

Dopamine Agonists Upregulate IL-6 and IL-8 Production in Human Keratinocytes

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Key Words

Dopamine agonists · Cytokine · IL-6 · IL-8 · Keratinocyte

Abstract

Aim: Catecholamines regulate functions of the nervous, neuroendocrine and immune systems. Dopamine may modulate the activity of keratinocytes, which play a role in secreting cytokines and chemokines. The aim of this study was to evaluate the effect of dopaminergic agonists on the production of IL-6 and IL-8 by a non-tumoral human keratinocyte cell line (HaCaT). **Methods:** Cells were stimulated with dopamine and the D₂ dopamine receptor agonist cabergoline. Levels of IL-6 and IL-8 in culture supernatants were then determined. Cell proliferation was also assessed. Assays were carried out in the presence or absence of the dopaminergic and β -adrenergic receptor antagonists (sulpiride and propranolol, respectively) and ascorbic acid. **Results:** Dopamine stimulated the production of IL-6 and IL-8 in a concentration-dependent manner. The effects observed on the secretion of IL-6 were more potent than those corresponding to IL-8 and were reduced by ascorbic acid. The dopamine-induced IL-6 secretion was partially reduced by sulpiride and abrogated by propranolol. The latter drug was able to block the effect of dopamine on the secretion of IL-8. The cabergoline-induced IL-6 release was reduced by sulpiride. Cell viability was not affected by any of the drugs. **Conclusions:** Dopaminergic agonists can stimulate keratinocytes to produce IL-6 and IL-8 which are related to inflammatory cutaneous pro-

cesses. These effects are mediated by dopaminergic and β -adrenergic receptors and by receptor-independent oxidative mechanisms.

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Introduction

Catecholamines (dopamine, adrenaline and noradrenaline) are known to regulate many functions of the central nervous system and the neuroendocrine axis. They also modulate functions of the immune system [1, 2]. It is known that several immune cell types express neurotransmitter receptors [3, 4]. Dopamine can modulate proliferation, differentiation, apoptosis and cytokine production by immune cells by means of its interaction with dopaminergic, α - and β -adrenergic receptors located on the cell surface and by intracellular oxidative mechanisms as well [4–6].

In the skin immune system, keratinocytes are connected with resident immune cells such as lymphocytes, Langerhans cells and macrophages, to contribute to innate immunity and inflammatory processes. Exposure to agents causing inflammation as well as environmental aggressors can induce the secretion of proinflammatory cytokines and chemokines (IL-1, IL-6, IL-8, IL-10, IL-18) by keratinocytes. These soluble mediators, in turn, carry out their functions in paracrine and autocrine manners [7, 8]. The proinflammatory cytokine IL-6 is known to be in-

volved in the proliferation and differentiation of epidermal cells [9]. Modifications in the regulatory pathways of IL-6 are related to abnormal inflammatory and proliferative processes, such as keloid formation during the wound healing process [10]. The main function of IL-8 is to stimulate the chemotaxis of neutrophils which secrete antimicrobial peptides, i.e. defensin and cathelicidin, which play a crucial role in defense mechanisms of the skin [7]. The latter chemokine is also implicated in proliferation and chemotaxis of keratinocytes [11]. Moreover, several studies have demonstrated that keratinocytes belonging to psoriatic patients secrete high levels of IL-8 [12].

The ability of keratinocytes to synthesize and degrade catecholamines, as well as their capacity to express dopaminergic and α/β -adrenergic receptors on their surface, has been demonstrated [13–15]. These receptors have been proven to be implicated in the pathogeny of skin inflammatory diseases such as vitiligo, psoriasis and atopic dermatitis [13, 16] and in the regulation of wound healing [17–19]. However, the participation of the dopaminergic pathways in the physiology of the skin immune system has not been studied so far.

Catecholamines can modulate the immune function through oxidative processes in addition to its interaction with catecholaminergic receptors. Keratinocytes have many antioxidant systems (enzymes and molecules such as tocopherol, glutathione and ascorbic acid) which protect cells from oxidative damages generated by environmental factors [20]. In the HaCaT human keratinocyte cell line, the presence of efficient systems for the intracellular accumulation of ascorbic acid have been demonstrated [21]. This non-tumoral cell line is an excellent model to perform studies under physiological conditions, since these cells have phenotypical features that are similar to those of normal keratinocytes [22, 23]. Since studies dealing with the research of dopaminergic pathways operating in the skin immune system are scarce, the aim of the present work was to evaluate the effect of dopamine and the dopaminergic agonist cabergoline in the regulation of IL-6 and IL-8 production. Further, in order to assess whether the effect was mediated by dopaminergic/ β -adrenergic receptors or oxidative mechanisms, we used catecholaminergic receptor antagonists as well as ascorbic acid as antioxidant.

