Modification of Cardiac Na\(^+\) Current by RWJ 24517 and Its Enantiomers in Guinea Pig Ventricular Myocytes

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ABSTRACT

We examined the effects of the cardiotonic agent RWJ 24517 (Crasatrin, racemate) and its (S)- and (R)-enantiomers on action potential duration, Na\(^+\) current (I\(_{Na}\)), and delayed rectifier K\(^+\) current (I\(_{K}\)) of guinea pig ventricular myocytes. RWJ 24517 (0.1 and 1 μM) prolongation of action potential duration could not be accounted for by suppression of either the (R) or slow I\(_{Na}\) component of I\(_{Ca}\), although RWJ 24517 did reduce I\(_{K}\) at concentrations of 1 μM. A more dramatic effect of RWJ 24517 (0.1-1 μM) and the (S)-enantiomer of RW 24517 (0.1-3 μM) was an increase in peak I\(_{Na}\) and slowing of the rate of I\(_{Na}\) decay, eliciting a large steady-state current. Neither RWJ 24517 nor the (S)-enantiomer affected the fast time constant for I\(_{Na}\) decay, but both significantly increased the slow time constant, in addition to increasing the proportion of I\(_{Na}\) decaying at the slow rate. Both agents elicited a use-dependent decrease of peak I\(_{Na}\) (3-10 μM), which probably resulted from a slowing of both fast and slow rates of recovery from inactivation. In contrast, the (R)-enantiomer of RWJ 24517 did not induce a steady-state component I\(_{Na}\) or increase peak I\(_{Na}\) up to 10 μM, but it decreased peak I\(_{Na}\) at 30 μM. The (R)-enantiomer displayed little use-dependent reduction of I\(_{Na}\) during trains of repetitive pulses and had no effect on rates of inactivation or recovery from inactivation. These actions of the racemate and the (S)-stereoisomer to slow inactivation and to prolong both Na\(^+\) influx and action potential duration may contribute to the positive inotropic actions of these agents because the resulting accumulation of intracellular Na\(^+\) would increase intracellular Ca\(^{2+}\) via Na\(^+\)/Ca\(^{2+}\) exchange.

In recent years, a variety of inotropic agents have been developed that alter the kinetics of Na\(^+\) current inactivation, thus increasing the amount and rate of Na\(^+\) influx. The resulting prolongation of the action potential (AP) duration (APD) produces both a positive inotropic effect and a potential class III antiarrhythmic action. One of the most widely studied of these agents, DPI 201-106 (DPI; 4-[3-(4-benzhydryl-1-piperazinyl)-2-hydroxypropoxy]-1H-indole-2-carbonitrile), has been shown to prolong the APD (Buggisch et al., 1988) and slow the inactivation of the Na\(^+\) current (I\(_{Na}\); Kohlhardt et al., 1986, 1987; Wang et al., 1990). The inotropic action of DPI has been suggested to result from an increased accumulation of intracellular Na\(^+\), leading to an increase in intracellular Ca\(^{2+}\) through the Na\(^+\)/Ca\(^{2+}\) exchanger (Kohlhardt et al., 1986; Romey et al., 1987; Gwathmey et al., 1988).

DPI has been shown to exert stereospecific effects on cardiac I\(_{Na}\) (Romey et al., 1987; Wang et al., 1990). At low concentrations (≤3 μM), (S)-DPI increased peak I\(_{Na}\) and produced a marked slowing of I\(_{Na}\) decay, resulting in a large steady-state current. The (R)-enantiomer blocked I\(_{Na}\) and had little effect on I\(_{Na}\) decay. The racemic compound exerted similar effects to (S)-DPI, except that at higher concentrations (>10 μM), the racemate blocked I\(_{Na}\). In addition to modifying cardiac I\(_{Na}\), DPI has been shown to block cardiac L-type calcium current (I\(_{Ca}\); Holek and Osterrieder, 1988; Siegel et al., 1988; Ravens et al., 1991), inward rectifying K\(^+\) currents (I\(_{K1}\)), and delayed rectifier K\(^+\) currents (I\(_{K}\); Amos and Ravens, 1994). Unlike the effects on I\(_{Na}\), however, the blocking effects of DPI on I\(_{Ca}\) were not stereoselective (Siegell et al., 1988; Ravens et al., 1991).

ABBREVIATIONS: AP, action potential; APD, action potential duration; DPI 201-106, DPI; 4-[3-(4-benzhydryl-1-piperazinyl)-2-hydroxypropoxy]-1H-indole-2-carbonitrile; RWJ 24517, Crasatrin, 6-[1-[1-bis(4-fluorophenyl)methyl]piperazin-4-yl]-2-hydroxy-3-propanylthiopurine; (S)-RWJ, (S)-enantiomer of RWJ 24517; (R)-RWJ, (R)-enantiomer of RWJ 24517; I-V, current-voltage; I\(_{Na}\), sodium current; t\(_{f}\), fast time constant; t\(_{s}\), slow time constant; STX, saxitoxin; I\(_{Ca}\), L-type calcium; I\(_{K}\), delayed rectifier K\(^+\) current.
RWJ 24517 (Carsatrin; 6-[1-[1-bis(4-fluorophenyl)ethyl]-piperazin-4-yl]-2-hydroxy-3-propanylthio]purine) is a new positive inotropic agent that increases twitch tension and prolongs the APD of ventricular muscle without affecting the Na+,K+-ATPase, adenylyl cyclase, phosphodiesterase isozymes, or cardiac myofilaments (Salata et al., 1991; Press et al., 1992; Kawamura et al., 1993). Its positive inotropic effect can be prevented by tetrodotoxin but not by the adrenergic antagonists timolol, yohimbine, or prazosin (Salata et al., 1991). RWJ 24517 is structurally related to DPI (Fig. 1), and it has been postulated that RWJ 24517 acts on INa similarly to DPI (Salata et al., 1991; Press et al., 1992; Krafte et al., 1994). However, it has recently been proposed that the primary method by which RWJ 24517 prolongs APD occurs via an action to block the delayed rectifier K+ current IK and Ca2+-activated K+ channel based on changes in AP waveform (Kawamura et al., 1993). No comprehensive study has been conducted to examine the effects of RWJ 24517 on cardiac ion channels.

