Fluoxetine and all other SSRIs are 5-HT<sub>2B</sub> Agonists - Importance for their Therapeutic Effects

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Abstract: Fluoxetine and other serotonin-specific re-uptake inhibitors (SSRIs) are generally thought to owe their therapeutic potency to inhibition of the serotonin transporter (SERT). However, research in our laboratory showed that it affects, with relatively high affinity the 5-HT<sub>2B</sub> receptor in cultured astrocytes; this finding was confirmed by independent observations showing that fluoxetine loses its ability to elicit SSRI-like responses in behavioral assays in mice in which the 5-HT<sub>2B</sub> receptor was knocked-out genetically or inhibited pharmacologically. All clinically used SSRIs are approximately equipotent towards 5-HT<sub>2B</sub> receptors and exert their effect on cultured astrocytes at concentrations similar to those used clinically, a substantial difference from their effect on SERT. We have demonstrated up-regulation and editing of astrocytic genes for ADAR2, the kainate receptor GluK2, cPLA<sub>2</sub> and the 5-HT<sub>2B</sub> receptor itself after chronic treatment of cultures, which do not express SERT and after treatment of mice (expressing SERT) for 2 weeks with fluoxetine, followed by isolation of astrocytic and neuronal cell fractionation. Affected genes were identical in both experimental paradigms. Fluoxetine treatment also altered Ca<sup>2+</sup> homeostatic cascades, in a specific way that differs from that seen after treatment with the anti-bipolar drugs carbamazepine, lithium, or valproic acid. All changes occurred after a lag period similar to what is seen for fluoxetine’s clinical effects, and some of the genes were altered in the opposite direction by mild chronic inescapable stress, known to cause anhedonia, a component of major depression. In the anhedonic mice these changes were reversed by treatment with SSRIs.

Keyword: Astrocytes, gene expression, 5-HT<sub>2A</sub> receptor, 5-HT<sub>2B</sub> receptor, SSRIs.

INTRODUCTION

It is generally assumed that fluoxetine and the other so-called ‘serotonin-specific re-uptake inhibitors’ (SSRIs) do not act on serotonin receptors at therapeutically relevant concentrations. At the time they were introduced clinically [1] it had been shown that SSRIs inhibited reuptake of serotonin (5-HT), but not of any other known transmitter, from the synaptic cleft in rat brain, decreased neuronal firing in the midbrain, and caused a rapid decrease in serotonin turnover [2]. Detailed studies showed very low affinity for any known receptor for serotonin or any other neurotransmitter [3,4]. The 5-HT<sub>2B</sub> receptor was unknown at that time, because it was discovered in 1992 [5], and it was classified as the 5-HT<sub>2B</sub> receptor only in 1994 [6]. Nevertheless, interaction of fluoxetine with a serotonin receptor on astrocytes had been shown already in 1979 by Hertz et al. [7], and the identity of this receptor as a 5-HT<sub>2B</sub> receptor was demonstrated by Kong et al. in 2002 [8], after it erroneously (like many other effects on the 5-HT<sub>2B</sub> receptor) had been identified as a 5-HT<sub>2C</sub> receptor [9]. In contrast to all other serotonin (5-HT) receptors, the 5-HT<sub>2B</sub> receptor has since been shown to have sufficiently high affinity for fluoxetine [10] to be activated by therapeutically relevant concentrations of these drugs (Table I).

