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Research paper

Functional characterization of sodium- and chloride-dependent taurine transport in human keratinocytes☆

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Abstract

In the skin, taurine acts as an important osmolyte required for keratinocyte hydration. It has antioxidant effects, protects cells from UV-induced stress and has effects on cell proliferation, inflammation and collagenogenesis. This study was performed to find and characterize functionally a taurine transport system in keratinocytes and to establish a cell culture model for skin taurine transport studies. Uptake of [^{3}H]taurine was studied both in the human adult low calcium high temperature (HaCaT) cell line and in human native epidermal keratinocytes. Uptake of [^{3}H]taurine in HaCaT cells was strictly dependent on extracellular sodium and chloride. The taurine uptake rate was saturable and indicated participation of a single transport system with kinetic parameters of $K_t = 5.1 \pm 0.2$ μM and $V_{max} = 320.5 \pm 2.8$ pmol/10 min per mg of protein. Uptake was strongly inhibited by β-amino acids (taurine, β-alanine, hypotaurine, β-guanidinopropionic acid), whereas α- and γ-amino acids had little or no effect. Taurine uptake in normal keratinocytes was very similar to that in HaCaT cells with respect to substrate specificity and affinity. We conclude that keratinocytes express the Na^+ and Cl^- dependent, high-affinity taurine transporter. This system accepts β- and certain γ-amino acids as substrates.

Keywords: Human adult low calcium high temperature cells; Keratinocytes; NHEK; Skin; Taurine transport; TAUT

1. Introduction

Taurine is one of the most abundant free amino acids in almost all mammalian tissues. In the intracellular space it is present in millimolar concentrations [2–5]. Though this amino acid is not incorporated into proteins and is known to participate in only very few metabolic reactions, available evidence indicates that taurine might participate in a variety of cellular functions and even be an essential nutrient during development. There are several biological functions of taurine under discussion, such as modulation of calcium levels, synaptic activity neuromodulator [6], inhibitory neurotransmitter [7] and membrane-stabilizing effects [2]. Its function as osmoregulator is of crucial importance because control of intracellular solute concentrations and cell volume is required for optimal cell function [3,4,8]. Taurine also has effects on thermoregulation, converts lipids and lipid-soluble substances into a water-soluble state and promotes the bioavailability of the lipid-soluble vitamins [3,4,9]. In the skin, taurine acts as an important osmolyte required for keratinocyte hydration [1]. It has antioxidant effects, protects cells from UV-induced stress and has effects on cell proliferation, membrane stability, inflammation and collagenogenesis [1,10–13].

Taurine deficiency can be manifested as a wide range of clinical symptoms, like retina degeneration and skin abnormalities. It has recently been proposed to be clinically significant in psoriasis [14]. Taurine deficiency could be caused, e.g. by defects of participating enzymes or transporters, by stress or demanding situations or by insufficient taurine intake. Because of its relevance for healthy skin, a large number of patents concerning taurine and skin function have been claimed. Değim et al. [15]

☆ During the preparation of this manuscript Janeke et al. [1] reported the presence of taurine transport and of the taurine transporter TAUT in human keratinocytes.

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described a dermal taurine formulation, which significantly increased wound tensile strength by decreasing the lipid peroxide formation—malondialdehyde and increasing hydroxyproline levels.

Recent studies have described a saturable transport system for taurine in many tissues such as liver, placenta, intestine, retina, kidney, choroid plexus and at the blood-brain barrier (for review see Refs. [16–18]). The taurine transport system has been cloned from several species. The transporter (TAUT, SLC6A6) belongs to the family of the sodium- and chloride-dependent neurotransmitter SLC6 (for review see Ref. [19]). Recently, a TAUT knockout mouse has been established [20]. The animals exhibited retinal degeneration and a marked impairment of reproduction. It has to be noted that taurine is also transported by other systems such as the proton-coupled amino acid transport system PAT1 but with much lower affinity [21].

Very recently, a paper appeared demonstrating the presence of TAUT mRNA in human keratinocytes and describing the biological function of taurine in these cells [1]. The localization of TAUT in normal human skin was done by western blot analysis using a polyclonal anti-TAUT antibody. TAUT was detected in the epidermis but not in the dermis. After preincubation of the cells with taurine and either osmotic or UV-induced stress the number of apoptotic cells was reduced. The authors concluded that taurine behaves as an important epidermal osmolyte required for keratinocyte hydration in a dry environment. This study was the first publication on the taurine transport system in skin. This is very surprising considering the relevance of taurine in this organ. What was known before was that in the epidermis taurine shows a specific pattern of distribution. It is highly concentrated in the epidermis (about 5 μmol/g fresh tissue) whereas the amount in the isolated stratum corneum is extremely low (<0.07 μmol/g) [22]. This report led us to suggest that keratinocytes express an active taurine carrier able to accumulate taurine against a concentration gradient.

