ABSTRACT

Indapamide is a diuretic agent with direct electrophysiological effects on ionic currents involved in cardiac repolarization. In particular, indapamide blocks the slow component of delayed rectifier potassium current. In contrast, most class III antiarrhythmic agents, such as dl-sotalol, block the rapid component of delayed rectifier potassium current. Computer simulations have suggested potentiating effects of drug effects on cardiac repolarization by the combined block of the rapid component of delayed rectifier potassium current and the slow component of delayed rectifier potassium current. Therefore, the objective of our study was to evaluate the modulation of cardiac electrophysiological effects of dl-sotalol by indapamide. Two indices of cardiac repolarization, monophasic action potential duration at 90% repolarization and effective refractory period, at two basic cycle lengths (800 and 400 msec) were determined in 24 anesthetized open-chest dogs. In two treatment groups (n = 6/group), data were obtained at base line and every 2 min during steadily increasing concentrations of dl-sotalol (0–40 μg/ml) either alone or in the presence of indapamide (500 ng/ml). Data were also obtained in dogs receiving either a low-dose (500 ng/ml) or a high-dose (up to 7.5 μg/ml) infusion regimen of indapamide alone. Administration of dl-sotalol was associated with concentration-dependent increases in monophasic action potential duration at 90% repolarization and effective refractory period, whereas repolarization was only slightly altered by the administration of indapamide alone. However, concentration-response curves of dl-sotalol were shifted to the left in dogs treated with the combination of dl-sotalol and indapamide, and the EC50 values of dl-sotalol estimated for the prolongation of monophasic action potential duration at 90% repolarization and effective refractory period were decreased 3-fold during the coadministration of both drugs (P < .05 vs. dl-sotalol alone). Thus, under conditions of normal K+ levels, clinically relevant concentrations of indapamide modulate dl-sotalol effects on cardiac repolarization. Additional block of cardiac Kv1 currents, especially the rapid component of delayed rectifier potassium current and the slow component of delayed rectifier potassium current could explain these observations.

Block of IKS by the Diuretic Agent Indapamide Modulates Cardiac Electrophysiological Effects of the Class III Antiarrhythmic Drug dl-Sotalol

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ABBREVIATIONS: BCL400, a basic cycle length of stimulation of 400 msec; BCL800, a basic cycle length of stimulation of 800 msec; EADs, early afterdepolarizations; ERP, effective refractory period; IKS, delayed rectifier potassium current; IKr, the rapid component of IK; IKs, the slow component of IK; MAPD90, monophasic action potential duration at 90% repolarization.
indicated delayed repolarization in the human (Wang et al., 1996; Bennett et al., 1995; Roden et al., 1995; Wang et al., 1995; Curran et al., 1995; Trudeau et al., 1995). On the other hand, drug-induced excessive lengthening of action potential duration is most often associated with compounds that alter cardiac repolarization through blockade of K⁺ channel proteins (Roden, 1993; Campbell and Loaiza, 1992; Roden, 1988; Colatsky et al., 1990).

Potassium currents responsible for limiting cardiac action potential duration vary depending on species and cell types. In guinea pig, dog and human ventricular myocytes, I_{Kr} is a major outward K⁺ current responsible for termination of the action potential plateau phase (Li et al., 1996; Liu et al., 1993; Matsuura et al., 1987). In these species, I_{Kr} comprises both an I_{Ks} and an I_{Kd} (Li et al., 1996; Gintant, 1996; Sanguinetti and Jurkiewicz, 1990). Although I_{Ks} and I_{Kd} exhibit interspecies differences in their microscopic constant characteristics, the macroscopic characteristics of I_{Kr} and I_{Ks} are preserved (Li et al., 1996; Gintant, 1996; Daleau and Turgeon, 1994a; Sanguinetti and Jurkiewicz, 1990). I_{Kr} is usually described as a small current (=1 pA/μF at 0 mV; peak current) that activates rapidly (compared with I_{Kd}). The current exhibits voltage-dependent fast inactivation that results in a decrease in peak I_{Kr} activation current at potentials positive to 0 mV (Spector et al., 1996b). A human ether-a-go-go-related gene (HERG) encodes subunits of a K⁺ channel with biophysical characteristics and sensitivity to methanesulfonamide derivatives similar to those of I_{Ks} (Snyders and Chaudhary, 1996; Spector et al., 1996a; Sanguinetti et al., 1995). On the other hand, I_{Kd} is characterized by a delayed onset of activation that occurs over a voltage range typical of the classically described cardiac I_{Ks}. Recent studies have also demonstrated that two proteins, K_{LQT1} and I_{Ks} (the minimal K⁺ channel; minK), coassemble to form the I_{Ks} cardiac K⁺ current (Sanguinetti et al., 1996; Barhanin et al., 1996). Consequently, both currents (I_{Ks} and I_{Kd}) are expected to play a major role in cardiac repolarization (Zeng et al., 1995; Courtney et al., 1992). In particular, it was proposed that excessive lengthening of cardiac repolarization will be observed during the coadministration of I_{Kd} and I_{Ks} blockers as a result of the potentiation of drug effects (Zeng et al., 1995; Courtney et al., 1992).

