

Sodium Bicarbonate Infusion Test: A New Method for Evaluating Parathyroid Function

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Abstract. We have developed a new test for estimating the secretory capacity of parathyroid hormone (PTH) from the parathyroid gland. Sodium bicarbonate solution [8.4% (w/v); 35 ml/m² body surface area] was infused for 2 min, and blood samples for the determination of plasma ionized calcium, plasma PTH (intact, midregion, carboxy-terminus) and related parameters were serially obtained. In 8 healthy volunteers, the mean (\pm SE) plasma ionized calcium fell promptly and significantly (from 1.21 ± 0.01 to 1.11 ± 0.01 mmol/L) after the sodium bicarbonate infusion. The mean (\pm SE) plasma intact PTH increased promptly and significantly, by more than four fold (42.3 ± 4.2 to 182.4 ± 34.7 pg/ml), and then gradually returned to basal levels. In patients with partial hypoparathyroidism who have detectable basal plasma levels of PTH, the absolute increment in PTH levels was much less, and in the plasma obtained from patients with complete hypoparathyroidism, absolutely no response was observed. Plasma obtained from patients diagnosed with primary hyperparathyroidism (parathyroid adenoma or hyperplasia) has high basal PTH levels. The response to the sodium bicarbonate infusion in these patients was markedly blunted (less than a two-fold increase in all cases examined). No significant adverse effects were observed during the procedure. Therefore, the sodium bicarbonate infusion test is a simple and sensitive method to stimulate PTH release, and is clinically useful for evaluating parathyroid gland function.

Key words: Parathyroid hormone, Parathyroid gland, Hyperparathyroidism, Hypoparathyroidism, Calcium

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A variety of provocative tests using regulatory factors have been developed and are being used clinically for evaluating endocrine functions. For example, glucose loading is applied for the diagnosis of diabetes mellitus, and hypothalamic releasing hormones such as corticotropin-releasing hormone or growth hormone-releasing hormone are used for evaluating anterior pituitary function. These tests using physiological

stimuli are quite valuable not only for the diagnosis of a disease, but also in characterizing the function of endocrine organs in both the healthy and diseased states.

Regarding the parathyroid gland, however, few provocation tests have been used and/or examined clinically. A physiological regulator in determining the presence of parathyroid hormone (PTH) is extracellular ionized calcium, a decrease of which, through calcium-sensing receptors, stimulates the release of PTH and vice versa, thereby showing an inverse sigmoid curve-like relationship [1, 2]. Ethylenediamine-tetraacetic acid (EDTA) infusion has previously been developed for stimulating PTH release by lowering ionized calcium [3, 4], although it is not widely used

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now because of its' weak stimulatory potency and adverse cardiovascular effects.

In the present study, we propose a new test for evaluating parathyroid gland function using sodium bicarbonate. The principle is that an acute infusion of a relatively small amount of sodium bicarbonate solution causes a transient rise in blood pH followed by decreased ionized calcium concentration, which in turn potently stimulates the release of this hormone. We found that this procedure is a simple and safe way to stimulate PTH release, and can be used to evaluate the secretory reserve as well as the differential diagnosis of various parathyroid gland disorders.

Patients and Methods

Subjects

We studied eight healthy volunteers and ten patients with various parathyroid diseases (four patients with idiopathic or post-surgical hypoparathyroidism and six patients with primary hyperparathyroidism). Healthy volunteers [mean (\pm SD) age, 34.0 ± 4.1 years (range, 29 to 43)] enrolled as control subjects in this study were found to have normal serum total calcium, serum inorganic phosphate, plasma ionized calcium, plasma PTH (intact, midregion, and carboxy-terminus), and no known diseases including those of an endocrine nature. Patients with primary hypoparathyroidism originally had hypocalcemia and hyperphosphatemia

