Voltage- and time-dependent inhibitory effects on rat aortic and porcine coronary artery contraction induced by propafenone and quinidine

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1 Class I antiarrhythmic drugs (e.g. Na⁺ channel blockers) such as propafenone and quinidine also inhibit voltage-gated Ca²⁺ and K⁺ channels. In the present paper the voltage- and time-dependent inhibitory effects of propafenone and quinidine were studied on depolarization-induced vascular contractions and ⁴⁵Ca²⁺ uptake in isolated endothelium denuded rat aorta and pig left descending coronary artery.

2 Quinidine and propafenone (10⁻⁷ M–5 × 10⁻⁵ M) produced a concentration-dependent relaxation of the contractions induced by 80 mM KCl. Propafenone was significantly more potent (*P* < 0.05) than quinidine in both rat aorta and pig coronary arteries but both drugs were more potent (*P* < 0.05) in relaxing rat aorta than pig coronary arteries. In rat aortic rings, the relaxant effects of propafenone were unaffected by pretreatment with the Na⁺ channel blocker, tetrodotoxin.

3 The degree of inhibition produced after prolonged exposure (40 min) to propafenone and quinidine differed as the time of depolarization with 80 mM KCl was increased. Quinidine (3 × 10⁻⁶ M, 10⁻⁵ M and 3 × 10⁻⁵ M) not only produced an inhibition at the very early stage of contraction, but also a time-dependent inhibition was observed. In contrast, propafenone (10⁻⁶ M, 3 × 10⁻⁶ M and 10⁻⁵ M) produced a more marked concentration-dependent early block but only a mild time-dependent inhibition.

4 The voltage-dependence of propafenone- and quinidine-induced inhibition, was studied in rat aorta and coronary arteries which had been incubated in 5 or 40 mM KCl Ca²⁺-free solution and then contracted by changing the bath solution to 100 mM KCl and 2 mM CaCl₂ solution. The inhibitor effects of quinidine were significantly enhanced (*P* < 0.05) when the preparations were preincubated in 40 mM KCl (depolarizing) solution. In contrast, the effects of propafenone were quite similar in 5 or in 40 mM KCl solution.

5 Quinidine, 10⁻⁵ M, produced a greater inhibition (*P* < 0.05) of 100 mM KCl-stimulated ⁴⁵Ca²⁺ uptake in aortic rings preincubated in depolarizing as compared to normal solution. In contrast, the inhibition produced by 3 × 10⁻⁶ M propafenone was similar in aortic rings incubated in 5 or 40 mM KCl solution.

6 It is concluded that both quinidine and propafenone inhibited vascular smooth muscle contraction which could be attributed to reduced Ca²⁺ entry. The voltage- and time-dependent inhibitory effects of quinidine may reflect an increased binding of the drug to Ca²⁺ channels at depolarized potentials.

Keywords: Propafenone; quinidine; rat aorta; pig coronary

Introduction

Although the main antiarrhythmic mechanism of action of class I antiarrhythmic drugs results from the inhibition of cardiac voltage-gated Na⁺ channels (Hondeghe & Katzung, 1984; Grant *et al.*, 1984; Tamargo *et al.*, 1992), other voltage-gated cation channels can also be blocked by these drugs which may influence their antiarrhythmic actions (Colatsky *et al.*, 1990; Nattel, 1991; Tamargo *et al.*, 1992). Quinidine and propafenone are two class I antiarrhythmic agents widely used for the treatment of cardiac arrhythmias. Both drugs inhibit the inward cardiac Na⁺ current (*I*_{Na}) in a voltage- and use-dependent manner and thus, they are considered as Na⁺ channel blockers (Hondeghe & Katzung, 1984; Nattel, 1991; Tamargo *et al.*, 1992). In addition, they also inhibit Ca²⁺ (Satoh & Hashimoto, 1984; Salata & Wasserstrom, 1988; Scamps *et al.*, 1989; Delgado *et al.*, 1993; Fei *et al.*, 1993) and K⁺ cardiac currents (Satoh & Hashimoto, 1984; Balser *et al.*, 1991; Duan *et al.*, 1993; Delpón *et al.*, 1993) and exert indirect effects on the cardiovascular system, including anticholinergic and α-adrenoceptor blockade (quinidine, Schmid *et al.*, 1974; Motulsky *et al.*, 1984) or

β-adrenoceptor blocking properties (propafenone, Funk-Brentano *et al.*, 1990).

