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Effects of orexin A on proliferation, survival, apoptosis and differentiation of 3T3-L1 preadipocytes into mature adipocytes

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ABSTRACT

Metabolic activities of orexin A (OXA) in mature adipocytes are mediated via PI3K/PKB and PPARγ. However, the effects of OXA on preadipocytes are largely unknown. We report here that OXA stimulates the proliferation and viability of 3T3-L1 preadipocytes and protects them from apoptosis via ERK1/2, but not through PKB. OXA reduces proapoptotic activity of caspase-3 via ERK1/2. Inhibition of ERK1/2 prevents the differentiation of preadipocytes into adipocytes. Unlike insulin, neither short-term nor prolonged exposure of 3T3-L1 preadipocytes to OXA induces preadipocyte differentiation to adipocytes, despite increased ERK1/2 phosphorylation. Unlike insulin, OXA fails to activate PKB, which explains its inability to induce the differentiation of preadipocytes.

1. Introduction

The differentiation of fibroblastic precursor cells (preadipocytes) into adipocytes is controlled by the orchestration of multiple transcription factors [1]. PPARγ and C/EBP are indispensable transcription factors for adipocytes differentiation. Exposure of 3T3-L1 fibroblasts to stimuli of cAMP production (IBMX), together with PI3K/PKB activators, and dexamethasone (DEXA) leads to differentiation of these cells into mature adipocytes [2–4].

Recently, we and others showed that the neuropeptide orexin A (OXA) regulates metabolic and endocrine functions in human and rodent mature adipocytes [5,6]. OXA also increased lipid accumulation, adiponectin secretion, glucose uptake and inhibited lipolysis in 3T3-L1 adipocytes through a PPARγ-dependent pathway [5]. Since OXA also stimulated 3T3-L1 fibroblasts proliferation [7], we assumed that OXA also influences the differentiation of preadipocytes into mature adipocytes. To test this hypothesis, we studied whether OXA can induce the adipocytes differentiation and characterized the mechanisms underlying the proliferative activity of OXA in 3T3-L1 preadipocytes.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, all reagents were from Sigma–Aldrich (Deisenhofen, Germany). LY294002, U0126 (both used at 10 μM) and MTT were from Calbiochem (San Diego, CA, USA). DMEM and supplements for cell culture were from GIBCO Invitrogen (Karlsruhe, Germany). Specifications of all antibodies are given in Supplementary Table 1.

Abbreviations: AKT, protein kinase B (PKB); BrDU, 5-bromo-2′-deoxyuridine; C/EBPα, CCAAT/enhancer binding protein alpha; cAMP, cyclic adenosine monophosphate; DEXA, dexamethasone; ERK1/2, extracellular signal-regulated kinase 1/2; FCS, fetal calf serum; HPRT, hypoxanthine–guanine phosphoribosyltransferase; IBMX, 3-isobutyl-1-methylxanthine; IGF-1, insulin-like growth factor 1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ORO, oil red O; OXA, orexin A; PI3K, phosphatidylinositol 3-kinase; PPARγ, peroxisome proliferator-activated receptor gamma; SDM, standard differentiation medium; SGM, standard growth medium

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2.2. Cell culture

3T3-L1 and NIH/3T3 fibroblasts (ATCC, LGC Standards, Wesel, Germany) were maintained in a standard growth medium (SGM: DMEM, 10% FCS, 100 kU/l penicillin, 100 mg/l streptomycin) at 37 °C in a humidified atmosphere (5% CO_2, 95% O_2).

2.3. Isolation and differentiation of rat primary preadipocytes

Rat preadipocytes were isolated as previously described [8]. Differentiation was induced by treatment with DMEM/F12 medium supplemented with 2 nM T3, 167 nM insulin and 30 nM dexamethasone with or without 100 nM OXA for 5 days.

2.4. 3T3-L1 fibroblasts differentiation

Two days post-confluent 3T3-L1 preadipocytes were differentiated in SGM supplemented with 1 μM DEXA and 500 μM IBMX with or without 100 nM OXA, or 1 μM insulin. After 2 days, medium was replaced by DMEM (10% FCS) containing either 100 nM OXA or 1 μM insulin, and cells were incubated for an additional 2 and 4 days, respectively. In a parallel study evaluating ERK1/2 regulation, cells were incubated in standard differentiation medium (SDM: DMEM, 10% FCS, 1 μM DEXA, 500 μM IBMX) in the presence or absence of 10 μM U0126 for 2 days. Two additional groups were incubated in SDM supplemented with insulin (1 μM) or OXA (100 nM). Then cells were harvested for the quantification of *Pparγ* and *C/ebpα* mRNA levels or cultured in SGM for an additional 2 and 4 days, respectively. Cellular triacylglycerol content was then measured.

2.5. Proliferation

3T3-L1, NIH/3T3 or isolated rat primary preadipocytes were seeded (2×10^3 cells/well) in 96-well plates and cultured for 24 h. To synchronize cell cycle, cells were serum-deprived for 24 h and then treated with test agents for a further 24 h. BrdU solution (10 μM) was then added and cells were incubated for 2.5 h. The BrdU incorporation into the DNA was measured by Cell Proliferation ELISA BrdU colorimetric kit (Roche Diagnostics, Penzberg, Germany).

