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Veratridine Blocks Voltage-Gated Potassium Current in Human T Lymphocytes and in Mouse Neuroblastoma Cells

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Abstract. (i) Effects of veratridine on ionic conductances of human peripheral blood T lymphocytes have been investigated using the whole-cell patch-clamp technique. (ii) Veratridine reduces the net outward current evoked by membrane depolarizations. The reduction originates from block of a 4-aminopyridine-sensitive, voltage-gated K^+ current. (iii) Human T lymphocytes do not appear to express voltage-gated Na^+ channels, since inward currents are observed neither in control nor in veratridine- and bretylium-exposed lymphocytes. (iv) The effect of veratridine consists of an increase in the rate of decay of the voltage-gated K^+ current and a reduction of the peak current amplitude. Both effects depend on veratridine concentration. Half-maximum block occurs at 97 μM and the time constant of decay is reduced by 50% at 54 μM of veratridine. (v) Possible mechanisms of veratridine action are discussed. The increased rate of K^+ current decay is most likely due to open channel block. The decrease of current amplitude may involve an additional mechanism. (vi) In cultured mouse neuroblastoma N1E-115 cells, veratridine blocks a component of voltage-gated K^+ current, in addition to its effect on voltage-gated Na^+ current. This result shows that the novel effect of veratridine is not confined to lymphocytes.

Key words: Na^+ channel — K^+ channel — Patch clamp — Veratridine — Lymphocyte — Neuroblastoma

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Introduction

Since the application of the patch-clamp technique (Hamill et al., 1981), considerable progress has been made in identifying the ionic conductances in lymphocytes and their functional relevance. The membrane conductance of resting lymphocytes is mainly determined by voltage-gated K^+ channels with characteristics of delayed rectifier K^+ channels of muscle and nerve cells. Substantial evidence supports a role of these channels in the proliferative response of T lymphocytes (*reviewed in*: Gallin, 1991). Although voltage-gated K^+ channels have been extensively characterized in lymphocytes (DeCoursey et al., 1984; Fukushima, Hagiwara & Henkart, 1984; Matteson & Deutsch, 1984; Cahalan et al., 1985; DeCoursey et al., 1985; Lee & Deutsch, 1990; Pahapill & Schlichter, 1990), the presence of Na^+ channels in lymphocytes remains a matter of controversy.

Damjanovich and coworkers reported that bretylium tosylate, a Na^+ channel opener in mucosal epithelium cells of the frog *Rana pipiens* (Ilani, Lichtstein & Bacaner, 1982), causes repolarization of the membrane potential of depolarized human, rat and mouse T and B lymphocytes (Pieri et al., 1989; Trón et al., 1990). This hyperpolarizing effect showed a dependence on the extracellular Na^+ concentration and was sensitive to amiloride, ouabain, tetrodotoxin, azide and temperature. The effect was interpreted as an increased activity of the electrogenic Na^+/K^+ ATPase triggered by Na^+ influx, which was presumed to be mediated by bretylium-activated Na^+ channels (Gáspár et al., 1992). However, ubiquitous expression of Na^+ channels in human lymphocytes is not supported by electrophysiological evidence. Inward currents, attributed to a Na^+

conductance, were observed in only a small fraction of human lymphocytes, i.e., 3 out of 90 human T cells (Cahalan et al., 1985; DeCoursey et al., 1985) and 5 out of 28 human thymocytes (Schlichter, Sidell & Hagiwara, 1986). The seemingly conflicting results might be explained by the presence of silent Na^+ channels in lymphocytes.

Various nonexcitable cells have been shown to express tetrodotoxin-sensitive Na^+ channels, which can be activated only in the presence of veratridine and other compounds that enhance the activated state of the Na^+ channel (Romey et al., 1979; Reiser & Hamprecht, 1983). Veratridine is widely used to chemically enhance ion flux through Na^+ channels, for it stabilizes the Na^+ channel open state. This effect causes prolongation of the voltage-gated Na^+ current and the appearance of large, slowly decaying Na^+ tail currents at the end of membrane depolarizations (Ulbricht, 1969; Barnes & Hille, 1988). In addition, veratridine and several other lipid-soluble toxins, which modify Na^+ channel gating in a similar manner, have been reported to block voltage-gated Ca^{2+} channels in mouse neuroblastoma cells (Romey & Lazdunski, 1982).

Veratridine was used to investigate the presence of silent Na^+ channels in lymphocytes. Here, we report the effects of veratridine on ionic conductances in human T lymphocytes and comparable effects in cultured neuroblastoma cells.

Materials and Methods

Cell Isolation and Culture

Human peripheral blood lymphocytes were isolated from heparinized blood of healthy volunteers using the standard Ficoll-Paque sedimentation technique. The resulting cell population contained >75% T lymphocytes as assessed by CD4/CD8 fluorescent labeling. The isolated cells were kept at 4°C in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and the following amino acids (in mM): cysteine·HCl 0.3, L-alanine 0.4, L-asparagine 0.45, L-aspartic acid 0.4, L-proline 0.4 and L-glutamic acid 0.4. Cells were used the day after isolation. Before the experiments, cells were diluted in extracellular solution and allowed to settle for 5–10 min on the glass bottom of a 35-mm dish. Generally, the phenotype of the cell was determined at the end of the electrophysiological experiment. Results obtained with CD4 and with CD8 positive T-cells could not be distinguished.

Mouse neuroblastoma cells of the clone N1E-115 were cultured as described previously (Amano, Richelson & Nirenberg, 1972). Cells were grown in DMEM, supplemented with 7.5% FCS and with the same amino acids as above. Cultures were maintained at 37°C in a humidified atmosphere containing 7.5% CO_2. Cells were trypsinized every five days and plated on 35-mm tissue culture dishes. Differentiation was initiated two days after plating by changing the culture medium to DMEM supplemented with 1.5% FCS, the above-listed amino acids and 1% (v/v) dimethylsulfoxide (DMSO). Cells were used for experiments 4–6 days after differentiation.

