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Differential Response of Dopamine Mediated by β-Adrenergic Receptors in Human Keratinocytes and Macrophages: Potential Implication in Wound Healing

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Keywords
Dopamine · β-Adrenergic receptors · Keratinocytes · Macrophages · Cytokines · Metalloproteinase · NFκB

Abstract
Objective: Dopamine is an immunomodulatory neurotransmitter. In the skin, keratinocytes and macrophages produce proinflammatory cytokines and metalloproteinases (MMPs) which participate in wound healing. These cells have a catecholaminergic system that modulates skin pathophysiologic processes. We have demonstrated that dopamine modulates cytokine production in keratinocytes via dopaminergic and adrenergic receptors (ARs). The aim of this study was to evaluate the effect of dopamine and its interaction with β-ARs in human HaCaT keratinocytes and THP-1 macrophages. We evaluated the production of inflammatory mediators implicated in wound healing. ***Methods:*** Cells were stimulated with dopamine in the absence or presence of the β-adrenergic antagonist propranolol. Wound closure, MMP activity, and the production of IL-8, IL-1β, and IκB/NFκB pathway activation were determined in stimulated cells. ***Results:*** Dopamine did not affect the wound closure in human keratinocytes, but diminished the propranolol stimulatory effect, thus delaying cell migration. Similarly, dopamine significantly decreased MMP-9 activity and the propranolol-induced MMP activity. Dopamine significantly increased the p65-NFκB subunit levels in the nuclear extracts, which were reduced in the presence of propranolol in keratinocytes. On the other hand, dopamine significantly increased MMP-9 activity in THP-1 macrophages, but did not modify the propranolol-increased enzymatic activity. Dopamine significantly increased IL-8 production in human macrophages, an effect that was partially reduced by propranolol. Dopamine did not modify the p65-NFκB levels in the nuclear extracts in THP-1 macrophages. ***Conclusion:*** We suggest that the effect of dopamine via β-ARs depends on the physiological condition and the cell type involved, thus contributing to either improve or interfere with the healing process.

Introduction

Dopamine is a key neurotransmitter connecting the nervous and the immune systems as well as a mediator produced and released by immune cells [1, 2]. It has been demonstrated that dopamine and catecholamines, adren-

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aline and noradrenaline, modulate proliferation, apoptosis, and cytokine production in immune cells [3, 4]. Both dopaminergic, α- and β-adrenergic receptors (ARs), and intracellular oxidative mechanisms are involved in these processes [4, 5].

The skin is the first immunological barrier to environmental microbial and chemical agents. The so-called "skin immune system" is composed of a complex network of immune and nonimmune cells, such as keratinocytes, macrophages, Langerhans cells, and lymphocytes. Keratinocytes and macrophages produce proinflammatory cytokines (IL-1α, IL-1β, TNF-α, IL-6), chemokines (IL-8, MCP-1), and metalloproteases in response to external agents to promote an immune response [6, 7]. The expression of these inflammatory mediators is regulated by several signal transduction pathways, including the activation of NFκB, which is an important proinflammatory transcription factor expressed in many cell types, including macrophages and keratinocytes [3, 8].

The skin is innervated by sympathetic nerves [9]. It has been suggested that keratinocytes and macrophages have a catecholaminergic system like the cells of the central nervous system. The activity of such receptors can be regulated in response to several stimuli [10]. In addition, keratinocytes and macrophages can synthesize and degrade catecholamines and express dopaminergic and α/β-ARs on their surface [11]. At the epidermal level, electrophysiological studies have suggested that dopamine accelerates the skin barrier recovery [12]. In contrast, at the dermal level, dopamine antagonists, which block the action of the endogenous dopamine, stimulate angiogenesis, thus promoting wound healing [13]. Recent studies have indicated that the activation of β-ARs induces the migration of keratinocytes and the reepithelization of wounds [14–16]. Catecholamines can exert significant effects on the inflammatory response mediated by ARs in macrophages [17]. At the skin level, the inflammatory activity of macrophages would modulate the wound healing development [18]. This process comprises dynamic phases including an inflammatory state featuring the secretion of proinflammatory cytokines and chemokines (like IL-1, IL-6, IL-8, TNF-α, VEGF, TGF-β), tissue formation (reepithelization, angiogenesis), and a final phase of tissue remodeling [19]. It has been reported that metalloproteinases (MMPs) play a key role in many physiological and pathological remodeling processes. Particularly, MMP-2 and MMP-9 play an essential role in the regulation of cell migration, tissue remodeling, and angiogenesis during the wound-healing process [20].

In human keratinocytes, we have previously demonstrated that dopamine agonists stimulate the secretion of IL-6 and IL-8, acting on β-ARs and dopaminergic receptors as well as by an oxidative mechanism [5]. In the present study, we have assessed the effect of dopamine mediated by β-ARs in human keratinocytes and macrophages and the potential implication in the wound-healing process. These studies demonstrate a differential effect of dopamine on both cell types, human keratinocytes and macrophages. We postulate that this effect varies according to the cell type, its physiological condition, and the catecholaminergic receptors involved.

