Effects of captopril, an angiotensin converting enzyme inhibitor on TAME-esterase induced contractions in rat aorta strips in vitro.

Anwar Hussein Subratty* and Fawzia B.H.Gunny.
Department of Health and Medical Sciences, Faculty of Science, University of Mauritius, Reduit, Mauritius, (* author for correspondence)

Abstract

The in-vitro effects of Captopril an angiotensin converting enzyme inhibitor were studied on TAME-esterase, a component of the Kinin-Kallikrein system, induced contractions on rat aorta strips. Our results showed that contractile responses induced by TAME (EC\textsubscript{50} 6 x 10\textsuperscript{-14} M) were significantly attenuated when the aorta strips were pre-incubated with 1M Captopril (EC\textsubscript{50} 1.8 x 10\textsuperscript{-10} M). Based on our data we concluded that TAME-esterase induced contractions involve degradation of kinins by kinases.

Key Words: Kinin, Kallikrein, ACE inhibitor, TAME, aorta.

In vitro koşullarda çan aort striplerinde angiotensini dönüştüren enzim inhibitörü kaptoprilin, TAME-esterazin uyardyği kasımlara etkileri

Özet

Angiotensini dönüştüren enzim inhibitörü kaptoprilin in vitro etkileri çan aort striplerinin kasılması uyaran kinin-kallikrein sistemi komponenti olan TAME-esterazda araştırıldı. Sonuçlarımız, aort stripleri önceden1M (EC50 1-8 x 10-10m) kaptopril ile inhibe edildiği zaman, TAME (EC50 6 x 10-14m) tarafından uyarılan kasıma cevaplarının kayda değer bir şekilde azaldığını gösterdi. Verilerimiz dayanarak, TAME-esterazın uyardyğı, kasımanın kinazlar tarafından kininlerin yıkılması ile ilgili olduğu sonucuna varıldı.

Anahtar sözcükler: Kinin, Kallikrein, ACE inhibitör, TAME, aort

Introduction

Angiotensin-converting enzyme (ACE) inhibitors possess anti-ischaemic effects and are used in the treatment of heart failure (Linz W et al., 1992). It has been reported that an increase in the generation of angiotensin II and a decrease in the accumulation of kinins in tissues that result from an increase in ACE will have detrimental consequences (Vanhoutte PM et al., 1993). The latter, however, may be alleviated during therapy with ACE inhibitors. For example, kinins mediate the anti-ischaemic actions of nonsulphurhydryl ACE inhibitors in animal models (Linz W et al., 1992). Actually during myocardial ischaemia there is an increase in kinin generation in the coronary circulation (Hashimoto K, et al., 1978)

Kinins are peptide hormones that are formed as part of the kinin-kallikrein system (KKS). They were discovered in 1909 when Abelous reported an acute fall in blood pressure induced by experimental injection of urine. Later Frey & Werle discovered that a high molecular weight, thermolabile constituent was responsible for this effect. Kallikreins were later identified as proteases that could cleave off human
nonapeptide bradykinin and the human decapeptide kallidin from the larger kininogen precursors. Kinins play a significant role as mediators of inflammation, for example, by provoking pain and oedema (Scholkens, 1996). Nevertheless, because of their vasodilatory effects, they have always been suspected to be involved in cardiovascular regulation as well. The diuretic and natriuretic effect of kinins in particular suggests that the renally produced kallikreins may be significant for the regulation of blood pressure (Bhoola et al., 1992).

The endothelium controls vascular smooth muscle tone by secreting relaxing and contracting factors in response to shear stress and several vasoactive substances (Furchgott et al., 1980, De Mery et al., 1990). Kininogens, the precursors of kinins, are present in the plasma, but can also be produced by endothelial and vascular smooth muscles (Schmaier et al. 1988, Figuera et al., 1992). A variety of kininogenases exist in the blood and in the vascular tissues with the important varieties being plasma and tissue kallikreins (Andreas et al., 1999). Plasma kallikreins is activated by Hageman factor and is regulated by circulating serine protease inhibitors (Bhoola et al., 1992). They are secreted as proenzymes and are activated by proteases in the submaxillary gland and in the kidney. Certain serine protease inhibitors also inhibit tissue kallikrein. In addition to the generation of kinins, tissue kallikreins may also process other hormones and enzymes involved in the vascular tone (Bhoola et al., 1992).

N-α-tosyl L-arginine methyl ester [TAME]-esterase has been demonstrated to be an enzyme which is involved during the sequence of events leading to the activation of the kinin-kallikrein system (Subratty & Moonsamy, 1998). Furthermore, we have previously reported that TAME-esterase induced contraction in toad ileal strips *in-vitro* is mediated via a nitric oxide-citric GMP pathway (Subratty & Hossany, 1999). Thus our results tend to show that TAME-esterase activity has a significant contribution during contraction of smooth muscles in-vitro. Reports from the literature also describe the major contribution of ACE in the termination of the action of bradykinin relative to the other inactivation processes (including carboxypeptidases and internalization) that determine the degree of potentiation of the response to kinins observed with ACE inhibitors. The present study was undertaken to further elucidate the role of TAME-esterase as a component of the KKS system since ACE inhibitors can prevent both the activation of angiotensin I to angiotensin II and the cleavage of kinins to inactive fragments. Furthermore, kinins are hydrolysed by a variety of intracellular and extracellular peptidyl peptidases and ACE is a major kininase at the surface of endothelial cells.

