Ionic Mechanism of Ibutilide in Human Atrium: Evidence for a Drug-Induced Na\(^+\) Current Through a Nifedipine Inhibited Inward Channel

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Accepted for publication March 31, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

This study examined the ionic mechanism of ibutilide, a class III antiarrhythmic in clinical use, on freshly isolated human atrial cells. Cells had resting potentials of \(-71.4 \pm 2.4\) mV, action potentials with overshoot of \(36.8 \pm 1.8\) mV, duration of 265 \pm 89 msec at 90% repolarization and slow repolarization (\(n = 16\)). Ibutilide, at \(10^{-7}\) M, markedly increased action potential duration. Four types of outward currents were detected: \(I_{to}\), \(I_{so}\), a delayed rectifier and \(I_{Kr}\). Ibutilide had no inhibitory effect on these outward currents at \(10^{-7}\) M (\(n = 28\)). In K\(^-\)free solutions and \(-40\) mV holding potential, mean peak inward current at 20 mV was \(-1478 \pm 103\) pA (\(n = 12\)). Ibutilide increased this current to \(-2347 \pm 75\) pA at \(10^{-7}\) M, with half maximal effect (\(K_d\)) of 0.1 to 0.9 nM between \(-10\) and \(+40\) mV (\(n = 21\)). At similar concentrations, the drug increased APD, with \(K_d\) of 0.7 and 0.23 nM at 70 and 90% repolarization, respectively (\(n = 8\)). Ibutilide shifted the midpoint of the steady-state inactivation curve from \(-21\) to \(-12.2\) mV (\(n = 6\)), and reduced current decline during repetitive depolarization (\(n = 5\)). The drug induced inward current was carried by Na\(^+\), through a nifedipine inhibited inward channel because Na\(^+\) removal eliminated the effect, and nifedipine abolished the inward current and the drug induced APD prolongation. We propose that a Na\(^+\) current through the L-type Ca\(^{2+}\) channel mediates ibutilide’s potent clinical class III antiarrhythmic action.

Inward and outward ionic currents play critical roles in regulating action potentials and effective refractory period in cardiac tissues. In human heart cells, the major inward current responsible for shaping the action potential plateau is the L-type Ca\(^{2+}\) current (Grand et al., 1990). The presence of T-type Ca\(^{2+}\) current (Grand et al., 1990) has not been established. In contrast, multiple K\(^+\) currents in the plateau region were reported. The most often described is the transient outward current, \(I_{to}\) (Amos et al., 1996, Escande et al., 1987, Shibata et al., 1989). This current is inactivated by positive holding potentials and is Ca\(^{2+}\) dependent or independent (Escande et al., 1987). Also, there is a rapidly activating, slowly inactivating, sustained outward current, \(I_{so}\) (Amos et al., 1996, Wang et al., 1993) (or \(I_{Kur}\)) (Amos et al., 1990). It is blocked by micromolar 4-AP, and is present in human atrial, but not in ventricular cells. In several studies, a delayed outward current, \(I_{K}\) (Beuckelmann et al., 1993, Wang et al., 1993) was described, which can be subdivided into a rapid, \(I_{Kr}\), and a slow, \(I_{Ks}\) current (Wang et al., 1994, Li et al., 1996). In addition, there is the inward rectifier, \(I_{K1}\) (Beuckelmann et al., 1993). This current is markedly reduced in the ventricular cells of patients with terminal heart failure.

Blockade of the K\(^+\) currents, as by class III antiarrhythmic compounds, can effectively prolong the APD and may terminate re-entrant arrhythmia and atrial arrhythmia (Singh and Nademanee, 1985). The projected clinical usefulness of such a mechanism has spurred intense efforts searching for these compounds. Sotalol, which is approved for use in human, is a good example. In animal heart, sotalol, like most class III antiarrhythmic compounds, blocks the delayed rectifier, specifically, the rapidly activating outward potassium current, \(I_{Kr}\) (Sanguinetti and Jurkiewicz, 1990). In human atrial cells, however, sotalol’s action is unknown because \(I_{Kr}\) has been difficult to resolve in human heart tissues. Only one laboratory has reported this currents in considerable detail (Li et al., 1996, Wang et al., 1994). Other investigators (Amos et al., 1996, Escande et al., 1987, Shibata et al., 1989), including a recent study of ventricular myocytes in nonfailing human hearts (Konarzewski et al., 1995), have not observed \(I_{Kr}\). This is not to imply that \(I_{Kr}\) plays no role in APD prolongation because in human papillary muscles, E-4031, a specific \(I_{Kr}\) blocker, clearly prolongs APD (Ohler and Ravens, 1994).

