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The Role of K^+, Ca^{2+} channels, Some Endothelium Hyperpolarizing Factors in Taurine Induced Vasorelaxation in Rats Aorta

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Abstract. The current study included the relaxant effect of taurine on rat's aortic rings and the mechanism behind this relaxation. Taurine produced a potent spasmolytic effect on aortic rings at concentrations from zero to 80 mM. The results of K^+ channel subtypes using specific blockers indicated that the Kv channel has a considerable role in taurine-induced relaxation, while K_{ATP} has a limited role, Exposure of aortic rings to combinations of two K^+ blockers showed that K_{Ca}, K_v, and K_{IR} play important role in taurine mediated relaxation. The endothelium-derived hyperpolarizing factors used showed responses to a variable extent in taurine mediated relaxation; since NO and cGMP played a major role whereas PGS played a minor role in taurine mediated relaxation. Finally, the results also indicated that taurine-mediated relaxation is endothelium-dependent.

Keywords: K^+ channels, Ca^{2+} channels, endothelium hyperpolarizing factors, aorta, taurine, vasorelaxation.

1. Introduction

Taurine is also known as 2-aminoethanesulfonic acid [1], is one of the abundant free sulfur-containing amino acids in animal tissues [2], and bile acid [3]. Taurine is found relatively in a large quantity in humans, Huxtable [4] reported that the total taurine rate is approximately 1g / 1kg body weight and in the human plasma, its concentration is about 50 µM [2]. It has been shown that taurine can protect the endothelium cells from damage produced by oxidized low-density lipoprotein (LDL), hyperglycemia, and nitric oxide (NO) of inflammatory cells [5]. Also, it is well known for its capability to reduce muscle fatigue during exercises [6] and for its capability to increase the production of vasodilator NO [7]. Taurine is also essential for the muscles, heart, retina, and nervous system to preserve cellular integrity [8] and its protective effect against arteriosclerosis [9]. Studies have shown that taurine has pharmaceutical effects on hypertension, heart failure, and heart ischemia [10]. Furthermore, it also contributes to neuromodulation, calcium homeostasis, cytoprotection, osmoregulation, and detoxification [9].

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The present study aimed to investigate the vasodilation effects of taurine on isolated aortic rings of the rats and clarifying the mechanism(s) behind these effects by using different EDHF inhibitors and K^+ and Ca^{2+} channel blockers.

2. Material and Methods

2.1 Animal model and tissue preparation

All experiments were performed on healthy adult male Wistar rats (mean 290 ± 4g), which were housed in Animal House facilities at the University of Zakho. Rats were maintained at a mean room temperature of 23.5 ± 0.5°C, mean humidity of 26.2 ± 1.2%, and exposed to a regular diurnal photoperiod cycle with free access to water and food *ad libitum*.

The rats were killed by cervical dislocation and about 2.5-3.0 cm from the thoracic aorta was gently isolated and placed in a Petri dish containing cold Krebs-Henseleit solution. The aorta was cleaned from adipose tissue and cut into 4 rings, each of 3-5 mm in length. Subsequently, the aortic ring was fixed from one end to the tissue holder and from the other end to the isometric force transducer and immersed in the glass tissue chamber of PanLab organ bath containing warm Krebs-Henseleit solution bubbled with carbogen (95% O_2 and 5% CO_2) and kept at a constant temperature of 37°C and a pH of 7.4. The aortic ring resting tension was set at 2 g, left for equilibration for 60 min in the Panlab 4-chambered organ bath system. During this period, the tissue bath solution was replaced at 15 minutes intervals. The tissue viability and the endothelial integrity were confirmed by using 1µM phenylephrine (PE) and 10µM acetylcholine (ACh), respectively. The aortic rings contractile responses were recorded and digitized by LabChart 7 software and saved on the computer and used later for statistical analysis.

