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Ultraviolet Irradiation Selectively Disrupts the γ-Aminobutyric Acid/Benzodiazepine Receptor-Linked Chloride Ionophore

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SUMMARY

The ability of UV light to affect radioligand binding and $^{36}Cl^-$ uptake at the γ-aminobutyric acid$_A$ ($GABA_A$) receptor-chloride channel complex was examined. Exposure to 302 nm UV light produced a rapid ($t_{½}$ = 4 min) reduction in [^{35}S]*t*-butylbicyclophosphorothionate binding (assayed in the presence of 200 mM chloride) to sites associated with the $GABA_A$ receptor-coupled chloride ionophore. Saturation analysis revealed that this effect could be attributed entirely to a decrease in the maximum number of binding sites. Exposure to UV irradiation at lower (254 nm) and higher (366 nm) wavelengths also inhibited [^{35}S]*t*-butylbicyclophosphorothionate binding, but the respective rates of inactivation were 8- and 27-fold slower, compared with 302 nm. Other anion-dependent interactions at the $GABA_A$ receptor complex were disrupted in a similar manner. In the absence of permeant anion, [^{3}H]flunitrazepam binding to benzodiazepine receptors was unaffected by 302 nm UV irradiation, whereas chloride-enhanced [^{3}H]flunitrazepam binding was inhibited markedly. In the presence of 250–500 mM chloride, [^{3}H]methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate binding to benzodiazepine receptors was also inhibited after UV exposure. Basal $^{36}Cl^-$ uptake into synaptoneurosomes was nearly doubled after 15 min of exposure to 302 nm light, whereas pentobarbital- and muscimol-stimulated $^{36}Cl^-$ uptake were reduced significantly. UV irradiation at 302 nm appears to disrupt selectively the anion-dependent functional interactions at the $GABA_A$ receptor complex. The apparent wavelength specificity suggests that the gating structure (channel) may contain tryptophan and/or tyrosine residues vital to the regulation of anion movement through the ionophore portion of this supramolecular receptor-ion channel complex.

BZs, barbiturates, and other clinically useful drugs produce their distinctive neuropharmacological effects by modulating the activity of GABA-gated Cl^- channels (1, 2). A functional association between these channels and radioligand binding to BZ receptors was first suggested by studies reporting that small anions (such as Cl^-) increased the apparent affinity of [^{3}H]BZs for their receptors (3–5). Subsequent investigations have shown that anions that are permeant through Cl^- channels can modulate radioligand binding to other components of the $GABA_A$ receptor complex. Thus, barbiturate and pyrazolopyridine enhancement of radioligand binding to BZ and GABA receptors is anion dependent (6–9), as is the binding of the "cage" convulsant [^{35}S]TBPS (10, 11). Electrophysiological studies suggest that specific anion binding sites within GABA-gated Cl^- channels impart selectivity to these channels, determining the type of anions gated when ligands occupy the multiple, allosterically interacting, recognition sites on this supramolecular complex (12–14). Several lines of neurochemical evidence, including robust correlations between the potencies of a series of monovalent anions to support the binding of [^{35}S]TBPS and BZ receptor ligands and their permeabilities through GABA-gated Cl^- channels, suggest that these anion recognition sites also modulate anion-dependent radioligand binding to the constituent recognition sites on the $GABA_A$ receptor complex (3, 11, 15, 16).

Photoaffinity labeling techniques have proven useful in the characterization of the $GABA_A$/BZ receptor complex (17–19). During the course of studies attempting to photolabel selectively the Cl^- ionophore portion of the $GABA_A$ receptor complex, we observed that a brief exposure to 302 nm UV irradiation abolished [^{35}S]TBPS binding. This finding and the subsequent demonstration that other anion-modulated events at the $GABA_A$ receptor complex are disrupted suggest that tyrosine and tryptophan residues constitute part of a specific anion recognition site that is a target of UV irradiation.

Methods

Animals. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) (250–350 g) were housed in a vivarium with a 12 hr light/dark cycle (lights on, 0700). Rat chow and water were available *ad libitum*. Experiments were initiated between 0800 and 0930. Animals were sacrificed by decapitation, and their brains were immediately removed for use in either radioligand binding or $^{36}Cl^-$ uptake studies.

