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MODIFICATION OF ELECTROPLAX EXCITABILITY BY VERATRIDINE

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SUMMARY

Veratridine influences membrane-potential changes arising both from the action potential and from the application of external cholinergic agonists in the isolated monocellular electroplax preparation. The action potential shows a long depolarizing after-potential in the presence of veratridine. The effects of various pharmacological agents and of external ion changes on this after-potential are similar to those reported for other nerve and muscle fibers and are consistent with the view that veratridine acts chiefly to increase the Na^+ conductance.

Membrane depolarizations by cholinergic agonists are inhibited by veratridine at pH 7 but strikingly amplified at pH 9. The former effect appears to involve interaction with the cholinergic receptor at the surface of the membrane, while the latter potentiation parallels the increase in the spike after-potential at pH 9 and presumably arises from a Na^+ conductance increase.

Veratridine appears to interact with the component involved in the Na^+ conductance in the interior membrane phase. The possible localization of this component in both the conducting and synaptic membrane is discussed.

INTRODUCTION

Naturally occurring toxic compounds have become useful probes in the analysis of molecular interactions in a variety of biological systems. A number of such toxins have been shown to interact specifically with the acetylcholine receptor of excitable membranes. These include the snake venom neurotoxins, *e.g.* α-bungarotoxin and the α-toxin of *Naja naja*[1,2]. Other toxins, including the marine types tetrodotoxin and saxitoxin[3] and batrachotoxin from the skin of a small Colombian frog[4] act specifically on Na^+ pathways in these membranes. Batrachotoxin is reported to act by increasing Na^+ conductances; a similar action has been attributed to veratridine, a major alkaloid component of the alkaloid mixture veratrine obtained from the seeds of *Schoenocoulon officinale* of the lily family. A comprehensive review of the effect of veratridine on excitable membranes has been written by Ulbricht[5].

The study presented here describes several aspects of the action of veratridine

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on the monocellular electroplax preparation[6]. The electroplax preparation is a unique tool for studying components in excitable membranes. It is a large flat cell, roughly 5 mm × 10 mm, with approximately 20000–50000 synapses. Therefore it can be mounted in a chamber and used to separate two pools. Both the transcellular potential and, through the use of an internal microelectrode, the potential across either cell face can be monitored independently. The potential is generated by a ouabain-sensitive (Na^+, K^+)-ATPase[7,8]. Only the membrane on the innervated posterior face is excitable.

The innervated face of the electroplax may be excited and elicit an action potential both directly by an imposed outward current flow and indirectly by stimulation of nerve terminals at synapses on the innervated face[9,10]. Synaptic activation results in graded postsynaptic potentials and constitutes a non-propagated activation system which may be distinguished operationally from the electrically activated conducting-membrane system[11]. External applications of cholinergic agonists in the solution bathing the innervated face result in depolarizations of the innervated membrane by synaptic activation; the magnitudes of these depolarizations may be related quantitatively to the concentration of agonist in the bath and indicate that depolarization results from a receptor–agonist complex. Antagonists may be added to compete with the agonist for the receptor site and thus to inhibit the depolarizations[12,13]. The conductance increases arising from synaptic activation involve both Na^+ and K^+ (ref. 14), while those resulting from electrical stimulation of the conducting membrane involve mainly Na^+ (ref. 15).

Despite the operational distinctions between the synaptic and conducting membranes, the chemical mechanisms responsible for activation in the two systems may be similar[16]. Acetylcholinesterase, which terminates the activation arising from the physiological agonist acetylcholine, is localized in both synaptic and conducting membranes[17]. The localization of the receptor has been more difficult to demonstrate by electron microscopy. Experimentally, the properties of the receptor have been defined only in the synaptic membrane; however, the failure of certain cholinergic antagonists to block the action potential of the conducting membrane has been shown, in the squid giant axon, to result from membrane structural barriers which prevent access to a putative receptor in the conducting membrane[18]. The specific ion channels responsible for conductance changes in the synaptic and conducting membranes have been termed ionophores[19]. Although little is known about the molecular basis for the action, tetrodotoxin appears to distinguish between ionophores in the two membranes by preventing Na^+ conductance increases only in the conducting membrane[20,15].

A recent report[21] indicated that veratrine acts by increasing the Na^+ conductance in the isolated electroplax preparation. In the present study veratridine is shown to have an effect on membrane potential changes arising both from the action potential and from the application of external cholinergic agonists. The use of veratridine as a probe of synaptic and conducting membrane ionophores is discussed.

