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Stimulation rate modulates effects of the dihydropyridine CGP 28 392 on cardiac calcium-dependent action potentials

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1 Calcium (Ca^{2+})-dependent action potentials were recorded from 22 mM potassium (K^+)-depolarized guinea-pig papillary muscle at several different pacing frequencies in the absence and presence of CGP 28 392 (10 μM), a Ca^{2+} channel agonist. The maximum upstroke velocity ($\dot{V}_{max}$) of the slow response action potential was measured to determine relative changes in Ca^{2+} current as a function of pacing frequency.

2 CGP 28 392 increased $\dot{V}_{max}$ more than two fold at low rates of stimulation (1 or 12 pulses min^{-1}), but had no significant effect on $\dot{V}_{max}$ during rapid pulsing (200 pulses min^{-1}).

3 The enhancement of $\dot{V}_{max}$ was dependent upon extracellular [K^+]. Increasing extracellular [K^+] from 22 mM to 27 mM suppressed the frequency-dependent agonist effects and increased the antagonist effects on $\dot{V}_{max}$.

4 These results indicate that CGP 28 392 is a partial Ca^{2+}-channel agonist and suggest that its effects on Ca^{2+} current are voltage-dependent.

Introduction

Dihydropyridine calcium (Ca^{2+}) antagonists have been shown to block the movement of Ca^{2+} through voltage-sensitive channels of a diverse group of cell types, including smooth muscle (Karaki & Weiss, 1984), cardiac muscle (Lee & Tsien, 1983) and several cultured cell lines (Freedman & Miller, 1984). Ca^{2+} current block by the dihydropyridines is modulated by voltage, as evidenced by the finding that block by nitrendipine and nisoldipine is enhanced at depolarized potentials (Sanguinetti & Kass, 1984a).

The discovery of structural analogues of these dihydropyridines (Schramm *et al.*, 1983) that compete for common high affinity binding sites (Janis *et al.*, 1984; Vaghy *et al.*, 1984a,b), yet enhance rather than block Ca^{2+} current (e.g. Bay K 8644, CGP 28 392) has led to speculation about basic mechanisms that regulate Ca^{2+}-channel gating (Hess *et al.*, 1984). Patch-clamp studies of isolated cardiac cells have demonstrated that enhancement of Ca^{2+}-channel current by Bay K 8644 (Hess *et al.*, 1984; Ochi *et al.*, 1984) and CGP 28 392 (Kokubun & Reuter, 1984) results from a drug-induced prolongation of single channel open time and not from an increase in amplitude of unitary channel openings.

It is now quite apparent that many of the dihydropyridines should be referred to as partial agonists. For example, low concentrations of the 'Ca^{2+} antagonists' nifedipine and nitrendipine actually increase the contractility of cat papillary muscle (Strauer, 1974) and of guinea-pig isolated perfused hearts (Thomas *et al.*, 1984). Furthermore, high concentrations of the 'Ca^{2+} agonist' Bay K 8644 decrease contractility of isolated hearts (Thomas *et al.*, 1984) and inhibit $^{45}Ca^{2+}$ influx into cultured cells (Freedman & Miller, 1984). In addition, the modulation of Ca^{2+} current by Bay K 8644 is voltage-dependent. In voltage-clamped cardiac Purkinje fibres Bay K 8644 (0.2–2.0 μM) acts as a Ca^{2+}-channel agonist when clamp pulses are applied from holding potentials negative to approximately −50 mV, but rapidly reverses to an antagonist at more depolarized holding potentials (Sanguinetti & Kass, 1984b). The agonist effect of Bay K 8644 on Ca^{2+} current can also be reversed by rapid pulsing (Sanguinetti & Kass, 1985). Thus, Bay K 8644 can act as either an agonist or antagonist at a given concentration depending upon cellular membrane potential.

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The full range of effects on cardiac Ca^{2+} current produced by a second novel dihydropyridine, CGP 28 392, have not been described. Therefore, we have studied the effect of CGP 28 392 on slow response action potentials recorded from K^+-depolarized papillary muscles including protocols designed to enhance any possible Ca^{2+} antagonism.

