Electrophysiological Characterization of the Prokinetic Agents Cisapride and Mosapride in Vivo and in Vitro: Implications for Proarrhythmic Potential?

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ABSTRACT
In the present study the electrophysiological characteristics and the proarrhythmic potential of cisapride and a structurally related drug, mosapride, were compared. In the anesthetized guinea pig, cisapride and d-sotalol (0.01–10 μmol/kg i.v., n = 6) dose-dependently prolonged the duration of the monophasic action potential recorded from the left ventricle. The maximal lengthening was 18 ± 3.2% at 1.0 μmol/kg (mean ± S.E.M., P < .01 vs. base line) and 19 ± 2.5% at 10 μmol/kg (P < .001) for cisapride and d-sotalol, respectively. In contrast, mosapride did not increase this variable. In a rabbit model of the acquired long QT syndrome, infusion of cisapride (0.3 μmol/kg/min for 10 min maximum, n = 6), but not mosapride or vehicle, was associated with a significant lengthening of the QTU interval (43 ± 3.8 ms, P < .01). Furthermore, torsades de pointes appeared in two of the six rabbits given cisapride. In isolated rabbit Purkinje fibers (PF), cisapride increased the action potential duration (48 ± 5.6% at 0.1 μmol/l, P < .01 vs. control, n = 4). Mosapride did not significantly influence the action potential duration (3 ± 2.0% increase at 1.0 μmol/l, n = 6). However, after mosapride was washed out, the addition of cisapride (0.1 μmol/l) caused a 46 ± 3.2% lengthening of the action potential duration (P < .01 vs. 1.0 μmol/l mosapride). Early afterdepolarizations and triggered activity appeared in four of eight cisapride-supersurfused PF stimulated at a very low frequency (0.1 Hz). In isolated rabbit cardiomyocytes, cisapride concentration-dependently blocked (IC50 = 9 nmol/l) the rapid component of the delayed rectifying K+ current (I-Kr). Mosapride was approximately 1000-fold less potent in blocking I-Kr (IC50 = 4 μmol/l). It is concluded that the electrophysiological characteristics of cisapride may explain the recently reported propensity to prolong the QT interval and to induce torsades de pointes in susceptible patients, although a structurally related benzamide, mosapride, did not appear to have electrophysiological features of relevance for induction of torsades de pointes in common with cisapride.

Cisapride and mosapride are two structurally related benzamide derivatives (fig. 1) which facilitate or restore motility in the gastrointestinal tract (Wiseman and Faulds, 1994; Yoshida et al., 1993). Cisapride has been on the market for several years, whereas mosapride is presently undergoing extensive preclinical and clinical evaluation as a novel prokinetic agent (Sjövall and Abrahamsson, 1996; Suyama et al., 1993). Although it is not completely understood, agonistic action at 5-HT4 receptors, and thereby facilitation of cholinergic excitatory neurotransmission, has been suggested as the mechanism by which these agents enhance motility (Briejer et al., 1995; Wiseman and Faulds, 1994; Yoshida et al., 1993). Recently, it was reported that treatment with cisapride may occasionally give rise to excessive lengthening of the QT interval, torsades de pointes and/or ventricular fibrillation (ADRA C, 1996; Ahmad and Wolfe, 1995; Bran et al., 1995; Lewin et al., 1996; Warning on cisapride interactions, 1996; Wysowski and Baacsany, 1996). Most of these instances were in patients receiving concomitant medication with certain oral antifungals and macrolide antibacterials or with known risk factors for torsades de pointes.

Torsades de pointes is a characteristic type of polymorphic ventricular tachycardia in which the QRS axis of each successive beat differs slightly from the preceding one and seems to rotate around the isoelectric line. In clinical practice, antiarrhythmic agents, and particularly ones that delay the repolarization (i.e., class Ia and III), are most commonly implicated in torsades de pointes (Tan et al., 1995). Puisieux and co-workers (1996) recently demonstrated a concentration-dependent prolongation of the action potential duration of the QT interval, torsades de pointes and/or ventricular fibrillation (ADRA C, 1996; Ahmad and Wolfe, 1995; Bran et al., 1995; Lewin et al., 1996; Warning on cisapride interactions, 1996; Wysowski and Baacsany, 1996). Most of these instances were in patients receiving concomitant medication with certain oral antifungals and macrolide antibacterials or with known risk factors for torsades de pointes.