SSRIs AS 5-HT<sub>2B</sub> RECEPTOR AGONISTS

Subsequently it was shown that all clinically used SSRIs are not only specific 5-HT<sub>2B</sub> receptor agonists [11], but also are virtually equipotent, in contrast to a large and important difference from the widespread difference in their potency as SERT inhibitors. Moreover, it was demonstrated that cultured astrocytes do not express the serotonin transporter, SERT [8]. Later Diaz et al. [12] found that effects of SSRIs seen after long-term treatment as well as the enhanced neurogenesis normally seen in fluoxetine-treated animals [13] can be prevented by genetic deletion or pharmacological inhibition of 5-HT<sub>2B</sub> receptors; moreover stimulation of 5-HT<sub>2B</sub> receptors by compounds that are not SSRIs induced SSRI-like responses in behavioral assays. They also showed expression of the 5-HT<sub>2B</sub> receptor and SERT in raphe serotonergic neurons, and that SSRIs increased extracellular serotonin concentration in hippocampus. This increase was normally detectable after 20 min and peaked in 60 min after drug administration, and it was strongly reduced when 5-HT<sub>2B</sub> receptors were deleted (Fig. 1). These findings led to a conclusion that 5-HT<sub>2B</sub> receptor expression on the raphe neurons is required for the therapeutic actions of SSRIs. Experiments by Launay et al. [14], performed on neuronal cultures from raphe nuclei (and serotonergic
neurons induced from the 1C11 cell line) demonstrated that 5-HT<sub>2B</sub> receptor-PKC coupling promotes phosphorylations of SERT that control SERT activity. More specifically, it was shown that 5-HT<sub>2B</sub> receptor stimulation in the absence of added serotonin approximately doubled phosphorylation of SERT serotonin transport, a result very different from the generally accepted ability of fluoxetine to reduce SERT activity [14]. This pronounced difference may be explained by substantial differences between the immature and the mature brain [15, 16]. This age-dependent difference in fluoxetine effects is, however, in concordance with the finding by Sarkar et al. [17] that post-natal fluoxetine exposure results in the development of perturbed emotionality. Diaz et al. [12] also showed that fluoxetine was unable to elicit SSRI-like responses in behavioral assays in mice in which SERT was knocked out, but in these animals there is also a substantial depletion of serotonin [18].

5-HT<sub>2B</sub> receptors are present in many brain cells. Their expression was first identified in Purkinje cells by Choi and Maroteaux [19] and subsequently was demonstrated by mRNAs identification in freshly isolated fractions of neurons and astrocytes (Fig. 2) from adult mice in which cell-specific markers were linked with differently fluorescent compounds that allowed cell separation [20] using fluorescence-activated cell sorting (FACS). Since the same primers were used for neurons and astrocytes, in Fig. 2 the heights of the columns provide direct information about the density of expression, which for all three 5-HT<sub>2</sub> receptor genes, but

Table 1. K<sub>I</sub> values for 5-HT binding by selected SSRIs.

| Drug      | 5-HT<sub>1A</sub> | 5-HT<sub>1B</sub> | 5-HT<sub>1D</sub> | 5-HT<sub>2A</sub> | 5-HT<sub>2B</sub> | 5-HT<sub>2C</sub> | 5-HT<sub>3</sub> |
|-----------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Fluoxetine| 11,000            | 6,200             | 4,300             | 1,800             | 70*               | 270               | 19,000            |
| Citalopram| 16,600            | EC<sub>50</sub>&gt;10,000 | 13,500           | 5,400             | 3,300             | EC<sub>50</sub>&gt;10,000 |                   |
| Paroxetine| EC<sub>50</sub>&gt;10,000 | 25,000           | 4,900             | 10,200            | 17,400            | 2,600             |                   |

*From Hertz et al. [10]. All other values from Wong et al. [4].

Fig. (1). Serotonin-selective reuptake inhibitor (SSRI)-induced increase in extracellular serotonin (5-HT) concentration in hippocampus of freely moving mice is greatly reduced when 5-HT<sub>2B</sub> receptors are absent. After 4 h of equilibration, the SSRI paroxetine (2 mg/kg, i.p.) was administered at time zero (0): hippocampal samples of extracellular fluid, obtained by microdialysis, were collected each 20 min for 3 h, and 5-HT concentrations were measured by high-pressure liquid chromatography. The peak observed at one hour after paroxetine injection was significantly higher in wild-type (WT) mice than in 5-HT<sub>2B</sub>−/− mice or in WT mice pretreated with the 5-HT<sub>2B</sub> antagonist RS127445 15 min before the paroxetine injection. Inset represents the area under the curve (AUC) for each experimental group. Basal extracellular serotonin levels before the injection were identical under all three conditions. (From Diaz et al. [12]).
especially the 5-HT_{2B} receptor, is higher in astrocytes than in neurons.

