Effects of propafenone on ^{45}Ca movements and contractile responses in vascular smooth muscle

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1 In rat isolated aorta the class Ic antiarrhythmic drug, propafenone, dose-dependently inhibited the contractile responses induced by high K (80 mM) and noradrenaline (NA, 10^{-5} M), the IC_{50} s being $2.5 \pm 0.7 \times 10^{-6}$ M and $8.7 \pm 0.8 \times 10^{-6}$ M, respectively. These inhibitory actions were also observed with propafenone added after the induced contractions.

2 Contractile responses induced by addition of Ca to 0 Ca high-K solution were also inhibited by propafenone ($\text{IC}_{50} = 2.5 \pm 0.8 \times 10^{-6}$ M). Moreover, propafenone inhibited the contractile responses elicited by NA in strips incubated in 0 Ca ($\text{IC}_{50} = 1.9 \pm 0.9 \times 10^{-6}$ M).

3 Propafenone also inhibited ($\text{IC}_{50} = 1.2 \pm 0.4 \times 10^{-5}$ M) the development of spontaneous mechanical activity in portal vein segments.

4 Propafenone, 5×10^{-6} M and 10^{-5} M, inhibited ^{45}Ca uptake stimulated by high K or NA without altering ^{45}Ca uptake in resting strips.

5 These results indicated that in rat isolated aortae and portal veins propafenone inhibited Ca entry through voltage-operated channels and NA-induced Ca uptake as well as Ca release from intracellular stores. As a consequence it would reduce the concentration of intracellular free Ca available at the contractile apparatus for vascular smooth muscle contraction.

Keywords: Propafenone; rat aorta; ^{45}Ca movements

Introduction

Propafenone hydrochloride is a class Ic antiarrhythmic drug effective both in experimental and clinical ventricular and supraventricular tachyarrhythmias (Seipel & Breidhardt, 1980; Connolly *et al.*, 1983; Harron & Brogden, 1987; Somberg *et al.*, 1988; Funk-Brentano *et al.*, 1990). In isolated cardiac ventricular muscle propafenone exhibited a high affinity for the inactivated and activated states of the Na channel and dissociated very slowly from the Na channel receptor site (Kohlhardt & Seifert, 1983; Kohlhardt, 1984; Valenzuela *et al.*, 1989). Moreover, propafenone also inhibited the slow contractions and action potentials elicited by isoprenaline or histamine in ventricular muscle fibres partially depolarized with high K (Ledda *et al.*, 1981; Dukes & Vaughan Williams, 1984; Tamargo & Delgado, 1985; Delgado *et al.*, 1985). These results suggest that propafenone may also inhibit the slow inward Ca current in cardiac tissues. In fact, in voltage-clamp experiments it reduced the slow inward Ca current of the rabbit sino-atrial node (Sato & Hashimoto, 1984) and cat ventricular muscle fibres (Kohlhardt, 1977).

In addition, propafenone inhibited contractile responses induced by high K depolarization in bovine coronary arteries (Hapke & Prigge, 1976) and rat aorta (Philipsborn *et al.*, 1984), produced a relaxant effect in tracheal muscle strips and guinea-pig isolated colon (Hapke & Prigge, 1976; Kukovetz *et al.*, 1977) and inhibited the tetraethylammonium (TEA)-induced action potentials in vascular smooth muscle of canine coronary arteries (Harder & Bellardinelli, 1980). These results may be explained through an inhibition of Ca entry into vascular and non-vascular smooth muscle fibres. However, the possibility that the calcium antagonist properties of propafenone may be responsible for the relaxation of vascular and non-vascular smooth muscle has not been studied yet (Funk-Brentano *et al.*, 1990). Therefore, the present study was undertaken to examine the effects of propafenone on contractile

responses and ^{45}Ca uptake in rat isolated aortic strips and portal veins.

Methods

Experimental procedure

Sprague-Dawley rats of either sex (200–250 g) were killed by a blow on the head. The thoracic aorta were dissected and placed immediately in a modified Krebs-Henseleit solution (KS) of the following composition (mM): NaCl 118, KCl 4.75, NaHCO_3 25, MgSO_4 1.2, CaCl_2 1.8, KH_2PO_4 1.2 and glucose 11. After excess of fat and connective tissue were removed, the aortae were cut into helical strips of about 2.0 mm width and 20 mm in length. Aortic strips and vein segments were mounted vertically under 1 g of tension in organ baths containing 25 ml of KS as previously described (Barrigón & Tamargo, 1986). The tissue bath was maintained at 34°C and bubbled with 95% O_2 –5% CO_2 gas mixture. Contractile responses were recorded isometrically through a force-displacement transducer (Grass FT03) connected to a Grass polygraph (Tamargo & Tejerina, 1989). Each preparation was allowed to equilibrate for at least 2 h before the start of experiments and for this period the incubation medium was changed every 15 min.

