Communications to the Editor

Modulation of $GABA_A$ Receptor Function by Benz[e]indenes and Phenanthrenes

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γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain.[1] Binding of GABA to postsynaptic $GABA_A$ receptors results in the opening of a chloride-permeable ion channel that is an integral part of the $GABA_A$ receptor/chloride channel complex. In addition to the GABA binding site of this receptor/channel complex, there are additional binding sites for ligands that may either allosterically modulate the gating actions of GABA or, in some cases, initiate chloride flux in the absence of GABA. These additional sites have been defined pharmacologically by their respective ligands as the benzodiazepine,[2,3] barbiturate,[4] picrotoxin,[5] and steroid binding sites.[6-10] Drugs binding to these allosteric sites are useful anxiolytics, sedative hypnotics, anticonvulsants, and anesthetics.

Currently, there is considerable interest in developing new drugs that modulate $GABA_A$ receptor function via the steroid binding site.[8-10] In part, this interest has arisen because of studies showing that the anesthetic activity of alphaxalone[11] (3α-hydroxy-5α-pregnane-11,20-dione) and other anesthetic steroids described initially by Selye[12] can be explained by an ability to increase chloride conductance through the $GABA_A$ receptor/channel complex.[6,7] This interest is sustained by other studies suggesting that the endogeneous steroids, 3α-hydroxy-5α-pregnan-20-one (1) and 3α,21-dihydroxy-5α-pregnan-20-one (2), are important physiological regulators of $GABA_A$ receptor function.[13]

We are engaged in the development of novel nonsteroid ligands for the steroid binding site of the $GABA_A$ receptor. We present here preliminary results obtained with tricyclic compounds that contain either the B, C, D rings or the A, B, C rings of known steroid modulators[14,15] of the $GABA_A$ receptor complex. The goal of the study was to determine if flexibility at opposite ends of tricyclic mimics of the steroid molecule have different effects on $GABA_A$ receptor function. Thus, we describe the synthesis and evaluation of both dodecahydrobenz[e]indenes (5-7) and tetradecahydrophenanthrenes (20-22). As discussed (vida infra), the functional response to each class of compounds at the $GABA_A$ receptor complex differs significantly.

Compounds 5-7 were prepared from benz[e]indene 3[16,17] by the route outlined in Chart I. Reduction of 3 with DIBALH in toluene (3 h at 4 °C) gave diol 4 (93%; mp 145-147 °C; IR 3279 cm^{-1}; ^{13}C NMR δ 82.6, 60.8). Oxidation of diol 4 with NaOCl in HOAc[18] (1 h at room temperature) gave hydroxy ketone 5 (64%; mp 38-40 °C; IR 3435, 1739 cm^{-1}; ^{13}C NMR δ 222.1, 60.1). Treatment of 4 with 1.0 M t-BuOK in dimethoxyethane and tosylmethyl isocyanide[19] (3 h at room temperature) gave the hydroxy nitrile 6[20] (27%; mp 82-83 °C; IR 3294, 2233 cm^{-1}; ^{13}C NMR δ 121.4, 60.3). Hydroxy nitrile 6 when treated with CH_3MgI in EtOEt/THF[21] (24 h at reflux) gave hydroxy ketone 7 (90%, mp 61-62 °C; IR 3391, 1705 cm^{-1}; ^{13}C NMR δ 210.3, 60.5).

The synthesis of phenanthrenes 20-22 from phenanthrene 8[22] is also outlined in Chart I. Inversion of the configuration of the hydroxyl group of 8 was performed by way of the Mitsunobu reaction[23,24] to give diester 9 (68%; mp 98-99 °C; IR 1734 cm^{-1}; ^{13}C NMR δ 179.9, 171.2). Methanolysis of diester 9 with AcCl/MeOH (overnight at 50 °C) gave hydroxy ester 10 (97%; mp 97-98 °C; IR 3401 cm^{-1}; ^{13}C NMR δ 179.7, 66.3). Protection of the hydroxyl group of 10 as a *tert*-butyl ether was accomplished using BF_3-EtOEt, H_3PO_4, and isobutylene[25] (2 days at room temperature) yielding compound 11 (65%; mp 112-113