2.6. Cell viability

3T3-L1 preadipocytes were seeded (2×10^3 cells/well) in 96-well plates and cultured for 24 h. Following incubation in a serum-free DMEM supplemented with test agents for 48 h, MTT solution (0.5 mg/ml) was added. After an additional 3 h, the culture medium was removed and the formed formazan crystals were dissolved in 100 μl DMSO. Optical density was measured by a plate reader (SpectraMax Plus^{384} microplate reader, Molecular Devices, Ismaning, Germany) at 570 nm and 650 nm (reference wave length).

2.7. Preparation and administration of palmitic acid

Palmitic acid solution was prepared and administrated as previously described [9].

2.8. Cell death

3T3-L1 fibroblasts were cultured in six-well plates to 80% confluency and treated with 100 nM OXA with or without 10 μM U0126 for 48 h in serum-free DMEM with or without PA. Cell death was assessed by quantifying the cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes), using Cell Death Detection ELISA kit (Roche Diagnostics, Penzberg Germany).

2.9. Caspase-3 activity

Preadipocytes were cultured in a serum-free medium in six-well plates (1.5×10^5 cells/well). Caspase-3 activity was assessed using a Caspase-3 Colorimetric Assay Kit (BioVision Inc., Headquarters, Milipitas, CA, USA).

2.10. Western blot

Differentiated (day 8 post-differentiation) or non-differentiated 3T3-L1 cells were cultured in six-well plates and serum-deprived for 24 h before treatment with test agents. Cell lysates, protein isolation and Western blots were performed essentially, as described [10]. The signal intensities of phosphorylated ERK1/2 or PKB were measured using Quantity One 1-D Analysis software (Bio-Rad Laboratories, Munich, Germany) and the results were normalized against total ERK1/2 or total PKB.

2.11. Oil red O staining

Differentiated 3T3-L1 adipocytes were stained with ORO, as described [5]. The absorbance reading of the eluate was performed at 500 nm wave length using SpectraMax Plus^{384} microplate reader (Molecular Devices, Ismaning, Germany).

2.12. Intracellular triacylglycerol content

After exposure to test peptides, cells were washed with PBS, scraped and sonicated in a triacylglycerol assay buffer containing 50 mM Tris–HCl (pH 7.4) and 1 mM EDTA. Triacylglycerol was determined using a colorimetric triacylglycerol kit (Cypress Diagnostics, Langdorp, Belgium) and normalized to protein content.

2.13. Real-time PCR

Total RNA isolation, purification and quantitative RT-PCR were performed as described [8]. Sequences of gene-specific primers are given in Supplementary Table 2. Data were normalized to *Hprt* mRNA.

2.14. Statistical analysis

If not indicated, then data are mean ± S.E.M. of at least three independent experiments, performed in triplicates. Statistical analysis was performed using unpaired Student's *t* test; $^{*}P < 0.05$; $^{**}P < 0.01$ vs. appropriate control group.

3. Results

3.1. OXA enhances 3T3-L1 fibroblasts proliferation and viability

OXA (1–100 nM) increased 3T3-L1 cell proliferation in both, serum-free (Fig. 1A) and serum-containing medium (Fig. 1B). Moreover, OXA (100 nM) potentiated insulin-stimulated proliferation (Fig. 1C) and dose-dependently enhanced cell viability (Fig. 1D). Notably, OXA dose-dependently stimulated the proliferation of NIH/3T3 fibroblasts (Supplementary Fig. 2).

3.2. OXA increases 3T3-L1 fibroblasts proliferation and viability via ERK1/2-dependent mechanism

OXA stimulated ERK1/2 phosphorylation by ~4-fold over basal in non-differentiated 3T3-L1 cells, after 5 and 10 min (Fig. 2A), and by ~2-fold over basal after 10 min in mature 3T3-L1 adipocytes (Fig. 2B). Pharmacological blockade of ERK1/2 but not PI3K

