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Difference in the *In Vivo* Influence of Serotonin$_{1A}$ Autoreceptors on Serotonin Release in Prefrontal Cortex and Dorsal Hippocampus of the Same Rat Treated with Fluoxetine

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Abstract

Recent studies have demonstrated that antagonism of somatodendritic serotonin$_{1A}$ (5-HT$_{1A}$) autoreceptors can potentiate the increase of extracellular 5-HT concentrations induced by selective serotonin reuptake inhibitors including fluoxetine. The present study was conducted to uncover any functional difference between the 5-HT$_{1A}$ autoreceptors located on the cell bodies of 5-HT neurons in the dorsal (DRN) and median (MRN) raphe nuclei. The investigational approach used in the present study was to detect extracellular 5-HT concentrations in two terminal areas, prefrontal cortex (Pfc) and dorsal hippocampus (Dhp), which are mainly innervated by the 5-HT neurons located in the DRN and MRN respectively. To avoid possible variation between individual animals a dual-probe microdialysis procedure was applied to determine 5-HT concentrations in both brain areas of the same rat. Fluoxetine (10 mg/kg, s.c.) alone produced a smaller increase in the extracellular 5-HT concentration in the Pfc than Dhp of the same rat (maximal 5-HT concentrations were 183% and 223% of the baseline values in Pfc and Dhp respectively). However, an antagonist of 5-HT$_{1A}$ receptors, WAY100635, subsequently injected (s.c.) at 1 mg/kg brought the 5-HT concentrations to similar levels in the Pfc (332%) and Dhp (308%). Since the 5-HT concentrations immediately before the injection of WAY100635 were lower in the Pfc (102%) than Dhp (186%), WAY100635 induced a larger 5-HT net increase in the Pfc (332%−102%=230%) than Dhp (308%−186%=122%). On the other hand, WAY100635 alone did not significantly change the extracellular 5-HT concentrations in both areas. Furthermore, extracellular concentrations of dopamine (DA) and two DA metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, in both areas were not altered by the administrations of fluoxetine and WAY100635. In conclusion, the present study demonstrated that the antagonist of 5-HT$_{1A}$ receptors, WAY100635, produced a more robust potentiation in the fluoxetine-induced 5-HT increases in the Pfc than Dhp. Since Pfc and Dhp are predominately innervated by 5-HT neurons located in the DRN and MRN respectively, this result may indicate a functional difference between the 5-HT$_{1A}$ autoreceptors located on the cell bodies of 5-HT neurons in the DRN and MRN.

Key Words: fluoxetine, WAY100635, monoamines, prefrontal cortex, dorsal hippocampus, a dual-probe microdialysis procedure

Introduction

It has been well demonstrated that somatodendritic serotonin$_{1A}$ (5-HT$_{1A}$) autoreceptors play an important role in the control of 5-HT release in terminal areas (23). Antagonists of 5-HT$_{1A}$ receptors, therefore, have been proved to potentiate the increases of extracellular 5-HT concentrations induced by selective

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serotonin reuptake inhibitors (SSRIs) including fluoxetine (5, 29), paroxetine (9) and citalopram (21).

The 5-HT_{1A} autoreceptor-mediated control of 5-HT release may be different in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) 5-HT pathways. This view was supported by several lines of evidence. First of all, 5-HT neurons in the DRN and MRN are morphologically distinct (11), which may explain the findings that there are more 5-HT_{1A} receptors (27) and 5-HT reuptake sites (8) in the DRN than the MRN. Secondly, 5-HT_{1A} receptor agonist given into the DRN and MRN produced different behavioral and biochemical responsiveness (2, 7). Thirdly, the feedback inhibition mediated by the 5-HT_{1A} autoreceptors is more potent in the DRN than the MRN (21). Finally, desensitization of 5-HT_{1A} autoreceptors induced by repeated treatment with a 5-HT_{1A} agonist, 8-OH-DPAT, developed more rapidly and intensely in the DRN than the MRN (12).

One of the approaches to assess the functional difference between the 5-HT_{1A} autoreceptors in the DRN and MRN 5-HT pathways is to monitor 5-HT release in specific terminal areas under the manipulation of raphe 5-HT neuron activity. Although most brain areas are innervated by the ascending serotonergic pathways originating from both DRN and MRN (17), some brain areas are preferentially innervated by one of the nuclei. For example, prefrontal cortex (Pfc) and striatum are preferentially innervated by 5-HT axon terminals projecting from the DRN, while dorsal and ventral hippocampus is predominantly innervated by 5-HT axon terminals originating from the MRN (1, 3, 16). Therefore, Pfc and dorsal hippocampus (Dhp) were chosen in the present study to compare the functional difference of 5-HT_{1A} autoreceptors located in the DRN and MRN.

