Br. J. Pharmac. (1985), **86**, 765–775

Electrophysiological effects of propafenone in untreated and propafenone-pretreated guinea-pig atrial and ventricular muscle fibres

C. Delgado, J. Tamargo & T. Tejerina

Department of Pharmacology, School of Medicine, Universidad Complutense, 28040 Madrid, Spain

1 The electrophysiological effects of propafenone (10^{-7} to 10^{-4} M) were studied on guinea-pig isolated atrial and ventricular muscle fibres obtained from untreated animals and animals pretreated with propafenone, 3 and 10 mg kg^{-1}, for 28 days.

2 In untreated atria propafenone produced a dose-dependent decrease in the rate and maximum following frequency, prolonged the sinus node recovery time and reduced the maximum chronotropic responses to isoprenaline.

3 In untreated atrial and ventricular muscle fibres propafenone depressed action potential amplitude and V_{max}, reduced the resting membrane potential and prolonged the action potential duration (APD) and the effective refractory period, lengthening the effective refractory period relative to APD.

4 Propafenone depressed the amplitude and V_{max} and shortened the duration of the slow action potentials induced by isoprenaline and caffeine in K-depolarized papillary muscles.

5 Pretreatment with propafenone reduced atrial rate, but did not modify the action potential characteristics compared to the values obtained in untreated atria. Further addition of propafenone produced similar but more marked changes in untreated atria.

6 In ventricular muscle fibres pretreated with 3 mg kg^{-1}, action potential characteristics before and after further addition of propafenone were similar to those obtained in untreated fibres. However, muscles pretreated with 10 mg kg^{-1} exhibited a significant prolongation of the APD compared to that in untreated muscles or those pretreated with 3 mg kg^{-1}; further addition of propafenone shortened the APD even when this parameter was of similar value to those observed in the other two series of experiments.

7 It is concluded that even though the effects of propafenone are similar to those of quinidine (class I antiarrhythmic), it also exhibited class II and class IV actions. In pretreated animals a prolongation of the APD (class III action) could also be involved in the antiarrhythmic effects of the drug.

Introduction

Propafenone (2′-[2-hydroxy-3-propylaminopropoxy]-3-phenyl-propiophenone hydrochloride), is a new antiarrhythmic drug which has been found to be clinically effective in the treatment of supraventricular and ventricular arrhythmias (Beck *et al.*, 1975; Hochrein *et al.*, 1977; Seipel & Breithardt, 1980; Conolly *et al.*, 1983; Karagueuzian *et al.*, 1983; Schlepper & Olsson, 1983). The electrophysiological effects of propafenone have been previously described in atrial (Ledda *et al.*, 1981; Delgado *et al.*, 1984; Dukes & Vaughan Williams, 1984) and ventricular (Bergmann & Holte, 1977; Kohlhardt, 1977; 1982; Kohlhardt *et al.*, 1983; Dukes & Vaughan Williams, 1984; Calleja *et al.*, 1985) muscle fibres and in Purkinje fibres (Ledda *et al.*, 1981; Dukes & Vaughan Williams, 1984; Tamargo *et al.*, 1984; Calleja *et al.*, 1985). In these preparations propafenone reduced amplitude and V_{max} of the action potential which suggests that it inhibited the fast inward Na current (I_{Na}). Propafenone also exerted some β-adrenoceptor mediated sympatholytic actions (Hapke & Prigge, 1976; Ledda *et al.*, 1981) and it has been demonstrated in voltage clamp experiments that at high concentrations propafenone inhibits the slow inward Ca current (I_{si}) in cat ventricular muscle fibres (Kohlhardt, 1977). Thus, propafenone exhibits class I (membrane-stabilizing), class II (antisympathetic) and class IV (Ca antagonist) antiarrhythmic actions according to the classification

of Vaughan Williams (1975). In addition, recent studies have demonstrated that though propafenone shortens the duration of the action potential in Purkinje fibres (Ledda *et al.*, 1981), it does not modify or prolong the duration of the action potential in atria (Delgado *et al.*, 1984) or ventricular muscle fibres (Dukes & Vaughan Williams, 1984; Calleja *et al.*, 1985). Furthermore, Delgado *et al.* (1984) suggested that propafenone exhibited a class III antiarrhythmic action and that this effect was strengthened in fibres chronically pretreated with propafenone. However, until now the electrophysiological effects of chronic treatment with propafenone in isolated cardiac fibres have not been studied. Therefore, we have investigated the electrophysiological effects of a wide range of concentrations of propafenone in isolated atrial and ventricular muscle fibres obtained from untreated guinea-pigs and guinea-pigs treated for 28 days with propafenone.

Methods

General procedure

Guinea-pigs (350–500 g) of either sex were killed by a blow on the head and their hearts rapidly excised. Left and right atria and papillary muscles were dissected and pinned to the bottom of a 10 ml Lucite chamber through which Tyrode solution (32°C) gassed with 95% O_2 plus 5% CO_2 flowed continuously at a rate of 7 ml min^{-1}. Under these conditions right atria beat spontaneously. Left atria and papillary muscles were stimulated at a basal rate of 1 Hz. The drive stimuli (1 ms duration, twice threshold strength) were applied through Teflon-coated platinum electrodes and delivered from a multipurpose stimulator (Cibertec Model CS-220) able to be programmed. Each preparation was allowed to equilibrate for at least 30 min before control measurements were made. The techniques used and definitions of the sinus node recovery time (SNRT), maximum following frequency and strength-duration curves were as previously described (Tamargo, 1980). Following the control records, propafenone was added to the perfusate in concentrations between 10^{-7} and 10^{-4} M. For each dose-response curve propafenone was added cumulatively and the steady-state effects were recorded at least 30 min after each concentration level was attained.

Intracellular recordings

Transmembrane potentials were recorded with conventional glass microelectrodes (Rodriguez & Tamargo, 1980; Manzanares & Tamargo, 1983) filled with 3 M KCl, having resistances of 10–20 MΩ, displayed, via high-impedance capacity neutralizing amplifiers (WPI) on an oscilloscope (Tektronix 5103N) and photographed. Action potential characteristics measured included: action potential amplitude, maximum rate of rise of the upstroke (V_{max}), resting membrane potential and action poten-