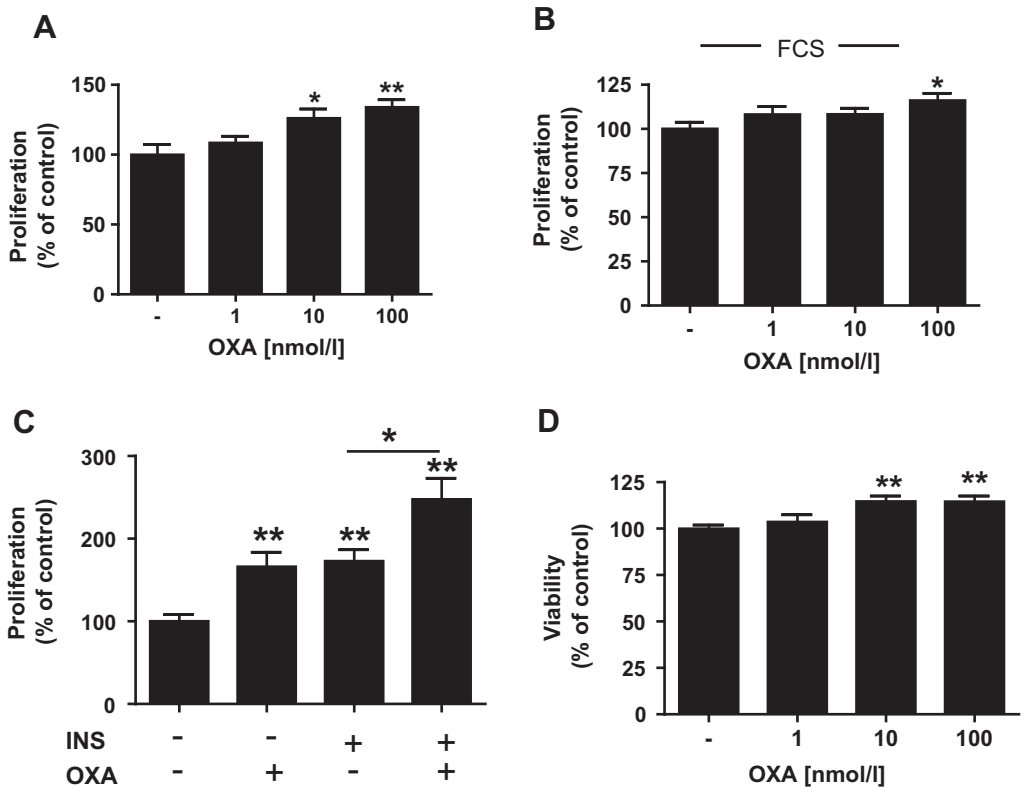

Fig. 1. OXA stimulates 3T3-L1 fibroblast proliferation and viability. 3T3-L1 preadipocytes were treated with OXA (1–100 nM) for 24 h in the absence (A) or presence of 10% FCS (B). (C) 3T3-L1 preadipocytes were exposed for 24 h to either 100 nM OXA or 10 nM insulin (INS), or both. Cell proliferation was assessed by BrdU assay (A–C). (D) 3T3-L1 cells were incubated with 100 nM OXA for 48 h and the cell viability was detected by MTT assay.

prevented OXA in stimulating ERK1/2 phosphorylation (Fig. 2C and D). OXA failed to stimulate 3T3-L1 proliferation and viability in the presence of ERK1/2 blocker U0126 but not in the presence of PI3K blocker LY294002 (Fig. 2E and F). These data suggest that ERK1/2 participates in OXA-induced stimulation of proliferation and viability.

3.3. OXA protects from serum deprivation and palmitic acid-induced apoptosis

Serum deprivation-induced apoptotic death of 3T3-L1 fibroblasts was prevented by OXA (Fig. 3A). OXA failed to protect fibroblasts against apoptosis in the presence of U0126 (Fig. 3B). Serum starvation-induced apoptosis was associated with caspase-3 activation, consistent with the results of a previous study [11] and Fig. 3C. OXA inhibited serum deprivation-stimulated caspase-3 activity (Fig. 3C), but was ineffective in the presence of U0126 (Fig. 3C). These findings suggest that OXA protects 3T3-L1 fibroblasts against apoptosis and blocks the activation of the pro-apoptotic executor protease caspase-3 via ERK1/2.

In obesity and type 2 diabetes increased FFA levels contribute to apoptosis. We therefore tested whether OXA protects 3T3-L1 fibroblasts against palmitic acid (PA)-induced apoptosis. PA-stimulated apoptosis and activation of caspase-3 in 3T3-L1 fibroblasts were attenuated by OXA (Fig. 3D and E).

3.4. ERK1/2 stimulates preadipocytes differentiation

Due to inconsistencies in the literature [12], we initially clarified the role of ERK1/2 in regulating the differentiation of preadipocytes. Incubation of 3T3-L1 cells in the SDM containing IBMX/DEXA for 48 h resulted in and a ~10-fold (*C/ebpα*) a ~14-fold (*Pparγ*) increase of mRNA expression of both surrogate markers of adipocyte differentiation (Fig. 4A and B). In the presence of U0126 the increases of *Pparγ* and *C/ebpα* mRNA expression were lost (Fig. 4A and B), indicating that ERK1/2 enhances the early differentiation process.

3.5. OXA fails to enhance the differentiation of 3T3-L1 fibroblasts into mature adipocytes

Since OXA increased pERK1/2 in 3T3-L1 preadipocytes, we tested whether OXA enhances the IBMX/DEXA-induced early differentiation. *Pparγ* and *C/ebpα* mRNA expression levels in 3T3-L1 fibroblasts incubated for 48 h (short-term) in the SDM containing 100 nM OXA were comparable to that measured in cells incubated in the SDM (contains IBMX/DEXA), only (Fig. 4A and B). In contrast, inclusion of insulin (1 μM) in the SDM potentiated the increases of both *Pparγ* and *C/ebpα* mRNA in differentiating 3T3-L1 cells (Fig. 4A and B).

