Effects of Ritanserin on Transmembrane Action Potentials in Canine Purkinje Fibers

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Abstract. **Ritanserin has been reported to be a potential antiarrhythmic. We studied the cellular electrophysiologic effects of ritanserin in canine Purkinje fibers. Ritanserin produced significant depressant effects on transmembrane action potentials elicited in canine Purkinje fibers. At concentrations of 10 and 40 mg/liter, ritanserin decreased $\dot{V}_{max}$ (the upstroke velocity) of action potential in a dose-dependent fashion and shortened the duration of fast response action potential. These concentrations of ritanserin also reduced the amplitude and duration of the slow response action potentials induced in Purkinje fibers treated with isoproterenol (10^{-5} *M*) and high K^+ (22 m*M*). These *in vitro* results suggest that the cellular electrophysiologic actions of ritanserin may be due to its direct actions on cardiac sodium and calcium channels, which, in turn, may account for its antiarrhythmic effects.** [P.S.E.B.M. 1992, Vol 199]

Ritanserin is a 5-hydroxytryptamine (5-HT_2) receptor antagonist similar to ketanserin. Unlike ketanserin, it is devoid of α_1-adrenergic receptor blocking properties (1). Ritanserin did not produce any significant effect on systemic blood pressure in either hypertensive patients (2) or animals (3), although it reduced portal pressure in cirrhotic dogs with portal hypertension (4). Recently, ritanserin was shown to be effective in suppressing ventricular ectopies and fibrillation induced by coronary artery ligation, with or without subsequent reperfusion, in the rat (5, 6). In order to explore the cardiac electrophysiologic effects and underlying basis for this antiarrhythmic action, we assessed the effects of ritanserin on the fast and slow response action potentials elicited in canine Purkinje fibers.

Materials and Methods

Mongrel dogs were anesthetized with sodium pentobarbital (30–35 mg/kg, iv). The heart was rapidly removed through a right thoracotomy and placed in a cool, oxygenated Tyrode's solution containing (in m*M*): NaCl, 137; $NaHCO_3$, 18; dextrose, 5.5; NaH_2PO_4, 1.8; $MgCl_2$, 0.5; $CaCl_2$, 2.7; and KCl, 4.0. Free-running Purkinje fibers were excised and mounted in a Lucite chamber (vol = 4 ml), paced with a bipolar electrode at 400- and 1000-msec cycle lengths, and superfused by warmed Tyrode's solution (37° ± 0.5°C) at a flow rate of 10 ml/min. The Tyrode's solution was gassed with 95% O_2 and 5% CO_2 and had a pH of 7.4.

Transmembrane action potentials were recorded through glass microelectrodes filled with 3 *M* KCl having resistance of 10–30 megohm. The microelectrodes were connected through Ag-AgCl junctions to amplifiers with a high-input resistance and capacity neutralization. An operational amplifier was used to obtain the first-time derivative of Phase 0 or the maximum rate of rise of the upstroke of the action potential, $\dot{V}_{max}$. Sawtooth signals (100–1000 V/sec) with 100-mV amplitude were injected between the tissue chamber and the ground to permit calibration of the $\dot{V}_{max}$. Both action potential and $\dot{V}_{max}$ traces were displayed on a Tektronix 5113 oscilloscope. The methods used to record and measure action potential amplitude, resting membrane potential, $\dot{V}_{max}$, and action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization have been described previously (7, 8). For the study of slow response action potentials, the tissue was driven by field stimulation at a cycle length of 2000 msec in the Tyrode's solution, containing 5.4–8.1 m*M* $CaCl_2$,

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22 mM KCl, 10^{-5} M isoproterenol, and 5×10^{-5} M EDTA (9). Following a period of 20 min of accommodation for the impalement to stabilize, ritanserin (10 mg/ml; Janssen Pharmaceutica, Belgium) was added to the reservoir of the Tyrode's solution to give the final concentrations of 10 and 40 mg/liter. The displayed action potentials were photographed on Polaroid films during the predrug control period and at 20–30 min after wash-in of the drug. No more than two Purkinje fibers were used from the same animal. Data obtained from single impalements that were maintained through both the control and drug exposure periods were used for analysis.