**FLUOXETINE TREATMENT DOWN-REGULATES EXPRESSION OF 5-HT_{1A} RECEPTORS**

A putative explanation for the long time lag between the onset of therapy with SSRIs and the clinical effect was first suggested by Blier and de Montigny [21] after administration of another serotonin reuptake blocker, zimelidine (no longer on the market). Administration of this drug not only increased extracellular serotonin concentration, but also strongly reduced electrical activity of serotonergic neurons, and this inhibition was gradually reduced during continued drug treatment. The authors therefore concluded that administration of a serotonin reuptake inhibitor may not result in enhanced serotonergic activity until normal excitability has been restored and it may thus have no therapeutic effect until then. The reason for the normalization of excitability was found to be down-regulation of activity of 5-HT_{1A} autoreceptors, which generally are homoautoreceptors, located proximally in the neurons [22]. Czachura and Rasmussen [23] examined the effects of administration of different fluoxetine doses on the recovery of activity of serotonergic neurons in the dorsal rat raphe nucleus during a 21-day exposure. They found that i) acute intravenous, subcutaneous and intraperitoneal administration of fluoxetine inhibited the activity of serotonergic neurons; ii) chronic administration of fluoxetine at clinically relevant doses caused recovery of the activity of the neurons to baseline levels over the course of 14–21 days independently of either plasma or cerebrospinal fluid levels of fluoxetine or norfluoxetine; and iii) a non-parallel shift in their dose-response curve to the 5-HT_{1A} agonist 8-OH-DPAT occurred over the 21 days of treatment, indicating 5-HT_{1A} receptor desensitization. Rasmussen [23] and Artigas et al. [26] found that inhibition of this receptor could decrease the firing rate of serotonergic neurons in the dorsal raphe nucleus and that the 5-HT_{1A} antagonist WAY-100635 restores the firing rate to basal values. Whether or not inhibition of 5-HT_{1A} receptors can accelerate the onset of clinical effect after beginning of the therapy with SSRI is un-resolved. Artigas et al. [26] found that inhibition of this receptor could decrease the lag period from administration of an SSRI till therapeutic effect. That this is not the case has, however, also repeatedly been reported [27, 28]. Also, in the study by Czachura and Rasmussen [23], firing recovery did not correlate with levels of fluoxetine or norfluoxetine in plasma or cerebrospinal fluid. This is in contrast to a report by Önder and Tural [28] of faster clinical effect of higher than lower doses of fluoxetine and may accordingly disagree with the hypothesis that 5-HT_{1A} receptor desensitization and recovery of firing of 5-HT cells in the dorsal raphe nucleus is of importance in the delayed therapeutic onset of fluoxetine. Finally, prevailing models suggest that 5-HT autoreceptors become desensitized not only in response to antidepressant administration but also in response to stress, two seemingly opposite manipulations [22]. Thus, questions remain about the role of the autoreceptors in determination of the time when the response to SSRIs becomes manifest.

**FURTHER STUDY IN CULTURED AND FRESHLY DISSOCIATED ASTROCYTES**

**Acute Effects**

The demonstration that fluoxetine acutely stimulates the 5-HT_{2B} receptor in astrocyte cultures [8] was followed by...
identification of the signal pathway that was activated in the cultured cells by Li et al. [29]. A slightly expanded version of this pathway is shown in Fig. 3. It has previously been shown that fluoxetine acutely stimulates glycogenolysis, an effect that is secondary to an increase in [Ca2+]i [9, 30]. Involvement of 5-HT2B receptor-stimulated glycogenolysis has also been demonstrated during establishment of memory, where acute administration of serotonin can rescue long-term learning in a one trial aversive learning paradigm in day-old chickens under conditions when the aversive stimulus was otherwise too weak to establish more than transient long-term memory retention [31, 32]. Fluoxetine and paroxetine have a similar effect in this paradigm and are equipotent, indicating that the rescue was not due to inhibition of SERT (where different SSRIs have widely different potencies), and the rescuing effect was inhibited by an inhibitor of glycogenolysis [32]. In contrast to high concentrations of 5-HT itself, which also stimulate 5-HT1A receptors and thereby can inhibit learning, fluoxetine and paroxetine at high levels have no inhibitory effect on learning [32]. Fluoxetine might also affect glycogen synthesis, since the AKT pathway (Fig. 3) is stimulated leading to AKT phosphorylation (Fig. 4). AKT phosphorylation in turn stimulates GSK phosphorylation (Fig. 3), making these in vitro findings consistent with demonstrations by Jope and coworkers [33-34] that administration of fluoxetine in brain cortex increases phosphorylation of GSK, and that serotonergic stimulation of GSK3 has mood effects.

