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Adaptive regulation of taurine and β-alanine uptake in a human kidney cell line from the proximal tubule

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Abstract

1. The underlying mechanisms involved in the adaptive regulation of β-amino acid uptake in the human proximal tubule were examined by use of an immortalized human embryonic kidney epithelial cell line (IHKE). 2. The results indicated that the adaptive response to maintain whole-body taurine homeostasis occurs predominantly via changes in the activity of the high-affinity taurine transport system by alterations in the uptake capacity and with an unaffected half-saturation constant. An adaptive response was not observed for the structurally related β-alanine. 3. Only colchicine, which interferes with microtubule organization, was capable of blocking the response to alterations of taurine in cell medium, whereas inhibition of protein and nucleic acid synthesis by cycloheximide and actinomycin D, respectively, did not change the adaptive pattern. 4. Phorbol 12-myristate 13-acetate (PMA), mimicking the effects of diacylglycerol, induced inhibition of both β-alanine and taurine uptake. By contrast, the Ca^{2+}-ionophore A23187, mimicking the effects of IP_3, only stimulated the uptake of taurine but not the influx of β-alanine. However, the effect of PMA down-regulation and A23187 up-regulation was rapid and short-lived in contrast to the adaptive response, suggesting that the inositol phospholipid pathway involving diacetylglycerol and IP_3 is less likely to be linked directly to the adaptive regulation, but rather plays a role in short-term regulation.

Keywords: Taurine; β-Alanine; Sodium ion-dependent uptake; Phorbol ester; Ca^{2+}-ionophore; Kidney cell, human

1. Introduction

The adaptive response of the renal tubules to dietary change have been studied with increasing interest the past ten to twenty years. Among the amino acids investigated, especially the β-amino acid taurine has received considerable attention. Taurine is the major free intracellular amino acid in most mammalian tissue and seems to play an important physiological role by being implicated as an agent in, e.g., bile acid conjugation, osmoregulation, membrane protection, calcium modulation, antioxidation, and detoxification (for further details see Refs. [1–3]). Substantial evidence indicates that this amino acid is a conserved metabolite and that the whole body homeostasis in mammals is regulated by changes in the activity of β-amino acid transport system(s) located in luminal membranes of renal proximal tubule [4–9]. Thus, the renal tubular epithelium is able to increase the uptake of taurine during periods of low dietary intake and vice versa.

Abbreviations: IHKE, immortalized human kidney epithelial cell line (embryonic); NHKE, normal human kidney epithelial cell line (embryonic, primary culture); Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid; MES, 2-(*N*-morpholino)ethanesulphonic acid; FCS, fetal calf serum

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Previous reports concerning the renal handling of taurine have been based mainly on experiments using renal cortex slices [1,10], isolated renal tubules [11,12] and filtration techniques [4,6,13–19]. However, in the past years, there has been an increasing focus on the use of epithelial cell cultures because of its suitability to study cellular regulation of amino acid transport under well-defined conditions. Especially the LLC-PK_1 cell line, which is derived from proximal tubule of pig kidney, have been examined in detail in regard to the renal reabsorption of taurine. Thus, it was initially shown that the uptake of taurine into confluent monolayers of LLC-PK_1 cells was mediated by a single, β-amino acid specific transport component highly dependent upon Na^+ and Cl^-. It was demonstrated that the LLC-PK_1 cells exhibited the same adaptive response to alterations in cell medium concentrations of taurine as mentioned above by changing the uptake capacity (V_{max}) rather than the apparent K_m for taurine and without changes in amino acid efflux [5]. Addition of cycloheximide or colchicine to the cell medium prevented the increase of taurine influx in response to taurine-free medium, whereas actinomycin D had no effect. Treatment of the LLC-PK_1 cell monolayers with phorbol 12-myristate 13-acetate and phorbol 12,13-dibutyrate reduced the taurine uptake, and this inhibitory effect could be blocked with staurosporin, indicating the involvement of protein kinase C [20]. However, a recent study concerning the polarity of taurine transport in the LLC-PK_1 cell line showed that in contrast to previous reports the uptake of amino acid occurs by two Na^+-dependent transport components at both the luminal and basolateral surfaces [21]. This raises the question whether it is only the activity of the high-affinity carrier or both systems, which is up-regulated at low dietary intake of taurine. Moreover, even though the LLC-PK_1 cell line has retained several characteristics of the proximal tubule, the LLC-PK_1 cells in contrast to normal kidney cells have almost no parathyroidea hormone (PTH) receptors, the Na^+-dependent phosphate uptake is not controlled by PTH or cAMP and a transport component for cystine/lysine seems to be absent.

