Parathyroid hormone induces adipocyte lipolysis via PKA-mediated phosphorylation of hormone-sensitive lipase

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Abstract

Parathyroid hormone (PTH) is secreted from the parathyroid glands in response to low plasma calcium levels. Besides its classical actions on bone and kidney, PTH may have other important effects, including metabolic effects, as suggested for instance by an increased prevalence of insulin resistance and type 2 diabetes in patients with primary hyperparathyroidism. Moreover, secondary hyperparathyroidism may contribute to the metabolic derangements that characterize states of vitamin D deficiency. PTH has been shown to induce adipose tissue lipolysis, but the details of the lipolytic action of PTH have not been described. Here we used primary mouse adipocytes to show that intact PTH (1-84) as well as the N-terminal fragment (1-37) acutely stimulated lipolysis in a dose-dependent manner, whereas the C-terminal fragment (38-84) was without lipolytic effect. The lipolytic action of PTH was paralleled by phosphorylation of known protein kinase A (PKA) substrates, i.e. hormone-sensitive lipase (HSL) and perilipin. The phosphorylation of HSL in response to PTH occurred at the known PKA sites S563 and S660, but not at the non-PKA site S565. PTH-induced lipolysis, as well as phosphorylation of HSL at S563 and S660, was blocked by both the PKA-inhibitor H89 and the adenylyl cyclase inhibitor MDL-12330A, whereas inhibitors of extracellular-regulated kinase (ERK), protein kinase B (PKB), AMP-activated protein kinase (AMPK) and Ca2+/calmodulin-dependent protein kinase (CaMK) had little or no effect. Inhibition of phosphodiesterase 4 (PDE4) strongly potentiated the lipolytic action of PTH, whereas inhibition of PDE3 had no effect. Our results show that the lipolytic action of PTH is mediated by the PKA signaling pathway with no or minor contribution of other signaling pathways and, furthermore, that the lipolytic action of PTH is limited by simultaneous activation of PDE4. Knowledge of the signaling pathways involved in the lipolytic action of PTH is important for our understanding of how metabolic derangements develop in states of hyperparathyroidism, including vitamin D deficiency.

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1. Introduction

In addition to its classical role in the regulation of blood calcium levels, there is accumulating evidence that parathyroid hormone (PTH) exerts other actions that may be of relevance for the development of extraskeletal disorders, including metabolic diseases. Positive correlations between circulating levels of glucose and PTH have been demonstrated in several studies [1–3]. An inverse relationship between PTH levels and insulin sensitivity has also been observed and an increased prevalence of diabetes is found in patients with high levels of PTH [4,5]. The association between insulin resistance and high levels of PTH is further supported by intervention studies with parathyroidectomy, which resulted in improved insulin sensitivity and normalized blood glucose levels [6–8]. High levels of PTH are not only associated with hallmarks of diabetes, but also with cardiovascular disease, as shown in several observational studies [9–11].

Primary hyperparathyroidism is a common endocrine disorder, especially among postmenopausal women. It is typically caused by the presence of one or several benign adenomas in the parathyroid glands secreting large amounts of PTH. Secondary hyperparathyroidism develops in response to low calcium levels in the blood, most commonly as a result of chronic kidney disease or vitamin D deficiency. During the last decade, the literature describing the association between vitamin D deficiency and metabolic disorders has grown immensely. It is likely that the hyperparathyroidism that accompanies vitamin D deficiency contributes to, or even accounts for, some of the many metabolic derangements that characterize vitamin D deficient states, as supported by a couple of recent reports demonstrating that vitamin D status and PTH levels are independent determinants of insulin sensitivity [12–14].

Abbreviations: AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; CaMK, Ca2+/calmodulin-dependent protein kinase; ERK, extracellular-regulated kinase; HSL, hormone-sensitive lipase; PKA, protein kinase A; PKB, protein kinase B; PTH, parathyroid hormone; PTHrP, PTH-related protein; PTH1R, PTH type 1 receptor; PTH2R, PTH type 2 receptor.

