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Photoisomerization of fluvoxamine generates an isomer that has reduced activity on the 5-hydroxytryptamine transporter and does not affect cell proliferation

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Abstract

Fluvoxamine, a selective serotonin re-uptake inhibitor, is used as antidepressant/anxiolytic. The presence of a C=N double bond in the structure of fluvoxamine implies the existence of two geometric isomers: E- (*trans*) and Z- (*cis*), and suggests the hypothetical susceptibility of the molecule to photoisomerization. Clinically effective fluvoxamine is in its *trans* form. UVB (ultraviolet light, class B, wavelength range 290–320 nm) irradiation of aqueous solutions of fluvoxamine generated a photoproduct, which was isolated and analyzed by nuclear magnetic resonance (NMR) and mass spectrometry (MS), and identified as the *cis* isomer of fluvoxamine. This *cis*-isomer lost capacity to inhibit serotonin uptake, suggesting that light exposure might reduce the clinical efficacy of fluvoxamine. Alternatively, the photoproduct could be used as an inactive isomer in the studies of antidepressant mechanisms. Recent proposal suggests that antidepressants increase neurogenesis in the adult brain, whereas either an inhibitory or a stimulatory action of antidepressants on [^{3}H]thymidine uptake in vitro has been attributed to their interaction with serotonergic mechanisms. Lower concentrations (i.e., 2 μM) of fluvoxamine and fluoxetine (another selective serotonin re-uptake inhibitor) stimulated [^{3}H]thymidine uptake in mature, but inhibited it in immature cultures of rat cerebellar granule cells; the photoproduct was ineffective. A high concentration of fluvoxamine (i.e., 20 μM) but not the photoproduct was toxic to both immature and mature cultures. We suggest that a mechanism sensitive to fluvoxamine photoisomerization might be involved in the action of antidepressants on cell proliferation.

Keywords: Antidepressant; Cell proliferation; Fluvoxamine; Photoisomerization; 5-HT (5-hydroxytryptamine, serotonin) uptake; 5-HT (5-hydroxytryptamine, serotonin) re-uptake inhibitor, selective

1. Introduction

The interaction of ultraviolet (UV) light with pharmacologically active molecules often results in molecular alterations that may significantly modify and/or reduce the biological activity of drugs and lead to unwanted effects, such as phototoxicity (Beijersbergen van Henegouwen, 1997). Since light therapy has also been used in treating depression (Lam et al., 2000), it would be useful to have a better understanding of possible interaction between light and antidepressants. For instance, Wang et al. (1992) investigated the potential phototoxicity of various antidepressants and proposed that there might be an increased risk for light damage to the lens and retina if patients are treated concurrently with antidepressants and light therapy.

Phototoxic reactions have been observed with a number of compounds acting on the central nervous system (CNS), e.g., antipsychotics, anxiolytics, and antidepressants (Harth and Rapoport, 1996; Glass et al., 1998). Viola et al. (2000) also found that two antidepressants, amitriptyline and imipramine, were moderately phototoxic to various substrates (3T3 murine fibroblasts, lipids). On the other side, it is known that the phototoxic effects of drugs are not limited to the organs directly exposed to light (for instance, skin and

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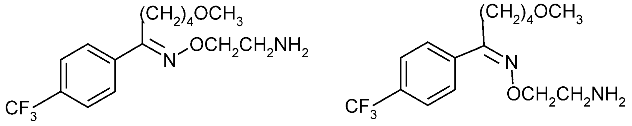

Fig. 1. Molecular structure of fluvoxamine and its *cis*-photoproduct. Note the C═N double bond which undergoes isomerization.

eyes). Thus, UV–visible radiation is able to penetrate the skin to different extent, depending on its wavelength and skin type. This radiation can reach capillaries and the circulating blood, causing systemic phototoxicity (Parrish, 1982).

Fluvoxamine is a selective inhibitor of neuronal serotonin (5-hydroxytryptamine, 5-HT) re-uptake, and has clinically established antidepressant/anxiolytic activity (Edwards and Anderson, 1999; Figgitt and McClellan, 2000). There are no reports of phototoxicity in the clinical use of fluvoxamine, but the presence in its molecule of a C═N double bond implies the existence of two different geometric isomers, E (*trans*) and Z (*cis*) (Fig. 1), of which only the former is marketed as a drug. It appears that *trans* to *cis* transformation does not occur during metabolism of fluvoxamine in vivo (Ruijten et al., 1984), but photoisomerization of C═N double bonds under suitable wavelengths is a well-known event (Turro, 1965; Fig. 1).