with normal or undetectable basal plasma PTH levels. Patients 1 and 3 in our study were diagnosed as having idiopathic hypoparathyroidism without a family history or any other abnormalities, whereas Patients 2 and 4 who had undergone thyro-parathyroidectomy for the removal of a thyroid neoplasm developed post-surgical hypoparathyroidism. However, subcutaneous transplantation of the parathyroid gland was carried out in Patient 2 and thus PTH secretion remained. Patients 3 and 4 were on a low dose vitamin D₃ and calcium lactate replacement therapy to maintain normal serum calcium levels, although administration of these medications was prohibited in the morning of the examination. Patients with primary hyperparathyroidism, in contrast, were shown to have hypercalcemia and hypophosphatemia with high basal plasma PTH, the diagnosis being confirmed by subsequent histological examinations (parathyroid adenoma in Patients 6 to 10, and hyperplasia in Patient 5). Clinical profiles of the patients enrolled are summarized in Table 1. Informed consent was obtained from all patients and healthy subjects enrolled in this study. The protocol for this study was reviewed and approved by the Ethical Committee at the Nagoya University School of Medicine.

Clinical studies

The test was performed as follows. In fasting or at least 4 h postprandial states, 35 ml per body surface

Table 1. Clinical profiles of the patients with parathyroid diseases

Patients	Age (years)	Gender	Diagnosis	Serum calcium ^{a)} (mg/dl)	Serum inorganic phosphate ^{b)} (mg/dl)	Parathyroid hormone intact ^{c)} (pg/ml)
1	28	Male	hypoparathyroidism (partial, idiopathic)	9.6	4.5	11.3
2	43	Female	hypoparathyroidism (partial, postoperative)	8.9	3.7	10
3	49	Male	hypoparathyroidism (complete, idiopathic) ^{d)}	8.9	3.9	UD ^{e)}
4	48	Female	hypoparathyroidism (complete, postoperative) ^{d)}	10.2	3.6	UD ^{e)}
5	61	Female	parathyroid hyperplasia	10.8	2.7	464
6	52	Male	parathyroid adenoma	10.4	2.6	295
7	54	Female	parathyroid adenoma	10.4	3.5	180
8	71	Female	parathyroid adenoma	10.9	2.5	144
9	22	Female	parathyroid adenoma	11.2	2.0	118
10	72	Female	parathyroid adenoma	10.9	2.8	80

^{a)} Normal range, 8.4–10.2 mg/dl; ^{b)} Normal range, 2.9–4.5 mg/dl; ^{c)} Normal range, 10–65 pg/ml; ^{d)} Under Vitamin D₃ replacement therapy; ^{e)} UD, undetectable.

Urinary calcium excretion (calcium/creatinine ratio) is not determined in this study.

area (m^2) of 8.4% (w/v) sodium bicarbonate solution (Otsuka Pharmaceutical Co., Tokyo, Japan) was injected into the ante-cubital vein over 2 min. Blood samples were obtained from the ante-cubital vein of the other arm before and 3, 5, 10, 30, and 60 min following the start of infusion. Total serum calcium and other biochemical parameters were measured by automated techniques. Samples for the determination of ionized plasma calcium and blood bicarbonate ion concentrations were collected into heparinized 1 ml syringes for immediate measurements. Blood samples to be used in the analysis of plasma PTH (intact, midregion, and C-terminus) concentrations were collected into tubes containing EDTA, centrifuged, and kept frozen at -30°C for subsequent analysis. Body surface area was calculated by the equation: $A = W^{0.444} \times H^{0.663} \times 88.83$ (A, body surface area [m^2]; W, body weight [kg]; H, body height [cm]) [5].

Biochemical analyses

Ionized plasma calcium was measured with an Easy-Ca analyzer (Medica corporation, Bedford, MA, USA). Blood pH and bicarbonate ion concentrations were measured with an ABI 320 blood gas analyzer (Radiometer, Copenhagen, Denmark). Plasma PTH was determined by three different assays: Intact PTH IRMA kit 'Mitsubishi' (Mitsubishi Chemical Co., Tokyo, Japan; normal range 14–66 pg/ml) [6], a double-antibody immunoradiometric assay for the determination of intact PTH; a PTH RIA kit 'YAMASA' using antiserum (CH9) against the mid-region of PTH (Yamasa Shoyu, Tokyo, Japan; normal range, 90–270 pg/ml) [7] was used in the analysis of the midregion PTH; and a PTH-C RIA kit using antiserum against the carboxy-terminus of PTH (Immuno-Nuclear Co., Tokyo, Japan; normal range, below 0.6 ng/ml) [8] was used in the analysis of the carboxy-terminus PTH. In addition, intact PTH was measured with an Allegro Intact PTH radioimmuno-metric assay kit (Nicholas Institute Diagnostics, San Juan Capistrano, CA, USA) [9, 10] which provides virtually identical results to those obtained by the 'Mitsubishi' assay.

