Role of Intracellular Taurine in Monensin-induced Na^+, Ca^{++} Accumulation and Mechanical Dysfunction in Isolated Rat Hearts

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It has been postulated that the intracellular taurine is co-transported with Na^+ down a concentration gradient and prevents the intracellular accumulation of sodium. It is therefore, expected that an elevated level of intracellular taurine prevents the sodium-promoted calcium influx to protect the cellular damages associated with sodium and calcium overload. In the present study, we evaluated the effects of intra- and extracellular taurine on the myocardial Na^+ and Ca^{++} contents and the cardiac functions in isolated rat hearts which were loaded with sodium by monensin, a Na^+-ionophore. Monensin caused a dose-dependent increase in intracellular Na^+ accompanied with a subsequent increase in intracellular Ca^{++} and a mechanical dysfunction. In this monensin-treated heart, myocardial taurine content was decreased with a concomittent increase in the release of taurine. The monensin-induced increases in intracellular Na^+, Ca^{++} and depression of cardiac function were prevented in the hearts of which taurine content had been increased by high-taurine diet. Conversely, in the hearts of which taurine concentration gradient had been decreased by addition of taurine in the perfusate, the monensin-induced increases in Na^+, Ca^{++} and functional depression were accelerated. These results suggest that taurine, depending on the intra-extracellular concentration gradient, can affect intracellular sodium and calcium concentrations, and that an increased intracellular taurine may play a role in protection of myocardial dysfunction associated with the sodium and calcium overload.

Key Words: Taurine, Monensin, Na^+, Ca^{++}, Cardiac function

INTRODUCTION

Taurine(2-aminoethane sulfonic acid) is a free β-amino acid which is highly abundunt in the mammalian heart cells. In rat hearts, intracellular taurine is as high as $20 \sim 40$ μ mole/g wet weight, which is more than 100 times higher than that of plasma (Huxtable, 1992). Although the myocardial taurine is maintained fairly constant in the normal heart, it's physiological role has not been established yet. It has been reported that a reduction of myocardial taurine content is accompanied with myocardial ischemia

(Cooper & Lombardini, 1981) and cardiomyopathy (Pion et al, 1987), and that the administration of exogenous taurine protects the hearts in various pathologic conditions, including calcium paradox (Kramer et al, 1981), isoproterenol- (Ohta et al, 1986) or doxorubicin-induced cardiotoxicity (Hamaguchi et al, 1988), ischemia or hypoxia (Franconi et al, 1985; Sawamura et al, 1986) and congestive heart failure (Azuma et al, 1992).

Most of the intracellular taurine is taken up from the dietary sources by an active transport mechanism. The transport of taurine across the cell membrane is affected by the transmembrane concentration gradient of Na^+, probably via a Na/taurine co-transport mechanism. A lowered transmembrane Na^+-gradient (an increased intracellular Na^+) induces the efflux of taurine, while an elevated gradient suppresses the

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efflux (Chapman et al, 1993; Schaffer et al, 1981). The existence of Na^+/taurine co-transport mechanism has been supported by the measurement of Na^+/taurine co-transport current in the isolated cardiomyocytes (Chapman et al, 1993; Earm et al, 1993; Suleiman et al, 1992). If a Na^+/taurine co-transport is responsible for the fall in tissue taurine associated with the reduction of the Na^+-gradient, it would be expected that changes in the transmembrane gradient for taurine will also affect the intracellular Na^+ concentration reversely. That is, an elevated concentration gradient for taurine (an increased intracellular taurine) would induce the Na^+-efflux coupled with taurine and the eventual lowering of intracellular Na^+ concentration. If this is the case, intracellular taurine may play an important role in various pathologic conditions associated with an intracellular Na^+-overload. Intracellular accumulation of Na^+ is followed by Ca^{++} accumulation via the Na^+-Ca^{++} exchange mechanism, contributing to the development of cellular damages and dysfunctions in various cardiac pathologies such as ischemia-reperfusion injury (Tani & Neely, 1989; Grinwald & Brosnahan, 1987) and Ca^{++}-paradox (Chapman et al, 1984). It is, therefore, not unreasonable to expect that an elevated transmembrane gradient of taurine will also reduce the increased level of intracellular Ca^{++} promoted by Na^+-overload. In the present study, we tried to confirm this plausible role of taurine modulating the intracellular Na^+, Ca^{++} and contractile function. The transmembrane concentration gradient of taurine was altered by an administration of exogenous taurine in isolated rat heart loaded with Na^+ by a Na-ionophore, monensin.

