Subacute infusion of physiological doses of parathyroid hormone raises blood pressure in humans

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Abstract

**Background.** Acute administration of parathyroid hormone (PTH) causes vasodilation and blood pressure decrease in experimental animals. This effect contrasts with the putative role of secondary hyperparathyroidism in the pathogenesis of hypertension of patients with renal failure. Uraemia is characterized by insulin resistance and hyperinsulinaemia. We therefore investigated whether subacute administration of physiological doses of human 1,34-PTH affects blood pressure under conditions of controlled insulin levels (euglycaemic clamp technique) in humans.

**Methods.** In a double-blind cross-over design 10 healthy male subjects received, on two occasions, in random order, for 2 h, either a sham infusion or an infusion of 200 units of 1,34-PTH.

**Results.** Mean ionized calcium concentration increased significantly \(P < 0.01\) within the normal range during euglycaemic hyperinsulinaemia, both with sham infusion (from \(1.25 \pm 0.04\) to \(1.29 \pm 0.02\) mmol/l) and with infusion of 1,34-PTH, but the increase was more marked with 1,34-PTH administration (from \(1.26 \pm 0.05\) to \(1.33 \pm 0.07\) mmol/l). In addition, mean platelet intracellular calcium concentration (by fluorescence spectroscopy) was unchanged with sham infusion (49.9 \(\pm 4.1\) versus 50.3 \(\pm 5.0\) mmol), but increased significantly \(P < 0.05\;\text{paired } t\)-test) after 1,34-PTH infusion (from \(49.8 \pm 5.0\) to \(52.8 \pm 5.8\) mmol). The infusion of 1,34-PTH resulted in a significant \(P < 0.01\) increase in mean MAP (from \(84 \pm 5\) to \(88 \pm 5\) mmHg) as compared with sham infusion (85 \(\pm 4\) versus 86 \(\pm 4\)). The intra-individual changes in intracellular calcium concentration (\(\Delta [\text{Ca}^{2+}]_i\)) were significantly correlated to the changes in mean MAP (\(\Delta\text{MAP}\)) \(r = 0.87, P < 0.001\). In contrast to blood pressure, insulin sensitivity was not affected by 1,34-PTH infusion (M-value: 7.2 \(\pm 1.6\) mg/kg per min) as compared with sham infusion (7.3 \(\pm 1.4\)).

**Conclusion.** Subacute administration of physiological doses of parathyroid hormone under hyperinsulinaemic conditions significantly affects intracellular calcium and blood pressure in healthy subjects, but does not affect the action of insulin.

**Key words:** parathyroid hormone; blood pressure; euglycaemic clamp; hypertension; hyperparathyroidism; insulin sensitivity; intracellular calcium

Introduction

Chronically increased plasma intact parathyroid hormone (PTH) concentration and/or intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) may be of relevance in the genesis of high blood pressure. This hypothesis is based on observations that hypertension in uraemic patients with secondary hyperparathyroidism is associated with increased PTH and intracellular calcium concentrations [1]. Further, in patients with primary hyperparathyroidism a high prevalence of hypertension is found in association with increased [Ca\(^{2+}\)]\(_i\) [2,3].

In this regard it is of interest that studies in rats have shown that PTH has acute vasodilator and blood pressure lowering effects, probably via an increase in prostaglandin production and release [4,5]. Nevertheless, studies in humans and even studies in rats, have documented that PTH plays a (permissive?) role in the blood pressure increase in different models of hypertension, e.g. spontaneously hypertensive rats, etc. [5–7]. So far, no firm evidence has been presented from intervention studies that blood pressure in humans is specifically affected by PTH.

Patients with renal failure are characterized by derangements of glucose metabolism, i.e. insulin resistance and concomitant hyperinsulinaemia [8–10]. Some recent studies suggested that this is due, at least in part, to increased PTH and/or [Ca\(^{2+}\)]\(_i\) [11–13]. Insulin resistance is also commonly found in patients with primary hyperparathyroidism [14]. Insulin affects intracellular calcium [15,16] and it is not known whether there is an interaction between PTH and insulin with respect to intracellular calcium and blood pressure.