Materials and Methods

Chemicals

Dopamine (Sigma, St. Louis, Mo., USA), the β -adrenergic antagonist propranolol (Imperial Chemical, England), the dopaminergic antagonists haloperidol (Sigma) and sulpiride (Vipral,

Laboratorio IVAX Argentina, SA) were dissolved in 0.1 N acetic acid. Cabergoline (kindly provided by Laboratorios Beta, Argentina) is a D_2 -type dopaminergic agonist displaying low affinity for adrenergic receptors. This drug was dissolved in absolute ethanol plus 10% phosphate-buffered saline. Work dilutions were done in RPMI medium supplemented with fetal calf serum, 100 IU/ml penicillin, 100 μ g/ml streptomycin, 2 mM pyruvate and 2 mM glutamine (Gibco-BRL/Invitrogen, Argentina).

Cell Culture

HaCaT cells, a non-tumoral human epithelial keratinocyte cell line, were kindly provided by Prof. N.E. Fusening from the German Cancer Research Center (Heidelberg, Germany). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, penicillin, streptomycin, pyruvate and glutamine at 37°C under a 5% CO₂ atmosphere until confluence was reached.

Treatment

Keratinocytes (1×10^4 cells/well) were placed in 96-well microtiter plates and stimulated with different concentrations of dopaminergic agonists (dopamine 10^{-4} to 10^{-7} M, cabergoline 10^{-4} and 10^{-5} M) in the presence or absence of 0.1 mM ascorbic acid and in the presence or absence of dopaminergic (haloperidol, sulpiride at 10^{-5} and 10^{-4} M) and β -adrenergic (propranolol 10^{-5} M) antagonists and incubated for 24 h. Antagonists were added 1 h previous to the stimulation with dopamine or cabergoline. At the end of the incubation time, the supernatants were collected and kept at -40°C for subsequent determination of cytokine levels. Experiments were repeated 4–5 times each.

Cell Proliferation and Viability

The WST-1 tetrazolium salt was employed (Roche Applied Science, Argentina). Upon oxidation by the mitochondrial system, the color developed by this reagent is a measure of the proliferation index of cells. Briefly, after 24 h of culture, the salt was added and incubated at 37°C with a 5% CO₂ atmosphere for 1 h. The formazan produced upon cleavage of the salt was quantified in an ELISA reader (Meterterch Σ 960) at 450 nm (OD₄₅₀). The values plotted are the means \pm SEM of the optical density (OD₄₅₀) obtained from 4–5 independent experiments.

Cytokine Determination

Cytokine levels were determined in culture supernatants by capture ELISA employing commercial kits (R&D Systems for human IL-6, and BD OptEIA, Bioscience, for human IL-8), according to the manufacturer's directions. ODs were measured in an ELISA reader (Meterterch Σ 960). Cytokine concentrations were derived from standard curves. Detection limits were 2.3 and 4.0 pg/ml for IL-6 and IL-8, respectively. The values plotted are the means \pm SEM of the percentage values obtained from 4–5 independent experiments (considering the control value as 100%).

Statistical Analysis

The effect of dopaminergic agonists on the production of cytokines and on proliferation was analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni's a posteriori test. The effect of antagonists on the dopaminergic agonist-induced cytokine release was analyzed by one-way ANOVA followed by the a posteriori Student-Newman-Keuls test. A p value of <0.05 was considered significant.

