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Cis-Urocanic Acid Down-regulates Histamine-mediated Activation of Adenylate Cyclase in the Pig Epidermis

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Urocanic acid (UCA), one of the skin's major components for absorbing UV radiation, is present naturally in the stratum corneum as a *trans*-isomer. On absorption of UVB radiation either *in vitro* or in the skin, UCA undergoes *trans*- to *cis*-isomerization in a dose-dependent manner. Although the mechanism by which *cis*-UCA suppresses immunity remains unelucidated, recent studies have indicated that *cis*-UCA appears to inhibit the induction of cyclic AMP in fibroblasts, which suggests that this molecule plays an active role in modifications to the skin. Here, we report that although neither *trans*-UCA nor *cis*-UCA increases cyclic AMP in the pig epidermis, *cis*-UCA actively down-regulates the increase of cyclic AMP induced by histamine. The effects of *cis*-UCA on the pig epidermis are revealed through the modulation of the effects caused by histamine. These findings suggest that in the pig epidermis, the initial biochemical and cellular event for UVB-induced immune suppression – that is, the step immediately following the isomerization of *trans*-UCA to *cis*-UCA – is down-regulation of cyclic AMP brought about by the activity of *cis*-UCA. *Key words: urocanic acid; cyclic AMP; pig epidermis; histamine*.

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Urocanic acid (UCA) is present in the stratum corneum, predominantly as a *trans*-isomer (deaminated histidine) (1). On ultraviolet B (UVB) irradiation, it undergoes isomerization to a *cis*-isomer (2). UVB-induced immune suppression *in vivo* has been shown to be associated with modification of antigen- presenting cell function and the induction of T-suppressor cells. It has also been shown that *cis*-UCA administered to mice can induce similar modifications of antigen-presenting cells, thereby replacing the UVB component (3, 4). It is generally believed that *cis*-UCA mediates impairment of the induction of contact hypersensitivity to UVB (5). Although we have recently demonstrated that *cis*-UCA promotes tolerance and induces suppressor cells in mice (6), the precise mechanism by which *cis*-UCA alters the immune system is currently unknown. A study involving UCA analogues and histamine receptor antagonists in the modulation of the delayed hypersensitivity response to the herpes simplex virus has indicated that UCA may act through histamine-like receptors (7). UCA is structurally homologous to histamine, and a recent report has shown that both histamine and *trans*-UCA up-regulate intracellular cyclic AMP, and that such induction is down-regulated by *cis*-UCA in dermal fibroblasts (8). Keratinocytes express H1 and H2 receptors, and both receptor types play a role in mediating the responses of keratinocytes to *cis*-UCA and histamine (9). It has also been reported that in cultured human keratinocytes, *cis*-UCA synergizes production of PGE2 in response to histamine, and that this is linked to indomethacin-inhibitable UVB-induced immunosuppression (9). In this report, we here examine the effects of *trans*- and *cis*-UCA on adenyl cyclase, as measured by cyclic AMP formation in the pig epidermis.

MATERIALS AND METHODS

Reagents

Histamine, the *trans*-isomer of urocanic acid (*trans*-UCA) (4-imidasoleacrylic acid) and 3-isobutyl-1-methyl-xanthine (IBMX) were all purchased from Sigma (St. Louis, MO, USA). The *cis*-isomer of UCA was prepared by UVB irradiation (FL20SE30 fluorescent lamp; Clinical Supply Co., Tokyo, Japan). Briefly, a thinly spread solution of *trans*-UCA in a phosphate-buffered saline (PBS) solution was irradiated for 8 h at a distance of 46 cm. The irradiated solution was then diluted in PBS to reach a final concentration of 1 mg/ml (6). Analysis via high-performance liquid chromatography (HPLC) revealed approximately 50.5% of the irradiated UCA present as the *cis*-isomer. The *cis*-UCA was then purified by HPLC, using the method of Caron et al. (10) with some modifications. The solution of irradiated UCA was subjected to HPLC on an analytical reversed-phase column (10) with KH_2PO_4 (0.05 M)-acetonitrile (1 : 1, v/v) as the mobile phase. The *cis*-UCA fraction was collected, and this collection procedure was repeated. Contamination by the *trans*-form was not detectable in the purified *cis*-UCA fraction. All other chemicals were of reagent grade.

Preparation of the pig epidermis

Pig skin slices were obtained using a keratome adjusted to a depth of 0.2 mm. The skin slices were then treated with 1,000 U/ml of dispase in RPMI 1640 medium for 30 min at 37°C. After the dispase treatment, the pure epidermal sheets were peeled off with sharp forceps. The slices were cut into 5 × 5-mm squares and floated on RPMI 1640 medium at 37°C with the keratin layer up.

The pig epidermis for cyclic AMP measurements

After 20-min preincubation at 37°C in the presence of 1 mm IBMX (8) to standardize the initial cyclic AMP levels in the epidermal pieces (11), the specimens were incubated for 5 min with either 5×10^{-5} histamine (12), or various doses of UCA (*trans* or *cis* form). To examine the effect of the *trans*-UCA or *cis*-UCA concentration on the histamine adenylate cyclase response, we stimulated preincubated epidermal slices for 20 min with various concentrations of *trans*- or *cis*-UCA plus 1 mm IBMX, and for a further 5 min with 5×10^{-5}M histamine. The generation of cyclic AMP was terminated by quickly freezing the skin pieces between two plates of dry ice.