ABBREVIATIONS: BZ, benzodiazepine; GABA, γ-aminobutyric acid; TBPS, *t*-butylbicyclophosphorothionate; FNZ, flunitrazepam; DMCM, methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

Radioligand binding assays. Brains were placed into ice-cold 50 mM Tris-citrate buffer (pH 7.4), dissected, weighed, and disrupted in 50 volumes of the same buffer with a Brinkman Polytron (setting 5–6, 15 sec). Homogenates were centrifuged at 20,000 × *g* for 20 min at 4°. Tissues were resuspended and recentrifuged ("washed") five more times before final resuspension in 50 volumes of 50 mM Tris-citrate buffer.

[^{35}S]TBPS binding was assayed by a modification of the method of Squires *et al.* (10). Incubations consisted of 0.3 ml of tissue suspension (250–350 μg of protein), 0.1 ml of [^{35}S]TBPS (60–120 Ci/mmol) diluted in Tris-citrate buffer to a final concentration of 2–5 nM, 0.05 ml of 2 M NaCl (200 mM final), and 0.05 ml of drugs or buffer, in a final volume of 0.5 ml. In some experiments, saturation isotherms were constructed by diluting [^{35}S]TBPS with TBPS, 1:25, followed by serial dilution of the resulting radiolabeled/nonradiolabeled mixture to concentrations ranging between 0.1 and 4.0 μM. Incubations were initiated by addition of radioligand and terminated after 2 hr at room temperature by rapid filtration through Whatman GF/B glass fiber filters, followed by washing with two 5-ml aliquots of assay buffer, using a Brandel M-48R filtering manifold (Brandel Instruments, Gaithersburg, MD). Nonspecific binding was defined using 200 μM picrotoxinin and typically represented less than 10% of total binding at a concentration of 2–5 nM radioligand.

[^{3}H]DMCM binding was assayed using a modification of the method of Braestrup *et al.* (20). Incubations consisted of 0.2 ml of fresh tissue suspension (150–250 μg of protein), 0.1 ml of [^{3}H]DMCM (80.7 Ci/mmol, diluted in Tris-citrate buffer to a final concentration of ~1 nM), 0.1 ml of salt solutions, drugs, and/or Tris-citrate buffer, in a final volume of 1.0 ml. Incubations (0–4°) were initiated by addition of radioligand and terminated after 60 min by filtration through GF/B filters, followed by washing with three 5-ml aliquots of ice-cold assay buffer. Nonspecific binding was defined using 10 μM FNZ and represented approximately 30% of total binding in the absence of Cl^-. [^{3}H] FNZ binding (90 Ci/mmol) was assayed under identical conditions, except that 0.1 ml of tissue suspension (75–125 μg of protein) was utilized. Nonspecific binding (defined using 10 μM FNZ) was consistently less than 2% of total binding. All assays contained citrate anion (160–200 mM). Chloride was added as the sodium salt.

$^{36}Cl^-$ uptake. $^{36}Cl^-$ uptake was studied in synaptoneurosomes (21) that were prepared from rat cerebral cortex using a modification of the method described by Schwartz *et al.* (22). Cerebral cortices (typically ~4 g of tissue from five rats) were removed and homogenized (five strokes) in 2 volumes of a HEPES/salt buffer solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20 mM HEPES, and 10 mM Tris), pH 7.4. Homogenates were filtered through three layers of nylon mesh (160 μm; Lawshe Instrument Co., Rockville, MD) and a 10-μm filter (No. LCWP047; Millipore, Bedford MA). The filtrate was centrifuged for 15 min at 1000 × *g* (4°), gently resuspended in 30 ml, and recentrifuged. The resulting pellet was resuspended in an equal volume of buffer and, after exposure to UV irradiation, incubated at 37° for 20 min before $^{36}Cl^-$ uptake was measured. Reactions were performed in a volume of 0.5 ml, consisting of 0.4 ml of tissue suspension (~2 mg of protein) and 0.1 ml of a mixture of drug and 0.5 μCi of $^{36}Cl^-$ (13.8 mCi/g) in assay buffer. The reactions were initiated by addition of the drug/isotope mixture and terminated after 5 sec by filtration under vacuum through glass fiber filters (No. 30; Schleicher & Schuell, Keene, NH) and washing with 5 ml of buffer (0–4°) containing picrotoxin (100 μM). The filter was washed twice more with buffer and the $^{36}Cl^-$ retained on the filter was determined by liquid scintillation counting.