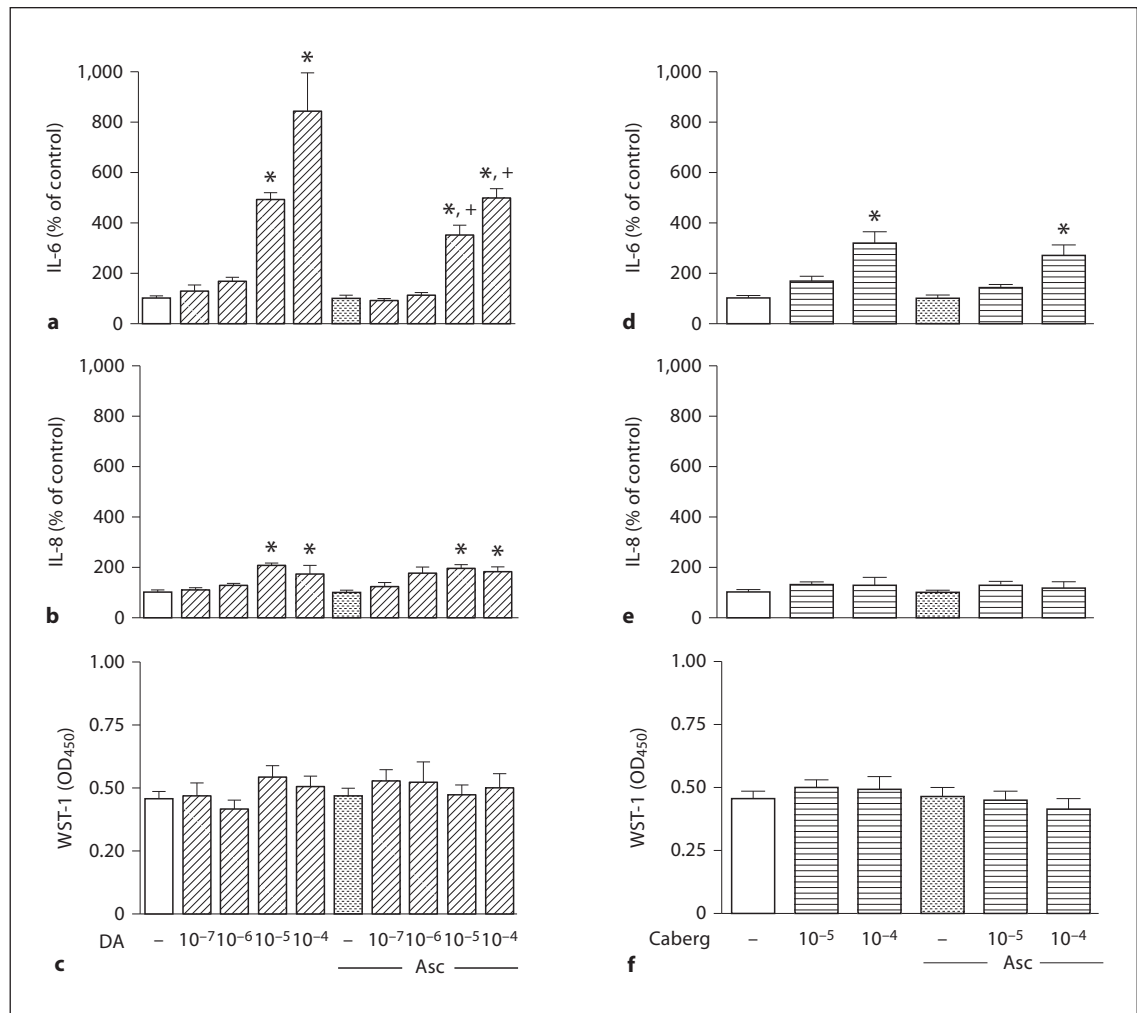


Fig. 1. Effect of the dopaminergic agonists dopamine (DA) and cabergoline (Caberg) on the secretion of cytokines and cell viability measured by WST-1 tetrazolium salt assay. Secretion of IL-6 (a, d), IL-8 (b, e) and cell viability (c, f) by HaCaT cells after 24 h of culture in the absence (RPMI) or presence of 0.1 mM ascorbic

acid (Asc). Cytokine levels were expressed as percentage of control (which was assigned a value of 100%) ± SEM. Cell viability was expressed as the average OD₄₅₀ ± SEM. * p < 0.05 versus control; + p < 0.05 versus dopamine.

Results

Effect of Dopaminergic Agonists on the Production of Cytokines by HaCaT Cells

In order to evaluate the effect of dopaminergic agonists on the production of IL-6 and IL-8, keratinocytes were stimulated with different concentrations of dopamine (10⁻⁷ to 10⁻⁴ M) and cabergoline (10⁻⁵ and 10⁻⁴ M) for 24 h.

