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COMPARATIVE EFFECTS OF NICARDIPINE AND VERAPAMIL ON THE STAIRCASE PHENOMENON IN THE ISOLATED PAPILLARY MUSCLE OF THE RABBIT

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In the isolated papillary muscle of the rabbit, stimulated by increasing frequencies of stimulation (0.1, 0.5, 1 and 2 Hz), the staircase phenomenon was produced in which higher frequencies of stimulation produced higher amplitudes of isometric contraction. In the presence of nicardipine (13 μmol. l^{-1}) the positive amplitude-frequency relationship was completely blocked. A three-fold increase in the concentration of calcium in the bathing medium did not restore the positive staircase phenomenon.

The positive pause-amplitude relationship was not affected by nicardipine (13 μmol. l^{-1}). An increase in the external calcium did not change this type of response qualitatively, but only quantitatively.

A sudden change in the frequency of stimulation produced a quick increase or decrease of the amplitude of contraction, depending on the frequency of stimulation. Nicardipine (13 μmol. l^{-1}) blocked this type of response.

Verapamil (13μmol. l^{-1}) produced a reversal of the positive staircase phenomenon into a negative one. An increase in the external calcium did not reverse this effect of verapamil. The pause-amplitude relationship was not significantly changed by verapamil.

Our results indicate that verapamil and nicardipine act on different types of calcium channels in the papillary muscle of the rabbit.

Key words: nicardipine, verapamil, the staircase phenomenon, papillary muscle

INTRODUCTION

Movements of calcium ions are known to be involved in excitation-contraction coupling in the mammalian heart musle. This is evident from studies of inotropic actions of drugs in various experimental models, such as steady-state strength-interval relationships, the staircase phenomenon, contractile transients, aftercontractions and postextrasystolic potentiation.

More over, calcium antagonists are known to inhibit the slow inward calcium current across the excited cardiac muscle cells and smooth muscle cells (Reuter, 1973). It has been already shown that verapamil and D 600, both calcium channel blockers, significantly affect the staircase phenomenon in the isolated cat papillary muscle (Bayer et al., 1975). Nicardipine, a dihydropyridine calcium channel blocking agent, differs from verapamil, not only in chemical structure, but also in some pharmacological characteristics.

It was therefore of interest to study the action of nicardipine on the staircase phenomenon of the isolated rabbit papillary muscle and to compare its effect with that of verapamil.

MATERIAL AND METHODS

The papillary muscle was taken from rabbits of both sexes (1.6 to 2.8 kg). The muscle was dissected from the left ventricle and prepared for recording according to the method of Schüman et al. (1974). The preparation was mounted in an isolated organ bath of 15 ml capacity, containing Tyrode solution of the following composition (in mmol. l^{-1}): NaCl 136. 7, KCl 2.81, $CaCl_2$ 1.8, MgC_2 0.105, NaH_2PO_4 0.417, $NaHCO_3$ 11.9 and sucrose 11.1. The Tyrode solution was oxygenated with a mixture of oxygen (97%) and carbon dioxide (3%). The temperature of the bath was kept around 37^0C.

The initial loading of the isolated papillary muscle was between 0.13-0.18 g. Electrical stimulation was carried out using square wave pulses of 0.4 ms duration and the following frequencies: 0.1, 0.5, 1, 2 and 3 Hz. The voltage output was usually kept at about 20% above the threshold. The electrodes (for direct electrical stimulation) were made of palladore (30% palladium and 70% silver). The isometric contractions of the muscle were recorded by a microdynamometer 7001 (Ugo Basile) and displayed on paper moving at various speeds (for more details see Matić et al., 1989; 1992).

The equilibrium of the muscle lasted 60 min. The first three or four (0.1, 0.5, 1, 2 and 3 Hz) of five frequencies usually produced a typical staircase phenomenon in which a higher frequency produced a higher amplitude of the isometric contraction of the muscle. Stimulation with one frequency lasted for 20 s, after which period a higher frequency was immediately switched on. The same procedure was repeated in the presence of calcium antagonists (nicardipine or verapamil).

In a separate series of experiments, continuous direct electrical stimulation (1 Hz) was stopped for 2, 4 and 8 s, respectively, and then a single electrical stimulation was applied using the same frequency (1 Hz).

Another type of stimulation was also applied in which continuous stimulation with 0.1 Hz was suddenly changed to 1 Hz, and *vice versa.*

In order to evaluate the role of calcium in the responses of the papillary muscle to direct electrical stimulation, the concentrations of calcium in the bathing medium were alternatively changed from 0.9 to 5.4 mmol. l^{-1}.