Statistical analysis

Estimates of change over time were made with repeated-measures analysis of variance (ANOVA).

All statistical tests were two-tailed. A p-value of below 0.05 was considered significant.

Results

In eight healthy volunteers, the mean blood pH and bicarbonate ion concentrations were found to be significantly increased immediately following the sodium bicarbonate injection, after which a decrease in the mean (\pm SE) ionized plasma calcium concentration was observed (from 1.21 ± 0.01 to 1.11 ± 0.01 mmol/L) (Fig. 1). The significant decrease in ionized calcium was maintained throughout the test period (until 60 min after the injection).

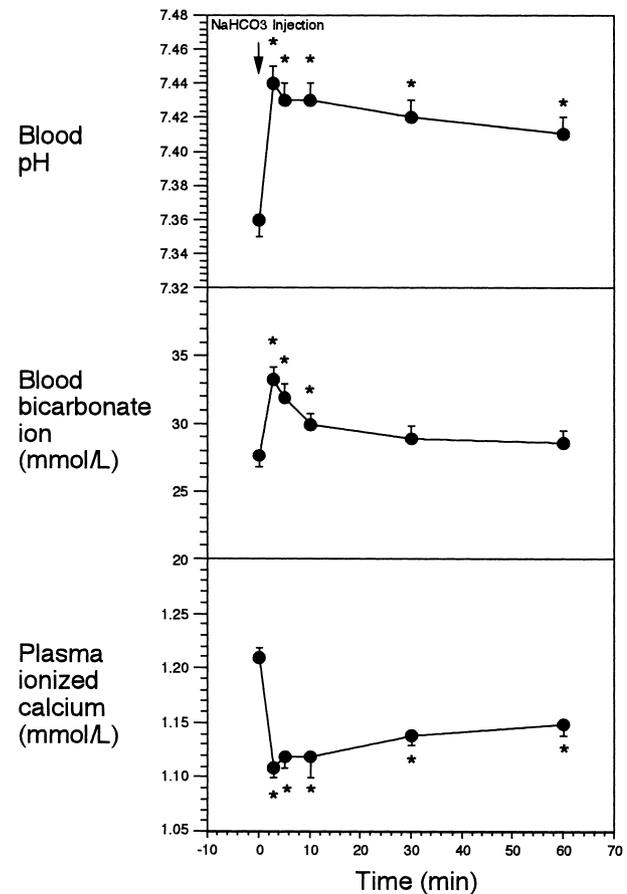


Fig. 1. Effects of sodium bicarbonate infusion on plasma ionized calcium and related parameters in eight healthy volunteers. Upper, middle, and lower panels show the mean (\pm SE) blood pH, blood bicarbonate ion, and plasma ionized calcium, respectively, before and after the sodium bicarbonate infusion. * $p < 0.05$ vs. the value at time zero.

In response to the decrease in plasma ionized calcium, a prompt and marked rise in the mean (\pm SE) intact plasma PTH levels (42.3 ± 4.2 to 182.4 ± 34.7 pg/ml) was observed already 3 min after the start of the sodium bicarbonate infusion, and rapidly returned to basal levels (Fig. 2). Similar responses of mid-region and carboxy-terminus PTH levels were observed, although the fold increases were less than that of intact PTH.

In plasma samples obtained from patients diagnosed with hypoparathyroidism (Patients 1 to 4), on the other hand, a diminished or no response in intact PTH was observed (Figs. 3A and 3B). The response was absent in two patients (Patients 3 and 4) with complete hypoparathyroidism with undetectable basal plasma PTH levels. Furthermore, in two patients with *partial* hypo-

parathyroidism (Patients 1 and 2) with near-normal basal plasma PTH levels, minimal or virtually no increment in hormone levels (peak value minus basal value) was observed in response to sodium bicarbonate infusion (Fig. 3B). Changes in ionized plasma calcium and blood bicarbonate concentrations during the test in each patient are shown in Table 2.