In the present study, we examined the effects of RWJ 24517 on APD, IK, and INa of guinea pig ventricular myocytes. We further conducted a detailed examination of RWJ 24517 and its (S)- and (R)-enantiomers [(S)- and (R)-RWJ, respectively] on INa and compared them with the effects of DPI. In particular, we focused on their effects to alter peak amplitude, induce use-dependent changes in peak amplitude, promote a slowly inactivating current, induce use-dependent changes in peak amplitude, and influence the rates of development of and recovery from inactivation.

### Materials and Methods

**AP and Potassium Current Measurements.** Guinea pig ventricular myocytes were isolated by collagenase perfusion of the coronary arteries with a Langendorff apparatus. Action potential recording and voltage-clamp techniques were similar to those in previous studies (Sanguinetti and Jurkiewicz, 1990). Microelectrodes were made from square bore (1.0 mm o.d.) borosilicate capillary tubing (Glass Co. of America, Bargaintown, NJ). Electrodes were filled with 0.5 M K+ gluconate, 25 mM KCl, and 5 mM K2ATP and had resistances of 3 to 7 MΩ (average, 5.5 ± 0.3 MΩ). A List EPC-7 amplifier was used in the voltage-clamp or current-clamp mode to record currents or APs, respectively, in the isolated cells. Series resistance was compensated 40 to 70%, and current was low pass filtered at a cut-off frequency of 1 kHz.

APs were elicited using 2-ms current pulses at a stimulus frequency of 0.2 Hz. Only cells showing normal AP configurations and resting membrane potential greater than −80 mV were used in this study (cf. Fig. 2). AP were studied after a control period of at least 5 min and after a minimum of 5 min of superfusion with differing test agents. For each condition, after reaching a steady-state effect, at least five individual APs were sampled, digitally averaged, and then measured.

Voltage-clamp was performed in whole-cell recording mode, and perfusion of the cells was minimized by maintaining constant negative pressure on the electrode using a 1-ml gas-tight syringe attached to the suction port of the microelectrode holder via air-tight tubing. Outward potassium currents were measured during superfusion of the cells at a rate of 2 ml/min with Ca2+-free modified Tyrode’s solution (35°C) containing 0.4 μM nisoldipine to block ICa. Cells were voltage-clamped at a holding potential (Vh) of −40 mV to inactivate IK. In some experiments, 20 μM tetrodotoxin or 3 μM saxitoxin (STX) was used to block residual INa. Time-dependent delayed rectifier K+ current amplitude, IK, was measured as the difference from the initial instantaneous current, after the settling of the capacitance transient, to the final (steady-state) current level during depolarizing voltage steps to various test potentials (Vt). Tail current amplitude, IKtail, was measured as the difference from the holding current level to the peak tail current amplitude on return to Vh. Data acquisition and analysis were performed using pCLAMP software (Axon Instruments, Foster City, CA) on a PC.

![Fig. 1](image1.png)

**Fig. 1.** Structural formulae for RWJ 24517 and DPI 201-106. *+, asymmetric carbon atom.

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**Fig. 2.** Effects of RWJ 24517 on APs of guinea pig isolated ventricular myocytes. A–E, RWJ 24517 prolonged APD in the absence but not in the presence of sodium channel blockade with 3 μM STX. Bottom, effects on APD at 90% of repolarization (APD90) in the presence and after washout of 3 μM STX. Data expressed as percentage change from control are mean ± S.E. (n = 5). Stimulus frequency of 0.2 Hz at 35°C.
**INa Measurements.** Cardiac INa was recorded using the whole-cell patch-clamp technique. The superfusion medium consisted of 5 mM NaCl, 5 mM CsCl, 1.8 mM CaCl2, 1.2 mM MgCl2, 125 mM tetramethylammonium chloride, 11 mM glucose, and 20 mM HEPES, adjusted to pH 7.4 with tetramethylammonium-OH. The internal (pipette) solution contained 145 mM CsF, 5 mM NaF, and 5 mM CsOH. Pipette resistance was ~0.8 MΩ. Ca²⁺ currents were blocked by internal and external Cs⁺ (Clarkson, 1990). All experiments were performed at 16 ± 1°C. The reduced temperature and external Na⁺ allowed for better voltage control for measuring INa. A custom-designed voltage-clamp amplifier was used as described previously (Sakakibara et al., 1992). Series resistance compensation was used but was usually not required with this amplifier due to low access resistance obtained after sealing and was found to have very little effect on the decay rate of capacitative current under our experimental conditions (Sakakibara et al., 1992). With peak current amplitudes of < 6 nA, the voltage error was < 5 mV even in the absence of series resistance compensation. Data were filtered at 5 kHz, digitized at 10 kHz, and stored on a PDP 11/73 computer. Capacity transients and linear leak were subtracted digitally offline. Off-line analysis was performed using locally developed algorithms.

**Mathematical Analysis.** Cell capacitance (C) was calculated using the equation

\[ C = \frac{Q}{V} \]  

(1)

where Q is the total charge movement determined by integrating the area defined by the capacitance transient from a 10-mV step depolarization from -140 mV. The mean cell capacitance was 216 ± 14 pF (n = 30).

The time course of INa decay and the rate of INa recovery from inactivation were fitted to an equation describing the sum of two exponentials:

\[ I_{Na} = A_1 \exp(-t/\tau_p) + A_2 \exp(-t/\tau_0) \]  

(2)

where \( \tau_p \) and \( \tau_0 \) are the fast and slow time constants, and \( A_1 \) and \( A_2 \) are the amplitudes of the fast and slow components, respectively. Curves for steady-state inactivation (h) were initially fitted with the following equation describing a single Boltzmann distribution using a sum of the least-squares algorithm:

\[ h_a = \left(1 - B\right)/(1 + \exp\left(V_e - V_{1/2}/k\right)) + B \]  

(3)

where \( V_c \) is conditioning potential, \( V_{1/2} \) is half-inactivation voltage, \( k \) is slope factor, and \( B \) is the steady-state component. In some instances, \( h_a \) curves were fitted best to a double Boltzmann distribution:

\[ h_a = A(1 + \exp\left(V_e - V_{1/2}/k_1\right)) \]

\[ + \left(1 - A - B\right)/(1 + \exp\left(V_e - V_{1/2}/k_2\right)) + B \]  