Materials and Methods

Reagents

DMEM (Dulbecco's modified Eagle's medium) and RPMI-1640, penicillin/streptomycin (P/S), and pyruvate/glutamine (P/G) were from Gibco/Invitrogen (Argentina). Fetal bovine serum (FBS) was from Natocor (Argentina). Dopamine (Sigma, St. Louis, MO, USA), was dissolved in 0.1 N acetic acid and 0.1 mM ascorbic acid. The β-AR antagonist propranolol (Imperial Chemical, UK) was dissolved in 0.1 N acetic acid. Both drugs were aliquoted and stored at –20 °C until used. Lipopolysaccharide (LPS) was from Sigma (Argentina). Work dilutions were done in culture medium supplemented with 10% FBS, 100 IU/mL penicillin, 100 μg/mL streptomycin, 2 mM pyruvate, and 2 mM glutamine. The antibodies used were: a goat polyclonal anti-NFκB p65 (C-20) IgG, a rabbit anti-IκBα (FL) polyclonal IgG, a goat anti-actin (C-11) polyclonal IgG, and a mouse anti-histone H1 (AE-4) monoclonal IgG2a, (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The secondary HRP-conjugated bovine anti-goat IgG, the HRP-conjugated sheep anti-mouse IgG, and the HRP-conjugated goat anti-rabbit IgG (H+L) were purchased from Jakson Immuno Research (USA).

Cell Culture

The nontumoral human epithelial keratinocyte cell line HaCaT was kindly provided by Prof. N.E. Fusening from the German Cancer Research Center (Heidelberg, Germany). Cells were cultured in DMEM medium supplemented with 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin, 2 mM pyruvate, and 2 mM glutamine at 37 °C in 5% CO_2 in a humidified incubator. The cells were harvested using EDTA-trypsin (Gibco BRL) every 48 h. For all experiments, cells were grown to 70–80% confluence, for no more than 10 cell generations. The human monocytic cell line THP-1 (ATCC™ TIB-202™, Rockville, MD, USA) was also employed. Cells were cultured in RPMI-1640 medium, supplemented with 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin, 2 mM pyruvate and 2 mM glutamine at 37 °C in 5% CO_2 in a humidified incubator. In the presence of phorbol esters, such as phorbol myristate acetate (PMA), these monocytic cells differentiate into macrophages that present biological characteristics comparable to those of peripheral blood mononuclear cell (PBMC)-derived macrophages [21]. Therefore, in all experiments, the THP-1 cells were used after treatment with PMA (40 ng/mL) for a period

of 72 h. The cells obtained with this procedure are termed THP-1 macrophages [22]. All the studies of this work were carried out using cell lines; therefore, the approval of the institutional ethics committee was not requested.

Cell Culture and in vitro Stimulation

Keratinocytes were placed in 6-well tissue culture plates for the analysis of cell migration (scratch assay) and for metalloprotease activity measurements (zymography; 5×10^{-5} cells/well). Cells were placed into a 100-mm culture dish at a density of 2.5×10^6 cells/dish for protein extraction and Western blot. For all experiments, keratinocytes were grown for 24 h and then stimulated. After PMA-induced differentiation, THP-1 macrophages were seeded into 24-well tissue culture plates at a density of 9×10^5 cells/mL for ELISA and zymography, and into a 100-mm culture dish at a density of 2.5×10^6 cells/dish for Western blot. Keratinocytes and the adherent macrophages were stimulated with dopamine (1×10^{-6} to 1×10^{-4} M) in the absence or the presence of propranolol (1×10^{-5} M) over different time periods depending on the experiment. The antagonist propranolol was added 1 h before the stimulation with dopamine. Cells were harvested for Western blot and culture supernatants were collected for ELISA and zymography.

Gelatin-Zymography Analysis

Culture supernatants were collected for gelatin zymography [23] to determine the levels of matrix metalloprotease-9 (gelatinase B activity). Samples were diluted in 5x sample buffer and loaded onto a 7.5% polyacrylamide gel containing 2 mg/mL gelatin, under nonreducing conditions. The proteins present in the gel were renatured by incubation with 2.5% Triton X-100 at room temperature for 1 h. The proteolytic activity was detected by incubating gel slabs in enzymatic buffer (25 mM Tris, pH 7.5, 0.9% NaCl, 5 mM $CaCl_2$) overnight at 37 °C. The gel was then stained with a solution of 0.5% Coomasie Brilliant Blue R-250. Gelatin-degrading enzymes were visualized as clear zones against a blue background. The identification of MMP-9 activity bands was made by comparison with molecular weight markers. All experiments were performed 4 times.

