Glucocorticoid receptor antagonists hasten and augment neurochemical responses to an SSRI antidepressant.

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Abstract

Background: Selective serotonin reuptake inhibitor (SSRI) antidepressants have a delayed onset and commonly produce an incomplete therapeutic response. The therapeutic actions of SSRIs are thought to depend on increased forebrain extracellular 5-HT, following desensitization of somatodendritic 5-HT₁A autoreceptors. Here we determined whether concurrent glucocorticoid receptor (GR) blockade enhances these neurochemical responses to the SSRI, fluoxetine.

Methods: Male rats were treated (3, 7, or 14 days) with either fluoxetine (10 mg/kg i.p.) or vehicle once daily, in combination with either a GR antagonist (Org 34850 15 mg/kg s.c. or Org 34517 25 mg/kg s.c.) or vehicle twice daily. Following treatment, 5-HT in the medial prefrontal cortex was measured by microdialysis.

Results: Chronic fluoxetine treatment (14 days) raised basal 5-HT and also attenuated the fall in 5-HT following acute systemic administration of fluoxetine (10 mg/kg i.p.) indicating desensitization of 5-HT₁A autoreceptors. Concurrent chronic administration (14 days) of Org 34850, or Org 34517, enhanced the fluoxetine-induced increase in basal 5-HT. Org 34850 also hastened the 5-HT₁A autoreceptor desensitization induced by chronic fluoxetine treatment. Org 34850 alone (14 days) failed to alter basal 5-HT or 5-HT₁A autoreceptor desensitization.

Conclusion: Antidepressant response is proposed to depend on 5-HT₁A autoreceptor desensitization and elevation of forebrain 5-HT. These data suggest adjunctive GR antagonists may both hasten and enhance antidepressant responses to SSRIs.
Introduction

The goal of antidepressant therapy is rapid and complete relief of depressive symptoms in all patients. However, the clinical profile of established antidepressant drugs is far from optimal: A delay in onset of therapeutic response (1) and partial responding in many patients (2) are two particular problems.

When administered chronically, the majority of effective antidepressants elevate extracellular 5-HT levels in the forebrain (3-5). The finding that depletion of the 5-HT precursor tryptophan both reduces the SSRI-induced elevation of 5-HT in the rat forebrain (6) and leads to a rapid return of depressive symptoms in SSRI-treated patients (7), suggests that this common action is crucial for antidepressant efficacy.

In recent years, preclinical studies of the autoregulatory control of 5-HT neurotransmission have provided a plausible explanation for the delay in antidepressant onset. Thus, the acute administration of selective serotonin reuptake inhibitors (SSRIs) elevates 5-HT in the dorsal raphe nucleus (DRN): the site of the somata of ascending 5-HT neurones (8-10). This increase in 5-HT activates inhibitory somatodendritic 5-HT$_{1A}$ autoreceptors, resulting in a decrease in 5-HT release in the forebrain (11, 12). The decrease in release means that, although terminal 5-HT reuptake is inhibited, the SSRI is unable to elevate forebrain extracellular 5-HT. However, chronic SSRI treatment desensitizes the inhibitory somatodendritic 5-HT$_{1A}$ autoreceptors (13-16), allowing DRN 5-HT neuronal firing and 5-HT release to be restored and, with terminal reuptake inhibited, forebrain extracellular 5-HT increases (15, 17, 18). The hypothesis that the delay in therapeutic onset of SSRIs reflects the time taken for DRN 5-HT$_{1A}$ receptors to desensitize has gained general acceptance, and treatments which aim to block 5-HT$_{1A}$
autoreceptors or hasten their desensitization, in addition to providing 5-HT uptake blockade, are in development (19-23).

The problem of incomplete therapeutic response has received less attention and remains puzzling. However, one possible explanation is that in some patients the neurochemical response is of insufficient magnitude to elicit a full antidepressant response. Thus, in these patients there may be factors which reduce the ability of the antidepressant to elevate forebrain 5-HT.

