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Convulsant-induced depression of amino acid responses in cultured mouse spinal neurones studied under voltage clamp

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1 A two-microelectrode voltage-clamp technique was used to record membrane current responses induced by the application of the neutral amino acids, γ-aminobutyric acid (GABA) and glycine, to mouse spinal neurones maintained in cell culture.

2 Membrane current responses to GABA were depressed by the convulsant agents, bicuculline, picrotoxin and pentylenetetrazole. Membrane current responses to glycine were depressed by the convulsant compound, strychnine.

3 Analysis of the fluctuations in membrane current about the mean response during prolonged applications of GABA revealed that neither the conductance nor the average open-time of GABA-activated ion-channels was altered in the presence of picrotoxin or bicuculline. Pentylenetetrazole caused a reduction in the open-time of GABA-activated ion-channels but this effect alone was too small to account for the depression of the current response.

4 A similar analysis of glycine-induced responses in the presence of strychnine revealed that the depression of glycine responses by strychnine was not associated with any changes in the conductance or average open-time of glycine-activated ion-channels.

5 We therefore conclude that the convulsant compounds act by reducing the rate of activation of ion-channels by the neutral amino acids either through a reduction in the number of free receptors or by a change in the kinetics of channel activation.

Introduction

Various drugs that induce convulsions *in vivo*, including bicuculline, picrotoxin, pentylenetetrazole, and strychnine, are known to depress synaptic potentials recorded in both pre- (Barker, Padjen & Nicoll, 1975a; Davidoff & Hackman, 1978) and postsynaptic elements (Scholfield, 1980) in the vertebrate central nervous system (CNS). Pharmacological applications of certain neutral amino acids can mimic some of the synaptic actions evoked physiologically at both pre- and postsynaptic sites and these pharmacological actions can be blocked by convulsant drugs at spinal and supraspinal levels (Barker, Padjen & Nicoll, 1975b; Nicoll, Padjen & Barker, 1976; Davidoff & Hackman, 1978; Constanti & Nistri, 1979; Simmonds, 1978; 1980; Homma & Rovainen, 1978; Gallagher, Higashi & Nishi, 1978; Scholfield, 1980). These findings have led to the conclusion that the convulsant antagonism of certain synaptic potentials does not involve actions on transmitter release processes, but rather occurs through an interaction between the convulsants and receptor-coupled ion-channels in the membrane of target cells.

Detailed study of this interaction has been difficult owing to the complexity and relative inaccessibility of vertebrate CNS preparations to high resolution electrophysiological techniques. However, during the last 10 years methods have become available for growing dissociated CNS neurones in cell culture (see Nelson & Liebermann, 1981). Cultured neurones possess many properties characteristic of cells *in vivo* including electrical and chemical excitability and spontaneous synaptic activity. Convulsants transform the randomly occurring electrical activity normally recorded in most cultured spinal neurones into

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patterns of activity which superficially resemble the electrical activity recorded *in vivo* in the presence of convulsants (MacDonald & Barker, 1979; Barker & MacDonald, 1980). Furthermore, in cultured mouse and avian spinal cord cells, convulsant drugs depress both Cl^--dependent responses to neutral amino acids and certain types of inhibitory, Cl^--dependent synaptic potentials (MacDonald & Barker, 1979; Choi, Farb & Fischbach, 1981; Barker, MacDonald, Mathers, McBurney & Oertel, 1981). Cultured neurones thus appear suitable for examining details of the interaction between convulsant drugs and membrane responses to neutral amino acids. In this study we have used the voltage-clamp technique in conjunction with fluctuation (or 'noise') analysis to study some details of the effects of convulsants on membrane currents evoked by γ-aminobutyric acid (GABA) and glycine in cultured mouse spinal neurones. A preliminary account of these results has appeared in abstract form (Barker, Mathers, McBurney & Vaughn, 1980).

Methods

Tissue culture

Neurones were dissociated from embryonic mouse spinal cords and grown in cell culture according to methods previously described (Ransom, Neale, Henkart, Bullock & Nelson, 1977; Barker & Ransom, 1978). After 4–6 weeks in culture the cells were large enough (25–35 μm diameter cell body) to allow sustained (> 30 min) recording with two intracellular microelectrodes.

Experimental preparation

Experiments were carried out under phase contrast optics on the modified stage of an inverted phase microscope. The 250× magnification of the monolayer culture allowed clear visibility of cells and accurate placement of two intracellular microelectrodes. All recordings were made at room temperature (24 ± 1.5 °C) using microelectrodes filled with 3 M KCl. The bathing medium consisted of Hank's Balanced Salt solution containing 10 mM $MgCl_2$ to suppress spontaneous synaptic activity and allow clearer examination of the pharmacological responses.