**Chronic Effects on 5-HT-Receptor and Related Proteins in Fluoxetine-Treated Animals and Cultures**

Fig. 2 shows that only one astrocytic 5-HT2 receptor, the 5-HT2B receptor is up-regulated by 14 days of in vivo treatment with fluoxetine, as also indicated in Table 2. This receptor is also up-regulated in whole brain [20]. The astrocytic 5-HT2A and 5-HT2C receptors are unaltered, but one neuronal 5-HT2 receptor, the 5-HT2C receptor, is also up-regulated in whole brain [20]. In addition the 5-HT2B
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receptor sites are normally unedited in both astrocytes and neurons, but after 2 weeks of treatment up to one quarter of each of 8 different editing sites become edited, i.e., undergo shifts in base pair composition, as indicated in Table 2. The importance of this is unknown, but for the 5-HT2C receptor editing can change G protein coupling [35]. Experiments in cultured astrocytes [36] have shown that upregulation of the 5-HT2B receptor itself in contrast with the changes in gene expression of ADAR2, cPLA2 and GluK2 and in Ca2+ homeostasis (these all will be discussed below) occurs very slowly (Fig. 5, A, B), but with the usual dependence on the fluoxetine concentration, i.e., an effect of 1 μM after 2 weeks. For comparison, the combined extracellular concentrations of fluoxetine and norfluoxetine in treated patients may reach up to 3 μM [37]. In contrast editing of the receptor (Fig. 5C) was obvious after 3 days of treatment and thus precedes up-regulation. After 7 days the edited receptor no longer responded to serotonin with an increase in IP3 turnover measured as described in the legend to the Fig. 5D. To ascertain that this was a direct result of receptor editing, and not due to other effects by chronic fluoxetine administration, COS-7 cells were infected with receptor plasmids of either normal 5-HT2B receptors or receptors with 8 RNA sites RNA edited, and a similar inhibition was shown (Fig. 5E). Thus an important result of chronic exposure to fluoxetine is to alter the normal response to serotonin.

Diaz et al. [12] showed that abrogation of the enhanced neurogenesis occurred in fluoxetine-treated animals after inactivation of 5-HT2B receptors, and a prominent feature in Fig. 3 is growth factor release transactivating the epidermal growth factor receptor (EGFR). For these reasons mRNA expression of EGFR was studied in freshly isolated neurons and astrocytes from both untreated control mice and fluoxetine-treated mice. Fig. 6 shows that its expression level was approximately 4 times higher in freshly isolated mouse brain astrocytes than in neurons obtained from the same brain. It was not increased after 14 days of in vivo treatment with fluoxetine (10 mg/kg per day ip.) in any of the two cell types. However, entry into neurons and especially astrocytes of nucleoside precursors for synthesis of DNA and RNA via the equilibrative nucleoside transporter ENT2 was increased during fluoxetine treatment as indicated by an elevation in its mRNA expression in both astrocytes and neurons – if anything most in astrocytes – after 14 day of fluoxetine treatment in the mouse [38]. Since participation of the 5-HT1A receptor so often has been anticipated in the process leading to manifestation of the therapeutic effect of SSRIs, and since this receptor is generally regarded as confined to the cell bodies of serotonergic neurons, its expression was also studied in the two cell types. Fig. 7 shows that 5-HT1A receptors are not confined to cell bodies of serotonergic neurons but also expressed in neurons from the cerebral hemispheres and even – although at lower density – in astrocytes. This is consistent with previous observations in astrocyte cultures and in striatal astrocytes [39-40]. Finally, the cell culture finding that SERT is absent in astrocytes was confirmed in freshly isolated astrocytes from the cerebral hemispheres. As can be seen in Fig. 8 this also applies to neurons from this location, although a minor expression cannot be excluded.
The effects of SSRIs that are important in connection with major depression are the chronic effects, since the effect of clinical treatment takes several weeks to appear. The cells obtained by FACS directly from the brains of fluoxetine-treated or control animals are intact enough that gene expression can be reliably determined, but for many functional studies they are probably not sufficiently well preserved. Both types of studies are easily carried out in cultured astrocytes, but astrocyte cultures have often been used rather uncritically. Our cultures are different from most in (i) originating from neonatal mice, (ii) being very clean, because the cells have been filtered through dense meshes, and (iii) being treated with dibutyryl cyclic AMP from the third week to replace a noradrenergic signal they would have received, had they remained in the brain. Comparison of effects of chronic treatment with fluoxetine on up-regulation of selected genes showed in all studied cases similar effects in these cultures and in astrocytes freshly isolated from animals chronically treated with fluoxetine, 10 mg/kg per day, the only SSRI studied in astrocytes in vivo (Table 2). They have also been similar to those found by other authors or ourselves in total brain of treated animals with the exception of gene expression of 5-HT2C, sPLA2 and GluK4, genes that were up-regulated in neurons of the treated animals and accordingly also in whole brain (reviewed by Li et al. [20]). Moreover, genes for cfos and fosB were up-regulated in both neurons and astrocytes (Table 2).

