Clinical Medicine Insights: Endocrinology and Diabetes

ORIGINAL RESEARCH

Parathyroid Hormone's Acute Effect on Vasodilatory Function

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Abstract: Parathyroid hormone (PTH) seems to affect the risk of cardiovascular disease. The aim of the present study was to investigate PTH's acute effect on endothelial vasodilatory function in forearm resistance vessels. Ten healthy subjects underwent forearm venous occlusion plethysmography. We measured forearm blood flow at baseline and at a stable, locally increased PTH level after intra-arterial infusion of metacholine and nitroprusside. The contralateral arm served as a control. Ionized calcium (Ca++) and PTH values were normal in all subjects at baseline (1.26 ± 0.02 mM/L, 3.6 ± 1.2 pM/L). After 30 minutes of PTH infusion, the PTH level increased in the active arm (13.8 ± 4.0 pM/L $P < 0.01$), while the Ca++ level was unchanged (1.25 ± 0.04; mM/L). Both the PTH and the Ca++ level in the contralateral arm remained unchanged, which indicates no systemic influence. The endothelial-dependent vasodilation was inversely correlated to the Ca++ level at baseline ($r = -0.75$, $P < 0.05$) and after PTH infusion ($r = -0.68$, $P < 0.05$). The vasodilatory function was not affected during PTH-infusion.

Keywords: parathyroid hormone, endothelial function, forearm venous occlusion plethysmography

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Introduction

It is well recognised that, through its impact on bones and kidneys, parathyroid hormone (PTH) plays a critical role in maintaining an adequate calcium-phosphorus homeostasis. PTH receptors are expressed, not only in these target organs, but also in a wide variety of other tissues, including the cardiovascular system and vascular endothelium.[1,2] Interest in PTH's cardiovascular effects has been stimulated by growing evidence of increased cardiovascular mortality and morbidity in patients with primary hyperparathyroidism (pHPT).[3,4] Endothelial dysfunction, an independent predictor of cardiovascular events, has been reported in pHPT.[5–8] The vasoactive actions of PTH, which seem to be complicated, have been studied in a few trials, with conflicting results. While the effect of short-term PTH infusion is vasodilation, prolonged infusion has resulted in hypertension.[9–11] Recently, a PTH fragment (the N-terminally located amino acids 1–34) was reported to activate production of NO, a potent vasorelaxing agent, in endothelial cells.[12] The aim of the present study was to investigate PTH's acute effects on endothelial vasodilatory function in forearm resistance vessels.

Methods

Forearm blood flow (FBF) measurement

Forearm venous occlusion plethysmography is a well-established method for measuring FBF.[13–15]

The method is based on measurement of the increase in forearm volume after the venous return has been suppressed by placing an inflated cuff (at a pressure of 30–40 mm Hg for 7 seconds) round the upper arm. At this pressure, venous occlusion occurs without any modification of the arterial inflow. The change in upper arm circumference is recorded by a mercury-in-silastic strain gauge placed around the forearm and connected to a calibrated plethysmograph. FBF is expressed graphically and is calculated (slope of the curve) as the mean of at least five consecutive recordings. With this method, cannulation of the brachial artery can be used to infuse vasoactive drugs in one arm without causing any alterations in systemic haemodynamics or in blood flow in the contralateral arm.[14,15] FBF is then measured in both forearms and the contralateral arm serves as a control. Arterial infusion of metacholine (MCh), a muscarinic receptor agonist that causes an endothelium-mediated vasodilator response, is used for evaluation of endothelium-dependent vasodilation (EDV). Arterial infusion of sodium nitroprusside (SNP), which directly affects the vascular smooth muscle cells, is used for evaluation of endothelium-independent vasodilation (EIDV). The endothelial function index is calculated as the ratio of forearm blood flow during the highest dose of MCh, 4 μg/min (EDV) to the highest dose of SNP, 10 μg/min (EIDV). This index expresses the contribution of endothelial NO release to the vasodilatory process. Evaluation of the reproducibility of measurements of EDV and EIDV using this technique has shown a variation of 5%–8% in the short- (2 h) and long-term (3 weeks) perspectives.[14] The authors' previous studies have shown that local intra-arterial infusion of saline did not significantly change either baseline FBF or the responses to MCh and SNP and the endothelial function index in the active arm.[16]

