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Effects of 5-hydroxy-propafenone in guinea-pig atrial fibres

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1 The effects of 5-hydroxy-propafenone (5-OH-P), an active metabolite of propafenone, were studied on isolated atrial muscle fibres obtained from non-treated guinea-pigs and from animals pretreated with 5-OH-P, 3 mg kg^{-1}, for 28 days.

2 In untreated atria 5-OH-P, 10^{-8} M–10^{-4} M, produced a dose-dependent decrease in amplitude and df/dt_{max} and reduced the amplitude of the slow contractions induced by isoprenaline and histamine in high K^+ media.

3 5-OH-P also produced a dose-dependent decrease in atrial rate, prolonged the sinus node recovery time and reduced the maximum chronotropic responses to isoprenaline.

4 In untreated atrial muscle fibres 5-OH-P depressed action potential amplitude and V_{max}, reduced the resting membrane potential and prolonged the action potential duration (ADP) and the effective refractory period, lengthening the effective refractory period relative to APD.

5 Pretreatment with 5-OH-P reduced atrial rate and increased contractile force, but did not modify the action potential characteristics from the values obtained in untreated atria. Further addition of 5-OH-P produced similar but more marked changes than in untreated atria.

6 It is concluded that in guinea-pig isolated atrial muscle fibres 5-OH-P, like propafenone, exhibits class I (membrane stabilizing), class II (antisympathetic) and class IV (Ca antagonistic) antiarrhythmic actions. Therefore, this metabolite could be responsible, at least in part, for some of the antiarrhythmic effects previously attributed to propafenone.

Introduction

Propafenone is a new antiarrhythmic drug which has proved to be effective for treating and preventing supraventricular and ventricular arrhythmias (Seipel & Breidhardt, 1980; Conolly *et al.*, 1983; Schleper & Olsson, 1983). The antiarrhythmic effects of propafenone seem to be a consequence of its ability to inhibit the fast inward Na current, I_{Na} (Kohlhardt, 1977; Kohlhardt & Seifert, 1980; Ledda *et al.*, 1981) and, therefore, it has been included in group Ic of the antiarrhythmic drugs (Dukes & Vaughan Williams, 1984). However, in atrial fibres propafenone not only exhibits class I (membrance stabilizing) but also class II (antisympathetic) and class IV (Ca antagonist) antiarrhythmic actions (Ledda *et al.*, 1981; Dukes & Vaughan Williams, 1984; Delgado *et al.*, 1984; 1985a,b; Tamargo & Delgado, 1985). Furthermore, recently it has been demonstrated that in atrial fibres from animals pretreated chronically with propafenone, a prolongation of the action potential duration (class III) could also be involved in the antiarrhythmic effects of the drug (Delgado *et al.*, 1985b). Propafenone is extensively metabolized, with less than 1% of the oral dose excreted unchanged in urine (Conolly *et al.*, 1983; Siddoway *et al.*, 1984). It undergoes oxidative metabolism to form 5-hydroxy-propafenone (5-OH-P; Hollman *et al.*, 1983; Hege *et al.*, 1984a,b) an active metabolite which in preliminary experiments exhibited local anaesthetic properties and marked antiarrhythmic effects on cardiac arrhythmias of rats induced by aconitine or by isoprenaline as well as on arrhythmias following coronary occlusion in dogs (Philipsborn *et al.*, 1983; 1984). These results suggest that some of the cardiodepressant effects of propafenone previously described in atrial fibres (Dukes & Vaughan Williams, 1984; Delgado *et al.*, 1985b) as well as the potentiation of its cardiac effects observed in atrial fibres from animals pretreated with propafenone (Delgado *et al.*, 1985b) could be due, at least in part, to its main metabolite, 5-OH-P. This possibility has not been tested previously in isolated atria.

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Therefore, the present work was undertaken to study the electrophysiological effects of 5-OH-P in guinea-pig isolated atria obtained from untreated animals and from animals pretreated with 5-OH-P for 24 days. This would allow us to compare the effects of 5-OH-P with those previously reported with propafenone in guinea-pig atria under similar experimental conditions (Delgado *et al.*, 1985b).