To confirm the role of ERK1/2 in regulating the early-stage preadipocytes differentiation (first 48 h), we studied the consequences of ERK1/2 blockade on triacylglycerol accumulation (additional surrogate parameter of adipocytes differentiation) [13]. Four (Fig. 4C) or six days (Fig. 4D) after the onset of differentiation strongly increased triacylglycerol contents were detected in preadipocytes incubated in SDM. The blockade of the ERK1/2 pathway during the first 48 h of differentiation attenuated cellular triacylglycerol accumulation after 4 (Fig. 4C) or 6 days (Fig. 4D) of differentiation. In contrast, OXA failed to enhance the triacylglycerol content in 3T3-L1 preadipocytes (Fig. 4C and D). These results show that the activation of ERK1/2 pathway increases the

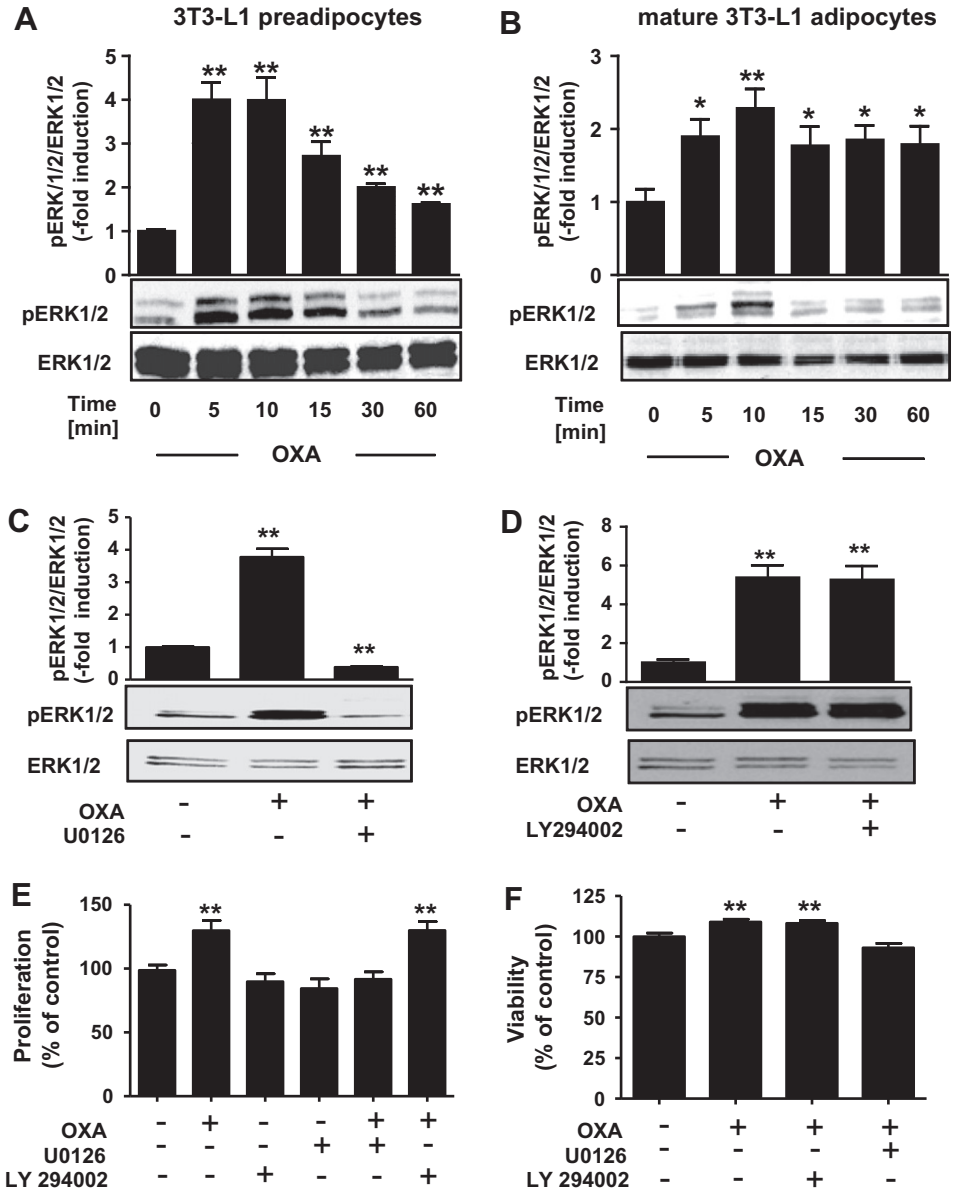

Fig. 2. OXA increases 3T3-L1 fibroblast proliferation and viability via ERK1/2 dependent mechanism. 3T3-L1 fibroblasts (A) and differentiated 3T3-L1 adipocytes (B) were treated with 100 nM OXA. ERK1/2 phosphorylation (pERK1/2) was measured by Western blots. Effect of the ERK1/2 inhibitor U0126 (10 μM) (C) or the PI3K inhibitor LY 294002 (10 μM) (D) on 100 nM OXA-stimulated (5 min) ERK1/2 phosphorylation in 3T3-L1 fibroblasts. The signal intensity of the pERK1/2 (corresponds to 42 and 44 kDa) was normalized against the total ERK1/2 (lower bands of the representative Western blot). The relative expressions of pERK1/2 vs. total ERK1/2 (±S.E.M.) (*x*-fold induction) are shown (upper panel). Lower panel: Representative Western blots of pERK1/2 (upper band) and total ERK1/2 (lower band). Effect of U0126 (10 μM) or LY 294002 (10 μM) on OXA-stimulated 3T3-L1 fibroblasts proliferation after 24 h (E) or cell viability after 48 h (F).

differentiation of 3T3-L1 fibroblasts into adipocytes, whereas short-term (48 h) treatment with OXA – despite stimulation of ERK1/2 – fails to enhance the differentiation.

