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Stimulation of ERK2 by taurine with enhanced alkaline phosphatase activity and collagen synthesis in osteoblast-like UMR-106 cells

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Abstract

Taurine is present in a variety of tissues and exhibits many important physiological functions in the cell. Even though its functions are well documented in many tissues, its actions on bone cells are largely unknown. Considering a recent finding that taurine is present in the bone, we wished to determine if taurine could have any effects on osteoblast cells. Taurine (10 mM) stimulated alkaline phosphatase activity as well as collagen synthesis. Taurine also stimulated tyrosine phosphorylation of a number of cellular proteins including a 42-kDa protein. The 42-kDa protein was identified as extracellular signal regulated protein kinase 2 (ERK2). A mitogen-activated protein kinase kinase (MEK) inhibitor blocked the taurine-stimulated alkaline phosphatase activity and collagen synthesis. These results suggest that taurine could regulate osteoblast metabolism via ERK2 activation.

Keywords: Taurine; Alkaline phosphatase; Collagen; ERK2; Osteoblast

1. Introduction

Taurine, 2-aminoethanesulfonic acid, is present in high concentrations in the brain, heart, eye, muscle, and liver. It can be synthesized from cysteic acid or hypotaurine, which is derived from methionine and cysteine in the body [1]. Since the activity of cysteinsulfinic acid decarboxylase, a major regulator of taurine synthesis, is relatively low in humans, taurine is supplied primarily through diets. Taurine exerts a variety of actions such as regulation of neuronal activity, stabilization of membranes, regulation of platelet aggregation, osmoregulation, antioxidation, and regulation of phosphorylation [2]. Taurine is very safe in terms of tissue toxicity and is used as a supplement to drugs, baby milk, or health foods by pharmaceutical companies. Recently, taurine was identified in the bone [3,4]. Considering that a significant amount of taurine is transported to bone tissues, it is reasonable to propose that taurine may play an important role in bone metabolism. Interestingly, taurine has been found to inhibit experimental bone resorption and osteoclast formation and survival [5]. ERK or MAP kinase plays an essential role in signal transduction following extracellular stimuli [6]. Regulation of ERK involves activation of a series of signaling molecules such as Grb2/SOS, ras, raf, and MEK [7–12]. The MEKs (MEK1, MEK2, MEK3) are dual specificity protein kinases that phosphorylate and activate ERKs on tyrosine and threonine residues [13–16]. ERK1/ERK2 is present mainly in the cytoplasm; however, upon activation, it undergoes translocation into the nucleus and phosphorylates transcription factors and enzymes involved in transcription such as AP-1, Elk-1, TAL1, and RNA polymerase in many cells [17–19]. In the present study, we designed a series of experiments to determine whether taurine has regulatory effects on osteoblast functions. The potential roles of ERK2 in the taurine-mediated effects in the UMR-106 cells are discussed.

2. Materials and methods

2.1. Materials

Taurine was purchased from the Sigma Chemical Co. The reagents used for polyacrylamide gel electrophoresis

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Abbreviations: ERK, extracellular signal regulated protein kinase; MAP, mitogen-activated protein; MEK, MAP kinase kinase; and MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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were from Bio-Rad. Nitrocelluose filters (0.45 μm) were purchased from Schleicher & Schuell. Antibodies to p44/p42 MAP kinase (ERK1/2), phospho-p44/p42 MAP kinase, and the MEK inhibitor (PD98059) were purchased from New England Biolab. An ECL kit was purchased from Amersham. UMR-106 cells were purchased from the ATCC. All other chemicals were purchased from Sigma.

2.2. Cell culture

UMR-106 cells were grown in DMEM medium with 10% fetal bovine serum in 5% humidified CO_2 atmosphere at 37°.

2.3. MTT assay

UMR-106 cell viability was measured using an MTT assay. The cells were plated in 96-well plates at a density of 1×10^5 cells/well. Following taurine treatment for 24 hr, the cells were treated with MTT (0.5 mg/mL) and allowed to incubate for 3 hr. The culture medium was removed, the cells were subjected to lysis in the presence of 100 μL of DMSO and 10 μL of Sorenson glycine buffer, and absorbance was measured with a spectrophotometer at 540 nm [20].