Besides K\(^+\) channel blockers, inward current activator can also promote class III activity. A typical example is ibutilide currently in clinical use. Ibutilide increases a Na\(^+\) sensitive

ABBREVIATIONS: APD, action potential duration; \(I_{to}\), transient outward potassium current; \(I_{so}\), sustained outward potassium current; \(I_{K1}\), inwardly rectifying potassium current; \(I_{Kr}\), rapid outward potassium current; \(I_{Ks}\), slow outward potassium current; TTX, tetrodotoxin; 4-AP, 4-aminopyridine.
inward current in guinea pig ventricular cells (Lee, 1992).
This current appears at the plateau potential positive to −20 mV and peaks at 20 mV. It shares many characteristics of the “L”-type Ca
superscript + current with the exception that it inactivates more slowly, has a more positive peak-current potential, and is removed upon external Na
superscript + removal. In Na
superscript + and Ca
superscript + containing external solution, ibutilide can increase this inward current at very low concentrations of 10−9 to 10−7 M.
Removing external Na
superscript + abolishes ibutilide’s effect. The remaining “L” type Ca
superscript + current is relatively insensitive to the drug. At similar low concentrations of 10−9 to 10−7 M, ibutilide prolongs APD (Lee, 1992). Thus, the close agreement between ibutilide’s effect on the Na
superscript + sensitive inward current and the APD prolongation suggests that the inward current is responsible for ibutilide’s class III activity. Ibutilide also blocks I Na in cultured tumor atrial cells, the AT-1 cells (Yang et al., 1995), and may contribute to APD prolongation.

In human heart cells, there is no mechanistic information on ibutilide. The purposes of this study were: 1) to establish experimental conditions for obtaining single human atrial cells that are suitable for stable recordings of action potentials and ionic currents by the suction pipette method; 2) to characterize ibutilide’s ionic mechanism of action on human atrium.

Methods

Cell Preparations

Atrial tissues procurement procedures were approved by the ethics committee of Bronson Methodist Hospital (Kalamazoo, MI). Segments of atrial appendages removed from patients undergoing heart surgery were immediately immersed in Ca
superscript + free-Tyrode’s solution at 37°C. The solution contained, in mM: NaCl, 137; KCl, 4; MgCl2, 1.05; glucose, 5; Tris HCl, 3.17; Tris base 0.41; pH at 7.47 and saturated with 100% O2 gas. Cell dissociation procedures began 10 to 20 min after tissue removal. The tissue was washed twice in Ca
superscript + free-Tyrode’s solution saturated with O2 and contained 30 mM 2,3-butanedionemonoxine (Peeters et al., 1995). This compound, although increased cell yield, reduced cell quality, and its use was discontinued shortly in this study. The cell dissociation procedure was very similar to that for the dissociation of coronary smooth muscle cells (Wilde and Lee, 1989). Briefly, atrial tissue submerged in Ca
superscript + free-Tyrode’s solution was minced by a pair of scissors to about 1-mm pieces and then washed twice with the same solution. The minced tissues were then transferred to the same Ca
superscript + free-Tyrode’s solution that contained 350 U/ml collagenase (type II, Sigma Chemical Co., St. Louis, MO), 34 U/ml elastase (type II, aqueous suspension, Sigma), 200 U/ml soybean trypsin inhibitor, 0.1% taurocholic acid, and 0.1% bovine serum albumin. In this enzymatic solution, the minced tissues were incubated at 35°C while stirred at 330 r.p.m. with a stir bar. A drop of incubation solution was drawn for examination of single cells every 10 min until cells appeared. Then the solution was decanted for centrifugation at 1380 rpm for 1 min. The pellet was resuspended in a wash solution of 300 μM Ca
superscript + and 1% BSA at room temperature for electrophysiological recording in the next 4 to 6 hr.

Electrophysiological Recordings

Membrane currents of single human atrial cells were recorded in whole-cell configuration using the suction pipette method. Pipettes were fabricated from borosilicate glass capillaries (Scientific America, Evanston, IL) with a P.80/PC, Flaming Brown puller (Sutter Instruments, Novato, CA). Pipette resistance was 0.8 to 1 MΩ when filled with experimental solutions. Whole-cell currents were recorded using an AXOPATCH-1D amplifier (Axon Instruments, Inc., Foster City, CA) interfaced with a personal computer. A commercial software “pClamp” (Version 6.0.4, Axon Instruments, Inc.) was used for data acquisition and analysis. Using the suction pipette, spontaneous action potentials were elicited by brief positive current pulses in current-clamp mode. In voltage-clamp mode, the cell capacitance was calculated from the capacitance transient elicited by a −10 mV pulse. The seal resistance was between 50 to 2 GΩ. The series resistance was compensated by about 90%. Currents were filtered at 2 KHz and digitized at 1 to 2 KHz. Cells were held at −40 mV throughout during experiments and dialyzed internally with either K
superscript + or Ca
superscript + solutions for 10 to 15 min to reach equilibrium before recording. Current amplitudes were not corrected for leak.

Solutions and drugs. For resting and action potentials: A, Pipette solution, in mM: K-glutamate, 115; K-aspartate, 10; KCl, 5; CaCl2, 1; EGTA, 10; MOPS, 10; glucose, 20; Mg
superscript + -ATP, 5; creatine phosphate, sodium salt, 5; pH adjusted to 7.2 with KOH. B, External normal Tyrode’s solution, in mM: NaCl, 137; KCl, 4; CaCl2, 2; MgCl2 1.05; N-2-hydroxyethylpiperazin-N’-2-ethanesulfonic acid (HEPES), 10; pH adjusted to 7.5 with NaOH.