METHODS

Experimental protocol and monensin treatment

Hearts were isolated from male, Sprague-Dawley rats, weighing 200~300 g. Experimental groups were divided into three: 1) control hearts isolated from normal rats, 2) high-taurine hearts isolated from rats fed with taurine-containing(5% w/w) rat chow for 10 weeks, and 3) isolated hearts perfused with taurine-containing(10 mM) physiologic salt solution. The isolated hearts were perfused with Krebs-Henseleit (K-H) buffer solution (in mmol/l: NaCl 118.0, KCl

4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $CaCl_2$ 1.25, and glucose 10.0, pH 7.4) at a perfusion pressure of 80 cm H_2O by the non-recirculating Langendorff method. The buffer solution was saturated with a gas mixture of 95% O_2-5% CO_2 at 37°C. A Na^+-ionopheore, monensin was administered to induce intracellular Na^+ loading. After a stabilization period of 15min, the heart was perfused with monensin(0.5, 2.0, or 5.0 µM dissolved in ethanol)-containing K-H solution for 15min, and then switched to the normal K-H solution for 30min.

Measurement of cardiac mechanical function

Left ventricular pressure and heart rate were continuously monitored throughout the perfusion period. For measurement of left ventricular pressure, a rubber baloon-tipped catheter was inserted into left ventricle via mitral valve and connected to a physiograph (Grass 79E) via a pressure transducer(P23XL). The baloon was inflated until the resting left ventricular end-diastolic pressure(LVEDP) reached 5 mmHg. Heart rate(HR) was measured as the numbers of recorded contractions for one minute. The product of left ventricular developed pressure(LVDP; the difference between ventricular end-systolic pressure, LVESP and LVEDP) and HR was calculated as the cardiac functional index.

Measurement of myocardial intracellular Na^+ and Ca^{++} content

After the perfusion, the heart was perfused and washed with ice-cold 350 mM sucrose-5 mM histidine solution to minimize the contamination of extracellular cations. The atria were trimmed away, and the ventricles were blotted and weighed. The ventricular tissue samples were immediately frozen in liquid nitrogen, and were homogenized in 2% perchloric acid solution with Polytron tissue homogenizer(Brinkman, USA). After centrifugation at 12000 g for 30 min, the supernatant was taken and used for cation measurements. Na^+ concentration was measured with a Na^+-selective electrode, and Ca^{++} with a Ca^{++}-selective electrode. For correcting the inevitably contaminated extracellular cations, extracellular fluid volume was measured in another group of hearts by using ^{14}C-sorbitol(0.2 µM) method. The calculated extracellular Na^+ and Ca^{++} concentrations were sub-

tracted from the measured values.

Taurine assay

Myocardial taurine content was measured with HPLC method as described by Shihabi et al (1979). The tissue homogenate prepared as above was diluted ten times with distilled water. A 20 μl of aliquot was mixed with 20 μl of 200 mM phosphate buffer(pH 7.8) and 100 μl fluorescamine reagent(250 mg fluorescamine/l of acetone). After 15 minutes of incubation, a 20 μl of the reaction mixture was injected into a reverse phase column(C18, Microsorb-MV, 250 mm × 4.6 mm). The mobile phase was composed of acetonitrile and 15 mM phosphate buffer (28:72), pH 2.8, and the flow rate was 1 ml/min. Taurine peak was observed with a fluorescence detector equipped with 390 nm excitation and 450 nm emission filter. For measuring the taurine concentration in perfusate, the perfusion fluid was collected throughout the experiment with 5 ∼ 10 minutes interval, and processed as same as above.

RESULTS

Myocardial taurine content and release in monensin-treated hearts

The myocardial taurine content in normal rat heart was 28.8 ± 1.2 μmole/g wet wt, and was not altered significantly even after 60 minutes of normal K-H perfusion. In the hearts treated with monensin for 15min and perfused subsequently with normal K-H solution for 30min, the taurine levels were decreased, showing 74.7, 46.9 and 24.3% of the control level in 0.5, 2.0 and 5.0 μM monensin-treated hearts, respectively. In these hearts taurine was released into the coronary perfusate during the monensin treatment and the subsequent perfusion. Total amounts of taurine released throughout the whole perfusion period were equivalent to 21.9, 43.8 and 83.0% of the control myocardial content in the respective monensin-treated hearts (Table 1, Fig. 1).

The myocardial taurine content was increased to 32.6 ± 1.1 μmole/g wet wt in the hearts isolated from rats fed with high-taurine(5% w/w) diet for 10 weeks.