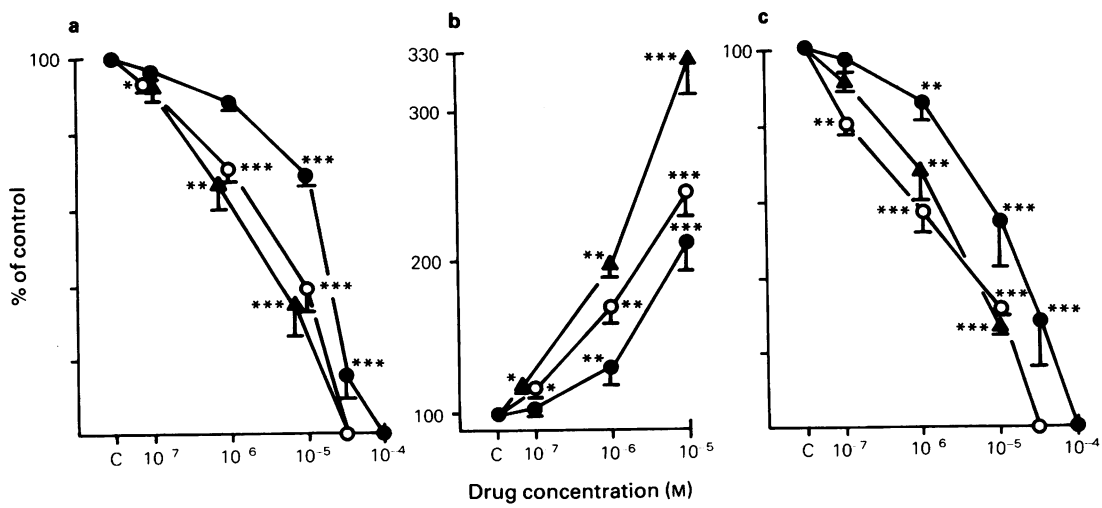

Figure 1 Effect of propafenone on (a) atrial rate, (b) sinus node recovery time and (c) maximum following frequency in guinea-pig right atria obtained from untreated (●) and propafenone-treated: (○) 3 mg kg^{-1}, (▲) 10 mg kg^{-1}, i.p. for 28 days. Ordinate scales: % of control values. Abscissa scales: drug concentration (M). Each point represents the mean of 8–18 experiments; vertical lines show s.e.mean. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

tial duration measured at both 50% (APD_{50}) and 90% (APD_{90}) of repolarization. The effective refractory period (ERP) and the recovery time (RT) were measured by introducing premature test-stimuli of twice threshold strength at different invervals from the preceding basic action potential. Interpolation and shift along the voltage axis were carried after every eighth basic drive stimulus.

Ca-mediated slow action potentials were elicited in papillary muscles partially depolarized by 27 mM K-Tyrode solution by adding isoprenaline (10^{-6} M) and driven at a basal rate of 0.12 Hz (Pappano, 1970). In some experiments the slow action potentials were induced by adding 5 mM caffeine.

Experiments in propafenone-pretreated animals

In further studies, guinea-pigs were injected with propafenone (3 and 10 mg kg^{-1} i.p. daily) for 28 days. The animals were killed 16–18 h after the final injection and both right and left atria and papillary muscles were dissected and set up for intracellular recording as described above. In these two series of pretreated animals plasma concentrations of propafenone were measured with a high-pressure liquid chromatographic method as described by Keller *et al.* (1978). This method allowed sufficiently precise determinations of levels down to 1–2 ng ml^{-1}.

Drugs

Drugs used were: propafenone hydrochloride (Knoll Ibérica), caffeine and isoprenaline hydrochloride (Sigma). Propafenone was dissolved in deionized distilled water. Further dilutions were carried out in Tyrode solution to obtain final concentrations between 10^{-7} and 10^{-4} M (0.037–37.8 μg ml^{-1}). To compare the results, the values obtained after 30 min of equilibration in each experiment were set to 100% and compared to those obtained 30 min after each increment in propafenone concentration. Throughout the paper data have been presented as mean ± s.e.mean. Statistical significance was determined by Student's t test and differences were considered significant when $P < 0.05$.

Results

Effect on atrial rate and sinus node recovery time

The effects of propafenone (10^{-7}–10^{-4} M) on atrial rate and sinus node recovery time (SNRT) are shown in Figure 1. In spontaneously beating untreated right atria the control values for both parameters averaged 194.6 ± 14.3 beats min^{-1} ($n = 18$) and 383.5 ± 33.4 ms ($n = 9$), respectively. Propafenone produced a dose-dependent decrease in atrial rate (Figure 1a), this effect being statistically significant at concentrations higher than 10^{-6} M. At the highest concentration tested, 10^{-4} M, all preparations became inexcitable within 5 min. Propafenone also prolonged the SNRT (Figure 1b), but at concentrations higher than 10^{-5} M it was impossible to determine the SNRT because the atria resumed their spontaneous activity more than 10 s after being electrically paced. The onset of the negative chronotropic effect appears after 1 min and the maximal effect was usually observed within 20–25 min. The effects produced by concentrations $< 10^{-6}$ M were reversed by washing the atria with Tyrode solution, whereas at concentrations $> 10^{-5}$ M the effects of the drug were only partly reversible after washing.

In atria pretreated with 3 mg kg^{-1} the control values for the spontaneous atrial rate and SNRT were not significantly different from those obtained in untreated atria (178.8 ± 11.7 beats min^{-1} and 355.6 ± 16.2 ms, respectively; $n = 8$; $P > 0.05$), whereas in atria pretreated with 10 mg kg^{-1} atrial rate was significantly reduced (112.3 ± 18.9 beats min^{-1}; $n = 8$; $P < 0.001$) and the SNRT prolonged (540.0 ± 90.0 ms; $P < 0.05$) compared to control values obtained in untreated atria. In pretreated atria propafenone produced a more marked negative chronotropic effect and suppressed the spontaneous activity at 5×10^{-5} M instead of at 10^{-4} M, as occurred in untreated atria (Figure 1a). At all concentrations tested, propafenone also produced a dose-dependent prolongation of the recovery of the sinus function. This effect was more evident in atria pretreated with 10 mg kg^{-1} than in those pretreated with 3 mg kg^{-1} and more marked in both series than in untreated atria (Figure 1b).

Effect on atrial excitability and maximum following frequency

The effect of propafenone on excitability was studied in both untreated and propafenone-treated atria in which strength-duration curves were obtained under control conditions and in the presence of propafenone (Figure 2). In untreated atria propafenone, 10^{-6} and 10^{-5} M, increased the minimum current required to evoke a response for each stimulus duration, i.e. it depressed atrial excitability, and the curve was shifted upward with an increase in the rheobase. In atria pretreated with 3 and 10 mg kg^{-1} the strength-duration curve was similar to that obtained in untreated atria. However, under these conditions 10^{-5} M propafenone produced an upward shift of the curve which was significantly greater ($P < 0.05$) than that observed in untreated atria at all durations of the stimulus tested. Nevertheless, there were no significant differences found in the effects produced by propafen-

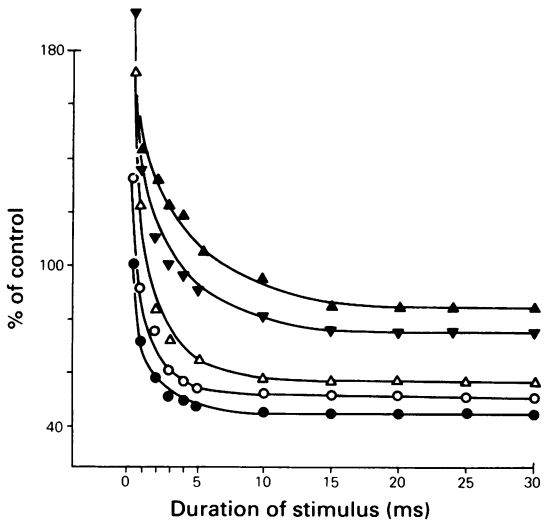

Figure 2 Effect of propafenone (P) on the strength-duration curve in electrically driven left atria. Ordinate scale: minimum current required to evoke a contractile response (% of control values). Abscissa scale: duration of the stimuli at that time (ms). Each point represents the mean of 8 experiments. (●) Controls. P, 10^{-6} M (○) and 10^{-5} M (△) in untreated atria. P, 10^{-5} M, in atria pretreated with P, 3 (▼) and 10 mg kg^{-1} (▲).

