Amino Acids (2000) 19: 615–623

Amino
Acids

Effects of chronic taurine treatment on reactivity of the rat aorta

W. Abebe and **M. S. Mozaffari**

Department of Oral Biology and Maxillofacial Pathology School of Dentistry, Medical College of Georgia, Augusta, Georgia, U.S.A.

Accepted January 9, 2000

Summary. The effects of chronic taurine treatment on the reactivity of the aorta form male Wistar-Kyoto rats were investigated. Contractile responses to norepinephrine (NE) and potassium chloride (KCl) were attenuated in aortic rings from taurine-treated rats as compared to controls both in the absence and presence of endothelium. However, the degree of attenuation was greater in endothelium-intact tissues contracted with NE. Acetylcholine (Ach)-induced relaxation responses were augmented in endothelium-intact vessels from rats supplemented with taurine compared to the responses observed in control preparations. Relaxation responses of the aortae from control and taurine-treated rats to sodium nitroprusside (SNP) were not different from each other. Our results suggest that taurine treatment attenuates vascular contractility nonspecifically and this effect is partly mediated via the endothelium.

Key words: Amino acids – Taurine – Rat aorta – Vascular reactivity – Endothelium

Introduction

Taurine (2-aminoethanesulphonic acid) is a ubiquitous amino acid found in high concentration in muscle, brain, heart and blood. It plays an important role in neuromodulation, osmoregulation, thermoregulation and regulation of calcium-dependent processes (Huxtable, 1992; Schaffer et al., 1980; Schaffer and Azuma, 1992). With regards to the cardiovascular system, the administration of taurine has been shown to cause a reduction of blood pressure in hypertensive patients (Fujita et al., 1987) as well as rats (Fujita and Sato, 1984; Paakkari et al., 1983). However, the mechanism(s) for this effect of taurine is still not fully understood. In an effort to address this issue, several studies have focussed on determination of effects of taurine on the reactivity of blood vessels from normotensive and hypertensive animals. In this regard, Li et al. (1996) demonstrated that the contractile responses of mesenteric arteries from taurine-treated stroke-prone spontaneously hypertensive rats (SHRSP)

but not Wistar Kyoto (WKY) rats to norepinephrine (NE) were decreased. In contrast, the responses of the arteries from either the hypertensive or normotensive animals to angiotensin II or potassium chloride (KCl) were not altered by taurine treatment. Further, *in vitro* exposure to taurine has been shown to exert a selective inhibition of NE-induced contraction of the tissues from SHRSP (Li et al., 1996). On the other hand, reports by Ristori and Verdetti (1991) indicate that taurine, with *in vitro* application, causes a reduction of both NE and KCl-induced contraction of aortae from normotensive rats. In addition, Franconi et al. (1982) found that taurine could also inhibit the contractile responses of the rabbit ear artery to KCl but not NE. However, the results of these studies, aside from being inconsistent, do not provide adequate information on the mechanisms of action of taurine on blood vessels, including the potential role of the endothelium. Some of the factors believed to contribute to the disparate observations include variations in species of animals (SHRSP, WKY or Wistar rats vs. rabbits), vascular preparations (mesenteric arteries or aortae vs. ear arteries) used and methods of taurine supplementation.

Functionally, vascular smooth muscle responds to vasoactive agents with either contraction or relaxation. Contraction can be elicited by agents activating specific receptors on the plasma membrane, leading to increased intracellular calcium levels. Depolarization-mediated activation of plasma membrane calcium channels also elicits a similar effect. The endothelium exerts an inhibitory influence on contraction of the vascular smooth muscle. This effect has been attributed to the spontaneous release of endothelium-derived relaxing factors (EDRF), which result in sustained, low elevation of cGMP content in vascular smooth muscle (MacLeod et al., 1987). In addition, substances that stimulate EDRF release from the endothelium cause relaxation of blood vessels. Therefore, factors that interact with or influence the function of the endothelium can modulate the contractile responsiveness of blood vessels. Vascular smooth muscle relaxation can also be induced independent of the endothelium by vasoactive substances that directly act on the smooth muscle.