Methods

Papillary muscles were isolated from right ventricles of guinea-pigs (250–350 g, of either sex) and placed in a temperature-controlled (37°C) tissue bath superfused with Tyrode solution of the following composition (mM): NaCl 127, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.43, $NaHCO_3$ 23.8, glucose 5.5. After equilibration in normal solution, the tissue was exposed to a Tyrode solution containing 22 or 27 mM KCl, with an equimolar reduction in NaCl plus 0.5 mM $BaCl_2$. The muscles were field stimulated at a pulse duration of 5 ms with a pair of platinum foil electrodes. The intensity of stimulation was adjusted to maintain a constant delay between the stimulus artifact and the peak dV/dt ($\dot{V}_{max}$) of the slow response action potential upstroke. Standard intracellular electrical recording techniques were used (Sanguinetti & West, 1982) and a computer (DEC, PDP 11/23) was used to make on-line measurements of $\dot{V}_{max}$ and action potential duration at 50% (APD_{50}) and 90% (APD_{90}) of full repolarization. Membrane voltage and dV/dt (analog) signals were displayed on a storage oscilloscope and photographed with an oscilloscope camera.

After a 1 h equilibration period in the high K^+-Tyrode solution, the muscle was stimulated at rates of 1, 12, 30, 60, 120 and 200 pulses min^{-1}. Records were taken of $\dot{V}_{max}$, APD_{50} and APD_{90} after steady-state conditions were reached at each stimulation rate. The tissue was then exposed to 10 μM CGP 28 392 for 50 min before repeating the same measurements. A single continuous electrode impalement was maintained throughout each experiment.

CGP 28 392 4-[2-(difluormethoxy) phenyl]-1,4,5,7-tetrahydro-2- methyl-5-oxofuro [3,4-6] byridine-3 car-

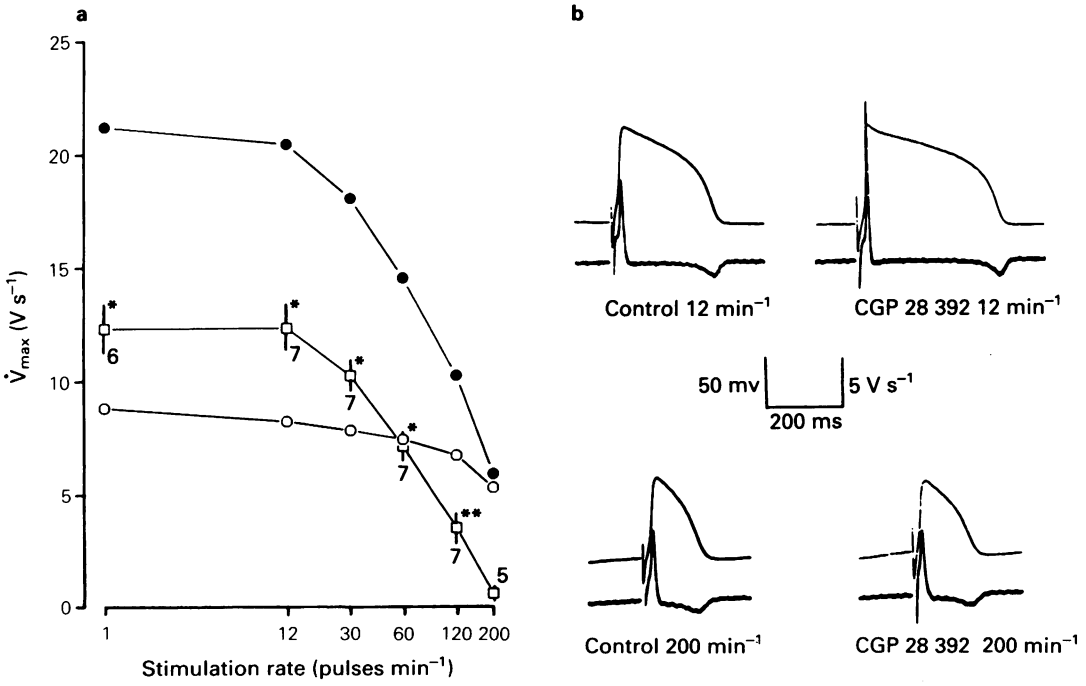

Figure 1 Rate-dependent changes in the maximum upstroke velocity ($\dot{V}_{max}$) of slow response action potentials after exposure of papillary muscles to CGP 28 392. (a) The average $\dot{V}_{max}$ of 7 preparations before (○) and after exposure to 10 μM CGP 28 392 (●), plotted as a function of stimulation rate (logarithmic scale). The mean difference (± s.e.mean) between paired control and drug observations are shown (□). The number of paired observations is indicated for each stimulation rate. (b) The action potentials and dV/dt recorded from a single cell during steady-state stimulation at 12 pulses min^{-1} and 200 pulses min^{-1} before and after 10 μM CGP 28 392. * $P < 0.0005$, ** $P < 0.005$.

