Oxytocin modulates proliferation and stress responses of human skin cells: implications for atopic dermatitis

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Abstract: The neuropeptide hormone oxytocin (OXT) mediates a wide spectrum of tissue specific actions, ranging from cell growth, cell differentiation, sodium excretion to stress responses, reproduction and complex social behaviour. Recently, OXT expression was detected in keratinocytes, but expression of its receptor and function are still unexplored in human skin. Here, we showed that both OXT and its receptor are expressed in primary human dermal fibroblasts and keratinocytes. OXT induced dose dependent calcium fluxes in both cell types demonstrating that the OXT receptor (OXTR) is functionally expressed. We also showed that OXT decreases proliferation of dermal fibroblasts and keratinocytes in a dose dependent manner. In order to further investigate OXT mediated functions in skin cells, we performed OXTR knockdown experiments. OXTR knockdown in dermal fibroblasts and keratinocytes led to elevated levels of reactive oxygen species and reduced levels of glutathione (GSH). Moreover, OXTR depleted keratinocytes exhibited an increased release of the pro inflammatory cytokines IL6, CCL5 and CXCL10. Our data indicate that the OXT system modulates key processes which are dysregulated in atopic dermatitis (AD) such as proliferation, inflammation and oxidative stress responses. Furthermore, we detected a downregulation of the OXT system in peri lesional and lesional atopic skin. Taken together, these data suggest that the OXT system is a novel neuroendocrine mediator in human skin homeoestasis and clinically relevant to stressed skin conditions like AD.

Key words: atopic dermatitis – inflammation – oxidative stress – oxytocin system – proliferation

Introduction
The nonapeptide hormone oxytocin (OXT) plays a key role in certain kinds of behavioural regulation, such as social recognition, attachment and anxiety (1,2), as well as in physiological functions, including milk ejection during lactation and uterine contraction during labour (3). OXT is mainly produced in the paraventricular and supraoptic nuclei of the hypothalamus and released from hypothalamic nerve terminals of the posterior pituitary into the blood stream (4,5). Apart from this, OXT is also synthesized in uterus, ovary, testis and myocardial tissue (3).

Oxytocin acts through its receptor, which belongs to the rhodopsin type class I G protein coupled receptor superfamily (6). The OXT receptor (OXTR) is differentially expressed in various tissues correlating with the pattern of sex steroids (3), and its expression is regulated by cytokines (7,8). Differential pharmacological profiles of the OXTR in the brain and peripheral tissues are associated with the existence of OXTR subtypes (9,10). Furthermore, the OXTR displays promiscuous coupling to Gq, Gi and G protein isoforms forming heterotrimERIC complexes, depending on the localization of the receptor within the plasma membrane. The variety of OXTR subtypes coupling with different G protein isoforms results in the activation of multiple signalling pathways, and consequently, of diverse physiological functions of OXT in different cell types (11,12). In fact, OXT is able to promote, to inhibit or to have no effect on proliferation of various cell types (13,14). However, the proliferative effects are altered by cholesterol depletion of membranes or changes in OXTR caveolar interaction (15), resulting in contrary observations (16,17). Inhibitory effects of OXT are mediated by activation of a cAMP kinase A pathway, whereas mitogenic effects are associated with an increase of intracellular calcium and tyrosine phosphorylation (14). Further functions of OXT are the modulation of neuroendocrine stress responses and inflammatory processes (18,19). OXT dampens the hypothalamo pituitary adrenal (HPA) axis by lowering corticosterone/cortisol levels in response to acute exposure to stressors (18,20). Human skin takes part in the local as well as the systemic neuroendocrine network (21). For instance, all key mediators of the HPA axis including the neuropeptides CRH, POMC derived ACTH, αMSH, β endorphin and cortisol/corticosterone are synthesized by skin cells (21 23). In term myometrium, CRH receptor function is inhibited by OXT via activation of protein kinase C (24). In view of possible cross talks, CRH receptors are also expressed by dermal fibroblasts and keratinocytes (22,25,26). However, with respect to inflammation, subcutaneously given OXT has been demonstrated to prevent sepsis induced depletion of glutathione (GSH) contents in colonic and uterine tissues of rats (27). Furthermore, wound healing, which involves a well organized inflammatory phase, has been shown to be facilitated by OXT administration (28,29). Moreover, in a rat model of dried latex induced paw oedema, OXT treatment showed anti inflammatory and antinociceptive activity, thus pointing to a role of OXT in skin inflammation (30). Inflammation is typically accompanied by oxidative stress. In an in vitro model, OXT decreased not only IL6 secretion, but also NADPH dependent superoxide production in vascular cells and THP 1 macrophages suggesting that OXT attenuates vascular oxidative stress (31).

