Positive allosteric modulation by ultraviolet irradiation on $GABA_A$, but not $GABA_C$, receptors expressed in *Xenopus* oocytes

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1. Recombinant rat $GABA_A$ ($\alpha1\beta2$, $\alpha1\beta2\gamma2$, $\beta2\gamma2$) and human $GABA_C$ ($\rho1$) receptors were expressed in *Xenopus* oocytes to examine the effect of ultraviolet (UV) light on receptor function.

2. GABA-induced currents in individual oocytes expressing GABA receptors were tested by two-electrode voltage clamp before, and immediately after, 312 nm UV irradiation.

3. UV irradiation significantly potentiated 10 μM GABA-induced currents in $\alpha1\beta2\gamma2$ GABA receptors. The modulation was irradiation dose dependent, with a maximum potentiation of more than 3-fold.

4. The potentiation was partially reversible and decayed exponentially with a time constant of 8.2 ± 1.2 min toward a steady-state level which was still significantly elevated (2.7 ± 0.3-fold) compared to the control level.

5. The effect of UV irradiation on $GABA_A$ receptors varied with receptor subunit composition. UV irradiation decreased the EC_{50} of the $\alpha1\beta2$, $\alpha1\beta2\gamma2$ and $\beta2\gamma2$ $GABA_A$ receptors, but exhibited no significant effect on the $\rho1$ $GABA_C$ receptor.

6. UV irradiation also significantly increased the maximum current 2-fold in $\alpha1\beta2$ $GABA_A$ receptors with little effect on the maximum of $\alpha1\beta2\gamma2$ (1.1-fold) or $\beta2\gamma2$ (1.1-fold) $GABA_A$ receptors.

7. The effect of UV irradiation on $GABA_A$ receptors did not overlap the effect of the GABA receptor- allosteric modulator, diazepam.

8. The UV effect on $GABA_A$ receptors was not prevented by the treatment of the oocytes before and during UV irradiation with one of the following free-radical scavengers: 40 mM D-mannitol, 40 mM imidazole or 40 mM sodium azide. In addition, the effect was not mimicked by the free-radical generator, H_2O_2.

9. Potential significance and mechanism(s) of the UV effect on GABA receptors are discussed.

γ-Aminobutyric acid (GABA)-gated chloride channels mediate fast inhibitory synaptic transmission, thereby controlling neuronal excitability. These chloride channels can be classified into $GABA_A$ and $GABA_C$ receptors according to their pharmacological properties. $GABA_A$ receptors are allosterically modulated by benzodiazepines, barbiturates and neurosteroids, and antagonized by bicuculline, whereas $GABA_C$ receptors are insensitive to these compounds (Woodward *et al.* 1992; Feigenspan *et al.* 1993; Qian & Dowling, 1993; Macdonald & Olsen, 1994; Johnston, 1996; Lukasiewicz, 1996; Ueno *et al.* 1997). These two types of GABA receptors also differ in their physiological properties and distribution. For example, $GABA_A$ receptors have fast activation and deactivation kinetics and show significant desensitization during prolonged agonist exposure, whereas $GABA_C$ receptors have slow kinetics without significant desensitization (Cutting *et al.* 1991, Amin & Weiss, 1994). While $GABA_A$ receptors are widely distributed in the central nervous system and retina, $GABA_C$ receptors are mainly localized in the retina (Enz *et al.* 1995, 1996; Yeh *et al.* 1996; Albrecht *et al.* 1997; Wegelius *et al.* 1998).

Molecular cloning has revealed several GABA-gated ion channel subunits and their isoforms α1-6, β1-4, γ1-3, ρ1-3, δ, ϵ, π and χ (Barnard *et al.* 1987; Schofield *et al.* 1987; Khrestchatisky *et al.* 1989; Olsen & Tobin, 1990; Cutting *et al.* 1991; Garret *et al.* 1997; Hedblom & Kirkness, 1997; Whiting *et al.* 1997). The exogenous expression of GABA receptors simplifies studies by avoiding the complication of heterogeneous populations of GABA receptor subtypes in the same cell. Such studies have revealed that the distinct physiological and pharmacological properties of $GABA_A$ and $GABA_C$

receptors are due to the differences in their subunit compositions. Recombinant $\alpha\beta\gamma$ GABA receptors have pharmacological and physiological properties similar to native $GABA_A$ receptors (Levitan *et al.* 1988; Pritchett *et al.* 1989; Malherbe *et al.* 1990; Sigel *et al.* 1990; Verdoorn *et al.* 1990), whereas exogenously expressed ρ1 homomeric GABA receptors have properties similar to native $GABA_C$ receptors (Cutting *et al.* 1991). The distinct pharmacological properties and spatial distribution for receptors with different subunit composition is the basis for developing subunit-specific therapeutic approaches for the treatment of insomnia, epilepsy and other neurological disorders.