Previous data have shown that a 5-HT_{1A} antagonist, N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(pyridinyl)cyclohexanecarboxamide (WAY100635), potentiated the fluoxetine-induced increases of extracellular 5-HT concentration in the hypothalamus (5, 29) and frontal cortex (4), but not in ventral hippocampus (14). Since those studies were conducted in separate animals in various laboratories, the data are not ideal for comparative analysis on the 5-HT_{1A} antagonist-potentiated 5-HT increases induced by fluoxetine in different brain areas. The present study was aimed to fill this knowledge gap by applying a dual-probe microdialysis procedure to detect extracellular 5-HT concentrations in the two brain areas (Pfc and Dhp) of the same rat, which can avoid the variation between individual animals and various laboratories under different experimental conditions.

Materials and Methods

Animals

Male Sprague-Dawley rats with body weight of 260 - 300 g from Charles River Laboratories (Portage, MI) were used. The rats were housed (4 rats per cage) in a temperature-controlled (22 ± 1°C) laboratory for one week before use and had free access to water and dry lab chow. Light cycle is 7 AM (light on) - 7 PM (light off).

Dialysate Probe Implantation

Home-made plastic dialysis probes of a loop-type (13, 19) were made by using cellulose dialysis membrane (C-D Medical, Miami, FL) with molecular weight cut-off of 5000. The length of exposed tubing of dialysis probe was 2 - 3 mm with a diameter of 0.6 mm. For this type of probes, the in vitro recovery across the dialysis membrane at a flow rate of 1.5 µl/min was about 20% for monoamines (19).

Anesthesia was induced with 170 mg/kg of chloral hydrate and 36 mg/kg of pentobarbital in 30% propylene glycol and 14% ethanol. Dialysis probes were slowly implanted into the Pfc and the Dhp of the same rat brain. The coordinates used for the brain areas were: 3.2 mm anterior to bregma, 0.8 mm lateral to the mid-sagittal suture, 5.0 mm ventral from dura for the Pfc; and 3.8 mm posterior to bregma, 1.8 mm lateral to the mid-sagittal suture, 4.0 mm ventral from dura for the Dhp (18). The probes were secured to the skull by using two screws and cranioplastic cement (Plastics One, Roanoke, VA).

Dialysate Sample Collection

Microdialysis experiments were performed two days after surgery to allow the rats to fully recover from the operation. The rat was placed in a plastic bowl and connected to a liquid swivel system for freely moving animals (CMA/120, BioAnalytical Systems, West Lafayette, IN). The input tube of the dialysis probe was connected to a syringe pump (Harvard Instruments, Model 22, South Natick, MA) which delivered an unbuffered artificial cerebrospinal fluid containing 150 mM NaCl, 3 mM KCl, 1.7 mM $CaCl_2$ and 0.9 mM $MgCl_2$ (pH 6.0) to the probe at a rate of 2 µl/min. The output tube from the swivel was connected to two microfraction collectors (CMA 142) which collected the dialysate samples from up to four rats at the same time.

Simultaneous collection of dialysate samples (60 µl) from the Pfc and Dhp of the same rat was for 30 min. The dialysate samples were transferred within 1 hr after the collection into a temperature controlled

(2°C) sample tray on an automatic HPLC sample injector (Gilson model 231) attached to a ten-port valve and analyzed within 4 hr after the collection. Each dialysate sample was injected once onto analytical column. Less than 20% variation of the basal monoamine concentrations was obtained 2 - 3 hr after the start of experiments. Each experiment lasted for 8-10 hr.

Drug Treatments

Drugs were given after at least three stable baseline samples were obtained. Fluoxetine (Eli Lilly) and WAY100635 (RBI) were dissolved in distilled water at a concentration of 10 mg/ml and 1 mg/ml respectively. The drugs were administered at a volume of 1 ml/kg through an implanted subcutaneous tube to avoid handling the rats during the experiments.

Although our previous data have shown that vehicle alone did not induce significant change in the baseline values of monoamines in the hypothalamus under the same experimental conditions (20), possible influence of saline injection on monoamine levels in the Pfc and Dhp was determined in the present study.

