Picrotoxin Convulsions Involve Synaptic and Nonsynaptic Mechanisms on Cultured Mouse Spinal Neurons

Abstract. *The cellular mechanisms underlying picrotoxin-induced convulsive activity were studied by using mouse spinal neurons growing in tissue culture. Picrotoxin-induced convulsive activity in most but not all of the cells studied. The activity could be inverted by polarizing to positive potentials and eliminated either by decreasing the ratio of calcium to magnesium or by applying tetrodotoxin. When applied locally to individual cells, picrotoxin lowered spike threshold and induced spontaneous firing in some but not all cells tested. The results suggest that picrotoxin-induced convulsive activity involves rapidly summating synaptic activity which may be evoked by high-frequency repetitive firing.*

One cellular event associated with pharmacologically induced epileptic foci in vivo is the paroxysmal depolarizing shift (PDS) (*1*, *2*). The PDS is an abrupt membrane depolarization with repetitive spiking and is thought to be either synaptic or nonsynaptic in origin (*3*, *4*). In some supraspinal areas of the central nervous system (CNS) these depolarizing events appear to have an inversion potential (*1*, *3*), a property characteristic of conventional synaptic activity (*5*). In the hippocampus, PDS-like activity consists of synaptically triggered spike bursts (*4*, *6*). Thus, while abrupt depolarizing shifts are a cellular correlate of convulsive activity evoked in different CNS areas, the mechanisms underlying their generation may not be identical. The precise mechanisms involved in the events have been difficult to study in vivo owing to the relative complexity of the intact mammalian CNS. Convulsive activity can be readily induced in monolayer cultures of mouse spinal neurons by a variety of convulsants active in vivo, including penicillin, pentylenetetrazole, picrotoxin, bicuculline, and strychnine (*7*). Monolayer cultures may thus be a suitable model system to resolve some of the mechanisms underlying convulsive activity. We report here that (i) picrotoxin-induced convulsive activity is generated by rapidly summating excitatory synaptic activity, and (ii) picrotoxin lowers spike threshold and induces spontaneous firing. The results indicate that picrotoxin-induced convulsive activity has both synaptic and nonsynaptic components.

