The Osmolyte Strategy of Normal Human Keratinocytes in Maintaining Cell Homeostasis

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Compatible organic osmolytes, such as betaine, myoinositol, and taurine, are involved in cell volume homeostasis as well as in cell protection, for example, against oxidative stress. This socalled osmolyte strategy requires the expression of specific osmolyte transporting systems such as the betaine/γ-amino-n-butyric acid (GABA) transporter, the sodium-dependent myoinositol transporter and the taurine transporter (TAUT). In contrast to liver, kidney, and neural cells, nothing is known about osmolytes in the skin. Here we report that primary normal human keratinocytes (NHK) express mRNA specific for the betaine/GABA transporter, for the sodium-dependent myoinositol transporter and for the TAUT. In comparison to normoosmotic (305 mosmol per L) controls, a 3–5-fold induction of mRNA expression for the betaine/GABA-, the sodium-dependent myoinositol- and the TAUT was observed within 6–24 h after hyperosmotic exposure (405 mosmol per L). Expression of osmolyte transporters was associated with an increased uptake of radiolabeled osmolytes. Conversely, hypoosmotic (205 mosmol per L) stimulation induced significant efflux of these osmolytes. Exposure to ultraviolet B (290–315 nm) or ultraviolet A (340–400 nm) radiation, which are major sources of oxidative stress in skin, significantly stimulated osmolyte uptake. Increased osmolyte uptake was associated with upregulation of mRNA steady-state levels for osmolyte transporters in irradiated cells. These studies demonstrate that NHK possess an osmolyte strategy, which is important for their capacity to maintain cell volume homeostasis and seems to be part of their response to UV radiation.

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Compatible organic osmolytes are compounds which are specifically accumulated by cells in response to hyperosmotic stress and which are rapidly released from cells after hypoosmotic exposure (Kwon and Handler, 1995; Häussinger, 1996). Organic osmolytes need to be non-perturbing solutes that do not interfere with protein function even when occurring at high intracellular concentrations (Burg *et al*, 1997). Hyperosmotic osmolyte accumulation inside the cells is accomplished by an osmosensitive expression of osmolyte transporters, such as the betaine-/γ-amino-n-butyric acid (GABA) transporter (BGT-1) for betaine, the sodium/myoinositol cotransporter (SMIT) for myoinositol and taurine transporter (TAUT) for taurine. The osmolyte strategy is of special importance in renal medullary cells, which are exposed to strong fluctuations in ambient osmolarity during antidiuresis and diuresis (Graf, 1992), in liver cells, where osmolytes can interfere with important cell functions such as gene expression (Zhang *et al*, 1996a, b; Warskulat *et al*, 1997a, c; Weik *et al*, 1998), and in brain in which—due to encapsulation in rigid bone—cell volume homeostasis requires delicate control (Häussinger *et al*, 1994). Human peripheral blood monocytes use myoinositol and betaine as compatible organic osmolytes as well (Denkert *et al*, 1998). But nothing is known about osmolytes in the skin although the functional relevance of water transport and cell hydration is well known in skin epidermis (Warner *et al*, 1988; Wolff *et al*, 1999; Sougrat *et al*, 2002). In this regard it is important to note that osmolytes do not only serve a role in cell volume homeostasis, but have additionally been found to protect other cell types—for example, by virtue of their capacity to stabilize proteins (Qu *et al*, 1998)—against various different injuries. Assessment of the osmolyte strategies of human skin cells and, in particular, of epidermal keratinocytes, which represent the primary cellular targets for environmental noxae, may thus provide new insights into previously unrecognized, endogenous protection strategies of the skin.

Former studies have shown that, in dog and rat epidermis, taurine is present at high concentrations in the granular and upper spinous layers (Lobo *et al*, 2001), but its function was not examined. Furthermore, hyperosmotic stimulation has previously been reported to elevate intracellular calcium levels and to inhibit proliferation of the spontaneously immortalized human keratinocyte cell line HaCaT (Dascalu *et al*, 2000). In HeLa epithelial cells, UV radiation and osmotic stress, in an essentially identical manner, induce cell membrane receptor clustering and subsequent activation of the c-Jun amino-terminal protein kinase (JNK) cascade,