ABBREVIATIONS: EADs, early afterdepolarizations; I-Kr, rapidly activating delayed rectifier K+ current; MAP, monophasic action potential; MAPD75, monophasic action potential duration at the 75% repolarization level; ECG, electrocardiogram; HEPES, N-2-hydroxyethylpiperazine-N’-ethanesulfonic acid.
and the induction of EADs by cisapride in the isolated rabbit Purkinje fiber. EADs, defined as single or multiple repetitive oscillations of the transmembrane voltage in the setting of prolonged action potential duration, were hitherto the most likely candidates for the initiation of torsades de pointes (Carlsson et al., 1993; Jackman et al., 1988; Tan et al., 1995). EADs may occur as a consequence of an imbalance between net inward and outward currents, which may lead to failure of normal membrane repolarization and, if reaching threshold, such EADs may induce triggered upstrokes which manifest as torsades de pointes on the surface ECG. This study sought to elucidate the cellular and ionic mechanisms for the cisapride-induced long QT syndrome. Furthermore, cisapride was compared with mosapride to get an indication of a possible structure-activity relationship for benzamide derivatives and action potential-prolonging potential.

Materials and Methods

**Class III effects in the anesthetized guinea pig.** This methodology was recently described in detail (Fazekas et al., 1996). Male guinea pigs (831 ± 22.4 g b.wt., n = 18) were anesthetized with pentobarbital (40–50 mg/kg i.p.) and then instrumented for blood pressure recording, blood sampling, drug infusion and ECG recording (lead II). A tracheotomy was performed, and the animal was artificially ventilated with room air. To reduce any potential autonomic reflexes by the investigational drugs, both vagi were cut in the neck, and 2 μmol/kg propranolol was given intravenously 15 min before the start of the experimental protocol. After a left-sided thoracotomy a custom-designed suction electrode for recording of a MAP was applied to the left ventricle. A bipolar electrode was clipped to the left atrial appendage for pacing (2 ms duration, twice the diastolic threshold). The pacing rate was set just above the spontaneous sinus rate. The MAP signal and the ECG were recorded on a Minograph ink-jet recorder (Siemens-Elema, Solna, Sweden). All signals were collected on a computer during the last 10 s of each pacing sequence and the last 10 s of the following minute of sinus rhythm. (The sampling frequency was 1000 Hz, and each sampling period was 10 s.) Finally, the signals were analyzed with a custom-designed computer program, PC-lab (Axenborg and Hirsch, 1993).

The test procedure consisted of two basal control recordings, 5 min apart, during both pacing and sinus rhythm. After the second control recording the first dose of the drug under investigation (i.e., cisapride, d-sotalol or mosapride) was infused into the jugular vein catheter for 30 s (infusion volume, 0.2 ml). Three minutes later pacing was started and a new recording was made. Five minutes after the previous dose the next dose of the test substance was administered. Ten consecutive doses (varying between 0.01 μmol/kg and 10 μmol/kg) were given during each experiment. The mean of the two control recordings was used as a reference value and set to 100%, and the effects recorded after consecutive doses of the three agents were expressed as percentage changes from this value. In six separate animals administered mosapride, arterial blood (approximately 1 ml) was drawn via the right carotid artery for subsequent analysis of plasma concentrations of mosapride. The sample was collected immediately after the last dose was administered, and the electrophysiological parameters were recorded.

**Class III effects and proarrhythmic properties in the anesthetized rabbit.** This model was described extensively in previous studies (Carlsson et al., 1990, 1993). Male New Zealand White rabbits (2.9 ± 0.09 kg b.wt., n = 18) were anesthetized with methohexitol-sodium (5 mg/kg i.v.) and α-chloralose (80–90 mg/kg i.v.). After tracheotomy the animals were mechanically respirated with room air and catheters implanted into the right carotid artery and into the marginal ear veins for recording of arterial blood pressure, blood sampling and infusion of drugs, respectively. ECGs (leads I–III, aVR, aVL and aVF) were recorded on a Mingograph ink-jet recorder, and arterial blood pressure and heart rate were recorded on a Grass polygraph (Grass Instruments Co., Quincy, MA). In addition, ECGs (leads I and II), blood pressure and heart rate were recorded at predetermined intervals on a personal computer. The signals were sampled at a frequency of 500 Hz, and each sampling period lasted for 5 s. Finally, data were processed with use of the PC-lab program (Axenborg and Hirsch, 1993).

