Chapter 4
Vascular Modulation of Rat Aorta by Taurine

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Abstract Taurine is found in high concentration in smooth muscle and heart muscle (approximately 10–20 mM). We found that taurine affects NE- and KCl-induced vasoconstriction. The mechanisms regulating these vasoconstrictions mostly involve Ca^{2+} channels and EDRF(NO). Taurine exerted either a vasodilation or vasoconstriction depending on cellular Ca^{2+} concentration. When vascular tone was excessively low, taurine promoted vasoconstriction allowing the maintenance of blood pressure. On the other hand, taurine dilates vessels to increase blood flow during ischemia or hypoxia. Thus, taurine modulates vascular wall tone to maintain blood flow. These results indicate that taurine plays an important homeostatic function on vascular smooth muscles as well as cardiac muscle.

Abbreviations $[Ca^{2+}]_o$, extracellular Ca^{2+} concentration; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; *CHF*, congestive heart failure; *EDHF*, endothelium-derived hyperpolarizing factors; *EDRF*, endothelium-derived releasing factor; *EGTA*, ethylene glycol-O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid; *HOCl*, hypochlorous acid; *LDL*, low density lipoprotein; *L-NAME*, Nω-nitro-L-arginine methyl ester; *NE*, norepinephrine; *SPC*, sphingosylphosphorylcholine; *TEA*, tetraethylammonium; *VLDL*, very low density lipoprotein

4.1 Introduction

The sulfur amino acid, taurine, plays an important role in the maintenance of cardiac function during hypoxia, ischemia and cardiac failure. Patients in congestive heart failure (CHF) with less than 50% ejection fraction were treated with 3 g taurine a day (Azuma et al. 1992). After 6 weeks, a significant improvement in systolic function was observed. This improvement is thought to be caused the modulation of cardiac ion channels. At low intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), taurine enhanced L-type Ca^{2+} channel current (I_{Ca}) but inhibited I_{Ca} at high $[Ca^{2+}]_i$ (Satoh

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and Sperelakis 1993, 1998; Satoh and Horie 1997). Taurine also inhibited the fast Na^+ current (TTX-sensitive), an effect which might contribute to the antiarrhythmic activity of taurine (Satoh and Sperelakis 1992; Satoh 1994d). Thus, taurine can regulate $[Ca^{2+}]_i$ through the modulation of ionic channels (such as Ca^{2+}, K^+ and Na^+ channels) and secondly through the modulation of Na/Ca exchange (Satoh et al. 1992; Sperelakis and Satoh et al. 1993; Satoh and Sperelakis 1998). Therefore, taurine acts to maintain homeostasis in the presence of low $[Ca^{2+}]_i$ as well as Ca^{2+} overload.

Similarly, taurine could also modulate ionic channels of vascular smooth muscle cells. Not only does the aorta maintain high levels of taurine (126 μg/g wet) (Song et al. 1998; Satoh et al. 2002) but it dilates rabbit ear arteries in the presence of high-K^+ medium (Franconi et al. 1982) and has a similar effect on rat aorta induced to contract in the presence of either high K^+ or NE (Risori and Verdetti 1991). Thus, taurine mediated dilation of vascular smooth muscle involves either the inhibition of Ca^{2+} channels or some other undetermined mechanisms.

The basis underlying the treatment of heart failure consists of (1) redution in the workload of the heart, (2) protection of the cardiomyocyte, and (3) restriction of plasma volume and sodium. In order to reduce both pre-load and afterload, the dilation of arterioles and veins is strongly recommended when filling pressure is elevated. Therefore, taurine could serve as a therapeutic agent to not only modulate ionic channel function of the heart but also to regulate vascular tone. The effects of taurine on the vasculature are not mediated by α- and β-adrenoceptors and muscarinic receptors. Furthermore, unlike the heart (Satoh 1994a, b, c, 1995a, b, c 1966, 1998a, b, c, 1999) taurine's actions are independent of the endothelium and extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) (Risori and Verdetti 1991). Because few studies have examined the effects of taurine on vascular smooth muscle the present study was initiated to elucidate the pharmacological effects of taurine on vascular smooth muscle.

4.2 Materials and Methods

All experiments were carried out according to the guidelines laid down by the Nara Medical University Animal Welfare Committee, and also under the terms of the Declaration of Helsinki.