(4)

**Results**

**Basis for AP Prolongation with RWJ 24517.** RWJ 24517 increased APD of guinea pig isolated ventricular myocytes in a concentration-dependent manner similar to the effects observed in multicellular preparations (Salata et al., 1991; Kawamura et al., 1993). RWJ 24517 at 0.01 and 0.1 μM increased APD₀ by ~30 and 45%, respectively (Fig. 2 and Table 1). Preliminary studies indicated that RWJ 24517 increased “late” INa, which could be the mechanism for the increase in APD (Salata et al., 1991). We tested this hypothesis by examining the effects of RWJ 24517 after pretreatment of the myocytes with STX to block INa. STX alone (3 μM) decreased APD slightly but nonsignificantly (Fig. 2, Table 1). The addition of 0.1 μM RWJ 24517 in the continued presence of STX had little or no effect on APD, but an RWJ 24517-induced prolongation of APD became manifest after washout of the STX. This RWJ 24517-induced prolongation of APD was almost fully reversible after washout of the RWJ 24517.

**Drug Effects on “Late” INa.** Figure 3 shows drug effects on whole-cell currents measured during 600-ms voltage steps from a holding potential of -80 mV to a test potential of -10 mV. Currents were measured during superfusion with Ca²⁺-free external solution (35°C) with 0.4 μM nisoldipine to block Ica. Extracellular Na⁺ was at a near-physiological level of 132 mM. During control, there was a large rapid inward INa that inactivated almost fully within 50 ms; thereafter, the

\[ V_i \text{ and } V_o \text{ are half-inactivation voltages for the two components, } k_i \text{ and } k_o \text{ are the slope factors for the first and second components, and } A \text{ is the amplitude of the first component.} \]

**Drugs.** RWJ 24517 and its (S)- and (R)-enantiomers (RWJ 25320 and RWJ 25319, respectively) were generously provided by R.W. Johnson Pharmaceutical Research Institute (Spring House, PA). The purity of the enantiomers was >99%. The drugs were initially prepared as a 1 mM stock in 20% ethanol and then added to the superfusion medium to obtain the desired concentrations (0.1–30 μM). The concentration of ethanol never exceeded 0.6%, which had little effect on INa amplitude. DPI was generously provided by Merck Research Laboratories (Rahway, NJ). DPI was prepared as a 1 mM stock in 20% dimethyl sulfoxide. The final concentration of dimethyl sulfoxide never exceeded 0.2%, which had no effect on INa amplitude or kinetics. Each concentration of RWJ or DPI was superfused for ≥10 min before INa was recorded.

**Statistical Analysis.** The data were initially analyzed using a one-way ANOVA and post hoc by a Newman-Keuls or Dunnett test to determine significant differences between mean values. The minimum level of statistical significance was p < .05. All data are expressed as mean ± S.E.

**Table 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>RMP</th>
<th>AMP</th>
<th>APD₀</th>
<th>APD₂₀</th>
<th>APD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-82</td>
<td>136</td>
<td>132</td>
<td>208</td>
<td>230</td>
</tr>
<tr>
<td>3 μM STX</td>
<td>-83</td>
<td>132</td>
<td>126</td>
<td>190</td>
<td>210</td>
</tr>
<tr>
<td>3 μM STX + 0.1 μM RWJ 24517</td>
<td>-83</td>
<td>130</td>
<td>130</td>
<td>200</td>
<td>221</td>
</tr>
<tr>
<td>0.1 μM RWJ 24517</td>
<td>-83</td>
<td>135</td>
<td>184</td>
<td>303</td>
<td>333</td>
</tr>
<tr>
<td>Washout</td>
<td>-82</td>
<td>133</td>
<td>119</td>
<td>214</td>
<td>242</td>
</tr>
</tbody>
</table>

**Legend:**
- RMP, resting membrane potential; AMP, AP amplitude; APD₀, APD₂₀, and APD₅₀, APDs measured at 20, 50, and 90% of repolarization, respectively. Data are expressed as mean ± S.E. (n = 5).
- Statistical analysis was performed with ANOVA.
- *p < .05 with Dunnett’s t test for post hoc comparison of the individual mean values with the control mean value. 

<table>
<thead>
<tr>
<th>M</th>
<th>STX</th>
<th>RWJ 24517</th>
<th>RWJ 25320</th>
<th>RWJ 25319</th>
</tr>
</thead>
<tbody>
<tr>
<td>M STX</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>24517</td>
<td>25320</td>
<td>25319</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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net current during the step became outward (Fig. 3 and Table 2). The application of 0.01 and 0.1 μM RWJ 24517 induced a large concentration-dependent inward shift in the current throughout the depolarizing step. The addition of 3 μM STX abolished the inward shift in the current produced by RWJ 24517.

Effects of RWJ 24517 on \( I_{\text{Na}} \). We then investigated the direct effects of RWJ 24517 on cardiac \( I_{\text{Na}} \). The concentration-dependent effects of RWJ 24517 on cardiac \( I_{\text{Na}} \) are shown in Fig. 4A and summarized in Fig. 5A. Myocytes were held at \( V_h = -140 \) mV and depolarized to −20 mV for 100 ms. Under control conditions, \( I_{\text{Na}} \) activated rapidly and inactivated within 20 ms. RWJ 24517 (3 μM) modestly increased peak \( I_{\text{Na}} \) but more noticeably slowed the rate of \( I_{\text{Na}} \) decay, leaving a substantial noninactivating (steady-state) component of \( I_{\text{Na}} \) even after 100 ms (summarized in Fig. 5B and inset). At 10 μM, RWJ 24517 decreased peak \( I_{\text{Na}} \) slightly but increased steady-state current. At 30 μM, RWJ 24517 markedly decreased peak current, but steady-state \( I_{\text{Na}} \) was still increased compared with control (Fig. 5B). DPI had comparable effects on steady-state \( I_{\text{Na}} \) (Figs. 4B and 5B) at even lower concentrations than RWJ 24517 but did not increase peak \( I_{\text{Na}} \) (Fig. 5A).

