Electrocardiographic interactions between pinacidil, a potassium channel opener and class I antiarrhythmic agents in guinea-pig isolated perfused heart

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1 Drugs that shorten action potential duration could decrease the Na-channel blocking effect of class I antiarrhythmic agents by reducing the availability of Na channel in the inactivated state.

2 This hypothesis was tested in guinea-pig perfused heart, measuring the surface ECG effects of three class I drugs endowed with different binding kinetics (15 μ M mexiletine, 10 μ M quinidine and 3 μ M flecainide) in the presence of increasing concentrations of pinacidil (10 μ M, 30 μ M, 50 μ M), a potassium channel opener that shortens action potential duration.

3 The ECG parameters measured were: the QRS interval, i.e. the intraventricular conduction time; the JT interval, which reflects the duration of ventricular repolarization; the ratio between JTpeak (the time from the end of QRS and the peak of T wave) and JT interval, which quantifies changes in the morphology of the T wave.

4 At the concentrations tested all the antiarrhythmic drugs widened the QRS complex by 55–60%. Flecainide did not significantly change JT interval, but quinidine prolonged and mexiletine shortened it. Mexiletine also decreased the JTpeak/JT ratio. Pinacidil by itself decreased the JT interval and the JT peak/JT ratio in a dose-dependent way, but did not affect QRS duration.

5 In the presence of fixed antiarrhythmic drug concentrations, however, pinacidil decreased the QRS prolongation induced by mexiletine (–17%) and quinidine (–8%), but not that induced by flecainide: this effect was already maximal at the lower concentration tested (10 μ M) and there was no relationship between pinacidil-induced JT shortening and QRS changes. To explain this unexpected result it has been supposed that, at the driving frequency used (4 Hz), myocardial cells were partially depolarized and that pinacidil could repolarize them, thus decreasing the number of inactivated Na channels and the effects of drugs that (mainly or partly) block the channels in the inactivated state. In agreement with this hypothesis, an additional series of experiments carried out with 15 μ M mexiletine at a lower stimulation rate (2 Hz) showed only a negligible loss of QRS effect (–2.3%) at any pinacidil concentration.

6 Flecainide, but not quinidine and mexiletine, antagonized the JT shortening induced by pinacidil; furthermore, no drug modified the JTp/JT decrease induced by pinacidil.

7 These results indicate that: (a) an antagonism between class I antiarrhythmic drugs and pinacidil is possible; (b) mexiletine is the most involved among the drugs tested; (c) the interaction is not related to pinacidil-induced repolarization shortening, but probably to changes in membrane resting potential. The possible clinical implications need to be defined.

Keywords: Pinacidil; mexiletine; quinidine; flecainide; interaction; class I antiarrhythmic agents; guinea-pig isolated heart

Introduction

Potassium channel openers (KCO), as a class of drugs, have been widely studied in the last decade because of their wide-spectrum pharmacological effects (Cook, 1988) and clinical applications (Andersson, 1992). Recently, some papers (Wollenbreg *et al.*, 1989; Chi *et al.*, 1990; Padrini *et al.*, 1992) pointed out the potential proarrhythmic effect of these drugs both in acute ischaemia and in normoxic conditions. However, the effect of K channel openers on heart rhythmicity is not unequivocal. On the one hand, these drugs favour re-entry arrhythmias by shortening action potential duration (APD) and increasing refractoriness dispersion (Di Diego & Antzelevitch, 1993). On the other hand, however, they have proved to be effective, even at low concentrations, in suppressing arrhythmias triggered by early afterdepolarizations (Fish *et al.*, 1990). The interplay between KCO and arrhythmias, may be further complicated by a potential interaction between KCOs and the class I antiarrhythmic drugs (Na channel blockers). On the basis of the 'modulated receptor' (Hondeghe & Katzung, 1977) or 'guarded receptor'

(Starmer *et al.*, 1984) hypotheses, shortening of APD is expected to reduce the availability of sodium channels in the inactivated state and, hence, to decrease the electrophysiological effects of those antiarrhythmic agents that predominantly block the inactivated sodium channels.

Since there is little information about this interaction, we studied the influence of pinacidil on the electrophysiological actions of three commonly used class I antiarrhythmic drugs, which differ as to receptor binding kinetics and channel state preferentially blocked (Campbell, 1983; Kodama *et al.*, 1987; Weirich & Antoni, 1990): mexiletine (fast kinetics, inactivated channel blocked), flecainide (slow kinetics, open channel blocked) and quinidine (intermediate kinetics, open channel blocked).

