0026-895X/92/06952-06$03.00/0

MOLECULAR PHARMACOLOGY, 42:952-957

ACCELERATED COMMUNICATION

Effects of a Benz[e]indene on γ-Aminobutyric Acid-Gated Chloride Currents in Cultured Postnatal Rat Hippocampal Neurons

NANCY T. RODGERS-NEAME, DOUGLAS F. COVEY, YUEFEI HU, KEITH E. ISENBERG, and CHARLES F. ZORUMSKI

Departments of Psychiatry (N.T.R.-N., K.E.I., C.F.Z.), Molecular Biology and Pharmacology (D.F.C., Y.H.), and Anatomy and Neurobiology (C.F.Z.), Washington University School of Medicine, St. Louis, Missouri 63110

Received August 13, 1992; Accepted September 11, 1992

SUMMARY

Benz[e]indenes (BIs) are tricyclic molecules that can be envisioned as steroids without an A-ring. Because certain steroids are known to alter γ-aminobutyric acid (GABA) responses in central neurons, we examined the effects of a substituted BI resembling 3α-hydroxy-5α-pregnan-20-one (3α-OH-DHP) on GABA-gated chloride currents in cultured postnatal rat hippocampal neurons. The compound, BI-1, reversibly potentiated GABA currents at concentrations of >10 nM, with an EC_{50} value of 0.2 μM. BI-1 increased the apparent affinity of GABA for its receptor, decreasing the GABA EC_{50} from 9 μM to 3 μM. BI-1 had no effect on the shape of the GABA current-voltage relationship and did not alter the GABA reversal potential. The effects of BI-1 were not altered by benzodiazepine or picrotoxin site antagonists. At concentrations up to 10 μM, where maximal effects on GABA currents were seen, BI-1 did not directly activate a membrane current. This contrasts with the effects of 3α-OH-DHP, which activated chloride currents at concentrations that were subsaturating for GABA potentiation. These results suggest that the BIs may be useful for determining the mechanisms by which steroids potentiate GABA responses and directly gate chloride channels.

Certain steroids, including 3α-OH-DHP and alphaxalone, are potent modifiers of responses mediated by GABA-linked chloride channels (1, 2). These agents potentiate responses to GABA administered exogenously (3) or released endogenously at inhibitory synapses (4). Additionally, at slightly higher concentrations these steroids directly gate chloride channels in a bicuculline-sensitive fashion (5). The steroid effects appear to be mediated by sites that are distinct from the GABA, picrotoxin, and BDZ binding sites (6, 7). Furthermore, although the steroids and barbiturates have similar actions on GABA responses, they appear to act at distinct sites within the $GABA_A$ receptor complex (8) and exert different actions on recombinant GABA-gated chloride channels expressed in human embryonic kidney cells (9).

The structural features crucial for steroid actions at the $GABA_A$ receptor are incompletely understood. Two essential features appear to be the presence of a 5α or 5β reduced tetracyclic steroid skeleton and a hydroxyl group in the 3α-position (10). Substitution of the 3α-hydroxyl group with other functional groups produces compounds that are largely devoid of activity (10). Although a number of studies have examined steroid structure-activity issues (6, 10, 11), there is little information regarding the necessity of the tetracyclic steroid nucleus. It is believed that the steroids interact with GABA receptors at a site that is within the membrane lipid bilayer (12), but the orientation of the steroids at their recognition site and the nature of the steroid binding site are unknown.

In an effort to examine the importance of the tetracyclic structure as well as to create potential steroid site ligands with greater molecular flexibility, we synthesized a series of substituted BIs. The BIs are tricyclic molecules that can be envisioned as steroid-like but that contain only a portion of the steroid A-ring. In this paper, we examine the actions of the most effective of these compounds synthesized to date, [3*S*-(3α, 3aα,5aβ,7α,9aα,9bβ)]-1-[7-(2-hydroxyethyl)dodecahydro-3a-methyl-1*H*-benz[*e*]inden-3-yl]ethanone, which we refer to as BI-1 (Fig. 1), and compare its actions on GABA-gated

This work was supported in part by Grants GM47969, HD19746, and NS14834 from the National Institutes of Health and National Institute of Mental Health Research Scientist Development Award MH00964 (C.F.Z.). N.T.R.-N. was supported by National Institute on Alcohol Abuse and Alcoholism Training Grant AA07466.

ABBREVIATIONS: 3α-OH-DHP, 3α-dihydroprogesterone (3α-hydroxy-5α-pregnane-20-one); GABA, γ-aminobutyric acid; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; BI, ben[e]indene; BDZ, benzodiazepine; αIMGBL, α-isopropyl-α-methyl-γ-butyrolactone; αEMTBL, α-ethyl-α-methyl-γ-thiobutyrolactone.