Electrophysiological recording and fluctuation analysis

After successful penetration with two microelectrodes the cell was voltage-clamped using a multistage feedback circuit (for details, see Smith, Barker, Smith & Colburn, 1980). Fluctuations in membrane current under baseline conditions and during agonist-induced membrane current responses were analysed with the aid of PDP 11/10, 11/23 and 11/40 computers according to a protocol outlined previously (Barker, McBurney & MacDonald, 1982). The fluctuations were analysed assuming: that they reflect the activity of a population of two-state ion-channels each of whose conductance is constant but whose open-state lifetimes are exponentially distributed; that the probability of any single channel being open at any instant is small; and, that the channels operate randomly and independently of each other (Neher & Stevens, 1977). Briefly, the membrane current signal was first amplified and filtered and then digitized at an appropriate rate (usually either 1, 2 or 5 ms per sample). The average variance in 6144-point epochs of data was obtained by calculating the variance in 17 successive, unique 2048-point records, each of which overlapped by 1792 points. Spectral analysis of the filtered membrane current record was carried out using a Fast Fourier Transform routine and the spectrum computed for each epoch of data. Baseline variance and its associated spectrum were subtracted from values obtained during agonist-induced responses to yield the variance and spectrum corresponding to the agonist-induced response.

Provided that the assumptions outlined above are valid, fluctuation analysis permits estimates of the average electrical properties of ion channels. The open-state conductance of a single channel, γ, can be calculated from $\gamma = \sigma^2/(\Delta I.V_D)$ where σ^2 is the variance, ΔI is the membrane current response and V_D is the driving force acting on the conductance (Neher & Stevens, 1977). The average open-time of a single channel, τ, can be derived from spectral analysis of the fluctuations in membrane current using the relationship $\tau = (2\pi f_c)^{-1}$ where f_c is the corner frequency (at which spectral intensity falls by 50%) of the power density spectrum of agonist-induced fluctuations. Comparison of estimates of channel properties made from analysis of fluctuations induced by GABA in cultured mouse spinal neurones (Barker *et al.*, 1982) has been made with properties calculated from direct measurements of single ion-channel current pulses activated by GABA in patches of cultured mouse spinal neurones recorded under the same experimental conditions (Jackson, Lecar, Mathers & Barker, 1982). The patch-clamp results show that GABA can activate two-state ion-channels whose conductance appears to be close to that predicted from analysis of fluctuations arising in a population of active channels. Histograms of open single-channel lifetimes indicate that there may be another, faster component in channel activity in addition to the slower component that corresponds to estimates de-

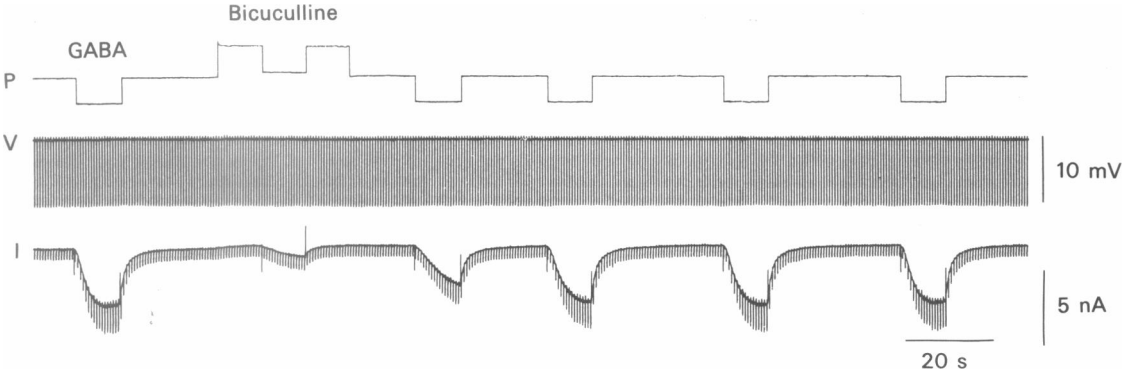

Figure 1 Bicuculline depresses membrane responses to GABA evoked in a cultured mouse spinal neurone under voltage clamp. The membrane potential was held at −70 mV and 10 mV-50 ms hyperpolarizing commands applied repetitively (trace marked V). GABA was applied by iontophoresis from a closely positioned pipette containing 1 M amino acid. Application periods are indicated by downward deflections in the top trace (marked P). Bicuculline (50 μM) was applied by pressure during the period shown by the upward deflection in the top trace. Membrane current responses to GABA are illustrated in the bottom trace (marked I). Bicuculline markedly attentuates the current response and conductance increase to GABA in a reversible manner. The conductance increase evoked by GABA is reduced from about 100 nS to about 10 nS during bicuculline.

