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Interaction between taurine and angiotensin II: Modulation of calcium transport and myocardial contractile function

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Summary. Angiotensin II modulates several aspects of cardiac function, including myocardial contractility, heart rate and myocyte growth. Most of these actions are intimately associated with alterations in calcium transport. Since taurine also modulates calcium transport, we examined possible interactions between taurine and angiotensin II at the level of the major cellular extruder of calcium, the Na^+-Ca^{2+} exchanger. Over a concentration range of 0.5–25 mM, taurine served as an effective inhibitor of angiotensin II-mediated stimulation of the exchanger. An Arrhenius plot of Na^+-Ca^{2+} exchange activity revealed that angiotensin II (2 nM) increased transporter activity by reducing the activation energy of the transport process. Taurine (25 mM) inhibited the angiotensin II effect by partially preventing the reduction in activation energy. However, neither agent significantly altered the transition temperature, ruling out a change in membrane fluidity or an alteration in the rate limiting step of the transporter as a cause of the observed effects. Since the Na^+-Ca^{2+} exchanger plays an important role in the handling of $[Ca^{2+}]_i$ by the myocardium, the effect of taurine on angiotensin II's modulation of contractile function was also examined. Hearts perfused with buffer containing angiotensin II experienced a slight positive inotropic effect in the absence of taurine but this was converted to a negative inotropic effect in the presence of taurine. The data suggest that taurine inhibits some, but not all of the actions of angiotensin II. The possibility that a phosphorylation event is the site of the angiotensin II-taurine interaction is discussed.

Keywords: Amino acids – Taurine – Angiotensin II – α-Adrenergic agonists – Na^+-Ca^{2+} exchange – Heart – Contraction

Introduction

The amino acid, taurine, is found in very high concentration in the heart (Huxtable, 1992). One of its major functions is the regulation of cell volume (Atlas et al., 1984; Rasmusson et al., 1993), however, it also appears to alter cellular sodium, calcium and pH homeostasis (Schaffer et al., 1994). In addition, taurine appears to serve as an important modulator of other cellular effectors (Fujimoto et al., 1976; Pham et al., 1987; Failli et al., 1992).

One group of effectors altered by taurine is the α-adrenergic agents. Franconi et al. (1986) have reported that guinea pig ventricular strips treated with taurine exhibit a reduction in the positive inotropic effect of the α-agonist, phenylephrine. Although it has been reported that taurine may affect the binding of the agonist to the α-adrenergic receptor, this concept remains controversial (Franconi et al., 1986; Endoh et al., 1989). A more likely site of taurine action is one of the reactions in the signal transduction pathway of the effector.

The α-adrenergic agonists are thought to exert their influence through the activation of phospholipase C and the generation of two cellular messengers, calcium and inositol triphosphate. Another effector, which activates a similar signal transduction cascade, is angiotensin II (Baker et al., 1989). Like the α-adrenergic agonists, angiotensin II is capable of modulating sodium, hydrogen and calcium transport, regulating myocardial mechanical function and stimulating protein synthesis (Lindpaintner and Ganten, 1991; Baker et al., 1992). Since taurine alters the activity of the α-adrenergic agonists, whose myocardial actions and signalling pathway resembles that of angiotensin II, we tested the hypothesis that taurine might also attenuate the activity of angiotensin II. The study focuses on phenomena affected by both taurine and angiotensin II; namely, myocardial contraction and calcium transport.

Methods

Heart perfusions

Hearts from 240–280 g male Wistar rats were perfused within 45 seconds following decapitation. The standard working heart apparatus was used, in which the coronary system of the heart was perfused from a reservoir placed 100 cm above the aortic cannula, while the left atrium received fluid from an atrial reservoir maintained at a fixed pressure head of 13 cm water. Left ventricular pressure was measured with a Statham P23Gb pressure transducer by inserting a 22 gauge needle through the ventricle wall.