In humans, chronically inflamed skin is, amongst others, evident in atopic dermatitis (AD). The skin of patients suffering
from AD shows lichenification, erythema and pruritic lesions. AD is a multifactorial skin disease: genetic predisposition, bacterial infections, skin barrier disruption, immunological alterations and psychological stress have all been discussed as causes and aggravation factors (32,33). Epidermal hyperplasia is a common feature of AD and attributed to T cell derived cytokines such as tumor necrosis factor α and interferon γ which are potent inducers of epidermal growth factors and their receptor (34 36). Additionally, these cytokines initiate inflammatory cascades and have been shown to induce an abnormal chemokine production profile in keratinocytes of AD patients (37).

A recent study showed that in human skin, OXT is also produced by epidermal keratinocytes (38). However, its role in cutaneous homoeostasis remains unknown. The aim of the present study was the characterization of the OXT system in human skin and the analysis of its functional activity and relevance in skin physiology. We showed that dermal fibroblasts and keratinocytes express OXT and its receptor. Additionally, we revealed the role of OXT in the modulation of cutaneous proliferation, inflammation and oxidative stress responses with possible implications for AD.

**Methods**

**In vivo studies**

All *in vivo* studies were conducted according to the Declaration of Helsinki and approved by the ethics committees of the Medical Associations at Hamburg, Kiel and Freiburg, Germany. All study participants gave written informed consent. AD patients displayed a local SCORAD of 5.8 ± 0.3 on test arm area. Ages of healthy (*n* = 32) and AD (*n* = 24) participants were equivalent (healthy: 36.6 ± 2.1, atopic: 35.0 ± 1.7 years). Punch biopsies were taken under local anaesthesia from peri lesional/lesional areas of the forearm of atopic patients and healthy volunteers. Suction blisters were prepared as described by Kiistala (39). In an additional study, suction blister fluids were taken from the inner upper arms of six healthy women and six healthy men aged 20-40 at *t*₀ and *t₁*. At *t₁*, participants caressed themselves with a soft brush every 30 min for 5 min during 3.4 h around the investigated area (tactile stimulation) leaving the other arm unstimulated (control).

**Isolation and culture of primary human dermal fibroblasts and keratinocytes**

Skin cells were isolated from biopsies and cultured as described by Roggenkamp et al. (40), and used at passages 1-5. As determined by ELISA, basal OXT concentrations in culture medium for fibroblasts and keratinocytes were 66 pM and 2.7 nM, respectively. Physiological plasma OXT levels range from 5 to 300 pM (41 43). In knockdown experiments, no additional OXT was applied. OXT doses used in the experiments to determine Ca²⁺ fluxes and proliferation have been previously elaborated (44).

**Immunofluorescence analyses**

Cells grown on glass chamber slides (Thermo Fischer Scientific, Waltham, MA, USA) were fixed with 4% paraformaldehyde (Sigma Aldrich, Munich, Germany) in PBS and permeabilized with 0.1% Triton X 100 (Sigma Aldrich) in PBS. Cryosections of human skin were fixed with ice cold acetone. Cells and sections were blocked with 1% BSA in PBS and incubated with antibodies directed against human OXT (fibroblasts, keratinocytes: 1:200, LS C37953; LifeSpan BioSciences (Seattle, WA, USA), skin: 1:500, MAB5296; Millipore, Billerica, MA, USA) and OXTR (1:200, SYC592; Eurogentec, Seraing, Belgium). For staining of nuclei and visualization, cells and sections were incubated with Alexa 488 conjugated secondary antibodies (1:1000; Invitrogen, Darmstadt, Germany) mixed with DAPI (1:2000, 5 mg/ml stock; Sigma Aldrich). Microscopy was performed with an Axiovert S100 (Zeiss, Jena, Germany).