The concentration dependence of RWJ 24517 on the peak \( I_{\text{Na}} \)–voltage (I-V) relationship is shown in Fig. 4A (bottom) and summarized in Fig. 5A. Under control conditions, \( I_{\text{Na}} \) was activated at −60 mV and reached its maximal inward amplitude between −40 and −80 mV. The 10-mV negative shift in the activation voltage of \( I_{\text{Na}} \) was most likely due to a time-dependent shift during whole-cell recording, as described by other investigators using cardiac myocytes (Kunze et al., 1985; Hanck and Sheets, 1992; Wasserstrom et al., 1993a), rather than a direct drug effect. RWJ 24517 (3 and 10 μM) caused a slight increase in peak \( I_{\text{Na}} \) over the test voltage range of −70 to +40 mV but decreased peak \( I_{\text{Na}} \) at 30 μM. In contrast, the I-V relationship for DPI showed only a decrease in peak \( I_{\text{Na}} \) (Fig. 4B, bottom).

To determine whether the results of RWJ 24517 on cardiac \( I_{\text{Na}} \) were due to stereospecific effects of its enantiomers, we examined the effects of (S)-RWJ and (R)-RWJ on \( I_{\text{Na}} \) (Fig. 4, C and D). The concentration-dependent effects of (S)-RWJ on \( I_{\text{Na}} \) were very similar to those of RWJ 24517 (Fig. 5), as were the effects on the I-V relationship (Fig. 4C). In contrast, (R)-RWJ only decreased peak \( I_{\text{Na}} \) especially at higher concentrations, and had little effect on \( I_{\text{Na}} \) decay (Figs. 4D and 5B). Reversal potential was not altered by these compounds, suggesting that they did not affect ionic selectivity.

In contrast to RWJ 24517, DPI significantly decreased peak \( I_{\text{Na}} \): 3 μM decreased peak current by 38 ± 6% (n = 6, p < .01), whereas 10 μM reduced current by 83 ± 4% (n = 3, p < .01). However, like RWJ 24517, DPI also had profound concentration-dependent effects to increase steady-state current (Figs. 4B, top, and 5B).

To quantify the concentration-dependent effects of the different compounds on the kinetics of \( I_{\text{Na}} \) decay, the rates of \( I_{\text{Na}} \) decay were measured using equation 2 described in Materials and Methods. Currents elicited at −20 mV were fitted to a double exponential function. RWJ 24517 (10 μM) had no significant effect on \( \tau_p \) of \( I_{\text{Na}} \) decay (3.9 ± 0.4 ms in control compared with 2.7 ± 0.2 ms after drug, n = 12). Similarly, (S)-RWJ, (R)-RWJ, and DPI did not affect \( \tau_p \) at any concentration tested. However, RWJ 24517 (10 μM) increased \( \tau_g \) from 28 ± 6 to 434 ± 52 ms (n = 12, p < .01), as did (S)-RWJ (26 ± 9 to 452 ± 76 ms; n = 9–11, p < .01). Similar effects were observed with DPI. In contrast, (R)-RWJ elicited a small but nonsignificant increase in \( \tau_g \) (from 37 ± 8 to 142 ± 42 ms, n = 12). There also was a concentration-dependent increase in the fraction of \( I_{\text{Na}} \) that inactivated at the slow time constant with the three effective agents (Fig. 6).

### Table 2

Effects of RWJ 24517 on late \( I_{\text{Na}} \) in the presence and absence of 3 μM STX

<table>
<thead>
<tr>
<th>Current</th>
<th>( I_h ) at 50 ms</th>
<th>( I_{\text{test}} ) at 100 ms</th>
<th>( I_{\text{test}} ) at 250 ms</th>
<th>( I_{\text{test}} ) at 500 ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>58 ± 25</td>
<td>−15 ± 13</td>
<td>18 ± 14</td>
<td>46 ± 14</td>
</tr>
<tr>
<td>0.01 μM RWJ 24517 (n = 3)</td>
<td>79 ± 34</td>
<td>−155 ± 33</td>
<td>−107 ± 26</td>
<td>−48 ± 17</td>
</tr>
<tr>
<td>0.1 μM RWJ 24517 (n = 6)</td>
<td>53 ± 22</td>
<td>−1052 ± 173*</td>
<td>−961 ± 157*</td>
<td>−774 ± 131*</td>
</tr>
<tr>
<td>0.1 μM RWJ 24517 + 3 μM STX (n = 3)</td>
<td>103 ± 30</td>
<td>2 ± 5</td>
<td>17 ± 3</td>
<td>33 ± 1</td>
</tr>
</tbody>
</table>

\( I_h \) holding current at −80 mV; \( I_{\text{test}} \) current at varying times during test pulse to −10 mV. Data are expressed as mean ± S.E. Statistical analysis was performed with ANOVA.

* \( p < .05 \) with Dunnett’s \( t \) test for post hoc comparison of the individual mean values with the control mean value.
results show that RWJ 24517, (S)-RWJ, and DPI slow the rate of INa decay by increasing $\tau_S$ and increasing the proportion of INa decaying at the slower rate.

Figure 7A shows the voltage dependence of steady-state inactivation ($h_v$) in the absence and presence of RWJ 24517 (3 and 10 $\mu$M). The control $h_v$ curves were best fitted with a single Boltzmann equation with a $V_{1/2}$ of $-82.4 \pm 3.0$ mV and a slope factor ($k$) of $5.3 \pm 0.2$ mV ($n = 12$). All channels were completely inactivated at a $V_c$ of $-60$ mV (i.e., $h_v = 0$). At 3 $\mu$M RWJ 24517, 17 $\pm$ 2% of total INa remained available even after 1-s conditioning steps to voltages as positive as $-20$ mV. Moreover, the $h_v$ curve was best described by a double Boltzmann equation, indicating that two distinct voltage-dependent processes determine steady-state inactivation after Na$^+$ channel modification with RWJ 24517. Thus, there were two values of $V_{1/2}$ ($-97.3 \pm 2.9$ and $-65.2 \pm 3.4$ mV) and $k$ ($5.3 \pm 0.4$ and $9.2 \pm 1.2$ mV; $n = 12$), with the second component (representing drug-modified channels) accounting for 45 $\pm$ 4% of the fitted curve. There was a $15$-mV hyperpolarizing shift in the first $V_{1/2}$ value of the $h_v$ curve, which probably represents a time-dependent negative shift in the voltage dependence of activation (Fig. 4). In the presence of 10 $\mu$M RWJ 24517, the $V_{1/2}$ values were $-100.5 \pm 2.6$ and $-62.2 \pm 1.9$ mV, and the $k$ values were $5.5 \pm 0.3$ and