After equilibration the following experiments were performed: (1) In some experiments aortic strips were exposed to single submaximal concentrations of KCl (80 mM) and noradrenaline (NA, 10^{-5} M). An initial 25 min control contraction was obtained in each experiment with the appropriate stimulatory agent and strips were then rested for a minimum of 30 min. Control contractile responses for each agonist were obtained at the beginning of the experiment until two successive responses were almost identical in height. This was followed by exposure to propafenone for a 30 min period in both cases before addition of KCl or NA and during the 25 min re-exposure to stimulatory agents. Only one agonist was used

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in each experiment. The results of these experiments are expressed as a percentage of the maximal control agonist-induced contractile responses. (2) To determine whether propafenone could relax an existing contraction, aortic strips were contracted by single submaximal concentrations of NA or KCl. When the contractile response to either agonist was maximum, propafenone was added in progressively increasing cumulative concentrations (10^{-7} M– 10^{-4} M). Strips were allowed to reach a new steady-state tension before each successive concentration of the relaxant drug. The results are expressed as a percentage of the maximal control agonist-induced responses. (3) In additional experiments dose-response curves to CaCl_2 (1–5 mM) were obtained in aortic strips incubated in Ca-free KS containing 2 mM EGTA for 2 h and then in Ca-free high-K (80 mM) solution for 5 min before addition of drugs (Barrigón *et al.*, 1984). The results are expressed as a percentage of the maximal contractile response induced by 5 mM CaCl_2 . (4) To study the effects of propafenone on a membrane store of Ca mobilized by NA, the experimental protocol employed was similar to that previously described by Ebeigbe & Aloamaka (1985). Strips incubated in Ca-free KS were stimulated by successive additions of 10^{-5} M NA every 30 min ('Ca-washing'). When the contractile response to NA was suppressed the strips were exposed for 5 min to 2.7 mM Ca KS (Ca-loading) and then exposed to Ca-washing KS for 2–3 min, followed by 10^{-5} M NA stimulation. The above protocol was repeated in the presence of propafenone added at the end of the 'Ca-loading' period. Control experiments were performed using the same four experimental protocols but omitting propafenone.

Measurements of ^{45}Ca movements

Techniques employed to estimate ^{45}Ca movements in aortic strips were as previously described by Godfraind (1983) and Barrigón *et al.* (1984). To determine ^{45}Ca uptake, aortic strips were bisected and in each experiment half of the aorta served as the control and the other half as the experimental strip. Both halves were equilibrated for 1 h under 1 g of tension in KS of the following composition (mM): NaCl 122, KCl 5, NaHCO_3 25, CaCl_2 1.25, MgSO_4 1.2 and glucose 11.5. This solution was adjusted to pH 7.4 and gassed with 95% O_2 –5% CO_2 . After equilibration, experimental strips were treated with propafenone. Then both experimental and control strips were incubated for 5 min in KS containing ^{45}Ca (specific activity $2 \mu\text{Ci ml}^{-1}$; Amersham International, Buckinghamshire) either containing or not containing propafenone and then for another 5 min in the same solution with or without the addition of 80 mM KCl or 10^{-5} M NA. At this time strips were washed for 5 min at 0°C to remove extracellular bound Ca in a lanthanum solution (mM): NaCl 122, KCl 5.9, MgCl_2 1.25, glucose 11, LaCl_3 50 and Tris maleate 15 (pH 6.8). Afterwards strips were removed, blotted and weighed. The strips were then placed in scintillation vials and 0.5 ml of Protosol (Dupont) added and the strips digested overnight at 60°C . Radioactivity was assayed in a liquid scintillation counter (LKB Wallac 1211 Rack) as previously described (Barrigón & Tamargo, 1986).

Drugs

The following drugs were used: propafenone (Knoll-Made, S.A.) and noradrenaline bitartrate (Sigma, London). Propafenone as a powder was initially dissolved in distilled deionized water and further dilutions were made in KS. Ascorbic acid (10^{-4} M) was added to each solution of NA, made up freshly every day. Throughout the paper values are expressed as the mean $\pm$ s.e.mean and statistical analysis was performed with Student's *t* test. Differences between control and experimental values were considered significant when $P < 0.05$. Dose-response slopes were analyzed to give the concentration of propafenone producing a 50% inhibition of the maximal contractile response (IC_{50}) by linear regression analysis (method of least squares).

