0026-895X/93/030642-07$03.00/0

MOLECULAR PHARMACOLOGY, 44:642-648

Verapamil Blocks a Rapidly Activating Delayed Rectifier K^+ Channel Cloned from Human Heart

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Received April 6, 1993; Accepted June 9, 1993

SUMMARY

Verapamil is an antagonist of L-type Ca^{2+} channels, and part of its binding site is located in the sixth transmembrane segment (S_6) in the fourth repeat of the protein. Verapamil also blocks K^+ channels, which are members of the same supergene family as Ca^{2+} channels. We examined the effects of verapamil on a rapidly activating delayed rectifier K^+ channel (designated fHK) cloned from human heart. Verapamil inhibited $^{86}Rb^+$ efflux from fHK-transfected human embryonic kidney cells with an EC_{50} of 4.5×10^{-5} M. Whole-cell patch-clamp experiments revealed that verapamil induced a rapid component of fHK current inactivation but was without effect on activation. The effect was concentration and voltage dependent and was attributed to open channel blockade. The apparent association and dissociation rate constants measured at +50 mV were about 1.65×10^5 M^{-1} sec^{-1} and 3.48 sec^{-1}, respectively. S_6 of fHK has significant homology to that portion of the verapamil binding site identified in Ca^{2+} channels, and S_6 is thought to form part of the inner mouth of K^+ channel pores. The data support a role for verapamil as a blocker of the inner pore of voltage-dependent K^+ channels in human myocardium.

Voltage-dependent K^+ channels are important in controlling electrical activity in a wide variety of excitable cells. In the heart, these channels play a major role in determining the duration of the cardiac action potential. For this reason, voltage-dependent K^+ channels are currently being examined as targets for antiarrhythmic drug therapy (1, 2). However, pharmacological studies of voltage-dependent K^+ channels are complicated by the existence of multiple channel types. Furthermore, these channels may be regionally distributed within the heart and show species specificity (for review, see Ref. 3).

In an attempt to overcome some of these difficulties we have cloned a rapidly activating delayed rectifier K^+ channel (designated fHK) from human heart and expressed this channel in a human embryonic kidney cell line (4). This channel is highly homologous to hPCN1, originally cloned from a human insulinoma (5), and to HK2, a delayed rectifier channel cloned from human ventricle (6).

Voltage-dependent K^+ channels belong to the same gene family as Ca^{2+} and Na^+ channels (7, 8). Certain K^+ channels have been reported to be sensitive to verapamil, a Ca^{2+} channel antagonist (9). The present study was undertaken to examine the effects of verapamil on fHK and to explore its mechanism of action. We established that verapamil blocks open fHK channels from the cytoplasmic surface in a manner similar to its blockade of Ca^{2+} channels; in addition, fHK channels contain a sequence that is homologous to the sequence of a verapamil binding site in Ca^{2+} channels (10). The locations of these sequences in topographical models of K^+ and Ca^{2+} channels are similar (10), and in K^+ channels S_6 may line the inner mouth of the ion conduction pathway or pore (11, 12).

Materials and Methods

Cell culture. The human embryonic kidney cell line HEK was used for all experiments. These cells were transfected with a human fetal heart K^+ channel cDNA as described previously (4). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO BRL, Grand Island, NY), in an atmosphere of 95% air/5% CO_2. This medium also contained penicillin-streptomycin-fungizone and G418 (0.5 mg/ml; GIBCO BRL).

$^{86}Rb^+$ efflux assays. $^{86}Rb^+$ efflux experiments were carried out essentially as described previously (13). Briefly, transfected and untransfected HEK cells were grown to confluency in 35-mm culture dishes and were incubated with 2–5 μCi of $^{86}RbCl$ (New England Nuclear, Boston, MA) for 18–20 hr. After this time the cells were removed from the incubator and washed three times with PSS containing the following (in mM): NaCl, 140; KCl, 2.5; $MgCl_2$, 1.0; $CaCl_2$, 1.8; and glucose, 10; pH 7.4 with Tris base. After this wash 3 ml of PSS were added to the dishes, and the cells were allowed to equilibrate at room temperature for 4 min. After this time two 100-μl aliquots were taken from each dish (time 0). After this, 60 μl of PSS (final KCl concentration, 2.5 mM) or 60 μl of 2.5 M KCl (final KCl concentration,

ABBREVIATIONS: S_6, sixth transmembrane segment; HEK, HEK-293; PSS, physiological saline solution; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

55 mM) were added to the dishes. After 1 min, four 100-μl aliquots were taken and the remaining solution was suctioned off. The cells were then digested with 0.5% NaOH to determine the amount of radioactivity associated with the cells at the end of the flux experiments. Radioactivity was quantitated using liquid scintillation counting. $^{86}Rb^+$ efflux was expressed as the percentage of radioactivity released during the 1-min flux period. The IC_{50} value for verapamil was obtained by nonlinear least-squares fit of the data (GraphPAD Software, San Diego, CA).