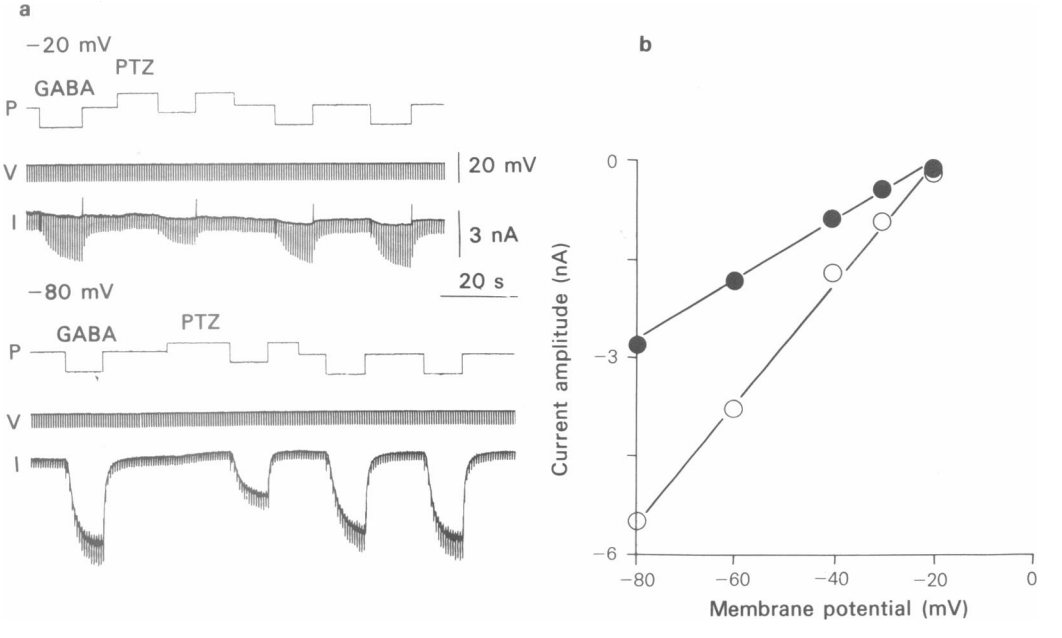

Figure 2 Pentylenetetrazole (PTZ) blocks the membrane response to GABA without changing its inversion potential. Membrane potential was held at different potentials over the range −80 to −20 mV and 12 mV-50 ms hyperpolarizing commands superimposed (traces marked V in (a)). At each potential GABA was iontophoresed (during periods marked by downward deflections in top traces marked P in (a)) and PTZ (10 mM) was applied by pressure (marked by upward deflections in P traces in (a)). (a) Shows that PTZ blocks membrane current responses (traces marked I) and conductance increases to GABA at −80 and −20 mV in a reversible manner; (b) is a plot of current response amplitude as a function of membrane potential under control conditions (○) and in the presence of PTZ (●). From these observations it is evident that PTZ does not shift the inversion potential of the GABA response (about −20 mV) while markedly depressing the amplitude of the GABA-induced current.

rived from spectral analysis. These comparisons provide evidence that fluctuation analysis yields relatively accurate estimates of the average electrical properties of the ion-channels underlying current responses generated by GABA in these cells.

Drug applications

All drug applications were made onto the cell body of visualized neurone level within 10 μm of the cell surface. GABA and glycine (Sigma, St. Louis) were applied either by iontophoresis from pipettes containing 1M solution or by pressure from pipettes containing 20 μM amino acid in bathing medium. Bicuculline methiodide, picrotoxin and strychnine sulphate (Sigma) were applied either by passive diffusion from pipettes containing 50–100 μM drug, which were positioned adjacent to the amino acid-containing pipette, or by low pressure (<1 lb/in^2) at 50 μM distance from the cell under study. Pentylenetetrazole (Sigma) was applied by passive diffusion or low pressure from pipettes containing 5–20 mM drug.

Presentation of results

Numerical results are presented in the Tables as mean ± s.d. An asterisk beside a pair of measure-