2.2 Experimental protocol

2.2.1 The role of K^+ and Ca^{2+}channels in taurine-mediated relaxation of isolated aortic rings

To investigate the role and the mechanism behind the relaxant effects of taurine on the aorta, the aortic rings were preincubated with one L-type Ca^{2+} channel- and four types of K^+ channel blockers, alone or in a combination of two K^+ blockers, for 10-20 minutes before precontraction with PE 1µM and before the cumulative addition of taurine at concentrations (20, 40, 60, 80 mM) to the organ bath chambers. The K^+ channel blockers were the K_{IR} channel blocker ($BaCl_2$, 1 mM for 10min)), K_V channel blocker (4-AP, 1 mM for10 min), K_{ATP} channel blocker (Glib 10 µM for 20min), and K_{Ca} channel blocker (TEA 10 mM for 10min). Furthermore, the L-type Ca^{2+} channel blocker nifedipine (10µM for 20min) was used.

2.2.2 The role of NO, and prostaglandins, and cGC on taurine-mediated relaxation of aortic rings

To study the role of NO and prostaglandins in the relaxant effects of taurine, the rings were incubated with their respective inhibitors for 10-20 minutes before precontraction with PE 1µM and before the cumulative addition of taurine at concentrations (20, 40, 60, 80 mM). The inhibitors used were L-NAME (nitric oxide synthase inhibitor) (100µM, 10min), indomethacin (cyclooxygenase inhibitor) (10µM, 20min), and Methylene blue (MB) (soluble guanylyl cyclase inhibitor) (10µM, 10min).

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2.2.3 The effects of endothelial denudation on taurine-mediated relaxation of aortic rings

To study the role of the endothelium on taurine-induced relaxation, the endothelium of the aortic rings was gently removed by mechanical rubbing using wooden toothpicks Subsequently, aortic rings were precontracted with 1 µM PE, and to 10 µM ACh as a relaxant agent to confirm the complete removal of endothelial. Any tissue that showed less than 10% relaxation was selected to be endothelium-denuded and any tissue that showed more than 90% relaxation was considered as an endothelium intact tissue. After confirmation, aortic rings were precontracted with 1 µM PE and when the tissues were stable, taurine was added cumulatively at concentrations (20, 40, 60, 80 mM) tissue chambers and the effects were recorded.

2.3 Statistical analysis

The obtained data were expressed as mean ± SEM and analyzed using student's t-test and one-way AVOVA with Dunnett's multiple comparison test were used after the baseline correction was performed. Data are analyzed and demonstrate as graphs by using GraphPad Prism 8 software. A *p*-value <0.05 is considered significant.

3. Results and Discussions

3.1 The role of K^+ and Ca^{2+} channels in taurine-mediated relaxation of isolated aortic rings

The opening of potassium channels leads to potassium efflux which causes membrane hyperpolarization and subsequent smooth muscle cell relaxation [11]. It is known that K_{ATP}, K_{IR}, and Kv, channels are specifically blocked by using 10 µM Glib [12], 1 mM BaCl2, and 1 mM 4-AP, respectively [13]. We tried to find out which effect of potassium channel blockers either alone or in combinations of two K^+ channel blockers can inhibit taurine-mediated vasorelaxation.

Aortic relaxation mediated by taurine showed a variable degree induced response since the taurine-induced relaxation curves in the presence of TEA and $BaCl_2$ were close to those of the control. This reflects that both K_{Ca} and K_{IR} channels played no direct role in the relaxation induced by taurine (Figure 1A-D and Table 1). However, in the presence of 4-AP and Glib, the relaxant responses were inhibited in a dose-dependent way, but the amplitude of inhibition was not the same since 4-AP produced a considerable inhibition in dose-response-curve (DRC) which was very close to the baseline, while in the presence of Glib, the inhibitory effect was much smaller. This indicates that the Kv channel has a considerable role in the taurine-induced relaxation whereas K_{ATP} has a limited role in the induced relaxation.