**ADARs**

The up-regulation of ADAR2 by chronic treatment with fluoxetine is of special importance since editing of other genes depends on ADAR2 as shown for up-regulation of cPLA2 [41]. ADARs constitute a family of adenosine deaminases, which catalyze deamination of adenosine to inosine in double-stranded regions of mRNAs. This changes the amino acids in the translated protein sequence, since
**Fig. (6).** mRNA expression measured by RT-PCR of epidermal growth factor receptor (EGFR) in astrocytes and neurons isolated by FACS from cerebral hemispheres in vivo from similar fluoxetine-treated mice as used in Fig. 2. (A) A representative experiment showing mRNAs for EGFR and for TBP, as a house-keeping gene, in control animals and the corresponding results in fluoxetine-treated animals. The sizes of the PCR products of EGFR is 305 bp and of TBP 236 bp. (B) Means ± SEM of scanned ratios between EGFR and TBP. n = 3 (neurons) or 4 (astrocytes). *P<0.05 between neuron group and astrocyte group. (Unpublished experiments by B. Li, L. Hertz and L. Peng, using similar technique as in Li et al. [20]).

**Fig. (7).** mRNA expression measured by RT-PCR of 5-HT<sub>1A</sub> receptor in astrocytes and neurons isolated by FACS from cerebral hemispheres in vivo from similar fluoxetine-treated mice as used in Fig. 2. (A) A representative experiment showing mRNAs for 5-HT<sub>1A</sub> receptor (5-HT<sub>1A</sub>R) and for TBP, as a house-keeping gene in control animals and the corresponding results in fluoxetine-treated animals. The sizes of the PCR products of the 5-HT<sub>1A</sub> receptor is 401 bp and of TBP 236 bp. (B) Means ± SEM of scanned ratios between 5-HT<sub>1A</sub> receptor and TBP. n = 3 (neurons) or 4 (astrocytes). *P<0.05 between neuron group and astrocyte group. (Unpublished experiments by B. Li, L. Hertz and L. Peng, using similar technique as in Li et al. [20]).
inosine is perceived by the cells as guanosine [42]. The ADAR family comprises ADAR1, ADAR2 and ADAR3 [43], which all are expressed in the brain [44]. In the brain ADAR2 is expressed in hippocampal pyramidal neurons and cerebellar Purkinje cells and Bergmann glial cells, but ADAR 1 and 3 are sparsely expressed [45]. ADAR2 up-regulation in fluoxetine-treated mice is subtype- and astrocyte-specific. In cultured astrocytes mRNA and protein expression of ADAR2 almost doubled within 3 days which can be prevented by treatment with 5-HT2B receptor siRNA [46]. However sPLA2 is up-regulated by fluoxetine in neurons and in whole brain (Table 2).

**Calcium-Dependent Phospholipase 2 (cPLA2)**

This enzyme in the brain in vivo is strongly expressed in astrocytes [47-49]. Its activation releases the unsaturated fatty acid arachidonic acid from the sn-2 position of membrane-bound phospholipid substrate in neural preparations [50-53], including glioma cells [54]. Arachidonic acid strongly stimulates glucose metabolism in cultured astrocytes [55]. So does treatment with 10 μM fluoxetine for 24 h, which might have sufficed to induce an increase in cPLA2 [56]. In contrast, acute exposure of astrocyte cultures to fluoxetine has no similar effect (L. Peng and L. Hertz, unpublished experiments). Arachidonic acid also stimulates glycogenolysis [57, 58].

Rapoport and coworkers [59-61] showed that chronic administration of fluoxetine leads to stimulation of cPLA2 activity and enhanced mRNA and protein expression of its gene in rat brain. Neither of the two other phospholipases A2 (secretory PLA2 [sPLA2] and intracellular PLA2 [iPLA2]) was similarly affected. Li et al. [41] confirmed a slow and selective up-regulation of mRNA and protein expression of cPLA2a, the major isoform of cPLA2, in mouse astrocytes in primary cultures during chronic exposure to 1 or 10 μM fluoxetine. The up-regulation was prevented by the 5-HT_{2B} antagonist SB 204741, the metalloproteinase inhibitor GM6001 and the inhibitor of EGFr receptor tyrosine phosphorylation AG1478 and by U0126, the inhibitor of ERK1/2 phosphorylation, all inhibitors of the signaling pathway shown in Fig. 3 for fluoxetine. Thus, up-regulation of mRNA and protein of cPLA2 were inhibited by the same drugs that acutely inhibit ERK1/2 phosphorylation and by inhibition of the phosphorylation itself. As shown in Table 2, up-regulation, specifically of cPLA2a, has been confirmed in freshly dissociated astrocytes isolated by FACS after 2 weeks treatment of rats with fluoxetine, whereas no corresponding effect was found in neurons [20]. Accordingly, the enhanced cPLA2 activity demonstrated in whole brain after chronic fluoxetine treatment [60] is likely to selectively occur in astrocytes. However, as already mentioned, sPLA2 is up-regulated by fluoxetine in neurons and in whole brain [20].

