

Increase in P_{Na} and P_K of Cultured Heart Cells Produced by Veratridine

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ABSTRACT Noninnervated cultured chick embryonic heart cells are depolarized by veratridine (10^{-5} – 10^{-6} g/ml) within a few minutes to membrane potentials of -12 ± 2 mv. Action potentials and beating cease. Before depolarization begins, the repolarizing phase of the action potential is prolonged and leads to a long-lasting depolarizing afterpotential, probably due to a holding open of Na^+ channels. There is no direct effect on automaticity. Maximum rate of rise of the action potential decreases as a function of the depolarization. The inexcitability is transiently reversed by repolarizing current pulses and by 5 mM Ba^{++} (but not Sr^{++}) which increases membrane resistance (R_m) and produces a small transient repolarization. Cocaine does not reverse the depolarization. The depolarization also occurs in Cl^- -free Ringer and in Na^+ -free Li^+ -Ringer, but not in Na^+ -free sucrose-Ringer. In most cases, R_m , measured in the presence and absence of Cl^- , initially decreases but sometimes increases. Some of the decrease or increase in g_K may be indirectly produced by anomalous or delayed rectification, respectively. Tetrodotoxin, although having no effect on the action potential magnitude or rate of rise, prevents the depolarizing action of veratridine but not its effect on decreasing R_m . It is concluded that veratridine depolarizes by increasing the resting Na^+ permeability (P_{Na}); it also tends to increase P_K , but this action may be obscured by anomalous rectification when E_m is allowed to change. The equilibrium potential for veratridine action is about halfway between E_{Na} and E_K , similar to that of acetylcholine at the vertebrate neuromuscular junction.

Cultured heart cells, both atrial and ventricular, from embryonic and newly hatched chicks are remarkably unresponsive to those autonomic agents which normally produce alterations in the spontaneous frequency of discharge of intact hearts (17, 19). The membrane potentials are insensitive to acetylcholine, carbachol, epinephrine, norepinephrine, atropine, *d*-tubocurarine, histamine, and aconitine (17, 19). The action potentials normally depend on Na^+ and not on Ca^{++} , although there is evidence that Ba^{++} and Sr^{++} may participate in spike electrogenesis (21, and unpublished observations). However, tetrodotoxin does not influence the rate of rise or magnitude of the

action potential (19). Like cells in intact adult myocardium, however, cultured heart cells are slowly depolarized and rendered inexcitable by the cardiac glycoside, ouabain, and by the local anesthetics, cocaine and tetracaine (21). The depolarization produced by ouabain and the local anesthetics appears to be due to inhibition of active ion transport. Addition of Ba^{++} and Sr^{++} produces a rapid repolarization and a transient restoration of excitability in these cells. This reversal of the depression produced by cardiac glycosides and local anesthetics is attributed to stimulation of the Na^+ pump, perhaps by stimulation of the transport ATPase (21, 31).

The veratrine alkaloids have pronounced actions on excitable membranes as described by Krayer and Acheson (7) and by Shanes (15), including peripheral nerve (7, 24), skeletal muscle (8), and cardiac muscle (1). These alkaloids, which are structurally similar to the cardiac aglycones, prolong the repolarizing phase of the action potential, giving rise to pronounced depolarizing afterpotentials, sometimes with repetitive spikes superimposed. Depolarization occurs at higher concentrations. The major cardiovascular effects of veratrine alkaloids are all reflex in nature (2). Sensory receptors are activated by veratrine, the pressure receptors of the circulatory system being especially susceptible. In intact mammalian hearts, veratrine in moderate doses decreases excitability, prolongs the refractory periods, slows conduction, and sometimes raises the threshold of vulnerability to fibrillation; however, there is almost no effect on automaticity (25). Strength and duration of contraction are sometimes increased (2), apparently due to prolongation of the action potential (29), and release of catecholamines. With higher doses, there is a greater tendency to spontaneous firing and arrhythmias which induce ventricular fibrillation. Atrial arrhythmias are abolished by atropine (2), thus indicating that some of the actions are due to release of acetylcholine from nerve endings.

Because cultured heart cells are insensitive to many cardioactive agents, it was of interest to determine whether these cells, like intact myocardium, are sensitive to veratridine. Since the cells are grown in monolayer sheets devoid of neurons, this preparation permits examination of the direct effects of veratridine on the membrane properties of myocardial cells. It was found that veratridine rapidly and nearly completely depolarizes mainly due to an increase in resting Na^+ permeability; the K^+ permeability appears also to be increased. Tetrodotoxin prevents the increase in resting P_{Na} produced by veratridine but not the increase in P_K .

METHODS

The tissue culture and electrophysiological techniques employed have been described previously (17). Briefly, ventricles of 7-15 day chick embryos were treated with 0.025% trypsin in a Ca^{++} - and Mg^{++} -free solution to disperse the cells. The cells

were grown on microscope cover slips and incubated at 37°C for 3–28 days. The incubation medium was a modified Puck solution containing 15% (v/v) horse serum, 40% synthetic nutrient medium (N-16 medium from Microbiological Associates, Bethesda, Md.), 45% Ringer solution, antibiotics (penicillin, 50 U/ml, and streptomycin, 50 U/ml), and a fungicide (Fungizone, E. R. Squibb & Sons, New York, N. Y. 2.5 ml/liter). The ionic composition of the medium was (in mM): 4.6 K⁺, 145 Na⁺, 1.9 Ca⁺⁺, 0.7 Mg⁺⁺, 143 Cl⁻, 9.7 HCO₃⁻, 0.5 H₂PO₄⁻, and 0.8 SO₄⁻.

The single cells usually assembled into monolayer groups attached to the surface of the culture dish, and most of the cultures studied consisted of such monolayers of cells organized into loose reticular networks. The flattened ribbon-like cells varied in size from culture to culture, but a typical cell was about 3–5 μ thick, 15 μ wide, and 100–200 μ long. When in contact with one another, neighboring cells contracted synchronously. Contiguous cells are functionally connected by junctions that appear to be of high resistance (17). It is unlikely that veratrine acted primarily on the junctional membranes.