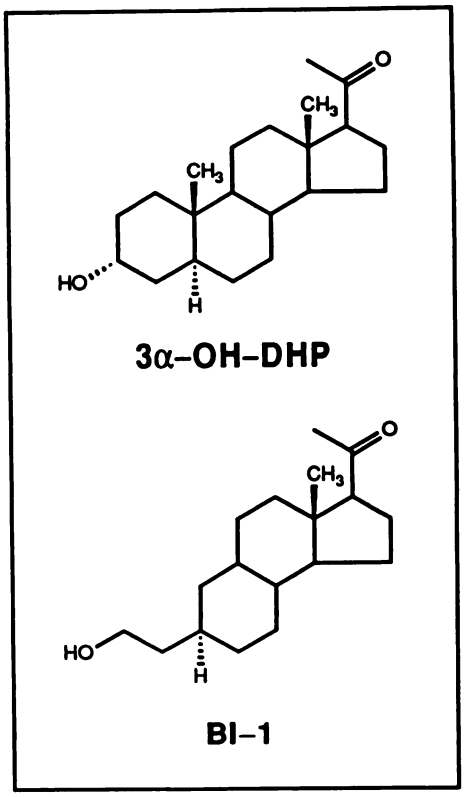

Fig. 1. Structures of 3α-OH-DHP and BI-1.

chloride currents in cultured postnatal rat hippocampal neurons with responses produced by 3α-OH-DHP.

Materials and Methods

Cell culture. Primary hippocampal cultures were prepared using established methods (13). The hippocampi were dissected from 1–2-day-old albino rat pups, sliced transversely into approximately 400-μm-thick sections, and placed in oxygenated Leibovitz L-15 medium containing 0.2 mg/ml fatty acid-free bovine serum albumin and 1 mg/ml papain at 35°. After 30 min in the papain solution, the slices were placed in Eagle's minimal essential medium containing 5% (v/v) fetal calf serum, 5% (v/v) horse serum, 400 μM glutamine, 50 μg/ml streptomycin, 50 units/ml penicillin, and 17 mM glucose. Single cells were isolated by gentle trituration using fire-polished Pasteur pipettes. The resulting cell suspension was plated in 35-mm collagen-coated tissue culture dishes at a density of about 300,000 cells/ml and was incubated in a humidified 5% CO_2 atmosphere. After 72 hr in culture, the cells were treated with 10^{-5} M cytosine arabinoside to retard the growth of glia. Experiments were performed on cells grown in culture for 3–14 days.

Electrophysiology. Experiments were carried out at room temperature (~22°) on the stage of an inverted microscope equipped with phase contrast optics. At the time of an experiment the growth medium was exchanged for a solution containing (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 10 HEPES, and 0.001 tetrodotoxin, with pH adjusted to 7.3. Tetrodotoxin was included to block voltage-gated Na^+ currents and to diminish spontaneous synaptic currents. Recording electrodes were fashioned from 1.2-mm borosilicate glass capillaries (World Precision Instruments) using a four-stage pull on a Flaming-Brown P-87 horizontal pipette puller (Sutter Instruments) and were filled with a solution containing (in mM) 140 CsCl, 4 NaCl, 4 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, and 5 EGTA, with pH adjusted to 7.3 using CsOH. In some experiments, the CsCl was replaced by 140 mM cesium methanesulfonate and the $MgCl_2$ was omitted. This substitution changes the chloride equilibrium potential from 0 mV to −86 mV. When filled with these solutions, fire-polished recording pipettes had resistances of 5–8 MΩ. Neurons were recorded in voltage clamp using the whole-cell patch-clamp technique (14). During an experiment the series resistance was typically 10–15 MΩ and was compensated by 50%. To ensure an adequate spatial voltage clamp, analyses were restricted to those cells in which GABA activated smoothly rising and decaying currents free of notches, spikes, or distortions. Currents were filtered at 1.5 kHz and were digitized at 0.25 kHz using pCLAMP version 5.5 (Axon Instruments).

Drug delivery. GABA stock solutions were prepared by dissolution in the extracellular solution. Steroid, BDZ, and bicuculline stock solutions were prepared in ethanol or DMSO and were diluted with the extracellular solution at the time of an experiment. The final ethanol or DMSO concentration was no greater than 0.2% and was usually less. At 0.2%, ethanol and DMSO do not alter GABA currents in hippocampal neurons. All concentrated stock solutions were stored at 4°. Dilute stocks and combinations of drugs for delivery were prepared fresh in the extracellular solution for each experiment.