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PTH is released from the parathyroid glands in response to lowered blood calcium levels. Intact PTH is an 84 amino acid polypeptide (PTH 1-84) with a half-life of approximately 5 min in the circulation. It is processed in the Kupffer cells of the liver to an N-terminal fragment (PTH 1-37), with an even shorter half-life than intact PTH, and a C-terminal fragment (PTH 38-84), with a half-life of several hours. All of the classical biological activities of PTH are contained within the N-terminal fragment, whereas the biological roles of the long-lived C-terminal fragment is less known, but with regard to effects on bone they appear to be opposite to those of PTH 1-37 (for a review see [15]). PTH 1-84, as well as PTH 1-37, exerts their effects following binding to the PTH type 1 and 2 receptors (PTH1R and PTH2R), members of the G-protein coupled receptor family [16], whereas PTH 38-84 binds to a yet unknown receptor [8]. PTH-related protein (PTHrP), encoded by a gene distinct from the PTH gene, is expressed in many normal and malignant tissues and causes hypercalcemia in certain malignancies by acting through the PTH1R [17].

Ectopic lipid deposition plays a critical role in the development of insulin resistance, a hallmark of type 2 diabetes, and defects in adipocyte lipolysis may account for ectopic lipid deposition through increased flux of fatty acids to non-adipose organs [18]. Several hormones and cytokines have been shown to be involved in the regulation of lipolysis, with catecholamines being the major lipolytic hormones and insulin being the major anti-lipolytic hormone [19]. PTH has been shown to act lipolytically several decades ago in studies performed in humans as well as other primates [20–23]. Since these studies were performed, much has been learnt regarding both signaling pathways and lipases involved in the lipolytic process [24]. Thus, the aim of this study was to describe the lipolytic action of PTH on basis of the current knowledge of the molecular control of lipolysis.

2. Materials and methods

2.1. Materials

C57Bl/6J BomTac mice were obtained fromTacoon, Denmark. Endothelin-1, isoprenaline hydrochloride, free glycero reagent and glyc erol standard were from Sigma, fatty acid-free BSA from Roche diagnostics GmbH (Mannheim, Germany) and amplex ultra red and pre-cast Novex gels fromInvitrogen (Carlsbad, CA, USA). PTH (1-37), PTH (1-84) and PTHrP were from Bachem (Bubendorf, Switzerland) and PTH (38-84) was obtained from GL Biochem (Shanghai, China). MDL-12330A hydrochloride and KN-93 was from Calbiochem (San Diego, CA, USA). H89 was from Enzo lifescience (AH Diagnostics AB, Solna, Sweden), MK 2206 from Active Biochem (Bonn, Germany), MDL-12330A hydrochloride and KN-93 was from Calbiochem (San Diego, CA, USA), H89 was from Enzo lifescience (AH Diagnostics AB, Solna, Sweden), MK 2206 from Active Biochem (Bonn, Germany), RO-20-1724 from Biomol Research Labs (Plymouth Meeting, PA, USA) and OPC3911 from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan), MRT 199665 was a gift from Kristopher Clark, University of Dundee, UK. PD 0325901 was provided by the Division of Signal Transduction Therapy (University of Dundee, UK) and insulin was from Novo Nordisk A/S (Måløv, Denmark).

2.2. Antibodies

The antibody against total HSL protein was an affinity-purified rabbit anti-rat HSL made in house against homogenous recombinant rat HSL [25]. The phospho-specific antibodies against HSL (S563, S565 and S660), anti-PKB, anti-phospho ERK 1/2 S202/Y204, anti-ERK 1/2, anti-ACC and anti-phospho ACC S79 were obtained from Cell Signaling Technology (Beverly, MA). Anti-perilipin and anti-phospho perilipin S522 were purchased from Vala Sciences (San Diego, CA, USA), anti-phospho PKB S473 from Invitrogen (Carlsbad, CA, USA) and anti-phospho adipocyte triglyceride lipase (ATGL) S406 from Abcam (Cambridge, UK). Horseradish peroxidase (HRP)-conjugated secondary anti-mouse antibody was from GE Healthcare (Uppsala, Sweden) and HRP-conjugated secondary anti-rabbit antibody was from Pierce/Thermo Fisher Scientific (Waltham, MA, USA).