While 48 h treatment with OXA was ineffective, we tested whether a prolonged (up to 6 days) exposure to OXA enhances triacylglycerol accumulation in preadipocytes (Fig. 4E and F). 3T3-L1 fibroblasts were incubated in a SDM supplemented either with 1 μM insulin (positive control) or 100 nM OXA for 2 days. Thereafter, cells were switched to a SGM and divided into three different treatment groups. Cells were either continuously incubated either with OXA or insulin, or in the SGM for 2 or 4 days. During the first 2, 4 and 6 days of incubation with OXA, no further increase of cellular triacylglycerol accumulation was detected, as compared to 3T3-L1 preadipocytes incubated in the SGM without OXA (Fig. 4E and F). Expectedly, preadipocytes differentiated in the presence of insulin (positive control) showed an increase of intracellular triacylglycerol content (Fig. 4E and F). These data indicate that neither short- nor long-term incubation in SDM supplemented with OXA potentiates the differentiation of 3T3-L1 preadipocytes. Expectedly, also OXA alone was ineffective at inducing 3T3-L1 cell differentiation (Supplementary Fig. 1A–D).

Activation of PI3K/PKB signaling is required for insulin-induced adipocytes differentiation [4,14,15]. In mature adipocytes, OXA can increase PKB phosphorylation [5], however, this has not yet been investigated in preadipocytes. We therefore tested the ability of OXA to induce PKB phosphorylation in 3T3-L1 cells during the differentiation procedure (Fig. 4G). Expectedly, insulin stimulated PKB phosphorylation in preadipocytes during the differentiation