Methods

General procedure

Guinea-pigs of either sex weighing 350–400 g were killed by a blow on the head. The hearts were rapidly removed and left and right atria were dissected. Preparations were placed in a chamber and superfused continuously with Tyrode solution (34°C) equilibrated with 95% O_2: 5% CO_2. Under these conditions right atria beat spontaneously. Left atria were stimulated at a basal rate of 1 Hz. The drive stimuli (1 ms duration, twice threshold strength) were applied through bipolar platinum electrodes and delivered from a multipurpose programmable stimulator (Cibertec Model CS-220). The frequency and amplitude of contractions were recorded isometrically by means of a Grass FT03 force-displacement transducer on a Grass polygraph. The techniques and definitions of the parameters of isometric contractions, sinus node recovery time (SNRT) and strength-duration curves were as previously described (Tamargo, 1980; Delgado *et al.*, 1985b). Slow contractions were obtained in atria partially depolarized by high K^+ (27 mM) Tyrode solution by adding isoprenaline (10^{-6} M) and driving at a basal rate of 0.12 Hz. After 1 h of equilibration, control measurements were made. Then, 5-OH-P was added cumulatively to the perfusate in concentrations between 10^{-8} M and 10^{-4} M and steady-state effects were recorded 30 min (10^{-8} M–10^{-6} M) to 60 min (10^{-5} M and 5×10^{-5} M) after each concentration level was attained. The β-adrenoceptor blocking activity was evaluated by the ability of 5-OH-P to antagonize the dose-response curve for positive chronotropic effect of isoprenaline in spontaneously beating right atria. The pA_2 values were calculated according to the method of Van Rossum (1977).

In further studies guinea-pigs were injected with 5-OH-P (3 mg kg^{-1}, i.p. daily) for 28 days. The animals were killed 24 h after the final injection and both right and left atria were dissected and mounted as described above.

Intracellular recordings

Transmembrane potentials were recorded through standard glass microelectrodes filled with 3 M KCl, having resistances of 10–20 MΩ, displayed via high-impedance, capacity neutralizing amplifiers (WPI) on an oscilloscope (Tektronix 5104N) and photographed on film (Rodriguez & Tamargo, 1980). The maximum upstroke velocity (V_{max}) was obtained by electronic differentiation (Tamargo & Delgado, 1985). The effective refractory period (ERP) and the recovery time (RT) were measured by introducing after the eighth basic stimulus a premature test-stimulus of twice threshold strength at different intervals from the preceding basic action potential.

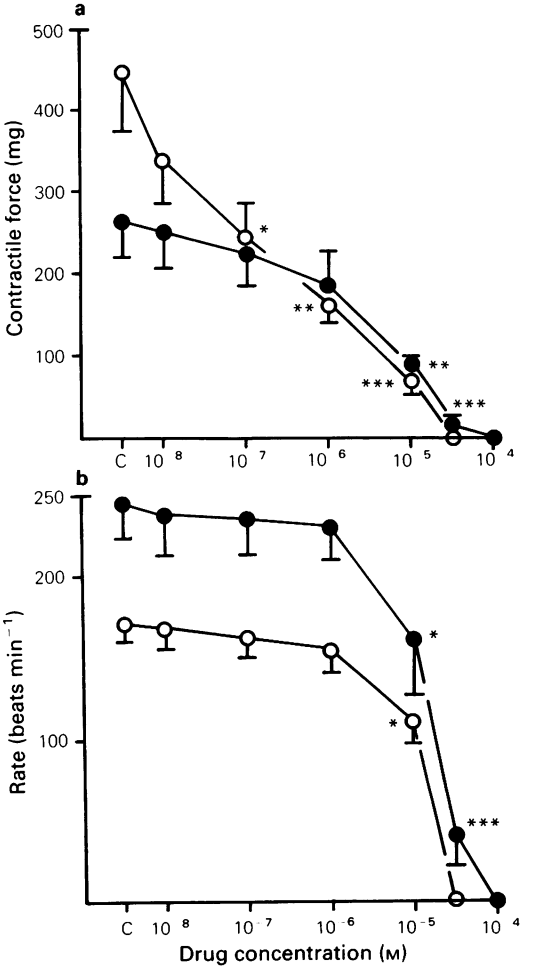

Figure 1 Effect of 5-hydroxy-propafenone (5-OH-P) on (a) peak contractile force and (b) rate of spontaneous contractions in right atria obtained from untreated (●) and 5-OH-P pretreated (○) guinea-pigs. Each point represents the mean of 8–10 experiments; vertical lines show s.e.mean. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Drugs