2.3. Isolation of primary mouse adipocytes

Adipocytes were isolated by collagenase digestion from epidymal adipose tissue of fed male C57BL/6J BomTac mice at the age of 9–12 weeks. The preparation was performed according to [26] except that the collagenase concentration was lowered from 1 mg/ml to 0.6 mg/ml and the incubation time was prolonged from 30 min to 75 min. The study was approved by the local Animal Ethics Committee, Lund University, Sweden.

2.4. Lipolysis assay and sample preparation

Cells were diluted to a concentration of 5% in Krebs Ringer buffer containing 25 mM Hepes, 1% fatty acid-free BSA, 200 nM adenosine and 2 mM glucose. In experiments with inhibitors, cells were pre-incubated with the respective inhibitor for 1 h (5 μM MK 2206, 3 μM MRT 199665 or 0.2 μM PD 0325901) or 30 min (50 μM H89, 25 μM MDL-12330A or 10 μM KN-93) at 37 °C in a shaking incubator at 60 rpm. Lipolysis was modulated by the addition of hormones as follows: PTH in concentrations ranging from 1 to 100 nM, insulin at 10 nM and isoprenaline at 20 nM. Cells were incubated at 37 °C in a shaking incubator, 150 rpm, for different time periods as indicated. Experiments were ended by incubating the cells on ice for 30 min before an aliquot of the incubation media was taken for analysis of glycerol release used as an index of lipolysis. Glycerol was measured using a commercially available kit with the addition of Amplex Ultra Red, a hydrogen peroxide sensitive fluorescence dye, described by Clark et al. [27]. The rest of the media was discarded and cells were dissolved in lysis buffer (50 mM Tris–HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, 1% NP40, 1 mM Na-orthovanadate, 40 mM NaF, 4 mM Na-pyrophosphate, 0.27 M sucrose, 500 mM DTT, 20 μg/ml leupeptin, 10 μg/ml antipain and 1 μg/ml pepstatin), followed by a 5 min centrifugation at 13,000 g to remove the fat layer.

2.5. Western blot analysis

An equal volume of lysate from each sample was subjected to Novex pre-cast SDS-PAGE gels, 4–12%, and electrophoresed to nitrocellulose membranes. The membranes were blocked in 2% BSA and 2% skimmed milk powder for 30 min before probing with primary antibodies overnight at 4 °C. Proteins were detected using horseradish-peroxidase conjugated secondary antibodies together with a chemiluminescence reagent: PTH in concentrations ranging from 1 to 100 nM, insulin at 10 nM and isoprenaline at 20 nM. Cells were incubated at 37 °C in a shaking incubator, 150 rpm, for different time periods as indicated. Experiments were ended by incubating the cells on ice for 30 min before an aliquot of the incubation media was taken for analysis of glycerol release used as an index of lipolysis. Glycerol was measured using a commercially available kit with the addition of Amplex Ultra Red, a hydrogen peroxide sensitive fluorescence dye, described by Clark et al. [27]. The rest of the media was discarded and cells were dissolved in lysis buffer (50 mM Tris–HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, 1% NP40, 1 mM Na-orthovanadate, 40 mM NaF, 4 mM Na-pyrophosphate, 0.27 M sucrose, 500 mM DTT, 20 μg/ml leupeptin, 10 μg/ml antipain and 1 μg/ml pepstatin), followed by a 5 min centrifugation at 13,000 g to remove the fat layer.

2.6. Human protein atlas

Images of immunohistochemically stained tissue samples were downloaded from the human protein atlas (www.proteinatlas.org) [28,29] and used according to their data usage policy. mRNA-sequencing data from the human protein atlas is based on The Human Protein Atlas version 13 and Ensembl version 75.37 [30].

2.7. GTEx portal and GEO profiles database

mRNA-sequencing data (reads per kilobase of transcript per million mapped reads [RPKM]) for PTH1R and PTH2R in human tissues was extracted from the data set: dbGaP accession number phs000424.v4.p1 downloaded 04/16/2015 [31] according to GTEx Data Release and Publication Policy (v05-08-15; table updated May 8, 2015) (http://www.gtexportal.org/home/). mRNA sequencing data (normalized read counts [NRG]) for mouse (C57BL/6J) epidymal adipose tissue was extracted from the GEO profiles database [32]; GEO accession GSE65976 [33].
2.8. Sequence alignment

Sequences |P12272|37-177 and |P01270|32-115 (UniProtKB) representing mature PTHrP and PTH, respectively, were compared by pairwise alignment using the EMBOSS needle (global) and water (local) programs [34].