The heart was perfused with Krebs-Henseleit buffer (37°C) supplemented with 10 mM glucose and 0.6 mM calcium. In some hearts, 20 mM taurine was also included in the perfusion medium. At time 0, angiotensin II (75–750 nM) was added to the perfusion medium. Left ventricular pressure and coronary flow were monitored before and after addition of angiotensin II. To eliminate variability due to changes in beating rate, all hearts were paced at 300 beats/min.

Na^+-Ca^{2+} exchanger assay

Sarcolemmal vesicles were isolated from male Wistar rats by the methods of Pitts (1979). Based on the degree of NaN_3 and ruthenium red inhibition of Ca^{2+} transport, the iso-

lated sarcolemmal preparation contained <2% mitochondrial contamination. Similarly, sarcoplasmic reticular contamination of the membrane preparation was <6%, as both oxalate-facilitated and p-nitrophenylphosphate supported Ca^{2+} accumulation were minimal. To assay for Na^{+}-Ca^{2+} exchanger activity, the vesicles were loaded with buffer containing 160 mM NaCl, 20 mM MOPS, 100 μM Gpp(NH)p and 1 mM $MgCl_2$. After a preincubation period to allow temperature stabilization (10–45°C), angiotensin II (2 nM), 2 nM angiotensin II plus 25 mM taurine, 100 μM phenylephrine or 100 μM phenylephrine plus 25 mM taurine was added to the medium. The membranes were incubated with these effectors for 10 min before initiation of the Na^{+}-Ca^{2+} exchange reaction. To assay Na^{+}-Ca^{2+} exchange activity, the membranes (5–7 μg) were added to 500 μl of a potassium buffer containing 160 mM KCl, 20 mM MOPS, 40 μM $^{45}CaCl_2$ and 5 μM valinomycin. After 2 sec, the reaction was terminated by the addition of 3 ml of ice-cold MOPS buffer containing 160 mM KCl and 1 mM $LaCl_3$ followed by rapid filtration. The filters were washed five times with the $LaCl_3$ containing buffer before being dried and counted. All data were corrected for nonspecific binding and expressed as nmol/mg/sec.

Results

Initial studies focused on the contractile effects of angiotensin II. We found that angiotensin II (75–750 nM) had no significant effect on left ventricular pressure of the working rat heart perfused with buffer containing 1.25 mM Ca^{2+}. However, a reduction in the calcium content of the perfusion medium to 0.6 mM reduced left ventricular pressure from 163 ± 3 to 116 ± 5 cm H_2O (n = 5) and enhanced the angiotensin II effect. At the lower calcium concentration, 250 nM angiotensin II mediated a modest positive inotropic effect, increasing left ventricular pressure by 8 ± 2% (Fig. 1). Further increases in angiotensin II concentration caused no additional change in contractile function.

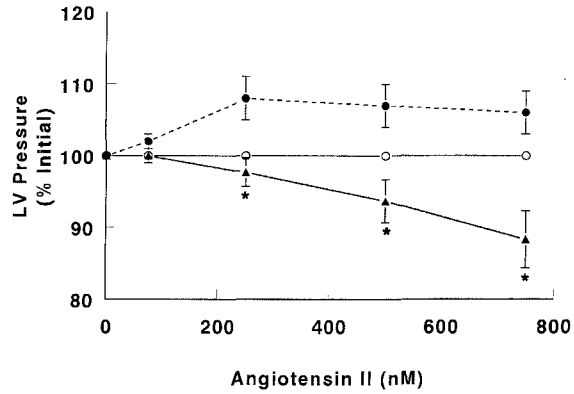

Fig. 1. Effect of angiotensin II and taurine on contractile function of the isolated rat heart. Hearts of male Wistar rats were perfused with Krebs-Henseleit buffer containing 0.6 mM calcium and either 0 (●-●, ○-○) or 20 mM (▲-▲) taurine. Following a 15 min stabilization period, the hearts were treated with varying concentrations of angiotensin II and left ventricular pressure monitored. Control, untreated hearts are represented by the 100% line (○-○). Values shown represent the mean ± S.E.M. of 5 hearts. Asterisks denote significant difference between taurine plus angiotensin II treated hearts and angiotensin II treated heart ($p < 0.05$)