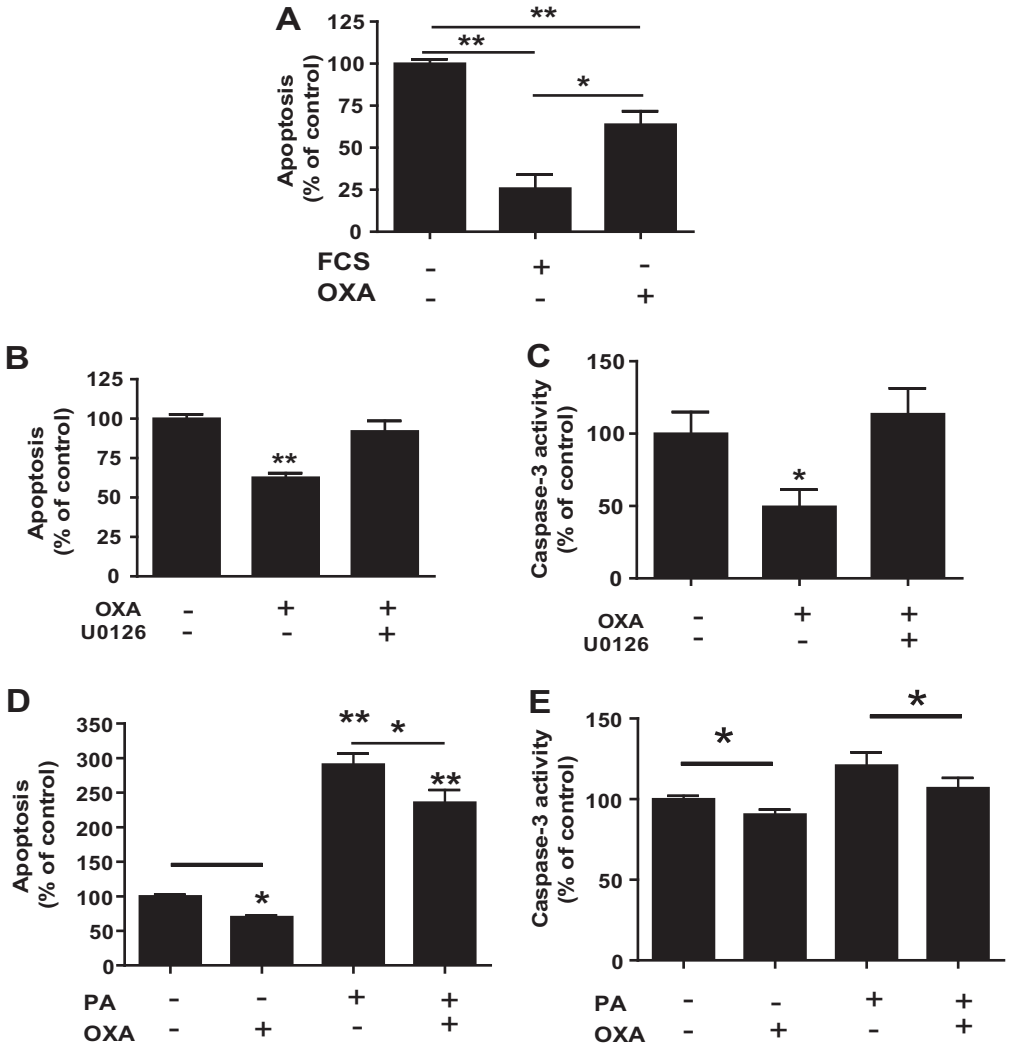

Fig. 3. OXA protects 3T3-L1 fibroblasts against serum deprivation- or palmitic acid-induced apoptosis. Effect of 100 nM OXA on serum-deprivation stimulated apoptosis in the presence or absence of the ERK1/2 blocker U0126 (10 μM) (A and B). Reduction of caspase-3 activity by OXA (48 h treatment) in the absence of U0126 (C). *Note:* U0126 prevents OXA at protecting cells against apoptosis and against attenuation of caspase-3 activity (B and C). Attenuation of 0.2 mM palmitic acid-stimulated apoptosis by OXA (D). Reduction of palmitic acid-stimulated caspase-3 activity by OXA (E).

process, whereas OXA was ineffective. Thus, the failure of OXA to stimulate preadipocytes differentiation is possibly due to the lack of PKB activation.

3.6. OXA stimulates the proliferation of isolated rat primary preadipocytes without influencing their differentiation

We studied the effects of OXA on the proliferation and differentiation of white preadipocytes in more physiological settings using isolated rat primary preadipocytes. OXA enhanced the proliferation of rat primary adipocytes (Fig. 5A), however the differentiation into mature adipocytes, as judged by the morphology and intracellular triacylglycerol content, was not influenced by OXA (Fig. 5B–D). These findings confirm our observations in 3T3-L1 and NIH/3T3 cells.

4. Discussion

3T3-L1 fibroblasts proliferation is controlled by ERK1/2 pathway [16,17], however contradictory results (stimulation vs. inhibition) were reported (reviewed in [12]). In agreement with studies performed on endothelial and adrenocortical cells [18,19], we report that OXA activates ERK1/2 pathway. OXA stimulates 3T3-L1 fibroblasts proliferation and viability, and protects against apoptotic cell death via ERK1/2-dependent mechanism. An earlier study demonstrated that ERK1/2 activation protects 3T3-L1 fibroblasts from serum starvation-induced apoptosis [16]. Consistent with this observation, prolonged (48 h) incubation of 3T3-L1 fibroblasts with OXA enhanced cell viability and prevented from serum-deprivation- and PA-induced apoptotic death. These effects of OXA were reversed by blocking the ERK1/2 pathway, indicating that OXA stimulates 3T3-L1 cell proliferation and viability, and protects against apoptotic cell death via the ERK1/2.

3T3-L1 cell proliferation and viability is also stimulated by PI3K/PKB activators [16,20]. We recently reported that OXA stimulates PI3K/PKB phosphorylation in mature adipocytes [5] and glucagon-producing cells [10]. In the current study we show that OXA fails to activate PI3K/PKB pathway and that PI3K/PKB is dispensable for OXA-induced stimulation of 3T3-L1 preadipocytes proliferation, and viability. These results suggest that OXA regulates PI3K/PKB in a cell-type specific manner (preadipocytes vs. adipocytes).

Some studies showed that ERK1/2 activation leads to decreased PPARγ activity, thereby inhibiting preadipocytes differentiation