Drug delivery was accomplished using pressure ejection pipettes fashioned in a manner similar to that used for the recording pipettes except that the tip was not fire-polished and the opening was approximately 1–2 μm. Each pipette tip was examined microscopically for homogeneity and size, resulting in little variability of response between drug delivery pipettes. Pipettes were positioned within 5 μm of the recorded neuron and drugs were applied for 100–500 msec by a jet of compressed air at 10–20 psi. This system allows no discernable drug leakage between applications and affords reliable repeated drug delivery. The concentrations of drugs reported are those in the pipette. The actual concentration at the cell is likely to be less due to diffusion and the fact that the entire cell is not uniformly exposed to the pipette contents.

Data analysis. Data were analyzed using pCLAMP version 5.5, Sigmaplot version 4.0, and routines written in Axobasic. Peak currents obtained by 200-msec applications of drugs at various concentrations were fit to a dose-response equation of the form:

$$\text{Response} = \text{Response}_{max} \times \frac{[\text{Conc}]^n}{[\text{Conc}]^n + \text{EC}^{n}_{50}}$$

where Response_{max} is the maximum effect, [Conc] is the drug concentration, EC_{50} is the half-maximal effective concentration, and n is the Hill coefficient. The data in this paper represent mean ± standard error.

Materials. GABA, 3α-OH-DHP, and most salts were obtained from Sigma Chemical Co. Exceptions to this were NaCl (Mallinckrodt Chemical Co.) and cesium methanesulfonate (Aldrich Chemical Co.). The complete experimental details for the synthesis of BI-1 and other BI analogs will be reported separately. Briefly, the 15-step synthesis of BI-1 starts with commercially available 17β-hydroxyestr-4-en-3-one (19-nortestosterone). The A-ring double bond is reduced, establishing the 5α-configuration for the A,B-ring fusion, and then the synthesis proceeds with cleavage of the A-ring between C_2 and C_3 and sequential removal of C_2 and C_1. The synthesis ends with the introduction of the acetyl side chain on the five-membered ring of BI-1. The compound, BI-1, is a white crystalline solid (m.p., 61–62°) having IR and NMR (both ^1H and ^{13}C) spectra consistent with the stated structure. Elemental analysis for $C_{18}H_{30}O_2$ was follows: calculated: C, 77.65; H, 10.86; found: C, 77.68; H, 10.83.

Results

Using the pressure ejection drug delivery system, GABA activates chloride currents in cultured postnatal rat hippocampal neurons with an EC_{50} value of 9 ± 1 μM and saturation at about 30 μM (Hill coefficient = 1.9). The GABA currents are potently and effectively augmented by 3α-OH-DHP (Fig. 2).

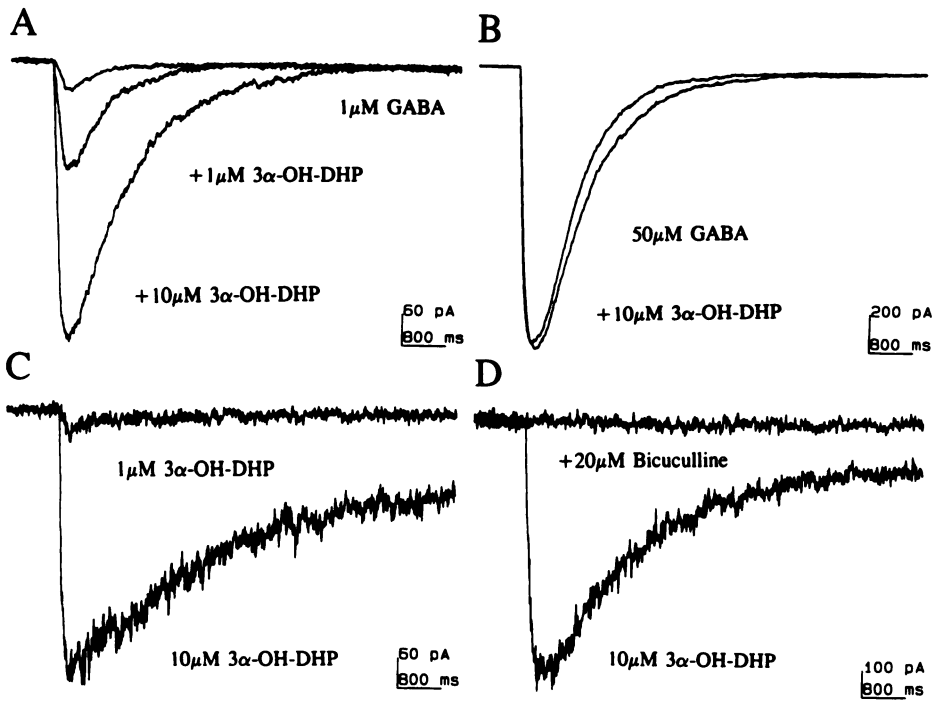

Fig. 2. 3α-OH-DHP potentiates GABA currents in hippocampal neurons. A, The *traces* show the effect of 1 μM and 10 μM 3α-OH-DHP on the response of a hippocampal neuron to a 200-msec application of 1 μM GABA. B, When GABA is applied at 50 μM, 3α-OH-DHP has little effect on the peak response. C, 3α-OH-DHP directly gates chloride currents in the absence of GABA. For these experiments, 3α-OH-DHP was applied at 1 μM and 10 μM for 200 msec. D, Bicuculline inhibits the currents gated by 10 μM 3α-OH-DHP. In all panels of this figure, the neurons were voltage clamped at −50 mV.

