Allosteric Modulation by Benzodiazepine Receptor Ligands of the $GABA_A$ Receptor Channel Expressed in *Xenopus* Oocytes

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Chick brain mRNA was isolated and injected into *Xenopus* oocytes. This led to the expression in the surface membrane of functional GABA-activated channels with properties reminiscent of vertebrate $GABA_A$ channels. The GABA-induced current was analyzed quantitatively under voltage-clamp conditions. Picrotoxin inhibited this current in a concentration-dependent manner with IC_{50} = 0.6 μM. The allosteric modulation of GABA currents by a number of drugs acting at the benzodiazepine binding site was characterized quantitatively. In the presence of the benzodiazepine receptor ligands diazepam and clorazepate, GABA responses were enhanced, and in the presence of the convulsant β-carboline compound methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), they were depressed. Maximal stimulation of the response elicited by 10 μM GABA was 160% with diazepam and 90% with clorazepate, and maximal inhibition was 42% with DMCM, 30% with methyl β-carboline-3-carboxylate (β-CCM), 15% with ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate (Ro 15-1788), and 12% with ethyl β-carboline-3-carboxylate (β-CCE). Half-maximal stimulation was observed with 20 nM diazepam and 390 nM clorazepate, respectively, and half-maximal inhibition with 6 nM DMCM. β-CCM had a similar effect to DMCM, whereas β-CCE and Ro 15-1788 showed only small inhibition at low concentrations (<1 μM). All the tested carboline compounds and Ro 15-1788 showed a biphasic action and stimulated GABA current at concentrations higher than 1 μM. The modulatory drugs did not affect the reversal potential of the current or the maximal GABA response. Diazepam shifted the K_a of the GABA dose–response curve from 21 to 11 μM without altering the slope, whereas DMCM changed the K_a to 48 μM and decreased the apparent slope from 1.7 to 1.4.

GABA is the major inhibitory neurotransmitter in mammalian brain and is thought to mediate its effects through a postsynaptic membrane protein containing an anion channel (Nistri and Constanti, 1979). A considerable body of electrophysiological, pharmacological, and biochemical observation has suggested that the anxiolytic and anticonvulsant drugs of the benzodiazepine type and the convulsants of the β-carboline type exert their effects via the GABA receptor (Olsen, 1982; Turner and Whittle, 1983). A protein complex of M_r 220,000, retaining binding activity for GABA, benzodiazepines, β-carbolines, picrotoxin, and pentobarbital has been purified to homogeneity from bovine cerebral cortex (Sigel et al., 1983; Sigel and Barnard, 1984).

The nature of the action on the GABA-induced conductance or on membrane potential changes by the clinically used benzodiazepines has received wide attention. There is consensus about the potentiating effect of these drugs. Another class of drugs of the β-carboline type has more recently been described as binding to the same receptor sites as the relaxant benzodiazepines, but with an opposite, convulsive effect (Braestrup et al., 1983). This type of drug has been reported to decrease GABA-induced conductance changes (Polc et al., 1981; Skovgaard Jensen and Lambert, 1983, 1986; Skerritt and MacDonald, 1984; Chan and Farb, 1985). Both benzodiazepine and β-carboline actions are thought to be antagonized by the compound Ro 15-1788 (Hunkeler et al., 1981).

Most of the electrophysiological studies on the action of benzodiazepine receptor ligands have been performed on primary neuronal cell cultures. An alternative model system for the study of neuronal channels has been developed in recent years. *Xenopus* oocytes are injected with mRNA isolated from the tissue of interest, and subsequently acquire membrane proteins typical for this origin of the mRNA (Barnard et al., 1982). It has been shown previously that GABA receptor channels expressed by this technique retain properties similar to those in the brain (Miledi et al., 1982; Smart et al., 1983; Gundersen et al., 1984; Houamed et al., 1984; Parker et al., 1986). Here we describe quantitatively the GABA-induced current and its positive and negative allosteric modulation by some drugs that act via the benzodiazepine binding site. For these types of drugs, the terms "benzodiazepine agonist," "benzodiazepine inverse agonist," and "benzodiazepine antagonist" have also been used in the literature.