Results

Effects on resting tension and spontaneous myogenic activity

At concentrations between 10^{-7} M and 10^{-4} M, propafenone had no effect on base-line tension in aortic strips. However, at this range of concentrations propafenone inhibited the amplitude but not the frequency of the spontaneous myogenic activity in portal veins. Thus, at 10^{-5} M propafenone inhibited the amplitude of the spontaneous contractions by $44.6 \pm 9.9\%$ ($n = 4$), whereas at 5×10^{-5} M it completely suppressed the spontaneous activity, the IC_{50} for the inhibition being $1.2 \pm 0.4 \times 10^{-5}$ M.

Effect on contractions induced by KCl and noradrenaline

The inhibitory effect of propafenone on contractile responses induced by KCl (80 mM) and NA (10^{-5} M) were quantified and compared in isolated aortic strips. Both agonists produced submaximal contractile responses of 435.4 ± 79.7 mg and 289.6 ± 44.9 , respectively ($n = 8$), these values representing $65.0 \pm 5.2\%$ and $95.0 \pm 2.0\%$ of the maximal responses induced by 140 mM KCl and 10^{-4} M NA, respectively. In untreated strips the contractile responses induced by successive applications of both stimulatory agents did not change by more than $6.8 \pm 3.5\%$. The effects of propafenone, 10^{-7} M– 5×10^{-4} M, on the contractile responses induced by KCl- and NA are shown in Figure 1a. At concentrations $\geq 10^{-6}$ M, propafenone produced a concentration-dependent inhibition of the contractile response induced by both stimulatory agents. However, whereas 10^{-4} M propafenone suppressed the contractile response induced by NA, this concentration left unaltered about $12.0 \pm 2.0\%$ of the response induced by high K. This contractile response was completely suppressed by 4×10^{-4} M propafenone. The IC_{50} values for propafenone depression of high-K and NA-induced responses were $2.5 \pm 0.7 \times 10^{-6}$ M and $8.7 \pm 0.8 \times 10^{-6}$ M, respectively, which indicates that propafenone was slightly more potent ($P < 0.05$) in inhibiting the contractile responses induced by high K than those induced by NA. The inhibitory effects of propafenone were reversed by washing with drug-free KS.

Figure 1b shows the relaxant ability of cumulatively increasing concentrations of propafenone on aortic strips previously contracted with either 80 mM KCl or 10^{-5} M NA. In untreated aortic strips contractile responses induced by both stimulatory agents remained stable for more than 3 h. Cumulative increases in the concentration of propafenone (10^{-6} M– 10^{-4} M) to strips previously contracted resulted in a dose-dependent relaxation. However, whereas in the presence

Figure 1 Effect of propafenone, 10^{-6} M– 10^{-4} M, on the contractile responses induced by high K (●, 80 mM) and noradrenaline (▲, 10^{-5} M) in rat aortae when added either before (a) or after the induced contractions (b). Ordinate scale: relaxation (% of control values). Abscissa scale: propafenone concentration (M). Each point represents the mean of 8 experiments; vertical bars show s.e.means. The asterisks denote significant differences ($*P < 0.05$) from control values.

of 10^{-4} M propafenone the responses to NA were completely suppressed, this concentration inhibited by $74.5 \pm 5.1\%$ the contractions elicited by high K. Thus, the IC_{50} s for inhibition of the contractions induced by high K and NA were significantly different ($6.7 \pm 0.7 \times 10^{-6}$ M and $2.0 \pm 0.6 \times 10^{-6}$ M, respectively. $P < 0.05$).

Effects on Ca-induced contractions

The inhibitory effects of propafenone on the $[Ca]_0$ -dependent contractile responses are shown in Figure 2. Following the incubation for 2 h in a Ca-free high-K (80 mM) KS, the addition of Ca to the bathing media (1–5 mM) caused a concentration-dependent contraction of the aortic strips. The maximum contractile response induced by 5 mM $CaCl_2$ was 210.3 ± 45.9 mg, not significantly different from that induced by 10^{-5} M NA (289.6 ± 44.9 , $n = 8$). Propafenone, 10^{-6} M and 5×10^{-6} M not only produced a progressive decrease in the amplitude of Ca-induced contractions of K-depolarized aortic strips but also caused a progressive shift of Ca concentration-response curves downward and to the right. Regression analysis of the inhibition yielded an IC_{50} of $2.5 \pm 0.9 \times 10^{-6}$ M.