At concentrations of >10 nM, 3α-OH-DHP potentiates GABA responses in a dose-dependent fashion, with an EC_{50} value of 1.2 ± 0.1 μM and maximal effects at about 10 μM. At a saturating concentration, 3α-OH-DHP (10 μM) increases the peak current gated by 1 μM GABA to 518 ± 57% of control (n = 9). The degree of potentiation is dependent on the GABA concentration, with greater effects at low (<10 μM) agonist levels and less effect at saturating agonist concentrations.

In addition to potentiating GABA currents, 3α-OH-DHP at concentrations of ≥1 μM directly gates a current in the absence of GABA. The steroid-gated currents are completely inhibited by 20 μM bicuculline (n = 3), suggesting that they result from the activation of GABA-linked chloride channels (Fig. 2). At 10 μM, 3α-OH-DHP gates a current that is 30 ± 6% (n = 5) of the current gated by 1 μM GABA in the same cells. One micromolar is a concentration at the foot of the GABA dose-response curve in hippocampal neurons.

The BI BI-1 also reversibly potentiates GABA currents at concentrations of >10 nM (Fig. 3). The EC_{50} value for the BI-1 effect is 0.2 ± 0.03 μM, with maximal effects at about 1 μM (Fig. 4). Like 3α-OH-DHP, BI-1 also potentiates GABA currents to a greater extent at lower GABA concentrations, with less effect on peak currents at saturating agonist concentrations (Fig. 3). At saturating GABA concentrations, BI-1, like 3α-OH-DHP, appears to prolong the agonist-gated response. BI-1 increases the apparent affinity of GABA for its receptor, changing the agonist EC_{50} from 9 μM to 3 μM.

In comparison with 3α-OH-DHP, BI-1 is ineffective at gating chloride channels in the absence of GABA. At concentrations up to 10 μM, where maximal potentiation of GABA currents occurs, BI-1 produces no macroscopic current when administered alone. At 100 μM a small response to BI-1 can be recorded in some cells (Fig. 5). The current gated by 100 μM BI-1 is 76 ± 8% of that gated by 10 μM 3α-OH-DHP in cells exposed to both agents (n = 4) (Fig. 5).

BI-1 does not alter the shape of the GABA current-voltage (I-V) relationship or the GABA reversal potential. In six neurons, BI-1 produced a change of +2 ± 2 mV in the reversal potential of currents gated by GABA.

BI-1 does not appear to act at either the BDZ or picrotoxin site to potentiate GABA currents. The BDZ antagonist flumazenil (RO 15-1788) (15) inhibits the potentiation of GABA

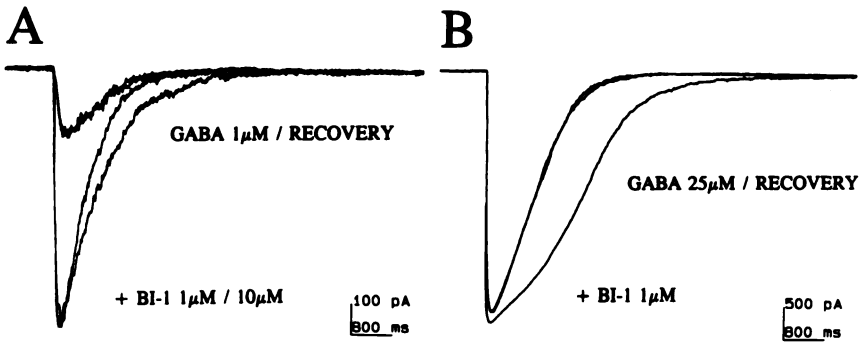

Fig. 3. BI-1 potentiates GABA currents. A, The effect of 1 μM and 10 μM BI-1 on the response to 1 μM GABA of a hippocampal neuron voltage-clamped at −50 mV is shown. The BI-1-induced potentiation is completely reversible. B, BI-1 (1 μM) has less effect on the peak response to 25 μM GABA, although prolongation of the response is seen.