Recently, we have characterized an immortalized human embryonic kidney epithelial cell line (IHKE), which exhibits microvilli and enzymes specific for the proximal tubule. We have shown that this cell line has retained the capacity and specificity to transport α-amino isobutyric acid by comparing results with those found in normal human embryonic kidney epithelial cells (NHKE) [22]. The uptake of taurine and β-alanine was characterized by two Na^+-dependent transport components, and competition experiments revealed that taurine and β-alanine drastically reduced the uptake of one another by the high-affinity transport system. Further investigation of the high-affinity taurine carrier revealed a specific requirement for Cl^- with a probable coupling ratio of 3 Na^+/1 Cl^-/1 amino acid and preliminary experiments indicated that the IHKE cell line also up-regulates the influx of taurine in response to taurine-free cell medium [23]. Comparison of the results obtained for normal (NHKE) and immortalized (IHKE) embryonic cells with those obtained for primary cell cultures from proximal tubule of human adults (AHKE) suggested an unaltered expression of the types of transport carriers for neutral α-and β-amino acids. However, the results also indicated a relative increase in the uptake capacity of the above-mentioned transport proteins during maturation, which may explain, at least partly, the phenomenon of physiologic amino aciduria in neonates (for further details, see Ref. [24]).

Thus, the purpose of the present study was to examine the underlying mechanisms involved in the adaptive regulation of taurine uptake in the human proximal tubule by use of the IHKE cell line. We demonstrate that it is predominantly the activity of the high-affinity transport component, which is increased in the presence of a taurine-free cell medium and that a similar response is absent for the structurally related β-alanine. Furthermore, the role of protein and nucleic acid synthesis, microtubule organization, and the two branches of the inositol phospholipid signalling pathway were examined by addition of specific pharmacological agents to human kidney cells.

2. Materials and methods

2.1. Materials

[1,2-^{3}H]taurine was purchased from Amersham and β-[3-^{3}H(N)]alanine was obtained from Dupont, NEN

research Products (Boston, MA). Fetal calf serum (FCS), growth medium, transferrin and antibiotics were from Gibco, NY, USA. Hydrocortisone (H0135), epidermal growth factor (E-4127), insulin (I-1882), phorbol 12-myristate 13-acetate (PMA) (P-8139), 4α-phorbol 12,13-didecanoate (PDD) (P8014), the calcium ionophore A23187 (C-7522), cycloheximide (C-6255), colchicine (C-9754), actinomycin D (A1410), taurine (T-0625), and β-alanine (A-7752) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of the highest purity available.

2.2. Cell culture

IHKE cells were kindly provided by Dr. Aage Haugen, Department of Toxicology, National Institute of Occupational Health, Oslo, Norway, and grown in Dulbecco's MEM with glutamax containing 1% of FCS. The medium was supplemented with epidermal growth factor (10^{-5} g/l), insulin (5×10^{-3} g/l), hydrocortisone (4×10^{-5} g/l), transferrin (5×10^{-3} g/l), 2 mM glutamine, penicillin (10^{2} IU/l) and streptomycin (1×10^{-1} g/l) and Na_2SeO_3 (5×10^{-3} g/l). The cultures were grown at 37°C (pH 7.4) in an atmosphere of 5% CO_2 in air.