2.9. Statistical analysis

Data are expressed as the mean ± S.D. Statistical significance was determined using unpaired two-tailed Student’s t-tests, unless otherwise indicated. The differences were considered statistically significant if \( p < 0.05 \). Statistical analysis was performed using Graph Pad Prism version 6 software (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. PTH induces lipolysis in mouse adipocytes in a dose-dependent manner

In order to describe the details of the lipolytic action of PTH, primary mouse adipocytes were stimulated with PTH and, for comparison, the non-selective \( \beta \)-adrenergic agonist isoprenaline. As an index of lipolysis, glycerol release into the medium was measured at three different time points. PTH was used in three different molecular forms; intact PTH (1-84), the N-terminal fragment (1-37) and the C-terminal fragment (38-84). As shown in Fig. 1, the N-terminal fragment of PTH as well as intact PTH stimulated lipolysis, whereas the C-terminal fragment exhibited no lipolytic effect. A clear dose-dependency was observed upon increasing the concentration from 1 to 100 nM, whereas little additional effect was observed upon raising the PTH concentration to 100 nM. In subsequent experiments, PTH (1-37) at a concentration of 10 nM was used. Compared to isoprenaline, PTH exerted a maximal effect that was approximately 25% of the effect of an isoprenaline concentration that in our hands gave maximal induction of lipolysis.

3.2. Stimulation of mouse adipocytes with PTH (1-37) induces phosphorylation of known PKA targets

After having established that PTH acts lipolytically in our experimental system, we next went on to investigate if the lipolytic action of PTH was paralleled by phosphorylation of known targets of protein kinase A (PKA). The rationale for this was two-fold. First, PKA is known to play a critical role in the molecular control of lipolysis through its phosphorylation of several of the key components, including perilipin 1, which coats the adipocyte lipid droplets and thereby regulates lipase access. It also phosphorylates hormone-sensitive lipase (HSL), which acts in concert with adipose triglyceride lipase (ATGL) to hydrolyze stored triacylglycerols (for a review see [35]). Secondly, PTH (1-37) is a ligand of the G-protein coupled PTH receptors which activate PKA pathways in target tissues. Perilipin 1 is known to be phosphorylated by PKA on at least three serine residues, including S522 [36]. HSL is phosphorylated at three serine residues by PKA, with S563 being the quantitatively dominating site and S660 being the activity-controlling site [37]. As shown in Fig. 2, stimulation of mouse adipocytes with PTH (1-37) induced phosphorylation of PKA sites of both perilipin 1 and HSL. The phosphorylation of HSL at S565, a site presumably phosphorylated by AMP-activated protein kinase (AMPK) and with an anti-lipolytic role [38], was reduced upon stimulation with PTH, as was also the case upon stimulation with isoprenaline.

ATGL was recently described to be phosphorylated by PKA at S406 and phosphorylation of this site following \( \beta \)-adrenergic stimulation of adipocytes was proposed to be coupled to a moderate increase in ATGL activity and thus part of the molecular control of lipolysis [39]. Using a commercially available phospho-specific antibody against S406 of ATGL, we found no evidence for phosphorylation of this site in response to stimulation of mouse adipocytes with either isoprenaline or PTH (data not shown). The reason for this discrepancy against published data is not clear. However, we observed that the antibody in addition to ATGL also recognized a slightly larger and very abundant phosphoprotein.