During the experiments, Puck's medium was replaced with normal Ringer solution having an ionic composition of (in mM): 2.7 K⁺, 149 Na⁺, 1.8 Ca⁺⁺, 1.0 Mg⁺⁺, 145 Cl⁻, 11.9 HCO₃⁻, and 0.4 H₂PO₄⁻. In some experiments, the culture was washed several times and completely replaced by a medium of different ion composition, such as Cl⁻-free solution (methyl sulfonate substitution). Ion concentrations (Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺, Li⁺, Sr⁺⁺, Ba⁺⁺) in the various bathing solutions were measured with an atomic absorption spectrophotometer (Perkin-Elmer model 290). The following drugs were used: veratridine (C₃₆H₅₁N₁O₁₁, mol wt of 673.8), veratrine·SO₄ (Nutritional Biochemicals), tetrodotoxin (Sankyo, crystallized powder obtained from Calbiochem Co.), and cocaine·HCl (Merck). All drugs were dissolved in distilled water, except veratridine which was dissolved in an equimolar amount of dilute HCl. Following recording during a control period, a small volume (0.05–0.20 ml) of a concentrated solution of the drug was added to the culture bath (volume of about 1.5 ml). Since the bath could not be stirred because the microelectrode would become dislodged from the cell, there was a lag period of about 1–3 min for complete mixing. Mixing occurred as a result of mechanical disturbance and turbulence produced by the added solution and diffusion.

The cells were impaled while observed with a long working distance objective (X 32, Leitz) at a magnification of 640 times. The temperature of the culture bath was maintained at 35°C during the experiments. The microelectrode, mounted on a de Fonbrune micromanipulator, passed through the sidearm of the culture flask at an angle of approximately 12° from the horizontal. The glass capillary microelectrodes were filled with 3 M KCl and had resistances of 20–35 MΩ (tip diameter of 0.5 μ or less). The reference electrode was an agar-Ringer salt bridge, and both electrodes were connected to Ag:AgCl half-cells. Potentials were amplified by an electrometer-input dc preamplifier having capacity neutralization and recorded on a dual-beam cathode ray oscilloscope. A bridge circuit was used so that the microelectrode could simultaneously pass polarizing current pulses and record voltage (18). The bridge was balanced with the microelectrode extracellular before each penetration by applying rectangular current pulses (duration of about 1 sec) and adjusting the variable

bridge resistance until there was no DC deflection; balance was rechecked after withdrawal of the electrode. The second oscilloscope channel was used to record the first derivative of the action potential or to monitor the applied current as the voltage drop across a known resistance.

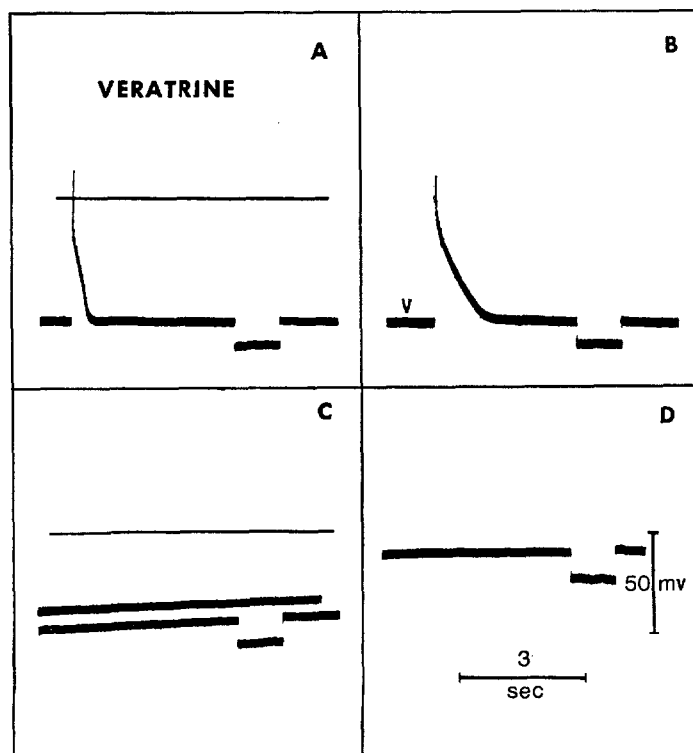


FIGURE 1. Effect of veratrine on membrane potentials and input resistance of a cultured heart cell bathed in normal Ringer solution. Calibrations for voltage (V), time (t), apply to all photos. Horizontal lines in A and C mark zero potential level. Anodal current pulses of 1 na applied in all photos. A, 1 min after addition of veratrine (final concentration 1.4×10^{-4} g/ml); no change from control conditions had occurred. B, prolongation of repolarization phase of action potential without change in resting potential at 2.5 min after veratrine. C-D, successive photos at 4-5 min; two sweeps superimposed in C.

RESULTS

Prolongation of Action Potential Repolarization and Depolarizing Afterpotentials

Addition of veratrine ($0.2-4 \times 10^{-4}$ g/ml) or veratridine ($0.2-5 \times 10^{-5}$ g/ml) to the medium bathing cultured heart cells rapidly produced changes in the shape of the action potentials (Fig. 1). These changes, which occurred in the absence of Cl^- as well as in its presence, appeared within 1-3 min. The late phase of repolarization was the first region to become prolonged (Fig. 1 B), and this change was first observed before any change in resting

potential (E_m). The prolongation of repolarization was not always apparent, especially when depolarization was rapid.