Systemic administration of quinidine causes mild hypotension, forearm vasodilatation and attenuates the vasoconstrictor response to sympathetic nerve stimulation (Schmid *et al.*, 1974; Nelson *et al.*, 1974; Walsh & Horwitz, 1979; Mariano *et al.*, 1992). This vasodilator response persisted in denervation tissues, suggesting that it is mediated both by a direct effect and by inhibition of α-adrenoceptor-mediated vasoconstriction (Schmid *et al.*, 1974). Ca²⁺ entry through voltage-gated L-type channels is the main mechanism that controls long-term vascular smooth muscle tone. In cardiac tissues both quinidine (Salata & Wasserstrom, 1988; Scamps *et al.*, 1989) and propafenone (Satoh & Hashimoto, 1984; Delgado *et al.*, 1993; Fei *et al.*, 1993) inhibit L-type Ca²⁺ channels. However, information on the inhibitory effect of antiarrhythmic drugs in vascular smooth muscle is sparse. Recently, it has been found that the inhibitory effect exerted by two class Ic antiarrhythmic drugs, propafenone and flecainide, in rat isolated aorta was accompanied by inhibition of ⁴⁵Ca²⁺ influx (Carrón *et al.*, 1991; Pérez-Vizcaino *et al.*, 1991). Thus, it is possible that the hypotensive effect of these antiarrhythmic agents may be related to the inhibition

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of vascular smooth muscle Ca^{2+} channels. The blockade of Ca^{2+} channels (Bean *et al.*, 1986; Nelson & Worley, 1989) as well as the vasodilator effect (Morel & Godfraind, 1987; Nelson & Worley, 1989; Salomone & Godfraind, 1993) of some dihydropyridine Ca^{2+} channel blockers (CCBs) have been shown to be voltage- and time-dependent. Therefore, the aim of the present paper was to study the voltage- and time-dependent inhibitory effects of propafenone and quinidine on depolarization-induced vascular contractions in endothelium-denuded rat aorta and pig coronary artery.

Methods

Tissue preparation

Male Sprague-Dawley rats (300–350 g) from Interfauna (Barcelona) and young pigs (5–7 kg) from the local abattoir were used for these experiments. Porcine hearts, still beating, were immersed in cold (4°C) physiological saline solution (PSS) of the following composition (mM): NaCl 118, KCl 5, NaHCO_3 25, MgSO_4 1.2, HCl 2, KH_2PO_4 1.2 and glucose 11, previously bubbled with 95% O_2 :5% CO_2 gas mixture. The left descending coronary artery was dissected in PSS and transported immediately to the laboratory. Rats were killed by a blow on the head and exsanguinated. The descending thoracic aorta was rapidly dissected and placed in PSS. After excess fat and surrounding tissue had been removed, the aorta and coronary arteries were cut into rings (3–4 mm in length) as described elsewhere (Pérez-Vizcaino *et al.*, 1991; Tejerina *et al.*, 1992; Pérez-Vizcaino *et al.*, 1993). Endothelium was removed by gentle rubbing of the internal surface of the vessels with a small metal rod. At the beginning of the experiments, the absence of functional endothelium was confirmed by the inability of the preparation precontracted with 10^{-7} M noradrenaline to relax in response to 10^{-6} M acetylcholine.

Mechanical force recording

Arterial rings were mounted in 5 ml organ baths containing PSS under tension (2 g for the aorta and 0.5 g for coronary rings) by two parallel L-shaped stainless-steel holders (diameter 0.8 mm for aorta and 0.2 mm for coronary rings) inserted into the lumen. Holders were attached to force-displacement transducers (Grass FT03, Grass, Quincy, MA, U.S.A.) and connected to a polygraph (Grass Model 7) to measure isometric contractile force as previously described (Pérez-Vizcaino *et al.*, 1991, 1993). Tissue baths were filled with PSS maintained at 37°C and bubbled with 95% O_2 :5% CO_2 gas mixture. Each preparation was allowed to equilibrate for 90 min prior to initiation of experimental procedures and during this period the incubation medium was changed every 30 min.

Depolarization-induced responses

After equilibration, aortic and coronary rings were exposed to 80 mM KCl. When the contractile responses to KCl reached steady state, concentration-response curves were obtained for quinidine and propafenone by cumulative addition of the drugs. The relaxant effect of each concentration was allowed to reach a stable level (normally after 25–35 min) before the next concentration was added. In parallel time controls, contractile force decreased by less than 5% during the study.