After basic measurements a continuous infusion (for 20 min at most) of the α1 agonist methoxamine (70 nmol/kg/min) was started (Carlsson et al., 1990, 1993). Ten minutes later cisapride (0.3 μmol/kg/min), mosapride (0.3 μmol/kg/min) or vehicle (tartaric acid, ethanol and saline; 7%-3%-90%, 0.1 ml/kg/min) was continuously infused for 10 min at most and the ECG was continuously monitored. The ECG analysis was subsequently performed on averaged ECG complexes (including at least 10 consecutive beats). The T wave usually had a bifid appearance in the rabbit (TU complex), and the QTU interval was defined as the time between the first deviation from the isoelectric line during the PR interval and the second peak of the TU complex. Torsades de pointes was defined as a transient tachyarrhythmia seen in the presence of QTU interval lengthening, with a typical initiation (“short-long-short sequence”) and more than five consecutive undulating peaks of sequential QRS complexes observed in at least two leads.
Class III effects in isolated rabbit Purkinje fibers and ventricular muscle. Male New Zealand White rabbits were anesthetized with pentobarbital sodium (60 mg/kg i.v.). After excision of the heart, the right ventricular anterior papillary muscle with its free-running Purkinje fibers was dissected out, mounted in a 2-ml organ bath and superfused with a modified Tyrode’s solution. The temperature was kept constant at 37°C, and the preparation was stimulated at a frequency of 1 Hz (50% above threshold). The preparation was then left to stabilize for approximately 2 h before the experimental protocol was initiated. A set-up of two microelectrodes (filled with 3 mol/l KCl) was used to make simultaneous recordings of transmembrane action potentials from ventricular muscle cells and Purkinje fibers. The signals were recorded and analyzed in a way similar to that described for the in vivo experiments (see above). The amplified transmembrane potentials were also recorded on a strip chart recorder.

After a period of control recordings, the lowest concentration of the test compound was added to the superfusion medium. The preparation was exposed to each concentration for 30 min. The effect of cisapride was examined at 0.01 and 0.1 μmol/l and mosapride at 0.01, 0.1 and 1.0 μmol/l. In addition, when the highest concentration of mosapride had been studied, the solution was washed out for 30 min and the tissue superfused with 0.1 μmol/l cisapride for 10 min. In eight of the preparations superfused with cisapride (0.1 μmol/l), the stimulation frequency was reduced from 1.0 Hz to 0.1 Hz.

Voltage clamp studies in isolated rabbit ventricular myocytes. The continuous single-electrode, whole-cell voltage clamp technique was applied to measure the rapidly activating delayed rectifier K⁺ current (I_{Kr}) of ventricular myocytes from male New Zealand White rabbits, enzymatically isolated as described previously (Carlsson et al., 1992). Voltage control was achieved by use of an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Axo-data (Axon Instruments) running on a Macintosh Quadra 700 computer connected via an A/D-converter (ITC-16 Computer Interface, Intrutech Corporation, Elmont, NY) was used for amplifier control and data acquisition. Current recordings were filtered at 1 KHz with a lowpass Bessel filter (constructed at Astra Hässle AB) and digitized at 333 Hz. Axograph (Axon Instruments) was used for experimental analysis. Nonfilamented borosilicate glass electrodes (Clark Electromedical Instruments, Reading, England; inner diameter, 0.69 mm; outer diameter, 1.2 mm), with a resistance of 1 to 3 megohm when filled with the electrode solution (in mmol/l: KCl, 120; MgCl₂, 6; Na₂ATP, 5; HEPES, 10; ethyleneglycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 5; CaCl₂, 0.15, pH 7.2, adjusted with KOH) were used. The superfusion solution (in mmol/l: NaCl, 140; KCl, 5.4; MgCl₂, 0.5; CaCl₂, 1.8; CdCl₂, 0.1; HEPES, 10; glucose, 5.0, pH 7.4, adjusted with NaOH) was warmed to 37°C with an inline heater.

Upon rupture of the cell membrane, cell capacitance and series resistance (up to 80%) were compensated. Series resistance was held to less than 10 megohm; leak current subtraction was not attempted because of the difficulties in determining leak conductance in cardiac myocytes. An initial 3-min period was allowed for cell and current amplitude stabilization before beginning an experiment. Preliminary time-matched control experiments demonstrated only marginal rundown after this initial 3-min period. Substance effects were evaluated after 5 min superfusion, which was sufficient time for the effects of the substances to reach steady state. The holding potential in all experiments was −60 mV, a 50-ms prepulse to −40 mV was performed to inactivate I_{Na}, a 2-s test pulse to +10 mV was then used to activate I_{Kr}. Subsequent tail currents were elicited by clamping back to −40 mV for 1 s and the frequency was 0.1 Hz. The amplitude of the tail current was considered representative of the amplitude of I_{Kr} activated by the test pulse. This was obtained by subtracting the amplitude of the nondeactivating current amplitude at −40 mV from the maximum tail current amplitude observed directly after clamping back to −40 mV.