In patients with depression, dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis is common (24, 25). Circulating glucocorticoids influence several aspects of 5-HT neurotransmission including 5-HT$_{1A}$ autoreceptor function (26-29). Thus, we reasoned that the elevation of nadir cortisol levels seen in depressed patients (30, 31) may influence the neurochemical response and, hence, the antidepressant response to SSRIs. In support of this we showed that flattening the glucocorticoid rhythm in the rat reduces the ability of an SSRI to elevate forebrain 5-HT levels (5). Furthermore, a recent clinical study in depressed patients showed that HPA axis dysfunction was associated with reduced SSRI efficacy (32). In a further clinical study concurrent treatment with metyrapone (a steroid hormone synthesis inhibitor) increased both speed of onset and the response rate to a serotonergic antidepressant (33). Interestingly, the effect of metyrapone was independent of basal cortisol levels, indicating that antiglucocorticoid therapy may enhance antidepressant efficacy even in patients without overt HPA axis dysfunction.

The actions of glucocorticoid hormones in the brain are mediated in part by glucocorticoid receptors (GRs) (for review see 34). In the present preclinical study we
sought to determine whether concurrent blockade of GRs, using GR antagonists Org 34850 and Org 34517, would influence the neurochemical responses to an SSRI. Our two primary outcome measures were the elevation of extracellular 5-HT in the prefrontal cortex (PFC) and the SSRI-induced desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors. In three sequential *in vivo* microdialysis experiments we determined: i) the time course of the emergence of neurochemical effects following combined Org 34850 and fluoxetine treatment compared to fluoxetine alone, ii) whether Org 34850 alone had any effect, and iii) whether a second GR antagonist Org 34517 replicated the effects of Org 34850. Some of these data have been presented as abstracts to the British Association for Psychopharmacology and European College for Neuropsychopharmacology.

**Methods and materials**

**Animals**

All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986. Adult male Hooded Lister rats (Charles River UK) were group housed in controlled environmental conditions on a 12 h light/dark cycle (lights on 7:00) with food and water available *ad libitum*.

**Chronic treatments**

The study comprised three separate experiments (see below). For all three experiments the treatment protocol was as follows:-
the GR antagonists Org 34850 (15 mg/kg s.c.) or Org 34517 (25 mg/kg s.c.), or the vehicle (30% DMSO/70% PEG) were administered twice daily (7:00-8:00 and 18:00-19:00). Fluoxetine (10 mg/kg i.p.), or its vehicle (sterile water), was administered once daily (7:00-8:00). All injections were given at a volume of 1 ml/kg. Groups comprised 8-12 animals.

**Experiment 1**

The time course of emergence of neurochemical effects in animals undergoing combined treatment with Org 34850 and fluoxetine was compared to that in animals treated with fluoxetine alone. Paired groups of animals received Org 34850 and fluoxetine (Org 34850/fluoxetine) or vehicle and fluoxetine (vehicle/fluoxetine) for 3, 7 or 14 days. Comparisons were also made with an untreated (control) group.

**Experiment 2**

This experiment examined whether Org 34850 alone had any effect on the neurochemical outcome measures independent of an interaction with fluoxetine. A two-by-two design was used in which groups of animals were treated for 14 days with vehicle/vehicle, Org 34850/vehicle, vehicle/fluoxetine, or Org 34850/fluoxetine.