I_{Kr} is a particularly important target for antiarrhythmic drugs that prolong action potential. For example, N-acetylprocainamide, d-sotalol, E-4031, dofetilide and most methanesulfonanilide derivatives selectively block I_{Kr} (Gwilt et al., 1991; Turgeon et al., 1990; Sanguinetti and Jurkiewicz, 1990; Komeichi et al., 1990). However, I_{Kr} is also the target of “non-antiarrhythmic agents” such as histamine H1 receptor antagonists and diuretic agents (Khalifa et al., 1995; Salata et al., 1995; Turgeon et al., 1994; Daleau and Turgeon, 1994b; Woosley et al., 1993). Recent data from our laboratory suggest that diuretic derivatives such as indapamide exhibit direct cardiac electrophysiological effects (Turgeon et al., 1994). Using isolated guinea pig ventricular myocytes, we demonstrated that indapamide selectively inhibits I_{Ks} (Turgeon et al., 1994).

Factors that predispose the individual to pharmacologically induced long QT syndrome include slow HR, low serum levels of K⁺ and Mg²⁺ and concomitant administration of diuretics (Siegel et al., 1992; Roden, 1988; Neuvonen et al., 1982; Redleaf and Lerner, 1968; Ramee et al., 1985; Moro et al., 1986; Fofar and Gribbin, 1984; Karen et al., 1981; McKibbin et al., 1984). So far, drug interactions between diuretics and drugs that prolong action potential have been explained mainly in terms of diuretic-induced low K⁺ levels on the basis of both in vitro and in vivo models (Roden and Hoffman, 1985; Roden et al., 1986). However, our recent electrophysiological studies with indapamide have led us to propose that the electrophysiological effects of class III antiarrhythmic drugs could be directly modulated by diuretics, even under conditions of normal K⁺ levels.

Modulation of class III drug electrophysiological effects by diuretics such as indapamide would offer an additional explanation, besides diuretic-induced hypokalemia, why proarrhythmic events may occur in patients undergoing concomitant diuretic treatment and therapy with a drug that prolongs action potential. Therefore, the objective of the present study was to compare, in an in vivo dog model, the cardiac electrophysiological effects of indapamide alone, of dl-sotalol alone and of the combination of these two agents.

### Materials and Methods

Experiments were performed in accordance with our institutional guidelines on animal use in research. Animals were housed and maintained in compliance with the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

### Surgical Procedure

Mongrel dogs of either sex (20–25 kg) were anesthetized with an i.v. bolus dose (30 mg/kg) and a maintenance infusion (65 mg/hr) of sodium pentobarbital. Dogs were ventilated with room air supplemented with oxygen via an endotracheal tube with a Harvard respirometer. Arterial blood gases were measured and kept in the physiological range (pH: 7.35–7.45; pO₂: 80–100 mm Hg). Arterial blood pressure was monitored through a catheter advanced into the aorta via the left femoral artery. A temperature-sensitive probe was inserted into the body via the esophagus and connected to a digital thermometer. Temperature was maintained between 37°C and 38°C with heating blankets placed underneath the animal.

