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Short Communication

Multiple Mechanisms of Antagonism of γ-Aminobutyric Acid (GABA) Responses

N. Akaike,[1] T. Yakushiji,[1] N. Tokutomi,[1] and D. O. Carpenter[1,2]

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SUMMARY

1. Gamma-aminobutyric acid (GABA) is one of the most important neurotransmitters in the brain. In an effort to understand the operation of the GABA receptor–ionophore complex, the antagonism of GABA responses by four substances was studied in bullfrog dorsal root ganglion cells by concentration-clamp and internal-perfusion techniques.

2. Two antagonists (bicuculline and Zn^{2+}) were competitive; two (picrotoxin and penicillin) were noncompetitive. However, significant changes in the kinetics of activation and inactivation were produced by the antagonists, including those that were competitive.

3. The causes of these changes may be important clues to the structure and operation of the GABA receptor–ionophore complex.

INTRODUCTION

Gamma-aminobutyric acid (GABA) is thought to be the neurotransmitter at the majority of inhibitory interneurons in the mammalian central nervous system (McGeer *et al.*, 1981). Dysfunction at GABA receptors is a central and perhaps causative feature of several human diseases of the nervous system (Ribak *et al.*, 1981).

[1] Department of Physiology, Kyushu University, Faculty of Medicine, Fukuoka 812, Japan.

[2] To whom correspondence should be addressed at Wadsworth Center for Laboratories and Research, New York State Department of Health, and School of Public Health Sciences, State University of New York at Albany, Albany, New York 12201.

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We have attempted to determine how several antagonists of GABA responses act by using a technique which provides rigorous control of the internal and external media.

METHODS

Frog dorsal root ganglion cells were isolated as previously described (Hattori *et al.*, 1984) and recorded with an electrode in a whole-cell patch technique with internal perfusion. The electrode, with the cell at its tip, was placed into a hole in the side of a small pipette through which extracellular bathing fluid was constantly drawn. This allowed instantaneous application of solutions of altered composition or containing drugs (concentration clamp) (Akaike *et al.*, 1986). The internal perfusion medium contained 95 m*M* CsCl, 10 m*M* Cs-aspartate, 25 m*M* TEA–Cl, and 0.5 m*M* EGTA. The external solution contained 83 m*M* Tris–Cl, 2 m*M* CsCl, 5 m*M* $MgCl_2$, 25 m*M* TEA–Cl, 3 m*M* 4-aminopyridine, and 5 m*M* glucose. The pH of each solution was adjusted to 7.4 with tris(hydroxymethyl)aminomethane–base (Tris–base) or *N*-2-hydroxyethylpiperazine-*N*-2-ethane-sulfonic acid (HEPES).

Since neither the external nor the internal solution contained Na^+, K^+, or Ca^{2+}, the Cl^- currents could be recorded in isolation. Fast and slow time constants of activation and inactivation were plotted by curve-peeling from tape-recorded data through a program run on an NEC computer, Type PC-9801E.

RESULTS

The recording techniques are shown in diagrammatic form in Fig. 1A. The dose–response curves for GABA, alone and in the presence of each antagonist, are shown in Fig. 1B. After essentially instantaneous GABA application the recorded current rose to a peak and then fell due to receptor desensitization. The currents plotted are peak currents, which varied with GABA concentration in a sigmoidal relation. The application of an antagonist (pretreated for 1 min) mixed with GABA decreased the responses. Bicuculline and Zn^{2+} dose–response curves were shifted to the right without depression of the peak current. This pattern indicates competitive inhibition. The peaks for picrotoxin and penicillin were depressed, indicative of noncompetitive inhibition.

The currents produced by GABA in the presence of these antagonists revealed differences that are not reflected in the measurement of the peak current (for examples see Fig. 2). In all of these studies the antagonist was used at a concentration which depressed the peak response by 30–50%, then the response was recorded with a gain that matched the peaks in the absence and presence of the antagonist. This technique allows an analysis of how the kinetics of the response was changed by the antagonist.