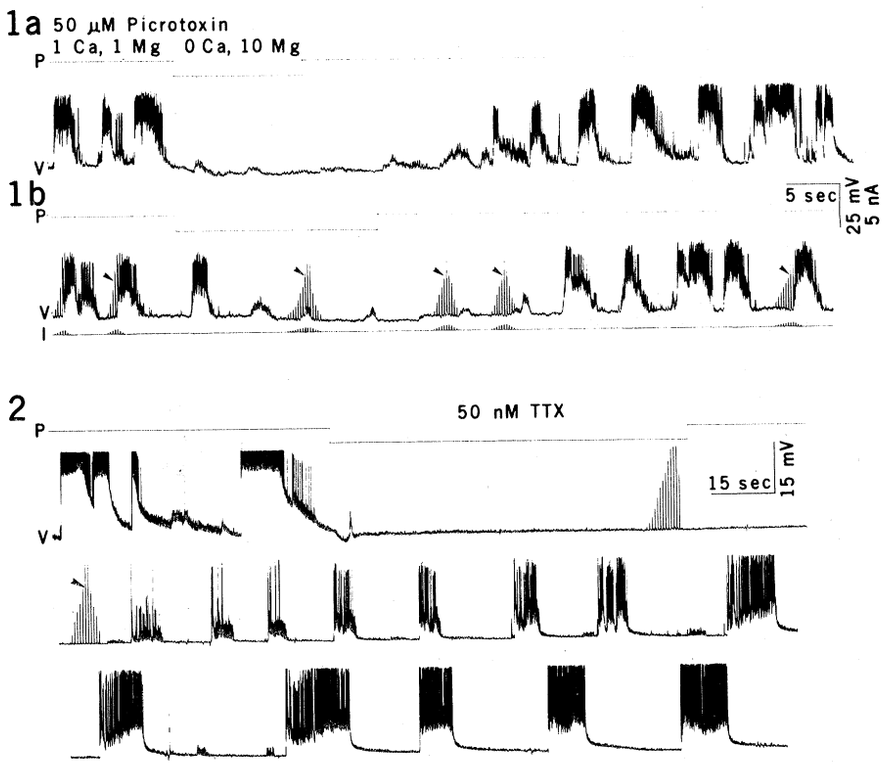

Fig. 1. Convulsive activity requires Ca^{2+} and is tetrodotoxin (TTX)-sensitive. This figure shows potassium acetate recordings from two different spinal cord neurons bathed in a solution containing 50 μM picrotoxin; *P* marks trace monitoring pressure, *V* is membrane voltage, and *I* is current injected intracellularly. (Panel 1a) Local pressure application of a bathing solution containing 0 m*M* $CaCl_2$, 10 m*M* $MgCl_2$, and 50 μM picrotoxin from a nearby pipette rapidly and reversibly eliminates convulsive activity. (Panel 1b) This effect occurs with little change in the spike threshold (marked by arrowheads) of the cell under study. Bursts of excitatory synaptic activity of low amplitude can be seen during and after the application period before the convulsive activity is fully restored. (Panel 2) Continuous membrane potential trace showing that pressure application of a bathing solution containing 50 μM picrotoxin and 50 n*M* TTX eliminates both spike and convulsive activity (upper trace) in a reversible manner. Upward-going events toward the end of TTX application and at the beginning of middle trace are voltage responses to depolarizing current stimuli used to test for the presence of spikes. Arrowhead delineates spike from subthreshold voltage responses (middle trace). Convulsive activity returns in a graded fashion. Initially it is comprised of synaptic bursts which barely trigger spikes (middle trace). The synaptic input gradually increases and summates to provide a sustained depolarization sufficient to repetitively excite the cell (lower trace). Baseline membrane potentials (in millivolts): −57 (panel 1) and −54 (panel 2).

Mouse spinal neurons were grown in dissociated cell culture according to methods previously described (*8*). By means of conventional or voltage clamp techniques we obtained intracellular recordings using 4*M* potassium acetate micropipettes. Picrotoxin (Sigma) was either added to the bathing medium or applied to individual cells under study by pressure from pipettes positioned close to the cell surface. Divalent cation concentrations in the vicinity of individual cells were changed by microperfusion from closely positioned pipettes. Tetrodotoxin (TTX, Calbiochem) was also applied to individual cells by pressure from nearby pipettes.

Most of the cultured spinal cord cells (72 of 98) generated convulsive activity in the presence of picrotoxin (Fig. 1). The activity consisted of paroxysmal depolarizing events (PDE's) superimposed on a baseline relatively devoid of synaptic activity. The PDE's were characterized by abrupt membrane depolarizations 10 to 30 mV in amplitude that were associated with sustained repetitive action potential activity. Less than 10 percent of cells recorded in the normal bathing medium generate membrane potential behavior that resembles this type of activity. The PDE's were blocked in a reversible manner by local perfusion of bathing solutions that contained elevated magnesium ion concentrations and were nominally free of calcium ions (Fig. 1, panel 1a). This result was obtained on all eight cells tested. Microperfusion of bathing solutions containing elevated magnesium ion concentrations and the

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normal complement of calcium ions blocked PDE generation in only 2 of 20 cells tested. The complete block of the convulsive activity could be obtained with little, if any, change in either the input resistance of the cell or in the threshold for spike generation (Fig. 1, panel 1b) or repetitive firing, although microperfusion with solutions containing more than 10 m*M* magnesium was usually associated with an increase in spike threshold and depression of repetitive firing. Both spike and convulsive activity were completely and reversibly blocked by microperfusion of bathing solutions containing TTX on all seven cells tested (Fig. 1, panel 2). The PDE's recovered as action potential generation was restored.