Wistar male rats (5–14 weeks old) were anesthetized with ether and euthanized by exsanguinations using methods similar to those described previously (Nishida and Satoh 2003, 2004). The thoracic aorta was quickly removed and the isolated aorta was cut into 3-mm rings in length. The rings were suspended between two triangular-shaped stainless steel stirrups in a jacketed organ chamber filled with 20 ml modified Krebs-Henseleit solution. The modified solution was, in mM: 118 NaCl, 4.6 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose, 27.2 $NaHCO_3$, 0.03 ethylene glycol-O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), and 1.8 $CaCl_2$. The chamber solution was kept at 36.5°C and oxygenated with 95% O_2 and 5% CO_2. The lower stirrup was anchored and the upper stirrup was attached to a

force-displacement transducer (TB-652T, Nihon Kohden, Tokyo, Japan) to record the isometric force. All rings were stretched to generate a resting tension of 1.2 g.

After a 40 min-rest period, 1 μM norepinephrine (NE), different concentrations of KCl (30 to 60 mM), or 30 nM sphingosylphosphorylcholine (SPC, a Rho-kinase activator) was added to the bath to induce vasoconstriction. After a steady state contractile response was achieved, taurine was added to the bath. The responses were measured 6–10 min later. The relaxation response was analyzed as a percent decrease in maximal contraction induced by NE. Pretreatment with L-NAME (a NO synthase inhibitor), nicardipine and TEA (an inhibitor of Ca^{2+}-activated K^+ channel) were carried out to evaluate the role of key modulators of contractile function.

4.3 Results

4.3.1 Effects on NE-Induced Vasoconstriction

Taurine dilated aortic strips which were induced to contract with NE (1 μM). However, the effect of taurine depended both $[Ca^{2+}]_o$ and taurine concentration. In normal Krebs solution (1.8 mM Ca^{2+}), taurine had no effect on NE-induced vasoconstriction, but at high $[Ca^{2+}]_o$ (3.6 and 5.4 mM), it mediated a vasodilatory response. At the high $[Ca^{2+}]_o$ of 5.4 mM, the vasodilating effect of taurine was weaker than that seen at 3.6 mM $[Ca^{2+}]_o$ (Fig. 4.1)

4.3.2 Effects in the Presence of Some Inhibitors

To examine the involvement of the voltage-dependent Ca^{2+} channels, pretreatment with nicardipine was carried out. In the presence of the inhibitor, the effect of taurine on the NE-induced vasoconstriction was examined at 3.6 mM $[Ca^{2+}]_o$.

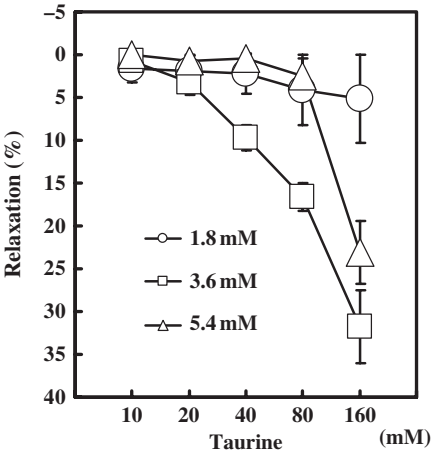

Fig. 4.1 Effect of taurine on NE-induced vasoconstriction at different $[Ca^{2+}]_o$ (1.8 to 5.4 mM)

Table 4.1 Modulation by inhibitors of the vasodilation induced by taurine

		Taurine (mM)				
	n	10	20	40	80	160
Control	6	0.7±0.7	3.2±1.4	9.6±1.5	16.6±1.6	31.7±4.7
Nicardipine 0.1 μM	6	0±0	0±0	2.5±1.7[a]	4.7±2.2[a]	10.8±2.7[b]
L-NAME 100 μM	6	1.4±1.2	4.3±1.9	6.3±2.3	10.5±2.7[a]	30.2±2.1
TEA 10 μM	6	1.1±0.8	7.1±3.3	10.5±2.7	20.8±2.3	32.7±3.8

Values shown represent % taurine-mediated vasorelaxation of aortic strips exposed to NE 3.6 mM Ca^{2+} in the presence of the indicated inhibitor.
[a]: $P<0.05$, [b]: $P<0.01$.

Taurine-mediated vasodilation was attenuated by nicardipine (0.1 μM); it fell from 31.7 ± 4.6 to 10.8 ± 2.7% ($n = 6$, $p<0.01$) at 160 mM taurine.

