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THE EFFECTS OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS (ENALAPRIL AND CAPTOPRIL) ON THE RESPONSES OF THE ISOLATED PAPILLARY MUSCLE OF THE RABBIT TO NIFEDIPINE

MILICA PROSTRAN*, IVANA MATIĆ*, Z. TODOROVIĆ*, MILANKA JEZDIMIROVIĆ**, M. Š. PROSTRAN***, Z. VASILJEVIĆ**** and V. M. VARAGIĆ*

*Department of Pharmacology, Faculty of Medicine, P. O. Box 662, University of Belgrade,
**Department of Pharmacology, Faculty of Veterinary Medicine, Bulevar JNA 18,
***Department of Anatomy, Faculty of Medicine, Dr Subotića 4/II,
****University Institute for Cardiovascular Diseases, YU-1000 Belgrade, Yugoslavia

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In the present experiments the interaction between nifedipine, a dihydropyridine calcium channel blocker, and angiotensin- converting enzyme inhibitors (captopril and enalapril) on the isolated electrically stimulated papillary muscle of the rabbit was studied.

Nifedipine (1.3 μmol. l^{-1}, 3 min) completely abolished the staircase phenomenon. This concentration of nifedipine produced a general, frequency-dependent depression of the response of the papillary muscle by 26-54%.

Both enalapril and captopril (3.37 μmol. l^{-1}, 90 s) by themselves did not qualitatively alter the staircase phenomenon. This means that in the presence of enalapril or captopril in the bathing medium this phenomenon was present, but evidently at a lower level, i. e. the response of the muscle to each frequency of the electrical stimulation was always depressed in comparison with the control response (with no drug in the medium).

In the presence of nifedipine and captopril or enalapril, the staircase phenomenon was completely abolished or preserved, respectively.

Our results indicate a possible implication of calcium in the depressive action of both ACE inhibitors on the electrically paced heart muscle of the rabbit. Also, our results suggest that only enalapril possesses protective action against nifedipine-induced changes of the staircase phenomenon.

Key words: nifedipine, captopril, enalapril, the staircase phenomenon, papillary muscle

INTRODUCTION

Captopril and enalapril are inhibitors of angiotensin-converting enzyme (ACE), an enzyme which converts angiotensin I to angiotensin II (the powerful vasoconstrictor octapeptide). Consequently, ACE inhibitors block angiotensin II formation, which accounts, in part, for the vasodilatator response of these agents. ACE is functionally identical with kininase II which destroys kinins (Regoli and Barnabe, 1980). Components of the renin-angiotensin system have been described in many different tissues. This means that angiotensin II is not exclusively generated in the plasma. The presence of angiotensin I and angiotensin II has been demonstrated in the heart, the adrenal glands, the liver and the brain (Marshall et al., 1993).

The antihypertensive effects of enalapril and captopril are explaind mainly by the inhibition of ACE (Gomez et. al., 1985).

It has been previously shown that both captopril and enalapril produced a dose-dependent depression of the isometric contraction of isolated, spontaneously beating guinea-pig heart atria (Prostran, 1989; Prostran et al., 1989). Also, it has been found that both drugs did not qualitatively alter the staircase phenomenon, a very sensitive biological response of the rabbit papillary muscle (Prostran et. al., 1991).

The importance of calcium ions in excitation-contraction coupling in the mammalian heart muscle has been well documented (Siegl, 1986). This is evident from studies of the inotropic drugs in various experimental models, such as the staircase phenomenon, contractile transients, aftercontractions etc. (Bayer et al., 1975). These authors have shown that verapamil and D 600, both calcium channel blockers, significantly affect the staircase phenomenon in the isolated cat papillary muscle. A dihydropyridine calcium channel blocking drug, nicardipine differs from verapamil, not only in chemical structure, but also in some pharmacological characteristics (Matić et al., 1989; 1994).

For several reasons, an increasing number of patients with hypertension are treated with ACE inhibitors + calcium channel blockers (Eber et al., 1992). In other words, ACE inhibitors and calcium antagonists are now used widely as the first line therapy for high blood pressure (Singer et al., 1991). This gradual move from beta-blockers and diuretics is, in part due to fewer or different side effects and the perceived lack of deleterious metabolic effects. Calcium antagonists may be more effective in elderly and low-renin patients with a higher initial pressure. The clinical efficacy of ACE inhibitors is related to the initial level of plasma renin or angiotensin II activity.