Materials and Methods

Total mRNA was prepared from forebrain of 2-d-old chick following the procedures of Cathala et al. (1983) with the previously described modifications (Sigel, 1987a), except that fresh tissue was used in the initial homogenization. Injection of mRNA into the oocytes and the removal of cellular layers surrounding the membrane have been described elsewhere (Sigel, 1987a). Measurements were performed on denuded oocytes, i.e., the follicular layers were removed prior to the current measurement. The oocytes were placed in a 0.4 ml bath on a nylon grid. The bath was perfused at a rate of 6 ml/min with a medium containing 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM HEPES-NaOH (pH 7.4). Where indicated, perfusion was switched to the identical medium, with the addition of GABA, picrotoxin, and clorazepate. β-Carbolines were dissolved in ethanol. The final ethanol concentration in the experiment was always below 0.5%. Ethanol concentrations up to 1% and propylene glycol up to 0.2% failed to affect

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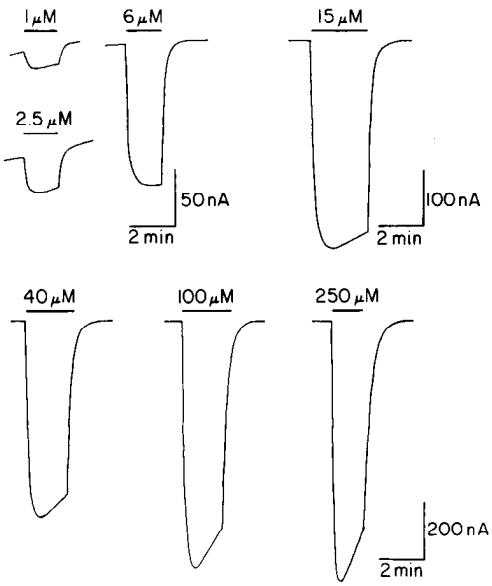

Figure 1. Membrane current in response to different concentrations of GABA. The oocyte was kept at −80 mV under voltage-clamp. The temperature was 6°C. Continuous perfusion was switched for the periods of time indicated by the *bar* to the same medium containing GABA.

the current elicited by GABA. Diazepam and Ro 15-1788 were diluted into standard medium from preparations used for intravenous application (Valium, 17.6 mM in propylene glycol/ethanol/H_2O; Anexate, 0.33 mM NaCl/EDTA/Na-acetate buffer). If diazepam was diluted from a stock ethanol solution, stimulation of GABA responses had a similar concentration dependence, but the maximal effect was found to be smaller (80%) than that with the dilution from the propylene glycol solution (160%), presumably because of solubility problems. Each application of a drug was followed by perfusion with standard medium for a period long enough to allow full recovery of the channel from desensitization (4–15 min, depending on the GABA concentration used). All experiments were carried out at 20°C, with the exception of the GABA dose–response curves, which were performed at 6°C. For the current measurement under voltage-clamp, the oocyte was impaled with 2 conventional electrodes as described (Sigel, 1987a), and the membrane potential was held at −100 or −80 mV, as indicated in the figure legends. Current amplitudes were either read as the peak currents or, in the case

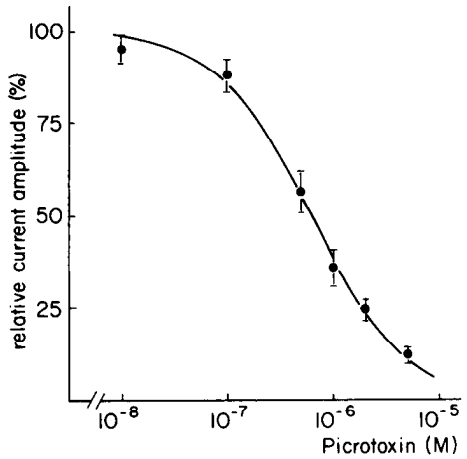

Figure 2. Inhibition of the current by picrotoxin. The membrane potential was kept at −100 mV and currents were measured at 20°C. The current amplitude evoked by application of medium containing 10 μM GABA and different concentrations of picrotoxin was determined. Values for the relative current amplitudes from 4 experiments were averaged and fitted with the equation $I_{rel} = (1 + [\text{picrotoxin}]/K_i)^{-1}$.