In this study, we observed that UV irradiation has differential effects on recombinant $GABA_A$ and $GABA_C$ receptors. $\alpha1\beta2$, $\beta2\gamma2$ and $\alpha1\beta2\gamma2$ GABA receptors can be potentiated by UV irradiation whereas ρ1 GABA receptors are insensitive to UV light. The potentiation of $GABA_A$ receptors by UV irradiation does not overlap with the effect of the $GABA_A$ receptor-allosteric modulator, diazepam. The elucidation of the molecular mechanism could facilitate the identification of new structural element(s) crucial for $GABA_A$ receptor function. It could also help us to understand the pathophysiology of UV-induced retina damage, and facilitate the development of strategies to prevent or reverse the damage.

METHODS

cDNA and cRNA preparation

cDNAs encoding rat GABA receptor subunits α1, β2 and γ2L were cloned into pAlter-1 in the SP6 orientation (Amin *et al.* 1994) and the human ρ1 GABA receptor subunit was cloned into pAlter-1 in the T7 orientation (Amin & Weiss, 1994). The cDNAs were linearized by *Ssp*I (α, β and γ) or *Nhe*I (ρ1). cRNAs were transcribed by standard *in vitro* transcription protocols. Briefly, RNase-free DNA templates were prepared by treating linearized DNA with proteinase K. The cRNAs then were transcribed by SP6 RNA polymerase (α, β and γ subunits) or T7 RNA polymerase (ρ1 subunit). After degradation of the DNA template by RNase-free DNase I, the cRNAs were purified and resuspended in diethyl pyrocarbonate (DEPC)-treated water. cRNA yield and integrity were examined on a 1% agarose gel.

Oocyte preparation and receptor expression

Female *Xenopus laevis* (Xenopus I, Ann Arbor, MI, USA) were anaesthetized by 0.2% MS-222 (Sigma, St Louis, MO, USA). The ovarian lobes were surgically removed from the frog and placed in calcium-free OR2 incubation solution consisting of (mM): 92.5 NaCl, 2.5 KCl, 1 $MgCl_2$, 1 Na_2HPO_4, and 5 Hepes; plus 50 U ml^{-1} penicillin and 50 μg ml^{-1} streptomycin, pH 7.5. The frog was then allowed to recover from surgery before being returned to the incubation tank. After the third surgery, the frog was killed by decapitation while still under anaesthesia. This procedure was carried out in accordance with the rules and regulations set forth by the UAB Animal Care Committee. The ovarian lobes were cut into small pieces, and digested with 0.3% Collagenase A (Boehringer-Mannheim Biochemicals, Indianapolis, IN, USA) with constant stirring at room temperature for 1.5–2 h. The dispersed oocytes were thoroughly rinsed with the above solution plus 1 mM $CaCl_2$. The stage VI oocytes were selected and the follicular layer (if still present) was manually removed with fine forceps. The oocytes were incubated at 18°C before injection.

Micropipettes for cRNA injection were pulled from borosilicate glass (Drummond Scientific, Broomall, PA, USA) on a Sutter P87 horizontal puller, and the tips were cut with forceps to ~40 μm in diameter. The cRNA, with proper dilution in DEPC-treated water, was drawn up into the micropipette and injected into oocytes with a Nanoject micro-injection system (Drummond Scientific) at a total volume of 20–60 nl.

Electrophysiology

One to three days after cRNA injection, the oocyte was placed in a 100 μl chamber with continuous OR2 perfusion. The oocytes were voltage clamped at −70 mV to measure GABA-induced currents using a GeneClamp 500 (Axon Instruments, Foster City, CA, USA). The OR2 was used as an extracellular solution, which consisted of the following (mM): 92.5 NaCl, 2.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 5 Hepes, pH 7.5.

Ultraviolet irradiation

After recording control GABA-induced currents, each oocyte was taken out of the recording chamber and placed onto a FBTIV-614 transilluminator (Fisher Scientific, Pittsburgh, PA, USA). Except for the UV dose–response experiments, the oocyte was irradiated by UVB (312 nm) at 8 mW cm^{-2} for 1 min. The oocyte was then placed back into the recording chamber to assess the UV effect with the same set of GABA concentrations. For spectral response comparison, an ELC-403 UV curing light gun (Edmund Optics, Barrington, NJ, USA) emitting UVA (340–380 nm, peak at 365 nm) with a minimum output of 40 mW cm^{-2} at 2.54 cm distance (Edmund Optics) was used. The oocyte was taken out of the recording chamber and placed onto a fused silica glass window mounted in the bottom of a Petri dish. The UV gun was directed from the bottom of the fused silica glass window for 1 min at the oocyte. For visible light stimulation, an illuminator with an EKE type 150 W halogen lamp (Southern Micro Instruments, Marietta, GA, USA) was used with a fibre optic guide pointed towards the oocyte in the recording chamber. After voltage clamping the oocyte, all the lights were turned off for 10 min and a 3 μM GABA-induced current was monitored at 5 min intervals before and after a 5 min exposure to light at 80% of the maximum intensity.