The left femoral and jugular veins were cannulated for the i.v. administration of drugs (dl-sotalol and/or indapamide). Lead II of the surface ECG was monitored. ECG, blood pressure and monophasic action potential signals were recorded continuously throughout the experiment. Electrolytes (K⁺, Na⁺ and Cl⁻) were also monitored. Serum K⁺ levels were maintained between 3.5 and 4.5 mM with 10–20 mEq/L of KCl added to a dextrose/water 5% perfusate.

A left thoracotomy was performed in the fifth intercostal space, the epicardium was opened, the heart was exposed and a pericardial cradle was created. Two stainless steel wires were sutured to the epicardial surface of the ventricles. Complete AV block was obtained by injection of 37% formaldehyde (0.1–0.3 mL) into the AV node (Shindo et al., 1982). Ventricular pacing at a basic cycle length of 600 msec (100 beats/min) was performed for the remainder of the surgical procedure.

A 6F pacing monophasic action potential catheter (quadripolar contact electrode, model No. 1650, 100 cm long; EP Technologies Inc., Sunnyvale, CA) was positioned into the left ventricle via the left carotid artery to measure local repolarization time, to control ventricular rate during the experiment and to determine local refractory periods (Franz et al., 1990). Another monophasic action potential catheter was placed into the right ventricle via the right femoral vein to measure local repolarization time.

### Pharmacokinetic Studies

Pharmacokinetic parameters of dl-sotalol and indapamide were determined in two groups of three mongrel dogs (20–25 kg) of either...
sex. Animals were anesthetized and underwent surgery as described in the previous section. Thirty minutes after the end of surgery, a bolus of either dl-sotalol (5 mg/kg over 1 min) or indapamide (1 mg/kg over 1 min) was administered via the left femoral vein. Serial blood samples were obtained for the next 12 hr, and plasma was separated by centrifugation within 30 minutes and frozen at −20°C until analysis. Plasma concentrations of dl-sotalol and indapamide were determined by HPLC (Miller et al., 1993; Fiset et al., 1993a). Data on plasma concentrations vs. time were analyzed using compartmental analysis, and pharmacokinetic parameters were obtained for dl-sotalol and indapamide. Thereafter, loading and maintenance dose regimens were defined to achieve desired concentrations of the drugs during electrophysiological protocols.

**Electrophysiological Studies**

Four groups of six dogs received a treatment that consisted of dl-sotalol alone (treatment 1), indapamide alone at a low-dose infusion regimen (treatment 2), indapamide alone at a high-dose infusion regimen (treatment 3) or dl-sotalol and indapamide administered in combination (treatment 4). All dogs were subjected to the surgical procedure described previously. Monophasic action potential signals were set at the beginning of the electrophysiological study, and catheters were not repositioned during the study.

**Treatment 1 (dl-sotalol alone).** After a 30-min equilibrium period that followed surgery, baseline electrophysiological recordings [ECG, blood pressure, monophasic action potential signals and effective refractory period (ERP)] were obtained at BCL900 and BCL400. Blood samples were drawn for measurement of baseline drug concentration and electrolyte determination were obtained. Dosing regimens of dl-sotalol were designed to cause continuously increasing concentrations of the drug in order to reach dl-sotalol concentrations of 7.5 µg/ml at 1 hr, 20 µg/ml at 1.5 hr and 40 µg/ml at 2 hr. dl-Sotalol i.v. infusion rates were as follows: 1) 83.3 µg/kg/min × 60 min; 2) 250 µg/kg/min × 30 min; 3) 500 µg/kg/min × 30 min.

At the beginning of the dl-sotalol infusion (time zero), heart was paced at BCL900. At 1 min, ECG, blood pressure and monophasic action potential signals were recorded. Between 1 and 2 min, ERP was determined. At 2 min, pacing at BCL900 was started. At 3 min, ECG, blood pressure and monophasic action potential signals were recorded. Between 3 and 4 min, ERP was determined. At 5 min, pacing at BCL900 was restarted, and so forth. Data (every 2 min) and blood samples (every 4 min) were obtained for determination of dl-sotalol and electrolytes obtained for a total of 120 min.