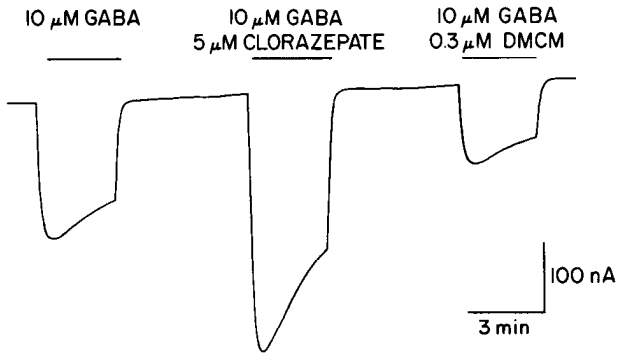

Figure 3. Effect of the benzodiazepine clorazepate and the β-carboline DMCM on the GABA response. The experiment was carried out under standard conditions at a membrane potential of −100 mV. The modulatory drugs were applied together with GABA.

of the GABA dose–response curves, were obtained by back-extrapolation of the current trace to the time when the current reached 50% of the maximal response. The maximal correction that had to be applied to a measured current amplitude was 11%. Dose–response curves were fitted with the equation given in Results, using a nonlinear least-squares method (Gauss–Newton–Marquardt). The desensitization of the GABA current was fitted, using the same method, to a single- or double-exponential decay plus a constant.

Methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), ethyl β-carboline-3-carboxylate (β-CCE), and methyl-β-carboline-3-carboxylate (β-CCM) were from Research Biochemicals (Konstanz, Germany); diazepam (Valium) and ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate (Ro 15-1788; Anexate) were from Hofmann-La Roche (Basel); and clorazepate was a gift from Boehringer-Ingelheim (UK).

Results

Concentration and voltage dependence of GABA currents

After injection with mRNA isolated from brain tissue of 2-d-old chicks, oocytes acquired ion channels with properties typical

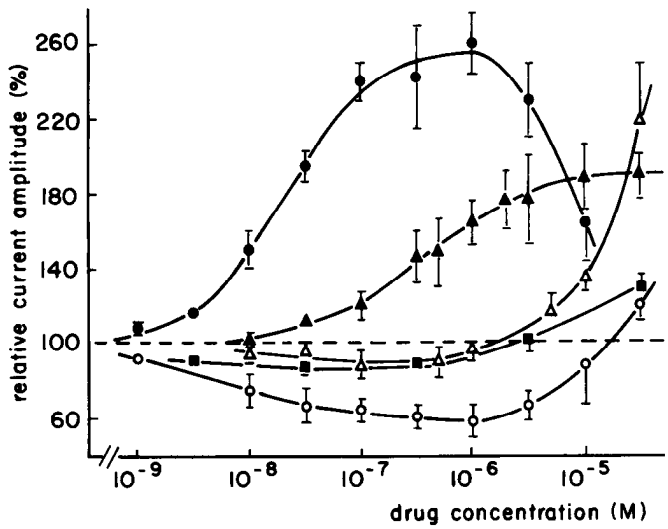

Figure 4. Concentration dependence of different modulators of the GABA channel. All measurements were standardized by assigning 100% to the current amplitude elicited by 10 μM GABA (see Fig. 3). Drugs were applied together with 10 μM GABA and omitted in the washout intervals. For each point, mean ± SD is given for at least 4 determinations performed on different oocytes. ●, Diazepam; ▲, clorazepate; △, β-CCE; ○, DMCM; ■, Ro 15-1788. Values obtained for β-CCM are omitted for clarity; these values are between those for β-CCE and DMCM at all drug concentrations.

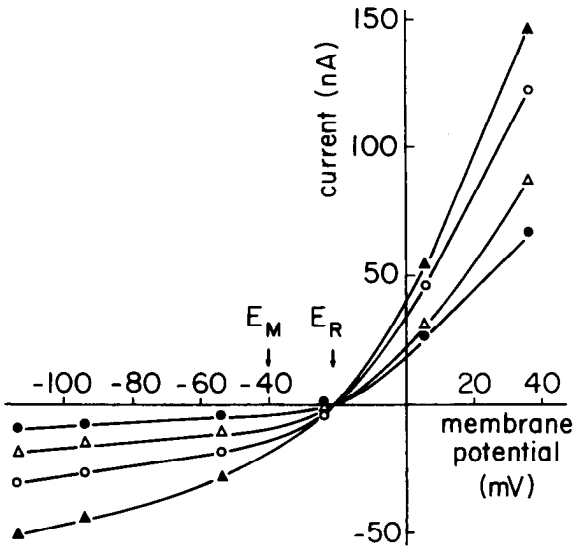

Figure 5. Current–voltage relationship of the GABA-induced current. Data were obtained from an experiment of the type shown in Figure 3. Before application of the drugs and during peak current amplitude, a discontinuous voltage ramp of 100 msec step duration was applied from a holding potential of −100 mV. The current amplitude measured at the end of the step was plotted against membrane potential. ●, Resting conditions; ○, GABA (10 μM); ▲, GABA (10 μM) and 5 μM clorazepate; △, GABA (10 μM) and 0.3 μM DMCM. E_M and E_R indicate the membrane potential of the oocyte and the reversal potential of the current, respectively.