Inclusion of 20 mM taurine in the perfusion medium significantly increased cardiac work (22 ± 4%) in hearts perfused with buffer containing 0.6 mM Ca^{2+} but had no significant effect when the buffer Ca^{2+} concentration was increased to 1.25 mM. Most importantly, inclusion of taurine in the perfusion medium containing 0.6 mM Ca^{2+} prevented the positive inotropic effect of angiotensin II (Fig. 1). Instead, angiotensin II exerted a negative inotropic effect in the presence of 20 mM taurine. This effect was concentration dependent, with the decline in left ventricular pressure reaching 12% at an angiotensin II concentration of 750 nM.

The inotropic effect of angiotensin II has been largely attributed to changes in calcium movement (Dosemeci et al., 1988; Wikman-Coffelt et al., 1991; Sempe et al., 1994). One of the calcium transporters altered by angiotensin II is the Na^+-Ca^{2+} exchanger (Ballard and Schaffer, 1996). As seen in Fig. 2, isolated sarcolemma loaded with sodium containing buffer supplemented with the GTP analog, 5′ guanylyl imidodiphosphate [Gpp(NH)p] experienced a 65% increase in Na^+-Ca^{2+} exchange activity following exposure to 2 nM angiotensin II. Although taurine alone had no effect on Na^+-Ca^{2+} exchanger activity (data not shown), it significantly reduced the stimulatory effect of angiotensin II (Fig. 2). At taurine concentrations of 5 mM and 25 mM, the angiotensin II effect was reduced by 25% and 42%, respectively. Interestingly, the effect of taurine on angiotensin II was comparable to its effect on the α-adrenergic agonist, phenylephrine. Like angiotensin II, phenylephrine (100 μM) significantly stimulated Na^+-Ca^{2+} exchanger activity. Inclusion of taurine (25 mM) in the incubation medium reduced the phenylephrine response by about 45% (Fig. 3).

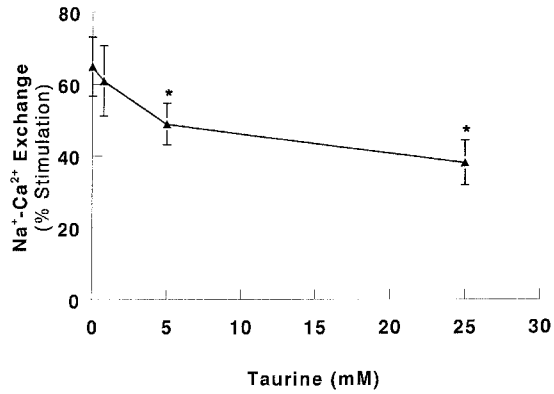

Fig. 2. Effect of taurine on angiotensin II-mediated stimulation of the Na^+-Ca^{2+} exchanger. Enriched sarcolemmal vesicles from rat heart were prepared and loaded with buffer containing 160 mM sodium, 100 μM Gpp(NH)p and the appropriate concentration of taurine. After incubation with 2 nM angiotensin II, Na^+-Ca^{2+} exchanger activity was measured. The stimulation of the Na^+-Ca^{2+} exchanger by angiotensin II at various concentrations of medium taurine is expressed as % stimulation, with the activity of the unstimulated exchanger being 2.4 nmol/mg/sec. Values shown represent the mean ± S.E.M. of 3 to 5 preparations. Asterisks denote significant difference between taurine treated and untreated membrane ($p < 0.05$)

