Mechanisms of Verapamil Inhibition of Action Potential Firing in Rat Intracardiac Ganglion Neurons

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ABSTRACT
The effects of verapamil and related phenylalkylamines on neuronal excitability were investigated in isolated neurons of rat intracardiac ganglia using whole-cell perforated patch-clamp recording. Verapamil (≥10 μM) inhibits tonic firing observed in response to depolarizing current pulses at 22°C. The inhibition of discharge activity is not due to block of voltage-dependent Ca^{2+} channels because firing is not affected by 100 μM Cd^{2+}. The K^+ channel inhibitors charybdotoxin (100 nM), 4-aminopyridine (0.5 mM), apamin (30–100 nM), and tetraethylammonium ions (1 mM) also have no effect on firing behavior at 22°C. Verapamil does not antagonize the acetylcholine-induced inhibition of the muscarine-sensitive K^+ current (M-current) in rat intracardiac neurons. Verapamil inhibits the delayed outwardly rectifying K^+ current with an IC_{50} value of 11 μM, which is approximately 7-fold more potent than its inhibition of high voltage-activated Ca^{2+} channel currents. These data suggest that verapamil inhibits tonic firing in rat intracardiac neurons primarily via inhibition of delayed outwardly rectifying K^+ current. Verapamil inhibition of action potential firing in intracardiac neurons may contribute, in part, to verapamil-induced tachycardia.

Postganglionic neurons of mammalian intracardiac ganglia mediate vagal innervation of the heart, forming functional afferent, efferent, and local circuits responding to neural and humoral substances to influence heart rate (Moravec and Moravec, 1989; Armour, 1991). In neonatal rat intracardiac ganglia, >90% of neurons display tonic or slowly adapting action potential firing in response to a depolarizing current pulse at 22°C (Cuevas et al., 1997). At 37°C, these neurons display rapidly adapting action potential firing to a depolarizing current pulse. The discharge activity of intracardiac neurons can be modulated by acetylcholine (ACh), which is the primary neurotransmitter involved in the vagal innervation of the heart.

Verapamil, a derivative of papaverine, is a class IV antiarrhythmic commonly used for the treatment of supraventricular tachyarrhythmias. The antiarrhythmic effects of verapamil are most marked on the atrioventricular node, shortening the refractory period of the atrioventricular node (for a review, see Nademanee and Singh, 1988).

Several studies have recently reported that verapamil also inhibits delayed rectifier K^+ currents [I_{K(DR)}] in a number of tissues, including rat alveolar epithelial cells (DeCoursery, 1995), chick embryo dorsal root ganglion neurons (Trequattrini et al., 1996, 1998a), K^+ channels cloned from human heart (Rampe et al., 1993), and mouse mKv1.3 channels (Rauer and Grissmer, 1996). In the present study, the effects of verapamil on action potential firing behavior and tonic currents were investigated in isolated neurons from neonatal rat intracardiac ganglia. Verapamil inhibits tonic action potential firing observed at 22°C and in the presence of muscarinic ACh receptor stimulation. Although verapamil partially inhibits high voltage-activated Ca^{2+} channel currents in rat intracardiac neurons, it attenuates action potential firing at low concentrations (≤10 μM), primarily through inhibition of I_{K(DR)}. Preliminary reports of some of these results have been published (Hogg and Adams, 1997; Trequattrini et al., 1998b).

Materials and Methods
Cell Preparation. Parasympathetic neurons from neonatal rat intracardiac ganglia were isolated and cultured as described previ-

ABBREVIATIONS: ACh, acetylcholine; I_{K(DR)}, delayed rectifier K^+ currents; I_{M}, muscarine-sensitive K^+ current; TTX, tetrodotoxin; TEA, tetraethylammonium ions; D600, methoxyverapamil hydrochloride; 4-AP, 4-aminopyridine; ChTX, charybdotoxin.
ouslly (Xu and Adams, 1992a). Briefly, 2- to 8-day-old rats were stunned and decapitated, and the hearts were excised and placed in a saline solution containing 140 mM NaCl, 3 mM KCl, 2.5 mM CaCl₂, 0.6 mM MgCl₂, 7.7 mM glucose, and 10 mM histidine, pH 7.2. The atria were separated, and the medial region containing the pulmonary veins and superior vena cava was identified, isolated, and incubated in the above saline solution, containing 1 mg·ml⁻¹ collagenase (Type 2, activity ~200 U·mg⁻¹; Worthington Biochemical Corp., Freehold, NJ). After enzyme digestion, the ganglia were removed, and neurons were dispersed by trituration in a high glucose culture medium [Dulbecco's modified Eagle's medium, containing 10% (v/v) FCS, 100 U·ml⁻¹ penicillin, and 0.1 mg·ml⁻¹ streptomycin] using a fire-polished pasteur pipette. The dissociated neurons were plated onto laminin-coated glass coverslips and incubated at 37°C in a 95% air/5% CO₂ atmosphere for 24 to 72 h. For experimentation, coverslips containing dissociated neurons were transferred to a perfusion chamber (0.5 ml volume) mounted on an inverted microscope, and individual cells were identified under 400× magnification using phase-contrast optics.