of neural tissue. Channel expression was highest for voltage-dependent Na channels and GABA channels, but Ca channels or kainate-sensitive channels could also be reproducibly induced. Perfusion of mRNA-injected oocytes clamped at −80 mV with GABA produced an inward current that was GABA-concentration-dependent (Fig. 1). The current showed time-dependent inactivation in the presence of GABA. This desensitization was faster at higher concentrations of GABA. Typical current amplitudes elicited by 100 μM GABA 5 d after mRNA injection were between 1 and 2 μA. The convulsant picrotoxin inhibited the GABA-induced current in a concentration-dependent manner. Inhibition by 50% was observed at 0.6 μM concentration (Fig. 2). The voltage dependence of the chick brain mRNA-induced GABA-activated current was similar to that described earlier (Miledi et al., 1982; Parker et al., 1986). The reversal potential was −22 mV (Fig. 5), similar to the chloride equilibrium potential in the *Xenopus* oocyte (Barish, 1983).

Effect of benzodiazepine binding site ligands on the GABA current

The water-soluble benzodiazepine ligand clorazepate increased and the β-carboline compound DMCM decreased the size of the current elicited by 10 μM GABA (Fig. 3). The concentration dependence of both the positive and negative modulation was studied further (Fig. 4). The concentration dependence of the drug action reached an extreme at low drug concentrations, except for the water-soluble clorazepate (Fig. 4, Table 1). The curves were fitted as indicated in Materials and Methods. The values for half-maximal stimulation or inhibition are shown in Table 1. The concentration of the drugs exhibiting half-maximal effect increased in the following sequence: Ro 15-1788 < DMCM <diazepam < clorazepate. The data on Ro 15-1788 were obtained from an experiment described below. The absolute values determined here for half-maximal effects are similar to the known binding constants of these drugs at the benzodiazepine binding site (Möhler and Richards, 1981; Braestrup et al., 1983). At concentrations higher than 1 μM, all drug actions were smaller than at the optimal concentration, and some drugs even switched from inhibition to stimulation. The largest effect of this type was seen with β-CCE, which changed from 12% inhibition to 120% stimulation. The compound Ro 15-1788, usually cited as a benzodiazepine antagonist in the literature, exhibited a similar effect to that of β-CCE, except that the stimulation at high drug concentration was small (30% at 30 μM). Each point in Figure 4 represents the average of 4 experiments carried out with 4 different oocytes. If oocytes were exposed to the perfusion of any of the modulators in the absence of GABA, no current response could be detected. The size of the modulatory effect of the drugs was found to be dependent on the GABA concentration (see below).

Table 1. Action of positive and negative modulatory drugs on the current amplitude elicited by 10 μM GABA

Drug	K_a (or K_i) (nM)	Optimal effect (%)	Effect at 30 μM (%)
Diazepam	20	160	63[c]
Clorazepate	390	90	190
Ro 15-1788	0.6[b]	15	30
β-CCE	<100	−12	120
β-CCM	<100	30	90
DMCM	6	42	20

The curves obtained in the experiment shown in Figure 4 were evaluated as indicated in the text. All the drug actions, except that of chlorazepate, show an extreme at low drug concentration, which is called the "optimal effect."

[a] The effect of a drug is expressed as percentage stimulation or inhibition of the current amplitude elicited by 10 μM GABA on the same oocyte.

[b] This value was obtained, as indicated in the text, from the antagonism of the stimulation by clorazepate.

[c] Measured at 10 μM diazepam.

Effect of modulators on the reversal potential of the GABA response

The presence of positive or negative modulators (5 μM clorazepate or 0.3 μM DMCM) that act at the benzodiazepine binding site did not alter the reversal potential of the GABA-induced current response (Fig. 5). The shape of the current–voltage relationship was not changed by the modulator compounds, indicating that the ion selectivity of the channel was not affected.

