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Modulation of Neurotransmitter Action: Control of the γ-Aminobutyric Acid Response through the Benzodiazepine Receptor[1]

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Abstract

The ability of several homologous benzodiazepine and heterologous nonbenzodiazepine ligands to alter the conductance increase induced in spinal cord neurons by γ-aminobutyric acid (GABA) was determined. Complete dose-response curves were carried out on individual neurons, reducing error introduced by cell-to-cell variability. The efficacies of modulation differ for "classical" benzodiazepines and novel nonbenzodiazepine drugs in a manner consistent with a model of control of GABA receptor action through a common receptor. There was no apparent correlation between efficacy and potency. CL 218,872, a triazolopyridazine, gave the lowest efficacy of the tranquilizers tested. Ro 15-1788 (an imidazobenzodiazepine) potentiated g_{GABA} with high potency and low efficacy, consistent with an action as a partial agonist and an "antagonist" of classical benzodiazepine action. In order of increasing efficacy, Ro 15-1788, CL 218,872, and flurazepam are partial agonists, whereas clonazepam, chlordiazepoxide, diazepam, and flunitrazepam are full agonists. The β-carboline drugs methyl-β-carboline-3-carboxylate (β-CCM) and methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) are "anxiogenics" and convulsants that were found to exert through the benzodiazepine receptor-inhibitory and apparently insurmountable control of g_{GABA}. β-CCM and DMCM display large negative efficacies and act like effectors at a site distinct from the picrotoxin-sensitive chloride ionophore and coincident with the benzodiazepine site. The actions of these different benzodiazepine receptor ligands *in vivo* range from anxiolytic and anticonvulsant to anxiogenic and convulsant. Efficacy rather than potency almost certainly determines the qualitative nature of the pharmacological actions of these drugs.

The existing data strongly suggest that γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter in the CNS (Obata, 1977; Nistri and Constanti, 1979) and in primary monolayer cell cultures of embryonic chick spinal cord (Farb et al., 1979; Choi et al., 1981b; Rando et al., 1981). The GABA receptor is a complex receptor containing a benzodiazepine-binding site/receptor as an integral component. This is consistent with the hypothesis that the anti-anxiety, muscle-relaxing, and anticonvulsant actions of benzodiazepine agonists are mediated through the GABA receptor complex. Benzodiazepine tranquilizers potentiate GABA action in neuronal chick spinal cord cultures (Choi et al., 1977; Chan et al., 1983) and *in vivo* (Gallager, 1978; Schlosser and Franco, 1979). The discovery of drugs that appear to distinguish between multiple classes of benzodiazepine-binding sites and exhibit varied pharmacological effects raises questions of whether such ligands act through multiple receptor subtypes or with different efficacies through a single benzodiazepine receptor (BZD-R). For example, the triazolopyridazine drug, CL 218,872, binds with high affinity to at least one subset of benzodiazepine sites and, in keeping with this selectivity, is reportedly an effective tranquilizer with relatively few side effects (Klepner et al., 1979; Lippa et al., 1979). In contrast, the imidazobenzodiazepine, Ro 15-1788, antagonizes the binding and physiological effects of benzodiazepines (Hunkeler et al., 1981). Remarkably, certain β-carbolines exert novel effects on the CNS, opposing the actions of benzodiazepines while producing anxiety and convulsant activity in the intact CNS (Möhler and Richards, 1981; Braestrup et al., 1982; Ninan et al., 1982). Do the β-carboline drugs, methyl-β-carboline-3-carboxylate (β-CCM) and methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), that produce such dramatic pharmacological effects act independently as agonists or as antagonists?

Direct electrophysiological measurement of the actions of BZD-R ligands are needed to determine whether differences in the pharmacological effects of such drugs are due to differential effects upon multiple classes of BZD-Rs or to different efficacies at BZD-Rs. Described here are some properties of BZD-R ligands as potentiators and inhibitors of GABA-induced conductance increases. Preliminary accounts of these results have been presented (Chan et al., 1982; Farb et al., 1984).

Materials and Methods

Benzodiazepines were a gift of Dr. W. Scott of F. Hoffman-La Roche (Nutley, NJ). β-CCM and DMCM were a gift of Dr. Claus Braestrup of A/S Ferrosan (Soeborg, Denmark), and CL 218,872 was a gift of Lederle Laboratories (Pearl River, NY). All other chemicals were obtained from commercial sources.

Neurons were dissociated from spinal cords of 7-day chick embryos, plated on collagen-coated 35-mm plastic tissue culture dishes (plating density 4×10^5 cells/dish), and maintained in culture for 2 to 6 weeks as previously described (Farb et al., 1979; Choi et al., 1981a). During electrophysiological experiments, cells were perfused (1 ml/min; bath volume, 1.5 ml) with recording medium (Earle's balanced salts solution (BSS) supplemented with 3.2 mM $CaCl_2$, 16 mM glucose, and 0.25% horse serum, pH 7.4) at 37°C under a stream of 5% CO_2/95% air. Neurons were penetrated with glass

