0026-895X/95/061189-08$3.00/0

MOLECULAR PHARMACOLOGY, 47:1189–1196 (1995).

Modulation of the γ-Aminobutyric Acid Type A Receptor by the Antiepileptic Drugs Carbamazepine and Phenytoin

PATRICK GRANGER, BRUNO BITON, CECILE FAURE, XAVIER VIGE, HENRI DEPOORTERE, DAVID GRAHAM, SALOMON Z. LANGER, BERNARD SCATTON, and PATRICK AVENET

Synthélabo Recherche, Preclinical Research Department, 92220 Bagneux, France

Received October 13, 1994; Accepted March 23, 1995

SUMMARY

We report here that carbamazepine and phenytoin, two widely used antiepileptic drugs, potentiate γ-aminobutyric acid (GABA)-induced Cl^- currents in human embryonic kidney cells transiently expressing the α1β2γ2 subtype of the $GABA_A$ receptor and in cultured rat cortical neurons. In cortical neuron recordings, the current induced by 1 μM GABA was enhanced by carbamazepine and phenytoin with EC_{50} values of 24.5 nM and 19.6 nM and maximal potentiations of 45.6% and 90%, respectively. The potentiation by these compounds was dependent upon the concentration of GABA, suggesting an allosteric modulation of the receptor, but was not antagonized by the benzodiazepine (ω) modulatory site antagonist flumazenil. Carbamazepine and phenytoin did not modify GABA-induced currents in human embryonic kidney cells transiently expressing binary α1β2 recombinant $GABA_A$ receptors. The α1β2 recombinant is known to possess functional barbiturate, steroid, and picrotoxin sites, indicating that these sites are not involved in the modulatory effects of carbamazepine and phenytoin. When tested in cells containing recombinant α1β2γ2, α3β2γ2, or α5β2γ2 $GABA_A$ receptors, carbamazepine and phenytoin potentiated the GABA-induced current only in those cells expressing the α1β2γ2 receptor subtype. This indicates that the nature of the α subunit isoform plays a critical role in determining the carbamazepine/phenytoin pharmacophore. Our results therefore illustrate the existence of one or more new allosteric regulatory sites for carbamazepine and phenytoin on the $GABA_A$ receptor. These sites could be implicated in the known anticonvulsant properties of these drugs and thus may offer new targets in the search for novel antiepileptic drugs.

Antiepileptic drugs or drugs showing anticonvulsant properties in animal models have been suggested to act through various mechanisms (1, 2). Carbamazepine, phenytoin, valproate, lamotrigine, and flunarizine have been shown to inhibit voltage-activated Na^+ channels, thereby reducing the firing of depolarized neurons (3–8). Also, T-type Ca^{2+} channels implicated in rhythmic neuronal firing have been reported to be antagonized by phenytoin, ethosuximide, and flunarizine (2). In addition, carbamazepine has recently been shown to block the *N*-methyl-D-aspartate receptor, albeit at high concentrations (9).

Compounds that produce membrane hyperpolarization, such as those that potentiate chloride fluxes through the $GABA_A$ receptor, have long been known to possess anticonvulsant properties. This is the case for pentobarbital and the BZs, which act at distinct modulatory sites on the $GABA_A$ receptor. Recently, another anticonvulsant, loreclezole, has been shown to potentiate GABA-induced chloride fluxes via an interaction with a novel binding site on the $GABA_A$ receptor complex (10). Such data reinforce the idea that the $GABA_A$ receptor may also be considered a major target for antiepileptic drugs.

The present study was designed to determine whether the two most widely used antiepileptic drugs, carbamazepine and phenytoin, could modulate $GABA_A$ receptor function. Phenytoin has been shown in some, but not all, neuronal preparations to enhance GABA-ergic neurotransmission (11–13). This discrepancy in the effects of phenytoin observed in different neuronal preparations may well be explained by $GABA_A$ receptor heterogeneity.

The $GABA_A$ receptor is an ionotropic receptor, probably pentameric, formed by the combination of α, β, γ, δ, and ρ subunits (14). Several isoforms of these subunits, numbered α1–6, β1–3, δ, γ1–3, and ρ1 and -2, have been cloned and have been shown to exhibit different localization patterns in the brain (15). Different combinations of these isoforms produce multiple subtypes of $GABA_A$ receptors with defined pharmacological properties, as shown in heterologous expression experiments (16, 17).

A main characteristic of the $GABA_A$ receptor is the presence on this macromolecular complex of several allosteric modulatory sites that regulate GABA affinity (18). These modulatory sites include the BZ site (which recognizes BZs, imidazopyridines, and cyclopyrrolones and is antagonized by

ABBREVIATIONS: GABA, γ-aminobutyric acid; BZ, benzodiazepine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HEK, human embryonic kidney; DMSO, dimethylsulfoxide.

flumazenil) as well as distinct binding sites for barbiturates, picrotoxin, and steroids. In addition, several other sites have been discovered, i.e., the peripheral BZ binding site for ligands such as Ro5–4864 (4′-chlorodiazepam) and PK 11195 (19–21), the anticonvulsant loreclezole binding site (10), and sites for the general anesthetic propofol (22) as well as some pyrazinone derivatives (23). Studies using recombinant $GABA_A$ receptors have shown that some of these sites, such as the BZ site or the binding site for Ro5–4864, are present only if ternary $\alpha\beta\gamma$ subunits are coexpressed (19). In contrast, functional picrotoxin, barbiturate, steroid, or loreclezole binding sites can be formed using binary receptors composed of α and β subunits (10, 19, 24, 25).

In this study, we demonstrate that carbamazepine and phenytoin positively modulate $GABA_A$ receptors present in cultured rat cortical neurons. To further characterize the site(s) involved in this potentiation, we also used different recombinant receptors transiently expressed in HEK 293 cells. Carbamazepine and phenytoin potentiated the GABA-induced current only with the $\alpha1\beta2\gamma2$ subunit combination. Some of these results have been presented elsewhere, in abstract form (26).

Materials and Methods

Cultured neurons. Primary cultures of neonatal rat cortical neurons were prepared from 1-day-old pups according to the method of Vigé *et al.* (27). Briefly, slices of cerebral tissue were trypsinized and the cells were dissociated by gentle trituration. Cells were resuspended in basal Eagle's culture medium containing 10% fetal bovine serum, 25 mM KCl, 2 mM glutamine, and 100 μg/ml gentamicin, then seeded on poly-D-lysine-coated glass coverslips (0.25×10^6 cells/coverslip), and placed in 12-well Corning dishes. Cells were incubated at 37° in a humidified atmosphere containing 5% CO_2/95% air. Cytosine β-D-arabinofuranoside (1 μM) was added the day after seeding, to prevent the replication of non-neuronal cells. The coverslips were transferred to the experimental chambers for patch-clamp experiments after 7–10 days in culture.