**Experiment 3**

This experiment tested whether a second GR antagonist Org 34517 would replicate the effects of Org 34850. Groups of animals were treated for 14 days with vehicle/vehicle, vehicle/fluoxetine, Org 34850/fluoxetine, or Org 34517/fluoxetine.
Microdialysis.
Following chronic treatment animals underwent in vivo microdialysis. Between 8:00 and 9:00 (≈25 h following the last fluoxetine or vehicle injection) animals were anaesthetised with chloral hydrate (500 mg/kg i.p. plus supplementary doses as necessary). A concentric dialysis probe (3.0-3.5 mm dialysing window of Hospal membrane) was stereotaxically implanted into the right PFC (coordinates: 3.2 mm anterior, 1.0 mm lateral to bregma and 4.6 mm ventral to the dura surface (35)). The probe was perfused (2.3 µl/min) with an artificial CSF (see 5). Dialysate samples were collected over 20 min periods and assayed for 5-HT by high-pressure liquid chromatography (5). Dialysate samples were taken for 180 min. The aCSF was then switched to one containing fluoxetine (10 µM) which was perfused for a further 200 min. In Experiments 1 and 2, fluoxetine (10 mg/kg) was injected i.p. 80 min following the switch of perfusion. At the end of the experiments animals were killed and the adrenal glands were removed and weighed.

Basal samples were used to assess the effect of treatments on extracellular 5-HT levels. Dialysate 5-HT following local infusion of fluoxetine allowed assessment of the effect of the treatments on 5-HT release. Finally, the fall in 5-HT levels following systemic injection of fluoxetine was used as an index of the sensitivity of somatodendritic 5-HT<sub>1A</sub> autoreceptors.

Data Analysis
For statistical analysis, the dialysis data were reduced to three measures:

- ‘Basal’ dialysate 5-HT levels - average of samples 1-3,
- Dialysate 5-HT levels following local application of fluoxetine - average of samples 7 and 8 (Experiments 1 and 2) or samples 12 and 13 (Experiment 3).
  [Note that although in Experiments 1 and 2, sample 8 was collected after the systemic administration of fluoxetine, previous experiments have shown that the effect of i.p. fluoxetine is slow in onset (5)].
- The fall in 5-HT level following an acute systemic injection of fluoxetine (an index of 5-HT\textsubscript{1A} receptor sensitivity) - average of samples 7 and 8 minus the average of samples 12 and 13).

Experiment 1: data were analysed using a two-way ANOVA with treatment (vehicle or Org 34850) and duration (3, 7 or 14 days) as between-subjects factors. One-way ANOVA with a post-hoc (LSD) analysis was also used to analyse data within treatment groups. Experiment 2: data were analysed by two-way ANOVA. Experiment 3: data were analysed by two-way ANOVA as well as one-way ANOVA (fluoxetine treated groups only) with post-hoc LSD tests. Individual planned comparisons were also made by t test. Significance at the 95% level is reported.

**Results**

**Animal health**
Animals appeared healthy and maintained a normal increase in body weight throughout treatments. The only noted effect was that animals treated with the GR antagonists for 14 days exhibited a slight thickening of the skin around the injection sites.

**Experiment 1.**

To examine the time course of the emergence of neurochemical effects following Org 34850 and fluoxetine treatment compared to fluoxetine alone, groups of animals were treated for 3, 7 or 14 days with Org 34850/fluoxetine or vehicle/fluoxetine or were untreated controls (indicated as 0 days). Figure 1 shows the full microdialysis time course for this experiment.

**Basal 5-HT levels**

As shown in Figure 2, following treatment with vehicle/fluoxetine basal dialysate 5-HT levels rose slightly but not significantly (p>0.05, one-way ANOVA). In comparison, following Org 34850/fluoxetine treatment there was a marked and significant elevation in basal 5-HT levels ($F_{3,33}=4.6$ p=0.009, one-way ANOVA). This elevation was greater with increasing treatment duration and was significant at both 7 and 14 days (p=0.021 and p=0.001, respectively, compared to the untreated controls). A two-way ANOVA revealed a main effect of Org 34850 which was approaching significance ($F_{1,49}=4.0$; p=0.051) but no main effect of duration and no significant duration x Org 34850 interaction.

**5-HT release**
Changing the perfusion to one containing fluoxetine (10 μM) markedly increased dialysate 5-HT (Figure 1). Dialysate 5-HT levels after 60-80 min of local fluoxetine perfusion did not differ between groups (one-way and two-way ANOVA).

5-HT\textsubscript{1A} autoreceptor sensitivity.