Subjects

Three healthy volunteers (1 man, 2 women), aged 22–25 years, were included in a pilot study to determine an appropriate intra-arterial infusion rate of Preotact® (Nycomed), a full-length parathyroid hormone (PTH 1-84), for attaining a locally increased PTH level in the active arm. Thereafter, FBF was analysed in ten healthy volunteers (5 men and 5 women, aged 21–28 years). Vasoreactivity was analysed before and after 30 minutes of PTH infusion and locally increased PTH levels. Female subjects underwent a pregnancy test before inclusion. None of the subjects were taking any medication, had a history of any disease known to affect the cardiovascular system or used nicotine regularly. Each subject gave written consent to participate in the study, which was approved by the Ethics Committee of Uppsala University.

Experimental procedure

The subjects were investigated after an overnight fast in the supine position in a room maintained at a constant temperature. An arterial cannula was inserted in the brachial artery (active arm) for administration of the study drugs (PTH, SNP och MCh). The contralateral arm was used as control. The levels of PTH and ionized calcium (Ca++) were analyzed in the active arm at baseline and after 30 min of PTH infusion. FBF was measured at baseline before drug infusions. Thereafter, vasodilation in the active arm

was achieved by infusion of one of the vasodilatory drugs (SNP or MCh) for 10 min. The drugs were given in a random order at a maximum rate of 1 ml/min. The infused dosages were 2 and 4 μg/min for MCh (5 min each) and 5 and 10 μg/min for SNP. FBF was recorded at the end of the infusion. This procedure was repeated with the other vasodilatory drug after 20 min of washout time. The PTH infusion was started after these baseline measurements. After 30 min of PTH infusion, the blood flow measurements were repeated as described above.

Intra-arterial infusion of PTH

We used Preotact® (Nycomed), a full-length parathyroid hormone (PTH 1–84), diluted with saline in three steps to 0.2 μg/ ml. In a pilot study with 3 volunteers, we determined the intra-arterial infusion rate that was needed to attain a PTH level above the normal upper range (6.9 pM/L) in the active arm without any systemic influence. Guided by the results of the pilot study, a dose rate of 70 ml/h during 30 minutes was chosen for the PTH infusion.

Biochemistry and statistics

Anaerobic venous samples from the active arm were analysed at baseline and after PTH infusion, for Ca++ (i-STAT 1-Abbott, normal range 1.1–1.3 mM/L) and intact plasma PTH (Immunolite 2500, Diagnostics Product Company LA,CA, USA; intact P-PTH, normal range 1.1–6.9 pM/L). Plasma PTH and Ca++ levels were also analysed in the contralateral arm in 4 subjects to exclude a systemic effect during the infusions. Statistical analysis was performed with the SPSS for Windows statistical package 16.0 (SPSS Inc; Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD) and compared by Student's paired *t*-tests. Pearson's correlation coefficient was used to analyse relationships between pairs of variables. All tests were done two-tailed and $P < 0.05$ was considered to be statistically significant.

Results

Biochemical data, resting FBF, EDV and EIDV values are presented in Table 1 and Figure 1 for all study subjects. Ca++ and PTH values were normal in all subjects at baseline. After 30 minutes of PTH infusion, the PTH level in the active arm increased significantly while the Ca++ remained unchanged (Table 1).

The levels of Ca++ (baseline 1.26 ± 0.02, after PTH infusion 1.28 ± 0.02, mM/L, P = ns) and PTH (baseline 3.23 ± 0,86, after PTH infusion 3.53 ± 0.39, nM/L, P = ns) measured in the contralateral arm in 4 subjects were unchanged, which indicates no systemic influence. FBF and EDV or EIDV did not increase during PTH infusion. EDV at baseline and after PTH-infusion correlated inversely to Ca++ in the active arm (baseline r = –0.75; $P < 0.05$, after PTH infusion, r = –0.68; $P < 0.05$) (Figure 2).

