Activation of Ca^{2+}/calmodulin-dependent protein kinase II is involved in hyperosmotic induction of the human taurine transporter

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Received 12 April 2004; accepted 4 May 2004

Available online 5 June 2004

Edited by Felix Wieland

Abstract **We investigated the signaling pathways participating in hyperosmotic regulation of the human taurine transporter (TAUT) by using specific inhibitors of various intracellular signaling molecules. Among them, the specific inhibitor of calcium/calmodulin-dependent protein kinase II (Ca^{2+}/CaM kinase II) completely repressed the hyperosmotic regulation of TAUT. The osmosensitive upregulation of TAUT was also significantly inhibited by calmodulin antagonists and calcium-chelators. The increased expression level of TAUT mRNA by hypertonicity was repressed by the specific Ca^{2+}/CaM kinase II inhibitor. The activated form of Ca^{2+}/CaM kinase II protein could only be detected in Caco-2 cells under hypertonic conditions.**

Keywords: Taurine; Transporter; Osmoregulation; Calcium/calmodulin-dependent protein kinase II (Ca^{2+}/CaM kinase II); Caco-2

1. Introduction

Extracellular hypertonicity is balanced by maintaining a high intracellular content of non-perturbing osmolytes like betaine, *myo*-inositol, glycerophosphorylcholine, sorbitol, and taurine in mammalian cells [1]. The intracellular accumulation of such osmolytes as betaine, *myo*-inositol, and taurine is performed by betaine/γ-amino-*n*-butyric acid transporter 1 (BGT1), the *myo*-inositol transporter (SMIT), and the taurine transporter (TAUT) [2]. It has been found in many cells that hypertonicity induced the activity and expression level of these osmolyte transporters [3]. Aldose reductase (AR), which is involved in the synthesis of sorbitol from glucose, is also known to be upregulated under hypertonic conditions [4], such an osmotic response being performed to maintain the cell volume against exposure to a hypertonic environment.

The osmotic regulation of osmolyte transporters and synthetic enzymes has been studied in several tissues [5–7]. Since the small intestine, especially the intestinal epithelial cells, and the kidney mudulla are exposed to extreme changes of hypertonicity, we have previously reported that taurine would be likely to behave as an osmolyte and that hypertonicity stimulated the activity and gene expression of TAUT in human intestinal Caco-2 cells [8] and the rat small intestine [9]. We have also reported the osmoregulation of TAUT and cysteine dioxygenase, one of the key enzymes of taurine biosynthesis, in a human hepatic HepG2 cell line [10].

Although the intracellular signaling pathways leading to the upregulation of several osmolyte transporters and synthetic enzymes have recently been investigated, the intracellular regulatory mechanism of TAUT under hypertonic conditions has not yet been elucidated. In mammalian cells, hypertonicity induces three enzymes in the mitogen-activated protein kinase (MAP kinase) family: extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), and p38 [11,12]. Studies on the relationship between the MAP kinase pathway and osmotic regulation of BGT1, SMIT, and AR have been reported [13–19], although there has not yet been any report about the signal cascade including MAP kinase that is involved in the osmoregulation of TAUT.

We investigated in the present study the signaling pathway involved in the hyperosmotic induction of human TAUT in human intestinal Caco-2 cells. Specific inhibitors of various intracellular signaling molecules, including the MAP kinase family, were utilized in this analysis.

2. Materials and methods

2.1. Materials

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo, Japan) and fetal calf serum (FCS) was from Gibco (Gaithersburg, MD, USA). [1,2-^{3}H] taurine (specific radioactivity of 29 Ci/mmol) and [α-32P] dCTP were from Amersham Pharmacia Biotech (Little Chalfont, England). PD98059, SB203580, wortmannin, 1-O octadecyl-2-O-methyl-rac-glycero-3-phosphorylcholine (Et-18-OCH_3), rapamycin,

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Abbreviations: AR, aldose reductase; BGT1, betaine/γ-amino-*n*-butyric acid transporter 1; Ca^{2+}/CaM kinase II, calcium/calmodulin-dependent protein kinase II; CREB, cAMP-responsive element-binding protein; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular signal-regulated kinase; Et-18-OCH_3, 1-O octadecyl-2-O-methyl-rac-glycero-3-phosphorylcholine; FCS, fetal calf serum; HBSS, Hank's balanced salt solution; JNK, Jun N-terminal kinase; LPC, lysophosphatidylcholine; MAP kinase, mitogen-activated protein kinase; PBS, phosphate-buffered saline; PDTC, pyrrolidine dithiocarbamate; PKA, protein kinase A; PKC, protein kinase C; SMIT, *myo*-inositol transporter; TAUT, taurine transporter

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doi:10.1016/j.febslet.2004.05.062

KN-62, KN-93, W-7, and calmidazolium were all purchased from CAL Biochemicals (San Diego, CA, USA). 4,4′-Diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) was from Sigma (St. Louis, MO, USA) and the anti-active Ca^{2+}/CaM kinase II polyclonal antibody was purchased from Promega (Madison, WI, USA).