Chart I

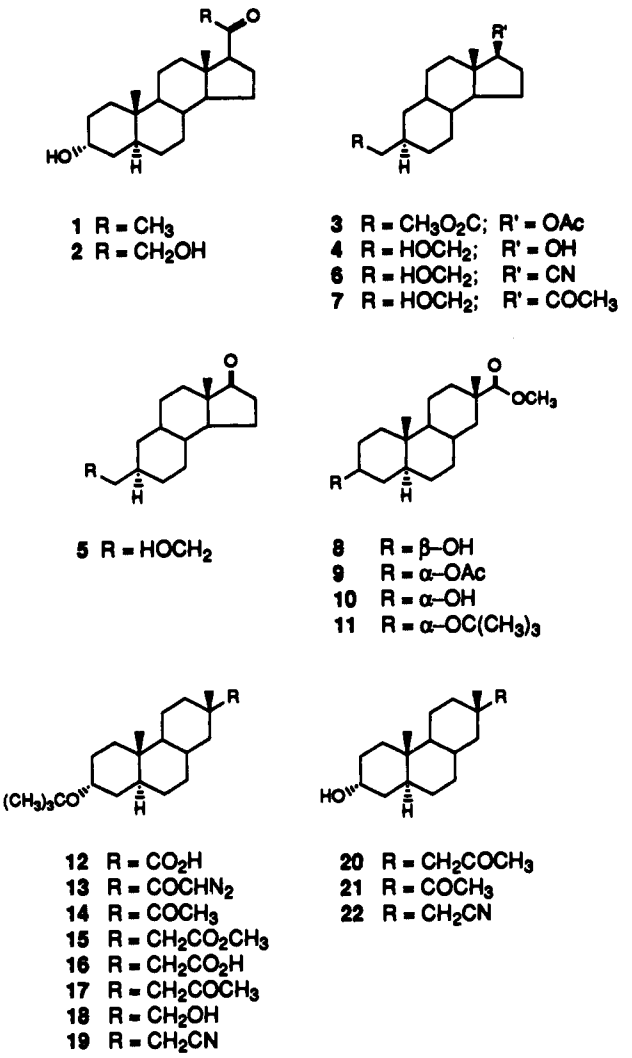

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Table I. Effects of Benz[*e*]indenes and Phenanthrenes on GABA-Mediated Currents, Current Activation in the Absence of GABA, and ^{35}[S]-TBPS Binding[a,b]

compound	% response relative to current produced by GABA[c]	compound-gated current[d]	^{35}[S]-TBPS Displacement		Scatchard analysis of ^{35}[S]-TBPS binding[e]	
			IC_{50} (μM)	nHill	K_d (nM)	B_{max} (pmol/mg protein)
^{35}[S]-TBPS					51.2 ± 0.9 (3)	1.55 ± 0.04 (3)
1	195 ± 16 (9)[f]	30 ± 6 (5)	0.18 ± 0.03 (3)[g]	0.88 ± 0.07 (3)	80.0 ± 8.1* (3)	1.22 ± 0.07* (3)
5	127 ± 4 (6)	NR (6)[h]	44.1 ± 6.1 (2)	1.13 ± 0.04 (2)		
6	346 ± 2 (6)	NR (6)	4.50 ± 0.01 (2)	0.94 ± 0.02 (2)		
7	489 ± 19 (12)	NR (12)	5.82 ± 0.76 (3)	0.93 ± 0.03 (3)	82.4 ± 3.2** (3)	1.04 ± 0.09** (3)
20	113 ± 3 (4)	1 ± 0.5 (3)	3.98 ± 0.19 (3)	1.26 ± 0.01 (3)	80.7 ± 4.5** (3)	1.15 ± 0.05** (3)
21	108 ± 4 (5)	NR (5)	7.06 ± 0.58 (3)	1.26 ± 0.05 (3)		
22	106 ± 3 (4)	8 ± 2 (4)	3.27 ± 0.23 (2)	1.15 ± 0.00 (2)		