Electrophysiological experiments. HEK cells (J10 subclone) transfected with fHK were seeded on glass coverslips 24–48 hr before electrophysiological recording. Whole-cell ion currents were recorded at room temperature via the gigaseal patch-clamp technique, as described by Hamill *et al.* (14), using an Axopatch-1D amplifier (Axon Instruments, Burlingame, CA). Electrodes were fashioned from TW150 glass capillary tubes (World Precision Instruments, New Haven, CT) and had resistances of 2–3 MΩ when filled with internal solution. Pipettes used for intracellular recording were filled with the following solution (in mM): potassium aspartate, 120; KCl, 20; Na_2ATP, 4.0; HEPES, 5.0; $MgCl_2$, 1.0; pH 7.2 with KOH. The external recording solution contained the following (in mM): NaCl, 130; KCl, 5.0; sodium acetate, 2.8; $MgCl_2$, 1.0; HEPES, 10; glucose, 10; and $CaCl_2$, 1.0; pH 7.4 with 1 N NaOH. Series resistance was compensated after rupture of the seal, to provide the fastest possible capacity transient without ringing. Cell currents were conditioned by a four-pole low-pass filter with a cutoff frequency of 1 kHz. Currents were stored and analyzed using a Compaq Deskpro computer and pCLAMP software (Axon Instruments).

Results

Fig. 1A shows the effects of K^+ stimulation on $^{86}Rb^+$ efflux in HEK cells. $^{86}Rb^+$ efflux in untransfected cells averaged about 1% of total and, furthermore, was not enhanced when cells were stimulated for 1 min with high (55 mM) extracellular K^+. In contrast, HEK cells that were transfected with fHK released 55.7 ± 4.3% (four experiments) of their total $^{86}Rb^+$ upon exposure to 55 mM extracellular K^+. We found that this $^{86}Rb^+$ efflux could be inhibited by verapamil at concentrations of ≥3 μM (Fig. 1B). The IC_{50} value for verapamil inhibition of K^+-stimulated $^{86}Rb^+$ efflux in these cells was 4.5×10^{-5} M.

We next turned to the whole-cell patch-clamp technique to directly assess the effects of verapamil on fHK current. Fig. 2 shows the effects of 50 μM verapamil on fHK current induced by a 1-sec depolarizing pulse to +30 mV from a holding potential of −80 mV. As can be seen, the decay rate of fHK was accelerated in the presence of verapamil. When measured at the end of a 1-sec pulse (steady state) to +30 mV, 50 μM verapamil inhibited fHK current by 63 ± 6% (five experiments). These effects took approximately 2 min to reach equilibrium and were reversible upon washing of the cell with drug-free solution.

Fig. 3 shows the effects of verapamil on fHK current inactivation at +30 mV during a prolonged (10-sec) pulse. Under control conditions, inactivation was well fitted to a single-exponential function, with a time constant of 3386 ± 278 msec (four experiments). After verapamil (50 μM) treatment, inactivation was clearly a biexponential process, with fast and slow time constants of 204 ± 31 msec and 3817 ± 426 msec (four experiments), respectively. The fast, drug-induced component of inactivation comprised 77% of the total current under these conditions. Although verapamil accelerated inactivation, it had no apparent effect on current activation. Fig. 4 shows the effects of verapamil on fHK current activation. At a test potential of

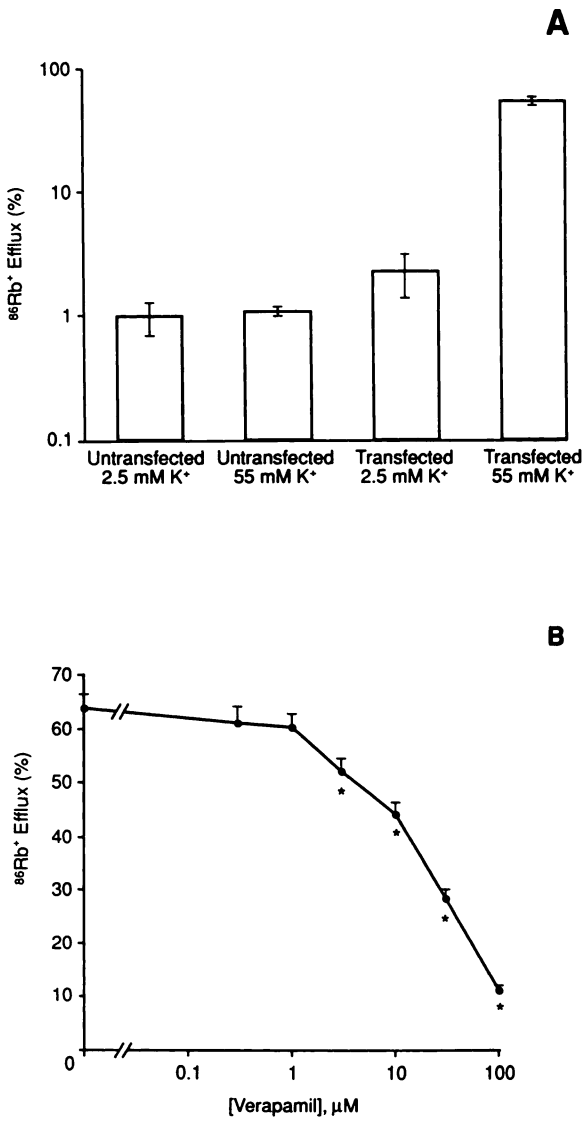

Fig. 1. Effects of verapamil on $^{86}Rb^+$ efflux in HEK cells. A, Effects of extracellular K^+ levels on $^{86}Rb^+$ efflux from control HEK cells and those transfected with fHK. The percentage of $^{86}Rb^+$ efflux from the cells was measured over a 1-min time interval, as described in Materials and Methods, and is plotted on a logarithmic scale. B, Dose-response relationship for verapamil inhibition of $^{86}Rb^+$ efflux from fHK-transfected HEK cells stimulated with 55 mM extracellular K^+. *, Significantly different from efflux in the absence of verapamil ($p < 0.02$, t test). *Error bars*, standard errors (four experiments). The IC_{50} value for verapamil was 4.5×10^{-5} M.