Stimulation of glucose metabolism may be important in the pharmacological treatment of depressive illness. In patients suffering from unipolar depression brain glucose
metabolism is reduced in many regions, primarily in the fronto-temporal parts [62-65], with a correlation between the degree of hypometabolism and severity of the illness [66], and normalization following treatment with an SSRI [67-69]. Sublette et al. [70] showed that arachidonic acid may play a role in determining rates of cerebral glucose metabolism. This could be seen from a rectilinear correlation in depressed patients between plasma concentration of arachidonic acid and rate of cerebral glucose utilization in a region affected metabolically. That major depression may even be related to an astrocyte-specific energy failure was suggested by Hundal [71]. Arachidonic acid metabolites, including prostaglandins, exert additional beneficial effects important for the amelioration of depression and are not only important for induction of inflammation [72]. Thus, prostaglandin synthesis inhibitors in doses used to treat pain may cause fear, agitation, and affective liability [73, 74], and one euthymic bipolar patient repeatedly developed a depression during such exposure [75]. This should in no way be taken as a suggestion that inflammatory events do not contribute to major depression, since such a contribution is well known, as described by Müller [76]. However, the lack of up-regulation of cPLA2 in neurons by fluoxetine suggests that inflammatory effects on neurons may not be enhanced by fluoxetine. Whatever the reasons are, genetic associations are found between cPLA2 and major depression [77, 78].

### The Calcium Channel Gene Ca1,2 and Ca2+ Homeostasis

Free intracellular Ca2+ concentration ([Ca2+]i) in astrocytes is low in resting cells (~100 nM, compared to 1-2 mM in the extracellular fluid), but [Ca2+]i increases are a necessary and essential component of all astrocytic activities (glycogenolysis, release of transmitter ATP, formation of glutamate and activities of many transmitters [36, 58, 79]. Increases in [Ca2+]i can also spread between astrocytes as Ca2+ waves [80]. Inside the cell Ca2+ can be accumulated into and released from intracellular organelles (endoplasmic reticulum or ER, mitochondria). Ca2+ transport across the cell membrane is therefore of utmost importance for astrocytic functions. In contrast to the ability of fluoxetine [9] (and many transmitters) acutely to cause a increase in [Ca2+]i, in astrocytes, chronic treatment with fluoxetine rapidly abolishes or reduces transmitter and fluoxetine-induced [Ca2+]i increase [46]. However, a corresponding increase in astrocytic [Ca2+]i by elevation of extracellular K+ concentrations above 15 mM [81] is not reduced, but increased, by chronic treatment with fluoxetine [82-83]. The reason for this is a fluoxetine-mediated up-regulation of the L-channel gene Cav1.2 [83], shown both in cultured cells and in astrocytes freshly obtained from fluoxetine-treated animals (Table 2). This overcompensates for a down-regulation of the store-operated (or capacitative) Ca2+ entry (SOCE) occurring via store-operated channels, SOCS [82, 84] as shown by the increase in astrocytic [Ca2+]i [83]. In contrast, the Ca1,3 gene, which plays a smaller role in astrocytes than Ca1,2, is unaffected by treatment of mice with fluoxetine for 2 weeks [83]. SOCS are very important for regulation of intracellular Ca2+, especially for the levels in the ER, which controls the amount of Ca2+ released by activation of inositoltrisphosphate (InsP3) receptors, InsP3R and ryanodine receptors (RyR). The 'transient receptor potential channel' (TRPC) protein TRPC1 is a major component of SOCS [84-86]. In cells in which TRPC1 had been knocked down by treatment with antisense oligonucleotides TRPC1 antibody the capacitative Ca2+ uptake is greatly reduced [82, 87]. The same occurs after short-lasting chronic treatment with fluoxetine and many other drugs (see below) and reduces or abrogates the ability of transmitters to increase astrocytic [Ca2+]i [82]. In conclusion, chronic treatment with