Table 1. Myocardial taurine content and release in monensin-treated isolated hearts of rats

	N	Taurine, μ mole/g.wet wt		Change, %	
		Content[a]	Release[b]	Content	Release[c]
Control[d]	6	28.8 ± 1.2	—	100	—
⁺Monensin					
0.5 μ M	5	21.5 ± 3.6*	6.3 ± 1.6*	74.7	21.9
2.0 μ M	6	13.5 ± 1.0*	12.6 ± 1.8*	46.9	43.8
5.0 μ M	5	7.0 ± 1.6*	23.9 ± 2.4*	24.3	83.0
Taurine-perfused[e]					
+ Monensin					
2.0 μ M	6	12.4 ± 0.7*	—	43.1	—
Taurine-fed[f]	6	32.6 ± 1.1	—	100	—
+ Monensin					
2.0 μ M	6	17.4 ± 2.0⁺	—	53.4	—

a. Taurine contents measured after 30 min of normal perfusion following monensin treatment for 15 min.

b. Amount of the taurine released during the whole perfusion period(45 min).

c. The released taurine expressed as percent of the control taurine content.

d. The myocardial taurine contents were measured immediately after sacrifice.

e. Taurine (10 mM) was perfused throughout the whole perfusion period.

f. High-taurin (5%, w/w) diet was fed for 10 weeks.

*: $p < 0.05$ vs. control, ⁺:$p < 0.05$ vs. Taurine-fed.

In this high-taurine heart, monensin(2 µM) treatment induced a reduction of the taurine content by about 50%. The hearts perfused with taurine(10 mM)-containing K-H solution throughout the whole perfusion(45 min) showed no significant change in the myocardial taurine content. In this taurine-perfused heart, the extent of monensin(2 µM)-induced reduction in the taurine content was not different from that in the control hearts treated with same amount of monensin (Table 1).

Effect of taurine on monensin-induced intracellular Na^+ accumulation

The treatment of hearts with monensin increased the intracellular Na^+ content in a dose-dependent fashion. Compared to Na^+ concentration of the normal fresh heart (6.5 ± 0.5 µmole/g wet wt), that of the hearts treated with 2.0 and 5.0 µM monensin were significicaltly increased, showing 1.8 and 3.1 times as high with the respective treatment of monensin. At 30 minutes after the transition to normal K-H perfusion following the monensin treatment, the Na^+ levels were somewhat decreased, but still higher than the normal level (Fig. 2). The high-taurine hearts isolated from taurine-fed rats showed an elevated Na^+ content (11.1 ± 0.9 µmole/g wet wt) after monensin
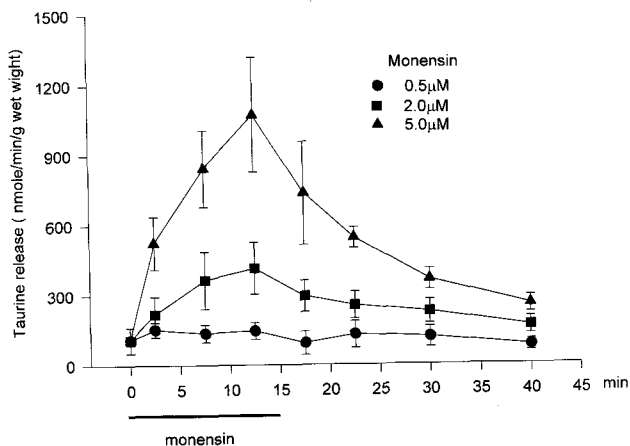

Fig. 1. Monensin-induced taurine release in isolated hearts of rats. The isolated hearts perfused by Langendorff method were treated with monensin for 15 min followed by normal perfusion for 30 min. The concentrations of taurine in perfusate collected during the indicated time interval were measured by HPLC method. Values represent mean ± S. E. of 6 experiments.
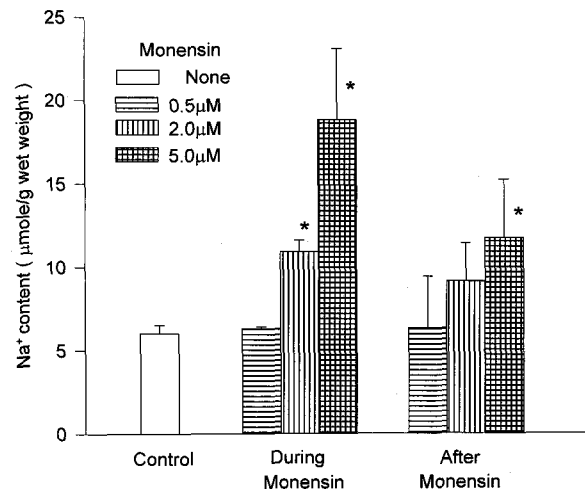