2.3. Transport studies

Uptake studies were performed on monolayers in 35-mm single dishes (NUNC, Denmark) as soon as the cells had obtained confluence. Transport of taurine and β-alanine was measured by the following techniques: 24 h before each experiment taurine or β-alanine was added to the cell medium, reaching a final concentration of 0, 50 or 500 μM unless otherwise stated. After removal of medium, each dish was washed twice at 37°C with 2 ml Earle's Balanced Salt Solution (EBSS), containing 5 mM D-glucose (pH 7.4), and incubated for 90 min with the same solution in order to minimize any trans-effects due to the intracellular pool of amino acids. They were then incubated with 0.75 ml of incubation medium containing EBSS (pH 7.4, 37°C), radiolabelled ligand and other constituents as required. The incubation was terminated after 30 min by aspiration of the incubation media and immediately washing the cells three times with 2 ml ice-cold EBSS (pH 7.4). After removal of the last volume of the ice-cold stop solution, 0.6 ml of 1% SDS in 0.2 N NaOH was added to solubilize the cells. The radioactivity in the cells was determined by withdrawal of three samples of 0.1 ml, which were counted in a liquid scintillation counter (Packard Tri-carb liquid scintillation analyzer) after addition of 3 ml scintillation fluid. Correction for non-specific uptake was made in control experiments by estimating the amount of radiolabelled taurine retained in the extracellular space or bound to the cell surface after incubation with ice-cold incubation medium. Thus, these parallel experiments were performed in exactly the same way as the other experiments with the only exception that the incubation medium, kept on ice, was added to the Petri dishes for 10 s before withdrawal and the cells were three times with ice-cold stop solution. All uptake studies were performed in an atmosphere of 5% CO_2 in air and the EBSS were supplemented with 15 mM Hepes/KOH (pH 7.4) in order to stabilize extracellular pH.

The amount of total cell protein was determined by the method of Lowry et al. with bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) as a standard. All solutions in this study were sterilized before use.

2.4. Calculations

Kinetics: The results of the saturation experiments were analyzed by Michaelis-Menten kinetics according to the following equation:

$$\text{Uptake} = \frac{V_{\max 1}[\text{S}]}{K_{\text{m}1} + [\text{S}]} + \frac{V_{\max 2}[\text{S}]}{K_{\text{m}2} + [\text{S}]}$$

where K_{m} represents the substrate concentration that gives half-maximal uptake, $V_{\max}$ denotes maximal rate and [S] indicates the concentration of substrate in the medium. Index 1 and 2 refer to the first and a second transport system, respectively. In the case where $K_{\text{m}2} \gg [\text{S}]$, the equation is simplified to:

$$\text{Uptake} = \frac{V_{\max 1}[\text{S}]}{K_{\text{m}1} + [\text{S}]} + \text{P}[\text{S}]$$

where P is a constant. This equation incorporates one transport system in parallel with a non-enzymatic leak mechanism. Theoretical saturation curves were

fitted to the experimental data by using a computer-analyzed statistical iteration procedure [25].

3. Results

Initially, we examined whether or not taurine starvation up-regulates the activity of both the high and low affinity taurine transport systems in the IHKE cell line. To do so, we measured the kinetics of taurine uptake after the cells had been incubated in cell media containing 0, 50 or 500 μM taurine 24 h prior to addition of increasing concentrations of radiolabelled taurine (for details see Section 2). The results are shown in Fig. 1A, where it is seen that the lower the taurine concentration in the cell medium the higher the radiolabelled taurine uptake is at a given taurine concentration. In all three cases, the kinetic analyses of the data indicate the presence of more than one transport component. On the assumption that 50 μM taurine reflects the physiological level of amino acid, the results in Fig. 1B are given as the uptakes obtained by starved cells (0 μM taurine) relatively to the uptakes found for the non-starved cells (50 μM taurine). The hyperbolic curve clearly indicates that the increased amino acid uptake is caused predominantly by an up-regulation in the activity of the high affinity transport component. Since numerous studies have indicated that the renal uptake of taurine occurs by a specific β-amino acid transport component [13,18,19,26,27], similar experiments were carried out in which the radiolabelled taurine was replaced by the structurally related β-alanine. By contrast to results shown in Fig. 1B, the absence of β-alanine in the cell medium for 24 h did not affect the uptake of radiolabelled β-alanine within the concentration range studied (Fig. 1C). Nor did incubation of the IHKE cells in cell media containing 0, 50 or 500 μM taurine result in any increase in the uptake of radiolabelled β-alanine.