It was therefore of interest to study the possible interaction between nifedipine, a dihydropyrydine calcium channel antagonist and captopril and enalapril on the isolated papillary muscle of the rabbit. This muscle is a particularly suitable tissue for studying the action of drugs on the heart muscle because of the regular orientation of muscle fibers, thus making the analysis of drug effects on it more easy.

MATERIAL AND METHODS

The papillary muscle, taken from rabbits of both sexes (1.6-2.8 kg), was dissected from the left ventricle and prepared for recording according to the method of Schümann et al. (1974). The muscle was removed from the left ventricle as quickly as possible and mounted in a 15 ml organ bath containing Tyrode solution of the following composition (mmol. l^{-1}): NaCl 136.7, KCl 2.8, $CaCl_2$ 1.8, $MgCl_2$ 0.105, NaH_2PO_4 0.417, $NaHCO_3$ 11.9 and dextrose 11.1. The Tyrode solution was oxygenated with a mixture of 97% O_2 and 3% CO_2. The temperature of the bathing medium was kept at 37^0C. The initial loading of the isolated papillary muscle was between 0.13-0.18 g.

Electrical stimulation was carried out using a square wave pulse of 0.4 ms duration and the following frequencies: 0.1, 0.5, 1, 2 and 3 Hz. The voltage output was usually kept about 20% above the threshold. The electrodes (for direct electrical stimulation) were made of palladore (30% palladium and 30% silver). The isometric contraction of the muscle was recorded by a microdynamometer 7001 (Ugo Basile) and displayed on paper moving at various speeds.

The equilibrium of the muscle lasted 60 min. In the present study all five frequencies used (0.1, 0.5, 1, 2 and 3 Hz) usually produced a typical staircase phenomenon in which higher frequency produced a higher amplitude of the isometric contraction of the isolated papillary muscle. Stimulation with one frequency lasted for 20 s, after which period a higher frequency was immediately switched on (applied).

The results are expressed as the mean $\pm$ s.e. of n determinations, and the difference between means was assessed for significance by Student's t-test.

The following substances were used: nifedipine (Lek, Ljubljana), calcium chloride (Merck), captopril (Zorka, Šabac) and enalapril maleate (Krka, Novo Mesto). All drugs, except nifedipine, were dissolved in distilled water. The stock solution of nifedipine (15 mg/ml ethanol) was made every 2nd or 3rd day, but further dilutions with distilled water were made before starting the experiments. All experiment with nifedipine were performed in a dark room because of quick decomposition of the drug if its solution is exposed to daylight.

RESULTS

The amplitude-frequency relationship (the staircase phenomenon). – The increasing frequencies of electrical stimulation (0.1, 0.5, 1 and 2 Hz) in the majority of experiments produced a typical staircase phenomenon in which higher frequency of stimulation produced a higher amplitude of the isometric contraction of the isolated papillary muscle of the rabbit. Meanwhile, a frequency of 3 Hz also produced a further increase in the amplitude of contractions. The results are shown in Figure 1 (A and B).

The effect of nifedipine on the amplitude-frequency relationship. – In the presence of nifedipine in the bathing medium (1.3 μmol. l^{-1}, 3 min) the positive amplitude-frequency relationship was completely abolished. This concentration