Discussion

PTH infusion into the brachial artery resulted in a local elevation of the PTH level in the forearm without affecting the systemic level. Measurement of the blood-flow response to intra-arterial infusion of vasoactive agents using the forearm model is the golden standard for assessing endothelial function

Table 1. Forearm blood flow and biochemical data in all subjects at baseline and after parathyroid hormone infusion.

	Baseline n = 10	**After PTH infusion n = 10**	**Baseline vs. after PTH infusion *P***
Ca++ (1.1–1.3 mM/L)	1.26 ± 0.02	1.25 ± 0.04	ns
PTH (1.1–6.9 pM/L)	3.6 ± 1.2	13.8 ± 4.0	$P < 0.01$
Resting FBF (ml/min/100 ml tissue)	4.5 ± 1.5	5.0 ± 2.3	ns
EDV (MCh, 2 μg/min)	20.0 ± 13.4	21.5 ± 13.0	ns
EDV (MCh 4 μg/min)	24.7 ± 13.1	29.1 ± 15.9	ns
EIDV (SNP 5 μg/min)	16.2 ± 3.5	18.3 ± 8.2	ns
EIDV (SNP 10 μg/min)	22.2 ± 7.9	24.0 ± 9.7	ns
Endothelial function index	1.09 ± 0.41	1.25 ± 0.57	ns

The endothelial function index the ratio EDV (MCh 4 μg/min)/ EIDV (SNP 10 μg/min). Data are means ± SD.
Abbreviations: Ca++, ionized calcium; PTH, parathyroid hormone; FBF, forearm blood flow; EDV, endothelium-dependent vasodilation during intra-arterial infusion of metacholine (MCh); EIDV, endothelium-independent vasodilation during intra-arterial infusion of sodium nitroprusside (SNP).

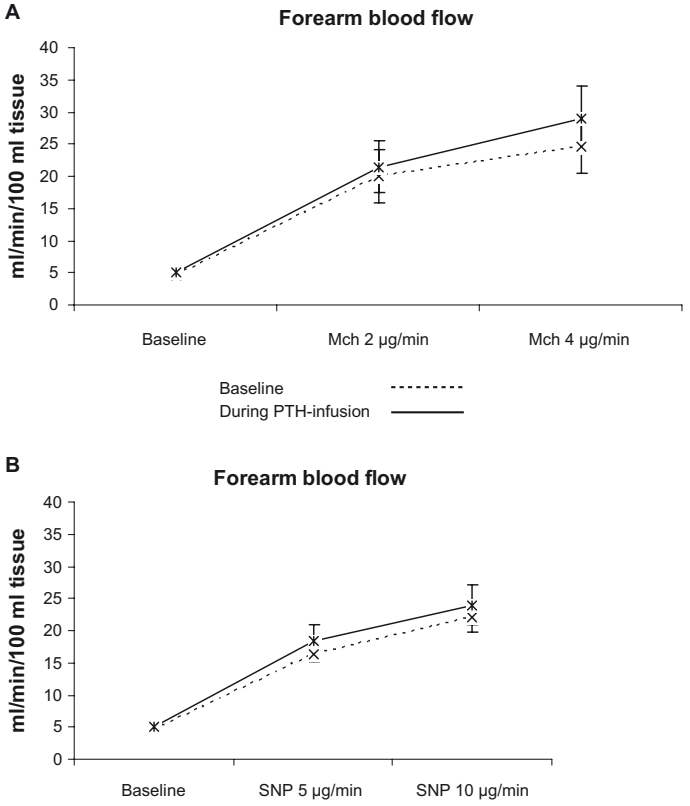

Figure 1. Forearm blood flow (FBF), endothelium-dependent vasodilation (metacholine, MCh 2 and 4 µg/min, Fig 1a) and endothelium-independent vasodilation (sodium nitroprusside, SNP 5 and 10 µg/min, Fig 1b) before and at steady state of local intra-arterial administration of parathyroid hormone (PTH).

in resistance arteries. To our knowledge, this is the first time this model has been used for the present purpose. The main advantage of the method is that the risk of confounding by systemic effects can be disregarded. Our results do not support the notion that PTH alone mediates any acute effects on endothelial vasodilatory function in forearm resistance vessels.