UV irradiation. Membranes in 50 mM Tris-citrate were poured into Petri dishes, to a depth of no more than 0.5 cm, and placed on ice. A 254 nm UV light source (Spectroline Model EB-280C UV lamp; Spectronics Corp., Westbury, NY) or a 302/366 nm source (Mineralight UVSL-58 Multiband UV lamp; UV Products, San Gabriel, CA) was positioned 5 cm from the top of the membrane layer and illuminated for the indicated length of time. Control (nonirradiated) membrane preparations were treated identically except that the UV source was not turned on. Except where noted, membranes were irradiated after the washing procedure and assayed immediately for binding studies. For uptake studies, synaptoneurosomes were irradiated immediately before their incubation at 37°.

Protein content was determined by the Miller (23) modification of the method of Lowry *et al.* (24) using bovine serum albumin as a standard. The radioactivity retained by the filters was measured using a Beckman LS 5801 liquid scintillation counter. K_d and B_{max} values were estimated using both linear and nonlinear fitting methods (Lundon Software, Cleveland, OH, and MLAB, National Institutes of Health). EC_{50} and E_{max} values were calculated by nonlinear regression using the sigmoidal function $f(x) = A + (B - A)/(1 + ((10^X)^D/(10^C)^D)$, where $f(x)$ = binding at a given concentration, x, of drug or anion, A = minimum binding, B = maximum binding, C = concentration of drug or anion producing half-maximal effect (EC_{50}), and D = Hill slope (positive for activation, negative for inhibition) (25) (GraphPad; ISI Software). Half-lives were calculated by fitting time-course data to the equation $f(x) = Ae^{-BX}$, where $f(x)$ = binding at time x, A = binding at time zero, and B = time constant equal to $\ln 2/t_{1/2}$ (25) (GraphPad; ISI Software). Data are expressed as means ± standard errors. Significance was calculated by Student's t test (two-tailed, unpaired).

Materials. $^{36}Cl^-$, [^{3}H]FNZ, and [^{3}H]DMCM were obtained from New England Nuclear (Boston, MA). Picrotoxin, picrotoxinin, and muscimol were obtained from Sigma Chemical Co. (St. Louis, MO). Unlabeled FNZ was donated by Hoffman-LaRoche (Nutley, NJ).

Results

Effect of UV irradiation on [^{35}S]TBPS binding. The effect of UV irradiation on [^{35}S]TBPS binding to rat cerebral cortical membranes exposed to varying wavelengths (254, 302, or 366 nm) of UV light is illustrated in Fig. 1. All three wavelengths produced a time-dependent reduction in the amount of [^{35}S]TBPS bound in the presence of 200 mM NaCl. The loss of radioligand binding was a monoexponential function of the length of exposure to UV irradiation ($t_{1/2}$ = 32.4, 4.0, and 107.1 min for 254, 302, and 366 nm, respectively). Rates of reduction of [^{35}S]TBPS binding were not affected by the addition of up to 500 mM NaCl or NaBr to membrane preparations before irradiation, nor were there significant differences between experiments in which membranes were washed before or after UV exposure (data not shown). All subsequent experi-

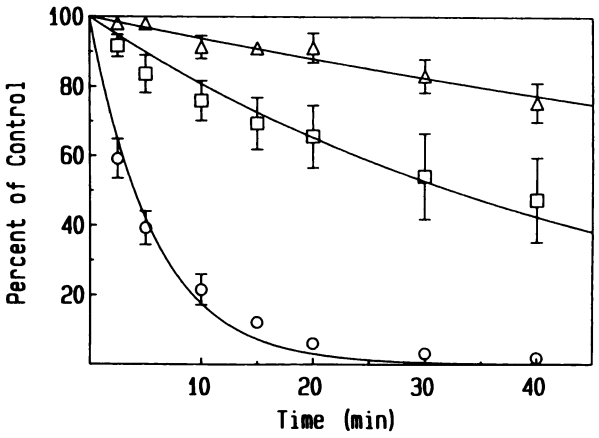

Fig. 1. Effect of time and wavelength of UV irradiation on [^{35}S]TBPS binding in the presence of 200 mM Cl^-. Rat cortical membranes were exposed for indicated times at wavelengths of 254 (□), 302 (○), or 366 nm (△). Results are expressed as percentage of control and represent mean ± standard error (control = 46.9 fmol/mg of protein, three experiments). The concentration of [^{35}S]TBPS was 2.8 nM. Two-way ANOVA indicates significant ($p < .001$) effect of time, wavelength, and interaction.

ments utilized 302 nm UV irradiation of washed membranes in 50 mM Tris-citrate buffer.