**Real time semi quantitative PCR**

Total RNA from fibroblasts, keratinocytes and suction blister derived epidermis was isolated using RNeasy Kit (Qiagen, Hilden, Germany). Concentration and purity of RNA were assessed utilizing a NanoDrop 1000 (Peqlab, Erlangen, Germany). RNA was subjected to cDNA synthesis with the high capacity cDNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany). Expression levels were detected by semiquantitative real time polyme rease chain reaction (RT PCR) using gene expression assays mixed with TaqMan Master Mix performed in a 7900HT Fast RT PCR System (all from Applied Biosystems). The gene expression assays Hs00792417 g1 (OXT) and Hs00168573 m1 (OXTR) were utilized. Expression was quantified by the comparison ΔΔCT method using 18S expression as endogenous control.

**Detection of oxytocin and cytokine protein levels**

OXT concentrations in suction blister fluids were measured using an OXT ELISA (Enzo Life Sciences, Lörrach, Germany) and normalized to total protein contents determined by BCA quantification (Interchim, Montluçon, France). Cytokine concentrations were measured using a 27 Plex (BioRad, Munich, Germany).

**Oxytocin receptor knockdown**

One day after seeding cells at a density of 2 × 10⁵/well into six well plates, they were transfected with 20 nM (fibroblasts) or 50 nM (keratinocytes) of scrambled (No.1027281; Qiagen) or OXTR specific (AM16708, ID1766; Invitrogen) siRNA using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer’s instructions.

**Measurement of Ca²⁺ currents**

Intracellular calcium concentrations were measured by flow cytometry as described previously (45). Briefly, cells were incubated with PBS containing 3 µg/ml Fluor 4 AM, 10 µg/ml Fura Red, 0.02% Pluronid Detergent F 127 and 4 µg/ml Probenecid and 10% FCS (all from Invitrogen). Ca²⁺ currents were induced by OXT (Tocris, Bristol, UK) diluted in 10% FCS in PBS and recorded using the flow cytometer FACS Canto® II (BD Bioscience, Heidelberg, Germany). Inhibition of OXT induced Ca²⁺ currents was performed by pre addition of the OXTR specific antagonist L371,257 (Tocris). Data were analysed using FlowJo v. 7.2.1 (BD Bioscience). Ca²⁺ currents were normalized to cell number. Imaging of Ca²⁺ fluxes was carried out using the confocal microscope SPS (Leica, Bensheim, Germany).

**Proliferation assay**

Fibroblasts and keratinocytes were seeded in 96 well plates. One day after plating, cells were treated daily with OXT alone or in combination with the OXTR specific antagonist L371,257. At day 4 and 7 after initial treatment, cells were fixed and stained with 6.2 µg/ml propidium iodide (46) (Invitrogen) and 17% ethanol in PBS. Proliferation was quantified by cell counting using an Axiovert 200M (Zeiss).

**UV irradiation**

For UV exposure (47), cells grown in six well plates were irradiated in 1 ml PBS/well. Fibroblasts were irradiated with 5 J/cm² UVA light using a Psorisan 900/5164 H1 (Dr. Hönle Medizintechnik, Kaufering, Germany). Keratinocytes were irradiated with solar simulated light (SSR) using an Oriel 1600W (Newport Corporation, Stratford, CT, USA) at a dose of 100 mJ/cm² UVB. UV doses were determined with an IL1700 Research Radiometer (Interna tional light, Newburyport, MA, USA). The UV spectra of the light...
sistol 3 phosphate signalling, leading to increased intracellular Ca\textsuperscript{2+}. Directly after irradiation, cells were subjected to reactive oxygen species (ROS) and GSH determination assays.

**Results**

**Detection of reactive oxygen species**

Cells were incubated with 150 ng/ml 2',7' dichlorodihydrofluorescein dichloride (Invitrogen) in PBS according to the manufacturer’s instructions. Fluorescence intensities were measured at 535 nm using a Safire reader (Tecan, Crailsheim, Germany) and normalized to cell number.

**Detection of glutathione**

Cells were incubated with 20 μM ThioTrackerman® Violet dye (Invitrogen) in PBS according to the manufacturer’s instructions. This dye was used to estimate the cellular level of reduced GSH, because it represents the majority of intracellular free thiols in the cell. Fluorescence intensities were measured at 526 nm using a Safire reader (Tecan) and normalized to cell number.