Drugs used were: 5-hydroxy-propafenone (Knoll AG), isoprenaline hydrochloride (Sigma) and histamine dihydrochloride (Sigma). 5-OH-P was dissolved in distilled deionized water. Further dilutions were carried out in Tyrode solution to obtain final concentrations between 10^{-8} M and 10^{-4} M (0.004–40.0 μg ml^{-1}).

Throughout the paper the data are presented as mean ± s.e.mean. Statistical analysis of the data was performed by analysis of variance and Bonferroni's method was used to compensate for multiple pair-wise comparisons (Wallenstein *et al.*, 1980). A P value of less than 0.05 was considered as significant.

Results

Effect on spontaneously beating right atria

Figure 1 shows concentration-response curves for the effects of 5-OH-P (10^{-8} M–10^{-4} M) on rate and amplitude of spontaneous contractions in 10 control, untreated, atria. Control values for both parameters were 227.0 ± 24.0 beats min^{-1} and 280.7 ± 43.7 mg, respectively. 5-OH-P at concentrations above 10^{-8} M produced a dose-dependent decrease in contractile force which at concentrations higher than 10^{-6} M was accompanied by a significant negative chronotropic effect. Thus, at 10^{-5} M 5-OH-P suppressed the spontaneous activity in 2 atria, in 3 at 5×10^{-5} M and in the remaining 5 at 10^{-4} M. The onset of the negative inotropic and chronotropic effects appears after 2–3 min and the maximal effect was usually evident within 20–25 min. In 8 pretreated right atria, control values for rate and peak contractile force were significantly different (172.2 ± 11.8 beats min^{-1} and 452.4 ± 81.0 mg, respectively; $P < 0.05$) from those observed in untreated atria. As is shown in Figure 1, 5-OH-P (10^{-8} M–10^{-4} M) produced a significant negative inotropic and chronotropic effect which was more marked than that observed in untreated atria. In fact, spontaneous activity was suppressed in all atria within 10 min after addition of 5-OH-P, 5×10^{-5} M, to the bath.

The negative chronotropic effect of 5-OH-P was accompanied by a prolongation of the SNRT. In 9 untreated atria, control values of the SNRT averaged 198.8 ± 16.9 ms and in the presence of 5-OH-P, 10^{-7} M, 10^{-6} M and 10^{-5} M, increased by 10.6 ± 3.2% ($P > 0.05$), 17.2 ± 5.8% ($P < 0.05$) and 116.0 ± 29.0% ($P < 0.001$), respectively. In 6 pretreated atria the control values for the SNRT were not statistically different from those obtained in untreated atria (378.0 ± 48.0 ms, $P > 0.05$); 5-OH-P, 10^{-7} M–10^{-5} M, prolonged the SNRT by 14.6 ± 5.3% ($P > 0.05$), 26.0 ± 5.6% ($P < 0.05$) and 91.0 ± 20.0% ($P < 0.001$), respectively. Therefore, the prolongation of the SNRT produced by 5-OH-P was similar in untreated and in treated atria.

Effects on electrically driven left atria

The effects of 5-OH-P were also studied in 9 left atria driven at a constant rate of 1 Hz. Control values for the different parameters of isometric contractions were as follows: peak contractile force: 228.8 ± 28.0 mg, df/dt_{max}: 7.2 ± 0.8 mg ms^{-1}, time to peak tension: 52.7 ± 4.7 ms and time for total contraction: 191.2 ± 12.9 ms. At concentrations between 10^{-8} M and 10^{-4} M (Figure 2), 5-OH-P produced a significant and parallel decrease of peak contractile force and df/dt_{max} (not shown). However, the drug had no significant effects on the time to peak tension and time for total contraction. Atrial fibres became inexcitable 5–10 min after 5-OH-P 10^{-4} M was added to the bath. Resting tension was not modified at any of the concentrations of 5-OH-P tested. In 8 pretreated atria, control values for peak contractile force and df/dt_{max} (366.5 ± 33.0 mg and 9.2 ± 1.2 mg ms^{-1}, respectively) were significantly greater ($P < 0.05$) than those obtained in untreated atria. However, no significant differences between untreated and pretreated