Abbreviations: BGT-1, betaine-/γ-amino-n-butyric acid (GABA) transporter; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPRT, hypoxanthine-guanine-phosphoribosyl-transferase; NHK, normal human keratinocytes; SMIT, sodium-dependent myoinositol transporter; TAUT, taurine transporter; UV, ultraviolet; UVA, ultraviolet A; UVB, ultraviolet B

thereby causing induction of many target genes (Rosette and Karin, 1996). In this regard, osmotic shock modulates epidermal growth factor receptor transactivation (Rosette and Karin, 1996), which is mediated by the stress kinase $p38^{MAPK}$ in HaCaT keratinocytes (Cheng *et al*, 2002). Interestingly, $p38^{MAPK}$ is also involved in the hyperosmotic induction of BGT-1 and SMIT in human peripheral blood monocytes (Denkert *et al*, 1998). Preliminary evidence suggests that hyperosmotic stimulation can upregulate the expression of UVB-inducible genes in long-term cultured, normal human keratinocytes (NHK).[1] Because sunlight is the most important natural source of oxidative stress for skin (Bender *et al*, 1997; Peus *et al*, 1998; Krutmann, 2001), UV radiation is well accepted as a surrogate for oxidative stress in keratinocytes (Grether-Beck *et al*, 1996, 2000; Heck *et al*, 2003).

In this report we demonstrate that primary NHK possess an osmolyte strategy, which is important for their capacity to maintain cell volume homeostasis and seems to be part of their response to UV radiation.

Results

Expression of BGT-1, SMIT, and TAUT mRNA in NHK Real-time RT-PCR analysis revealed that under normoosmotic (305 mosmol per L) conditions, NHK expressed mRNA specific for BGT-1, SMIT, and TAUT ($0.1 \pm < 0.1$, 3.6 ± 0.3 and $0.1 \pm < 0.1$ mRNA copies/hypoxanthine-guanine-phosphoribosyl-transferase (HPRT) mRNA copy). Hyperosmotic (405 mosmol per L) exposure of NHK led to a 3–5-fold time-dependent induction of BGT-1, SMIT, and TAUT mRNA levels (Fig 1*A*). Maximal responses were obtained for SMIT and TAUT mRNA expression after 9 h and for BGT-1 after 3 h of hyperosmotic exposure.

Organic osmolyte transport in NHK The functional significance of hyperosmotic stress-induced osmolyte transporter mRNA expression was assessed by measuring uptake of radiolabeled osmolytes in NHK. Under normoosmotic (305 mosmol per L) conditions, NHK took up betaine, myoinositol, and taurine at low levels (Table I). Under these conditions, taurine uptake was 2.6- and 2.2-fold higher than uptakes of betaine and myoinositol over a 2 h time period. Culture of NHK in osmolyte-free, normo- *versus* hyperosmotic media for 16 h and a subsequent exposure for 2 h to media containing 100 μM betaine, myoinositol, or taurine caused a significant increase in osmolyte uptake in hyperosmotically stressed cells with 50% for betaine, 53% for myoinositol and 160% for taurine. It should be noted that 2 h uptake values were chosen in order to minimize potential osmolyte effects on a substrate-induced transporter downregulation which was previously seen to occur in other cell types (Warskulat *et al*, 1997c). Also, due to the slow accumulation of osmolytes into cells these 2 h uptake values do not reflect intracellular steady-state levels, but represent a roughly initial uptake rates and thereby reflect transporter activity.