UV irradiation produced a time-dependent reduction in the number of [^{35}S]TBPS binding sites in both cerebral cortex and cerebellum (Fig. 2; Table 1). Thus, 5 or 15 min of exposure reduced the B_{max} for [^{35}S]TBPS in both cortical and cerebellar membranes by 38–91%. UV irradiation had no effect on the apparent affinity (K_d) of [^{35}S]TBPS in either brain region.

Effect of UV irradiation on synaptoneurosomal $^{36}Cl^-$ uptake. Synaptoneurosomes prepared from rat cortex were irradiated for 15 min, followed by assay of basal and muscimol- and pentobarbital-stimulated $^{36}Cl^-$ uptake (Fig. 3). Whereas 15 min of UV exposure doubled basal $^{36}Cl^-$ uptake (15.6 ± 1.3 versus 31.4 ± 1.0 nmol/assay for controls and irradiated tissues, respectively; six experiments; $p < 0.001$), both muscimol- and pentobarbital-promoted $^{36}Cl^-$ uptake (Fig. 3) were blunted significantly. Thus, in the absence of irradiation, muscimol (2–50 μM) and pentobarbital (0.1–1 mM) both stimulated net $^{36}Cl^-$ uptake by 80–300% whereas, after irradiation, maximal stimulation was reduced to 27 and 59%. There was no effect on the potency of either compound.

Effect of UV irradiation on radioligand binding to benzodiazepine receptors. Cortical membranes were irradiated for 10 min, followed by assay of binding of the BZ agonist [^{3}H]FNZ and the inverse agonist [^{3}H]DMCM at Cl^- concentrations between 0 and 500 mM. Basal [^{3}H]FNZ binding was unaffected by 10 min of UV exposure (control = 608 ± 37 fmol/mg versus UV = 652 ± 51; three experiments; see also Fig. 4A); Cl^--enhanced [^{3}H]FNZ binding was reduced by as much as 70%. Similarly, [^{3}H]DMCM binding in the presence of 250–500 mM Cl^- was also diminished after UV irradiation (Fig. 4B). However, in contrast to [^{3}H]FNZ binding, [^{3}H]DMCM binding in the absence of permeant anion was increased 3-fold after UV exposure for 10 min (control = 44.7 ± 1.8 fmol/mg versus UV = 135.4 ± 6.0; three experiments; $p < 0.001$). This increase in basal binding was time dependent (175 ± 9 and 199 ± 33% of control after 2.5 and 10 min of irradiation, respectively; $p < 0.002$) and occurred also in cerebellar membranes (203 ± 13

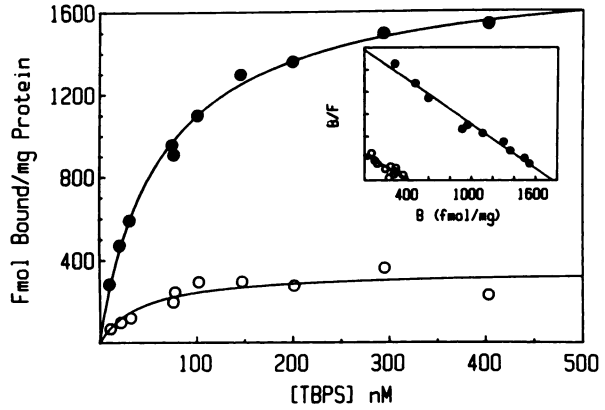

Fig. 2. Effect of UV irradiation on [^{35}S]TBPS binding. Cortical membranes were exposed for 0 (●) or 15 min (○) followed by determination of TBPS binding in the presence of 200 mM Cl^-. *Inset*, Scatchard transformation of the same data. A typical experiment is shown. See Table 1 for K_d, and B_{max} values.

TABLE 1

Effect of UV irradiation on [^{35}S]TBPS binding

Rat cortical and cerebellar membranes were prepared as described in Methods and exposed to 302 nm UV light for the indicated times. Radioligand binding was determined at 0.01–4.0 μM TBPS in the presence of 200 mM Cl^-; K_d (nM) and B_{max} (fmol/mg of protein) values were calculated from the resulting saturation isotherms, as described in Methods. Values represent mean ± standard error (three experiments).