+50 mV, fHK current activated with a time constant of 2.03 ± 0.14 msec (four experiments). After the addition of 50 μM verapamil this value was not significantly changed and averaged 1.95 ± 0.11 msec.

Fig. 5 illustrates the effects of verapamil on the fHK current-voltage (I-V) relationship. Verapamil (20 μM) reduced current throughout the I-V curve, when measured at steady state (Fig. 5C). This steady state block exhibited a shallow voltage dependence at test potentials of +30 mV and higher (Fig. 5D). Inhibition of fHK current by verapamil was significantly ($p < 0.01$, t test) greater at +60 mV than at +30 mV (nine experiments).

We next examined whether verapamil displayed any fre-

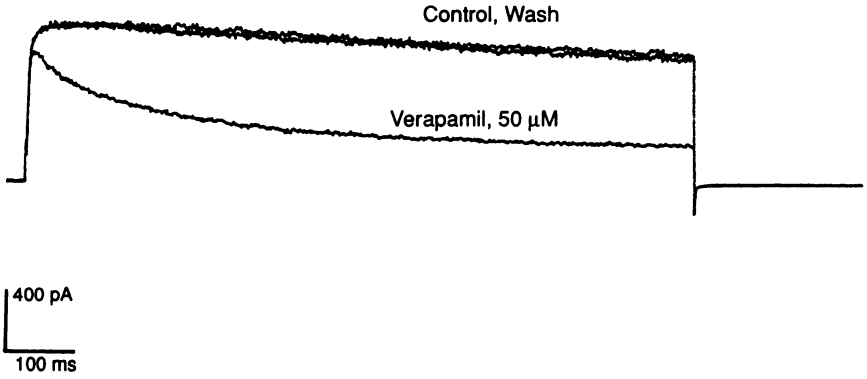

Fig. 2. Effects of verapamil on whole-cell K^+ currents in fHK-transfected HEK cells. Currents were elicited by a 1-sec test pulse to +30 mV from a holding potential of −80 mV. Control current, current in the presence of 50 μM verapamil, and current after washout are indicated. Washout took approximately 2 min (chamber volume, 500 μl; flow rate, 3 ml/min). When measured at the end of the 1-sec pulse, verapamil (50 μM) inhibited current by 63 ± 6% (five experiments).

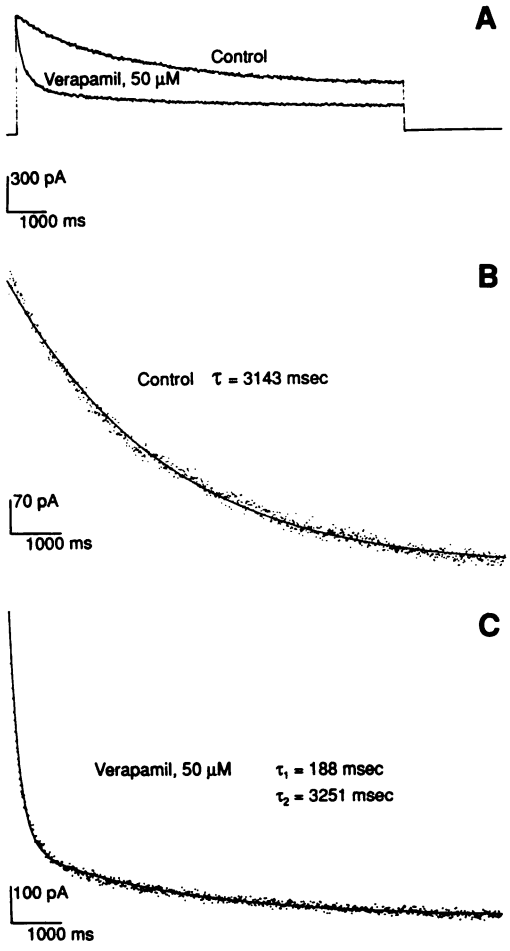

Fig. 3. Effects of verapamil on fHK inactivation kinetics. A, Whole-cell currents were elicited by a 10-sec depolarizing pulse to +30 mV from a holding potential of −80 mV under control conditions and after the addition of 50 μM verapamil. B, Inactivation under control conditions was well fitted by a single-exponential decay with a time constant of approximately 3 sec. C, After verapamil treatment current inactivation was greatly enhanced and could be fit as the sum of two exponentials, a fast drug-induced component and a much slower component similar to control.