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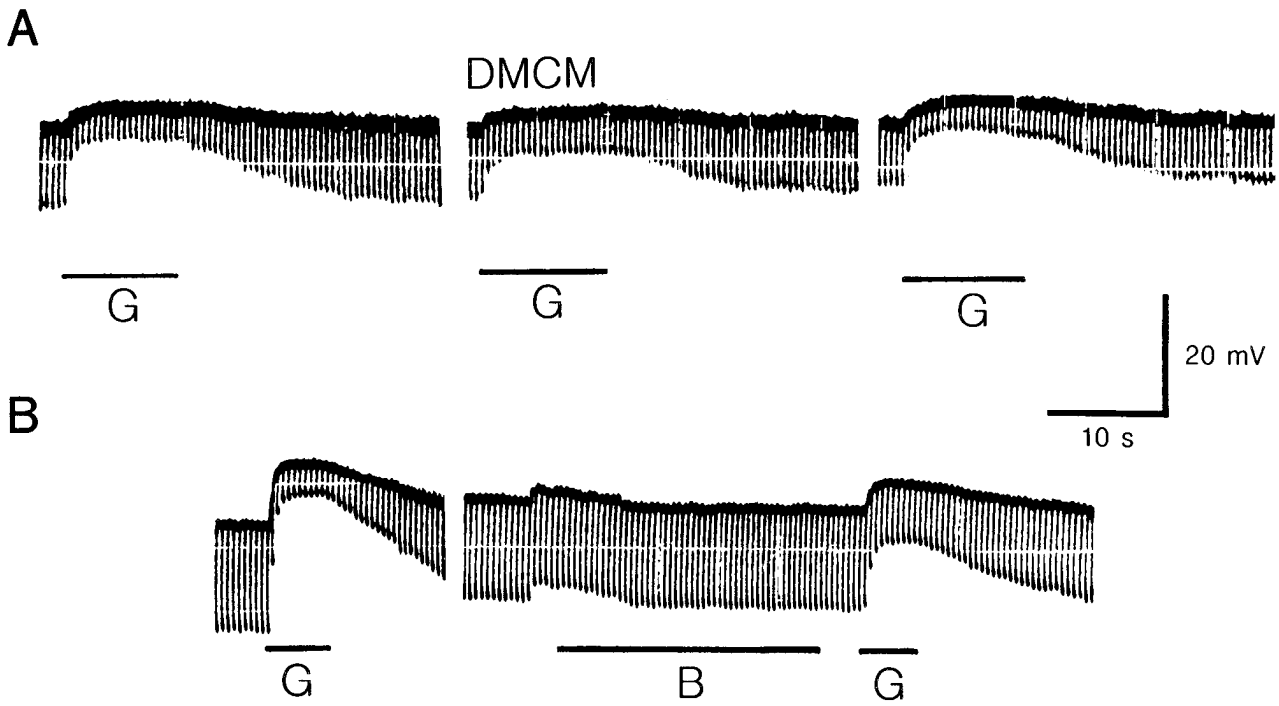

Figure 1. DMCM and β-CCM inhibit the responses of spinal cord neurons to GABA. The effect of pressure ejection of 100 nM DMCM and 1 μM β-CCM (*B*) for 20 sec prior to GABA application on the GABA responses of spinal cord neurons to 17 μM GABA (G) are shown in *A* and *B*. DMCM and β-CCM inhibited the GABA response. Downgoing deflections of the oscilloscope trace are produced by periodic (100 msec, 2 Hz) injection of current (*A*, 0.14 nA; *B*, 0.16 nA). No direct action of drugs on membrane potential or conductance was observed. In *B* a small spontaneous depolarization preceded β-CCM application and returned to base line during β-CCM pulse. Breaks in traces reflect recovery periods of 1 min. *Bars* below traces show duration of pressure pulses.