Figure 4 reveals the temperature dependence of the Na^+-Ca^{2+} exchanger in the presence and absence of angiotensin II, as well as in the presence of both taurine and angiotensin II. The data are plotted according to the Arrhenius method in order to obtain information on the transition temperature, the temperature at which the two lines of differing slope intersect, and activation energy of the reaction. Incubation of sarcolemmal vesicles with angiotensin II (2nM) increased exchanger activity at all temperatures examined, but the largest effect occurred at the lower temperatures. A transition temperature for the Na^+-Ca^{2+} exchanger occurred at 15°C (1000/T = 3.47) in

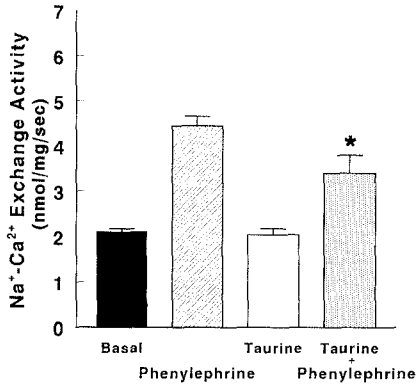

Fig. 3. Effect of taurine on phenylephrine-mediated stimulation of the Na^+-Ca^{2+} exchanger. The experiment was performed as described in Fig. 2 except 100μM phenylephrine was used in place of 2nM angiotensin II and the taurine concentration employed was 25mM. Values shown represent the mean ± S.E.M. of 4 preparations. Asterisk denotes significant difference between the α-adrenergic agonist alone and the combination of taurine and the α-adrenergic agonist ($p < 0.05$)

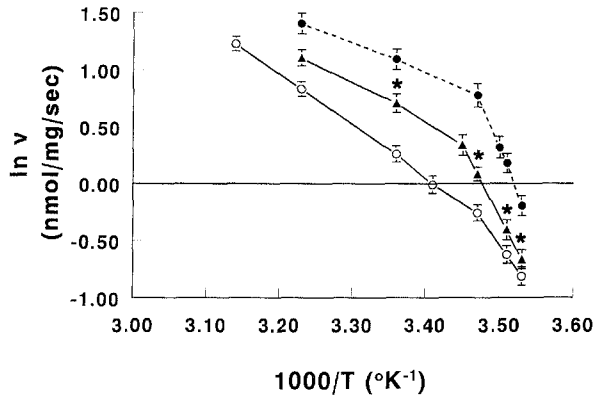

Fig. 4. Temperature dependence of the Na^+-Ca^{2+} exchanger plotted according to the method of Arrhenius. Na^+-Ca^{2+} exchanger activity expressed as ln velocity was assayed at the indicated inverse temperatures ($°K^{-1}$) in medium containing no addition (○-○), 2nM angiotensin II (●-●) or 2nM angiotensin II and 25mM taurine (▲-▲). Values shown represent the means of 4 preparations. Asterisks denote significant difference between angiotensin II treated membrane in the presence and absence of taurine ($p < 0.05$)

the presence and absence of angiotensin II (Fig. 4). Although taurine caused a slight shift in the transition temperature to 17°C (1000/T = 3.44), the major effect of taurine was to attenuate the angiotensin II-mediated decrease in activation energy of the exchanger. Based on standard calculations, the activation energy for the Na^+-Ca^{2+} exchanger in control, angiotensin-treated and taurine plus angiotensin II treated membrane was 9.09 Kcal/mol, 5.18 Kcal/mol and 7.38 Kcal/mol, respectively.

Discussion

The most important finding of this study is that taurine inhibits several actions of angiotensin II. Although the present study focused on the inotropic and Ca^{2+} transport effects of angiotensin II, the octapeptide also exhibits chronotropic actions, promotes cardiac growth and is involved in ventricular remodeling (Lindpaintner and Ganten, 1991; Baker et al., 1992). The latter two effects have received the most attention because of their obvious involvement in the development of congestive heart failure. However, angiotensin II has also been implicated in myocardial ischemia injury, with the ACE inhibitors serving as effective cardioprotective agents (Lonn et al., 1994).