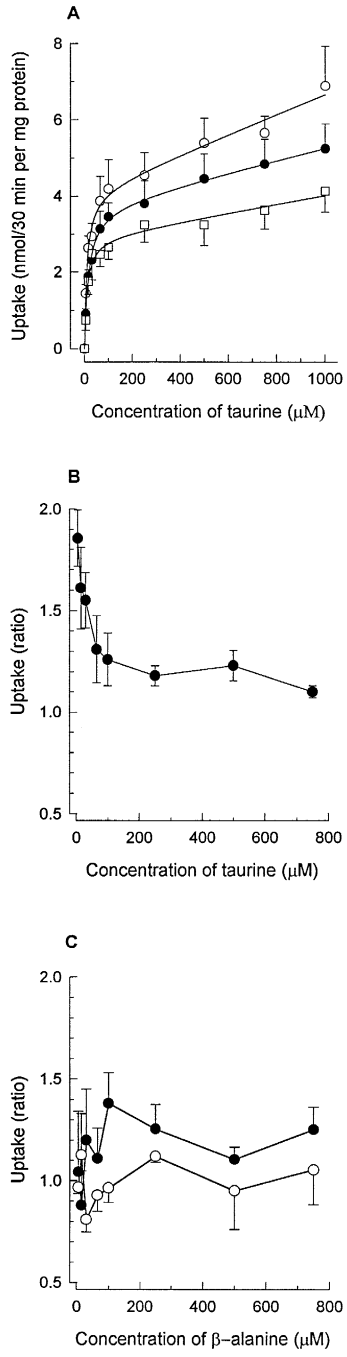

Fig. 1. Kinetics of taurine and β-alanine uptake by IHKE cells preincubated with taurine or β-alanine. (A) Monolayers of IHKE cells were preincubated with 0 (○), 50 (●) and 500 (□) μM taurine for 24 hrs (for further details, see Section 2). The cells were incubated in EBSS, 40 nM [1,2-^{3}H]taurine and various concentrations of unlabelled taurine ranging from 0–1.0 mM. (B) The uptake of taurine illustrated as the uptake ratio between cells preincubated with 0 μM and 50 μM taurine for 24 h. (C) The uptake of β-alanine illustrated as the uptake ratio between cells preincubated with 0 μM and 50 μM β-alanine (○) or with 0 μM and 50 μM taurine (●) for 24 h. Uptake was measured as described above substituting taurine with 109 nM β[3-^{3}H(N)]alanine and various concentrations of unlabelled β-alanine ranging from 0–1.0 mM. The results given are the mean values ± S.D. of four experiments. In those cases where vertical bars are absent, S.D. were smaller than graphical representation of the mean.

Table 1 shows the kinetic constants for the high affinity taurine transport system found by computer analysis [25] of the uptake data given in Fig. 1A. The half-saturation constants K_{m1} are almost identical in all three cases, whereas there seems to be a tendency of increasing the uptake capacity of amino acid in the absence of taurine in the cell medium. The calculated constants for the low affinity system(s) could not be determined satisfactorily because of the lack of saturation of the transport component(s) over the concentration range studied. Calculations using the one-transport one-leak model resulted in exactly the same kinetic parameters as those given in Table 1 and the standard deviation of the fit was identical to the one found for the two site model.