In this work, we established that UVB light (wavelength range 290–320 nm) is indeed capable of transforming fluvoxamine from *trans*- into *cis*-isomer and we characterized some of the biological effects of fluvoxamine and its photoproduct.

2. Materials and methods

2.1. Compounds

Fluvoxamine maleate, (*E*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]1-pentanone *O*-(2-aminoethyl)oxime maleic acid salt, was a kind gift by Solvay (Solvay Pharmaceutical, Weesp, The Netherlands). Fluoxetine hydrochloride was obtained from Eli Lilly & Co. (Indianapolis, IN, USA). Desipramine, pargyline, phentolamine, and prazosin were purchased from Sigma (St. Louis, MO, USA). All other reagents and solvents were of analytical grade.

2.2. Animals

Adult male Sprague–Dawley rats (200–250 g) were obtained from Harian–Nossan (S. Pietro al Natisone, Italy) and housed under controlled temperature (23 ± 2 °C) and illumination (12 h light–12 h dark, 18:00–06:00). Experiments were performed between 10:00 and 17:00.

Rat pups were obtained from Harlan (Indianapolis, IN, USA). The experiments have been carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

2.3. Irradiation procedure

Two Philips PL-S 9W/12 UVB lamps (Minicells), emitting mainly at 312 nm were used for irradiation experiments. The total energy was 0.4 J/cm^2/min, as measured with a Cole Palmer 97503-00 radiometer (Niles, IL, USA). Water solutions of fluvoxamine were irradiated in quartz cuvettes maintained at room temperature.

2.4. Chromatographic analysis

The irradiated solution of fluvoxamine was analyzed by both high performance liquid chromatography (HPLC) and thin layer chromatography (TLC).

HPLC was performed on a Perkin Elmer Series 410 LC pump equipped with an LC-235 Diode Array Detector. A reverse phase column was used (Waters Spherisorb S5 ODS2, 250 × 4.6 mm) eluted isocratically with an acetonitrile–methanol–tetrahydrofuran–water–triethylamine mixture (79:20:0.63:0.12:0.063) at a flow rate of 1.0 ml/min.

For TLC analysis, the irradiated solution was lyophilized, and the residue dissolved in methanol was loaded onto silica gel plates 60 F254 (E. Merck, Darmstadt, Germany) developed with methanol–ethyl acetate (70:30).

The bands were scraped and extracted with methanol. After removal of the solvent, the residue was crystallized from isopropanol and used for spectrometric characterization (nuclear magnetic resonance (NMR): Bruker AMX-300; mass spectroscopy (MS): Varian MAT 112 and PerSeptive Biosystems Mariner 5220), as well as for biological tests.

2.5. Synaptosomal preparation

Brain synaptosomes were isolated according to Whittaker and Barker (1972). Rats were decapitated, the brains rapidly excised, and the frontal cortex dissected out on ice. The tissue was homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose and then centrifuged at 1800 × *g* for 10 min (4 °C). The pellet was discarded and the supernatant centrifuged at 17 000 × *g* for 60 min (4 °C). The pellet was resuspended in a small volume of 0.32 M sucrose, layered on a 1.2–0.8 M sucrose gradient and centrifuged at 37 000 × *g* for 60 min (4 °C). The synaptosomal fraction at the interface between 0.8 and 1.2 M sucrose was collected and further centrifuged al 17 000 × *g* for 30 min. The pellet was resuspended in buffer (composition in mM: NaCl 115, KCl 4.97, $CaCl_2$ 1, $MgSO_4$ 1.22, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11.1, pH 7.4, plus 0.01 mM pargyline) and immediately used. Protein content was determined according to Lowry et al. (1951) with bovine serum albumin as standard.