In another group of experiments the effects of propafenone were studied on the contractile responses induced by NA in strips which were incubated in Ca-free KS, then in 'Ca-loading' KS for 5 min and finally in Ca-free KS for 2–3 min. At this time stimulation with NA (10^{-5} M) resulted in a phasic contraction of an amplitude of 131.0 ± 38.0 mg ($n = 6$), presumed to reflect Ca mobilization from a membrane-bound store (Karaki *et al.*, 1979). In 6 experiments propafenone, 10^{-6} M and 5×10^{-6} M, when applied after 'Ca-loading' inhibited by $29.7 \pm 3.3\%$ ($P < 0.001$) and $81.0 \pm 1.7\%$, ($P < 0.001$), respectively, the magnitude of the NA-induced phasic contraction, the IC_{50} value being $1.9 \pm 0.9 \times 10^{-6}$ M.

Effects on ^{45}Ca uptake

From the previous experiments two concentrations of propafenone (5×10^{-6} M and 10^{-5} M) were selected to study the effects of the drug on the La-resistant ^{45}Ca uptake in resting, non-stimulated, as well as in aortic strips stimulated by 80 mM

KCl and 10^{-5} M NA. Propafenone, 10^{-5} M, had no significant effects on ^{45}Ca uptake in resting, non-stimulated aortic strips (85.4 ± 3.5 vs. $76.2 \pm 5.2 \mu\text{mol kg}^{-1}$ aorta; $n = 12$; $P > 0.05$). Addition of high K and NA increased ^{45}Ca uptake to 231.6 ± 12.4 ($n = 12$; $P < 0.001$) and $161.6 \pm 10.4 \mu\text{mol kg}^{-1}$ aorta ($n = 12$; $P < 0.001$), respectively. At 5×10^{-6} M and 10^{-5} M propafenone significantly decreased ^{45}Ca uptake stimulated either by KCl to 166.3 ± 10.1 ($n = 12$; $P < 0.05$) and $133.5 \pm 11.8 \mu\text{mol kg}^{-1}$ aorta ($n = 12$; $P < 0.001$) and by NA to 119.0 ± 11.3 ($n = 12$; $P < 0.01$) and $91.2 \pm 12.1 \mu\text{mol kg}^{-1}$ aorta ($n = 12$; $P < 0.001$).

Discussion

The results of this work demonstrated that in rat isolated aortic strips and portal veins propafenone, a class Ic anti-arrhythmic drug, inhibited: (1) the contractile responses induced by high K or by increases of extracellular Ca in K depolarized aortic strips, (2) NA-induced contractions both in a Ca-containing or 0Ca medium, (3) ^{45}Ca uptake in aortic strips stimulated by high K and NA, and (4) the spontaneous myogenic contractions recorded in portal veins. These results confirmed and extended previous experimental evidence showing that propafenone exhibited spasmolytic effects in vascular (Hapke & Prigge, 1976; Harder & Bellardinelli, 1980; Philipsborn *et al.*, 1984) and non-vascular smooth muscle fibres (Hapke & Prigge, 1976; Kukovetz *et al.*, 1977).

In rat isolated aorta NA- and high K-induced contractions have been resolved into a phasic and a tonic component (Hudgins & Weiss, 1968; Godfraind & Kaba, 1969). An increase in Ca entry through receptor-operated channels (ROCs) seems to determine the tonic component of NA-induced contractions (Hudgins & Weiss, 1968; Deth & Van Breemen, 1974; Bolton, 1979), whereas both contractile responses induced by high K or by increasing Ca concentration in K-depolarized aortae are thought to be entirely dependent upon the influx of extracellular Ca through voltage-sensitive channels (VSCs) (Hudgins & Weiss, 1968; Godfraind & Kaba, 1969; Bolton, 1979). Propafenone inhibited in a concentration-dependent manner the contractile responses induced by high K depolarization or by Ca which have been used to provide a simple means of studying drugs with possible Ca entry blocking properties (Haeusler, 1972; Godfraind *et al.*, 1986) as well as the tonic component of NA-induced responses. Moreover, in the present experiments propafenone decreased high K- as well as NA-induced contractions which indicated that it may inhibit Ca entry both through VSCs and ROCs. Another two groups of experiments seem to agree with this hypothesis. Firstly, propafenone inhibited the spontaneous myogenic activity in rat portal veins, the IC_{50} value being quite similar to that obtained in K-depolarized aortic strips. Since myogenic activity has been attributed to depolarization of the membrane which activated VSCs (Sigurdsson *et al.*, 1975), the inhibitory effect of propafenone may be explained through its ability to decrease Ca entry through VSCs. Secondly, at the same range of concentrations at which propafenone inhibited the amplitude of the tonic component of high K- and NA-induced contractions, it also inhibited ^{45}Ca uptake stimulated by both agonists. In fact, the percentage of inhibition for high K- and NA-induced contractions and ^{45}Ca uptake were almost superimposed, thus suggesting that propafenone-induced inhibition can be related to the blockade of Ca entry through VSCs and ROCs, respectively. However, if at the high concentrations used in this study NA depolarized the membrane potential of aortic smooth muscle fibres, the inhibitory effects of propafenone on NA-induced contractions and ^{45}Ca uptake could be attributed to inhibition of Ca entry through both ROCs and VSCs.