[a] Electrophysiological determinations were carried out by the whole cell patch-clamp technique on rat hippocampal cells as described in detail elsewhere.[29] [b] ^{35}[S]-TBPS binding (IC_{50} determinations) and Scatchard analysis were done with rat brain membranes as described in detail elsewhere[35] except that the compounds were dissolved in DMSO and that IC_{50} values were determined by logistic analysis using the program SigmaPlot purchased from Jandel Scientific, San Rafael, CA. The final concentration of DMSO in the assay was 0.25%, and control experiments showed that this concentration of DMSO had no effect on ^{35}[S]-TBPS binding. [c] Percent response relative to current (i) produced by 1 μM GABA = $i_{(GABA + 1\ \mu M\ cmpd)}/i_{GABA} \times 100$. [d] Current gated by 10 μM compound in the absence of GABA is reported as percent relative to current produced by 1 μM GABA on the same cell. [e] The assay was carried out in triplicate with triplicate determinations of each data point within an assay. K_d and B_{max} values were determined from linear regressions of the data after the Scatchard transformation. These linear regressions were carried out with the program SigmaPlot. Statistical significance (TBPS alone vs TBPS and test compound) was determined by Student's t test, *$p < 0.02$, **$p < 0.01$. [f] Number reported is the mean ± SEM. Number in parentheses indicates number of determinations. [g] This value is comparable to a reported value of 275 ± 29 nM obtained with rat brain synaptosomes using ethanol as solvent for the steroid.[31] [h] NR = no response.

°C; IR 1731 cm^{-1}; ^{13}C NMR δ 179.7, 28.2). Saponification of **11** with NaOH in aqueous MeOH (overnight at reflux) gave carboxylic acid **12** (96%; mp 191–192 °C; IR 1699 cm^{-1}; ^{13}C NMR δ 185.5) which was sequentially treated with oxalyl chloride (2.5 h at room temperature) and diazomethane to give diazo ketone **13** (85%; oil; IR 2102, 1629 cm^{-1}; ^{13}C NMR δ 202.4, 51.6). Diazo ketone **13** was treated with either 47% HI[26] (~3 min at room temperature) to give ketone **14** (80%; mp 77–78 °C; IR 1705 cm^{-1}; ^{13}C NMR δ 215.3, 24.4) or a solution of AgOBz[27] in $(Et)_3N$ (~2 h at room temperature) to give ester **15** (85%; mp 41–42 °C; IR 1739 cm^{-1}; ^{13}C NMR δ 173.0). Saponification of **15** (NaOH in aqueous MeOH, 4 days at reflux) gave acid **16** (90%; mp 134–135 °C; IR 1705 cm^{-1}; ^{13}C NMR δ 178.5) which was sequentially treated with oxalyl chloride (1.5 h at room temperature), diazomethane, and 47% HI (~3 min at room temperature) to give ketone **17** (38% overall; 78–79 °C; IR 1720 cm^{-1}; ^{13}C NMR δ 209.7).

Reduction of ester **15** with DIBALH (1 h at −20 °C) to give alcohol **18** (96%, mp 123–124 °C; IR 3368 cm^{-1}; ^{13}C NMR δ 74.8), and then treatment of the mesylate of **18** with KCN in DMSO (overnight at 90 °C) gave nitrile **19** (54% overall; mp 102–105 °C; IR 2242 cm^{-1}; ^{13}C NMR δ 118.5). Deprotection of **17**, **14**, and **19** with TMSI[28] in CH_2Cl_2 (typically 3–5 min at room temperature) gave the corresponding ketone **20** (73%; mp 105–106 °C; IR 3401, 1708 cm^{-1}; ^{13}C NMR δ 209.6, 66.4), ketone **21** (30%; mp 134–136 °C; IR 3435, 1703 cm^{-1}; ^{13}C NMR δ 215.3, 66.4), and nitrile **22** (76%; mp 114–115 °C; IR 3400, 2219 cm^{-1}; ^{13}C NMR δ 118.3, 66.4), respectively.

Electrophysiological effects of compounds **1**, **5–7**, and **20–22** are shown in Table I. At 1 μM, steroid **1** has been shown to potentiate GABA-mediated current and to directly activate a small current in the absence of GABA.[7,29] At 1 μM, benz[*e*]indenes **5–7** also potentiate GABA-mediated currents.[30] However, even at a 10-fold higher concentration none of these compounds directly activate a current in the absence of GABA. These results suggest that analogs having increased flexibility in the region of the A ring of a steroid molecule will have a diminished ability to activate chloride currents in the absence of GABA, but not a diminished ability to potentiate GABA-mediated chloride currents.

The electrophysiological results obtained with compounds **20–22**, analogs having increased flexibility in the region of the D ring of a steroid molecule, are strikingly different (Table I). At 1 μM, these compounds are largely devoid of GABA-potentiating effects, and at 10 μM their channel-activating effects are also weak. Thus, increasing ligand flexibility in this manner seems to produce only compounds with decreased potency for both effects.