![Figure 4](http://jpet.aspetjournals.org/lookup/fig/4)
Although there was no significant difference in the \( V_{1/2} \) and \( k \) values between 3 and 10 \( \mu M \) RWJ 24517, the fraction of modified channels increased to 62 ± 3%, whereas the noninactivated component remained the same (17 ± 2%). DPI produced similar effects on steady-state inactivation. At 3 \( \mu M \), \( h_{\infty} \) curves were best fitted with a double Boltzmann function with \( V_{1/2} \) values of 210.4 ± 3.8 mV (\( k = 6.2 \pm 0.2 \) mV) and −63.7 ± 3.3 mV (\( k = 6.7 \pm 0.6 \) mV; \( n = 7 \)), with 35 ± 7% of the channels modified by DPI.

(S)-RWJ produced similar effects on steady-state inactivation (Fig. 7B). In the absence of (S)-RWJ, the \( V_{1/2} \) and \( k \) values were −78.2 ± 2.7 and 5.1 ± 0.1 mV (\( n = 9 \)), respectively. In the presence of drug, \( h_{\infty} \) curves were best described by a double Boltzmann equation in the presence of 3 and 10 \( \mu M \) (S)-RWJ. The \( V_{1/2} \) and \( k \) values were −98.9 ± 3.6 mV (\( k = 5.2 \pm 0.4 \) mV) and −60.9 ± 3.4 mV (\( k = 8.7 \pm 1.3 \) mV) in the presence of 3 \( \mu M \) (S)-RWJ. Similar \( V_{1/2} \) and \( k \) values were observed at 10 \( \mu M \) (S)-RWJ. The fraction of channels modified by (S)-RWJ were 39 ± 4 and 70 ± 1% at 3 and 10 \( \mu M \), respectively. The noninactivated components were 17 ± 2 and 19 ± 3% at 3 and 10 \( \mu M \), respectively.

In contrast, (R)-RWJ had little effect on steady-state inactivation (Fig. 7C). The \( h_{\infty} \) curves for control and 3 and 10 \( \mu M \)
(R)-RWJ were all best fitted with a single Boltzmann equation with $V_{1/2}$ values of $-81.8 \pm 1.2$, $-99.5 \pm 3.0$, and $-103.6 \pm 3.1$ mV and $k$ values of $5.2 \pm 0.2$, $5.8 \pm 0.3$, and $6.0 \pm 0.3$ mV ($n = 10$ and $n = 11$), respectively. Furthermore, there was very little change in the noninactivating component [1 ± 1% for control, 3 ± 2% for 3 μM (R)-RWJ, and 4 ± 3% for 10 μM (R)-RWJ]. These observations demonstrate that RWJ 24517, its (S)-enantiomer, and DPI alter the voltage dependence of steady-state inactivation such that there are two distinct voltage-dependent processes. The first component of the $h_c$ curve represents unmodified Na$^+$ channels, whereas the second component reflects drug-modified channels.

One important characteristic of Na$^+$ channel modifiers is a use-dependent decline in current amplitude. Figure 8A illustrates the use-dependent effects of RWJ 24517 on peak $I_{Na}$. Cardiac myocytes were stimulated with a train of 20 depolarizing pulses to $-20$ mV from a $V_h$ value of $-140$ mV at a constant interpulse interval of 500 ms with pulse durations of 10 or 100 ms. In control, there was very little use-dependent decrease in $I_{Na}$ (2–3%; $n = 12$) at either pulse duration. At a 10-ms pulse duration, RWJ 24517 (3 μM) produced a slight decrease in $I_{Na}$ (5 ± 1%, $n = 12$), measured at the 20th pulse (data not shown). With a 100-ms pulse duration, there was a 20 ± 2% ($n = 12$, $p < .05$) use-dependent decrease in peak $I_{Na}$. Use-dependent decrease in $I_{Na}$ magnitude was enhanced in the presence of 10 μM RWJ 24517; pulse durations of 10 and 100 ms elicited a 14 ± 1 and 43 ± 3% use-dependent reduction in $I_{Na}$, respectively ($n = 12$, $p < .01$; Fig. 8A). The extent of use-dependent decline was significantly greater ($p < .01$) with a 100-ms than with a 10-ms depolarizing step pulse at both 3 and 10 μM. In addition, the use-dependent decrease of $I_{Na}$ became progressively greater with RWJ 24517 as the interpulse interval was shortened from 1000 to 500 to 333 ms (data not shown). (S)-RWJ caused comparable use-dependent decline in $I_{Na}$ to that of RWJ 24517 (Fig. 8B). DPI (3 μM) elicited a significantly greater use-dependent decline in peak $I_{Na}$ than 3 μM RWJ 24517 at both pulse durations (21 ± 5 and 50 ± 7% reduction at 10 and 100 ms, respectively; $n = 6$, $p < .05$ compared with RWJ 24517). In contrast, 10 μM (R)-RWJ reduced $I_{Na}$ by only 7 ± 2 and 12 ± 2% ($n = 8$) for 10- and 100-ms depolarizing step pulse, respectively ($p < .01$ compared with control; Fig. 8C). Although 10 μM (R)-RWJ produced a significant degree of use-dependent decrease at both pulse durations, these effects were very modest compared with either RWJ 24517 or its (S)-enantiomer.