Transfection of HEK 293 cells. cDNAs encoding the $\alpha1$, $\alpha3$, $\alpha5$, $\beta2$, and $\gamma2S$ subunits of the rat $GABA_A$ receptor were supplied by P. H. Seeburg (University of Heidelberg). These cDNAs had been subcloned into pCDM8 expression vectors (Invitrogen, San Diego, CA) using standard recombinant DNA procedures.

The transfection procedure followed that described by Faure-Halley *et al.* (17). Briefly, each plasmid was purified by ion exchange chromatography (Qiagen) and the DNAs were combined as either $\alpha1\beta2\gamma2$, $\alpha3\beta2\gamma2$, $\alpha5\beta2\gamma2$, or $\alpha1\beta2$ to transfect exponentially growing, adenovirus-transformed HEK 293 cells. Cells were harvested 48 hr after the addition of DNA, removed from the culture dish, and allowed to settle in the recording chamber for patch-clamp experiments.

Electrophysiology. The chambers containing the cell preparation (volume, 500 μl) were placed on the stage of an inverted microscope (Olympus IMT2) equipped with Hoffman optics (Modulation Contrast, Greenvale, NY) and were viewed at a total magnification of 400×. Cells were continuously superfused (flow rate, 3–5 ml/min) with the standard extracellular solution (see below for composition) via polyethylene tubing (500-μm internal diameter), which was brought to within 3 mm of the cell under investigation. The tubing was connected to a gravity-feed solution distributor (internal volume, 15 μl) receiving eight separate lines, thus allowing constant-flow rate solution exchange. The use, in some cases, of two separate distributors with separate outlets for control and test solutions gave identical results.

We used the whole-cell configuration of the patch-clamp technique. Pipettes were pulled from thick-walled, borosilicate glass capillaries (Phymep, Paris, France) on a two-stage puller and had resistances of 5–10 MΩ when filled with the pipette solution. Pipettes were brought to the cells with a three-dimensional hydraulic micromanipulator (WR89; Narishige). Whole-cell currents were recorded with an Axopatch 1D amplifier (Axon Instruments) connected to a 386 DX personal computer driven by pCLAMP software (Axon Instruments). In plated cultured neurons under whole-cell, voltage-clamp recording conditions, large GABA-induced chloride currents can affect the chloride concentration in unstirred layers and possibly in the intracellular space of thin neurites, giving rise to apparent desensitization. In addition, after large GABA-induced currents, currents in the opposite direction can sometimes be observed, probably due to Cl^- ion redistribution. These phenomena can occur even if low (1 μM) concentrations of GABA are applied. To minimize these chloride fluxes, we used a −20-mV holding voltage in most experiments. Whole-cell currents were recorded on videotape, through a digital audio processor (Vetter, Rebersburg, PA), for off-line analysis. Values were expressed as means ± standard errors of the number (n) of cells tested.

Solutions and reagents. The standard extracellular solution (pH 7.4) contained 147 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES/Tris-OH. The standard pipette solution (pH 7.2) contained 140 mM CsCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM HEPES/Tris-OH, and 4 mM Na_2ATP. The free Ca^{2+} concentration was 10 nM.

The following chemicals were used: carbamazepine, phenytoin, and picrotoxin (Sigma); pentobarbital (Sanofi); diazepam and flumazenil (Hoffmann LaRoche); and zolpidem (Synthelabo). These compounds were diluted in DMSO to a final concentration of 0.08% DMSO. Control solutions contained the same final DMSO concentration.

Results

Effects of carbamazepine and phenytoin on $GABA_A$ receptors in cultured cortical neurons. Carbamazepine or phenytoin at concentrations up to 10 μM did not affect the holding current of cortical neurons voltage-clamped at −20 mV, whereas 1 μM GABA typically induced inward currents ranging from 10 to 400 pA (Fig. 1A). In contrast, when GABA was applied in the presence of 10 μM carbamazepine or 10 μM phenytoin, the GABA-induced current was reversibly increased. The amplitudes of the potentiation measured with 10 μM carbamazepine ranged from 14 to 119% (mean, 45.6 ± 6.3%; n = 17) and with 10 μM phenytoin from 20 to 100% (mean, 50.8 ± 7.6%; n = 11). Although highly variable in amplitude, these effects could be measured in every neuron tested. However, compared with the effects of other compounds that positively modulate the $GABA_A$ receptor, the amplitudes of the potentiation obtained with carbamazepine and phenytoin were always smaller. This is illustrated in Fig. 1B, where, using the same neuron, the modulatory effects of carbamazepine and phenytoin were compared with those obtained using a similar concentration of the BZ site agonist zolpidem or the barbiturate site agonist pentobarbital (see also Fig. 7B).

The concentration-response relationships shown in Fig. 2 indicate similar affinities of 24.5 and 19.6 nM for carbamazepine and phenytoin, respectively, but a higher maximal effect for phenytoin (90%), compared with carbamazepine (50%). In the case of phenytoin, the maximal potentiation obtained with this compound was decreased at concentrations above 1 μM (Fig. 2C). The Hill coefficients of the concentration-dependence curves were 0.42 and 0.52 for carbamazepine and phenytoin, respectively.