**Treatment 2 (low-dose infusion regimen of indapamide alone).** Dogs received loading and maintenance infusions of indapamide in order to obtain a stable plasma concentration of indapamide of 500 ng/ml at 45 min and for the following 2 hr. Infusion rates of indapamide were as follows: 1) 60 µg/kg/min × 5 min; 2) 10 µg/kg/min × 10 min; 3) 30 µg/kg/min × 10 min; 4) 50 µg/kg/min × 15 min; 5) 3 µg/kg/min × 30 min; 6) 2 µg/kg/min × 30 min; 7) 1.33 µg/kg/min × 30 min; 8) 1.2 µg/kg/min × 30 min. Data (every 2 min) and blood samples (every 4 min) for determination of indapamide and electrolytes were obtained for a total of 165 min (45-min loading and 120-min maintenance infusions).

**Treatment 3 (high-dose infusion regimen of indapamide alone).** In this treatment group, the indapamide infusion regimen was designed to cause continuously increasing concentrations of the drug. Targeted concentrations were 1 µg/ml at 1 hr, 2.5 µg/ml at 1.5 hr and 7.5 µg/ml at 2 hr. Infusion rates were as follows: 1) 16.6 µg/kg/min × 60 min; 2) 50 µg/kg/min × 30 min; 3) 150 µg/kg/min × 30 min. Otherwise, the electrophysiological protocol for this treatment group was identical to that described for the dl-sotalol alone treatment group (treatment 1).

**Treatment 4 (dl-sotalol and indapamide).** After surgery, dogs received loading and maintenance infusions of indapamide identical to those described for treatment 2 (low-dose infusion regimen of indapamide alone). Infusions of dl-sotalol that caused steadily increasing concentrations of the drug were started 45 min after the beginning of indapamide infusion. dl-Sotalol infusion regimens were similar to those described for treatment 1. Electrophysiological recordings (ECG, blood pressure, monophasic action potential signals and ERP) for every 2 min and blood samples for dl-sotalol, indapamide and electrolyte determinations were obtained as described for treatment 1 during a 120-min period.

**Electrophysiological Measurements**

Data (surface ECG lead II, arterial blood pressure, monophasic action potential signals and ERP) were acquired at BCL900 and BCL400. Hearts were paced with a square-wave stimulus (2-msec duration) at twice diastolic threshold. Data were recorded with a E/M VR-12 physiological recorder, digitized (sampling rate of 1 KHz) and stored on a hard disk for analysis. S1-S1 and S1-S2 pulses were generated from an EP2 Clinical Stimulator (Medtronic, Minneapolis, MN). Ventricular ERP was defined as the longest S1-S2 that failed to result in a ventricular depolarization. Premature beats were induced in an incremental fashion by steps of 2 msec until ventricular depolarization was produced (Beauregard et al., 1990; Morady et al., 1989). Monophasic action potential signals were acquired using a DC amplifier (signals were amplified 100 times), digitized at a sampling rate of 1 KHz, filtered at 100 Hz and stored on hard disk before analysis. MAPD90 was determined automatically by a routine using the program CVRP92 (Cardiovascular Research Partner, Datton System Enr. Quebec, Canada). At least six complexes were used for each measurement. MAPD90 values were determined and analyzed until it was possible to detect a positive voltage deflection (slope >0) that interrupted the smooth contour of phase 2 or phase 3 repolarization (Rubart et al., 1993). EADs and ventricular premature complexes were recorded throughout the experiments.

**Pharmacodynamic Analysis**

Drug administration regimens for dl-sotalol and indapamide (high-dose infusion regimen) were designed to allow complete characterization of the concentration-effect relationship of the drugs. The concentration-effect relationship was estimated via a sigmoidal E_{max} model (Hill’s equation) using all concentration points from 0 to maximum concentration of dl-sotalol before the development of EADs (Lalonde, 1992; Holford and Sheiner, 1991). Changes in electrophysiological parameters (expressed as percent change from baseline) induced by dl-sotalol alone or indapamide alone were compared to those observed during the dl-sotalol/indapamide combined therapy in order to detect modulation of electrophysiological effects.