For outward currents: The pipette solution of (A) was used. C, External solution, full-Na, in mM: NaCl, 126; KCl, 5; MgCl2, 0.8; CaCl2, 0.2; HEPES, 10; glucose, 5.5; CsCl, 2 (or 1 μM nifedipine); pH to 7.4 by Tris base. D, External solution, Na
superscript + free, in mM: choline Cl, 126; KCl, 5; MgCl2, 0.8; CaCl2, 1; NaHPO4, 0.33; HEPES, 10; glucose, 5.5; COCl2, 2; pH to 7.4 by Tris base.

For inward currents: E, Pipette solution, K
superscript + free, in mM: CsOH, 151; L-aspartic acid, 10; glutamic acid, 10; taurocholic acid, 20; glucose, 10; EGTA, 10; TEA, 20; 4-AP, 5; Mg-ATP, 5; Creatinine, free base, 10; pH adjusted to 7.2 with H2PO4 (Lee, 1992). (E1) External solution, full-Na
superscript +, K
superscript + free, in mM: NaCl, 137; CsCl, 4; CaCl2, 2; MgCl2, 1.05; glucose, 5; pH adjusted to 7.5 with Tris-HCl, 6.34; Tris-Base, 1.65. (E2) External solution, Na
superscript + free, K
superscript + free, in mM: Choline Cl or tетraethylammonium chloride (TEA-Cl), 137; CsCl, 4; CaCl2, 2; MgCl2, 1.05; glucose, 5; Tris-HCl, 6.34; Tris-Base, 1.65; pH at 7.5.

Ibutilide, N-(4-(4-ethylheptylamino)-1-hydroxybutyl]benzyl]-methane-sulfonamide, (E)-2-butenedioate (2:1 salt) was dissolved in distilled water to make 10−3 M stock solution which was diluted to all subsequent concentrations for testing.

In voltage clamp experiments, depolarization steps were delivered to the cell at 0.5 Hz frequency or as indicated. For pulse-train stimulations, 16 identical pulses to a given voltage were delivered to the cell at 2 Hz. All experiments were carried out at 36 to 37°C. Data presented were mean ± S.E.M. The statistical significance of the difference between mean values was determined by a paired T test and P < .05 indicates significant differences whereas P > .05, insignificant.

Results

In this study, 110 atrial tissues were processed for single cell dissociation. Tissues from 35 patients with various medical conditions and medications yielded successful experiments. Among this group, 12 had hypertension; 5 had coronary artery diseases; 11 had diabetes, including insulin-dependent and noninsulin-dependent diabetes mellitus; 4 had chronic obstructive pulmonary diseases; 2 had myocar-
Fig. 1. Three types of human atrial action potentials. Action potentials shown were obtained from three internally dialyzed cells using the suction pipette method and solutions described in "Methods." The spike preceding each action potential is the stimulus artifact in response to a 0.5 to 0.9 nA stimulus lasting 5 to 10 msec. The action potentials shown were elicited by the last pulse in a pulse train with 16 identical stimuli delivered to the cell at 1 Hz frequency. No outward holding current was injected into the cell to artificially hyperpolarize the membrane potential and to generate the repolarization phase (temperature, 37°C).

Fig. 2. A, Typical effect of ibutilide on a triangular action potential (type 3). Again, suction pipette and solutions for action potentials similar to figure 1 was used. For a steady-state effect, the cell was stimulated by a pulse-train containing 16 pulses delivered to the cell at 1 Hz frequency. The first pulse-train was delivered to the cell in the absence of drug; then after 5 min in drug exposure, a second pulse train was delivered; this was followed by a third pulse train after a 10-min wash in the control solution. Action potentials shown were elicited by the last pulse of each train. Temperature was at 37°C. Note the drug specifically generated a plateau at about \(-10\) mV in these triangular action potentials, but without delaying either the early or late repolarization phase. B, Current traces and I-V relationship (current amplitudes measured at the end of the 2-sec step) obtained in 2 mM 4-AP, 100 nM atropine, 1 \(\mu\)M nifedipine for the isolation of \(I_{K}\) and \(I_{Na}\) (Wang et al., 1994). Currents were elicited every 10 sec from holding potential of \(-50\) mV to various step (2 sec long) potentials as indicated. Open symbols, control; solid symbols, in dofetilide (temperature, 36°C).
dial infarction and 1 had atrial fibrillation. They were treated with one or more types of medications such as aspirin, insulin, Ca$^{2+}$ channel blockers (diltiazem, nicardipine), heparin, nitroglycerine, glybenclamide and alprazolam. Most were smokers. Due to the small sample for each condition, we were unable to establish a correlation of cell yield or drug response with any of these conditions. The age of the 35 patients ranged from 41 to 82 yr old, with a mean of 64.5 ± 7.2 yr. Tissues from younger patients yielded more and better cells with shiny appearance and clear striations. Other patients produced no viable cells or cells with low resting membrane potential and rapid rundown of inward currents.