	Cortex		Cerebellum	
	K_d	B_{max}	K_d	B_{max}
	nM	fmol/mg of protein	nM	fmol/mg of protein
Control	28.5 ± 1.7	1612 ± 239	32.6 ± 3.5	1480 ± 78
UV				
5 min	35.8 ± 3.1	1007 ± 179	35.7 ± 0.8	738 ± 119[a]
15 min	29.0 ± 1.1	236 ± 55[b]	35.0 ± 3.0	138 ± 49[b]

[a] $p < 0.05$.
[b] $p < 0.02$ compared with control (paired t test).

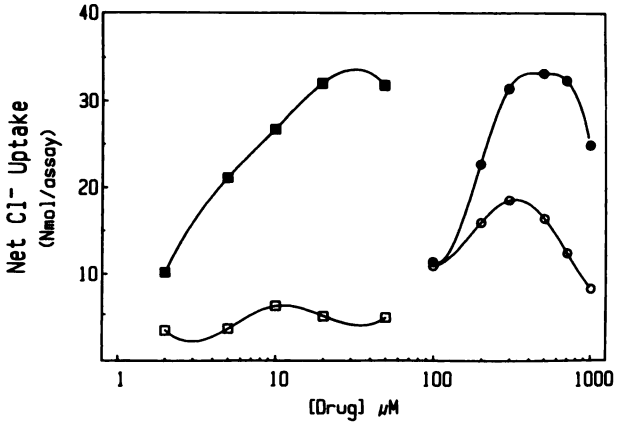

Fig. 3. Effect of UV irradiation on $^{36}Cl^-$ uptake into cortical synaptoneurosomes. Tissue was exposed for 0 (*filled symbols*) or 15 min (*open symbols*), followed by assay of $^{36}Cl^-$ uptake in the presence of indicated concentrations of muscimol (*squares*) or pentobarbital (*circles*). Results are expressed as net stimulated Cl^- uptake above basal levels and represent the mean of three experiments. Two-way ANOVA indicates significant ($p < 0.05$ or better) effect of UV irradiation, pentobarbital or muscimol, and their interaction.

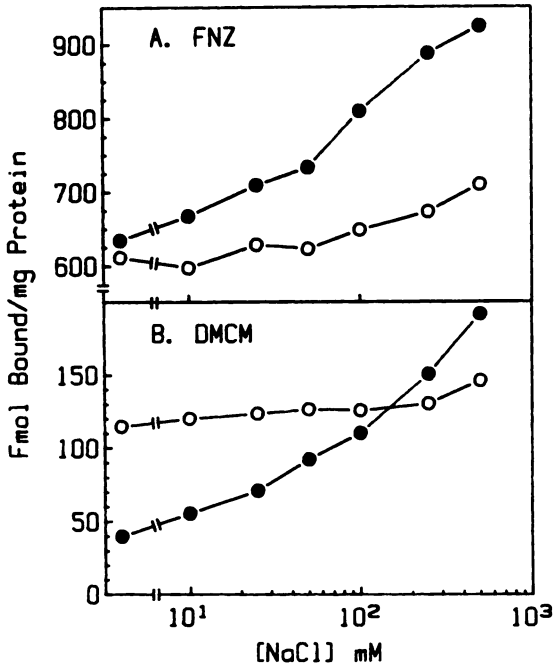

Fig. 4. Effect of UV irradiation on binding of [^{3}H]FNZ (A) and [^{3}H]DMCM (B). Cortices were irradiated for 0 (●) or 10 min (○). Results depict typical results from three determinations. Concentrations of radioligand were 1.3 (A) and 0.9 nM (B).

and 319 ± 33% of control after 2.5 and 10 min; $p < 0.05$). This UV-induced increase appeared to be due to an increase in affinity (decreased K_d) for the ligand (Fig. 5; Table 2), although the magnitude of the effect did not reach statistical significance. Reduction of chloride-mediated stimulation of [^{3}H]DMCM binding after UV irradiation appeared to be due to reversal of the Cl^- effect on both maximal binding and affinity. Thus, in control membranes, Cl^- appeared to increase both affinity (reduced K_d; $p < 0.05$ versus no Cl^-) and B_{max} ($p < 0.05$) for [^{3}H]DMCM; in irradiated membranes, the affinity and B_{max} increases were both attenuated ($p < 0.02$ versus no UV).