In order to address this issue we measured blood pressure under stable hyperinsulinaemic conditions...
(euglycaemic hyperinsulinaemic clamp technique) in a placebo-controlled study with or without administration of physiological doses of human 1,34-PTH in healthy normotensive volunteers. In parallel, whole blood ionized calcium concentration and intracellular calcium concentration in platelets were measured.

Participants and methods

Participants

Ten healthy normotensive white male subjects (mean age 25 ± 3 years, mean body mass index (BMI) 22.4 ± 2.0 kg/m²) were recruited. All were normotensive non-smokers who were within 10% of ideal body weight and took no medication. Their family histories were negative for hypertension or metabolic diseases. At the beginning of the study they underwent a physical examination, routine chemical tests and urine analysis. Normal glucose tolerance according to WHO criteria was confirmed in all volunteers by a 100-g oral glucose tolerance test.

Protocol

The protocol was approved by the Ethics Committee of the University of Heidelberg. Written informed consent was given by all participants. A randomized double-blind crossover design was used. The volunteers were instructed to adhere for the duration of the study to a diet standardized with respect to carbohydrate and NaCl content. They maintained constant body weight (±0.5%) for at least 4 weeks before and during the study; alcohol consumption was not allowed and physical activity was maintained at its usual level throughout the investigation. All volunteers were studied twice within an interval of 7 days under euglycaemic conditions. During a standard 2-h euglycaemic clamp the subjects received, in random order, either a sham infusion, Phosphate concentrations were measured with an autoanalyzer (Hitachi 705, Critikon Inc., USA) at the start and at the end of each clamp and urine collection was done during the clamp examinations in order to assess sodium, calcium and phosphate excretion.

Measurements and calculations

The amount of glucose infused to maintain euglycaemia (glucose infusion rate) was evaluated in the last 40 min of the clamp, i.e. after a steady state of glucose infusion was achieved. The glucose disposal rate (M-value) was calculated for this period in order to assess insulin sensitivity [17]. Plasma insulin concentrations were measured enzymatically (ES 22®, Boehringer Mannheim, Germany) and arterialized standard bicarbonate levels with a blood–gas analyser (Periquant 803®, Güttingen Medizin Elektronik GmbH, Germany) at the start and at the end of the clamp. Mean arterial blood pressure (MAP) was measured automatically (Dinamap®, Critikon Inc., USA) at regular intervals before and during the clamp experiments. A urine collection was separated from the dye by passage through a Sepharose-2B-CL column (XK 16®, Pharmacia Biotech, Germany) at room temperature. The column was prepared with a solution containing 145 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l MgSO₄, 0.5 mmol/l Na₂HPO₄; 10 mmol/l HEPES (pH 7.4; osmolality 300 mosmol). Samples of 1 ml were pooled, the platelets counted and their concentration adjusted to 4.0 x 10⁷/ml. The platelets were re-incubated in the above