Drug preparation

GABA (Calbiochem, La Jolla, CA, USA) stock solution (100 mM) was prepared daily from the solid form. Diazepam (Sigma) stock solution (50 mM) was prepared in DMSO and stored at −20 °C. Imidazole (Fisher Scientific, Pittsburgh, PA, USA), D-mannitol (Sigma) and sodium azide (Sigma) solutions were freshly prepared from the solid form. The H_2O_2 solution was freshly diluted before use (J. T. Baker, Phillipsburg, NJ, USA).

Data analysis

The dose–response relationship of the GABA-induced current in recombinant $GABA_A$ or $GABA_C$ receptors before and after UV irradiation was analysed by a least-squares fit to the following Hill equation:

$$I = \frac{I_{max}}{1 + (EC_{50}/[GABA])^{n_H}}, \quad (1)$$

where the GABA-induced current (I) is a function of the GABA concentration, EC_{50} is the GABA concentration required for inducing a half-maximal current, n_H is the Hill coefficient, and I_{max} is the maximum current. The maximum current was then used to normalize the dose–response curve for each individual oocyte. For the GABA dose–response relationship after UV irradiation, the normalization was achieved by using the maximum current before irradiation. The average of the normalized currents for each GABA concentration was used to plot the data. All the data are presented as means ± S.E.M. (standard error of the mean).

The UV irradiation dose-dependent potentiation was normalized as follows:

$$P = (I_{UV} - I_{control})/I_{control}, \quad (2)$$

where the fraction of potentiation, P, was calculated from the current response before ($I_{control}$) and after (I_{UV}) UV exposure. The potentiation data were least squares fitted with the Hill equation in the following form:

$$P = \frac{P_{max}}{1 + (ED_{50}/time)^{n_H}} \times 100, \quad (3)$$

where the percentage potentiation P is a function of the time of UV irradiation. The ED_{50} is the time required for 50% of the maximum potentiation.

For the recovery of the UV effect, the following exponential equation was used:

$$I = I_0 \exp(-t/\tau) + I_s, \quad (4)$$

where the 10 μM GABA-induced current (I) was measured repeatedly at 10 min intervals. I_0 is the current amplitude immediately after UV exposure (time zero) and I_S is the steady-state level of potentiation (i.e. the irreversible component).

RESULTS

UV irradiation potentiated GABA-induced currents in recombinant $GABA_A$ receptors

Figure 1*A* shows that the GABA-induced current (10 μM GABA) in an oocyte expressing $\alpha1\beta2\gamma2$ GABA receptors can be potentiated by 1 min UV irradiation using a transilluminator (left). The GABA-induced current did not change in control oocytes placed on the same surface, but without UV irradiation (right). Note that the GABA-induced current trace shows no desensitization before UV irradiation. After UV irradiation, however, the increased current shows some desensitization, suggesting an increase in GABA sensitivity. Figure 1*B* is a bar graph presentation of the averaged data from three oocytes.

UV enhancement of the GABA-induced current was dose dependent

Since we could not accurately vary the UV intensity to test the dose–response relationship, we varied the exposure time at a fixed intensity. Figure 2*A* shows that the increase in the GABA-induced current (10 μM GABA) in oocytes expressing $\alpha1\beta2\gamma2$ GABA receptors is irradiation dose dependent. The ED_{50} (half-saturation dose) was 12.4 s × intensity (max) with a Hill coefficient of 1.8 (Fig. 2*B*). Since the effect was observed in different oocytes for each exposure time, the large variability precluded a detailed analysis of the dose–response relationship (i.e. the significance of the co-operative Hill coefficient). Nevertheless, the dependence of the potentiation of the GABA-induced current on the UV exposure time further confirms that UV irradiation can potentiate $GABA_A$ receptors.

The UV effect on $GABA_A$ receptors was partially reversible

Figure 3 shows that immediately after UV irradiation, the GABA-induced current in $\alpha1\beta2\gamma2$ GABA receptors was potentiated 3.4 ± 0.2-fold ($n = 3$). However, when we monitored the current for 1 h, the amplitude decreased as a single exponential toward a steady-state level which was still 2.7 ± 0.3-fold higher than the control value. The time constant of the decay was 8.2 ± 1.2 min. Long term monitoring can be complicated by changes in expression level. New GABA receptor expression will result in a mixed population of UV-irradiated and non-irradiated GABA receptors. Therefore, we cannot exclude the possibility of a much slower component of recovery than that observed in Fig. 3. Nevertheless, these data suggest that the UV effect on $GABA_A$ receptors was only partially reversible.