**Statistics**

Data were analysed with Prism 5 (GraphPad Software, San Diego, CA, USA) and are presented as the mean ± SEM. Normality was checked by the Shapiro-Wilk test. If the hypothesis of normality was rejected, groups were compared using the Mann Whitney U test (unpaired data) or the Wilcoxon signed rank test (paired data). Otherwise, Student’s t test (paired/unpaired data) was used for group comparison. P values < 0.05 were considered statistically significant.

**Results**

The oxytocin system is functionally expressed in primary human skin cells

Oxytocin expression in keratinocytes has recently been reported (38), but expression of the OXT receptor (OXTR) and its role in human skin have not been investigated so far. Immunofluorescence analyses revealed that both OXT and the OXTR are expressed in dermal fibroblasts and keratinocytes (Fig. 1a-d). Furthermore, OXT is localized throughout the epidermis (Fig. 1e). In contrast, the OXTR is mainly detected in the basal layer of the epidermis (Fig. 1f). RT PCR analyses confirmed OXT and OXTR expression in skin cells (Fig. 1g-h). The highest mRNA expression levels of OXT and the OXTR were detected in fibroblasts compared to keratinocytes or epidermis tissue. OXT expression in keratinocytes was eightfold higher than in the epidermis. This was presumably due to a higher expression of OXT in proliferating keratinocytes (38), which were predominant in the in vitro monoculture, compared to differentiated keratinocytes, predominant in the epidermis in vivo/ex vivo. Hypothalamic mRNA served as positive control for OXT/OXTR expression.

To examine OXT protein levels in human skin in vivo, we analysed suction blister fluids, taken from both arms of 12 healthy volunteers. With respect to gender, OXT levels were equivalent (males: 6.5 ± 0.6 pg OXT/mg protein, females: 6.4 ± 1 pg OXT/mg protein) (Fig. 1b). As higher OXT concentrations are found in the saliva upon tactile stimulation (48), we investigated whether OXT is also released locally by tactile stimulation from skin tissue. To this end, suction blister fluids were taken either after repeated tactile stimulation or from the corresponding, unstimulated area. Significantly higher OXT concentrations were observed in suction blister fluids taken from stimulated areas (6.8 ± 0.7 pg OXT/mg protein) compared to unstimulated areas (5.9 ± 0.4 pg OXT/mg protein) (Fig. 1b), indicating a local OXT release from tactile stimulated skin.

OXTRs predominantly couple to G\textsubscript{i} proteins and enhance inositol 1,4,5-trisphosphate signalling, leading to increased intracellular Ca\textsuperscript{2+} fluxes (3). In order to test, whether OXTR activation leads to increased Ca\textsuperscript{2+} fluxes in dermal fibroblasts and keratinocytes, we performed flow cytometry analyses. In both cell types, OXT led to a significant increase in Ca\textsuperscript{2+} currents in a dose-dependent manner, indicating that the OXTR is functional (Fig. 2a-c). Induction with 1 μM OXT resulted in a twofold increase in Ca\textsuperscript{2+} currents compared to baseline. This effect was significantly inhibited by OXTR knockdown or by the OXTR antagonist L371,257. Visualization of OXT induced Ca\textsuperscript{2+} fluxes via fluorescence analyses confirmed OXTR signalling in dermal fibroblasts and keratinocytes (Fig. 2d,e).
Oxytocin treatment of fibroblasts and keratinocytes significantly decreased their proliferation rate (Fig. 2e,f). At day 7 of daily treatment with 1 nM OXT, proliferation of fibroblasts was significantly reduced (14 ± 2%) (Fig. 2c). This growth inhibiting effect of OXT was dose dependently more pronounced using higher concentrations (1 μM OXT: 37 ± 6% decrease). Cotreatment with the OXTR antagonist L371,257 (1 μM) counteracted the antiproliferative effects of 1, 10 and 100 nM OXT. In comparison to fibroblasts, keratinocytes responded to OXT treatment with a less pronounced but significant decrease in cell proliferation (10 nM OXT: 7 ± 3%, 10 μM OXT: 22 ± 4%) (Fig. 2f). Cotreatment with the OXTR antagonist L371,257 (10 μM) significantly antagonized the antiproliferative effect of OXT.