Fig. 2. Effects of monensin on sodium content of isolated rat hearts. Sodium content were measured at the end of monensin treatment for 15 min (During) and after 30 min of normal perfusion following cessation of the monensin treatment (After). Control content was measured immediately after sacrifice. Values represent the mean ± S. E. of 6 experiments. *: p < 0.05 vs control.
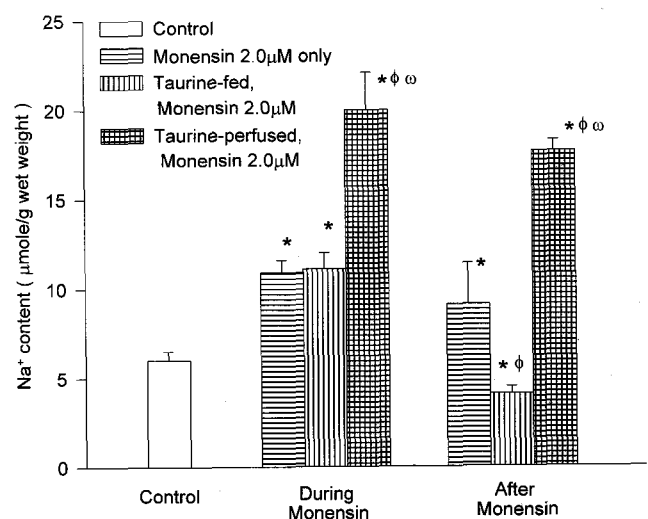

Fig. 3. Monensin-induced changes in sodium content of hearts isolated from taurine-fed rats and taurine-perfused hearts. Taurine-fed rats were fed with high-taurine(5 % w/w) diet for 10 weeks. Taurine-perfused hearts were isolated from normal rats and perfused with K-H solution containing 10 mM taurine throughout the experimental periods. Sodium content were measured after the monensin(2 µM) treatment for 15 min (During) and after 30 min of normal perfusion following cessation of the monensin treatment (After). Control content was measured immediately after sacrifice. Values represent the mean ± S. E. of 6 experiments. *: p < 0.05 vs control, φ: p < 0.05 vs monensin only, ω: p<0.05 vs taurine-fed.
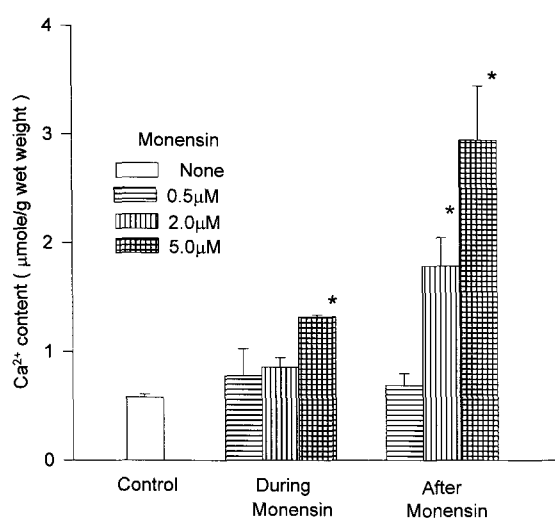
Fig. 4. Effects of monensin on calcium content of isolated rat hearts. Calcium content were measured after the monensin treatment for 15 min (During) and after 30 min of normal perfusion following cessation of the monensin treatment (After). Control content was measured immediately after sacrifice. Values represent the mean ± S. E. of 6 experiments. *: $p < 0.05$ vs control.
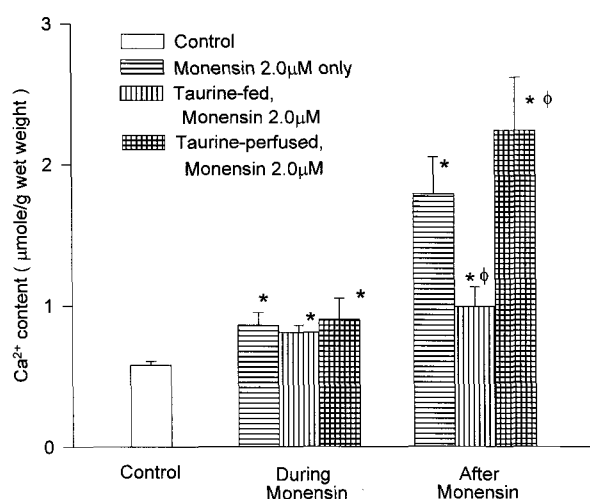
Fig. 5. Monensin-induced changes in calcium content of hearts isolated from taurine-fed rats and taurine-perfused hearts. Taurine-fed rats were fed with high-taurine(5% w/w) diet for 10 weeks. Taurine-perfused hearts were isolated from normal rats and perfused with perfusate containing 10 mM taurine throughout the experimental periods. Calcium content were measured immediately after the monensin(2 µM) treatment for 15 min (During) and after 30 min of normal perfusion following cessation of the monensin treatment (After). Control content was measured immediately after sacrifice. Values represent the mean ± S. E. of 6 experiments. *: $p < 0.05$ vs control, ϕ: $p < 0.05$ vs monensin only, ω: $p < 0.05$ vs taurine-fed.