2. Material and methods

2.1. Materials

Cell culture media and supplements and trypsin solution were purchased from Life Technologies, Inc. (Germany), fetal bovine serum from Biochrom AG (Germany). [1,2-^{3}H]Taurine (specific activity 32.0 Ci/mmol) was obtained from Amersham International (UK). Taurine, hypotaurine, β-alanine, leucine, serotonin, proline, γ-aminobutyric acid (GABA), choline, α-(methylamino)-isobutyric acid (MeAIB), 3-amino-1-propanesulfonic acid, β-guanidinopropionic acid (GPA) and γ-hydroxybutyric acid were purchased from Sigma (Germany). All other chemicals were of analytical grade.

2.2. Cell culture

HaCaT (human adult low calcium high temperature keratinocytes) cells (passages 36–50) established by Boukamp and coworkers [23] were routinely cultured in 75-cm^2 culture flasks with Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, gentamicin (44 μg/ml), 1% glutamine and calcium solution (0.09 mM) [16,17,24,25]. Subconfluent cultures were treated with Dulbecco's phosphate-buffered saline for 5 min followed by a 2-min incubation with 0.05% trypsin–0.02% EDTA solution. For most experiments, the cells were seeded in 35 mm disposable petri dishes (Becton Dickinson, UK) at a density of 0.8×10^6 cells per dish. The uptake measurements were performed on the second day after confluence. Normal keratinocytes were isolated from neonatal foreskins according to the standard procedure described by Life Technologies and cultured in serum-free keratinocyte medium supplemented with bovine pituitary extract (25 μg/ml) and recombinant epidermal growth factor (0.1 ng/ml). Subconfluent cells were washed with Dulbecco's phosphate-buffered saline and isolated using trypsin–EDTA at 37 °C. Cells were seeded at passage 2 at a cell density of 0.12×10^6 cells per dish. The uptake measurements were performed on days 13–14 after seeding.

2.3. Transport studies

Uptake of [^{3}H]taurine in cells cultured on plastic dishes was measured at room temperature [16,17]. In most cases, the uptake buffer contained 25 mM Hepes/Tris (pH 7.5), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, 10 nM [^{3}H]taurine and increasing concentrations of unlabeled substances (0–10 mM). After incubation for the most part, 10 min, the cells were quickly washed four times with ice-cold buffer, solubilized with 1 ml of a lysis buffer containing 50 mM Tris, 140 mM NaCl, 1.5 mM $MgSO_4$, 0.5% Igepal-Ca-630 and 0.2% sodium dodecyl sulfate and prepared for liquid scintillation spectrometry.

2.4. Data analysis

Experiments were done in duplicate or triplicate and each experiment was repeated two to three times. Results are given as means ± SEM. IC_{50} values (i.e. concentration of the unlabeled compounds necessary to inhibit 50% of [^{3}H]taurine carrier-mediated uptake) were determined by non-linear regression using the four parameter logistic equation as described [24,25].

3. Results

To determine whether keratinocytes take up taurine by a carrier-mediated process, we first investigated the taurine

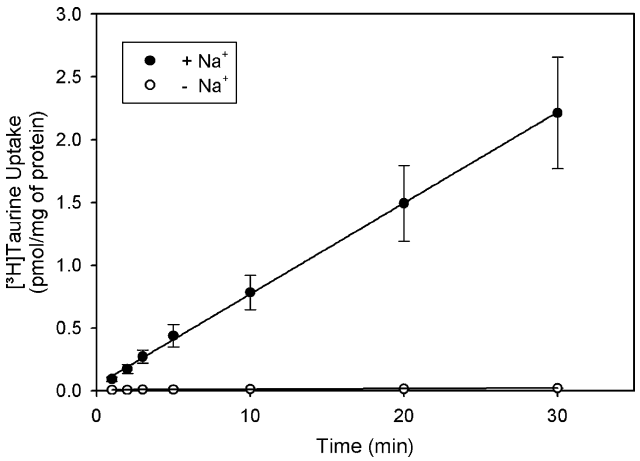

Fig. 1. Time course of [^{3}H]taurine uptake in HaCaT cells. Uptake of [^{3}H]taurine (10 nM) was measured in buffer (pH 7.5) containing NaCl 140 mM (closed circles) or choline chloride 140 mM (open circles). Data are shown as mean ± SEM, $n \geq 3$.