The response to GABA in the presence of 1×10^{-4} *M* Zn^{2+} differed relatively little from the control in either the rising or the decaying phase. This similarity would be expected of a true competitive antagonist that removes a portion of the receptors from activity but does not alter the functioning of the remainder. There was, however, a small but significant slowing of the rate of current rise, which was seen most clearly on the expanded sweep in the presence of Zn^{2+}. The rise time course of the current was

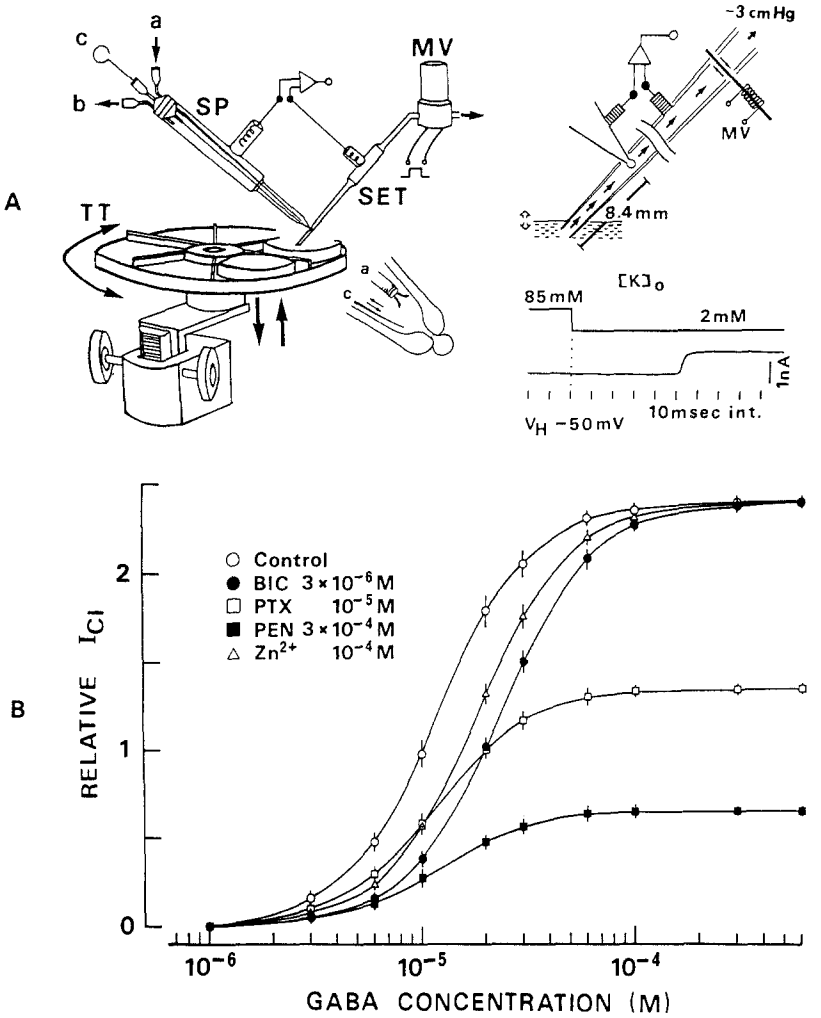

Fig. 1. (A) Schematic illustration of the concentration-clamp technique, which combines internal perfusion via a suction pipette with a rapid change of the external solution (Akaike *et al.*, 1986). SP, suction pipette; SET, solution exchange tube (polyethylene tube); MV, electromagnetic valve driven by 24 DC V; a, internal solution inlet (the internal perfusion speed during experiment is 1 drop/min); b, solution outlet connected to negative pressure (−30 cm Hg); c, Pt–Ir puncture wire for small cells, such as DRG and adrenergic ganglion cells having diameters of 30 to 40 μm. This wire is used only to clean the tip of the electrode after each experiment. The cell diameter is 30–40 μm. The interior diameter at the tip of the glass pipette, where it attaches to part of cell, is about 7 μm. (B) Dose–response curves in the presence of antagonists. Each point is a mean ± SD for 7–10 experiments. Each antagonist was perfused for 1 min before being applied at various concentrations with GABA. The holding potential (V_H) was −10 mV throughout the experiments. E_{GABA} is +4.2 ± 0.38 mV (mean ± SD; $N = 10$), quite close to the theoretical chloride equilibrium potential (E_{Cl}) of +3.6 mV calculated from the Nernst equation. The external solution contained 120 m*M* Cl^-, with an estimated activity coefficient of 0.73. The internal solution contained 120 m*M* Cl^-, with an estimated activity coefficient of 0.84. The Cl^- activities in test solutions were estimated using a F1012Cl Cl^- electrode connected to an ION85 ion analyzer (Radiometer A/S, Copenhagen). In the control the K_d for GABA was 1.17×10^{-5} *M*, and the I_{max} was 2.4. In bicuculline the K_d was 2.3×10^{-5} *M*, and the I_{max} was 2.4. In Zn^{2+} the K_d was 1.8×10^{-5} *M*, and the I_{max} was 2.4. In picrotoxin the K_d was 1.17×10^{-5} *M*, and the I_{max} was 1.35. In penicillin the K_d was 1.17×10^{-5} *M*, and the I_{max} was 0.65.

