic hypoparathyroidism, and in normal controls. Basal concentrations of plasma cAMP ($17 \text{ nmole/l} \pm 1, 6 \text{ SEM}$) and cGMP ($8,7 \text{ nmole/l} \pm 1, 3 \text{ SEM}$) were the same in all studied children. Plasma cAMP in normal and idiopathic hypoparathyroid children significantly (30-fold, $P < 0.001$) and con-

stantly rose with a peak value ($537 \text{ nmole/l} \pm 210 \text{ SEM}$) observed 5-10 min after bPTH injection. By contrast, no significant change in plasma cAMP occurred in children with pseudohypoparathyroidism. The data confirmed further the inability of pseudohypoparathyroid children to increase cAMP after exogenous PTH, while the cGMP response did not appear to be significantly modified. It is suggested that an injection of 150 USP/m2 bPTH with plasma samples for cAMP assay taken before and 10 min after hormone administration represents a simplified assessment of Ellsworth-Howard's test.

The syndrome of pseudohypoparathyroidism clinically characterized by short stature, mental retardation, rounded face and short stubby hands, and an apparent target organ resistance to parathyroid hormone, was first described by ALBRIGHT¹. CHASE subsequently demonstrated that patients with pseudohypoparathyroidism had defective urinary cAMP excretion after receiving bovine parathyroid hormone (BPTH)². The role of cAMP as an intracellular mediator in the action of PTH has been well established, while the physiological role of cGMP is still unclear.

These two nucleotides seem to have antagonistic effects in a number of biological systems⁴. PTH has been shown to increase urinary cGMP and cAMP excretion, but few studies have considered the phenomenon for cyclic nucleotide plasma exit from specific tissues^{5,9}.

We studied the effect of B.PTH Injection on plasma cAMP and cGMP in three groups of children: 1) pseudohypoparathyroid patients, 2) hypoparathyroid patients and 3) normal controls, in an effort to observe differences among them and to make practical recommendations for the investigation of hypocalcemia. Preliminary results have been previously reported⁶.

PATIENTS AND METHODS

Six healthy, seven hypoparathyroid and two pseudohypoparathyroid children were studied (table 1) informed parental consent was obtained. Plasma cAMP determination was performed by radiocompetition using a binding protein purified from bovine muscle⁷. Radioassay of cAMP by radioimmunoassay (AMERSHAM) kits. Plasma IPTH determination was performed with a radioimmunoassay previously described⁸ using BW 211/32 antiserum (WELCOM) and the standard B.PTH supplied by the Medical Research Council (Hampstead, ENGLAND). 150 USP units/m² of body surface area of BPTH⁹ were administered intravenously. 1 ml EDTA¹⁰ blood samples were drawn immediately before, 5, 10, 20, 30, 40, 60 and 90 minutes after BPTH injection. Statistical analysis was performed with the MANN-WHITNEY test.

RESULTS

Basal concentrations of plasma cAMP ($17 \text{ n mol/l} \pm 1.6 \text{ SEM}$) and cGMP ($8.7 \text{ n mol/l} \pm 1.3 \text{ SEM}$) were similar in all three groups. After BPTH injection, plasma cAMP increased two to three fold, the peak occurring 10 to 30 minutes after injection without any significant difference among the three groups. In contrast in normal and idiopathic hypoparathyroid children, plasma cAMP rose significantly (30 fold, $p < 0.001$) and constantly, with a peak value at 5 to 10 minutes after BPTH injection (controls: $537 \text{ n mol/l} \pm 210 \text{ SEM}$ and hypoparathyroid children: $490 \text{ n mol/l} \pm 150 \text{ SEM}$). Conversely, children with pseudohypoparathyroidism showed no significant change in plasma cAMP (case 1: 40 n mol/l , case 2: 36 n mol/l at 60 minutes) (fig. 1).

DISCUSSION AND CONCLUSIONS

These results further confirm the inability of pseudohypoparathyroid children to increase cAMP following administration of exogenous PTH, while retaining an apparently unmodified cGMP response. The validity of this method as a PTH renal responsiveness test is relative to the fact that the marked effect of PTH upon cAMP is chiefly due to the increment from the kidney. Indeed KAMINSKY did not observe any modification of plasma cAMP in anephric patients⁵.

As suggested by TOMLINSON⁹, our observations indicate that cAMP assays with 1 ml blood samples taken before and 10 minutes after BPTH injection are a simple substitute for the classical ELLSWORTH-HOWARD test¹⁰ precluding the necessity of collecting urine samples.

§ Eli and Lilly Co

§§ Vacutainer (Beckton-Dickinson)

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 - 11- Requests for reprints should be addressed to E. MALLET, Md Université d'explorations fonctionnelles - Pavillon de Pédiatrie - Hôpital Charles Nicolle - Centre Hospitalo-Universitaire de ROUEN.
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Abbreviations used :

IPTH immunoreactive parathyroid hormone
BPTH bovine parathyroid hormone
cAMP adenosine 3'5' monophosphate
cGMP guanosine 3'5' monophosphate
EDTA éthylène diamine tétraacétate

	NORMAL CONTROLS	IDIOPATHIC HYPOPARATHYROIDISM	PSEUDO- HYPOPARATHYROIDISM
N	6	7	2
Plasma Ca mg/dl range	9.2 - 10.2	4.8 - 6.1	5.3 and 8.6
" " P mg/dl " "	4.1 - 5.5	6.5 - 10	5.3 " 6.9
" " IPTH ng/ml	0.8 - 1.4	undetectable - 0.9	2.3 " 3.2

Table I Children studied

IPTH normal values $1.2 \pm 0.3 \text{ ng/ng}$ (mean $\pm 1 \text{ SD}$)

Figure 1 : Plasma cAMP and cGMP responsiveness to Parathyroid hormone in children with hypoparathyroidism and pseudo hypoparathyroidism.