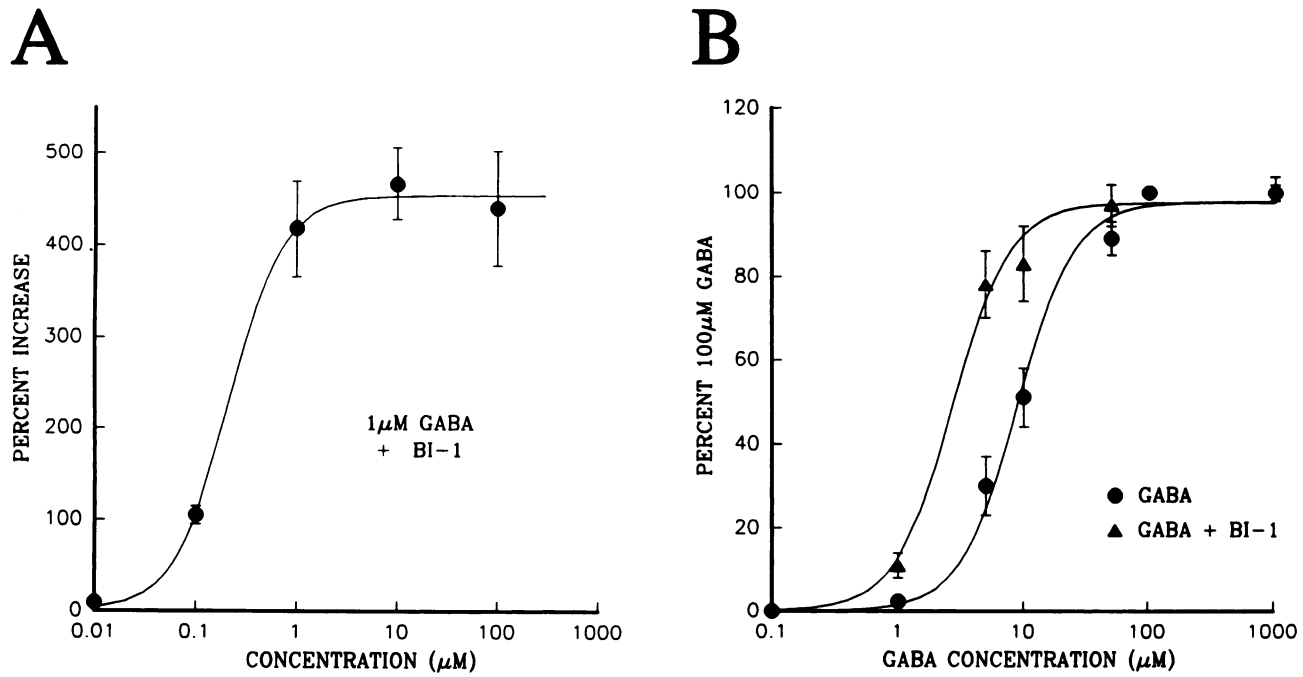

Fig. 4. BI-1 potentiates GABA currents in a dose-dependent fashion. A, The graph shows the percent increase in peak currents gated by 1 μM GABA in the presence of various concentrations of BI-1 in neurons voltage clamped at −50 mV and exposed to 200-msec applications of drugs. The individual *points* represent the mean ± standard error for 4–16 cells. *Solid line*, best fit of the experimental points to the dose-response equation specified in Materials and Methods. For these data, $Response_{max}$ = 454 ± 11%, EC_{50} = 0.2 ± 0.03 μM, and n = 1.6 ± 0.2. B, The graph shows the effect of BI-1 on the GABA dose-response curve. The *points* represent the mean ± standard error for 3–10 cells and are normalized with respect to the peak 100 μM GABA response. The GABA dose-response (●) was fit to the dose-response equation with $Response_{max}$ = 98 ± 2%, ED_{50} = 9 ± 1 μM, and n = 1.9 ± 0.4. After treatment with BI-1 (▲), the EC_{50} shifted to 3 ± 1 μM.

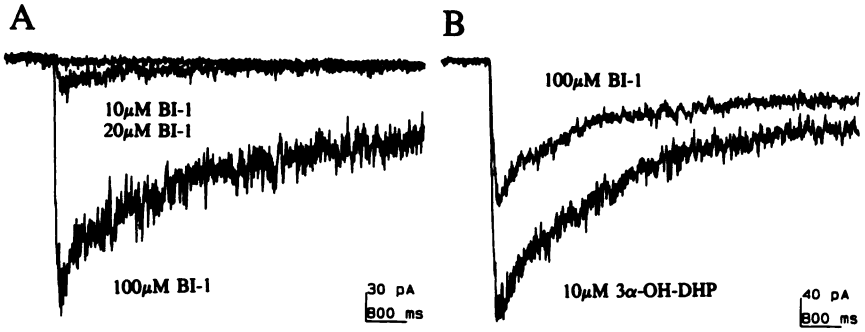

Fig. 5. BI-1 gates a current at high concentrations. A, A hippocampal neuron was voltage-clamped at −50 mV and exposed to 200-msec applications of 10 μM, 20 μM, and 100 μM BI-1 in the absence of GABA. B, The *traces* show the currents gated by 100 μM BI-1 and 10 μM 3α-OH-DHP on the same neuron. In all cells studied, 100 μM BI-1 gave a smaller response than 10 μM 3α-OH-DHP (n = 4).