There is excellent rationale for assuming that the effects of angiotensin II on Na^+-Ca^{2+} exchanger activity contribute to the pathology of both the ischemic-reperfused and failing myocardium. One of the prominent mechanisms advanced to account for ischemic-reperfusion injury is the "pH paradox" (Karmazyn and Moffat, 1993). According to this theory, H^+ accumulate during ischemia, initiating Na^+ influx via the Na^+-H^+ exchanger and promoting Ca^{2+} influx via the Na^+-Ca^{2+} exchanger. The resulting Ca^{2+} overload leads to a series of adverse responses, which are ultimately lethal to the cell. In this setting inhibition of Na^+-Ca^{2+} exchange flux by taurine should benefit the ischemic myocardium.

Taurine would also affect the failing myocardium, in which the activity of the sarcoplasmic reticular Ca^{2+} pump is reduced while the activity of the Na^+-Ca^{2+} exchanger is elevated (Beuckelmann et al., 1995; O'Brien et al., 1995). In the failing human heart, reduced sarcoplasmic reticular Ca^{2+} pump activity causes prolongation of the Ca^{2+} transient, contributing to impaired myocardial relaxation (Gwathmey et al., 1987; Pieske et al., 1995). The activation of the Na^+-Ca^{2+} exchanger partially compensates for the relaxation defect; however, it also results in a net extrusion of Ca^{2+} from the cell. Consequently, Ca^{2+} cycling is reduced and myocardial contractility falls (Pieske et al., 1995). We believe that taurine would modulate this response by inhibiting the activation of the Na^+-Ca^{2+} exchanger by angiotensin II. While this action of taurine would decrease the rate of Ca^{2+} removal from the cytoplasm during diastole, the decline in Na^+-Ca^{2+} exchanger activity would tend to elevate $[Ca^{2+}]_i$ by partially restoring the balance between the sarcoplasmic reticular Ca^{2+} pump and the Na^+-Ca^{2+} exchanger. In addition, taurine might modulate angiotensin II-mediated alterations in ventricular remodeling. This supposition is based on the evidence that angiotensin II-mediated alterations in Ca^{2+}

transport play a central role in several biochemical processes involved in ventricular remodeling (Baker and Aceto, 1990; Booz et al., 1994; Sadoshima et al., 1995). In this regard, it is significant that taurine benefits patients suffering from congestive heart failure (Azuma et al., 1983). A key question that remains unanswered is whether taurine benefits congestive heart failure patients by inhibiting the Ca^{2+} transport and/or ventricular remodeling effects of angiotensin II.

In contrast to the diseased myocardium, the effects of taurine on angiotensin II activity would probably have minimal influence on normal cardiac function. Angiotensin II does mediate a modest positive inotropic effect in many species (Lindpaintner and Ganten, 1991; Baker et al., 1992). In the present study we found that angiotensin II stimulates contractile function in the hemodynamic rat heart in the absence of taurine, but reduces contractile function in the presence of taurine. This finding indicates the complexity of angiotensin II's inotropic action in the rat heart, a conclusion also supported by the diverse results reported in the literature (Wikman-Coffelt et al., 1991; Feolde et al., 1993; Sempe et al., 1994).

The nature of the angiotensin II response appears dependent upon the balance between opposing actions of the octapeptide. According to Baker et al. (1989), the positive inotropic effect of angiotensin II is linked to an increase in $[Ca^{2+}]_i$. Since taurine inhibits this elevation in $[Ca^{2+}]_i$ (Takahashi et al., 1996), it is not surprising that it also blocks the positive inotropic effect of angiotensin II. Presumably, by inhibiting the favorable effects of angiotensin II on calcium transport, taurine exposes the negative inotropic effects of angiotensin II.