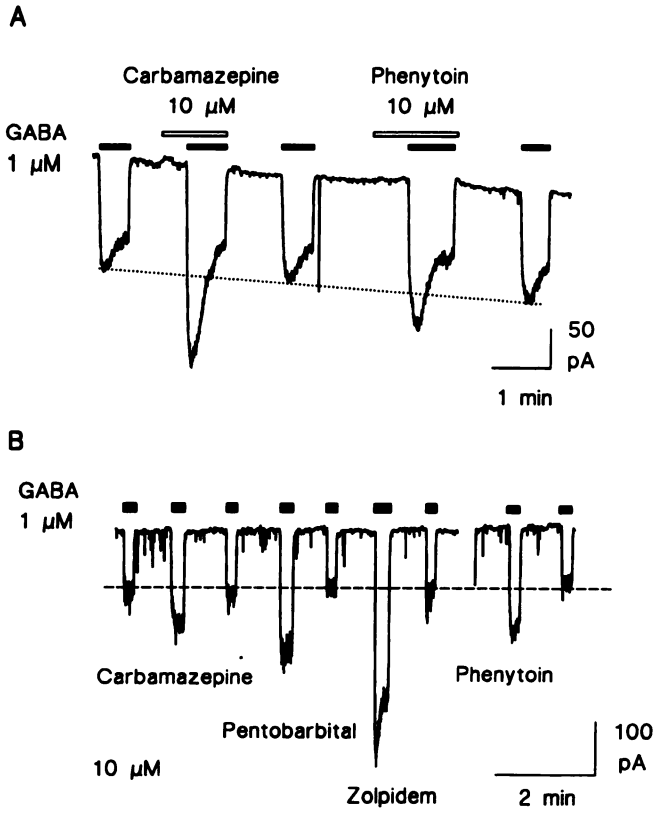

Fig. 1. Potentiation by carbamazepine and phenytoin of GABA-induced whole-cell currents in cultured rat cortical neurons. In A and B the whole-cell currents were recorded at a −20-mV holding potential. A, Experiment showing that exposure of the neuron to carbamazepine or phenytoin (*white bars*) did not affect the holding current, whereas the response to 1 μM GABA (*black bars*) was enhanced in the presence of the compounds. B, Comparison of the potentiation of the GABA-induced current by carbamazepine and phenytoin with that by pentobarbital and zolpidem, in the same neuron.

The similarity between the parameters for the potentiation produced by the two anticonvulsants and those observed with BZ site agonists led us to test the effect of the BZ site antagonist flumazenil on carbamazepine and phenytoin responses. Flumazenil (10 μM) reduced the potentiation obtained with 10 μM zolpidem by 91.7 ± 3.2% (n = 7). In the presence of 10 μM flumazenil the potentiation by 10 μM carabamazepine and phenytoin averaged 47 ± 4.9% (n = 6) and 37 ± 5% (n = 6), respectively, values that were not significantly different from those observed in the absence of flumazenil (see above and Fig. 3A). Furthermore, potentiations by lower concentrations of carbamazepine (1 μM) and phenytoin (0.1 μM) were still observed in the presence of 10 μM flumazenil (Fig. 3, B and C, respectively).

The modulatory action of BZ site agonists is known to produce a leftward shift of the GABA concentration-dependence curve. We examined whether a similar allosteric mechanism might be involved in the potentiations produced by carbamazepine and phenytoin, by studying the effects of these compounds on chloride currents induced by various concentrations of GABA in the presence of 10 μM flumazenil. Fig. 4 shows that the potentiations of the chloride current produced by both compounds are strongly dependent on the GABA concentration. Each concentration-response relationship could be reasonably fitted with an equation derived from

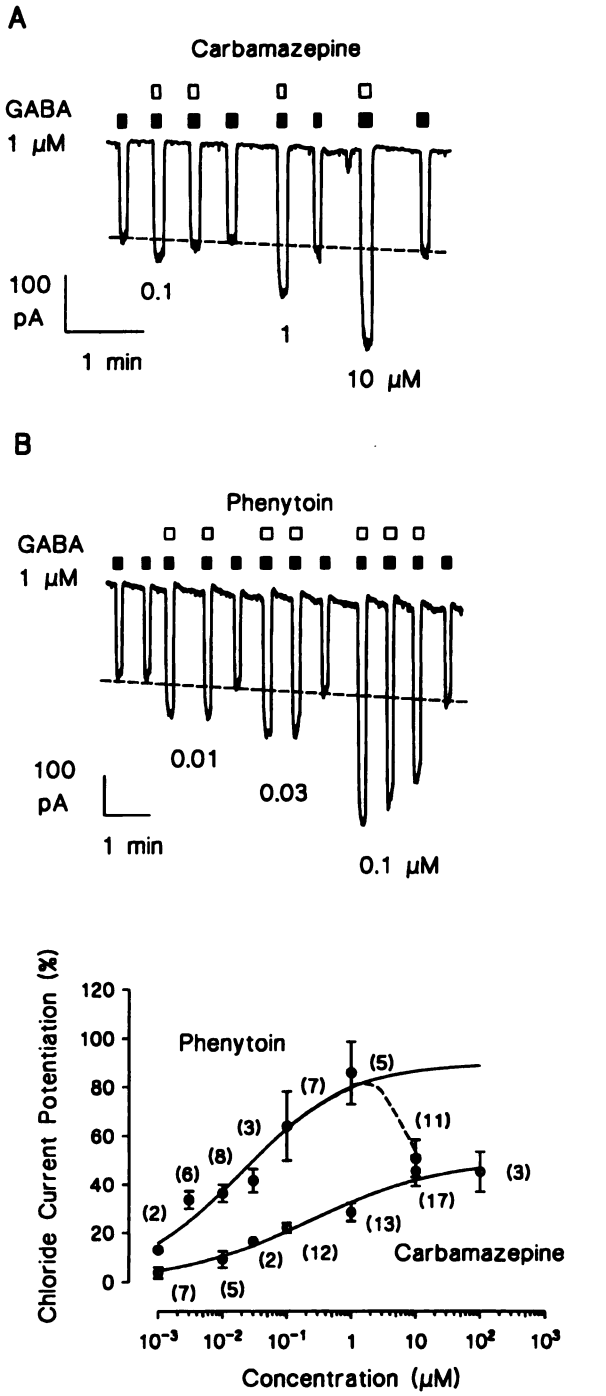

Fig. 2. Concentration dependence of the potentiation by carbamazepine and phenytoin of the GABA-induced whole-cell current in cultured rat cortical neurons. A and B, Examples of recordings used to construct the concentration-response curve. *Black bars*, exposure of the neuron to GABA 1 μM; *white bars*, exposure of the neuron to carbamazepine or phenytoin. All neurons were voltage-clamped at −20 mV. The current potentiation was measured as the increase of the current with respect to the control GABA response preceding and following the response in the presence of the compounds. C, Current potentiations averaged from the number of neurons indicated in parentheses. *Continuous lines*, data fitted with the equation $(P_{max} \cdot C^n)/(C^n + EC_{50}^n)$, with P_{max} being the maximal potentiation, C the compound concentration, and n the Hill coefficient. In the case of phenytoin, the data obtained at 10 μM were not used for the fit. Carbamazepine and phenytoin gave EC_{50} values of 24.5 nM and 19.6 nM and P_{max} values of 50.8% and 90%, respectively.