currents produced by diazepam but does not alter the response to BI-1 (Fig. 6). In the presence of 1 μM flumazenil the potentiation of 1 μM GABA currents by 10 μM diazepam is inhibited by 97 ± 6% (n = 12), whereas currents gated in the presence of 1 μM GABA, 10 μM BI-1, and 1 μM flumazenil are 101 ± 3% (n = 8) of those in the presence of 1 μM GABA plus 10 μM BI-1. Additionally, the picrotoxin site antagonist αIMGBL (3 mM) (16) fails to alter BI-1-induced potentiation of GABA currents but reverses the augmentation produced by αEMTBL, an agent that has previously been shown to potentiate GABA responses via an action at the picrotoxin site (17) (Fig. 6). In the presence of 3 mM αIMGBL, currents gated by 1 μM GABA plus 10 μM BI-1 are 110 ± 4% (n = 4) of control, whereas 3 mM αIMGBL inhibits the potentiation of 1 μM GABA currents produced by 200 μM αEMTBL by 69 ± 8% (n = 3). In support of an action of BI-1 at the steroid site on $GABA_A$ receptors, we found that saturating concentrations of BI-1 do not augment GABA currents that are maximally potentiated by 3α-OH-DHP (Fig. 7). Similarly, 3α-OH-DHP does not produce further potentiation of GABA currents in the presence of saturating concentrations of BI-1. In the presence of 1 μM GABA plus 10 μM BI-1 and 10 μM 3α-OH-DHP, currents are 104 ± 3% (n = 6) of those in the presence of 1 μM GABA plus either agent alone. BI-1 also does not potentiate currents directly gated by 3α-OH-DHP. Responses in the presence of both 10 μM 3α-OH-DHP and 10 μM BI-1 are 101 ± 3% (n = 6) of those produced by 3α-OH-DHP alone.

Discussion

We examined the actions of the substituted BI BI-1 on GABA-gated chloride currents in cultured hippocampal neurons and compared its actions with those of 3α-OH-DHP. Both compounds are effective potentiators of GABA responses, producing comparable effects at high nanomolar to low micromolar concentrations. However, unlike 3α-OH-DHP, BI-1 is much less effective at directly gating chloride channels in the absence

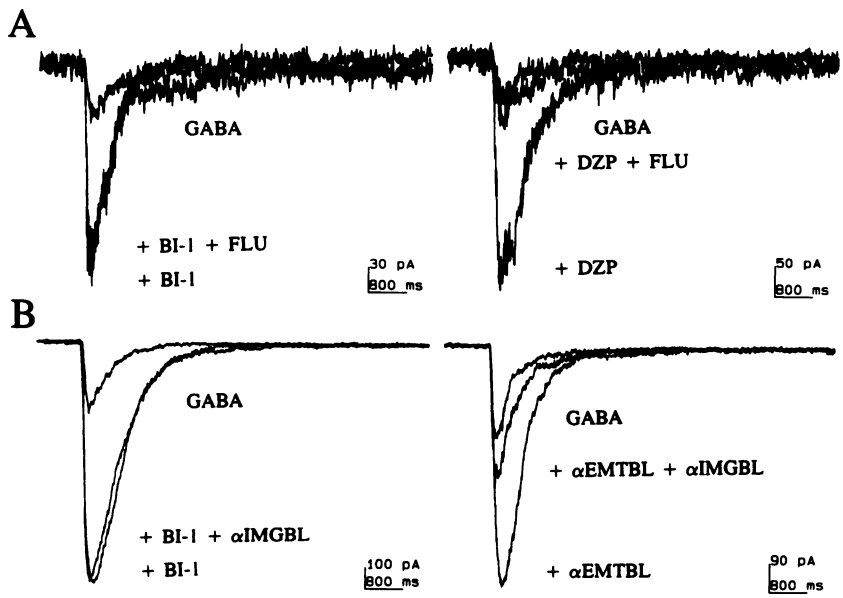

Fig. 6. BI-1 does not act at the BDZ or picrotoxin site on GABA receptors. A, The effect of 10 μM BI-1 on currents gated by 1 μM GABA in the absence and presence of 1 μM flumazenil (*FLU*), a BDZ antagonist, is shown (*left*). In contrast to the lack of effect on BI-1, 1 μM flumazenil markedly inhibits the potentiation of GABA currents produced by 10 μM diazepam (*DZP*) (*right*). B, αIMGBL (3 mM), a picrotoxin site antagonist (16), does not affect the response to 10 μM BI-1 (*left*) but markedly diminishes the potentiation of GABA currents produced by 200 μM αEMTBL (*right*), a picrotoxin site inverse agonist (17).