METHODS

The electroplax preparation has been described previously[6,22]. Action potentials were generated either directly by cathodal or indirectly by anodal stimulation with 0.1-ms monophasic pulses[20,10] and were recorded extracellularly on a Tektronix storage oscilloscope. Resting potentials were monitored intracellularly[23].

The effects of various agents on the excitable membrane were determined by their addition to the solution bathing only the innervated face unless otherwise stated. Dose–response curves for agonists and antagonists were obtained as in a previous report[12]. The composition of the eel–Ringer's solution in mM was: NaCl, 160; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; H_2NaPO_4, 0.3; HNa_2PO_4, 1.2; glucose, 10; pH 7.0. If the pH was raised to 9.0 all experiments were conducted in Tris–Ringer's solution, in which the phosphate buffer was replaced by 2 mM Tris.

Veratridine (mol. wt 674) was obtained from Penick Chemical, New York; tetrodotoxin was from Sankyo Ltd, Tokyo; and the remaining reagents were readily available commercially.

RESULTS

Effect of veratridine on the resting and action potential

Veratridine applied to the innervated face of the electroplax affects neither the rate nor the amplitude of the rising phase of the action potential singificantly, but causes both a decrease in the negative slope of the falling phase and a long depolarizing after-potential. In contrast to nerve fiber preparations[5] the resting potential is not affected by the drug even at concentrations of up to 1 mM for as long as 40 min unless the cell is stimulated. Application of 1 mM veratridine to the non-innervated face for 15 min has no effect on either the resting or the action potential. Fig. 1 demonstrates the effect of repetitive stimulation on a cell preincubated in 20 μM (13.5 μg/ml) veratridine. The amplitude of the first action potential is only slightly reduced compared to the control. The remaining spikes were elicited during the latency period when the cell retains a depolarizing after-potential of about 10 mV; the duration and amplitude of the action potentials progressively decrease, and the spike can be blocked with further stimulation. The after-potentials are reduced when the cell is stimulated in the state of depolarization. Similar observations have been made on frog fibers[24].

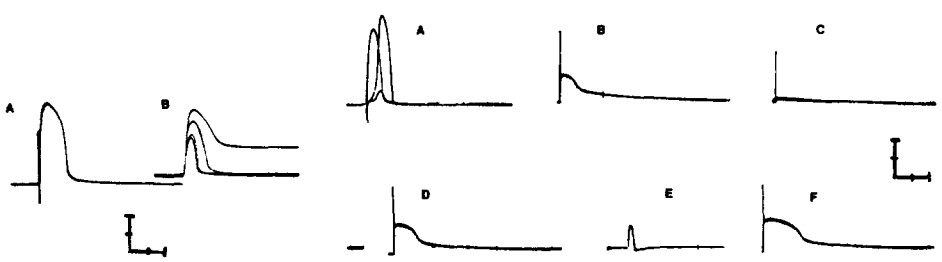

Fig. 1. (Left) Effect of repetitive stimulation in the presence of veratridine on the action potential. Direct spikes were recorded as outlined in the Methods section. A, control; B, cell was exposed to 20 μM veratridine for 60 min prior to the recording; four externally recorded action potentials evoked by stimulation at a frequency of 2 per s are superimposed. Calibration unit: 25 mV and 2 ms.

Fig. 2. (Right) Action of veratridine on cell depolarized by increased K^+ concentration. Sequential measurements without interruption were made at pH 7.2 on a single cell in normal Ringer's solution whose KCl concentration on the innervated face had been altered as described below. A, control; direct and indirect spikes. B–F, *plus* 20 μM veratridine; the KCl concentration and time of exposure prior to measurement were as follows: B, normal Ringer's solution for 12 min; C, 10 mM KCl for 4 min; D, normal Ringer's solution for 9 min; E, 15 mM KCl for 2.5 min; F, normal Ringer's solution. Calibration unit: 25 mV; A, 2 ms; B–F, 200 ms.

If the frequency of the repetitive stimulation is increased, the after-potentials are summed and result in larger depolarizations of the cell. If the cell is given a single stimulus while exposed to 1 mM veratridine, the after-potential can last as long as 10 min, and the action potential is sometimes blocked irreversibly. Recovery of the action potential after a short exposure to concentrations of veratridine up to 20 μM is complete; after long exposures or higher concentrations, recovery is incomplete and a small after-potential persists (see Figs 10A and 10I).