Maximal enhancement of [^{3}H]FNZ binding by pentobarbital (in the presence and absence of 50 mM NaCl) was reduced modestly (18–19%) following 10 min of UV exposure (Fig. 6; Table 3). The potency of pentobarbital was not altered significantly. GABAergic augmentation of [^{3}H]FNZ binding was also attenuated by UV irradiation (Fig. 7; Table 3). The maximal effect of GABA was reduced by 17–35% following 10 min of UV exposure. The potency of GABA was also decreased by UV irradiation when enhancement was measured in the absence of Cl^-. However, in the presence of Cl^-, the EC_{50} for GABA was increased slightly.

Discussion

The effector component of the GABA/BZ receptor complex is an ionophore that exhibits selective permeability to small, negatively charged anions such as Cl^-. Electrophysiological studies indicate clearly that anions differ greatly with respect

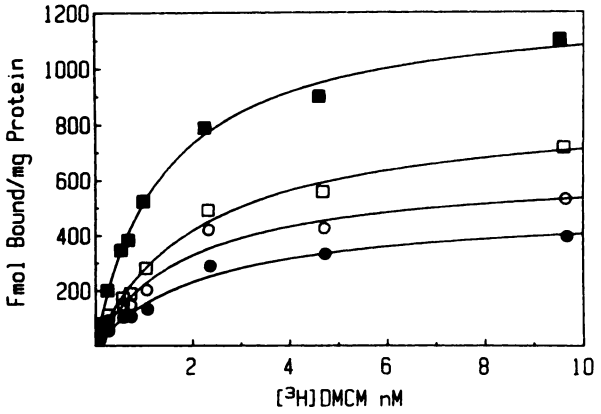

Fig. 5. Effect of UV irradiation on [^{3}H]DMCM binding. Cortical membranes were exposed for 0 (*filled symbols*) or 10 min (*open symbols*), followed by assay of [^{3}H]DMCM binding in the presence (*squares*) or absence (*circles*) of 250 mM NaCl. A typical experiment is shown. See Table 2 for K_d and B_{max} values.

TABLE 2

Effect of UV irradiation on [^{3}H]DMCM binding

Rat cortical membranes were prepared and treated as described in Methods and Table 1. K_d (nM) and B_{max} (fmol/mg of protein) values were calculated from the resulting saturation isotherms, as described in Methods, and correspond to results depicted in Fig. 5. Values represent mean ± standard error (three experiments).

	K_d		B_{max}	
	No Cl^-	250 mM Cl^-	No Cl^-	250 mM Cl^-
	nM		*fmol/mg of protein*	
Control	2.00 ± 0.50	1.08 ± 0.19[a]	658 ± 72	1286 ± 85[a]
UV—10 min	1.32 ± 0.38	1.74 ± 0.36[b]	644 ± 72	923 ± 83[b]

[a] $p < 0.02$ compared with no Cl^- (same row).
[b] $p < 0.05$ compared with no UV (same column).

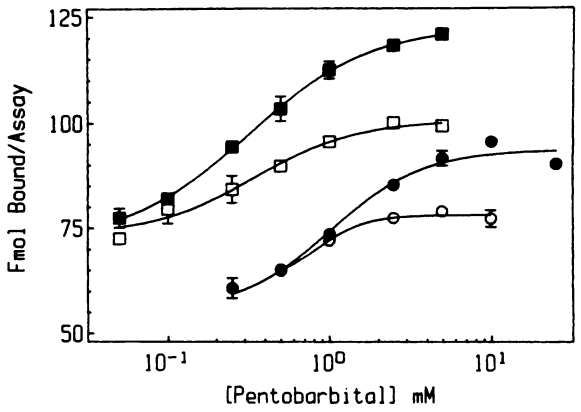

Fig. 6. Effect of UV irradiation on modulation of [^{3}H]FNZ binding by pentobarbital. Tissues were exposed for 0 (*filled symbols*) or 10 min (*open symbols*), followed by assay of [^{3}H]FNZ binding in the presence (*squares*) or absence (*circles*) of 50 mM NaCl. Results are expressed as fmol bound/assay and depict typical results from three determinations. The concentration of [^{3}H]FNZ was 1.6 nM. See Table 3 for EC_{50} and E_{max} values.

TABLE 3

Effect of UV irradiation on modulation of [^{3}H]FNZ binding

Rat cortical and cerebellar membranes were prepared and treated as described in Methods and Table 1. Radioligand binding was determined at 1.5–1.6 nM [^{3}H]FNZ. EC_{50} (mM or μM as indicated) and E_{max} (fmol/assay) values were calculated from data in Figs. 6 and 7 as described in Methods. Values represent mean ± standard error (three experiments).