The objective of the present investigation was to determine the effects of chronic taurine treatment on the functions of the smooth muscle and endothelium of the aorta from the rat. These functions were assessed by measuring smooth muscle contractility as well as endothelium-dependent and independent relaxations of the aortae. Contractility was induced by receptor and potential-dependent mechanisms using NE and KCl, respectively. Endothelium-dependent and independent relaxations were produced by acetylcholine (Ach) and sodium nitroprusside (SNP), respectively.

Materials and methods

Animals and tissue preparation

Six-week-old male WKY rats (97–118 g, Harlan Laboratories, Indianapolis, IN) were given either tap water (control) or 1% taurine solution (taurine-treated) for 7–8 weeks.

These animals had free access to food and drinking fluid. Thereafter, the rats were sacrificed by decapitation and the thoracic aorta was quickly removed from each animal and placed in Krebs buffer. The composition of the buffer was the same as reported previously (Abebe et al., 1993). The aorta was carefully cleaned of adherent fat and connective tissue. Intraluminal blood was removed by gentle lavage in the Krebs solution. During this time, care was taken to ensure that the endothelial layer was not damaged. The vessel was then cut into rings of ~3 mm length. The endothelium was removed from some of the rings by gently abrading the lumen with forceps while the rest of the rings were left intact (Abebe et al., 1993).

Isolated tissue bath experiments

The rings from both control and taurine-treated rats were suspended in isolated tissue baths containing oxygenated (95% O_2/5% CO_2) Krebs solution at 37°C for isometric tension measurements as previously described (Abebe et al., 1993). The rings were placed under a resting tension of 2 g, a tension found to be optimal for this tissue. The rings were then equilibrated for 90 min before starting the experiments. During this period, the bathing solution was replaced every 15–20 min. All experiments were performed in the presence of the beta-adrenergic blocker timolol (1 μM), the neuronal uptake blocker desipramine (0.1 μM) and the extraneuronal uptake blocker hydrocortisone (2 μM). This was necessary in order to minimize variations in responsiveness of tissues due to effects of beta-adrenoceptor stimulation, and neuronal and extraneuronal uptake. After equilibration, cumulative concentration-response curves to NE and KCl were obtained for each preparation. After completion of the KCl concentration-response curves, tissues were contracted with a submaximal concentration of NE (10^{-7} M), and cumulative concentration-response curves to Ach and then SNP were obtained. Aortic rings were washed with Krebs solution and equilibrated for at least 60 min between each concentration-response curve. This procedure did not affect the responsiveness of tissues to the drugs. After completing the experiments, rings were blotted dry and weighed. Contractile responses of each preparation to NE and KCl were calculated as an increase in tension (g) in response to each concentration of the drug per mg tissue weight. Relaxation responses to Ach and SNP were calculated as the percentage of decreased tension in response to each concentration of these drugs.

Drugs and chemicals

Norepinephrine, Ach, and SNP were purchased from Sigma Chemical Company (St. Louis, MO). Drug solutions were prepared fresh daily in distilled water. Stock solution of NE was prepared in the presence of 2 mg/ml ascorbic acid to prevent oxidation of NE.

Statistical analysis

Data are expressed as mean ± SEM. Statistical differences between groups were assessed as described previously (Abebe et al., 1993) using Student t-test, and a 95% confidence level ($p < 0.05$) was accepted significant.

Results

Contractile responses to NE and KCl

The cumulative addition of NE and KCl contracted all the aortic rings from control and taurine-treated rats in a concentration-dependent manner (Figs. 1