Western Blot Analysis

HaCaT and THP-1 macrophages were incubated for 1 h with dopamine in the absence or the presence of propranolol as described above. After treatment, lysates were obtained as described previously [24]. The cytosolic fractions were obtained by resuspending the cell pellet in lysis buffer A (10 mM HEPES, pH 7.6, 0.1 mM EDTA, 0.1 mM EGTA, 10 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 2 μg/mL each of leupeptin, aprotinin, and pepstatin) and 10% NP-40. Nuclear extracts were obtained by resuspending the pellets in hypertonic buffer C (20 mM HEPES, pH 7.6, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM Na_2MO_4, and 2 μg/mL each of leupeptin, aprotinin, and pepstatin). The total protein content in nuclear and cytoplasmic extracts was determined by the Bradford protein assay (Bio-Rad, USA). Equal amounts of protein (35 μg/lane) were run on 10% SDS polyacrylamide gels and transferred onto nitrocellulose membranes by electroblotting. Membranes were blocked overnight at 4 °C, and then incubated overnight at 4 °C with a goat anti-NFκB p65 polyclonal IgG (1:800), a rabbit anti-IκB-α polyclonal IgG (1:800), a goat anti-actin polyclonal IgG (1:1,000), or a monoclonal anti-histone H1 IgG2a (1:700) as the primary antibody, and an HRP-conjugated anti-goat bovine IgG (1:2,500), an anti-mouse HRP-conjugated sheep IgG (1:2,500), or an anti-rabbit HRP-conjugated goat IgG (1:2,000) as the secondary antibody, for 3 h at room temperature. Detection of immune-reactive bands was carried out by an enhanced chemiluminescence detection kit as described by the manufacturer (Thermo Scientific, USA). All experiments were performed 3 times.

Measurement of Cytokine Production

Cells were incubated with different concentrations of drugs for 24 h. Cytokine levels were determined in culture supernatants by capture ELISA employing commercial kits (OptEIA, BD-Bioscience, for human IL-8, and R&D Systems for human IL-1β), according to the manufacturer's directions. Optical densities were measured in an ELISA reader (Multiskan EX). Cytokine concentrations were derived from standard curves. The values plotted are the means ± SEM of the fold induction values from the control obtained from 4–5 independent experiments (considering the control value as 1). Detection limits were 3.1 and 3.9 pg/mL for IL-8 and IL-1β, respectively. All experiments were performed 3 times.

Analysis of Cell Migration (Scratch Assay)

Keratinocytes were grown to confluence in multiwall plates. The cells were then deprived of FBS for 24 h before stimulation. They were either left untreated (control) or treated with dopamine in the absence or presence of propranolol. A sterile pipette tip was used to scratch a wound along the well. The cells were then washed with PBS, and 3 demarcated areas of each wound were photographed on an inverted Olympus CK3 inverted microscope at the time of wounding (time 0) and at 24 h after wounding. Image J software was used to measure the wound area at each demarcated area at time 0 and at 24 h to calculate the percentage of healing at the 3 positions for each wound. All experiments were performed 3 times.

Statistical Analysis

Data were analyzed by 1-way analysis of variance (ANOVA) followed by the post hoc Student Newman-Keuls test (Tukey test). All calculations were carried out using GraphPad Prism Software version 5.0. A p value <0.05 was considered statistically significant.

Results

Dopamine Decreased the Propranolol-Induced Wound Closure in Confluent Keratinocyte Culture

Since keratinocyte migration from the wound edge is critical for the wound-healing process, we used scratch wound assays to determine whether dopamine (1×10^{-5} and 1×10^{-4} M) can modulate human keratinocytes mobility in a wound environment. None of the dopamine concentrations used in our study induced a significant modification of the scratch wound closure at 24 h when compared to nontreated keratinocytes. Recent studies indicate the potential role of β_2-ARs in wound healing by

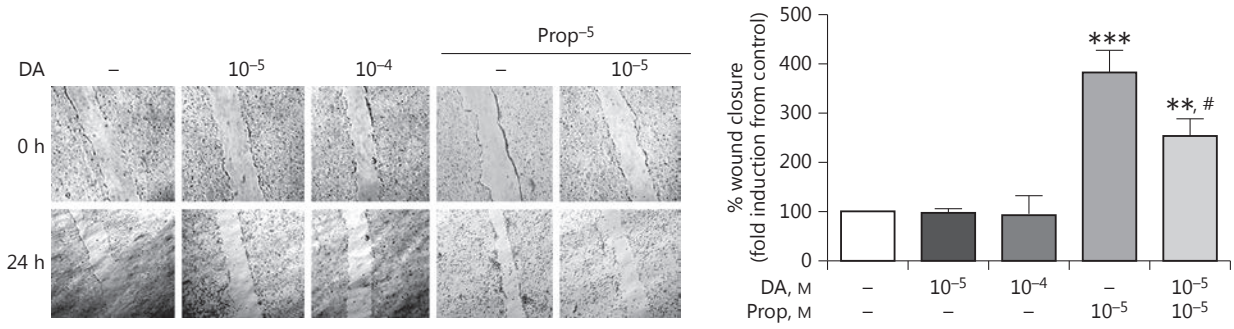

Fig. 1. Effect of dopamine on the wound healing of keratinocytes. HaCaT cell monolayers were treated with dopamine (DA, 1×10^{-5} and 1×10^{-4} M) or propranolol (Prop, 1×10^{-5} M), and then wounded by a scratch, as described in Materials and Methods. The defined areas were photographed at 0 and 24 h after wounding. Values represent the percentage of healing for each condition as the mean ± SEM. ** $p < 0.01$; *** $p < 0.001$ versus the control; # $p < 0.05$ versus propranolol.