transport in HaCaT cells by measuring the time-dependent uptake of [^{3}H]taurine (10 nM) in the absence or presence of Na^+. Fig. 1 describes the time course of intracellular taurine accumulation in the presence of either NaCl or choline chloride. Extracellular Na^+ stimulated the total taurine uptake by a factor of 60 (at a 10 min uptake) indicating that the taurine transport in HaCaT cells is strictly Na^+ coupled. The time-dependent uptake was linear for up to 30 min ($r^2 = 0.9997$ and 0.9828, respectively) and occurred at a rate of 72.5 ± 0.8 fmol/min per mg protein in the presence of sodium versus 0.47 ± 0.04 fmol/min per mg protein in the absence of sodium. Extracellular sodium and an incubation period of 10 min were chosen for further uptake measurements. [^{3}H]Taurine uptake was found to be little dependent on extracellular pH as shown in Fig. 2. The uptake of [^{3}H]taurine (10 nM) was highest between 6.0 and 7.5.

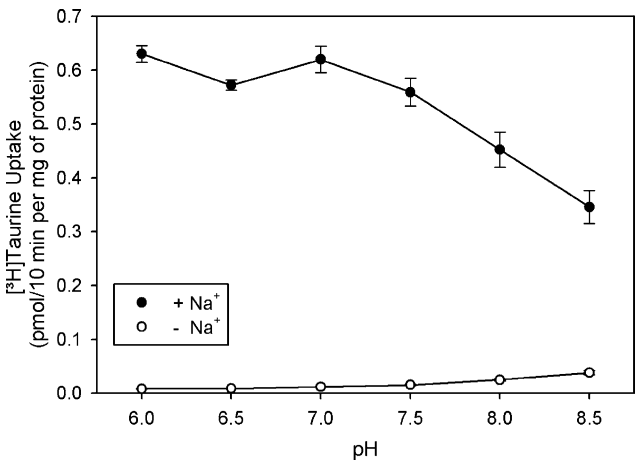

Fig. 2. Effect of extracellular pH on [^{3}H]taurine uptake in HaCaT cells. Uptake of [^{3}H]taurine (10 nM) was measured in buffer with different pH values containing NaCl (closed circles) or choline chloride (open circles) for 10 min. Data are shown as mean ± SEM, $n \geq 3$.

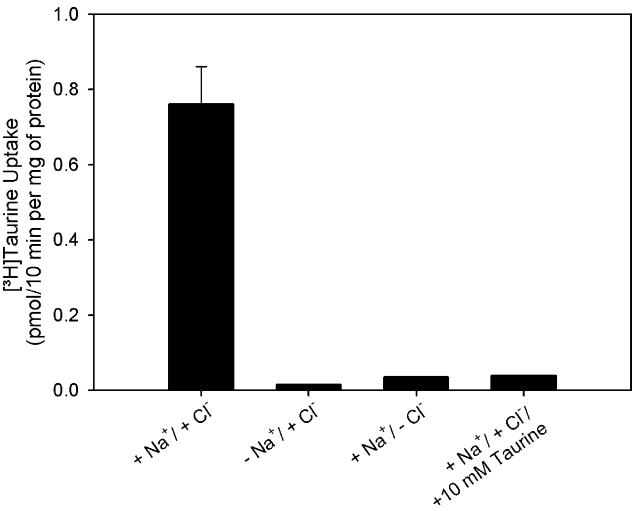

Fig. 3. Effect of extracellular Na^+ and Cl^- on specific [^{3}H]taurine uptake in HaCaT cells. Uptake of [^{3}H]taurine (10 nM) was measured in buffer (pH 7.5) with or without extracellular Na^+ or Cl^- and in the presence or absence of 10 mM unlabeled taurine for 10 min. For Na^+-free buffers, NaCl was replaced by choline chloride. Data are shown as mean ± SEM, $n \geq 3$.