Levels of IL-6 in culture supernatants increased after stimulation with dopamine in a dose-dependent manner, reaching significant levels for 10⁻⁵ and 10⁻⁴ M dopamine

(fig. 1a). The latter effect was reduced by the addition of 0.1 mM ascorbic acid. There were no significant differences between control levels of IL-6 in the presence or absence of ascorbic acid (23.83 ± 1.49 and 34.07 ± 3.69 pg/ml, respectively).

Levels of IL-8 in culture supernatants of HaCaT cells were also increased in response to the stimulation with 10⁻⁵ and 10⁻⁴ M dopamine. Although this increase was less pronounced than the one obtained for IL-6, IL-8 reached levels that doubled those of controls. Besides, the increase in IL-8 was not reduced by the addition of ascorbic acid (fig. 1b). Again, no differences were found be-

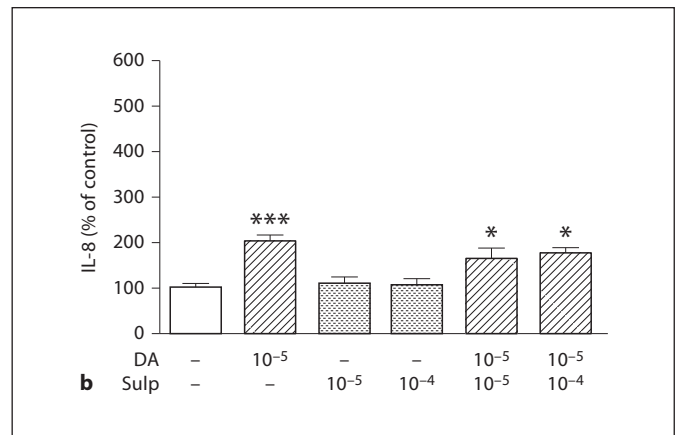
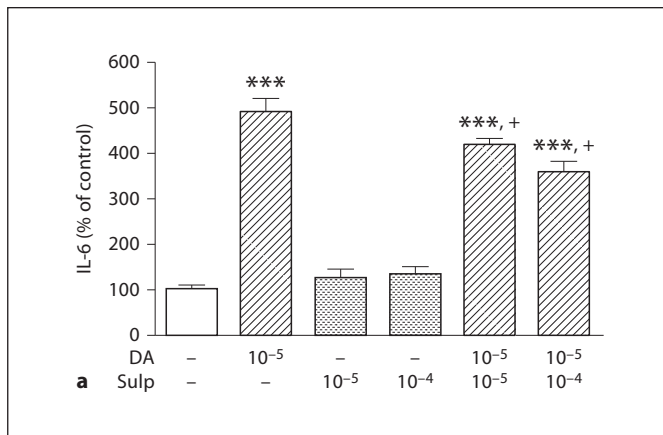


Fig. 2. Effect of the dopaminergic antagonist sulpiride (Sulp) on the production of IL-6 (a) and IL-8 (b) after treatment of HaCaT cells with dopamine (DA). Data are expressed as percentage of control values \pm SEM. * $p < 0.05$ versus control; *** $p < 0.001$ versus control; + $p < 0.05$ versus dopamine.

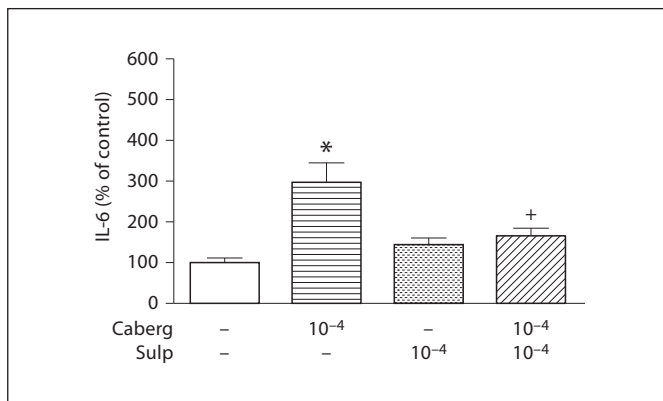


Fig. 3. Effect of the dopaminergic antagonist sulpiride (Sulp) on the production of IL-6 after treatment of HaCaT cells with cabergoline (Caberg). Data are expressed as percentage of control values \pm SEM. * $p < 0.05$ versus control; + $p < 0.05$ versus cabergoline.

tween basal levels of this cytokine in the presence or absence of ascorbic acid (69.02 ± 4.36 and 78.28 ± 3.66 pg/ml, respectively).