Time-dependence of drug-induced inhibition

To study the time-dependent inhibitory effect of propafenone and quinidine on KCl-induced contractions, following the equilibration period rat aortic rings were contracted with 80 mM KCl PSS. The contractile response was recorded for

40 min and then the preparations were washed in normal PSS. Thereafter, rings were exposed to propafenone (10^{-6} M, 3×10^{-6} M or 10^{-5} M), quinidine (3×10^{-6} M, 10^{-5} M or 3×10^{-5} M) or vehicle (PSS) for 40 min and finally rings were again contracted by addition of 80 mM KCl PSS for another 40 min in the continuous presence or absence of the drug. The contractile responses evoked during the second application of 80 mM KCl were expressed as a percentage of either the maximal tension (Figures 2a and 3a) or the tension at the corresponding time interval (Figures 2b and 3b) obtained in the same ring during the first contraction.

Voltage-dependence of drug-induced inhibition

To examine the pattern of voltage-dependence of propafenone- and quinidine-induced inhibition, in rat aorta and coronary artery the effect of the drugs was studied at different membrane potentials which were controlled by changing the extracellular concentration of KCl (i.e., 'potassium clamp'; Burges *et al.*, 1987; Nelson & Worley, 1989; Godfraind & Salomone, 1991; Salomone & Godfraind, 1993). Aortic and coronary rings were incubated for 30 min in Ca^{2+} -free (5 mM KCl) or in Ca^{2+} -free depolarizing (40 mM KCl) PSS (with isotonic replacement of NaCl by KCl). Then the preparations were contracted by a short-duration (2 min) depolarizing pulse by changing the bath solution to a solution containing 100 mM KCl and 2 mM CaCl_2 . The duration of the pulse was short enough to avoid the drugs coming into equilibrium with the Ca^{2+} channels at the membrane potential set by 100 mM KCl (Nelson & Worley, 1989). The preparations were then incubated for 30 min in PSS containing 5 or 40 mM. Three control contractile responses to 100 mM KCl PSS containing 2 mM CaCl_2 were obtained at the beginning of the experiment at 30 min intervals. This was followed by exposure to propafenone (10^{-6} M, 3×10^{-6} M and 10^{-5} M) or quinidine (3×10^{-6} M, 10^{-5} M and 3×10^{-5} M) for 40 min. Thereafter, the contractile response to 100 mM KCl was elicited in the presence of the drug. In each preparation only one drug and one concentration (5 or 40 mM) of KCl were tested. In parallel time controls, responses were in the range of 94 to 108% of the initial contractions. The results of these experiments were expressed as a percentage of the initial control contractile responses.

Measurement of $^{45}\text{Ca}^{2+}$ influx

The effect of the drugs on the $^{45}\text{Ca}^{2+}$ influx in aortic rings was quantified by means of the fraction of $^{45}\text{Ca}^{2+}$ uptake resistant to displacement by La^{3+} (Pérez-Vizcaino *et al.*, 1991). Rings weighing between 6–10 mg were equilibrated for 90 min in PSS bubbled with a 95% O_2 :5% CO_2 gas mixture. All the solutions employed afterwards contained no Ca^{2+} or phosphates in order to minimize $^{45}\text{Ca}^{2+}$ precipitation. After equilibration, rings were transferred to a 5 or 40 mM KCl PSS in the presence or absence of propafenone (3×10^{-6} M), quinidine (10^{-5} M) or verapamil (10^{-4} M) for 40 min. At this time both experimental and control rings were incubated for 2 min in 100 mM KCl PSS containing $^{45}\text{Ca}^{2+}$ (specific activity $2 \mu\text{Ci ml}^{-1}$) in the presence or absence of quinidine, propafenone or verapamil and then washed for 5 min in an ice-cold lanthanum solution (composition in mM: NaCl 122, KCl 5.9, MgCl_2 1.25, glucose 11, LaCl_3 50 and Tris maleate 15; pH 6.8 at 0°C). Afterwards the rings were removed, dried between two sheets of filter paper, weighed and digested in a perchloric acid (35%)- H_2O_2 (33 volumes) mixture (1:1, v/v) at 75°C for 20 min. Radioactivity of the samples was assayed in a liquid scintillation counter as previously described (Pérez-Vizcaino *et al.*, 1991).