HPLC Assays of Monoamines

A BDS-Hypersil 3μ C18 analytical column (2×150 mm, i.d. from Keystone Scientific, Bellefonte, PA) was used for the separation. A small sample clean-up column (BDS-Hypersil 3μ C18, 2×10 mm, i. d.) was connected to the analytical column to trap late-eluting peaks contained in the dialysate samples. The analytical column was maintained at 40°C with a column heater, while the sample clean-up column was maintained at room temperature. A ten-port HPLC valve with a 20 μl sample loop was configured with the analytical column. The mobile phase for both columns was the same and consisted of 75 mM sodium phosphate monobasic, 350 mg/L 1-octane-sulfonic acid sodium salt, 0.5 mM EDTA, 0.9% tetrahydrofuran (HPLC grade, inhibitor-free) and 9% acetonitrile at pH 3.0 (adjusted with phosphoric acid). The flow rate for both columns was 0.20 ml/min.

An electrochemical detector (EG & G PARC, Princeton, NJ) with dual glassy carbon electrodes (E1=700 mV, E2=0 mV, range=0.5 nA on both electrodes) was used for the detection. 5-HT was detected at E1 and dopamine (DA) was detected at E2. The sensitivity was approximately 0.1 pmol/ml dialysate for 5-HT and DA and 10 pmol/ml for 5-HIAA, DOPAC and HVA. The data was collected by a Compaq 486/33 chromatography data system (Ezchrom Scientific Software, San Ramon, CA) which calculated peak heights and sample concentrations.

Histological Verification of Probe Location

Probe placement was checked by infusing a dye (2,3,4-triphenyl-2H-tetrazolium chloride) through the dialysate probe at the end of experiments. The animal brains were removed 10 min later, frozen and sliced until the probe dye spot was observed.

Statistical Analysis

Rats with improper probe location and monoamine baseline values (2 times of the mean values) were not included in the statistical tests. Monoamine concentrations were calculated as pmol/ml dialysate without correction for recovery across the dialysate membrane. Since the baseline values may vary between individual animals, the data included in statistical analysis and figures were expressed as percent of respective baseline values.

For statistical analysis, a two-way (treatment and time) analysis of variance (ANOVA) followed by Fisher's protected least square different (PLSD) test and Scheffe F-test was used to determine the statistical significance of difference among the several treatment groups, in terms of the nature of independent data obtained from the in vivo study (28). The minimal level for statistical significance was set at $P < 0.05$.

Results

Effects on 5-HT and Metabolite

As shown in Fig. 1, fluoxetine given at 10 mg/kg (s.c.) induced significant increases in the extracellular 5-HT concentrations in the Pfc ($F_{10,89} = 3.297$, $P = 0.0120$) and Dhp ($F_{10,95} = 2.820$, $P = 0.0048$). The fluoxetine-induced 5-HT increases were smaller in the Pfc (Fig. 1A) than that in the Dhp (Fig. 1B), as seen from the maximal 5-HT concentration elevation caused by fluoxetine in the Pfc (183% of the baseline value, Fig. 1A) and Dhp (223% of the baseline value, Fig. 1B). The 5-HT concentration in the Pfc reached a peak level 60 min after the administration of fluoxetine and started to decline afterward (Fig. 1A), while the 5-HT concentration in the Dhp was continuously elevated (Fig. 1B).

Subsequent administration of the antagonist of 5-HT$_{1A}$ receptors, WAY100635, at 1 mg/kg (s.c.) 150 min after the injection of fluoxetine increased the 5-HT concentrations to similar levels in both areas, since the maximal 5-HT concentrations induced 120 min after the administration of WAY100635 were similar in the Pfc (332% of the baseline value, Fig. 1A) and Dhp (308% of the baseline value, Fig. 1B). The 5-HT concentrations immediately before the