Cell size varied markedly, capacitance ranged from 63 to 207 pF with a mean of 115.5 ± 6.28 pF (n = 36). The cells were rod-shape, many with irregular branches. About 60% of the cells had low resting potential of −60 to −40 mV, and full size action potentials were difficult to elicit (Amos et al., 1996). The other cells had normal resting membrane potentials of −71.4 ± 2.4 mV (n = 16). At 37°C, brief current stimulation of these cells at 1 Hz elicited full size action potentials with overshoot of 36.8 ± 1.8 mV and duration (APD) of 265 ± 89 msec at 90% repolarization. The resting potential, action potential overshoot and duration remained stable in 30 to 60 min recording time. Figure 1 shows three types of representative action potentials that have different plateau heights. Type 1 had an early plateau and a late plateau phase; type 2 had a late plateau, and type 3, had no plateau (Wang et al., 1993). In all cases, the late repolarization phase was slow. Action potentials were recorded from the 16 cells. Type 1 was obtained from three cells, all from a smoking patient with diabetes and treated with glybenclamide and insulin. Type 2 was from five cells obtained in

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**Fig. 3.** Separation of $I_{Na}$ from $I_{p}$ using 4-aminopyridine (4-AP) and depolarization prepulses. A. The cell was held at −40 mV and step depolarized from −20 to 50 mV, at 10 mV increments. The cell was bathed in 0.2 mM Ca$^{2+}$ Tyrode’s solution with 1 μM nifedipine (solution C). The 4-AP sensitive net current traces were obtained by subtracting the current in 0.1 mM 4-AP from the control. Open symbols (○, □) are in 0.1 mM 4-AP; the dotted line on the I-V curve represents net 4-AP sensitive current, $I_{Na}$. Vertical scale bar = 400 pA; horizontal bar = 100 msec. B. Concentration response curve of $I_{Na}$ (○) and $I_{p}$ (●). The current amplitude was normalized against the peak current amplitude in control; $I_{Na,max}$ peak control current amplitude; $I_{p}$ current amplitude at various 4-AP concentrations as indicated on the abscissa. $IC_{50}$ was obtained by fitting the data to the equation: $a-d/(1+(x/c)^{b})+d$ where $a$ = asymptotic maximum; $b$ = slope; $c$ = $IC_{50}$; $d$ = asymptotic minimum; $x$ = drug concentration. C, prepulses lasting 2 sec from −70 to −10 mV, at 10 mV increments, were used to inactivate $I_{Na}$ with a 5-msec return to −40 mV preceding the 50 mV test pulse. D, Normalized steady-state inactivation curves of $I_{Na}$ (○) and $I_{p}$ (●). $I_{Na,max}$ is the current amplitude at prepulse potential of −70 mV and $I_{p}$ current amplitudes at prepulse potentials positive to −70 mV as indicated on the abscissa. The data of $I_{Na}$ were best-fitted by the Boltzmann function: $1/(1+exp(V_{V_{0.5}})/K)+C$ where $V_{0.5}$ is prepulse potentials; $V_{V_{0.5}}$ potential at 50% of maximal current; $K$, the slope factor; and $C$, the inactivating component. $V_{V_{0.5}} = −70 mV$ and slope = 3.4 at 36°C. For $I_{Na}$, due to incomplete inactivation, the curve can not be fitted properly.
two patients, one with myocardial infarction and coronary artery disease, treated with heparin, diltiazem and the other with hypertension, treated with nitroglycerine. Type 3 was observed in eight cells, all from hypertensive patients on chronic Ca$\text{_{11}}$ channel blockers (diltiazem and nifedipine), nitroglycerine. Due to the small patient sample with multiple diseases and drug treatments, it was not possible to relate the action potential shape with any particular patients or treatment. However, the hypertensive patients treated with Ca$\text{_{11}}$ channel blockers more often developed type 3 action potentials. For unknown reasons, type 1 and 2 action potentials can convert to type 3 spontaneously.

Ibutilide elevates and prolongs the plateau of human atrial action potentials, in the absence of IKr. Type 3 action potential is of special interest, because, according to Wang et al. (1993), it has no IKr. If ibutilide were to prolong APD by blocking this current, then it should not affect type 3 action potentials. Nevertheless, figure 2A shows that ibutilide induced a robust and reversible APD prolongation. The effect is specific, by generating a plateau at $-15$ to $-10$ mV, and this was observed in all experiments of this kind. The action potential result suggests, although indirectly, that in human atrial cells, ibutilide’s class III effect may be independent of IKr activity.

Voltage-clamp experiments of type 3 action potentials, also using the same pipette solution for recording action potentials, failed to detect IKr. Figure 2B illustrates currents elicited by 2-sec long voltage steps in 2 mM 4-AP, 100 nM atropine, 1 µM nifedipine (Wang et al., 1994) and step depolarized from a holding potential of $-50$ mV to various step potentials as indicated for 2 sec long, at 10 mV increments. Ibutilide ($10^{-7}$ M) was applied for 5 to 10 min before data were taken. I-V are current amplitudes measured at the end of the 2-sec step vs. voltage (temperature, 37°C).