The role of extracellular ions on specific taurine transport uptake was studied further by measuring Na^+ or Cl^- dependence in the absence or presence of unlabeled taurine (Fig. 3). Again, extracellular Na^+ stimulates total taurine uptake in HaCaT cells 47-fold. The taurine uptake also proved to be dependent on extracellular Cl^-: substitution of Cl^- with gluconate inhibited the specific [^{3}H]taurine uptake completely. In this series of studies, we also determined the saturability of the [^{3}H]taurine uptake. Unlabeled taurine at a concentration of 10 mM reduced the [^{3}H]taurine uptake remarkably by 95%. The remaining 5% represents non-saturable uptake components such as unspecific binding and/or simple diffusion. The same result was shown for normal human epidermal keratinocytes where the absence of sodium reduced the uptake of [^{3}H]taurine by 97%. With 10 mM unlabeled taurine the [^{3}H]taurine uptake was also decreased by 97% (data not shown).

To calculate the kinetic parameters of the uptake process we examined taurine transport in HaCaT cells as a function of substrate concentration in the range of 0.1–50 μM (Fig. 4). The study was performed in the presence of extracellular Na^+ at 22 °C for 10 min. The non-saturable diffusional component was determined by measuring the uptake of [^{3}H]taurine (10 nM) in the presence of 10 mM unlabeled taurine. This value was subtracted from the total transport rates to calculate the saturable, carrier-mediated component. The apparent affinity constant K_t (Michaelis–Menten constant) and the maximal velocity V_{max} of the Na^+-dependent taurine transporter were 5.1 ± 0.2 μM and 320.5 ± 2.8 pmol/10 min per mg of protein, respectively. When the results were expressed in the form of an Eadie–Hofstee plot (uptake rate/substrate concentration versus uptake rate), a straight line ($r^2 = 0.9975$) was obtained.

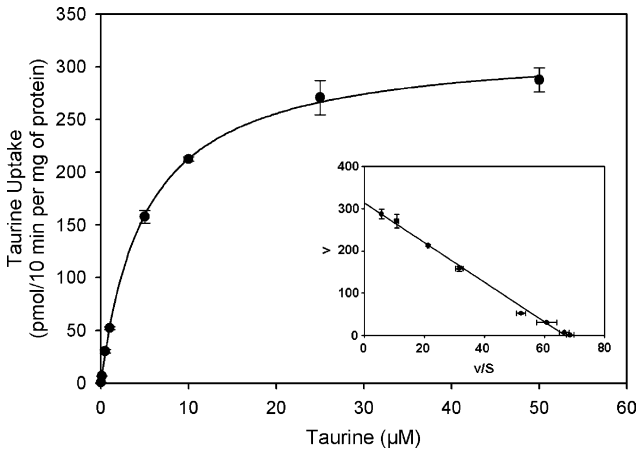

Fig. 4. Saturation kinetics of taurine uptake in HaCaT cells. Uptake of [^{3}H]taurine (10 nM) was measured in the presence of increasing concentrations of unlabeled taurine at pH 7.5 for 10 min. The results represent specific (saturable) uptake values after subtraction of the non-mediated component of transport ([^{3}H]taurine uptake in the presence of 10 mM unlabeled taurine). The results are shown as mean ± SEM, $n \geq 3$. Inset: Eadie–Hofstee transformation of the data.

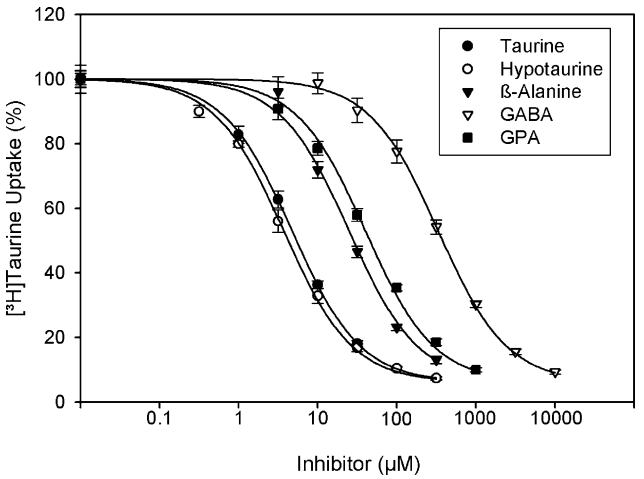

Fig. 5. Dose–response relationship for the inhibition of [^{3}H]taurine uptake in HaCaT cells. Uptake of [^{3}H]taurine (10 nM) was measured in the absence (control) or presence of increasing concentrations of various unlabeled substances at pH 7.5 for 10 min. The results are shown as mean ± SEM, $n \geq 3$.