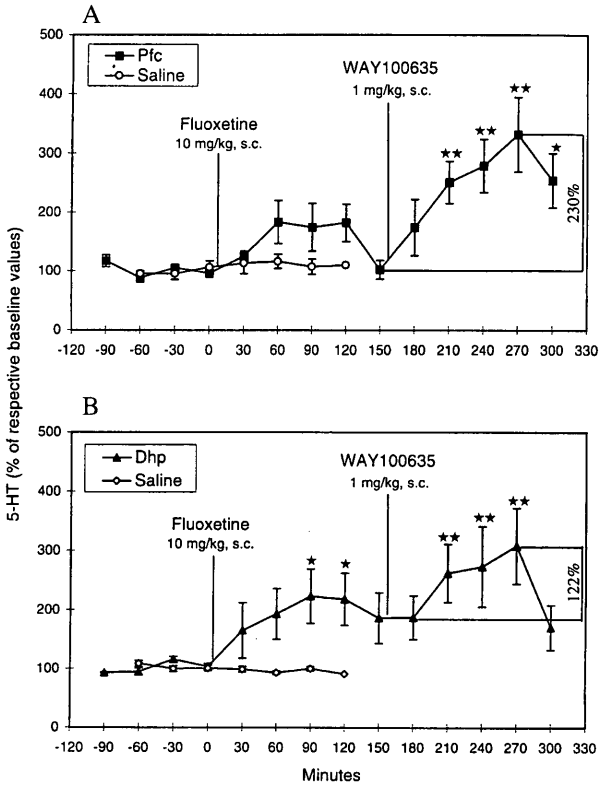

Fig. 1. Comparison of changes in extracellular 5-HT concentrations in rat prefrontal cortex (Pfc, 1A) and dorsal hippocampus (Dhp, 1B) induced by sequential injections of fluoxetine and WAY100635. Two dialysis probes were implanted into the Pfc and Dhp of the same rat brain. Data are represented as percent (means ± S.E.M.) of respective baseline values. The 5-HT baseline values were 0.19 ± 0.02 pmol/ml (means ± S.E.M.) in the Pfc and 0.37 ± 0.05 in the Dhp. n = 5–8 rats. *P<0.05 and **P<0.01 versus respective baseline values according to a two-way ANOVA followed by Fisher's PLSD test and Scheffe F-test. The influence of saline injection (1 ml/kg, s.c.) on the extracellular 5-HT concentrations in rat Pfc and Dhp was tested in a separated experiment (n = 4 rats). No significance was found 2 hr after the injection of saline.

injection of WAY100635 were 102% in the Pfc (Fig. 1A) and 186% in the Dhp (Fig. 1B). The net 5-HT increases (the difference between the 5-HT concentrations immediately before the injection of WAY100635 and the maximal 5-HT concentrations after the injection of WAY100635) induced by WAY100635 were, therefore, 230% (from 102% to 332%) in the Pfc and 122% (from 186% to 308%) in the Dhp. Thus, WAY100635 produced a more robust potentiation in the fluoxetine-induced 5-HT increases in the Pfc than in the Dhp.

The extracellular concentrations of a 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in both brain areas were decreased by fluoxetine, which reached significance ($F_{10,99} = 5.240$, $P = 0.0001$ for the Pfc and $F_{10,71} = 6.776$, $P = 0.0001$ for the Dhp) at 150 min after the administration of fluoxetine (Fig. 2). The subsequent injection of WAY100635 further decreased the 5-HIAA concentrations especially at 150 min after the administration of WAY100635 (Fig. 2). Saline injection (1 ml/kg, s.c.) did not induce significant change in the extracellular 5-HT concentrations in the Pfc and Dhp during a 120-min period after the injection (Fig. 1A and 1B). The 5-HT baseline values were 0.19 ± 0.02 pmol/ml (mean ± SEM) in the Pfc and 0.37 ± 0.05 in the Dhp, and the 5-HIAA baseline values were 157 ± 17 in the Pfc and 448 ± 62 in the Dhp.

Effects on DA and Metabolites

The sequential injections of fluoxetine and WAY100635 did not significantly change the extracellular concentrations of DA and two DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the Pfc (Fig. 3A) and Dhp (Fig. 3B).

Effects of WAY100635 Alone

The extracellular concentrations of 5-HT and DA in the Pfc and Dhp were not significantly altered by WAY100635 injected at 1 mg/kg (s.c.) alone during a 90-min period after the administration (Fig. 4).

Discussion

The present in vivo study showed that fluoxetine produced a slightly smaller increase in the extracellular 5-HT concentration in the Pfc than Dhp of the same rat when acting alone, but increased the 5-HT concentrations to similar levels in both areas when