2.6. [^{3}H]5-hydroxytryptamine (5-HT) and [^{3}H]norepinephrine uptake in synaptosomes

Synaptosomes (200–250 μg protein) were preincubated for 5 min at 37 °C in the absence or in the presence of different concentrations of drugs. Uptake was started by addition of 5 nM [^{3}H]5-HT (specific activity 27.3 Ci/mmol) or [^{3}H]norepinephrine (specific activity 14.9 Ci/mmol) (both from New England Nuclear, Boston, MA); incubation continued for 6 min. Control samples were incubated at 0 °C to evaluate membrane diffusion. The reaction was stopped by cooling the tubes on ice. The samples were then filtered through Whatman GF/C glass fibre filters (Whatman, Clifton, NJ, USA) and washed twice with 150 mM Tris–HCl, pH 7.4. Filter-bound radioactivity was counted by liquid scintillation spectrometry with Filter Count (Packard). The difference in [^{3}H]5-HT or [^{3}H]norepinephrine accumulation at 37 and 0 °C was taken as a measure of active uptake. K_i ($\pm$ S.E.M.) values were calculated by a computer-assisted curve fitting program (EBDA) (Mc Pherson, 1987).

2.7. Cerebellar neural cultures

The cerebellar neural cultures were prepared from the cerebella of 7-day-old rat pups; they were grown in a serum-free medium and in 24-well plates (300 000 cells/0.5 ml medium/well) (Manev et al., 2001). The *trans*- and *cis*-fluvoxamine and fluoxetine were dissolved in dimethyl sulfoxide (DMSO); 1 μl/well was added directly from stock solutions under the red photo-safety light; controls were treated with DMSO. Thereafter, the cultures were maintained in regular culture conditions but protected from light. Two experimental designs were used: (a) treatment initiated 24 h after plating (1 day in vitro), and (b) treatment initiated 10 days after plating (10 days in vitro). Cell proliferation was assayed 48 h later (i.e., at 3 and 13 days in vitro, respectively) as incorporation of [^{3}H]thymidine/well (6 h exposure to 1 μl [^{3}H]thymidine; 1 μCi/ml, Amersham; TRK120) (Manev et al., 2001), and is expressed as a percentage of the corresponding DMSO-treated control. Cell morphology was examined by light microscopy. For statistical analyses, analysis of variance (ANOVA) was followed by the Student's *t*-test or the Dunnett's test for multiple comparison with control. $^*P < 0.05$ was taken as significant.

3. Results

3.1. Photostability of fluvoxamine

Fig. 2 shows how the UV absorption spectrum of fluvoxamine (1×10^{-5} M in water) changes as a function of the UVB (ultraviolet light, class B, wavelength range 290–320 nm) dose administered. The absorption band at 245 nm disappears, and the presence of two isosbestic points at 218 and 224 nm suggests that a single photoproduct forms in response to irradiation.

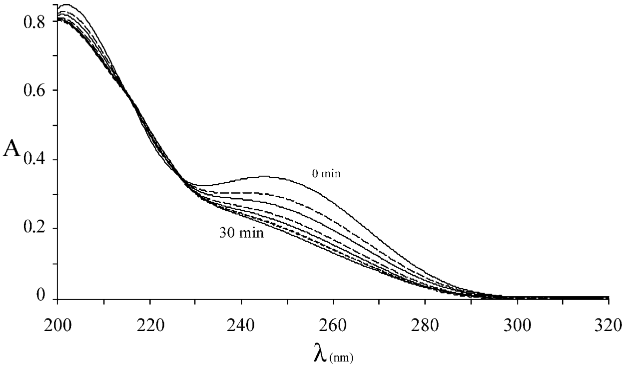

Fig. 2. UV absorption spectrum of fluvoxamine in aqueous solution (upper curve). Increasing UVB doses (spectra recorded after 3, 5, 8, 10, 15, 20, and 30 min, i.e., 10 J/cm^2) produces a gradual decrease in the absorption peak at 245 nm indicating photomodification of the drug.

Photolysis of a 1×10^{-2} M solution was also followed by reverse-phase HPLC. In the chromatogram, in addition to the peak of the original fluvoxamine ($T_r = 6.5$ min), only one new peak appears ($T_r = 5.0$ min) and gradually increases on irradiation.

Fig. 3 shows the decrease of the peak area of fluvoxamine and the increase of the peak area of the photoproduct.

3.2. Isolation of the photoproduct

After irradiation with 10 J/cm^2—which induces about 70% photolysis—the fluvoxamine solution was lyophilized and the residue submitted to TLC. On the plates, only two spots were detected having R_f values of 0.22 (parent fluvoxamine, as the free base) and 0.72 (photoproduct), respectively. The latter band was scraped and extracted with methanol. Removal of the solvent gave a residue which was crystallized from isopropanol and used for analytical determinations.