2.4. Measurement of alkaline phosphatase activity

After incubation with insulin for 24 hr, the cell layer was washed with PBS and solubilized with 0.1% Triton X-100. Following brief sonication, aliquots of the total cell extract were used for measurement of alkaline phosphatase activity as previously described [21] and for determination of collagen levels.

2.5. Measurement of collagen synthesis

Collagen synthesis was analyzed by measuring hydroxyproline levels as follows. Sonicated cells were mixed with concentrated HCl, with final concentrations being adjusted to 6 N HCl, and incubated in a 130° oven for 3 hr. The reaction mixture was adjusted to pH 6, 1 mL of chloramine T solution was added, and the mixture was incubated at room temperature for 20 min for oxidation. The oxidation reaction was stopped by adding 1 mL of 3.15 M perchloric acid, and the reaction mixture was incubated for 5 min at room temperature. One milliliter of *p*-dimethylaminobenzaldehyde was added, and the reaction mixture was incubated at 60° for 20 min. Following filtration through a 0.22 μm filter, the filtrate was subjected to measurement with a spectrophotometer at 557 nm [22].

2.6. Western blot analysis

Electrotransfer of proteins from the gels to nitrocelluose paper (Schleicher & Schuell) was carried out for 1 hr at 100 V (constant) as described by Towbin *et al.* [23]. The filter papers were preincubated for 1 hr at 23° with PBS containing 0.1% Tween 20 and 3% bovine serum albumin and washed with PBS containing 0.1% Tween 20 three times for 10 min each. The blots were probed with primary antibodies (ERK2 or phospho-ERK2) for 1 hr at 23°. The blots were then incubated with horseradish peroxidase-conjugated anti-rabbit IgG for 30 min and washed with PBS containing Tween 20 five times for 10 min each. The detection of immobilized specific antigens was carried out by enhanced chemiluminescence (ECL) (NEN).

2.7. Statistics

All values are expressed as means ± SD. Comparison between groups was performed by Student's *t*-test.

3. Results

3.1. Effect of taurine on UMR-106 cell viability

To determine if taurine has any cytotoxic effects on UMR-106 cells, MTT assays were carried out. Taurine in the 0.001 to 10 mM concentration range had little cytotoxic effect on the cells as compared with the control (data not shown).

3.2. Effect of taurine on alkaline phosphatase activity

Alkaline phosphatase is regarded as an index of osteoblastic differentiation and plays an important role in bone mineralization. Taking this into consideration, we determined whether taurine has any effect on alkaline phosphatase activity in UMR-106 cells. Taurine stimulated alkaline phosphatase activity by 32–87% in the 1–20 mM concentration range (Fig. 1).

3.3. Effect of taurine on collagen synthesis

Considering that hydroxyproline consists of approximately 14% collagen and this ratio is constant, the collagen synthesis rate could be analyzed by measuring cellular hydroxyproline levels. Taurine had little effect on hydroxyproline levels in UMR-106 cells below 1 mM; however, it increased hydroxyproline levels in these cells at 5, 10, 15, and 20 mM by 37, 65, 65, and 59%, respectively (Fig. 2).

3.4. Effects of taurine on tyrosine phosphorylation

Since tyrosine phosphorylation is an important reaction for cellular signaling, the effects of taurine on tyrosine phosphorylation were explored. Taurine stimulated the tyrosine phosphorylation of a number of proteins such as 63-, 53-, 42-, and 38-kDa proteins (Fig. 3). One of the tyrosine-phosphorylated proteins was identified as ERK2, as evi-