Drugs

The following drugs were used: propafenone hydrochloride (Knoll AG, Ludwigshafen, Germany), quinidine sulphate,

(-)-noradrenaline bitartrate, acetylcholine chloride, tetrodotoxin (Sigma Ltd. Co., London). All drugs were dissolved in distilled deionized water to prepare a 1 mM stock solution and further dilutions were made in PSS. Ascorbic acid (Merk) 0.1 mM was added to the solution of noradrenaline. $^{45}\text{CaCl}_2$ was obtained from Amersham International plc (Buckinghamshire, England).

Statistics

Throughout the paper, values are expressed as mean $\pm$ s.e.mean. Statistical analysis was performed with Student's unpaired *t* test. The differences between control and experimental values were considered significant when $P < 0.05$. The negative logarithm of the half-maximal effective concentration (pD_2) was calculated from the concentration-response curves by fitting the experimental data to a logistic equation by non-linear regression analysis.

Results

Concentration-response curves

Figure 1 shows the relaxant effects of quinidine and propafenone when added at the plateau of the contractile responses evoked by 80 mM KCl. Addition of 80 mM KCl to rat aortic and pig coronary rings gradually induced a contractile response which averaged 2793 ± 208 ($n = 16$) and 2101 ± 268 mg ($n = 12$), respectively. Cumulative increases of quinidine and propafenone (10^{-7} M– 5×10^{-5} M) resulted in a concentration-dependent relaxation. The pD_2 values for the relaxant effects of quinidine and propafenone are shown in Table 1. It can be observed that propafenone was significantly more potent ($P < 0.05$) than quinidine in both rat aorta and pig coronary arteries but both drugs were more potent ($P < 0.05$) in relaxing rat aorta than pig coronary arteries.

In rat aortic rings, the Na^+ channel blocker tetrodotoxin (3×10^{-5} M), when added at the plateau of the KCl-induced contraction had no effect on tension. Furthermore, in these preparations the relaxant effects of propafenone were unaffected by the pretreatment with tetrodotoxin (Table 1).

Time-dependent effects in rat aorta

To study the time-dependent of the effects of propafenone and quinidine, aortic rings were contracted with 80 mM KCl and the evoked contraction was monitored for 40 min. Then the rings were incubated for a 40 min period in the absence or presence of the drugs and finally, contracted with 80 mM KCl for another 40 min. Figures 2 and 3 show the time course of the contractions induced by 80 mM KCl after the incubation for 40 min with vehicle, propafenone (10^{-6} M, 3×10^{-6} M and 10^{-5} M) and quinidine (3×10^{-6} M, 10^{-5} M and 3×10^{-5} M), respectively. The contractile response recorded in the presence of each drug was compared to that

Figure 1 Effects of (a) propafenone and (b) quinidine in rat aortic (●) and pig coronary arterial rings (■) precontracted with KCl 80 mM. After the contraction induced by 80 mM KCl reached its plateau, concentration-response curves were performed by cumulative addition of the drugs. Results are expressed as a percentage of the initial control tension. Each symbol represents the mean $\pm$ s.e.mean of 6–7 rings from different animals.

Table 1 pD_2 values for the relaxant response of propafenone and quinidine on 80 mM KCl-induced contractions in rat aorta and pig coronary arteries

		Propafenone	Quinidine
Rat aorta	– TTX	5.64 ± 0.02 (6)	$5.26 \pm 0.05^*$ (7)
	+ TTX	5.49 ± 0.01 (3)	ND
Pig coronary		5.34 ± 0.06 (6)	$4.65 - 0.03^*$ (6)

TTX = 3×10^{-5} M tetrodotoxin, $^*P < 0.05$ vs propafenone, the number of experiments is shown in parenthesis; ND not determined.