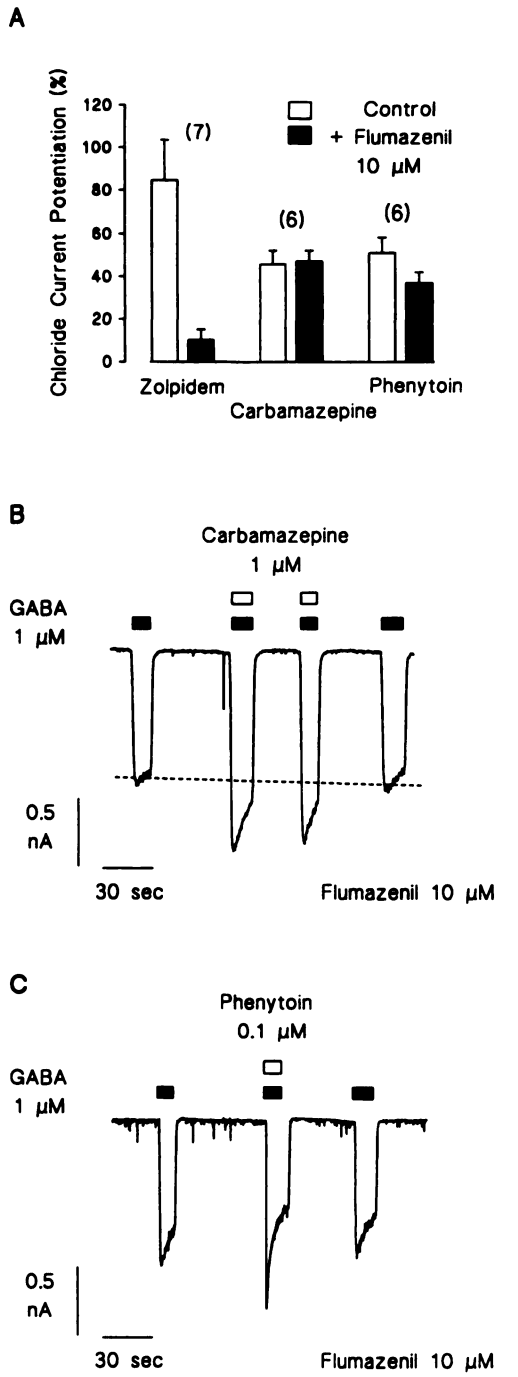

Fig. 3. Lack of flumazenil antagonism of the carbamazepine- and phenytoin-induced current potentiation in cultured rat neurons. A, The potentiation by zolpidem, carbamazepine, and phenytoin of the current induced by 1 μM GABA was measured in the absence (control) or presence of 10 μM flumazenil. Note that only the potentiation induced by zolpidem was reduced in the presence of flumazenil. B and C, In the presence of 10 μM flumazenil, currents induced by 1 μM GABA (*black bars*) were potentiated by carbamazepine (B) or phenytoin (C) (*white bars*) applied conjointly with GABA. In A, B, and C, the whole-cell voltage was clamped at −20 mV.

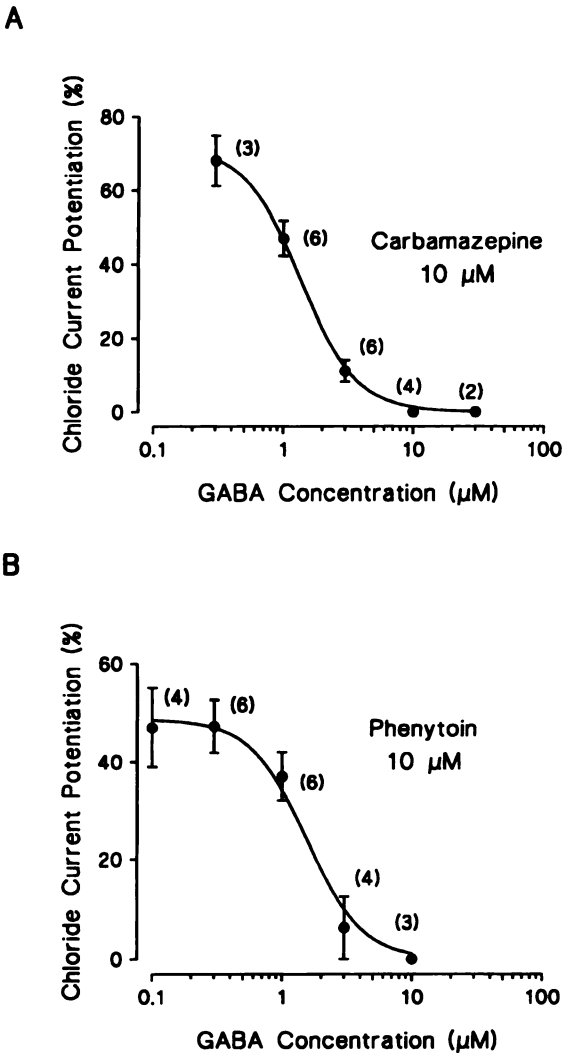

Fig. 4. GABA concentration dependence of carbamazepine (A) and phenytoin (B) potentiation of GABA-induced current in cultured rat neurons. Potentiations of the current induced by different GABA concentrations were measured in the presence of 10 μM flumazenil, at a holding voltage of −20 mV. Data were fitted (*continuous line*) using the equation $P\% = 100\ [(C^n + EC_{50}^n)/(C^n + EC_{50}'^n) - 1]$, where $P\%$ is percentage increase of the GABA-induced current, C the GABA concentration, n the Hill coefficient of the GABA concentration-dependence relationship, EC_{50} its value under control conditions, and EC'_{50} the shifted value in the presence of carabamazepine or phenytoin. Reasonable fits were obtained by using a constant Hill coefficient value of 2 for all GABA concentration-response relationships.

a model based on a leftward shift of the EC_{50} for GABA. In two series of experiments, EC_{50} values for GABA were shifted from 1.76 to 1.34 μM (Fig. 4A) and from 1.85 to 1.52 μM (Fig. 4B) upon the application of 10 μM carbamazepine and phenytoin, respectively.

The potentiations of GABA-induced current by carbamazepine and phenytoin were independent of the holding voltage, as shown in Fig. 5A, where the cell was maintained at a positive voltage of +20 mV. As shown in the voltage ramps of Fig. 5, B and C, the reversal potential of the current in the presence of carbamazepine or phenytoin was identical to that obtained with GABA alone.