![Fig. 4. Effect of ibutilide on outward K$^+$ currents. A, Superimposed current traces on the left were controls and at right, 5 min in 10$^{-7}$ M ibutilide. The cell was held at -40 mV, without (Aa, top panels) and with a 2-sec prepulse to 0 mV (Ab, middle panels). Net I_{net} current (Ac, bottom) was obtained by subtracting Ab from Aa. Currents lasting 150 msec were elicited from -50 to 60 mV at 10 mV increments, and repolarized to -20 mV for 50 msec for detection of tail currents. Aa, total current; Ab, I_{net}; Ac, I_{net}. Open symbols are controls and solid symbols, in ibutilide. Vertical bar = 400 pA. B, Left, I_{net}, measured at 10 and 150 ms into the step before (□, □) and 5 min after (●, ■) 10$^{-7}$ M ibutilide application. Right, I_{net} before (○, ○) and after (▲, ▲) 10$^{-7}$ M ibutilide application. C, Ibutilide has no effect on the IKs-like current. External solution contained 2 mM 4-AP, 100 nM atropine, 1 µM nifedipine (Wang et al., 1994) and step depolarized from a holding potential of $-50$ mV to various step potentials as indicated for 2 sec long, at 10 mV increments. Ibutilide ($10^{-7}$ M) was applied for 5 to 10 min before data were taken. I-V are current amplitudes measured at the end of the 2-sec step vs. voltage (temperature, 37°C).]
Fig. 5. Inward currents and their response to ibutilide. K$^+$-free solutions with TEA and 4-AP were used to remove the K$^+$ currents (see "Methods"). Both Na$^+$ and Ca$^{2+}$ were present in the external solution. Cell was held at −40 mV and depolarized to potentials as indicated. A. Ramp depolarization at 1.2 mV/msec was delivered to the cell at potentials as indicated. Upper current trace is the control and lower trace is in ibutilide. Note: at negative potentials, the fast inward Na$^+$ current failed to appear probably due to slow recovery from inactivation. This was consistent in human atrial cell experiments. B. The time course of current increase at 10 mV in response to 10$^{-7}$ M ibutilide application. Open symbols are control current amplitudes at the peak (○) and at 15 msec into the step (□); solid symbols are in ibutilide. Dotted lines indicate control current level. C. Another cell, inward current elicited by step depolarization. Steps to potentials as indicated were delivered to the cell at 0.5 Hz. Two to four I-V controls were taken to make sure the current was stable, then ibutilide was applied to the cell bath under gravity while the cell was continuously stimulated to a fixed potential of 10 mV at 0.5 Hz. After 2 to 5 min, the same I-V run was performed. Currents were not corrected for leak. Holding potential, −40 mV and temperature, 37°C.
us to conclude that under our experimental conditions, these human atrial cells have undetectable I_{Kr}. Our results agree with earlier reports that I_{Kr} is absent or is too small to be detected in human atrial cells (Amos et al., 1996, Escande et al., 1987, Shibata et al., 1989). We did not systematically examine the IKr issue further because regardless of the action potential shape, or the cell size, I_{Kr} was not routinely observed.

In the absence of 4-AP, a transient outward current and a sustained outward current were always present (fig. 3A). The sustained outward current, I_{so}, can be separated pharmacologically from the transient, I_{to}, by using μM 4-aminopyridine because I_{so} had a IC_{50} of 0.09 mM (○, n = 6) whereas I_{to} had a IC_{50} of 1.7 mM (○, n = 6) (fig. 3B). Similarly, these current can be separated by positive prepulse potentials (fig. 3C). The half steady-state inactivation potential (V_{0.5}) for I_{to} was −30 mV (○, n = 5) whereas I_{so} inactivated only partially (□, n = 5) (fig. 3D). These currents are therefore similar to the I_{to} and I_{so} described earlier (Amos et al., 1996). Furthermore, there was the inward rectifier, I_{K1}, which was very small in human atrial cells (fig. 2B, current trace not shown).

Ibutilide does not inhibit human atrial I_{to}, I_{so}, I_{K1}, and I_{Ks}-like currents at moderate concentrations. To examine if the APD prolongation by ibutilide is caused by K^+
current blockade, we separated $I_{h}$ from $I_{so}$ by using a 2-sec, −10 mV prepulse to inactivate $I_{so}$ (fig. 3C). Also, in a separate set of experiments, we isolated the $I_{Ks}$-like current using the 2 mM 4-AP, 100 nM atropine and 1 μM nifedipine protocol (fig. 2B) (Wang et al., 1994). We then chose the concentration of $10^{-7}$ M that caused a robust APD prolongation (fig. 2A). In control at 20 mV, for example, $I_{to}$, $I_{so}$ and $I_{Ks}$ measured $312 \pm 80$, $219 \pm 60$ and $122.6 \pm 23$ pA, respectively; in $10^{-7}$ M ibutilide, the corresponding values became $395 \pm 60$ (n = 8, P > .05), $214 \pm 75$ (n = 8, P > .05) and $116 \pm 19$ pA (n = 4, P > .05). Figure 4A shows total outward current (upper panels), $I_{so}$ (middle panels) and $I_{h}$ (lower panels) before (left) and after (right) ibutilide application. Figure 4B are I-V plots of $I_{so}$ and $I_{h}$ before and after drug application. Figure 4C further illustrates experiments on the $I_{Ks}$-like current. In the physiological temperature and potential range tested, ibutilide did not inhibit these currents. Finally, we examined ibutilide’s effect ($10^{-7}$ M) on the inward rectifier, $I_{K1}$. Again, the result was negative. For example, at −100 mV, $I_{K1}$ measured $−214 \pm 20$ pA at 2 sec into the depolarization step; in $10^{-7}$ M ibutilide, it became $−188 \pm 18$ pA (n = 8, P > .05). These results demonstrate that ibutilide, at a concentration that prolongs APD (fig. 2A), produces no apparent changes on these $K^{+}$ currents. At higher concentration of $10^{-6}$ M and above, ibutilide blocked $I_{so}$ dose dependently (data not shown). However, the high concentration effect can not explain ibutilide’s APD prolongation seen at $10^{-7}$ M. This point will become more apparent later in this report.