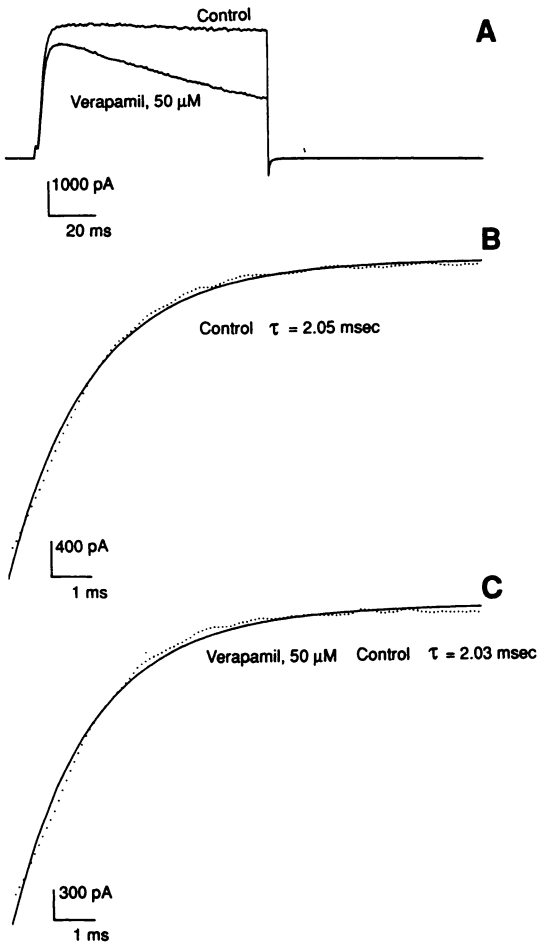

Fig. 4. Effects of verapamil on fHK current activation. A, Currents were induced by 100-msec clamp pulses to +50 mV in the presence or absence of 50 μM verapamil. B and C, Current activations in the absence (B) and presence (C) of 50 μM verapamil were fit to single exponentials.

quency-dependent effects on fHK current. Fig. 6A shows control currents elicited by 100-msec test pulses to +50 mV from a holding potential of −80 mV at a frequency of 10 Hz. At this frequency, control currents decreased by 4.0 ± 1.4% between pulses 1 and 20 (five experiments). After an approximately 2-

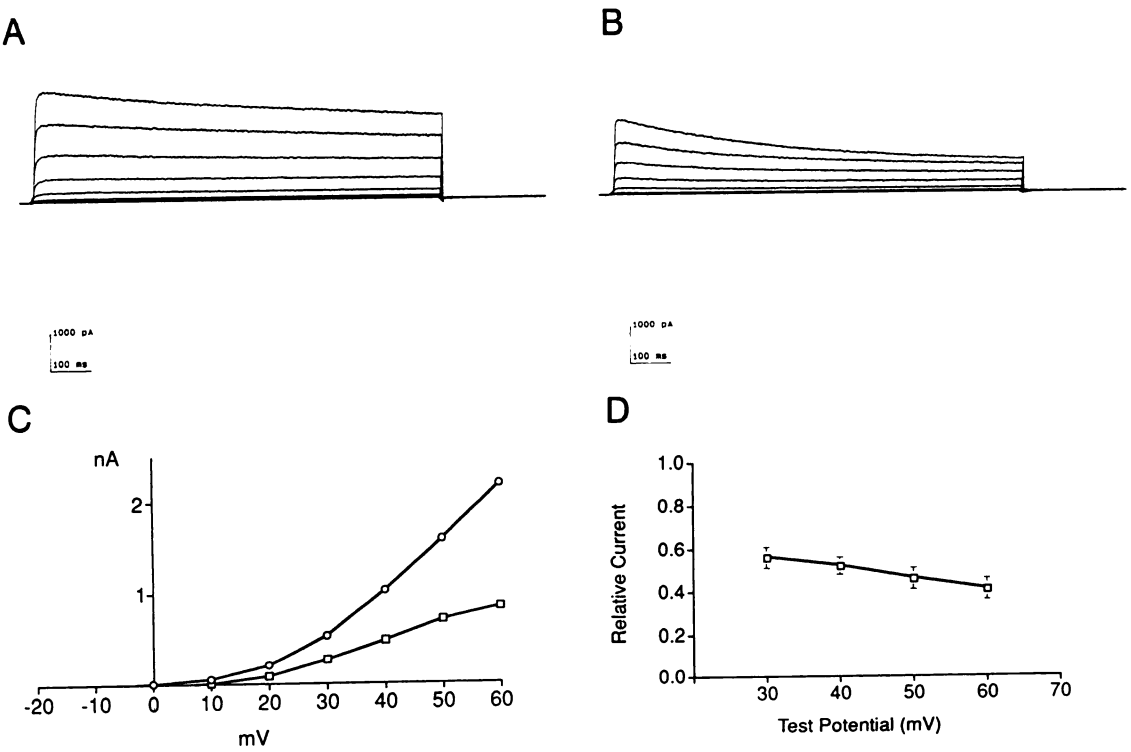

Fig. 5. Effects of verapamil on fHK current-voltage (I-V) relationship. A and B, Currents elicited by 1-sec test pulses to potentials between −20 and +60 mV under control conditions (A) and after the addition of 20 μM verapamil (B). C, Resultant I-V relationships taken at the end of the test pulses. ●, Control data; □, current after addition of verapamil. D, Inhibition of fHK current by verapamil at test potentials of +30 mV to +60 mV. *Error bars*, standard errors (nine experiments).

min exposure to 10 μM verapamil, we repeated this pulse protocol. When measured at the end of pulse 1, verapamil decreased current by 24.0 ± 4.4%, compared with control. Furthermore, an additional 23.6 ± 1.9% decrease in current was observed by the end of pulse 20. Most of this additional block was apparent within the first four or five pulses (Fig. 6, B and C) and was due to equilibration of verapamil with the open state during the train of pulses.