Analysis of mosapride in arterial blood from the guinea pig. After centrifugation the blood plasma was frozen at −20°C until analysis. Subsequently, frozen plasma was allowed to thaw at room temperature and an internal standard added. Mosapride and the internal standard were then extracted from the plasma at pH 12 with a mixture of hexane and dichloromethane (1:1). After evaporation, the organic extract was redissolved in mobile phase and the substances were separated by reverse phase liquid chromatography and monitored by fluorescence detection.

Drugs. The following drugs were used: cisapride (kindly provided by Janssen Pharmaceutica NV, Beerse, Belgium), d-sotalol hydrochloride (synthesized by Astra Hässle AB, Mölndal, Sweden), mosapride citrate dihydrate (provided by Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO), methoxamine hydrochloride (Sigma). All drugs were freshly prepared on the day of use, and all doses in the text refer to bases of the compounds. Propranolol, methoxamine and d-sotalol were all dissolved in distilled water. Cisapride and mosapride were prepared as concentrated stock solutions by dissolving the agents in 0.1 mol/l tartaric acid, ethanol and saline; 7%;3%-90%, pH 2.3. The stock solutions were then further diluted with saline to the final concentration.

Statistics. Results are presented as means ± S.E.M. and n indicates number of observations. Student's t test (paired observations, with correction for multiplicity by the Bonferroni procedure as appropriate) and Dunnet’s t test were used to test the significance of differences. A two-tailed value of P < .05 was considered as statistically significant.

Results

Class III effects in the anesthetized guinea pig. The effects of cisapride, mosapride and d-sotalol (0.01–10 μmol/kg) on the MAPD$_{75}$, AV conduction time and RR interval are illustrated in figure 2. Both MAPD$_{75}$ and AV conduction time were measured during atrial pacing. In animals administered cisapride, atrial pacing was not possible at cumulative doses above 3 μmol/kg. The interstimulus interval for the different groups of animals did not differ statistically (242 ± 5.3 ms for cisapride, 233 ± 8.3 ms for mosapride and 242 ± 8.3 ms for d-sotalol, respectively). Cisapride and d-sotalol caused a dose-dependent lengthening of the MAPD$_{75}$. The maximal prolongation was 18 ± 3.2% for cisapride (at 1 μmol/kg, n = 5, P < .01 vs. base line) and 19 ± 2.5% for d-sotalol (at 10 μmol/kg, n = 6, P < .001), respectively. Administration of mosapride was not associated with any increase in MAPD$_{75}$. On the contrary, at the highest dose of mosapride (10 μmol/kg, n = 6), the MAPD$_{75}$ was reduced by 7 ± 1.8% (P < .05). AV time was not significantly influenced by any of the agents tested. The RR interval at sinus rhythm was increased in a dose-dependent manner by both cisapride and d-sotalol. Hence, at 1 μmol/kg cisapride, the RR interval was increased by 21 ± 3.5% (P < .01), whereas the highest dose of d-sotalol caused an increase of the RR interval of 20 ± 4.4% (P < .05). Mosapride did not significantly influence the RR interval.

In six separate guinea pigs given mosapride, the plasma levels of mosapride were determined after the last dose was administered. These plasma concentrations amounted to 101 ± 25.7 μmol/l. The results from the electrophysiological parameters measured in these animals did not significantly differ from the ones reported above.

Class III effects and proarrhythmic properties in the anesthetized rabbit. Three groups of rabbits (six rabbits in...
Class III effects in isolated rabbit Purkinje fibers and ventricular muscle. Superfusion with cisapride caused a prolongation of the action potential duration (at 90% repolarization) both in the ventricular muscle and in the Purkinje fibre (fig. 6). At the highest concentration studied (0.1 μmol/l), the action potential duration was increased by 22 ± 1.5% and by 48 ± 5.6% in the ventricular muscle and in the Purkinje fibre, respectively (P < .01 vs. control, n = 4). Mosapride, in a concentration range between 0.01 and 1.0 μmol/l, did not significantly influence the action potential duration in either tissue (n = 4, figs. 7 and 8). However, after mosapride had been washed out for 30 min, 10 min superfusion with cisapride (0.1 μmol/l) caused a substantial lengthening of the QTU interval after cisapride.