**Inward current increased by ibutilide.** We then examined the inward currents at the plateau potential region, hereafter referred to as the inward current. The current was recorded by bathing the cell in $K^{+}$-free solutions containing $K^{+}$ channel blockers (solutions E and E1, see “Methods”). In addition, holding potential was at −40 mV throughout to inactivate the fast inward $Na^{+}$ current and the T-type $Ca^{2+}$ current. Because stable inward currents were critical for this study, we carefully selected cells that had less than 5% “run-down” in the first 10 min of recording time. In a typical good experiment, the inward currents remained stable for 30 min or longer. Figure 5A shows the inward current elicited by a ramp depolarization. Application of $10^{-7}$ M ibutilide caused the peak current to increase significantly over the plateau potential range. Figure 5B is the time course of current increase in response to ibutilide application. In most cells, the onset of drug effect was slow, requiring 10 to 15 min, and took 15 to 20 min to washout. Figure 5C illustrates inward currents elicited by depolarization steps in the absence and presence of ibutilide. At 20 mV depolarization, for example, the peak inward current was increased from $−1478 \pm 103$ to $−2347 \pm 75$ pA (n = 12, P < .05). The sustained inward current, measured at 50 msec into the step, was increased from $−296 \pm 56$ to $−425 \pm 87$ pA (n = 12, P < .05). At the physiological temperature, ibutilide increased both the peak and the sustained inward current. The sustained effect was most evident at −10 mV (fig. 5C). In addition, ibutilide slowed the time course of the inward current inactivation. Double exponential fit to the inactivation time course showed that the average fast time constant at −10 mV was increased from a control of 3.2 ± 0.5 msec to 4.7 ± 0.3, 5.2 ± 0.24 and 4.9 ± 0.2 msec in $10^{-9}$, $10^{-8}$ and $10^{-7}$ M ibutilide, respectively; the average slow time constant was also increased from a control of 26 ± 5 msec to 30 ± 8, 44 ± 4 and 41 ± 3 msec, respectively (n = 12). Qualitatively, the human atrial
cell result is similar to the guinea pig ventricular cells at room temperature (Lee, 1992).

Ibutilide concentration dependently increases the inward current, effective at $10^{-2}$ M. Figure 6 illustrates a continuous response of the inward current of a cell to sequential ibutilide application from $10^{-2}$ to $10^{-6}$ M, then back to $10^{-8}$ M before washing out the drug. Figure 6A shows peak inward current increase with time in response to cumulative drug application. The experiment lasted for about 75 min until it was terminated after a long washout. The increase reached a peak at $10^{-6}$ M. However, subsequent lowering of the drug concentration to $10^{-8}$ M increased the current further. The drug was difficult to washout, requiring 15 to 20 min. Most cells did not last that long. Figure 6B are superimposed I-V curves of inward current obtained from the same cell. Both the peak inward current (left) and the late inward current (right) measured at 50 msec into the step are shown. In the presence of external Na$^{+}$, the inward current, especially the late component, consistently has a more positive I-V relationship than the "L" type Ca$^{2+}$ current recorded in Na$^{+}$-free external solution. The positive shift is also obvious at slower rate of depolarization as illustrated by the ramp experiment (fig. 5A).

Figure 7A summarizes peak inward current densities at increasing concentrations of the drug. By expressing the increase as a percent of control peak current, and by fitting the data to the Hill equation, we obtained half maximal effective concentrations, $K_{d}$, of 0.1, 0.26, 0.65, 0.22, 0.69 and 0.9 nM at $-10$, 0, 10, 20, 30 and 40 mV, respectively. The $K_{d}$s plotted against step potentials (fig. 7B) are below $10^{-9}$ M in the plateau potential range. Curve-fit of the $K_{d}$s to the Woodhull equation indicates that the drug effect is slightly voltage dependent, at potentials negative to 10 mV.

The inward current and APD prolongation have similar concentration response curves. Although ibutilide is highly effective on the inward current at very low concentrations, there is no evidence yet that this effect can directly contribute to APD prolongation. We therefore examined ibutilide's effect on APD at similarly low concentrations. Figure 8 (left panels) illustrates that ibutilide, at these low concentrations, elevated the plateau to prolong APD. For example, at 70 and 90% repolarization, the controls were $75 \pm 9.8$ and $223 \pm 53$ msec, respectively; in $10^{-8}$ M ibutilide, the corresponding values became $117 \pm 19$ msec (56% increase) and $263 \pm 47$ msec (18% increase) ($n = 4$). Thus, the greatest effect appeared at the plateau phase. Curve-fit anal-
Identity of the ibutilide sensitive inward current. In human atrial cells, the ibutilide sensitive inward current resembles the L-type Ca\(^{2+}\) current. In our earlier study on guinea pig ventricular cells (Lee, 1992), it was suspected to be a new type of inward current because removal of external Na\(^{+}\) removes this drug induced inward current. However, subsequent experiments indicate that it is blocked by nifedipine. In human atrial cells, similar observations were made consistently. Figure 11 is a good example, demonstrating, on the same cell, that the drug induced Na\(^{+}\) current is through a nifedipine inhibited channel. By using this protocol, figure 11 highlights the importance of Na\(^{+}\) for ibutilide’s effect. Four other cells showed similar response. By pooling data from different cells, in Na-free solution, the peak inward current amplitude was \(-1244 \pm 65\) pA; and in ibutilide (10\(^{-7}\) M), it remained relatively unchanged at \(-1302 \pm 108\) pA (n = 8, P < .05). In full-Na solution, peak inward current amplitude was slightly larger, at \(-1344 \pm 98\) pA (n = 17); and in ibutilide (10\(^{-7}\) M), the current increased to \(-2173 \pm 116\) pA (n = 26, P < .05). TTX, of up to 10 \(\mu\)M, did not affect the currents (data not shown).