Sometimes the prolongation of repolarization was so pronounced that it

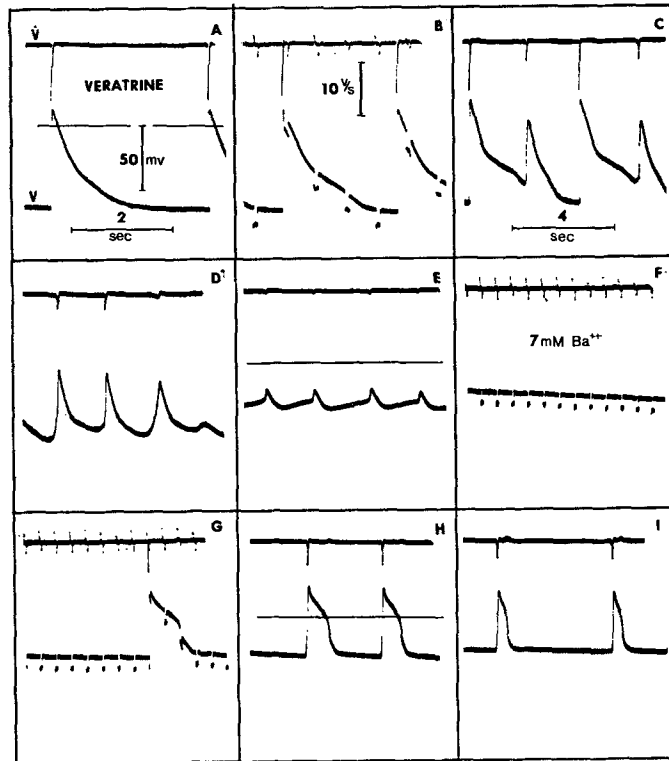


FIGURE 2. Effect of veratrine on membrane potentials of a cell bathed in Cl^- -containing Ringer, and the antagonism of the veratrine-induced inexcitability by Ba^{++} . Calibrations for V and $+\dot{V}_{\text{max}}$ apply to all photos; time calibration in A applies to A and B, that in C applies to C-I. Horizontal lines in A, D, and H mark zero potential level. Anodal current pulses of 1 na applied in B, and F-G. A, 3 min after addition of veratrine (1×10^{-4} g/ml). B-E, 4 (B), 5.5 (C), 6 (D), and 7 min (E) after addition of veratrine. F-G, within 30 sec (F) after addition of 7 mM Ba^{++} , hyperpolarization begins and spontaneous action potentials begin at 45 sec (G). H-I, 1 and 2 min after Ba^{++} , the interspike interval increases while action potential duration decreases; spontaneous action potentials ceased at 2.5 min.

resembled a long-lasting depolarizing (negative) afterpotential (Fig. 2 A, B). A single "premature" action potential sometimes occurred during the late phase of the afterpotential; as illustrated in Fig. 2 C, the amplitude of the premature action potential and its maximum rate of rise ($+\dot{V}_{\text{max}}$) were depressed. It sometimes appeared that the depolarizing afterpotential became indefinitely sustained, with repetitive small action potentials superimposed (Fig. 2 D).

The sustained depolarizing afterpotential induced by veratrine was similar to that produced by Ba^{++} in smooth muscle (23) and cultured heart cells (20). Fig. 3 illustrates this action of Ba^{++} (5 mM) on a quiescent cultured heart cell. Ba^{++} initially produced a small depolarization leading to oscillations of E_m (Fig. 3 A). Then action potentials with large and prolonged

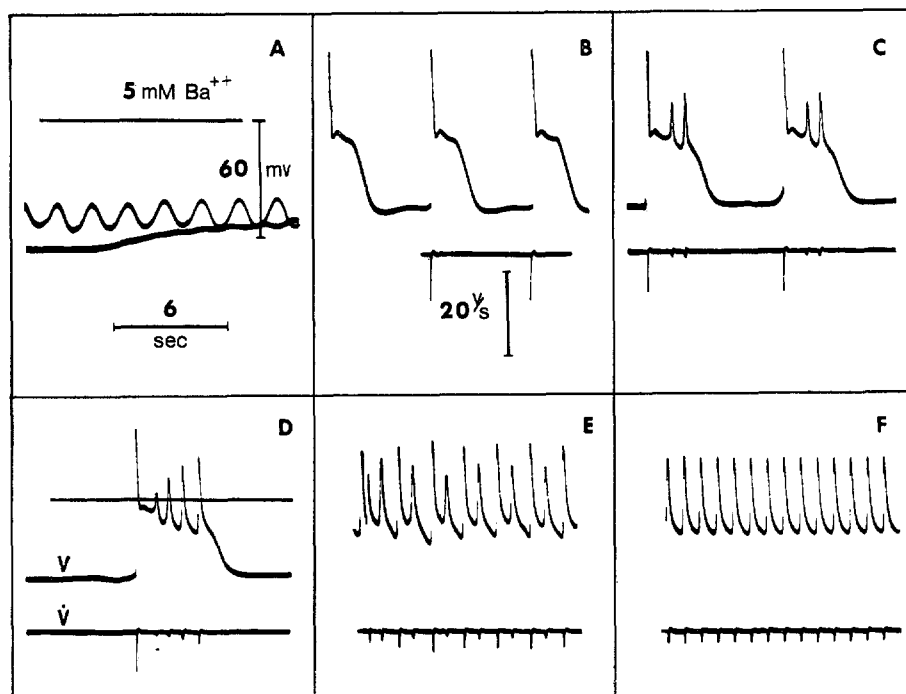


FIGURE 3. Sustained depolarizing afterpotentials produced in one cell in the presence of Ba^{++} . Calibrations for V , t , and $+\dot{V}_{max}$ apply to all photos; horizontal lines in A and D mark zero potential. A, two successive sweeps superimposed at 20 sec after addition of Ba^{++} (5 mM) showing onset of depolarization and oscillations. B, at 1 min after Ba^{++} , action potentials with long plateaus appear. C-D, at 2 and 3 min, the plateau becomes more prolonged and has repetitive action potentials superimposed. E-F, at 5 and 7 min, rhythmically firing action potentials arise from a sustained plateau.

depolarizing afterpotentials occurred (Fig. 3 B). The afterpotentials had repetitive spikes superimposed (Fig. 3 C, D), and became progressively longer leading to an indefinitely sustained afterpotential or "plateau" (Fig. 3 E, F). Sometimes the sustained plateau state shifted to the resting state either with the application of hyperpolarizing current pulses or spontaneously; i.e., there were two stable states.

Sr^{++} (5 mM) also produced prominent depolarizing afterpotentials, sometimes with one or more spikes superimposed (20). In addition, moderate sized depolarizing afterpotentials were occasionally observed in normal

media, and they became augmented in media made hypertonic with sucrose (13).