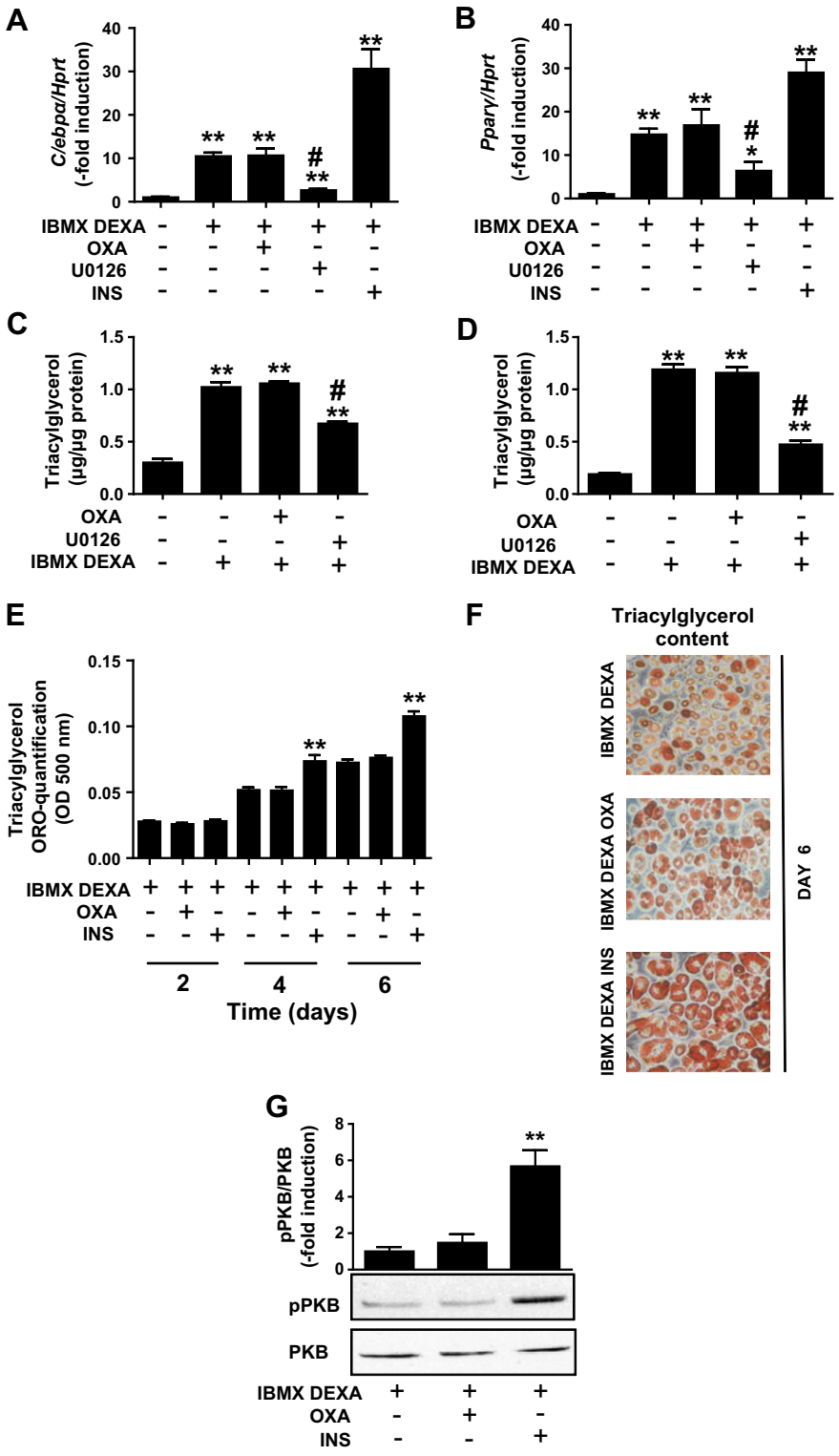

Fig. 4. OXA increases ERK1/2 phosphorylation but fails to affect PKB phosphorylation and the differentiation of 3T3-L1 preadipocytes into mature adipocytes. (A and B) 3T3-L1 cell differentiation was induced by incubating the cells in a SDM in the absence or presence of the ERK1/2 inhibitor (10 μM) for 48 h. Two groups were incubated in SDM with 100 nM OXA or 1 μM insulin. Thereafter the expressions of *Pparγ* and *C/ebpα* mRNA were assessed by quantitative RT-PCR. (C and D) In 2-days post-confluent 3T3-L1 cells the differentiation was induced as described in (A) and (B). Four (C) or six days (D) days after the initiation of the differentiation process cellular triacylglycerol was determined. (E) 3T3-L1 fibroblasts were differentiated and 2, 4 or 6 days of the onset of the differentiation procedure cellular triacylglycerol content was measured using ORO staining. (F) Corresponding representative images of ORO-stained cells were taken after 6 days of differentiation at a 20-fold magnification. Cellular triacylglycerol content was detected by quantifying ORO-stained cells. (G) Two days postconfluent 3T3-L1 cells were incubated in the SDM alone or supplemented with 100 nM OXA or 1 μM insulin for 10 min. Phosphorylated PKB and total PKB (~60 kDa) were detected by Western blots.

[21,22]. However, others questioned these observations by showing that an early activation of ERK1/2 is crucial for adipocytes differentiation [23,24]. Control experiments addressing these discrepancies, utilizing U0126, show clearly that ERK1/2

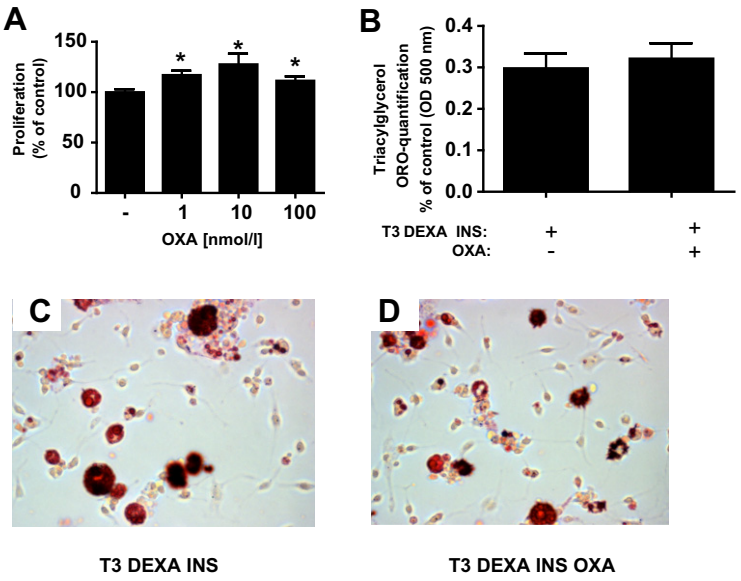

Fig. 5. OXA stimulates proliferation but fails to enhance differentiation of isolated primary rat preadipocytes. (A) Isolated primary rat preadipocytes were exposed to OXA (1–100 nM) for 24 h. Cell proliferation was assessed by BrdU assay. (B–D) Isolated white rat preadipocytes were differentiated as described in Section 2 in the presence or absence of 100 nM OXA for a total period 5 days. Thereafter, ORO staining of triacylglycerol was performed and quantified (B), and cells were photographed (C and D).