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

The following substances were used: nicardipine hydrochloride (Lek, Ljubljana), verapamil hydrochloride (Lek, Ljubljana) and calcium chloride (Merck).

RESULTS

The amplitude-frequency relationship.- The increasing frequencies of stimulation (0.1, 0.5, 1 and 2 Hz) in the majority of experiments produced a staircase phenomenon in which higher frequencies of stimulation produced higher amplitudes of the isometric contractions of the isolated papillary muscle. Meanwhile, a frequency of 3 Hz did not produce any further increase in the amplitude of contractions, but rather a decrease. Thus, the typical staircase phenomenon was limited to a range of frequencies between 0.1 and 2 Hz. The results are shown in Figure 1a.

The effect of nicardipine on the amplitude-frequency relationship.- In the presence of nicardipine (13 μmol). l^{-1}) the positive amplitude-frequency relationship was completely blocked. This concentration of nicardipine produced a universal depression of the response of the papillary muscle by about 19-65 per cent, no matter which frequency of stimulation was used. In other words, in the presence of 13 μmol. l^{-1} nicardipine, all the frequencies used (0.1, 0.5, 1 and 2 Hz) produced almost the same amplitude of contractions.

A three-fold increase in the concentrations of calcium in the bathing medium did not restore the positive amplitude-frequency relationship. Instead, an almost identical increase in the amplitude of contractions was obtained after all frequencies of stimulation (Figure 1a).

The effect of nicardipine on the pause-amplitude relationship. – A constant stimulation with a frequency of 1 Hz, but after 2, 4 and 8 seconds pause, respectively, produced the staircase phenomenon in which the highest amplitude was recorded after a longer duration of pause. This positive pause-amplitude relationship was not affected by nicardipine (13 μmol. l^{-1}).

If stimulation was done in the presence of both nicardipine and a three-fold increased amount of calcium, the response was not affected qualitatively but quantitatively. The positive pause-amplitude relationship was stil present, but the responses after all pauses were potentiated. The level of statistical significance (in comparison with the control value) was reached after 8 s pause, as shown in Figure 1b.

The effect of nicardipine on the sudden step changes in the frequency of stimulation. – In a preparation continuously stimulated with 0.1 Hz, a sudden change to 1 Hz produced a significant increase in the amplitude of contractions. In the same way, a sudden change from 1 Hz to 0.1 Hz produced a reversal to the control values of amplitude (Figure 1c).