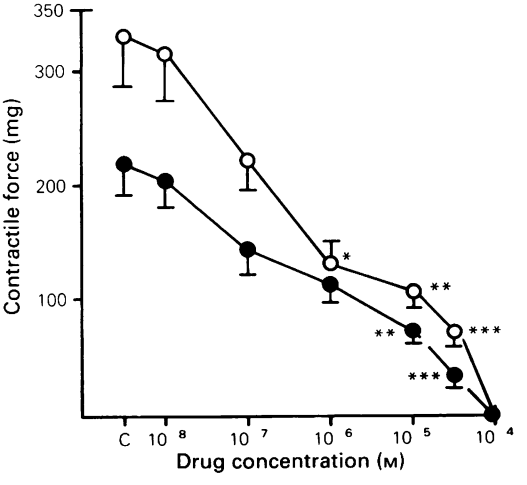

Figure 2 Effect of 5-hydroxy-propafenone (5-OH-P) on peak contractile force in electrically driven left atria obtained from untreated (●) and 5-OH-P pretreated (○) guinea-pigs. Ordinate scale: contractile force (mg). Abscissa scale: drug concentration (M). Each point represents the mean of 8–9 experiments; vertical lines show s.e.mean. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

atria were found in the time to peak tension (55.8 ± 4.4 ms, $P > 0.05$) and time for total contraction (182.5 ± 10.2 ms, $P > 0.05$). 5-OH-P, 10^{-8} M–10^{-4} M, produced a dose-dependent decrease in contractile force (Figure 2) and df/dt_{max} more marked than in untreated atria, without altering the time to peak tension and time for total contraction.

In another 10 left atria following equilibration, the K concentration of Tyrode solution was increased to 27 mM and the preparations became enexcitable because of the voltage-dependent inactivation of the fast Na channels. Isoprenaline (10^{-6} M) and histamine (10^{-6} M) restored the excitability, i.e. slow contractions, when the atria were driven at a basal rate of 0.12 Hz. 5-OH-P (10^{-8} M–10^{-4} M) dose-dependently decreased peak contractile force of the slow contractions induced by both agonists (Figure 3) which were suppressed within 5 min of exposure to 10^{-4} M. The effect of 5-OH-P was completely reversed by increasing the $[Ca]_o$ from 1.8 (control) to 5.4 mM.

Effect on atrial excitability

The effects of 5-OH-P were studied in untreated ($n = 6$) and pretreated ($n = 5$) atria in which strength-duration curves were obtained. In untreated atria, 5-OH-P (10^{-6} M and 10^{-5} M) increased the stimulus current required to evoke a contraction at each stimulus duration, i.e. excitability decreased, and the curves were shifted dose-dependently upwards (Figure 4).

In pretreated atria, strength-duration control curves were similar to those obtained in untreated atria (not shown). At 10^{-6} M 5-OH-P produced similar effects to those obtained in untreated atria, whereas at 10^{-5} M it produced an upward shift of the curve which only reached significant values ($P < 0.05$) at a stimulus duration between 0.5 and 2 ms (Figure 4).

β-Adrenoceptor blocking (class II) action

The effects of 5-OH-P were also studied on the dose-response curves for the positive chronotropic and inotropic responses to isoprenaline in isolated right atria. Both curves were dose-dependently shifted to the right in a competitive way after treatment with 5-OH-P, 10^{-6} M and 10^{-5} M, for 30 min. From these experiments the pA_2 for the blockade of the positive chronotropic responses of isoprenaline was found to be 5.23 ± 0.05 ($n = 8$). Under similar conditions the