boxylic acid ethylester was dissolved in polyethylene glycol 400 to make a 10 mM stock solution. Appropriate aliquots of this solution were added directly to the Tyrode solution to obtain the final desired concentrations. The concentration of polyethylene glycol 400 present had no effect on $\dot{V}_{max}$, APD_{50}, or APD_{90}. Data are expressed as means ± s.e.mean (n = number of preparations). Statistical significance was determined where appropriate using the paired Student's t test.

Results

Exposure of the papillary muscles to 22 mM extracellular K^+ depolarized the membrane potential from approximately − 85 mV to − 50 ± mV (n = 7).

In the absence of drug, a rate-dependent reduction in $\dot{V}_{max}$ was observed at stimulation rates greater than 30 pulses min^{-1}, such that at 200 pulses min^{-1} $\dot{V}_{max}$ was 60% of that measured when the muscle was pulsed at a rate of 1 pulse min^{-1} (Figure 1a). An increase in stimulation rate also reduced both APD_{50} and APD_{90} (Table 1).

After the control records were obtained, the stimulation rate was set at 12 pulses min^{-1} during exposure to 10 μM CGP 28 392. After a 10 min exposure to CGP 28 392, the stimulation threshold voltage decreased approximately 65%, and the $\dot{V}_{max}$ increased two fold. Steady-state conditions (no further change in $\dot{V}_{max}$, APD_{50} or APD_{90}) were obtained after about 40 min, and the effects of stimulation frequency were again determined after a total of 50 min exposure to the drug.

The average data for $\dot{V}_{max}$ from 7 experiments are shown in Figure 1a, which illustrates that CGP 28 392 markedly enhances $\dot{V}_{max}$ at low rates of stimulation, but has almost no effect at a stimulation rate of 200 pulses min^{-1}. Figure 1b shows representative action potentials and dV/dt recorded while pacing the tissue at a low and a high stimulation rate, before and after the addition of CGP 28 392. In the presence of the drug, APD_{50} and APD_{90} were significantly prolonged at all rates of stimulation except at 200 pulses min^{-1}. As observed with effects on $\dot{V}_{max}$, the magnitude of the drug effects on APD_{50} and APD_{90} was inversely related to stimulation frequency (Table 1).

In one experiment nifedipine (1 μM) was added to a preparation exposed to 10 μM CGP 28 392 for 50 min. Nifedipine, in the continued presence of CGP 28 392, caused a 50% decrease in $\dot{V}_{max}$ after 8 min, and completely blocked the slow response in 24 min when the stimulation rate was kept constant at 12 pulses min^{-1}.

We next determined the effect of extracellular $[K^+]$ on the changes in slow response action potentials caused by CGP 28 392. The influence of stimulation rate on $\dot{V}_{max}$ was determined in a muscle depolarized by 22 mM extracellular K^+ and then repeated after equilibration in 27 mM K^+ Tyrode solution. In the absence of drug, increasing extracellular $[K^+]$ from 22 mM to 27 mM caused an additional 10 mV depolarization and an increase in $\dot{V}_{max}$ from 6.7 V s^{-1} to 7.5 V s^{-1} at 1 pulse min^{-1}, but it caused a greater rate-dependent depression of $\dot{V}_{max}$ (Figure 2). The preparation was then exposed to 10 μM CGP 28 392 (in 22 mM K^+-Tyrode) for 50 min. As shown previously, in 22 mM K^+-Tyrode solution, CGP 28 392 increased $\dot{V}_{max}$ maximally (186%) at a stimulation rate of 1 pulse min^{-1}, and much less so at higher frequencies (Figure 2a). In contrast, raising the extracellular $[K^+]$ to 27 mM in the presence of CGP 28 392 resulted in only a 55% increase of $\dot{V}_{max}$ over control at 1 pulse min^{-1}, and caused a decrease in $\dot{V}_{max}$ compared to control at 120 and 200 pulses min^{-1} (Figure 2b).