**Electrophysiological Recording.** Current and voltage recordings were made using the whole-cell recording configuration of the patch-clamp technique (Hamill et al., 1981). Electrical access to the cell interior was obtained using either the perforated-patch configuration (Horn and Marty, 1988) or the conventional (dialyzed) whole-cell recording configuration for recording voltage-dependent Na⁺ and Ca²⁺ currents. The perforated-patch configuration allows electrical access to the cell interior without the loss of cytoplasmic components, which is important in maintaining functional responses in these cells. A stock solution of 60 mg·ml⁻¹ amphotericin B in dimethyl sulfoxide was prepared on the day of the experiment and was diluted in the pipette solution to yield a final concentration of 240 μg·ml⁻¹ amphotericin B in 0.4% dimethyl sulfoxide. The tip of the pipette first was filled with antibiotic-free solution to prevent any disruption of seal formation and then was backfilled with the amphotericin B-containing solution. Pipettes were pulled from thin-walled borosilicate glass (Clark Electromedical Instruments, Reading, UK) using a Sutter instruments P-87 pipette puller and after fire polishing had resistances of ~1 MΩ. Access resistances using the perforated patch configuration were typically 4 to 8 MΩ before series resistance compensation of 60% to 80%.

Membrane currents and voltages were recorded using an Axopatch 200A patch-clamp amplifier (Axon Instruments Inc., Foster City, CA), filtered to 2 to 10 kHz (−3 dB, Bessel filter), digitized at 10 to 50 kHz (Digidata 1200 interface; Axon Instruments Inc.), and stored on a PC (Pentium, 100 MHz) for viewing and analysis. Voltage and current protocols were applied, and data acquisition and analysis were carried out using pClamp software (Version 6.1.2; Axon Instruments Inc.). Depolarization-activated ionic currents were elicited with voltage steps from a holding potential of −100 to −60 mV to more positive potentials. Capacitive transients were minimized by using analog circuitry of the amplifier, and leak currents were subtracted with a −P/4 protocol. Dose-response curves were obtained by measuring either the peak inward Ba²⁺ current or steady-state outward K⁺ current amplitude at each antagonist concentration, and the experimental data points were fit using the equation:

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + (\frac{[A]}{IC_{50}})} + I_0
\]

where \(I_{\text{max}}\) is the relative current, \([A]\) is the antagonist concentration, \(IC_{50}\) is the concentration giving half-maximal inhibition, \(n\) is the Hill coefficient, and \(I_0\) is the current remaining in the presence of a maximally effective concentration of antagonist.

**Numerical data are presented as the mean ± S.E. (where \(n\) is number of observations).**

**Solutions.** The pipette filling solution for perforated patch experiments contained 75 mM K₂SO₄, 55 mM KCl, 5 mM MgSO₄, and 10 mM HEPES, titrated with N-methyl-d-glucamine to pH 7.2. In the dialyzed whole-cell recording configuration, the pipette solution contained 140 mM KCl, 2 mM MgATP, 10 mM K₁,2-bis(2-aminophenoyl)-ethane-N,N',N''-tetraacetic acid, and 10 mM HEPES-KOH, pH 7.2. The pipette solution used to record voltage-activated Ca²⁺ channel currents using the conventional whole-cell configuration contained 100 mM CsCl, 10 mM Na₁,2-bis(2-aminophenoyl)-ethane-N,N',N''-tetraacetic acid, 2 mM MgATP, 0.2 mM GTP, and 40 mM HEPES-CsOH, pH 7.2. The control extracellular solution contained 140 mM NaCl, 3 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 7.7 mM glucose, and 10 mM HEPES-NaOH, pH 7.2. In experiments to examine \(I_{\text{Kr}}\), 300 mM tetrodotoxin (TTX) and 0.1 mM CdCl₂ were included in the extracellular solution. Depolarization-activated Ca²⁺ channel currents were isolated in the presence of 300 nM TTX, 5 mM tetrathylenamonium ions (TEA)-Cl, and 10 mM BaCl₂. The muscarine-sensitive K⁺ current (M-current \(I_{\text{M}}\)) was isolated as described previously using extracellular solutions containing 3 mM CsCl, 1 mM 4-aminoypyridine, and 300 mM TTX (Cuevas et al., 1997). Experiments were carried out at 22°C except where indicated otherwise. The osmolality of all solutions was monitored with a vapor pressure osmometer (5500; Wescor, Logan, UT) and was in the range of 285 to 295 mmol·kg⁻¹.