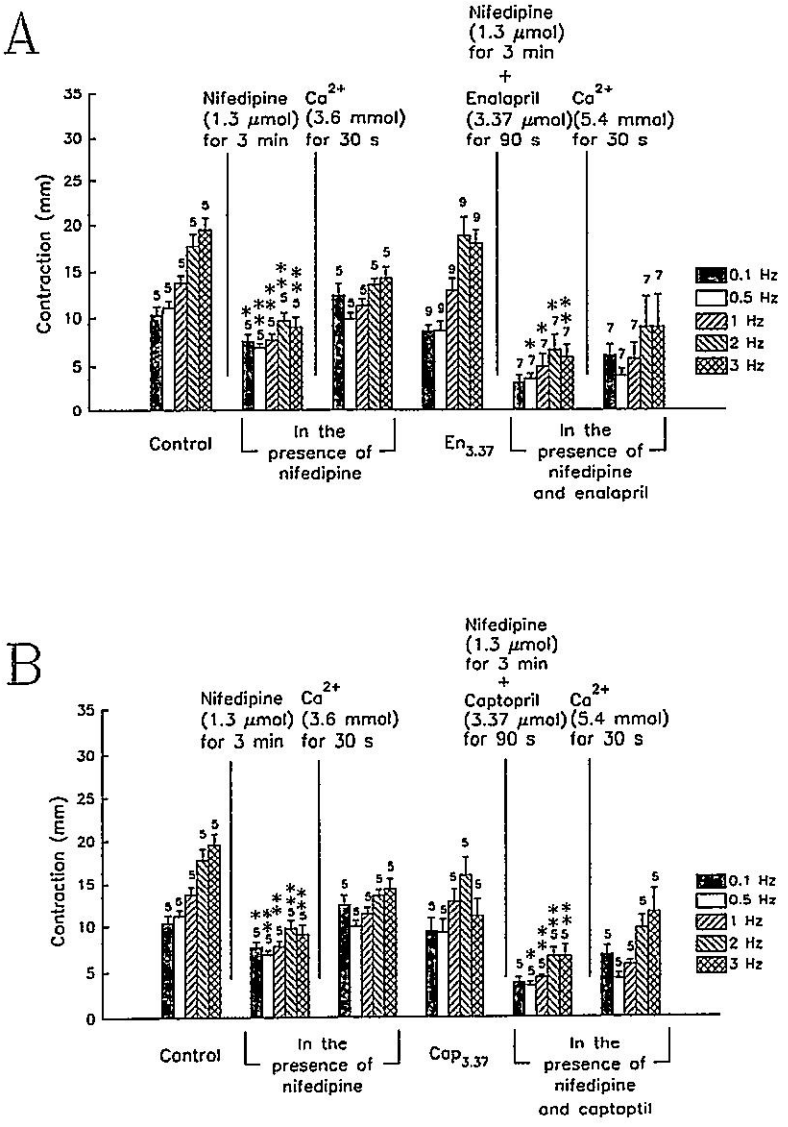

Figure 1. The interaction between nifedipine and enalapril maleate (A) and captopril (B) on the amplitude-frequency relationship (the staircase phenomenon) of the isolated papillary muscle of the rabbit. Each column represents the mean ± s.e.m. (the vertical bars) of 5-7 experiments. * and ** - Significantly different from the corresponding control response ($P < 0.05$ and $P < 0.01$, respectively, Student's *t*-test). Corresponding control responses for the effect of captopril or enalapril maleate are not shown, as well as corresponding control responses for the effect of captopril or enalapril maleate in the presence of nifedipine.

of nifedipine produced a general depression of the response of the papillary muscle by 26-54%. The depression of the isometric contraction was *frequency-dependent*, i.e. the smallest depression was produced with the smallest frequency of stimulation of 0.1 Hz and the highest depression of 54% was produced with the highest frequency of stimulation of 3 Hz.

A two-fold increase in the concentration of calcium (the final concentration of $CaCl_2$ in the bathing medium was 3.6 mmol. l^{-1}) did not completely restore the positive amplitude-frequency relationship.

The effect of captopril and enalapril on the amplitude-frequency relationship. – Both captopril (3.37 μmol. l^{-1}, 90 s) and enalapril maleate (3.37 μmol. l^{-1}, 90 s) *did not qualitatively alter the staircase phenomenon* produced by increasing frequencies of the electrical stimulation (0.1, 0.5, 1 and 2 Hz). Both drugs produced a nonsignificant depression of the response of the papillary muscle to all frequencies of stimulation, the depression produced by captopril being more pronounced. Also, it should be pointed out that although captopril produced depression (statistically nonsignificant) of the response of the papillary muscle to all frequencies of stimulation, the depression of the response to the 3 Hz frequency of stimulation was more pronounced.

The interaction between nifedipine and enalapril maleate – In the presence of nifedipine (1.3 μmol. l^{-1}, 3 min) and enalapril maleate (3.37 μmol. l^{-1}, 90 s) (enalapril maleate was added to the bathing medium 3 min after the addition of nifedipine), the amplitude-frequency relationship was preserved (Figure 1 A).

It should be stressed that in the presence of both drugs in the bathing medium, the staircase phenomenon was significantly depressed in comparison not only with the corresponding control phenomenon (as indicated in Figure 1 A), but also in comparison with the effect of nifedipine (0.1 and 0.5 Hz) and enalapril maleate alone (all frequencies of stimulation) (not indicated in Figure 1 A).

A three-fold increase in the concentration of calcium in the bathing medium did not restore the usual response of the papillary muscle to increasing frequencies of stimulation.