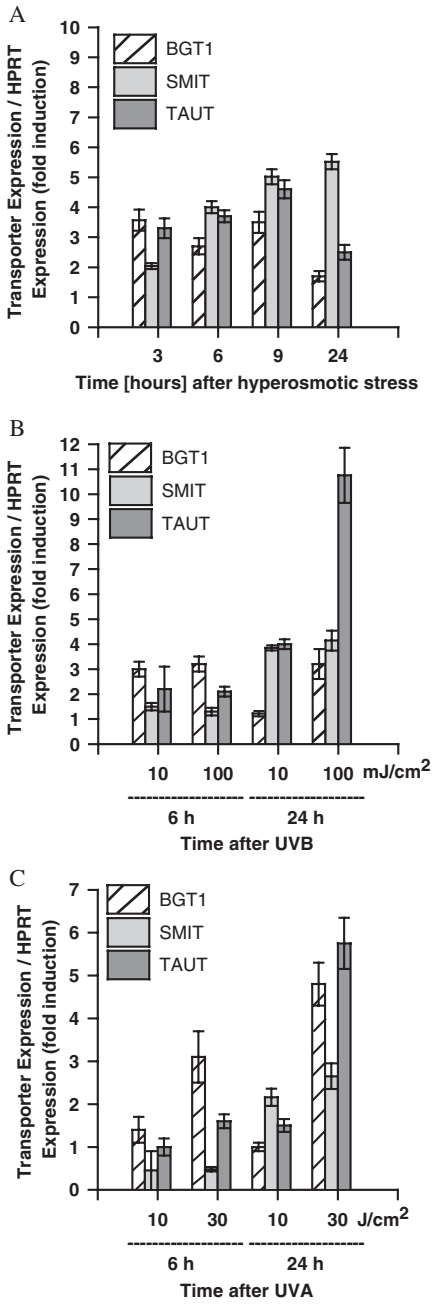

Figure 1
Induction of BGT-1, SMIT, TAUT in NHK after hyperosmotic stress (*A*), UVB (*B*) or UVA radiation (*C*) in NHK as detected by real-time PCR. (*A*) NHK were exposed for the indicated time periods to hyperosmotic (405 mosmol per L) medium or cultured under normoosmotic (305 mosmol per L) conditions. Osmolarity changes were performed by appropriate addition of NaCl. (*B*) NHK were exposed to two doses of UVB radiation (10 or 100 mJ per cm^2) and harvested at 6 and 24 h after UVB treatment. (*C*) NHK cells were exposed to two doses of UVA radiation (10 or 30 J per cm^2) and harvested at 6 and 24 h after UVA treatment. Results are given as fold induction of gene of interest (BGT-1: hatched barr, SMIT: light grey barr; TAUT: dark grey barr) based on HPRT (= housekeeping) gene expression in stimulated *versus* unstimulated (= normoosmotic, sham-irradiated) cells. Gene expression under normoosmotic conditions in sham-irradiated cells was assessed at all indicated time points and arbitrarily set as one. Real-time PCR was done as described in Materials and Methods. Data are given as means ± SEM and are from three independent experiments.

[1]Felsner I, Grether-Beck S, Schmitt H, Sies H, Krutmann J: Induction of the human heme oxygenase-1 gene by ultraviolet A radiation (UVAR) and osmotic stress. Arch Derm Res 290:78, 1998 (abstr.).

Table I. Organic osmolyte transport in NHK

	Osmolyte uptake (nmol per mg protein)		
Osmolyte	**Betaine**	**Myoinositol**	**Taurine**
Test conditions			
305 mosmol per L	1.3 ± 0.1	1.5 ± 0.1	3.8 ± 0.3
405 mosmol per L	2.1 ± 0.5[a]	2.6 ± 0.1[a]	9.6 ± 2.9[a]
305 mosmol per L + 50 mJ per cm^2 UVB	2.5 ± 1.3	2.1 ± 0.1[a]	8.4 ± 1.5[a]
305 mosmol per L + 10 J per cm^2 UVA	1.3 ± 0.1	1.7 ± 0.3	5.3 ± 0.1
305 mosmol per L + 30 J per cm^2 UVA	2.1 ± 0.5[a]	3.4 ± 0.2[a]	6.2 ± 0.2[a]

Cells were ultraviolet-irradiated or sham-irradiated as indicated and then incubated for 16 h in normo (305 mosmol per L)- or hyperosmotic medium (405 mosmol per L). Osmolarity changes were performed by addition of 50 mM NaCl. Thereafter, the uptake of 100 μM [^{3}H]taurine, [^{14}C]betaine or [^{3}H]myoinositol was measured for 2 h in medium with the same osmolarity. Data are given as means ± SEM (n = 3–6).
[a]Significantly different from the control condition (305 mosmol per L, sham-irradiated), $p < 0.05$.

Efflux of osmolytes after hypoosmotic exposure NHK were allowed to accumulate osmolytes (added at a concentration of 10 μM) for 16 h. Thereafter, cells were washed twice and incubated in normo- (305 mosmol per L) or hypoosmotic (205 mosmol per L) buffer for 5 min to 1 h. This hypoosmotic stimulation induced a significant efflux of osmolytes (Table II). The release of betaine in isoosmotic medium was only 34% after 1 h, whereas in hypoosmotic medium it was 44% after 5 min and 68% after 1 h. Efflux of myoinositol and taurine in isoosmotic medium was 13% and 24% after 1 h, but 27% and 77% in hypoosmotic medium, respectively (Table II).