UV irradiation shifts the GABA dose–response curve to the left for $GABA_A$, but not $GABA_C$, receptors

Since 10 μM GABA was not a saturating concentration for $\alpha1\beta2\gamma2$ GABA receptors, the increase in GABA-induced current could be due to an increase in sensitivity to GABA or to an increase in the maximum current (maximum-open probability or single-channel conductance) or both. This question can be addressed by comparing dose– response relationships before and after UV irradiation. Note that due to the slow recovery process, the dose– response relationship constructed by multiple GABA concentrations tested over about 15 min would be partially recovered. Nevertheless, Fig. 4*A* shows that the $\alpha1\beta2\gamma2$ GABA

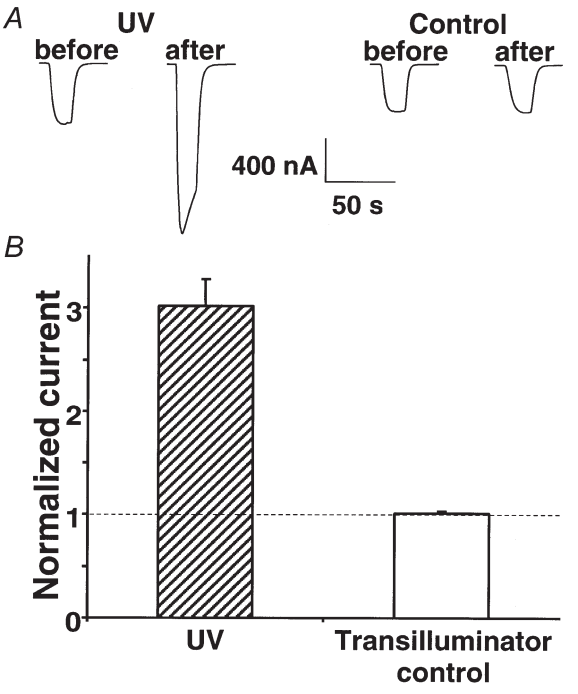

Figure 1. UV irradiation potentiated GABA-induced currents (10 μM GABA) in oocytes expressing $\alpha1\beta2\gamma2$ GABA receptors

A, left, the GABA-mediated current (10 μM GABA) was potentiated 3.0 ± 0.3-fold upon UV irradiation for 1 min on a transilluminator ($n = 3$). Right, in contrast, no potentiation was observed (1.0 ± 0.0) after 1 min on the transilluminator with the UV lamp off ($n = 3$). *B*, averaged data presented as a bar graph.

Table 1. UV effect on dose–response relationships of recombinant $GABA_A$ and $GABA_C$ receptors

	Before UV			After UV			
Subunit combinations	I_{max} (nA)	EC_{50} (μM)	Hill coeff	I_{max} (nA)	EC_{50} (μM)	Hill coeff	Number of oocytes
$\alpha1\beta2\gamma2$	922 ± 460	44.2 ± 3.3	1.6 ± 0.1	1093 ± 601	23.2 ± 3.1	1.3 ± 0.1	3
$\alpha1\beta2$	178 ± 77	5.9 ± 1.4	1.1 ± 0.1	375 ± 200	1.5 ± 0.1	1.1 ± 0.1	3
$\beta2\gamma2$	380 ± 91	51.3 ± 9.3	1.3 ± 0.2	400 ± 103	25.1 ± 2.8	1.1 ± 0.1	4
$\rho1$	182 ± 91	1.0 ± 0.1	2.8 ± 0.1	197 ± 96	1.2 ± 0.1	2.0 ± 0.2	3

receptor dose–response relationship was shifted by UV irradiation to the left by about 2-fold (from 44.2 ± 5.7 to 23.2 ± 5.4 μM). The maximum current, however, was only slightly increased by UV irradiation. A similar EC_{50} shift without a significant change in the maximum was observed for $\beta2\gamma2$ GABA receptors (Fig. 4*B*). For $\alpha1\beta2$ receptors, UV irradiation significantly increased the maximum current (2-fold), and decreased the EC_{50} (Fig. 4*C*). In contrast to $\alpha1\beta2\gamma2$, $\beta2\gamma2$, and $\alpha1\beta2$ receptors, homomeric $\rho1$ $GABA_C$ receptors were insensitive to UV irradiation (Fig. 4*D*). The maximum current, EC_{50} and Hill coefficients before and after UV irradiation are listed in Table 1.