2.2. Cell culture

Caco-2 cells were cultured with a medium consisting of DMEM, 10% FCS, 1% non-essential amino acids, 2% glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin and an appropriate amount of sodium bicarbonate. The cells were incubated at 37 °C under a humidified atmosphere of 5% CO_2 in air. The uptake experiments used Caco-2 cells cultured in 24-well plates that had been precoated with collagen at a density of 1.4×10^5 cells/well, cell monolayers being taken after 14 days of culture.

2.3. Uptake experiments

The uptake experiments have been described previously [20]. The [^{3}H] taurine uptake experiments were performed in the absence (total uptake) or presence (non-specific uptake) of 50 mM unlabeled taurine, this allowing the specific uptake to be calculated by subtraction. The Caco-2 monolayers were washed twice for 5 min with 700 μl of Hank's balanced salt solution (HBSS) containing 4 mM sodium bicarbonate and 10 mM HEPES, the pH value being adjusted to 7.4 with KOH (uptake buffer). The cells were next incubated with 0.3 μCi of [^{3}H] taurine in 300 μl of the uptake buffer, with or without excess (50 mM) taurine, at 37 °C for 10 min. At the end of the incubation period, the buffer was removed and each monolayer was carefully washed three times with 700 μl of ice-cold phosphate-buffered saline (PBS) containing 0.05% sodium azide for 5 min. To each well was then added 250 μl of 0.1% Triton X-100, before the dissolved cells were taken into 3 ml of a scintillation cocktail. The tritium content of each monolayer was finally determined with an LSC 5100 liquid scintillation analyzer (Aloka, Tokyo, Japan).

2.4. Northern blot analysis

Five μg of poly(A^+) RNA fractionated on 1% agarose gel containing 2.2 M formaldehyde was transferred to a nylon filter (Hybond-N; Amersham) according to the manufacturer's instructions. The filter was hybridized with the RT-PCR product of the human taurine transporter and labeled by random priming with an [α-32P] dCTP labeling kit (Multiprime; Amersham). After treating in a hybridization solution (Rapid; Amersham) at 65 °C for 3 h, the filter was washed in 0.1 × NaCl/Cit containing 0.1% SDS at 65 °C.

2.5. Detection of activated Ca^{2+}/CaM kinase II by a Western blot analysis

Caco-2 cells cultured in 6-well plates for 14 days were washed twice with ice-cold PBS. The cells were scraped off and then homogenized with a buffer containing 20 mM Tris–HCl (pH 8.0), 2 mM EDTA, 2 mM EGTA, 2 mM DTT, 1% NP-40, 1 mM PMSF, 1 μg/ml of leupeptin, 10 μg/ml of pepstatin A, and 1 mM sodium orthovanadate. The homogenate was incubated for 20 min at 4 °C and then centrifuged at $12\,000 \times g$ for 20 min at 4 °C. The supernatant was fractionated by SDS/10% PAGE and transferred to a PVDF membrane (Millipore). The membrane was blocked overnight at 4 °C by PBS-T containing 5% non-fat dried milk. The membrane was then incubated for 2 h with the anti-active Ca^{2+}/CaM kinase II antibody (1:1000 dilution). The blot was washed in PBS-T and incubated for 1 h with goat anti-rabbit IgG-HRP (1:2500 dilution). A chemiluminescent substrate (ECL; Amersham Biosciences) was used for detection.

2.6. Statistical analysis

Each result is expressed as the mean ± S.E. Differences among the experimental data were assessed by ANOVA.

3. Results

3.1. Effect of specific inhibitors of the intracellular signaling molecule on the hyperosmotic regulation of TAUT

Among various inhibitors of the intracellular signaling molecule, we first examined the effect of PD98059, the MEK-specific inhibitor, and of SB203580, the specific inhibitor of p38, on hypertonicity-induced upregulation of the taurine uptake in Caco-2 cells. The cells were incubated with a medium containing PD98059 (20 μM) or SB203580 (20 μM) for 3 h and further cultured for 48 h with or without 100 mM raffinose in the presence of PD98059 or SB203580. However, neither PD98059 (20 μM) nor SB203580 (20 μM) had any effect on the osmotic regulation of TAUT in Caco-2 cell monolayers (Fig. 1A and B).