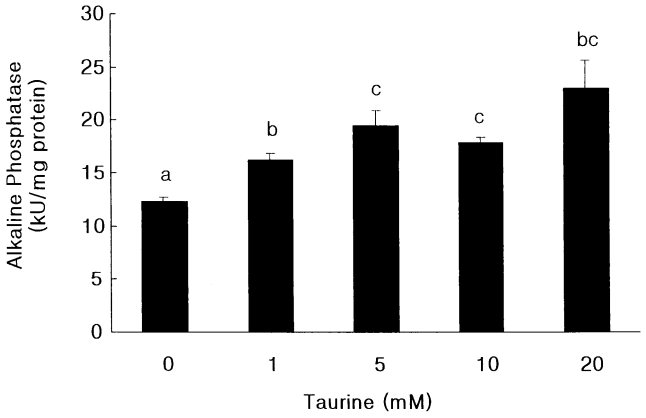

Fig. 1. Effect of taurine on alkaline phosphatase activity in UMR-106 cells. UMR-106 cells were treated with taurine (1–20 mM) for 24 hr. Alkaline phosphatase activity was determined in the cytosolic fraction, using an assay kit, and was corrected for protein content. Values are means ± SD, N = 5. Bars not sharing a common letter are significantly different from each other at $P < 0.05$ (by Duncan's multiple range test).

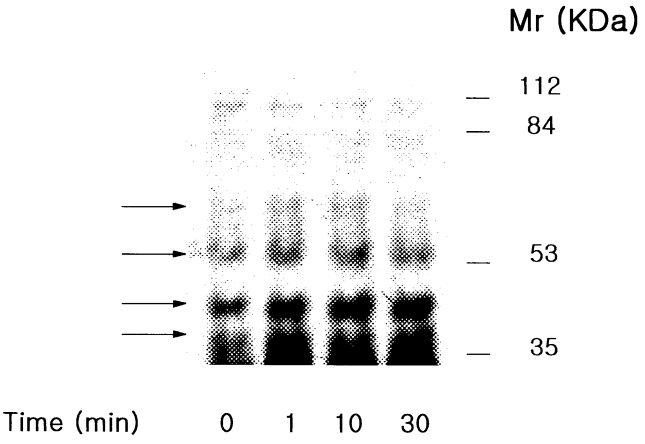

Fig. 3. Effect of taurine on tyrosine phosphorylation in UMR-106 cells. UMR-106 cells were maintained in DMEM medium containing 0.1% bovine serum albumin instead of fetal bovine serum and then were stimulated with 10 mM taurine for the indicated times before protein extraction. Western blot analysis was performed with specific antibodies against phosphorylated tyrosine. This experiment was done five times.

denced by western blot analysis using anti-ERK and anti-phospho-ERK antibodies (Fig. 4). Taurine stimulated tyrosine phosphorylation of ERK2 from as early as 1 min, and the tyrosine-phosphorylated ERK2 remained activated for 30 min. The tyrosine phosphorylation of ERK2 following taurine treatment began to occur at 0.01 mM, and it was stimulated further by increasing taurine concentrations (Fig. 5).

3.5. Effects of an MEK inhibitor on taurine-induced alkaline phosphatase activation and collagen synthesis

To delineate the mechanism of taurine-induced alkaline phosphatase activation and collagen synthesis, an inhibitor of MEK (PD98059) that specifically inhibits ERK2 was employed. When the cells were preincubated with the MEK inhibitor, taurine failed to stimulate alkaline phosphatase activity and collagen synthesis (Fig. 6). These results suggest that stimulation of alkaline phosphatase activity and collagen synthesis induced by taurine is mediated by ERK2.

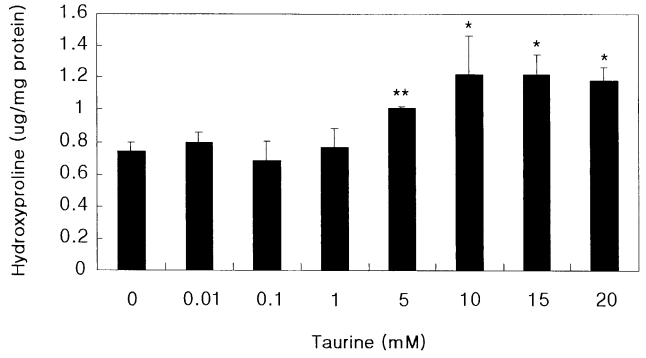

Fig. 2. Effect of taurine on the content of hydroxyproline in UMR-106 cells. UMR-106 cells were maintained in DMEM medium containing 0.1% bovine serum albumin instead of fetal bovine serum and then stimulated with 0.01 to 20 mM taurine for 24 hr. The concentration of hydroxyproline was measured in the cell lysate after hydrolysis of peptides by acid. Values are means ± SD, N = 6. Asterisks indicate values significantly different from control (*, $P < 0.05$; **, $P < 0.01$).