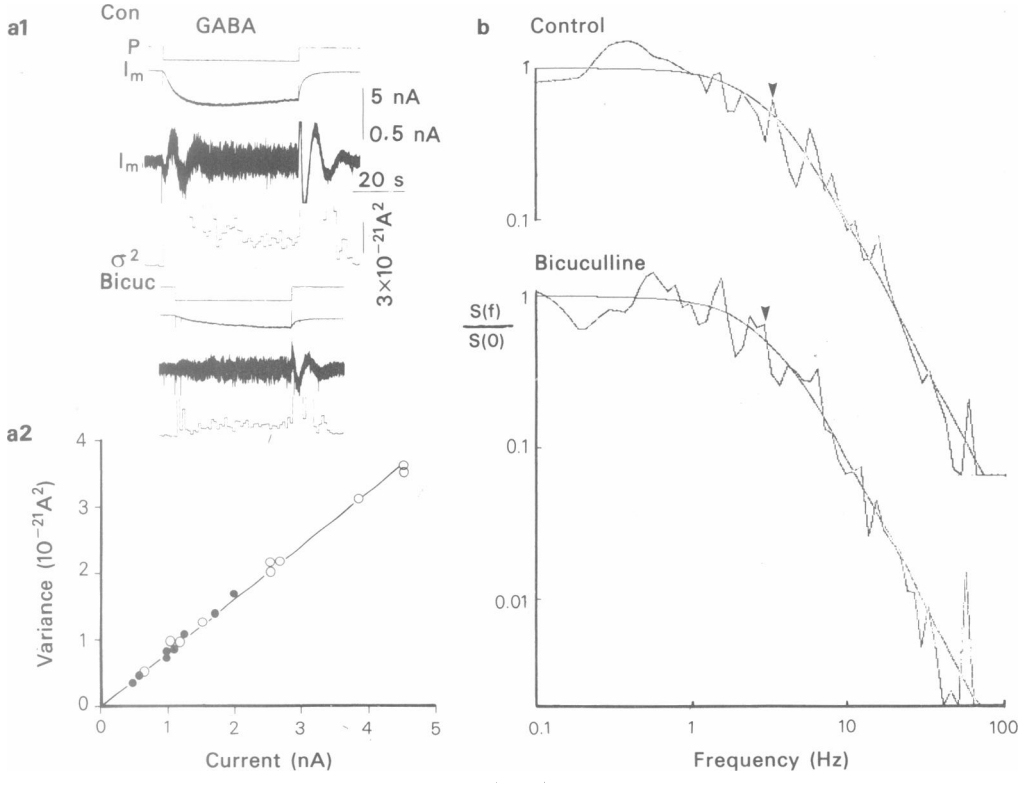

Figure 3 Fluctuation analysis of GABA responses depressed by bicuculline. The cell's membrane potential was held at −70 mV and GABA applied by iontophoresis (during periods indicated by the downward deflections in the top traces marked P of (a1)). The membrane current signal is displayed as a d.c.-coupled trace marked Im and then again amplified 10 × as an a.c.-coupled trace (filtered between 0.1 and 100 Hz) marked Im. The bottom trace, σ^2, is the membrane current variance derived from the filtered current signal, integrated every second and displayed as a voltage. The membrane current response evoked by GABA and its associated variance are markedly attenuated by 50 μM bicuculline (Bicuc) applied by pressure. (a2) Plot of current variance as a function of current response amplitude under control conditions (○) and in the presence of bicuculline (●). The slope of the plot yields a single channel conductance of 15 pS (given a driving force of 50 mV) and this is the same under control conditions and in the presence of bicuculline. (b) Normalized power spectra of GABA-induced fluctuations under control conditions and during bicuculline application. The jagged lines represents the data while the solid smooth lines are (computer-fitted) Lorentzian equations of the form $S(f)/S(0) = [1 + (f/f_c)^2]^{-1}$ whose corner frequencies, f_c, are marked by arrowheads. The average duration, τ, of single channels can be derived from $\tau = (2\pi f_c)^{-1}$ (see text). F_c was 4 Hz in control and 4.2 Hz in bicuculline, which gives τ_{con} = 39.8 ms and τ_{Bic} = 37.9 ms.

ments indicates a significant difference between them at the 5% level or less, as determined by a Student's *t* test.

Results

The results to be discussed were obtained from experiments conducted on 29 cultured mouse spinal neurones.

Antagonism of GABA and glycine responses

Membrane current responses and conductance increases evoked by GABA on cultured mouse spinal neurones were uniformly and reversibly depressed by the convulsants bicuculline (Figure 1), picrotoxin and pentylenetetrazole. The depressant actions of the drugs did not change the reversal potential of the current response (Figure 2) and thus did not alter the driving force associated with the conductance increase to Cl^- ions. In similar manner strychnine depressed membrane current responses and conductance increases to Cl^- ions evoked by glycine without changing the inversion potential for the response (not shown).

Fluctuation analysis of GABA responses depressed by convulsants

Membrane current responses evoked by GABA at −70 mV were always associated with additional membrane current variance (Figure 3a1) that was directly proportional to the amplitude of the current response (Figure 3a2). Membrane current variance was attenuated by bicuculline, picrotoxin and pentylenetetrazole in direct proportion to the convulsant-induced depression of the membrane current response evoked by GABA. The relationship between current variance and current amplitude was similar under control conditions and in the presence of a convulsant (Figure 3a2). Since the convulsants did not change V_D nor alter the ratio $\sigma^2/\Delta I$, it is evident that convulsant-induced depression of the membrane current response evoked by GABA is not associated with consistent and significant changes in the conductance of the ion-channels activated by GABA (Table 1 A, B and C).

Spectral analysis of membrane current fluctuations associated with GABA responses under control conditions could usually be described by a single Lorentzian equation of the form $S(f)/S(0) = [1 + (f/f_c)^2]^{-1}$ (see Methods). Values of f_c obtained from the majority of responses depressed by either bicuculline (Figure 3) or picrotoxin (not shown) were not significantly different from those observed under control conditions. Therefore, depression of GABA responses by these two drugs was not associated with any consistently significant changes in the average open-state lifetimes of GABA-activated ion-channels (Table 1 A and B). However, in five of eight cells tested, f_c values of spectra calculated for responses markedly depressed by pentylenetetrazole were shifted to higher frequencies (Figure 4), resulting in estimates of τ (see Methods) that were significantly shorter than those obtained under control conditions (Table 1C) Although the stoichiometry between the estimated degree of shortening in the average open-time of GABA-activated channels and the degree of depression in the amplitude of the current response caused by pentylenetetrazole was not examined in detail, the apparent shortening of channel open-time could not completely account for the depression observed in the amplitude of the current response, since responses were depressed to a greater extent than the estimated degree of channel shortening.