There appeared to be no effect of the veratrine alkaloids on automaticity. Quiescent and infrequently firing nonpacemaker cells were not converted to

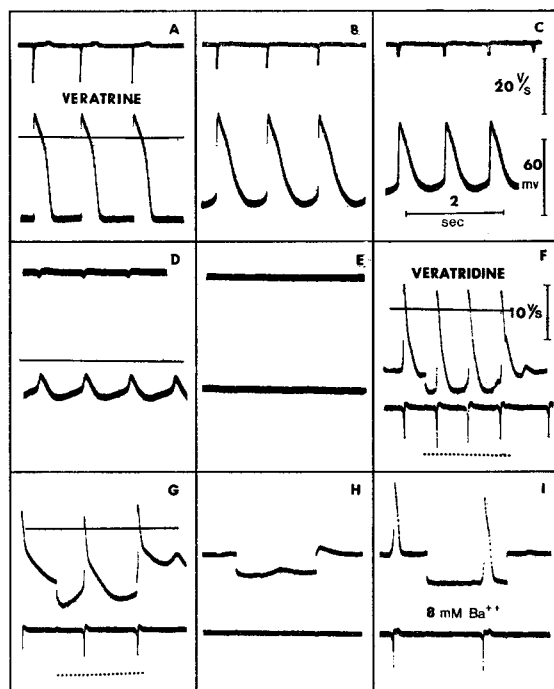


FIGURE 4. A-E, effect of veratrine on resting and action potentials of a rhythmically driven nonpacemaker cell in normal Ringer solution. Calibrations for V , $+V_{\max}$, and t apply to all photos. Horizontal lines in A and D mark zero potential. A, control response obtained 1.5 min after addition of veratrine (1×10^{-4} g/ml). B-E, records obtained at 2, 2.5, 3, and 4 min. F-I, reversal of veratridine-induced inexcitability by hyperpolarizing current pulses and by Ba^{++} in a pacemaker cell. Calibrations apply to all photos; zero potential level marked by horizontal lines in F and G. Anodal current pulse of 1.5 na applied in F-I; dotted lines mark pulse application. F, control record obtained at 2 min after addition of veratridine (2×10^{-6} g/ml). G-H, at 4 and 7 min after veratridine. I, action potentials obtained at 1 min after the addition of Ba^{++} (8 mM).

pacemaker cells, as shown in Fig. 1 C-D. The frequency of firing of rhythmically driven nonpacemaker cells was not increased by veratrine, even during the induced depolarization (Fig. 4 A-E). However, sometimes the frequency became decreased indirectly when the action potentials were prolonged. Even pacemaker cells, which normally increase their rate of firing with electrotonic depolarization, did not increase their rate with the veratrine depolarization. In fact, their frequency decreased concomitant with increased duration of each action potential (Fig. 4 F-I).

Depolarization and Loss of Excitability

Experiments were performed in Cl^- -free solutions to determine whether Cl^- was involved in the veratrine-induced depolarization. A period of 20–30 min was allowed for equilibration. The veratrine alkaloids rapidly depolarized all cells both in the presence and absence of Cl^- . Depolarization began within 1–4 min and was complete within 4–7 min (Fig. 5). In the presence of Cl^- , the final value of the resting potential was -12 ± 2 mv (measurements were

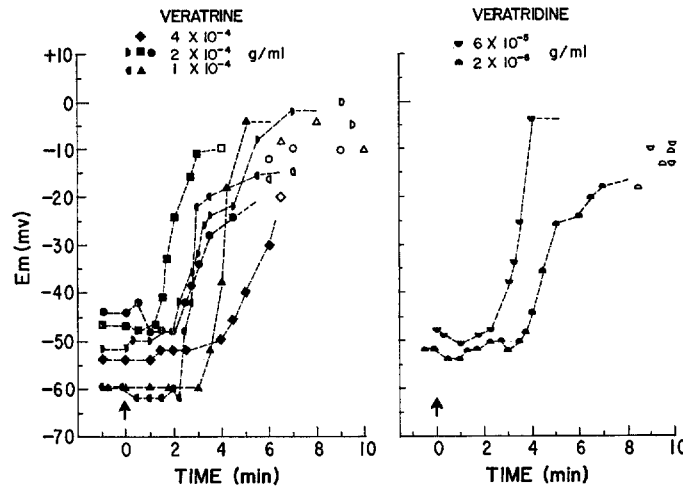


FIGURE 5. Plot of time course of depolarization produced by various concentrations of veratrine and veratridine in cultured heart cells. Ordinate, resting E_m in millivolts; abscissa, time in minutes. The drugs were applied at zero time (marked by arrows). Each type of closed symbol refers to multiple measurements made on an individual cell. The open symbols represent single measurements made on other cells in the same culture.

made in several cells in each of 11 different cultures). In Cl^- -free media, the final resting E_m of -14 ± 2 mv (15 cells in 3 cultures) was not significantly different from the value obtained in the presence of Cl^- . No difference in degree of depolarization was observed in pacemaker (Fig. 4 F-I) vs. non-pacemaker cells (Fig. 4 A-E). All drug concentrations used had a substantial depolarizing action.

Measurements of input resistance (r_{in}) during the course of veratrine-induced depolarization showed variable changes (Fig. 6 B). During the initial period of depolarization, r_{in} was diminished in three experiments, unchanged in one experiment, and increased in two experiments. At peak depolarization, r_{in} was increased in four out of six experiments. The data plotted in Fig. 6 were from experiments done in the presence of Cl^- ; similar results were obtained in the absence of Cl^- . Since in Cl^- -free solution, $g_{\text{Cl}} = 0$, measure-

ments of r_{in} should reflect more clearly any changes in g_K . The large depolarization produced by veratrine could have contributed to the changes in r_{in} , as illustrated in Fig. 1, in which r_{in} did not change (A-C) until con-