4. Discussion

Taurine is a water-soluble, two-carbon atom amino acid. Besides its well-known function in bile salt amidation, taurine is involved in osmolarity regulation, cellular proliferation, and modulation of neuronal excitability, and is antiepileptic. However, its action in bone cells remains to be characterized. The fact that taurine has been identified recently in bone, and has been found to inhibit experimental bone resorption and osteoclast formation and survival, prompted us to explore if taurine has any effects on osteoblasts. We have presented evidence that taurine has stimulatory actions on alkaline phosphatase and collagen synthesis, and that these actions are mediated by ERK2.

The amount of taurine found in bone is currently unknown. Based on the results of other studies utilizing tau-

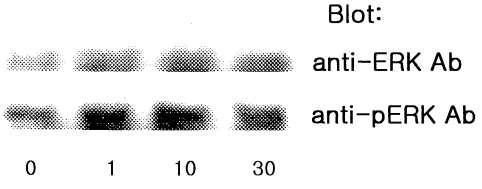

Fig. 4. Time course of ERK2 activation by taurine in UMR-106 cells. UMR-106 cells were maintained in DMEM medium containing 0.1% bovine serum albumin instead of fetal bovine serum and then were stimulated with 10 mM taurine for the indicated time before protein extraction. Western blot analysis was performed with specific antibodies against ERK2 and phosphorylated ERK2. This experiment was done five times.

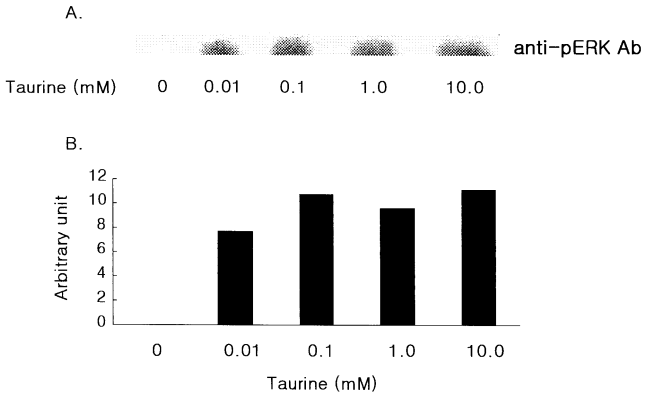

Fig. 5. Effect of taurine concentration on ERK2 activity in UMR-106 cells. UMR-106 cells were maintained in DMEM medium containing 0.1% bovine serum albumin instead of fetal bovine serum and then were stimulated with 0.01 to 10 mM taurine for 10 min before protein extraction. Western blot analysis was performed with specific antibodies against phosphorylated ERK2 (A). The content of ERK2 was quantified by scanning densitometry (B). This experiment was done five times.