| Gene                     | FACS Astrocytes | Cultured Astrocytes |
|--------------------------|-----------------|---------------------|
| 5-HT2B receptor expression | up              | up                  |
| 5-HT2B editing           | up              | up                  |
| 5-HT2C receptor expression | unchanged       | unchanged           |
| ADAR2                    | up              | up                  |
| sPLA2                    | unaltered       | unaltered           |
| Ca1,2                    | up              | up                  |
| efos expression          | up              | up                  |
| fosB expression          | up              | up                  |
| GluK2 expression         | up              | up                  |
| GluK2 editing            | up              | up                  |
| GluK4 expression         | unaltered       | unaltered           |

All data except those for Ca1,2 are from Li et al. [20] and those for Ca1,2 from Du et al. [83].
SSRIs inhibits the ability of transmitters but, on account of the up-regulation of Ca,1.2, not that of elevated K+ concentrations to increase astrocytic [Ca2+]i, and thus glycogenolysis [79]. K+-mediated stimulation of glycogenolysis has been shown both in intact brain tissue [88] and in cultured cells [89] to increase with the magnitude of the K+ elevation. The effect on K+ stimulation by fluoxetine is opposite to that seen after chronic treatment with any of the 3 anti-bipolar drugs lithium, carbamazepine or valproic acid, which inhibits both transmitter- and K+-mediated Ca2+ uptake [81].

The Immediate Early Genes cfos and fosB

As shown in Table 2 these two genes are also up-regulated by chronic treatment with fluoxetine, and this happens in both neurons and astrocytes. In astrocytes the up-regulation depends on normal operation of the signaling pathway shown in Fig. 3 [29]. The mechanism for up-regulation in neurons is unknown. It may or may not be secondary to the effect in astrocytes and could be secondary to growth factor release as shown in Fig. 9 for a different tissue [90].

The Kainate Receptors GluK2 and GluK4 and other Glutamate Receptors or Transports

The role of glutamate in major depression and its drug treatment has repeatedly been discussed [10, 20, 91-92], but the precise roles of different receptors are far from resolved. However, astrocytes are essential for all aspects of glutamate homeostasis in brain (synthesis, uptake and oxidation) as reviewed by Hertz and Zielke [93] and Schousboe et al. [94]. Many aspects of glutamatergic transmission including astrocyte-specific glutamate transporters are affected in depressive illness [95-96]. Correlations between drug effects on major depression and on glutamatergic activities have attracted special interest in connection with the rapid but short-lasting therapeutic effects of ketamine and riluzole in depressed patients [95]. Recently the effects of riluzole and of ketamine have also been reviewed by Murrough and colleagues [97, 98]. The paper by Lapidus et al. [97] mainly discusses neuronal effects, although disregarding the GluK4 receptor but mentioning mGluRs. However, as seen from Table 2, GluK4 becomes up-regulated in neurons after chronic fluoxetine treatment [20], whereas at least mGluR5 is virtually unaffected by fluoxetine treatment [36]. It is also often assumed that increase in [Ca2+]i, in astrocytes is mediated by the metabotropic glutamate receptor mGluR5, a response which however is present only in the immature brain [15]. Murrough et al. [98] notes very interesting correlations between ketamine, major depression and cognitive function. This is reminiscent of the finding that stimulation of 5-HT2B receptor activity is important not only for the mechanism of action of SSRIs but also during learning [32].

The kainate receptor GluK2 can operate not only in an inotropic, but also in a metabotropic form [99]. It is up-regulated and edited in astrocytes both in culture and in the brains of mice treated with fluoxetine for 14 days [20, 46]. The genome-encoded GluK2 mRNA can be edited at 3 sites, the I/V site, the Y/C site, and the Q/R site. The editing was increased at all three sites by chronic treatment with fluoxetine [46]. Fluoxetine-mediated changes in GluK2 editing are consistent with a previous observation by the Barbon group, but they found editing of the Q/R site in intact brain to be slightly decreased by chronic fluoxetine treatment in a brain preparation including white matter [100]. Inclusion of subunits containing the edited R form of the Q/R site often lowers Ca2+ permeability [101], and in fluoxetine-treated cultures a normally occurring increase in free cytosolic Ca2+ concentration to 300-400% of control value in response to 100 μM glutamate was abolished by the fluoxetine treatment [46]. This increase is evoked by the GluK2 receptor operating in its metabotropic manner [10]. mRNA expression analysis has demonstrated that the human GluK2c splice variant in brain is mainly expressed in non-neuronal cells and barely expressed in neurons [102]. Knock-out of the GluK2 receptor in mice results in less anxious or more risk-taking type behavior and less manifestation of despair [103]. Obsessive-compulsive disorder is also genetically linked to abnormalities in Grik2, the gene coding for GluK2 [104, 105]. GluK4 is up-regulated in neurons after chronic treatment with fluoxetine [20].

