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SPECIAL REPORT

Cisapride-induced prolongation of cardiac action potential and early afterdepolarizations in rabbit Purkinje fibres

[1]François L. Puisieux, Monique M. Adamantidis, Bérengère M. Dumotier & Bernard A. Dupuis

Laboratoire de Pharmacologie, Faculté de Médecine, 1, Place de Verdun, 59045 Lille Cédex, France

Cisapride, a gastrointestinal prokinetic agent, has been associated with cases of Torsades de Pointes but its effects on the cardiac action potential have not been described. We investigated its electrophysiological effects on rabbit isolated Purkinje fibres. The results demonstrated that cisapride (0.01–10 μM) lengthened concentration-dependently the action potential duration without modifying other parameters and induced early after depolarizations and subsequent triggered activity. This typical class III antiarrhythmic effect, that showed 'reverse' rate-dependence and was reduced by increasing external K concentration, can account for clinical arrhythmogenesis.

Keywords: Cisapride; Torsades de Pointes; cardiac Purkinje fibres; Class III antiarrhythmic effects

Introduction Cisapride (Prepulsid or Propulsid), a widely used gastrointestinal prokinetic agent, has been recently associated with long QT syndrome (Bran *et al.*, 1995) and reported to cause Torsades de Pointes (TdP) life-threatening arrhythmias (Ahmad & Wolfe, 1995). Because the cardiac cellular effects of cisapride have not been described, an explanation for the cause of arrhythmogenesis is lacking. Therefore we investigated its electrophysiological effects on rabbit Purkinje fibres and the likelihood of its causing early afterdepolarizations (EADs) and subsequent triggered activity that are believed to be the cellular basis for initiating TdP (Antzelevitch & Sicouri, 1994).

Methods As previously described in detail (Adamantidis *et al.*, 1995) Purkinje fibres were isolated from New Zealand rabbit hearts, then superfused with oxygenated (95% O_2, 5% CO_2) Tyrode solution of the following composition (mM): NaCl 108.2, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 1.8, $NaHCO_3$ 25.0 and glucose 11.0; pH 7.35±0.05 at 36.5±0.5°C; and electrically stimulated by rectangular pulses of 1 ms duration with an intensity 50% above threshold at the basal frequency of 1 Hz. Action potentials were recorded with conventional glass microelectrodes. The concentration-dependent effects were studied by adding cisapride to Tyrode solution in increasing cumulative concentrations (0.01–10 μM) each of which was superfused for 30 min. In control (drug-free) conditions and then in the presence of the drug, the pacing rate was decreased from 1 to 0.2 Hz between the 20th and the 22nd min at each drug concentration. In other preparations, we investigated the rate-dependence of the cisapride effects on APD by increasing pacing rates (0.2–3 Hz) using a protocol applied in control conditions then in the presence of cisapride. In each series of experiments, electrophysiological measurements were made just before changing to the next concentration or the next rate frequency. Then we evaluated the time-dependent effects of cisapride in a separate series of experiments by exposing Purkinje fibres, bathed in Tyrode solution containing either 4 mM KCl or 5.4 mM KCl, to a low stable cisapride concentration for 2 h. Action potentials were recorded and analysed every 30 min. The data are expressed as mean±s.e.mean. Statistical analysis was calculated by Student's paired and unpaired t test. A probability value of $P<0.05$ was considered significant.

Results Cisapride increased the action potential duration (APD) concentration-dependently (Figure 1a,d) without altering significantly the resting membrane potential, action potential amplitude and maximal rate of phase 0 depolarization (data not shown). The APD lengthening was so marked that in 5 of 8 fibres, a cellular one-to-one response to pacing could no longer be elicited at cisapride concentrations ranged from 1 to 10 μM. In these cases, the APD data collected for Figure 1d have been estimated to 800 ms and 1000 ms for APD measured at 50% (APD_{50}) and 90% (APD_{90}) repolarization respectively, to visualize the striking lengthening of the repolarization phase. In addition (Figure 1c) abrupt reduction of pacing rate from 1 to 0.2 Hz increased cisapride-induced effects on APD and single or multiple EADs initiating subsequent sustained triggered activity (defined as more than 6 consecutive EADs) developed at plateau level. Furthermore the prolonging effect induced by 0.3 μM cisapride ($n=6$) showed 'reverse rate-dependence' (Figure 1e) that is, increasing the pacing rate from 0.016 to 3 Hz reduced APD lengthening progressively; nevertheless this still remained significant at 3 Hz.