Ultraviolet B and A radiation increased osmolyte uptake in human keratinocytes Since UVB radiation is a major source of oxidative stress (Bender *et al*, 1997; Peus *et al*, 1998), which may result in cell hydration changes (Hallbrucker *et al*, 1993; Saha *et al*, 1993), and since UVB and osmotic stress induce similar signalling events (Rosette and Karin, 1996), we next assessed the influence of UVB radiation on osmolyte transport. Biological effects which are caused by wavelengths below 300 nm mainly result from direct DNA damage such as the formation of cyclobutane pyrimidine dimers (Stege *et al*, 2000). At wavelengths above 300 nm, however, in addition to the direct formation of DNA photoproducts, the generation of oxidative stress is becoming increasingly important (Kochevar, 1985). Therefore, in this study, a UVB irradiation device was used which primarily emitted in the long wave UVB range >300 nm with an emission maximum around 312 nm.

Ultraviolet B radiation (50 mJ per cm^2) significantly stimulated myoinositol and taurine uptake in NHK (Table I). In contrast to hypoosmotic exposure, UVB radiation did not affect osmolyte efflux from NHK cells (Table II). UVB radiation resulted in a 3-fold upregulation of BGT-1 mRNA expression at 6 and 24 h post-exposure (Fig 1*B*) whereas increased SMIT mRNA expression could only be observed 24 h after UVB treatment. TAUT mRNA expression was found to be upregulated 2-fold 6 h after UVB irradiation and either 4-fold (10 mJ per cm^2) or 11-fold (100 mJ per cm^2) 24 h after exposure.

Modulation of osmolyte uptake and osmolyte transporter expression was not specific for UVB, but was also observed after UVA irradiation. Accordingly, UVA radiation resulted in upregulation of all transporters tested (Fig 1*C*): expression of BGT-1 mRNA was induced 3-fold 6 h and 5-fold 24 h after irradiation with a dose of 30 J per cm^2 UVA. SMIT mRNA expression increased 2-fold at 10 J per cm^2 or 3-fold at 30 J

Table II. Osmolyte efflux from NHK following hypoosmotic exposure and UVB radiation

	Osmolyte efflux (% of total radioactivity)					
Osmolyte	**Betaine**		**Myoinositol**		**Taurine**	
Time (min)	**5**	**60**	**5**	**60**	**5**	**60**
Test conditions						
305 mosmol per L	19 ± 2	34 ± 1	10 ± 3	13 ± 3	14 ± 2	24 ± 4
205 mosmol per L	44 ± 5[a]	68 ± 7[a]	15 ± 2	27 ± 2[a]	54 ± 8[a]	77 ± 7[a]
305 mosmol per L + 100 mJ per cm^2 UVB	28 ± 4	40 ± 4	15 ± 4	18 ± 6	18 ± 5	29 ± 7

Cells were allowed to accumulate [^{14}C]betaine, [^{3}H]myoinositol, or [^{3}H]taurine (added at a concentration of 10 μM) for 16 h, washed thrice, UVB- or sham-irradiated as indicated and thereafter exposed to osmolyte-free normo (305 mosmol per L)- or hypoosmotic (205 mosmol per L) Krebs–Henseleit buffer. [^{3}H]taurine, [^{14}C]betaine and [^{3}H]myoinositol appearence in the supernatant was measured and expressed as the percentage of total radioactivity (contained in cells plus supernatant). Data are given as means ± SEM and are from 4 separate experiments.
[a]Significantly different from the control condition (305 mosmol per L, sham-irradiated), $p < 0.01$.

per cm^2 after 24 h. TAUT mRNA expression was concentration dependently increased at both time points. Upregulation of osmolyte transporter mRNA expression was associated with a significantly increased uptake of the respective osmolytes in UVA-irradiated NHK cells (Table I).