In plasma samples obtained from patients diagnosed with primary hyperparathyroidism (Patients 5 to 10), intact PTH responses to sodium bicarbonate infusion were maintained in most of the patients (Fig. 4A). However, the fold increases (peak value divided by the basal value) of the hormone were much lower (1.9-fold or less) than those of healthy volunteers (4.3-fold increase on average) (Fig. 4B). In one patient with parathyroid adenoma (Patient 9), absolutely no re-

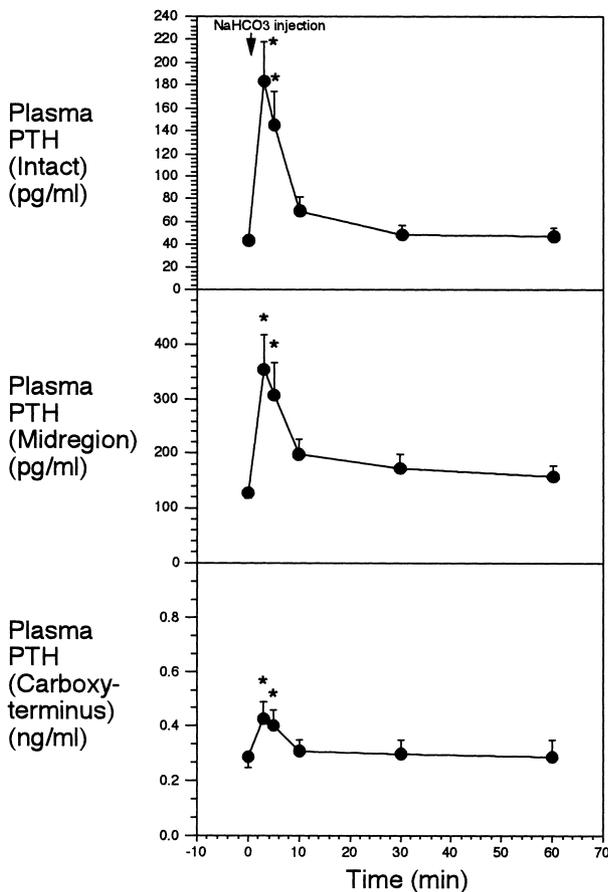


Fig. 2. Effects of sodium bicarbonate infusion on plasma PTH levels in eight healthy volunteers. Upper, middle, and lower panels show the mean (\pm SE) intact, midregion, and carboxy-terminus PTH concentrations, respectively, before and after the sodium bicarbonate infusion. * $p < 0.05$ vs. the value at time zero.