(2.0 µM) treatment. The elevation was as same degree as in the monensin-treated control hearts. In the taurine-perfused hearts, the myocardial Na⁺ content was increased more significantly to 20.0 ± 2.1 µ mole/g wet wt, which was 3.3 fold higher than the Na⁺ content of normal fresh heart and 2 fold higher than that of the monensin-treated control hearts. At 30 minutes after transition to normal K-H perfusion following the monensin treatment, the Na⁺ content of high-taurine heart was reduced to 45% of that of the monensin-treated control hearts, while that of taurine-perfused heart showed still 2 fold higher than the level of the control heart (Fig. 3).

Effect of taurine on intracellular Ca⁺⁺ concentration in monensin-treated hearts

Intracellular Ca⁺⁺ levels were increased to 0.78, 0.86, and 1.32 µmole/g wet wt during the respective 0.5, 2.0 and 5.0 µM monensin treatment. The increased Ca⁺⁺ concentrations were equivalent to 1.3 ~2.3 fold the normal control level (0.58 ± 0.3 µ mole/g wet wt). After switching to normal perfusion following the monensin treatment, Ca⁺⁺ levels increased further to 1.79 and 2.95 µmole/g wet wt. in
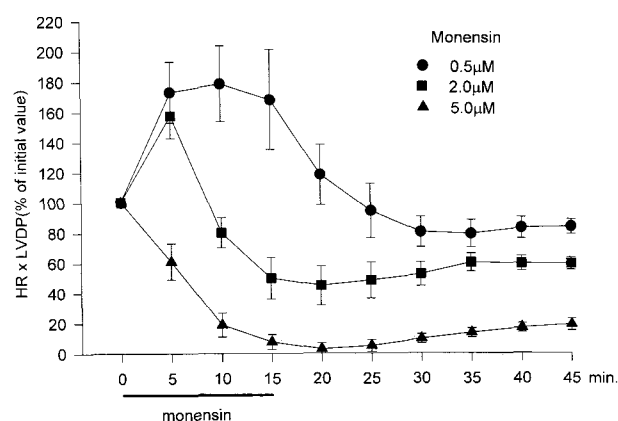

Fig. 6. Effects of monensin on cardiac function of isolated rat hearts. The isolated hearts perfused by Langendorff method were treated with monensin for 15 min followed by normal perfusion for 30 min. The product of HR and LVDP was calculated as cardiac function index. Values (mean ± S. E. of 6 experiments) were presented as percent of the initial cardiac function measured before the monensin treatment.
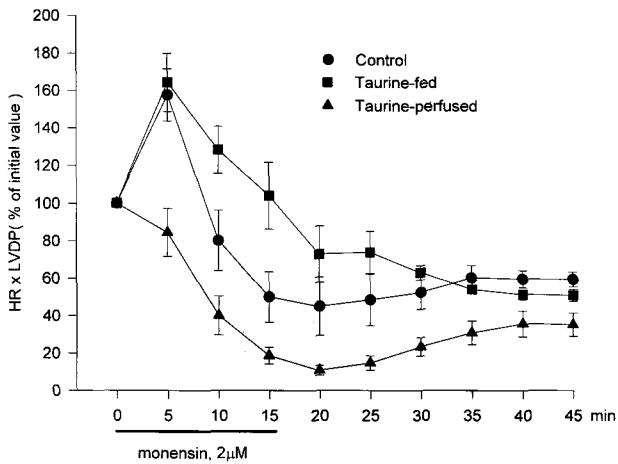
Fig. 7. Monensin-induced changes in cardiac function of hearts isolated from taurine-fed rats and taurine-perfused hearts. Taurine-fed rats were fed with high-taurine(5% w/w) diet for 10 weeks. Taurine-perfused hearts were isolated from normal rats and perfused with perfusate containing 10 mM taurine throughout the experimental periods. The isolated hearts perfused by Langendorff method were treated with monensin for 15 min followed by normal perfusion for 30 min. The product of HR and LVDP was calculated as cardiac function index. Values (mean ± S. E. of 6 experiments) were presented as percent of the initial cardiac function measured before the monensin treatment.
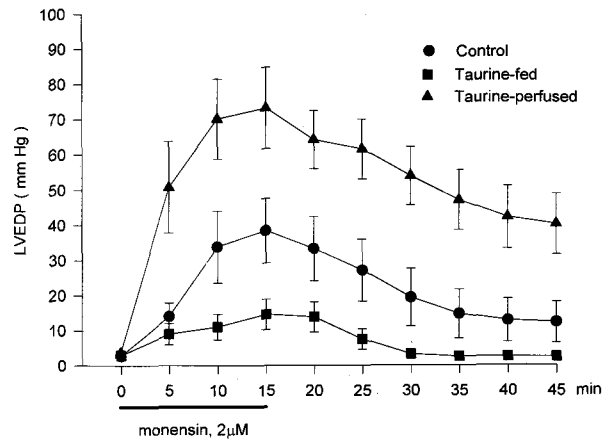