At the concentrations employed, dopamine did not modify cell viability, as assessed by WST-1 (fig. 1c).

In order to evaluate the role of D_2 receptors in cytokine production, cabergoline was employed, since this drug displays high affinity for these receptors. Upon stimulation of HaCaT cells with 10^{-5} and 10^{-4} M cabergoline, a dose-dependent increase in the release of IL-6 was observed. Values were significantly different from controls

when 10^{-4} M cabergoline was employed; however, the maximum effect obtained was lower than that obtained with dopamine (fig. 1d). No significant increase in the production of IL-8 was observed after stimulation with the same dopaminergic agonist (fig. 1e). The addition of ascorbic acid did not modify the cytokine secretion profile after cabergoline stimulation (fig. 1d, e). Furthermore, at the concentrations of cabergoline employed, cell viability was not affected (fig. 1f).

Effect of the Dopaminergic Antagonist Sulpiride on the Dopaminergic Agonist-Induced IL-6 and IL-8 Secretion

In order to determine the type of receptor involved in the production of cytokines induced by dopaminergic agonists, the D_2 antagonist sulpiride was employed. Unlike haloperidol (10^{-4} M) which induced a decrease in cell viability (data not shown), the addition of sulpiride at 10^{-5} and 10^{-4} M did not have toxic effects on HaCaT cells.

As shown in figure 2a, the addition of the antagonist sulpiride 10^{-5} and 10^{-4} M induced a low but significant decrease in the production of IL-6 when keratinocytes were stimulated with dopamine at 10^{-5} M in the absence of ascorbic acid, a condition under which the maximum cytokine release was obtained. On the other hand, the receptor blockade induced by sulpiride did not affect the dopamine-induced secretion of IL-8 (fig. 2b). As expected, the addition of sulpiride at 10^{-4} M induced a significant decrease in the cabergoline-induced secretion of IL-6 (fig. 3).

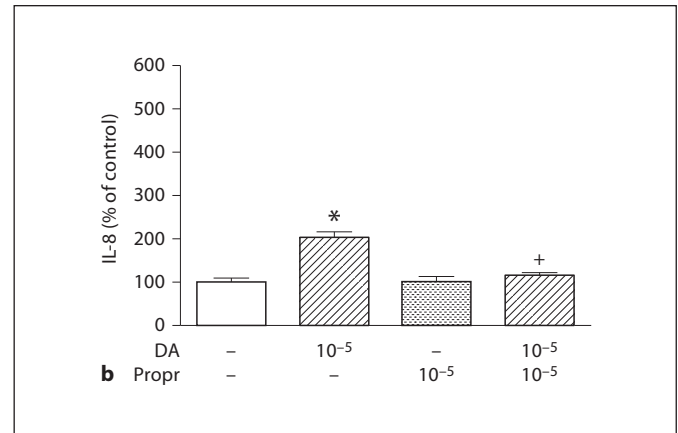
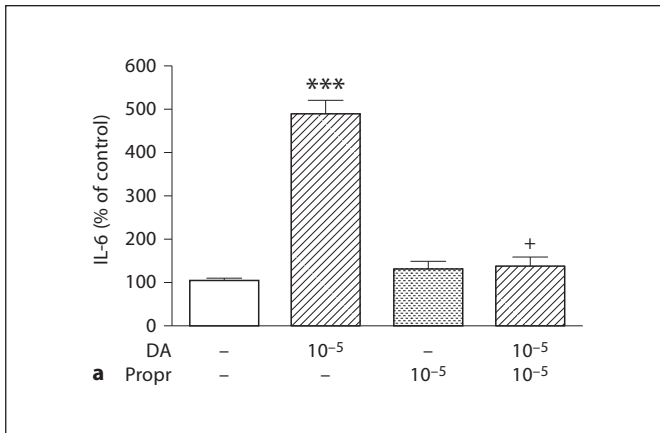


Fig. 4. Effect of the β -adrenergic antagonist propranolol (Propr) on the production of IL-6 (a) and IL-8 (b) after the treatment of HaCaT cells with dopamine (DA). Data are expressed as percentage of control values \pm SEM. * $p < 0.05$ versus control; *** $p < 0.001$ versus control; + $p < 0.05$ versus dopamine.