The [^{1}H]-NMR spectrum of fluvoxamine maleate (Fig. 4B) has previously been reported (Foda et al., 1996). The spectrum of the photoproduct (Fig. 4A) lacks the signals of maleic acid (singlet at 6.24 ppm and broad singlet at 10 ppm), while a broad singlet appears at 2.1 ppm belonging to the two aminic protons of the free base. The other signals match exactly those of fluvoxamine.

The [^{13}C]-NMR spectrum of the photoproduct was also recorded and fully corresponded to the literature data for fluvoxamine (Foda et al., 1996).

Using the electron impact technique, the mass spectrum of the photoproduct lacks the molecular peak and shows main fragments at $m/z = 258$, 200, 187, 172, 71, in close agreement with literature data (Foda et al., 1996). When the photoproduct was analyzed with the electrospray technique, the $[M+H]^+$ ion was found at $m/z = 319$, corresponding to fluvoxamine (free base).

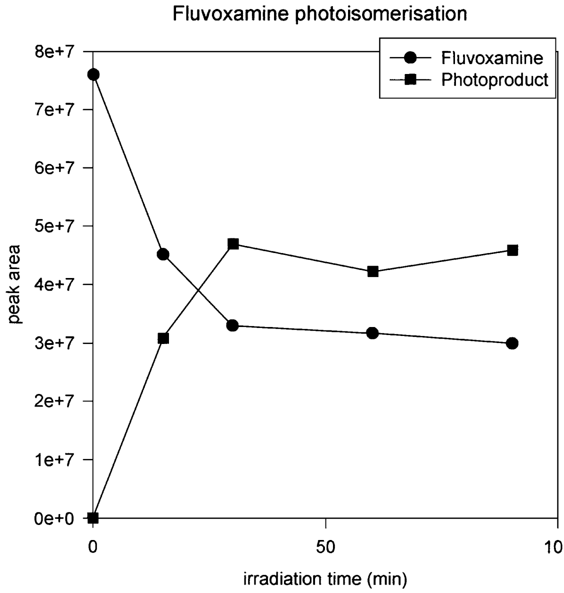

Fig. 3. Photoisomerization of fluvoxamine in response to UVB irradiation: time course. An aqueous solution of fluvoxamine was irradiated for indicated periods of time and 20-μl aliquots were analyzed by HPLC. The results are expressed as the area of peaks corresponding to fluvoxamine and its photoproduct at each indicated time point.

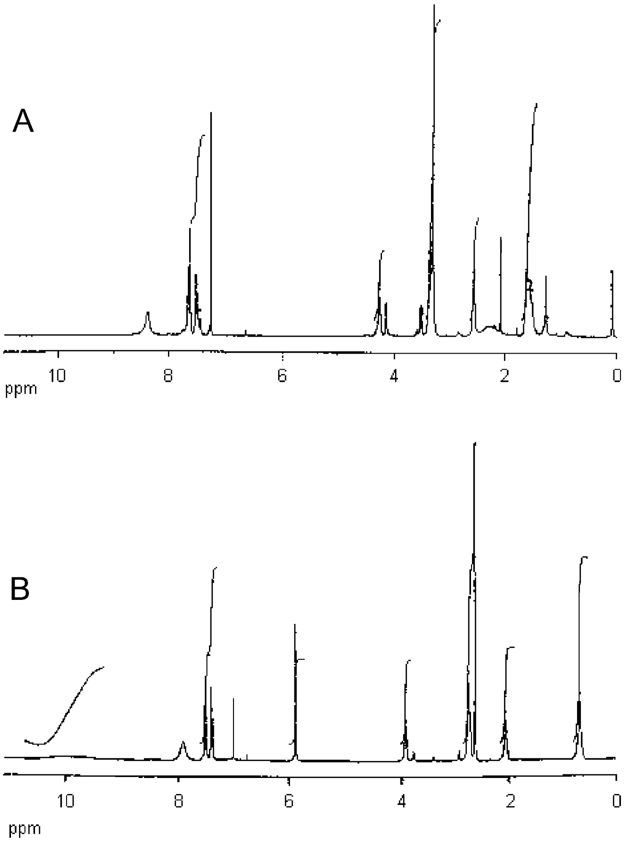

Fig. 4. [^{1}H]-NMR spectrum of fluvoxamine maleate (upper panel) and its photoisomer (lower panel). Note that the difference is only due to the loss of the maleate (whose peaks are marked with *) during chromatographic separation, while the other protons remain unchanged.