A dose–response curve showing the effects of veratridine concentration on the spike after-potential could not be obtained because of the lack of a quantitative cell response. The after-potential appears to be multi-phasic; after an initial lag phase the after-potential decay consists of both a fast and a slow phase (see Fig. 2). Each phase appears to have a somewhat different dependence on the veratridine concentration. If the area approximated by the product of the amplitude and the duration of the depolarizing after-potential is used as a rough measure of the veratridine effect, the cell response to veratridine increases sharply between 10 and 30 μM at pH 7.

Modifications of the veratridine response arising from changes in external ion concentrations.

Potassium. Variations in the concentration of KCl bathing the innervated face affect the veratridine response as shown in Figs 2 and 3. Each recording is the result of a single stimulus applied after exposure to the described solution. The cumulative effect of veratridine with time is also indicated in these figures (*cf.* Figs 2B, 2D and 2F).

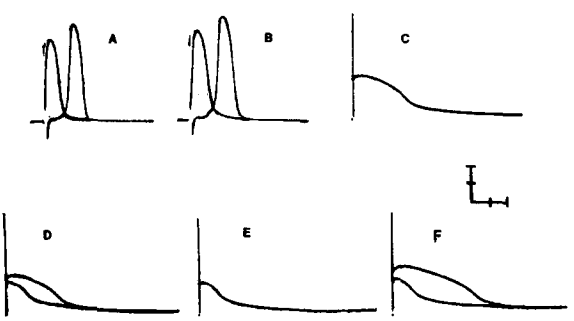

Fig. 3. Action of veratridine on cell hyperpolarized by decreased K^+ concentration. Measurements as in Fig. 2, pH 7.0. A, control. B, 1 mM KCl for 5 min. C–F, *plus* 15 μM veratridine: C, 1 mM KCl for 3 min; D, lower trace, normal Ringer's solution for 3 min, superimposed on C; E, normal Ringer's solution for 5 min; F, upper trace, 1 mM KCl for 2.5 min, superimposed on E. Calibration unit: 25 mV; A and B, 2 ms; C–F, 200 ms.

Increased K^+ concentrations reduce the veratridine-induced after-potential, and decreased K^+ concentrations enhance it. Similar observations have been made on crustacean and squid axons by Shanes[25]. These variations have been attributed to K^+-induced changes in the resting potential by Ulbricht[5], who demonstrated that similar variations could be induced by displacing the resting potential with constant currents.

Calcium. A 5-fold increase in the Ca^{2+} concentration decreases the duration of the after-potential (Fig. 4). A decrease in the Ca^{2+} concentration has no detectable effect on the veratridine response. In nerve fiber preparations high external Ca^{2+}

has been found to antagonize veratridine-induced spontaneous depolarizations, but variable Ca^{2+} effects have been reported on the after-potential induced at lower veratridine concentrations[5].

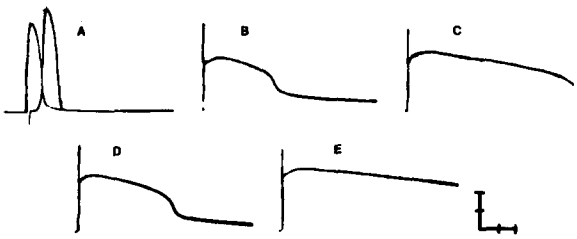

Fig. 4. Reduction of veratridine-induced after-potential at increased Ca^{2+} concentrations. Measurements as in Fig. 2, pH 7.0, except that the Ca^{2+} concentrations were altered. A, control. B–E, *plus* 20 μM veratridine: B, 10 mM $CaCl_2$ for 5 min; C, normal Ringer's solution for 5 min; D, 10 mM $CaCl_2$ for 5 min; E, normal Ringer's solution for 7.5 min. Calibration unit: 25 mV; A, 2 ms; B–E, 200 ms.

Sodium. Since the action potential is abolished when other ions are substituted for Na^+ (ref. 15), the effects of both twice and one-half the normal NaCl concentrations were investigated. In Fig. 5 the cell is exposed to hypertonic NaCl on both faces for 15-min periods. The resting potential is unchanged, but an increase in amplitude and a decrease in duration of the action potential are observed. Action potential thresholds are increased, and the indirect spike is sometimes blocked even though the post-synaptic potential is unchanged. All of these effects are completely reversed in normal Ringer's solution. Slightly higher concentrations of hypertonic NaCl were observed to depolarize a different cell irreversibly after exposure for 2 min. The effect of veratridine on the after-potential is decreased in hypertonic NaCl.