When the membrane voltage was clamped to −50 mV the convulsive membrane potential activity was replaced by abrupt paroxysmal episodes of inward membrane currents superimposed on a relatively quiet baseline (Fig. 2, panel 1a). These paroxysmal episodes were composed of many smaller discrete current events (Fig. 2, panel 1b) that were frequently present at such a high frequency (> 50 Hz) that they summated, causing a sustained change in membrane current which then abruptly relaxed (Fig. 2, panel 2b). Sometimes the frequency of occurrence and summating effect of the events declined gradually, eventually resolving into distinct bursts that became less and less frequent (Fig. 2, panel 1a). The events in these bursts typically occurred at a frequency that could be recorded on the pen-recorder (< 50 Hz) (Fig. 2, panel 1b). The small events themselves closely resembled synaptic currents (*9*) (Fig. 2, panel 1c) in that they had a rapid rise time and exponential decay. Both the summated currents and the discrete synaptic-like events inverted in polarity between −20 and 0 mV on the nine cells where this was examined (Fig. 2, panel 2) (*10*). Although the steady-state current-voltage relations were nonlinear over the −80 to +30 mV range, a region of negative resistance, a membrane property characteristic of "bursting" pacemaker (*11*) was not observed in any of the cells (Fig. 2, panel 2a). Thus, a convulsive episode could never be triggered by simply depolarizing the cell with extrinsic current unless that current also evoked intensive synaptic activity in a recurrent pathway.

When picrotoxin was applied to individual cells bathed in a medium containing elevated magnesium ion concentrations to prevent ongoing evoked synaptic activity, it induced spontaneous action potential activity in 16 of 28 cells tested in a rapid and easily reversible manner (Fig. 3). This activity was either random in appearance or occurred as brief bursts of spikes (Fig. 3, panel 1). In half of the cells excited by picrotoxin, the spontaneous activity was associated with several millivolts of depolarization, but little detectable change in input resistance (Fig. 3, panel 2). The induction of spontaneous activity was always associated with a lowering of spike threshold (see Fig. 3, panel 3, where picrotoxin application was adjusted to lower spike threshold without inducing spontaneous action potential activity). Picrotoxin application also lowered the current necessary to induce repetitive spike firing (not shown). These effects on spike threshold were observed with little change in either spike amplitude (Fig. 3, panel 5) or resting membrane properties (Fig. 3, panel 3).

These results show that (i) picrotoxin-induced convulsive activity is reversibly blocked by simultanesouly lowering calcium and raising magnesium ion concentrations or by applying TTX, (ii) under voltage clamp the convulsive episodes are replaced by rapidly summating synaptic-like currents which invert between −20 and 0 mV, and (iii) application of picrotoxin to the cell bodies of individual cells induces spontaneous firing by lowering spike threshold. The PDE's in culture thus appear to reflect intense excitatory synaptic activity, because they