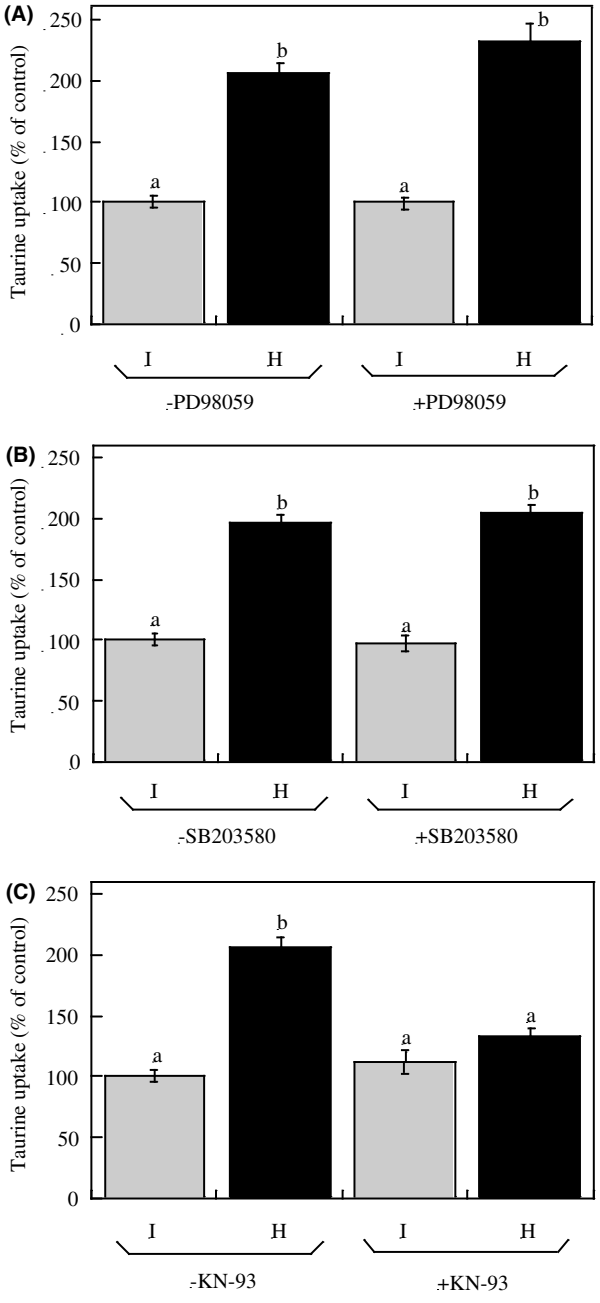

Fig. 1. Effect of various inhibitors on the hypertonicity-induced upregulation of the taurine uptake by Caco-2 monolayers. Caco-2 cells, which had been cultured for 14 days, were incubated with (A) PD98059 (20 μM), (B) SB203580 (20 μM), or (C) KN-93 (5 μM) for 3 h, and further incubated for 48 h in a hypertonic (containing 100 mM raffinose; H) or isotonic medium (I) in the presence or absence of an inhibitor. The uptake experiments were then performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 6$), $P < 0.01$.

Various specific inhibitors of such intracellular signaling molecules as PI3-kinase (wortmannin), phospholipase C (Et-18-OCH3), Ca^{2+}/CaM kinase II (KN-93), and mTOR signaling (rapamycin) were tested for the osmotic regulation of taurine uptake in Caco-2 cells. Among these, the treatment with KN-93 at 5 μM significantly blocked the hypertonicity-induced upregulation of taurine uptake (Fig. 1C), whereas the other inhibitors had no significant effect (data not shown).

3.2. Dose-dependent effect of the Ca^{2+}/CaM kinase II inhibitors on the osmotic regulation of TAUT

Fig. 2A shows that the hypertonicity-induced TAUT activity decreased as the concentration of KN-93 was increased, and that the treatment with KN-93 at 10 μM completely blocked the osmotic induction of TAUT. The effect of KN-62, another Ca^{2+}/CaM kinase II inhibitor, on the osmotic regulation of TAUT in Caco-2 cells was also studied. As shown in Fig. 2B, KN-62 significantly repressed the hypertonicity-induced upregulation of TAUT in a concentration-dependent manner and completely blocked the hypertonicity-induced upregulation of TAUT in Caco-2 cells at a concentration of 5 μM. These results suggest that Ca^{2+}/CaM kinase II was involved in the hypertonicity-induced upregulation of TAUT in intestinal epithelial Caco-2 cells.