The interaction between nifedipin and captopril. – In the presence of nifedipine (1. 3 μmol. l^{-1}, 3 min) and captopril (3.37 μmol. l^{-1}, 90 s) (captopril was added to the bathing medium 3 min after addition of nifedipine), the amplitude-frequency relationship was completely abolished (Figure 1 B).

Depression of the response of the papillary muscle to almost all frequencies of stimulation was significantly potentiated, both in comparison with the effect of nifedipine alone (0.1, 0.5, 1 and 2 Hz) as well as in comparison with the effect of captopril alone (0.1, 0.5, 1 and 2 Hz) (not indicated in Figure 1 B).

A three-fold increase in the concentration of calcium in the bathing medium (the final concentration of $CaCl_2$ was 5.4 mmol. l^{-1}) did not restore the staircase phenomenon, but the response of the muscle was significantly potentiated in comparison with the effect of nifedipine + captopril (0.1 and 1 Hz).

DISCUSSION

It has already been shown that the ACE inhibitors enalapril and captopril produced a negative inotropic effect on isolated, spontaneously beating guinea-pig atria (Prostran, 1989; Prostran et al., 1989). The depressant action of both enalapril and captopril on the isometric contractility of isolated, spontaneously beating guinea-pig atria may be explained (at least in part) by the effect of these substances on calcium balance (Prostran et al., 1991).

Also, it has been reported that both enalapril and captopril did not qualitatively alter the staircase phenomenon produced by increasing the frequencies of electrical stimulation of the rabbit papillary muscle (Prostran et al., 1991; 1993a).

On the other hand, nifedipine produced a concentration-dependent depression of the isometric contractility and the atrial rate or isolated, spontaneously beating quinea-pig atria (Prostran et al., 1985). Bayer et al. (1981) demonstrated that nifedipine decreases force development in isotonically contracting cat papillary muscle. The negative inotropic effect of nifedipine depends on the concentration of calcium in the bathing medium, being more pronounced at lower levels of calcium.

Nicardipine, another dihydropyridine calcium channel antogonist, has also been found to produce a concentration-dependent depression of the isometric contraction of the isolated papillary muscle of the rabbit (Matić et al., 1989).

Many patients require a combination of more than one drug to control their blood pressure. In spite of the fact that the combination of a converting enzyme inhibitor with either dihydropyridine or a benzothiazepine calcium antagonist is a particularly effective approach to the treatment of patients with more severe essential hypertension (Eber et al., 1992), very little is known about their interactions.

Tabrizchi and Triggle (1992) investigated the interrelationship between the effects of captopril and nifedipine on alpha-mediated vasoconstriction elicited by administration of the full and the partial $alpha_1$ - adrenoceptor agonists (St 587 and cirazoline, respectively) and the $alpha_2$ - adrenoceptor agonist B-HT 920 in pithed normotensive rats.

Treatment with captopril was found to attenuate pressor responses produced by the administration of either $alpha_1$ - or $alpha_2$ - adrenoceptor agonists. The maximum response was unaltered.

Nifedipine displaced the dose-response curves for all three alpha-agonists to the right. Following treatment with nifedipine, however, the maximum responses were significantly reduced.

A combination of captopril and nifedipine did not result in any significant additive increase in the ED_{50} values compared to those obtained with captopril and nifedipine alone. However, the inhibition of the maximum response to B-HT 920 by a combination of captopril and nifedipine was additive.

It has already been found that in the presence of nifedipine in the bathing medium the staircase phenomenon-a very sensitive biological phenomenon-was abolished (Prostran et al., 1993b; 1993c).

In the present experiments, once again, enalapril and captopril (in equimolar concentrations of 3.37 μmol. l^{-1}, 90 s) did not qualitatively alter the staircase phenomenon produced under control conditions by increasing frequencies of the electrical stimulation (0.1, 0.5, 1, 2 and 3 Hz). This means that in the presence of both enalapril and captopril the staircase phenomenon was present, but evidently at a lower level, i.e. the response to each single stimulation frequency was always depressed in comparison with the control response (with no enalapril or captopril in the bathing medium).

The depressive effect of captopril on the staircase phenomenon was slightly more pronounced than the depressive effect of enalapril. It should be noted that in the presence of both enalapril or captopril in the bathing medium, the positive staircase phenomenon was produced by 4 out of 5 frequencies used (0.1, 0.5, 1 and 2 Hz). Under control conditions (with no drug in the bathing medium) the same phenomenon was poduced by all 5 frequencies of electrical stimulation used (0.1, 0.5, 1,2 and 3 Hz).