The electrophysiological effects were evaluated in terms of surface ECG changes, in guinea-pig Langendorff perfused hearts. This experimental model, as well as providing physiological perfusion conditions, has also been shown in several studies (Gillis & Kates, 1988; Bova *et al.*, 1989; Nielsen *et al.*, 1989; Gillis & Keashly, 1991; Padrini *et al.*, 1992) to be suitable for measuring the effects of drugs on ventricular repolarization (QT interval) and on intraventricular conduction time (QRS).

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Methods

Guinea-pigs of either sex (body weight 250–350 g) were stunned by a blow to the neck and exsanguinated. The heart was rapidly removed, put in ice-cold buffer solution and perfused retrogradely at a constant flow rate (10 ml min^{-1}) with a Gilson peristaltic micropump. The perfusion medium was a modified Krebs-Henseleit solution (mM: NaCl 118, KCl 4.7, CaCl_2 2.5, MgSO_4 1.2, NaCO_3 25, KH_2PO_4 1.2, glucose 11.1 and pyruvate 2) bubble with $\text{O}_2\text{:CO}_2$ gas mixture (95%:5%) and maintained at 37°C ($\text{pH } 7.46 \pm 0.01$). Atrial free walls and septum were removed and platinum electrodes were fitted over the upper border of the interventricular septum to by-pass the AV node and stimulate the His bundle. A driving frequency of 4 Hz was chosen in order to magnify the effect of class I antiarrhythmic drug on the QRS interval (use-dependent effect) (Grant *et al.*, 1984). In some experiments frequency was set at 2 Hz to investigate the influence of rate on drug interaction (see Results). The stimulation parameters (S88 Grass stimulator) were: amplitude 2–5 V ($= \times 1.5$ threshold value); duration 0.5 ms.

ECG measurements

Surface ECG was recorded by means of two electrodes, one placed on the crux cordis and the other on the left ventricle free wall. The signal was monitored with a storage oscilloscope (Tektronix Mod. 5115) and, when required, the ECG tracing was 'frozen' on the screen and photographed with a polaroid camera. The following time intervals were measured (Figure 1): (1) The intraventricular conduction time (QRS interval), i.e. the interval from the stimulus artifact (corresponding to the His bundle activation) to the end of ventricular activation. (2) The duration of ventricular repolarization (QT interval): the interval from the stimulus artifact to the end of T wave. (3) The time from the stimulus artifact to the T wave peak (QTpeak interval). In order to improve the accuracy of the ECG interval measurements, two different recording speeds were used, according to the duration of the interval to be measured: 0.2 mm ms^{-1} for the QT and QTpeak intervals; 0.5 mm ms^{-1} for the QRS interval.

Using the values of QRS, QT and QTpeak other parameters were calculated: the JT interval (QT minus QRS interval), which reflects more specifically than QT pure repolarization changes; the JTpeak interval (QTpeak minus QRS interval); JTpeak/JT ratio, which is an index of changes in the T wave shape. This ratio has already been shown to decrease following exposure to pinacidil (Padrini *et al.*, 1992). Since changes in the QT interval, at constant frequency, induce the opposite changes in the duration of the electrical diastole, the diastolic interval (DI) was also measured as the difference between cycle length (250 ms) and QT.

Drug treatment

Hearts were first exposed to $15 \mu\text{M}$ mexiletine hydrochloride, $10 \mu\text{M}$ quinidine sulphate or $3 \mu\text{M}$ flecainide hydrochloride. Preliminary experiments have shown that these concentrations produced a similar QRS enlargement (about 55–60%). After reaching steady state ECG effects, pinacidil monohydrate was added in steps to obtain a concentration of 10, 30 and $50 \mu\text{M}$. In a separate series of experiments the effects of the same pinacidil concentrations were evaluated without antiarrhythmic drugs.

Statistical analysis

All data are expressed as mean $\pm$ s.e. The ECG changes from the baseline induced by pinacidil, mexiletine, quinidine and flecainide were analysed by means of Student's paired *t* test. Comparisons of the effect of pinacidil alone and in the presence of antiarrhythmic drugs were carried out by two-way analysis of variance (ANOVA) followed by multiple

Student's *t* test with the Bonferroni correction. Possible correlations between QRS and other ECG parameters were tested by means of linear regression analysis. The statistical analysis was carried out on a PC (SuntaQ AC-486) using the 'Excel' statistical package. Statistical significance was set at $P < 0.05$.