Blood samples for measurements of plasma glucose levels were taken from a retrograde dorsal hand vein canula throughout the clamp at 5 min intervals. The hand was rested in a heated box (∼50°C) to arterialize the venous blood. Plasma glucose concentrations were measured with the Glucohyslycaemic clamp experiment was performed in a quiet room from 8 a.m. to 10 a.m. after an overnight 12-h fast. The hyperinsulinaemic euglycaemic clamp-technique was used as described in detail elsewhere [17]. In brief, first a priming bolus infusion of 100 mU insulin/m² per min (H-Insulin®, Hoechst AG, Germany) was given over 2 min. Thereafter the rate of infusion was gradually decreased to a constant rate of 40 mU/m² per min. Plasma insulin levels were raised to about 600 pmol/l. In order to prevent adsorption of insulin to the infusion line, 2 ml of the volunteer’s own blood were added to the infusion. The infusion rate (25 ml/h) and consequently the volume infused were the same with both infusion modalities. Both the volunteers and the investigator performing the clamp were blinded with respect to the type of infusion. Urine was collected for 24 h by all volunteers on the day before the clamp experiments in order to assess sodium, calcium and phosphate excretion. All participants were admitted to the clinic at 8 p.m. on the day before the clamp experiments. On the morning of the next day a euglycaemic clamp experiment was performed in a quiet room from 8 a.m. to 10 a.m. after an overnight 12-h fast. The hyperinsulinaemic euglycaemic clamp-technique was used as described in detail elsewhere [17]. In brief, first a priming bolus infusion of 100 mU insulin/m² per min (H-Insulin®, Hoechst AG, Germany) was given over 2 min. Thereafter the rate of infusion was gradually decreased to a constant rate of 40 mU/m² per min. Plasma insulin levels were raised to about 600 pmol/l. In order to prevent adsorption of insulin to the infusion line, 2 ml of the volunteer’s own blood was added to the insulin infusion. At 4 min after the start of the insulin infusion a 20%-glucose infusion (Glucosteril 20%, Fresenius AG, Germany) was started.
HEPES buffer with CaCl₂ in order to achieve a calcium concentration in the solution of 1 mmol. The [Ca²⁺] measurement was performed with a luminescence spectrophotometer (LS 50B®, Perkin–Elmer, UK), while the platelets were continuously stirred in a quartz cuvette maintained at 37°C. Fluorescence was read at an emission wavelength of 510 nm and sequential excitation wavelengths of 340 and 380 nm. The cells were lysed with 0.1% Triton X-100 in order to obtain maximum fluorescence; minimal fluorescence was determined by adding EGTA. The cytosolic free calcium concentration ([Ca²⁺]) was calculated according to the equation by Grynkiewicz et al. [19]: 

\[
K_d \times (R - R_{\text{max}})/(R_{\text{max}} - R)
\]

where \( R_{\text{max}} \) stands for the ratio F340/380 after addition of Triton X-100, \( R \) means the ratio F340/380 after addition of EGTA and \( K_d \) is the dissociation constant of fura 2 for calcium (224 nmol/l). The coefficient of variation of [Ca²⁺] measurement in the same subjects was less than 8% as tested in five subjects.

**Statistical analysis**

The primary study endpoint was the mean arterial blood pressure during the clamp studies. The mean of five consecutive MAP measurements before the start and at the end of the clamp experiments was taken for analysis. Intra-individual MAP values at the start and at the end of the clamp experiments with sham and with 1,34-PTH infusion were compared using the \( t \)-test for paired samples. In addition, intra-individual data at the start and at the end of each clamp were also compared using the paired \( t \)-test. Bonferroni correction was applied in order to account for multiple comparisons. The differences were regarded as significant at \( P < 0.05 \). All data are presented as mean ± SD. The correlation of the intra-individual changes in intracellular calcium concentration (Δ[Ca²⁺]) and the changes in mean MAP (ΔMAP) with infusion of 1,34-PTH was calculated using the method after Pearson.

**Results**

The infusion of 1,34-PTH over a period of 2 h resulted in a significant increase in mean MAP, whereas MAP remained unchanged with sham infusion (Table 1). The intra-individual changes of MAP with sham and with PTH infusion are shown on Fig. 1. Mean ionized calcium concentrations increased somewhat with sham infusion. The increase was marked and significant with infusion of 1,34-PTH, however. Mean intracellular calcium concentrations remained unchanged with sham infusion, but a significant increase was documented with infusion of 1,34-PTH. The increase in intracellular calcium concentration of platelets, i.e. Δ[Ca²⁺], was significantly correlated to the increase in mean MAP (ΔMAP) (\( r = 0.87, P < 0.001 \)). Mean muscle blood flow was unchanged with placebo or PTH administration.