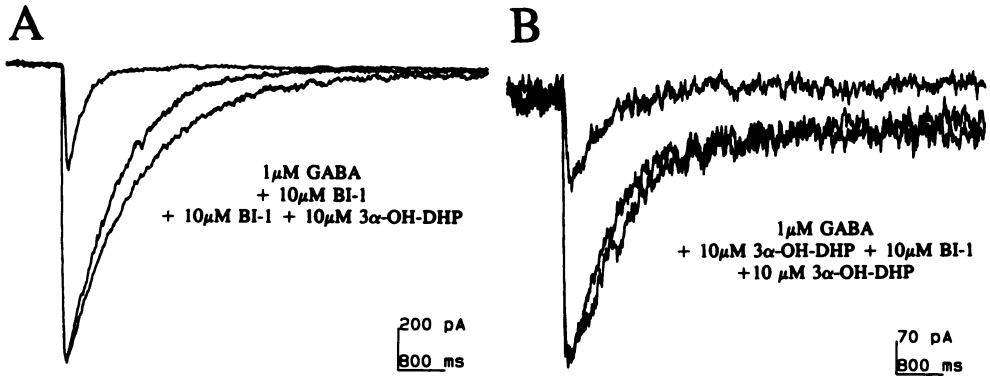

Fig. 7. Effects of coapplications of saturating concentrations of BI-1 and 3α-OH-DHP on GABA currents. A, At 10 μM, BI-1 markedly potentiates the response of a neuron to 1 μM GABA. However, addition of 10 μM 3α-OH-DHP produces no further augmentation. B, In another hippocampal neuron, 10 μM 3α-OH-DHP markedly potentiates the response to 1 μM GABA but no further potentiation is seen upon addition of 10 μM BI-1.

of GABA. We have found similar actions of other BIs,[1] suggesting that the effects reported here are characteristic of this class of drugs.

Previous studies have suggested that steroids may act at several loci within the $GABA_A$ complex. In this study we chose to compare the effects of BI-1 with those of 3α-OH-DHP because BI-1 differs from the steroid primarily in the alteration of the A-ring, with similar substitutions on the D-ring. The failure of BDZ and picrotoxin site antagonists to block the BI-1 effects, coupled with the inability of BI-1 to potentiate GABA currents in the presence of high concentrations of 3α-OH-DHP and vice versa, suggests that the BI acts at the steroid site on $GABA_A$ receptors. Clearly, removal of the steroid A-ring does not eliminate GABA-potentiating ability but markedly alters the ability to gate chloride channels directly. It is possible that the GABA-potentiating and direct chloride channel-gating effects may be mediated by separate sites. If this is the case, then the BIs may prove useful for distinguishing between these sites, because they are at least 1 order of magnitude more potent in potentiating than activating currents.

Previously, Schulz and Macdonald (18) suggested that differences in the potency of barbiturates for potentiating GABA currents versus gating chloride channels directly may account for the clinical observation that some barbiturates are better anticonvulsants, whereas others are better anesthetics. The anesthetic pentobarbital potentiates and directly gates chloride channels at comparable concentrations, whereas the anticonvulsant phenobarbital potentiates GABA currents but is considerably less potent at channel activation. It is interesting that a number of steroids have been used clinically as anesthetics (10). These agents, including 3α-OH-DHP, both potentiate GABA currents and directly gate chloride currents. In cultured hippocampal neurons, 3α-OH-DHP produces these effects at similar concentrations, with clear channel gating occurring at concentrations that are subsaturating for GABA potentiation. In contrast, the potentiation of GABA currents by BI-1 is maximal at about 1 μM but the drug does not directly gate chloride currents at concentrations up to 10 μM. At concentrations near 100 μM small chloride currents are produced by BI-1. The Schulz and Macdonald hypothesis (18) predicts that BI-1 will be more useful as an anticonvulsant than as an anesthetic. Consistent with this, preliminary *in vivo* experiments indicate that the dose of BI-1 that blocks pentylenetetrazole seizures in mice is 4-fold lower than the dose that produces ataxia.[1] This degree of separation of anticonvulsant and sedative effects is comparable to the effects of phenobarbital.