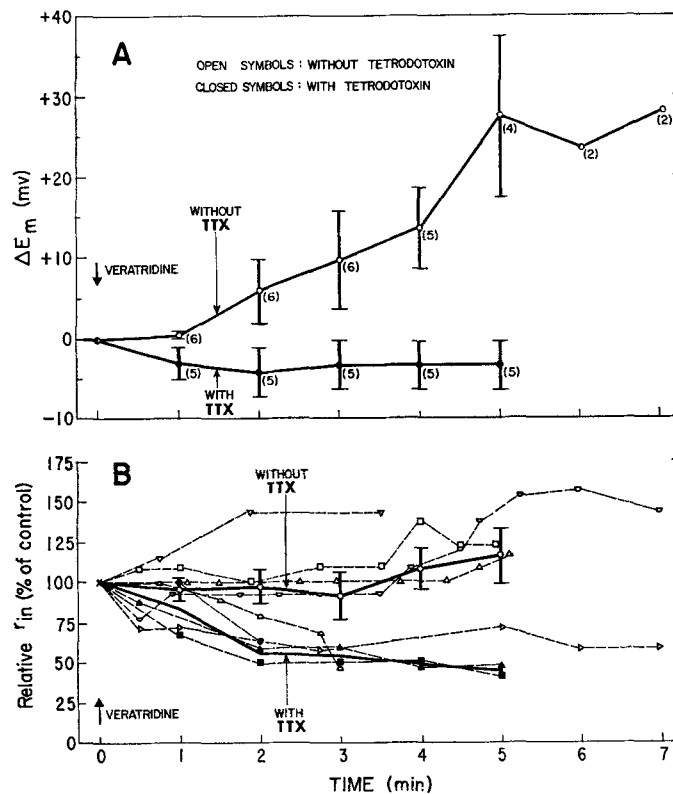


FIGURE 6. Influence of tetrodotoxin ($1-2 \times 10^{-6}$ g/ml) on depolarization (A) and changes in input resistance, relative r_{in} (B) produced by veratridine (10^{-6} – 10^{-5} g/ml). Open symbols, in the absence of tetrodotoxin (TTX); closed symbols, in the presence of TTX. A, time course of change in resting E_m (mean \pm SEM) after administration of veratridine at zero time (at arrow). Number of measurements represented by each point indicated in parentheses. B, symbols connected by heavy continuous line pass through the mean values; vertical bars represent the SEM. Symbols connected by light dashed lines are multiple measurements from individual cells. Number of measurements made in the absence of TTX same as those in part A; however, in the presence of TTX, data from only three cells are plotted. In the presence of TTX, veratridine produces a small hyperpolarization while r_{in} is simultaneously reduced.

siderable depolarization had occurred (D). Because of this complication, experiments were designed to examine the effect of veratrine on r_{in} when changes in resting E_m were prevented (see section below on tetrodotoxin).

The steady-state voltage/current curves determined at peak veratrine depolarization mainly for the anodal branch were linear and had a slope of

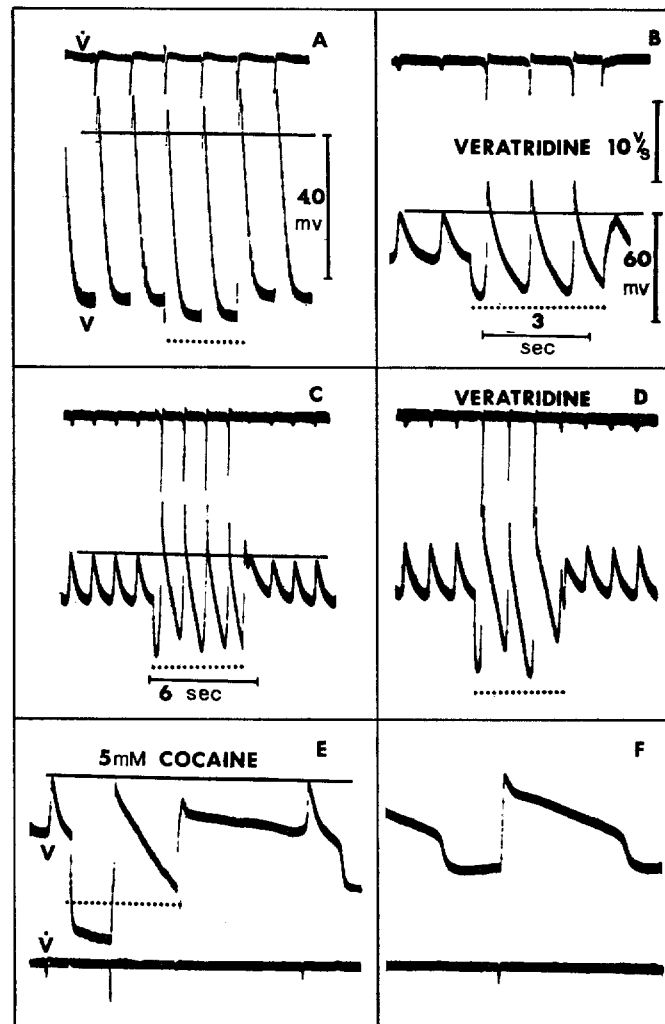


FIGURE 7. Restoration of action potentials by anodal current pulses in a cell partially depolarized by veratridine. Calibration for $+\dot{V}_{\max}$ in B applies to all photos. Time calibration in B applies to A-B; that in C to C-F. Calibration for voltage in A applies only to A, that in B applies to B-F. Horizontal lines in A, B, C, and E mark the zero potentials. A, control obtained in Cl^- -free Ringer; hyperpolarizing current pulse of 1 na applied. B-C, at 50 and 53 min after addition of veratridine (4×10^{-6} g/ml); anodal current pulses of 3 and 6 na applied, respectively. D, at 2 min after second dose of veratridine (8×10^{-6} g/ml total concentration) was added; hyperpolarizing current pulse of 6 na applied. E, 2 min after cocaine (5 mM); appearance of action potentials with prominent plateaus. F, plateau phase still prominent at 4 min after cocaine; resting E_m later declined.

about 12 M Ω , which is similar to the resistance determined under normal conditions (18). It was also found that r_{in} was not substantially different in Cl⁻-free solution, indicating that P_{Cl} is low relative to P_K .