Table 1
Kinetic parameters for the uptake of taurine by IHKE cells preincubated with 0, 50 and 500 μM taurine

Taurine (μM)	V_{max1} (nmol/30 min per mg protein)	K_{max1} (μM)
0	4.28 ± 1.57	10.4 ± 3.29
50	2.50 ± 1.59	14.9 ± 8.43
500	2.30 ± 0.94	12.7 ± 10.74

The kinetic constants for the high affinity taurine transport system were determined by computer-analysed statistical iteration procedure [25] of the uptake data given in Fig. 1A.

In order to further examine the adaptive response regarding the renal handling of taurine, a series of experiments was performed in which the cell monolayers were pretreated with either actinomycin D, cycloheximide, or colchicine. The results are shown in Fig. 2, where the uptakes are given as percentage of the taurine influx measured in IHKE cells incubated in cell media containing 50 μM taurine (designated as 100%). Actinomycin D, which inhibits nucleic acid synthesis (transcription and chain elongation), did not affect the adaptive phenomenon, although actinomycin D in general reduced the uptake of taurine (Fig. 2A). Exactly the same picture emerged, when cycloheximide, inhibiting the protein synthesis by binding to the 80-s ribosome, substituted actinomycin D in the pretreatment period (Fig. 2B).

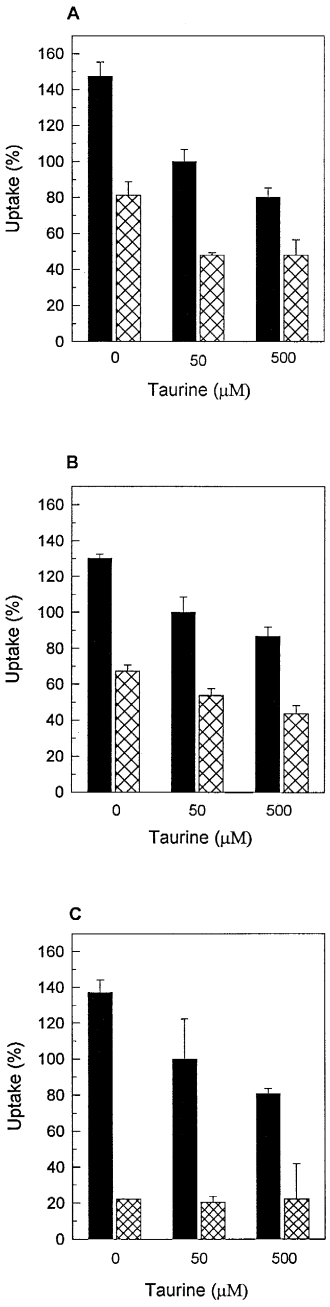

Fig. 2. Effect of actinomycin D, cycloheximide and colchicine on the uptake of taurine. (A) IHKE cell monolayers were preincubated for 2 h with and without addition of actinomycin D (0.5 μg/ml), (B) cycloheximide (0.1 μM) or (C) colchicine (50 μM) to the cell medium containing 0, 50 or 500 μM taurine. The uptake was measured as described in Section 2 and the media contained 255–700 nM [1,2-^{3}H]taurine and sufficiently unlabelled taurine to reach a total concentration of 1 μM. The uptakes are given in percentage of taurine influx measured in cells preincubated in media containing 50 μM taurine (100%). Solid bars without inhibitors, hatched bars with inhibitors. Results are given as mean values of at least 3 determinations $\pm$ S.D.

Increasing the concentration of cycloheximide from 0.1 μM to 70 μM and actinomycin D from 0.5 μg/ml to 50 μg/ml did not alter the pattern apart from an increase in the general reduction of taurine influx (results not shown). By contrast, colchicine had a marked effect on the adaptive response to changes in the taurine concentration in the cell medium. Colchicine binds specifically to tubulin and interferes with microtubule organization, depolarizes the microtrabecular network and prevents protein exocytosis but not endocytosis. The data illustrated in Fig. 2C show that addition of colchicine results in almost identical uptake values at the cell medium concentration of 0, 50 and 500 μM taurine. Thus, the adaptive response at low taurine concentrations is no longer observed.