One possible explanation for the use-dependent decline in $I_{Na}$ amplitude is a slowing in the rate of $I_{Na}$ recovery between pulses within a train. The rate of recovery from inactivation was measured using the double pulse protocol shown in Fig. 9, bottom right. The test current ($I_{Test}$) was normalized to its corresponding conditioning current ($I_{Condition}$) and plotted as a function of the recovery interval (top) and of $1 - (I_{Test}/I_{Condition})$ (Fig. 9, bottom). Under control conditions, the recovery of $I_{Na}$ was completed within ~200 ms and was best described by the sum of two exponentials. RWJ 24517 and (S)-RWJ both produced a similar degree of concentration-dependent prolongation of both the fast ($τ_F$) and slow ($τ_S$) time constants for recovery. Both agents increased $τ_F$, noticeably prolonged $τ_S$ (Fig. 9, A and B), and decreased the proportion of $I_{Na}$ that recovered at the fast rate ($% τ_F$ in Table 3, $p < .01$). In contrast, (R)-RWJ had a much smaller effect on $τ_F$ and $τ_S$ than RWJ 24517 (Fig. 9C, Table 3). A similar effect was observed with DPI (3 μM), which caused a more profound effect than RWJ 24517. (R)-RWJ had less effect on $τ_S$ at both 3 and 10 μM than RWJ 24517. These results suggest that the use-dependent effects of RWJ 24517, (S)-RWJ, and DPI may result from prolongation of the slow rate of recovery of $I_{Na}$ with an increase in the proportion of $I_{Na}$ recovering at the slow rate. The greater use-dependent decrease of peak $I_{Na}$ with DPI is reflected in the greater lengthening of the slow time constant of recovery. Furthermore, (R)-RWJ elicits no use-dependent reduction in $I_{Na}$ due to its lack of effect on the rate of recovery of $I_{Na}$ from inactivation.

**Effects of RWJ 24517 on Delayed Rectifier K$^+$ Currents.** The effects of RWJ 24517 on the I-V relationships for outward K$^+$ currents are shown in Fig. 10. Currents were elicited using 550-ms depolarizing voltage steps between −30 and +60 mV from a holding potential of −40 mV. Figure 10A shows the effects of 10 μM RWJ 24517 on the time-dependent and repolarizing tail currents ($I_{Kstn}$). As shown by the drug-sensitive difference current at a test potential of 0 mV (Fig. 10A, bottom), relatively high concentrations of RWJ 24517...
TABLE 3
Effects of RWJ 24517, (S)-RWJ, (R)-RWJ, and DPI on the time constants (τ_f and τ_r) for recovery from inactivation

<table>
<thead>
<tr>
<th></th>
<th>τ_f</th>
<th>τ_r</th>
<th>ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWJ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.1 ± 0.4</td>
<td>92 ± 2</td>
<td>90 ± 25</td>
</tr>
<tr>
<td>3 μM</td>
<td>14.3 ± 4.7^a</td>
<td>72 ± 1^b</td>
<td>1318 ± 311^b</td>
</tr>
<tr>
<td>10 μM</td>
<td>32.2 ± 10.8^b</td>
<td>40 ± 3^b</td>
<td>2414 ± 468^b</td>
</tr>
<tr>
<td>(S)-RWJ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.6 ± 0.1</td>
<td>93 ± 3</td>
<td>58 ± 15</td>
</tr>
<tr>
<td>3 μM</td>
<td>11.9 ± 2.4</td>
<td>58 ± 7^c</td>
<td>1797 ± 283^c</td>
</tr>
<tr>
<td>10 μM</td>
<td>31.3 ± 5.4^b</td>
<td>31 ± 4^b</td>
<td>3066 ± 256^b</td>
</tr>
<tr>
<td>(R)-RWJ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.3 ± 0.6</td>
<td>87 ± 1</td>
<td>72 ± 15</td>
</tr>
<tr>
<td>3 μM</td>
<td>16.9 ± 6.6</td>
<td>83 ± 3</td>
<td>235 ± 56^f</td>
</tr>
<tr>
<td>10 μM</td>
<td>26.0 ± 9.5^b</td>
<td>73 ± 2</td>
<td>722 ± 248^b</td>
</tr>
<tr>
<td>DPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.0 ± 0.6^d</td>
<td>87 ± 2</td>
<td>115 ± 25</td>
</tr>
<tr>
<td>3 μM</td>
<td>39.2 ± 11.9^bd</td>
<td>22 ± 4^bd</td>
<td>3118 ± 28^bd</td>
</tr>
</tbody>
</table>

^a p < .05 compared with control.
^b p < .01 compared with control.
^c p < .05 compared with RWJ 24517.
^d p < .01 compared with RWJ 24517.

(10 μM) inhibited a rapidly activating time-dependent outward current during depolarization and a prominent tail current on repolarization to −40 mV. Inhibition of the time-dependent current occurred at test voltages between −30 and +30 mV (Fig. 10B), whereas the inhibition of I_{Kstral} occurred over the entire voltage range (Fig. 10C). This profile of block of I_K is essentially identical with the effects produced by methanesulfonanilide class III antiarrhythmic agents like E-4031 and dofetilide that selectively block the rapidly activating component of the delayed rectifier K⁺ current, I_{Kr} (Sanguinetti and Jurkiewicz, 1990). The similarities to I_{Kr} include a drug-sensitive current that is maximal at −0 mV and decreases progressively at more positive potentials, consistent with the inwardly rectifying property of I_{Kr}. Finally, an analysis of the concentration dependence of block of the I_{Kr} tail current revealed an EC_{50} value of ~0.9 μM, making this agent less potent than the methanesulfonanilides (Sanguinetti and Jurkiewicz, 1990).

Discussion

The major findings of this work are that RWJ 24517 prolongs the APD and elicits a profound slowing of I_{Na} decay, thus eliciting a large steady-state current. This latter effect is stereospecific because only the (S)-enantiomer shared the same properties on I_{Na} decay as RWJ 24517, whereas (R)-RWJ had little effect on either peak I_{Na} or the rate of I_{Na} decay. RWJ 24517 and (S)-RWJ produced a duration- and frequency-dependent decrease in I_{Na} amplitude, which was enhanced by prolonging pulse duration or shortening interpulse interval and which presumably is the result of a slowing of the rate of recovery from inactivation. (R)-RWJ lacked any substantial use-dependent effects on I_{Na} and had little effect on rate of recovery. RWJ 24517 also inhibited I_{Kr} but not I_{Ks}, suggesting that its effects are not limited to the sodium channel. Therefore, prolongation of APD is induced by a slowing in the decay of the I_{Na} and possibly by inhibition of I_{Kr}, especially at higher drug concentrations.