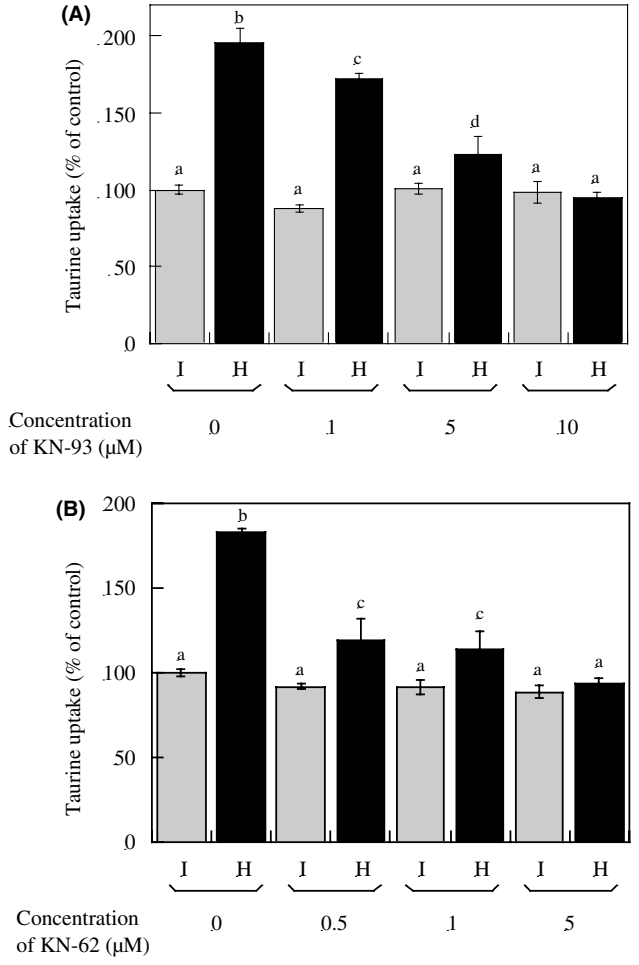

Fig. 2. Inhibitory effect of Ca^{2+}/CaM kinase II inhibitors on the osmosensitive upregulation of TAUT activity in Caco-2 monolayers. Caco-2 cells were incubated with (A) 1, 5, or 10 μM of KN-93 or (B) 0.5, 1, or 5 μM of KN-62 for 3 h, and further incubated for 48 h in a hypertonic (containing 100 mM raffinose; H) or isotonic medium (I) in the presence or absence of KN-93 or KN-62. The uptake experiments were performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 6$), $P < 0.01$.

3.3. Effect of the calmodulin antagonist and EGTA on the hyperosmotic regulation of TAUT

Cells were treated with the calmodulin antagonist, W-7 or calmidazolium, to suppress the Ca^{2+}/CaM kinase II activation. Exposure to W-7 (at 20 μM) and to calmidazolium (at 10 μM) each significantly repressed the activation of TAUT activity under hypertonic conditions (Fig. 3A). The effect of the calcium-chelating reagent, EGTA, on the osmotic regulation of TAUT was also investigated. Caco-2 cells were incubated with 5 mM EGTA for 3 h and further cultured for 48 h with or without 100 mM raffinose in the presence of EGTA. Fig. 3B shows that the EGTA treatment completely blocked the hy-

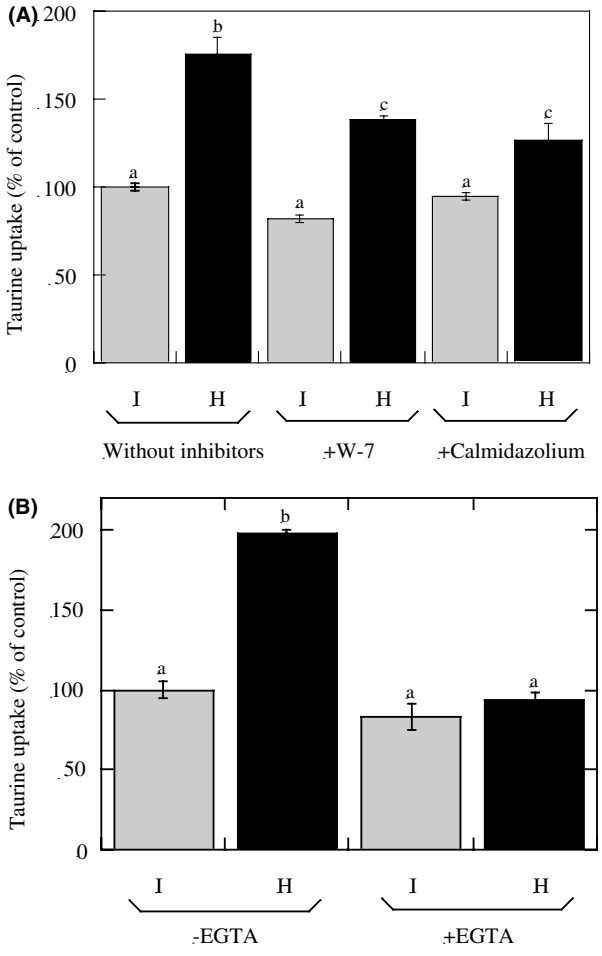

Fig. 3. Effect of the Ca^{2+}/calmodulin antagonist and EGTA on the upregulation of taurine uptake by hypertonicity. (A) Caco-2 cells were incubated with the Ca^{2+}/calmodulin antagonist, W-7 (20 μM), and calmidazolium (10 μM) for 3 h, and further incubated for 48 h in a hypertonic (containing 100 mM raffinose; H) or isotonic medium (I) in the presence or absence of the antagonist. Uptake experiments were then performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 6$). (B) Caco-2 cells were incubated with 5 mM EGTA for 3 h and further incubated for 48 h with a hypertonic (containing 100 mM raffinose; H) or isotonic medium (I) in the presence or absence of EGTA. Uptake experiments were performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 6$), $P < 0.01$.

pertonicity-induced upregulation of TAUT activity. The possible involvement of Ca^{2+}/CaM kinase II in the osmotic regulation of TAUT in Caco-2 cells was thus demonstrated.