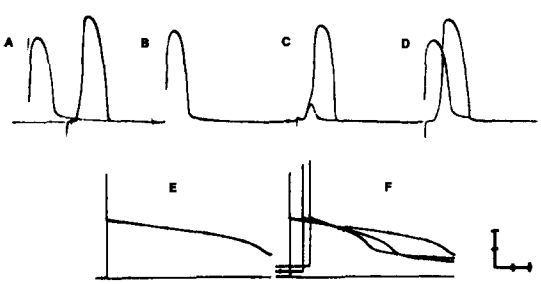

Fig. 5. Effect of doubling the NaCl concentration on the normal and veratridine-altered action potential. Sequential measurements without interruption were made at pH 7.0 on a single cell in normal Ringer's solution whose NaCl concentration on both the innervated and non-innervated faces had been altered as described below. The stimulating voltage thresholds for the appearance of the action potential evoked by direct and indirect stimulation, respectively, are also given. The NaCl concentration and time of exposure prior to measurement were as follows: A, control normal Ringer's solution; thresholds 0.5 and 15 V. B, 320 mM NaCl for 15 min; direct stimulation, threshold 0.8 V. C, cell as in B; indirect stimulation, sub-threshold (post-synaptic potential) 100 V, spike threshold 150 V. D, normal Ringer's solution for 13 min; thresholds 0.5 and 15 V. E, normal Ringer's solution *plus* 10 μM veratridine for 4 min; direct stimulation threshold 0.5 V. F (baseline and delay were changed to distinguish the spikes), second and third action potentials in 320 mM NaCl *plus* 10 μM veratridine for 2 and 14 min, respectively; direct stimulation thresholds 0.5 V; superimposed on E.

When the concentration of NaCl in normal Ringer's solution is reduced to 80 mM on both faces of the electroplax, additional components must be added to maintain osmolarity. If no such addition is made, the membrane is reversibly depolarized by 10–20 mV in the hypotonic solution. No component was tested as a substitute for 80 mM NaCl which did not give rise to both a decrease in the carbamylcholine-induced depolarization (Fig. 6) and a few mV reduction of the action potential. Compounds tested as substitutes were Tris, glucose, sucrose, choline chloride, $CaCl_2$ and $MgCl_2$. Furthermore, similar decreases in the response to carbamylcholine and in the action potential are observed when 160 mosM concentrations of the same components are added to normal Ringer's solution (Fig. 6). The veratridine-induced after-potential is diminished either by substitution or addition of the above components.

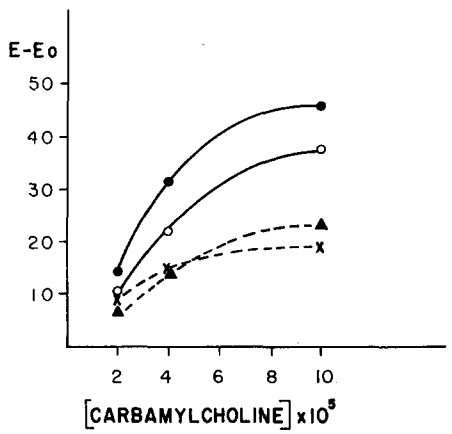

Fig. 6. Reduction of carbamylcholine-induced depolarization by sucrose. The mV depolarization of the innervated face (E–E_0) is plotted against the molar carbamylcholine concentration, as outlined in the Methods section. Curves were generated on the same cell at pH 7.0 in the following order: ●—●, normal Ringer's solution; ×– – –×, normal Ringer's solution in which 80 mM NaCl was replaced by 160 mM sucrose; ○—○, normal Ringer's solution; ▲—▲, normal Ringer's solution *plus* 160 mM sucrose. Modifications of the normal Ringer's solution were made on both the innervated and the non-innervated faces simultaneously.

A reduction of the veratrine response in solutions with a 50% reduction in NaCl concentration has been observed in frog muscle preparations[26]. However, Ulbricht and Flacke[24] showed that brief exposures of veratridine-treated frog fibers to hypertonic NaCl for a maximum of 15 s resulted in after-potentials which were markedly increased over those in normal NaCl. This result is in contrast to the one in Fig. 5, where the cell is exposed to hypertonic NaCl for a much longer period.