modulating the proinflammatory response and impairing wound reepithelialization [25, 26]. To analyze the implication of β-AR, HaCaT cells were incubated with propranolol 1×10^{-5} M (a β-adrenergic antagonist) in the absence or presence of 1×10^{-5} M dopamine. As expected, the antagonist significantly increased the wound closure, as compared to the control. This effect was reduced when cells were treated with dopamine and propranolol simultaneously (Fig. 1).

Dopamine Decreased the Metalloproteinase Activity via β-AR in Human Keratinocytes but Not in THP-1 Macrophages

MMPs promote cell migration and tissue remodeling, and participate in all stages of the wound-healing process. To study the effect of dopamine on the activity of the collagenase MMP-9, HaCaT cells and THP-1-derived macrophages were stimulated with dopamine (1×10^{-5} and 1×10^{-4} M) and/or propranolol (1×10^{-5} M).

The treatment of keratinocytes with 1×10^{-5} M, but not with 1×10^{-4} M dopamine, induced a significant decrease in the activity of MMP-9 when compared to untreated cells. The treatment with the β-adrenergic antagonist significantly increased the MMP activity, as compared to the control. In the presence of dopamine, the induction of MMP-9 activity was significantly reduced (Fig. 2a).

On the other hand, in THP-1 macrophages, the MMP-9 activity increased significantly in the presence of dopamine (1×10^{-4} to 1×10^{-5} M) as well as propranolol (Fig. 2b). Additionally, the propranolol-induced activity of MMP-9 was not modified when cells were treated with propranolol and dopamine simultaneously.

Dopamine Increased IL-8, but Not IL-1β Levels, via β-AR in Human THP-1 Macrophages

It is known that certain cytokines are activated during inflammatory processes, infections, or immunological disorders and they are mainly involved in the repair of damaged tissues and the restoration of homeostasis [27]. Therefore, to evaluate the effects of dopamine on cytokine production, THP-1 macrophages were stimulated with dopamine (1×10^{-6} to 1×10^{-4} M). Results showed that the treatment with 1×10^{-6} M dopamine did not modify either IL-8 or IL-1β levels. However, the treatment with 1×10^{-5} and 1×10^{-4} M dopamine significantly increased the levels of IL-8 (Fig. 3a), without affecting the levels of IL-1β (Fig. 3b).

We then analyzed whether the dopamine-induced production of IL-8 was mediated by β-AR, as we previously described for keratinocytes [5]. While 1×10^{-5} M propranolol did not modify the IL-8 levels, the treatment with the β-adrenergic antagonist significantly reduced the IL-8 production induced by 1×10^{-5} M dopamine in THP-1 macrophages (Fig. 3c).

Dopamine Decreased NFκB Expression via β-AR in Human Keratinocytes but Not in THP-1 Macrophages

Since NFκB plays a key role in the regulation of proinflammatory cytokine-activated responses, we investigated whether NFκB plays a role in the dopamine-mediated