Table 1
Drug-induced inhibition of [^{3}H]5-HT uptake in rat cortical synaptosomes

Drug	K_i (nM)
Fluvoxamine	5.5 ± 0.48
Photoproduct	803 ± 93
Fluoxetine	4.9 ± 0.46
Desipramine	138 ± 6.5

Data are means ± S.E.M. from five different experiments, each performed in triplicate.

The identity of the NMR and mass spectra and the difference of both UV absorption and chromatographic behavior can only be accounted for by *trans* to *cis* (E to Z) photoisomerization.

3.3. [^{3}H]5-HT uptake by isolated synaptosomes

The biological activity of fluvoxamine photoproduct (i.e., its *cis*-isomer) was tested in cortical synaptosomes. In this preparation, incubation of synaptosomes in the presence of fluvoxamine or another specific 5-HT uptake inhibitor, fluoxetine, potently and dose-dependently inhibits the [^{3}H]5-HT uptake. An inhibitory effect was also observed with desipramine [a norepinephrine uptake inhibitor] and with the photoproduct, albeit only with high concentrations of these compounds. The K_i values, estimated from the concentration of a drug required to inhibit 50% of [^{3}H]5-HT uptake, are shown in Table 1. The synaptosomal uptake of [^{3}H]5-HT was most strongly reduced by fluvoxamine and fluoxetine, whereas the photoproduct and desipramine, although effective, were clearly less potent (Table 1). Thus, the *cis* isomer of fluvoxamine is about 150 times less effective in inhibiting [^{3}H]5-HT uptake by isolated synaptosomes than the clinically used *trans* isomer.

3.4. [^{3}H]norepinephrine uptake by isolated synaptosomes

In alternative experiments, isolated synaptosomes were used to investigate the effects of the above-noted compounds on [^{3}H]norepinephrine uptake. Again, all four compounds tested, i.e., desipramine, fluoxetine, fluvoxamine, and its *cis*-isomer, inhibited dose-dependently [^{3}H]norepinephrine uptake. However, the K_i values estimated from the concentration of drug required to inhibit 50% of [^{3}H]norepinephrine confirmed that, as expected, this uptake was

Table 2
Drug-induced inhibition of [^{3}H]-norepinephrine uptake in rat cortical synaptosomes

Drug	K_i (nM)
Fluvoxamine	535 ± 16
Photoproduct	550 ± 24
Fluoxetine	320 ± 15
Desipramine	0.53 ± 0.0041

Data are means ± S.E.M. from five different experiments each performed in triplicate.

most potently reduced by desipramine. All three other compounds, including the *cis*-isomer of fluvoxamine, had about 1000 times greater K_i values. This rather nonspecific action of fluvoxamine was not affected by photoisomerization; both fluvoxamine and the photoproduct exhibited almost identical K_i values for [^{3}H]norepinephrine uptake inhibition (Table 2).

3.5. Effects of fluvoxamine photoisomers on cell proliferation in rat cerebellar cultures

The biological activity of fluvoxamine and its *cis* photoisomer was characterized in a model of in vitro neural cell proliferation. The results of this study confirm recent observation (Manev et al., 2001) that 1–2 μM fluoxetine reduces proliferation in younger cultures of cerebellar granule cells (1–3 days in vitro), whereas in older cultures (10–13 days in vitro), fluoxetine stimulates cell proliferation (Fig. 5). A comparable concentration (i.e., 2 μM) of fluvoxamine produced a similar dual effect in younger vs. older cultures, whereas the photoproduct was inactive (Fig. 5). Lower concentrations, i.e., 0.02 μM of either fluvoxamine or its *cis*-isomer, did not alter cell proliferation. On the other hand, a higher concentration of 20 μM fluvoxamine reduced thymidine incorporation both in the younger and the older cultures, the photoproduct being inactive (Fig. 5). The effect on thymidine incorporation of 20 μM fluvoxamine appears to be due to toxicity. Whereas even a prolonged (12 days) exposure to the photoproduct (20 μM) did not kill cells, fluvoxamine completely destroyed the morphology of the culture (similar effect was observed with fluoxetine; not shown). Co-treatment with the *cis*-isomer did not prevent the toxic effect of the high concentration of fluvoxamine, nor did it affect the inhibitory effect on cell proliferation of 2 μM fluvoxamine (not shown).