Fig. 9. Monensin-induced changes in left ventricular end-diastolic pressure (LVEDP) of hearts isolated from taurine-fed rats and taurine-perfused hearts. Taurine-fed rats were fed with high-taurine(5% w/w) diet for 10 weeks. Taurine-perfused hearts were isolated from normal rats and perfused with perfusate containing 10 mM taurine throughout the experimental periods. The initial LVEDP was adjusted to 5 mmHg. Values represent the mean ± S. E. of 6 experiments.
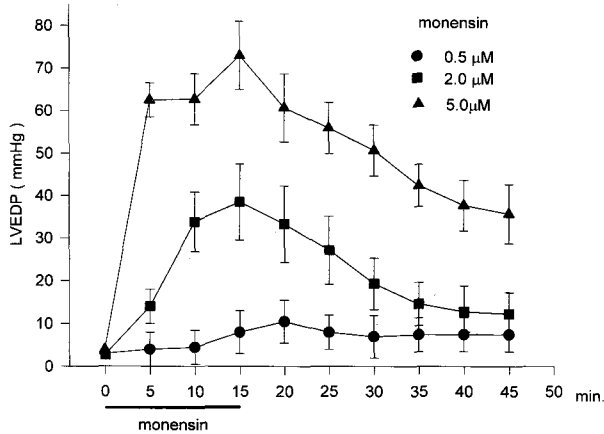

Fig. 8. Effects of monensin on left ventricular end-diastolic pressure (LVEDP) of isolated rat hearts. The isolated hearts perfused by Langendorff method were treated with monensin for 15 min followed by normal perfusion for 30 min. The initial LVEDP was adjusted to 5 mmHg. Values represent mean ± S. E. of 6 experiments.
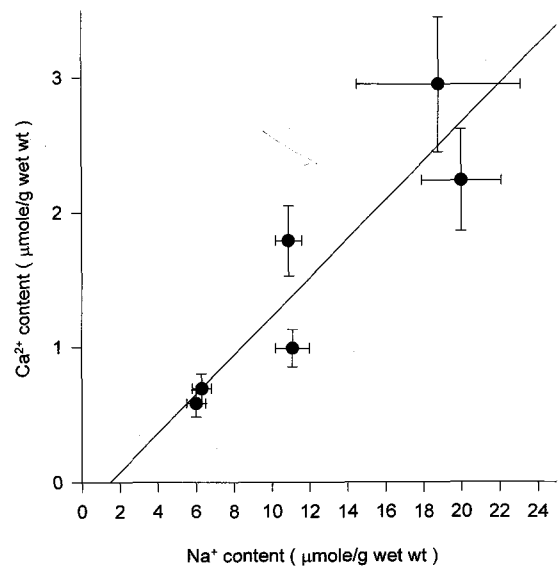