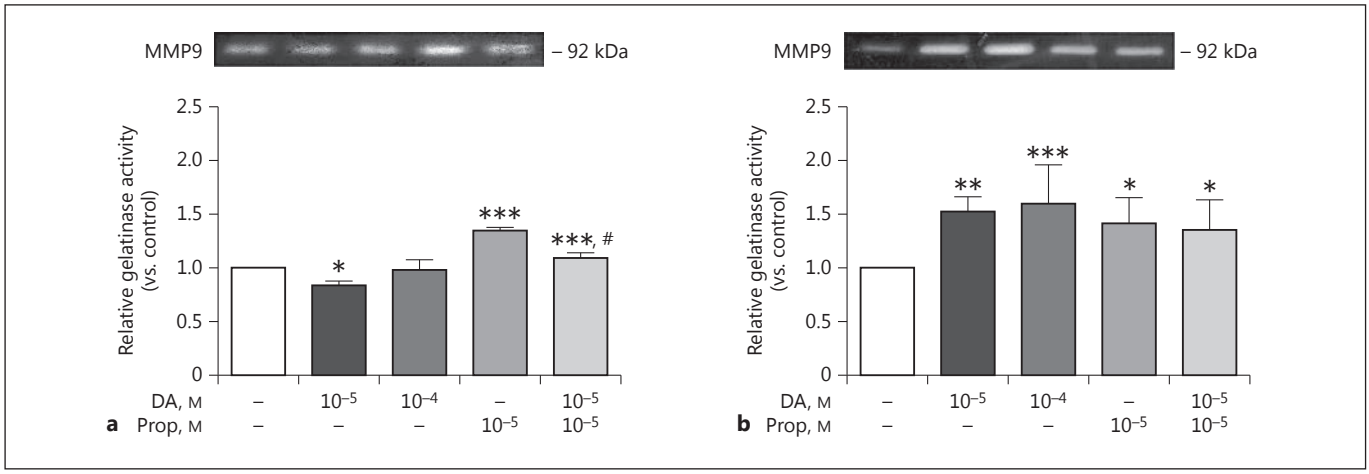

Fig. 2. Effect of dopamine on MMP-9 activity in HaCaT keratinocytes (**a**) and THP-1 macrophages (**b**). Both cell types were treated with either dopamine (DA, 1×10^{-5} to 1×10^{-4} M) or propranolol (Prop, 1×10^{-5} M), and then the MMP activity was evaluated by zymography. The densitometric data were plotted as the fold induction versus the control (mean ± SEM). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus the control; # $p < 0.05$ versus propranolol.

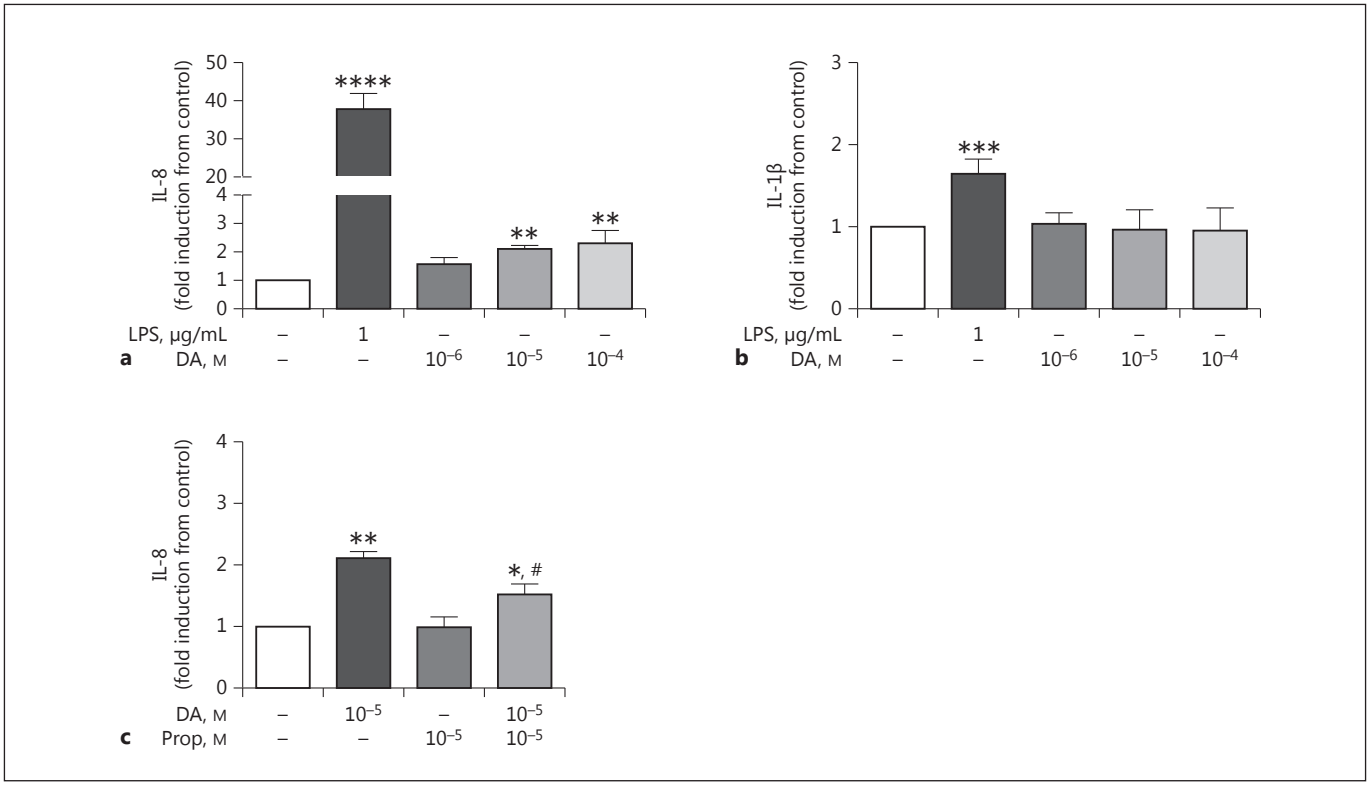

Fig. 3. Effect of dopamine on the production of IL-8 (**a**, **c**) and IL-1β (**b**) in THP-1 macrophages. Cells were treated with LPS (1 µg/mL, positive control), 1×10^{-5} to 1×10^{-4} M dopamine (DA), and/or with 1×10^{-5} M propranolol (Prop), and cytokine levels were measured by ELISA. Optical densities are represented as induction times versus the control (mean ± SEM). **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ versus the control; # $p < 0.05$ versus dopamine.