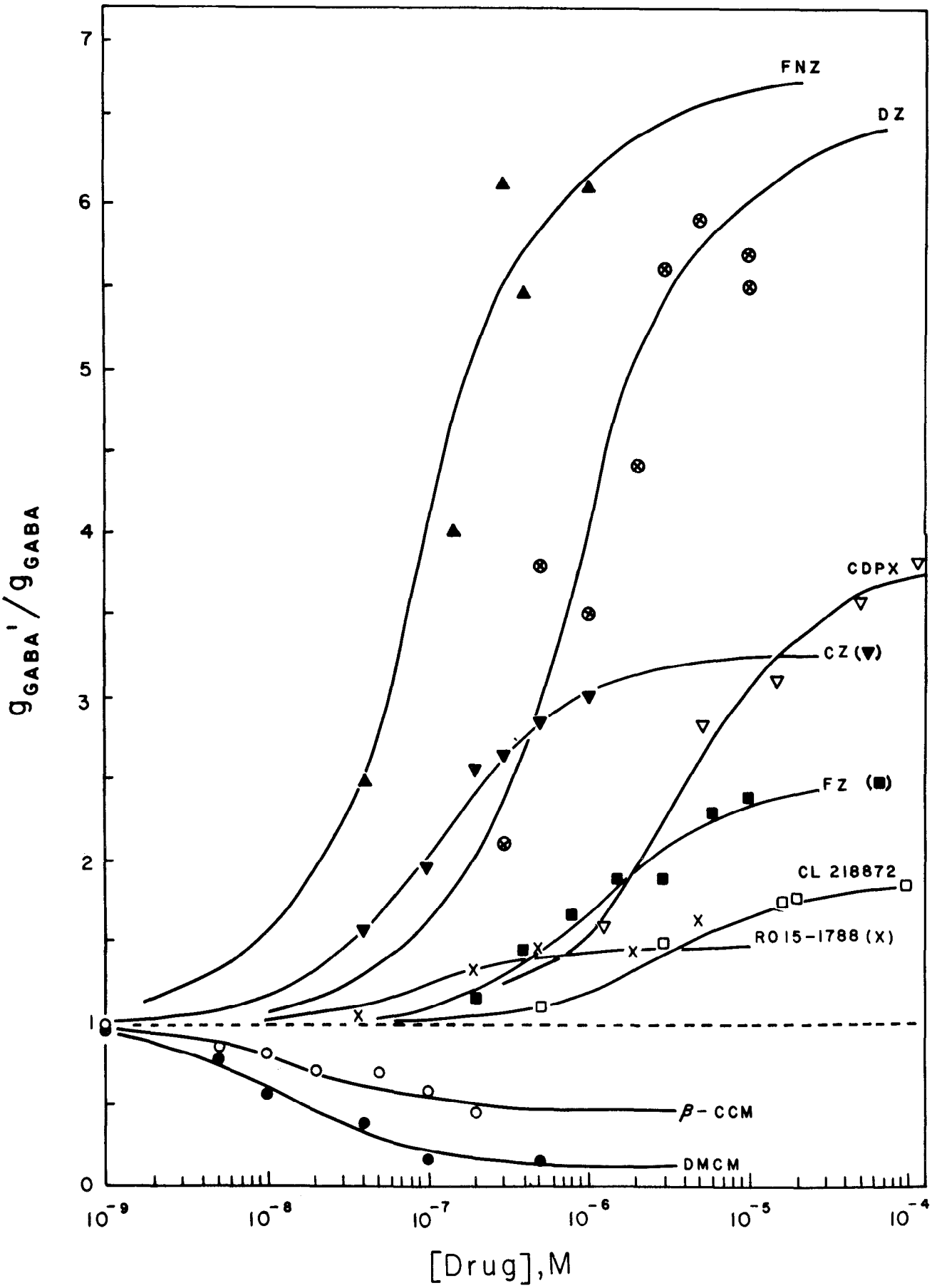

Figure 2. Plot to show comparison of representative dose-response curves of "classical" benzodiazepines and "nonbenzodiazepine" drugs, each carried out on a single spinal cord neuron, illustrating the efficacies and potencies for negative and positive modulators of GABA responses. Each single neuron dose-response curve shown was selected as most representative of a mean of three to eight independent replicate experiments (see Table I for average values from all experiments). Data are plotted as g_{GABA}'/g_{GABA} against drug concentration to show differing efficacies and potencies of negative (DMCM, β-CCM), partial positive (Ro 15-1788, CL 218,872, and flurazepam (*FZ*), and positive modulators (*CDPX*, chlordiazepoxide, clonazepam (*CZ*), diazepam (*DZ*), and flunitrazepam (*FNZ*). *Solid lines* are best theoretical one-site fits to data as determined by nonlinear least squares regression analysis. Michaelis-Menten plots of the same data shown demonstrate that g_{GABA}'/g_{GABA} "saturates" at maximal drug concentrations.

TABLE I

Efficacies and potencies for enhancement and inhibition of the GABA response by benzodiazepines and nonbenzodiazepine drugs

Values of α_{max} and EC_{50} are means ± SEM of values determined from N independent dose-response curves, each determined on a single neuron as described under "Materials and Methods" (see Fig. 2 for representative curves).

	α_{max} (%)	EC_{50} (nM)	N
Flunitrazepam	516 ± 207	180 ± 40	4
Diazepam	453 ± 136	570 ± 170	4
Clonazepam	348 ± 10	140 ± 50	5
Chlordiazepoxide	322 ± 50	18,000 ± 4,800	8
Flurazepam	164 ± 30	930 ± 17	5
CL 218,872	121 ± 13	3,300 ± 1,400	5
Ro 15-1788	71 ± 11	130 ± 30	3
β-CCM	−55 ± 9	18 ± 4	3
DMCM	−66 ± 8	10 ± 4	4

microelectrodes (R = 60 to 100 megohms, 0.5 M potassium acetate). Stable resting potentials of at least −50 mV, linear current-voltage relationships, and unambiguous bridge balance (after penetration) were required of recordings to be accepted for analysis.

Membrane conductance at rest (g_m) was determined from the amplitude of the voltage response elicited by constant current pulses (0.1 nA, 100 msec, 2 Hz) injected across the membrane by means of a bridge circuit. GABA and drugs were dissolved in recording medium and expelled from seven-barrel pipettes (5 to 7 μm tip diameter/barrel) positioned within 50 μm of the neuronal perikaryon. The perfusion medium surrounding the target neuron is effectively replaced during pressure ejection (Choi et al., 1981a). The conductance change due to GABA application (g_{GABA}) is calculated as the observed conductance in the presence of GABA (g_{obs}) minus the membrane conductance at rest: $g_{GABA} = g_{obs} - g_m$. [GABA] (2 to 5 μm) was used to give g_{GABA} = 1 to 6 nS for potentiators, whereas higher [GABA] (17 μM; g_{GABA} = 50 to 70 nS) was used with inhibitors to increase the precision of measurement. The degree of inhibition by β-CCM or DMCM is not a function of [GABA] (see "Results"), unlike potentiation which decreases at high GABA (see Fig. 5). The percentage of potentiation or inhibition of the GABA response (α or $-\alpha$) is given by the equation $\alpha = [(g_{GABA}'/g_{GABA}) - 1] \times 100$, where g_{GABA}' is determined after drug application. Values of α or g_{GABA}'/g_{GABA} are, respectively, >0 or >1 for potentiators; 0 or 1 for conditions without effect; and <0 or <1 for inhibitors of g_{GABA}. In the presentation of results and discussion below, α is generally used when it is more informative to use percentages for expression of data. For each neuron, the EC_{50} and $(g_{GABA}'/g_{GABA})_{max}$ were estimated by weighted Eadie-Hofstee analysis and by nonlinear regression. Water-insoluble drugs were dissolved in dimethyl sulfoxide and diluted to a final concentration of 0.2% dimethyl sulfoxide that did not alter g_m or g_{GABA}.

Results

β-CCM and DMCM inhibit GABA-induced conductance increases. Figure 1 shows that DMCM (100 nM) and β-CCM (1 μM) reduced the GABA (17 μM) response. When applied alone, β-CCM did not exhibit direct effects on input resistance or resting potential. Inhibition of g_{GABA} depended on ligand concentration, was saturable, and obeyed Michaelis-Menten kinetics (Hill slope 1). The results are consistent with a specific, single class of functional β-carboline sites (β-CCM: EC_{50} = 18 nM, α_{max} = −55%; DMCM: EC_{50} = 10 nM; α_{max} = −66%; see Fig. 2).