stimulates 3T3-L1 preadipocytes differentiation as judged upon the stimulation of *Pparγ* and *C/ebpα* mRNA expression, as well as triacylglycerol accumulation. In line with our data, blockade of ERK1/2 reduced insulin-stimulated triacylglycerol accumulation in 3T3-L1 cells incubated in the SDM [25]. These results suggest that ERK1/2 stimulates 3T3-L1 adipocytes differentiation.

PPARγ is known to stimulate adipocytes differentiation [26]. Since OXA stimulates ERK1/2 and *Pparγ* expression in mature adipocytes [5,6], we predicted that OXA is able to enhance the differentiation of preadipocytes. Unexpectedly, and in contrast to insulin, OXA failed to enhance the differentiation of 3T3-L1 cells, despite stimulation of ERK1/2 phosphorylation. Stimuli of preadipocytes differentiation such as insulin or IGF-1 are known to increase ERK1/2 phosphorylation in 3T3-L1 cells [27,28]. However, it has been suggested that the ability of insulin to enhance the differentiation of preadipocytes depends upon the activation of the PI3K/PKB pathway. Earlier studies clearly showed that the blocking of PI3K/PKB signaling prevents the differentiation of 3T3-L1 preadipocytes into mature adipocytes [29,30]. In support of this observation, overactivity of PKB triggered the spontaneous differentiation of 3T3-L1 fibroblasts into mature adipocytes [31]. Our current data reveals that OXA is unable to induce PKB phosphorylation in 3T3-L1 preadipocytes during early differentiation. In contrast, stimulation of PKB phosphorylation by insulin leads to a full differentiation. Thus, the results of our experiments suggest that ERK1/2 activity is required for the induction of preadipocytes differentiation at an early stage; however PKB plays an essential role in achieving full differentiation. The lack of induction of 3T3-L1 preadipocytes differentiation into adipocytes by OXA is in line with the recently published in vivo study showing that orexin-deficient mice have changes of adipogenesis in brown but not in white adipocytes [32]. Of note, genetic mice studies showed that orexin improves insulin sensitivity [33,34]. In this context orexin's antiapoptotic and mitogenic actions on preadipocytes may be of physiological relevance. The higher content of large, hypertrophic adipocytes rather than small sized adipocytes is associated with metabolic abnormalities (hyperglycemia, hyperlipidemia, insulin resistance) [35]. Increased content of small adipocytes is the improvement of insulin sensitivity [36,37]. Although OXA fails to induce the differentiation of preadipocytes into adipocytes, through an increase of preadipocytes proliferation and the protection against apoptosis, OXA may remodel the adipose tissue composition, without affecting fat tissue content. These alterations may lead to improved insulin sensitivity [34]. Recently, a few groups reported that increased adipocytes death is a hallmark of obesity [38,39]. Pro-apoptotic phenotype of adipocytes is associated with increased secretion of pro-inflammatory cytokines and macrophages infiltration, contributing to insulin resistance. In line with these data it has been shown that the inhibition of adipocytes apoptosis is associated with reduced macrophages infiltration and lower insulin resistance, independent of body weight [38]. Therefore, it appears that the inhibition of apoptosis of adipocytes (e.g. by OXA) may represent a novel target in the therapy of obesity-associated metabolic abnormalities.

Finally, by reporting that OXA stimulates NIH/3T3 cells (Supplementary Fig. 2) as well as rat preadipocytes proliferation, without affecting their differentiation, we conclude that OXA's activities on these cell types are a rather general phenomenon.

In summary, our study demonstrates that OXA regulates 3T3-L1 preadipocytes proliferation and survival, reduces the pro-apoptotic caspase-3 activity, and also protects against apoptotic death induced by serum-deprivation or PA via the ERK1/2 pathway. 3T3-L1 fibroblasts differentiation into adipocytes depends upon ERK1/2. Despite increasing ERK1/2 phosphorylation OXA fails to enhance the differentiation of preadipocytes into adipocytes. In contrast to insulin, OXA fails to increase PKB phosphorylation in 3T3-L1 preadipocytes, an event that we previously reported in OXA-treated fully differentiated 3T3-L1 adipocytes [5]. The lack of stimulation of PKB possibly explains the inability of OXA in enhancing preadipocytes differentiation. Since the effects of OXA on two different cell lines which are able to differentiate into adipocytes are transferable to primary preadipocytes, the findings of this study may be of physiological significance. Based on our recent and current results we conclude that OXA cell-type specifically regulates the functions of preadipocytes and mature adipocytes, thereby potentially contributing to improvement of glucose and lipid homeostasis as reported by recent in vivo studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.10.013.

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