Perhaps the critical link between the inward current mechanism and APD prolongation is established by data shown in figure 12. Here, nifedipine, at 10\(^{-6}\) M, almost completely eliminated ibutilide-induced plateau, without affecting either the early or late repolarization. Four other cells showed similar results. These data imply that, under normal physiological conditions, ibutilide promotes Na\(^{+}\) influx through a nifedipine sensitive inward channel that causes APD prolongation in human atrium.

Discussion

The main body of knowledge concerning ionic mechanisms of class III antiarrhythmic compounds is derived from animal heart cells. Few studies (Wang et al., 1995) have systematically examined the action of clinically useful drugs on human heart cells. One reason may be the low accessibility to viable human heart tissues that yield cells suitable for electrophysiological experiments. An alternative approach has been the cloning of channels from human heart libraries that has yielded valuable information (Kiehn et al., 1995). However, the physiological and pharmacological properties of these cloned ion channels have to be carefully validated against native human heart ion channels which are not readily available. In this study, we have examined ibutilide’s effect on the K\(^{+}\) currents and plateau inward currents in freshly isolated human atrial cells. At low concentrations less than 10\(^{-6}\) M,
Ibutilide is ineffective on the K$^{+}$ currents. Instead, it specifically increases a plateau inward current with half maximal concentration ($K_d$) of about 0.1 nM at -10 mV. The $K_d$ for the inward current matches with the $K_d$ for APD prolongation. In most cells, however, the drug induced plateau appears below or at the inward current threshold of -20 mV, probably due to large I_{Kr}. However, in other cells bathed in high concentration of ibutilide, a large plateau near 0 mV could be observed (fig. 12) which was removed by nifedipine. The agreement suggests that a nifedipine sensitive inward current is mainly responsible for mediating ibutilide's class III antiarrhythmic action, although contribution by I_{Kr} cannot be ruled out at present. The other finding of this study is the ability of ibutilide to reduce the amount of inward current inactivation. This could help maintain the drug effect at high heart rates.

**Human atrial cells have variable action potentials shapes.** Internally dialyzed cells with resting potentials of
–70 mV generate full size action potentials in response to brief positive current stimulations. Using our internal solution, the action potential and its duration remained relatively stable. They resemble that of the intact tissues recorded by the microelectrode technique (Gelband et al., 1972). Wang et al. (1993) reported that the shape of human atrial action potentials can vary quite extensively from triangular shape to that with a full plateau. The triangular shape is the majority and may be consequential to the patient’s chronic use of Ca\textsuperscript{2+} channel blockers. A study of isolated human atrial cells by Grand et al. (1990) has documented that chronic use of nifedipine, nicardipine or diltiazem could triangulate the action potential as a result of Ca\textsuperscript{2+} channel blockade. In agreement, about half the cells with triangular action potentials and small inward current we examined were from patients treated with Ca\textsuperscript{2+} channel blockers.

In addition to Ca\textsuperscript{2+} currents, it is also possible that the shape may be determined by delayed rectifiers. Absence of delayed rectifiers may cause triangulation (Wang et al., 1993). Our inability to detect I\textsubscript{Kr} current support this contention. We think this is not due to the dialysis technique because on guinea pig atrial cells, the same technique consistently recorded I\textsubscript{Kr}. More likely, its absence could be a result of human cardiac diseases. Method of isolation could be another important factor. Li et al. (1996) suggested that I\textsubscript{Kr} was better preserved if whole heart enzymatic perfusion was used as oppose to minced tissue enzymatic digestion that we used in this study. Until I\textsubscript{Kr} can be reliably recorded from these human atrial cells, its physiological and pharmacological role can not be determined. As for the other human atrial K\textsuperscript{+} currents, I\textsubscript{to} and I\textsubscript{so}, ibutilide has insignificant effect at therapeutic concentrations. Thus, ibutilide differs from flecainide, quinidine and 4-aminopyridine that block I\textsubscript{to} at micromolar concentrations; the latter two also blocked I\textsubscript{so} at similar concentrations (Wang et al., 1995).