	EC_{50}		E_{max}	
	Control	UV, 10 min	Control	UV, 10 min
	mM		*fmol/assay*	
Pentobarbital	1.04 ± 0.10	0.75 ± 0.05	94.2 ± 1.0	76.4 ± 1.0[a]
Pentobarbital + 50 mM NaCl	0.30 ± 0.02[b]	0.33 ± 0.10[b]	122.0 ± 2.0[b]	100.3 ± 1.0[a,b]
GABA μM	2.26 ± 0.23	3.09 ± 0.20	94.2 ± 16.0	78.5 ± 10.1
GABA + 50 mM NaCl	3.84 ± 0.26[b]	0.51 ± 0.02[a]	164.0 ± 16.0[b]	106.0 ± 14.5

[a] $p < 0.05$ compared with control (same row).
[b] $p < 0.05$ compared with no NaCl (same column).

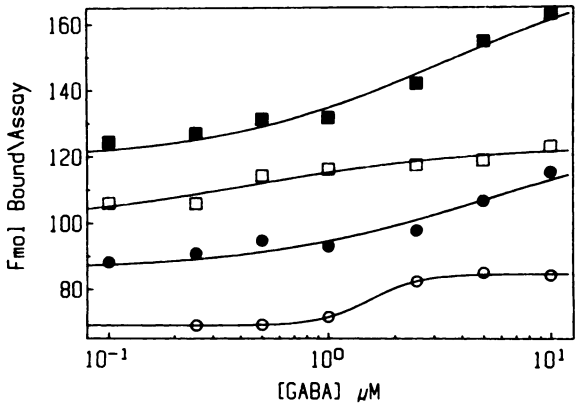

Fig. 7. Effect of UV irradiation on modulation of [^{3}H]FNZ binding by GABA. Tissues were exposed for 0 (*filled symbols*) or 10 min (*open symbols*), followed by assay of [^{3}H]FNZ binding in the presence (*squares*) or absence (*circles*) of 50 mM NaCl. Results are expressed as fmol bound/assay and depict typical results from three determinations. The concentration of [^{3}H]FNZ was 1.5 nM. See Table 3 for EC_{50} and E_{max} values.

to their permeabilities through GABA-regulated Cl^- channels, indicating the presence of a selectivity filter within these complexes (12–14). In addition, anion permeabilities deduced from such studies are highly correlated with their potencies to enhance the binding of both the cage convulsant [^{35}S]TBPS and BZ agonist and inverse agonists (also referred to as positive and negative BZ ligands) (11, 16, 26). These observations strongly suggest that ligand occupation of various recognition components of the supramolecular complex is regulated by the same anion binding site that selectively "gates" anions at the ionophore portion of this complex. The present findings suggest that UV irradiation selectively disrupts this anion binding site, affecting its ability to gate anions and modulate binding to receptor sites within the supramolecular complex.

After a brief (5 min) exposure to 302 nm light, the number of [^{35}S]TBPS binding sites was reduced by 38–50% in cortical and cerebellar membranes. The apparent affinity of [^{35}S]TBPS was unchanged, suggesting an "all or none" phenomenon in which UV irradiation selectively destroys the ability of the GABA-gated Cl^- channel (where cage convulsants such as TBPS are thought to act) to accommodate the radioligand. UV inactivation was unaffected by the presence of endogenous GABA (unwashed membranes) or of up to 500 mM chloride or bromide during irradiation, suggesting that ligands for GABA and anion binding sites fail to protect the [^{35}S]TBPS binding site from the destructive effect of UV irradiation.

Brief periods of UV exposure, which diminished [^{35}S]TBPS binding, had no significant effect on the binding of [^{3}H]FNZ in the absence of permeant anion. Thus, the wavelength and target (receptor site) specificity suggest an action of UV irradiation on a distinct component of the $GABA_A$ receptor, rather than a generalized effect on all radioligand binding sites. Moreover, the dramatic reduction in Cl^--mediated promotion of [^{3}H]FNZ binding reinforces the premise that UV irradiation disrupts the anion binding site and confirms previous suggestions that anions act to promote BZ agonist binding at a site closely associated with the [^{35}S]TBPS binding site (16, 26).