The UVA or visible light did not significantly potentiate $GABA_A$ receptors

Since $GABA_A$ receptors can be modulated by UV, it is possible that they might be modulated by other wavelengths of light. To test this possibility, we examined the effects of visible light and UVA (340–380 nm) on $\alpha1\beta2\gamma2$ $GABA_A$ receptors. Oocytes were placed in the dark for 10 min and then exposed to visible light for 5 min. At the

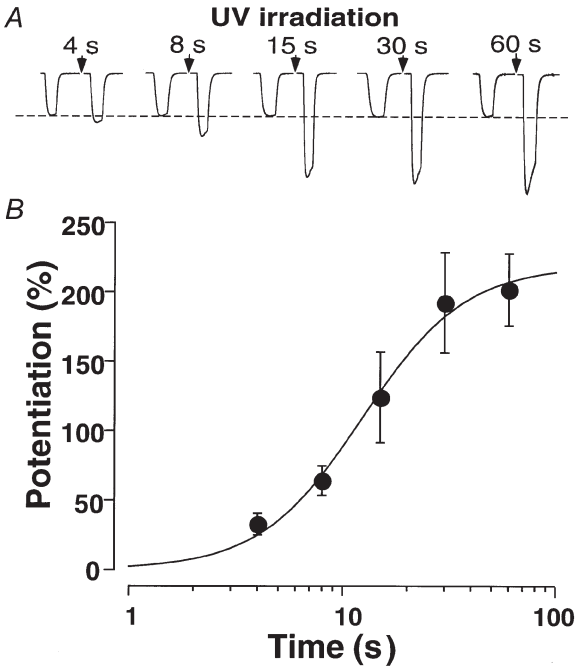

Figure 2. The effect of UV irradiation on recombinant $GABA_A$ receptors was irradiation dose dependent

A, the normalized current traces before and immediately after UV irradiation. The UV dose was controlled by varying the exposure time as indicated with a fixed intensity (maximum, see Methods). Each oocyte was exposed to UV irradiation only once. *B*, the difference in the currents before and after UV irradiation for each oocyte was calculated and normalized to its own control (before UV). Each data point is the average of three oocytes. The continuous line is the least-squares fit to the averaged data points using eqn (3). The fit resulted in an EC_{50} of 12.4 s × intensity(max) and a Hill coefficient of 1.8.

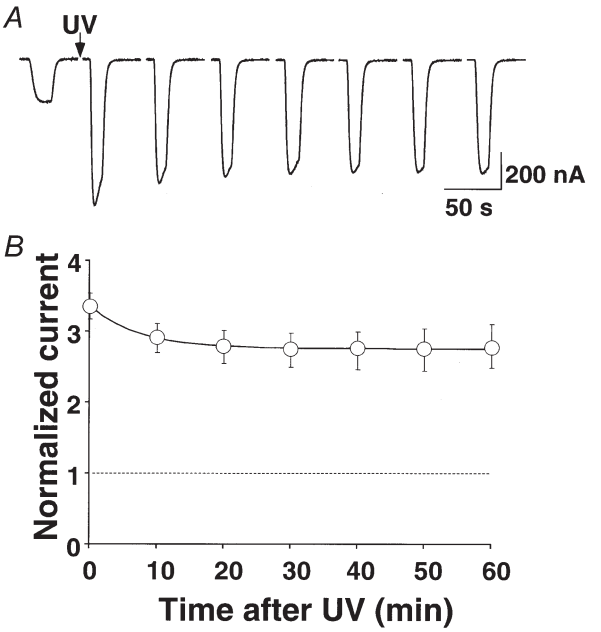

Figure 3. UV-mediated potentiation of GABA-induced currents on $\alpha1\beta2\gamma2$ GABA receptors was partially reversible

A, examples of current traces before and after UV irradiation with a 1 min exposure at the maximum intensity (see Methods). A 10 μM GABA test pulse was applied every 10 min. The potentiation decreased over time as a single exponential. *B*, the normalized (to control current) and averaged 10 μM GABA-test pulse was plotted against time. The fitting of the data points to eqn (4) resulted in a time constant of 8.2 ± 1.2 min ($n = 3$). Note that the GABA-induced current slowly decreased in amplitude towards a steady-state level, which was still significantly elevated (2.7 ± 0.3-fold).

end of this 5 min exposure, the current amplitude in response to 3 μM GABA (normalized to control) was 1.02 ± 0.01 ($n = 3$). We also examined the GABA-activated current after a 1 min exposure to UVA irradiation. In this case the amplitude of the GABA (10 μM)-activated current was 1.08 ± 0.07 ($n = 4$) with respect to the control. These results suggest that the $\alpha1\beta2\gamma2$ $GABA_A$ receptors are much less sensitive, if at all, to light with a wavelength longer than 340 nm.