It has been proposed that one of the major factors contributing to the negative inotropic effect of angiotensin II is severe coronary artery vasoconstriction (Lindpaintner and Ganten, 1991). However, in the present study, coronary flow did not decrease over the angiotensin II concentration range of 100 to 750 nM. The vasoconstrictor effect of angiotensin II was minimized in the present study by utilizing perfusion medium containing low concentrations of calcium (0.6 mM), which reduced vascular resistance and lowered myocardial oxygen demand by reducing contractile function. Inclusion of taurine in the perfusion medium under these conditions had little effect on coronary flow despite the reduction in contractile function.

Another potential mechanism contributing to the observed negative inotropic effect of angiotensin II is the activation of protein kinase C. Phorbol esters, which are potent activators of protein kinase C, have been reported to mediate a positive inotropic effect at low concentrations and a negative inotropic effect at higher concentrations (Ward and Moffat, 1992). Interestingly, at higher concentrations the phorbol esters also function as potent vasoconstrictors. Thus, angiotensin II and the phorbol esters share many of the same properties. According to Capogrossi et al. (1990), protein kinase C activators, such as the phorbol esters, reduce contractile function in part through a reduction in $[Ca^{2+}]_i$. Since the Na^+-Ca^{2+} exchanger is the premier transporter extruding Ca^{2+} from the cell, Capogrossi et al. (1990) proposed that the phorbol esters must activate the exchanger, causing enhanced Ca^{2+}

efflux and decreasing $[Ca^{2+}]_i$. However, the present data are inconsistent with this theory. Instead of the predicted improvement in contractile function accompanying the inhibition of Na^+-Ca^{2+} exchanger activity, taurine converted the positive inotropic effect of angiotensin II into a negative inotropic effect. While this action of taurine is incompletely understood, it may be related to the direct effects of taurine on contractile function. Clearly, further studies are required to clarify this complex phenomenon.

At the present time, the mechanism underlying the actions of angiotensin II and taurine on the Na^+-Ca^{2+} exchanger is inadequately understood. Figure 4 reveals that neither agent significantly alters the transition temperature of the Na^+-Ca^{2+} exchanger, ruling out a change in membrane fluidity or an alteration in the rate-limiting step of the transporter as a cause of the observed effects. The major effect of angiotensin II is a reduction in the activation energy of the exchanger. Taurine partially reverses this effect.

Although the basis underlying the reduction in activation energy has not been established, three lines of evidence have implicated a protein phosphorylation event in the stimulation of the exchanger by angiotensin II. First, the protein kinase C inhibitor, chelerythrine, is a potent inhibitor of the angiotensin II effect (Ballard and Schaffer, 1996). Second, other protein kinase C activators are capable of stimulating the exchanger (Ballard and Schaffer, 1996). Third, the similarities between the angiotensin II and phenylephrine responses are striking, suggesting that taurine must act at some point in their signal transduction pathways. Interestingly, protein kinase C is a common step in the two signalling cascades.

The phosphorylation hypothesis is attractive because it provides a logical explanation for the interaction between angiotensin II and taurine. According to this hypothesis, angiotensin II activates the exchanger through a phosphorylation event, while taurine reverses the angiotensin II effect either by inhibiting the phosphorylation step or by promoting the dephosphorylation of the regulatory phosphorylation site. Significantly, there is precedence for the idea that taurine can modulate a phosphorylation event. Lombardini (1992) and Schaffer et al. (1990) have observed changes in the phosphorylation of several cardiac proteins by taurine. Although the nature of the taurine sensitive protein kinase has not been identified, both calmodulin-dependent protein kinase and protein kinase C have been implicated in the taurine regulation (Schaffer et al., 1990; Li and Lombardini, 1991). It remains to be determined whether the taurine sensitive protein kinase is capable of activating the Na^+-Ca^{2+} exchanger.

In summary, taurine inhibits several actions of angiotensin II, which are likely to have important implications for the functioning of the diseased myocardium.

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