The time-course of the response to acute systemic administration of fluoxetine is shown in Figure 1 and the response expressed as ‘change in 5-HT levels’ is shown in Figure 3. In the control group, systemic fluoxetine (10 mg/kg i.p.) caused a large fall in dialysate 5-HT levels over the following 120 min. In the vehicle/fluoxetine groups this response was attenuated. Although the attenuation was not significant by one-way ANOVA across the 0, 3, 7 and 14 days groups (p=0.098), direct comparison by t test between the control and 14 day fluoxetine groups was statistically significant (p=0.016).

In the Org 34850/ fluoxetine groups there was a highly significant attenuation of the response to systemic fluoxetine (F\textsubscript{3,33}=8.8; p<0.0001, one-way ANOVA). The attenuation was both earlier in onset and of greater magnitude than in the vehicle/fluoxetine groups. The response was significantly different from the control group by 3 days (p=0.01), as well as at 7 (p<0.0001) and 14 days (p<0.0001, post-hoc LSD) (Figure 3). A two-way ANOVA revealed a significant effect of Org 34850 (F\textsubscript{1,49}=4.7; p=0.034), but no significant main effect of duration and no duration x Org 34850 interaction.

Adrenal weights.
The (combined) weights (in mg) of the adrenal glands were: $57.7 \pm 2.2$ in the controls; $67.0 \pm 2.5$, $60.9 \pm 5.6$, and $66.4 \pm 1.4$ in the vehicle/fluoxetine groups treated for 3, 7 and 14 days respectively; and $62.4 \pm 2.5$, $77.1 \pm 2.4$, and $76.1 \pm 4.6$ in the Org 34850/fluoxetine groups treated for 3, 7 and 14 days, respectively. Vehicle/fluoxetine treatment had no effect on adrenal weight ($p>0.05$, one-way ANOVA). In contrast, Org 34850/fluoxetine treatment significantly increased adrenal weight (one-way ANOVA $F_{6,57}=5.9$; $p=0.0001$; 7 days: $p<0.0001$, 14 days: $p<0.0001$, post-hoc LSD). Two-way ANOVA showed a significant main effect of Org 34850 ($F_{1,49}=7.3$; $p=0.009$) as well as an Org 34850 x duration interaction ($F_{2,49}=5.8$; $p=0.005$).

**Experiment 2.**

The objective of this experiment was to determine whether Org 34850 alone could alter the neurochemical outcome measures independent of an interaction with fluoxetine. On the basis of data from Experiment 1, we chose a fixed 14 day treatment period. Animals were treated for 14 days with vehicle/vehicle, vehicle/fluoxetine, Org 34850/vehicle, or Org 34850/fluoxetine. Figure 4 shows the full microdialysis time course.

**Basal 5-HT levels**

Basal 5-HT levels for the four groups are shown in Figure 5. Chronic fluoxetine caused a highly significant elevation of basal 5-HT as reflected in a significant main effect in a two-way ANOVA ($F_{1,30}=44.4$; $p=0.0001$). There was no significant main effect of Org 34850 and, whilst basal 5-HT levels were highest in the Org 34850/fluoxetine group, the Org 34850 x fluoxetine interaction was not significant (two-way ANOVA).
comparisons between groups showed no difference between the vehicle/vehicle and Org 34850/vehicle, however, basal 5-HT was significantly higher in the Org 34850/fluoxetine group than the vehicle/fluoxetine group (t-test, p=0.046).

5-HT release
Local perfusion of fluoxetine (10 μM) markedly increased dialysate 5-HT levels (Figure 4). However, 60-80 min following local application of fluoxetine, dialysate 5-HT levels did not differ between groups. Two-way ANOVA confirmed no significant effect of either treatment and no significant interaction between treatments.