**Oxytocin receptor knockdown induces oxidative stress.** Reduces glutathione levels and leads to enhanced pro-inflammatory cytokine release of skin cells.

To elucidate OXT-mediated functions in the skin, we performed transient OXTR knockdown in dermal fibroblasts and keratinocytes. Next, we investigated whether OXTR knockdown in skin cells affects their oxidative stress status. Determination of ROS levels revealed significantly increased intracellular ROS concentrations in OXTR depleted fibroblasts (22 ± 7%) and keratinocytes (30 ± 9%) (Fig. 2a). As expected, both cell types responded to UV irradiation with increased ROS formation (fibroblasts: 60 ± 19%, keratinocytes: 25 ± 7%). OXTR knockdown significantly further aggravated the ROS status of irradiated skin cells (fibroblasts: 68 ± 30%, keratinocytes: 26 ± 11% increase).

Additionally, we determined intracellular GSH concentrations to elucidate whether higher ROS levels correlate with lower GSH levels in OXTR depleted dermal fibroblasts and keratinocytes. In OXTR knockdown fibroblasts, GSH levels decreased significantly (38 ± 5%), whereas only a slight decrease was observed in OXTR knockdown keratinocytes (Fig. 3b). Irradiation of fibroblasts with 5 J/cm² UVA light diminished GSH concentrations significantly (40 ± 4%). Irradiation of keratinocytes with 100 mJ/cm² SSR light led to a marginal lowering of GSH level (4 ± 6%). Nevertheless, irradiated OXTR knockdown cells were more susceptible to GSH depletion than control cells. They exhibited a significant further decrease of GSH contents by approximately 13%.

OXTR modulates inflammatory processes (19,31,49). In order to investigate whether the OXT system might be involved in the inflammatory stress response of the skin, we screened 27 cytokines concerning their regulation in OXTR knockdown skin cells. Out of these cytokines, the release of IL 6, CCL5 and CXCL10 was upregulated in keratinocytes in response to OXTR knockdown (Fig. 3c). The enhanced release was significant for all three cytokines and the strongest effect was observed for CXCL10 (IL6: 164 ± 65%, CCL5: 226 ± 90%, CXCL10: 1297 ± 632%). In contrast, secretion of these cytokines was not modulated in OXTR depleted fibroblasts.

The oxytocin system is downregulated in atopic skin.

Our data revealed modulation of cytokine release, oxidative stress status and proliferation by the OXT system in skin cells (Figs 2c-e and 3). As these parameters are aberrant in AD (50,51), we explored whether the OXT system might be dysregulated in atopic skin. Thus, we analysed mRNA expression of OXT and its receptor in dermal fibroblasts and keratinocytes derived from biopsies of healthy volunteers and atopic skin patients. RT PCR analyses...
significantly decreased in both, atopic peri lesional and lesional fibroblasts (5 J/cm² UVA and irradiation with 5 J/cm² UVA or 100 mL/cm² SSR). (c) GSH levels in fibroblasts (n = 6) and keratinocytes (n = 6) were determined directly after sham irradiation and irradiation with 5 J/cm² UVA or 100 mL/cm² SSR. (c) Cytokine concentrations in culture supernatants of OXT knockdown fibroblasts (n = 4) and keratinocytes (n = 11) relative to siControl were determined. Supernatants were collected 24 h after addition of fresh media at day 4 post transfection. Paire d t test, *p < 0.05; **p < 0.01; ***p < 0.001.

revealed significantly reduced OXT expression in atopic lesional fibroblasts (2.8 ± 2.6%) and in atopic peri lesional keratinocytes (0.9 ± 0.1%) compared to healthy controls (Fig. 4a). Atopic peri lesional fibroblasts and atopic lesional keratinocytes also showed reduced OXT expression. Additionally, OXTR expression was significantly decreased in both, atopic peri lesional and lesional fibroblasts (620 ± 52%, 560 ± 65%, respectively), compared to healthy fibroblasts (Fig. 4b). Atopic keratinocytes also showed a decreased OXTR expression (Fig. 4b). Accordingly, a similar trend to reduced OXTR expression levels was detected in epidermis derived from atopic peri lesional suction blisters (Fig. 4b). To evaluate OXT levels in atopic versus healthy skin in vivo, we measured OXT concentrations in suction blister fluids (SBF) of atopic lesional and healthy skin were detected. Unpaired t test, Mann-Whitney U test, *p < 0.05; **p < 0.01.