The results of the effect of combinations of two K^+ channel blockers also showed to some extent variable responses. Thus, the combinations of (TEA+4-AP) and (TEA+Glib) cause mild inhibition in which the responses were very closed to that of the control except at the highest taurine concentration (80 mM) in which a considerable blocking occurs. This indicates that K_{Ca}, Kv, and K_{IR} play important roles in relaxation induced by taurine but at the highest taurine concentration (80mM) used (Figure 2 and Table 2). However, exposure of aortic rings to (TEA+$BaCl_2$), ($BaCl_2$+ 4-AP) and (Glib+ 4-AP) showed a considerable blocking in taurine induced relaxation at all applied doses except at the highest dose used (80mM) at which the blocking effect was diminished, and the induced response returned to the levels of the control. This also indicates that the above combinations activated K_{Ca}, K_{IR}, Kv, and K_{ATP} channels and play important roles in induced relaxation at all doses used except at the highest dose (80 mM) used. On contrary, taurine-induced relaxation in aortic rings showed no effect on the DRC in the presence of ($BaCl_2$+Glib) and the produced response was close to those of the control.

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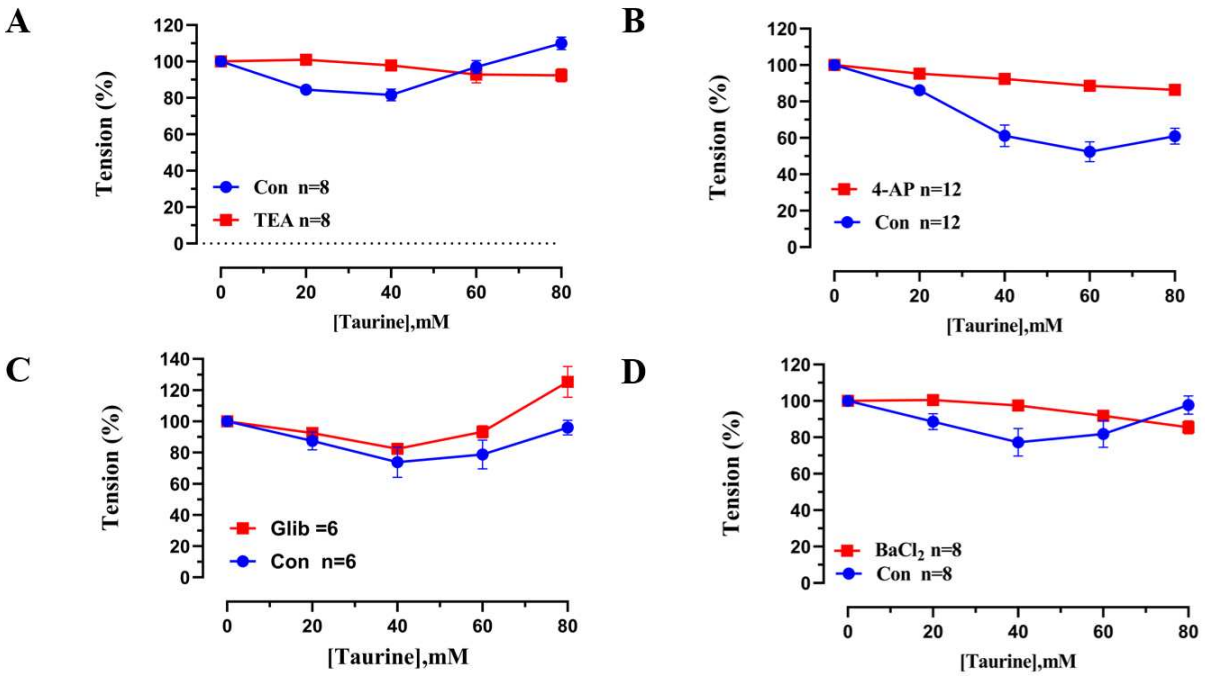

Figure 1. The role of K^+ channels in taurine-mediated relaxation in the isolated aortic rings treated with individual K^+ channel blockers (A-D). Each point is the mean ± S.E.M, of 6–12 aortic rings obtained from different individuals.