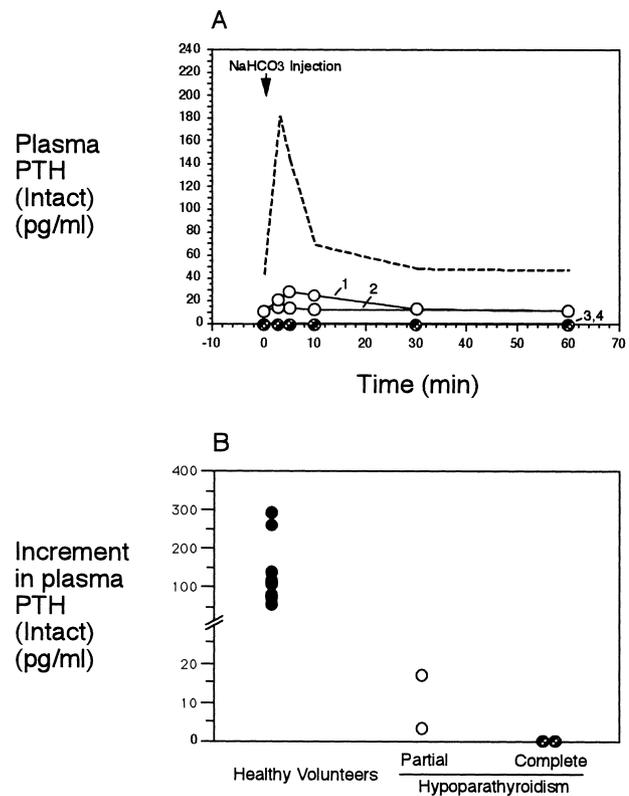


Fig. 3. Effects of sodium bicarbonate infusion on plasma PTH levels in four patients with primary hypoparathyroidism. Panel A shows the time course of the plasma intact PTH concentrations in each patient during the test. Dotted line shows the mean response in healthy volunteers. Panel B shows the absolute increment in plasma intact PTH (peak value minus value at time zero) in patients (right, middle) and in healthy volunteers (left).

Table 2. Change in the plasma ionized calcium and blood bicarbonate ion concentrations during the sodium bicarbonate infusion in patients with parathyroid diseases

Patients	Plasma ionized calcium (mmol/L)			Blood bicarbonate ion (mmol/L)		
	Basal	nadir	decrement	Basal	peak	increment
1	1.10	1.01	0.09	ND ^{a)}	ND	–
2	1.10	1.03	0.07	25.9	30.6	4.5
3	1.12	1.05	0.07	26.8	31.2	4.4
4	1.17	1.08	0.09	22.1	28.1	6.0
5	1.27	1.19	0.08	22.4	28.2	5.8
6	1.27	1.18	0.09	ND	ND	–
7	1.29	1.19	0.10	26.5	31.2	4.7
8	1.42	1.30	0.12	23.2	29.8	6.6
9	1.39	1.32	0.07	25.9	29.1	3.2
10	ND	ND	ND	ND	ND	–

^{a)} ND, not determined

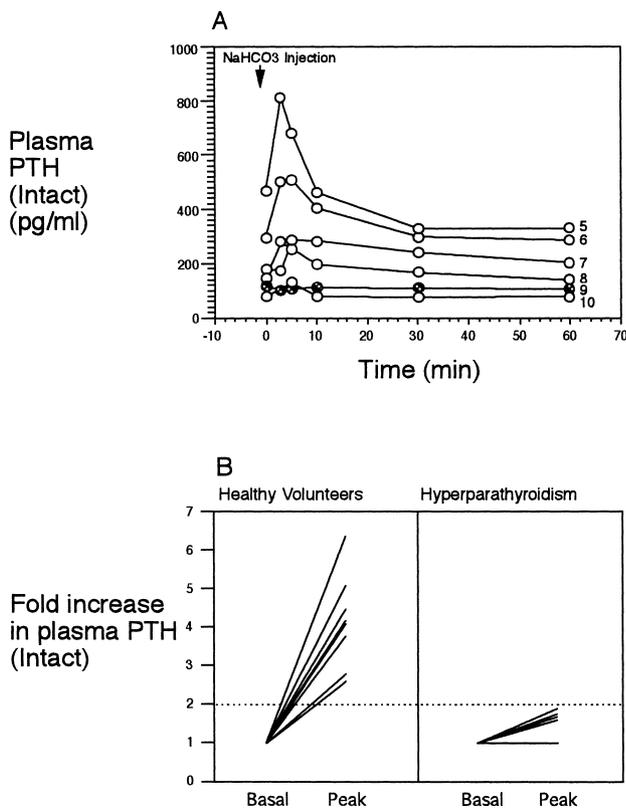


Fig. 4. Effects of sodium bicarbonate infusion on plasma PTH levels in six patients with primary hyperparathyroidism. Panel A shows the time course of the plasma intact PTH concentrations in each patient during the test. Shaded circles represent the data obtained in Patient 9, in which no PTH responses were observed. Panel B shows the fold increase of the hormone in patients (right) and in healthy volunteers (left).

sponse in intact plasma PTH was observed. Similar results were also obtained in midregion and carboxy-terminus PTHs (data not shown). Changes in plasma ionized calcium and blood bicarbonate concentrations during the test in each patient are summarized in Table 2.

No significant side effect was observed during the test, except that transient oral paresthesia was recognized in some subjects (both in patients and in healthy volunteers).