Effects of carbamazepine and phenytoin on $GABA_A$ receptors transiently expressed in HEK 293 cells. Fig. 6 compares the effects of diazepam, pentobarbital, phenytoin, and carbamazepine in HEK 293 cells transfected with the $\alpha1\beta2\gamma2$ (Fig. 6, A–C) and $\alpha1\beta2$ (Fig. 6, D–G) combinations of subunits. As expected, diazepam potentiated GABA-induced currents in cells transfected with the $\alpha1\beta2\gamma2$ combination (Fig. 6A) but not in those transfected with the $\alpha1\beta2$ combi-

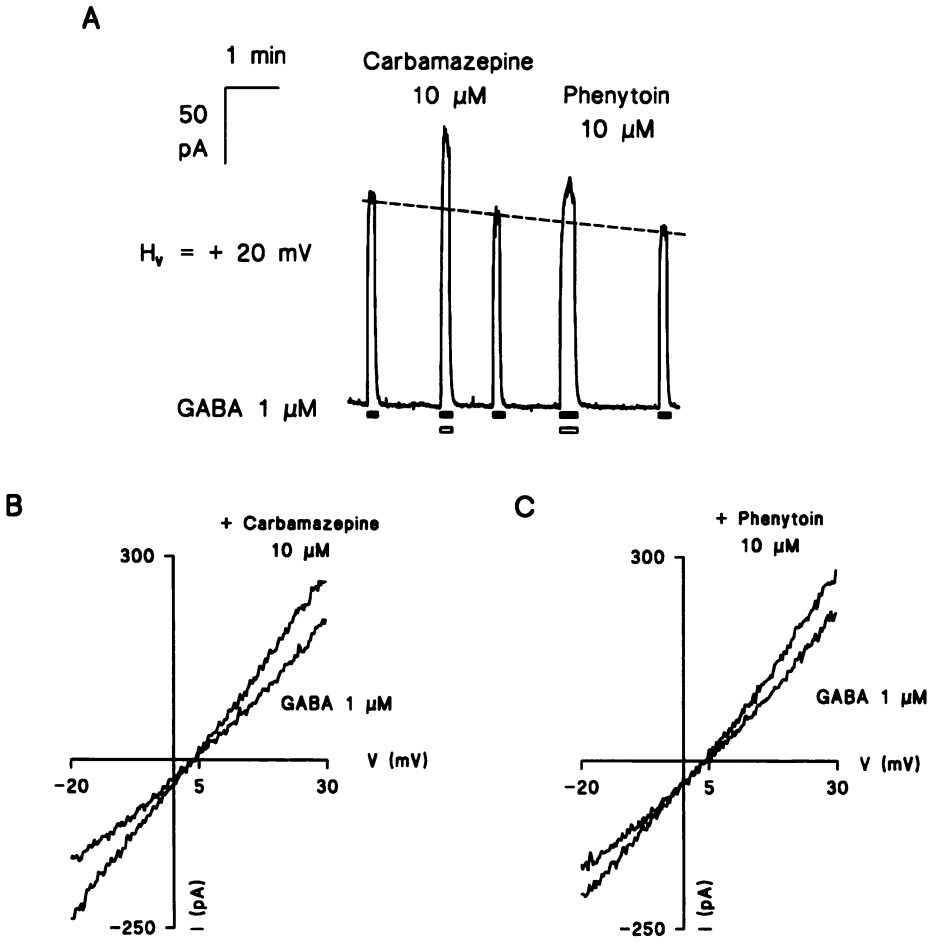

Fig. 5. Voltage dependence of the potentiation by carbamazepine and phenytoin of the GABA-induced current in cultured rat cortical neurons. A, Example of recording of the potentiation by carbamazepine and phenytoin of the whole-cell current of a neuron voltage-clamped at +20 mV. *Black bars*, exposure of the neuron to GABA 1 μM; *white bars*, exposure of the neuron to carbamasepine or phenytoin. B and C, Whole-cell current responses to a voltage ramp from −20 mV to +30 mV produced in the presence of 1 μM GABA, in the presence or absence of 10 μM carbamazepine (A) or 10 μM phenytoin (C). Note that the reversal potentials are identical for the currents induced by GABA alone and those induced by GABA in the presence of carbamazepine or phenytoin.

nation (Fig. 6D), whereas pentobarbital was effective in both preparations (Fig. 6, B and E). Picrotoxin inhibited the GABA-induced Cl^- current with both $\alpha1\beta2\gamma2$ (data not shown) and $\alpha1\beta2$ (Fig. 6E) receptor combinations. Phenytoin and carbamazepine both potentiated the GABA-induced current in $\alpha1\beta2\gamma2$-transfected cells. In contrast, no effect on the GABA-induced response was detected in the $\alpha1\beta2$-transfected cells, indicating that coexpression of the γ subunit is required for the potentiation by carbamazepine and phenytoin of GABA-induced Cl^- current.

In contrast to what we observed with $\alpha1\beta2\gamma2$ receptors, carbamazepine and phenytoin did not potentiate GABA-induced currents with $\alpha3\beta2\gamma2$ or $\alpha5\beta2\gamma2$ receptor combinations (Fig. 7). As expected, diazepam and pentobarbital potentiated the GABA-induced currents in these latter two preparations. Fig. 7B summarizes the potentiation of the GABA-induced current we obtained with the different receptor combinations, in comparison with that observed in cultured cortical neurons. From these results, it appears that carbamazepine and phenytoin are effective only with GABA$_A$ receptors formed by the $\alpha1\beta2\gamma2$ subunits and with those present in cultured neurons.

Discussion

Phenytoin and carbamazepine are structurally different compounds but have similar spectra of anticonvulsant activities in animal seizure models. Furthermore, these compounds exhibit a similar therapeutic efficacy in partial and generalized tonic-clonic seizures (2). One common site of action for carbamazepine and phenytoin is the voltage-operated Na^+ channel, which is voltage-dependently antagonized by these drugs. The results of the present study show that carbamazepine and phenytoin also potentiate the GABA-induced Cl^- current of one particular subtype of GABA$_A$ receptors. A modulatory effect of phenytoin on GABA responses was previously demonstrated by Aickin *et al.* (11) with crayfish stretch receptors. In that study, phenytoin, at concentrations ranging from 1 nM to 100 μM, enhanced spontaneous inhibitory postsynaptic potentials as well as responses produced by iontophoretic application of GABA or muscimol. Phenytoin at 40 μM (but not at lower concentrations) also potentiated the postsynaptic response produced by iontophoretically applied GABA in mouse spinal cord neurons in cell culture (28) and in some cells of the rat cuneate nucleus (29). An enhancement of GABA-ergic transmission by phenytoin was not confirmed, however, in mammalian preparations such as the rat hippocampus (12) or rat sensory ganglion cells (13). This variability in the effects of phenytoin was attributed to a possible diversity of GABA$_A$ receptors (28). The heterogeneity of GABA$_A$ receptors has since been largely confirmed, and the fact that carbamazepine and phenytoin, in our experiments, were without effect on certain subtypes of recombinant receptors may explain the lack of effect observed by some authors with certain neuronal preparations. Although corresponding data dealing with carbamazepine are not available, our study suggests a similar action of this compound on GABA-ergic neurotransmission.