Ro 15-1788 and CL 218,872 are partial agonists. Surprisingly, whereas Ro 15-1788 is an antagonist of benzodiazepine action *in vivo*, it potentiated g_{GABA} (Table I). However, Ro 15-1788 displayed a low efficacy (α_{max} = 72%) and high potency (EC_{50} = 0.13 μM) as compared with diazepam (EC_{50} = 0.57 μM; α_{max} = 453%) (Table I, Figs. 2 and 3), in keeping with its actions as an antagonist of the pharmacological actions of classical benzodiazepines (Hunkeler et al., 1981; Bonetti et al., 1982; Darragh et al., 1982). CL 218,872 gave a moderate potency (EC_{50} = 3.3 μM) and the lowest efficacy (α_{max} = 121%) of the tranquilizers tested (Figs. 2 and 3), but not unlike the values obtained with flurazepam (EC_{50} = 0.93 μM; α_{max} = 164%). Although the efficacy of CL 218,872 is only 1.7-fold greater than that of Ro 15-1788, a seemingly small difference, the former drug is a tranquilizer, whereas the latter exerts little pharmacological activity (Klepner et al., 1979; Lippa et al., 1979; Hunkeler et al., 1981). However, recent experiments are consistent with the prediction from these results that Ro 15-1788 should be a partial agonist in behavioral tests (Prado de Carvalho et al., 1983).

Efficacies of "classical" benzodiazepines differ. Differences among the efficacies of BZD-R ligands were not restricted to nonbenzodiazepines. The "classical" benzodiazepines flunitrazepam, diazepam, chlordiazepoxide, clonazepam, and flurazepam, listed in order of decreasing efficacy, exhibited widely differing efficacies (Table I), as illustrated in Figure 2 by representative single-neuron dose-response curves.

Correlations. There was no clear correlation of efficacy and potency. However, three general groups of ligands can be identified, exhibiting (*1*) full efficacy of potentiation with high potency (flunitrazepam, diazepam, and clonazepam) or low potency (chlordiazepoxide), (*2*) partial efficacy of potentiation with intermediate potency (flurazepam and CL 218,872) or high potency (Ro 15-1788), and (*3*) partial efficacy of inhibition with very high potency (β-CCM and DMCM). A good correlation (Fig. 3) was observed between the efficacy $(g_{GABA}'/g_{GABA})_{max}$ for potentiation or inhibition of g_{GABA} and the GABA ratios determined in binding assays (Braestrup et al., 1982). The GABA ratio, {IC_{50} for competing ligand in the presence of muscimol}/{IC_{50} in the absence of muscimol}, reflects the efficacy of enhancement by GABA agonists of modulator ligand binding to membrane homogenates at 0°C. The fact that a good correlation was found supports the hypothesis that the benzodiazepine and nonbenzodiazepine ligands that modulate g_{GABA} with positive and negative efficacy act through a common receptor mechanism.

Picrotoxin and β-CCM inhibit g_{GABA} through different sites. Would picrotoxin, itself a noncompetitive inhibitor of GABA action, block inhibition of g_{GABA} by β-CCM? β-CCM inhibited g_{GABA} (at 100 μM GABA) to the same extent in the presence or absence of picrotoxin (30 to 100 μM) (Fig. 4), indicating the independence of the two inhibitory mechanisms and arguing that β-CCM and picrotoxin act at different sites. Picrotoxin was able to completely inhibit g_{GABA} (at 100 μM GABA: α = −92 ± 2%, n = 11, at 30 μM picrotoxin; and α = −98 ± 1%, n = 3 at 70 μM picrotoxin), unlike β-CCM and DMCM which exhibited saturable and partial inhibition.

GABA does not surmount the inhibition of g_{GABA} by β-CCM or DMCM. β-CCM inhibited g_{GABA} to about the same extent at all of the GABA concentrations tested (see Fig. 5). A saturating concentration of β-CCM (1 μM; EC_{50} = 0.018 μM) reduced g_{GABA} by 53 ± 9% at 17 μM GABA (n = 4), by 50 ± 5% at 100 μM GABA (n = 6), and by 46% at 500 μM GABA (n = 2). When a complete experiment was carried out on a single neuron, 1 μM β-CCM inhibited the responses to 17 μM and 100 μM GABA identically, to within error (Student's t test, $p > 0.2$). Similarly, GABA did not surmount the inhibition of g_{GABA} by DMCM: maximal inhibition of g_{GABA} was 68 ± 6% (n = 4) (DMCM, 500 nM; GABA, 17 μM). Using 30 nM DMCM (subsaturating), the decrease in g_{GABA} was 55% (n = 2) at 50 μM GABA, and 40 ± 5% (n = 7) at 200 μM GABA. Thus, the activities of β-CCM and DMCM were apparently insurmountable by GABA, suggesting that both β-carbolines are either partial noncompetitive or uncompetitive inhibitors of g_{GABA}.

GABA surmounts benzodiazepine potentiation of g_{GABA}. Figure 5 shows that increasing [GABA] decreased α for chlordiazepoxide, as expected since chlordiazepoxide decreases the GABA EC_{50} from 17 μM to 8 μM with no change in $(g_{GABA})_{max}$ (Choi et al., 1981a). Similar results were observed with other benzodiazepines: at 2 μM and 100 μM GABA the α_{max} for flunitrazepam was, respectively, 516% and 48%; similarly, the α_{max} for clonazepam was 348% ± 10 (n = 5) at 2 to 5 μM GABA and undetectable (~0%, n = 2) at 200 μM GABA. In contrast, GABA did not surmount the activity of β-carbolines (see above).

Competition between ligands of positive and negative efficacy. Could a benzodiazepine-positive modulator reverse the inhibition of g_{GABA} exerted by a β-carboline? Clonazepam (30 μM) reversed the ability of DMCM to inhibit g_{GABA} (50 μM GABA) (Fig. 6). Competition was also obtained when the experiment was repeated on the same neuron using 200 μM GABA. This experiment was repeated twice on different neurons with identical results. Similarly, DMCM inhibited g_{GABA} at 500 μM GABA by 32% (n = 2), and g_{GABA} was restored to control in the presence of clonazepam. No enhancement of g_{GABA} past control was seen with 200 μM or 500 μM GABA, consistent with the result that clonazepam does not augment maximal g_{GABA}. Finally, the partial agonist Ro 15-1788 surmounted the inhibition of g_{GABA} ([GABA], 10 μM) by β-CCM (Table II): in three experiments mean values for α in the presence of β-CCM, Ro 15-1788, and a mixture of β-CCM plus Ro 15-1788 were, respectively, −56%, +94%, and +44%.