Discussion

This report describes betaine, myoinositol, and taurine as compatible organic osmolytes in NHK. This conclusion is based upon the following findings: (i) NHK expressed mRNA specific for BGT-1, SMIT, and TAUT and this expression was upregulated upon hyperosmotic exposure (Fig 1); (ii) mRNA expression of these osmolyte transporters was associated with uptake of the respective osmolytes, and osmolyte uptake was significantly increased in these cells under hyperosmotic stress (Table I); and (iii) incubation of keratinocytes in hypoosmotic medium led to a rapid efflux of betaine, myoinositol, and taurine from cells (Table II). It should be noted that the experiments performed do not allow to estimate the net changes of osmolyte flux. Determination of osmolyte efflux and uptake was done using a standard prodedure where radioactively labelled osmolytes were detected either in the medium or in the cell lysate. As the analysis of the efflux is preceded by loading of the cells with the radioactively labelled osmolyte in a concentration of 10 μM for 16 h the efflux data cannot be compared to the uptake data—even when the cells have been kept under the same osmotic conditions and exposed to the same dose of irradiation.

Betaine, myoinositol, and taurine were already identified as osmolytes in the brain, kidney, and liver and interfere with cell volume regulation and cell function (for reviews see Kwon and Handler, 1995; Häussinger, 1996, 1998). It is of great interest that compatible organic osmolytes are not only important for cell volume homeostasis but also for cell protection, for example, against oxidative stress (Yancey *et al*, 1982; Huxtable, 1992). They can act as "chemical chaperones" and stabilize native protein structures and protein function (Liu and Bolen, 1995; Welch and Brown, 1996). In this regard, betaine and taurine can protect cells *in vitro* against various types of injury (Chapman *et al*, 1993; Welch and Brown, 1996; Wettstein and Häussinger, 2000; Alfieri *et al*, 2002). Altered osmolyte contents are described for important diseases, for example, hepatic encephalopathy, diabetes, polycystic kidney disease, and Gaucher disease (Häussinger *et al*, 1994; Ogbron *et al*, 1997; Bluml *et al*, 1998; Vom Dahl *et al*, 2000; Hanson, 2001). Also, a high taurine concentration was shown to be present in dog and rat epidermis (Lobo *et al*, 2001). Taken together with the results from this study it seems likely that, similar to kidney, liver, and neural cells, epidermal keratinocytes possess an osmolyte strategy which may be important for maintenance of cell volume homeostasis and reflect part of their stress response towards environmental noxae such as UV radiation. We propose that this newly identified property of keratinocytes is of general relevance for their cellular integrity under conditions which are characterized by a disturbance of cell volume homeostasis including intraepidermal edema formation (spongiosis) which may occur during acute inflammation, or epidermal dehydration, which may result from an increased transepidermal water loss (Wolff *et al*, 1999). In addition, any major disturbance of epidermal integrity as a consequence of mechanical or thermal stress as well as subsequent wound-healing processes are expected to be associated with profound changes in cell hydration. Our concept is supported by the observation that taurine may be effective in wound healing of mouse skin (Degim *et al*, 2002). In addition, TAUT mRNA expression increases not only by hyperosmolarity and UV radiation, but also by the known skin irritant sodium lauryl sulfate (SLS), as was recently shown by means of DNA microship analysis in studies employing the EpiDerm skin model (Fletcher *et al*, 2001). Also, topical betaine application reduced skin-irritating effects of SLS in the oral mucosa (Soderling *et al*, 1998).

In this study, we have focused on the analysis of osmolyte strategies in epidermal keratinocytes because these cells build the outermost barrier of the skin towards the environment. In future studies, it will be of interest to assess whether other epidermal and possibly also dermal cell populations are equipped with similar or even different osmolyte strategies. Accordingly, heterogeneity of osmolyte transport systems among different cell types and their different expression upon stimulation has previously been shown to exist for other organs including the liver (for reviews see Häussinger, 1996, 1998).