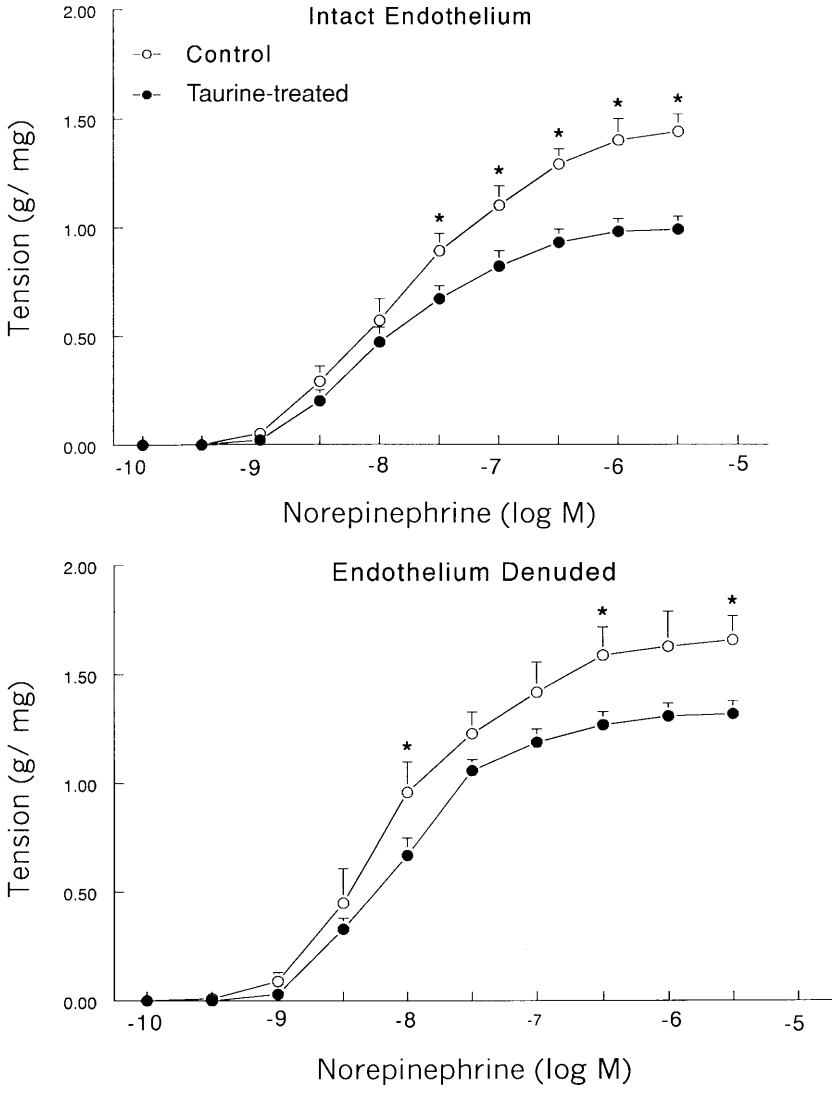

Fig. 1. Contractile responses of aortae from control and taurine-treated rats to norepinephrine (NE) in the presence (top panel) and absence (bottom panel) of endothelium. Each point represents the mean ± SEM of data from 8–10 aortic rings obtained from 4–6 animals. *Significantly different from taurine-treated groups ($p < 0.05$)

and 2). Removal of the endothelium resulted in enhanced contractile responses of tissues from both groups of animals to NE and KCl (Figs. 1 and 2). In both endothelium-intact and denuded preparations, contractile responses to NE and KCl were attenuated by taurine supplementation (Figs. 1 and 2). However, the magnitude of attenuation was greater in endothelium-intact aortic rings contracted with NE (i.e., 33.2 ± 4.2% inhibition of maximum contraction in intact vs. 20.2 ± 3.2% inhibition of maximum contraction in denuded tissues; $p < 0.05$).