5-HT$_{1A}$ autoreceptor sensitivity
Following systemic administration of fluoxetine, dialysate 5-HT levels fell (Figure 4). This response to fluoxetine injection was attenuated by fluoxetine (two-way ANOVA: significant main effect of fluoxetine ($F_{1,30}$=31.4; p<0.001)) but there was no effect of Org 34850 and no significant fluoxetine x Org 34850 interaction (Figure 6). Direct comparisons between Org 34850/vehicle and vehicle/vehicle and between Org 34850/fluoxetine and vehicle/fluoxetine groups by t test also showed no significant effect.

Adrenal weight
Adrenal weights (mg) were: vehicle/vehicle: 56.7 ± 1.7, vehicle/fluoxetine: 61.0 ± 3.4, Org 34850/vehicle: 68.3 ± 1.8, and Org 34850/fluoxetine 71.7 ± 1.8. Statistical analysis revealed that whilst fluoxetine was without effect Org 34850 increased adrenal weight
(two-way ANOVA: significant main effect of Org 34850 (F\(_{1,30}=24.2, \ p=0.0001\)). There was no significant Org 34850 x fluoxetine interaction.

**Experiment 3**

In Experiment 3 we aimed to test whether the ability of Org 34850 to augment the fluoxetine-induced elevation of basal 5-HT observed in Experiments 1 and 2, could be replicated by a second GR antagonist Org 34517. We also examined the response to local fluoxetine perfusion over an extended time period to confirm a lack of effect on 5-HT release. Groups of animals were treated for 14 days with vehicle/vehicle, vehicle/fluoxetine, Org 34850/fluoxetine, or Org 34517/fluoxetine. Figure 7 shows the full microdialysis time course for this experiment.

**Basal 5-HT levels**

Basal 5-HT levels for the four groups are shown in Figure 8. Chronic fluoxetine elevated basal 5-HT as revealed by a significant main effect of fluoxetine in a two-way ANOVA (F\(_{1,36}=7.5; \ p=0.01\)). However, there was also a significant main effect of GR antagonist (F\(_{2,36}=4.7; \ p=0.016\), two-way ANOVA). Further analysis of the fluoxetine treated groups showed that both Org 34850 and Org 34517 enhanced the effect of fluoxetine compared to vehicle/fluoxetine treatment (F\(_{2,27}=4.2, \ p=0.027\), one-way ANOVA, Org 34850/fluoxetine p=0.013, Org 34517/fluoxetine p=0.043 post-hoc LSD)

**5-HT release**
As shown in figure 7, local fluoxetine perfusion (10 μM) markedly increased dialysate 5-HT. Mean dialysate 5-HT levels 160-180 min following local application of fluoxetine did not differ between groups. Thus, two-way ANOVA showed no effect of fluoxetine or GR antagonist and one-way ANOVA (in fluoxetine treated groups) confirmed no significant effect of either Org 34850 or Org 34517 on 5-HT release compared to vehicle.

Adrenal weights

Adrenal weights (mg) were: vehicle/vehicle: 63.7 ± 2.7; vehicle/fluoxetine: 62.2 ± 1.1; Org 34850/vehicle: 80.3 ± 2.5; Org 34517/fluoxetine: 78.2 ± 3.5. Two-way ANOVA revealed no significant effect of fluoxetine but a highly significant main effect of GR antagonist (F<sub>3,45</sub>=11.7; p=0.0001). One-way ANOVA (F<sub>4,45</sub>=10.8, p<0.0001) with post-hoc LSD showed that both Org 34850 (p<0.0001) and Org 34517 (p=0.0001) significantly increased adrenal weight compared to vehicle.