Electrophysiology

Membrane currents were recorded in the whole-cell patch-clamp configuration (Hamill et al., 1981) using an EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Germany). Recording pipettes (Clark; GC150 borosilicate glass) with a resistance of 2–7 MΩ were used. The external solution contained (in mM): NaCl 160, KCl 4.5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5, adjusted to pH 7.4 with NaOH and to 325–345 mOsm with 25 mM sucrose. Unless stated otherwise, the pipette solution contained (in mM): KCl 100, KF 50, HEPES 5, EGTA 10, ATP 1, GTP 0.1, adjusted to pH 7.2 with KOH and to 300–320 mOsm with 30 mM sucrose. Cells were placed on the stage of an inverted phase-contrast/fluorescent microscope (Carl Zeiss, Axiovert 35M). A 0.5 mm diameter superfusion pipette was positioned within a distance of 0.5 mm from the cell. The pipette was connected to a four-way valve to allow switching between different solutions. Superfusion of the cell, with a flow rate of about 0.5 ml/min, was started before voltage clamping the cell and was maintained throughout the experiment. External solution, to which 1 mM of the K^+ channel blocker 4-aminopyridine (4AP) was added, was routinely superfused at the end of an experiment, and the time course of K^+ current block was used as a control for adequate superfusion. Voltage-gated K^+ currents were evoked by 400 msec depolarizing pulses to +60 mV from a holding potential of −80 mV, unless stated otherwise. Series resistance was compensated for 70%. Linear components of capacitative and leakage currents were subtracted, using a P/10 protocol. P/10 pulse trains were applied at 100 sec intervals to allow complete recovery from inactivation. Linear voltage ramps from −140 to +60 mV with a duration of 200 msec were applied after every four P/10 pulse trains to obtain instantaneous current-voltage relations. Since properties of the K^+ current may vary during the first minutes after breaking into the cell (Cahalan et al., 1985; Deutsch, Krause & Lee, 1986) at least 10 min were allowed for control currents to stabilize. All experiments were conducted at room temperature (21–27°C).

Data Acquisition and Analysis

The membrane currents were low-pass filtered (Butterworth filter; −3 dB at 2 kHz; 12 dB/octave), digitized (12 bits; 1,024 points/record) and stored on magnetic disk for off-line computer analysis. Curve fitting was performed using a Levenberg-Marquardt nonlinear least-squares algorithm (Marquardt, 1963). Current decay was fitted by the exponential function:

$$i_t = i_\infty + i_0 \cdot e^{-t/\tau}$$

and concentration-effect curves were fitted by the function:

$$E = E_{max}/(1 + \{EC50/[C]\}^n)$$

Amplitudes and time constants are presented as mean ± SD calculated from data obtained from different cells and parameters of concentration dependence as mean ± estimated SD, as obtained from the fitting procedure. Results were compared using the two-tailed Student's *t*-test.

Chemicals

Veratridine (purity by thin layer chromatography >99%), 4-aminopyridine (4AP) and tetrodotoxin (TTX) were purchased from Sigma Chemical, St. Louis, MO; Ficoll-Paque from Pharmacia, Sweden; Anti-Human Leu-3a (CD4) PE and Anti-Human Leu-2a (CD8) FITC from Becton and Dickinson, San José, CA; bretylium tosylate from

Wellcome Pharmaceuticals, Utrecht, The Netherlands. Veratridine was dissolved in DMSO as a 10 mM stock solution. Superfusion with external solution containing up to 2.5% DMSO, the maximum present in veratridine experiments, did not affect K^+ currents.

Results

Lymphocytes

Effects on Whole-Cell Current

In voltage-clamped human T lymphocytes, the presence of voltage-gated Na^+ channels was examined. Depolarizing voltage steps of more than 40 mV from the holding potential of −80 mV evoked a transient outward current. Inward currents were never detected in the human T lymphocytes ($n > 500$). To reveal the possible presence of silent voltage-gated Na^+ channels, we applied the Na^+ channel opener veratridine. Superfusion with veratridine (1–250 μM) did not induce any membrane current when monitored in the whole-cell configuration at a holding potential of −80 mV. Furthermore, membrane depolarization in the presence of veratridine did not activate any inward current, while also no tail current was observed upon repolarization ($n = 52$). However, veratridine reduced the peak amplitude of the voltage-gated outward current in lymphocytes and increased the rate of the outward current decay. At a concentration of 100 μM, veratridine inhibited the peak current amplitude to 47.4 ± 12% of the control value ($n = 7$) and decreased the time constant of decay to 34.4 ± 8.4% of the control value ($n = 8$, Fig. 1*A*). Additional experiments were performed to determine whether voltage-gated Na^+ channels are involved in this effect of veratridine. The possibility that a small inward current is masked by a larger outward current was examined using a pipette solution in which all K^+ ions were replaced by Cs^+ ions. Under these conditions, neither outward nor inward current was observed in control solution or in the presence of veratridine when the membrane was depolarized to 0 mV (Fig. 1*B*). This implicates that the net outward current is completely carried by K^+ channels, which are blocked by internal Cs^+, and that voltage-gated Na^+ current is absent. Moreover, in the presence of 0.5 μM TTX, the effect of veratridine on the outward current evoked by a membrane depolarization to +60 mV was the same as in control experiments (Fig. 1*C*).