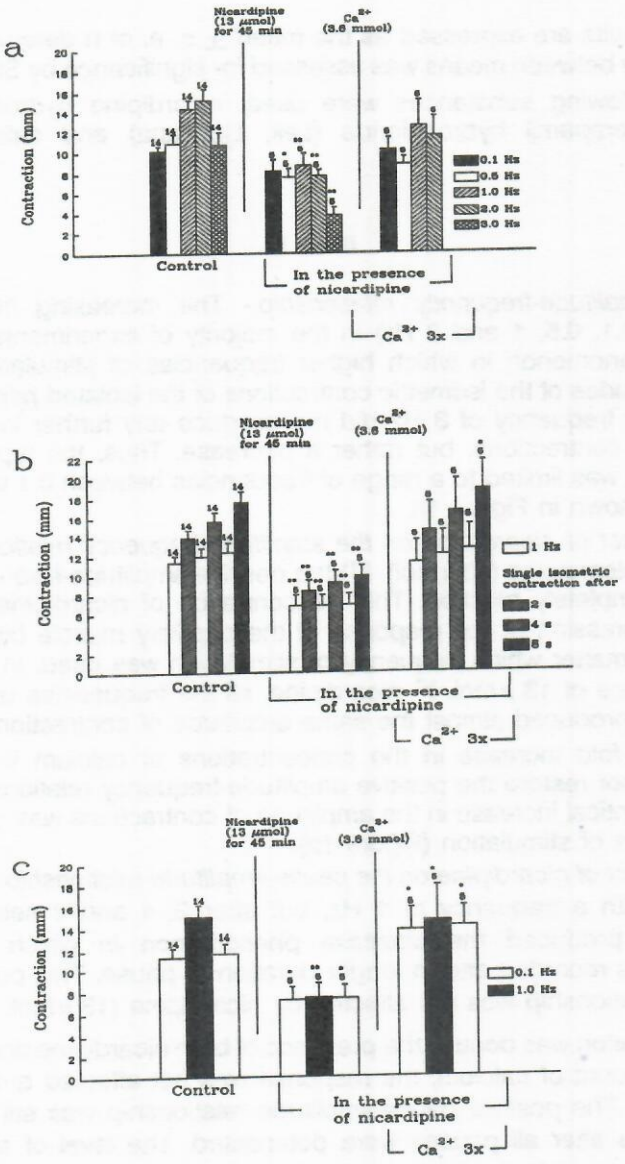

Figure 1. a. The effect of nicardipine on the amplitude-frequency relationship (the staircase phenomenon) of the isolated papillary muscle of the rabbit. Each column represents the mean ± s.e.m. (the vertical bars) of 5-14 experiments. * and ** – Significantly different from the corresponding control responses ($P < 0.05$ and 0.005, respectively, Student's *t*-test). b. The effect of nicardipine on the single isometric contraction of the isolated papillary muscle of the rabbit elicited after 2, 4 and 8 s pause, respectively, during continuous stimulation with 1 Hz frequency. c. The effect of nicardipine on the isometric contraction of the isolated papillary muscle of the rabbit after sudden step changes in the frequency of stimulation (0.1 Hz to 1 Hz, and back to 0.1 Hz).

In the presence of nicardipine (13 μmol. l^{-1}, for 45 min), there was no change in the amplitude of contractions when the stimulation frequency was suddenly changed to 1 Hz from 0.1 Hz. A three- fold increase in the concentration of calcium in the bathing medium did not restore the usual response of the papillary muscle to a sudden change to higher frequency of stimulation.

Nevertheless, in the presence of a three-fold increased concentration of calcium, the responses to both 0.1 Hz and 1 Hz stimulation were significantly increased, as also shown in Figure 1c (right columns).

The effect of verapamil on the amplitude-frequency and pause-amplitude relationship. – Verapamil has already been found to reverse the positive staircase phenomenon into a negative one, and to produce a significant potentiation of the papillary muscle response after a shortlasting resting interval, i. e. to cause a positive pause-amplitude relationship (Bayer et al., 1975). The same authors have also found that verapamil leaves contraction amplitudes nearly unchanged at 6/min stimulation frequency, whereas at 60/min stimulation frequency more than 90% depression occurred.

Our experimental set up was different to some extent from those described by Bayer et al. (1975), but the results were basically the same.

This series of experiments had to be done in order to compare nicardipine and verapamil, two calcium channel blockers from different chemical groups.

It was found that verapamil, in a concentration equimolar to that of nicardipine (13 μmol. l^{-1}) produced a reversal of the positive staircase phenomenon into a negative one. Increasing the calcium concentration in the bathing medium qualitatively did not change the effect of verapamil. Instead, calcium produced an increase in the amplitudes as a response to all frequencies of stimulation (Figure 1a).

The same concentration of verapamil did not affect the response of the papillary muscle to 0.1 Hz frequency of stimulation, but highly depressed the response to 2 Hz stimulation frequency. Once again, a three-fold increase in the concentration of calcium in the bathing medium, did not reverse this effect of verapamil.

The single isometric contraction of the papillary muscle elicited after 2, 4 and 8 s pause, respectively, during continuous electrical stimulation with 1 Hz frequency (for 20 s) was not qualitatively changed by verapamil (13 μmol. l^{-1}). On the contrary, the responses after all pauses were significantly increased. This effect of verapamil was not changed by two – and three-fold increase of the calcium concentration in the bathing medium (Figure 2b).

The effect of verapamil on the sudden step changes in the frequency of stimulation. – In a continuously stimulated preparation with 0.1 Hz, sudden change to 1 Hz produced a significant increase in the amplitude of isometric contractions. In the same way, a sudden change from 1 Hz to 0.1 Hz produced a reversal to the control values of amplitude (Figure 2c). In the presence of verapamil (13 μmol. l^{-1}, for 45 min), a sudden change of the stimulation frequency