**Reagents.** All chemicals used were of analytical grade. All drugs were applied by bath application except where indicated. The drugs methoxyverapamil hydrochloride (D600), (+)-verapamil hydrochloride, acetylcysteine chloride, mecamylamine hydrochloride, apamin, TEA, ATP, and GTP were supplied by Sigma Chemical Co. (St. Louis, MO). 4-Aminopyridine (4-AP) was from Aldrich Chemical Co. (Milwaukee, WI). Charybotoxin (ChTx) was from Auspep (Parkville, Victoria, Australia). D890 (LU 44280) was from Knoell AG-Ludwigshafen. TTX was from Calbiochem-Novabiochem Pty. Ltd.

**Results**

**Effects of Verapamil on Action Potential and Firing Discharge Activity**

The effect of verapamil on action potential firing at 22°C was investigated using the perforated patch recording configuration in >70 neonatal rat intracardiac neurons. The average resting membrane potential was ~52 mV and was unchanged in the presence of 10 to 100 μM verapamil (data not shown). At 22°C, neonatal rat intracardiac neurons respond to suprathreshold depolarizing current pulses with either a slowly adapting or a tonic discharge (Fig. 1A; Cuevas et al., 1997). Bath application of verapamil (≥10 μM) reversibly reduced the number of action potentials in response to a 100-pa depolarizing current pulse (Fig. 1, B and C). Verapamil also altered the action potential waveform, whereby the peak amplitude of the afterhyperpolarization is reduced and a slower rate of depolarization during the interspike interval was observed (Fig. 1D). A mild slowing of the rate of action potential repolarization (14.4 versus 19.6 mV·ms⁻¹) was also consistently observed.

In contrast, at 37°C, no changes in the rate of firing were observed in response to a depolarizing current step in the absence or presence of 100 μM verapamil (Fig. 2, A and B). Phasic action potential firing in rat intracardiac neurons in response to a depolarizing current pulse has previously been attributed to the presence of an \(I_{\text{M}}\) at 37°C (Xi-Moy and Dun, 1995; Cuevas et al., 1997). The application of 100 μM ACh together with 3 μM mecamylamine (to block neuronal nictinic ACh receptor activation) to the cell soma at 37°C inhibits \(I_{\text{M}}\) and induces tonic firing (Fig. 2C). Tonic firing in the presence of ACh is reversibly inhibited in the presence of 100 μM verapamil, as shown in Fig. 2, D and E. Given that verapamil has been reported to competitively antagonize
muscarnic ACh receptors (Karlner et al., 1982; Baumgold, 1986), the effect of verapamil on $I_{\text{M}}$ in the absence and presence of ACh was examined at both 22°C and 37°C. At 22°C, the kinetics of $I_{\text{M}}$ are slowed and its amplitude is reduced, resulting in tonic firing. In five cells, bath application of verapamil (100 μM) did not affect $I_{\text{M}}$ or its inhibition by ACh (100 μM ACh plus 3 μM mecamylamine). ACh reduced $I_{\text{M}}$ by approximately 30% in the absence and presence of 100 μM verapamil at both temperatures ($n = 5$, data not shown).

To assess the effect of a Ca$^{2+}$ channel blocker on action potential firing at 22°C, 0.1 mM Cd$^{2+}$, which completely blocks depolarization-activated Ca$^{2+}$ currents in rat intracardiac neurons (Xu and Adams, 1992b; Jeong and Wurster, 1997), was bath applied and had no effect on firing behavior ($n = 6$, data not shown). Similarly, the K$^+$ channel inhibitors ChTX (100 nM), 4-AP (0.5 mM), apamin (30–100 nM), and TEA (1 mM) also had no effect on action potential firing (Cuevas et al., 1997). The effect of verapamil on voltagedependent Na$^+$ currents was also examined in rat intracardiac neurons, but these currents were unaffected by bath application of 10 to 100 μM verapamil ($n = 4$; data not shown).