Cellular hydration can change after oxidative stress. Oxidative stress exerted by hydroperoxides or intracellular generation of H_2O_2 was shown to induce cell shrinkage due to an opening of K^+ channels (Hallbrucker *et al*, 1993; Saha *et al*, 1993). In addition, oxidative stress led to alterations of the osmolyte content in brain (Brand *et al*, 1999). Under physiological conditions, epidermal keratinocytes are exposed to oxidative stress from various sources among which sunlight is the most important one (Krutmann, 2001). Solar UV radiation comprises both UVB (290–315 nm) and UVA (315–400 nm) radiation, and both types of irradiation have previously been shown to exert biological effects in NHK through the generation of oxidative stress (Lim and Soter, 1993; Grether-Beck *et al*, 1997; Bender *et al*, 1997; Heck *et al*, 2003). In particular, UVB and UVA radiation-induced intracellular signalling was found to involve the generation of H_2O_2 (Peus and Pittelkow, 2001) and singlet oxygen (Grether-Beck *et al*, 1996, 2000), respectively, and it has recently been demonstrated that in the epithelial HeLa cell line, UVB radiation-induced signalling events could be mimicked by hyperosmotic stimulation of cells (Rosette and Karin, 1996). Taken together it is thus tempting to speculate that UVB and UVA radiation, similar to hyperosmotic stress, may cause keratinocyte shrinkage and the subsequent uptake of osmolytes. This concept is supported by the present observation that UVB irradiation, similar to hyperosmotic stress, increases the uptake of myoinositol and taurine in NHK (Table I). Taurine was the most affected osmolyte under the stimulation conditions tested and this osmolyte is therefore most likely of particular relevance for UVB responses of human keratinocytes. In contrast, UVA irradiation induced transporter mRNA expression and uptake of all three osmolytes to a similar extent, which may thus be equally important for the UVA response of keratinocytes.

The UVB and UVA radiation doses employed in this study did not affect cell viability (MTT assay; data not shown). In

addition, increased osmolyte uptake after UVB and UVA irradiation or hyperosmotic stress in NHK was associated with upregulation of osmolyte transporter mRNA expression. This indicates that the UVB-, UVA-, as well as hyperosmolarity-induced increase in osmolyte uptake most likely reflects an active process such as an increased synthesis of transporter proteins rather than the short-term activation of pre-existing transporters. Similarly, long-term induction of osmolyte transporter expression and osmolyte uptake has previously been reported for hyperosmotically stressed rat parenchymal and non-parenchymal liver cells (Warskulat *et al*, 1997a–c; Weik *et al*, 1998) as well as human monocytes and macrophages (Denkert *et al*, 1998).

Because of their function as "chemical chaperones", it is thus likely that increased osmolyte uptake by UV-irradiated keratinocytes may be part of their defense strategy against detrimental effects induced by UV irradiation. Accordingly, Janeke *et al* (2003) reported that keratinocytes express a functionally active TAUT transporter. The authors also provide *in vivo* evidence for the existence of a gradient for TAUT expression in the epidermis with maximal TAUT levels in the outermost granular keratinocyte layer and lower levels in the stratum corneum and demonstrate *in vitro* that taurine protects keratinocytes against UVB radiation-induced apoptosis. Taurine (Aruoma *et al*, 1988; Devamanoharan *et al*, 1998) and betaine (Metha *et al*, 2002; Patrick, 2002) are known to exert antioxidative activities and it will therefore be of great interest to assess in future studies whether these osmolytes—through this or additional functions—are capable of protecting human keratinocytes against other UV radiation-induced biological effects as well. Ongoing studies employing TAUT knockout mice (Heller-Stilb *et al*, 2002) will test this hypothesis more directly.

Materials and Methods

Cells Long-term cultured, NHK were prepared from neonatal foreskins obtained from surgery at the time of operations due to unrelated circumcision with informed consent of the donors. The NHK were cultured under serum-free conditions as described (Grether-Beck *et al*, 1996).

Osmolarity changes Osmolarity changes were achieved by varying the NaCl concentration in medium, which is a widely used method to characterize osmolyte strategies. In this regard, many studies showed that regulation of mRNA steady-state levels for TAUT, BGT-1 and SMIT by osmolarity is independent from Na^+ and Cl^- concentration, for example, in kidney cells, parenchymal, and non-parenchymal liver cells as well as human monocytes and macrophages (Zhang *et al*, 1996a; Burg *et al*, 1997; Denkert *et al*, 1998; Weik *et al*, 1998). In preliminary experiments we have found that, stimulation of osmolyte uptake occurred regardless of whether hyperosmolarity was instituted by addition of NaCl or by raffinose (data not shown), indicating that effects on osmolyte transport were caused by hyperosmolarity and not by an increase in extracellular Na^+ and Cl^- concentration. Manipulation of NaCl, but not of raffinose levels, allows to induce both hyper- and hypoosmotic stress, and therefore in this study, only the first approach was used.