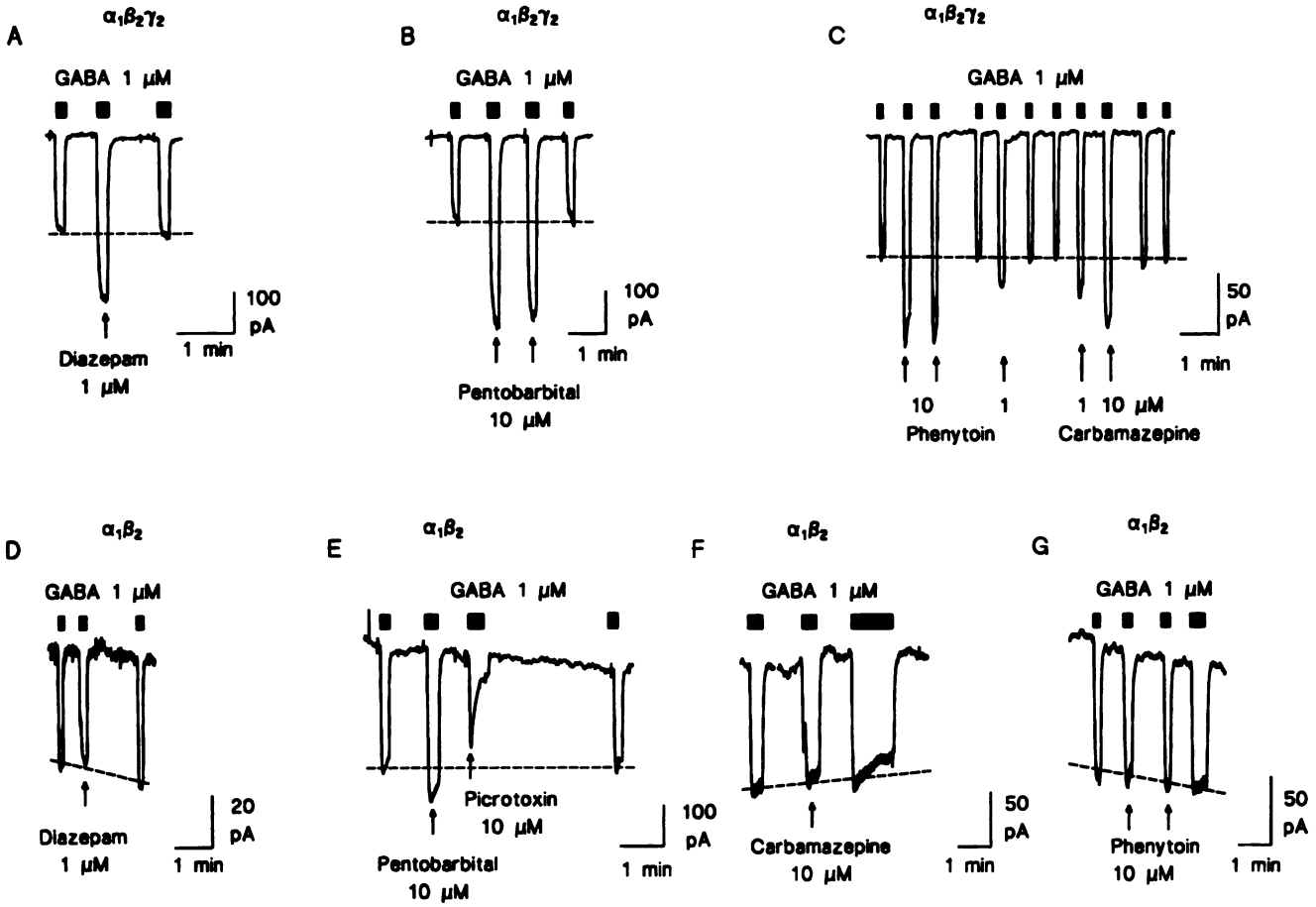

Fig. 6. Potentiation by carbamazepine and phenytoin of the GABA-induced whole-cell current in HEK 293 cells expressing $\alpha1\beta2\gamma2$ (A-C) or $\alpha1\beta2$ (D-G) $GABA_A$ receptor subunit combinations. Cells were voltage-clamped at −20 mV. In the $\alpha1\beta2\gamma2$ receptor combination, diazepam, pentobarbital, phenytoin, and carbamazepine potentiated the current induced by 1 μM GABA (*black bars*). With the $\alpha1\beta2$ receptor assembly only pentobarbital and picrotoxin were effective.

The potentiations we observed with carbamazepine and phenytoin are of smaller amplitude than those that are obtained with full, positive, allosteric, BZ modulators or with compounds acting at the barbiturate site. The potentiations displayed by carbamazepine and phenytoin are, however, comparable to those obtained with partial BZ agonists and, as such, are likely to affect GABA-ergic neurotransmission. Furthermore, the potentiations occur at concentrations in the namomolar range, far below the cerebral concentrations of 5–10 μM measured for carbamazepine and phenytoin at therapeutic doses and far below those needed to antagonize voltage-operated Na^+ channels. The potentiation of GABA-ergic neurotransmission by these drugs would stabilize the postsynaptic membrane potential at hyperpolarized values, thereby preventing the spread of epileptic discharges throughout the brain.

The mechanism by which GABA-induced currents are potentiated by carbamazepine and phenytoin resembles that of allosteric modulators such as BZs, loreclezole, and the recently described pyrazinone derivatives U-92813 and U-94863, in that the potentiation is GABA concentration dependent and, therefore, likely involves an increase in the affinity of GABA (10, 23). However, this potentiation does not occur through the BZ site, because it is not blocked by flumazenil, a BZ antagonist.

In an attempt to further characterize the sites on the $GABA_A$ receptor involved in the potentiation of the GABA-induced Cl^- current by carbamazepine and phenytoin, we studied the effects of these two compounds on various receptor subunit combinations. Heterologous expression of $GABA_A$ receptors has shown that, among the different modulatory sites, the barbiturate, picrotoxin, steroid, and loreclezole sites, as well as the site involved in the potentiation of GABA responses by pyrazinone derivatives, remain functional in binary receptor combinations composed of α and β subunits (10, 16, 23, 25, 30). In contrast, we show in this study that expression of the γ subunit is an additional requirement for the potentiation of GABA responses by carbamazepine and phenytoin. A similar requirement for the γ subunit has been described for the proconvulsant compound Ro5–4864, which inhibits GABA-induced currents via a flumazenil-insensitive binding site in cortical neurons (19).