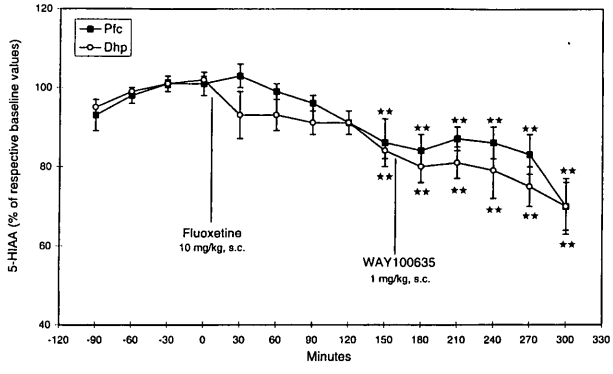

Fig. 2. Effects of sequential injections of fluoxetine and WAY100635 on extracellular concentrations of a 5-HT metabolite, 5-HIAA, in rat prefrontal cortex (Pfc) and dorsal hippocampus (Dhp) as studied by a dual-probe microdialysis procedure. Data are represented as percent (means ± SEM) of respective baseline values. The 5-HIAA baseline values were 157 ± 17 pmol/ml (means ± SEM) in the Pfc and 448 ± 62 in the Dhp. n = 5–8 rats. **P<0.01 versus respective baseline values according to a two-way ANOVA followed by Fisher's PLSD test and Scheffe F-test.

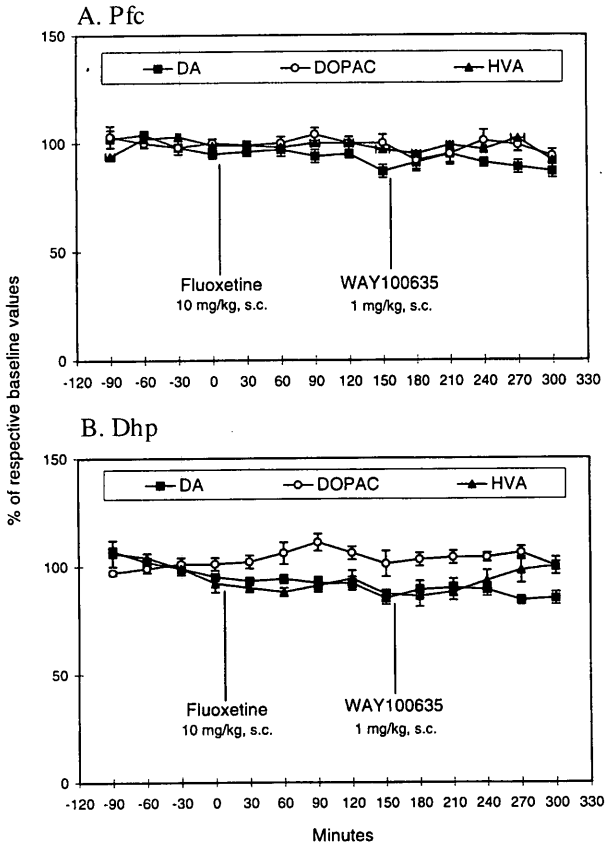

Fig. 3. Effects of sequential injections of fluoxetine and WAY100635 on extracellular concentrations of DA and two DA metabolites, DOPAC and HVA, in rat prefrontal cortex (Pfc, 3A) and dorsal hippocampus (Dhp, 3B) as studied by a dual-probe microdialysis procedure. Data are represented as percent (means ± SEM) of respective baseline values. n = 5–8 rats. No significance was found among the treatments according to a two-way ANOVA followed by Fisher's PLSD test and Scheffe F-test.

the 5-HT$_{1A}$ autoreceptor-mediated inhibition of 5-HT release was removed by the 5-HT$_{1A}$ antagonist, WAY100635. Thus, 5-HT release appears to be controlled by 5-HT$_{1A}$ autoreceptors more potently in the Pfc than Dhp. Since the comparison was done in the same rat by means of a dual-probe microdialysis procedure, it avoided possible variation between individual animals and separate experiments. Therefore, the present study provides a piece of direct in vivo evidence showing the difference between the mechanism(s) controlling 5-HT release in the Pfc and Dhp.

The present findings are in agreement with many previous reports. First of all, a SSRI, citalopram, induced a larger increase in the extracellular 5-HT concentrations in the Dhp than frontal cortex (9). Furthermore, WAY100635 potentiated the citalopram-induced 5-HT increase in the frontal cortex and striatum, areas innervated by the DRN 5-HT pathway, but not in the Dhp, area innervated by the MRN 5-HT pathway (9).

Secondly, WAY100635 was found to potentiate the 5-HT increases induced by another SSRI, paroxetine, in a regionally dependent manner with a more robust potentiation of the 5-HT increases in the striatum and frontal cortex than the Dhp (21), which is in line with the present finding with the combination of fluoxetine and WAY100635.