Both, euglycaemia and hyperinsulinaemia during the clamp studies were similar with sham and with PTH infusion (Table 2). Intravenous administration of 1,34-PTH did not influence insulin sensitivity; the difference in mean M-value between PTH and sham infusion was not significant. As expected, mean plasma phosphate concentrations decreased significantly during euglycaemic hyperinsulinaemia. The decrease was similar with both infusion protocols. The mean intact PTH level was significantly lower after sham and after 1,34-PTH infusion.

**Table 1.** Haemodynamic variables and parameters of calcium metabolism during the clamp experiments in healthy volunteers (\( n = 10 \)) receiving sham and 1,34-PTH (200 U) infusion

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>1,34-PTH</th>
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<tbody>
<tr>
<td></td>
<td>Start of clamp periods</td>
<td>End</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>85 ± 4</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>MBF (ml/100 g per min)</td>
<td>16.5 ± 3.3</td>
<td>16.7 ± 2.2</td>
</tr>
<tr>
<td>( [\text{Ca}^{2+}] ) (mmol/l)</td>
<td>1.25 ± 0.04</td>
<td>1.29 ± 0.04²</td>
</tr>
<tr>
<td>( [\text{Ca}^{2+}] ) (nmol)</td>
<td>49.9 ± 4.4</td>
<td>50.3 ± 5.0</td>
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<tr>
<td>PTH (ng/l)</td>
<td>24 ± 11</td>
<td>16 ± 6²</td>
</tr>
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MAP, mean arterial blood pressure; MBF, muscle blood flow; \( [\text{Ca}^{2+}] \), whole blood ionized calcium concentration; \( [\text{Ca}^{2+}] \), platelet intracellular calcium concentration; PTH, plasma intact parathyroid hormone. \( ^1 P < 0.05 \), \( ^2 P < 0.01 \), intra-individual comparison of data at the start and at the end of the clamps; \( ^3 P < 0.05 \), \( ^4 P < 0.01 \), intra-individual comparison of data between sham and 1,34-PTH infusion.
infusion; but the decrease was more marked after 1,34-PTH infusion. Mean urinary sodium excretion during the clamps was not affected by 1,34-PTH infusion, whereas mean calcium excretion was significantly lower and phosphate excretion higher with PTH administration as compared with sham infusion (Table 2).

The mean 24-h urinary sodium, calcium and phosphate excretions on the day before the clamp experiments were comparable to those on the day before sham infusion (171 ± 39, 4.9 ± 1.4 and 24 ± 4 mmol/24-h) and the day before infusion of 1,34-PTH (176 ± 45, 4.9 ± 2.0 and 23 ± 6 mmol/24-h).

### Discussion

A salient feature of the present study is the demonstration that subacute administration of physiological doses of human 1,34-PTH increased blood pressure in healthy volunteers under euglycaemic conditions in the presence of controlled circulating insulin levels. In parallel, a significant and consistent increase in ionized calcium concentration and in \([\text{Ca}^{2+}]\), was observed with administration of PTH, and the increase in blood pressure was strongly correlated to changes in \([\text{Ca}^{2+}]\). This observation is not surprising, since changes in \([\text{Ca}^{2+}]\), are the final common pathway through which many of the effects of parathyroid hormone are mediated [5,12]. Our findings suggest that an increase in PTH levels and/or \([\text{Ca}^{2+}]\), may be of relevance in the genesis of high blood pressure. This speculation is corroborated by observations that in uraemic patients with secondary hyperparathyroidism, as well as in patients with essential hypertension and even in genetically pre-hypertensive subjects, a positive correlation is found between blood pressure on the one hand and intact PTH concentrations and \([\text{Ca}^{2+}]\), on the other [1,20–22].