The recombinant receptor study led us to conclude that carbamazepine and phenytoin act at sites on the $GABA_A$ receptor that are different from those described previously. In the absence of a selective antagonist, we are unable to conclude whether both compounds act at the same recognition site. However, the similar recombinant receptor selectivity patterns obtained with these compounds suggest that these two compounds probably interact with a common recognition site. The affinities of 24 and 19 nM obtained for carbamazepine and phenytoin, respectively, were similar,

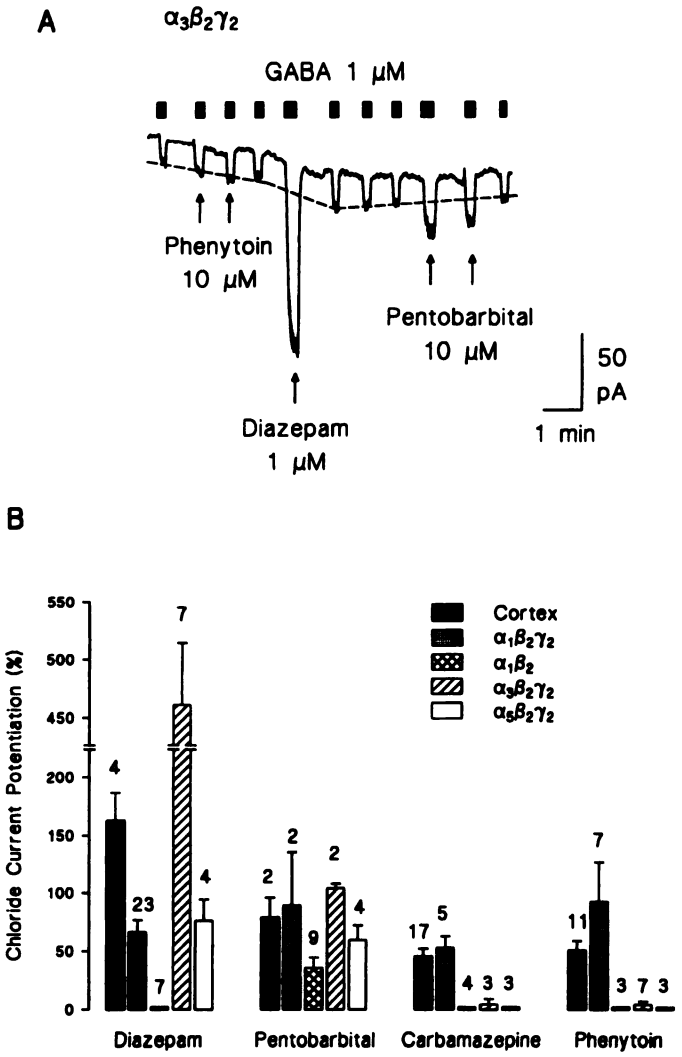

Fig. 7. Comparison of potentiation amplitudes produced by diazepam, pentobarbital, phenytoin, and carbamazepine on GABA-induced currents of recombinant GABA$_A$ receptors and of GABA$_A$ receptors of cultured neurons. A, Example of recording of the GABA-induced current of a HEK 293 cell expressing the $\alpha 3\beta 2\gamma 2$ receptor subtype, voltage-clamped at −20 mV. Note that phenytoin had no effect on this receptor combination. B, Mean potentiation obtained with different receptor subtypes, using the protocol shown in A. *Numbers above the bars*, number of cells studied.

but phenytoin exhibited a 2-fold greater intrinsic activity than did carbamazepine. Therefore, if the hypothesis of a single site for both compounds is correct, then one would also have to invoke the notion of partial agonism at this novel site, in analogy to that described for the BZ site.

Our study was restricted to a few GABA$_A$ receptor subtypes. It remains to be determined whether the apparent selectivity of carbamazepine and phenytoin for the $\alpha 1$-containing receptor subtype would be maintained in comparisons with $\alpha 2$- and $\alpha 6$-containing receptors. Because all cortical neurons responded to some extent to carbamazepine and phenytoin, the possibility exists that other receptor subtypes, containing other α isoforms or combinations of different α isoforms, are sensitive to the two anticonvulsants. The large variability in potentiation amplitude may also indicate that a mixture of receptors, which are sensitive or insensitive to carbamazepine and phenytoin, are expressed in cultured neurons. Further studies with more subunit combinations are also needed to investigate the influence of different γ and β subunit isoforms.

In conclusion, our present results suggest that carbamazepine and phenytoin potentiate GABA-induced current through a novel site on the GABA$_A$ receptor that is different from previously described binding domains on this heterooligomeric protein. In addition to their previously described properties as voltage-dependent Na^+ channel blockers, the interaction of these compounds with a certain subpopulation of GABA$_A$ receptors is likely to contribute to their antiepileptic activity.

Acknowledgments

We thank Dr. J. Benavides for helpful comments on the manuscript, Dr. G. Perrault for discussion on antiepileptic and anticonvulsant drugs, M. Vasseur for excellent technical assistance, and M. Gilles for construction of equipment and electronics.