Positive modulation of UV irradiation on the $GABA_A$ receptor was independent of the effect of diazepam

Modulation of $GABA_A$, but not $GABA_C$, receptors by UV irradiation raised the possibility that the mechanism of UV potentiation might overlap with that of the $GABA_A$ receptor allosteric modulator, diazepam. To investigate this possibility, we tested diazepam after potentiation by UV irradiation. Figure 5 shows that UV irradiation shifted the GABA dose–response curve to the left. The EC_{50} decreased from a control value of 34.5 ± 2.6 to 18.7 ± 4.7 μM after UV irradiation ($n = 3$). Diazepam (1 μM) further shifted the dose–response curve to the left with an EC_{50} of 8.2 ± 1.1 μM. The additional 2.3-fold decrease in the EC_{50} by diazepam after a saturating UV irradiation was comparable to the 2.5-fold shift by the same diazepam concentration without UV (Ghansah & Weiss, 1999) indicating that the potentiation by irradiation does not occlude potentiation by diazepam.

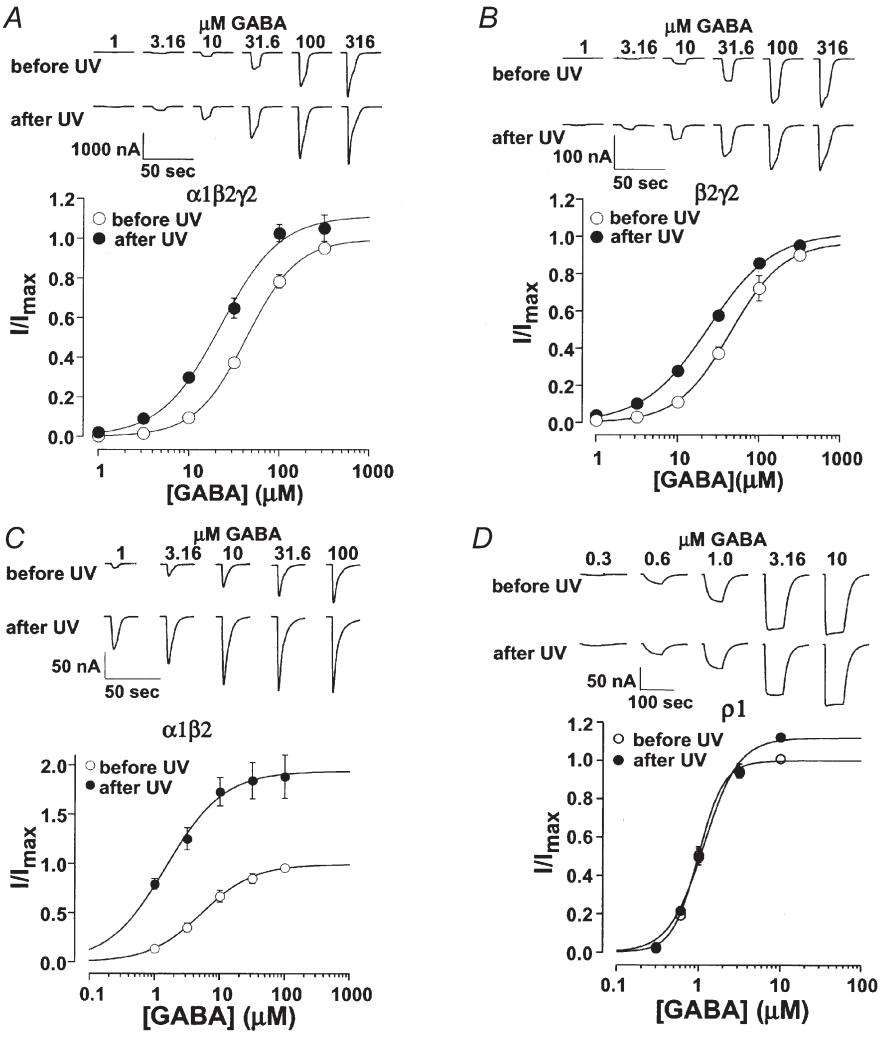

Figure 4. The effect of UV irradiation on the GABA dose–response curve of GABA receptors

A, examples of current traces induced by a range of GABA concentrations before and after UV irradiation. The average of the normalized current was plotted against GABA concentration. Continuous lines are from least-squares fits of the data points to eqn (1). The resulting EC_{50} values and Hill coefficients are listed in Table 1. Note that UV irradiation shifted the GABA dose–response curve to the left about 2-fold, without a significant change in the maximum current. *B* and *C*, similar to *A*, but for $\beta2\gamma2$ and $\alpha1\beta2$ $GABA_A$ receptors. Note that for $\alpha1\beta2$, UV irradiation shifted the GABA dose–response curve to the left ~3.8-fold, as well as increased the maximum current (~2-fold). *D*, in contrast to $\alpha1\beta2\gamma2$, $\beta2\gamma2$ and $\alpha1\beta2$ receptors, homomeric $\rho1$ $GABA_C$ receptors were insensitive to UV irradiation.