A paired t test with Bonferroni adjustment (10) was used to compare the effect among treatments or between treatments and predrug controls.

Results

Action potential recording in canine Purkinje fibers that can be studied for a period of 4 hr has been documented previously (11–14). Sham recordings over the same study period remained stable and essentially unchanged.

At concentrations of 10 and 40 mg/liter, ritanserin significantly altered the configuration of Purkinje action potentials (Fig. 1). It decreased the upstroke and amplitude of the action potential and shortened the APD_{50} in a dose-related fashion (Table I, Fig. 1). It also reduced the plateau height of the action potential. At 40 mg/liter, the $\dot{V}_{max}$ at 400-msec pacing cycle length was significantly lower than that at 1000 msec ($P < 0.05$) and thus was considered to be use dependent. The effects of ritanserin on APD_{90} were more variable; eleven of the 20 fibers studied (11/20) showed prolongation, six showed shortening, and the other three were unchanged. At a pacing cycle length of 400 msec, the resting membrane potential was reduced by both concentrations of ritanserin, whereas at the 1000-msec cycle length, the resting membrane potential was decreased only at the high dose (40 mg/liter) (Table I). The effects of ritanserin persisted following a washout period of 90–120 min (Fig. 1).

The effect of ritanserin on slow response action potential was also investigated to study the role of inward calcium current on the lowering of action potential plateau and the shortening of action potential duration. At a pacing cycle of 2000 msec, ritanserin significantly decreased the slow response action potential amplitude and duration (APD_{50}) at the concentration of 10 mg/liter (Table II). APD_{90} tended to decrease at this concentration, but did not reach the level of significance. At 40 mg/liter, the amplitude, duration, and resting membrane potential of the slow response action potentials were significantly depressed, as compared with the control ($P < 0.05$) (Table II, Fig. 2).

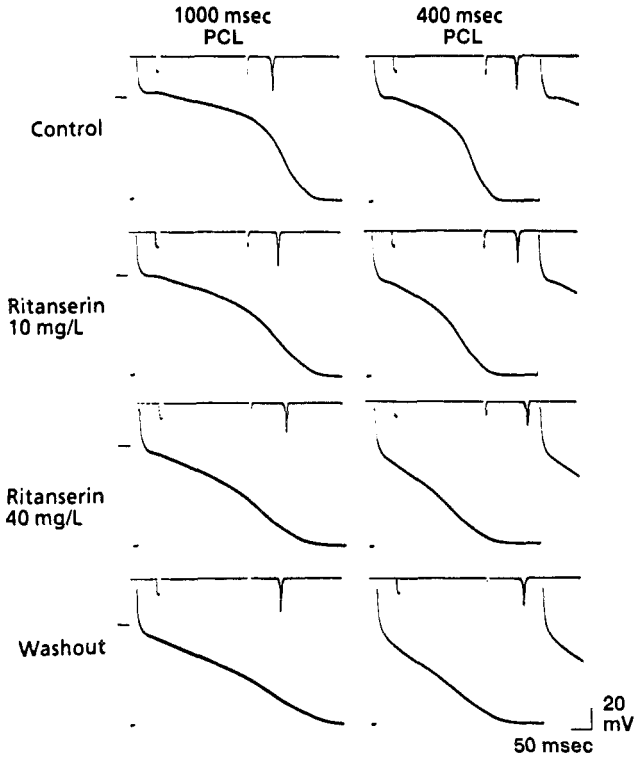

Figure 1. Effect of ritanserin on transmembrane action potentials recorded from canine Purkinje fiber. Records arrayed in the left-hand column are at 1000-msec pacing cycle length (PCL), whereas those in the right are at 400-msec PCL. Zero membrane potential levels (0 mV) are indicated by the horizontal bars in the left margin. The $\dot{V}_{max}$ trace on the top of each panel is preceded by a calibration of 400-V/sec sawtooth reference. Records were taken at 30 min following each concentration of ritanserin and 100 min into washout. Time base and voltage calibrations for action potentials appear at the lower right (vertical bar, 20 mV; horizontal bar, 50 msec).