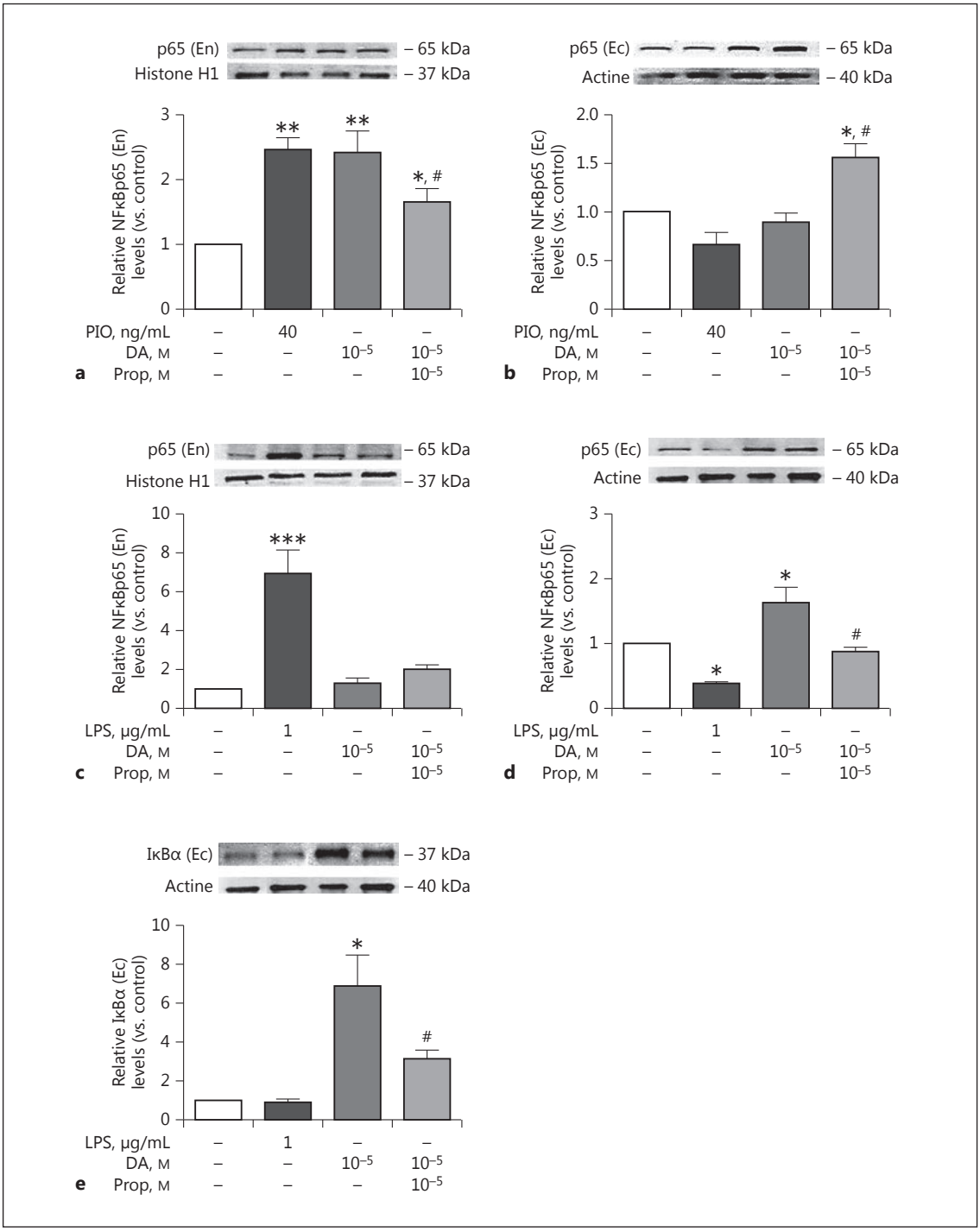

Fig. 4. Keratinocytes (**a**, **b**) and THP-1 cells (**c–e**) were either untreated (control) or treated with either PMA/ionomycin (PIO, 40 ng/mL), LPS (1 µg/mL), or dopamine (DA) in the absence or presence of the β-adrenergic antagonist propranolol (Prop) for 1 h at 37 °C. After treatment, the nuclear (En) and cytosolic (Ec) extracts were obtained, and the expression of NFκB was determined by Western blot as described in Materials and Methods. Values are expressed as the fold change versus the control (means ± SEM). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control; # $p < 0.05$ versus dopamine.

effects observed in keratinocytes and THP-1 macrophages.

The treatment of keratinocytes with 1×10^{-5} M dopamine induced a significant increase in the levels of the NFκB p65 subunit in the nuclear extracts, which were reduced when cells were incubated with dopamine in the presence of propranolol (Fig. 4a). These results correlate with the increased p65 levels in the cytosolic extracts obtained from cells treated with both drugs (Fig. 4b). These results suggest the existence of a dopamine-induced translocation of NFκB to the nucleus driven by β-AR on HaCaT keratinocytes.