Each group were given a concomitant infusion of methoxamine (70 nmol/kg/min) and cisapride (0.3 μmol/kg/min), mosapride (0.3 μmol/kg/min) or vehicle. Cisapride, mosapride and the vehicle were administered for 10 min maximum, and the effects of these compounds on QTU interval, RR interval and mean arterial blood pressure (all variables measured once a minute) are illustrated in figures 3 and 4. Administration of cisapride was associated with a rapid lengthening of the QTU interval (from 117 ± 4.2 ms to 160 ± 7.0 ms after 10 min of infusion, P < .01 vs. base line, fig. 3). Mosapride or vehicle did not significantly influence the QTU interval (from 126 ± 6.4 ms to 131 ± 8.7 ms and from 122 ± 4.5 ms to 126 ± 6.2 ms, respectively). A continuous increase (not statistically significant) in the RR interval was observed in all three groups of animals (fig. 4). At the end of the infusion, the RR intervals had increased from 289 ± 14.4 ms to 313 ± 20.7 ms (cisapride), from 271 ± 20.4 ms to 315 ± 32.8 ms (mosapride) and from 280 ± 17.7 ms to 351 ± 46.2 ms (vehicle), respectively. The concomitant infusion of methoxamine and mosapride or vehicle was associated with a significant increase in mean arterial blood pressure. During the initial 10 min of infusion with methoxamine the mean arterial blood pressure increased from 73 ± 6.6 to 95 ± 5.4 mm Hg, from 78 ± 8.5 to 102 ± 6.2 mm Hg and from 91 ± 5.5 to 115 ± 36 mm Hg (all P < .05) in animals subsequently administered vehicle, mosapride or cisapride, respectively. During the following 10 min infusion of vehicle or mosapride, the blood pressure further increased to 123 ± 3.9 mm Hg and to 119 ± 4.8 mm Hg, respectively (fig. 4). In contrast, in the cisapride-treated rabbits the blood pressure fell to 90 ± 7.4 mm Hg (P < .01).

No proarrhythmias related to drug effects on the repolarization were observed in the rabbits given mosapride or vehicle. However, in the animals administered cisapride, the lengthening of the QTU interval was associated with the appearance of ventricular extrasystoles and, in two of the six rabbits, the induction of torsades de pointes (after cumulative doses of 1.1 and 1.3 μmol/kg, respectively; fig. 5).

![Fig. 2. Dose-dependent effects (Δ%) of cisapride (filled squares), d-sotalol (filled circles) and mosapride (filled triangles) on the MAPD75 (top panel), the atrioventricular conduction time (AV-time, middle panel) and the RR interval at sinus rhythm (bottom panel) in the anesthetized guinea pig. Before the drugs were given, the MAPD75 was 128 ± 3.9 ms, 127 ± 4.8 ms and 117 ± 4.1 ms, the AV-time was 93 ± 5.5 ms, 87 ± 1.9 ms and 86 ± 3.2 ms and the RR interval was 265 ± 6.2 ms, 263 ± 7.0 ms and 255 ± 7.3 ms for the groups of animals subsequently treated with cisapride, d-sotalol and mosapride, respectively. Values shown are means, S.E.M. omitted for clarity, n = 6 within each group of animals.](image)

![Fig. 3. Effects of cisapride, mosapride and vehicle on the QTU interval (delta ms, left panel) in the anesthetized rabbit. The agents were infused for 10 min maximum, and the QTU intervals were measured once a minute. Six rabbits were studied within each group. Two rabbits given cisapride developed torsades de pointes during the infusion. Values shown are means ± S.E.M. The right panels show average ECGs from two rabbits before drug infusion and after administration of 3.0 μmol/kg mosapride (top panel) or 0.9 μmol/kg cisapride (bottom panel), respectively. Note the marked prolongation of the QTU interval after cisapride.](image)
ening of the action potential duration in the Purkinje fiber (46 ± 3.2%, P < .01 vs. 1.0 μmol/l mosapride, fig. 7). In four of eight preparations superfused with cisapride, a reduction in the stimulation frequency from 1 to 0.1 Hz was associated with the appearance of early afterdepolarizations and occasionally triggered activity (fig. 9).