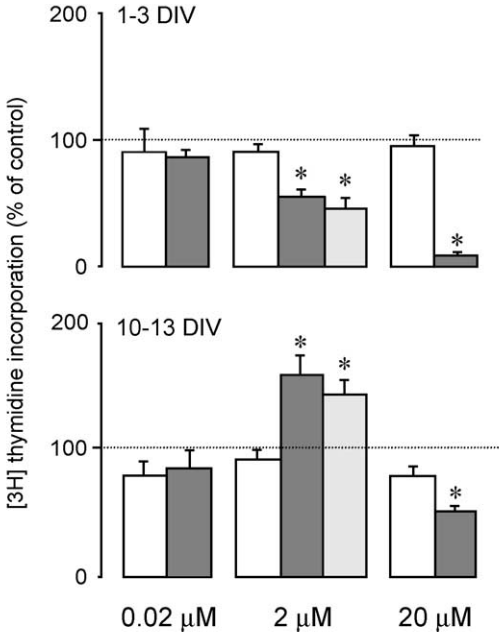

Fig. 5. Dual effects of a lower concentration of fluvoxamine or fluoxetine on the uptake of [^{3}H]thymidine (a marker of proliferation) in rat cerebellar granule cells, and the absence of the effect with the fluvoxamine photoproduct (i.e., *cis*-isomer). Upper panel: 48-h-treatment started at 1 day in vitro (DIV) with fluvoxamine photoproduct (open bars; 0.02–20 μM); fluvoxamine (hatched bars; 0.02–20 μM); and fluoxetine (dotted bars; 2 μM). Both fluvoxamine and fluoxetine inhibited [^{3}H]thymidine uptake in immature cultures; the fluvoxamine photoproduct was inactive. Lower panel: stimulatory effect of 2 μM fluvoxamine and fluoxetine in older cultures (48-h treatment started at 10 DIV); the fluvoxamine photoproduct was inactive and 20 μM fluvoxamine was toxic. Results (mean ± S.E.M.) are expressed as percentage of corresponding control ($n = 6–12$ wells per treatment; $^*P < 0.05$ vs. 100% control). Note the absence of any effect with fluvoxamine photoproduct both at 1 and 10 DIV. The inhibitory effect of a high concentration of fluvoxamine (20 μM) both at 1 and 10 DIV is due to toxicity, observed as the destruction of neural network and collapse of cell bodies (not shown).

4. Discussion

Our present findings confirmed that fluvoxamine is photounstable and that UVB irradiation of a fluvoxamine solution gives rise to a single photoproduct. The [^{1}H]- and [^{13}C]-NMR spectra, as well as the mass spectrum of this product, indicate that it has the same general formula of fluvoxamine. However, despite this similarity, the UV absorption spectrum and the chromatographic behavior of the photoproduct differed significantly from fluvoxamine. This difference suggests structural diversity that can only be attributed to the geometrical isomerization of the C═N double bond in the molecule of fluvoxamine; i.e., *trans* to *cis* conversion.

Often, UVB irradiation of drugs leads to cytotoxicity. Experiments in cell cultures demonstrated phototoxicity of imipramine, amitriptyline (Viola et al., 2000), and some phenothiazines (Miolo et al., 1999). In these conditions, fluvoxamine was not phototoxic; for example, experiments with irradiation of murine 3T3 fibroblasts in the presence of fluvoxamine failed to induce detectable toxic effects (unpublished observation). Nevertheless, compared with the parent molecule, the *cis*-isomer (i.e., photoproduct) lost the specific inhibitory activity of fluvoxamine on 5-HT uptake. This indicates that the *trans* configuration is of great importance for binding of fluvoxamine to the 5-HT transporter. Similarly, the importance of *trans* configuration of a molecule for its specific binding properties was previously observed with a ligand for opiate receptors (Nitz et al., 1986). In contrast, other less specific binding properties of fluvoxamine, for example, to idazoxan-labeled α2 adrenergic receptors, were not sensitive to *trans* to *cis* isomerization (unpublished data). Thus, the K_i values for both *trans* and *cis*-isomers in displacing idazoxan binding were similar but about 20 times lower than the K_i value of another selective serotonin re-uptake inhibitor, fluoxetine (unpublished data). In addition, norepinephrine uptake was equally affected by both fluvoxamine isomers.