Potent effect of ibutilide on a plateau inward current. In contrast to the K\textsuperscript{+} currents, the inward current recorded in Na\textsuperscript{+} and Ca\textsuperscript{2+} solutions is sensitive to subnano-

Fig. 11. External Na\textsuperscript{+} removal and nifedipine effect on the inward currents. Upper panels are superimposed current traces obtained under conditions as indicated. Currents were elicited every 15 sec by a 150-msec step to 10 mV from a holding potential of –40 mV. Below are peak inward current amplitudes plotted against time under conditions as indicated. Full-Na is the E1 solution; Na-free is the E2 solution (see “Methods”). In some cells, choline-Cl was used instead, but the results remained similar. The current amplitude of this cell is smaller than the average. Symbols represent current traces as shown. In most cells, external Na\textsuperscript{+} removal shifts the holding current outward. This shift occurs rapidly and its I-V relationship is linear and time-independent. Hence, although not done here, it can be subtracted from the voltage and time-dependent inward current by using a –10 mV leak current scaled up to the corresponding step potential (temperature, 36°C).
molar ibutilide. To rule out that the drug effect is not contaminated by the short “run-up” followed by “run-down” commonly observed in internally dialyzed cells, we applied the drug only after the “run-up” was completed. In fact, most drug application took place during the “run-down” phase. The ability of ibutilide to reduce inward current inactivation is interesting. This may have the net effect for preserving the drug effect on fast pacing, and potentially more arrhythmogenic tissues. By comparison, agents that increase inactivation, such as Bay K-8644 (Sanguinetti et al., 1986) would be less effective on fast pacing cells, but more effective on slower pacing, normal cells. Such agent with “reverse use-dependence” may be inherently more proarrhythmic.

In about 20% of the cells, the inward current was either small (less than 5 pA/pF) or was unstable, and ibutilide failed to increase the current. These cells may have been damaged by the isolation procedures or were from patients with various heart diseases such as those with low ejection fractions. According to a recent paper by Piot et al. (1996), the L-type Ca\(^{2+}\) current from such cardiac patients is small and failed to potentiate by repetitive depolarization, a widespread phenomenon that was originally described by Lee (1987) in isolated, dialyzed heart cells. The same patient conditions may reduce or alter the inward currents and may explain why ibutilide’s effect is highly variable on cells with small or unstable currents.

The potency of ibutilide on both the inward current and the action potential is very high. The half maximal activation concentrations (\(K_D\)) for the inward current is between 0.1 and 0.9 nM. This agrees well with the \(K_D\) for action potential that has \(K_D\) of 0.23 to 0.7 nM. Its high potency is seen in clinical situations where i.v. administration of 0.015 mg/kg is fully effective for terminating human atrial arrhythmia (Ellenbogen et al., 1996). However, since this study represents only a subset of cells isolated from patients over a 9-mo period, its clinical significance is uncertain at this time.

**Ionic identity of the Na\(^+\)-sensitive inward current.** In guinea pig ventricular cells, we have shown that in the presence of ibutilide, the inward current consists of two components: a “L” type Ca\(^{2+}\) current and a Na\(^+\)-sensitive component (Lee, 1992). Our data on human resemble that of the guinea pig. However, over the years, we have not been successful in separating it from the L-type Ca\(^{2+}\) channel because application of nifedipine would remove the drug effect, on both the inward current (fig. 11) and APD prolongation (fig. 12). Furthermore, neither the current-voltage relationship nor the steady-state inactivation differs substantially from that of the L-type channel. The logical interpretation of our result would be that ibutilide promotes Na\(^+\) permeability through the L-type Ca\(^{2+}\) channels that are normally impermeable to this ion (Lee and Tsien, 1984). While a drug induced change in Na\(^+\) permeability through
the L-type Ca\(^{2+}\) channel has not been reported previously, it is well established that such changes can be brought about simply by lowering external Ca\(^{2+}\) (Hess and Tsien, 1984), thereby reducing occupancy of the high affinity Ca\(^{2+}\) binding sites by Ca\(^{2+}\), permitting monovalent cations to bind and permeate the channel. Subsequent site-directed mutagenesis indicates that the L-type Ca\(^{2+}\) channel selectivity is determined by four conserved glutamate residues in equivalent positions in the pore lining regions of repeats I-IV in the Ca\(^{2+}\) channel α\(_1\) subunit (Yang et al., 1993). Neutralization of the glutamic acid sites in repeats II and IV by substitution with alanine or glutamine resulted in 10-fold reduction in affinity of the channel for Ca\(^{2+}\) (Yantani et al., 1994) allowing Na\(^{+}\) to permeate the channel. Interestingly, at neutral pH, ibutilide is positively charged. It is possible that the drug may alter the polar fields of the glutamate residues, thereby modifying channel selectivity in due process.

In conclusion, we have shown that in human atrial cells, ibutilide is a potent activator of an inward Na\(^{+}\) current, possibly, through the L-type Ca\(^{2+}\) channel. The drug’s similar potency on the inward current and the action potential prompts us to suggest that this current, at least in part, is responsible for the class III antiarrhythmic actions seen in human atrium (Ellenbogen et al., 1996).

**Acknowledgments**

The authors thank Michael P. Halpin, M.D., Mark L. Marbury, M.D. and other surgical staffs of Bronson Methodist Hospital, Kalamazoo, MI for their generous support and supply of human atrial tissues; Lucy R. Sun for her experiments in the initial phase of this study and, Xiao-Dong Sun, Ph.D. for his experiments on action potentials in ibutilide and nifedipine.

**References**