The onset and reversibility of veratridine effects are shown in Fig. 1*D*. Superfusion with external solution containing 100 μM veratridine was started at various times within the 100 sec period between the last recording of control current and the first effect. Regardless of the moment of application of veratridine, the effects on peak amplitude and current kinetics hardly changed between the first and second stimulation after superfusion. This indicates that the onset of the effect of veratridine is rapid and that the steady effect is reached at the first membrane depolarization. Both effects were at least partially reversed by washing with control external solution.

Concomitant with veratridine application, a decrease of the small net inward current at the holding potential of −80 mV was observed in 62% of the cells ($n = 13$), which showed any measurable holding current. This decrease tended to be transient at 1–10 μM and sustained at 10–100 μM veratridine. The nature of this reduction was not further examined.

Since veratridine appeared unable to unmask Na^+ current, experiments were also performed with bretylium tosylate. In lymphocytes superfused with external solutions containing 2–3 mM bretylium tosylate for periods of up to 10 min, neither bretylium- nor voltage-gated Na^+ currents were detected. Instead, bretylium tosylate reduced the outward as well as the inward K^+ currents evoked by voltage ramps in normal and high (30–60 mM) K^+ external solutions (*results not shown*).

Effects on Voltage-gated K^+ Current

The transient outward current evoked by membrane depolarization was identified as the "n-type" K^+ current (DeCoursey et al., 1984; Cahalan et al., 1985) on the basis of its voltage dependence (Fig. 2*A*) and sensitivity to block by 4AP (Fig. 2*B*), as well as by the use-dependent inactivation and the reversal potential of the tail current, which ranged between −80 and −90 mV (*data not shown*). The peak amplitude of the voltage-gated K^+ current evoked by step depolarizations to +60 mV varied between 20 and 2,500 pA (602 ± 412 pA; $n = 95$). Inactivation of these currents could be fitted by a single exponential function and a nonzero steady level. The time constant of inactivation, as obtained from 58 cells, with peak current amplitudes between 300 and 1,500 pA, amounted to 179 ± 33 msec (range 120–244 msec). The amplitude of the fitted steady level varied between cells and in some cases in the course of an experiment.

The voltage dependence of the effects of veratridine, i.e., decrease of the peak outward current amplitude and increase of the rate of decay, was examined. Figure 3 illustrates the effects of 100 μM veratridine on voltage-gated K^+ currents evoked by step depolarizations of varying amplitude. The reduction of peak amplitude of the K^+ current by veratridine occurred over a wide range of membrane potentials and did not appear to be voltage dependent (Fig. 3*A*). Although the percentage of block by veratridine slightly increased at higher membrane potentials (0.14%/mV in the range of 10–110 mV), this effect was too small to be distinguished from the slow rundown of K^+ current in the

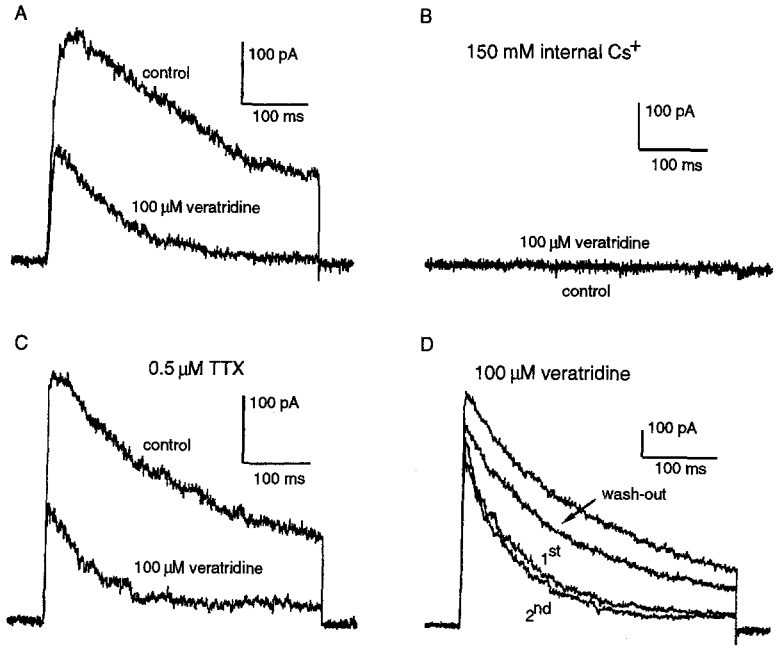

Fig. 1. Whole-cell currents of human T lymphocytes during depolarizing voltage steps from a holding potential of −80 mV in four different cells. (*A*) The outward current evoked by a depolarizing step to 0 mV, recorded in control solution and 10 min after switching to a solution containing 100 μM veratridine. (*B*) Superimposed traces of membrane currents evoked by depolarizing voltage steps to 0 mV after replacing all K^+ ions in the pipette solution by Cs^+. No current was observed in control external solution nor in the presence of 100 μM veratridine. (*C*) The presence of 0.5 μM TTX did not affect the blocking effect of veratridine on the outward current evoked by depolarizing steps to +60 mV. (*D*) Time course of the action of veratridine. Currents were evoked by depolarizations to +60 mV before, 100 and 200 sec after switching from control to 100 μM veratridine-containing external solution and 100 sec after returning to control external solution. Both steady-state block and maximum recovery were reached within 100 sec.