Once again, in the present experiments nifedipine (1.3 umol. l-1) abolished the same phenomenon already 3 min after its addition to the bathing medium. A two-fold increase of calcium concentration in the bathing medium attenuated the depression of the isometric contractility, but did not restore the phenomenon.

In the presence of nifedipine and captopril (captopril was added to the medium 3 min after addition of nifedipine), the staircase phenomenon was completely abolished. Depression of the response of the papillary muscle to almost all frequencies of the electrical stimulation was significantly potentiated, both in comparison with the effect of nifedipine alone (0.1, 0.5, 1 and 2 Hz) as well as in comparison with the effect of captopril alone (0.1, 0.5, 1 and 1 Hz) (not indicated in Figure 1 B).

In the presence of nifedipine and enalapril (enalapril was added to the medium 3 min after addition of nifedipine), the staircase phenomenon was preserved. Depression of the response of the papillary muscle was significantly potentiated, both in comparison with the effect of nifedipine alone (0.1 and 0.5 Hz) as well as in comparison with the effect of enalapril alone (all frequencies of stimulation).

Our results indicate that only enalapril possesses protective action against nifedipine-induced changes of the staircase phenomenon. Also, our results indicate a possible implication of calcium on the depressive effects of both enalapril and captopril on the electrically paced heart muscle of the rabbit. It has been found that captopril, a suphydril ACE inhibitor, reduced contractile force in guinea-pig isolated hearts (Walker et al., 1988), an effect which in isolated ventricular cells has been attributed to an inhibition of Ca^{2+} entry through voltage-dependent L-type channels, $I_{Ca,L}$ (Bryant et al., 1991). However, it is unknown whether this negative inotropic effect is related or not to the chemical structure (-SH) of captopril.

On the other hand, it has been indicated (Valenzuela et al., 1993) that the negative inotropic effect of lisinopril, a non sulphydryl ACE inhibitor, cannot be explained by a decrease in Ca^{2+} entry through L-type calcium channels. These authors suggested that lisinopril may possibly act at an intracellular site to reduce contractile force.

These results, as well as our results, are in agreement with the data obtained by Gill et al. (1992) who also found that ACE inhibitors (particularly lisinopril) may possess calcium channel/calcium mobilization blocking properties.

Also, it seems that the staircase phenomenon is a very sensitive biological response when studying the action of dihydropyridine calcium channel blockers as well as their interactions with ACE inhibitors on the heart muscle.

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EFEKT INHIBITORA ANGIOTENZIN-KONVERTIRAJUĆEG ENZIMA ENALAPRILA I KAPTOPRILA NA ODGOVOR IZOLOVANOG PAPILARNOG MIŠIĆA KUNIĆA PREMA NIFEDIPINU

MILICA PROSTRAN, IVANA MATIĆ, Z. TODOROVIĆ, MILANKA JEZDIMIROVIĆ, M. Š. PROSTRAN, ZORANA VASILJEVIĆ i V. M. VARAGIĆ

SADRŽAJ

U ovom radu izučavana je interakcija između nifedipina, dihidropiridinskog blokatora kalcijumskih kanala i enalaprila i kaptoprila, inhibitora angiotenzin-konvertirajućeg enzima na izolovanom, električki stimulisanom papilarnom mišiću kunića.

U prisustvu nifedipina (1.3 μmol. l^{-1}, 3 min) fenomen stepenica više ne postoji. Ta koncentracija nifedipina je dovela do univerzalne, frekvencija-zavisne depresije odgovora papilarnog mišića na električnu stimulaciju od 26-54%.

Enalapril i kaptopril (3.37 μmol. l^{-1}, 90 s) ne menjaju kvalitativno fenomen stepenica. To znači da u prisustvu bilo kojeg od ova dva leka, fenomen stepenica postoji, ali je deprimiran, tj. odgovor mišića na svaku pojedinačnu frekvenciju stimulacije je snižen u odnosu na kontrolne uslove (bez lekova u kupatilu).

U prisustvu nifedipina i kaptoprila fenomen stepenica ne postoji, ali u prisustvu nifedipina i enalaprila taj fenomen je sačuvan.

Naši rezultati ukazuju da je kalcijum uključen u depresivno dejstvo enalaprila i kaptoprila na izolovanom, električki stimulisanom papilarnom mišiću kunića. Takođe, naši rezultati sugerišu da samo enalapril poseduje zaštitno dejstvo protiv promena fenomena stepenica izazvanih nifedipinom.