The prevalence of hypertension is known to be high in patients with primary hyperparathyroidism [2]. In line with these observations are data from the study of Hulter et al. [23] who administered supraphysiological doses of human 1,34-PTH for 12 days to healthy subjects. The infusion of 1,34-PTH induced hypercalcaemia and in parallel a significant increase in blood pressure. Both, hypercalcaemia and hypertension normalized after the infusion was stopped [23]. In our study a dose of PTH was chosen that induced only a minor increase in ionized calcium concentration within the normal range; nevertheless a modest but significant increase in blood pressure was seen. This observation is in agreement with results of several experimental studies, which have shown that PTH potentiates the pressor-effect of calcium by enhancing its entry into vascular smooth muscle cells [5,24]. There are good arguments that the acute vasodilator and blood pressure lowering effects of PTH result from enhanced vascular prostaglandin production [4,5]. This is in apparent contrast to the present (sub)acute study in our volunteers plethysmographically measured leg muscle blood flow did not change significantly with 1,34-PTH infusion. This observation is compatible with the notion that the increase in blood pressure seems not to be mediated by vasoconstriction in the skeletal muscle microvasculature. We cannot rule out the possibility, however, that minor changes in muscle blood flow were undetectable with plethysmography. Further, the discrepancy between the animal data and the present human data may potentially relate to different contributions between species of the PTH/PTHrp and the novel PTH receptor and/or to different coupling to adenylate cyclase and the PKC/Ca pathway, respectively.

We controlled plasma insulin concentrations because of its known vasodilator effects [25]. It is interesting that during sham infusion under conditions of the euglycaemic hyperinsulinaemic clamp, ionized calcium increased significantly while plasma phosphate (and potassium) decreased, as has been shown previously in our laboratory [26]. This was associated with a significant decrease of intact PTH concentration [26]. The latter is not an artefact resulting from circadian

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**Table 2.** Metabolic variables and urinary electrolyte excretion during the clamp experiments in healthy volunteers (n = 10) receiving sham and 1,34-PTH (200 U) infusion

<table>
<thead>
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<th>Sham</th>
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<tr>
<td></td>
<td>Start of clamp periods</td>
<td>End</td>
<td>Start of clamp periods</td>
<td>End</td>
</tr>
<tr>
<td>P-insulin (pmol/l)</td>
<td>55 ± 10</td>
<td>562 ± 93&lt;sup&gt;2&lt;/sup&gt;</td>
<td>54 ± 13</td>
<td>566 ± 96&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>P-glucose (mmol/l)</td>
<td>4.8 ± 0.3</td>
<td>4.7 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>P-phosphate (mmol/l)</td>
<td>1.27 ± 0.21</td>
<td>0.91 ± 0.19&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.29 ± 0.18</td>
<td>0.91 ± 0.13&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>M-value (mg/kg per min)</td>
<td>7.2 ± 1.6</td>
<td>7.2 ± 1.6</td>
<td>7.3 ± 1.4</td>
<td>7.3 ± 1.4</td>
</tr>
<tr>
<td>P-HCO&lt;sub&gt;3&lt;/sub&gt; (mmol/l)</td>
<td>25.8 ± 1.2</td>
<td>25.5 ± 1.5</td>
<td>25.5 ± 1.7</td>
<td>25.0 ± 1.6</td>
</tr>
<tr>
<td>U-Na (mmol/h)</td>
<td>11 ± 4</td>
<td>11 ± 3</td>
<td>11 ± 3</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>U-Ca (mmol/h)</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.14</td>
<td>0.2 ± 0.14</td>
<td>0.2 ± 0.14</td>
</tr>
<tr>
<td>U-P (mmol/h)</td>
<td>1.5 ± 0.3</td>
<td>2 ± 0.5&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
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</table>