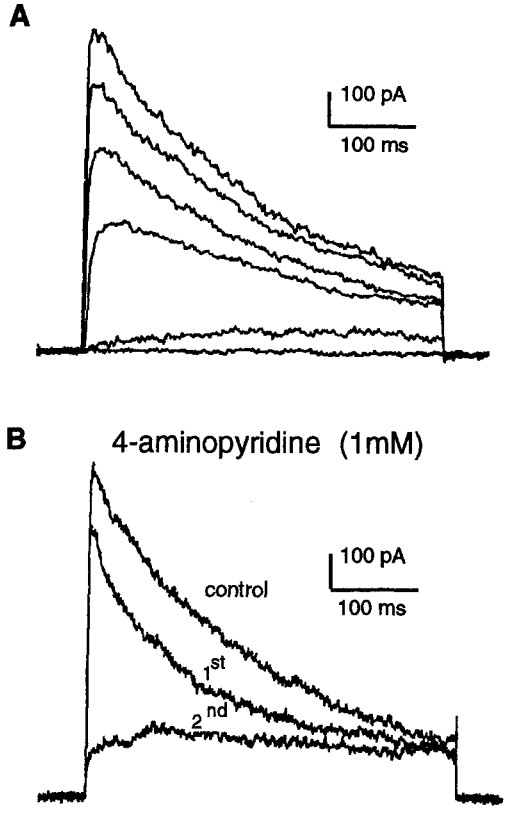

Fig. 2. Characterization of the n-type K^+ current in T lymphocytes. (*A*) A family of currents evoked by depolarizing voltage steps of 20 to 170 mV with 30 mV increments from a holding potential of −80 mV. (*B*) Use-dependent block after superfusion with 1 mM 4AP. The response to the first depolarizing step in the presence of 4AP showed an increased rate of decay and steady block was reached at the second depolarization to +60 mV.

course of the experiments, which lasted for >1 hr. The acceleration of K^+ current decay by veratridine also occurred over the entire range of membrane potentials. In addition, the marked voltage dependence of the time constant of decay, which was observed under control conditions in the membrane potential range below +20 mV, disappeared in the presence of veratridine (Fig. 3*B*). K^+ current decay in the presence of veratridine showed only minor voltage dependence.

Intracellular veratridine, applied by adding 100 μM of the toxin to the pipette solution before the experiment, also accelerated K^+ current decay. At 2 min after establishing the whole-cell configuration the time constant of decay was 95 ± 32 msec (n = 3), which was markedly reduced as compared to the mean value obtained with control pipette solution ($P < 0.001$). In time, K^+ current decay was gradually further accelerated and the time constant reached a steady value of 73 ± 30 msec (n = 3) after about 10 min (*result not shown*). This effect on the time constant is not significantly different from that during external application of 100 μM veratridine ($P = 0.15$). The slower onset of the effect on internal application may be due to limited diffusion of veratridine from the pipette to the cell membrane. Subsequent superfusion with external solution also containing 100 μM veratridine resulted in a further reduction of the time constant of K^+ current decay. This means that veratridine is effective both from the extracellular and the intracellular side, which is to be expected in view of the lipid solubility of this molecule. Because no intrinsic control currents can be recorded with veratridine in the pipette, the effect on the amplitude was not determined in these experiments.

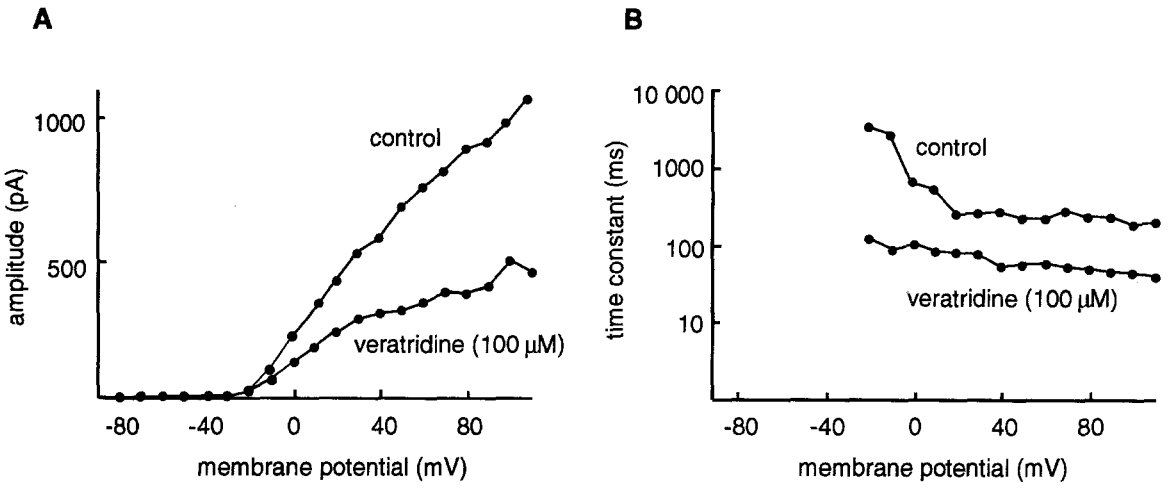

Fig. 3. Voltage dependence of the veratridine effects. Currents were recorded with voltage steps from 0 to 190 mV in 10 mV increments from a holding potential of −80 mV. (*A*) The *I-V* relationship of the peak outward current amplitude before and during application of 100 μM veratridine. Control current traces of the same lymphocyte are depicted in Fig. 2*A*. (*B*) The exponential time constants of decay fitted to the outward currents of the same lymphocyte as used in *A*.

Concentration Dependence

Veratridine affected voltage-gated K^+ current in lymphocytes in a concentration-dependent manner. Block of the peak amplitude of K^+ current as well as the acceleration of decay increased with augmenting concentrations of 10–250 μM veratridine (Fig. 4*A*). In contrast to the effects of veratridine at concentrations beyond 10 μM, the effects of 1–10 μM veratridine on K^+ current kinetics were transient in most cases (Fig. 4*B*). These transient effects occurred in six out of eight cells at 1 μM and in five out of seven cells at 10 μM veratridine. The transient effects appeared to be more pronounced than the sustained effects observed in some of the cells at the same concentrations of veratridine (Table). The transient effects at 1 and 10 μM did not significantly differ from each other ($P = 0.27$) and appeared unrelated to the transient effects on holding current mentioned before. The concentration dependence of the effects of veratridine is summarized in Fig. 5. Fitted concentration-effect curves yield EC50 values for the reduction of the time constant of 54 ± 10 μM veratridine, and for the block of the peak amplitude of 97 ± 6 μM veratridine.