The UV effect on $GABA_A$ receptors cannot be prevented by free radical scavengers, nor mimicked by a free radical generator

UV irradiation can generate free radicals, which in turn can activate protein kinases, thereby modulating protein function (Klotz *et al.* 1997). To test the possibility of UV irradiation acting through free radicals, we used the following free radical scavengers: the singlet oxygen quenchers, sodium azide (40 mM) and imidazole (40 mM), or the hydroxyl radical scavenger, D-mannitol (40 mM). These compounds have been shown to block the UV irradiation effects on c-Jun-N-terminal kinase (Klotz *et al.* 1997) at similar concentrations. For UV irradiation, we used an exposure (20 s × max) that achieved about 75% of the maximum potentiation on $GABA_A$ receptors and the oocytes were both preincubated in the compounds and incubated during UV exposure. The level of potentiation was unaltered by these compounds with a fractional potentiation of 2.80 ± 0.20 ($n = 5$), 2.88 ± 0.19 ($n = 3$), 3.05 ± 0.14 ($n = 3$) and 2.61 ± 0.25 ($n = 3$) for normal OR2 (control), sodium azide, D-mannitol and imidazole, respectively. In addition 1% H_2O_2 (free radical generator) did not potentiate the GABA-induced current. In fact, the current was slightly decreased after H_2O_2 treatment to 0.80 ± 0.01 of the control value ($n = 3$). This oxidation stress has an inhibitory effect on $GABA_A$ receptors similar to the effect of the oxidizing agent, 5-5′-dithiobis-2-nitrobenzoic acid (Pan *et al.* 2000). These two pieces of evidence suggest that UV light exerts its effect on $GABA_A$ receptors by means other than through free radicals.

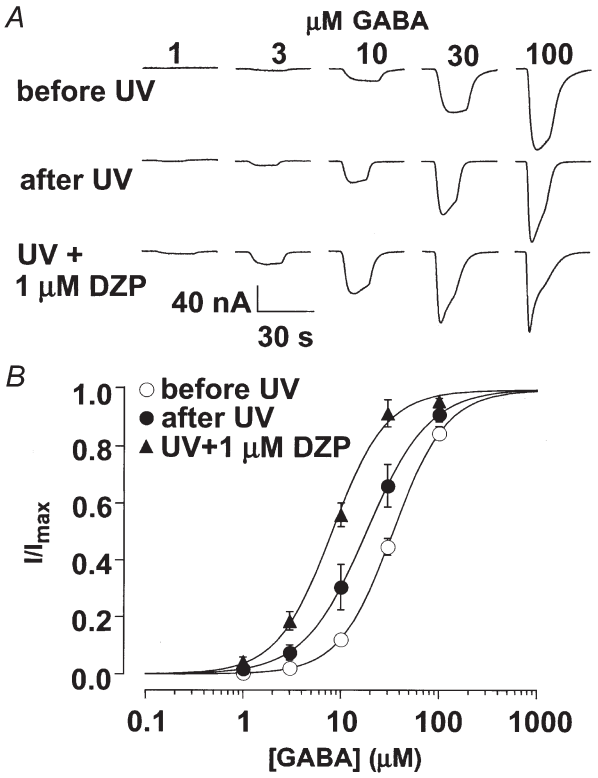

Figure 5. UV-mediated modulation of $GABA_A$ receptors was distinct from benzodiazepine-mediated modulation

A, current traces induced by a range of GABA concentrations before irradiation, after irradiation, and after UV irradiation in the presence of 1 μM diazepam. *B*, the normalized currents were plotted against GABA concentration. The continuous lines are least-squares fits of the data points to eqn (1). Note that after a saturating UV exposure, diazepam still shifted the GABA dose–response curve to the left (~2.3 fold). This is similar to the 2.5-fold shift by the same diazepam concentration without UV (Ghansah & Weiss, 1999).

DISCUSSION

$GABA_A$ and $GABA_C$ receptors have distinct activation and pharmacological properties. In this study, we have provided evidence that they are also distinct in UV-mediated modulation. UV irradiation has positive allosteric modulatory effects on $\alpha1\beta2$, $\alpha1\beta2\gamma2$ and $\beta2\gamma2$ $GABA_A$ receptors, but not on $\rho1$ $GABA_C$ receptors. The UV potentiation of $\alpha1\beta2\gamma2$ GABA receptors was only partially reversible, did not appear to overlap the effect of the benzodiazepine diazepam and did not appear to be mediated by singlet oxygen or hydroxyl radicals.