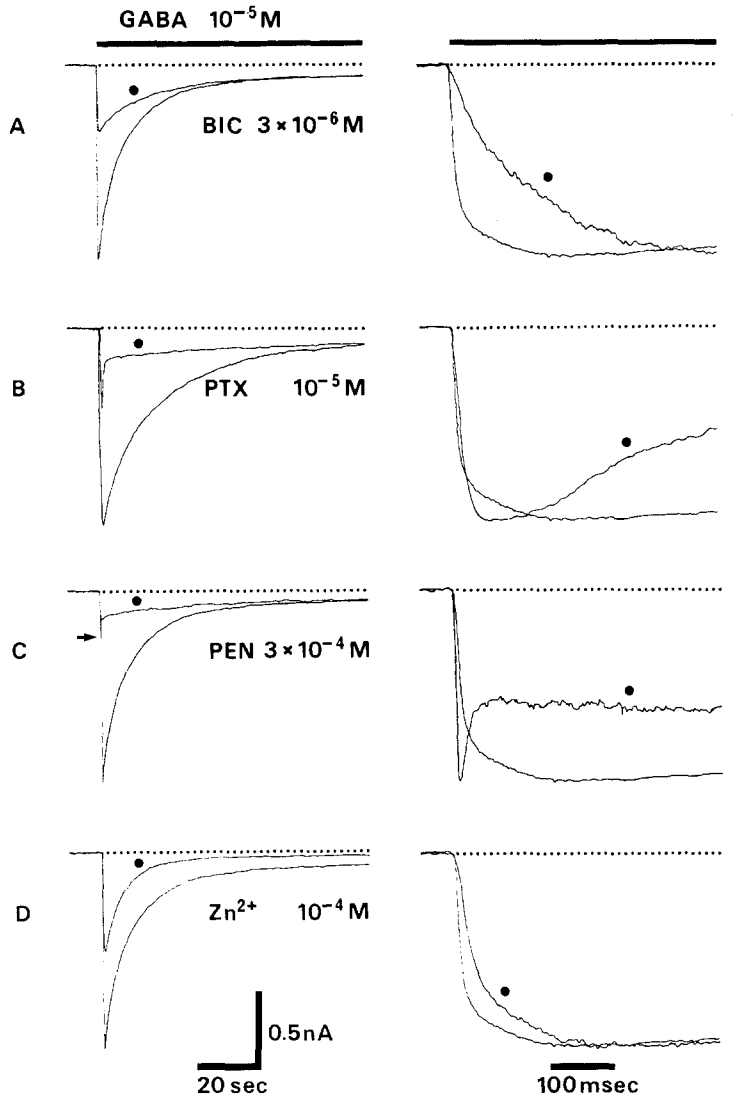

Fig. 2. Effects of antagonists on activation and inactivation of GABA responses. Records on the left show control GABA responses in the presence of each of the four antagonists at a concentration sufficient to depress the peak by about 50%. Records on the right are at a faster sweep, with the peak response in the presence of the antagonist matched in amplitude to the control. Note antagonist-induced alterations in both the rising (right) and the falling (left) phases of the response.

even more dramatically slowed by bicuculline. In contrast, the rising phase with picrotoxin was rapid but fell off very quickly. The response to penicillin had an initial rapid peak, with a rise time similar to that of the control, and then a sudden decrease in current, followed by a plateau response.