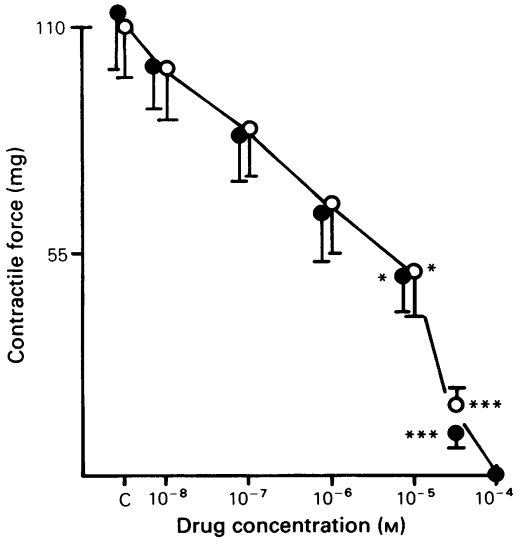

Figure 3 Effect of 5-hydroxy-propafenone (5-OH-P) on the slow contractions induced by isoprenaline (●, 10^{-6} M) and histamine (○, 10^{-6} M) in atrial fibres depolarized with 27 mM K^+ Tyrode solution. Ordinate scale: contractile force (mg). Abscissa scale: drug concentration (M). Each point represents the mean of 6 experiments; vertical lines show s.e.mean. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

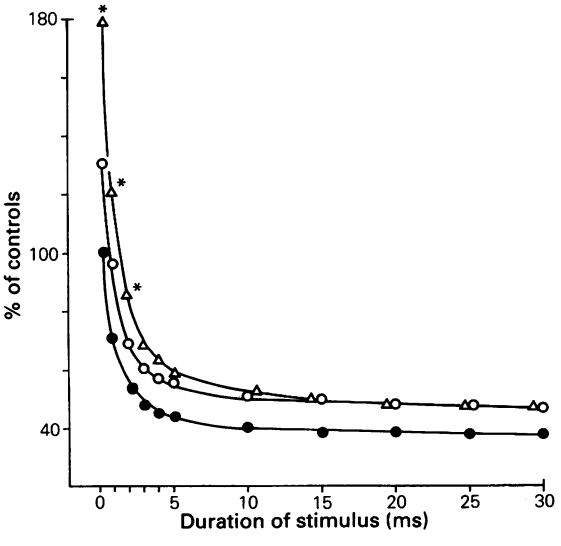

Figure 4 Effect of 5-hydroxy-propafenone (5-OH-P) on strength-duration curves in electrically driven atria. Ordinate scale: minimum current required to evoke a contraction (% of control values). Abscissa scale: duration of the stimuli at that time (ms). Each point represents the mean of 5 experiments. (●) Controls; 5-OH-P in untreated (○) and in atria pretreated with 5-OH-P, 3 mg kg^{-1} (△). $*P < 0.05$.

pA_2 for propafenone was 6.04 ± 0.02 ($n = 8$).

Ca antagonism (class IV) action

To determine whether 5-OH-P exhibited some Ca antagonistic properties the effects of the drug were studied on the positive inotropic responses to increases in extracellular Ca concentration (1.8–7.0 mM). As is shown in Figure 5, 5-OH-P (10^{-6} M and 10^{-5} M) produced a progressive shift of the dose-response curve to Ca to the right. These results suggested that 5-OH-P might have some Ca antagonistic activity in guinea-pig isolated atria.

Effect on transmembrane action potentials

The effects of 5-OH-P in a range of concentrations between 10^{-8} M and 10^{-4} M were examined in untreated and pretreated left atria driven at a basal rate of 1 Hz. Results are summarized in Figure 6. Data obtained with 10^{-8} M and 10^{-7} M were omitted from the figure because they were not statistically different from controls. At 10^{-6} M, 5-OH-P significantly reduced the V_{max}, whereas at higher concentrations it also produced a significant reduction of the amplitude and V_{max} of the action potential which was accompanied by a progressive shift of the resting membrane potential to less negative values. At 10^{-4} M the resting membrance potential was depolarized to −81.0 ± 1.1 mV and all fibres became inexcitable within 10 min. At concentrations higher than 10^{-6} M the slope of phases 2 and 3 decreased and the duration of phase 3 increased which led to a progressive prolongation in the action potential duration at both 50% (APD_{50}) and 90% (APD_{90}) levels of repolarization. This prolongation reached significant values ($P < 0.05$) at 10^{-5} M for APD_{90} and at 5×10^{-5} M for APD_{50} values. The effects of concentrations of 5-OH-P up to 10^{-5} M were reversible within 30–60 min of perfusion with Tyrode solution. At higher concentrations the effects of 5-OH-P were only partly reversed during washout.