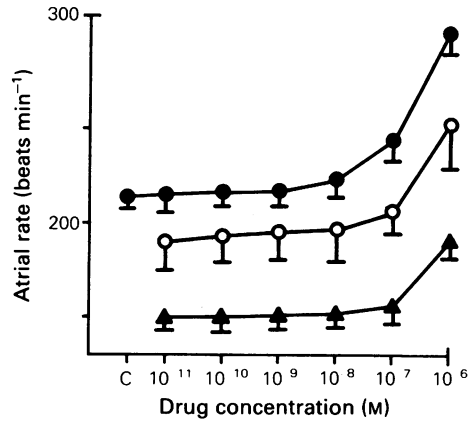

Figure 3 Effect of propafenone (P) on chronotropic responses of guinea-pig atria to isoprenaline. (●) Control; (a) P, 10^{-6} M; (▲) P, 10^{-5} M. Ordinate scale: atrial rate (beats min^{-1}). Abscissa scale: isoprenaline concentration (M). Each point is the mean of 8 experiments; vertical lines represent the s.e.mean.

one between the two series of atrial fibres pretreated with propafenone.

The maximum following frequency at which the atria can be electrically driven averaged 848.9 ± 54.9 beats min^{-1} in 8 untreated atria. Similar values were obtained in atria treated with 3 mg kg^{-1} (870.0 ± 55.0 beats min^{-1}; $n = 8$; $P > 0.05$), whereas in atria pretreated with 10 mg kg^{-1} the maximum following frequency was significantly reduced (560.0 ± 66.0 beats min^{-1}; $n = 8$; $P < 0.01$) compared to values obtained in untreated atria. As is shown in Figure 1c, propafenone produced a dose-dependent reduction of the maximum following frequency in all three series, but its effect was more evident in pretreated atria than in untreated atria, even when no significative differences were found between atria pretreated with 3 or 10 mg kg^{-1}.

Effect of propafenone on the chronotropic responses to isoprenaline

The effect of propafenone on the positive chronotropic effect of isoprenaline was evaluated on a paired basis in 8 spontaneously beating right atria. Dose-response curves were obtained by cumulative addition of isoprenaline (10^{-11}–10^{-6} M) to the bath under control conditions and in the presence of propafenone. As shown in Figure 3 in the presence of propafenone, 10^{-6} M and 10^{-5} M, the maximum chronotropic response produced by each concentration of isoprenaline in the control period was consistently higher than the chronotropic response produced by isoprenaline during exposure to propafenone. Thus, under control conditions 10^{-6} M isoprenaline increased atrial rate by 42.6 ± 3.9% (from 213.7 ± 7.8 to 301.5 ± 13.6 beats min^{-1}), whereas in the presence of propafenone the maximum chronotropic response was 31.6 ± 6.3% ($P < 0.05$) and 27.5 ± 3.4% ($P < 0.01$), respectively.

Electrophysiological effects of propafenone on atrial fibres

The effects of propafenone, 10^{-7}–10^{-4} M, were studied in untreated and treated guinea-pig atria. The effects of the drug were usually apparent within 5 min and reached steady state values within 30 min. Control values of the measured parameters and values obtained after 30 min exposure to different concentrations of propafenone are shown in Figure 4. In 18 untreated atria propafenone 10^{-6} M slightly reduced ($P < 0.05$) the V_{max} of the action potential, but no changes were observed in amplitude and duration of the action potential or the resting potential. At higher concentrations propafenone produced a progressive decrease in the amplitude and V_{max} of the action potential and a reduction of the resting membrane potential. At 10^{-4} M the resting membrane potential was decreased to −68.7 ± 2.2 mV and all fibres

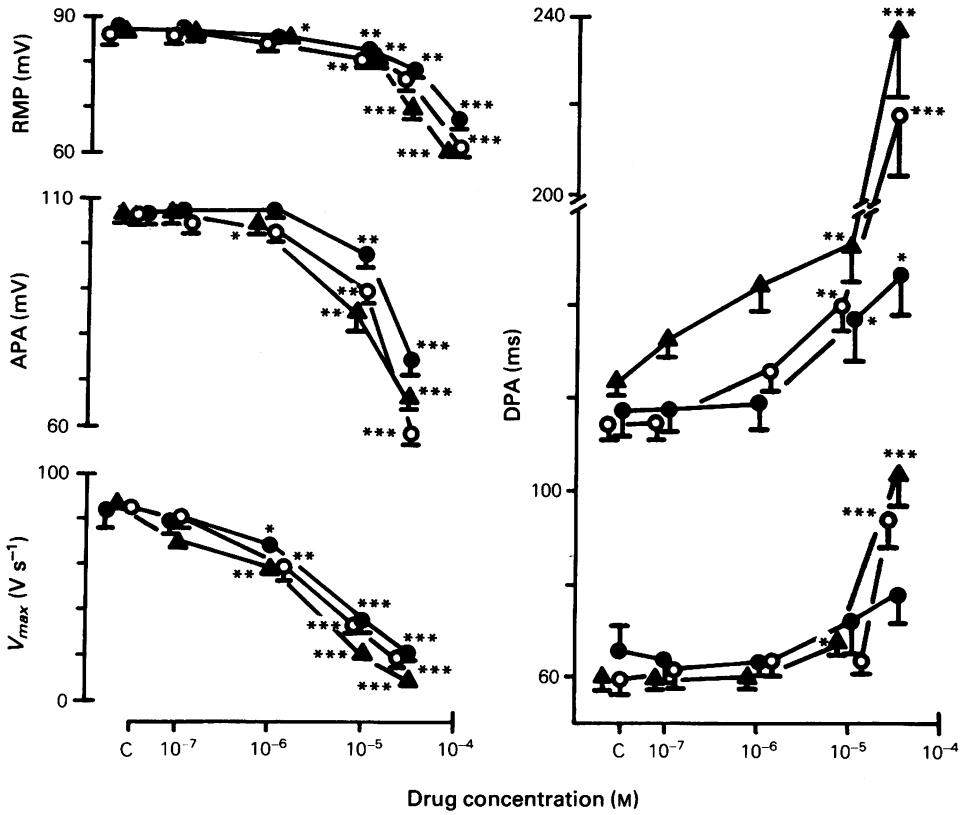

Figure 4 Electrophysiological effects of propafenone (P) in atrial fibres obtained from untreated (●) and P-treated (○ : 3 mg kg^{-1}. ▲ : 10 mg kg^{-1}) guinea-pigs. RMP = resting membrane potential. APA = action potential amplitude. APD = action potential durtion at 50% and 90% of repolarization. Each point represents the mean and vertical lines s.e.mean of 8–18 experiments. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

became inexcitable within 10 min of perfusion. Propafenone, at any of the concentrations tested, modified the APD_{50}, but at concentrations higher than 10^{-6} M, the slope of phase 3 decreased and the duration of phase 3 increased, which led to a slight prolongation of the APD_{90}. The effects produced by concentrations of propafenone $>10^{-5}$ M were only partly reversed after 60 min of washout with drug-free Tyrode solution.