Results (mean $\pm$ s.e.mean) were calculated from the experiments shown in Figure 1.

obtained before the addition of propafenone or quinidine. In the presence of propafenone (Figure 2a) and quinidine (Figure 3a) the contraction was inhibited in a concentration-dependent manner, but propafenone was more potent than quinidine ($P < 0.05$). More interestingly, Figure 2b and 3b show that the degree of inhibition produced by propafenone and quinidine differ as the time of depolarization was prolonged. At 3×10^{-6} M, quinidine did not produce any inhibition at the very early stage of contraction but it progressively developed reaching steady-state after 20 min depolarization, whereas in the presence of 10^{-5} M and 3×10^{-5} M the drug not only produced a marked early block but a great time-dependent inhibition was also observed (Figure 3b). Thus, the percentage of inhibition produced by the three concentrations of quinidine was significantly greater ($P < 0.01$) after 20 min than after 15 s. In contrast, propafenone (10^{-6} M, 3×10^{-6} M and 10^{-5} M) produced a more marked concentration-dependent early block but only a mild time-

Figure 2 Time-dependent inhibition of 80 mM KCl-induced contraction produced by propafenone in rat isolated aorta. A first control contraction was elicited for 40 min. Then the rings were incubated for a 40 min period in the absence (○) or in the presence of 10^{-6} M (■), 3×10^{-6} M (▲) and 10^{-5} M (◆) propafenone and, finally, contracted by KCl in the presence of the drug for another 40 min. In (a) results are expressed as a percentage of the maximal tension during the first contraction and in (b) as a percentage of the tension of the first contraction at each time interval. Each symbol represents the mean $\pm$ s.e. mean of 5–7 rings from different animals.

dependent inhibition, which began with a delay of 3 min, was observed (Figure 2b). Thus, only at the highest concentration of propafenone tested the percentage of inhibition observed after 20 min was significantly greater ($P < 0.05$) than after 15 s.

The time course of the inhibition for the two concentrations of quinidine which did not produce a high early block (3×10^{-6} M and 10^{-5} M) could be fitted by a non linear analysis to the equation:

$$B = B_{\max} (1 - e^{-(K_1[D] + K_{-1})t})$$

where $B_{\max}$ is the steady-state inhibition, B is the level of inhibition at the time t of depolarization and $[D]$ is the drug concentration. The estimated values for the association (K_1) and dissociation (K_{-1}) constants were $0.13 \mu\text{M}^{-1} \text{min}^{-1}$ and 0.19min^{-1} , respectively, and the calculated value of $K_D(K_{-1}/K_1 = 1.47 \times 10^{-6} \text{M})$ was in the range of the observed pD_2 values.

Voltage-dependence of drug-induced inhibition

In order to test if the effects of propafenone and quinidine were modified by depolarizing the membrane potential, aortic and coronary rings were incubated for 40 min in the continuous presence of the drugs and the membrane potential was controlled by changing the KCl concentration (5 or 40 mM) in the bathing media. Thereafter, the preparations

Figure 3 Time-dependent inhibition of 80 mM KCl-induced contraction produced by quinidine in rat isolated aorta. Results were obtained in the absence (○) or in the presence of 3×10^{-6} M (■), 10^{-5} M (▲) and 3×10^{-5} M (◆) quinidine. Controls were the same as in Figure 2. Each symbol represents the mean $\pm$ s.e. mean of 5–7 rings from different animals. See Figure 2 for explanation.

were contracted by changing for 2 min to a 100 mM KCl-depolarizing solution containing quinidine or propafenone. The previous concentration of KCl in the bathing media did not influence the amplitude of the contractile response induced by the 100 mM KCl in both, aortic (792 ± 82 and 708 ± 40 mg in 5 and 40 mM KCl, respectively, $n = 19$) or coronary rings (800 ± 104 and 759 ± 74 mg in 5 and 40 mM KCl, respectively, $n = 19$). Propafenone and quinidine inhibited these contractions in a concentration-dependent manner in rat aorta (Figure 4) and pig coronary artery (Figure 5). Furthermore, the inhibitory effects of quinidine in rat aorta (Figure 4b) and pig coronary arteries (Figure 5b) were significantly enhanced ($P < 0.05$ for all concentrations) when the preparations were preincubated in depolarizing (40 mM KCl) as compared to normal (5 mM KCl) PSS. For propafenone, a similar trend was also observed in normal and in depolarizing PSS (Figures 4a and 5a). However, differences for the inhibitory effects at 5 and 40 mM KCl were significant only at 10^{-5} M propafenone in pig coronary arteries. The pD_2 values for the inhibitory effects of quinidine and propafenone are summarized in Table 2.