Fig. 10. Correlation between the sodium content after monensin treatment for 15 min and the calcium content after 30 min of normal perfusion following cessation of the monensin treatment. Each point represents the mean ± S. E. of 6 experiments. Correlation coefficient was γ^2 =0.84.
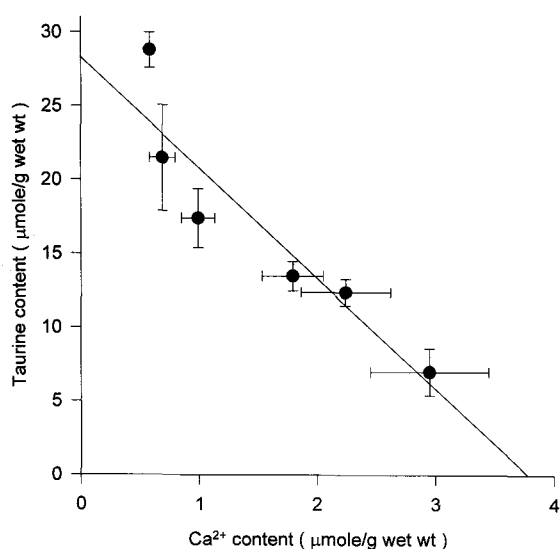
Fig. 11. Correlation between the sodium content after monensin treatment for 15 min and the taurine content after 30 min of normal perfusion following cessation of the monensin treatment. Each point represents the mean ± S. E. of 6 experiments. Correlation coefficient was γ^2=0.74.
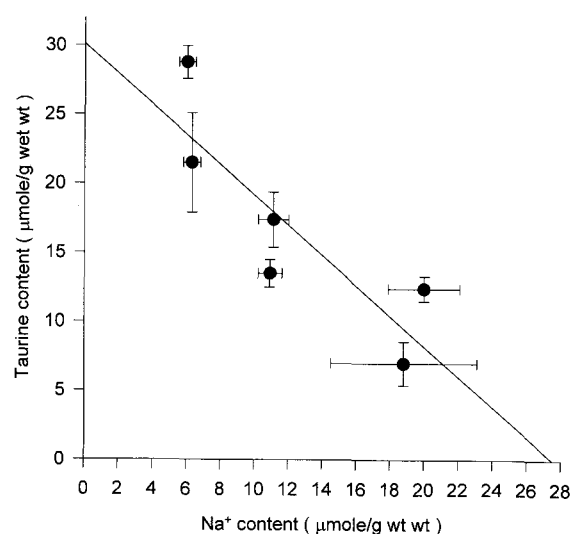

Fig. 12. Correlation between the calcium content and the taurine content after 30 min of normal perfusion following cessation of the monensin treatment. Each point represents the mean ± S. E. of 6 experiments. Correlation coefficient was γ^2=0.86.

2.0 and 5.0 μM monensin-treated hearts. These values indicated 3.1 and 5.1 times as high as than the normal control level (Fig. 4). Ca^{++} contents of the high-taurine hearts and the taurine-perfused hearts were increased during the monensin treatment, but there were no significant differences between them and the control hearts. However, after the transition to normal perfusion, a further increase in Ca^{++} level was recognized in the taurine-perfused hearts, while no change in the high-taurine hearts (Fig. 5).

Effect of taurine on the mechanical function in monensin-treated hearts

In the hearts treated with 0.5 and 2.0 μM monensin, the cardiac function (the pressure-rate index, LVDP × HR) was increased transiently during the initial periods of the treatments, and then reduced to 84% and 59% of the pretreatment value, respectively, at the end of the perfusion. The treatment with a higher concentration(5.0 μM) of monensin suppressed the cardiac function severely from the start of the treatment, showing 19% of the pretreatment value at end of the perfusion (Fig. 6). In the taurine-fed hearts, the monensin(2.0 μM) treatment induced a reduction of the cardiac function after an initial transient in-

crease similar to that in the monensin-treated control hearts. However, the development of the functional reduction was delayed as compared with that of the control hearts. On the other hand, in the taurine-perfused hearts, the cardiac function was depressed continuously from the beginning of the monensin treatment and throughout the following perfusion (Fig. 7). Left ventricular end-diastolic pressure (LVEDP) was elevated maximally to 8.0, 38.5 and 73.0 mmHg during the treatment of 0.5, 2.0, and 5.0 μM monensin, respectively. The LVEDPs were partially reduced after cessation of the monensin treatments, but were still higher than the pretreated value (Fig. 8). The elevation of LVEDP induced by monensin was singnificantly prevented in the taurine-fed hearts, while augmented in the taurine-perfused hearts (Fig. 9).

DICUSSION

A Na-ionophore, monensin caused a reduction of taurine content in the isolated hearts of rats. The loss of taurine content may be due to either an increased release out of the cell or an accelerated intracellular metabolism. It was proved in this study that the taurine release into the perfusate was increased by

monensin, and the total amount of released taurine was equivalent to that of taurine reduced in the myocardial tissue. Chapman & Tunstall (1987) reported in an earlier study that the taurine content of isolated guinea-pig heart was reduced with manipulations which caused an intracellular Na^+ overload, and that the taurine reduction was prevented by blocking the Na^+ influx. Christensen (1982) and Suleiman et al (1992), reported also that taurine release was potentiated by an elevation of intracellular Na^+, without any change in the membrane permeability. More recently, several electrophysiologic studies illustrated that the Na^+ current was altered by taurine in the isolated cardiomyocytes (Chapman et al, 1993; Earm et al, 1993). From these observations, it was suggested that the intracellular Na^+ played an important role in regulating the myocardial taurine content, probably via Na^+/ taurine co-transport system in the sarcolemmal membrane. Consistently, a negative correlation between the intracellular Na^+ and the taurine content demonstrated in this study (Fig. 11) also implicates that the monensin-induced taurine loss is involved with an increased intracellular Na^+ concentration via Na^+/ taurine co-transport mechanism.

