Amino Acids (2004) 26: 159–162
DOI 10.1007/s00726-003-0046-4

Amino Acids
Printed in Austria

Protective effect of taurine on the detachment of cultured cardiac fibroblasts from the substratum induced by calcium depletion

M. Azuma [1], **K. Takahashi** [1], **A. Kimura** [1], **T. Matsuda** [1], **S. W. Schaffer** [2], and **J. Azuma** [1]

[1] Department of Clinical Evaluation of Medicines and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan
[2] Department of Pharmacology, University of South Alabama, School of Medicine, Mobile, Alabama, U.S.A.

Received June 25, 2003
Accepted August 20, 2003
Published online October 17, 2003;

Summary. Removal of Ca^{2+} from the incubation medium of cultured rat cardiac fibroblasts causes cellular morphological changes, such as the formation of blebs, the ballooning of the cell membrane and the detachment from the culture dish. A 24 hr preincubation with 20 mM taurine blocked the Ca^{2+} depletion-induced detachment of the cardiac fibroblasts. However, taurine treatment did not prevent other morphological changes induced by Ca^{2+} depletion. The data suggest that taurine plays an important role in cell adhesion in the heart.

Keywords: Taurine – Calcium – Cardiac fibroblasts – Morphological change – Cell adhesion

Introduction

Taurine is an amino acid found in very high concentration in the heart, where it appears to regulate cellular volume, Ca^{2+} transport and contractile function (Huxtable, 1992; Rasmusson et al., 1993; Schaffer et al., 1994). Depending on the status of the myocardium, taurine is capable of either increasing or decreasing intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In hearts made hypodynamic by reductions in $[Ca^{2+}]_i$, taurine appears to enhance Ca^{2+} influx. On the other hand, taurine treatment reduces $[Ca^{2+}]_i$ in the Ca^{2+} overloaded myocardium. Since $[Ca^{2+}]_i$ plays a central role in the physiology and pathology of the heart, it is not surprising that the depletion of taurine leads to the development of a cardiomyopathy (Pion et al., 1987).

The high taurine content of the cardiac myocyte is maintained by a transporter that carries taurine into the cell against a concentration gradient. A similar transporter is expressed by the cardiac fibroblast (Takahashi et al., 2003). Although the cardiac fibroblast is the dominant cell of the myocardium, the role of taurine in the fibroblast is poorly defined. In accordance with its actions in the cardiac myocyte, one might also expect taurine to alter Ca^{2+} movement in the cardiac fibroblast. One of the important functions of Ca^{2+} in the fibroblast is the stabilization of cell adhesion molecules (Ko et al., 2001). Therefore, we reasoned that taurine might have an effect on cell adhesion. To test this idea, we examined the influence of taurine on morphological changes of cultured cardiac fibroblasts induced by extracellular Ca^{2+} depletion.

Material and methods

Preparation of cardiac myocytes and fibroblasts

The preparation of primary cardiac myocytes and non-myocytes cultures from 1-day old Wister rats was performed as described previously (Takahashi et al., 1997). This procedure yielded cultures with more than 90% fibroblasts, as assessed by three different chemical stains (acetylated-low density lipoprotein, a-smooth muscle actin, and neuron-specific nuclear protein). Cells were kept serum-containing culture medium, Dulbecco's modified Eagle's medium/F-12 (1:1 v/v) supplemented with pyruvic acid, ascorbic acid, insulin, transferring and selenium (DMEM/F-12), newborn calf serum (5%; for cardiac myocytes, 10%; for cardiac fibroblasts) for 2 days.

Assay

Cells were washed with phosphate buffered saline for 3 times and incubated in DMEM/F-12 containing either 0 or 20 mM taurine for 24 hr, and then exposed to the reaction medium containing either 0 or 20 mM taurine for 10 min. The reaction medium described by Wakabayashi et al. (Wakabayashi and Goshima, 1981) was modified and prepared as shown in Table 1. The morphological status of the cardiac fibroblasts was monitored with an inverted phase contrast microscope (IX70, Olimpas, Tokyo, Japan) and videomonitor equipped videocopy processor. The shape and location of each cell was photographically recorded before initiating the experiment. Cell detachment was estimated from the ratio of cells attached before and after exposure to Ca^{2+}-free medium.