Interference by Other Drugs

To determine whether specific K^+ current components are involved in the action of veratridine, we investigated interactions between the effects of veratridine, 4AP and Cd^{2+}.

The current remaining in the presence of 100 μM veratridine was still sensitive to 4AP and showed the characteristic use dependence of block by 4AP (Figs. 6*A* and 2*B*). The amplitude of the remaining current in the presence of 1 mM 4AP was further reduced by 100 μM veratridine, but the typical acceleration of K^+ current decay by veratridine did not occur (Fig. 6*B*). The small outward current component remaining in the simultaneous presence of the two drugs is consistent with the partial effects of the blockers applied individually at these concentrations.

To determine a possible involvement of Ca^{2+} ions in the effect of veratridine, we examined the influence of Cd^{2+}. Superfusion with external solution containing 300 μM Cd^{2+}, which inhibits Ca^{2+} fluxes in T lymphocytes (Kuno et al., 1986; Lewis & Cahalan, 1989), resulted in a decrease of the K^+ current amplitude and a slowing of inactivation kinetics (Fig. 7*A*). As evident from the current-voltage relationships in Fig. 7*B*, obtained by applying voltage ramps, this may be attributed to a shift of the voltage dependence to more positive potentials. The effect of veratridine on the remaining current was similar to the effect on K^+ current in the absence of Cd^{2+}. Because Cd^{2+} did not interfere with the action of veratridine, it seems unlikely that influx of extracellular Ca^{2+} is involved in the effect of veratridine. Since the pipette solution contained both F^- ions and the Ca^{2+} chelator EGTA, any effect of intracellular Ca^{2+} can also be excluded. Using a pipette solution without EGTA and Cl^- instead of F^- did not influence the effects of veratridine (*data not shown*).

Neuroblastoma Cells

To examine whether veratridine affects K^+ currents in other cell types as well, we also performed experiments on N1E-115 neuroblastoma cells. The same stimulation protocol used in lymphocytes activated both a fast in-

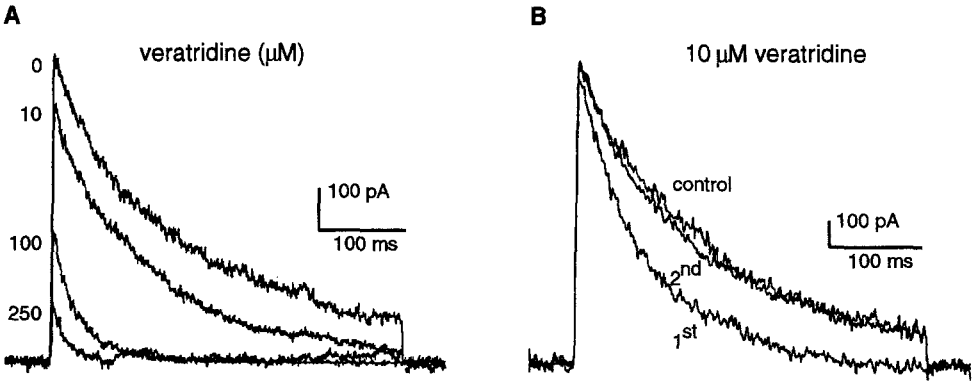

Fig. 4. Concentration dependence of veratridine effects. (*A*) Currents evoked by a depolarizing step to +60 mV in the presence of the indicated concentrations of veratridine (in μM) in the same cell. In each case the current was recorded 10 min after switching to a new concentration of veratridine. (*B*) An example of a transient response to a low concentration of veratridine. The acceleration of the outward current decay and the small decrease of the peak amplitude were only observed during the first depolarization after superfusion with 10 μM veratridine. At the second depolarization, these parameters had returned to control values.

Table. Transient and sustained effects of low concentrations of veratridine on voltage-gated K^+ current in human T lymphocytes

[Veratridine] μM	τ transient (% of control)	τ sustained (% of control)
1	73.5 ± 9.9 (6)	100.0 ± 0 (2)
10	65.2 ± 14 (5)	81.0 ± 2.8 (2)

Mean values ± SD for transient and sustained effects on the time constant of K^+ current decay (τ) are given for the number of experiments in parentheses. All parameters are expressed as percentages of control values in the same cell. K^+ currents were evoked by depolarizations from the holding potential of −80 to +60 mV.

ward and a delayed outward current in these neuroblastoma cells. The inward current is carried by Na^+ ions through TTX-sensitive Na^+ channels, whereas the outward current is a K^+ current (e.g., Moolenaar & Spector, 1978). Figure 8*A* shows that superfusion with external solution containing 100 μM veratridine resulted in the appearance of a large, slowly decaying tail current at the end of the membrane depolarization. The effect of veratridine on the initial Na^+ current, a reduction of the peak amplitude and a slowing of the inactivation, cannot be discerned at this time scale. The tail current is attributed to veratridine-modified Na^+ channels that remain open at the holding potential of −80 mV (Barnes & Hille, 1988). In addition, the net outward current was reduced in the presence of veratridine. In a similar experiment, performed in the presence of 0.5 μM TTX, the Na^+ current and the Na^+ tail current were completely blocked, but the outward current was still reduced in the presence of veratridine (Fig. 8*B*). As in lymphocytes, a marked acceleration of the kinetics of the outward K^+ current decay was observed. The peak amplitude of the K^+ current was reduced by 100 μM veratridine to 64 ± 13% of control