Voltage clamp studies in isolated rabbit ventricular myocytes. The amplitude of the rapidly activating delayed rectifier $I_{Kr}$ was markedly reduced after superfusion with low concentrations of cisapride (fig. 10A). Tail current amplitude was reduced from 114 ± 22 pA to 7 ± 3 pA ($n = 5$, P < .01) in the presence of 0.1 μmol/l cisapride. Block was reversible after washout. The amplitude of the cisapride-sensitive current was expressed as a fraction of the control current amplitude (to give fractional block) and plotted against the logarithm of the corresponding cisapride concentration (fig. 10C). A sigmoidal function (see legend to fig. 10 for formula) was then fitted to the data points. The concentration for half-maximum block (IC_{50}) was 9 nmol/l, and the Hill slope factor was 1.4. Mosapride also blocked $I_{Kr}$, but at markedly higher concentrations than cisapride (fig. 10B). Superfusion with mosapride (10 μmol/l) reduced tail current amplitude
This comparative study has demonstrated that cisapride, but not mosapride, has electrophysiological features resembling those of the novel class III antiarrhythmic agents, which prolong cardiac repolarization without slowing conduction (Colatsky and Argentieri, 1994). Most of the latter compounds act by selectively blocking the rapid component of the delayed rectifying K⁺ current (IK,r), which consequently leads to a lengthening of the action potential (Colatsky and Argentieri, 1994; Roden, 1993). In the present study, we observed that cisapride quite unexpectedly blocked IK,r in rabbit cardiomyocytes with a potency (IC₅₀ = 9 nmol/l) within the same range as dofetilide, an extremely potent class III antiarrhythmic agent currently undergoing clinical development for treatment of ventricular and supraventricular tachyarrhythmias (Carmeliet, 1992). In man, the peak plasma concentration of cisapride after oral dosing lies within the range of 100 to 200 nmol/l, of which 97 to 98% is bound to plasma proteins (MacCallum, 1991; Wiseman and Faulds, 1994). *In vitro* (i.e., rabbit preparations), the lengthening of the action potential duration, as well as the blockade of IK,r, was observed at drug concentrations of cisapride which fall within this plasma concentration range. Of further interest was the observation that the cisapride-induced lengthening of the action potential duration was more pronounced in the Purkinje fiber network than in the ventricular muscle, and the occasional appearance of EADs. Increased dispersion of repolarization and the induction of EADs are typical effects of most of the class III antiarrhythmic agents and have been suggested as key factors in the initiation of torsades de pointes (Abrahamsson et al., 1993; Antzelewitch et al., 1991; Jackman et al., 1988; Tan et al., 1995). Concordant results were recently presented by Puissieux and colleagues (1996). In isolated rabbit Purkinje fibers, superfusion with cisapride was associated with a significant lengthening of the action potential duration and the appearance of EADs and triggered activity. For mosapride the expected plasma concentrations will probably be in the range of 200 to 500 nmol/l, and with a protein binding of 99% the free plasma concentration will be 2 to 5 nmol/l (B. Hamelin, personal communication). In the present guinea pig experiments, a plasma concentration approximately 200 to 500 times higher than that seen in man was detected. Despite these high drug levels, mosapride did not delay the repolarization (*i.e.*, the monophasic action potential duration). These observations indicate a satisfactory safety margin in relation to rhythm abnormalities related to delayed repolarization even when the major degradation system for mosapride (cytochrome P4503A4) is suppressed by interacting agents.

In the acquired form of the long QT syndrome, torsades de pointes is a bradycardia-dependent and potentially life-threatening polymorphous ventricular tachyarrhythmia. In clinical practice, antiarrhythmic agents (and predominantly those subclassified as class Ia or class III) are most commonly implicated in torsades de pointes (Jackman et al., 1988; Roden, 1993; Tan et al., 1995). However, a large number of miscellaneous drugs, not primarily prescribed for treatment of arrhythmias, but which cause QT lengthening, also have the propensity to induce torsades de pointes (Jackman et al., 1988). Reports of ventricular tachyarrhythmias, including torsades de pointes associated with QT prolongation, in patients on cisapride medication have recently appeared (AD-RAC, 1996; Ahmad and Wolfe, 1995; Bran et al., 1995; Lewin et al., 1996; Warning on cisapride interactions, 1996; Wysowski and Bacsanyi, 1996). The Food and Drug Administration recently reported 57 cases of torsades de pointes and/or prolonged QT intervals in patients administered cisapride (Wysowski and Bacsanyi, 1996). Among these, four patients were reported to have died and 16 resuscitated after cardiopulmonary standstill. Seven of the patients were children. Of the patients experiencing torsades de pointes who were given cisapride, the majority had known risk factors for this kind of proarrhythmia or were put on concomitant treatment with oral antifungals and macrolide antiinfectives, drug regimens which recently have been contraindicated. Such combinations may result in markedly elevated plasma concentrations of cisapride because of inhibition of the he-

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**Fig. 8.** Original tracings of action potentials recorded from a Purkinje fiber of the rabbit. The preparation was first superfused with increasing concentrations of mosapride (0.01–1.0 µmol/l) and subsequently, after a period of washout, with cisapride (0.1 µmol/l). The action potentials shown were recorded in the control situation, after 1.0 µmol/l mosapride and after 0.1 µmol/l cisapride, respectively. Vm, membrane voltage. The recordings stem from three different impalements but from the same experiment.