Effect of modulators on the GABA-concentration dependence of the current

At normal temperature, the GABA response showed fast desensitization. In order to obtain reliable current amplitudes for GABA dose–response curves, the temperature was lowered to 6°C for these measurements. As shown in Figure 6, time-dependent inactivation was greatly slowed at this temperature. The GABA-concentration dependence of the current is shown in Figure 7 (closed circles). The data were obtained as follows: Each dose–response curve represents the average of 3 separate experiments performed with different oocytes and variable levels of channel expression. The individual dose–response curves were fitted (see Materials and Methods) with the equation $I(c)$ =

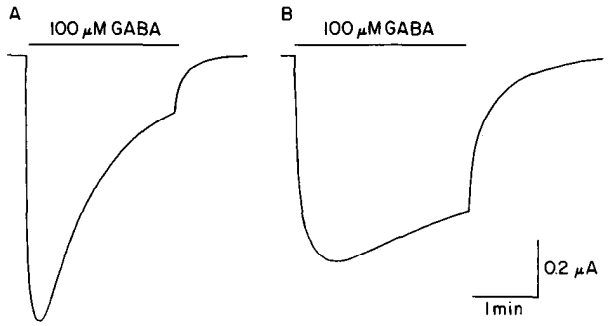

Figure 6. Effect of temperature on the desensitization of the GABA response. GABA, 100 μM, was applied twice to the same oocyte, with a 15 min washout interval. First exposure (*A*) was at 20°C, second exposure (*B*) at 6°C. Holding potential was −100 mV.

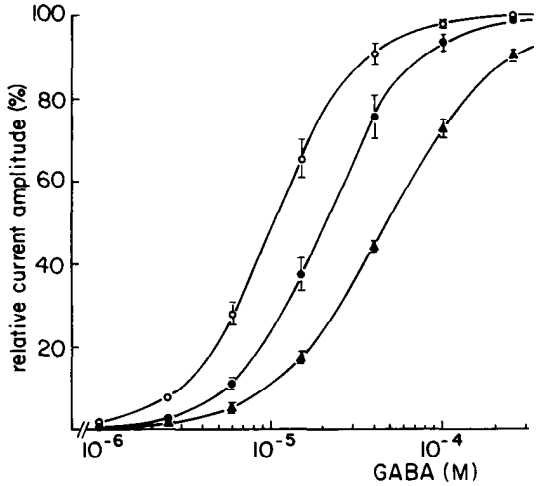

Figure 7. Effect of the modulators on the GABA-concentration dependence of the response. The experiment was carried out at 6°C and at a holding potential of −80 mV. The GABA dose–response curve is shown in the absence of drug (●), in the presence of 0.1 μM diazepam (○), and in the presence of 0.3 μM DMCM (▲). Drugs were added together with GABA and omitted during the washout intervals. The dose–response curve was obtained 3 times for each condition, with oocytes displaying different degrees of channel expression. Each curve was first fitted with the equation given in the text. Maximal current I_{max} was then arbitrarily set at 100% and the extrapolated current amplitudes (see Materials and Methods) were expressed in relative values. Each point represents the average of 3 determinations. The curves shown were obtained by reapplying the equation to the standardized average values.

$I_{max}c^n/(c^n + K_a^n)$, where I_{max} is the maximal current amplitude, c the GABA concentration, n the Hill number, and K_a the GABA concentration that elicits half-maximal current response. Individual curves were standardized by setting I_{max} = 100%, and the 3 experiments were then averaged and fitted again with the above equation to give the solid line in Figure 7. The values obtained for K_a and n in the absence and presence of 0.3 μM DMCM or of 0.1 μM diazepam in the medium are summarized in Table 2. While diazepam did not alter the Hill number, DMCM decreased this value.

Effect of modulators on the maximal GABA response

The procedure used above to standardize the current responses does not allow any conclusion to be drawn as to whether the modulator drugs also affect the maximal GABA response. To clarify this question, we studied the effect of the modulators on the response elicited by 100 μM GABA. This was the highest GABA concentration at which a reproducible response could be achieved within 15 min of the control application. These experiments were carried out at 6°C. In the presence of 0.1 μM diazepam the average response was 105 ± 9% of the GABA control (4 experiments), and the corresponding value for 0.3 μM DMCM was 82 ± 7% (5 experiments). Similar changes would be expected for a pure effect of the modulators on the K_a, with no effect on I_{max}.