The effects of 5-OH-P on action potential characteristics were also studied in 8 left atria obtained from animals pretreated with 5-OH-P for 28 days. As is shown in Figure 6 the control values for these characteristics were not significantly different from those obtained in untreated atria. In pretreated atria, 5-OH-P at concentrations between 10^{-6} M and 10^{-5} M produced a significant reduction ($P < 0.05$) in the amplitude and V_{max} of the action potential without altering the resting membrane potential. At 5×10^{-5} M the resting membrane potential was also decreased and thus, a further reduction in phase 0 characteristics was observed. Moreover, at concentrations higher than 10^{-6} M the effects of 5-OH-P on phase 0 characteristics and resting membrane potential were significantly higher ($P < 0.05$) in pretreated than in untreated atria. Furthermore, and in contrast to untreated atria, 5-OH-P only at 5×10^{-5} M produced a prolongation of the APD_{50} and APD_{90} values which was not statistically significant. At 10^{-4} M, fibres depolarized to −75.0 ± 2.1 mV and became inexcitable within 5–10 min.

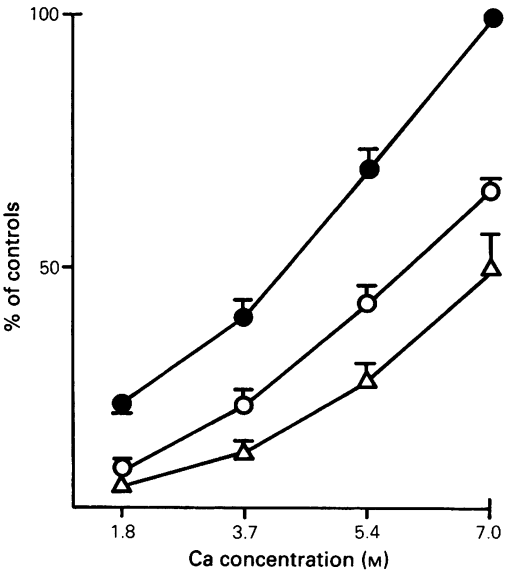

Figure 5 Effect of 5-hydroxy-propafenone (5-OH-P) on the positive inotropic effect of increasing Ca concentrations in electrically driven left atria. Ordinate scale: contractile force (% of control values). Abscissa scale: concentration of Ca (mM) in the bathing media. Each point represents the mean of 6 experiments; vertical lines show s.e.mean. (●) Controls; (○) 5-OH-P 10^{-6} M; (△) 5-OH-P, 10^{-5} M.

The effects of 5-OH-P on ERP, RT and the ERP/APD ratio were also studied in untreated ($n = 8$) and pretreated atria ($n = 6$). Control values for these parameters were similar in untreated (120.4 ± 6.5 ms, 162.1 ± 7.6 ms and 0.95 ± 0.02) and pretreated atria (131.0 ± 12.5 ms, 170.2 ± 10.6 ms and 0.90 ± 0.03, respectively). In untreated atria 5-OH-P at concentrations between 10^{-7} M and 10^{-6} M had no significant effects on the ERP. At 10^{-5} M it produced a significant prolongation of the ERP (140 ± 6.9 ms, $P < 0.05$) which paralleled the prolongation of the APD_{90} and thus, the ERP/APD ratio was not significantly different from control values (0.93 ± 0.02, $P > 0.05$). At 5×10^{-5} M it produced a progressive decrease in excitability which was accompanied by a significant prolongation of the ERP (605.6 ± 151.7 ms, $P < 0.001$) over the APD_{90} values (196.0 ± 3.4 ms) and