The effects of chronic treatment with 3 and 10 mg kg^{-1} propafenone on action potential characteristics were also studied in 16 and 8 atria, respectively. The control values for these characteristics were not significantly different in pretreated atria from those values obtained in untreated atria. In order to study whether the effects of propafenone were modified in pretreated atria, they were perfused with propafenone, 10^{-7}–10^{-4} M. Results obtained after 30 min exposure to the different concentrations are shown in Figure 4.

As in untreated atria, propafenone 10^{-6} M in atria pretreated with 3 mg kg^{-1} produced a reduction in the amplitude and V_{max} of the action potential without altering the resting membrane potential. At higher concentrations the resting membrane potential was also decreased and thus, a further reduction in phase 0 characteristics was observed. At 10^{-5} M propafenone also produced a prolongation in the APD_{90}, whereas at 5×10^{-5} M, it produced a prolongation in both the APD_{50} and APD_{90} values. In atria pretreated with 10 mg kg^{-1} concentrations of propafenone $>10^{-6}$ M, produced a dose-dependent decrease in amplitude and V_{max} of the action potential and in the resting membrane potential. At concentrations $>10^{-5}$ M these effects were accompanied by a progressive decrease in the slope of phases 2 and 3 of repolarization, which led to a progressive prolongation in the APD at 50% and 90% level of repolarization. In atria pretreated with 3 and 10 mg kg^{-1}, propafenone 10^{-4} M depolarized the

resting membrane potential to -63.6 ± 1.2 mV and -62.0 ± 2.3 mV, respectively.

Effect of propafenone on ventricular muscle fibres

The electrophysiological effects of propafenone, 10^{-7}–10^{-4} M, were also studied on action potential characteristics of papillary muscles obtained from untreated and treated animals. Results are summarized in Figure 5. In 12 untreated papillary muscles propafenone 10^{-6} M significantly reduced the amplitude and V_{max} of the action potential without altering the action potential duration or the resting membrane potential. At higher concentrations the dose-dependent decrease in amplitude and V_{max} of the action potential was accompanied by a progressive reduction of the resting membrane potential. Moreover, at concentrations between 10^{-7} and 10^{-5} M propafenone did not modify the APD_{50} but produced a slight prolongation of the APD_{90} which reached significant values ($P < 0.05$) at 10^{-5} M. At 5×10^{-5} M propafenone significantly reduced the height and duration of phase 2 which led to a significant shortening of the APD_{50} ($P < 0.05$) and increased the slope of phase 3 causing APD_{90} to return to the level of control values. The effects of chronic treatment with 3 and 10 mg kg^{-1} on action potential characteristics were studied in 17 and 16 guinea-pig papillary muscles, respectively. Pretreatment with 3 mg kg^{-1} did not modify the action potential characteristics from those obtained in untreated papillary muscles. In these experiments propafenone at concentrations $\geq 10^{-6}$ M produced a dose-dependent decrease in amplitude and V_{max} of the action potential which was accompanied by a depolarization of the resting potential similar to that found in untreated papillary muscles. At concentrations between 10^{-7} and 10^{-5} M propafenone also slowed the slope of phases 2 and 3 and prolonged the APD_{50} ($P < 0.05$) and APD_{90} values ($P < 0.01$). At 5×10^{-5} M propafenone produced a shortening of the APD in such a way that the APD_{50} reached values similar to the control ones while the APD_{90} was hardly affected.

In fibres pretreated with 10 mg kg^{-1} control values

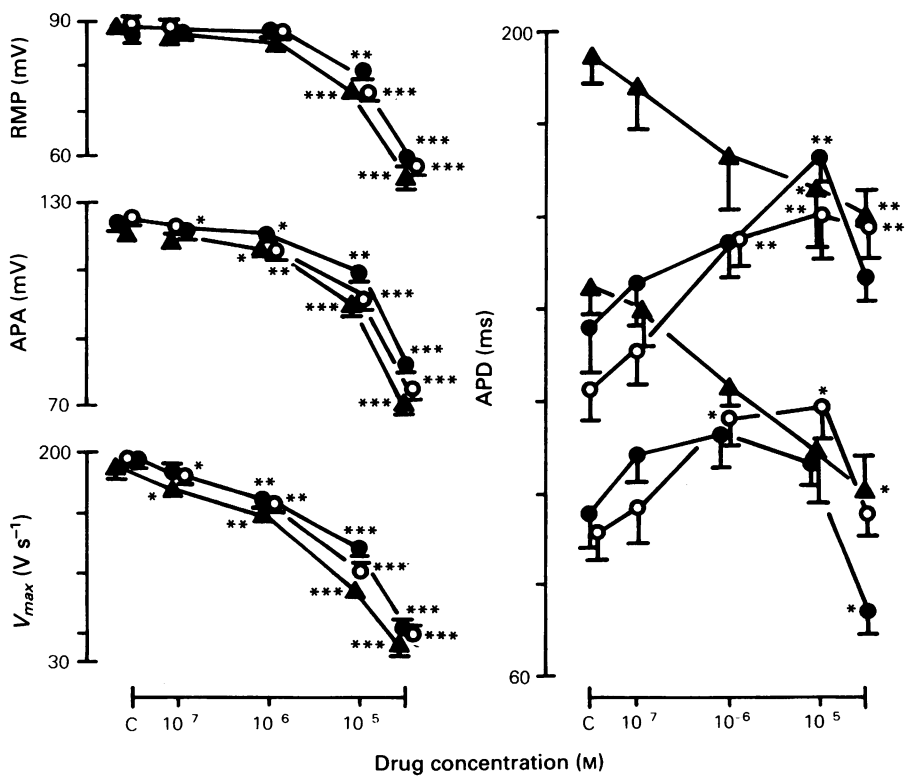

Figure 5 Electrophysiological effects of propafenone (P) in papillary muscles obtained from untreated (●) and P-treated (○: 3 mg kg^{-1}. ▲: 10 mg kg^{-1}) guinea-pigs. RMP = resting membrane potential. APA = action potential amplitude. APD = action potential duration at 50% and 90% of repolarization. Each point represents the mean and vertical lines s.e.mean of 8–18 experiments. $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