The rapid component of inactivation induced by verapamil (10–100 μM) was much faster (approximately 10-fold or more) than that observed in the absence of drug. Therefore, we used this drug-induced time constant (τ_D) as an approximation of the drug-channel interaction kinetics, as described previously (15) and as calculated according to the equation $1/\tau_D = k[D] + L$, where [D] is the concentration of verapamil and k and L are the apparent association and dissociation rates, respectively, for the drug. A plot of $1/\tau_D$ versus verapamil concentration at a test potential of +50 mV is shown in Fig. 7. The slope and intercept of the least squares fit to the data resulted in an apparent association rate k of 1.65×10^5 M^{-1} sec^{-1} and an apparent dissociation rate L of 3.48 sec^{-1}. The apparent K_d value, L/k, was 21.1 μM. The k, L, and K_d values obtained at a test potential of +40 mV were 1.02×10^5 M^{-1} sec^{-1}, 3.33 sec^{-1}, and 32.6 μM, respectively. Finally, these values were 4.87×10^4 M^{-1} sec^{-1}, 3.13 sec^{-1}, and 64.3 μM, respectively, when examined at a test potential of +30 mV.

We evaluated the effects of 50 μM verapamil at +50 mV with a simple three-state Markov model (16), ignoring the slower inactivation present in control currents. Fig. 8 shows fHK current before and after the addition of 50 μM verapamil overlaid on the corresponding computer-derived model. The values obtained with the model gave rate constants similar to those measured experimentally.

Discussion

Verapamil is widely used clinically to control a number of arrhythmias, especially those of supraventricular origin (17). This antiarrhythmic property undoubtedly arises mainly from the ability of verapamil to block L-type Ca^{2+} channels in specialized areas like the atrioventricular node. However, verapamil blockade of other cardiac ion channels, including delayed rectifier K^+ channels, has been reported. In calf Purkinje fibers, verapamil (10 μM) has been shown to partially block delayed outward currents (18). The verapamil analogue D600 (2 μM) produced a modest block of outward K^+ currents in cat ventricular muscle preparations (19). In frog atrial cells, K^+ currents were blocked by D600 but only at high (10^{-4} M to 10^{-3} M) concentrations (9). The existence of multiple classes of K^+ channels within a preparation and possible species-dependent variations in K^+ channel types complicate such studies. For these reasons, we have examined the effects of verapamil on a rapidly activating delayed rectifier K^+ channel cloned from human heart.

Both $^{86}Rb^+$ efflux studies and whole-cell patch-clamp experiments confirmed an inhibitory action of verapamil on fHK over the concentration range of 3–100 μM. Verapamil did not alter fHK current activation but dramatically accelerated inactivation. The data indicate that verapamil acts as an open channel blocker of fHK, and the Markov model presented in

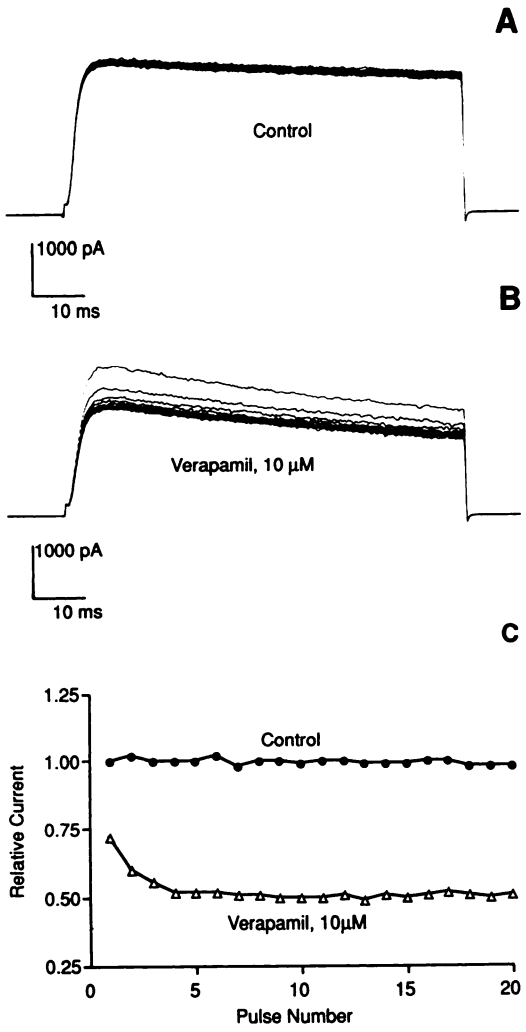

Fig. 6. Frequency-dependent effects of verapamil on fHK. A, Whole-cell current was induced by test pulses to +50 mV from a holding potential of −80 mV at a frequency of 10 Hz. A, Train of 20 pulses; current relative to pulse 1 is plotted (●) in C. B, After equilibration of the cell with 10 μM verapamil (a process that took approximately 2 min and was monitored at a pulse frequency of 0.05 Hz) the pulse train was repeated as described for A; current relative to control pulse 1 is plotted (Δ) in C. All currents were measured at the end of the 100-msec pulse.