**Fig. 9.** Original tracings showing early afterdepolarizations and triggered activity in a rabbit Purkinje fiber (PF) superfused with 0.1 µmol/l cisapride. Note propagated responses (arrows) in the ventricular muscle cell (VM). Stimulation frequency, 0.1 Hz.

from 68 ± 16 pA to 13 ± 5 pA (n = 4, P < .05). The block caused by mosapride was reversible after washout. The experimental data for mosapride were then analyzed as described for cisapride, giving an IC₅₀ of 4 µmol/l and a Hill slope factor of 1.4 (fig. 10C).

**Discussion**

This comparative study has demonstrated that cisapride, but not mosapride, has electrophysiological features resembling those of the novel class III antiarrhythmic agents, which prolong cardiac repolarization without slowing conduction (Colatsky and Argentieri, 1994). Most of the latter compounds act by selectively blocking the rapid component of the delayed rectifying K⁺ current (IK,r), which consequently leads to a lengthening of the action potential (Colatsky and Argentieri, 1994; Roden, 1993). In the present study, we observed that cisapride quite unexpectedly blocked IK,r in rabbit cardiomyocytes with a potency (IC₅₀ = 9 nmol/l) within the same range as dofetilide, an extremely potent class III antiarrhythmic agent currently undergoing clinical development for treatment of ventricular and supraventricular tachyarrhythmias (Carmeliet, 1992). In man, the peak plasma concentration of cisapride after oral dosing lies within the range of 100 to 200 nmol/l, of which 97 to 98% is bound to plasma proteins (MacCallum, 1991; Wiseman and Faulds, 1994). *In vitro* (i.e., rabbit preparations), the lengthening of the action potential duration, as well as the blockade of IK,r, was observed at drug concentrations of cisapride which fall within this plasma concentration range. Of further interest was the observation that the cisapride-induced lengthening of the action potential duration was more pronounced in the Purkinje fiber network than in the ventricular muscle, and the occasional appearance of EADs. Increased dispersion of repolarization and the induction of EADs are typical effects of most of the class III antiarrhythmic agents and have been suggested as key factors in the initiation of torsades de pointes (Abrahamsson et al., 1993; Antzelewitch et al., 1991; Jackman et al., 1988; Tan et al., 1995). Concordant results were recently presented by Puissieux and colleagues (1996). In isolated rabbit Purkinje fibers, superfusion with cisapride was associated with a significant lengthening of the action potential duration and the appearance of EADs and triggered activity. For mosapride the expected plasma concentrations will probably be in the range of 200 to 500 nmol/l, and with a protein binding of 99% the free plasma concentration will be 2 to 5 nmol/l (B. Hamelin, personal communication). In the present guinea pig experiments, a plasma concentration approximately 200 to 500 times higher than that seen in man was detected. Despite these high drug levels, mosapride did not delay the repolarization (*i.e.*, the monophasic action potential duration). These observations indicate a satisfactory safety margin in relation to rhythm abnormalities related to delayed repolarization even when the major degradation system for mosapride (cytochrome P4503A4) is suppressed by interacting agents.

In the acquired form of the long QT syndrome, torsades de pointes is a bradycardia-dependent and potentially life-threatening polymorphous ventricular tachyarrhythmia. In clinical practice, antiarrhythmic agents (and predominantly those subclassified as class Ia or class III) are most commonly implicated in torsades de pointes (Jackman et al., 1988; Roden, 1993; Tan et al., 1995). However, a large number of miscellaneous drugs, not primarily prescribed for treatment of arrhythmias, but which cause QT lengthening, also have the propensity to induce torsades de pointes (Jackman et al., 1988). Reports of ventricular tachyarrhythmias, including torsades de pointes associated with QT prolongation, in patients on cisapride medication have recently appeared (AD-RAC, 1996; Ahmad and Wolfe, 1995; Bran et al., 1995; Lewin et al., 1996; Warning on cisapride interactions, 1996; Wysowski and Bacsanyi, 1996). The Food and Drug Administration recently reported 57 cases of torsades de pointes and/or prolonged QT intervals in patients administered cisapride (Wysowski and Bacsanyi, 1996). Among these, four patients were reported to have died and 16 resuscitated after cardiopulmonary standstill. Seven of the patients were children. Of the patients experiencing torsades de pointes who were given cisapride, the majority had known risk factors for this kind of proarrhythmia or were put on concomitant treatment with oral antifungals and macrolide antiinfectives, drug regimens which recently have been contraindicated. Such combinations may result in markedly elevated plasma concentrations of cisapride because of inhibition of the he-
The markedly high potency of cisapride in blocking $I_{Kr}$, the underestimated blocking properties to induce torsades de pointes may be crucial importance of the methoxamine infusion, because the appearance of torsades de pointes (Buchanan et al., 1990, 1993; Carlsson et al., 1990, 1993). A limitation of the model is the crucial importance of the methoxamine infusion, because the propensity of an agent with ancillary alpha-1 adrenoceptor-blocking properties to induce torsades de pointes may be underestimated (Carlsson et al., 1990). In view of the remarkably high potency of cisapride in blocking $I_{Kr}$, the observed incidence of torsades de pointes (2 of 6 rabbits) was surprisingly low (Buchanan et al., 1993; Carlsson et al., 1990, 1993). However, cisapride, but not mosapride, has been reported to bind to alpha-1 adrenoceptors, with affinity in the same range as its binding to 5-HT$_2$ receptors, and this fact may at least partially contribute to the low incidence (Briejer et al., 1995; Cohen et al., 1996; Karasawa et al., 1990; Schuurkes et al., 1985). In the present study, an alpha-1 adrenoceptor blocking effect of cisapride was indirectly supported by the observation that when cisapride was added to methoxamine in the anesthetized rabbit, the blood pressure did not continue to increase (as seen in the mosapride- and vehicle-treated rabbits). On the contrary, the blood pressure actually decreased significantly once cisapride was administered. Hypotensive effects of cisapride in rats have been demonstrated previously (Onat et al., 1994).