Effects of various pharmacological agents

Tetrodotoxin is known to block the sodium conductance increase associated with activation of the conducting membrane of the electroplax[15] and has been shown to completely reverse the Na^+ conductance increase caused by veratridine in frog fiber nodes[27]. A similar blocking action is shown in Fig. 7, where a low concentration of tetrodotoxin which reversibly reduced but did not block the action potential prevents all but a very slight veratridine-induced after-potential. Despite somewhat

different exposure times, one may conclude that the antagonism is at least as strong at pH 9.0 as at pH 7.0 (Figs 7C and 7E). This observation is significant in view of the much higher potency of veratridine at pH 9.0 described below.

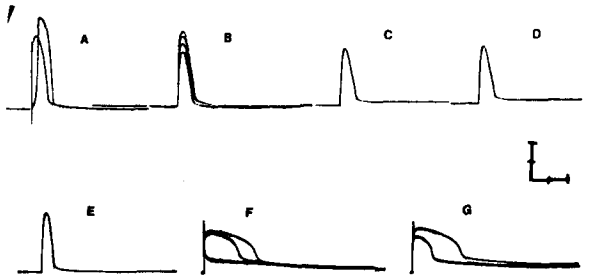

Fig. 7. Antagonism between veratridine and tetrodotoxin. Sequential measurements without interruption were made at the given pH on a single cell in either normal or Tris–Ringer's solution (see Methods) after exposure to either 5 ng/ml tetrodotoxin and/or 15 μM veratridine for the given period of time. A, pH 7.0, control; direct and indirect spike. B–G, direct spike. B, superimposed action potentials before and 1, 5, and 8 min after exposure to tetrodotoxin at pH 7.0. C, tetrodotoxin *plus* veratridine for 9 min at pH 7.0. D, tetrodotoxin for 10 min at pH 9.0. E, tetrodotoxin *plus* veratridine for 5 min at pH 9.0. F, veratridine for 1 min (lower trace), 5 min (middle trace), and 12 min (upper trace) at pH 9.0. G, tetrodotoxin *plus* veratridine for 1 min (upper trace) and 3 min (lower trace) at pH 9.0. Calibration unit: 25 mV, A–E, 2 ms; F and G, 200 ms.

The veratridine response is also reduced by tetracaine (Fig. 8). A small after-potential persists at tetracaine levels which greatly reduce the action potential. Eserine has an effect similar to tetracaine at concentrations which begin to decrease the action potential. Curare at concentrations up to 100 μM and dithiothreitol, which reduces disulfide bonds involved in the receptor[28], have no effect on the veratridine response.

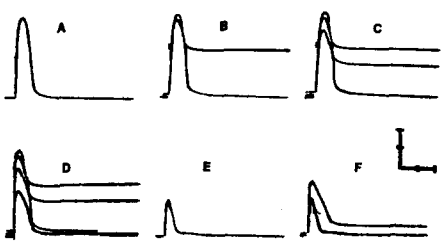

Fig. 8. Inhibition of veratridine-induced after-potential by tetracaine. Measurements as in Fig. 7 at pH 7.0 after exposure to either 15 μM veratridine or tetracaine for the given period of time. A–F, direct spike. A, control. B, veratridine for 4 min superimposed on A. C, veratridine *plus* 10 μM tetracaine for 2.5 min superimposed on B. D, veratridine *plus* 50 μM tetracaine for 3 min superimposed on C. E, veratridine *plus* 50 μM tetracaine for 6 min. F, veratridine *plus* 10 μM tetracaine for 3 min superimposed on E. Calibration unit: 25 mV, 2 ms.

Temperature dependence

The effect of temperature on the control and veratridine-modified action potential is shown in Fig. 9. Electroplax cells can withstand well temperatures between 5 °C and 40 °C if exposure to the extreme temperatures is brief. The duration and amplitude of the control action potential increases with a decrease in temperature, as has

been reported previously[29]; but the veratridine-induced after-potential decreases at lower temperatures. The post-synaptic potential and hence the indirect spike is reduced at 30 °C and blocked at 40 °C, in agreement with a previous observation that the post-synaptic potential is blocked above 32 °C[30].