Antagonism of clorazepate-stimulated GABA currents by Ro 15-1788

The concentration-dependent action of Ro 15-1788 on currents elicited by 10 μM GABA and 5 μM clorazepate is shown in Figure 8. Half-maximal inhibition of clorazepate (5 μM) stimulation was observed at about 8 nM Ro 15-1788. The inhibition of the current below the standard value of 100% chosen for the current response to 10 μM GABA is due to the direct inhibitory effect of Ro 15-1788 on the GABA response (see Fig. 4). It has previously been shown in binding studies that Ro 15-1788 competes with the classical benzodiazepines at a common binding site (Möhler and Richards, 1981). The data presented above suggest that binding of the modulatory drugs to this binding site directly relates to their electrophysiological effect. Therefore, the K_d for Ro 15-1788 may be estimated as $K_d = 0.6\ nM = IC_{50}/(1 + c/K_a)$, where c is the drug concentration of clorazepate used in this displacement experiment, K_a is the concentration of clorazepate that effects half-maximal stimulation (Fig. 4), and IC_{50} is the concentration of Ro 15-1788 that prevents 50% of the stimulation of the GABA response by clorazepate. The binding affinity for Ro 15-1788 is therefore higher than the K_a for diazepam or the K_i for DMCM. A similar conclusion has been drawn from binding studies of these drugs to the benzodiazepine binding site (Möhler and Richards, 1981; Braestrup et al., 1983).

Effect of modulators on desensitization

In Figure 1, it is clearly shown that even at 6°C there is considerable desensitization of the GABA current. Desensitization is more pronounced at higher concentrations of GABA (Figs. 1, 9*a*) and at a higher temperature (Fig. 6). We further investigated desensitization of the GABA current by applying different concentrations of GABA for 5 min in the presence or absence of modulators at 20°C. Figure 9*a* shows the result of such an experiment. The time-dependent decay of the current could be fitted with a single exponential function at GABA concentrations lower than 100 μM. At higher concentrations, the decay was characterized by double-exponential decay, with the time constants τ_f and τ_s for fast and slow desensitization. The follow-

Table 2. Effect of diazepam and DMCM on the GABA dose-response curve

Drugs applied	K_a (μM)	Hill coefficient
GABA	20.8 ± 2.6	1.69 ± 0.12
GABA + 0.1 μM diazepam	10.5 ± 1.0	1.72 ± 0.12
GABA + 0.3 μM DMCM	48.3 ± 2.2	1.36 ± 0.09

Three experiments using different oocytes were performed for each condition. The dose–response curves were fitted with the equation given in the text. Averages and standard deviations of the fitted values are given for each condition.

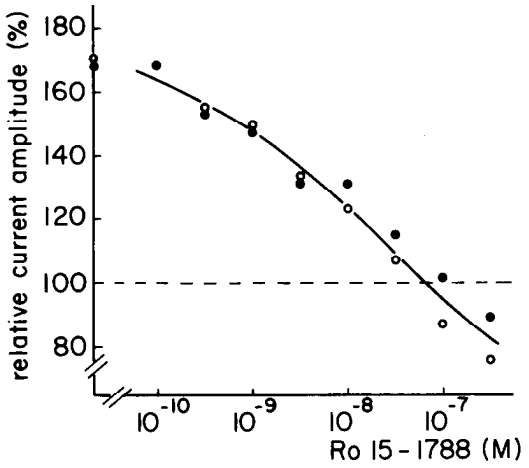

Figure 8. Effect of Ro 15-1788 on clorazepate-stimulated GABA currents. Varying concentrations of Ro 15-1788 were applied in combination with 10 μM GABA and 5 μM clorazepate. Peak current amplitudes were standardized as explained for Figure 4. The results of 2 experiments performed with different oocytes are shown.

ing limitations apply to our measurements. Owing to the slow decay of the current amplitude in the presence of GABA concentrations lower than 10 μM, no reliable evaluation could be made for a 5 min observation period. Another limitation was imposed by the application of GABA to the oocyte by perfusion. From the washout times of low GABA concentrations, it could be shown that the solution change was characterized by $\tau = 8$ sec. Any inactivation time constant near or below that value would be missed or biased. The GABA-concentration dependence of τ_f and τ_s is shown in Figure 9*b*. These results are qualitatively similar to data obtained by Akaike et al. (1986) for the GABA current in frog sensory neurons, but the absolute values are slower by a factor of about 10. Addition of 0.1 μM diazepam and 0.3 μM DMCM, respectively, had no significant effect on the desensitization time constants in the GABA concentration range between 20 and 250 μM (Fig. 9*b*). A time-independent component of the GABA current was also found in the presence or absence of modulatory drugs. This component saturated at low GABA concentration (<10 μM; Fig. 9*a*).