Effects of RWJ 24517 on Peak I_{Na}. RWJ 24517 (0.1–1 μM) and its (S)-enantiomer (0.1–3 μM) have a modest agonist action to increase peak I_{Na} by ~25%, whereas concentrations of >10 μM markedly reduced I_{Na}. In single L-type Ca²⁺ channel studies, Bay K 8644 also increased the frequency and duration of channel openings, resulting in larger ensemble currents (Brown et al., 1984; Hess et al., 1984; Kokubun and Reuter, 1984). A similar mechanism may explain the effects of RWJ 24517 and (S)-RWJ on I_{Na} because DPI has also been shown to increase the frequency and duration of single Na⁺ channel openings (Kohlhardt et al., 1986, 1987b; Nilius et al., 1989). Interestingly, no effect of I
or 10 μM RWJ 24517 was observed on the maximal upstroke velocity of the AP ($V_{max}$) in ferret (Salata et al., 1991) or guinea pig (Kawamura et al., 1993) papillary muscle. However, changes in $V_{max}$ may not directly reflect changes in $I_{Na}$ because a nonlinear relationship has been reported between $V_{max}$ and $I_{Na}$ (Cohen et al., 1984; Sheets et al., 1988).

DPI does not share the same enhancing effects as RWJ 24517 due to the simultaneous blocking actions of its ($R$)-enantiomer. We observed very little blocking effect of ($R$)-RWJ at 0.1 to 3 μM, and only at 30 μM did ($R$)-RWJ significantly decrease $I_{Na}$. In contrast, 3 μM ($R$)-DPI decreased $I_{Na}$ from ~35% (Wang et al., 1990) to 90% (Romey et al., 1987).

($R$)-RWJ thus appears to have considerably less blocking activity on peak $I_{Na}$, than ($R$)-DPI, suggesting that the inhibitory effect of ($R$)-RWJ provides little opposition to the enhancing activity of the ($S$)-enantiomer at concentrations ≤10 μM. At 30 μM, RWJ 24517 and both of its enantiomers reduced peak $I_{Na}$ to a comparable level, suggesting that non-specific interactions of the compounds with the channel or lipid environment may explain these results.

The most profound effect of Na$^+$ channel modification by RWJ 24517 and ($S$)-RWJ is a slowing of the rate of $I_{Na}$ decay, eliciting a nonactivating component (steady-state current) at the end of a 100-ms depolarizing pulse. In fact, the steady-state current continued to flow at the end of 1-s test voltage steps (cf. Fig. 7). The concentration-dependent increase in the steady-state current peaked at 10 μM with both RWJ 24517 and ($S$)-RWJ. Interestingly, Salata et al. (1991) observed a maximal effect of RWJ 24517 on isometric twitch tension of ferret papillary muscles at 10 μM. This suggests that there may be a causal relationship between the inotropic action of RWJ 24517 and its effect on $I_{Na}$ inactivation despite differences in experimental conditions. If such a relationship exists, then the larger steady-state currents produced by RWJ 24517 than DPI (Figs. 4 and 5) may also explain the greater potency of RWJ 24517 on contractile function of isolated papillary muscles and in vivo canine hearts compared with DPI (Press et al., 1992). The increase in steady-state current with RWJ 24517 and its ($S$)-isomer most likely arises as a result of an increase in the frequency and/or duration of Na$^+$ channel openings due to the removal of Na$^+$ channel inactivation, as demonstrated in single-channel studies with DPI (Kohlhardt et al., 1986, 1987b; Nilius et al., 1989). Removal of the inactivated state of the Na$^+$ channel permits the channel to cycle between the open and closed states, resulting in greater current flow throughout depolarization. ($R$)-RWJ elicited no steady-state current and thus lacks the enhancing properties of RWJ 24517 and ($S$)-RWJ. This result contrasts with ($R$)-DPI, which elicited a small noninactivating current that was most noticeable at 10 μM (Wang et al., 1990). Therefore, RWJ 24517 appears to have more agonistic actions on cardiac $I_{Na}$ and less blocking effects than DPI.

In addition to the increase in the slow time constant for $I_{Na}$ decay, RWJ 24517 and ($S$)-RWJ increased the proportion of $I_{Na}$ decaying at the slow rate, at the expense of current decaying at the fast rate. This effect is similar to that ob-

Fig. 10. Effects of RWJ 24517 on $I_{K}$. A, current traces recorded during control and after 10 μM RWJ 24517. Difference current (bottom) shows drug-sensitive current ($I_{Kr}$) during a voltage step from −40 to −10 mV. B, I-V plots of the relative time-dependent $I_{K}$ during 550-ms pulse measured from the settling of the initial capacitance spike to the end of the test pulse. C, I-V plots of the relative tail currents measured on return of the membrane potential to −40 mV from the indicated test potential. Data are mean ± S.E. (n = 5–6). B and C, ○, control; ▼, 1 μM RWJ 24517; ●, 10 μM RWJ 24517. Currents obtained in the presence of 0.4 μM nisoldipine at 35°C.
served with other \( \text{Na}^+ \) channel modifiers that slow or eliminate inactivation, such as the sea anemone toxins anthopleurin-A (Wasserstrom et al., 1993a) and ATX-II (El-Sherif et al., 1992) and \( \alpha \)-chymotrypsin (Clarkson, 1990). Although these \( \text{Na}^+ \) channel modifiers prolong both the fast and slow rates of \( I_{\text{Na}} \) decay, they also augment the proportion of current undergoing the slow rate of decay.

Agents that modify \( \text{Na}^+ \) channel inactivation have been shown to exert their effects in heart in a frequency-dependent manner, including veratridine (Honerjager and Reiter, 1975), ATX-II (Beress et al., 1982), anthopleurin-A (Wasserstrom et al., 1993a), and batrachotoxin (Wasserstrom et al., 1993b). Indeed, a frequency-dependent increase in ferret ventricular APD with RWJ 24517 has been reported (Salata et al., 1991) in which slower stimulation rates (1 Hz) led to a greater prolongation of the APD in the presence of 1 \( \mu \text{M} \) RWJ 24517 than at faster rates (2 and 3 Hz). We observed that RWJ 24517 and (S)-RWJ elicited a significant use-dependent decrease in peak \( I_{\text{Na}} \) that was dependent on stimulation frequency and pulse duration. Thus, the enhanced effects observed on ferret ventricular AP at slower frequencies may result from a greater number of drug-modified \( \text{Na}^+ \) channels contributing to prolongation of the APD.