Table 1 Effect of CGP 28 392 (10 μM) on APD_{50} and APD_{90} (mean average) of slow response action potentials at different rates of stimulation

		Stimulation rate (pulses min^{-1})					
		1	*12*	*30*	*60*	*120*	*200*
	Control	201	183	160	133	109	82
APD_{50}	CGP	261	233	189	145	110	82
(ms)	Δ	60 ± 9.6**	50 ± 7.7*	29 ± 5.8**	12 ± 4.7†	1 ± 4.2	0 ± 5.0
	Control	241	227	207	180	151	124
APD_{90}	CGP	307	279	237	194	161	127
(ms)	Δ	66 ± 8.9*	52 ± 6.8*	30 ± 4.9*	14 ± 3.9†	10 ± 4.3†	3 ± 3.8
n		6	7	7	7	7	5

APD_{50} and APD_{90} = action potential duration at 50% and 90% of full repolarization respectively.
The difference between control and drug treated (Δ) is expressed as the mean ± s.e.mean for n preparations.
* $P < 0.0005$, ** $P < 0.005$, † $P < 0.05$.

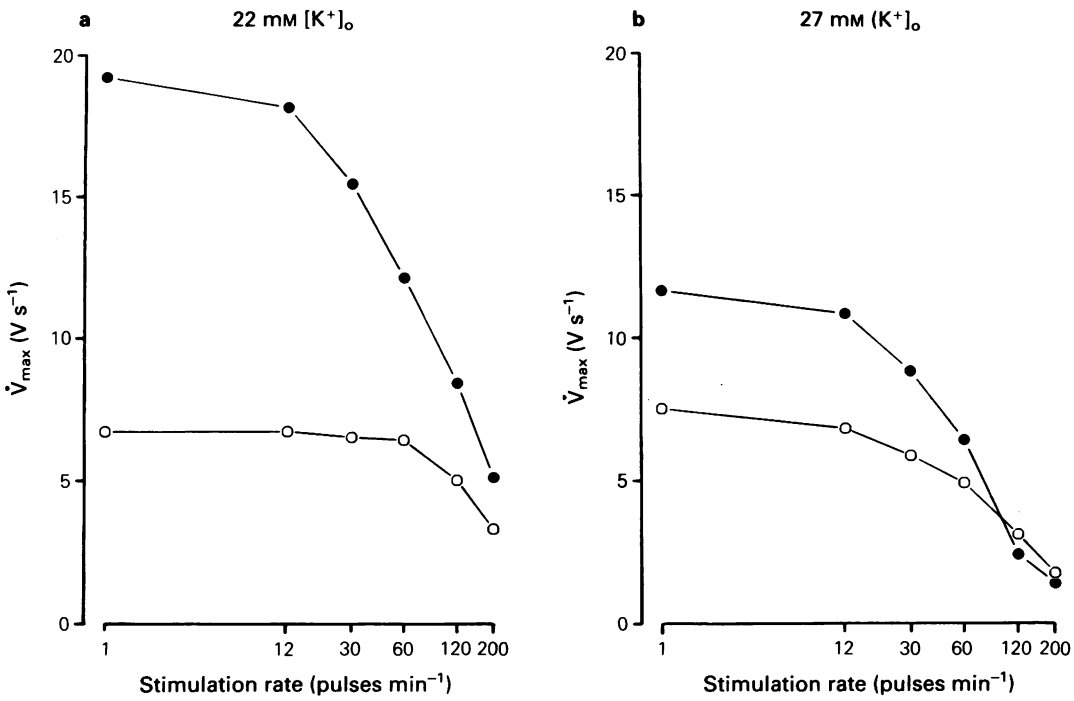

Figure 2 Effect of CGP 28 392 on rate-dependent changes in the maximum upstroke velocity ($\dot{V}_{max}$); influence of extracellular $[K^+]$. (a) The $\dot{V}_{max}$ before (○) and after 10 μM CGP 28 392 (●) when the extracellular $[K^+]$ was 22 mM. (b) Shows the corresponding data obtained during the same impalement as in (a), but with an extracellular $[K^+]$ of 27 mM.