Discussion

It has previously been shown that the *Xenopus* oocyte represents a useful model for the description of the pharmacological effects of drugs on neuronal channels by measuring whole-cell currents (Parker et al., 1986) or single-channel currents (Methfessel et al., 1986; Sigel, 1987b). The environment provided by the *Xenopus* oocyte in terms of the phospholipid composition of the membrane and the regulatory state seems appropriate for such studies. Specifically, in this type of measurement the concentrations of GABA can be well controlled and there is no contribution from presynaptic uptake or release systems, or from possible endogenous benzodiazepine ligands. Furthermore, in contrast to most studies performed on neuronal cell cultures, the oocytes containing the neuronal ion channels may easily be investigated under voltage-clamp. An experimental system offering similar advantages, and, in addition, a much faster method for the change of agonist concentrations, has recently been described by Akaike et al. (1986).

We have investigated, in the oocyte system, the effect of different compounds that act at the benzodiazepine binding site. Control experiments showed that the GABA current in the oocytes displays properties reminiscent of vertebrate CNS GABA currents (Fig. 1) with respect to GABA-concentration dependence (Fig. 7) and sensitivity to picrotoxin (Fig. 2), and the reversal potential is similar to the chloride equilibrium potential (Fig. 5).

Effect of modulators on the GABA dose–response curve

The main action of these modulatory drugs is to shift the GABA-concentration response curve to the left or to the right, depending on their nature. The maximal GABA response was not affected. The Hill coefficient was not altered by diazepam, and was slightly decreased by DMCM. The convulsant β-carboline DMCM shifted the K_a from 21 to 48 μM, and the anticonvulsant benzodiazepine diazepam to 11 μM (Fig. 7). These findings should be compared with the results obtained by Choi et al. (1981), who reported a 2-fold decrease in the K_a upon exposure to the benzodiazepine chlordiazepoxide. These results were obtained using spinal cord neurons in culture and measuring conductance changes. Earlier studies with compounds of the β-carboline type (Skerrit and MacDonald, 1983) have indicated that DMCM reduces the GABA-induced depolarizations of the membrane

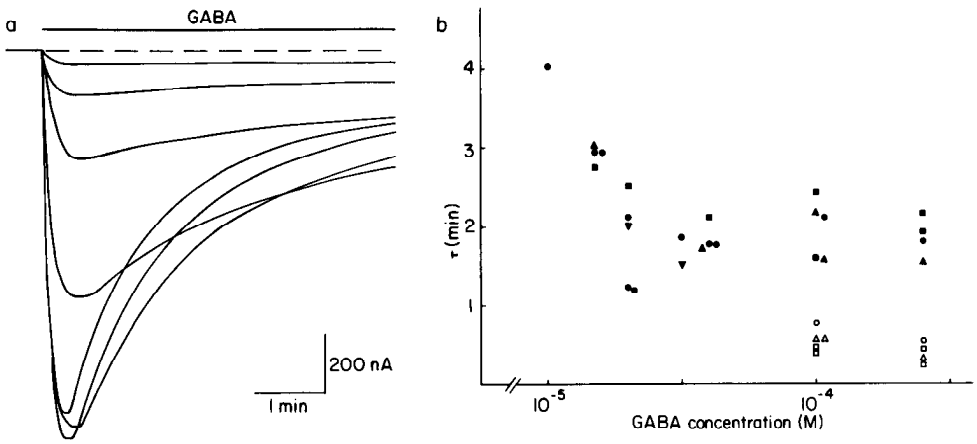

Figure 9. Effect of the modulators on the desensitization of the GABA current. *a*, Superimposed traces of GABA currents elicited by different concentrations of GABA on the same oocyte. *b*, GABA was applied for periods of 5–10 min in the absence or presence of modulatory drugs. The time course of the current decay was fitted with a single- (GABA < 100 μM) or a double-exponential function, characterized by the time constants τ_f (*open symbols*) and τ_s (*closed symbols*) for the fast and slow component, respectively. τ_f and τ_s are shown to be dependent on the GABA concentration. ●, ○, GABA; ■, □, GABA and 0.1 μM DMCM; ▲, △, GABA and 0.1 μM diazepam; ▼, GABA and 5 μM clorazepate.

of the same cell types mentioned above. In the same system, β-CCM and β-CCE did not alter the GABA response. Another group has shown that β-CCE antagonizes GABA action on hippocampal neurons (Polc et al., 1981). Skovgaard Jensen and Lambert (1986) have recently studied the effect of DMCM on cultured mouse CNS neurons. Under voltage-clamp conditions, they found that DMCM (10 μM) caused a 35% decrease of the GABA-induced current, with no change in the K_a. GABA was applied iontophoretically in those experiments. The discrepancies with our measurements can probably be explained by the inadequate control of the drug concentration, which cannot be avoided in studies with cultured neurons and iontophoretic application of drugs.