Table 1. Effect of taurine on morphological changes of cardiac fibroblasts induced by removing Ca^{2+} from the incubation medium

Reaction medium	Ionic composition (mM)						Blebs and ballooning	Detachment	Total
	Na^+	K^+	$Choline^+$	Ca^{2+}	EGTA	Cl^-			
1 (Control)	135	5	0	1	0	141	0	0	288
2	135	5	0	0	2	140	98 (45)	107 (49)	217
3	140	0	0	1	0	141	0	3	271
4	0	140	0	1	0	141	0	3	261
5	0	5	135	1	0	141	0	6	262
6	0	0	140	1	0	141	0	9	291
7	135	5	0	3	2	141	0	6	288
8 (Taurine)	135	5	0	0	2	140	96 (43)	85* (38)	221

All media contained 5.5 mM glucose and 10 mM HEPES. Parentheses represent the percentage of cells showing morphological changes. Total examined cells (217–291) were prepared from three different cultures. The number in the parenthesis represents % of morphologically altered cells/total observed cells. Asterisks denote significant differences from the Ca^{2+}-free medium (reaction medium 2) ($^*p < 0.001$)

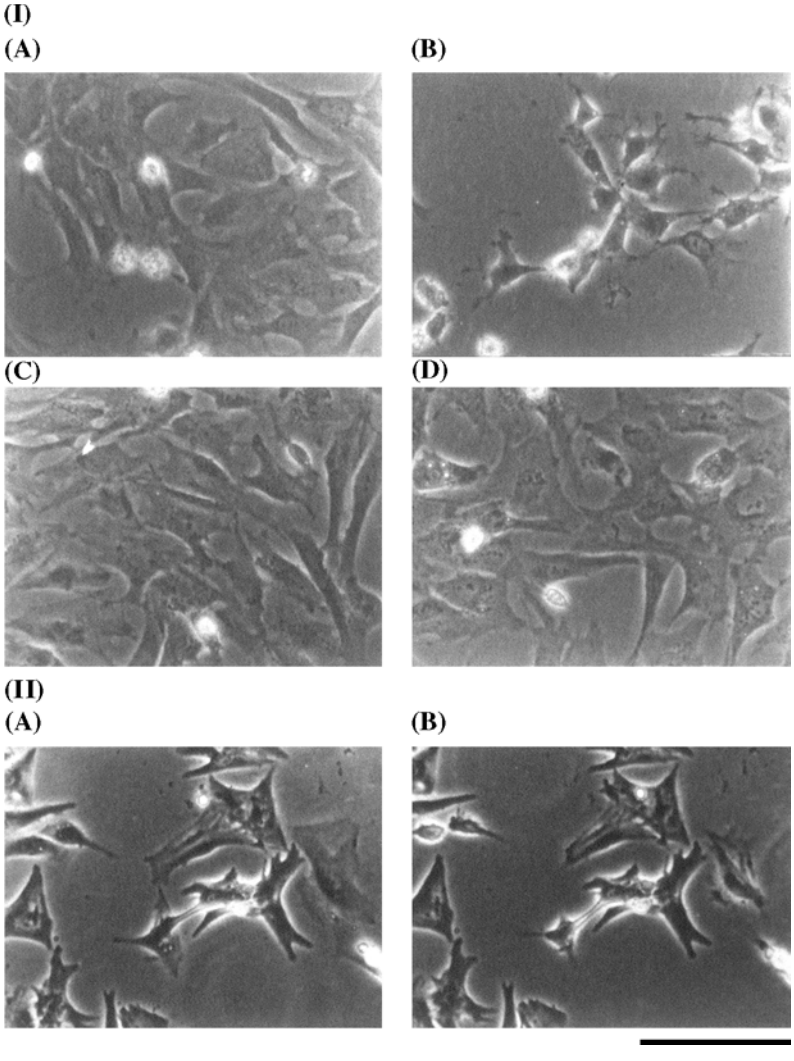

Fig. 1. Photomicrographs showing morphological changes of cultured cardiac cells. (**I**) Cardiac fibroblasts: (**A**) Unexposed cells (reaction medium 1). (**B**) Cells exposed to Ca^{2+}-free medium (reaction medium 2) for 10 min. (**C**) Cells exposed to K^+-free medium (reaction medium 3) for 10 min. (**D**) Cells exposed to Na^+-free medium (reaction medium 4) for 10 min. (**II**) Cardiac myocytes: (**A**) Unexposed cells (reaction medium 1). (**B**) Cells exposed to Ca^{2+}-free medium (reaction medium 2) for 10 min. Bar = 50 μm

Statistical analysis

Statistical significance was determined by χ^2 test. Differences were considered statistical when the calculated p value was less than 0.05.

Results

Cardiac fibroblasts exposed to Ca^{2+}-free medium (Table 1; medium No. 2), underwent morphological changes, including the formation of blebs, the ballooning of the cell membrane and the detachment from the culture dish (Fig. 1-I). However when the cardiac fibroblasts were exposed to reaction media lacking Na^+, K^+ or both Na^+ and K^+ (Table 1; medium No. 3–7), the cells rarely underwent the characteristic Ca^{2+}-depletion induced morphological changes. Moreover, in contrast to the fibroblast, cardiac myocytes did not detach from the culture dish, even upon exposure to Ca^{2+}-free medium (Fig. 1-II).