3.4. Involvement of Ca^{2+}/CaM kinase II in the osmotic regulation of TAUT in HepG2 cells

The results of our recent study have shown that hypertonicity induced TAUT activity in a human hepatoblastoma HepG2 cell line [10]. Experiments were performed to reveal whether Ca^{2+}/CaM kinase II would also be involved in the osmoregulation of TAUT in HepG2 cells or not. Fig. 4A shows that KN-93 also significantly repressed the hypertonicity-induced upregulation of TAUT activity, whereas SB203680 had no significant effect (Fig. 4B). This result suggests that Ca^{2+}/CaM kinase II, but not p38 MAP kinase, was involved in the osmotic regulation of TAUT in human hepatoma HepG2 cells, like that in Caco-2 cells.

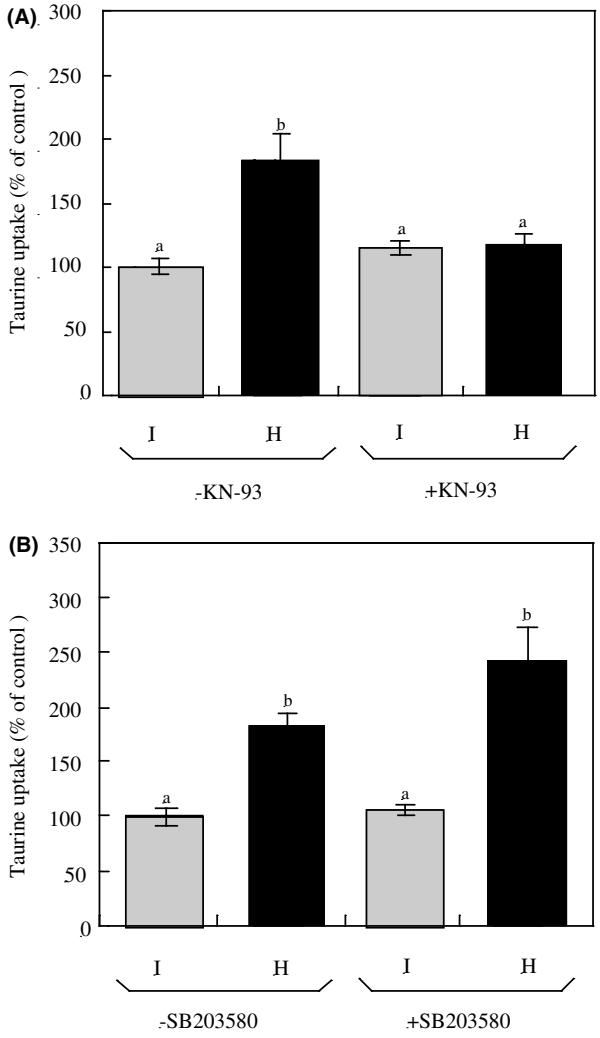

Fig. 4. Effect of KN-93 on the hypertonicity-induced TAUT activation in human hepatoma HepG2 cells. HepG2 cells were incubated with (A) KN-93 (10 μM) or (B) SB203580 (20 μM) for 3 h, and further incubated for 48 h in a hypertonic (containing 100 mM raffinose; H) or isotonic medium (I) in the presence or absence of the inhibitor. Uptake experiments were performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 6$), $P < 0.01$.

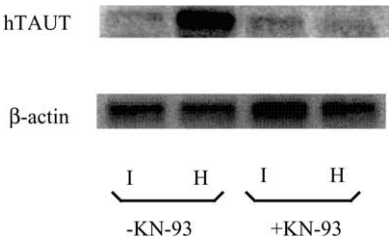

Fig. 5. Northern blot analysis of TAUT mRNA from Caco-2 cells cultured in the hypertonic medium with or without KN-93. Caco-2 cells were pre-cultured with 10 μM KN-93 for 3 h and further cultured (I) without or (H) with 100 mM raffinose for 48 h in the presence of KN-93. A 10 μg amount of poly(A)$^+$ RNA isolated from the Caco-2 cells was subjected to a Northern blot analysis. β-Actin was used as the control.

3.5. Effect of Ca^{2+}/CaM kinase II inhibitors on the expression of TAUT mRNA

The expression levels of TAUT mRNA in hypertonic and isotonic cells were examined in the presence of KN-93. Human TAUT cDNA obtained by RT-PCR was used as a probe to perform a Northern blot analysis. Five μg of poly(A)$^+$ RNA extracted from Caco-2 cells that had been cultured in a hypertonic or isotonic medium for 48 h with or without KN-93 was used. Fig. 5 shows that the mRNA level was markedly higher in the cells that had been cultured with the hypertonic medium than in those with the isotonic medium. In the presence of KN-93, however, the expression level of TAUT mRNA was not increased, even in the hypertonic cells, its expression level being similar to that in the isotonic cells (Fig. 5). This result supports the possibility of the participation of Ca^{2+}/CaM kinase II in the osmoregulation process. It also suggests that the Ca^{2+}/CaM kinase II pathway acted upstream of TAUT gene expression in this osmotic regulation.