These findings suggest that *trans* to *cis* photoisomerization of fluvoxamine produced a molecule, i.e., photoproduct that retained the less specific activity of fluvoxamine but lacked specific activity on the 5-HT transporter. Hence, fluvoxamine photoproduct could be used as a control compound in studies of the mechanisms of action of fluvoxamine and possibly other inhibitors of 5-HT re-uptake.

In primary cultures of cerebellar granule cells, antidepressants that block serotonin transporters appear to exert a dual effect on cell proliferation, i.e., they inhibit [^{3}H]thymidine uptake in immature proliferating cultures and stimulate [^{3}H]thymidine uptake in older cultures (Manev et al., 2001). Similar dual effects of fluoxetine were observed in vitro in human T-cell proliferation (Edgar et al., 1999), whereas in vivo administration of selective serotonin reuptake inhibitors to rats reduced mitogen-induced lymphocyte proliferation (Pellegrino and Bayer, 2000). In this study, we found that the concentration of 2 μM fluvoxamine produced the same dual effect on [^{3}H]thymidine uptake in rat cerebellar granule cell cultures as fluoxetine, whereas the fluvoxamine photoproduct was inactive.

Possibly, the observed dual effect of fluvoxamine and fluoxetine could involve an action of drugs on the 5-HT transporter. Hence, it has been shown that the pluripotent neural precursors express 5-HT transporter as early as embryonic day 10 (Hansson et al., 1999), whereas the adult cerebellum does not appear to express significant amounts of this protein (Bengel et al., 1997). However, it is unlikely that the effects of drugs observed in rat cerebellar granule cell cultures were mediated by their action on endogenous serotonin, namely, serotonin was not added to the culture medium and cerebellar granule cells do not synthesize it. Hence, our results suggest that a target mechanism affected by fluvoxamine but not by its photoproduct, for example, an intrinsic activity of the 5-HT transporter (e.g., ion fluxes (Beckman and Quick, 2001; Galli et al., 1997)), might be involved in the effects of antidepressants on proliferation of neural precursors. Moreover, if fluvoxamine is capable of entering into the cells, a direct intracellular action of the drug could also be a possible mechanism of action. It remains to be elucidated whether the in vitro effects of antidepressant drugs on [^{3}H]thymidine uptake are indicative of the recently described stimulatory action of antidepressants on neurogenesis in vivo (Jacobs et al., 2000; Malberg et al., 2000; Manev et al., 2001).

We found that *trans* to *cis* isomerization abolished the toxic in vitro effects of a high concentration of fluvoxamine. In vitro cytotoxicity was recently characterized for a number of antidepressants including fluoxetine, amitriptyline, imipramine, and tranylcypromine (Slamon and Pentreath, 1998). In our model of rat cerebellar granule cells, the toxicity of fluvoxamine was comparable to toxicity of fluoxetine (unpublished observation). Since this toxic effect was sensitive to photoisomerization of fluvoxamine, it is possible that it also involves a *trans*- but not a *cis*-isomer-sensitive mechanism common to other antidepressants.

In conclusion, interaction of UV light with fluvoxamine does not induce direct phototoxic effects, as in the case of other CNS drugs, but UVB radiation can generate the pharmacologically inactive *cis*-isomer. Considering that about 10% of the total blood volume is in the skin, and thus is susceptible to UV radiation (Parrish, 1982), and UVB is able to reach blood vessels in the dermis (Anderson and Parrish, 1982), it is conceivable that some photoisomerization of fluvoxamine may occur in vivo. Attempts have been made to measure the amount of UVB radiation reaching the earth surface (see, for example, Moore and Zhou, 1994; Gonzáles and Gonzáles, 1996; Parisi and Kimlin, 1999), but the results span over a wide range, depending on the exposure conditions (season, latitude, zenith angle, etc.). For example, Gonzáles and Gonzáles (1996) reported a value as high as 7.5 J/cm^2/h, a dose capable of isomerizing fluvoxamine in vitro.

Based only on our in vitro studies, we cannot conclude about possible effects of sun exposure on fluvoxamine in a patient population. Thus, the possibility of fluvoxamine photoisomerization in vivo requires further studies. Nevertheless, our findings suggest that the *cis*-isomer of fluvoxamine described in our present work could be used as a research tool (e.g., inactive control) in studies of the mechanism of action of antidepressant drugs.

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