References

1. Catterall, W. A. Common modes of drug action on Na^+ channels: local anesthetics, antiarrhythmics and anticonvulsants. *Trends Pharmacol. Sci.* **8**:57–65 (1987).
2. Rogawski, M. A., and R. J. Porter. Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacol. Rev.* **42**:223–286 (1990).
3. Willow, M., T. Gonoi, and W. A. Catterall. Voltage clamp analysis of the inhibitory actions of diphenylhydantoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. *Mol. Pharmacol.* **27**: 549–558 (1985).
4. Ragsdale, D. S., T. Scheuer, and W. A. Catterall. Frequency and voltage-dependent inhibition of type IIA Na^+ channels, expressed in a mammalian cell line, by local anesthetic, antiarrhythmic, and anticonvulsant drugs. *Mol. Pharmacol.* **40**:756–765 (1991).
5. McLean, M. J., and R. L. MacDonald. Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. *J. Pharmacol. Exp. Ther.* **237**:1001–1011 (1986).
6. Cheung, H., D. Kamp, and E. Harris. An *in vitro* investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. *Epilepsy Res.* **13**:107–112 (1992).
7. Lees, G., and M. J. Leach. Studies on the mechanism of action of the novel anticonvulsant lamotrigine (Lamictal) using primary neuroglial cultures from rat cortex. *Brain Res.* **612**:190–199 (1993).
8. Pauwels, P. J., J. E. Leysen, and P. A. J. Janssen. Ca and Na channels involved in neuronal cell death: protection by flunarizine. *Life Sci.* **48**: 1881–1893 (1991).
9. Lancaster, J. M., and J. A. Davies. Carbamazepine inhibits NMDA-induced depolarizations in cortical wedges prepared from DBA/2 mice. *Experientia (Basel)* **48**:751–753 (1992).
10. Wafford, K. A., C. J. Bain, K. Quirk, R. M. McKernan, P. B. Wingrove, P. J. Whiting, and J. A. Kemp. A novel allosteric modulatory site on the GABA$_A$ receptor β subunit. *Neuron* **12**:775–782 (1994).
11. Aickin, C. C., R. A. Deisz, and H. D. Lux. On the action of the anticonvulsant 5,5-diphenylhydantoin and the convulsant picrotoxin in crayfish stretch receptor. *J. Physiol. (Lond.)* **315**:157–173 (1981).
12. Hershkowitz, N., and G. F. Ayala. Effects of phenytoin on pyramidal neurons of the rat hippocampus. *Brain Res.* **208**:487–492 (1981).
13. Connors, B. W. A comparison of the effects of pentobarbital and diphenylhydantoin on the GABA sensitivity and excitability of adult sensory ganglion cells. *Brain Res.* **207**:357–369 (1981).
14. DeLorey, T. M., and R. W. Olsen. γ-Aminobutyric acid$_A$ receptor structure and function. *J. Biol. Chem.* **267**:16747–16750 (1992).
15. Wisden, W., D. J. Laurie, H. Monyer, and P. H. Seeburg. The distribution of 13 GABA$_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J. Neurosci.* **12**:1040–1062 (1992).
16. Pritchett, D. B., H. Lüddens, and P. H. Seeburg. Type I and type II GABA$_A$-benzodiazepine receptors produced in transfected cells. *Science (Washington D. C.)* **245**:1389–1392 (1989).
17. Faure-Halley, C., D. Graham, S. Arbilla, and S. Z. Langer. Expression and properties of recombinant $\alpha 1\beta 2\gamma 2$ and $\alpha 5\beta 2\gamma 2$ forms of the rat GABA$_A$ receptor. *Eur. J. Pharmacol.* **246**:283–287 (1993).
18. Sieghart, W. GABA$_A$ receptors: ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. *Trends Pharmacol. Sci.* **131**:446–450 (1992).
19. Puia, G., M. R. Santi, S. Vicini, D. B. Pritchett, P. H. Seeburg, and E. Costa. Differences in the negative allosteric modulation of γ-aminobutyric acid receptors elicited by 4′-chlorodiazepam and by a β-carboline-3-carboxylate ester: a study with natural and reconstituted receptors. *Proc. Natl. Acad. Sci. USA* **86**:7275–7279 (1989).

20. Gee, K. W., R. E. Brinton, and B. S. McEwen. Regional distribution of a Ro5–4864 binding site that is functionally coupled to the γ-aminobutyric acid/benzodiazepine receptor complex in rat brain. *J. Pharmacol. Exp. Ther.* **244**:379–383 (1987).
21. Gee, K. W. Phenylquinolines PK 8165 and PK 9084 allosterically modulate [^{35}S]t-butylbicyclophosphorothionate binding to a chloride ionophore in rat brain via a novel Ro5 4864 binding site. *J. Pharmacol. Exp. Ther.* **240**: 747–753 (1987).
22. Concas, A., G. Santoro, M. P. Mascia, E. Maciocco, L. Dazzi, and G. Biggio. Effects of propofol, pentobarbital and alphaxalone on *t*-[^{35}S]butylbicyclophosphorothionate binding in rat cerebral cortex. *Eur. J. Pharmacol.* **267**: 207–213 (1994).
23. Im, H. K., W. B. Im, T. M. Judge, R. B. Gammill, B. J. Hamilton, D. B. Carter, and J. F. Pregenzer. Substituted pyrazinones, a new class of allosteric modulators for γ-aminobutyric acid$_A$ receptors. *Mol. Pharmacol.* **44**:468–472 (1993).
24. Pritchett, D. B., H. Sontheimer, C. M. Gorman, H. Kettenmann, P. H. Seeburg, and P. R. Schofield. Transient expression shows ligand gating and allosteric potentiation of GABA$_A$ receptor subunits. *Science (Washington D. C.)* **242**:1306–1308 (1988).
25. Horne, A. L., P. C. Harkness, K. L. Hadingham, P. Whiting, and J. A. Kemp. The influence of the γ2L subunit on the modulation of responses to GABA$_A$ receptor activation. *Br. J. Pharmacol.* **108**:711–716 (1993).
26. Granger, P., B. Biton, P. Avenet, C. Faure, X. Vigé, D. Graham, S. Z. Langer, B. Scatton, and H. Depoortere. Modulation by phenytoin and carbamazepine of GABA-induced chloride currents. *Can. J. Physiol. Pharmacol.* **72** (Suppl. 1):396 (1994).
27. Vigé, X., A. Carreau, B. Scatton, and J. P. Nowicki. Antagonism by N^G-nitro-L-arginine of L-glutamate-induced neurotoxicity in cultured neonatal rat cortical neurons: prolonged application enhances neuroprotective efficacy. *Neuroscience* **55**:893–901 (1993).
28. McLean, M. J., and R. L. MacDonald. Multiple actions of phenytoin on mouse spinal cord neurons in cell culture. *J. Pharmacol. Exp. Ther.* **227**: 779–789 (1983).
29. Kaneko, S., T. Hirano, T. Kondo, K. Otani, Y. Fukushima, R. Hishada, and M. Matsunaga. Antiepileptic drug phenytoin potentiates GABA in the rat cuneate nucleus. *Jpn. J. Psychiatry Neurol.* **42**:643–645 (1988).
30. Puia, G., S. V. Vicini, P. H. Seeburg, and E. Costa. Different sites of action of neurosteroids and benzodiazepines on natural and recombinant GABA$_A$ receptors, in *GABAergic Synaptic Transmission* (G. Biggio, A. Concas, and E. Costa, eds.). Raven Press, New York, 103–110 (1992).

Send reprint requests to: Dr. P. Avenet, Synthélabo Recherche, Preclinical Research Department, 31 Av. P. Vaillant-Couturier, 92220 Bagneux, France.