We also investigated the role of the inositol phospholipid pathway in order to determine if protein kinase C or Ca^{2+}/calmodulin kinases are involved in the signal pathway of adaptive regulation of the uptake of β-amino acids in IHKE cells. The two branches of the inositol phospholipid pathway are based on the cleaving of PIP_2 to diacylglycerol and IP_3. Since the effects of diacylglycerol can be mimicked by phorbol esters, we examined the effect of phorbol 12-myristate 13-acetate (PMA) on the activity of the β-amino acid transporter in the IHKE cell line. Thus, PMA is a highly potent tumor promoter capable of activating protein kinase C [28]. The cell monolayers were treated with PMA (100 nM) for 20 to 120 min before addition of radiolabelled amino acid. For comparison, we also studied the effect of treating the monolayers with 4α-phorbol 12,13-didecanoate (PDD), which even though it is an analogue to PMA, is unable to activate protein kinase C [29,30]. Since stock solutions of the phorbol esters contained dimethyl sulphoxide (DMSO), control experiments were carried out in which cell monolayers were treated with equal concentrations of DMSO but without phorbol esters. We found that the concentration of DMSO used in the present study did not affect the uptake of amino acid under these conditions (results not shown). Fig. 3A illustrates that treatment of the IHKE cells with PMA, in contrast to PDD, reduced the uptake of taurine with a maximal inhibition of 60 min. Hereafter, the effect of PMA diminished and the amino acid uptake at 120 min has almost reached the same level as the uptake of taurine in the presence of

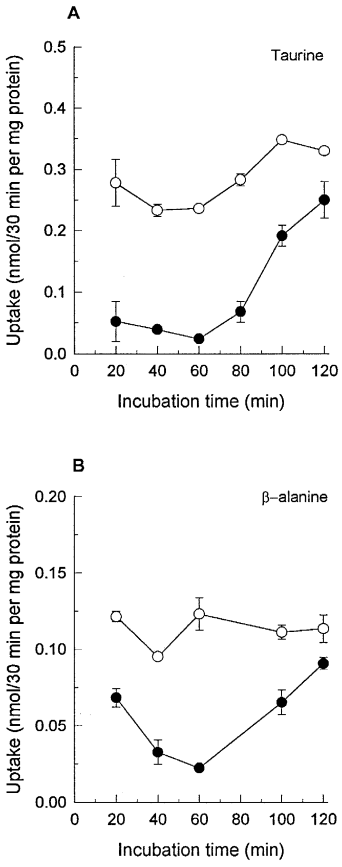

Fig. 3. Time course of the effect of PMA and PDD on the uptake of taurine and β-alanine. The IHKE cells were preincubated for 20–120 min in EBSS containing 5 mM D-glucose and 100 nM PMA (●) or PDD (○). (A) The uptake medium contained 255 nM [1,2-^{3}H]taurine, 1 μM taurine and 100 nM PMA or PDD and (B) 72 nM β[3-^{3}H(N)]alanine, 1 μM β-alanine and 100 nM PMA or PDD. Each point is defined by at least 3 determinations $\pm$S.D. In those cases where vertical bars are absent, standard deviations are smaller than the graphical representation of the mean.

PDD. Similar results were obtained by replacing radiolabelled taurine with equivalent amount of β-alanine as illustrated in Fig. 3B. Thus, our results indicate that the PMA-induced inhibition of the β-amino acid transport is caused by the involvement of protein kinase C since PDD had no effect on the amino acid uptake. However, the effect of PMA was rapid and short-lived as compared to the adaptive response seen by starved cells.