Speculation on the mechanism of the UV effect on GABA receptors

The possible mechanism of the UV-mediated potentiation of $GABA_A$ receptors could be a direct effect on the $GABA_A$ receptor or an indirect effect such as activation of intracellular signalling pathways, which in turn modulate channel function. The direct effect could be due to absorption of UV light by select amino acids such as tyrosine, phenylalanine, tryptophan or cysteine. The cross-linking or ionization of these residues could change the free energy landscape of the receptor and result in changes in receptor conformation and responsiveness to agonists. For example, redox modulation of recombinant $GABA_A$, but not $GABA_C$, receptors has been reported (Amato *et al.* 1999; Pan *et al.* 2000) which involved a cysteine residue in the M3 domain. We mutated the corresponding amino acid residue in the $\rho1$ subunit to cysteine (S330C) and, similar to the wild type $\rho1$ receptor, we failed to see an UV effect on this mutant $GABA_C$ receptor (data not shown). Furthermore, the reported redox effect on GABA receptors was mainly limited to an amplitude change without a significant change in GABA sensitivity (Amato *et al.* 1999; Pan *et al.* 2000). In contrast, the UV-mediated modulation we report here mainly changed the EC_{50} with little effect on the maximum current in $\alpha1\beta2\gamma2$ and $\beta2\gamma2$ GABA receptors. For $\alpha1\beta2$ GABA receptors, however, UV irradiation decreased the EC_{50} and increased the maximum current. This does not necessarily imply a different mechanism of modulation since an increase in the ratio of the opening and closing rate constants could shift the dose–response

relationship to the left and would also increase the maximum if the initial maximum open probability (before UV irradiation) was low, as has been reported for $\alpha\beta$ receptors (Serafini *et al.* 2000). This subunit-dependent potentiation of the maximum amplitude is similar to the reported dithiothreitol (DTT) effect (Amato *et al.* 1999; Pan *et al.* 2000). One difference, however, is that the UV potentiation has a much longer lifetime than the DTT effect, which is rapidly reversible (Pan *et al.* 2000). Since the apparent affinity of $GABA_A$ receptors increased upon UV irradiation, UV irradiation could also interact with residues in the GABA binding site. However, conservation of tyrosines in proposed GABA binding sites (Amin & Weiss, 1993, 1994) across $\alpha1$, $\beta2$, $\gamma2$ and $\rho1$ subunits suggests that it is unlikely that UV irradiation can selectively modulate tyrosine residues in $\alpha1$, $\beta2$ or $\gamma2$ subunits, but not in the highly homologous $\rho1$ subunit.

$GABA_A$ receptors can be modulated by interacting with other proteins (Brandon *et al.* 1999; Liu *et al.* 2000) or by phosphorylation (Moss *et al.* 1995; Yan & Surmeier, 1997; McDonald *et al.* 1998; Poisbeau *et al.* 1999; Filippova *et al.* 2000; Flores-Hernandez *et al.* 2000). Concerning a potential indirect effect of UV irradiation on GABA receptors, UV irradiation could activate a protein kinase, which in turn could modulate receptor function. There is evidence that many protein kinases can be activated by UV irradiation (Bender *et al.* 1997). The phosphorylation of tyrosine residues of several growth factor receptors is the early detectable cellular reaction after UV irradiation (Bender *et al.* 1997). However, 40 μM genistein, a tyrosine kinase inhibitor, did not block the UV-mediated modulation of the $GABA_A$ receptor (data not shown).

Potential significance of the finding

In this study we demonstrate that $GABA_A$ receptors can be modulated by UV irradiation. It has previously been demonstrated that light of wavelength < 324 nm potentiates NMDA receptors in isolated retinal neurons (Leszkiewicz *et al.* 2000). Thus, both excitatory and inhibitory synaptic transmission are potential targets for light-mediated modulation. The components of sunlight with wavelength shorter than 290 nm (UVC) do not reach the earth's surface because they are absorbed by the ozone layer (Madronich, 1993). UVB and UVA, however, do reach the earth's surface, and can cause significant damage to skin and eyes. Whether UVB and UVA can reach the human retina is still controversial. For example, it has been suggested that the young primate lens can transmit UVB (Gaillard *et al.* 2000). In contrast, Dillon *et al.* (2000) concluded that the anterior segment of young primates transmits almost no UV light. Therefore, there is a possibility that UV light may have an influence on $GABA_A$ receptors in the human retina, which may have potential physiological or pathophysiological significance. For example, stimulation of $GABA_A$ receptors may be neurotoxic in the retina (Chen *et al.* 1999). Furthermore, the elucidation of the mechanism of UV-mediated potentiation on $GABA_A$ receptors may provide a new way to modulate $GABA_A$ receptor function, although identification of the precise mechanism must await future studies.

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