3.6. Detection of activated Ca^{2+}/CaM kinase II in Caco-2 cells under hypertonic conditions

The expression of Ca^{2+}/CaM kinase II mRNA could be constitutively detected by RT-PCR (data not shown). We then examined whether the Ca^{2+}/CaM kinase II protein would be activated under hypertonic conditions or not.

A protein extract was collected every 2 h from Caco-2 cells cultured under either hypertonic or isotonic conditions and served for a Western blot analysis with the anti-active Ca^{2+}/CaM kinase II antibody. This antibody could only detect the activated form of the Ca^{2+}/CaM kinase II protein. As shown in Fig. 6, activated Ca^{2+}/CaM kinase II was detected in Caco-2 cells that had been cultured for 11 h under hypertonic conditions. This result confirms that the Ca^{2+}/CaM kinase II protein

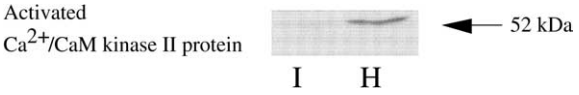

Fig. 6. Detection of activated Ca^{2+}/calmodulin-dependent protein kinase II in Caco-2 cells cultured under hypertonic conditions. Caco-2 cells were cultured (I) without or (H) with 100 mM raffinose for 11 h. The cells were then scraped off and a protein extract was prepared. A 100 μg amount of this protein extract was subjected to a Western blot analysis as described Section 2. The activated Ca^{2+}/CaM kinase II protein was detected at approximately 52 kDa.

was unequivocably activated by hypertonicity in human intestinal Caco-2 cells.

4. Discussion

We investigated in the present study the osmotic regulation of human TAUT in terms of the intracellular signaling pathway and revealed for the first time that the activation of Ca^{2+}/CaM kinase II was involved in the hypertonicity-induced upregulation of human TAUT.

It is not yet understood as to how the cells adapted to a change in extracellular osmolarity that led to the regulation of osmolyte transporters or synthetic osmolyte enzymes through the signal transduction pathway. However, several studies on signal transduction in the regulation of osmolyte transporters or synthetic enzymes have been reported, particularly in relation to the MAP kinase family [15–19]. Kwon et al. [15], who used MDCK cells cultured in a hypertonic medium, have reported that the transcription of genes responsible for the active transport of *myo*-inositol (SMIT) and betaine (BGT1) was markedly stimulated, resulting in an increase in SMIT and BGT1 mRNA, which in turn was followed by the increased activity of osmolyte transport. ERK, one of the MAP kinase family, was also stimulated in a PKC-dependent manner in hypertonic MDCK cells. However, SMIT and BGT1 mRNA were increased in hypertonic MDCK cells, whereas the activation of ERK was completely repressed by the downregulation of PKC. This result indicates that the activation of ERK was not involved in the transcriptional regulation of SMIT and BGT1 by hypertonicity. Furthermore, Kultz et al. [16] have reported that the osmoregulation of AR was accompanied by an increase in the transcription of the aldose reductase gene in PAP-HT25 cells. Hypertonicity also stimulated the activation of JNK and p38 in PAP-HT25 cells. However, they found that the activation of JNK or p38 was not necessary for the transcriptional regulation of AR by using PAP-HT25 cells transfected with the dominant negative JNK, SEK1-AL, or with the dominant negative p38 kinase, MKK3-AL. On the other hand, it has been reported that hypertonic exposure of human monocytes and macrophages led to the upregulation of BGT1 and SMIT in their mRNA levels, as well as to an increase in the betaine and *myo*-inositol uptake, and that this upregulation was significantly inhibited by SB203580, indicating that the activation of p38 was involved in the hyperosmolarity-induced upregulation of osmolyte transporters BGT-1 and SMIT [17]. Sheih-Hamad et al. [18] have also reported that p38 kinase activity was essential for the osmotic induction of BGT1 mRNA in MDCK cells. Our results show that neither PD98059 nor SB203580 inhibited the hypertonicity-induced upregulation of TAUT activity (Fig. 1A and B), suggesting that neither the ERK nor p38 pathway was involved in the osmotic regulation of human TAUT in Caco-2 cells. It is therefore presumed that the intracellular signal cascade involved in osmoregulation would be different among the osmosensitive genes; i.e., p38 kinase would be essential for BGT1 and SMIT, but not for TAUT.