rine, we used a 10 mM taurine concentration in the present study. It is known that taurine accumulates in the bone with a rate similar to that of the liver or kidney [3,4]. Hence, it is reasonable to speculate that the taurine content in bone may be similar to those of liver or kidney (2–11 mM/kg tissue) [2]. Taurine had little cytotoxic action on UMR-106 osteoblast-like cells at concentrations between 0.001 and 10 mM. Hence, we used this taurine concentration range to explore its actions on the cells. Alkaline phosphatase activity was stimulated by taurine in a concentration-dependent manner, whereas collagen synthesis was stimulated at a taurine concentration of 10 mM; these effects were observed when taurine was added for 24 hr. These results indicate that taurine may play a role in osteoblastic differentiation as well as bone matrix formation. Taurine is known to modulate the phosphorylation of cellular proteins [24] including the 44-kDa protein in heart mitochondria [25]. It was found recently that MAP kinases mediate the stimulation of bile acid secretion by tauroursodeoxycholate in rat liver [26]. In the present study, tyrosine phosphorylation of cellular proteins by taurine was stimulated significantly, and one of the proteins (42 kDa) was identified for the first time as ERK2. Taurine rapidly activated ERK2, and its activation occurred even at a 0.01 mM concentration, suggesting that osteoblasts are very sensitive to taurine. It was also shown recently that α-FAK, Jak2, and Stat5 are tyrosine phosphorylated by growth hormone in osteoblast-like cells [27,28]. We do not know the identities of the ~63-, ~53-, and ~38-kDa proteins that were tyrosine phosphorylated in the presence of taurine in the present study. However, based on molecular size, we can at least suggest that the 63-, 53-, and 38-kDa phosphoproteins are not related to α-FAK (125 kDa), Jak2 (120 kDa), or Stat5 (118 kDa). It has been shown that UMR-106 cells contain a large number of insulin receptors (80,000 binding sites per cell) [29]. However, taurine appears to have little effect on the tyrosine kinase activity of the 95-kDa β-subunit of insulin receptors since autophosphorylation of the insulin receptor β-subunit was not stimulated in response to taurine. It is likely that taurine may directly activate another cellular tyrosine kinase(s) such as MEK, which can phosphorylate and activate ERKs on tyrosine residues. The activation of alkaline phosphatase and the induction of collagen synthesis by taurine may be mediated by ERK2 activation, as evidenced by the experiments utilizing an MEK inhibitor. There were no significant effects of taurine (10 mM) on alkaline phosphatase activity and collagen synthesis at earlier time points such as 1, 10, 30, and 60 min (data not shown). Considering that taurine had no significant effect on alkaline phosphatase activity and collagen synthesis at the earlier time points, it is possible that following the rapid activation of ERK by taurine, there is activation of signaling cascades, which lead to the eventual activation of alkaline phosphatase and collagen synthesis. Taken together, these results suggest that taurine transported into the cytosol of UMR-106 cells rapidly activates ERK2 through tyrosine kinase activation, thereby stimulating alkaline phosphatase activity and collagen synthesis. ERK2 is present in the cytosol as well as the nucleus of UMR-106 cells [30], and the taurine-activated ERK2 may phosphorylate transcription factors, activating collagen gene transcription and protein synthesis. These taurine-in-

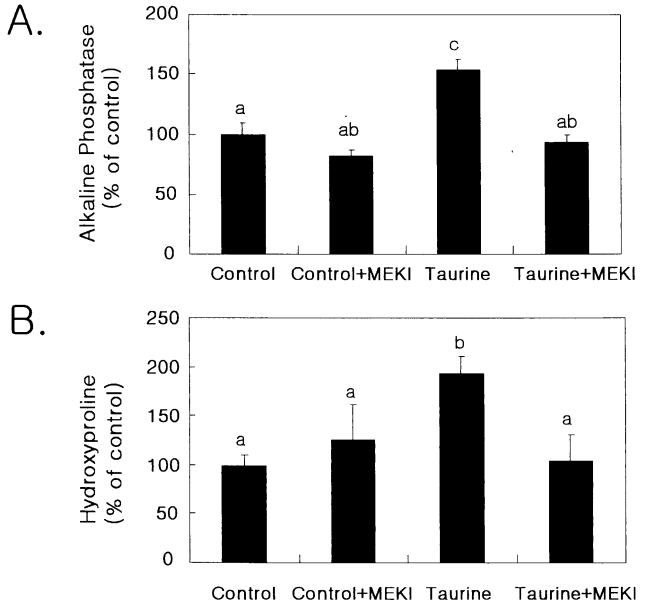

Fig. 6. Effect of taurine and an MEK inhibitor on alkaline phosphatase activity and hydroxyproline in UMR-106 cells. UMR-106 cells were treated with taurine (10 mM) and/or MEK inhibitor (50 μM) for 24 hr. (A) Alkaline phosphatase activity was determined in the cytosolic fraction, using an assay kit, and was corrected for protein content (100% = 12.5 kU/mg protein). (B) The concentration of hydroxyproline was measured in the cell lysate after hydrolysis of peptides by acid (100% = 0.74 μg/mg protein). Values are means ± SD, N = 5. Bars not sharing a common letter are significantly different from each other at $P < 0.05$ (by Duncan's multiple range test).

duced actions may be beneficial to osteoblastic differentiation and bone matrix formation.

Acknowledgments

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