Figure 4. Expression of oxytocin and oxytocin receptor is reduced in atopic skin. (a, b) Transcript levels for OXT and its receptor in healthy (H), peri lesional (pI) and lesional (L) dermal fibroblasts (FIB) and keratinocytes (KER) were transfected with either scrambled siControl or OXTR specific (siOXTR) siRNA. (a) OXT levels in fibroblasts (n = 6) and keratinocytes (n = 12) were determined directly after sham irradiation and irradiation with 5 J/cm² UVA or 100 mL/cm² SSR. (b) OXT levels in fibroblasts (n = 6) and keratinocytes (n = 6) were determined directly after sham irradiation and irradiation with 5 J/cm² UVA or 100 mL/cm² SSR. (c) OXYT expression in culture supernatants of OXTR knockdown fibroblasts (n = 4) and keratinocytes (n = 11) relative to siControl were determined. Supernatants were collected 24 h after addition of fresh media at day 4 post transfection. Paired t test, *p < 0.05; **p < 0.01; ***p < 0.001.

Discussion

The neurohypophyseal hormone OXT exerts a wide spectrum of central and peripheral effects, including facilitation of reproduction at several levels, establishment of complex social behaviour, neuroendocrine modulation and immunological processes (3,31). Apart from studies, investigating the impact of subcutaneously injected OXT on different inflammation models (29,30), the role of the OXT system in skin has not been described so far. Recently, Denda...
et al. (38) demonstrated that OXT is expressed in human skin keratinocytes and released in response to calcium influx via P2X receptors. In this study, we showed that OXT is expressed not only in keratinocytes, but also in human skin derived dermal fibroblasts. Moreover, we were able to detect mRNA transcripts of the OXTR in dermal fibroblasts and keratinocytes. A previous study reported OXTR expression in human foreskin fibroblasts (52). Our immunochemistry staining of OXT and its receptor in human skin showed an expression of OXT in all epidermal layers and a preferential expression of the OXTR in the basal layers (52). Our findings of cutaneous OXT expression, the release might also originate from skin cells such as dermal fibroblasts and keratinocytes.

Stimulation of dermal fibroblasts and keratinocytes with OXT induced an increase in intracellular Ca\(^{2+}\) demonstrating the functionality of the OXTR in these skin cells. The specificity of this response was confirmed by either blocking Ca\(^{2+}\) signalling via OXTR knockdown or application of the OXTR antagonist L371,257. In smooth muscle cells, activation of the OXTR also induces G\(_{q}\) mediated increases in Ca\(^{2+}\) leading to contraction (56).

Depending on cell type, the activation of the OXTR is either associated with stimulation (14,57) or inhibition (11,44) of proliferation. We found a suppressive effect of OXT on the proliferation of dermal fibroblasts and keratinocytes. The regulation of proliferation by OXT is complex. It depends on expression levels and coupling of G\(_{q}\) and G\(_{i}\) protein isoforms, OXT concentration and OXTR recruitment to caveolae and cholesterol content in the cell membrane (15,44,58). The regulatory mechanisms underlying the modulation of proliferation by OXT signalling in dermal fibroblasts and keratinocytes need to be elucidated in future studies.

By inhibiting OXTR signalling, our data revealed an impact of OXT on the modulation of oxidative stress, intracellular GSH levels and cytokine release in dermal fibroblasts and keratinocytes. OXTR knockdown skin cells showed increased susceptibility to oxidative stress. This might indicate a cytoprotective role of OXT with regard to oxidative stress, as the formation of ROS leads to damage of nucleic acids, proteins and membrane lipids (59). OXT has anti oxidative activity by scavenging radicals and preventing the oxidation of lipoproteins (60). Furthermore, OXT improves the anti oxidative state of colonic tissue in a colitis model in rat (49) and balances stress. This might indicate a cytoprotective role of OXT with regard to oxidative stress in the hearts of the newborns (65). In keratinocytes, the effects of OXTR knockdown on ROS formation and GSH levels were less pronounced than in dermal fibroblasts, indicating a cell type specific susceptibility of skin cells to oxidative stress and its modulation by OXT. In accordance with this finding, Marionnet et al. (66) reported a higher sensitivity to oxidative stress of dermal fibroblasts in comparison with keratinocytes in UV irradiated skin models.