**Effects of Verapamil on Voltage-Activated Ca$^{2+}$ and K$^+$ Channel Currents**

The effect of verapamil on the high voltage-activated Ca$^{2+}$ channel current in rat intracardiac neurons is shown in Fig. 3A. Bath application of 10 to 300 μM verapamil reversibly inhibited the Ba$^{2+}$ current elicited by step depolarization from −100 mV to +20 mV. Verapamil inhibits the Ba$^{2+}$ current in a concentration-dependent manner with an IC$_{50}$ value of 78 μM ($n \geq 4$; Fig. 3B). The inward Ba$^{2+}$ current remaining in the presence of 0.3 to 1 mM verapamil was completely inhibited by 100 μM Cd$^{2+}$ (Fig. 3A).

Bath application of verapamil inhibited $I_{\text{KDR}}$ in a concentration-dependent manner as shown in Fig. 4A. Verapamil induced a rapid, dose-dependent decay of the current to a new steady-state level without affecting its rising rate, suggesting a state-dependent block. A dose-response curve was obtained by applying depolarizing voltage pulses of sufficient duration (225–500 ms) to reach steady-state block in the presence of varying concentrations of verapamil. Half-maximal inhibition of $I_{\text{KDR}}$ by verapamil occurred at 11 μM ($n \geq 3$; Fig. 4B), which is approximately 7-fold more potent than that for verapamil inhibition of high voltage-activated Ca$^{2+}$ channel currents.

The effect of the related phenylalkylamines, D600 and its permanently charged quaternary derivative D890, on action potential firing and $I_{\text{KDR}}$ was also examined. Bath application of 100 μM D890 did not affect firing in response to a depolarizing current pulse (Fig. 5B), whereas 100 μM D600 attenuated discharge activity (Fig. 5C). The relative abilities of 100 μM D600 and D890 to inhibit $I_{\text{KDR}}$ are compared in Fig. 5D. Under voltage-clamp conditions, the amplitude of $I_{\text{KDR}}$ elicited on depolarization to +80 mV was reduced in the presence of D600 but not D890 (Fig. 5D). The ability of the phenylalkylamines to reduce action potential firing appears to correlate with their potency to inhibit $I_{\text{KDR}}$.

**Verapamil Block of Delayed-Rectifier K$^+$ Currents**

Given that verapamil block of $I_{\text{KDR}}$ appears to underlie the inhibition of action potential firing, the characteristics of verapamil block of $I_{\text{KDR}}$ were investigated. Figure 6 shows the effects of 30 μM verapamil on $I_{\text{KDR}}$ evoked by depolarizing pulses from a holding potential of −80 mV to test potentials between −40 and +80 mV. As also shown at voltages positive to 0 mV (see Fig. 4A), verapamil caused a dramatic change in the time course of $I_{\text{KDR}}$; the current activated to a maximum then decayed with time to a small fraction of its peak value (Fig. 6B). In contrast, no change was observed in the activation kinetics of the current. The decay of the current was well fitted by a single exponential.

Figure 6C shows the activation curve of the $I_{\text{KDR}}$ (filled symbols) together with the steady-state fractional block of the current as a function of the membrane potential (open
Block increased steeply between -20 mV and +20 mV, the voltage range of channel activation, whereas it was approximately stable at membrane potentials more positive than +40 mV, where all channels are open. This indicates that block may be directly linked to the gating process (compare with Snyders et al., 1991), as expected for a state-dependent block in which the drug can bind to the channel only in the open state according to the kinetic scheme:

\[ C \xrightarrow[\alpha]{k_{on}[B]} O \xrightarrow[\beta]{k_{off}} O \cdot B \] (2)

where C, O, and O-B are the closed, open, and open-blocked states; [B] is the blocker concentration; and \( \alpha, \beta, k_{on}, \) and \( k_{off} \) are the rate constants for the transitions between states. A prediction of this model is that the rate of current decay is dependent on the blocker concentration. This prediction is tested by an experiment of the type shown in Fig. 4A. The plot shown in Fig. 6D demonstrates that the decay rate (1/\( \tau \)) exhibits a linear dependence on verapamil concentration.