Results

The effects of $10 \mu\text{M}$ quinidine, $15 \mu\text{M}$ mexiletine and $3 \mu\text{M}$ flecainide observed on the QRS interval and the JT interval were consistent with their cellular electrophysiological effects. All the drugs widened the QRS interval (quinidine = +54.3%, $P < 0.001$; mexiletine = +56.4%, $P < 0.001$; flecainide = +59.2%, $P < 0.005$). In addition, quinidine prolonged the JT interval by +7.2% ($P < 0.05$), mexiletine shortened it by -2.4% ($P < 0.05$), while flecainide induced non significant changes (+4.2%, NS). The JTp/JT ratio was not changed by quinidine and flecainide, but was significantly decreased by mexiletine ($P < 0.05$).

The mean values ($\pm$ s.e.) of the ECG parameters given above, measured in control conditions and at steady state of drug effects are shown in Table 1.

The ECG effects of 10–30–50 μM pinacidil, both alone and in the presence of a fixed concentration of quinidine (10 μM), mexiletine (15 μM) and flecainide (3 μM), are shown in Figures 2–4.

Pinacidil alone shortened the JT interval (Figure 3, ●), decreased JTp/JT (Figure 4, ●) but did not affect the QRS interval duration (Figure 2, ●).

Although it had no direct effect on QRS, when in the presence of fixed antiarrhythmic drug concentrations pinacidil concentrations were increased stepwise (10–30–50 μM), the QRS prolongation induced by quinidine and mexiletine was curtailed, on average, by -17% and -8%, respectively; however, the QRS effect of flecainide was

Figure 1 Schematic drawing of the ECG signal, with the indication of the basic intervals measured (QRS, QT, JT and JTpeak).

Table 1 Electrocardiography parameters in control and treated hearts

		QRS (ms)	JT (ms)	JTp/JT
Quinidine	C	35.9 ± 2.0	143.2 ± 4.1	0.83 ± 0.03
	T	$55.4 \pm 3.3^{***}$	$153.7 \pm 5.8^*$	0.84 ± 0.01 NS
Mexiletine	C	33.6 ± 2.5	143.3 ± 5.2	0.84 ± 0.02
	T	$52.5 \pm 1.4^{***}$	$139.9 \pm 4.1^*$	$0.81 \pm 0.01^*$
Flecainide	C	36.0 ± 2.5	140.5 ± 2.9	0.85 ± 0.01
	T	$58.6 \pm 1.4^{**}$	146.5 ± 3.8 NS	0.84 ± 0.02 NS

Significance levels: $***P < 0.001$; $**P < 0.005$; $*P < 0.05$; NS = not significant. C: control; T: treated.

not modified (Figure 2). The antagonism of the QRS effect of quinidine and mexiletine by pinacidil was not concentration-dependent but it did appear to be an all-or-nothing response, at least in the range of concentrations tested. In fact, the QRS decrease observed was virtually the same at both the lowest and at the highest concentrations. Furthermore, there was no correlation either between the pinacidil-induced QRS changes and the corresponding JT (Figure 5) or QT shortening, or between QRS changes and diastolic interval (data not shown). To obtain more information on the mechanism of the interaction we carried out another set of experiments at a lower stimulation frequency (2 Hz). The new hypothesis being tested was that the high frequency formerly used (4 Hz) partially depolarized cardiac myocytes and that pinacidil could repolarize cell membrane and decrease the number of inactivated Na channels (see Discussion). Mexiletine was chosen for these experiments because it showed the most marked interaction with pinacidil.

At 2 Hz, mexiletine increased the QRS duration by $17.2 \pm 2.0\%$ (as compared with $56.4 \pm 3.4\%$ at 4 Hz). In

Figure 2 Relationship between pinacidil concentrations and mean percentage QRS changes obtained with pinacidil alone (control group: ●) and in the presence of $15 \mu\text{M}$ mexiletine (Δ), $10 \mu\text{M}$ quinidine ($\circ$) and $3 \mu\text{M}$ flecainide ($\diamond$). Each point is the mean $\pm$ s.e.mean of 5 experiments. Two-way analysis of variance (ANOVA) showed that the QRS effect was not different between the 3 concentration groups and that pinacidil significantly decreased QRS in the mexiletine and quinidine groups compared to the controls. The asterisks indicate significant differences when multiple *t* tests (with Bonferroni adjustment) were applied.