Discussion

The present study examined whether concurrent GR blockade influences the ability of chronic SSRI treatment to elevate extracellular 5-HT levels in the forebrain and desensitize 5-HT<sub>1A</sub> autoreceptors. Org 34850 and Org 34517 both enhanced the effect of fluoxetine on basal 5-HT levels in the PFC. Org 34850 hastened the fluoxetine-induced desensitization of 5-HT<sub>1A</sub> autoreceptors. Org 34850 alone affected neither basal 5-HT levels nor the sensitivity of 5-HT<sub>1A</sub> autoreceptors, demonstrating that Org 34850 has a synergistic effect with the SSRI. Both Org 34850 and Org 34517 have high affinity for GRs and show more than 300-fold selectivity for GRs versus mineralocorticoid receptors.
Fluoxetine-induced elevation of basal forebrain extracellular 5-HT is augmented by GR antagonists

Chronic treatment with fluoxetine alone increased basal 5-HT in the PFC. Although this effect was not statistically significant in Experiment 1, in Experiments 2 and 3 the elevation of 5-HT by fluoxetine was highly significant. This finding of raised basal extracellular 5-HT many hours following the final dose of a chronic treatment with fluoxetine is consistent with our own and others’ data (5, 9, 36). As the half-life of fluoxetine in the rat is 7-8 h (37), it is likely that reuptake blockade would be low when measurements were made. Hence, it seems likely that the elevation of basal 5-HT results from a down-regulation of the 5-HT transporter (38-41).

Chronic treatment with a GR antagonist alone had no effect on basal extracellular 5-HT in the PFC. However, concurrent administration of the GR antagonist Org 34850 enhanced the effect of fluoxetine on basal 5-HT levels, approximately doubling the effect of 14 days fluoxetine treatment. This effect was evident in all three experiments (although in Experiment 2 only a planned comparison by t test showed significance) and was reproduced by a second GR antagonist, Org 34517. The affinity of the drugs for 5-HT₁₅ receptors is unknown however, it seems highly unlikely that the GR antagonists are simply blocking 5-HT₁₅ receptors since chronic Org 34850 failed to block the response to acute systemic administration of fluoxetine (Experiment 2). We conclude...
that GR antagonism represents a novel mechanism by which SSRI efficacy may be modulated.

We also measured extracellular 5-HT during local perfusion of fluoxetine. As extracellular, and hence dialysate, levels of 5-HT are influenced primarily by the opposing processes of release and reuptake, measurements made in the presence of local reuptake blockade can be used as an index of release (11). We found no significant effects of any of the treatments on 5-HT levels during local fluoxetine, indicating that none of the treatments influence the terminal 5-HT release.

Fluoxetine-induced desensitization of 5-HT\textsubscript{1A} autoreceptors is hastened by a GR antagonist.

It is established that when local terminal reuptake is inhibited, systemic administration of SSRIs decreases dialysate 5-HT in terminal regions (11, 12, 42, 43, 44). This response is markedly attenuated by 5-HT\textsubscript{1A} receptor antagonists administered both systemically (11, 12, 42, 43) and locally into the DRN (44). The fall in dialysate 5-HT is thought to reflect the SSRI-induced inhibition of 5-HT neuronal firing and hence terminal release. Evidence from electrophysiological and microdialysis studies indicates that the effects of systemic SSRIs on 5-HT firing and release are mediated by somatodendritic 5-HT\textsubscript{1A} receptors and are not contributed to by postsynaptic feedback loops (45, 46). These data indicate that the decrease in dialysate 5-HT following systemic administration of fluoxetine is principally mediated by somatodendritic 5-HT\textsubscript{1A} receptors. Here we used the magnitude of the fall as an index of 5-HT\textsubscript{1A} autoreceptor sensitivity. In Experiment 2 after 14 days fluoxetine treatment the 5-HT\textsubscript{1A} autoreceptor-mediated response was
markedly desensitized consistent with previous reports (5, 15, 16). In Experiment 1 the desensitization induced by fluoxetine (alone) was less consistent and was not statistically significant by ANOVA. However, importantly, concurrent treatment with Org 34850 hastened the time-course of the desensitization in Experiment 1, such that a significant effect was observed after just 3 days of the combined treatment. In Experiment 2, the near complete desensitization with fluoxetine alone produced a floor effect and Org 34850 had no additional effect over that of fluoxetine. In contrast to our observation, Le Poul and colleagues (47) showed no effect of another GR antagonist (RU 38486) on 5-HT$_{1A}$ receptor desensitization induced by fluoxetine. It is not clear what factors underlie this discrepancy but they may include partial GR agonist effects of RU 38486 (48) and/or differences in treatment dose and duration or measures made.