**Statistical Analysis**

Data obtained in each treatment group were compared by one-way ANOVA with Duncan’s post-hoc test if the null hypothesis of equal means could be rejected at P < .05. Confidence intervals fixed at 95% were used to compare dl-sotalol concentration-effect relationships (MAPD90 and ERP) between the group that received dl-sotalol alone and the group that was treated with dl-sotalol and indapamide in combination. All results are reported as mean ± S.D.

**RESULTS**

**Pharmacokinetics**

Pharmacokinetics of dl-sotalol were determined in three anesthetized open-chest dogs after the administration of an i.v. bolus dose (5 mg/kg) of the drug. Mean decline in plasma concentrations of dl-sotalol was described by a two-compartment open model: C = 4.834e^{−5.58x10 + 0.831e^{−0.159x10.831e^{−0.159x10}}. Similarly, three anesthetized open-chest dogs received an i.v. bolus dose (1 mg/kg) of indapamide. Mean decline in plasma concentrations of indapamide was also described by a two-compartment open model: C = 1.452e^{−7.58x10 + 0.737e^{−0.092x10}}.
Infusion regimens used in our study were determined on the basis of these pharmacokinetic data.

Figure 1 illustrates plasma concentrations of dl-sotalol measured in dogs exposed to the drug either alone or in combination with indapamide. Measured plasma concentrations of dl-sotalol during the first 1.5 hr of drug infusion correlated fairly well with predicted plasma concentrations from our sham animals. However, from 1.5 to 2 hr, increased interdog variability was observed. This could be due either to physiological changes in our animals or to drug interactions, because plasma concentrations of dl-sotalol appeared to be higher than expected during the coadministration of indapamide. On the other hand, indapamide plasma concentrations in this treatment group were very stable between 45 min and 2.75 hr and correlated well with targeted plasma concentrations (500 ng/ml).

Pharmacodynamics

Values measured for serum concentrations of electrolytes, particularly serum levels of K⁺ ions (table 1), as well as pH, pCO₂ and pO₂ were within normal range throughout the experiments in all animals studied. There were no differences among the groups, and there were no significant changes in these parameters from the beginning to the end of the protocols.

Effects of dl-Sotalol, Indapamide and Their Combination on MAPD₀₉₀

Administration of dl-sotalol to anesthetized dogs according to our infusion protocol caused a concentration-dependent increase in MAPD₀₉₀. An example of raw data obtained for the prolongation of MAPD₀₉₀ in the left ventricle at BCL₅₀₀₀ after the administration of dl-sotalol is presented in figure 2. At this frequency of stimulation, MAPD₀₉₀ was prolonged approximately 80 msec, whereas at BCL₄₄₀₀, MAPD₀₉₀ was prolonged 46 to 60 msec (table 2). In contrast, indapamide administered alone, even at concentrations as high as 7.5 μg/ml, did not cause any significant changes in MAPD₀₉₀ measured in both ventricles at each stimulation frequency (table 2). Reverse frequency-dependence characteristics of the prolonging effects of dl-sotalol on MAPD₀₉₀ were observed in the absence as well as in the presence of indapamide. For both ventricles and at both pacing cycle lengths, maximum MAPD₀₉₀ prolongation observed before the development of EADs during the combined administration of dl-sotalol and indapamide did not differ from that observed during the administration of dl-sotalol alone.

The data presented in figure 2 show that the coadministration of indapamide shifted the concentration-response curve of dl-sotalol to the left. Results similar to those observed at BCL₅₀₀₀ in the left ventricle were noted at both basic cycle lengths in each ventricle. In fact, in all cases, concentration-effect (MAPD₀₉₀ prolongation) curves of dl-sotalol were shifted to the left by coadministration of indapamide. Concomitant administration of indapamide decreased the EC₅₀ of dl-sotalol for prolongation of MAPD₀₉₀ about 3-fold (table 2).