In contrast, the basal binding of the BZ inverse agonist [^{3}H]DMCM was increased after UV irradiation, whereas anion enhancement of binding was blocked, in a manner similar to that of the BZ agonist [^{3}H]FNZ. Recent studies have suggested that both BZ agonist and inverse agonist binding domains within the supramolecular complex are regulated by the same site that governs anion selectivity, demonstrated in electrophysiological and biochemical studies (16). However, there were important differences between the two types of BZ ligands, both in the magnitude of anion enhancement of their binding (i.e., the Cl^- effect on [^{3}H]DMCM binding is approximately 10-fold greater than the effect on [^{3}H]FNZ binding) and in the mechanism by which anions produce their results (increased affinity and number of sites for [^{3}H]DMCM versus only increased affinity for [^{3}H]FNZ). The apparent affinity of [^{3}H]DMCM binding was increased by UV irradiation when measured in the absence of Cl^-. In the presence of 250 mM NaCl, UV irradiation appeared to reduce the number of sites (the Cl^--induced B_{max} increase was only 43% for UV versus 95% for controls), as well as the apparent affinity for [^{3}H]DMCM (the K_d was higher than both control plus 250 mM Cl^- and UV with no Cl^-). Thus, it appears that there are important differences in the way in which BZ agonist and inverse agonist binding sites are influenced by the anion binding site but that anion enhancement of both types of binding is dependent on a common recognition site, which is disturbed by UV exposure.

The effect of UV irradiation on modulation of BZ binding by GABA-mimetic and barbiturate ligands also appears to be influenced by, but not completely dependent on, the anion binding domain of the Cl^- ionophore. Barbiturate- and GABA-stimulated [^{3}H]FNZ binding were diminished following UV irradiation, both in the presence and in the absence of 50 mM NaCl. The effect on pentobarbital-enhanced [^{3}H]FNZ binding was manifest as a decrease in the maximal effect of the drug, consistent with the decreased E_{max} for net $^{36}Cl^-$ uptake observed after UV irradiation. A similar parallel effect was observed for the efficacy of GABA to enhance $^{36}Cl^-$ uptake and [^{3}H]FNZ binding, except that shifts in GABA potency were also evident for the latter effect. These changes in the EC_{50} of GABA to enhance BZ binding could have been secondary to the E_{max} changes. The shift in efficacy but not potency of pentobarbital suggests that UV light may not directly affect the barbiturate binding site but, instead, that the result observed (decreased efficacy of pentobarbital to alter [^{3}H]FNZ binding) is a manifestation of the BZ receptor-anion binding site interaction. The lack of a suitable barbiturate radioligand precludes directly testing this hypothesis.

Functional evidence that UV irradiation perturbs the anion binding properties of the GABA/BZ receptor complex is provided by the data measuring Cl^- uptake into synaptoneurosomes (Fig. 3). Under basal conditions, Cl^- uptake was nearly doubled after 15 min of UV irradiation at 302 nm, whereas muscimol- and pentobarbital-mediated stimulation of Cl^- uptake were markedly decreased. These data suggest that perturbation by UV irradiation may "lock" the anion channel in an open conformation, disrupting the ability of GABA and barbiturate ligands to modulate the movements of anions such as Cl^- through the channel.

Because 302 nm irradiation disrupted [^{35}S]TBPS binding 8- to 27-fold more rapidly than 254 and 366 nm irradiation, it appears that the UV effect is not a function of the radiant energy applied but instead may be related to the absorptive properties of the target site(s). An absorption maximum closer to 300 nm than to 250 nm is characteristic of the aromatic amino acids tryptophan and tyrosine, but not phenylalanine (27). The recent determination of the amino acid sequences of the BZ and GABA receptor subunits (28) places significant numbers of these amino acids in positions that could have a significant impact on the movement of anions through the ionophore. Thus, both tyrosine and tryptophan residues are found in six of eight hydrophobic regions that are postulated to comprise the membrane-spanning domains constituting the ion channel (28). In addition, tyrosine residues are found within one to four positions of positively charged residues located in four regions thought to be located on the extracellular face of the complex near the channel opening. These positively charged regions could comprise part of the anion binding site, and the tyrosine residues contained in these sequences may play an important role in its tertiary structure. The ability of brief exposure to 302 nm UV light to disrupt anion-mediated events within the supramolecular complex strongly suggests a role for tyrosine and tryptophan in the putative anion binding site that regulates the specificity of anion movements gated by GABA and the BZs. Moreover, the use of 302 nm UV light to selectively disrupt anion-mediated events at GABA-gated Cl^- channels

may provide a useful tool in the further elucidation of the pharmacology and physiology of these sites.

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