Previous studies have shown that steroids having electrophysiological effects on $GABA_A$ receptor/channel function also potently displace [^{35}S]*tert*-butylbicyclophosphorothionate (TBPS) from the picrotoxin binding site on the $GABA_A$ receptor complex.[7,31,32] The IC_{50} values for ^{35}[S]-TBPS displacement by the compounds evaluated in this study are shown in Table I. All of the tricyclic compounds are at least 20-fold less potent displacers of ^{35}[S]-TBPS than steroid **1**. Since the flexible analogs should have a larger entropic term for their binding than the steroid, this is not a surprising result. Of some importance is the finding that 1 μM steroid **1**, which is the most potent displacer of ^{35}[S]-TBPS, does not potentiate 1 μM GABA-mediated currents as well as 1 μM benz[*e*]indenes **6** and **7**. Moreover, even though benz[*e*]indene **5** displaces ^{35}[S]-TBPS less potently than any of the phenanthrenes, it is a better potentiator of 1 μM GABA-mediated current than any of the phenanthrenes. These results suggest that IC_{50} values for ^{35}[S]-TBPS displacement are unlikely to be useful for identifying compounds that differentially potentiate GABA-mediated currents without directly activating currents in the absence of GABA.

An additional comparison of the picrotoxin receptor binding properties of compounds **1**, **7**, and **20** was provided by Scatchard analysis (Table I and Figure 1). This analysis also did not reveal any distinguishing characteristics of binding for the different compounds. None of the compounds are competitive displacers of TBPS, and all of them change both the K_d and B_{max} for ^{35}[S]-TBPS binding in a similar fashion. These results reinforce the conclusion made from the IC_{50} studies regarding the utility of TBPS binding studies.

In summary, we have shown that the electrophysiological effects of flexible analogs of steroid modulators of the

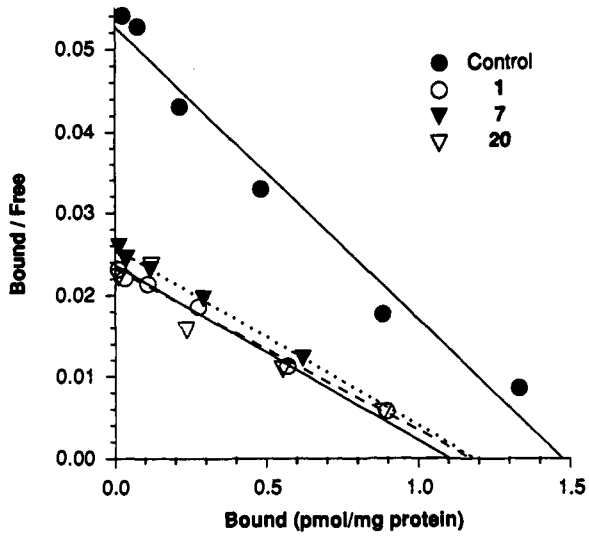

Figure 1. Typical Scatchard plots of ^{35}[S]-TBPS displacement by compounds **1**, **7**, and **20**. The control line is for ^{35}[S]-TBPS alone. Each compound was tested at its IC_{50} concentration reported in Table I.

$GABA_A$ receptor complex are very dependent on the region of flexibility within the analog. Flexibility in the region of a steroid A ring yields compounds that are excellent potentiators of GABA-mediated current, but diminished activators of current in the absence of GABA. If, as it has been suggested by Schulz and Macdonald[33] for the barbiturates, anticonvulsant activity correlates with potentiation of GABA-mediated currents; whereas anesthetic activity correlates with direct activation of the chloride current in the absence of GABA, the benz[*e*]indenes offer the prospect of being anticonvulsants/anxiolytics with diminished sedative hypnotic/anesthetic activity.[34] Flexibility in the region of a steroid D ring yields compounds of lower potency having little or no potentiating effects on GABA-mediated current when used at a concentration below that causing direct activation of the current. Further modification of the phenanthrene side chain may increase the potency of this class of compounds. If so, the Schultz and Macdonald hypothesis applied to the results given here would indicate that these phenanthrenes would be most useful as sedative hypnotics/anesthetics. Further studies are in progress to establish the merit of our speculations.

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