Figure 3 Relationship between pinacidil concentrations and mean percentage JT changes obtained with pinacidil alone (control group: ●) and in the presence of $15 \mu\text{M}$ mexiletine (Δ), $10 \mu\text{M}$ quinidine ($\circ$) and $3 \mu\text{M}$ flecainide ($\diamond$). Each point is the mean $\pm$ s.e.mean of 5 experiments. Two-way analysis of variance showed a significant concentration-dependent JT shortening in all groups, but the JT shortening was significantly less marked in the flecainide group than in the controls. The asterisks indicate significant differences when multiple *t* tests (with Bonferroni adjustment) were applied.

contrast with 4 Hz results, at the 2 Hz driving rate pinacidil induced only a negligible loss in the mexiletine effect at any concentration considered (-2.3% , on average) (Figure 6). Since the maximum JT shortening induced by pinacidil at 2 Hz was virtually the same ($-26.7 \pm 4.3\%$) as that obtained at 4 Hz ($-24.8 \pm 5.2\%$), it is likely that the resting membrane potential increase (rather than repolarization shortening) was responsible for the drug interaction.

The typical effects of quinidine and mexiletine on ventricular repolarization were also modified by pinacidil and the results of the interaction was the simple algebraic sum of the effect of each drug. However, in the presence of flecainide the JT shortening induced by pinacidil was significantly smaller (Figure 3). These results indicate that some class I antiarrhythmic drugs can, in their turn, interfere with the repolarization effects of pinacidil. The JTp/JT ratio decrease induced by pinacidil was not significantly modified by any of the antiarrhythmic drugs tested (Figure 4).

Discussion

QRS interval changes induced by class-I antiarrhythmic agents are believed to reflect changes in the Na inward current and a widely used clinical classification (Vaughan Williams, 1984) divides class-I drugs into three subgroups

Figure 4 Relationship between pinacidil concentrations and mean percentage JTp/JT ratio changes obtained with pinacidil alone (control group: ●) and in the presence of $15 \mu\text{M}$ mexiletine (Δ), $10 \mu\text{M}$ quinidine ($\circ$) and $3 \mu\text{M}$ flecainide ($\diamond$). Each point is the mean $\pm$ s.e.mean of 5 experiments. Two-way analysis of variance showed a significant concentration-dependent JTp/JT shortening for all the three drug groups; the JTp/JT effect was not significantly different from the controls.

Figure 5 Relationships between pinacidil-induced decreases in JT interval and the corresponding QRS changes, in the presence of $15 \mu\text{M}$ mexiletine (Δ), $10 \mu\text{M}$ quinidine ($\circ$) and $3 \mu\text{M}$ flecainide ($\diamond$). Each point is the mean $\pm$ s.e.mean of 5 experiments.

Figure 6 Pinacidil-mexiletine interaction at a 2 Hz stimulation frequency. (a) Relationships between pinacidil concentration and mean percentage changes in QRS interval (○). (b) Relationships between pinacidil concentration and mean percentage changes in JT interval (○). For comparison, the results obtained with mexiletine at 4 Hz stimulation frequency are also shown (dotted lines): controls (●) and 15 μM mexiletine (Δ). Two-way analysis of variance (ANOVA) showed that the QRS effect was not different between the 3 concentration groups and that pinacidil significantly decreased QRS compared to the control group. The asterisks indicate significant differences when multiple *t* tests (with Bonferroni adjustment) were applied.

(Ia, Ib, Ic) according to their ability to widen the QRS complex at sinus rhythm on surface ECG. Although theoretical and experimental evidence has indicated that the relationship between Na current and conduction velocity is not linear (Buchanan *et al.*, 1985), QRS interval is generally accepted as an approximate index of Na channel availability (provided that intraventricular conduction pathway remains constant).