On acute administration SSRIs, like fluoxetine, have limited effects on extracellular 5-HT in the forebrain (12, 49) probably because they indirectly activate 5-HT$_{1A}$ autoreceptors and, thereby, reduce terminal 5-HT release. Removal of this inhibitory feedback allows the SSRI to exert its full effect on forebrain extracellular 5-HT levels. Hence, the earlier desensitization of 5-HT$_{1A}$ autoreceptors in Org 34850/fluoxetine-treated animals may (at least partly) underlie the earlier increase in extracellular 5-HT observed in this group (but see below).

GR antagonists increased adrenal gland weight.

Both Org 34850 and Org 34517 increased adrenal gland weight. Here, the use of anaesthetic precluded determination of basal corticosterone levels, however, in a separate experiment, we found that while fluoxetine alone had no effect on morning (nadir)
corticosterone levels, Org 34850 moderately increased levels and the combination of Org 34850/fluoxetine had a greater effect (manuscript in preparation). Bachmann et al. (50) also found that the GR antagonists increased adrenal weight but neither Org 34850 nor Org 34517 elevated morning corticosterone levels. Raised corticosterone has been shown to attenuate 5-HT\textsubscript{1A} receptor sensitivity (26, 29). However, our finding that Org 34850 alone failed to alter 5-HT\textsubscript{1A} receptor sensitivity suggests that the 5-HT\textsubscript{1A} autoreceptor desensitization is not mediated by increased corticosterone.

**Putative mechanisms underlying the interactions between GR antagonists and SSRIs.**

Although we found a hastening of 5-HT\textsubscript{1A} autoreceptor desensitization, our finding that the effect of Org 34850/fluoxetine on basal 5-HT levels after 14 days occurred in the absence of any augmentation of this 5-HT\textsubscript{1A} autoreceptor desensitization suggests a second independent interaction between the two treatments. Thus, two distinct processes appear to be affected by a synergistic interaction between fluoxetine and GR agonists. As 5-HT release was not altered, the most likely mechanism underlying elevated basal 5-HT levels is a synergistic effect of fluoxetine and the GR antagonist on 5-HT reuptake.

The present study did not address the mechanisms underlying the synergistic interactions between GR antagonists and fluoxetine. However, the possibility of a pharmacokinetic interaction raising brain levels of fluoxetine seems unlikely, since the two drugs are chemically dissimilar and are metabolised by different routes (51; Organon unpublished data). More likely, the blockade of GRs - which are expressed in 5-HT neurones in the DRN (52, 53) - could influence receptor and/or transporter proteins within 5-HT neurones. Although Org 34850 had no effect on its own suggesting that
normal diurnal levels of glucocorticoids do not exert a GR-mediated tonic regulation on 5-HT neurones, the synergy might be explained by the fact that SSRIs elevate corticosterone (54). Thus the combination of the GR antagonist and SSRI may block the effect of this elevated corticosterone.

**Behavioural effects of GR blockade.**

Although the potential effects of the Org 34850 and Org 34517 on behaviour were not examined here, it is of note that behaviours relevant to anxiety and depression are reported to be influenced glucocorticoid receptor function. Thus, chronic GR antagonists reportedly reverse depression-associated behaviours induced by chronic mild stress in rats (55) although anxiety-like behaviour on the elevated plus maze is unaffected (56). GR knock-out mice have also been reported to show reduced anxiety behaviour in several tests (57, 58). These data suggest that reduced GR activation, like antidepressants, might promote ‘behavioural resilience’. Clearly, studies on the behavioural effects of Org 34850 and Org 34517 both alone and in combination with SSRIs are warranted.