**Materials and methods**

**Preparation of arterial rings.**

Adult male Sprague-Dawley rats weighing between 50-100 g were killed by a severe blow to the head. From these animals the aorta was carefully dissected, applying minimal traction to avoid stretching and taking care not to subject the intima to rubbing either with instruments or upon itself. After dissection, the aorta was quickly immersed in a petri dish containing 20 ml of Krebs-Henseleit solution. To prevent blood clot formation in the dissected aorta, 2 ml of Heparin (5,000 IU/L) was added to the buffer in the petri dish. The buffer was maintained at 37°C in the organ bath by a thermostated circulating water bath. All salts were dissolved in distilled water and the final volume made up to 1 litre.

Strips of the isolated aorta were prepared and mounted *in-vitro* as previously described by Subratty & Hossany (1999). In brief, before mounting the aorta strip, the organ bath was filled with 25 ml of Krebs-Henseleit solution at room temperature. Two stainless steel hooks were inserted into the lumen of the aorta strip. This strip was then held horizontally in the organ bath. One hook was pinned to a fixed point in the apparatus and the other one was connected to a force-displacement transducer (Model LB-5, Showa Shokki, Japan) of the apparatus. The transducer was plugged in a multipen recorder (Rikadenki Model R50; Japan) capable of recording isometric contractile responses on a rolling chart. The system was switched on and the resting tension was adjusted to 1.5g. The strip was first allowed to equilibrate in the buffer for at least 15 minutes until a baseline tone was achieved.

**Bathing solution and drugs.**

The bathing solution was Krebs-Henseleit solution of the following composition (mM): NaCl 118; KCl 4.7;
CaCl₂·H₂O 2.5, MgSO₄·7H₂O; KH₂PO₄ 25; Na₂EDTA 9.7 mg/l). Glucose (2g/l) was added to the buffer just before use and gassed with a 95 % O₂ and 5 % CO₂ mixture, resulting in a pH of 7.45. The buffer was maintained at 37°C in the organ bath by a thermostat circulating water bath.

Drugs used in this study were N-α-tosyl L-arginine methyl ester [TAME] and Captopril. All drugs were prepared as aqueous solutions. A 10⁻³ M stock solution of TAME was prepared by dissolving 0.0038g of TAME in 10 ml of distilled water. Aliquots of this stock solution were used to make serial dilutions ranging from 10⁻³ to 10⁻¹⁷ M respectively. Twelve aorta strips from 12 rats were used in this series of experiments. Each strip was challenged with 100 µL of TAME, beginning with the lowest dilution (10⁻¹⁷ M). The procedure was repeated with increasing concentration to establish a cumulative dose-response curve after stabilization of the strip following any contractile responses or after 3 minutes in case of no observed changes. The final concentration in the bath was 10⁻³ M.

In addition to TAME, the effects of different concentrations of Captopril, an Angiotensin Converting Enzyme (ACE) inhibitor were also studied on rat aorta strips. Aorta strips were pre-incubated with 500 µl of the respective dilution of the ACE inhibitor. Each concentration was studied separately on seven strips.

Control experiments

In each series of experiments, a parallel control strip was included and challenged with 100 µL of distilled water added at 3 minutes interval.

Statistics

All data manipulation and statistical analyses were performed using Excel software. Means and standard errors were calculated. Statistical differences were assessed using unpaired Student’s t-test. Differences were considered as significant for p < 0.05.

Results

EC₅₀ values for each drug used on the rat aorta strips were determined form cumulative concentration curves (Figure 1). Our results (Table 1) showed that TAME-esterase induced contractions were significantly inhibited (p < 0.05) when rat aorta strips were pre-incubated with 1.0 M of the ACE inhibitor (Captopril). However non-significant inhibition of contractile responses was noted when the 0.01 & 0.2 M pre-incubated strips were challenged with the various dilutions of TAME (p>0.05). Figure 2 shows typical tracings of contractile responses obtained from the experiments performed.

Discussion

The sequence of events leading to contraction of airway (Subratty et al., 1994) and non-airway smooth muscles have previously reported elsewhere (Subratty & Moonsamy, 1998). It is well known that kinins have vasorelaxation properties. The present study tend to
show that contractile responses induced by TAME-esterase could be the result of degradation of kinins by kininase. The latter hypothesis is confirmed from experiments using the ACE inhibitor. Findings from these experiments have shown that when strips were pre-incubated with Captopril (1M), there was a definite significant inhibition of the contractile responses previously seen with TAME alone.

Based on our findings we thus postulate the following mechanism for TAME-esterase induced contractions. During the conversion of high molecular weight kininogens to kinins in the presence of kallikreins whereby TAME-esterase is a zymogen, bradykinin which is released is degraded by a kininase (kininase II). In the presence of a sufficient dose of a kininase II inhibitor, namely Captopril, the contractile effects of the kininase is substantially attenuated to the extent that no significant contractions were observed.

As mentioned earlier, we have already reported two different mechanisms by which TAME-esterase induce contraction \textit{in-vitro}. Through the present study, we are describing a third pharmacological pathway showing that TAME-esterase induced contractions involve degradation of kinins by kininase.

We conclude that TAME-esterase induced contractions can be prevented by the use of ACE inhibitors. However the role of TAME-esterase in hypertension remains to be established.

\textbf{Acknowledgement}

We are grateful to the Tertiary Education Commission of Mauritius and the University of Mauritius for financial support.

\textbf{References}


Subratty AH and Hossany R. Does TAME induced contraction involve an endothelium nitric oxide-cyclic GMP mediated pathway? \textit{Indian. J. Exp. Biol.} 37: 406-
408, 1999.