**Kinetic Constants of Verapamil Block**

The block and unblock rate constants, \( k_{on} \) and \( k_{off} \), were estimated from the verapamil-induced current decay (Fig. 6D) as previously described for verapamil block of I_{K(DR)} (Rampe et al., 1993; Trequattrini et al., 1998a). Interference from the concurrent inactivation process (\( \tau \approx 3-4 \) s) can be neglected compared with the much faster current decay (\( \tau_{b} < 150 \) ms) induced by verapamil. Given that at high depolarizing voltages (+80 mV) channel activation occurs with a time constant (~4 ms) more than 3-fold lower than \( \tau_{b} \), the decay of the current in the presence of verapamil represents the process of channel block. Under these conditions, block and unblock rate constants can be estimated using the relation 1/\( \tau_{b} = (k_{on}[B] + k_{on}) \), where \( \tau_{b} \) is the time constant of verapamil-induced current decay. Using this relation, \( k_{on} \) and \( k_{off} \) values are obtained from the slope and the intercept, respectively, of the linear regression of 1/\( \tau \) versus verapamil concentration. The block and unblock rate constants obtained from five neurons were 0.67 ± 0.04 ms^{-1} \cdot \mu M^{-1} and 0.0075 ± 0.008 ms^{-1}, respectively. These rate constants give a value of
the addition of 30 activation process from block. The block rate constants, after would rapidly activate all channels, thus uncoupling the maximal under control conditions (Fig. 7A). This protocol pulse (120 mV, for 25 ms) where channel activation was 1 mV (15-mV steps) were preceded by a depolarizing pre-

trini et al., 1996), test pulses to voltages between introduced by the shift in gating equilibrium (see Trequat-
errors in the measurement of the voltage dependence of block

rate constants

Voltage Dependence of Block and Unblock Rate Constants

The voltage dependence of verapamil block was assessed from the rate of block, 1/τb, and from the steady-state block, IVerapamil/IControl, at different voltages. To minimize possible errors in the measurement of the voltage dependence of block introduced by the shift in gating equilibrium (see Trequat-

in the absence (control) and presence of verapamil. Verapamil concentra-
tions were applied cumulatively from 3 to 100 μM to the same neuron. Holding potential, −80 mV. B, dose-response relationship obtained for verapamil steady-state inhibition of normalized I K(DR) amplitude obtained by voltage steps to +80 mV from a holding potential of −80 mV. Data points represent mean ± S.E. obtained from at least three cells. Continuous line represents the best fit of the data by eq. 1 with an IC50 value of 11 μM (Hill coefficient = 0.9).

11 μM for the dissociation constant (Km = koff/kon), consistent with that obtained from steady-state measurements.

Verapamil (1–100 μM) reversibly attenuates tonic action potential firing observed in neonatal rat intracardiac neu-
rons both at 22°C and after muscarinic receptor stimulation at 37°C, which may occur in vivo during prolonged vagal stimulation. Verapamil, however, has no effect on rapidly adapting firing observed in response to depolarizing current step, but in the presence of ACh or a muscarinic receptor agonist, I M is depressed and a depolarizing current step elicits tonic action potential firing. I M is largely inactivated at 22°C, and in response to membrane depolarization, neurons fire repeti-
tively. Verapamil has been reported to antagonize the bind-
ing of agonists to muscarinic ACh receptors in rat brain (Baumgold, 1986; Katayama et al., 1987) and heart (Karliner et al., 1982) and muscarinic ACh receptor activation of K+ currents in guinea pig atrial myocytes (Ito et al., 1989). In rat intracardiac neurons, verapamil failed to have any effect on I M in the absence or presence of ACh, suggesting that vera-
Verapamil inhibition of ACh-induced tonic firing observed in these neurons at 22°C and 37°C is not due to antagonism of muscarinic receptor activation. The inhibition of ACh-induced firing by verapamil appears to involve a verapamil-sensitive current other than I_Na that may also modulate neural excitability in rat intracardiac ganglia.

The inhibition of discharge activity is not due to block of voltage-gated Ca^{2+} or Ca^{2+}-dependent K^{+} channels because firing is not affected by 0.1 mM Cd^{2+}, 100 nM ChTX, 0.5 mM 4-AP, 30–100 nM apamin, and 1 mM TEA. Given that voltage-dependent Na^{+} currents and I_Na are also unaffected by verapamil, inhibition of the discharge activity in these neurons by verapamil most likely occurs through block of I_{K_{DR}}. Inhibition of I_{K_{DR}} by verapamil occurs with an IC_{50} value of 11 μM, which is similar to that determined in chick DRG neurons (4 μM; Trequattrini et al., 1996) and for delayed rectifier K^{+} channels cloned from human heart (21 μM; Rampe et al., 1993).