On several cells, fluctuation analysis was applied to GABA-induced responses depressed by 10 mM penicillin, another convulsant that antagonizes GABA responses in cultured mouse spinal neurones (MacDonald & Barker, 1979). The results indicate that penicillin can induce complex changes in the kinetics of the channels activated by GABA (unpublished observations). These will be described at a later time.

Fluctuation analysis of glycine responses depressed by strychnine

Membrane current responses evoked by glycine at −70mV were always associated with additional membrane current variance (Figure 5a1) that was directly proportional to the amplitude of the membrane current response (Figure 5a2). Membrane current variance was attentuated by strychnine in direct proportion to the drug-induced depression of the membrane current response evoked by glycine and the relationship between membrane current variance and current response amplitude was similar under control conditions and in the presence of strychnine (Figure 5a2). Since strychnine did not alter the driving force underlying glycine-induced responses nor the ratio $\sigma^2/\Delta I$, the depression of the responses by strychnine was not associated with detectable changes in the conductance of ion channels activated by glycine (Table 2).

Spectral analysis of the membrane current fluctuations associated with glycine responses depressed by strychnine showed that the drug did not alter f_c (Figure 5b). Thus, depression of glycine responses by strychnine was not associated with any detectable change in the average open-state lifetime of glycine-activated ion channels (Table 2).

Table 1 Effects of convulsants on properties of GABA-activated channels

	Cell	Condition	n	Channel conductance (pS)	Open-state lifetime (ms)
A					
	1	Control	12	20.7±2.3	26.9±2.0
		Bicuculline	10	21.8±4.1	26.5±2.2
	2	Control	20	17.0±3.6	27.7±3.0*
		Bicuculline	7	20.8±4.1	29.2±1.5
	3	Control	13	7.5±1.0	27.8±3.5
		Bicuculline	4	7.8±2.1	31.1±4.1
	4	Control	4	15.0±4.9	37.8±4.6
		Bicuculline	5	16.0±6.5	35.4±3.6
	5	Control	4	11.4±4.1	26.5±2.8
		Bicuculline	4	14.3±2.8	29.6±3.4
	6	Control	11	20.5±3.5	40.5±2.3
		Bicuculline	5	18.5±2.3	43.1±8.1
	7	Control	7	12.3±3.0	38.4±9.1
		Bicuculline	4	14.9±4.7	41.7±7.3
B					
	1	Control	25	17.5±3.5*	13.3±3.1
		Picrotoxin	13	21.2±4.1	13.4±2.0
	2	Control	18	23.4±4.4	23.7±4.2*
		Picrotoxin	6	24.5±3.6	27.6±5.1
	3	Control	10	23.9±3.1	18.4±2.6
		Picrotoxin	4	23.3±4.0	18.2±3.0
	4	Control	39	14.4±4.0	20.7±4.9*
		Picrotoxin	8	16.9±6.5	17.3±1.5
	5	Control	17	14.2±2.4	21.7±3.0
		Picrotoxin	4	15.1±1.5	19.7±1.8
	6	Control	8	15.8±3.0	16.5±2.2*
		Picrotoxin	4	15.3±1.0	13.9±2.0
	7	Control	9	12.4±3.0	33.9±4.0
		Picrotoxin	3	12.4±1.0	34.7±5.0
	8	Control	5	9.8±3.6	30.5±4.3
		Picrotoxin	4	12.1±4.0	33.4±4.0
	9	Control	7	20.6±5.0	47.7±7.0
		Picrotoxin	6	22.4±5.0	43.9±6.1
	10	Control	21	12.6±4.1	41.8±5.0
		Picrotoxin	8	14.3±2.0	42.7±2.7
C					
	1	Control	13	11.3±2.4	15.4±3.0*
		Pentylenetetrazole	3	9.6±3.0	10.2±2.0
	2	Control	11	12.4±5.0	33.9±4.1
		Pentylenetetrazole	3	14.1±3.1	33.0±4.0
	3	Control	6	25.5±5.1	26.4±8.0*
		Pentylenetetrazole	3	22.1±2.0	16.1±4.0
	4	Control	15	15.8±4.7	39.8±3.1*
		Pentylenetetrazole	5	16.3±8.1	24.5±6.1
	5	Control	9	13.3±2.3	30.1±5.6
		Pentylenetetrazole	4	11.1±9.1	28.1±4.1
	6	Control	6	25.2±3.2	33.2±3.1*
		Pentylenetetrazole	4	23.1±2.8	28.1±3.1
	7	Control	8	18.8±4.3	37.2±6.2*
		Pentylenetetrazole	6	17.2±3.1	24.5±3.4
	8	Control	9	22.1±3.1	23.7±5.9
		Pentylenetetrazole	9	20.6±3.6	24.3±5.1