Inhibition of $^{45}\text{Ca}^{2+}$ influx

To determine whether the inhibitory effects of quinidine and propafenone on aortic contractility and its voltage-

Figure 4 Voltage-dependence of the effects of (a) propafenone (10^{-6} M, 3×10^{-6} M and 10^{-5} M) and (b) quinidine (3×10^{-6} M, 10^{-5} M and 3×10^{-5} M) in aortic rings. Preparations were initially incubated for 40 min in either 5 (○) or 40 mM KCl (●) Ca^{2+} -free PSS and then, contracted by changing the solution to 100 mM KCl, 2 mM Ca^{2+} PSS for 2 min in the continuous presence of the drugs. Results are expressed as a percentage of the control contractile response induced by 100 mM KCl, 2 mM Ca^{2+} PSS. Each symbol represents the mean $\pm$ s.e.mean of 6–7 contractions in different rings expressed as a percentage of the initial control in the absence of the drug. * $P < 0.05$ vs 5 mM KCl.

Table 2 pD_2 values for the inhibitory effect of propafenone and quinidine incubated in 5 or 40 mM KCl PSS on 100 mM KCl-induced contractions in rat aorta and pig coronary arteries

	[KCl] (mM)	Propafenone	Quinidine
Rat aorta	5	5.60 ± 0.03 (6)	4.79 ± 0.01 (7)
	40	5.69 ± 0.02 (6)	5.12 ± 0.03 (6)*
Pig coronary	5	5.47 ± 0.03 (6)	4.80 ± 0.01 (6)
	40	5.63 ± 0.07 (7)	5.11 ± 0.03 (7)*

* $P < 0.05$ vs 5 mM KCl, the number of experiments is shown in parenthesis.

Results (mean $\pm$ s.e.mean) were calculated from the experiments shown in Figures 4 and 5.

Figure 5 Voltage-dependence of the effects of (a) propafenone (10^{-6} M, 3×10^{-6} M and 10^{-5} M) and (b) quinidine (3×10^{-6} M, 10^{-5} M and 3×10^{-5} M) in coronary artery rings. The rings were incubated in either 5 (○) or 40 mM KCl (●) Ca^{2+} -free PSS and then contracted by changing the solution 100 mM KCl, 2 mM Ca^{2+} PSS for 2 min. Each symbol represents the mean $\pm$ s.e.mean of 6–7 contractions in different rings expressed as a percentage of the initial control in the absence of the drug. * $P < 0.05$ vs 5 mM KCl.

dependence were due to reduced Ca^{2+} entry, we measured the 100 mM KCl-stimulated $^{45}\text{Ca}^{2+}$ influx using a protocol similar to that described above to study the voltage-dependence of drug-induced inhibition of contractile responses. Concentrations of propafenone (3×10^{-6} M) and quinidine (10^{-5} M) producing about 50% inhibition of contraction were chosen for this study. $^{45}\text{Ca}^{2+}$ uptake remaining in the presence of 10^{-4} M verapamil was considered as the maximal Ca^{2+} antagonist insensitive Ca^{2+} entry. As shown in Figure 6, $^{45}\text{Ca}^{2+}$ uptake was similar in control aorta previously incubated in 5 or 40 mM KCl. Both 3×10^{-6} M propafenone and 10^{-5} M quinidine significantly inhibited $^{45}\text{Ca}^{2+}$ uptake ($P < 0.05$) in preparations previously incubated in 5 or 40 mM KCl. However, quinidine produced a greater inhibition ($P < 0.05$) of $^{45}\text{Ca}^{2+}$ uptake in aortic rings incubated in depolarizing (40 mM KCl) PSS as compared to normal PSS. In contrast, the inhibition of $^{45}\text{Ca}^{2+}$ uptake produced by 3×10^{-6} M propafenone was not different in aortic rings incubated in 5 or 40 mM KCl PSS.

Figure 6 Voltage-dependent inhibition of $^{45}\text{Ca}^{2+}$ uptake in rat aortic rings. Rings were incubated in 5 or 40 mM KCl Ca^{2+} -free PSS in the absence (C, open columns) or in the presence of 3×10^{-6} M propafenone (P, hatched columns), 10^{-5} M quinidine (Q, cross-hatched columns) or 10^{-4} M verapamil (V, solid columns) for 40 min. Then rings were stimulated by 100 mM KCl PSS in the presence of $2 \mu\text{Ci ml}^{-1}$ $^{45}\text{Ca}^{2+}$ for 2 min. The $^{45}\text{Ca}^{2+}$ uptake in the presence of 10^{-4} M verapamil was considered as the maximal Ca^{2+} antagonist-insensitive $^{45}\text{Ca}^{2+}$ entry. Ordinate scale: c.p.m. mg^{-1} of dry tissue. Each column represents the mean $\pm$ s.e. mean of 14–16 rings. * $P < 0.05$, ns = not significant 5 vs 40 mM KCl.