P, plasma; M-value, insulin sensitivity; U-Na, U-Ca, U-P, urinary electrolyte excretion; HCO<sub>3</sub>, arterIALIZED bicarbonate concentrations. *P < 0.05; *P < 0.01, intra-individual comparison of data at the start and at the end of the clamps; *P < 0.05; *P < 0.01, intra-individual comparison of data between sham and 1,34-PTH infusion.
changes, since parallel PTH measurements in volunteers without intervention failed to show a similar decrease of PTH at the same time of the day (data not shown). The decrease of intact PTH concentration during sham infusion is probably a direct effect of the increase in ionized calcium concentration, i.e. feedback inhibition of PTH secretion. Our observation of increased ionized calcium concentration during hyperinsulinaemia is compatible with the notion that insulin has effects on calcium metabolism. The known effects on intestinal calcium absorption [27] cannot account for this observation, since the volunteers were fasted for at least 12 h. The superimposition of PTH infusion caused a further increase in ionized calcium concentration, whereas plasma phosphate concentration remained unchanged.

In the present study we used platelets in order to measure [Ca\(^{2+}\)]. The question arises whether platelets are adequate surrogates for vascular smooth muscle cells, in order to study the relationship between [Ca\(^{2+}\)], and blood pressure. We observed a significant increase in platelet [Ca\(^{2+}\)], but we admit that such measurements do not address the possibility of compartmentation of intracellular calcium. Changes in platelet [Ca\(^{2+}\)], have been directly related to changes in blood pressure in several pathological conditions [1,13,16,20]. Most studies investigating the interaction between [Ca\(^{2+}\)], and blood pressure have been performed on blood cells, mainly platelets, since these cells and vascular smooth muscle cells exhibit similar calcium-regulated processes [28].

A further interesting aspect of our study was the demonstration that subacute administration of physiological doses of 1,34-PTH did not affect insulin sensitivity in healthy normotensive subjects despite a significant increase in platelet [Ca\(^{2+}\)], within the normal range. The lack of effect on insulin sensitivity was seen, although the expected biological actions of 1,34-PTH were noted, i.e. a significant decrease in urinary calcium excretion accompanied by an increase in blood ionized calcium concentration and a decrease in intact PTH concentration. The latter action is thought to result from direct inhibition of PTH secretion, as has been show previously with infusion of 1,84-PTH [29]. An increase in PTH concentration, and in parallel an increase in intracellular calcium concentration [Ca\(^{2+}\)], has been linked to impaired glucose metabolism in renal patients with secondary hyperparathyroidism [13,30]. The exact mechanism by which increased [Ca\(^{2+}\)], may reduce insulin sensitivity is not yet completely elucidated, but there are links between [Ca\(^{2+}\)], and insulin action. The insulin receptor contains Ca\(^{2+}\)-binding sites and its phosphorylation is Ca\(^{2+}\)-dependent [31]. Further, intracellular glucose transport and glucose-6-phosphatase activity is dependent on [Ca\(^{2+}\)], and calcium is thought to be the main intracellular signal for insulin-mediated processes [13]. It has been even proposed that an optimal range of [Ca\(^{2+}\)], is needed for effective insulin action and that an increase of [Ca\(^{2+}\)], beyond this range causes insulin resistance [32]. Our data clearly argue against the notion that insulin sensitivity is specifically affected by subacute infusion of 1,34-PTH. They do not rule out the possibility, however, that chronically increased PTH concentration and/or [Ca\(^{2+}\)], play a role in derangements of glucose metabolism seen in primary hyperparathyroidism and/or uraemia. In uraemic patients increased PTH concentrations and [Ca\(^{2+}\)], were accompanied by insulin resistance and glucose intolerance [13]. These abnormalities were normalized after calcitriol treatment and reversal of secondary hyperparathyroidism [30,33].

In summary, the present study provides evidence that in healthy subjects subacute administration of physiological doses of human 1,34-parathyroid hormone increased blood pressure under euglycaemic conditions with controlled circulating insulin concentrations. The effect of PTH is accompanied by an increase in intracellular calcium. The observation is compatible with a permissive role for PTH in the genesis of hypertension of patients with primary or secondary (renal) hyperparathyroidism.

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