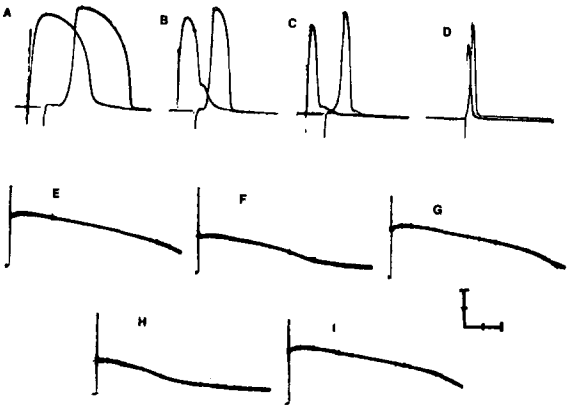

Fig. 9. Reduction of veratridine response at low temperature. The temperature of the pH 7.0 Ringer's solution was varied only on the innervated face of a single cell measured successively and action potentials were recorded immediately after the change. A–C, direct and indirect spikes at A, 10 °C; B, 20 °C; C, 30 °C. The post-synaptic potential appears on the falling phase of the direct spike in B and, in reduced form, in C but is absent in D. D, direct and "anodal break" direct spike (ref. 10, see also Methods). E–I, *plus* 20 μM veratridine. E, after 3.5 min at 20 °C. F, after 12 min at 10 °C. G, after 2 min at 20 °C. H, after 1.5 min at 10 °C. I, after 1.5 min at 20 °C. Calibration unit: 25 mV, A–D, 2 ms; E–I, 200 ms.

The pH dependence of veratridine effects on the depolarization by cholinergic agonists

The magnitude of the veratridine-induced after-potential increases sharply and reversibly as the pH is raised from 7.0 to 9.0 (Fig. 10). Recovery after washing in

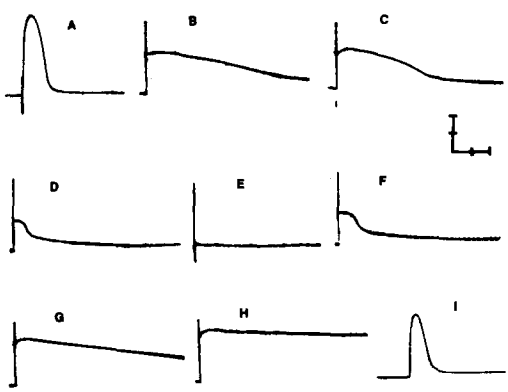

Fig. 10. Dependence of the veratridine-induced after-potential on pH. Sequential measurements without interruption were made at the given pH on a single cell in either normal or Tris–Ringer's solution (see Methods). The pH was altered only on the innervated face, the pool in contact with the non-innervated face remaining at pH 7.0. A–I, direct spike. A, control. B–H, *plus* 10 μM veratridine; each recording after exposure of the cell at the given pH for 5 min. B, pH 9.0. C, pH 8.0. D, pH 7.0. E, pH 6.0. F, pH 7.0. G, pH 8.0. H, pH 9.0. I, control, recovery after 40 min in normal Ringer's solution. Calibration unit: 25 mV; A and I, 2 ms; B–H, 200 ms.

normal Ringer's solution for 40 min is not complete, although a slight reduction in amplitude may be expected after exposure to Ringer's solution alone for 75 min. A determination of the dependence of the veratridine response at pH 9.0 on the veratridine concentration, in addition to the difficulties cited above for the determination at pH 7.0, was further complicated by frequent spontaneous depolarizations of 5–10 mV. These depolarizations are often triggered by stimulation and discourage an estimation of the quantitative effect of veratridine concentration. They are similar to depolarizations observed at pH 7.0 in the presence of strong inhibitors of acetylcholinesterase but apparently stem from a different mechanism, since preliminary experiments show that 10 μM veratridine does not inhibit soluble acetylcholinesterase activity appreciably (Chen, Y. T., unpublished observations).

At pH 7.0 veratridine acts as a weak receptor antagonist in inhibiting depolarization by carbamylcholine. The dose–response curve for carbamylcholine is shifted to the right in the presence of 20 μM veratridine (Fig. 11). In contrast to the veratridine-induced after-potential, the receptor antagonist action of veratridine reached a steady-state level of action very quickly. Repeated cycles in which carbamylcholine is applied and washed out in the presence of a constant concentration of veratridine result in the same depolarization in each cycle. This effect of veratridine is not blocked by tetrodotoxin and is completely reversible.