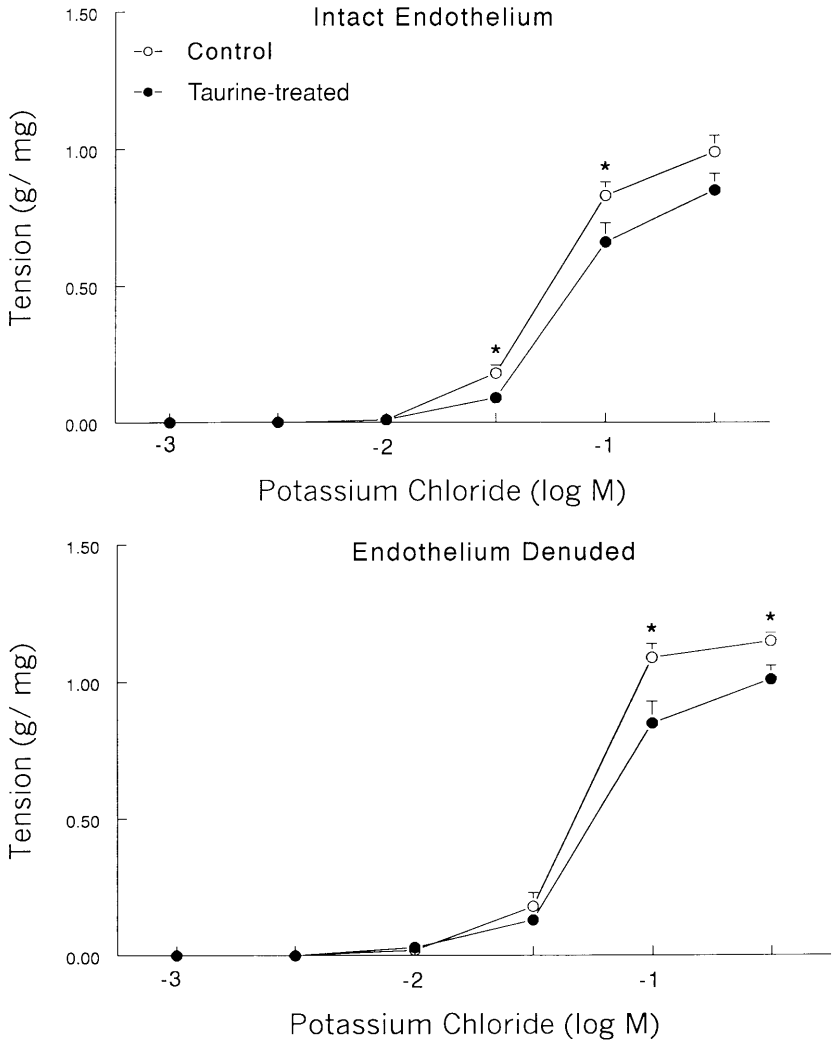

Fig. 2. Contractile responses of aortae from control and taurine-treated rats to potassium chloride (KCl) in the presence (top panel) and absence (bottom panel) of endothelium. Each point represents the mean ± SEM of data from 8–10 aortic rings obtained from 4–6 animals. *Significantly different from taurine-treated groups ($p < 0.05$)

Relaxation responses to Ach and SNP

Acetylcholine produced concentration-dependent relaxation of NE-contracted aortic rings from both control and taurine-treated rats in the presence of endothelium (Fig. 3). This effect was totally abolished in both types of vessels by removal of the endothelium (data not shown). The Ach-induced relaxation recorded in the intact tissues was augmented by treatment of the animals with taurine (Fig. 3). On the other hand, SNP relaxed all NE-contracted rings, with and without endothelium, in a concentration-dependent fashion (Fig. 4). No difference was observed between the intact