The ability of the BIs to differentiate GABA-potentiating actions from direct chloride channel gating may make these agents useful for developing a new class of anticonvulsant drugs. It is also possible that other alterations of the steroid

[1] C. F. Zorumski and D. F. Covey, unpublished observations.

molecule will allow a better definition of the structural features necessary for direct chloride channel gating and may allow the development of antagonists for the steroid recognition site(s).

Acknowledgments

The authors thank Ann Benz and Jianxin Que for technical assistance. The authors also thank Michael Rafferty, Mark Nevins, and Susan Nash of G. D. Searle and Co. (Skokie, IL) for the *in vivo* evaluation of BI-1.

References

1. Majewska, M. D. Neurosteroids: endogenous bimodal modulators of the $GABA_A$ receptor: mechanism of action and physiological significance. *Prog. Neurobiol.* **38**:379–395 (1992).
2. Paul, S. M., and R. H. Purdy. Neuroactive steroids. *FASEB J.* **6**:2311–2322 (1992).
3. Majewska, M. D., N. L. Harrison, R. D. Schwartz, J. L. Barker, and S. M. Paul. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science (Washington D. C.)* **232**:1004–1007 (1986).
4. Harrison, N. L., S. Vicini, and J. L. Barker. A steroid anesthetic prolongs inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J. Neurosci.* **7**:604–609 (1987).
5. Barker, J. L., N. L. Harrison, G. D. Lange, and D. G. Owen. Potentiation of γ-aminobutyric-acid-activated chloride conductance by a steroid anaesthetic in cultured rat spinal neurones. *J. Physiol. (Lond.)* **386**:485–501 (1987).
6. Gee, K. W., M. B. Bolger, R. E. Brinton, H. Coirini, and B. S. McEwen. Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. *J. Pharmacol. Exp. Ther.* **246**:803–812 (1988).
7. Cottrell, G. A., J. J. Lambert, and J. A. Peters. Modulation of $GABA_A$ receptor activity by alphaxalone. *Br. J. Pharmacol.* **90**:491–500 (1987).
8. Turner, D. M., R. W. Ransom, J. S. J. Yang, and R. W. Olsen. Steroid anesthetics and naturally occurring analogs modulate the γ-aminobutyric acid receptor complex at a site distinct from barbiturates. *J. Pharmacol. Exp. Ther.* **248**:960–966 (1989).
9. Puia, G., M. Santi, S. Vicini, D. B. Pritchett, R. H. Purdy, S. M. Paul, P. H. Seeburg, and E. Costa. Neurosteroids act on recombinant human $GABA_A$ receptors. *Neuron* **4**:759–765 (1990).
10. Phillipps, G. H. Structure-activity relationships in steroidal anaesthetics, in *Molecular Mechanisms in General Anaesthesia* (M. J. Halsey, R. A. Millar, and J. A. Sutton, eds.). Churchill Livingstone, Edinburgh, 32–47 (1974).
11. Harrison, N. L., M. D. Majewska, J. W. Harrington, and J. L. Barker. Structure-activity relationships for steroid interaction with the γ-aminobutyric $acid_A$ receptor complex. *J. Pharmacol. Exp. Ther.* **241**:346–353 (1987).
12. Lan, N. C., M. B. Bolger, and K. W. Gee. Identification and characterization of a pregnane steroid recognition site that is functionally coupled to an expressed $GABA_A$ receptor. *Neurochem. Res.* **16**:347–356 (1991).
13. Thio, L. L., D. B. Clifford, and C. F. Zorumski. Characterization of quisqualate receptor desensitization in cultured postnatal rat hippocampal neurons. *J. Neurosci.* **11**:3430–3441 (1991).
14. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch-clamp techniques for high resolution current recordings from cells and cell-free membrane patches. *Pflügers Arch.* **391**:85–100 (1981).
15. Richards, J. G., and H. Mohler. Benzodiazepine receptors. *Neuropharmacology* **23**:233–242 (1984).
16. Holland, K. D., K. W. Yoon, J. A. Ferrendelli, D. F. Covey, and S. M. Rothman. γ-Butyrolactone antagonism of the picrotoxin receptor: comparison of a pure antagonist and a mixed antagonist/inverse agonist. *Mol. Pharmacol.* **39**:79–84 (1991).
17. Holland, K. D., J. A. Ferrendelli, D. F. Covey, and S. M. Rothman. Physiological regulation of the picrotoxin receptor by γ-butyrolactones and γ-thiobutyrolactones in cultured hippocampal neurons. *J. Neurosci.* **10**:1719–1727 (1990).
18. Schulz, D. W., and R. L. Macdonald. Barbiturate enhancement of GABA-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions. *Brain Res.* **209**:177–188 (1981).

Send reprint requests to: Charles F. Zorumski, M.D., Department of Psychiatry, Washington University School of Medicine, 4940 Children's Place, St. Louis MO 63110.