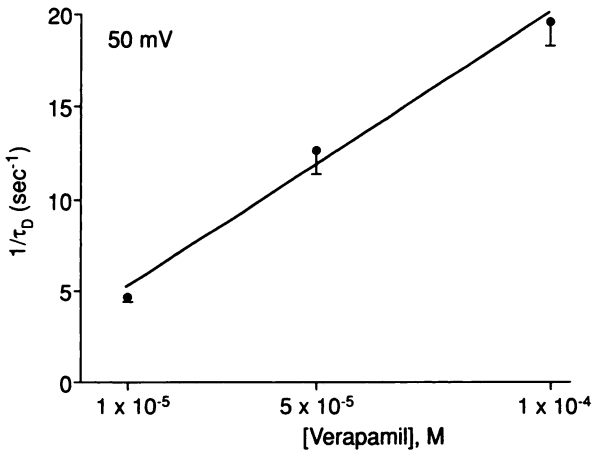

Fig. 7. Kinetics of verapamil block of fHK. The reciprocal of the fast drug-induced time constant ($1/\tau_D$) is plotted against verapamil concentration, according to the equation given in the text. The data shown are for currents induced by a 1-sec test pulse to +50 mV. Least squares fit of the data resulted in an apparent association rate constant (k) of 1.65×10^5 M^{-1} sec^{-1} and an apparent dissociation rate constant (L) of 3.48 sec^{-1}. The K_D value (k/L) was 21.1 μM. *Error bars*, standard error (four or five experiments).

Fig. 8 supports this interpretation. Open channel block of K^+ currents by verapamil has been reported in human lung carcinoma cells (20) and human tracheal cells (21). Because verapamil is membrane permeable and is positively charged at intracellular pH values (22), we feel that access to the channel is gained from the cytoplasm. The voltage dependence of the actions of verapamil and the enhancement of its association rate constant (k) with increasing membrane potential are consistent with this notion.

The effects of verapamil and other phenylalkylamines on L-type Ca^{2+} channels have been extensively studied. In heart cells D600 has been shown to have no effect on Ca^{2+} current activation but to accelerate its decay rate (23). These observations, combined with the ability of D600 to decrease the mean open time of single Ca^{2+} channels (24), demonstrate the ability of the phenylalkylamines to block the open state of the Ca^{2+} channel. The phenylalkylamines have also been shown to block L-type Ca^{2+} channels in a use-dependent manner (22, 25). The effects of permanently charged analogues of verapamil suggest an association of these compounds with the intracellular face of the channel (24). Finally, the association rate constant for D600 with the open state of the Ca^{2+} channel (6.16×10^4 M^{-1} sec^{-1}) is close to the values reported here for the fHK channel (26). Thus, in a number of respects the interactions of verapamil and related compounds with Ca^{2+} channels are similar to those observed here with fHK.

Because a region of the verapamil binding site has been localized to S_6 in Ca^{2+} channels and because S_6 may form part of the inner mouth of K^+ pores (11, 12), we searched fHK for the verapamil sequence. A sequence of significant homology (27) was identified at the carboxyl-terminal end of S_6 of fHK, the same region as identified in Ca^{2+} channels (8) (Fig. 9). We therefore suggest that this region may form part of the verapamil binding site in the pore of fHK. However, this sequence cannot by itself form the verapamil binding site. Thus, N-type Ca^{2+} channels are highly homologous to L-type Ca^{2+} channels in S_6 but cannot be blocked by verapamil. Moreover, another cardiac K^+ channel, KV2·1, has no significant homology in S_6 but is blocked by verapamil with the same potency as fHK.[1]

In humans, verapamil is infused in doses of 5–10 mg, intravenously, to control supraventricular tachyarrhythmias. After infusion, plasma levels of verapamil can reach into the low micromolar range (28, 29), and it is likely that localized levels in the heart during infusion are even higher. Still higher levels can be seen upon oral dosing in patients with liver disease (30) or in cases of overdose (31). Thus, plasma concentrations similar to those needed to block fHK are observed in a variety of clinical settings. Furthermore, block of cardiac K^+ channels by verapamil is likely to be enhanced during tachyarrhythmias due to the frequency dependence of its action. Speculatively, blockade of delayed rectifier K^+ currents in the human heart could attenuate the shortening of the action potential duration

[1] D. J. Arnold, W. J. Crumb, B. Wible, and A. M. Brown. Pharmacological properties of human cardiac K^+ channels. Manuscript in preparation.