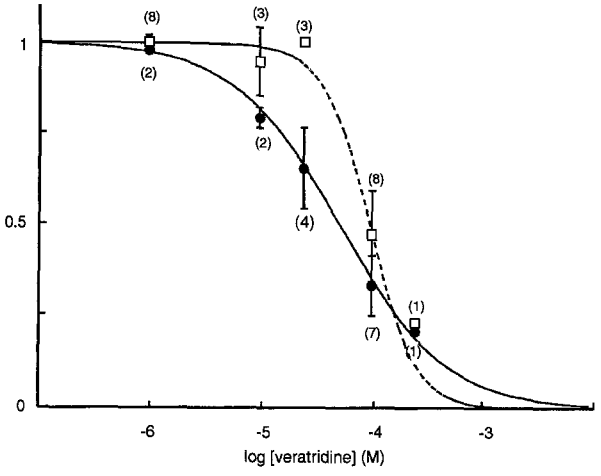

Fig. 5. Concentration-effect curves of the modification of voltage-gated K^+ current by veratridine. The ordinate represents the peak outward current amplitude (squares) and the exponential time constant of outward current decay (circles) at the indicated concentrations of veratridine. Both parameters were normalized to control values obtained from the same lymphocytes. Each point is the mean ± SD of the indicated number of measurements. Only sustained effects (*see text*) were used for curve fitting. The estimated values for the EC50 and the slope factor are 54 ± 10 μM and −0.92 ± 0.13 for the effect on the time constant and 97 ± 6 μM and −2.09 ± 0.55 for the effect on the amplitude of the outward current.

values (n = 5). Low concentrations of 1–10 μM veratridine did not affect the K^+ current in N1E–115 cells and increasing the concentration of veratridine from 100 to 250 μM did not further affect the outward current (Fig. 8*B*). Therefore, veratridine blocks part of the voltage-gated K^+ current in neuroblastoma cells with an EC50 value between 10 and 100 μM. The control K^+ current as well as the K^+ current remaining in the presence of veratridine were sensitive to block by 1 mM 4AP (*not shown*).

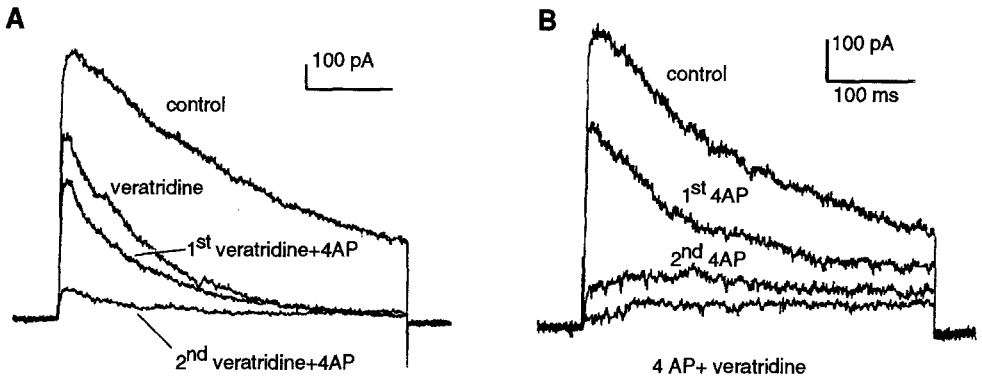

Fig. 6. The combined effects of 100 μM veratridine and 1 mM 4AP. (*A*) After 10 min superfusion with veratridine, the superfusate was switched to external solution containing both veratridine and 4AP. In the presence of veratridine, the open channel block by 4AP is very similar to that in control solutions (*compare* Figs. 2B and 6B). (*B*) After blocking the K^+ current with 4AP, superfusion with both 4AP and veratridine caused a further reduction of the K^+ current remaining in the presence of 4AP alone.