**Clinical implications.**

Evidence indicates that an increase in 5-HT neurotransmission is critical for antidepressant efficacy (7). However, this increase is manifest only after several weeks at a time when 5-HT$_{1A}$ autoreceptors have become desensitized (1). Our data, showing that GR antagonists can hasten the SSRI-induced desensitization of 5-HT$_{1A}$ autoreceptors, suggest that concurrent GR antagonist treatment may hasten the onset of clinical antidepressant effects of SSRIs. The clinical observation of partial response to SSRIs
may reflect an inadequate neurochemical response to the antidepressant in some patients. Our finding that GR antagonists can augment the magnitude of the effect of the SSRI on extracellular 5-HT after longer treatment duration suggests an adjunctive treatment combining GR antagonists and SSRIs might augment the clinical response in patients with residual depression. Finally, since our experiments were conducted in animals with normal HPA axis, our findings suggest that GR antagonist augmentation of SSRI treatment may be beneficial even in patients without HPA dysfunction.

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Figure 1. Experiment 1: Time course of changes in 5-HT levels for the untreated control group and the three vehicle/fluoxetine treatment groups (A) and the three Org 34850/fluoxetine treatment groups (B). Horizontal bar indicates switching of the perfusion medium to one containing fluoxetine (10 μM). The arrow represents a single systemic injection of fluoxetine (10 mg/kg i.p.). Data are mean dialysate 5-HT content. Error bars omitted for clarity.
Figure 2. Experiment 1: Basal 5-HT levels (average of samples 1-3) in the three Vehicle/fluoxetine and Org 34850/fluoxetine treatment groups and the untreated control group. Data are mean ± SEM. *p<0.05, one-way ANOVA and post-hoc LSD tests, see text for further statistical analysis.
Figure 3. Experiment 1: Responses to a systemic injection of fluoxetine (10 mg/kg i.p.) in the three Vehicle/fluoxetine and Org 34850/fluoxetine treatment groups and the untreated control group. Data represent the fall in 5-HT levels following fluoxetine injection (average of samples 7 and 8 minus the average of samples 12 and 13). *p<0.05, one-way ANOVA and post-hoc LSD tests, see text for further statistical analysis.
**Figure 4.** Experiment 2: Time course of changes in 5-HT levels for the vehicle/vehicle, Org 34850/vehicle, vehicle/fluoxetine and Org 34850/fluoxetine treatment groups. Horizontal bar indicates switching of the perfusion medium to one containing fluoxetine (10 μM). The arrow represents a single systemic injection of fluoxetine (10 mg/kg i.p.). Data are mean dialysate 5-HT values. Error bars omitted for clarity.
Figure 5. Experiment 2: Basal 5-HT levels (average of samples 1-3) in the vehicle/vehicle, vehicle/fluoxetine, Org 34850/vehicle and Org 34850/fluoxetine groups after 14 days of treatment. Data represent mean + SEM *p<0.05, t-test.
Figure 6. Experiment 2: Response to a systemic injection of fluoxetine (10 mg/kg i.p) in the vehicle/vehicle, vehicle/fluoxetine, Org 34850/vehicle and Org 34850/fluoxetine treatment groups. Data represent the fall in 5-HT (average of samples 7 and 8 minus the average of samples 12 and 13) following injection of fluoxetine. See text for full statistical analysis.
Figure 7. Experiment 3: Time course of changes in 5-HT levels for the vehicle/vehicle, vehicle/fluoxetine, Org 34850/fluoxetine and Org 34517/fluoxetine treatment groups. Horizontal bar indicates switching of the perfusion medium to one containing fluoxetine (10 μM). Data are mean dialysate 5-HT. Error bars omitted for clarity.
Figure 8. *Experiment 3*: Basal 5-HT levels in the vehicle, vehicle/fluoxetine, Org 34850/fluoxetine and Org 34517/fluoxetine groups after 14 days of treatment. Data represent mean ± SEM calculated from dialysate samples 1-3. (*p*<0.05, one-way ANOVA and *post-hoc* LSD tests) see text for further statistical analysis.