Effects of dl-Sotalol, Indapamide and Their Combination on ERP

A concentration-dependent increase in ERP was observed after the administration of dl-sotalol. ERP was prolonged 57 ± 20 msec at BCL₅₀₀₀ and 42 ± 15 msec at BCL₄₄₀₀ (table 3; both P < .05 vs. base line). In contrast, indapamide administered alone did not cause any significant changes in ERP at either stimulation frequency. In the low-dose infusion regimen (500 ng/ml), ERP determined 45 min after the beginning of drug administration at BCL₅₀₀₀ and BCL₄₄₀₀ were increased 1 ± 7 msec and 2 ± 7 msec from base line, respectively (table 3; both P > .05 vs. base line). Moreover, at the end of the high-dose infusion regimen of indapamide alone, ERP remained unchanged (table 3; both P > .05 vs. base line).

The concentration-dependent increase in ERP caused by dl-sotalol was altered by the coadministration of indapamide. In fact, EC₅₀ values estimated during the combined administration of dl-sotalol and indapamide were decreased 3-fold compared with the administration of dl-sotalol alone (table 3). Finally, prolongation of ERP caused by dl-sotalol administered either alone or combined with indapamide was reverse frequency-dependent; however, maximum increase in ERP before the development of EADs was not significantly different between the groups at either pacing cycle length (table 3).

Effects of Indapamide on dl-Sotalol-Induced EADs

Figure 3 illustrates typical ECG and monophasic action potential signals (BCL₅₀₀₀) obtained in dogs that received...
dl-sotalol either alone or in combination with indapamide. Signals were obtained during the control period and at the time the first EADs were observed on both ventricles. As shown in this figure, EADs arose before the completion of final repolarization and developed after significant lengthening of monophasic action potential duration. EADs were noted on both ventricles in 5 out of 6 dogs in the two groups. When EADs developed, they were observed first at BCL800. They became more and more prominent as drug concentration increased and were present until completion of the protocol.

EADs were noted at much lower dl-sotalol concentrations during combined dl-sotalol/indapamide administration than during the administration of dl-sotalol alone (6 ± 3 μg/ml vs. 15 ± 9 μg/ml; P < .05). In dogs that received dl-sotalol alone, EADs were observed at a lower concentration of dl-sotalol in the right ventricle (BCL400: 13 ± 11 μg/ml; BCL400, 12 ± 3 μg/ml) than in the left ventricle (BCL400: 18 ± 8 μg/ml; BCL400: 20 ± 7 μg/ml; P < .05). This heterogeneity between ventricles was not apparent during the coadministration of indapamide.

### Discussion

The major finding of this study is that prolongation of cardiac repolarization due to block of I\textsubscript{Ks} by agents such as dl-sotalol can be modulated by I\textsubscript{Ks} blockers such as indapamide. The unheralded nature of the drug interaction reflects the weak I\textsubscript{Ks} blocking potency of indapamide. The 3-fold decrease in EC\textsubscript{50} of dl-sotalol for the prolongation of MAPD\textsubscript{90} and ERP during concomitant administration of indapamide was observed under conditions of controlled ionic levels (especially, normal K\textsuperscript{+} levels) at clinically relevant concentrations (500 ng/ml) of indapamide and dl-sotalol (in the range of 1 to 10 μg/ml). Another very significant finding was that EADs developed at about 3 times lower concentrations of dl-sotalol when the drug was coadministered with indapamide. Thus these results suggest that diuretics such as indapamide can exert direct cardiac electrophysiological effects that modulate class III antiarrhythmic drug action on cardiac repolarization. This provides a new explanation of unexpected toxicity observed in patients with normal electrolyte levels during concomitant treatment with class III antiarrhythmic drugs and diuretic agents.

The experimental protocol and drug concentrations used in this study were chosen to reflect clinical situations and to allow characterization of pharmacodynamic parameters. The spectrum of dl-sotalol concentrations studied (0–40 μg/ml) covers the entire range of therapeutic and toxic concentrations of the drug (Woosley et al., 1990; Wang et al., 1986). Maximum effects of the drug on cardiac repolarization observed with the highest concentrations were necessary to determine precisely the EC\textsubscript{50} of dl-sotalol in all treatment groups. On the other hand, in the low-dose infusion regimen of indapamide, concentration was adjusted to 500 ng/ml (1.5 μM) in order to reproduce plasma concentrations reached during chronic indapamide therapy in the human (Chaffman et al., 1984). Characterization of the effects of indapamide on cardiac repolarization was sought by use of the high-dose infusion regimen.