Discussion

Our studies revealed that ritanserin produces significant alteration in the configuration of the transmembrane action potential in canine Purkinje fibers. It involves depression of the upstrokes and plateau phases of the action potential and shortening of action potential duration. At a similar concentration range, the ritanserin analog, ketanserin, was reported to produce similar effects on the action potential in the canine Purkinje fiber (15). The depressant effects of ritanserin on $\dot{V}_{max}$ could result in a slowing in conduction and suggests that it has sodium channel blocking properties. This observation is in agreement with the previous report that ritanserin, at concentrations ($1–3 \times 10^{-5}$ M) comparable to those used in the present study, reduces the maximal driving frequency of isolated rat atria and ventricular muscles (5).

The effects of ritanserin are not readily reversible during a washout period of 2 hr. Several factors may account for this phenomena: (i) the site(s) of action may be located inside of the cell membrane (e.g., activation and inactivation gates of sodium and calcium channels); or (ii) the drug may avidly bind to the

Table I. Effects of Ritanserin on Transmembrane Action Potentials in Canine Purkinje Fibers[a]

Treatment	PCL (msec)	AMP (mV)	RMP (mV)	APD_{90} (msec)	APD_{50} (msec)	$\dot{V}_{max}$ (V/sec)
Control	1000	125 ± 7	−92 ± 3	314 ± 64	218 ± 40	491 ± 160
Ritanserin (10 mg/liter)	1000	123 ± 9[b]	−91 ± 3	315 ± 72	179 ± 38[b]	429 ± 160[b]
Ritanserin (40 mg/liter)	1000	114 ± 12[b]	−87 ± 5[b]	301 ± 73[c]	126 ± 30[b,c]	337 ± 166[b,c]
Control	400	127 ± 8	−93 ± 3	243 ± 25	176 ± 32	497 ± 160
Ritanserin (10 mg/liter)	400	123 ± 11[b]	−91 ± 3[b]	253 ± 19[b]	138 ± 18[b]	417 ± 177[b]
Ritanserin (40 mg/liter)	400	111 ± 17[b]	−87 ± 6[b]	239 ± 22[c]	96 ± 22[b,c]	308 ± 160[b,c]

[a] Values are mean ± SD (n = 20). Abbreviations used in this table: PCL, pacing cycle length; AMP, action potential amplitude; RMP, resting membrane potential.
[b] Significantly different from the control of the same PCL and treatment group ($P < 0.05$).
[c] Significantly different from 10-mg/liter treatment group at the same PCL ($P < 0.05$).

Table II. Effects of Ritanserin on Slow Response Action Potentials at a Pacing Cycle Length of 2000 msec in Canine Purkinje Fibers[a]

Treatment	AMP (mV)	RMP (mV)	APD_{90} (msec)	APD_{50} (msec)
Control	68 ± 7	−49 ± 3	161 ± 16	116 ± 17
Ritanserin (10 mg/liter)	62 ± 11[b]	−50 ± 5	153 ± 11	113 ± 18[b]
Ritanserin (40 mg/liter)	38 ± 17[b]	−45 ± 4[b]	122 ± 23[b,c]	72 ± 16[b]

[a] Values are mean ± SD (n = 12). Abbreviations used in this table: AMP, action potential amplitude; RMP, resting membrane potential.
[b] Significantly different from the control of the same PCL and treatment group ($P < 0.05$).
[c] Significantly different from 10-mg/liter treatment group.

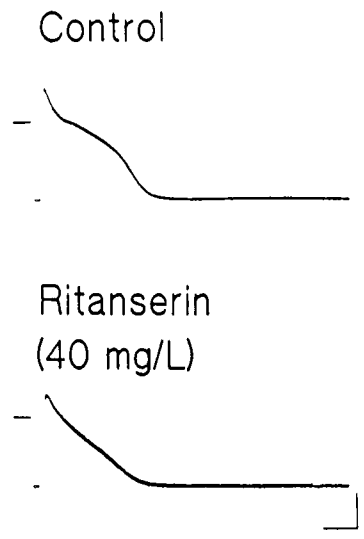

Figure 2. Effect of ritanserin on slow response action potentials elicited in canine Purkinje fiber. The tissue was driven by field stimulation at 2000-msec cycle length. Zero membrane potential levels (0 mV) are indicated by the horizontal bars in the left margin. Time base and voltage calibrations for action potentials appear at the lower right (vertical bar, 20 mV; horizontal bar, 50 msec).

channels and consequently become harder to wash out. This property of ritanserin seems to agree with its long duration of action in the *in vivo* system (16). The effects of antiarrhythmic agents bretylium (11) and clofilium (13) have also been shown to be unable to be washed out for a period of 4 hr under similar recording conditions in canine Purkinje fibers.