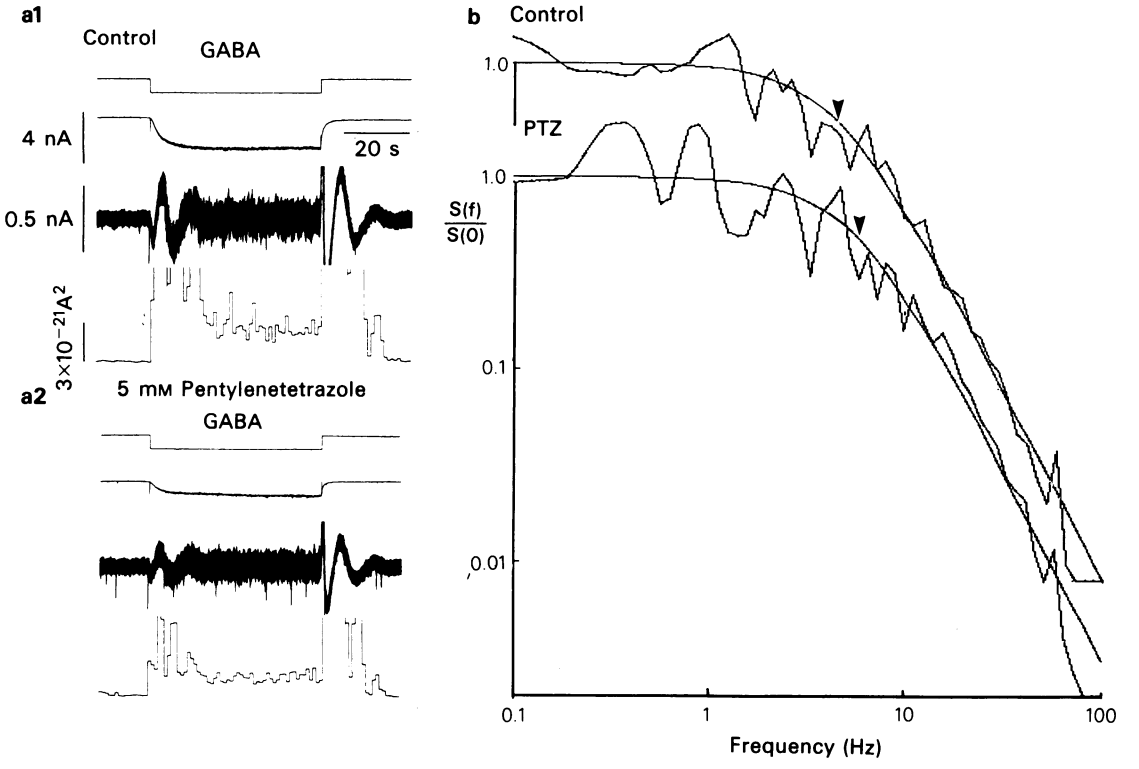

Figure 4 Spectral analysis of GABA responses depressed by pentylenetetrazole. The cell's membrane potential was held at −60 mV and GABA applied by iontophoresis (during periods indicated by the downward deflections in the top traces of (a)). The d.c. membrane current response is displayed above the a.c.-coupled current trace (amplified 10× and filtered between 0.1 and 100 Hz). The bottom trace reflects the membrane current variance integrated every second and displayed as a voltage. Pentylenetetrazole (PTZ) 5mM applied by pressure depresses the membrane current response to GABA and its associated variance (σ^2). (b) Normalized power spectra of GABA-induced variance under control conditions and during PTZ application. Arrowheads indicate that the corner frequency shifts from 4.4 Hz under control conditions to 5.5 Hz in the presence of PTZ. Thus, the open-state lifetime of GABA-activated channels shifts from a control value of 36.1 ms to 28.9 ms during depression by PTZ.

Discussion

The results presented here indicate that certain drugs with convulsant properties *in vivo* depress membrane responses to GABA and glycine with little, if any, consistent effect on the open-state conductance of the individual ion-channels comprising the response. Furthermore, bicuculline and picrotoxin do not significantly change the average open-state lifetime of channels activated by GABA, and strychnine does

Table 2 Effects of strychnine on properties of glycine-activated channels

Cell	*Condition*	n	*Channel conductance* (pS)	*Open-state lifetime* (ms)
1	Control	28	30.7±8.1	5.4±1.0
	Strychnine	14	28.4±7.1	4.9±1.0
2	Control	17	9.8±3.1	6.7±1.5
	Strychnine	5	10.1±2.2	5.6±1.0
3	Control	11	11.5±5.8	14.8±2.9
	Strychnine	5	9.4±2.0	13.1±1.4
4	Control	68	17.4±4.4	8.0±1.1
	Strychnine	39	17.3±4.9	8.1±0.8