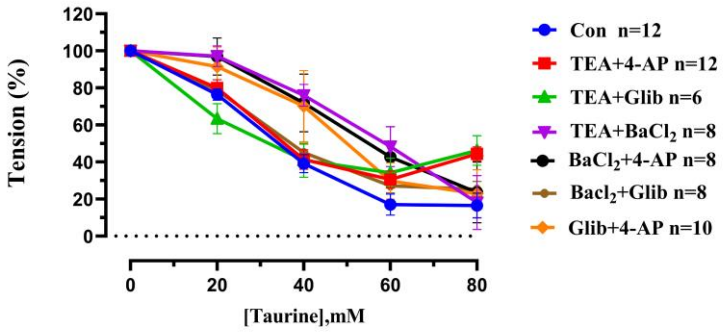

Figure 2. The role of K^+ channels on taurine-mediated relaxation in the isolated aortic rings treated with combinations of two K^+ channel blockers. Each point is the mean ± S.E.M, of 6–12 aortic rings obtained from several rats.

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Table 1. Statistical analysis results for aortic rings relaxed by taurine after preincubation with individual K^+ channel blockers and precontraction with 1µM phenylephrine (PE).

Test	M		Mean ± SEM	P-Value
	Control	Treatment		
TEA vs. Con	96.81	94.59	2.218 ± 5.502	0.70
Glib vs. Con	98.68	87.20	11.49 ± 8.768	0.23
BaCl2 vs. Con	95.07	89.10	5.969 ± 5.240	0.29
4-AP vs. Con	92.53	72.12	20.41 ± 9.290	0.06

Note: P-Value less than 0.05 considered significant

Table 1. Statistical analysis results for aortic rings relaxed by taurine after preincubation with the combination of K^+ channel blockers and precontraction with 1 µM phenylephrine (PE).

Test	M		Means diff.	P-Value
	Control	Treatment		
Con vs. TEA+4-AP	49.82	59.27	-9.447	>0.99
Con vs. TEA+ Glib	49.82	56.95	-7.134	>0.99
Con vs. TEA+BaCl2	49.82	67.92	-18.10	0.89
Con vs. Glib +4-AP	49.82	62.85	-13.03	0.97
Con vs. BaCl2+4-AP	49.82	67.02	-17.20	0.91
Con vs. Bacl2+ Glib	49.82	55.26	-5.435	>0.99

Note: P-Value less than 0.05 considered significant

The aortic relaxation mediated by taurine was not affected when incubated with L-type calcium channel blocker nifedipine (10 µM, 20 min) except at the last two concentrations used (60 and 80 mM) at which they showed a mild and non-significant ($p>0.05$) inhibition (Figure 3 and Table 3).

Taurine can inhibit calcium ion influx through L-type calcium channels in the neural and cardiac cells [14, 15]. However, Ristori and Verdetti [16] stated that extracellular calcium ion has no role in taurine vasorelaxation in a study carried on aortae of rats. Moreover, the current result also agrees with the previous result, because in this study taurine did not inhibit L-type calcium channels of the aortic smooth muscle cells when they were preincubated with L-type calcium channel blocker, NIF, suggesting that the relaxant effects of taurine that observed in the aortic smooth muscle cells were not mediated by blocking calcium ion influx through L-type calcium channels in the aortic smooth muscle cells.

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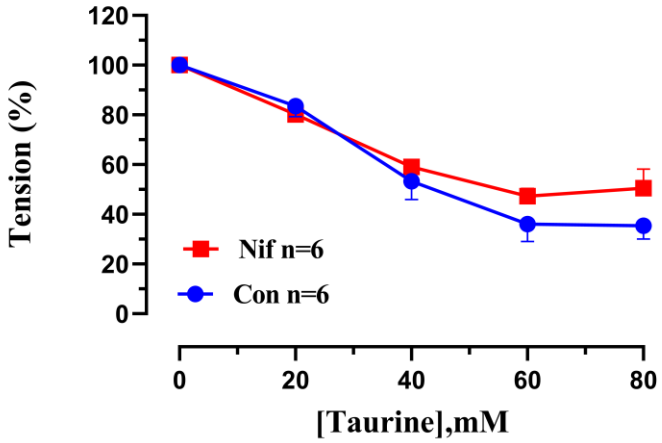

Figure 3. The role of L-type calcium channels on taurine-mediated relaxation in the isolated aortic rings. Each point is the mean ± S.E.M, of 6 aortic rings obtained from several rats.