The effects of the four antagonists on the rising, falling, and plateau currents are summarized in Table I. The rising (activation = *a*) and falling (inactivation = *i*) phases

Table I. Effects of Antagonists on GABA Current Activation and Inactivation[a]

Parameter	Control	Drug added			
	GABA (10^{-5} *M*)	Bicuculline (3×10^{-6} *M*)	Picrotoxin (10^{-5} *M*)	Penicillin (3×10^{-4} *M*)	Zn^{2+} (10^{-4} *M*)
N	22	6	6	5	6
I_{Cl} (nA)					
Peak	1.37 ± 0.13	0.51 ± 0.09***	0.63 ± 0.10***	0.37 ± 0.07***	0.78 ± 0.11***
Plateau	0.180 ± 0.021	0.158 ± 0.002**	0.149 ± 0.002***	0.146 ± 0.003***	0.041 ± 0.014
I_{Cl} ratio (drug:control)					
Peak	1.00	0.37	0.46	0.27	0.57
Plateau	1.00	0.88	0.83	0.81	0.23
Time constant (msec)					
Activation					
τa_f	18 ± 4.2	39 ± 6.7***	28 ± 5.0***	11 ± 2.1**	30 ± 5.1***
τa_s	125 ± 13.5	210 ± 25.0***	Disappear	Disappear	148 ± 16.0**
Inactivation					
τi_f	3,413 ± 205	4,048 ± 502***	275 ± 95**	17 ± 3***	3,174 ± 421
τi_s	10,232 ± 1,173	12,227 ± 1,504**	6,948 ± 1,831***	13,997 ± 2,231***	8,769 ± 1,287*

[a] All values are the average ± SD. The degree of significance was determined by Student's *t* test. "Disappear" indicates disappearance of the component.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

have rapid and slow phases, designated τa_f, τa_s, τi_f, and τi_s, respectively. Inactivation despite the continued presence of a transmitter is due to receptor desensitization and has been shown in several systems to have fast and slow components (Feltz *et al.*, 1982; Slater *et al.*, 1984). Fast and slow components of activation have not been previously described, because few studies with concentration jump have been performed. Analysis of these two components of activation is beyond the scope of the present study. It is clear that these data do not take into account the dose-dependent variations in rising and falling phases.

DISCUSSION

The effects of the competitive antagonists on activation were to alter the kinetics. This change was particularly dramatic for bicuculline, which caused a selective reduction of the fast components of both activation and inactivation. Picrotoxin and penicillin, in contrast, caused a pronounced alteration in the pattern of the current, with an apparent cutoff in the trajectory of activation, possibly as a result of channel-use-dependent blockade. However, differences between the patterns suggest that the mechanisms of blockade are not identical.

All four antagonists altered the process of desensitization in different ways and degrees. Drug effects on desensitization, including selective alteration of either the fast or the slow components of desensitization, have also been reported in *Aplysia* neurons (Slater *et al.*, 1985, 1986).

The change in the currents with bicuculline is of particular interest because of the usual assumption that a competitive antagonist must bind to the same site as the transmitter. The significance of the fast and slow phases of activation is not clear. GABA receptors have been reported to have Hill coefficients of 2 (Barker *et al.*, 1978) or 3 (Brookes *et al.*, 1973), suggesting that this number of GABA molecules must bind for channel opening. It is unlikely that the fast and slow rates are a function of the number of binding sites, since it is expected that all sites must be occupied before the channel opens. Competitive binding interactions with antagonists may occur even though the agonist and antagonist do not bind at exactly the same site (Carpenter *et al.*, 1976). In some systems bicuculline is a non-competitive inhibitor of the GABA response (Gallagher *et al.*, 1978), while in others it is competitive (Lebeda *et al.*, 1982). This fact suggests the possibilities that the bicuculline binding site is distinct from the receptor, occurs at varying distances from the GABA binding site, and has different interactions with the receptor in different preparations.

Penicillin (Hochner *et al.*, 1976; Pellmar *et al.*, 1977) and picrotoxin (Akaike *et al.*, 1985) have been proposed to have channel-blocking actions. Our results are consistent with this conclusion but provide new information on differences in their modes of action.

Our results raise as many questions as they answer. How, for example, is the difference in response between competitive and noncompetitive antagonists to be explained? Together with other information, such as the results of binding studies, the concentration-clamp data have considerable potential for analysis of these questions.

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