L-NAME (100 μM) pretreatment, which affects the endothelium, inhibited vasorelaxation induced by 80 mM taurine. TEA (10 μM), which examines the effect of Ca^{2+}-activated K^+ channels, did not affect taurine-induced relaxation (Table 4.1).

4.3.3 Effects on KCl-Induced Vasoconstriction

The effects of taurine on KCl-induced vasoconstriction were also investigated. In normal Krebs solution, taurine (10 to 160 mM) did not dilate KCl (30 mM)-induced vasoconstriction, but rather accentuated the degree of vasoconstriction (Fig. 4.2A). The taurine-mediated vasoconstriction was blocked by nicardipine (0.1 μM), but not by phentolamine (10 μM). On the other hand, taurine reduced the degree of vasoconstriction triggered by higher KCl (45 or 60 mM). Taurine mediated the most vasorelaxation at 45 mM, although it was not significant (Fig. 4.3A).

By contrast, at a medium concentration of 3.6 mM $[Ca^{2+}]_o$, taurine (10–80 mM) dilated KCl (30 mM)-induced vasoconstriction (Fig. 4.3B). However, even in the presence of 3.6 mM $[Ca^{2+}]_o$, 160 mM taurine triggered a vasoconstricting response. At a high KCl concentration (45 and 60 mM), taurine mediated vasodilation in contracted aortic slices.

When $[Ca^{2+}]_o$ was elevated to 5.4 mM, taurine similarly exhibited a biphasic response (Fig. 4.3C). Taurine (10 to 160 mM) potentiated 30 mM KCl-induced

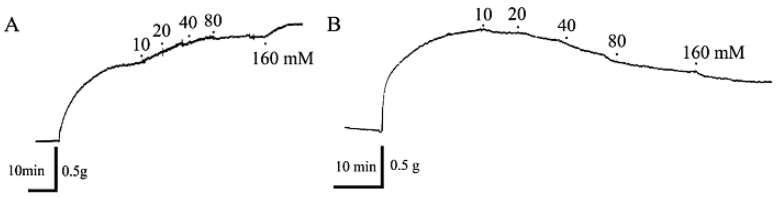

Fig. 4.2 Effect of taurine on KCl-induced vasoconstriction. (**A**) Vasoconstriction induced by taurine of aorta undergoing 30 mM KCl-induced vasoconstriction at 1.8 mM Ca^{2+}. (**B**) Vasorelaxation induced by taurine of aorta undergoing 60 mM KCl-induced vasoconstriction at 3.6 mM Ca^{2+}. In both experiments, KCl was added at time 0

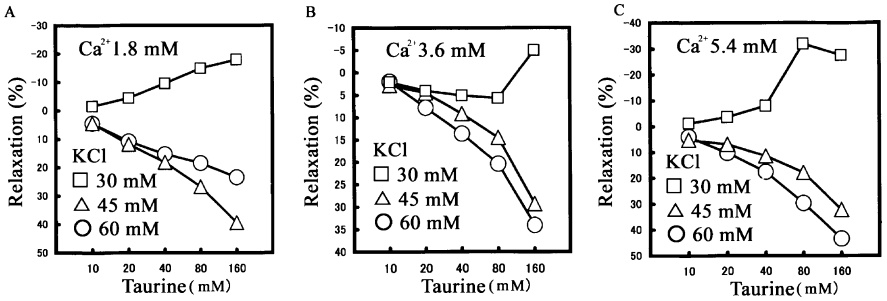

Fig. 4.3 Taurine-induced vasoresponses to KCl (30–60 mM)-induced vasoconstriction at different Ca^{2+} concentrations (1.8–5.4 mM)

vasoconstriction (Fig. 4.2B), but dilated vasoconstriction mediated by a high concentration of KCl (45 and 60 mM). Taurine 160 mM exerted a marked vasodilation (43.5 ± 8.6% ($n = 6$, $P < 0.01$) of aortic strips made to contract with 60 mM KCl at 5.4 mM $[Ca^{2+}]_o$.

4.3.4 Effects on Ca2+-independent constriction

For a Ca2+-independent vasoconstriction, the effect of taurine on SPC (a Rho-kinase activator)-induced vasoconstriction was examined. SPC alone caused a week vasoconstriction. But taurine did not dilate SPC-induced vasoconstriction but rather constricted it further (Fig. 4.4).