In other experiments, it was found that the APD lengthening induced by 0.3 μM cisapride developed with time of exposure but did not reach a steady-state after 2 h (Figure 2) and that two types of fibres could be identified, one with APD_{90} of less than 300 ms ('short APs') and the other with APD_{90} of more than 300 ms ('long APs'). Both types of fibre could be found in the same heart but the fibres with 'short APs' were excised near the septum and the fibres with 'long APs' more distally, between the free wall of the left ventricle and papillary muscles. In 4 mM KCl conditions, 'long APs' (6 of 19 fibres) were so prolonged by cisapride that after the first 30 min of exposure, all the fibres failed to respond to each stimulus and EADs developed. In 5.4 mM KCl medium, cisapride induced less pronounced effects on long APs (7 of 18 fibres) and a reduced incidence (3/7 versus 6/6) of EADs; however, in short APs, the prolonging effects exerted by cisapride were quite similar in 4 mM KCl or in 5.4 mM KCl medium.

Discussion The present study demonstrates that cisapride exerts typical class III antiarrhythmic effects (according to Vaughan-Williams' classification), i.e. it prolongs dose-dependently the repolarization phase without alteration of other parameters over a range of concentrations (0.1–1 μM) that are clinically relevant to the plasma concentrations found in healthy subjects following oral administration of a therapeutic dose (McCallum *et al.*, 1988). The prolonging effect was more

[1] Author for correspondence.