Endothelial dysfunction may result from an imbalanced release of endothelium-derived relaxing factors and endothelium-derived contracting factors.[17] The production of NO, the potent endothelium-derived relaxing factor, is catalyzed by the calcium-dependent enzyme nitric oxide synthase.[18] The endothelium-dependent contractions are probably mediated mostly by prostanoids, derived from the endothelial cyclooxygenase. For its function, the endothelial COX-1 isoform of cyclooxygenase requires an increase in the intracellular Ca++ concentration.[17] This phenomenon may explain our finding of an inverse correlation between Ca++ and EDV. The calcium ion serves as a regulator of a host of processes and the Ca++ mediated effects on endothelial function are complex. Calcium receptors are widely distributed and sensitive to small changes in Ca++.[19] In an earlier study with systemic and local infusion of calcium in healthy subjects, we found a dose-related impairment of endothelial vasodilatory function, an increased systolic blood pressure and a significant drop in the PTH level during systemic hypercalcemia but not during local hypercalcemia.[20] Unfortunately we were not able to simultaneously measure the production of NO or prostanoids.

Parathyroid hormone has been associated with both hypo- and hyper-tensive effects. Short-term PTH infusion caused a decrease in blood pressure when the calcium level was kept constant by clamp technique.[21] In contrast, long term infusion of PTH results in an elevated blood pressure and hypercalcemia.[10] The association between PTH and hypertension has been confirmed in several studies, both in pHPT and in epidemiological trials.[22–24] PTH seems to influence a wide variety of organ systems and the long-term effects of excess PTH may contribute to the increased cardiovascular mortality and morbidity, cardiac dysfunction and impaired endothelial function associated with pHPT.[25–28] PTH may stimulate renin-aldosteron activity, have inotropic and chronotropic effects on the heart and cause structural and functional alterations in the vascular wall.[21,29–31] Ohta suggested that the inhibitory effect of PTH on alpha-adrenoceptor-mediated contraction may result from an inhibition of the

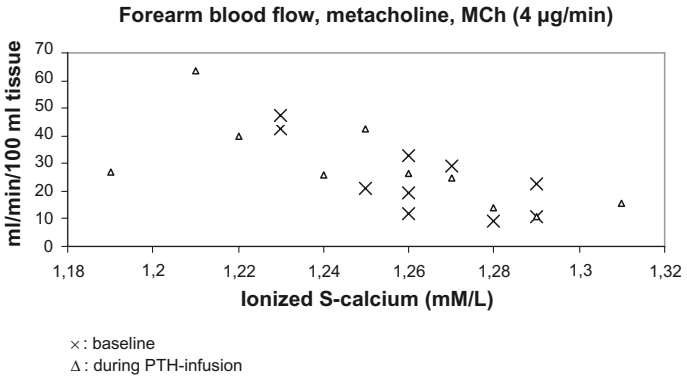

Figure 2. Forearm blood flow, endothelium-dependent vasodilation (EDV), during metacholine infusion (MCh 4 µg/min) before and at steady state of local intra-arterial administration of parathyroid hormone (PTH) correlated inversely to the ionized calcium level (baseline, $r = -0.75$, $P < 0.05$ and after PTH infusion, $r = -0.68$, $P < 0.05$).

influx of calcium ions through receptor-operated and voltage-gated calcium channels.[32] Experimental studies of the influence of PTH on the tension in ring preparations of rat arteries have demonstrated a dose-dependent relaxation caused by PTH regardless of the presence or absence of endothelium.[1] There are data suggesting that PTH increases the production and activity of endothelial nitric oxide synthase (eNOS), which involves availability of nitric oxide, a potent vasodilatory substance.[12,30] Reports of an endothelial expression of PTH receptors are to our knowledge limited to experimental studies of the genetic and physiologic expression in different endothelial cell lines and to immunolocalisation in the umbilical vein, where a parathyroid hormone-related protein paracrine system appears to exist [2,34,35]

To conclude, PTH's effects on systemic haemodynamics need further investigation. Our results do not support the notion that PTH alone mediates any acute effects on endothelial vasodilatory function in forearm resistance vessels.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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