In contrast to dermal fibroblasts, keratinocytes released larger quantities of the cytokines IL6, CCL5 and CXCL10 in response to OXTR knockdown, thus confirming a cell type differential response to impaired OXT signalling. OXT has been shown to decrease the release of interleukins and other inflammatory mediators in various tissues and on a systemic level (19,31,49). In myometrial cells, OXT treatment reduces CCL5, CXCL5 and CCL20 expression (67). Administration of OXT in humans reduces endotoxin induced increases in plasma cytokines amongst others IL6 and CXCL10 (68).

The impact of OXT on the modulation of oxidative and inflammatory stress responses in skin cells suggests a role in chronic inflammatory skin conditions such as AD. Actually, OXT and its receptor were downregulated in dermal fibroblasts and keratinocytes derived from atopic skin. In a translational approach, we confirmed the aberrant expression of the OXT system in atopic skin as suction blister derived epidermis and fluids from AD patients displayed decreased OXTR and OXT levels.

The dynamic modulation of OXT and OXTR expression has been demonstrated in various studies: The OXT system is highly regulated during gestation, parturition and lactation, thus enabling circulating OXT to target a particular organ depending on the precisely regulated tissue specific expression of the OXTR (69,70). Furthermore, the OXTR is regulated by oestrogen, foetal bovine serum and lysophospholipids in vitro (71,72). The OXTR promoter also displays potential interleukin response elements (73) explaining the negative regulation of OXTR gene expression by IL6, IL1β and interferon (7,8). Sepsis, which is associated with massive production of inflammatory mediators and neuroendocrine alterations, decreases OXT gene expression in the brain (74). As the skin of AD patients is chronically inflamed, displaying a plethora of up regulated cytokines (75 77), elevated levels of inflammatory mediators might account for the decreased OXT and OXTR gene expression in atopic skin. As a consequence, defects in OXT signalling possibly enhance oxidative stress and inflammation in atopic skin resulting in a vicious circle.

Altogether, we identified the OXT system as a novel neuroendocrine mediator of skin cell proliferation and stress responses in vitro. We speculate that the OXT system is also involved in skin neuroendocrine networks. Cutaneous CRH/POMC system activity is, amongst others, determined by cytokine release and is proposed to form a function analogous to the HPA axis (23,25,78). As OXT plays a role in modulating the HPA axis (79 81) and CRH signalling in term ‘myometrium’ (24), it is tempting to suggest that OXT might also interact with the skin’s local neuroendocrine mediators, such as CRH. Moreover, the OXT promoter can be stimulated by oestrogen receptors and thyroid hormone receptors (82,83), which are also produced by skin cells (21,84 86). In line with the finding that the skin shares numerous mediators with the CNS (22), OXT turns out to be a new candidate of a brain–skin derived neuropeptide. Additionally, as we were able to dem
onstrate OXTR expression by skin cells, either skin cell derived or plasma derived OXT might signal in an autocrine as well as in a paracrine way in the skin. This would link into the hypothesized bidirectional brain skin connection (22,87).

In summary, the OXT system was characterized in human skin demonstrating its relevance in skin physiology. Dermal fibroblasts and keratinocytes responded to OXT via Ca²⁺ influx, thus proving functional possibility of the OXTR in skin cells. OXTR knockdown via siRNA or inhibition by a specific antagonist revealed an impact of OXT on the modulation of proliferation, ROS formation, GSH content and cytokine release in dermal fibroblasts and keratinocyte cells. The effects of OXT signalling on skin cell physiology point to a role of OXT in cutaneous inflammation. Indeed, we found a reduced expression of the OXT system in AD skin indicating its clinical relevance to chronic inflammatory skin diseases.

Author contribution
V. D., J. K., F. S. and G. N. designed the research. V. D. and A. G. performed the research. V. D. and G. N. analysed the data. F. S. and H. W. contributed essential reagents and tools. V. D. and D. R. wrote the paper. A. B. and G. N. critically revised the manuscript. All authors have approved the submitted and final version of the manuscript and confirm that the submitted work complies with the ethical policies of Experimental Dermatology.

Conflict of interests
The authors have declared no conflicting interests.