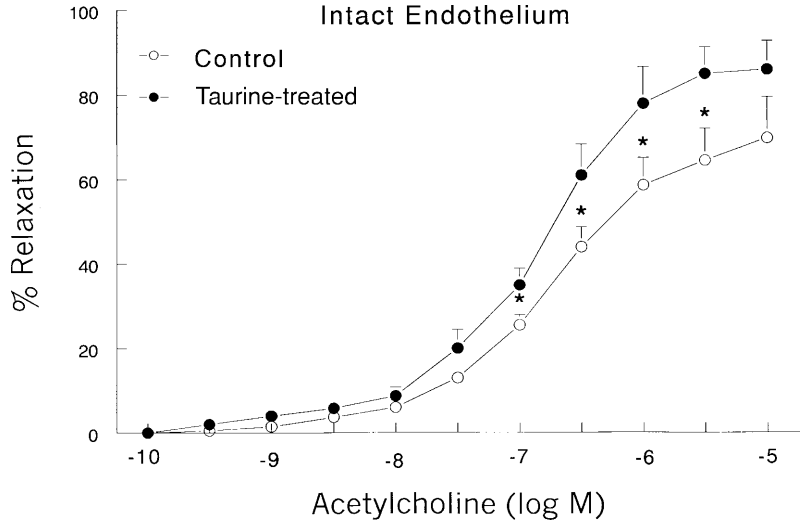

Fig. 3. Relaxation responses of aortae from control and taurine-treated rats to acetylcholine (Ach) in the presence of endothelium. Each point represents the mean ± SEM of data from 8–10 aortic rings obtained from 4–6 animals. *Significantly different from taurine-treated groups ($p < 0.05$)

and denuded vessels in response to SNP. The relaxation caused by SNP was also similar in aortae from both control and taurine-treated rats (Fig. 4).

Discussion

The focus of the present investigation was to examine the effects of chronic taurine treatment on the reactivity of the rat aorta by measuring functional responses of the smooth muscle and endothelium. In order to assess effects on smooth muscle function, we compared the contractile responses of tissues in which the influence of the endothelium was removed by denudation, and the endothelium-independent relaxation induced by SNP. Our results demonstrate that taurine supplementation attenuated contraction of the endothelium-denuded aortae in response to both NE and KCl. This indicates that the contractile ability of the aortic smooth muscle cells is depressed nonspecifically by taurine treatment. Assessment of relaxation by the endothelium-independent relaxing drug SNP showed that taurine did not have an effect on this response. This provides evidence that the relaxation properties of the vascular smooth muscle cells are not altered by taurine supplementation.

To evaluate the effects of taurine on the function of the endothelium, we tested contractile responsiveness and endothelium-mediated relaxation of aortic rings in which the endothelium was left intact. The results reveal that taurine treatment reduced contraction of the intact tissues in response to both NE and KCl. However, the magnitude of reduction caused by taurine was greater in the NE-contracted rings. In these tissues, since endothelium