Fig. 1. Lipolysis in response to stimulation with different PTH fragments. Primary mouse adipocytes were isolated and stimulated with (A) intact PTH (1-84), (B) the N-terminal fragment of PTH (1-37) or (C) the C-terminal fragment of PTH (38-84) in increasing concentrations from 1 to 100 nM for 3, 10 or 30 min. Untreated cells (control) and cells stimulated with 20 nM isoprenaline (iso) were used for comparison. Lipolysis was measured as glycerol concentration in the medium. The graphs represent mean values ± SD from three experiments per fragment. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \).
3.3. Inhibition of PKA completely blocks the lipolytic effect of PTH

After having established that known PKA targets are phosphorylated in response to PTH stimulation, we next investigated the effects of blocking the PKA signaling pathway on the lipolytic effect of PTH. Preincubating mouse adipocytes with H89, an inhibitor of PKA, was found to completely prevent the lipolytic response to both PTH and isoprenaline (Fig. 3a). For both agonists, this was accompanied by a reduction of the phosphorylation of the PKA sites of HSL (S563 and S660), whereas phosphorylation of the non-PKA site (S565) tended to be increased. To further validate the involvement of the PKA signaling pathway, we used an inhibitor of adenylate cyclase, MDL-12330A. This inhibitor was found to reverse the lipolytic response to both PTH and isoprenaline, although not to the same extent as H89 (Fig. 3b). As for H89, the reduction in lipolysis was accompanied by a reduction in the phosphorylation of the PKA sites of HSL.

3.4. Inhibition of ERK, PKB, AMPK or CaMK has little or no effect on the lipolytic action of PTH

Besides PKA, which is the key kinase in stimulation of lipolysis, other kinases have been implicated in the molecular control of lipolysis. Furthermore, at least in classical PTH target tissues, such as the kidney, binding of PTH to its receptor couples not only to Goαs and thus the adenylate cyclase-cAMP-PKA pathway, but also to Goαq, and thus signal transduction pathways involving phospholipase C, diacylglycerol, inositol triphosphate and calcium-dependent kinases. To investigate a possible contribution by other kinases than PKA to PTH-induced lipolysis, we used specific inhibitors to other relevant kinases. Extracellular-regulated kinase (ERK) has been implicated in both cAMP-dependent and cAMP-independent lipolysis and was in one study shown to phosphorylate HSL at a site (S600) distinct from the three sites already mentioned [40]. Pre-treatment with the selective ERK inhibitor PD 0325901 had no effect on PTH-stimulated lipolysis (Fig. 4a). In line with this, we observed no effects of PD 0325901 on the phosphorylation of HSL. Inhibition of endothelin-induced phosphorylation of ERK served as a control for functionality of the PD inhibitor in our experimental system. PKB is believed to be a key kinase in the antilipolytic effect of insulin through its phosphorylation and activation of PDE3B [41]. Pre-treatment with the selective PKB inhibitor MK 2206 [42] had no effect on PTH-induced lipolysis or phosphorylation of HSL, although it completely blocked the insulin-induced phosphorylation of PKB (Fig. 4b). AMPK activation has been shown to inhibit lipolysis and promote phosphorylation of HSL at S565, which prevents phosphorylation at the PKA sites [38]. Phosphorylation of HSL at S565 by AMPK has also been demonstrated in vitro [43]. Pre-treatment with the AMPK family inhibitor MRT 199665 tended to increase PTH-induced lipolysis (Fig. 4c). At the same time, phosphorylation at S565 was blunted, whereas...
phosphorylation at S563 and S660 was increased. The efficiency of the inhibitor was confirmed by its ability to markedly reduce phosphorylation of acetyl-CoA carboxylase, a known AMPK substrate. PTH induces activation of calcium-dependent kinases and calcium/calmodulin-dependent protein kinase (CaMK) has been shown to phosphorylate S565 of HSL, thereby preventing phosphorylation of the PKA sites[43]. Moreover, increasing the intracellular concentration of calcium has been shown to inhibit isoprenaline-induced lipolysis, presumably through activation of PDE and thereby reduction of cAMP levels and HSL phosphorylation[44]. Pre-treatment with 3 μM of the AMPK family inhibitor MRT 199665 for 60 min. Inhibition of phosphorylation of acetyl-CoA carboxylase A (ACC) served as positive control for MRT 199665. (D) Pre-treatment with 10 μM of the CaMK inhibitor KN-93 for 30 min. The graph illustrates mean values ± SD from 3–4 experiments, shown together with representative blots. *p < 0.05.