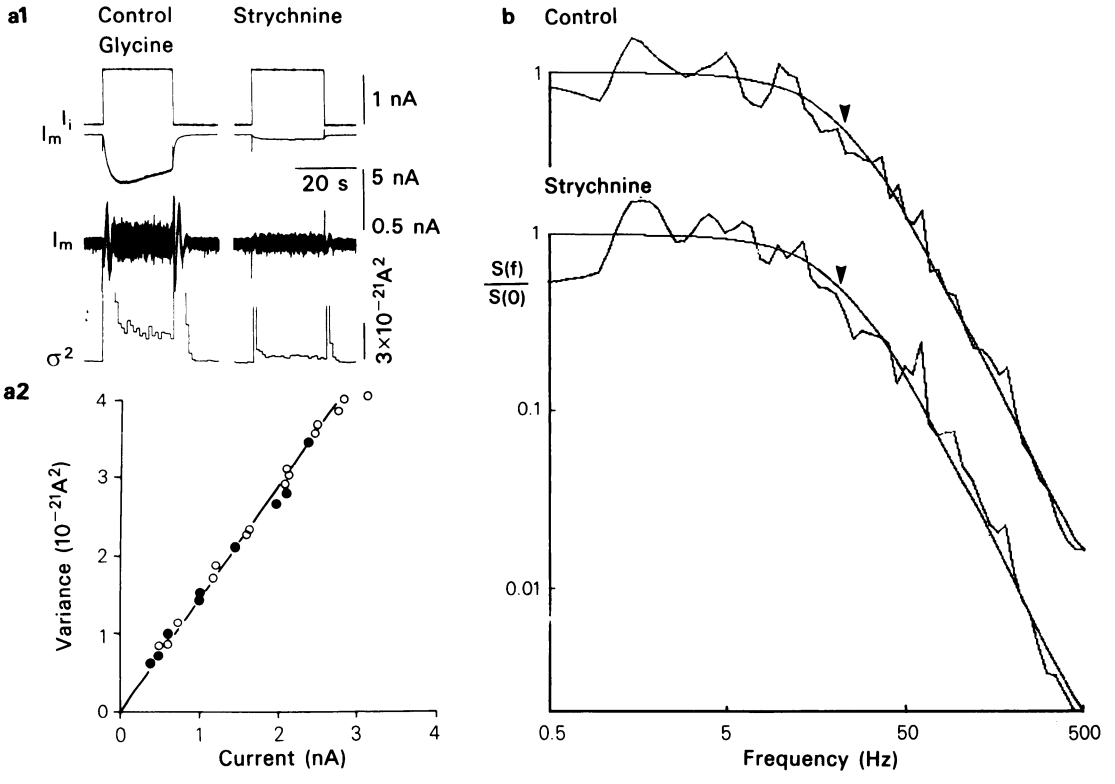

Figure 5 Fluctuation analysis of glycine responses depressed by strychnine. The membrane potential was held at −70mV and glycine iontophoresed during periods indicated on top traces (marked I_i) in panel (a1). The membrane current signal is displayed as a d.c.-coupled trace (Im) and then again below, amplified 10 × as an a.c.-coupled trace (filtered between 0.5 and 500 Hz). The bottom trace, σ^2, gives the current variance integrated every second. Strychnine (50 μM) markedly attenuates the membrane current response to glycine and its associated variance. (a2) Plot of current variance as a function of current response amplitude under control conditions (○) and in the presence of strychnine (●). The data points fall along the same slope which, with a driving force of 50 mV, gives a single channel conductance of 30 pS. (b) Normalized power spectra of glycine-induced fluctuations under control conditions and during strychnine application. Data are reflected in the jagged line, while a least squares fit to a Lorentzian equation (smooth line) is superimposed. Arrowheads mark f_c values which are both about 22 Hz in control and during strychnine application. This yields τ values of about 6 ms.

not significantly alter the average open-state lifetime of channels activated by glycine. We therefore conclude that these three drugs depress membrane responses to the amino acids by eliminating ion-channel activation in an all-or-none manner. An additional observation is that pentylenetetrazole depresses GABA-activated responses without changing the conductance of ion channels but with a variable effect on channel open-time. In some cells the open-state lifetime was shortened significantly. Although the relationship between the depression of current responses and the shortening in channel duration was not examined in detail, it was apparent that the change in this parameter could not completely account for the depression of the response.