An attempt to investigate the effect of changes in the cytosolic free calcium concentration on β-amino acid uptake was also done since the effects of IP_3 in

the inositol phospholipid pathway can be mimicked by using a Ca^{2+} ionophore allowing Ca^{2+} to move from the extracellular fluid into the cytosol. Thus, we evaluated the effect of the Ca^{2+} ionophore A23187 by comparing the uptakes of amino acid in the presence with the uptakes in the absence of A23187. The results for taurine are given in Fig. 4A, where the increase in ratio indicates a stimulation in the taurine uptake, which is maximal after an incubation time of 20 min (approx. 30% enhancement). Thereafter, the effect of the Ca^{2+} ionophore decreases approaching the normal influx level after 60 min, indicating that the effect of A23187 is rapid and relatively short-lived compared to the response obtained by starvation. By contrast, as seen from Fig. 4B, A23187 did not enhance the uptake of β-alanine, but rather decreased it. Thus, the amino acid influx in the presence of A23187 was only 0.7 times the uptakes in the absence of Ca^{2+} ionophore found after 60 min of incubation.

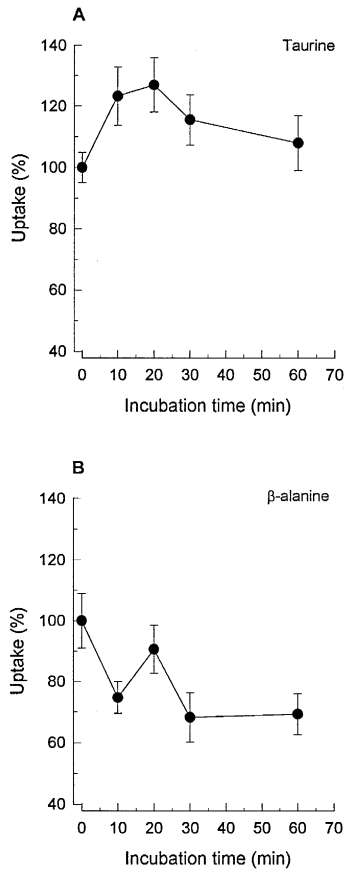

Fig. 4. Time course of the effect of the calcium ionophore A23187 on the uptake of taurine and β alanine. The IHKE cells were preincubated for 0–60 min in EBSS containing 5 mM D-glucose with and without 0.2 μM A23187. (A) The uptake medium contained 255 nM [1,2-^{3}H]taurine, 1 μM taurine and 0.2 μM A23187. (B) The uptake medium contained 72 nM β[3-^{3}H(N)]alanine, 1 μM β-alanine and 0.2 μM A23187. Results are given as mean values $\pm$ S.D. of triplicates.

4. Discussion

Our findings indicate that the adaptive response to maintain whole-body taurine homeostasis occurs predominantly via the high-affinity taurine carrier even though the amino acid uptake may be mediated by more than one Na^+-dependent transport system. Furthermore, the data given in Table 1 suggest that the adaption of membrane function is accompanied by changes in the uptake capacity (V_{max}), whereas the half-saturation constant (K_m) is unaffected. The same pattern has previously been reported for the LLC-PK_1 cell line and the K_m-value for the high-affinity component in these cells is identical to the one found in the present study for the IHKE cells [20]. Thus, these findings indicate that the adaptive response to changes in dietary taurine intake is the result of alterations in the number of active or available taurine carriers in the cell membrane. Since it is generally agreed that the influx of taurine and β-alanine at low substrate concentrations occurs via a β-amino acid-specific transport system, as also reported for the IHKE cell line [23], we examined the effect of depleting the cell medium of taurine or β-alanine on the uptake of β-alanine. However, no adaptive response was found under these conditions, emphasizing the importance of the sulfonate group in taurine versus the carboxylate group in β-alanine. Thus, although taurine and β-alanine have similarities, both having a positive and negative charge, there is a profound difference in the negative character of the carboxylate group compared to the sulfonate group.