Concomitant with depolarization, veratrine eventually abolished excitability, as shown in Figs. 2 and 4. The amplitude of the action potentials progressively diminished, and $+\dot{V}_{max}$ decreased as the cell depolarized (13); $+\dot{V}_{max}$ was not affected as long as the resting E_m remained unchanged. At the potential level at which all spontaneous activity ceased, the application of hyperpolarizing current pulses allowed action potentials to develop, as previously described (20). The amplitude of action potentials occurring during such artificial repolarization gradually declined during the later stages of depolarization and eventually failed (Fig. 4 H). A similar phenomenon was observed in cells only partially depolarized by low concentrations of veratridine; hyperpolarizing current pulses produced larger action potentials with faster rates of rise (Fig. 7 B-D).

Contractions ceased when the spontaneous action potentials disappeared, which usually occurred when the resting E_m reached -25 to -20 mv. However, small oscillations of E_m sometimes remained, and hyperpolarizing current pulses allowed the development of large action potentials with accompanying contractions.

Cocaine (2-5 mM) did not reverse the veratridine depolarization in two experiments, in contrast to the finding on nerve (24). Instead, the cells became further depolarized. In addition, in a cell only partially depolarized by a low concentration of veratridine (10⁻⁷ g/ml), cocaine prolonged the duration of the action potentials (Fig. 7 E-F). The action potentials which developed during hyperpolarizing pulses were longer in duration after cocaine (compare Fig. 7 E with D). There was evidence for a spontaneous transition between two stable states (right side of Fig. 7 E).

Reversal of Veratridine-Produced Inexcitability by Ba⁺⁺

In cells depolarized and rendered inexcitable by veratridine, Ba⁺⁺ (5-10 mM) rapidly restored excitability in each of three experiments (Figs. 2 F-I and 4 I). Concomitant with the restoration of spontaneous action potentials, there was a small repolarization of 5-10 mv (Fig. 2 F-H). These effects of Ba⁺⁺ were transient, disappearing with 2-10 min. Although additional increments of Ba⁺⁺ also hyperpolarized transiently, action potentials did not reappear; r_{in} increased slightly during each transient hyperpolarization.

Sr⁺⁺, unlike Ba⁺⁺, did not reverse veratrine-induced depolarization and inexcitability in five experiments. Furthermore, the prior presence of Sr⁺⁺ (5 mM) did not protect against subsequent veratrine depolarization in four experiments.

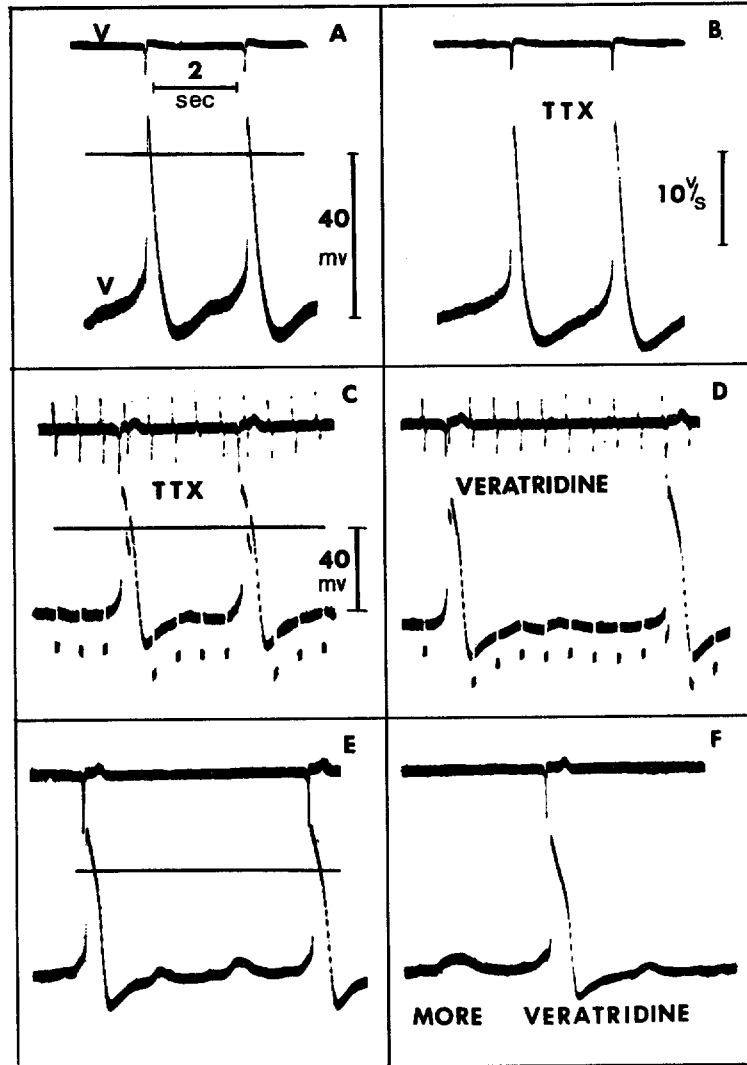


FIGURE 8. Protection by tetrodotoxin (TTX) against veratridine-induced depolarization and inexcitability. Calibrations for t and $+\dot{V}_{\max}$ apply to all photos. Voltage calibration in A applies to A-B, and that in C applies to C-F. Horizontal lines in A, C, and E mark zero potential. A-B, records obtained from single impalement. A, control in normal Ringer. B, unchanged pattern of electrical activity 10 min after addition of TTX (1.8×10^{-6} g/ml). C-F, records obtained from a single impalement in another culture. C, control response after preexposure to tetrodotoxin (2×10^{-6} g/ml) for 30 min; anodal current pulses of 0.6 na applied. D-E, records obtained at 0.5 min and 7 min, respectively, after addition of veratridine (9×10^{-6} g/ml); anodal pulses of 0.6 na. F, 8.5 min after second dose of veratridine (total concentration 1.7×10^{-5} g/ml).

Prevention of Veratridine Depolarization by Na⁺-Free Sucrose Solution and by Tetrodotoxin

NA⁺-FREE SOLUTIONS Two Na⁺-free solutions were used, NaCl being replaced by LiCl or by sucrose. In Li⁺-Ringer, veratrine produced its usual effects: it depolarized and abolished excitability within 3–6 min.