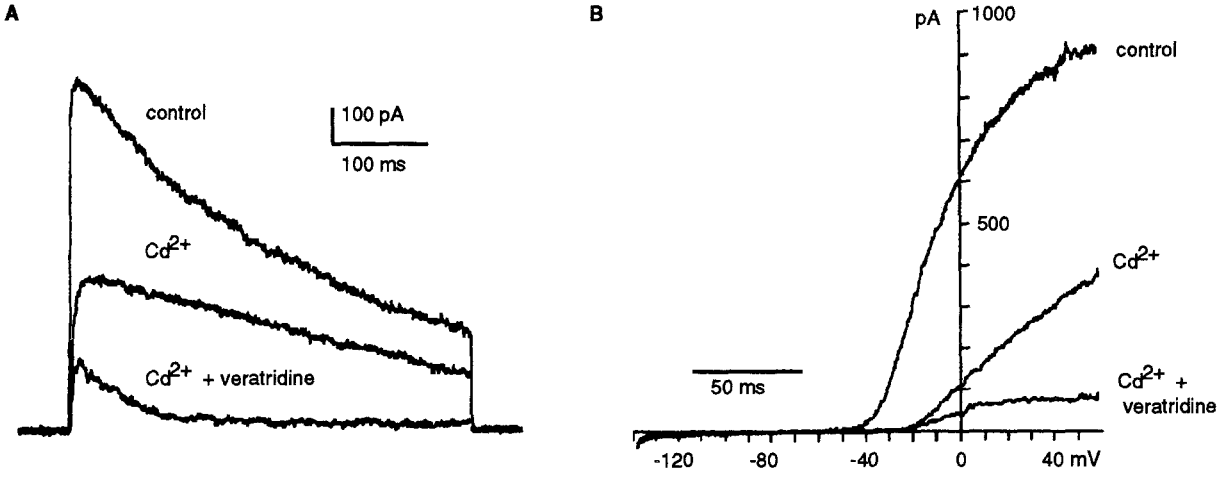

Fig. 7. The combined effects of 100 μM veratridine and 300 μM Cd^{2+}. (*A*) Superimposed currents evoked by voltage steps to +60 mV before and after superfusion with Cd^{2+}-containing external solution and after superfusion with external solution containing both veratridine and Cd^{2+}. The K^+ current remaining in the presence of Cd^{2+} was blocked by veratridine in a manner very similar to the block in control solutions (*compare* Figs. 1*A* and 4*A*). (*B*) The *I-V* relationships under these conditions, obtained by applying voltage ramps from −140 to +60 mV. Cd^{2+} shifted the *I-V* curve to more positive potential and reduced the slope, but the effect of veratridine was the same as under control conditions (*compare* Fig. 3*A*).

Discussion

The present results demonstrate that veratridine, in addition to modifying Na^+ channels (Ulbricht, 1969; Barnes & Hille, 1988) and Ca^{2+} channels (Romey & Lazdunski, 1982), affects voltage-gated K^+ channels. In cells of both neuronal and non-neuronal origin, i.e., mouse N1E-115 neuroblastoma cells and human peripheral blood T lymphocytes, voltage-gated K^+ currents were suppressed in the presence of veratridine. In lymphocytes, veratridine reduced the amplitude of outward voltage-gated current and accelerated the outward current decay. The possibility that this effect is caused by veratridine-activated Na^+ channels was excluded, since the block of outward currents also occurs in the presence of TTX. Furthermore, the K^+ channel blocker 4AP and internal Cs^+ did not unmask voltage-gated inward current in either control experiments or in the presence of 100 μM veratridine (Figs. 6*B*, 1*B*). These results demonstrate that veratridine impairs K^+ channel function in human T lymphocytes.

In N1E-115 neuroblastoma cells, 100 μM veratridine also decreases the peak amplitude and accelerates the decay of outward current in the presence of TTX. In contrast to lymphocytes, the K^+ current block in neuroblastoma cells saturates at 100 μM veratridine, leav-

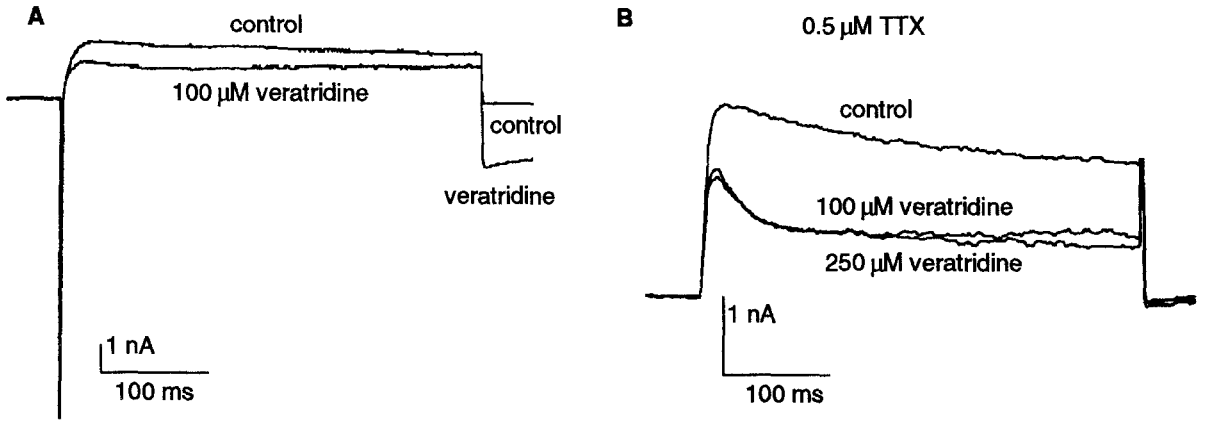

Fig. 8. Whole-cell currents in N1E-115 neuroblastoma cells evoked by depolarizing voltage steps to +60 mV from a holding potential of −80 mV. (*A*) Under control conditions, both Na^+ and K^+ currents can be distinguished. The fast inward peak is a voltage-gated Na^+ current, the outward current is a voltage-gated K^+ current. Veratridine induced a slow inward tail current and caused a decrease of the outward current compared to control. The effect of veratridine on the initial inward Na^+ current cannot be discerned at this time scale. (*B*) The effects of 100 and 250 μM veratridine on the voltage-gated current in the presence of 0.5 μM TTX. The initial inward Na^+ current and the veratridine-induced Na^+ tail current were blocked by TTX, but the decrease of the outward current was still observed in the presence of TTX.

ing a 4AP-sensitive K^+ current component unaffected, whose size is about half that of the control current amplitude. This suggests that in neuroblastoma cells, veratridine blocks a specific component of the K^+ current. In N1E-115 neuroblastoma cells, rapidly (100–700 msec) and slowly (2–15 sec) inactivating K^+ current components have been distinguished (Moolenaar & Spector, 1978). A combined whole-cell and single channel patch-clamp investigation of K^+ current in N1E-115 cells demonstrated that fast and slow K^+ channels with distinct single channel conductances contribute to the macroscopic voltage-gated K^+ current (Quandt, 1988). A possible explanation of the observed effects in neuroblastoma cells is that veratridine selectively blocks the slowly inactivating K^+ current component, leaving the faster inactivating component unaltered. Since lymphocytes appear to express a single type of voltage-gated K^+ channel only (Grissmer et al., 1990), a similar mechanism to explain the effects of veratridine in lymphocytes can be ruled out. Ca^{2+}-dependent K^+ current components in N1E-115 cells are not involved in the effect of veratridine, since the pipette solution contained a very low concentration of free Ca^{2+} (<2 nM, based on buffering by EGTA and the solubility product of CaF_2). In both lymphocytes and neuroblastoma cells, the K^+ currents blocked by veratridine have characteristics of delayed rectifier K^+ channels (Cahalan et al., 1985; Quandt, 1988). As selective blockers of the various subtypes of K^+ channels are scarce, veratridine might provide a candidate tool to distinguish delayed rectifier K^+ channels.