for phase 0 characteristics of the action potential and resting potential were similar to those observed in untreated muscles. In this series of experiments at concentrations $\geq 10^{-6}$ M propafenone produced a significant decrease of the amplitude and V_{max} of the action potential and depolarized the resting potential, similar to the results observed in fibres pretreated with 3 mg kg^{-1}. However, fibres pretreated with 10 mg kg^{-1} exhibited a significant prolongation of the APD_{50} (145.5 ± 9.3 ms; $P < 0.01$) and APD_{90} values (195.0 ± 8.8 ms; $P < 0.001$) compared to those obtained in untreated or in papillary muscles pretreated with 3 mg kg^{-1}. Moreover, in these fibres, contrary to what we had observed in the two preceding series, propafenone produced a dose-dependent shortening of the APD_{50} and APD_{90} values which reached significant values at the highest concentration tested, 5×10^{-5} M. In these three series of experiments perfusion with 10^{-4} M depolarized the resting membrane potential both in untreated (to − 68.0 ± 1.1 mV) and in fibres treated with 3 and 10 mg kg^{-1} (to − 68.7 ± 1.3 mV and − 67.8 ± 1.6 mV, respectively) and preparations became inexcitable within 5–10 min.

Effect of propafenone on the effective refractory period and recovery time in atrial and ventricular muscle fibres

The effects of propafenone on atrial ERP and RT were studied in untreated and propafenone-treated atria driven at a basal rate of 1 Hz. In 18 untreated atria control values for both parameters were 129.4 ± 6.9 ms and 156.7 ± 7.4 ms, respectively. At 10^{-5} M propafenone prolonged ($P < 0.05$) the ERP and RT to 154.4 ± 13.6 ms and 186.3 ± 18.3 ms, respectively. In 13 atria pretreated with 3 mg kg^{-1} control values for ERP and RT (116.9 ± 4.6 ms and 147.3 ± 4.8 ms, respectively) were not statistically different from those obtained in untreated atria. In 8 atria pretreated with 10 mg kg^{-1}, however, control values for ERP (145.6 ± 4.7 ms) and RT (185.5 ± 7.4 ms), were significantly longer ($P < 0.01$) than those in untreated atria. In both groups of pretreated atria, propafenone 10^{-7} and 10^{-6} M had no significant effects on these parameters. At 10^{-5} M, it depressed atrial excitability and 5 out of 16 atria pretreated with 3 mg kg^{-1} and 4 out of 8 atria pretreated with 10 mg kg^{-1} developed various degrees of conduction block and finally became inexcitable within 30 min of perfusion. In the remaining 11 atria pretreated with 3 mg kg^{-1}, propafenone significantly prolonged ($P < 0.01$) the ERP and RT values to 162.4 ± 7.1 ms and 188.4 ± 10.1 ms, respectively; in the remaining 4 atria pretreated with 10 mg kg^{-1}, it prolonged ($P < 0.001$) both values to 342.0 ± 41.3 ms and 352.8 ± 35.0 ms, respectively. Figure 6a shows the effects of propafenone on ERP/APD_{90} and ERP/RT ratios in untreated and pretreated atria. At concentrations $\geq 10^{-6}$ M, it had no effect on the ERP/APD_{90} ratio (Figure 6a). At 10^{-5} M, it did not modify this parameter in untreated atria. However, at this concentration propafenone significantly prolonged the ERP/APD_{90} ratio in atria pretreated with 3 mg kg^{-1} (from 0.93 ± 0.03 to 1.37 ± 0.22; $n = 13$. $P < 0.05$) and 10 mg kg^{-1} (from 0.90 ± 0.08 to 2.2 ± 0.2; $n = 5$; $P < 0.001$). In both untreated and pretreated atria propafenone (10^{-7}–10^{-5} M) did not modify the ERP/RT ratio (Figure 6a).

Figure 6b shows the effect of propafenone on the ERP/APD_{90} and ERP/RT ratios in untreated and propafenone-treated papillary muscles. The control values for the ERP and RT were similar in untreated (132.4 ± 7.9 ms and 156.8 ± 7.9 ms) and in muscles pretreated with 3 mg kg^{-1} (124.3 ± 5.6 ms and 151.5 ± 5.8 ms), whereas in muscles pretreated with 10 mg kg^{-1} both values were significantly prolonged compared to those of the control series and the series treated with 3 mg kg^{-1} (186.3 ± 11.3 ms and 217.0 ± 13.5 ms; $P < 0.001$). As is shown in Figure 6b propafenone, 10^{-7} and 10^{-6} M, had no effect on the ERP/APD_{90} ratio in any of the three series of experiments, but at 10^{-5} M it produced a significant prolongation of this ratio which was significantly greater in fibres pretreated with 10 mg kg^{-1} than in fibres pretreated with 3 mg kg^{-1} or in untreated muscle fibres ($P < 0.001$). However, propafenone did not modify the ERP/RT ratio in either untreated or propafenone-treated papillary muscles.

Effect of propafenone on the slow action potentials

The effect of propafenone on slow action potentials was studied in 9 papillary depolarized with 27 mM K-Tyrode solution to − 46.5 ± 1.9 mV to inactivate the fast Na channels. At this time preparations became inexcitable despite intense electrical stimulation, but excitability could be restored by adding 10^{-6} M isoprenaline to the perfusate. Propafenone, 10^{-7} M, had no effect on the slow action potentials. Between 10^{-6} and 10^{-5} M, it significantly reduced the amplitude and V_{max} of the upstroke but had no effect on the APD or the resting membrane potential. At 5×10^{-5} M these effects were accompanied by a significant shortening of the APD and a depolarization of the resting membrane potential. Within 10–20 min of perfusion with 10^{-4} M propafenone the resting membrane potential was shifted to − 36.3 ± 1.8 mV and the slow action potentials suppressed. Similar results were obtained in another 4 papillary muscles where the slow action potentials were induced by 5 mM caffeine.

Plasma concentrations of propafenone

The plasma levels of propafenone were determined after 28 days of pretreatment with 3 and 10 mg kg^{-1}.

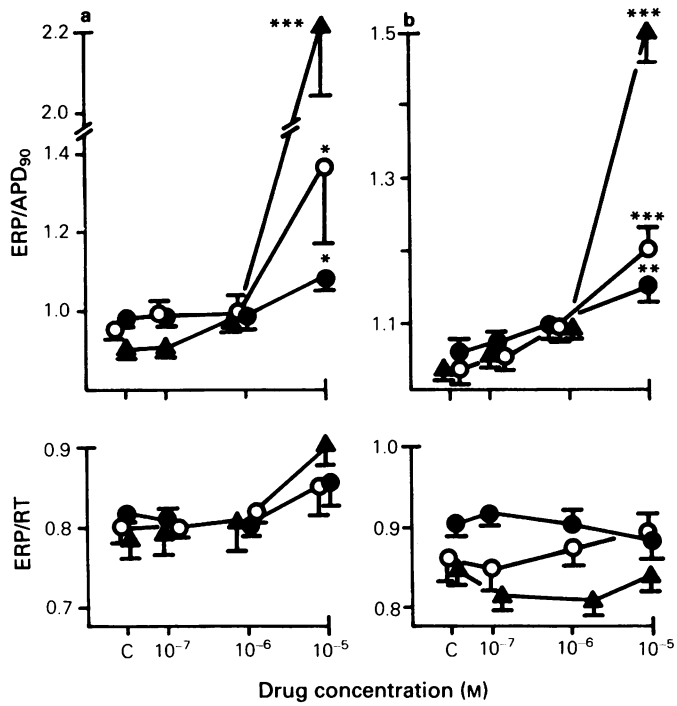

Figure 6 Effect of propafenone (P) on the ERP/APD_{90} and ERP/RT ratio in (a) atrial and (b) ventricular muscle fibres obtained from untreated (●) and P-treated (○: 3 mg kg^{-1}. ▲: 10 mg kg^{-1}) guinea-pigs. Each point represents the mean and vertical lines s.e.mean of at least 8 experiments. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. ERP = effective refractory period; RT = recovery time and APD_{90} = action potential duration at 90% of repolarization.