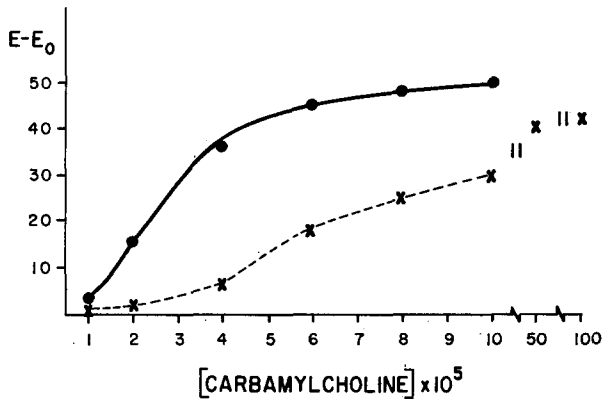

Fig. 11. Veratridine inhibition of depolarization by carbamylcholine at pH 7.0. The mV depolarization of the innervated face ($E-E_0$) is plotted against the molar carbamylcholine concentration, as outlined in the Methods section. ●—●, control; ×—×, *plus* 20 μM veratridine. Both curves were obtained on the same cell.

If the pH is shifted to 9.0 the effect of veratridine on depolarization by carbamylcholine becomes qualitatively different. This difference is illustrated in Fig. 12A, where a shift in pH from 6.5 to 9.0 without a change in the concentrations of either carbamylcholine or veratridine results in a striking depolarization of the cell at a very rapid rate. A similar pH shift in the absence of veratridine has no effect on the carbamylcholine depolarizations[31]. The pH shift has apparently converted veratridine from an inhibitor to a potentiator of the carbamylcholine depolarization. This potentiation is rapidly reversed by removing veratridine or restoring the pH to 7.0 while maintaining the carbamylcholine concentration at a constant level. The potentiation cannot be achieved by increasing the veratridine concentration at pH 7.0 (Fig. 12B).

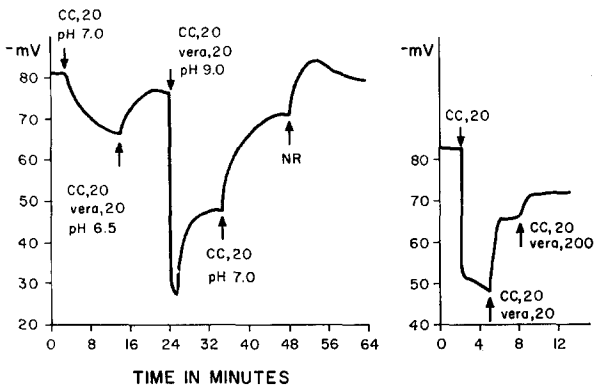

Fig. 12. Veratridine potentiation of depolarization by carbamylcholine at pH 9.0. Tracing of the membrane potential at the innervated face as a function of time in the presence of the indicated μM concentrations of carbamylcholine (CC) and veratridine (vera). A (left), pH changes are indicated; pH 6.5 and 7.0 are in normal Ringer's solution (NR) and pH 9.0 is in Tris–Ringer's solution (see Methods). B (right), pH 7.0.

DISCUSSION

Several characteristics of the veratridine effects on the action potential observed on the electroplax are similar to those seen with a variety of nerve and muscle fibers. Such similarities include the shape and duration of the depolarizing after-potential[5]; the decrease in the after-potential obtained when the resting membrane is depolarized, either by a summation of after-potentials following repetitive stimulation[24] or by an increase in the external K^+ concentration[25]; and the block of the after-potential resulting from the addition of tetrodotoxin[27]. The effects of local anesthetics and pH on the after-potential observed here are consistent with their effects on veratridine-induced depolarizations of nerve fibers[32,5]. Our observation that the after-potential is decreased in hypertonic NaCl does not confirm a previous report of increased after-potentials in veratridine-treated frog fibers[24]. The much longer period of exposure to hypertonic NaCl in our experiments, however, may prevent comparison of the two results.

The resting potential of the electroplax is not affected even by high concentrations of veratridine. This observation is in contrast to the large depolarizations seen with nerve fibers and corresponds with the failure of veratrine to depolarize the resting potential in frog muscle fibers[33].