In THP-1 macrophages, 10^{-5} M dopamine did not modify the NFκB p65 subunit levels in the nuclear extracts (Fig. 4c). However, a significant increase in the p65 subunit (Fig. 4d) and its inhibitor IκBα (Fig. 4e) was observed in the cytosolic extract. When cells were treated with dopamine and propranolol simultaneously, no changes in the NFκB p65 levels were observed in the nuclear fraction. Nevertheless, the p65 subunit and its inhibitor IκBα were reduced in the cytosolic fraction. This finding indicates that dopamine would not induce the translocation of NFκB transcription factor into the nucleus.

Discussion

Although dopamine is known for its role as a neurotransmitter, it has been described that this mediator can also modulate the immune response [1]. Cells of the cutaneous immune system express dopaminergic and ARs. These receptors can be activated by exogenous and endogenous catecholamines modulating the cellular activity [28]. In this work, we have demonstrated, in human keratinocytes and macrophages, that dopamine modulates the β-AR-mediated activity of MMP-9, the cytokine production, and the expression of the NFκB transcription factor. In addition, dopamine affects the keratinocyte migration.

It is known that catecholamines can modulate the migration of different cell types, such as lymphocytes [29] and stem cells [30], among others. Sivamani et al. [26] have shown that the catecholamines released in the wound site decrease the keratinocyte migration mediated by ARs. It has also been described that the activation of β_2-ARs leads to the in vitro and in vivo inhibition of keratinocytes migration [31, 32], and promotes the recruitment of polymorphonuclear cells by delaying the wound-healing process mediated by the increased IL-6 levels [33]. Furthermore, Shome et al. [34] suggested that the neurotransmitter dopamine inhibits the VEGF-induced migration of murine mesenchymal progenitor cells to the wound site in vitro. The latter effect is mediated by a mechanism that depends on D_2-dopamine receptors. In this work, we observed that dopamine did not affect the wound closure in a monolayer of human keratinocytes. However, dopamine was able to inhibit the stimulatory effect of the β-adrenergic blocker propranolol on the wound closure, as assessed by the scratch assay. In line with our results, other studies have shown that β-adrenergic antagonists stimulate the migration of keratinocytes in vitro, as well as the reepithelialization in vivo and the recovery of the epithelial barrier [14, 16, 35]. Under our experimental conditions, we observed that dopamine delayed the wound closure, thus suggesting that this neurotransmitter could exert an inhibitory effect on the migration of keratinocytes mediated by β-AR.

Similarly, catecholamines may either stimulate or inhibit the activity of MMPs, depending on the cell type and the pathophysiological condition [36]. For example, Chakroborty et al. [37] demonstrated that dopamine inhibits the expression and activity of MMP-9 in bone marrow in a tumor-bearing mouse model and hence it inhibits the migration of endothelial precursor cells. The latter effect was found to be mediated by the activation of the D_2-dopaminergic receptor, reducing the neovascularization and therefore tumor growth. In keratinocytes, the expression of MMP-9 is increased in response to tissue damage and it is involved in the migration of these cells [20]. In line with the results described by Chakroborty et al. [37], we also observed that dopamine significantly decreased MMP-9 activity in human keratinocytes. Besides, we studied the role of β-ARs in this effect. This inhibitory activity exerted by dopamine was also observed in the presence of the β-adrenergic antagonist propranolol, since dopamine significantly decreased the β-blocker-induced MMP-9 activity. It has been reported that β-adrenergic antagonists may alter the activity of MMPs. For example, the topical application of propranolol in wounds of diabetic mice induces the expression of MMP-9, which stimulates the reepithelialization in the early wound-healing stages [38]. Our results suggest that the inhibition of dopamine-induced MMP-9 activity in keratinocytes would be mediated, at least in part, by the activation of β-ARs.

On the other hand, the stimulation of hematopoietic progenitor cells with dopaminergic and adrenergic agonists has been demonstrated to induce the expression and

activity of different MMPs with the consequent migration of human and murine bone marrow precursor cells [36, 39]. In contrast to that observed in keratinocytes, dopamine increased the MMP-9 activity in human THP-1 macrophages. This result is in line with the findings of Giorelli et al. [40], who observed that dopamine induced the MMP-9 activity in PBMCs obtained from healthy individuals, but not in the peripheral monocytes from patients with multiple sclerosis. These authors did not analyze the receptors involved in that effect. We observed that the β-blocker propranolol increased the activity of MMP-9 similarly to dopamine in macrophages. We did not observe a synergistic or an antagonistic effect of β-blockers on the effect of this neurotransmitter, suggesting that the stimulating effect exerted by dopamine would not be mediated by β-ARs in this cell type.