Discussion

Slow response action potentials under certain conditions showed definite enhancement by CGP 28 392. However, under other conditions there is actually a depression of the slow response action potential by this compound. For example, at low stimulation rates in 22 mM K^+ the greatest enhancement by CGP 28 392 was observed. In more depolarized tissue (27 mM K^+) the enhancement was substantially less at the low stimulation rates and in addition, the highest stimulation rates tested resulted in a depression of the slow response action potential. Thus, our results show that CGP 28 392, at a fixed concentration, can either enhance or depress slow response action potentials depending upon the rate of stimulation and the extracellular $[K^+]$, the effects of the latter being perhaps secondary to the further depolarization induced. The normal heart rate of guinea-pigs is higher than the highest stimulation rate used in this experiment but unfortunately, higher stimulation rates were not possible under the given experimental conditions. However, this does not necessarily imply that CGP 28 392 would be a negative inotropic and chronotropic agent *in vivo*, since the Ca^{2+} antagonist effects of this drug were maximized by the high concentration used (10 μM) and by depolarizing the tissue, conditions known to enhance the Ca^{2+} channel blocking activity of these agents (Freedman & Miller, 1984; Sanguinetti & Kass, 1984b).

Interpretation of these results depends upon the validity of using $\dot{V}_{max}$ of slow response action potentials as a quantitative measure of relative magnitude of peak Ca^{2+} current, an issue that has been discussed elsewhere (Tritthart *et al.*, 1973; Ehara & Inazawa, 1980; Windisch & Tritthart, 1981; Sanguinetti & West, 1981). The reliability of $\dot{V}_{max}$ as a measure of Ca^{2+} current was enhanced in this study by using field stimulation (Sanguinetti & West, 1981) and by the use of Ba^{2+} which partially blocks outward K^+ currents (Armstrong *et al.*, 1982) which could otherwise seriously affect the relationship between $\dot{V}_{max}$ and peak Ca^{2+} current. Barium also acts to restore excitability to the K^+-depolarized tissue (Ehara & Inazawa, 1980).

The inferred changes in Ca^{2+} current caused by 10 μM CGP 28 392 are similar to results obtained with Bay K 8644 using voltage-clamped cardiac Purkinje fibres (Sanguinetti & Kass, 1984b). In those ex-

periments Bay K 8644, at a fixed concentration, increased Ca^{2+} current when measured from hyperpolarized holding potentials, but decreased Ca^{2+} current measured from depolarized holding potentials.

It has been proposed that dihydropyridines modulate Ca^{2+} channel gating by stabilizing either a channel gating mode characterized by very prolonged single channel openings (agonists) or by stabilizing the channels in the inactivated state (antagonists) (Hess *et al.*, 1984). Partial agonists could stabilize either mode, the predominant action being determined by membrane potential as well as by drug concentration.

Biochemical studies have demonstrated the existence of high affinity and low affinity dihydropyridine binding sites in cardiac tissue (Vaghy *et al.*, 1984b). It has been observed that the low affinity binding site has a K_D similar in magnitude to the IC_{50} for inhibition of cardiac contractility and the high affinity binding site has a K_D similar to the ED_{50} for enhancement of cardiac contractility (Marsh *et al.*, 1983; Vaghy *et al.*, 1984a,b). Thus, it has been suggested that compounds which are able to bind to both high and low affinity sites would be expected to act as partial agonists. However, this hypothesis does not seem to completely explain the voltage dependence of drug/channel interactions as described here and elsewhere (Sanguinetti & Kass, 1984a,b). Conceivably, changes in membrane potential and stimulation rate could alter the relative proportions of these high and low affinity sites, reflecting changes in state of the channel.

Our results cannot distinguish between the several possible mechanisms controlling the voltage-dependent modulation of Ca^{2+} current by CGP 28 392. The results do, however, indicate that under specific conditions (depolarized membrane potentials and variable stimulation rates) CGP 28 392 behaves as a partial Ca^{2+} channel agonist, and thus it is important to consider the dual actions of this compound in investigations where it is used as an experimental tool to enhance Ca^{2+} channel current.

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