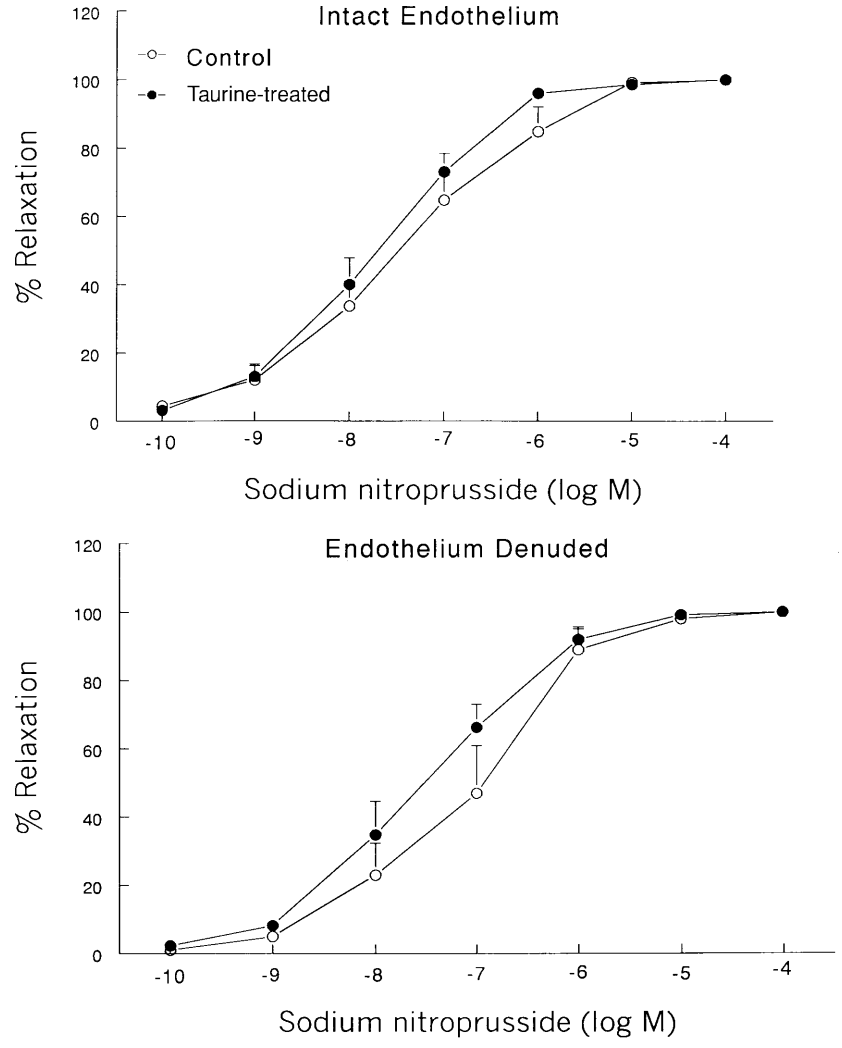

Fig. 4. Relaxation responses of aortae from control and taurine-treated rats to sodium nitroprusside (SNP) in the presence (top panel) and absence (bottom panel) of endothelium. Each point represents the mean ± SEM of data from 8–10 aortic rings obtained from 4–6 animals. Note that there are no significant differences between control and taurine-treated groups

removal induced greater enhancement of contraction compared to controls (i.e., 33% enhancement in taurine-treated vs. 15% enhancement in control tissues of maximum contraction), part of the hyporesponsive effect of taurine was endothelium-dependent. This observation suggests that taurine exerts a stimulatory influence on the endothelium, which results in inhibition of contraction of blood vessels in response to vasoactive agonists such as NE. Although taurine could attenuate the responses of the aortae to KCl, no endothelium-mediated effect was manifested by the amino acid in these vessels. The reason for this differential effect of taurine is not known. A more direct confirmation for the functional involvement of endothelium was

obtained by assessing the relaxation responses of the aortae to the endothelium-dependent relaxing agent Ach. The results demonstrate that tissues from rats treated with taurine responded with greater relaxation to Ach compared to control preparations. This supports our findings with NE in endothelium-intact tissues treated with taurine (see above). This observation substantiates the hypothesis that taurine enhances endothelial function and thus elicits part of its vasodilatory effect via this mechanism.

The present results regarding the effects of taurine on NE and KCl-induced contraction are similar to those reported by Ristori and Verdetti (1991) using aortic rings from Wistar rats. However, these investigators assessed the action of taurine with *in vitro* applications in organ baths. In addition, the effect of the amino acid on the relaxant responses of the aortic smooth muscle to vasorelaxant agents was not determined in the previous investigation. Therefore, the studies conducted in our laboratory were different from those of Ristori and Verdetti (1991) in some important aspects, and are considered to be more relevant to the *in vivo* situations. On the other hand, while our NE contractile data in the rat aortae were similar to those of Li et al. (1996) for mesenteric arteries from SHRSP, they were different from the responses of mesenteric arteries from WKY rats. Further, experiments by Li et al. (1996) have not demonstrated taurine-induced inhibition of KCl-mediated contraction of blood vessels from either SHRSP or WKY rats. However, similar to our findings, Franconi et al. (1982) reported that the intraluminal administration of taurine elicited concentration-dependent vasodilation in normotensive rabbit ear artery contracted with KCl; but in these studies, no effect of taurine was detected on the contractile responsiveness of the tissue to NE. Therefore, from the studies reported so far, the reason for the discrepancies between our data and those of others is not readily apparent. Nevertheless, it appears that this could be related, at least in part, to differences in the types of vascular preparations (e.g., aorta vs. ear or mesenteric artery) used and/or the methods of application of taurine (*in vivo* vs. *in vitro*).