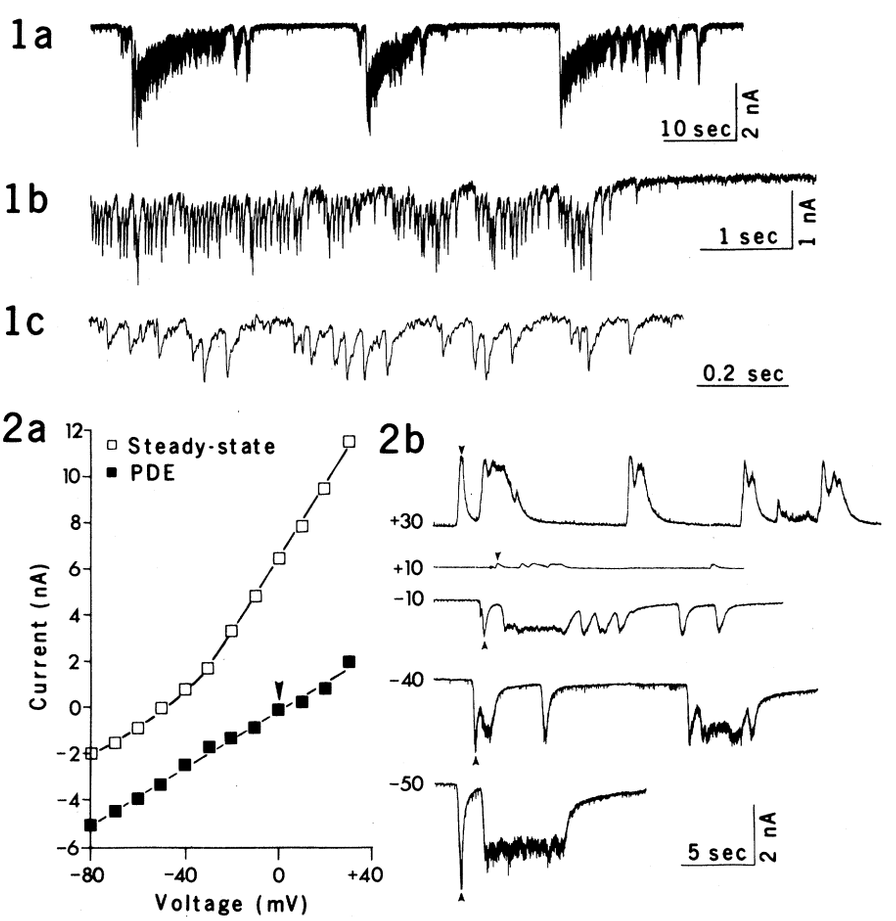

Fig. 2. Picrotoxin-induced convulsive activity is due to summating synaptic currents. This figure shows pen-recorder tracings of membrane current behavior recorded under voltage clamp from two different spinal neurons bathed in a solution containing 50 μM picrotoxin. Each cell generated convulsive activity in the unclamped state. (Panel 1a) Clamping the cell's membrane potential to −50 mV reveals the presence of episodic bursts of inward-going currents superimposed on a stable baseline relatively free of synaptic currents. Smaller, shorter bursts of activity occur toward the end of the larger, longer events (see panel 1, b and c). Traces at faster recording speeds show that the larger events are composed of many discrete smaller events that closely resemble synaptic currents. (Panel 2a) Plot of membrane current as a function of membrane voltage for steady-state membrane current and for the peak amplitude of the episodic current events (*PDE*). Arrowhead indicates the inversion potential of these events to be about 0 mV. (Panel 2b) Specimen records of membrane current activity at various membrane potentials. Small arrowheads mark the places on the traces which constitute the data points for the plot in panel 2a.

can be resolved into rapidly summating synaptic-like currents that have a distinct inversion potential and because they can be blocked by applying a calcium or magnesium solution that blocks evoked synaptic activity. The synaptic bombardment that comprises the PDE is probably derived from transmitter release evoked by a burst of spikes in cells presynaptic to the cell under study since the PDE's are eliminated by TTX, which blocks sodium-dependent spikes in spinal cord cells (*12*).

The effects of picrotoxin on active membrane properties would act to convert subthreshold depolarizing synaptic events into spike-firing potentials as well as increase the likelihood of sustained repetitive firing, which is associated with most PDE's. If the excitability-enhancing effects of picrotoxin extend to presynaptic terminal membranes, then high-frequency repetitive firing at presynaptic terminals could evoke the intense transmitter release involved in PDE generation. We have never observed such spike firing at the level of the cell body independent of that which is superimposed on the intense synaptic activity during a PDE. Thus, if repetitive firing does occur at presynaptic terminals under these conditions it is not propagated antidromically to invade the cell body. The suggestion that changes in presynaptic terminal activity occur during convulsive activity has been made before (*4*), and in fact penicillin produces antidromic bursts of activity originating in axon terminals at neuromuscular junctions (*13*).