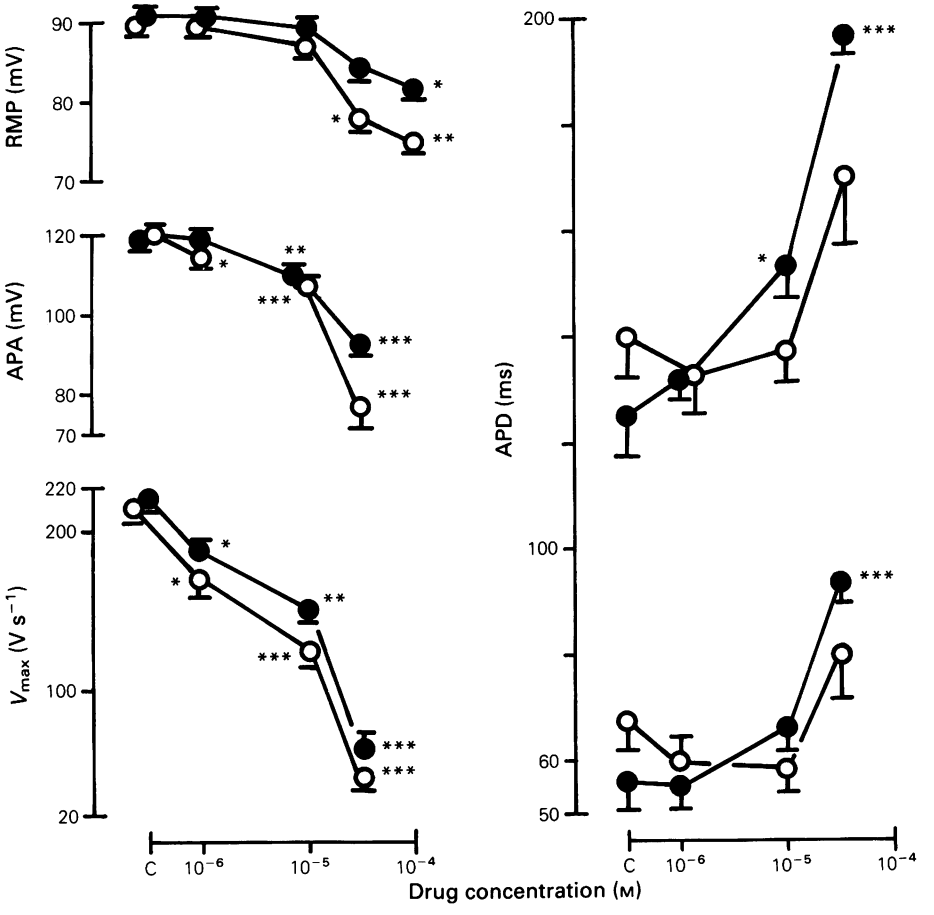

Figure 6 Electrophysiological effects of 5-hydroxy-propafenone (5-OH-P) in atrial fibres obtained from untreated (●) and 5-OH-P pretreated (○) guinea-pigs. RMP: resting membrane potential. APA: action potential amplitude. V_{max}: maximum rate of rise of the upstroke. APD: action potential duration at 50% and 90% of repolarization. Each point represents the mean of 8–13 experiments; vertical lines show s.e.mean. $*P<0.05$; $**P<0.01$; $***P<0.001$.

thus, the ERP/APD ratio was significantly increased to 3.42 ± 0.65 ($P<0.001$). In pretreated atria, 5-OH-P at concentrations between 10^{-7} M and 10^{-5} M, did not significantly modify the APD_{90} and ERP values over controls and the ERP/APD ratio remained unchanged. However, at 5×10^{-5} M the decrease in atrial excitability was accompanied by different degrees of conduction block and the ERP could not be determined.

In both untreated and pretreated atria 5-OH-P, 10^{-7} M–10^{-5} M, did not modify the ERP/RT ratio which suggests that it had no effect on the recovery from inactivation of the I_{Na}.

Discussion

In the present paper we studied the effects of 5-OH-P, the main metabolite of propafenone, on action potential characteristics and contractile force in guinea-pig atria obtained from untreated and pretreated animals. Pretreatment with 3 mg kg^{-1} daily for 24 days produced no remarkable alterations in general health and tissue appearance at necropsy. However, pretreated atria presented a significant reduction in sinus rate and a significant increase in peak contractile force. Even though we do not have an explanation for this increase in contractile force, it cannot be

attributed to cardiac hypertrophy since the ratio of the atrial weight to total heart weight or to body weight were similar in both pretreated and untreated animals (unpublished observations).