The efflux of taurine from the cells was also important when considering the intracellular content of taurine, although the efflux pathway for taurine has not yet been revealed. It has been reported for NIH3T3 mouse fibroblasts that the taurine efflux was significantly inhibited by 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), a specific inhibitor of the chloride channel, suggesting that the efflux of taurine was partly performed *via* the chloride channel [21,22]. Furthermore, the effect of the calmodulin antagonist, W-7, on swelling-induced taurine release has been reported, its effect being different among cell types. For example, the swelling-induced taurine release from Ehrlich cells and HeLa cells was inhibited in the presence of W-7 [22], whereas the taurine efflux from NIH-3T3 cells was potentiated by W-7 [21,23]. This opposing effect of W-7 is presumed to be due to the difference in tissues and species. In human intestinal Caco-2 cells, the efflux activity of taurine in response to a hypotonic milieu was also inhibited by DIDS. However, W-7 and KN-93 had no significant effect on the efflux activity of TAUT (data not shown), suggesting that activation of the Ca^{2+}/CaM kinase II cascade was initially involved in the taurine uptake and not in the taurine efflux in our present study.

The regulation of TAUT and its regulatory mechanism have been extensively investigated [24]. The upregulation of TAUT was observed under hypertonic conditions at the transcriptional level in several tissues, including intestinal epithelial cells (Fig. 5, [8,24]). Furthermore, the regulation of TAUT by such cytokines as TNF-α has also been reported, this regulation being performed at the transcriptional level [25]. On the other hand, TAUT was also regulated by protein kinase C at the post-translational level [26]. It has also been revealed that Ser-322 was a critical site for PKC regulation of the MDCK cell taurine transporter (pNCT) [27]. The activity of TAUT in intestinal epithelial cells has also been reported to be inhibited by lysophosphatidylcholine (LPC), the interaction of LPC with TAUT in the intestinal cell membrane being the cause of the reduced taurine uptake [28,29]. As shown in Fig. 5, TAUT mRNA was increased under hypertonic conditions. However, this result does not preclude the possibility of post-transcriptional regulation by hypertonicity; for example, the taurine uptake under hypertonic conditions may also include cellular trafficking of the transporter between the cytosol and plasma membrane, like the case of insulin-induced translocation of GLUT4 in the muscle and adipocyte [30,31]. More detailed experiments are needed to elucidate the mechanism for the osmoregulation of human TAUT *via* the activation of Ca^{2+}/CaM kinase II.

Ca^{2+}/CaM kinases are the most extensively characterized kinases to be directly modulated by calmodulin [32]. It is now understood that the Ca^{2+}/CaM kinase family has six isoforms (types I–VI), type II Ca^{2+}/CaM kinase being one of the most characterized enzymes among them. Ca^{2+}/CaM kinase II and the isozymes are strongly expressed in the brain, and are also expressed in most other tissues, including the small intestine [33,34]. This kinase has been reported to play vital roles in such functions as hippocampal long-term potentiation/memory [32,35] and insulin exocytosis [36,37], and is therefore called a "multifunctional protein kinase" [32]. The specific inhibitor of Ca^{2+}/CaM kinase II completely repressed the increased expression level of TAUT mRNA by hypertonicity (Fig. 5), suggesting that the activation of Ca^{2+}/CaM kinase II participated upstream of the transcriptional regulation of TAUT. A cyclic AMP-responsive element-binding protein (CREB), a transcriptional factor which is activated *via* the protein kinase A (PKA) pathway, is involved in these functions as a substrate of Ca^{2+}/CaM kinase II [32,38]. We therefore hypothesize a

pathway by which hypertonicity led to the activation of Ca^{2+}/CaM kinase II, and subsequent modulation of CREB that induced an increase in TAUT gene expression and finally increased the taurine uptake by Caco-2 cells. However, Br-cAMP exposure, which had been presumed to induce the activation of PKA and CREB, had no significant effect on the taurine uptake by Caco-2 cells (data not shown). This result suggests that the modulation of CREB was not involved in this Ca^{2+}/CaM kinase II-dependent osmoregulation of taurine uptake. We have recently cloned the promoter region of human TAUT and found the consensus element of AP-1 and nuclear factor-κB (NF-κB) in the promoter region. AP-1 and NF-κB may therefore be candidates for the signal cascade existing between Ca^{2+}/CaM kinase II activation and the increased expression of TAUT mRNA.

In conclusion, the involvement of Ca^{2+}/CaM kinase II in the hypertonicity-induced upregulation of human TAUT was demonstrated for the first time in this work. This result will provide more information for understanding the mechanism for the intracellular regulation of TAUT under hypertonic conditions, as well as a new function of Ca^{2+}/CaM kinase II in cellular regulation.

Acknowledgements: This study was partially supported by grant-in-aid for Creative Scientific Research by the Japan Society for the Promotion of Science (No. 13GS0015), and by grant-in-aid from Taisho Pharmaceutical Co. Ltd. (Tokyo, Japan). The uptake experiments in this work were performed at the Biotechnology Research Center of the University of Tokyo (Tokyo, Japan).