It has been reported that propafenone inhibited Ca entry through VSCs in cardiac muscle (Kohlhardt, 1977; Ledda *et al.*, 1981; Satoh & Hashimoto, 1984; Tamargo & Delgado,

Figure 2 Effects of propafenone on the restoration of the isometric contractions of aortic strips by addition of calcium (1–5 mM) to Ca-free high K (80 mM) medium. Ordinate scale: percentage of the maximum control contractions obtained with the highest concentration of Ca in each experiment. Abscissa scale: calcium concentration (mM). Each point represents the mean of 6 experiments; vertical bars show s.e.means. (●) Controls; (▲) after propafenone, 10^{-6} M and (■) 5×10^{-6} M. The asterisks denote significant differences between control responses and those after propafenone ($* P < 0.05$).

1985; Delgado *et al.*, 1985) and in fact in the present experiments propafenone inhibited Ca entry induced both by NA and high K. This lack of specificity is in contrast to what has been described with Ca antagonists, which in vascular smooth muscle fibres inhibited high K-induced contractions to a much greater extent than NA-induced contractions (Cauvin *et al.*, 1983; Tamargo, 1984; Godfraind *et al.*, 1986). However, like Ca antagonists (Cauvin *et al.*, 1983; Godfraind *et al.*, 1986) propafenone had no effect on ^{45}Ca uptake in resting aortic strips, which suggests that Ca entry through passive membrane leak channels seems to be insensitive to the drug.

Furthermore, propafenone also inhibited the phasic component of NA-induced contractions which, since they persisted in 0Ca medium have been attributed to the release of Ca from intracellular stores (Hudgins & Weiss, 1968; Godfraind & Kaba, 1969; Deth & Van Breemen, 1977). This finding suggested that propafenone in addition may also inhibit the release of Ca from an agonist releasable intracellular store. These phasic contractions have been attributed to mobilization of Ca from a membrane-bound store (Karaki *et al.*, 1979; Ebeigbe & Alaomaka, 1985). In the present study, propafenone when applied after 'Ca-loading' inhibited NA-induced phasic contractions elicited in Ca-free medium which suggested that the inhibitory effect of propafenone on NA-induced contractions can be at least partly related to a decrease in the release of Ca from an intracellular store. Another possibility is that in addition, propafenone enhances Ca extrusion from vascular smooth muscle fibres, but this possibility was not analyzed in the present experiments.

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The clinical relevance of the present results remains to be determined. In fact, propafenone is highly bound to plasma and cardiac proteins (Harron & Brogden, 1987) and is extensively hydroxylated to 5-hydroxypropafenone, a metabolite which presents similar properties to those of propafenone (Philipsborn *et al.*, 1984; Valenzuela *et al.*, 1987; 1988). Furthermore, a reduction in aortic blood pressure is not a constant finding either in experimental animals (Hapke & Prigge, 1976; Karagueuzian *et al.*, 1984) or in clinical studies (Connolly *et al.*, 1983; Harron & Brogden, 1987) even when a slight reduction in mean arterial pressure has also been described in some clinical studies (Bachour & Hochrein, 1977; Seipel & Breidhardt, 1980). A possible explanation for the low incidence of hypotension may be related to the negative inotropic effects of propafenone which may increase systemic vascular resistances, thus overriding the expected direct vasodilator and cardiodepressant effects of the drug. Therefore, caution should be exercised in the extrapolation of the present *in vitro* results to clinical situations.

In conclusion, the present data demonstrated that in rat isolated aortae and portal veins, propafenone inhibited Ca entry through voltage- and receptor-operated channels as well as Ca release from intracellular stores. As a consequence it would reduce the concentration of intracellular free Ca available at the contractile apparatus for vascular smooth muscle contraction.

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