Discussion

The concept that neuromodulation provides an important principle for nervous system function has found increasing support in recent years. All neurons have the capacity to transduce the presence of specific chemical substances into a useful function, primarily the alteration of membrane

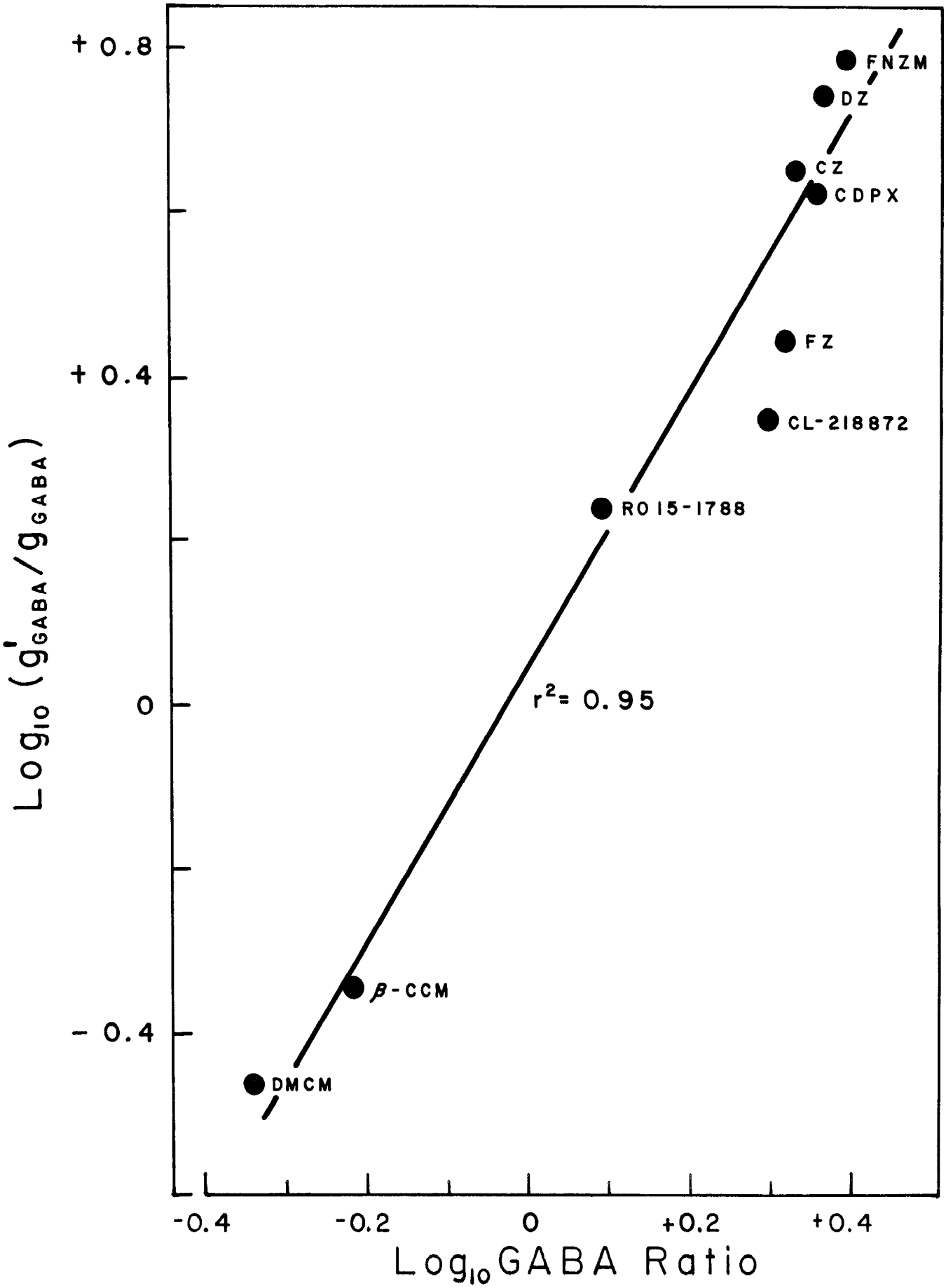

Figure 3. Efficacies for positive and negative modulation of GABA responses of spinal cord neurons (see Table I) correlate with GABA ratios from Braestrup et al. (1982) determined by radioligand binding. ($g_{GABA}'/g_{GABA})_{max}$ was determined as described under "Materials and Methods" and in the legend of Figure 2. GABA ratios, {the IC_{50} for competing ligand in the presence of muscimol}/{IC_{50} in the absence of muscimol}, were determined using membrane homogenates of adult rat brain; the value for flurazepam (*FZ*) was from Dr. C. Braestrup (personal communication). *CDPX*, chlordiazepoxide; *CZ*, clonazepam; *DZ*, diazepam; *FNZM*, flunitrazepam.