Although this level of experimental resolution has revealed that antagonism of neutral amino acid responses by structurally different convulsants is associated with little, if any detectable changes in the properties of Cl^- ion channels, the results do not reveal the site(s) at which the convulsants act to eliminate ion-channel activation. In biochemical assays using membrane fractions derived from CNS tissue bicuculline and picrotoxin interfere with the binding of GABA to putative receptor sites (for review, see Olsen, 1981). These binding sites for GABA derived from normally developed tissue may be relevant to the GABA-activated channels studied in cultured spinal neurones, since when structural analogues used in binding assays are investigated in

cultured neurones with fluctuation analysis, there is a highly significant correlation between the biochemical and biophysical data (Barker & Mathers, 1981). Bicuculline and picrotoxin may thus block channel activation by interacting directly or allosterically with a site where GABA binds to initiate the opening of ion-channels. Strychnine, whose binding to CNS membranes is displaced by glycine (De Feudis 1978; Muller & Snyder, 1978), may lower the frequency with which glycine activates Cl^- ion channels in a similar manner. Pentylenetetrazole does not interfere with the binding of GABA (Olsen, 1981). It thus may change the open-state lifetime of GABA-activated channels and lower the frequency of channel activation by interacting with site(s) removed from where GABA binds. However, the exact contributions of these effects to the drug-induced depression of the membrane current response have not been elucidated.

These findings may help to explain the actions of the drugs on Cl^--dependent synaptic potentials recorded in cultured avian sensory and spinal cord neurones (Choi *et al.*, 1981). Strychnine blocked inhibitory potentials of short duration (< 10 ms) while bicuculline and picrotoxin eliminated events of relatively long duration (> 15 ms). Using cultured mouse spinal neurones we have found that the electrical properties of Cl^- ion-channels activated by neutral amino acids depend on the structure of the amino acid (Barker & McBurney, 1979; Barker *et al.*, 1982). Channels activated by glycine are consistently shorter in duration and greater in conductance than those activated by GABA when the responses are compared in the same membrane, although there is considerable variability in values obtained in different cells. Furthermore, the amplitude of Cl^--dependent synaptic currents of relatively long duration (> 15 ms) is depressed by picrotoxin without change in time course (Barker *et al.* 1981). These observations suggest that the shorter-lasting (inhibitory) synaptic potentials observed in cultured neurones are mediated by glycine while the longer-lasting ones are mediated by GABA.

As mentioned before, convulsants can block inhibitory responses at concentrations that induce paroxysmal episodes of excitatory activity. Although antagonism of inhibitory synaptic activity should serve to enhance the ambient level of excitability, the exact relationship between these effects and the paroxysmal episodes of excitatory activity (or the convulsant effects seen *in vivo*) is not clear. Indeed, some drugs that are used therapeutically as anticonvulsants including phenobarbitone, diphenylhydantion, valproate and ethosuximide do not alter GABA-induced changes in membrane Cl^--conductance when applied at clinically relevant concentrations (Barker, Study & Owen, 1982). In all likelihood, previous findings of potentiation of GABA-evoked responses with iontophoretically applied phenobarbitone (MacDonald & Barker, 1979; Barker & McBurney, 1979) and valproate (MacDonald & Bergey, 1981) reflect concentrations considerably higher than those that occur clinically. Those studies examining the interaction between phenobarbitone and GABA report little effect, if any, at 100 μM (Nicoll & Wojtowicz, 1980; Simmonds, 1980), a clinically relevant concentration.

It should also be noted that some convulsants and anticonvulsants including phenobarbitone, diphenylhydantion and benzodiazepines have opposing actions on several types of electrically excitable membrane conductances (e.g. threshold for single and repetitive action potential activity) in cultured mouse spinal neurones at concentrations relevant to their convulsant-like effects in cultured neurones and *in vivo* (Barker & MacDonald, 1980; Barker, MacDonald & Mathers, 1980; Barker *et al.*, 1981; MacDonald & Barker, 1982). Convulsants and anticonvulsants have also been reported to have opposing effects on Ca^{2+}-dependent action potentials in cultured mammalian sensory and spinal neurones (Heyer & MacDonald, 1982; Heyer, Nowak & MacDonald, 1982). These actions on electrically-activated membrane properties would also be expected to contribute to the opposing effects of the drugs on CNS excitability *in vivo*. Finally, the excitability-enhancing actions of another convulsant, 4-aminopyridine (4-AP) have recently been recorded in cultured spinal and hippocampal neurones (Barker & Rogawski, 1982; Segal & Barker, 1982). 4-AP lowers the threshold for single and repetitive action potential discharge. Its actions are due primarily to depression of a transient voltage-dependent K^+-conductance. It remains to be seen whether other convulsants act on this K^+-conductance and, further, how depression of this conductance is related to the paroxysmal episodes of excitatory synaptic activity induced by convulsant drugs.

It is clear therefore that drug-induced convulsions might be mediated *in vivo* by a number of different mechanisms. Which of these mechanisms underlies epileptic seizures in man remains unclear as does the precise mode of action of drugs which are used clinically as anti-convulsants. Our study has revealed that some drugs which can induce seizures in man and animals do prevent the activation of Cl^- ion-channels by amino acids which are widely thought to be the inhibitory neurotransmitter substances at some synapses in the CNS. We cannot say precisely how our results relate to the actions of these and other convulsant drugs *in vivo*. More information is certainly needed on the concentration-effect relationships of these agents *in vivo* and *in vitro*.

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