The sodium-dependent taurine carrier expressed in other cells and tissues accepts taurine, hypotaurine and β-alanine as substrates. Therefore, we determined whether the taurine carrier expressed in HaCaT cells also recognizes these compounds (Table 1). The transport of radiolabeled taurine (10 nM) was strongly inhibited by 1 mM unlabeled taurine (to 7%), hypotaurine (to 7%), β-alanine (to 7%), GABA (to 29%), and GPA (to 12%), suggesting that these analogs are accepted by the keratinocyte taurine transporter. 3-Amino-1-propanesulfonic acid and serotonin inhibited the [^{3}H]taurine uptake to a smaller but significant extent. Unrelated compounds such as leucine, proline, choline, MeAIB and γ-hydroxybutyric acid had no effect on the taurine uptake into HaCaT cells. These results indicate that the taurine transport system in HaCaT cells is specific for taurine, hypotaurine, β-alanine, GPA, GABA, and 3-amino-1-propanesulfonic acid. To evaluate the affinity of these substrates the inhibitory constants versus taurine uptake were determined in competition assays and calculated from the displacement data shown in Fig. 5. The K_i values were 4.7 ± 0.1 μM for taurine, 3.7 ± 0.1 μM for hypotaurine, 24.8 ± 2.2 μM for β-alanine, 40.0 ± 2.2 μM for GPA, and 333.0 μM for GABA, respectively. The affinity constant of taurine for the taurine transport in human native keratinocytes was 3.3 ± 0.2 μM.

Table 1
Substrate specificity of [^{3}H]taurine uptake in keratinocytes cells

Inhibitor (1 mM)	[^{3}H]Taurine uptake (% ± SEM)	
	HaCaT cells	Native keratinocytes
Control	100 ± 2	100 ± 9
Taurine	6.5 ± 0.5	3.4 ± 0.6
Hypotaurine	6.8 ± 0.8	3.4 ± 0.1
β-Alanine	6.6 ± 0.2	3.1 ± 0.5
Serotonin	51 ± 1	n.d.
Leucine	88 ± 2	78 ± 3
Proline	93 ± 5	n.d.
γ-Aminobutyric acid	29 ± 1	12 ± 1
Choline	105 ± 4	n.d.
α-(Methylamino)-isobutyric acid	107 ± 2	n.d.
3-Amino-1-propanesulfonic acid	41 ± 2	n.d.
β-Guanidino-propionic acid	12 ± 1	n.d.
γ-Hydroxybutyric acid	102 ± 4	n.d.

Uptake was measured in the presence of NaCl at pH 7.5 for 10 min. Concentration of inhibitors was 1 mM. Data are shown as mean ± SEM, $n \geq 3$.

4. Discussion

The results of this investigation provide unequivocal evidence for carrier-mediated taurine transport in human keratinocytes. The system is functionally very similar to the taurine transport described in many tissues and organs such as liver, placenta, intestine, retina, kidney, choroid plexus and blood–brain barrier. As in other cells, in keratinocytes taurine uptake is strongly stimulated by extracellular Na^+ and Cl^- but not by extracellular H^+. Uptake of taurine by HaCaT as a function of substrate concentration can be described by a Michaelis–Menten-type kinetics with an affinity constant of $K_t = 5.1 \pm 0.2$ μM. Other authors have measured in different tissues and cells, K_t values between 1 and 232 μM, mostly in the 5–50 μM range.

Regarding the substrate specificity the Na^+/Cl^--dependent [^{3}H]taurine uptake can be inhibited by hypotaurine ≥ taurine > β-alanine > GPA > GABA > homotaurine. This result confirms that β-amino acids are recognized by

the taurine transporter with high affinity. α-Amino acids like proline or leucine and γ-amino acids have no or lower affinity for the transport system.

The HaCaT cells used in our study are human keratinocytes immortalized spontaneously [23]. To confirm that the taurine transport observed in these cells is not restricted to the cell line we demonstrated the same sodium dependence, substrate specificity and taurine affinity in native non-transformed human keratinocytes in primary culture.

The importance of taurine for skin function is well documented [1,10–13]. Taurine and several derivatives are used in preparations for skin care, cosmetic treatment and for treatment of dermal diseases. Approximately 90 different patents of pharmaceutical and cosmetical formulations containing taurine have been claimed.

Physiologically, the skin is supplied with taurine from the blood. Serum concentrations of 100–200 μM have been determined [26]. For the normal taurine concentration in the epidermis of rats, values of <0.07–5 μmol/g have been reported [22]. The system with a K_t value of 5 μM will transport taurine under physiological conditions efficiently and might also be responsible for the transport of taurine that is used in dermal and transdermal cosmetic and therapeutic preparations.

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