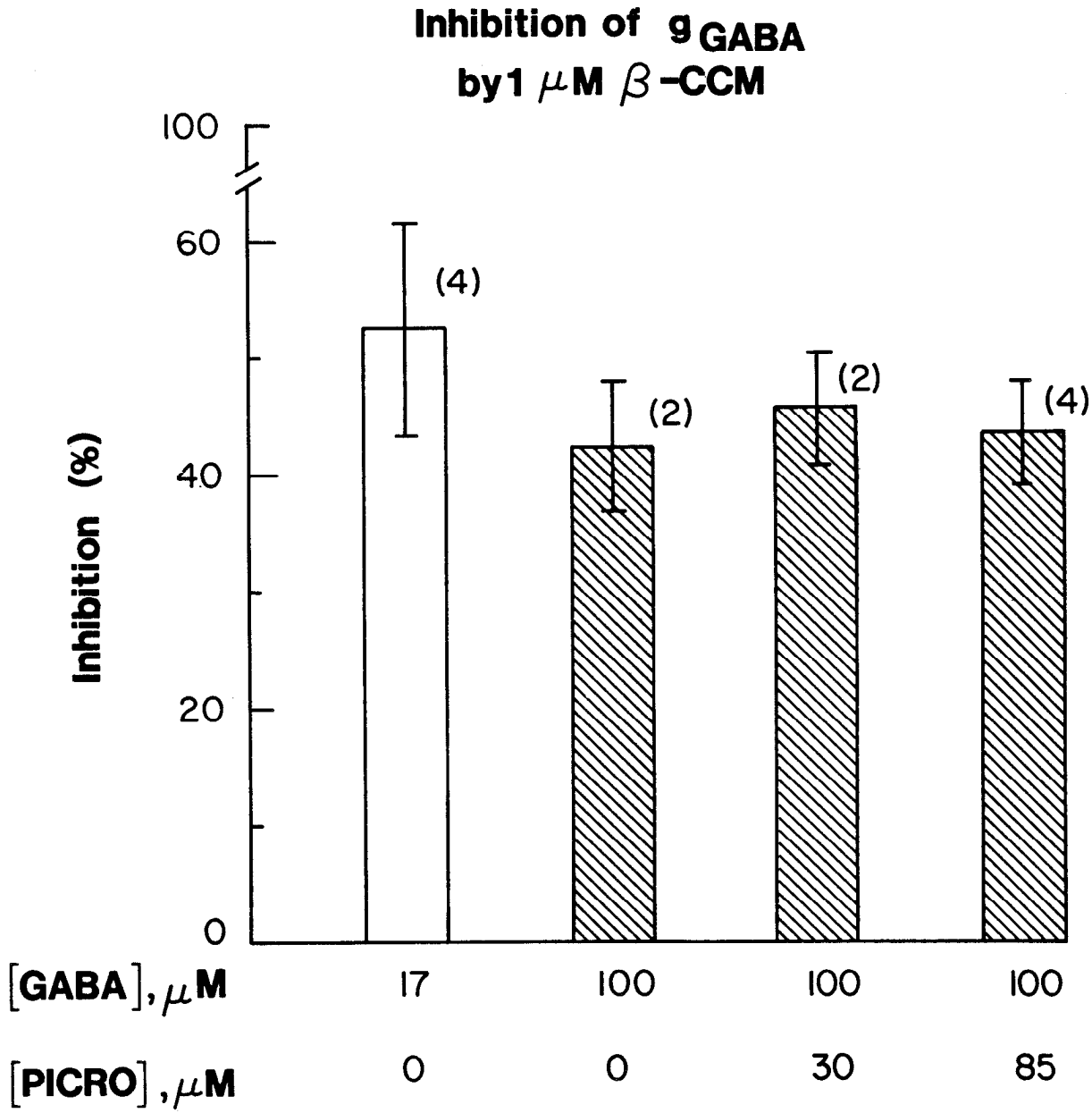

Figure 4. Inhibition of g_{GABA} by β-CCM is not antagonized by picrotoxin. The relative inhibition of g_{GABA} at 17 μM and 100 μM GABA by β-CCM (1 μM) was not reduced by 30 or 85 μM picrotoxin. Note that each *bar* of the graph has been normalized to correct for inhibition of g_{GABA} produced by picrotoxin itself. Actual g_{GABA} responses in the presence of 85 μM picrotoxin were about 10% of those observed in the absence of picrotoxin; thus, picrotoxin decreases g_{GABA} but does not diminish the action of β-CCM.