An effect attributable to I_{K_{DR}} inhibition is the reduced amplitude of the action potential afterhyperpolarization, which will slow the rate of Na^{+} channel (voltage-dependent) recovery from inactivation and increase the threshold for action potential firing. This effect of verapamil on Na^{+} current time course, secondary to I_{K_{DR}} inhibition, is consistent with a slower rate of depolarization and reduced overshoot of the subsequent action potential (see Fig. 1). The slower rate of depolarization associated with the increased interval between action potentials observed in the presence of verapamil may also be due, in part, to inhibition of the voltage-activated Ca^{2+} conductance, which would be reduced ~15% by 10 μM verapamil. An IC_{50} value of 78 μM was obtained for verapamil inhibition of the depolarization-activated Ba^{2+} current, indicating that verapamil is approximately 7-fold more potent in inhibiting I_{K_{DR}} compared with high voltage-activated Ca^{2+} channel currents.

The phenylalkylamine D600, but not the permanently charged D890, inhibited tonic action potential firing at 22°C with similar potency to their inhibitory effects on I_{K_{DR}}. The effectiveness of the different phenylalkylamines to block I_{K_{DR}} also correlates with their ability to cross the plasma membrane, suggesting that the binding site on the I_{K_{DR}} channel is not located on the external part of the channel. Externally applied verapamil has been proposed to reach an internal binding site by partitioning into the lipid phase of the membrane (DeCoursey, 1995). Furthermore, the verapamil binding site on I_{K_{DR}}, channels has been identified as part of the inner mouth of the K^{+} channel pore (Rampe et al., 1993).

The block of I_{K_{DR}} by verapamil is consistent with a state-dependent block in which the drug binds preferentially to the open state of the I_{K_{DR}} channel as reported previously (Rampe et al., 1993; DeCoursey, 1995; Trequattrini et al., 1996). However, the biexponential time course of recovery from block (see Fig. 7C) is not compatible with this simple three-state model unless an additional state, into which open-blocked channels can pass, is included. Previous studies have suggested such an additional state to be either an inactivated-blocked state (DeCoursey, 1995) or a closed-blocked state (Armstrong, 1971; Trequattrini et al., 1998a). The block of I_{K_{DR}} in rat intracardiac ganglion neurons has similar kinetics to that seen in chick embryo DRG neurons (Trequattrini et al., 1998a) and rat alveolar epithelial cells (DeCoursey, 1995).

Inhibition of action potential firing in parasympathetic neurons of intracardiac ganglia may be expected to reduce the bradycardia associated with vagal nerve stimulation in vivo, leading to tachycardia. Verapamil has been shown to cause a concentration-dependent increase in heart rate in conscious dogs (Nakaya et al., 1983) and has been reported to inhibit the bradycardic response to stimulation of the right vagal nerve in anesthetized neonatal pigs (Lee et al., 1985). Tachycardia, which is often observed on administration of verapamil to conscious animals, can be inhibited by autonomic block with atropine and propranolol but not with pro-
pranolol alone (Nakaya et al., 1983). The present data indicate that verapamil-induced inhibition of action potential firing in parasympathetic neurons may contribute, in part, to verapamil-induced tachycardia.

Fig. 7. Voltage dependence of verapamil block of $I_{K(DR)}$. A, $I_{K(DR)}$ elicited in response to a 25-ms depolarizing pulse to $+120$ mV from a holding potential of $-80$ mV, followed by a test pulse varying from $+105$ to $+45$ mV in 15-mV increments. Same stimulus protocol as in control after the addition of 30 µM verapamil. B, verapamil block [■] and unblock [■] rate constants, calculated from decay rate of the current and steady-state of block, respectively (n = 4), are plotted against membrane potential. Data are expressed as mean ± S.E.; error bars are within symbols. C, a double-pulse protocol used to assess recovery of $I_{K(DR)}$ after block by verapamil as function of time. A 200-ms conditioning pulse to $+80$ mV was separated from a second test pulse by a variable period during which the cell was repolarized to $-80$ mV. The percent of peak current recovered ($I_{peak}/I_{conditioning}$) is plotted against interpulse duration (n = 4 for each point). Data points were fitted by a double-exponential function giving time constants of $\tau_r = 0.4$ s and $\tau_f = 12.2$ s, respectively. Data are expressed as mean ± S.E.; error bars are within the symbols.