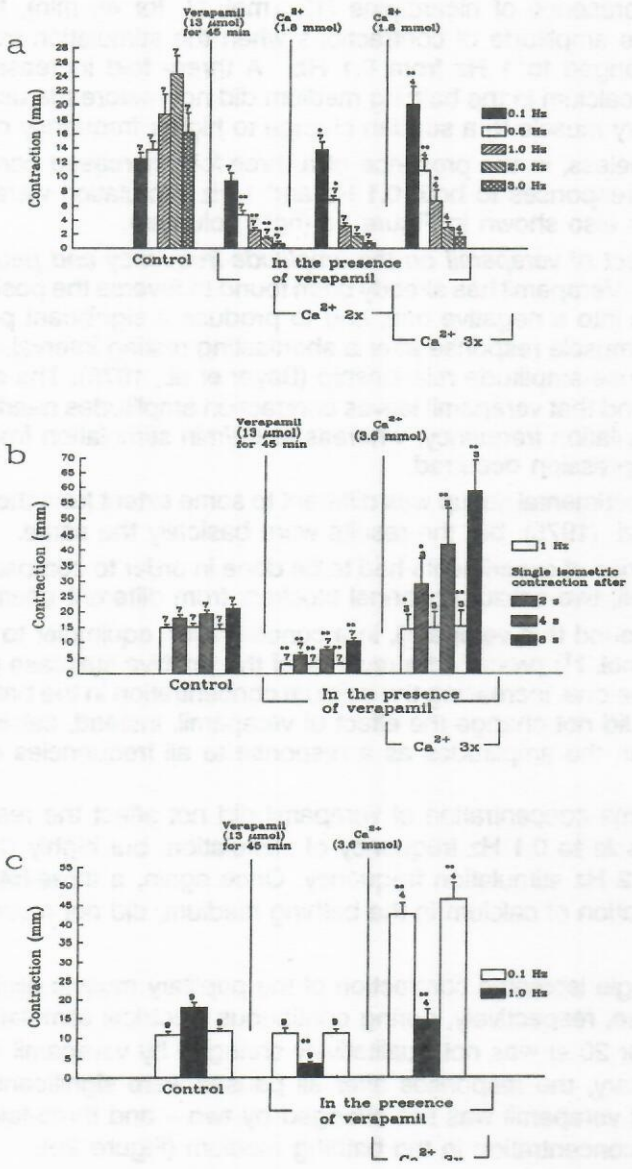

Figure 2. a. The effect of verapamil on the staircase phenomenon of the isolated papillary muscle of the rabbit. Calcium chloride was added twice. b. The effect of verapamil on the pause-amplitude relationship of the isolated papillary muscle of the rabbit. c. The effect of verapamil on the isometric contractions of the isolated papillary muscle of the rabbit after sudden step changes in the frequency of stimulation (from 0.1 Hz to 1 Hz, and back to 0.1 Hz).

from 0.1 to 1 Hz produced a significant depression of the isometric contraction of the isolated papillary muscle of the rabbit. A two- or three- fold increase in the concentration of calcium did not restore the usual response of the papillary muscle to a sudden change to higher frequency of stimulation.

DISCUSSION

The positive amplitude-frequency relationship, better known as the staircase phenomenon, has been described as an inherent characteristic of cardiac muscle. This phenomenon has never been adequately explained.

It was found in the present experiments that a stepwise range of frequencies of stimulation (0.1, 0.5, 1 and 2 Hz) usually produced a staircase-like increase in the amplitude of the isometric contraction of the isolated electrically stimulated papillary muscle of the rabbit. The highest frequency used in our experiments (3 Hz) did not produce a further increase in the amplitude of contraction, but rather a decrease, as compared to the frequencies of 1 and 2 Hz.

The importance of calcium in the excitation-contraction coupling in the heart muscle has been well documented (Siegl, 1986). It is known that the positive inotropic responses observed in the staircase phenomenon are associated with an increase in calcium uptake into the cardiac muscle cell (Haacke et al., 1970). Increased influx of calcium into the cardiac muscle cell is also possible by an indirect way, i. e. by alterations in sodium and potassium fluxes (Blesa et al., 1970). It may be, therefore, suggested that the increasing rates of stimulation of the papillary muscle produce higher concentrations of calcium at the critical sites, thus enabling the cardiac muscle to respond with an increased amplitude of the isometric contraction.

This suggestion is supported by previous findings that verapamil, a calcium channel blocking agent, produced a reversal of the positive staircase phenomenon into a negative one (Bayer et al., 1975) and also by the results of the present experiments in which nicardipine, a dihydropyridine calcium channel antagonist, blocks the positive amplitude-frequency relationship. In the presence of nicardipine (13 μmol. l^{-1}), all the frequencies which, under control conditions, produce the positive staircase phenomenon, now exhibit almost equal amplitudes of contractions.

A two-or three-fold increase in the calcium concentrations in the bathing medium does not restore the normal amplitude-frequency relationship. Actually, only a shift toward higher amplitudes of the isometric contraction was observed after all frequencies of stimulation used. This was also observed both after treatment with verapamil and D 600 (Bayer et al., 1975).