Gene Changes in Models of Major Depression

Since most experiments on SSRI action are carried out in normal animals or in cultures from normal animals it is important that some at least partial models of major depression exist. Chronic stress can induce anhedonia, the inability to experience pleasure from activities usually found enjoyable. Short-lasting exposure of experimental animals to moderately stressful experiences have been used to create anhedonia as a model of major depression, but anhedonia occurs only in a fraction of the stressed mice. However, mice displaying or not displaying anhedonia can be clearly separated into two distinct groups according to their preference of a sucrose solution over water, making this an even better test. Anhedonia is, however, only one of the components of major depression, and yet it causes changes opposite to those evoked by chronic exposure to fluoxetine in several of the genes shown in Table 2. This is illustrated in Fig. 10 and includes a down-regulation of the 5-HT2B receptor in astrocytes but not in neurons, but there is no effect on 5-HT2C expression in either neurons or astrocytes.
CONCLUDING REMARKS

Combination of our own findings with those of Diaz et al. [12] leaves no doubt that SSRIs act via the 5-HT$_{2B}$ receptor. However their interpretation of the mechanism differs widely from ours. In principle both could be operating, since the enhanced release of 5-HT envisaged by Diaz et al. [12] as the cause of the therapeutic effect also will act on astrocytic 5-HT$_{2B}$ receptors. That a direct, SERT-independent stimulation of this receptor is evoked in astrocytes by SSRIs is shown by the cell culture experiments and its in vivo relevance is proven by the similar effects on gene up-regulation and editing in the cultures (which express no SERT) and in astrocytes from fluoxetine-treated animals (expressing SERT in at least some neurons). The neurogenic effect by fluoxetine emphasized by Diaz et al. [12] is also consistent with the demonstrated signaling pathway for fluoxetine in astrocytes. Although this effect probably is not significant for the therapeutic effects of SSRIs [110], the release of growth factor may be important for the demonstrated gene effects of these drugs on neurons in intact animals. The claim by Diaz et al. [12] that fluoxetine cannot elicit SRI-like responses in behavioral assays in mice in which SERT is knocked out, is unconvincing on account of the concomitant severe depletion of serotonin in the SERT$^{-/-}$ mice [18]. Evidence for SERT-independent effects of fluoxetine has also been shown by Pinna and Rasmusson [111]. This opens the possibility that SERT-mediated inhibition may be an epiphenomenon rather than the mechanism by which they exert their therapeutic action.

5-HT$_{2B}$ receptors are involved in multiple biological functions besides brain activity, including cardiovascular function. There is a growing concern regarding adverse drug reactions, specifically cardiac valvulopathy associated with 5-HT$_{2B}$ agonists [112], as first seen in association with the anti-obesity drug fenfluramine [113]. The reason that similar side effects are not seen with SSRIs may be the high protein
binding of most of these drugs. However, there are other cardiovascular concerns, suggesting that 5-HT_{2B} receptors may be involved in apoptotic events associated with cardiac remodeling during increased adrenergic stimulation [114]. Jaffré et al. showed an interaction between autoimmune stimulation of AT_{1} receptors by angiotensin II and 5-HT_{2B} receptors in cardiac fibroblasts during β-adrenergic-dependent hypertrophic responses. There is also evidence that 5-HT_{2B} receptors may play a role in the development of DOCA-salt hypertension [115]. These effects may be the reason for a greatly increased mortality following acute coronary occlusion if the patients are treated with an SSRI, especially if the treatment is started after the coronary attack [116]. Paroxetine and possibly fluoxetine use in early pregnancy is also associated with a small increased risk for cardiovascular malformations, perinatal respiratory distress and persistent pulmonary hypertension [117]. Thus, as with most other drugs, it is important to remember that they have actions at more organs than the selected therapeutic target and that these actions in some cases can be adverse.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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