Discussion

In this study, we developed a new provocation test, sodium bicarbonate infusion, for the evaluation of parathyroid gland function. Using this procedure, we showed that, in healthy subjects, a prompt and brisk rise in plasma PTH occurred following a minimal decrease in plasma ionized calcium and an increase in blood pH. We also found that PTH responses were significantly altered in patients with hyper- or hypoparathyroidism. No noticeable side effects were observed during the test. Therefore, we believe that this test may be clinically useful in delineating decreased and/or disordered parathyroid gland function.

The secretion of PTH is well known to be negatively regulated by extracellular ionized calcium levels [1, 2, 10]. The principle of our new method is to stimulate PTH secretion by temporarily decreasing ionized calcium with the injection of sodium bicarbonate. Indeed, in healthy volunteers, marked PTH responses (on aver-

age 4.3-fold increase in plasma intact PTH) were identified immediately following the injection. Similar responses were observed in midregion and carboxy-terminus PTH levels, but less markedly, probably because of the differences in the metabolic clearance rate of each fragment [11, 12]; i.e., the slower the clearance, the higher the basal value and thus a diminished response of *de novo* secretion. It is interesting that the intact PTH response observed in this study was only transient despite a sustained decrease in ionized calcium during the test. These findings appear to suggest that PTH secretion is regulated not only by the absolute value but also by the rate of change of ionized calcium.

In hypoparathyroidism, decreased or no responses of intact PTH during the test was observed. Especially noteworthy was the fact that, in *partial* hypoparathyroidism with measurable basal intact PTH, very little or virtually no additional PTH release was observed. We believe that these observations may be caused by the decrease in parathyroid cell mass. In these patients, the near normal 'basal' PTH level seems to represent the almost maximally stimulated value as well, because of the diminished or abolished additional responses during the test. Since it has previously been difficult to evaluate the parathyroid gland reserve, this test should be particularly useful in the clinical setting.

In primary hyperparathyroidism, the PTH responses were preserved but blunted (less than two-fold) in all of the cases examined. The diminished responses may apparently be explained by the shifted range in the change of ionized calcium during the test. However, the inverse sigmoidal curve for the ionized calcium-PTH relationship itself is known to be deviated to the right as well in patients with parathyroid adenoma [13] and thus the decrease in ionized calcium in a higher range should also be a comparable stimulus for PTH release. The decreased response of this hormone might reflect abnormalities in the calcium responsiveness of hormone-secreting cells. For instance, the expression of calcium sensing receptors have been shown to be decreased in parathyroid adenomas [14, 15], and thus the variability in the diminished response might be partly explained by the degree of

decrease in the calcium sensing system of adenoma tissues. Another possibility is that the function of calcium sensing receptors is defective in tumor tissues because of the inactivating mutation of the receptor gene, although Cetani and colleagues recently showed no evidence of mutations in sporadic parathyroid adenoma [16].

An EDTA infusion test has been previously used for stimulating PTH release [3, 4]. However, it is not widely used now because of adverse cardiovascular effects. Instead, sodium citrate infusion is now being applied for the full characterization of the PTH-calcium relationship (inverted sigmoidal curve) [17]. The sodium bicarbonate infusion test described in this study does not provide the relationship because of the acuteness of the stimulation, and thus for this purpose, sodium citrate infusion appears to be more adequate [17]. In addition, our test is not recommended for hypoparathyroid patients with clinically overt hypocalcemia, because further decreases in ionized plasma calcium may worsen the symptoms of hypocalcemia, and in such cases, this test might better be done after near normalization of serum ionized calcium with adequate replacement therapy, as was performed successfully in this study.

In conclusion, the sodium bicarbonate infusion test is simple, safe, and suitable for rapid evaluation of parathyroid gland function. In particular, with this procedure, partial subclinical hypoparathyroidism, which was, to date, previously difficult to recognize, will be easily detected. This test may also be applicable for the diagnosis of other parathyroid disorders or for the estimation of residual or transplanted parathyroid gland function following surgery [18]. Further accumulation of clinical data will clarify the values and limits of this test.

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