3.2 The role of NO, prostaglandins, and sGC on taurine-mediated relaxation in aortic rings

The aortic-relaxation mediated by taurine incubated with L-NAME (100µ) (NO synthase inhibitor), MB (10µM) (sGC inhibitor), and indomethacin (10µM) (prostaglandin inhibitor) (Figure 4 A-C and Table 3) showed more than one response. Both L-NAME and MB produced highly significant ($P<0.01$) inhibition in DRC of aortic smooth muscle cells induced by taurine and with a superior inhibitory effect of L-NAME over that of MB. On contrary, indomethacin produced only a mild and non-significant inhibition only at the last two doses of taurine. These results indicated that activation sGC results in an elevation synthesis of cGMP which causes relaxation in the smooth muscle cell and vasodilation.

Prostaglandins are synthesized in nearly every organ and they have been associated with several roles within these organs such as in the cardiovascular system. Some prostaglandins are vasodilators whereas others are vasoconstrictors. Prostacyclin (PGI_2) and PGE_2, are vasodilators, while thromboxane A_2, is a vasoconstrictor. Results similar to those of the current study by Karabacak, Kaya [17], who observed that indomethacin did not inhibit taurine-induced relaxation in the rat's isolated aortic rings, suggesting that endothelial cyclooxygenase metabolites have no role in taurine-mediated vasorelaxation.

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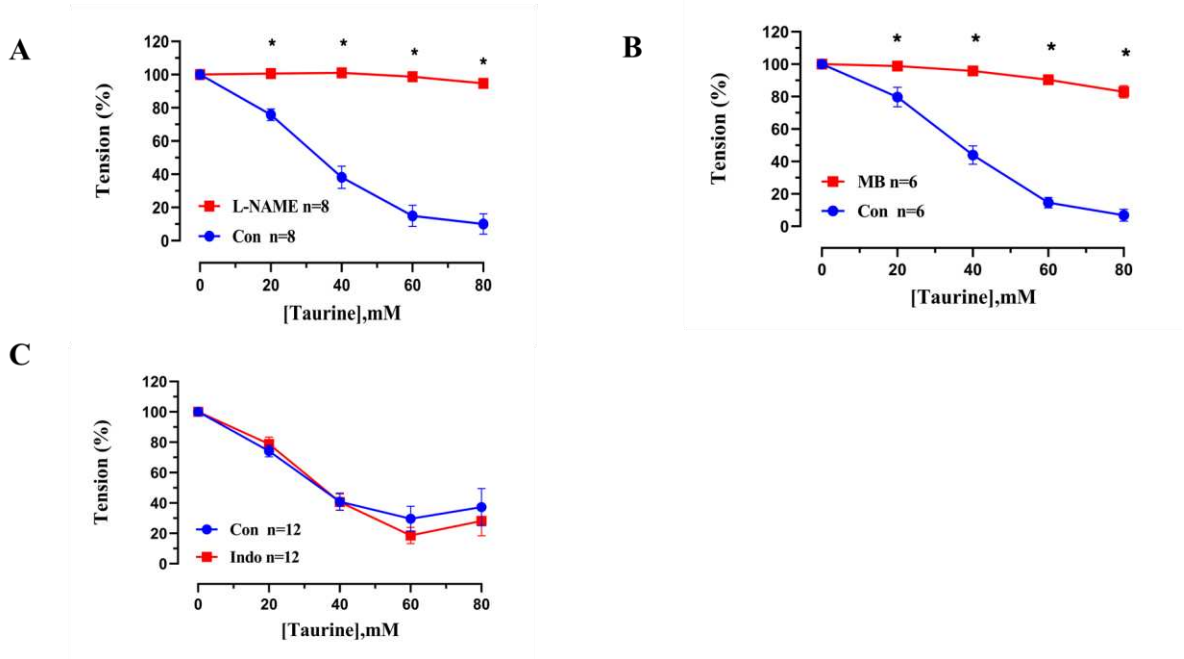

Figure 4. The role of NO, sGC, and prostaglandins on taurine-mediated relaxation in the isolated aortic rings (A-C). Each point is the mean ± S.E.M, of 6-12 aortic rings obtained from several rats. * =P < 0.05.