That all cells do not generate PDE's in picrotoxin and that all are not sensitive to the threshold-lowering effects of the convulsant suggests some specificity to these actions of the convulsant. In fact, all of the convulsants we have examined, including bicuculline, penicillin, pentylenetetrazole, and strychnine can lower spike threshold and induce spontaneous activity in a manner similar to picrotoxin, but these effects do not always occur on the same cells (*14*), suggesting that different receptors are involved. The present results do not explain the paroxysmal nature of the excitatory synaptic activity nor the relative absence of such activity between convulsive events. Furthermore, the precise role of convulsant antagonism of γ-aminobutyric acid–mediated postsynaptic inhibition in PDE generation is also unclear. Presumably such antagonism would increase the proportion of synaptic excitation to inhibition and thereby contribute to the predominance of synaptic excitation observed during convulsive activity. In conclusion, convulsive activity in tissue culture includes both synaptic and nonsynaptic components and these may underlie some forms of chemically induced epileptiform activity recorded in vivo.

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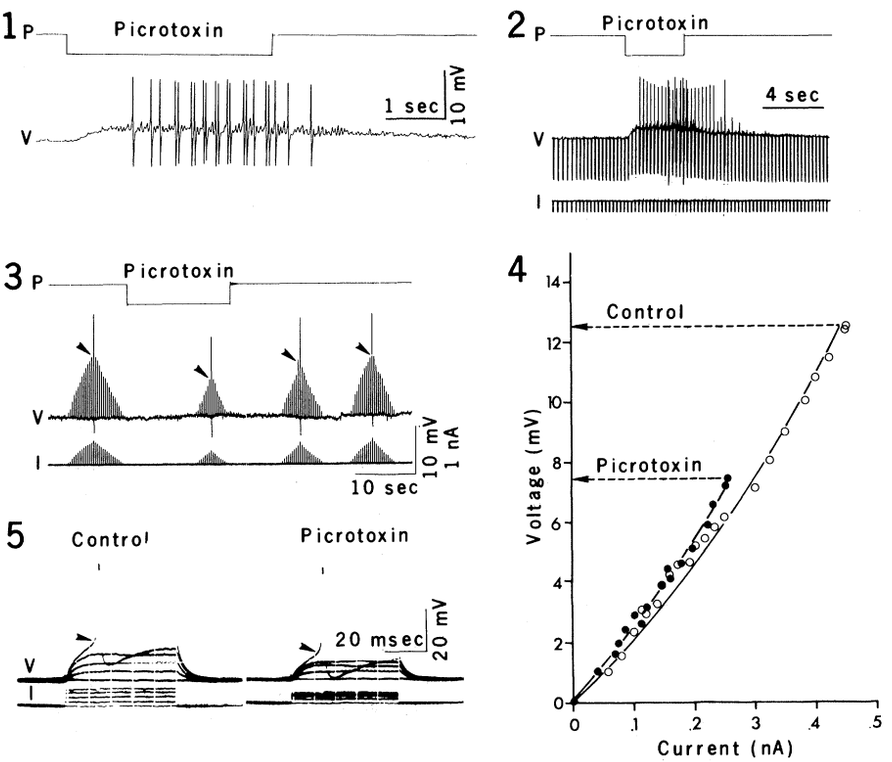

Fig. 3. Picrotoxin induces spontaneous firing by lowering spike threshold. This figure shows a potassium acetate recording from a spinal cord cell in bathing medium containing 10 m*M* $MgCl_2$. Picrotoxin was applied by pressure from a nearby pipette containing a 20 μM concentration of the drug. Traces show pressure (*P*), membrane voltage (*V*), and intracellularly injected current (*I*). (Panels 1 and 2) The pressure pipette is 10 μm from the cell surface. Picrotoxin application is associated with several millivolts of depolarization and the induction of spontaneous firing. The small membrane potential oscillations in panel 1 are associated with little, if any, change in the input resistance of the cell (panel 2). (Panels 3 to 5) The pressure pipette is now 50 μm from the surface. Picrotoxin application lowers spike threshold (marked by arrowheads) without depolarizing the cell. The time course of the effect is shown on the pen-recorder trace of panel 3. Current-voltage relations over the membrane potential range from resting membrane potential to the threshold potential for spike activation in control (open circles) and during picrotoxin application (filled circles) are plotted in panel 4 from data shown in panel 3. A 5-mV lowering of threshold during picrotoxin application with little change in the slope conductance of the membrane is evident. Resting membrane potential: −59 mV.

References and Notes

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