3.5. Expression of PTH receptors in adipose tissue

As shown in the lipolysis experiments displayed in Figs. 1 and 2, the maximal PTH-induced lipolysis was about 25% of the maximal isoprenaline-induced lipolysis. One possible explanation for this is that the number of PTH receptors is low. According to data deposited in the GTEx Portal and the Human Protein Atlas, the expression of PTH1R mRNA and protein is medium in human adipose tissue (Table 1 and Fig. 5a–b), whereas the expression of PTH2R is below detectable levels (Table 1 and Fig. 5c). With regard to data for mouse, a search of the GEO Profiles database showed that PTH1R is expressed in mouse epididymal adipose tissue, whereas the expression of PTH2R falls under the detection limit (Table 2).

3.6. Potentiation of PTH-induced lipolysis by inhibition of PDE4

Phosphodiesterases (PDEs), subdivided into 11 families, modulate the duration and intensity of the intracellular response to cAMP by hydrolyzing the cyclic nucleotide. Different cells show different expression patterns of PDEs and the different PDEs exhibit different intracellular distribution and catalytic and regulatory properties. PDE3B and PDE4 are the main PDEs expressed in adipocytes, the former having a key role in mediating insulin-induced inhibition of catecholamine-induced lipolysis. One possibility is that the difference in lipolytic capacity...
between PTH and isoprenaline is related to the activity and/or amount of PDE associated with the respective signaling complexes. Thus, to test the ability of PDE3 and PDE4 to restrain cAMP signaling initiated by the respective hormones, we utilized selective inhibitors for PDE3 and PDE4. Pre-treatment with OPC3911, a selective inhibitor of PDE3, was found to have no effect on either PTH-induced nor isoprenaline-induced lipolysis (Fig. 6a). Pre-treatment with RO-20-1724, a selective PDE4 inhibitor, on the contrary, was found to potentiate PTH-induced lipolysis by more than 100%. Also isoprenaline-induced lipolysis tended to be potentiated by RO-20-1724, although to a much lower degree and not statistically significant. In the presence of RO-20-1724, the lipolytic response to PTH was 56% of that in response to isoprenaline, compared to 27% in the absence of RO-20-1724 (Fig. 6a). Thus, both PTH and isoprenaline induce signaling pathways leading up to simultaneous cAMP formation and breakdown. However, the balance between these two pathways is different for the two agonists, with isoprenaline acting mainly lipolytically, whereas PTH appears to activate the two pathways to a similar extent.

Several studies have shown that the lipolytic effect exerted by isoprenaline, and other β-adrenergic agonists, can be completely blocked by insulin and that activation of PDE3B is the major mechanisms underlying this effect. Insulin, at concentrations found to completely block isoprenaline-induced lipolysis, was found also to completely block PTH-induced lipolysis. In both cases, the anti-lipolytic effect of insulin was paralleled by decreased phosphorylation of HSL (Fig. 6b). For both agonists, pre-treatment with OPC3911 blocked the anti-lipolytic effect of insulin, whereas RO-20-1724 did not (Fig. 6). Thus, PTH-induced lipolysis can be blocked by insulin-mediated activation of PDE3B, as is the case for isoprenaline-induced lipolysis, shown both here and previously [41].

3.7. Parathyroid hormone-related protein (PTHrP) exerts the same lipolytic action as PTH (1-37)

During the course of this work, PTHrP was shown to be involved in mediating energy wasting in fat tissues and to contribute to cancer cachexia [45]. Furthermore, using primary white and brown adipocytes differentiated from the inguinal stromal–vascular fraction of male mice, it was shown that PTHrP, as well as PTH, induced PKA-mediated phosphorylation of HSL that could be blocked by H89. PTHrP has a global sequence identity to PTH of 14% and a local sequence identity of 34.4% with the first 32 amino acids of the N-terminal fragment (Fig. 7a). To confirm that PTHrP acts lipolytically, we employed it in our experimental system and found it to behave identically to PTH (1-37) with regard to lipolytic potency at 10 nM (Fig. 7b).

4. Discussion

To the best of our knowledge this is the first study describing molecular details of the lipolytic action of PTH. PTH was found to exert its lipolytic effect through PKA-mediated phosphorylation of HSL, with minor or no contribution from other kinases. The maximal lipolytic effect of PTH was about 25% of that of isoprenaline, a non-selective β-adrenergic agonist. Simultaneous activation of the cAMP-PKA pathway and the anti-lipolytic pathway leading to activation of PDE4, rather than low expression of PTH receptors, explains the low maximal lipolytic effect of PTH, since the lipolytic effect of PTH in the presence of RO-20-1724, a PDE4 inhibitor, was comparable to that of isoprenaline.