Radioimmunoassay for cyclic AMP

After the frozen skin pieces were boiled and homogenized, the supernatant was succinylated and the cyclic AMP content measured by radioimmunoassay using a Yamasa cyclic AMP assay kit (Yamasa Shoyu Co., Tokyo, Japan) as previously described (13). The protein concentration was measured by the method of Lowry et al. (14).

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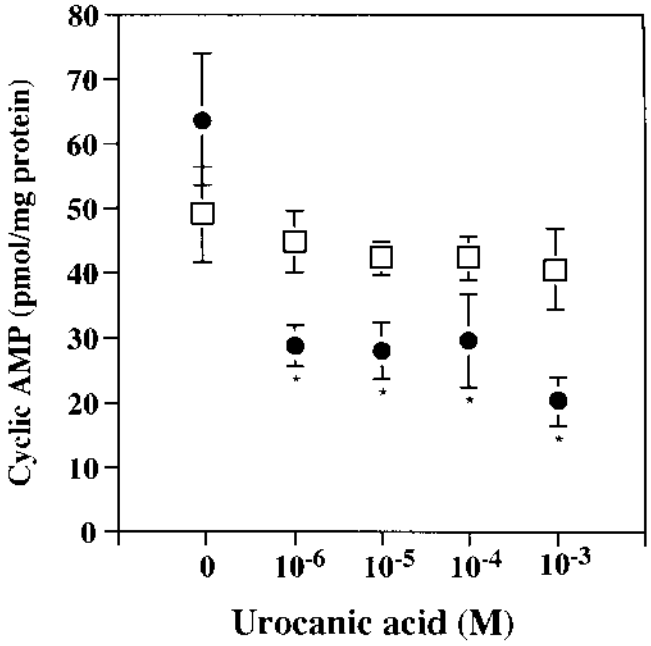

Fig. 1. The concentration effects of *trans*-UCA or *cis*-UCA on the histamine adenylate cyclase response in the pig epidermis. Epidermal slices preincubated for 20 min with various concentrations of *trans*- (□) or *cis*- (●) UCA plus 1 mm IBMX, were stimulated with 5×10^{-5}M histamine for 5 min. Data represent the mean ± SEM of three independent experiments. Results for *trans*- and *cis*-UCA are from separate experiments. For any results of *cis*- (●) UCA marked with an asterisk, a significant difference ($p < 0.05$) was detected between it and the control (5×10^{-5}M histamine stimulated without *cis*-UCA).

The pig epidermis trans- *or* cis-*UCA contents*

Homogenates of the pig epidermis were subjected to HPLC by the analytical method of Caron et al. (10).

RESULTS AND DISCUSSION

We first examined whether *trans*-UCA and *cis*-UCA could induce adenylate cyclase activation. Decreasing concentrations of purified *trans*- or *cis*-UCA were added to the pig epidermis, and the cyclic AMP levels were determined. Neither *trans*-UCA nor *cis*-UCA induced cyclic AMP at concentrations from 1×10^{-6}M to 1×10^{-3}M, but 5×10^{-5}M histamine did induce cAMP (data not shown).

Given that neither *trans*- nor *cis*-UCA induced cyclic AMP, we next attempted to determine whether UCA could down-regulate the induction of cyclic AMP brought about by histamine. Decreasing amounts of *trans*- or *cis*-UCA, together with a fixed amount of histamine (5×10^{-5}M), were added to the pig epidermis, and cyclic AMP induction was measured. Figure 1 shows that *cis*-UCA, at concentrations from 1×10^{-6}M to 1×10^{-3}M, inhibited the cyclic AMP-inducing effects of histamine, while *trans*-UCA did not. The effect of *cis*-UCA on the inhibition of the induction of cyclic AMP by histamine was not dose-dependent. A possible explanation for this result is that *cis*-UCA may have caused the uncoupling of the receptor from its signalling system, as Palaszynski et al. have reported (8). The original contents of *trans*-UCA and *cis*-UCA were 313 ± 33.3 and 198 ± 18.4 pmol/mg tissue, respectively.

There is increasing evidence that *cis*-UCA may be a mediator of at least some of the effects of UVB radiation, including immunosuppression. Using a murine model of the herpes simplex virus infection, Ross et al. have shown that *cis*-UCA is highly effective in suppressing delayed hypersensitivity responses to the virus (15). We have recently shown that *cis*-UCA induces tolerance and suppressor cells in mice (6). Other studies suggest that the immunosuppressive effects of *cis*-UCA occur through histamine or histamine-like receptor pathways (16, 17).

Although in the present study *cis*-UCA was found to inhibit histamine-mediated adenylate cyclase activation in the pig epidermis, neither *trans*- nor *cis*-UCA affected the transmembrane signals. Our results agree with a previous report that *cis*-UCA down-regulates the histamine response in cultured dermal fibroblasts (8). Those experiments, however, demonstrated that *trans*-UCA is biologically active in fibroblasts, and induces adenyl cyclase, as measured by cyclic AMP (8). The relation between *cis*-UCA and histamine is not simple. These discrepancies of induction of cyclic AMP by UCA may indicate that the *trans*- and *cis*-isoforms of UCA differ in their effects on various cell types in the skin. Such differential effects may also occur *in vivo* after exposure to UV irradiation.

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