Thirdly, in vivo neurochemical data have revealed that local injection of the 5-HT$_{1A}$ agonist, 8-OHDPAT, into the DRN mainly reduced the extracellular 5-HT concentration in the striatum, while it mainly reduced the 5-HT concentration in the hippocampus when locally injected into the MRN (3). This result showed the difference regarding 5-HT release in the DRN and MRN 5-HT pathways under activated condition. Fourthly, morphological data have shown that the Pfc is preferentially innervated by 5-HT neurons located in the DRN, while dorsal and ventral hippocampus is predominantly innervated by 5-HT neurons located in the MRN (1).

Furthermore, it was found that the 5-HT terminals projecting from the DRN are fine 5-HT axons and highly vulnerable to the neurotoxic effects of the amphetamine derivatives MDA and PCA, while the 5-HT terminals projecting from the MRN are beaded 5-HT axons and markedly resistant to MDA and PCA (15). Those findings provide an anatomical basis for the hypothesis that the DRN and MRN 5-HT pathways may function differently.

Finally, it has been reported that the DRN and MRN may differently influence several behavioral and biochemical functions including locomotor

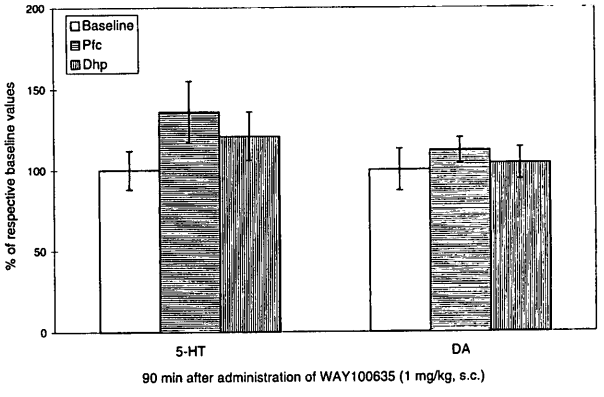

Fig. 4. Administration of WAY100635 alone (1 mg/kg, s.c.) did not significantly change extracellular 5-HT and DA concentrations in rat prefrontal cortex (Pfc) and dorsal hippocampus (Dhp) as studied by a dual-probe microdialysis procedure. Data obtained 90 min after the injection of WAY100635, are represented as percent (means ± S.E.M.) of respective baseline values. n = 4 rats. No significance was found among the treatments according to a two-way ANOVA followed by Fisher's PLSD test and Scheffe F-test.

activity (10), aggression (26), and hormone secretion (25). Therefore, the difference in the extracellular 5-HT concentrations in the Pfc and Dhp induced by the combination of fluoxetine and WAY100635 may in fact reflect a functional difference between the 5-HT_{1A} autoreceptors in the DRN and MRN 5-HT pathways.

The similar 5-HT concentrations induced by the combination of fluoxetine and WAY100635 in the Pfc and Dhp areas may be due to a ceiling effect on 5-HT release, which limits the total amount of 5-HT to be released in a certain period of time. Since the fluoxetine-induced 5-HT increases were smaller in the Pfc than Dhp, more room reserved for the Pfc area versus the Dhp area to further potentiate the 5-HT increases until the limit (ceiling) reached. However, the ceiling effect is not likely to explain the different 5-HT increases in the Pfc and Dhp areas induced by fluoxetine alone, which, in contrast, can be well explained by the feedback control mechanisms through the 5-HT_{1A} autoreceptors.

WAY 1001635 is a selective and silent 5-HT_{1A} antagonist (6). Consistent with the previous finding (22), the present study showed that WAY100635 alone produced no change in the extracellular 5-HT concentration. Those results indicate that under resting condition the somatodendritic 5-HT_{1A} autoreceptors are not tonically activated by 5-HT. In agreement with this view, it was reported that low concentrations of 5-HT did not influence the spontaneous activity of raphe 5-HT neurons (24). Because of the efficiency of the 5-HT transporters, any WAY100635-induced release of 5-HT may be re-uptaken into the terminals making detection of 5-HT increase difficult. Indeed, in rats pre-treated with fluoxetine the WAY100635-induced increases occurred and detected as demonstrated in the present study.

In summary, the present in vivo study clearly demonstrates that fluoxetine has weaker effect to increase the extracellular 5-HT concentration in the Pfc than Dhp, but has similar effect in both areas in the presence of an antagonist of 5-HT_{1A} receptors. Thus, the 5-HT_{1A} autoreceptor-mediated control of 5-HT release may have a variable impact on the SSRI-induced 5-HT increases in different 5-HT pathways. This issue may be critical to understand the changes in brain function induced by antidepressant and anxiolytic drugs.

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