Bathing the cells in Na⁺-free sucrose-Ringer abolished action potentials and contractions; large resting potentials of –40 to –50 mv were obtained. Even high concentrations of veratrine (4×10^{-4} g/ml) did not depolarize over observation periods of up to 30 min (in three out of three cultures).

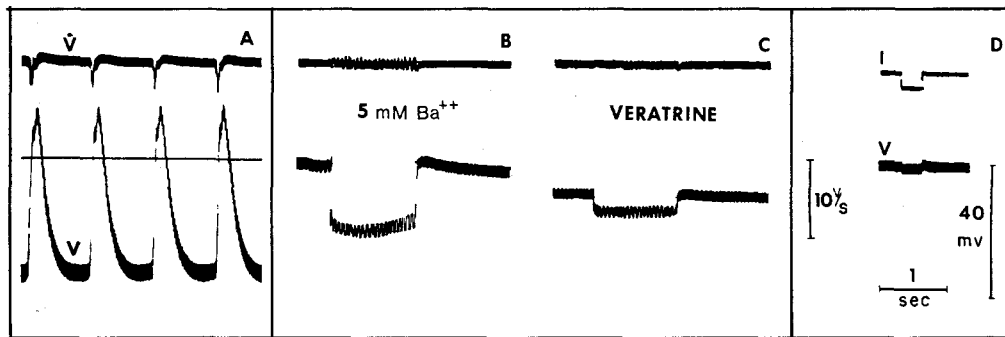


FIGURE 9. Decrease in input resistance (r_{in}) of a cell produced by veratridine following an initial increase in r_{in} produced by Ba^{++} . Calibrations for V , i , and $+\dot{V}_{max}$ apply to all photos; horizontal line in A marks zero potential level. Hyperpolarizing current pulses of 1 na applied in B–D. A, control in normal Ringer. B, depolarization and loss of action potentials at 4 min after addition of 5 mM Ba^{++} . C, at 1 min after addition of veratridine (2×10^{-4} g/ml); input resistance decreased as indicated by smaller ΔE_m produced by the anodal pulse. D, zero potential level shown after withdrawal of microelectrode.

TETRODOTOXIN No changes in resting E_m , in action potential amplitude, $+\dot{V}_{max}$, or frequency, and in spontaneous contractions were observed with tetrodotoxin, as illustrated in Fig. 8 A–B. In agreement with our previous report (19), the cells were insensitive to tetrodotoxin in very high concentrations. Tetrodotoxin was therefore used to determine whether changes in r_{in} would occur with veratridine in the absence of changes in membrane potential. In the prior presence of tetrodotoxin (2×10^{-5} g/ml) for about 20 min, the actions of veratridine (0.4 – 1.7×10^{-5} g/ml) on resting E_m (Fig. 6 A) and on excitability were prevented in all five experiments in which it was used. As illustrated in Fig. 8 C–F, veratridine did not change the action potential amplitude and $+\dot{V}_{max}$ over a period of 20 min. Spontaneous contractions were also unaffected, and they continued vigorously. However,

veratridine (10^{-5} – 10^{-4} g/ml) caused a reduction of r_{in} of about 50% within a few minutes in each of three experiments (Fig. 6 B). Although the active site of tetrodotoxin may be a guanidinium group (4), guanidine (4×10^{-4} M) was unable to protect against veratridine.

The prior addition of Ba^{++} (5 mM) was also used to demonstrate changes in r_{in} produced by veratridine, and is illustrated in Fig. 9. Ba^{++} produced nearly complete depolarization and markedly increased r_{in} (Fig. 9 B), as previously reported (19). The addition of veratrine (2×10^{-4} g/ml) reduced r_{in} without further depolarization (Fig. 9 C); a small repolarization of 10 mv accompanied the reduction of r_{in} in one experiment out of two.

DISCUSSION

These results clearly indicate that denervated cultured heart cells, like cells in intact adult hearts, are sensitive to veratrine alkaloids. As in other excitable tissues, these alkaloids produce depolarizing afterpotentials and depolarize. There are several mechanisms through which veratrine might depolarize heart cells and render them inexcitable: (a) by interfering with active $Na^+ : K^+$ transport (33); (b) by reducing g_K ; (c) by increasing g_{Na} ; (d) by a combination of increasing g_{Na} and decreasing g_K ; or (e) by increasing both g_{Na} and g_K . The mechanism underlying the depolarization must involve alterations in ion conductances in addition to any possible effect on ion pumping (5, 33). In preliminary experiments, veratrine did not significantly change the internal K^+ and Na^+ content of intact embryonic chick hearts in 15 min, arguing against inhibition of the cation pump.

Measurements of small changes in g_{Na} are not easily resolved by resistance measurements because g_{Na} makes a relatively small contribution to G_m . The evidence that veratrine increases P_{Na} includes the facts that depolarization does not occur in Na-free sucrose-Ringer solution or in the presence of TTX. The increase in the steady-state g_{Na} at an E_m of about -60 mv produced by aconitine (10^{-6} g/ml) in cardiac Purkinje fibers is also blocked by TTX (2×10^{-5} g/ml) (14). The action potentials of nerve and of some myocardial cells are sensitive to TTX, in contrast to the insensitivity demonstrated by cultured heart cells. There is considerable evidence that resting P_{Na} of nerve and muscle is increased by veratrine (8, 10, 24, 26, 27, 33).

There is also considerable evidence that veratrine alters the kinetics of the active P_{Na} changes during the action potential. In frog nodes, for example, veratridine (10^{-5} g/ml) prolongs the inward Na^+ current during activation for several seconds and then it depolarizes (26, 27). Lower concentrations (10^{-6} g/ml) produce large long-lasting depolarizing afterpotentials without an appreciable change in resting potential. Similar effects are also observed in squid and crustacean axons (10, 33) and in frog sartorius fibers (8). The normal return of P_{Na} to its resting value is prevented, according to Ulbricht

(26), by an extremely slow shutoff of the veratridine-induced P_{Na} . Lipid-soluble DDT also slows the turning-off process of the transient inward early current of nerve, but does not depolarize (3, 12).