UV irradiation Medium was replaced by phosphate-buffered saline (PBS), and cells were exposed to UVB radiation from a BIO-SUN irradiation device (LTF Labortechnik GmbH & Co KG, Wasserburg, Germany). This epoxy painted irradiation cabinet is equipped with a bank of two Philips TL-01 fluorescent tubes (Philips AEG, Springe, Germany) that allows exposure of cells to long wave UVB radiation with a maximum around 312 nm UVB. The UV intensity emitted by the bulbs is automatically controlled 6 × per second by means of internal silicon photoelectric cells.

For UVA radiation, medium was replaced by PBS, and cells were exposed at a distance of 30 cm to UVA radiation from a UVASUN 24,000 (System Dr. Sellmeier, Sellas GmbH, Gevelsberg, Germany) emitting in a range between 340 and 400 nm with a maximum around 360 nm. Dosimetry was done using a Waldmann UV meter type II (Herbert Waldmann GmbH & Co, Villingen-Schwenningen, Germany) with a spectral sensitivity matching the main emission spectrum around 350–400 nm.

For sham-irradiation, cells were not exposed to UVB or UVA radiation but otherwise treated identically.

Real-time PCR Total RNA from HNK was isolated using a total RNA extraction kit (Qiagen, Hilden, Germany) and reverse transcribed using a first strand cDNA synthesis kit (Roche, Mannheim, Germany). The levels of gene expression were measured by real-time SYBR Green PCR with the Gene Amp 5700 Sequence Detection System (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. The primers used for PCR were 5′-AAAGCTGTGACAATGATGCCG-3′ and 5′-CCAGTCCAAGCAAGAGAAGCA-3′ for TAUT, 5′-AACAGTGCCACCAGCTTTGTG-3′ and 5′-ACTCGGCCACTTCAGAAATGG-3′ for betaine transporter (BGT-1), 5′-TCAACCACATCATTCCCAACG-3′ and 5′-TGCTGTCTTCAGATT-TCCCGT-3′ for myoinositol transporter (SMIT), and 5′-AAGATGG-TCAAGGTCGCAAGC-3′ and 5′-GGTCCTTTTCACCAGCAAGCT-3′ for HPRT as internal standard. PCR conditions were: one cycle at 50°C for 2 min and 95°C for 10 min, and 40 cycles at 95°C for 15 s and 60°C for 1 min. The sample volume was 25 μL, respectively. After PCR, the specificity of the amplified DNA was assessed by recording dissociation curve to distinguish the fluorescence peak corresponding to the amplicon from the peak due to primer–dimer formation.

Uptake of betaine, myoinositol, and taurine Uptake of organic osmolytes was measured as described in a previous report (Warskulat *et al*, 1997c). Briefly, keratinocytes were incubated for 16 h in serum-free culture medium at the indicated osmolarity. Thereafter, medium was replaced by an identical volume of medium containing 100 μM [^{14}C]betaine (48.1 Ci per mmol), [^{3}H]taurine (24 Ci per mmol, both from New England Nuclear DuPont, Bad Homburg, Germany), or [^{3}H]myoinositol (22.3 Ci per mmol, ICN, Meckenheim, Germany) and cells were incubated for the indicated periods of time. Cultures were rinsed three times with ice-cold stop solution containing 10 mM Tris–HEPES (pH 7.4), 300 mM mannitol, and 300 mM NaCl. Cells were dried in air, lysed, and aliquots were taken for liquid scintillation counting and protein determination (BioRad protein assay, Bio-Rad laboratories, Hercules, California).

Efflux of betaine, myoinositol, and taurine Keratinocytes were loaded with 10 μM [^{14}C]betaine, [^{3}H]taurine, or [^{3}H]myoinositol in serum-free culture medium for 16 h. Cells were rinsed two times with PBS. After UVB irradiation, cells were incubated in betaine-, myoinositol- and taurine-free normo- (305 mosmol per L) or hypoosmotic (205 mosmol per L) culture medium for the indicated periods of time. Medium and cell lysates were collected and radioactivity was measured by scintillation counting. Efflux of [^{14}C]betaine, [^{3}H]myoinositol, and [^{3}H]taurine into the supernatant was expressed as percentage of total radioactivity in cell lysates plus supernatants.

Statistical analysis Statistical analysis was performed using two-tailed Student's *t* test. p values of <0.05 were considered to be significant.

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