It is known that catecholamines also modulate the production of cytokines and chemokines by immune cells [41, 42], which may contribute to the development of immune responses in the skin [43]. In addition to MMPs, keratinocytes and macrophages produce different inflammatory molecules such as cytokines [6, 44]. Gaskill et al. [11] demonstrated that dopamine (2×10^{-8} to 2×10^{-5} M) may either decrease or increase the production of cytokines (IL-6, CCCL-2, IL-8, IL-10, TNF-α) by PBMC-derived macrophages and depending on whether the cells are activated or not by bacterial LPS. However, it is known that the effect of dopamine depends not only on the activation status of the cell population involved, but also on the concentration of the catecholamine as well as on the mechanism involved (type of receptor/oxidative mechanism). In PBMCs and human monocytes, it has been demonstrated that high concentrations of dopamine, to the order of 10^{-4} M, can reduce the Con-A-stimulated secretion of IL-4, can inhibit the LPS-induced cell proliferation, and trigger apoptosis through a reactive oxygen species (ROS)-mediated mechanism. On the other hand, low concentrations of dopamine (less than 1×10^{-5} M) have been proven to decrease the generation of ROS and to inhibit apoptosis by a D_1-dopaminergic receptor-mediated mechanism [42, 45, 46]. Concerning the receptors involved in this process, it has been demonstrated that the D_2-dopaminergic antagonist haloperidol suppresses the secretion of IL-1β, IL-6, and IL-12p40 by inhibiting the NFκB pathway in murine dendritic cells and in LPS-stimulated macrophages [47]. However, other studies also performed on activated macrophages have shown that dopamine decreases IL-12p40 levels by a mechanism involving β-ARs without affecting cellular viability, even at high concentrations of dopamine (10^{-4} M) [48]. These findings contradict those reported by Cosentino et al. [46].

In THP-1 macrophages, we demonstrated that dopamine did not affect the production of the proinflammatory cytokine IL-1β, in the presence or not of the β-adrenergic antagonist, in any of the concentrations tested (1×10^{-6} and 1×10^{-4} M). Unlike the findings obtained in our model, other authors have demonstrated that dopamine can decrease LPS-induced levels of this cytokine by inhibiting NLRP3 inflammasome in activated macrophages, thus suggesting an anti-inflammatory effect of dopamine under those experimental conditions [49].

Besides, dopamine significantly increased the levels of the chemokine IL-8 in THP-1 macrophages, which is similar to the observations made by Gaskill [11] in LPS-stimulated human mononuclear-derived macrophages. The stimulatory effect of dopamine on the secretion of IL-8 was partially reversed by the β-adrenergic blocker propranolol, similar to the effect previously described in keratinocytes, where dopamine stimulated the production of IL-6 and IL-8 mainly mediated by β-ARs [5].

However, when we studied the NFKB/IκBα system, which is a pathway involved in cytokine production, we found that this pathway was induced in dopamine-stimulated keratinocytes but not in macrophages. The activation of catecholaminergic receptors is known to modulate the activity of different immune cells involving the activation of transcription factors such as NFκB. It has been described that adrenaline and noradrenaline induce the production of proinflammatory cytokines involving pathways that are either dependent [3] or independent of the transcription factor NFκB [50]. In this work, we have demonstrated, in THP-1 macrophages, that dopamine increases the production of IL-8 without increasing the levels of NFκB in the nuclear fraction. Besides, we observed a significant increase in both the p65 subunit and the IκBα inhibitor in the cytosolic fraction. The latter results suggest that the NFκB/IκBα signaling pathway would not be involved in the effect of dopamine in human macrophages. In line with our results, Brosnahan et al. [50] and Evans et al. [51] have demonstrated in epithelial cells that the catecholamine noradrenaline, via β-AR, increases IL-8 levels by mechanisms that are independent of NFκB, involving, among others, the activation of the adenylate cyclase, the mitogen-associated protein kinase, and the PI-3-kinase signaling pathway. Furthermore, the MAPK and ERK signaling pathways have been demonstrated to be involved in the dopamine-driven regulation of angiogenesis in normal and tumor tissues [34, 52].

Therefore, we suggest that other signaling pathways may be involved in the effects observed upon treatment of THP-1 macrophages with dopamine. Unlike macrophages, in human keratinocytes stimulated with dopamine, in which this neurotransmitter increases not only the production of IL-8 but also of IL-6 [5], we demonstrated that NFκB p65 levels were increased in the nuclear fraction with a corresponding decrease in the cytosol. These results suggest that the NFκB/IκB signaling pathway might be involved in the effect exerted by dopamine in keratinocytes.

In conclusion, in this work, we demonstrated a differential effect of dopamine in 2 cell types involved in the immune response of the cutaneous immune system. The neurotransmitter showed an inhibitory effect on the migration of keratinocytes in the scratch assay. Besides, we also observed a decrease in MMP-9 activity. Both actions would be mediated at least in part by β-AR and the activation of the transcription factor NFκB pathway. On the other hand, dopamine increased the activity of MMP-9 and the production of IL-8 in macrophages. The latter effects were partly mediated by β-ARs, involving mechanisms that are independent of the activation of NFκB. Therefore, we can conclude that the effect of dopamine via β-ARs depends on the physiological condition and the cell type involved. The effect of dopamine might contribute to either improving or delaying the healing process. The latter phenomenon is worth considering when novel therapeutic targets are developed for the treatment of skin diseases exacerbated by either stress conditions or infections.

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Disclosure Statement

The authors declare that they have no conflicts of interest regarding this work.

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