In 6 guinea-pigs pretreated with 3 mg kg^{-1} plasma concentrations were below the minimal detectable concentration with our method. In 7 animals pretreated with 10 mg kg^{-1} the range of concentrations was from 3.0 to 6.5 ng ml^{-1} (mean 4.7 ± 0.4 ng ml^{-1} equivalent to 1.2 ± 0.1 × 10^{-8} M), that is, far below the minimal concentration of propafenone which produced significant changes in the present experiments.

Discussion

In the present paper we have studied the effects of propafenone, a new antiarrhythmic drug, on the electrophysiological properties of guinea-pig atria and ventricular muscle fibres obtained from both untreated and pretreated animals (3 and 10 mg kg^{-1} for 28 days). The wide range of concentrations of propafenone tested (0.037–37.8 μg ml^{-1}) includes not

Table 1 Electrophysiological effects of propafenone on the slow transmembrane potentials in guinea-pig papillary muscles

Drug Concentration (M)	*Amplitude* (mV)	*Resting potential* (mV)	V_{max} ($V s^{-1}$)	APD_{50} (ms)	APD_{90} (ms)
0	75.2 ± 1.9	46.6 ± 1.8	9.2 ± 0.4	171.4 ± 12.4	190.8 ± 15.3
10^{-6}	72.4 ± 2.0*	46.0 ± 1.9	8.3 ± 0.4***	164.8 ± 13.6	184.8 ± 15.8
10^{-5}	65.4 ± 2.6**	45.0 ± 2.1*	6.9 ± 0.5***	153.5 ± 15.4	176.0 ± 15.5
5×10^{-5}	53.6 ± 3.4***	41.2 ± 1.0**	3.7 ± 0.5***	100.5 ± 10.8***	131.0 ± 12.6*
10^{-4}	—	36.6 ± 1.8***	—	—	—

Data shown are means ± s.e.means, $n = 9$.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

only the therapeutic plasma levels ($0.5-2\,\mu g\,ml^{-1}$: $1.3 \times 10^{-6}-5.3 \times 10^{-6}$M; Keller *et al.*, 1978; Karagueuzian *et al.*, 1983; Conolly *et al.*, 1983), but also the toxic plasma levels of the drug. The doses selected for pretreatment of animals were chosen from the results of Dieckmann *et al.* (1981). Pretreatment with $3\,mg\,kg^{-1}$ for 28 days produced no remarkable alterations in general health and tissue appearance at necropsy. However, pretreatment with $10\,mg\,kg^{-1}$ produced diarrhoea, reduction of body weight, food and water consumption and loss of hair. Plasma levels of propafenone after pretreatment with $3\,mg\,kg^{-1}$ were below the minimal detectable concentration; after pretreatment with $10\,mg\,kg^{-1}$ mean plasma levels were less than therapeutic plasma levels or the lowest concentration at which propafenone produced significant changes in our experiments. These results, therefore, indicate that propafenone was eliminated before the animals were killed and confirm the short half-life of the drug previously found by others (Keller *et al.*, 1978; Conolly *et al.*, 1983), even so the possibility that some drug is still present in cardiac fibres after 16 h cannot be discarded. Unfortunately, cardiac distribution of propafenone after chronic treatment has not yet been studied.

The most significant electrophysiological effect of propafenone on atrial and ventricular muscle fibres was a significant decrease in phase 0 characteristics of the action potential. In both untreated and pretreated fibres it produced a dose-dependent decrease in the V_{max} and amplitude of the upstroke, accompanied by a progressive reduction of the resting potential. Gradual depolarization of cardiac fibres is known to produce a reduction of V_{max} ((Weidmann, 1955), decrease the conduction velocity (Singer *et al.*, 1967) and decrease atrial excitability, shifting the strength-duration curves upward. When the resting potential reached about -75 mV the reduction in V_{max} was accompanied by different degrees of block and finally the fibres became inexcitable when the resting potential decreased to around -70 mV. All these effects confirm that propafenone inhibits the activation of the I_{Na} in atrial and ventricular muscle fibres, and that the drug exhibits class I antiarrhythmic actions (Kohlhardt, 1977; 1982). Like most class I antiarrhythmics propafenone exhibits local anaesthetic properties on nerves, its effects being similar to those of procaine (Hapke & Prigge, 1976). In both untreated and pretreated fibres propafenone (10^{-5} M) prolonged the ERP, RT and ERP/APD_{90} ratio but did not modify the ERP/RT ratio, which suggested that it had no effect on the recovery from inactivation (reactivation) of the I_{Na}. Similar results have been obtained in ventricular muscle fibres (Kohlhardt & Seifert, 1980; Kohlhardt, 1982; Kohlhardt *et al.*, 1983) and Purkinje fibres (Bergman & Bolte, 1977; Ledda *et al.*, 1981). In this respect, propafenone is similar to quinidine which, although it inhibits the activation of the I_{Na} does not modify its reactivation (Chen *et al.*, 1975). Because it reduced atrial conduction velocity and decreased excitability, propafenone reduced the maximum following frequency at which atria can be driven even at concentrations which did not modify the atrial ERP. The prolongation of the ERP and ERP/APD_{90} values increased with the pretreatment, an effect which might be explained on the basis that the effects of propafenone on the kinetics of the fast Na channels could be different in untreated and pretreated fibres. Since this is the first time the chronic effects of propafenone have been studied, further research is necessary in order to confirm this possibility.