Discussion

In the present paper we have analysed the effects of two Na^{+} channel blockers, propafenone and quinidine, on contractions and $^{45}\text{Ca}^{2+}$ influx induced by membrane depolarization (high KCl concentration) in endothelium-denuded rat aorta and pig coronary artery. Tonic contractions induced by depolarization with high concentrations of KCl are associated with Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels because they can be selectively blocked by Ca^{2+} channel blockers (CCBs) and are suppressed in Ca^{2+} -free solution (Cauvin *et al.*, 1983; Godfraind *et al.*, 1986). In the present experiments propafenone and quinidine inhibited the contractile responses and the increase in $^{45}\text{Ca}^{2+}$ influx induced by high KCl, propafenone being more potent than quinidine. Since Ca^{2+} ions play a determinant role in vascular contractions, these results strongly suggest that inhibition of arterial contractions induced by quinidine and propafenone may result from their inhibitory effect on Ca^{2+} entry through L-type channels. Moreover, they confirmed previous evidence suggesting that in rat isolated aorta the vasodilator effects of two class Ic antiarrhythmic drugs, propafenone and flecainide, can be attributed to their ability to inhibit Ca^{2+} entry through voltage-gated channels (Carrón *et al.*, 1991; Pérez-Vizcaíno *et al.*, 1991). The pD_2 values for inhibition of vascular contractions in the present experiments are very similar to those previously reported for inhibiting L-type Ca^{2+} channels in isolated cardiac myocytes using the patch-clamp technique for propafenone ($\text{pD}_2 = 5.3$ – 5.8 , Delgado *et al.*, 1993; Fei *et al.*, 1993) and quinidine ($\text{pD}_2 = 5.0$, Scamps *et al.*, 1989). The existence of Na^{+} channels either sensitive or relatively insensitive to tetrodotoxin in vascular smooth muscle have rarely been reported (Sturek & Hermesmyer, 1986). In the present experiments, 3×10^{-5} M tetrodotoxin did not affect aortic contractile force under depolarizing conditions or the relaxant response to propafenone. Therefore, a possible role for Na^{+} channel inhibition in the observed inhibitory effects can be excluded.

In *in vitro* experiments, CCBs inhibit high KCl-induced

contractions in vascular smooth muscle in a voltage- and time-dependent manner (Godfraind *et al.*, 1986). According to the modulated receptor hypothesis, binding of a drug to a receptor site located within the channel is modulated by the conformational state of the channel (rested, activated, inactivated) which is determined by the membrane potential (Hondeghem & Katzung, 1984). Radioligand binding (Burgess *et al.*, 1987; Godfraind & Salomone, 1991; Triggle, 1991; Salomone & Godfraind, 1993) and electrophysiological studies (Uehara & Hume, 1985; Bean *et al.*, 1986; Nelson & Worley, 1989) have found that most CCBs bind with greater affinity under depolarizing conditions. Since depolarization of the membrane potential shifts the conformation of Ca^{2+} channels into the activated and inactivated states, the voltage-dependence has been attributed to a preferential binding to these states of the channel (Uehara & Hume, 1985; Bean *et al.*, 1986; Tamargo & Delpón, 1991).