Concentration dependence of modulator action

We also studied the concentration dependence of the action of the modulatory drugs on the response to 10 μM GABA (Fig. 4). The stimulation by both clorazepate and diazepam was characterized by a Hill coefficient of about 1 (results not shown), indicating an interaction of the benzodiazepines with a single binding site. Similar observations have previously been made by Choi et al. (1981) using the benzodiazepine chlordiazepoxide. This group described channel activation with a Hill coefficient of 1.0 in the concentration range up to 0.1 mM chlordiazepoxide in primary cell cultures of embryonal chick spinal cord neurons. Using the same types of cells, an extensive study on a number of positive and negative modulators acting at the benzodiazepine binding site has recently been published (Chan and Farb, 1985). The experiments were carried out by measuring the GABA-induced membrane conductance change. Drugs were applied by pressure-ejection from pipettes. Our membrane current measurements under voltage-clamp are at least in qualitative agreement with this work, except that we found no effect of DMCM on GABA-induced I_{max}. A very detailed analysis has recently been done on the quantitative effects of diazepam on the GABA current in frog sensory neurons (Hattori et al., 1986) under carefully controlled conditions. This study also noted a decrease in the stimulatory properties of diazepam at higher concentrations, but both half-maximal and maximal stimulation were found at about 50-fold higher concentrations than we observed in the present work. Interestingly, we also found a biphasic behavior with all the negative modulators tested. If β-CCE, which acted as a weak negative modulator, was applied at concentrations above 1 μM, it became a marked stimulator. With the more potent negative modulators, the inhibitory effects were gradually lost if applied at concentrations higher than 1 μM, and even turned into stimulatory responses.

The reason for this biphasic action of positive and negative allosteric modulators is not known. A possible explanation is the presence on one protein complex of more than one binding site for benzodiazepines, each with a different affinity. Occupancy of more than one binding site by modulatory compounds may lead to the observed effects.

Effect of Ro 15-1788

Ro 15-1788 has been reported to bind with high affinity to the central benzodiazepine binding site and to antagonize a wide variety of benzodiazepine actions (Hunkeler et al., 1981). We report for the first time an inhibitory action of this drug on currents elicited by GABA. This inhibition was found reproducibly if Ro 15-1788 was applied at low concentrations (3–300 nM). At concentrations of Ro 15-1788 higher than 3 μM, a clear stimulation of the current response to 10 μM GABA was observed. A stimulation of the action of GABA on cervical sympathetic ganglia has previously also been observed, using high concentrations of Ro 15-1788 (Nutt et al., 1982). Weak stimulatory effects on GABA-induced responses have also been observed previously, with membrane potential changes in mouse spinal cord neurons (Skerritt and MacDonald, 1983) and with conductance changes in cultured cells from chick spinal cord (Chan and Farb, 1985). We conclude that, in spite of the wide use of the term "benzodiazepine antagonist" for the compound Ro 15-1788, this drug has, depending on the concentration, an inhibitory or an activating effect on GABA-induced chloride currents. This modulatory effect is, however, small and was never observed to affect the currents by more than 40% in the concentration range up to 30 μM. It is interesting that in the presence of the stimulatory compound clorazepate, relatively low concentrations of Ro 15-1788 cannot only reverse the stimulation, but also slightly inhibit the control response elicited by GABA (Fig. 8).

It should be stated in this context that clorazepate *in vivo* is rapidly converted to the more active compound N-desmethyldiazepam and probably does not correspond to the major active compound. Clorazepate was chosen here for its high water-solubility, which enabled rapid washout of the drug after application.

In conclusion, we have carried out an electrophysiological study on GABA-activated chloride channels expressed in *Xenopus* oocytes after injection with chick brain mRNA. Using this preparation, the currents can be studied quantitatively under voltage-clamp conditions. Drug concentrations can be well controlled by bath-perfusion. Presynaptic release of GABA or other regulatory substances can be excluded. In this system we have determined the effects on GABA currents of allosteric modulatory drugs that are known to act at the benzodiazepine binding site. The only effect of both positive and negative modulators was a shift in the K_a for GABA. The GABA–benzodiazepine receptor–ion channel complex expressed in *Xenopus* oocytes may represent a convenient assay system for the assessment of new drugs that interact with this protein complex. Furthermore, this system of expression, in combination with *in vitro* mutagenesis of the cloned DNA coding for the channel complex, will allow, in the future, the investigation of benzodiazepine action at a molecular level.

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