Based on literature information (Schaffer et al., 1980; Franconi et al., 1882; Ristori and Verdetti, 1991; Palmi et al., 1999), the mechanism(s) for the smooth muscle relaxant effect of taurine observed in our study and those of others appears to be linked to a reduction of cytosolic calcium levels. For both NE and KCl-mediated responses, this reduction could result from an increase in calcium uptake and/or from a decrease in extracellular calcium influx. Since NE is also known to generate contraction by releasing calcium from intracellular stores (Abebe et al., 1990), it was possible that taurine might have also produced its effect by inhibiting calcium release in aortic smooth muscle contracted with NE. This hypothesis is plausible in view of the fact that the vasodilatory action of the amino acid was found to be greater in NE than in KCl-contracted preparations. Our observation of enhanced endothelium-dependent relaxation by taurine and the augmented inhibitory effect of the amino acid on NE-induced contraction of the intact aortae could result from the release of EDRF from the endothelium. However, given the calcium requirement for EDRF release (MacLeod et al., 1987), it is difficult to propose

a mechanism for this effect of taurine on the basis of the calcium hypothesis (Schaffer et al., 1980; Franconi et al., 1882; Ristori and Verdetti, 1991; Palmi et al., 1999).

In conclusion, the present investigation provides evidence that chronic taurine treatment causes vasorelaxation in the rat aorta and this is partly mediated via the endothelium. This effect of taurine may contribute to its hypotensive action observed in hypertensive animals and humans in a number of previously cited studies.

Acknowledgements

This work was supported by grants from Taisho Pharmaceutical Company and the Medical College of Georgia School of Dentistry.

References

Abebe W, Harris KH, MacLeod KM (1990) Enhanced contractile responses of arteries from diabetic rats to alpha 1-adrenoceptor stimulation in the absence and presence of extracellular calcium. J Cardiovasc Pharmacol 16: 239–248

Abebe W, Cavallari N, Agrawal DK, Rowley J, Patricia E, Thorpe PE, Hunter WJ, Edwards JD (1993) Functional and morphological assessment of rat aorta stored in University of Wisconsin and Eurocollins solutions. Transplantation 56: 808–816

Franconi F, Giotti A, Manzini S, Martini F, Stendardi I, Zilletti L (1982) The effect of taurine on high potassium- and noradrenaline-induced contraction in rabbit ear artery. Br J Pharmacol 75: 605–612

Fujita T, Sato Y (1984) The antihypertensive effect of taurine in DOCA-salt rats. J Hypertension 2: 563–565

Fujita T, Ando K, Noda H, Ito Y, Sato Y (1987) Effects of increased adrenomedullary activity and taurine in young patients with borderline hypertension. Circulation 75: 525–532

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 75: 101–163

Li N, Sawamura M, Nara Y, Ikeda K, Yamori Y (1996) Direct inhibitory effects of taurine on norepinephrine-induced contraction in mesenteric artery of stroke-prone spontaneously hypertensive rats. Adv Exp Med Biol 403: 257–262

MacLeod KM, Ng DDW, Harris KH, Diamond J (1987) Evidence that cGMP is the mediator of endothelium-dependent inhibition of contractile responses of rat arteries to ∝-adrenoceptor stimulation. Mol Pharmacol 32: 59–65

Paakkari P, Paakkari I, Karppanene H, Paasonene MK (1983) Mechanisms of inhibitory cardiovascular effects of taurine and homotaurine. Acta Med Scand 677: 134–137

Palmi M, Youmbi GT, Fusi F, Sgaragli GP, Dixon HBF, Frosini M, Tipton KF (1999) Potentiation of mitochondrial Ca^{2+} sequestration by taurine. Biochem J 58: 1123–1131

Ristori MT, Verdetti J (1991) Effects of taurine on rat aorta *in vitro*. Fundam Clin Pharmacol 5: 245–258

Schaffer SW, Azuma A (1992) Review: myocardial physiological effects of taurine and their significance. Adv Exp Med Biol 315: 105–120

Schaffer SW, Kramer J, Chovan JP (1980) Regulation of calcium homeostasis in the heart by taurine. Fed Proc 39: 2691–2694

Authors' address: Dr. Worku Abebe, Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, CB-3710, Medical College of Georgia, Augusta, GA 30912-1128, U.S.A., e-mail: wabebe@mail.mcg.edu

Received December 20, 1999