Plasma concentrations of dl-sotalol appeared to be increased during the concomitant administration of indapamide, which suggested that a pharmacokinetic interaction had occurred. dl-Sotalol is not metabolized and is only weakly bound to plasma proteins (Fiset et al., 1993b; Hanyok, 1993). Therefore, a decrease in the renal clearance of dl-sotalol and/or a decrease in its volume of distribution is likely to explain increased plasma concentrations of the drug during concomitant administration of indapamide. In our study, time-related changes in cardiac repolarization during administration of dl-sotalol either alone or combined with indapamide were corrected for actual plasma concentrations of dl-sotalol. Consequently, concentration-effect curves should not be altered by a potential pharmacokinetic interaction between indapamide and dl-sotalol.

Indapamide is a selective but relatively weak blocker of I\textsubscript{Ks} (EC\textsubscript{50} = 100 μM) (Turgeon et al., 1994). In our study, administration of the drug at either targeted low concentration (500 ng/ml; 1.5 μM) or high concentration (7.5 μg/ml; 22 μM) was without apparent effect on cardiac repolarization. Results

### Table 1

<table>
<thead>
<tr>
<th>Time of Sampling</th>
<th>dl-Sotalol Alone</th>
<th>Indapamide Alone (low-dose infusion)</th>
<th>Indapamide Alone (high-dose infusion)</th>
<th>dl-sotalol and Indapamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning</td>
<td>3.6 ± 0.2</td>
<td>3.5 ± 0.5</td>
<td>3.8 ± 0.3</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>End</td>
<td>4.0 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>4.1 ± 0.7</td>
<td>4.4 ± 0.5</td>
</tr>
</tbody>
</table>

Values are reported as mean ± S.D.
could have been different at other pacing frequencies or with more potent agents, such as L-735,821 and chromanol 239-B (Busch et al., 1996; Salata et al., 1996). Nevertheless, cardiac electrophysiological properties of low-dose indapamide (500 ng/ml) were revealed by additional block of IKs during the administration of dl-sotalol, indicating the potentially unheralded nature of the drug interaction. It is likely that combined administration of potent IKs and IKCa blockers would cause even more potentiation of drug effects on cardiac repolarization.

Pharmacological effects of indapamide on the cardiovascular system are diverse (Campbell and Brackman, 1990). In addition to its diuretic effects, the drug exhibits direct vascular activity, including inhibition of smooth muscle cell contraction elicited by norepinephrine, epinephrine, angiotensin II and prostaglandin F2 (Miyazaki et al., 1985; Borkowski et al., 1981; Moore et al., 1977). Indapamide also has discrete effects on a number of interrelated systems that may protect the cardiovascular system (Campbell and Brackman, 1990). Nevertheless, the exact electrophysiological mechanisms underlying indapamide’s activity on vascular reactivity is unclear. In smooth muscle cells, the drug has been reported to inhibit the calcium-dependent K+ current (IKCa) with an EC50 of about 300 μM (90 μg/ml) (Mironneau, 1988). In contrast, inhibition of IKCa by hydrochlorothiazide was demonstrated in another study, but not with indapamide (Calder et al., 1992). A recent study has demonstrated that indapamide may bind to the slow inward calcium current, although it is 500 times less potent than nifedipine (Mironneau and Mironneau, 1981). Indapamide may also alter the phosphate balance of smooth muscle cells but does not modulate the ATP-dependent K+ current (IKATP) (Plante et al., 1988; Calder et al., 1992). Overall, some of these pharmacological effects of indapamide may explain the modulation of cardiac repolarization described in our study if the electrophysiological effects observed in smooth muscle cells can be extrapolated to cardiac tissue. On the other hand, potential block of IKCa by indapamide remains a probable explanation (Turgeon et al., 1994).