Table 3. Statistical analysis results for aortic rings relaxed by taurine after preincubation with L-type calcium channels, NO, and prostaglandins inhibitors and precontracted with 1µM phenylephrine (PE).

Test	M		Mean ± SEM	P-Value
	Treatment	Control		
L-NAME vs Con	99.02	47.80	51.22 ± 17.50	0.02
MB vs Con	93.62	49.05	44.57 ± 18.34	0.04
Indo vs Con	53.24	56.38	-3.142 ± 20.46	0.88
Nif vs Con	67.39	61.64	5.753 ± 16.36	0.73

Note: *P-Value* less than 0.05 considered significant

3.3 The effects of endothelial denudation on taurine-mediated relaxation of aortic rings

Taurine-mediated relaxation in the aortic rings of the rats significantly (P < 0.05) inhibited by denudation of the endothelial layer of aortic rings compared to the intact aortic rings. This indicates that taurine relaxation is endothelium-dependent in the aortic rings of the rats (Figure 5 and Table 4). According to Hu, Xu [18], taurine can increase serum concentrations of NO and NOS in the L-NAME-induced hypertensive model of rats. Furthermore, Yang, Lin [19] likewise, found that taurine, through increase signaling from the penile neuronal- and endothelial-dependent NO-cGMP signaling cascade, can improve penile vasorelaxation.

Last but not least, a study conducted recently on the right carotid artery and outer jugular vein of rats confirmed that the mechanism of taurine-induced relaxation in the smooth muscle of rats is endothelium-dependent through activation of the NO-cGMP pathway [20]. Consistent with the previous views, in the present work L-NAME (NOS inhibitor) and MB (sGC inhibitor) inhibit the relaxant effect of taurine in the isolated aortic rings of the rats. These results suggest that taurine relaxed aortic smooth muscle through the endothelium-dependent NO- cGMP pathway and this observation was supported by inhibiting taurine relaxation by denudation of endothelial layers of aortic rings.

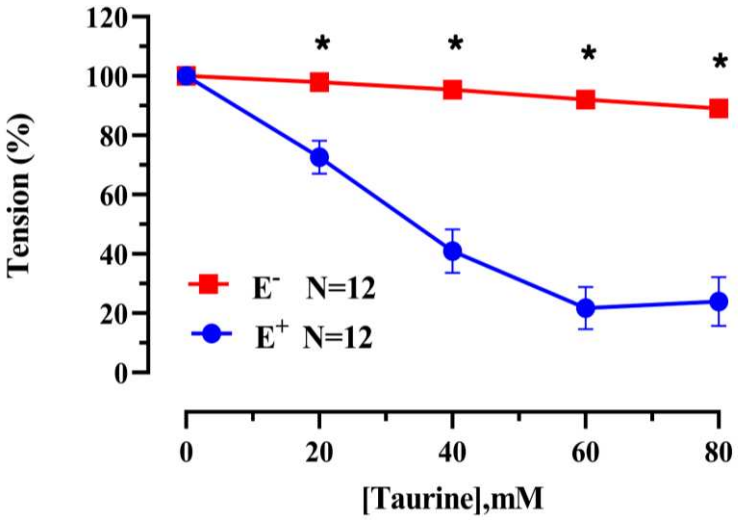

Figure 5. The relaxant effect of taurine on the dose-response curve for intact and denuded rats aortic rings precontracted with 1 uM PE. Each point represents the Mean±SEM.

Table 4. Statistical analysis results for taurine-mediated relaxation in denuded and intact aortic rings precontracted by 1µM phenylephrine (PE).

Test	Mean		Mean ± SEM	P-Value
	E-	E+		
E- vs E+	94.89	51.86	43.03 ± 15.22	0.02

Note: *E* stands for endothelium denuded (E-) and intact (E+), *P-Value* < 0.05 considered significant

4. Conclusion

From the current study, it was found that taurine induced a concentration-dependent relaxation in PE-pretreated rat's isolated aorta. Taurine-induced relaxation resulted from the activation of K_{Ca}, Kv, K_{ATP}, and K_{IR}, hyperpolarization of the smooth muscle cells, and subsequent relaxation. Furthermore, the induced relaxation was endothelial NO and cGMP dependent.

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