The prolongation of action potential repolarization and the depolarizing afterpotential in cultured heart cells could be due to a holding open of activated Na^+ channels, as described by Hille for nerve (3). Since the partially depolarized membrane produced by veratridine is capable of generating spikes, part of the Na^+ channels must remain capable of activation. This suggests that the veratrinized membrane has a number of open Na^+ channels, which are not inactivated, in parallel with the fast opening but transient Na^+ channels, as described for nerve (26). Apparently veratrine induces not only an increase in steady-state g_{Na} , but also affects the kinetics of the g_{Na} changes during the spike.

Because heart cells have a low resting g_{Cl} , the principal determinant of G_m is g_K . The change in r_{in} of cells depolarized by veratridine could reflect a voltage-dependent change in g_K as well as a voltage-independent change in P_{Na} and P_K . The primary action of the drug could be the increase in g_{Na} which causes depolarization, and this in turn could indirectly affect g_K . The increase in R_m sometimes observed, especially at later times and larger depolarizations, could be secondarily due to anomalous rectification (cf. reference 30) (although substantial rectification is not usually observed with moderate electrotonic depolarization of nonpacemaker cells [18]).

However, veratridine must have a primary action to increase g_K as well as g_{Na} , since TTX prevented the increase in g_{Na} and depolarization by veratridine, but yet R_m decreased. Of course, the relative change in g_K is much smaller than that in g_{Na} . It is concluded that veratridine depolarizes by increasing resting g_{Na} ; it also tends to increase g_K but this action may be obscured by anomalous rectification when E_m is allowed to change. Further support for this conclusion comes from the findings that veratridine: (a) decreases r_{in} (increases g_K) in Ba^{++} -depolarized cells, and (b) does not convert nonpacemaker cells to pacemaker cells, and the automaticity of pacemaker cells is not increased with the subsequent depolarization. Automaticity should decrease when g_K is augmented and increase when g_K is diminished (20).

There is also evidence from other investigations that veratrine increases P_K as well as P_{Na} (24, 33). The membrane readily repolarizes and actually hyperpolarizes beyond the original resting potential upon addition of cocaine (24), which produces Na^+ inactivation (30), suggesting that there is a significant increase in resting g_K which brings E_m closer to E_K . Veratridine increases K^+ efflux of guinea pig hearts (28) and of resting nerve (5).

Veratrine depolarization differs from that produced by ouabain, cocaine, and tetracaine in that it is: (a) more rapid, (b) greater in degree, and (c)

not antagonized by Sr^{++} . The depolarization produced by these other drugs may result from inhibition of the $\text{Na}^+:\text{K}^+$ pump (21). In common with one another, the inexcitability produced by all these agents (21) is transiently reversed during artificial repolarization. These data indicate that the early stage of inexcitability is simply a product of the depolarization. The transient reversal of the veratridine-produced inexcitability by Ba^{++} , concomitant with a slight repolarization, may be due to the participation of inward Ba^{++} current in the action potential. (Preliminary experiments indicate that Ba^{++} and Sr^{++} participate in spike electrogenesis.) It is unlikely that this effect of Ba^{++} is due to its direct action of decreasing g_{K} (20, 22) because further "depolarization" toward E_{Na} would be expected. However, the equilibrium potential for Ba^{++} action, in the presence of Cl^- , is also at an E_m of 0 to -10 mv (20). Even though Ba^{++} and veratridine depolarize to about the same level, Ba^{++} acts by specifically decreasing g_{K} whereas veratridine acts by increasing g_{Na} and g_{K} . The inability of Sr^{++} , which stimulates ion pumping under normal or depressed conditions, to hyperpolarize and restore excitability of cells depolarized by veratridine would occur if pumping were maximally stimulated under such conditions because of the depolarization and the increase in $[\text{Na}^+]_i$.

It is surprising that Sr^{++} does not prevent veratridine depolarization since elevated $[\text{Ca}^{++}]_o$ prevents or reduces veratridine depolarization in nerve supposedly by competition with veratridine for membrane sites (24, 33). The action of veratridine in producing physical instability of stearic acid monolayers is also antagonized by high Ca^{++} (16). Veratramine, an amine alkaloid rather than an ester alkaloid, prevents the effects of veratridine in many cells (2, 6), and antagonizes the positive chronotropic action of triiodothyronine on cultured chick heart cells (32).

Veratridine, which is lipid-soluble, depolarizes even when applied internally (10), whereas tetrodotoxin, which is lipid-insoluble, is not effective when applied internally (11). Weak bases, like veratridine, will be partly in their undissociated form at slightly alkaline pH, and therefore permeable. Perhaps acetylcholine and norepinephrine are ineffective in our cultured heart cells because of their lipid insolubility and a disappearance of receptors in culture. Therefore, the autonomic transmitters may not have access to key sites in the membrane at which they normally produce their effects on ion conductances.

There is a similarity between the actions of veratridine on the conductile membrane of cultured heart cells and of acetylcholine on the postsynaptic membrane at vertebrate neuromuscular junctions. In each case, the agent increases both P_{Na} and P_{K} , and the equilibrium potentials (E_{Ver} and E_{ACh}) are about halfway between E_{Na} and E_{K} , i.e., 0 to -20 mv. Therefore, there is some analogy between conductile and postsynaptic membranes with

respect to the ability of agents to alter resting permeabilities. The difference in rapidity of action could reside in the relative access times to the conductance-controlling sites.

The facts that veratridine increases both P_{Na} and P_K , but apparently not P_{Cl} , as with ACh⁺ action at the neuromuscular junction, and that E_{ver} is halfway between E_{Na} and E_K , might suggest that this agent opens additional negatively charged channels which allow passage of cations only. However, since veratridine increases resting P_K even though its influence on P_{Na} is prevented by TTX, this suggests that it opens separate channels for Na⁺ and for K⁺ rather than a more nonspecific cation channel. A similar conclusion regarding separate ion channels was reached for conductile (3) and post-synaptic membranes (9). Tetrodotoxin may bind at sites controlling resting g_{Na} , sites occupied by TTX being unavailable to veratridine. Since neither TTX nor veratridine appears to have a depressing effect on active P_{Na} , the antagonism between these two agents on resting P_{Na} suggests that the Na⁺ channels determining resting P_{Na} and those determining active P_{Na} are operationally separate.

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