In the present experiments, after prolonged exposure (40 min) to quinidine at normally polarized membrane potentials the degree of inhibition increased as the time of depolarization was prolonged (i.e., time-dependent inhibition), reaching a steady-state within 20 min of depolarization. Similar behaviour has been described previously with some CCBs of the dihydropyridine family (Morel & Godfraind, 1987; Wibo *et al.*, 1988; Godfraind & Salomone, 1991). Since depolarization shifts Ca^{2+} channels into the inactivated state, this time-dependent effect has been proposed to reflect an increased drug affinity for this state of the channel (Wibo *et al.*, 1988). The voltage-dependence of the inhibitory effects of CCBs on vascular smooth muscle have been reported in functional studies where the membrane potential is controlled by changing the extracellular concentration of KCl (i.e., 'potassium clamp'; Burgess *et al.*, 1987; Nelson & Worley, 1989; Godfraind & Salomone, 1991; Salomone & Godfraind, 1993). The voltage-dependence of the inhibitory effect of quinidine on contractions and $^{45}\text{Ca}^{2+}$ influx was confirmed by the significant increase in the inhibition observed in depolarized (40 mM KCl PSS) as compared to normally polarized arteries (5 mM KCl) when a short-duration depolarizing pulse (100 mM KCl PSS) was applied. Therefore, the voltage-dependence of the inhibitory effects of quinidine may reflect an increased binding of the drug to Ca^{2+} channels under depolarizing conditions. Because in preparations incubated in 5 mM KCl most of the Ca^{2+} channels will be in the resting state, whereas under depolarizing conditions (40 mM KCl) some channels will be in the inactivated and/or activated state we can speculate that quinidine preferentially binds to these states of the channel. In fact, in isolated ventricular myocytes, quinidine decreased L-type Ca^{2+} current in a voltage- and use-dependent manner and shifted the steady state inactivation curves towards hyperpolarization (Salata & Wasserstrom, 1988; Scamps *et al.*, 1989). However, at high concentration (3×10^{-5} M) quinidine significantly reduced the amplitude of the Ca^{2+} current of the first clamp step after a prolonged resting period, i.e. it also produced a tonic block (Salata & Wasserstrom, 1988; Scamps *et al.*, 1989).

Peak Ca^{2+} current through L-type channels occurs within milliseconds whereas the contractions in vascular smooth muscle take much longer to develop. Thus, during prolonged depolarization at least some of the Ca^{2+} channels must be in a conducting state implying that either a component of the current never inactivates or alternatively, that drug-free inactivated channels return to the conducting (activated) state (Imaizumi *et al.*, 1989). In fact, KCl-induced contractions are sustained, depend on extracellular Ca^{2+} and are selectively inhibited by CCBs (Cauvin *et al.*, 1983). After exposure to depolarizing (40 mM KCl) PSS a higher portion of Ca^{2+} channels would be in the inactivated (non-conducting) state and it could be expected that during the depolarizing pulse (100 mM KCl, 2 mM CaCl_2) there would be less Ca^{2+} influx and contraction. The finding that the amplitude of the contractions and $^{45}\text{Ca}^{2+}$ uptake stimulated by 100 mM KCl were

similar in arteries preincubated in 5 or 40 mM KCl PSS suggests that the contraction is dependent on this dihydropyridine-sensitive, non inactivating component of the Ca^{2+} current (Imaizumi *et al.*, 1989).

Propafenone also inhibited in a concentration-dependent manner the contractions induced by high KCl. However, and in contrast to quinidine, its inhibitory effect which was quite evident within the first 2 min, increased only slightly during prolonged depolarization. Furthermore, the inhibitory effects of propafenone on the contractions evoked by 100 mM KCl and 2 mM Ca^{2+} PSS were quite similar in aorta or coronary arteries preincubated in 5 or 40 mM KCl Ca^{2+} -free PSS. Thus, the affinity of propafenone for Ca^{2+} channels does not seem to depend on membrane potential.

The possible clinical relevance of the vasodilator effects and the inhibition of Ca^{2+} entry described in this paper for quinidine and propafenone in patients with cardiac arrhythmias is uncertain. However, these effects appeared at the same range of concentrations at which both antiarrhythmics exert their inhibitory effects on inward (Na^+ and Ca^{2+} currents) and outward K^+ currents in cardiac myocytes (Colat-

sky *et al.*, 1990; Nattel, 1991; Balser *et al.*, 1991; Tamargo *et al.*, 1992; Duan *et al.*, 1993; Delpón *et al.*, 1993). A decrease in systemic vascular resistance may reduce ventricular afterload, while a decrease in coronary vascular resistance might increase coronary blood flow. Thus, it is possible that in some patients these vasodilator actions may improve cardiac performance, overriding the cardiodepressant effects of propafenone and quinidine. In fact, the intravenous quinidine infusion is associated with a decrease in systemic vascular resistance and in cardiac filling pressure (Mariano *et al.*, 1992). Furthermore, the inhibition of Ca^{2+} entry may explain the previously described direct vasodilator effect of quinidine that together with its α -adrenoceptor blocking action (Schmid *et al.*, 1974; Motulsky *et al.*, 1984) is responsible for the hypotensive response observed following the parenteral administration of the drug (Hirschfield *et al.*, 1977; Mariano *et al.*, 1992).

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