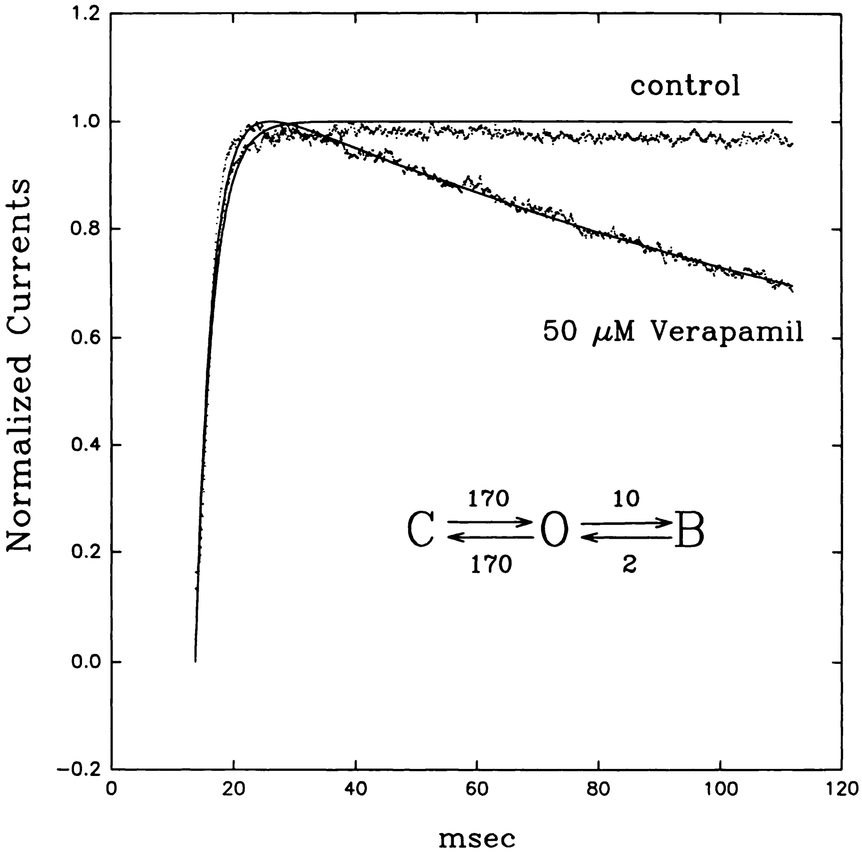

Fig. 8. Three-state Markov chain model of verapamil blocking kinetics for fHK. Rate constants are in sec^{-1}. The currents were measured at +50 mV in the presence and absence of 50 μM verapamil. The fit in control is offset slightly because the slow inactivation of fHK control currents (Fig. 3A) has been ignored.

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                                           Pore
                                  ______________________________   _____
fHK     AVYFAEADNQ  GTHFSSIPDA  FWWAVVTMTT  VGYGDMRPIT  VGGKIVGSLC      500
                                                              :
verap   ----------  ----------  ----------  --EEYTCGTN  FAYYYFISFY

             S6
        ____________________
fHK     AIAGVLTIAL  PVPVIVSNFN  YFYHRETDHE  EPAVLKEEQG  TQSQGPGLDR      550

              : : :   : ::  ::    :
verap   MLCAFLIINL  FVAVIMDNFD  YLTRDW----  ----------  ----------

fHK     GVQRKVSGSR  GSFCKAGGTL  ENADSARRGS  CPLEKCNVKA  KSNVDLRRSL      600

fHK     YALCLDTSRE  TDLX                                               614
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Fig. 9. Alignment of the proposed calcium channel verapamil binding site with fHK sequences. The 44-residue peptide from the α1 subunit of the skeletal muscle calcium channel (*verap*) that was identified as forming part of the verapamil binding site (10) is compared with fHK. :, Identical residues. *Thin bars*, putative pore and transmembrane S_6 regions of fHK. For verapamil binding to Ca^{2+} channels, S_6 and six residues beyond S_6 are thought to be most important (32).

caused by the inhibition of L-type Ca^{2+} channels by verapamil (9).

To our knowledge, this report is the first to examine the effects of verapamil on a delayed rectifier K^+ channel cloned from human heart. We find that verapamil inhibits this channel in a manner consistent with blockade of open channels. The results suggest that under certain clinical circumstances verapamil can act as a K^+ channel antagonist in human heart. The study of ion channels cloned from human cardiac tissue promises to aid in the development of new antiarrhythmic agents.

References

1. Colatsky, T. J., C. H. Follmer, and C. F. Starmer. Channel specificity in antiarrhythmic drug action: mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmias. *Circulation* **82:**2235–2242 (1990).
2. Hondeghem, L. M., and D. J. Snyders. Class III antiarrhythmic agents have a lot of potential but a long way to go: reduced effectiveness and dangers of reverse use dependence. *Circulation* **81:**686–690 (1990).
3. Anumonwo, J. M. B., L. C. Freeman, W. M. Kwok, and R. S. Kass. Potassium channels in the heart: electrophysiology and pharmacological regulation. *Cardiovasc. Drug Rev.* **9:**299–316 (1991).
4. Fedida, D., B. Wible, Z. Wang, B. Fermini, F. Faust, S. Nattel, and A. M. Brown. Identity of a novel delayed rectifier current from human heart with a cloned K^+ channel current. *Circ. Res.* **73:**210–216 (1993).
5. Philipson, L. H., R. E. Hice, K. Schaefer, J. LaMendola, G. I. Bell, D. J. Nelson, and D. F. Steiner. Sequence and functional expression in *Xenopus* oocytes of a human insulinoma and islet potassium channel. *Proc. Natl. Acad Sci. USA* **88:**53–57 (1991).
6. Tamkun, M. M., K. M. Knoth, J. A. Walbridge, H. Kroemer, D. M. Roden, and D. M. Glover. Molecular cloning and characterization of two voltage-gated K^+ channel cDNAs from human ventricle. *FASEB J.* **5:**331–337 (1991).
7. Catterall, W. A. Structure and function of voltage-sensitive ion channels. *Science (Washington D. C.)* **242:**50–61 (1988).
8. Jan, L. Y., and Y. N. Jan. Voltage-sensitive ion channels. *Cell* **56:**13–25 (1989).
9. Hume, J. R. Comparative interactions of organic Ca^{++} channel antagonists with myocardial Ca^{++} and K^+ channels. *J. Pharmacol. Exp. Ther.* **234:**134–140 (1985).
10. Striessnig, J., H. Hossmann, and W. A. Catterall. Identification of a phenylalkylamine binding region within the α_1 subunit of skeletal muscle Ca^{2+} channels. *Proc. Natl. Acad. Sci. USA* **87:**9108–9112 (1990).