Voltage clamp experiments performed on single nodes of frog motor fibers indicate that veratridine acts chiefly to increase the Na^+ conductance[5]. Such conductance increases are abolished in a Na^+-free medium or by tetrodotoxin. Current clamp and a. c. impedance measurements in the isolated electroplax show no veratrine effect on the resting conductance of the excitable membrane[21]. The prolonged after-potential caused by veratrine has the same Na^+ reversal potential characteristic of the peak of the action potential and arises from a Na^+ conductance increase.

The site of veratridine action is thought to be the membrane itself [5], and this idea is supported by internal perfusion experiments on axons in which the axoplasm

has been squeezed out[34]. Veratridine had the same effect when applied internally to the perfused axon as it had during external application to the intact axon.

Several aspects of veratridine action further support the idea that veratridine is acting in an interior, hydrophobic region of the membrane which is presumably associated with the Na^+ ionophore. The extremely long time required for veratridine to reach a steady-state level of action (ref. 5, see also Fig. 2) argues against an interaction at the membrane surface. Since veratridine is a weak base, its increased potency at higher pH values suggests that the neutral, uncharged form of the tertiary amine is the active form and may be acting in a hydrophobic region. This pH dependence is in sharp contrast to that shown by tertiary amines which act either as agonists or antagonists at the synapse or as local anesthetics on the conducting membrane[35]. These compounds more quickly attain a steady-state effect and are more potent in the charged form indicative of interaction at a receptor site on the membrane surface. Finally the decrease in veratridine potency on lowering the temperature to 10 °C (Fig. 9) is consistent with interaction in an interior membrane phase. Although the phase transition temperature of the innervated membrane of electroplax has not been studied, the phospholipid phase of the membrane at 10 °C would be expected to show more rigidity and to retard both the access and mobility of compounds approaching the interior of the membrane[36].

The reports of veratridine effects have been limited to conducting membrane, and no evidence of an effect on synaptic activation has been found. Kuffler[33] found that concentrations of veratrine which affect the nerve or muscle of the frog sartorius preparation profoundly have no appreciable influence on the sub-threshold end-plate potentials of the post-synaptic membrane. A similar conclusion was reached on the stellate ganglion of the cat [37]. On the electroplax at pH 7 veratridine has the properties of a typical antagonist of the receptor (Fig. 11). At pH 9, however, veratridine shows a large increase or potentiation of synaptic activation by carbamylcholine (Fig. 12A). An explanation of this pH-dependent difference can be based on the discussion in the preceding paragraph. Deprotonation of the tertiary amine group in veratridine by raising the pH to 9 shifts the distribution of veratridine binding to both the receptor and to the interior hydrophobic site associated with the ionophore in favor of the hydrophobic site.

The mechanism by which veratridine potentiates the carbamylcholine response at high pH is currently under study in this laboratory. The potentiation does not arise from the firing of an action potential, because the electroplax differs from muscle in its failure to fire any action potential as it is depolarized by externally applied agonist. Several explanations for the potentiation may be offered. Since current clamp and a.c. impedance measurements indicate that veratrine has no effect on the resting Na^+ conductance (data presumably recorded at pH 7), Ruiz-Manresa[21] concluded that veratrine acts specifically on Na^+ channels previously opened by an activation process. The potentiation of the agonist thus may occur by direct interaction of veratridine with the synaptic Na^+ ionophore opened by agonist depolarization. Alternatively, the potentiation may arise from a transitory activation of conducting membrane ionophores during agonist depolarization. This transitory activation could be so asynchronous as to fail to give rise to an action potential but could still allow veratridine to interact as it does during an action potential. A third explanation is offered by the work of Ulbricht[5], whose voltage clamp experiments on frog fibers

indicate that veratridine can interact with conducting membrane ionophores in the depolarized membrane without prior activation of the membrane. Veratridine-induced conductance increases are shown to be slowly reversible and to be a direct function of the membrane potential. If such a mechanism were operative in the electroplax at high pH, any agent which could partially depolarize the innervated membrane would be potentiated by veratridine.

The possibility that veratridine can directly interact with the synaptic as well as with the conducting membrane ionophore is important in determining the common features of activation in the synaptic and conducting membrane systems. No interactions of the synaptic membrane ionophore with a chemical probe have yet been reported; agents like tetrodotoxin which alter conducting membrane permeabilities have not yet been shown to affect synaptic membrane conductances.

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