The present results also suggest that, in addition, propafenone might exhibit class II (antisympathetic), class III (APD prolongation) and class IV (calcium antagonism) antiarrhythmic actions. In fact, some antagonism to the positive chronotropic effect of isoprenaline was demonstrated (Figure 3). These results confirm previous results which showed that propafenone produced a β-adrenoceptor mediated sympatholytic effect not only in cardiac muscle (Ledda *et al.*, 1981; Dukes & Vaughan Williams, 1984) but also in vascular and tracheal smooth muscle fibres (Hapke & Prigge, 1976; Paietta *et al.*, 1977). Since this class II action appears at concentrations equivalent to those that are effective on arrhythmias it may play a role in the antiarrhythmic effect of propafenone. Moreover, in untreated fibres propafenone also prolonged the APD (class III action). Because the APD is mainly determined by the activation of an outward K current (Carmeliet & Vereecke, 1979), the lengthening of the APD suggests a decrease in K conductance. However, the effects of propafenone are not similar to those of amiodarone, the prototype for class III antiarrhythmics. In fact, propafenone slightly prolonged the APD at concentrations at which it exerted potent chronotropic effects, prolonged the SNRT and reduced the amplitude and V_{max} of the upstroke (Singh & Vaughan Williams, 1970; Tamargo, 1980). However, in pretreated atria propafenone slowed down the rate of repolarization lengthening the APD_{50} and APD_{90} values. That is, although in untreated atria the class III action of propafenone is almost negligible, in pretreated atria it might play some role in the antiarrhythmic effects of the drug. A prolongation of the APD was also induced by therapeutic concentrations of propafenone in both untreated papillary muscles and those pretreated with $3\,mg\,kg^{-1}$. Following pretreatment with $10\,mg\,kg^{-1}$ the APD became significantly prolonged compared to values observed in untreated muscles or in those pretreated with $3\,mg\,kg^{-1}$. Paradoxically, propafenone produced a progressive shortening in these fibres, even though at the highest concentration tested, the duration of the AP was almost similar to that observed

in fibres pretreated with 3 mg kg^{-1} where propafenone produced a prolongation of the APD. All these results seem to indicate that propafenone also seems to produce a class III action in ventricular muscle fibres and that this effect is more marked in fibres chronically pretreated with propafenone. A prolongation of the APD has also been found during long-term treatment with β-adrenoceptor blocking drugs (Vaughan Williams *et al.*, 1975; Raine & Vaughan Williams, 1981). Moreover, long-term β-adrenoceptor blockade produces a lengthening of human APD, as measured with intracardiac suction electrodes (Edvardsson & Olsson, 1978), and prolonged Q–T interval in animals (Raine & Vaughan Williams, 1981) and man (Vaughan Williams *et al.*, 1980). This makes us wonder whether the lengthening of the APD produced by propafenone in pretreated muscles is the consequence of its accumulation in cardiac fibres or of its cardiac β-adrenoceptor blocking properties. Furthermore, propafenone also exhibits class IV effects, i.e. Ca antagonistic properties. In fact, it depressed the slow action potentials elicited in fibres partially depolarized with 27 mM K-Tyrode solution. Under these conditions the fast Na channels were voltage-inactivated, which resulted in a loss of excitability and mechanical failure. Excitability (slow action potentials) was restored by adding isoprenaline (Pappano, 1970) or caffeine (Schneider & Speralakis, 1975). The V_{max} of the slow action potentials thus represent an indirect measurement of the I_{si} (Tritthart *et al.*, 1973). Propafenone produced a dose-dependent decrease of the amplitude and V_{max} of the slow action potentials induced by isoprenaline and caffeine. Schneider & Speralakis (1975) have demonstrated that propranolol can block the isoprenaline-induced slow action potenpotentials without altering those induced by caffeine. Therefore, the present results suggest that at therapeutic concentrations propafenone may also reduce Ca influx via the slow inward Ca channel (class IV action) and that this effect is not simply due to its β-adrenoceptor blocking properties. According to these results, it may suppress supraventricular and ventricular arrhythmias by two mechanisms. Firstly, the decrease in conduction velocity and the prolongation of the ERP may be responsible for the conversion of an area of unidirectional conduction block into an area of bidirectional conduction block in the re-entrant pathway. These effects may be attributed to the decrease in I_{Na} and may be also responsible for the conduction disturbances (widening of the QRS, prolongation of the PR interval) produced by propafenone in the human ECG (Hochrein *et al.*, 1977; Conolly *et al.*, 1983; Schlepper & Olsson, 1983). In addition to these effects, propafenone may also act as an antiarrhythmic drug by depressing cardiac automaticity (Seipel & Breidhardt, 1980; Tamargo *et al.*, 1984). Because it is a weak Ca antagonist and a potent Na channel blocking agent, propafenone produced a negative chronotropic effect and prolonged the sinus node recovery time, i.e. depressed the sinus function. In fact, marked bradycardia, sino-atrial block and sinus arrest have been reported after the administration of propafenone (Schlepper & Olsson, 1983).

In conclusion, in untreated guinea-pig atria even when the electrophysiological effects of propafenone are similar to those of quinidine (class I action), it also exhibited class II (antisympathetic) and weak class IV (Ca antagonist) antiarrhythmic properties. In pretreated atria a prolongation of the APD (class III action) might also be involved in the antiarrhythmic effects of the drug.

The authors are grateful to Dr Margarita Arboix for the determination of propafenone plasmal levels. This work was supported by FISS and CAYCIT grants.

References

BECK, O., WITT, E. & HOCHREIN, H. (1975). Effect of the antiarrhythmic agent propafenone on cardiac conduction. *Z. Kardiol.*, **64**, 179–187.

BERGMANN, M. & BOLTE, H. (1977). Elektrophysiologische Untersuchmegen mit Propafenon an myokardialen Einzelfasern. In *Fortschritte in der Pharmakotherapie von Herzrhythmusstörungen*. ed. Hochrein, H., Hapke, H.J. & Beck, O. pp. 29–34. Stuttgart: Fischer.

CALLEJA, C., DELGADO, C. & TAMARGO, J. (1985). Effect of propafenone on the electrophysiological properties of sheep ventricular muscle and Purkinje fibres. *Br. J. Pharmac. Proc. Suppl.*, **84**, 172P.

CARMELIET, E. & VEREECKE, J. (1979). Electrogenesis of the action potential and automaticity. In *Handbook of Physiology. The Cardiovascular System. I. The heart*. ed. Berne, R. pp. 269–334. Bethesda: Am. Physiol. Soc.

CHEN, C., GETTES, L. & KATZUNG, B. (1975). Effect of lidocaine and quinidine on steady-state characteristics and recovery kinetics of (dv/dt)max in guinea-pig ventricular myocardium. *Circulation Res.*, **37**, 20–29.

CONOLLY, S., KATES, R., LEBSACK, C., HARRISON, D. & WINKLE, R. (1983). Clinical pharmacology of propafenone. *Circulation*, **68**, 589–596.

DELGADO, C., TAMARGO, J. & TEJERINA, T. (1984). The electrophysiological effects of propafenone on guinea-pig atrial muscle. *Br. J. Pharmac. Proc. Suppl.*, **81**, 171P.

DIEKMANN, W., FISCHER, A., HEIMAN, W., SCHNITZLEIN, W. & TRAGER, H. (1981). Exposé of propafenone HCl. Toxicological part. *Internal report VP/FBA 8126*. Lugwigshafen: Knoll AG.

DUKES, I. & VAUGHAN WILLIAMS, E. (1984). The multiple modes of action of propafenone. *Eur. J. Cardiol.*, **5**, 115–125.