11. Lopez, G. A., Y. N. Jan, and L. Y. Jan. Mutational analysis examining the potassium permeation pathway of the Shaker potassium channel. *Soc. Neurosci. Abstr.* **18**:921 (1992).
12. Choi, K. L., C. Mossman, J. Aube, and G. Yellen. The internal quaternary ammonium receptor site of *Shaker* potassium channels. *Neuron* **10**:533–541 (1993).
13. Schilling, W. P., A. K. Ritchie, L. T. Navarro, and S. G. Eskin. Bradykinin-stimulated calcium influx in cultured bovine aortic endothelial cells. *Am. J. Physiol.* **255**:H219-H227 (1988).
14. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391**:85–100 (1981).
15. Snyders, D. J., K. M. Knoth, S. L. Roberds, and M. M. Tamkun. Time-, voltage-, and state-dependent block by quinidine of a cloned human cardiac potassium channel. *Mol. Pharmacol.* **41**:322–330 (1992).
16. Colquhuon, D., and A. Hawkes. The principles of stochastic interpretation of ion channel mechanisms, in *Single Channel Recording* (B. Sakmann and E. Neher, eds.). Plenum Press, New York, 135–175 (1983).
17. Akhtar, M., P. Tchou, and M. Jazayeri. Use of calcium channel entry blockers in the treatment of cardiac arrhythmias. *Circulation* **80**:IV-31-IV-39 (1989).
18. Kass, R. S. Nisoldipine: a new, more selective calcium current blocker in cardiac Purkinje fibers. *J. Pharmacol. Exp. Ther.* **223**:446–456 (1982).
19. McDonald, T. F., D. Pelzer, and W. Trautwein. Cat ventricular muscle treated with D600: effects on calcium and potassium currents. *J. Physiol. (Lond.)* **352**:203–216 (1984).
20. Pancrazio, J. J., M. P. Viglione, R. J. Kleiman, and Y. I. Kim. Verapamil-induced blockade of voltage-activated K^+ current in small-cell lung cancer cells. *J. Pharmacol. Exp. Ther.* **257**:184–189 (1991).
21. Galietta, L. J. V., A. Rasola, V. Barone, D. C. Gruenert, and G. Romeo. A forskolin and verapamil sensitive K^+ current in human tracheal cells. *Biochem. Biophys. Res. Commun.* **179**:1155–1160 (1991).
22. Retzinger, G. S., L. Cohen, S. Lau, and F. J. Kezdy. Ionization and surface properties of verapamil and several verapamil analogues. *J. Pharm. Sci.* **75**:976–982 (1986).
23. Lee, K. S., and R. W. Tsien. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature (Lond.)* **302**:790–794 (1983).
24. Affolter, H., and R. Coronado. Sidedness of reconstituted calcium channels from muscle tranverse tubles as determined by D600 and D890 blockade. *Biophys. J.* **49**:767–771 (1986).
25. Klöckner, V., and G. Isenberg. Tiapamil reduces the calcium inward current of isolated smooth muscle cells: dependence on holding potential and pulse frequency. *Eur. J. Pharmacol.* **127**:165–171 (1986).
26. Timin, E. N., and S. Hering. A method for estimation of drug affinity constants to the open conformational state of calcium channels. *Biophys. J.* **63**:808–814 (1992).
27. Lawrence, C. E., and J. Goldman. Definition and identification of homology domains. *Comput. Appl. Biosci.* **4**:25–31 (1988).
28. Sung, R. J., B. Elser, and R. G. McAllister. Intravenous verapamil for termination of re-entrant supraventricular tachycardias: intracardiac studies correlated with plasma verapamil concentrations. *Ann. Int. Med.* **93**:682–689 (1980).
29. Reiter, M. J., D. G. Shand, and E. L. C. Pritchett. Comparison of intravenous and oral verapamil dosing. *Clin. Pharmacol. Ther.* **32**:711–720 (1982).
30. Stehle, G., J. Eibach, J. J. Lasserre, J. Buss, T. Plugge, I. Kehry, and D. L. Heene. Cardiogenic shock associated with verapamil in a patient with liver cirrhosis. *Lancet* **336**:1079 (1990).
31. Perkins, C. M. Serious verapamil poisoning: treatment with intravenous calcium gluconate. *Br. Med. J.* **2**:1127 (1978).
32. Nakayama, H., M. Taki, J. Striessnig, H. Glossman, W. A. Catterall, and Y. Kanaoka. Identification of 1,4-dihydropyridine binding regions within the $\alpha 1$ subunit of skeletal muscle Ca^{2+} channels by photoaffinity labeling with diazipine. *Proc. Natl. Acad. Sci USA* **88**:9203–9207 (1991).

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