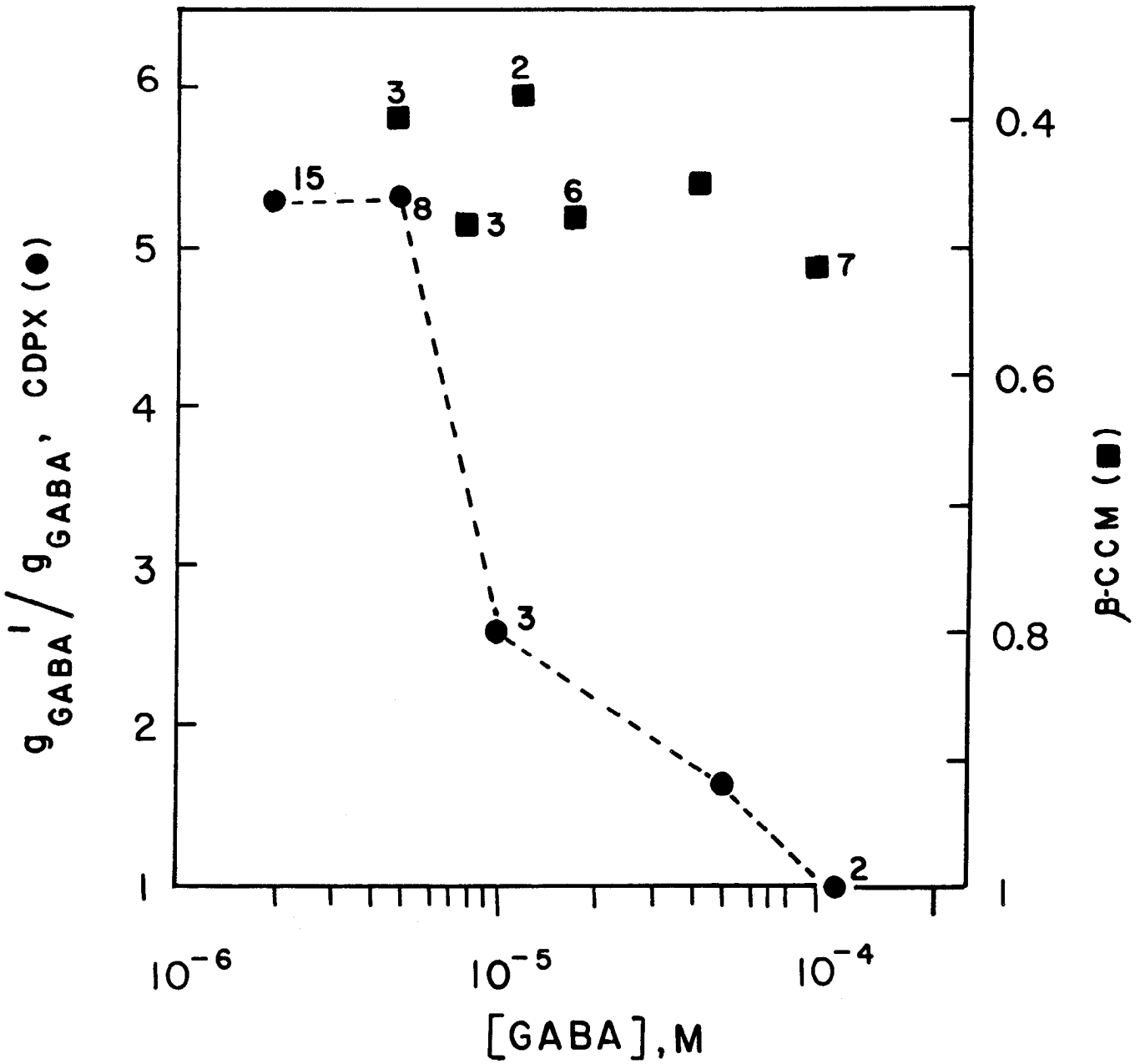

Figure 5. Plot to show the relation between extracellular GABA concentration and, respectively, potentiation or inhibition of the GABA response (g_{GABA}'/g_{GABA}) by 300 μM chlordiazepoxide (●, *CDPX*) or 1 μM β-CCM (■). Half-maximal decrease in g_{GABA}'/g_{GABA} for chlordiazepoxide is at ~8 μM GABA. The *numerals* adjacent to *symbols* indicate the number of neurons per data point.

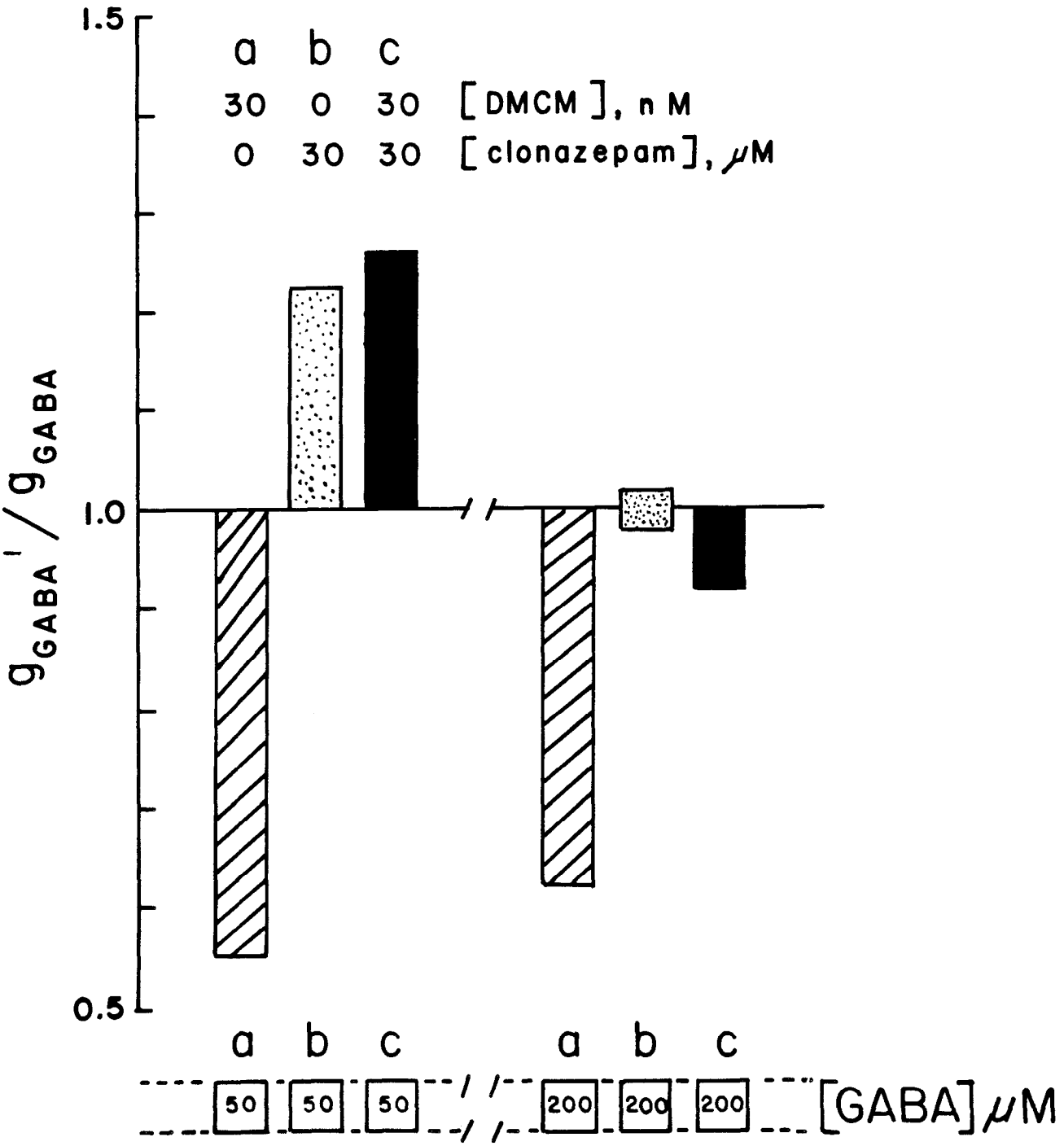

Figure 6. Clonazepam reverses the ability of DMCM to inhibit the GABA response. The *bar graph* shows data derived from an experiment carried out on a single neuron, as in Figure 1. Reading from left to right, following an initial test pulse of 50 μM GABA, drugs were applied, as indicated (a to c) prior to subsequent test pulses of GABA. *a*, DMCM inhibited g_{GABA}. *b*, Clonazepam potentiated g_{GABA} at 50 μM but was inactive at 200 μM GABA. *c*, Clonazepam reversed the ability of DMCM to inhibit both 50 μM and 200 μM GABA responses. The break in the *x* axis indicates the recovery period (20 min) prior to application of 200 μM GABA. The fractional occupancy, $[L]/([L] + EC_{50})$, for clonazepam (0.995) was chosen to favor competition with DMCM (0.75).