No change in maximum increase in ERP and MAPD90

![Fig. 3. ECG and monophasic action potential signals (BCL900) in dogs that received either dl-sotalol alone (upper panels) or dl-sotalol in combination with indapamide (lower panels). Signals were obtained in the control period (left panels) and at the time the first EADs were observed on both ventricles (right panels).](image-url)
before the development of EADs was observed in our study. In other words, EADs occurred when action potential durations were prolonged to the same degree. On the other hand, concentrations of the drug required to attain the threshold for the development of EADs have been predicted to be lowered by the coadministration of drugs with additive effects on cardiac repolarization (Zeng et al., 1995; Courtney et al., 1992). Results obtained in this study are in complete agreement with this statement.

The exact significance of EADs recorded from monophasic action potential signals is still unclear. If EADs recorded from monophasic action potential signals reflect true EADs, then surely only data obtained before the development of EADs should be analyzed for the determination of MAPD90. Their inclusion would lead to an artificial overestimate of the extent of MAPD prolongation. On the other hand, Antzelevitch and Sicouri have proposed that EADs recorded from monophasic action potential catheters reflect repolarization of M cells, which suggests that data with EADs should be included in the analysis (Antzelevitch and Sicouri, 1994). We have decided to conduct a conservative analysis of our results until this issue is resolved, so data with EADs were not included in the analysis.

A significant finding of our study is that EADs developed at 3 times lower concentrations of dl-sotalol when the drug was coadministered with indapamide. Although EADs were recorded, no sustained events such as polymorphic ventricular tachycardias, monomorphic ventricular tachycardia or ventricular fibrillation were observed. This indicates that conditions (slower HR, ventricular enlargement or hypertrophy, low K+ or Mg2+) in addition to prolonged repolarization are required to evoke proarrhythmic events. Nevertheless, EADs recorded in this study at clinically relevant concentrations of dl-sotalol during coadministration of indapamide are indicative of either heterogeneity in cardiac repolarization or non-driven action potentials (Takanaka and Singh, 1990; Roden and Hoffman, 1985; Antzelevitch and Sicouri, 1994), both of which may facilitate proarrhythmic events.

Another interesting observation made in our study is that EADs were observed at lower concentrations of dl-sotalol (12–13 µg/ml) in the right ventricle than in the left ventricle (18–20 µg/ml) when the drug was administered alone. During administration with indapamide, this difference was no longer apparent. We also observed that indapamide potentiated dl-sotalol effects on MAPD90 to a greater extent in the left ventricle than in the right ventricle (table 2). These observations suggest that the right ventricle is more sensitive than the left ventricle to the cardiac electrophysiological effects of dl-sotalol and that a “compensatory” mechanism (i.e., ionic current) present in the left ventricle is selectively eliminated by indapamide. Heterogeneity among right and left ventricles in the relative proportion of M cells, as well as heterogeneity in the relative proportions of ionic currents such as IKr and IKs among myocytes at the endocardial, M cell and epicardial layers, may explain these phenomena (Antzelevitch et al., 1991; Liu et al., 1993; Sicouri and Antzelevitch, 1991).

Several cases of torsades de pointes have been observed in patients undergoing concomitant therapy with thiazide diuretics and class III antiarrhythmic agents such as sotalol (Siegel et al., 1992; Roden, 1988; Neuvonen et al., 1982; Redleaf and Lerner, 1968; Fofar and Gribbin, 1984; McKibbin et al., 1984). It is also noteworthy that a pharmaceutical formulation allowing combined administration of dl-sotalol and hydrochlorothiazide, used in the early 1970s, led to striking occurrences of torsades de pointes (Jaattela, 1981; Reynaert, 1979). Our results suggest that, besides altered electrolyte serum concentrations that may have occurred in some of these patients, additional electrophysiological effects of dl-sotalol and diuretics on cardiac ionic currents may be responsible for some of the observed proarrhythmic events.

In summary, we have demonstrated that the coadministration of the diuretic agent indapamide modulates the electrophysiological effects of dl-sotalol on cardiac repolarization. This supports the hypothesis that weak IKr blockers possess unheralded cardiac electrophysiological effects that may be revealed under certain circumstances. These effects may potentiate actions of other drugs with IKs blocking properties, such as class III antiarrhythmic agents, erythromycin, histamine H1 receptor antagonists or antipsychotics, leading to excessive prolongation of cardiac repolarization and predisposing patients to proarrhythmic events.

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