EDVARDSSON, N. & OLSSON, S. (1978). Inverkan av akut och kronisk betablockad på repolarisationsfaseni höger kammare (abstr.). *Acta Scietatis Medicorum Suencanae*, **87**, 111.

HAPKE, H.J. & PRIGGE, E. (1976). Zur Pharmakologie von 2'-|2- Hydroxy-3-(propylamino)-propoxy|-3-phenylpropiophenon (Propafenon, SA 79)-hydrochlorid. *Arzneimittelforsch.* **26**, 1849–1857.

HOCHREIN, H., HAPKE, H-J. & BECK, O. (1977). *Forschritte in der Pharmakotherapie von Herzrhythmüsstorungen.* Stuttgart: Fischer.

KARAGUEUZIAN, H., FUJIMOTO, T., KOTOH, T., McKULLEN, A. & MANDEL, W. (1983). Suppression of ventricular arrhythmias by propafenone, a new antiarrhythmic agent, during acute myocardial infarction in the conscious dogs. A comparative study with lidocaine. *Circulation*, **66**, 1190–1198.

KELLER, R., MEYER-ESTORF, G., BECK, O. & HOCHREIN, H. (1978). Correlation between serum concentration and pharmacological effects on atrio-ventricular conduction time of the antiarrhythmic drug propafenone. *Eur. J. clin. Pharmac.*, **13**, 17–20.

KOHLHARDT, M. (1977). Der Finflup von Propafenon auf den transmembranören Na^+-und Ca^{++}-Strom der Warblütter-Myokard-fasermembran. In *Forschritte in der Pharmakotherapie von Herzrhythmusstörungen.* ed. Hochrein, H., Hapke, H.J. & Beck, O. pp. 35–38. Stuttgart: Fischer.

KOHLHARDT, M. (1982). A quantitative analysis of the Na^+-dependence of V_{max} of the fast action potential in mammalian ventricular myocardium. Saturation characteristics and the modulation of a drug induced INa blockade by $|Na^+|o$. *Pflugers Arch.*, **392**, 379–387.

KOHLHARDT, M., SEIFERT, C. & HONDEGHEM, L.M. (1983). Tonic and Phasic/Na blockade by antiarrhythmics. Different properties of drug binding to fast sodium channels as judged from V_{max} studies with propafenone and derivatives in mammalian ventricular myocardium. *Pflugers Arch.*, **396**, 199–209.

KOHLHARDT, M. & SEIFERT, C. (1980). Inhibition of V_{max} of the action potential by propafenone and its voltage-time and pH-dependence in mammalian ventricular myocardium. *Naunyn-Schmiedebergs Arch. Pharmac.*, **315**, 55–62.

LEDDA, F., MANTELLI, L., MANZINI, S., AMERINI, S. & MUGELLI, A. (1981). Electrophysiological and antiarrhythmic properties of propafenone in isolated cardiac preparations. *J. cardiovasc. Pharmac.*, **3**, 1162–1173.

MANZANARES, J. & TAMARGO, J. (1983). Electrophysiological effects of imipramine in non-treated and in imipramine pretreated rat atrial fibres. *Br. J. Pharmac.*, **79**, 167–175.

PAIETTA, E., POCH, O. & KUKOVETZ, W. (1977). Analyse der beta-blockerwirking von Propafenon (SA 79). In *Fortschritte in der Pharmakotherapic von Herzrhythmusstörungen.* ed. Hochrein, H., Hapke, H. & Beck, O. pp. 20–28. Stuttgart: Fischer.

PAPPANO, A. (1970). Calcium dependent action potential produced by catecholamines in guinea-pig atrial muscle depolarized by potassium. *Circulation Res.*, **27**, 379–390.

RAINE, A.E.G. & VAUGHAN WILLIAMS, E.M. (1981). Adaptation to prolonged beta-blockade of rabbit atrial, Purkinje and ventricular potentials, and of papillary muscle contraction. *Circulation. Res.*, **48**, 804–812.

RODRIGUEZ, S. & TAMARGO, J. (1980). Electrophysiological effects of imipramine on bovine ventricular muscle and Purkinje fibres. *Br. J. Pharmac.*, **70**, 15–23.

SCHLEPPER, M. & OLSSON, B. (1983). Cardiac arrhythmias. In *Proceedings 1st International Rytmonorm-Congress.* Berlin, Heidelberg, New York: Springer-Verlag.

SCHNEIDER, J. & SPERALAKIS, N. (1975). Slow Ca^{++} and Na^+ responses induced by isoproterenol and methylxanthines in isolated perfused guinea pig hearts exposed to elevated K^+. *J. mol. cell. Cardiol.*, **7**, 249–273.

SEIPEL, L. & BREITHARDT, G. (1980). Propafenone – A new antiarrhythmic drug. *Eur. Heart J.*, **1**, 309–313.

SINGER, D., LAZZARA, R. & HOFFMAN, B. (1967). Interrelationships between automaticity and conduction in Purkinje fibres. *Circulation Res.*, **21**, 537–558.

SINGH, B. & VAUGHAN WILLIAMS, E. (1970). The effect of amiodarone, a new antianginal drug, on intracellular potentials and other properties of isolated cardiac muscle. *Br. J. Pharmac.*, **39**, 657–667.

TAMARGO, J. (1980). Electrophysiological effects of bunaphtin on isolated rat atria. *Eur. J. Pharmac.*, **62**, 81–89.

TAMARGO, J., DELGADO, C. & TEJERINA, T. (1984). Effect of propafenone on ventricular automaticity. *Eur. Heart J.*, **5** (Suppl 1), 131.

TRITTHART, K., VOLKMER, R., WEISS, R. & FLECKENSTEIN, A. (1973). Calcium-mediated action potentials in mammalian myocardium. Alterations of membrane response as induced by changes of Ca_e or by promoters and inhibitors of transmembrane Ca inflow. *Naunyn-Schmiedebergs Arch., Pharmac.*, **280**, 239–252.

VAUGHAN WILLIAMS, E. (1975). Classification of antidysrhythmic drugs. *Pharmac. Ther.*, **B1**, 115–138.

VAUGHAN WILLIAMS, E.M., HASSAN, M.O., FLORAS, J.S., SLEIGHT, P. & JONES, J.V. (1980). Adaptation of hypertensives to treatment with cardioselective and non-selective beta blockers. Absence of correlation between bradycardia and blood-pressure control, and reduction in the slope of the Q-T/R-R relation. *Br. Heart J.*, **44**, 473–487.

VAUGHAN WILLIAMS, E.M., RAINE, A.E.G., CABRERA, A.A. & WHYTE, J.M. (1975). The effects of prolonged beta-adrenoceptor blockade on heart weight and cardiac intracellular potentials in rabbits. *Cardiovasc. Res.*, **9**, 579–592.

WEIDMANN, S. (1955). The effect of cardiac membrane potential on the rapid availability of the sodium-carrying system. *J. Physiol.*, **127**, 213–224.

(Received February 20, 1984.
Revised July 17, 1985.
Accepted August 5, 1985.)