References

[1] Yancey, P., Ckark, M., Hand, S., Bowlus, R. and Somero, R. (1982) Science 217, 1214–1222.
[2] Burg, M.B. (1994) J. Exp. Zool. 268, 171–175.
[3] Kwon, H. and Handler, J. (1995) Curr. Opin. Cell Biol. 7, 465–471.
[4] Smardo Jr., F., Burg, M. and Garcia-Perez, A. (1992) Am. J. Physiol. 262, C776–C782 (Cell Physiol. 31).
[5] Burg, M.B. (1995) Am. J. Physiol. 268, F983–F996 (Renal Fluid Electrolyte Physiol. 37).
[6] Uchida, S., Nakanishi, T., Kwon, H., Preston, A. and Handler, J. (1991) J. Clin. Invest. 88, 656–662.
[7] Moeckel, G., Lai, L.-W., Guder, W., Kwon, H. and Lien, Y.-H. (1997) Am. J. Physiol. 272, F100–F106 (Renal Physiol. 41).
[8] Satsu, H., Miyamoto, Y. and Shimizu, M. (1999) Biochim. Biophys. Acta 1419, 89–96.
[9] Shimizu, M. and Satsu, H. (2000) Amino Acids 19, 605–614.
[10] Satsu, H., Terasawa, E., Hosokawa, Y. and Shimizu, M. (2003) Biochem. J. 375, 441–447.
[11] Itoh, T., Yamauchi, A., Miyai, A., Yokoyama, K., Kamada, T., Ueda, N. and Fujiwara, Y. (1994) J. Clin. Invest. 93, 2387–2392.
[12] Matsuda, S., Kawasaki, H., Moriguchi, T., Gotoh, Y. and Nishida, E. (1995) J. Biol. Chem. 270, 12781–12786.
[13] Galcheva-Gargova, Z., Derijard, B., Wu, I.H. and Davis, R.J. (1994) Science, 806–808.
[14] Han, J., Lee, J.D., Bibbs, L. and Ulevitch, R.J. (1994) Science, 808–811.
[15] Kwon, H., Itoh, T., Rim, J. and Handler, J. (1995) Biochem. Biophys. Res. Commun. 213, 975–979.
[16] Kultz, D., Garcia-Perez, A., Ferraris, J.D. and Burg, M.B. (1997) J. Biol. Chem. 272, 13165–13170.
[17] Denkert, C., Warskulat, U., Hensel, F. and Haussinger, D. (1998) Arch. Biochem. Biophys. 354, 172–180.
[18] Sheikh-Hamad, D., Mari, J., Suki, W.N., Safirstein, R., Watts 3rd, B.A. and Rouse, D. (1998) J. Biol. Chem. 273, 1832–1837.
[19] Nahm, O., Woo, S., Handler, J. and Kwon, H. (2002) Am. J. Physiol. 282, C49–C58 (Cell Physiol.).
[20] Satsu, H., Watanabe, H., Arai, S. and Shimizu, M. (1997) J. Biochem. 121, 1082–1087.
[21] Lambert, I.H. and Sepulveda, F.V. (2000) Adv. Exp. Med. Biol. 483, 487–495.
[22] Lambert, I.H. (2004) Neurochem. Res. 29 (1), 27–63.
[23] Lambert, I.H. (2003) J. Membr. Biol. 192, 19–32.
[24] Tappaz, M.L. (2004) Neurochem. Res. 29 (1), 83–96.
[25] Mochizuki, T., Satsu, H. and Shimizu, M. (2002) FEBS Lett. 517, 92–96.
[26] Loo, D.D., Hirsch, J.R., Sarkar, H.K. and Wright, E.M. (1996) FEBS Lett. 392, 250–254.
[27] Han, X., Budreau, A.M. and Chesney, R.W. (1999) J. Am. Soc. Nephrol. 10, 1874–1879.
[28] Ishizuka, K., Kanayama, A., Satsu, H., Miyamoto, Y., Furihata, K. and Shimizu, M. (2000) Biosci. Biotechnol. Biochem. 64 (6), 1166–1172.
[29] Ishizuka, K., Miyamoto, Y., Satsu, H., Sato, R. and Shimizu, M. (2002) Biosci. Biotechnol. Biochem. 66, 730–736.
[30] Bryant, N.J., Govers, R. and James, D.E. (2002) Nat. Rev. Mol. Cell Biol. 3 (4), 267–277.
[31] Kanzaki, M. and Pessin, J.E. (2003) Curr. Biol. 13 (14), 574–576.
[32] Schulman, H. (1993) Curr. Opin. Cell Biol. 5, 247–253.
[33] Colbran, R.J. and Soderling, T.R. (1990) Curr. Topics Cell. Reg. 31, 181–221.
[34] Matovcik, L., Maranto, A., Soroka, C., Gorelick, F., Smith, J. and Goldenring, J. (1996) J. Histochem. Cytochem. 44, 1243–1250.
[35] Fukunaga, K. and Miyamoto, E. (2000) Neurosci. Res. 38, 3–17.
[36] Easom, R.A. (1999) Diabetes 48, 675–684.
[37] Niki, I., Okazaki, K., Saitoh, M., Niki, A., Niki, H., Tamagawa, T., Iguchi, A. and Hidaka, H. (1993) Biochem. Biophys. Res. Commun. 191, 255–261.
[38] Sheng, M., Thompson, M.A. and Greenberg, M.E. (1991) Science 252, 1427–1430.