In addition, high concentrations of taurine (160 mM) constricted resting aorta (lacking pretreatment with drugs). This vasoconstriction was attenuated by nicardipine (0.1 μM), but not by phentolamine (10 μM) (Fig. 4.5)

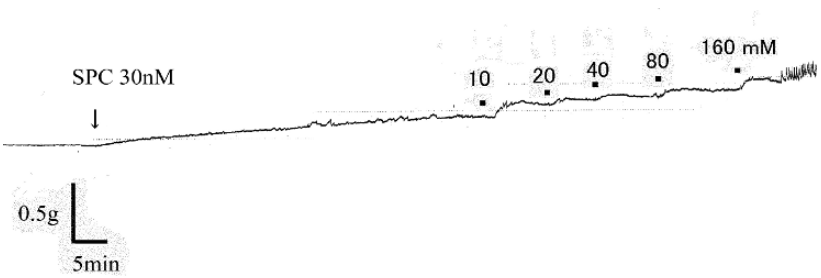

Fig. 4.4 Effect of taurine on SPC-induced vasoconstriction

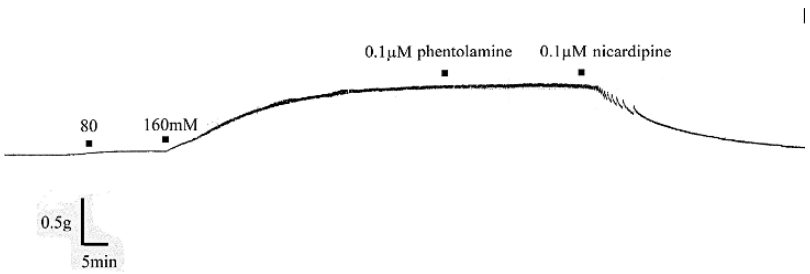

Fig. 4.5 Taurine-induced vasoconstriction of resting aorta

4.4 Discussion

4.4.1 Effects on NE-Induced Vasoconstriction

Taurine reduced the degree of NE-induced vasoconstriction in a concentration-dependent manner. Aorta contains about ten times the taurine content (10–20 mM) of cardiac muscle (Song et al. 1998; Satoh et al. 2002). Thus, the higher concentrations might be required to produce an effective response. The vasorelaxation was Ca^{2+}-dependent. At normal $[Ca^{2+}]_o$ (1.8 mM), taurine elicited a slight vasoconstriction, but at higher $[Ca^{2+}]_o$ it caused a marked vasorelaxation.

4.4.2 Ca^{2+}-Dependency of Taurine's Actions

Taurine's actions were modified by $[Ca^{2+}]_o$. In normal Krebs (1.8 mM Ca^{2+}), taurine did not dilate NE-induced vasoconstriction, but at a higher $[Ca^{2+}]$ (3.6 mM) it produces vasodilation, but the vasodilation at 5.4 mM Ca^{2+} was weaker than that at 3.6 mM Ca^{2+}. In KCl-induced vasoconstricted aorta, taurine mediated a Ca^{2+}-dependent vasorelaxation. In aorta constricted at 60 mM KCl taurine's vasorelaxation was clearly dependent on $[Ca^{2+}]_o$. With an increase in $[Ca^{2+}]_o$ from 1.8 to 5.4 mM, taurine-induced vasorelaxation became more obvious, but in 30 mM KCl-induced vasoconstricted aorta taurine-dependent relaxation was not observed at all $[Ca^{2+}]_o$.

Thus, taurine-induced vasorelaxation is only observed at high $[Ca^{2+}]_o$ and high KCl (45–60 mM). When vasoconstriction was induced by lower KCl (30 mM), taurine mediated a further constriction at 1.8 to 5.4 mM $[Ca^{2+}]_o$. Taurine also constricted the resting aorta. This constriction was reduced by nicardipine but not by phentolamine. Therefore, these results indicate that a major action of taurine is exerted by the modulation of Ca^{2+} channels. The present experiments show that taurine is strongly dependent on $[Ca^{2+}]_i$ and $[Ca^{2+}]_o$, different from the report of Risori and Verdetti (1991). However, the present results are consistent with the response of cardiac myocytes (Satoh and Horie 1997; Satoh 2001, 2003). Taurine activates the

Ca^{2+} channel at low $[Ca^{2+}]_i$, but inhibits it at high $[Ca^{2+}]_i$. The Ca^{2+} dependence should contribute to the regulation of vascular wall tone.