The conclusion that PTH exerts its lipolytic action via activation of the cAMP-PKA pathway with no or minor contribution from other signaling pathways is based on the finding that inhibitors of the cAMP-PKA pathway blocked the lipolytic action of PTH, whereas inhibitors of other kinases implicated in lipolysis regulation or PTH signaling, i.e. ERK, PKB, AMPK, and CaMK, did not. In fact, inhibition of AMPK tended to stimulate basal lipolysis as well as lipolysis induced by both PTH and isoprenaline. This was paralleled by complete abolishment of the phosphorylation of HSL at S565 and increased phosphorylation of the PKA sites (S563 and S660), well in line with the established anti-lipolytic role of S565 [38]. In classical PTH target tissues, PTH induces activation of calcium-dependent kinases and calcium/calmodulin-dependent protein kinase (CaMK) has been shown to phosphorylate S565 of HSL, thereby preventing phosphorylation of the PKA sites [43]. The fact that...
Fig. 5. Expression of PTH receptors in adipose tissue. (A) Representative images of human tissues stained using antibodies specific for PTH1R. Images were downloaded from www.proteinatlas.org and used according to their data usage policy. (B) Pthr1 and (C) Pthr2 mRNA expression levels in human tissues based on mRNA-seq data derived from the GTEx portal (in accordance with their data usage policy). Data is presented as Reads Per Kilobase of transcript per Million mapped reads (RPKM) ± standard deviation (log scale), n = 22–142 depending on tissue.
the CaMK inhibitor KN-93 was without effect on PTH-induced lipolysis suggests that CaMK is not induced in response to PTH stimulation of adipocytes and/or that CaMK does not phosphorylate HSL in vivo. In bone, and especially kidney, PTH has been shown to mediate some of its effects via binding to Gq-coupled receptors, which activate signaling cascades involving phospholipase C, the second messengers inositol-1,4,5-trisphosphate, calcium and diacylglycerol and protein kinase C [46,47]. Thus, although PTH appears to mediate effects on lipolysis mainly through coupling to Gs and thus activation of cAMP-PKA signaling cascades, minor contributions from signaling through Gq cannot be excluded.

![Fig. 6](image-url)

**Fig. 6.** Effects of inhibitors of PDE3B and PDE4 on PTH-induced lipolysis and HSL phosphorylation. Primary mouse adipocytes were isolated and pre-treated with or without 10 μM OPC3911 or 10 μM RO-20-1724 for 30 min. Cells were then stimulated with 10 nM PTH (1-37), 20 nM isoprenaline, or 10 nM insulin for 30 min or left untreated. Lipolysis was measured as glycerol concentration in the medium (A). Aliquots of whole cell lysates were subjected to Western blot analysis with antibodies recognizing HSL phosphorylated at S660, S563 and S565 and total HSL (B). The graph illustrates mean values ± SD from three experiments, shown together with representative blots. *p < 0.05, **p < 0.01, ***p < 0.001.

Data deposited in the GTEx Portal and the Human Protein Atlas showed that PTH1R is expressed at medium levels in adipose tissue, whereas PTH2R is expressed at low or non-detectable levels with a good correlation between mRNA and protein data. These data are in disagreement with a recently published paper on the expression of different G-protein coupled receptors in human adipose tissue, showing that both receptors type are expressed at only trace levels [48]. The reason for the discrepancy is not known but it should be pointed out that the data deposited in GTEx portal and human protein atlas is based on RNA sequencing data and immunohistochemical analyses, respectively, whereas the data in the paper by Amisten et al. [48] is based on mRNA quantification by qPCR. mRNA sequencing data deposited in the GEO Profiles Database showed that PTH1R is expressed also in mouse epididymal adipose tissue, whereas the expression of PTH2R is below the detection limit. Since the data in the GEO Profiles Database is from independent experiments, no comparisons to the expression levels in other mouse tissues can be made.