In order to gain further insight in the mechanisms involved in the adaptive response, monolayers of IHKE cells were exposed to actinomycin D, cycloheximide, or colchicine before addition of radiolabelled taurine. Only colchicine, which interferes with microtubule organization and prevents protein exocy-

tosis but not endocytosis, was capable of blocking the response to alterations of taurine in cell medium. By contrast, Jones and co-workers studying LLC-PK_1 cells could only demonstrate an effect of the agent in the presence of taurine-free medium but not on the response to high-medium taurine [20]. Inhibition of RNA transcription with actinomycin D did not affect the adaptive response of taurine influx in the IHKE cell line, which is in accordance with previous results regarding LLC-PK_1-cells [20]. On the other hand, inhibiting the protein synthesis with cycloheximide did not change the adaptive pattern of taurine uptake in the human cells, whereas similar experiments with LLC-PK_1 cells showed a marked effect on the response to taurine-free as well as taurine-high medium [20]. The discrepancy between the two cell lines is not known at present but variations in species/cell lines cannot be excluded.

We also examined the influence of the inositol phospholipid pathway on the uptake of β-amino acids in the IHKE cell line. Addition of PMA which activates protein kinase C by mimicking the effect of the physiologically diacylglycerols inhibited the uptake of both taurine and β-alanine in a time-dependent manner, reaching a maximum after 60 min. After 60 min, the influx of β-amino acid increased, indicating a reversible effect of PMA. Results from similar experiments with LLC-PK_1 cells and the human colon carcinoma cell line HT-29 showed that PMA had an inhibitory effect on the activity of the transport of taurine and both β-amino acids, respectively [20,31]. Addition of the isomer PDD, which is unable to activate the protein kinase C, did not affect the uptake of β-amino acids in the IHKE cell line. Nor did PDD reduce the uptake of amino acid in HT-29 cells [31]. Investigators have speculated whether or not the protein kinase C leads to phosphorylation of the taurine carrier, thereby inactivating the carrier [31]. This may be supported by the fact that the effect of PMA observed in the present study occurred relatively quickly and peaked after only 60 min. Exactly the same observation makes the theory that the enzyme may block the transcription and/or translation mechanisms, thereby inhibiting the synthesis of taurine carriers, less likely. Another hypothesis may be that protein kinase C reduces the transmembrane gradients of sodium and chloride [31]. Jones and co-workers have demonstrated that PMA increased taurine efflux from LLC-PK_1 cell monolayers, whereas PDD had no effect [20]. By contrast, the authors also reported in another study that the differences in uptake found after manipulation of medium taurine concentrations were not associated with changes in taurine efflux in the LLC-PK_1 cells [5]. The fact that PDD in contrast to PMA failed to alter the uptake of β-amino acids seems to indicate the involvement of protein kinase C in a short-term, rapid regulation of β-amino acid influx. Accordingly, PMA induced inhibition of both β-alanine and taurine uptake even though, in the present study, we could only demonstrate an adaptive regulation for the influx of taurine and not of β-alanine.

Since numerous studies have demonstrated that the influx of taurine and the structurally related β-alanine is mediated by the same specific β-amino acid transport system at low substrate concentrations, we anticipated that the Ca^{2+} ionophore A23187 mimicking the effects of IP_3 had similar effect on the uptake of both these amino acids. However, surprisingly, the A23187 only stimulated the transport of taurine transiently, whereas the uptake of β-alanine was unaffected. The reason for the discrepancy of the effect of A23187 on the uptake of the two β-amino acids is not clear. Thus, even though we observed a similar pattern for the adaptive response, suggesting that the complex regulation is more dependent on Ca^{2+}/calmodulin kinases rather than protein kinase C, there is a considerable time gap between the two phenomena, indicating that the pathway involving IP_3 is more likely to play a role in a short-term, rapid regulation of taurine uptake.

Taken together, the regulation of the high-affinity taurine transporter in the human IHKE cell line was affected by colchicine, indicating that transport of preformed carriers into the cell membrane may play an important part in the adaptive response to low dietary intake of taurine. Furthermore, the phenomenon of PMA down-regulation and A23187 up-regulation of taurine uptake was rapid and short-lived in contrast to the adaptive response. Consequently, it seems less likely that the inositol phospholipid pathway involving diacylglycerols and IP_3 is linked directly to the adaptive regulation, but rather plays a role in short-term regulation. However, an additional long-term, indirect effect cannot be excluded at present and needs to be further elucidated in the future.

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