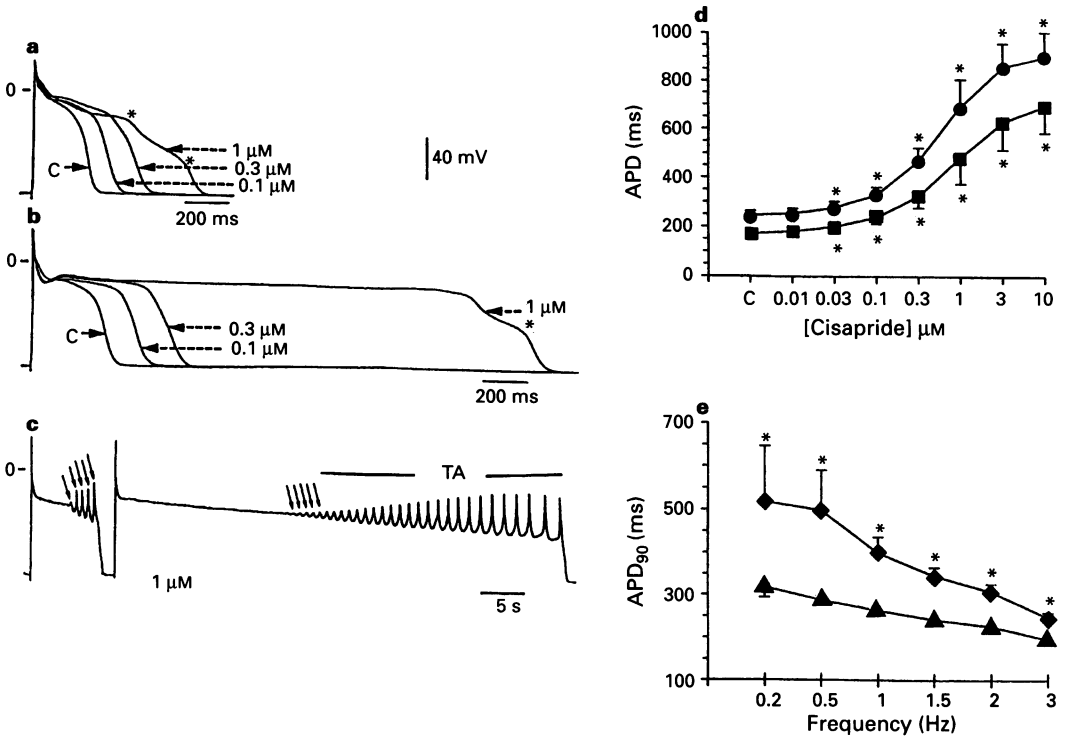

Figure 1 Concentration- and frequency-dependent effects of cisapride on action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization recorded in rabbit Purkinje fibres. (a,b,c): Representative examples of the prolonging effect exerted by increasing cumulative concentrations of cisapride on the same fibre stimulated at 1 Hz (a), 0.2 Hz (b) and 0.1 Hz (c). Early afterdepolarizations are labelled with asterisks in (a) and (b). In (c) small arrows indicate oscillations which initiated multiple EADs and triggered activity (TA). (d,e) Show quantitative results. Points represent means ± s.e.means. (d) Concentration-dependent effects of cisapride on APD_{50} (■) and APD_{90} (●), ($n = 8$; data were obtained from both short- and long-type cells); $^*P < 0.05$ vs control (c) values. (e) Influence of increasing stimulation frequency on APD_{90} in the absence (control, ▲) and in the presence (◆) of 0.3 μM cisapride ($n = 6$). The protocol of increasing stimulation frequency from 0.2 to 3 Hz (each step for 5 min) was applied before and after exposure to the drug. $^*P < 0.05$ cisapride vs control.

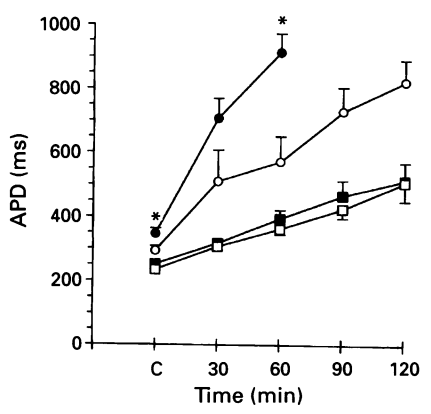

Figure 2 Time-dependent effects of 0.3 μM cisapride on action potential duration measured at 90% repolarization (APD_{90}) in separate series of Purkinje fibres stimulated at 1 Hz and bathed in 4 mM KCl or in 5.4 mM KCl Tyrode solution. 'Short' and 'long' action potentials were identified, depending on the control APD_{90} values measured in 4 mM KCl conditions (less or more than 300 ms, respectively). Points are means ± s.e.mean. $n = 13$ in short-4 mM KCl group (■), $n = 6$ in long-4 mM KCl group (●), $n = 11$ in short-5.4 mM KCl group (□) and $n = 7$ in long-5.4 mM KCl group (○). In the long 4 mM KCl group, cisapride-induced effects on APD were so pronounced that after the first 30 min of exposure, the 6 fibres no longer responded to stimulation at 1 Hz frequency. $^*P < 0.05$ 5.4 mM KCl versus 4 mM KCl conditions.

prominent at slow pacing rates and showed reverse rate-dependence, as widely described for class III drugs (Hondeghem & Snyders, 1990). Furthermore EADs developed at plateau level and triggered sustained rhythmic activities that are proposed as the cellular basis for initiating TdP (Antzelevitch & Sicouri, 1994). Although the precise mechanism of cisapride effects at ionic channel level cannot be determined from our results, they fit well with the clinical observations of a long QT syndrome associated with high doses of cisapride (Bran *et al.*, 1995) and of TdP in patients taking cisapride (Ahmad & Wolfe, 1995).

In addition, it is noteworthy that the effects of cisapride were much stronger in fibres with initial long APD (> 300 ms) but were attenuated by increasing extracellular K concentration. In the whole heart, it may be hypothesized that this may contribute to an increased dispersion of repolarization, thus providing an ideal substrate for re-entry which allows perpetuation of TdP (Surawicz, 1989). Although great care must be taken in the direct extrapolation from experimental study to clinical observations (Antzelevitch & Sicouri, 1994), this feature may be referred to the more marked effects of class III antiarrhythmic drugs in patients with an already prolonged cardiac repolarization i.e. patients with congenital or acquired long QT syndrome or with other predisposing factors to TdP such as bradycardia, hypokalaemia or hypomagnesaemia.

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