Since the data in the GTEx Portal and the Human Protein Atlas suggested that at least PTH1R is expressed at significant levels in adipose tissue, we next considered that the reason for the low maximal lipolytic effect of PTH was explained by properties of the PTH signaling rather than a low abundance of PTH receptors. In fact, blocking the activity of one of the major phosphodiesterases in adipose tissue, i.e. PDE4, significantly increased the lipolytic action of PTH, whereas it had a minor effect on the lipolytic action of isoprenaline. This indicates that the balance between lipolytic and anti-lipolytic action is very different for isoprenaline and PTH, with isoprenaline signaling predominantly through the lipolytic, cAMP-generating pathway, whereas PTH to a much larger extent activates also an anti-lipolytic, cAMP-hydrolyzing pathway. Thus, PTH exerts both lipolytic and anti-lipolytic actions and only in the presence of a PDE4 inhibitor is its full lipolytic action observed. The lipolytic effect of PTH can be antagonized by insulin in a PDE3B-dependent manner, as is the case also for isoprenaline.

The PTH levels in plasma are under normal physiological conditions in the picomolar range, and only under pathological conditions do they approach nanomolar concentrations. Secondary hyperparathyroidism as a result of severe kidney failure can be accompanied by very high levels of PTH, whereas secondary hyperparathyroidism, due to vitamin D deficiency without any kidney disease, and also primary hyperparathyroidism, due to the presence of adenomas of the parathyroid glands, usually are accompanied by more moderate elevations of PTH levels. In addition to elevated levels of PTH in different states of hyperparathyroidism, high, or even very high, levels of PTHrP are observed in tumor diseases, where it contributes to the development of cachexia, as recently demonstrated [45]. PTHrP binds to PTH1R and signals through the cAMP-PKA pathway, as shown both previously [45] and in the present study. In agreement with this, the lipolytic action exerted by PTHrP was comparable to that of PTH, as shown in the present study.

Elevated PTH levels may contribute to a metabolic phenotype in the different forms of hyperparathyroidism, where vitamin D deficiency presumably is the most prevalent condition accompanied by elevated PTH levels. Several clinical studies demonstrate that vitamin D status and PTH levels are independent determinants of insulin sensitivity with increased PTH levels being associated with decreased insulin sensitivity [4–8]. The mechanisms whereby PTH may decrease insulin sensitivity are not known, but one possible mechanism is that chronically elevated PTH levels, via its lipolytic action, lead to increased flux of fatty acid from adipose tissue and thereby ectopic lipid deposition, which plays a critical role in the development of insulin resistance [34]. Vitamin D deficiency and accompanying secondary hyperparathyroidism is common among obese individuals, possibly due to trapping of vitamin D in adipose tissue [16]. Obese individuals also exhibit decreased levels of PDE4 activity in subcutaneous and, even more pronounced, omental adipocytes compared to normal weight individuals [49]. Whether this is reflected in a more pronounced lipolytic action of PTH in obese than normal weight individuals is not known, but
constitutes an interesting question for future studies. Besides obesity, pharmacological inhibition of PDE4, currently in clinical use for maintenance treatment of chronic pulmonary obstructive disease [50], is another condition where the lipolytic action of PTH is potentiated. Again, whether this is the case in the clinical situation remains to be clarified.

5. Conclusions

In summary, we conclude that PTH induces lipolysis in primary mouse adipocytes via activation of the cAMP-PKA pathway, with no or little contribution from other kinases. The lipolytic action of PTH is weak compared to the non-selective β-adrenergic agonist isoprenaline. However, the lipolytic action of PTH is potentiated by more than 100% in the presence of a PDE4 inhibitor, whereas the lipolytic action of isoprenaline is not affected by the PDE4 inhibitor. The metabolic impact of elevated PTH levels in combination with decreased PTH levels is not yet understood. However, our findings suggest that in situations of elevated PTH levels in combination with decreased PTH activity in adipose tissue, such as in vitamin D deficiency in combination with obesity and/or treatment with PDE4 inhibitors, PTH could contribute to increased fatty acid flux from adipose tissue, which in turn may lead to the development of insulin resistance and other metabolic derangements.

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References