Veratridine affects the voltage-gated K^+ current in a concentration-dependent manner. In lymphocytes, the EC50 for block of outward current peak amplitude is 97 μM, and the time constant of decay was reduced to half of the control value by 54 μM veratridine (Fig. 5). In neuroblastoma cells, the EC50 for block of part of the voltage-dependent K^+ current is between 10 and 100 μM. These effects on K^+ channels occur at similar concentrations as reported for Na^+ channel opening (EC50 values of 44 μM in mouse neuroblastoma cells and 86 μM in rat C9 cells; Romey et al., 1979) and for block for voltage-dependent Ca^{2+} channels in neuroblastoma cells (EC50 of 26 μM; Romey & Lazdunski, 1982).

The effects of veratridine showed a rapid onset, and steady effects were reached at the first membrane depolarization following application of the drug (Fig. 1*D*). In contrast, several depolarizations are required to reach the steady block with 4AP (Figs. 2*B*, 6*B*). It has been demonstrated that the use dependence of block by 4AP in lymphocytes arises from open K^+ channel block and subsequent trapping of 4AP within the closed channel (Choquet & Korn, 1992). The action mechanism of veratridine clearly differs from that of 4AP. The effects of veratridine on K^+ current in human T lymphocytes resemble those of capsaicin, chlorpromazine, phencyclidine, quinidine and tetrahydroaminoacridine on a similar type of K^+ current in rat alveolar epithelial cells (Jacobs & DeCoursey, 1990). The latter compounds also caused acceleration of outward current decay and a reduction of outward current amplitude, which was explained by preferential block of open K^+ channels. The observed effects of veratridine are most readily explained by assuming the same mechanism of action. The observations that K^+ currents in the presence of veratridine always decay monoexponentially and that the time constant of decay gradually decreases with in-

creasing concentration of veratridine are consistent with open channel block. In addition, rapid open channel block by veratridine could account for the acceleration of K^+ current decay as well as for the observed change in voltage dependence of the time constant of decay (Fig. 3*B*). Alternative to open channel block, veratridine might accelerate outward current decay by modulating K^+ channel inactivation. However, two exponential components of current decay would be expected in case that drug-affected channels show an increased rate of inactivation and the relative contribution of the rapidly inactivating component should increase with the concentration of veratridine. In addition, a mere acceleration of inactivation cannot account for the observed decrease in peak amplitude (Figs. 1*A*, 4*A*). Extrapolation of the decaying outward current back to the beginning of the depolarizing step indicates that a fraction of the channels is already blocked at the holding potential. Different concentration-effect curves have been obtained for the effects of veratridine on K^+ current decay and on K^+ current amplitude (Fig. 5). This suggests that phasic and tonic block by veratridine involve two affinity states of the K^+ channel. Veratridine is effective from both the internal and external side of the membrane suggesting that veratridine modifies K^+ channels at a site accessible from within the membrane. More detailed experiments are required to establish the exact mechanism and the nature of the site(s) at which veratridine affects the K^+ channels in human T lymphocytes.

In lymphocytes, a reduction of the net inward holding current at a membrane potential of -80 mV was observed in addition to the effects on K^+ current evoked by membrane depolarizations. This suggests that the alkaloid also modifies a component of the membrane conductance at hyperpolarized potentials. Part of the holding current has been identified as a Ca^{2+} current (Lewis & Cahalan, 1989). An effect of veratridine on basal Ca^{2+} influx cannot be excluded, but would not interfere with the effects on voltage-gated K^+ current. Neither intracellular Ca^{2+} nor influx of extracellular Ca^{2+} appears to contribute to the effects of veratridine on K^+ current, since the effect of veratridine was still observed in the presence of 300 μM Cd^{2+} (Fig. 7), which is sufficient to block the Ca^{2+} conductance in lymphocytes (Kuno et al., 1986; Lewis and Cahalan, 1989), and the free Ca^{2+} concentration in the pipette solution was very low. Furthermore, the Ca^{2+} ionophore ionomycin and veratridine had opposite effects on the K^+ channels in phytohaemagglutinin-activated lymphocytes, which display both voltage-gated and Ca^{2+}-activated current components (J.A.H. Verheugen, *unpublished result*). Therefore, it is concluded that veratridine acts directly on the voltage-gated K^+ channels in lymphocytes.

Veratridine was initially used as a tool to reveal silent Na^+ channels in lymphocytes, whose presence has been suggested by effects of bretylium tosylate (Gáspár et al., 1992). However, we did not find any evidence for Na^+ channels in T lymphocytes, either in the presence of veratridine or bretylium. The present experiments confirm that bretylium causes K^+ channel block in lymphocytes (Gáspár et al., 1992). Apart from the opening of epithelial Na^+ channels (Ilani et al., 1982) many other effects of bretylium have been reported (*see* Bkaily et al., 1988). Among these effects, K^+ channel block is the more frequently observed.

Veratridine is often used as a depolarizing agent. The present observation that veratridine blocks K^+ channels, an effect that will cause depolarization in many cell types, may necessitate reinterpretation of some results obtained using this toxin to selectively enhance ion flux through voltage-gated Na^+ channels.

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