The amino acid, taurine, is known to modulate Ca^{2+} movement in the myocardium. Therefore, the effect of preincubating the fibroblasts for 24 hr with medium containing 20 mM taurine was examined. Taurine treatment had no effect on cell morphology prior to exposure to extracellular Ca^{2+} medium. The organic osmolyte also had no effect on blebbing and ballooning following exposure to Ca^{2+}-free medium. However, it significantly impeded the detachment of fibroblasts from the substratum of the culture dish (Table 1; medium No. 8).

Discussion

The major finding of the present study is that cardiac fibroblasts pretreated with taurine are resistant to the process of cellular detachment initiated by removal of Ca^{2+} from the incubation medium. It is known that Ca^{2+} can promote the attachment of cells to culture dishes through at least two mechanisms. First, Ca^{2+} is known to stabilize the active conformation of cell adhesion molecules, such as the cadherins, the secretins and the integrins. Drastic reductions in extracellular Ca^{2+} concentration induce conformational changes, in which the extracellular regions of the cell adhesion molecules become floppy and are rapidly degraded by proteolytic enzymes. Second, it has been reported that the cadherin–cadherin interaction induces $[Ca^{2+}]_i$ transients during cell–cell adhesion in fibroblasts, and that these Ca^{2+} signals regulate cell–cell adhesion through remodeling of cortical actin and the recruitment of cadherins and beta-catenin into the intercellular junction (Ko et al., 2001). Since cadherins play an important role, not only in cell–cell but also in cell-substratum adhesion, it is likely that the Ca^{2+} signal contributes to cell-substratum adhesion. In present study, Ca^{2+} depletion caused a 18% decrease in the $[Ca^{2+}]_i$ level of cardiac fibroblasts (not data shown). Taurine has been shown to regulate the level of $[Ca^{2+}]_i$ (Huxtable, 1992). Therefore, it is possible that the attenuation of Ca^{2+}-depletion-induced fibroblast detachment by taurine involves the retardation in $[Ca^{2+}]_i$ loss from the fibroblast.