The presumed increased availability of calcium produced by stepwise increase in the frequency of stimulation seems to occur very quickly, and, vice versa, restitution of the lower amounts of available calcium also occurs very quickly. This is evident from the sudden step changes in the frequency of stimulation from 0.1 Hz to 1 Hz, and vice versa. Under control conditions, the

sudden change of the stimulation frequency from 0.1 Hz to 1 Hz produced an almost instantaneous increase in the amplitude of contraction. If the sudden change of frequency was made the other way round, reversal to lower amplitude also occurs very quickly. Occasionally, this period of restitution of lower amplitudes might be prolonged for several seconds.

In the presence of nicardipine (13 μmol. l^{-1}) the positive amplitude-frequency relationship caused by the sudden change to higher frequency was completely blocked. This type of response to nicardipine is different from the effect of verapamil under similar experimental conditions.

Bayer et al. (1975) found that in the presence of verapamil a sudden increase in the stimulation frequency produced a drastic decrease in the amplitude of contraction. This difference between verapamil and nicardipine can be explained either by different kinetic characteristics while acting on the calcium channels, or by a difference in the mechanism of action on various types of calcium channels. Dihydropyridine calcium channel blockers have already been found to show little dependence on the frequency of stimulation (Murad, 1990), which is quite different from verapamil.

The positive pause-amplitude relationship, produced by constant stimulation with 1 Hz, but after 2, 4 and 8 s pause, respectively, was not qualitatively affected by nicardipine or verapamil. Doubling or even trebling of calcium in the bathing medium did not change, either the response to nicardipine or verapamil, or the usual positive pause-amplitude relationship. The only effect of calcium observed was a shift towards higher amplitudes of contraction. The positive pause-amplitude relationship, also called post-rest stimulation, most probably depended on the intracellular source of calcium (Siegl, 1986) because it was not altered by the calcium channel blocking agents (Lewartowski et al., 1978). Our finding with nicardipine is in agreement with the previous findings.

The most interesting finding in the present experiments was a striking difference between nicardipine and verapamil on the various patterns of amplitude-frequency relationship. It has already been found that cardiac myocytes contain L and T subtypes of calcium channels (Nilius et al., 1986). L channels are strongly blocked by either inorganic (e. g. cadmium) or organic (verapamil, diltiazem, nifedipine) calcium antagonists. On the other hand, T channels are relatively insensitive to the organic and most inorganic calcium antagonists, but they are blocked by nickel (Nowicky et al., 1985). It is easier to hypothesize than to explain the possibility that verapamil and nicardipine act on different types of calcium channels. Still, it has not been determined whether the various representatives of the organic calcium antagonists prefer any of the calcium channels known so far (Nayler, 1988).

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UPOREDNI EFEKTI NIKARDIPINA I VERAPAMILA NA FENOMEN STEPENICA U IZOLOVANOM PAPILARNOM MIŠIĆU KUNIĆA

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SADRŽAJ

U izolovanom papilarnom mišiću kunića, stimulisanom sa 0.1, 0.5, 1 i 2 Hz, proizveden je tipičan fenomen stepenica u kome više frekvencije prouzrokuju više amplitude izometrijske kontrakcije. U prisustvu nikardipina (13 μmol. l^{-1}) pozitivan odnos između amplitude i frekvencije stimulacije bio je kompletno blokiran. Trostruko povećanje koncentracije kalcijuma u medijumu ne uspostavlja pozitivni fenomen stepenica.

Pozitivan odnos između pauze i amplitude se ne menja pod dejstvom nikardipina (13 μmol. l^{-1}). Povećanje kalcijuma u medijumu ne utiče na ovaj efekt kvalitativno, već samo kvantitativno.

Brza promena u frekvenciji stimulacije prouzrokuje brzo povećanje ili smanjenje amplitude kontrakcija, zavisno od frekvencije stimulacije. Nikardipin blokira ovaj tip odgovora.

Verapamil (13 μ mol. l^{-1}) preobraća pozitivni fenomen stepenica u negativni. Povećanje koncentracije kalcijuma u medijumu ne otklanja ovaj efekt verapamila. Odnos između pauze i amplitude se ne menja značajno pod dejstvom verapamila.

Dobijeni rezultati ukazuju na mogućnost da verapamil i nikardipin deluju na različite tipove kalcijumskih kanala u papilarnom mišiću.