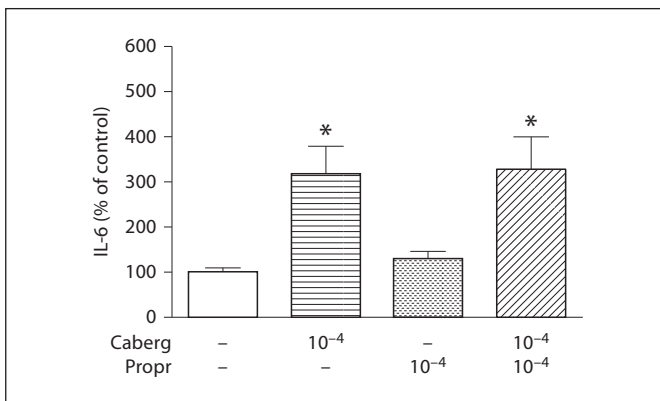


Fig. 5. Effect of the β -adrenergic antagonist propranolol (Propr) on the production of IL-6 after treatment with cabergoline (Caberg). Data are expressed as percentage of control values \pm SEM. * $p < 0.05$ versus control.

Effect of the β -Adrenergic Antagonist Propranolol on the Dopaminergic Agonist-Induced Production of IL-6 and IL-8

In order to determine whether the effect of the dopaminergic agonists on the production of cytokines was also mediated by β -adrenergic receptors, as described for cells of the immune system [24], keratinocytes were treated with propranolol, a selective β -adrenergic blocker. At 10^{-5} M, this antagonist was able to diminish significantly the stimulatory effect exerted by dopamine on the release of IL-6 and IL-8 (fig. 4). As expected, the addition of

10^{-4} M propranolol did not modify the stimulatory effect of cabergoline, which has a low affinity for β -adrenergic receptors, as regards the production of IL-6 (fig. 5).

In the absence of dopaminergic agonists, at the concentrations employed, propranolol did not modify the levels of cytokines released.

Discussion

In this study, we have demonstrated that dopaminergic agonists can stimulate keratinocytes to produce IL-6 and IL-8. This effect is mediated by β -adrenergic and dopaminergic receptors, together with the intervention of receptor-independent oxidative mechanisms.

The modulatory effect of catecholamines on the function of several cells of the immune system is well known [3, 4, 25]. It has recently been demonstrated that both epinephrine and norepinephrine can stimulate the production of proinflammatory cytokines [tumor necrosis factor- α (TNF- α), IL-1 β and IL-6] by macrophages which become activated by these catecholamines [26]. At the epidermal level, transformed cells like melanoma, can produce IL-6, IL-8 and vascular endothelial growth factor upon stimulation with norepinephrine, and the latter phenomenon is in association with cancer progression [27]. Our results demonstrated that dopamine can stimulate the production of IL-6 and IL-8 by the HaCaT keratinocyte cell line. It is noteworthy that the effect on the production of IL-6 was greater than that observed for IL-

8. In line with our results, it has also been demonstrated that dopamine has a stimulatory effect, inducing adrenal cells to secrete IL-6 [28], and that lung endothelial cells produce IL-8 upon stimulation [29].

The stimulatory effect on the production of IL-6 exerted by dopamine in keratinocytes was less marked in the presence of ascorbic acid. The latter phenomenon was not observed for IL-8. It is known that in this cell type, vitamin C contributes to counteract the oxidative stress [20] and diminishes the production of IL-6 induced by the exposure to UVA light [30].

Similarly to our observations, in monocytes obtained from adults, vitamin C decreases the production of the proinflammatory cytokines IL-6 and TNF- α without affecting the secretion of IL-8 [31]. However, these authors found opposite effects in blood cells of neonates induced by ascorbic acid [32], thus evidencing a differential modulatory effect of vitamin C on cytokine production. In agreement with these observations and employing UVB light as inflammatory stimulus, Kang et al. [33] have demonstrated that 2.5 mM ascorbic acid (a concentration higher than that employed in our study) can abrogate the increase in IL-8 and monocyte chemotactic protein-1 induced by suppression of the p38 MAPK pathway, thus regulating the inflammatory response. Although Beck et al. [29] have demonstrated in lung endothelial cells that vitamin C and other antioxidants can suppress the stimulatory effect of dopamine on the production of IL-8, a concentration similar to that employed in our study (0.1 mM) did not have any effect.