It is interesting that the cardiac myocyte, which contains higher levels of taurine than the fibroblast, is resistant to Ca^{2+}-depletion induced cellular detachment. The taurine content in cardiac fibroblasts was 84 ± 14 pmol/μg protein (Takahashi et al., 2003). ^{3}H-taurine (20 mM) incorporation into cardiac fibroblasts was 54 ± 16 pmol/μg protein for 0.5 h and subsequently showed a gradual increase for 24 h (not data shown). Presumably, taurine pretreatment of the fibroblast increases intracellular taurine content, thereby improving Ca^{2+} homeostasis and stabilizing adhesion molecules. This scenario would suggest that the active taurine transporter plays an important role in elevating taurine levels adequately to promote fibroblast adhesion (Takahashi et al., 2003). Although taurine exerts some effects, such as membrane stabilization, that one might have expected to minimize Ca^{2+} depletion-induced blebbing and ballooning, pretreatment with taurine failed to influence these membrane changes. The present study suggests that taurine protects the cardiac fibroblast from Ca^{2+} depletion-induced cellular detachment. Since the amino acid had no effect on membrane morphological changes, such as blebbing and ballooning, it is possible that the cardioprotective effects of taurine relate in part to the improvement in cellular adhesion.

In conclusion, taurine prevents the detachment of some cardiac fibroblasts from substratum following extracellular Ca^{2+} depletion. This result suggests that the heart maintains high levels of taurine to ensure proper regulation of Ca^{2+} homeostasis, in part to prevent damage to the cardiac fibroblast from perturbations in intracellular Ca^{2+} content.

References

Goshima K (1969) Synchronized beating of and electrotonic transmission between myocardial cells mediated by heterotypic strain cells in monolayer culture. Exp Cell Res 58: 420–426

Goshima K, Tonomura Y (1969) Synchronized beating of embryonic mouse myocardial cells mediated by FL cells in monolayer culture. Exp Cell Res 56: 387–392

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Ko KS, Arora PD, Bhide V, Chen A, McCulloch CA (2001) Cell–cell adhesion in human fibroblasts requires calcium signaling. J Cell Sci 114: 1155–1167

Long CS, Brown RD (2002) The cardiac fibroblast, another therapeutic target for mending the broken heart? J Mol Cell Cardiol 24: 1273–1278

Pion PD, Kittleson MD, Rogers QR, Morris JG (1987) Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. Science 237: 764–768

Rasmusson RL, Davis DG, Lieberman M (1993) Amino acid loss during volume regulatory decrease in cultured chick heart cells. Am J Physiol 264: C136–C145

Schaffer SW, Ballard C, Azuma J (1994) Mechanisms underlying physiological and pharmacological actions of taurine on myocardial calcium transport. In: Huxtable RJ, Michalk D (eds) Taurine health dis. Plenum, New York, pp 171–180

Smith KE, Borden LA, Wang CH, Hartig PR, Branchek TA, Weinshank RL (1994) Cloning and expression of a high affinity taurine transporter from rat brain. Mol Pharmacol 42: 563–569

Takahashi K, Azuma M, Taira K, Baba A, Yamamoto I, Schaffer SW, Azuma J (1997) Effect of taurine on angiotensin II-induced hypertrophy of neonatal rat cardiac cells. J Cardiovasc Pharmacol 30: 725–730

Takahashi K, Azuma M, Yamada T, Ohyabu Y, Takahashi K, Schaffer SW, Azuma J (2003) Taurine transporter in primary cultured neonatal rat heart cells: A comparison between cardiac myocytes and nonmyocytes. Biochem Pharmacol 65: 1181–1187

Wakabayashi S, Goshima K (1981) Kinetic studies on sodium-dependent calcium uptake by myocardial cells and neuroblastoma cells in culture. Biochim Biophys Acta 642: 158–172

Authors' address: Dr. Junichi Azuma, Department of Clinical Evaluation of Medicines and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, 565-0871, Osaka, Japan, Fax: +81-6-6879-8259, E-mail: azuma@phs.osaka-u.ac.jp