If the Na^+/taurine co-transport mechanism is responsible for the loss of taurine associated with an increased intracellular Na^+, it would be expected that changes in the intracellular content of taurine will also affect the cellular Na^+ concentration vice versa. In the present study, the transmembrane concentration gradient for myocardial taurine was alterted either by increasing the intracellular taurine content or by elevating the extracellular taurine concentration. When the rats were fed with high-taurine (5%, w/w) diet for 10 weeks, the myocardial taurine content was increased by 13%. In these hearts, of which transmembrane taurine gradient was enhanced, the intracellular Na^+ concentration was decreased after switching to normal K-H perfusion following the monensin treatment. Conversely, when the hearts were perfused with the taurine(10 mM)-containing solution to decrease the transmembrane taurine gradient, the monensin-induced increase in Na^+ concentration was more pronounced than in the control hearts. These data demonstrating an increased or a decreased intracellular Na^+ concentration depending on the taurine gradient suggest a regulatory role of taurine in control of the myocardial Na^+ concentration.

tration.

The monensin treatment induced an intracellular Ca^{++} accumulation. The Ca^{++} accumulation was delayed and more marked during the normal perfusion following monensin treatment. This result suggests that monensin does not induce the Ca^{++} influx directly, but promotes the Ca^{++} accumulation secondary to an increase in the Na^+ content, presumably by a Na^+-Ca^{++} exchange mechanism. A positive correlationship between the intracellular Na^+ and the Ca^{++} concentration in the monensin-treated hearts (Fig. 10) supports the involvement of Na^+-Ca^{++} exchange in the monensin-induced Ca^{++} accumulation.

Monensin suppressed the systolic and diastolic functions of the isolated hearts. This cardiac dysfunction may be resulted from a direct suppressive effect of monensin. However, the possibility of the direct effect on the myocardial contractile function could be excluded, because the decrease in cardiac functional index and the elevation of LVEDP were not completely recovered upon switching to the normal perfusion after cessation of the monensin administration. It has been reported by many investigators that in the various cardiac pathologies associated with Na^+- overload such as the hypoxia-reoxygenation and the ischemia-reperfusion injury of hearts, a Na-promoted Ca^{++} accumulation via Na^+-Ca^{++} exchange mechanism contribute to the development of myocardial cellular damage and functional abnormality (Grinwald and Brosnahan, 1987 ; Murphy et al, 1988 ; Tani and Neely, 1990). It is, therefore, considered that the monensin-induced mechanical dysfunction is also attributed to the intracelluar Ca^{++} accumulation secondary to the increase in Na^+ concentration via Na^+- Ca^{++} exchange system.

It has been reported that taurine produces a myocardial protective effect in various pathologic conditions associated with an intracellular Na^+-overload, including ischemia and hypoxia (Franconi et al, 1985; Sawamura et al, 1986). Although the mechanism of this protective action has not been elucidated several recent reports have suggested that a role of taurine in regulating the intracellular Na^+ is respinsoble for the myocardial protective effect. As shown in the reports of Chapman et al (1993), and Suleiman et al (1992), isolated guinea-pig ventricular cell, of which intracellular taurine level had been augmented during the course of isolation, showed a higher degree of viability and a resistance to increasing the cellular Na^+

level upon treatment of digitalis glycoside or elimination of divalent cations, which induced an increased Na$^+$-influx. In these cells, when taurine was added to the medium to elevate the extracellular taurine concentration, intracelluar Na$^+$ level was markedly elevated with a lower cellular viabilty. These reports led to the hypothesis that an increased intracellular conentration of taurine (or an increased transmembrane gradient) may play an important role in regulating the intracellular Na$^+$ level to protect the myocardial cells from damage caused by Na$^+$ overloading. An alteration of intracellular Na$^+$ concentration attributed to the change in the taurine gradient are presumed to affect the Na$^+$-Ca^{++} exchange mechanism and eventually intracellular Ca^{++} level as well as contractile function of heart. In the present study, when monensin was treated in the high-taurine hearts, a significant reduction of Ca^{++} accumulation and a prevention of mechanical dysfuntion accompanied with a reduction of Na$^+$ accumulation were observed, while an enhanced Ca^{++} accumulation and an accelerated mechanical dysfunction accompanied with an increased Na$^+$ in the taurine-perfused hearts. It is thought that these results are consistent with the hypothesis concerning about the cardioprotective mechanism of taurine. The negative correlation between the myocardial taurine content and the intracellular Na$^+$ and Ca^{++} concentrations in the monensin-treated hearts (Fig. 11, 12) supports the presumed role of taurine also.

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