4.4.3 Taurine-Induced Vasorelaxation and Vasoconstriction

Taurine dilates KCl-induced vasoconstriction. The vasoconstriction induced by taurine was inhibited by nicardipine. Since TEA did not affect taurine-induced vasorelaxation, the taurine effect does not appear to involve Ca^{2+}-activated K^+ channels. L-NAME partially modified taurine-induced vasodilation. Therefore, these results indicate that the vascular mechanisms are mainly caused by the inhibition of Ca^{2+} channels of smooth muscle cells, although they also involve endothelium-dependent relaxation, another effect that is inconsistent with the results of Risori and Verdetti (1991). The data suggest that taurine might affect endothelium-derived releasing factor (EDRF) and endothelium-derived hyperpolarizing factors (EDHF).

SPC activates Rho-kinase and constricts vascular muscle without an increase in $[Ca^{2+}]_i$, an effect likely involving the enhancement of Ca^{2+} sensitization (Shirao et al. 2002; Hirano 2007). In the present experiments, taurine did not dilate SPC-induced vasoconstriction, but rather caused further vasoconstriction in a concentration-dependent manner. Taurine also constricted resting aorta and this constriction was blocked by Ca^{2+} antagonists (Fig. 4.5). Thus, consistent with results in cardiac muscle, taurine-induced vasoconstriction in smooth muscle requires the activation of Ca^{2+} channels (Satoh 2001, 2003; Satoh and Sperelakis 1993; Satoh and Horie 1997). Taurine constricts vascular smooth muscles when $[Ca^{2+}]_i$ is not elevated, as in SPC-treated aorta or untreated resting aorta (under the low $[Ca^{2+}]_i$) (Fig. 4.6). On the other hand, taurine administration in the presence of high $[Ca^{2+}]_i$ dilates smooth muscle.

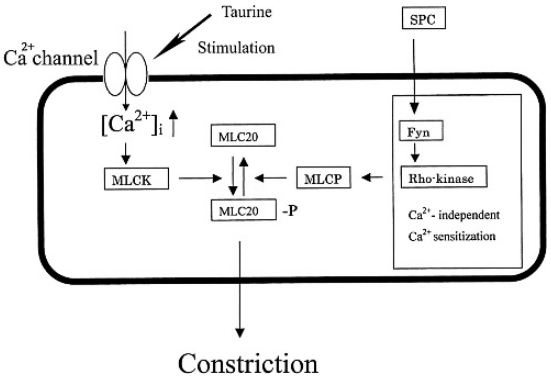

Fig. 4.6 Sheme of taurine's actions on vascular smooth muscle. SPC; sphingosylphosphorylcholine, Fyn; a member of SRC family tyrosine kinase. MLC20: 20-kDa regulatory myosin light chain. MLCK: Ca^{2+}-calmodulin-dependent myosin light chain kinase. MLCP: myosin light chain phosphatase

4.4.4 Vascular Regulation by Taurine

Induction of aortic regurgitation in rabbits leads to the development of CHF and a mortality rate of 53% after 8 weeks. However, daily administration of taurine (p.o. 100 mg/kg) reduced the mortality rate after 8 weeks to 10%. Cardiac function was maintained in the taurine-treated rabbits, but was depressed in the untreated rabbits (Takihara et al. 1986). Thus, taurine dilates vessels to maintain blood flow, improving cardiac function under these conditions.

Taurine treatment also reduces serum low density lipoprotein (LDL) and very low density lipoprotein (VLDL) by 44% in mice fed a high fat diet. Hypochlorous acid (HOCl) produced by myeloperoxidase in neutrophils and macrophages oxidizes LDL (Jerlich et al. 2000). By scavenging HOCl, taurine exerts a cytoprotective action (Kearns and Dawson 2000). Thus, taurine prevents atherosclerosis by inhibiting the oxidization of LDL. Simultaneously taurine prevents endothelial dysfunction. Taurine facilitates ACh-induced relaxation of the aorta in cholesterol-fed and streptozotocin-induced diabetic mice (Kamata et al. 1996). Therefore, taurine reduces vascular wall tone, and may act as an anti-atherosclerotic agent (Murakami et al. 1999).

4.5 Conclusion

Taurine has the potential to modulate vascular wall tone to maintain blood flow. If vascular tone is excessively low, as occurs in hypotension and bacterial shock (low $[Ca^{2+}]_i$ level), taurine can constrict vessels to maintain blood pressure. On the other hand, taurine dilates vessels to increase blood flow during ischemia or hypoxia (calcium overload). Therefore, taurine possesses homeostatic actions on vascular smooth muscles as well as cardiac muscle.

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