The action potential shortening effects of ritanserin in Purkinje fibers may be associated with the inhibition of inward sodium "window current" (17), calcium currents, or other plateau currents (18). Partially depolarized Purkinje fibers under the conditions of high K^+ supplemented with Ca^{2+} and isoproterenol were used to examine the effects of ritanserin on calcium-mediated action potentials through the slow channels. Under this partially depolarized state, slow inward calcium currents and the calcium channels are operative (19) and capable of blockade by calcium antagonists (19). In the present study, we showed that the calcium channel-mediated slow response action potentials were significantly suppressed by ritanserin. Thus, under our experimental conditions, ritanserin may act through inhibiting both sodium and calcium channels, thereby producing the profound action potential shortening response. Interestingly, ritanserin chemically shares a bis(4-fluorophenyl)piperidine structure with the novel calcium channel blocker AHR-5360 (9). This structural similarity may be linked to ritanserin's calcium channel-blocking properties.

The electrophysiologic action of ritanserin could be due to 5-HT_2 receptor antagonism and direct effects of ritanserin on the membrane channels. The existence of 5-HT_2 receptors has been demonstrated in the atrial and ventricular myocardium, in addition to the coronary vasculature (20–23). Since our results were obtained from isolated tissue without the presence of 5-HT, the depressant effects of ritanserin on Purkinje fibers are most likely due to direct actions on sodium and calcium channels, which, in turn, may account for

the antiarrhythmic effects of ritanserin observed previously in the rat (5, 6).

The concentration of ritanserin employed here is much higher than those shown to block 5-HT_2 receptors using isolated rat and rabbit vascular tissue, guinea pig trachea, or guinea pig ileum (24). This would suggest that interference with cardiac function by ritanserin is unlikely at doses that produce effective serotonergic blockade. However, in light of other evidence, ritanserin's effect on cardiac electrophysiology cannot be dismissed solely on the basis of this concentration difference. In contrast to the competitive nature of 5-HT_2 receptor blockade by ketanserin, ritanserin showed noncompetitive antagonism in the smooth muscle vasculature with a dose-related depression of the maximal response (24). A direct membrane effect by ritanserin has been suggested as the basis for its noncompetitive antagonistic action (24). Furthermore, the ritanserin-induced vascular contractions were resistant to washout, whereas the effect by ketanserin was readily reversible (24). The persistent action of ritanserin was also evident in our study and discussed above. The fact that ritanserin slowly dissociates from 5-HT_2 receptor sites and is not completely displaced from its binding by ketanserin (1) further suggests that accumulation of ritanserin in the cardiac tissue, resulting from slow dissociation, may yield drug concentrations capable of modifying cardiac action potential properties.

Ritanserin has been given to rats at doses up to 160 mg/kg (*in vivo* concentration is at approximately 10^{-6} *M*) to examine receptor binding and biogenic amine levels in the brain without resulting in any significant toxicities (1). This suggests that the ritanserin concentrations employed in this study are relevant and attainable *in vivo* without toxicity (LD_{50} in rats: 515–956 mg/kg, by mouth) in intact animals (16). Thus, while the implication of our findings for ritanserin could be considered toxicological, the data also provide useful insight into the understanding of cardiac electrophysiologic properties of this novel 5-HT_2 receptor antagonist. Furthermore, as ketanserin is emerging as a potential antiarrhythmic agent (15, 22), the present studies on ritanserin lend additional support to the notion that ketanserin's α_1-blocking properties play little role in its direct electrophysiologic effects on the heart.

We have shown that ritanserin, at the concentration range of 10–40 mg/liter, shortens action potential duration and depresses $\dot{V}_{max}$ in canine Purkinje fibers. While it is evident that other ion channels may also be involved, the action on sodium and calcium channels may contribute significantly to ritanserin's effects on the configuration of action potential.

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