conductance. In the classical model of drug-receptor interaction, as developed by Clark (1933), Gaddum (1937), and Stephenson (1956), the magnitude of the observed response was hypothesized to be a function, $f(S)$, of the stimulus S produced by occupancy of receptor, which is inactive at rest, by agonist (A) as the consequence of a simple binding equilibrium. Thus, response = $f(S)$ and $S = e \times \{[A]/([A] + K_d)\}$, where the efficacy factor, e, is an empirical constant that relates the maximal response obtained to receptor occupancy, and K_d is the equilibrium dissociation constant for ligand binding. Efficacy is generally assumed to be positive; however, if some receptor activity is present in the absence of agonist, it is theoretically possible for a drug to display negative efficacy by reducing the response below the resting level.

Until recently, the possibility of negative efficacy has remained a mathematical curiosity. In general, the receptors which have been extensively studied exhibit little activity in the absence of agonist. This is not surprising for receptors such as the acetylcholine or GABA receptors which are directly linked to ion channels. However, the neuromodulators that act through BZD-Rs do not by themselves produce a physiological response (Fig. 1*B* and Choi et al., 1981a), but control the sensitivity of the GABA receptor to its transmitter, GABA. In this system a combination of negative and positive modulation might prove advantageous, and many of the effects of benzodiazepines and related compounds (e.g., β-carbolines and triazolopyridazines) are explicable with such a model. For example, clinically effective benzodiazepine anxiolytics promote g_{GABA} (Table I) and bind with higher affinity in

TABLE II

Ro 15-1788 blocks the inhibitory action of β-CCM on the GABA response

The effect of the indicated conditions on the conductance increase produced by pressure ejection of 17 μM GABA was determined as in Figure 6. Fractional occupancies used for β-CCM (0.982) and Ro 15-1788 (0.996) were chosen to attempt to get competition at saturating drug concentrations; thus, α in condition 2 was intermediate between conditions 1 and 3. Values are means ± SEM from *N* neurons.

Condition	Drug (μM)	α (%)	*N*
1. β-CCM	1	−56 ± 6	3
2. β-CCM	1		
+ Ro 15-1788	40	+44 ± 19	3
3. Ro 15-1788	40	+94 ± 18	4

the presence of GABA. Similarly, the "anxiogenic" and convulsant β-carbolines inhibit g_{GABA} (Fig. 2), antagonize benzodiazepine action *in vivo*, and bind with lower affinity in the presence of GABA (Möhler and Richards, 1981; Braestrup and Nielsen, 1981).

Do β-carbolines act as agonists with negative efficacies at BZD-Rs by exhibiting an intrinsic inhibitory action on g_{GABA}, or as antagonists of GABA and/or benzodiazepine action? In the absence of an endogenous effector the use of the terms agonist and antagonist seems ambiguous. For example, the convulsant drug picrotoxin acts by inhibiting g_{GABA} (Takeuchi and Takeuchi, 1969), yet it is not classified as a negative agonist nor is it considered to be a benzodiazepine antagonist. β-Carbolines compete with benzodiazepines for binding and flunitrazepam, when used as a photoaffinity label for the BZD-R (Möhler et al., 1980; Chan et al., 1983) inhibits benzodiazepine binding while sparing β-carboline binding (Hirsch, 1982; Möhler, 1982). Thus, we wanted to determine whether β-carbolines might act as negative modulators of GABA action. First, β-CCM and DMCM decreased g_{GABA}, and increasing [GABA] did not surmount this inhibition. Second, clonazepam did not potentiate g_{GABA} at high [GABA] and reversed the inhibition of g_{GABA} by DMCM. Finally, a site distinct from the picrotoxin-sensitive chloride ionophore mediates the actions of these drugs since clonazepam does not reverse the inhibition of g_{GABA} by picrotoxin and picrotoxin does not inhibit the action of β-CCM or DMCM. β-CCM cannot, therefore, be classified as a classical competitive antagonist of GABA action, of benzodiazepine action, nor as a classical non-competitive antagonist of benzodiazepine action. Rather, β-CCM and DMCM are effectors that exhibit properties characteristic of negative agonists ($e < 0$) and opposite to those of benzodiazepines ($e > 0$). β-Carbolines reduce the GABA response in cultures that have been extensively washed by perfusion. These conditions would be expected to remove any reversibly bound ligands such as might exist, arguing that inhibition does not arise by displacement of a bound endogenous benzodiazepine agonist. Thus, direct electrophysiological evidence is presented supporting the idea that β-carbolines act as a novel class of negative modulators or inverse agonists (Braestrup et al., 1982). The results suggest that positive and negative modulators modify g_{GABA} in a qualitatively different manner; whereas the effects of chlordiazepoxide, flunitrazepam, and clonazepam could be surmounted by increasing GABA concentration, those of β-CCM and DMCM could not. Thus, it seems likely that β-carboline-negative modulators, unlike benzodiazepine positive modulators, are capable of changing the maximal GABA response.

Is there a common receptor that mediates the postsynaptic actions of classical benzodiazepines, CL 218,872, Ro 15-1788, and β-carbolines? Ro 15-1788 and clonazepam reversed the ability of β-CCM and DMCM to inhibit g_{GABA}, indicating functional competition among BZD-R ligands across the full range of efficacies observed. Efficacy but not potency correlates with the qualitative nature of the pharmacological actions of these drugs *in vivo*. The results of Figure 3 provide the first evidence supporting the idea that the ability of GABA to enhance/inhibit ligand binding to the benzodiazepine-binding site (*1*) is a valid measure of the ability of such ligands to modulate g_{GABA}, and (*2*) may be taken as an indicator of agonist/antagonist efficacy.

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