Our results suggest that the effect of dopamine on the production of IL-6 is mediated, at least in part, by oxidative mechanisms. The production of IL-8, on the contrary, is governed by less sensitive redox pathways.

High concentrations of dopamine have been proven to exert antiproliferative effects. These effects are mediated by dopaminergic receptors in lymphocytes [3] and by oxidative pathways in macrophages [34]. Our studies showed that the increase in the levels of IL-6 and IL-8 after the stimulation with dopaminergic agonists is not a consequence of changes in keratinocyte viability.

The behavior of cabergoline was different. This drug is a potent D₂ agonist employed in the treatment of hyperprolactinemia due to hypofiseal tumors and, at a lesser extent, in Parkinson's disease. This ergot derivative increased the production of IL-6 by keratinocytes without affecting cell viability. The effect was not modified by the presence of ascorbic acid. It has been suggested that cabergoline has antioxidant properties in the central nervous system which could act synergistically with endogenous

antioxidants such as vitamin E and ascorbic acid [35–37]. In the cell line employed herein, a synergistic effect of this drug with ascorbic acid was not observed, as regards the production of IL-6. The latter results could be due to a low concentration of the antioxidant employed (0.1 mM) and due to the model used to assess the effect of this dopaminergic agonist. The production of IL-8 was not affected by the presence of cabergoline. This is the first work dealing with the effect of cabergoline on the production of cytokines at the skin cell level.

From the results obtained in this work, it could be suggested that the effect of dopamine on the production of IL-6 by keratinocytes might be mediated by oxidative mechanisms triggered by the amine itself. This would not be the case for the production of IL-8. Other works have demonstrated that dopamine regulates the production of IL-12p40 and IL-10 by macrophages by both receptor-dependent and receptor-independent mechanisms, the latter ones being related to the autooxidation processes [24].

It is known that dopaminergic agonists can either induce or suppress the production of several cytokines, depending on the cell type, the mechanism involved and the physiological status [38, 39]. It has been suggested that dopaminergic agonists exert their effects through the binding to dopaminergic receptors when used at low concentrations, whereas when high doses are used, their effects are manifested through the binding to adrenergic receptors. Although the participation of catecholaminergic receptors in the modulatory effects of dopamine has been demonstrated in many cell types [3, 40], the receptor subtypes involved in the production of cytokines are still a matter of controversy. Our work demonstrated that, apart from the oxidative mechanisms present in keratinocytes, dopamine can stimulate the production of IL-6 by means of its interaction with dopaminergic and β -adrenergic receptors. Sulpiride, a D₂ antagonist, partially decreased the dopamine-induced production of IL-6 and, as expected, this drug suppressed the response induced by cabergoline, a potent D₂ agonist. This effect was not due to a toxic effect of sulpiride on keratinocytes, since no alterations in cell viability were detected. In line with our results, it has been demonstrated that in cells from the adrenal zona glomerulosa, dopamine can increase the D₂ receptor-mediated release of IL-6 and it can also suppress the production of TNF- α [28]. Haskó et al. [24] have observed that dopamine suppresses the production of IL-12 in macrophages and that this effect is mediated by β -adrenergic receptors. The observations made by these authors highlight an important modulatory role of dopamine in inflammation and during the immune

response. In our study, it was demonstrated that propranolol, a β -adrenergic antagonist, blocked the stimulatory effect of dopamine on the production of both IL-6 and IL-8. These results indicate that β -adrenergic receptors would also be involved in the effect of dopamine on keratinocytes.

It is known that IL-6 and IL-8 stimulate the proliferation of human keratinocytes and their overexpression is related to inflammatory and hyperproliferative disorders of the skin [9, 11, 12, 41]. Furthermore, dopaminergic and adrenergic receptors are known to be involved in the skin healing processes [17, 42]. IL-6 has also been demonstrated to be a potent mediator that facilitates wound healing of the corneous epithelium [43], and therefore, it could be hypothesized that dopamine could induce these effects through the induction of IL-6 secretion. However, this requires additional *in vivo* studies.

In summary, our results evidenced a modulatory role of dopaminergic agonists in keratinocyte functionality, affecting the production of proinflammatory cytokines or chemokines. The effect of dopamine at the cutaneous level should be borne in mind upon administration of dopamine agonists to treat neurological and neuroendocrine disorders.

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