A rich variety of structurally different compounds have been demonstrated to elicit class III electrophysiological activity. However, when structures of selective $I_{Kr}$-blocking agents are compared, a pattern emerges, and Morgan and Sullivan (1992) recently presented a general structure for such an agent. In this structure, a substituted phenyl ring is connected to a basic amine; among others, methanesulfonamido, cyano and nitro at the para position are the most effective (Cross et al., 1990). Although not a generally effective substituent, the fluoro substituent has been used, and examples of such agents have actually been demonstrated to induce QT lengthening as well as torsades de pointes in patients (e.g., lidoflazine, melperone and ketanserin; Hui et al., 1990; Tan et al., 1995). Given the facts presented above, it is not surprising that cisapride has class III electrophysiological features. Cisapride has a fluoro-containing phenyl ring (para-substituted) connected via a four-atom link to a tertiary amine (see fig. 1). In mosapride, however, the distance between the aromatic ring and the patic cytochrome P4503A4 enzyme system, a system predominantly involved in the metabolism of cisapride (Wiseman and Faulds, 1994).

In the present study, we used a rabbit model of the acquired long QT syndrome to evaluate the propensity of cisapride and mosapride to induce torsades de pointes. In this model, it was previously demonstrated that the concomitant infusion of the alpha-1 adrenoceptor agonist methoxamine and class III antiarrhythmic agents (e.g., dofetilide, E-4031, almokalant and clofilium) is associated with a consistent appearance of torsades de pointes (Buchanan et al., 1990, 1993; Carlsson et al., 1990, 1993). A limitation of the model is the crucial importance of the methoxamine infusion, because the propensity of an agent with ancillary alpha-1 adrenoceptor-blocking properties to induce torsades de pointes may be underestimated (Carlsson et al., 1990). In view of the remarkably high potency of cisapride in blocking $I_{Kr}$, the observed incidence of torsades de pointes (2 of 6 rabbits) was surprisingly low (Buchanan et al., 1993; Carlsson et al., 1990, 1993). However, cisapride, but not mosapride, has been reported to bind to alpha-1 adrenoceptors, with affinity in the same range as its binding to 5-HT$_2$ receptors, and this fact may at least partially contribute to the low incidence (Briejer et al., 1995; Cohen et al., 1996; Karasawa et al., 1990; Schuurkes et al., 1985). In the present study, an alpha-1 adrenoceptor blocking effect of cisapride was indirectly supported by the observation that when cisapride was added to methoxamine in the anesthetized rabbit, the blood pressure did not continue to increase (as seen in the mosapride- and vehicle-treated rabbits). On the contrary, the blood pressure actually decreased significantly once cisapride was adminis-
amine is probably too short (one carbon) to become a good class III pharmacophore.

It is concluded from the present set of experiments that cisapride has an electrophysiological and proarrhythmic profile resembling to some extent that of the novel and selective class III antiarrhythmic agents. It is conceivable that these features may explain the rare cases of torsades de pointes recently reported in the literature. Mosapride, on the other hand, does not demonstrate any repolarization-delaying characteristics or proclivity to induce repolarization-related proarrrhythmias.

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