We also found that these agents produced significant use-dependent decline in peak \( I_{\text{Na}} \) during pulse trains. The basis for this effect probably lies in the fact that both fast and slow rates of recovery in the biexponential process underlying recovery from inactivation in control (Clarkson, 1990) were significantly prolonged by RWJ 24517 and (S)-RWJ in a concentration-dependent manner. In addition, the fraction of \( I_{\text{Na}} \) recovering at the slower rate was enhanced. This overall slowing in the rate of recovery from inactivation prevents full recovery of the channel to a degree determined by the interpulse interval. In the present study, a recovery interval of 500 ms (same as the interpulse interval used in the use-dependent experiments) did not allow for full \( I_{\text{Na}} \) recovery; therefore, subsequent activation of \( I_{\text{Na}} \) would reveal less \( I_{\text{Na}} \) recovered and would allow less current flow. In contrast, (R)-RWJ produced very little use dependence and had little effect on the rate of recovery from inactivation. Interestingly, 3 \( \mu \text{M} \) DPI elicited a larger use-dependent decline in peak \( I_{\text{Na}} \) than 3 \( \mu \text{M} \) RWJ 24517. Both agents increased the steady-state \( I_{\text{Na}} \) and prolonged the slow time constant for \( I_{\text{Na}} \) decay to a similar extent; however, DPI had a larger effect on the slow rate of recovery from inactivation, which may explain its enhanced use-dependent decline of \( I_{\text{Na}} \), compared with RWJ 24517. Furthermore, 3 \( \mu \text{M} \) DPI significantly decreased peak \( I_{\text{Na}} \), whereas 3 \( \mu \text{M} \) RWJ 24517 had no effect on peak \( I_{\text{Na}} \). Therefore, the use-dependent decline in peak \( I_{\text{Na}} \) in the presence of DPI may also be related to selective use-dependent block by the (R)-isomer of DPI.

It is interesting that RWJ 24517 and (S)-RWJ slow both the decay of \( I_{\text{Na}} \) and the recovery from inactivation. Recent mutagenesis studies on sodium channels have demonstrated most mutations that slow the rate of current decay accelerate the recovery kinetics due to a destabilization of the inactivated state (Chahine et al., 1994; Yang et al., 1994; Hanna et al., 1996). The slowing in the recovery kinetics by RWJ 24517 and (S)-RWJ are curiously reminiscent of the effects of local anesthetic agents, such as lidocaine, which dramatically slow the recovery from inactivation (Bean et al., 1983). However, the action of local anesthetic agents is thought to be the result of stabilization of the inactivated state of the channel. We suggest that alterations in channel gating by RWJ 24517 may result in a destabilization of the fast-inactivated state but also shift a larger proportion of the channels into the slow-inactivated state. Recent studies have shown that partial (Townsend and Horn, 1997) or complete (Richmond et al., 1998) removal of fast inactivation in heterologously expressed cardiac sodium channels resulted in slow inactivation that was faster and more complete.

**Effects of RWJ on APD and Positive Inotropy.** The present study demonstrated that a major effect of RWJ 24517 is a slowing of \( I_{\text{Na}} \) inactivation. The positive inotropic effect that occurs with RWJ 24517 probably is the result of APD prolongation; the increase in slowly inactivating \( I_{\text{Na}} \) causes, first, a direct increase in intracellular \( \text{Na}^+ \) accumulation and, second, prolongation of depolarization, both of which reduce intracellular \( \text{Ca}^{2+} \) extrusion via \( \text{Na}^+\text{/Ca}^{2+} \) exchange. Blockade of \( I_{\text{K}} \) but not \( I_{\text{Na}} \) may also contribute to AP prolongation as suggested by Kawamura et al. (1993). However, our findings suggest that this action is unlikely to be the primary mode of action of RWJ 24517 on APD because \( I_{\text{Na}} \) modification is more sensitive to drug than \( I_{\text{K}} \) blockade. We found that the effective concentration for producing a noninactivating component of \( I_{\text{Na}} \) under nearly physiological conditions (37°C in K+-containing solutions) was 0.01 \( \mu \text{M} \), whereas the EC\(_{50}\) value for inhibition of \( I_{\text{K}} \) tails was nearly 1 \( \mu \text{M} \). More importantly, there was no change in APD with 10 times that concentration during \( I_{\text{Na}} \) channel blockade. Taken together, these results strongly suggest that AP prolongation by low concentrations of RWJ 24517 is likely to result from an effect on \( I_{\text{Na}} \) channels. This mechanism of increasing intracellular \( \text{Na}^+ \) activity by prolonging the open state of cardiac \( \text{Na}^+ \) channels may offer a promising avenue for the future development of new positive inotropic agents.

**References**


Cohen CJ, Bean BP and Tsien RW (1984) Maximal upstroke velocity (\( \nu_{\text{max}} \)) as an index of available sodium conductance: Comparison of \( V_{\text{Na}} \) and voltage clamp measurements of \( V_{\text{Na}} \). *J Gen Physiol* **84**:636–665.


Kohlhardt M, Frobe U and Herzig JW (1987b) Properties of normal and non-
Richmond JE, Featherstone DE, Hartmann HA and Ruben PC (1998) Slow inacti-
Krafte DS, Davison K, Dugrenier N, Estep K, Josef K, Barchi RL, Kallen RG, Silver
Kunze DL, Lacerda AE, Wilson DL and Brown AM (1985) Cardiac Na currents and
Wasserstrom JA, Liberty K, Kelly J, Santucci P and Myers MK (1993b) Modification of cardiac Na
Wagner G, Dugas M, Armah IB and Honerjaeger P (1990) Interaction between DPI 201-106 enantiomers at the cardiac sodium channel.

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