Our results show that pinacidil can antagonize the typical QRS widening effect of class I antiarrhythmic drugs, with differences that seem related to the binding kinetics of each individual drug. Flecainide, which is believed to block the Na channel mainly in the open state and is characterized by a slow recovery from block (Anno & Hondeghem, 1990), fully maintained its QRS effect even at the highest pinacidil concentration tested. Mexiletine, which has fast recovery kinetics and mainly interacts with the inactivated Na channel (Kodama *et al.*, 1987), lost about 17% of its QRS effect. Quinidine, which mainly is an open channel blocker with intermediate rate kinetics (Snyders & Hondeghem, 1990), but also binds to Na-channels during the slow inactivation phase (Hondeghem & Matsubara, 1988), lost only a small part of its effect (8%). The different degrees of interaction between drugs can be at first sight explained in the framework of the 'modulated receptor' or 'guarded receptor' hypotheses. Both these hypotheses predict (Grant *et al.*, 1984) that APD shortening and/or diastolic interval (DI) prolongation would decrease the effect of the drugs that bind the Na channel in

the inactivated state (i.e., during the plateau phase of AP), especially if the binding kinetics are fast. On the contrary, drugs that mainly block the open Na channel or drugs with slow binding kinetics, are not expected to feel the effect of APD/DI changes. However, in the case of mexiletine and quinidine, the lack of correlation between repolarization shortening (or DI increase) and QRS shortening does not fit into this model. One could speculate that as well as shortening APD, pinacidil may also hyperpolarize resting membrane potential (RMP), thus reducing the number of inactivated Na channels (Hondeghem, 1987). Wyse *et al.* (1993) have demonstrated that the effect of mexiletine on the maximum rate of depolarization is more affected by changes in RMP than is that of quinidine. The hyperpolarizing effect of pinacidil has been shown in smooth muscle cells (Southerton *et al.*, 1988; Weston, 1989), which have a much less negative RMP than the K equilibrium potential but it has not generally been seen in isolated ventricular myocytes (Imanishi *et al.*, 1984; Smallwood & Steinberg, 1988) where the RMP is more negative. It is, however, possible that in our experimental conditions, cardiac cells may be partially depolarized. In multicellular preparations high driving rates induce K accumulation in intercellular clefts and a negative shift in RMP (Attwell *et al.*, 1981). A similar mechanism was considered by Buchanan *et al.* (1985) to explain the progressive decrease in conduction velocity they observed in guinea-pig papillary muscle when driving frequency was increased in steps from 0.1 to 8 Hz.

To verify the hypothesis that cell hyperpolarization was the main mechanism underlying drug interaction, we repeated the experiments with mexiletine (the most sensitive among the drugs tested to pinacidil interaction) at a stimulation rate 50% lower (2 Hz) than that formerly used. At this frequency the QRS effect of mexiletine was diminished, but still present, (+17.2%). Although the ability of pinacidil to shorten repolarization was fully maintained, pinacidil only marginally antagonized the mexiletine effect (−2.3%). These findings strongly suggest that changes in RMP rather than in APD are responsible for the interaction.

Kojima & Ban (1988) studied the interaction between nicorandil (another KCO) and two antiarrhythmic agents, lignocaine (20 μM) and disopyramide (20 μM), in guinea-pig papillary muscles at different stimulation rates. They found that nicorandil reduced the V_{max} effect of lignocaine (an inactivated channel blocker with fast kinetics), but not that of disopyramide (an open channel blocker with slow kinetics) and that the interaction with lignocaine was evident only at driving frequencies higher than 2 Hz. In spite of the similarity with our results, the mechanism of the interaction was different in that RMP underwent little, non significant hyperpolarization while APD_{90} markedly shortened to about 30% of control. It could be speculated that the small size of the preparation used (2.3 × 1 mm) did not permit a marked interstitial K accumulation. Unfortunately, a full comparison with our data is prevented by the fact that only one, very high (1 mM), nicorandil concentration was tested.

Since the pinacidil hyperpolarizing effect occurs at lower concentrations than those required to shorten AP, assessment of the working mechanism of the interaction is a clinically relevant issue. If RMP hyperpolarization is the true mechanism, low (and possibly therapeutic) pinacidil concentrations would be enough to decrease the mexiletine antiarrhythmic effect during sustained high frequency arrhythmias; an important corollary is that pinacidil may induce a loss of mexiletine selectivity for pathologically depolarized myocardium (e.g. ischaemia), even at physiological heart rates.

In summary, an antagonism between pinacidil and class I antiarrhythmic agents is possible but considerable differences may exist between individual drugs. Mexiletine appears to be the most involved of all the drugs tested. The exact mechanism of such an interaction is not fully understood, but a high heart rate is essential for its occurrence. Since, at present, the only official therapeutic indication of pinacidil is

essential hypertension, it seems advisable that hypertensive patients requiring mexiletine treatment for ventricular arrhythmias are carefully checked for possible arrhythmic recurrences.

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