In both untreated and pretreated atria 5-OH-P reduced peak contractile force and df/dt_{max}, slowed atrial rate and prolonged the SNRT and reduced atrial excitability. But the most prominent electrophysiological effect of 5-OH-P was a significant decrease in phase 0 characteristics of the atrial action potential. At concentrations of 5-OH-P up to 10^{-5} M, the dose-dependent decrease in the V_{max} can be attributed to a direct inhibition of the I_{Na}, whereas at higher concentrations the reduction of V_{max} can also be attributed to inactivation of the I_{Na} due to the depolarization of the membrane potential. These effects indicated that 5-OH-P, like propafenone, exhibits class I antiarrhythmic action. Therefore, since the reduction of V_{max} produced by 5-OH-P and propafenone in guinea-pig atrial fibres appeared at the same range of concentrations (Delgado *et al.*, 1985b), the class I actions of 5-OH-P must play an important role in the antiarrhythmic effects of propafenone. Furthermore, 5-OH-P also prolonged the APD, ERP and ERP/APD ratio but did not modify the ERP/RT ratio. All these effects are similar to those previously described with propafenone in atrial fibres obtained from untreated (Ledda *et al.*, 1981; Dukes & Vaughan Williams, 1984; Delgado *et al.*, 1984; 1985a,b) and pretreated animals (Delgado *et al.*, 1984; 1985b), which suggests that 5-OH-P may be responsible, at least partly, for some of the electromechanical properties previously described with propafenone.

Furthermore, 5-OH-P also exhibited some β-adrenoceptor blocking (class II) actions. The pA_2 for blockade of isoprenaline-induced positive chronotropic responses was 5.3, a value which is about 10 times greater than the values found with propafenone in this paper and by others (Ledda *et al.*, 1981; Dukes & Vaughan Williams, 1984). This β-adrenoceptor blocking effect therefore would play a less important role in the antiarrhythmic action of 5-OH-P than in that of propafenone. Furthermore, the lesser potency of 5-OH-P in blocking β-adrenoceptors could explain why, in contrast to propafenone, 5-OH-P does not prolong the APD in pretreated atrial fibres. 5-OH-P also exhibited some Ca^{2+}-antagonistic (class IV) action. In fact, it shifted downward the dose-response curve to Ca at the same range of concentrations at which it inhibited the V_{max} of the slow action potentials as well as the slow contractions induced in K^+-depolarized atria (Delgado *et al.*, 1985a). However, these effects were observed only at high concentrations of 5-OH-P (above 10^{-5}M) which suggests that the class IV action does not play an important role in the antiarrhythmic effects of the drug.

When the effects of 5-OH-P are compared with those previously described with propafenone under similar experimental conditions (Delgado *et al.*, 1985b; Tamargo & Delgado, 1985) it is evident that some of the effects of propafenone cannot be attributed to its metabolite. There are three main differences. Firstly, even when both drugs prolonged the SNRT the effects of 5-OH-P were similar in untreated and pretreated atria, whereas the effects of propafenone were potentiated in pretreated atria. Thus, 5-OH-P does not seem to be responsible for the prolongation of the SNRT produced by propafenone in chronically treated animals (Delgado *et al.*, 1985b). Secondly, propafenone depolarized the resting membrane potential to less negative values (between −68 and −62 mV, Delgado *et al.*, 1985b) than 5-OH-P which may explain why propafenone produced a greater depression of phase 0 characteristics and excitability than 5-OH-P, particularly in pretreated atria. The third and most important difference between propafenone and 5-OH-P, was in their effects on APD. In pretreated atria both drugs dose-dependently prolonged the APD, an effect which was significantly potentiated in atria pretreated with propafenone (Delgado *et al.*, 1984; 1985b; Dukes & Vaughan Williams, 1984) but not with 5-OH-P. Thus, 5-OH-P is not responsible for the class III antiarrhythmic action exhibited by propafenone in pretreated atria.

In conclusion, the present experiments demonstrated that in guinea-pig isolated atria, 5-OH-P, the main metabolite of propafenone, exhibits class I, class II and class IV antiarrhythmic actions. Therefore, this active metabolite could be responsible, at least in part, for some of the electromechanical effects previously attributed to propafenone.

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