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Serotonin-2C antagonism augments the effect of citalopram on serotonin and dopamine levels in the ventral tegmental area and nucleus accumbens

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ABSTRACT

Many patients with major depression do not respond to selective serotonin reuptake inhibitors (SSRIs). Lack of response could be due to inhibition of dopamine (DA) release by serotonin (5-HT) through 5-HT_{2C} receptors. Combining an SSRI with a 5-HT_{2C} antagonist may result in improved efficacy by causing simultaneous increases of 5-HT and DA. In order to test this augmentation strategy, male Wistar rats were treated (s.c.) with an acute dose of the SSRI citalopram (Cit, 5 mg/kg), the 5-HT_{2C} antagonist SB 242084 (SB, 2 mg/kg), or Cit + SB, and the effect on 5-HT and DA release in the nucleus accumbens (NAcc) was assessed by microdialysis. In a separate experiment, animals were treated with vehicle, Cit (20 mg/kg/d), SB (2 mg/kg/d) or Cit + SB for a period of 2 days (s.c.), and the impact on the release of 5-HT and DA in the ventral tegmental area (VTA) and NAcc was studied. On the day of microdialysis, 5-HT_{2C} receptor sensitivity was assessed with an SB challenge. Acutely administered Cit + SB increased 5-HT release in the NAcc more than Cit alone. SB alone increased DA release in the NAcc (not in the VTA), but when administered together with Cit, this effect was abolished. A 2-day treatment with Cit or Cit + SB increased 5-HT release in both VTA and NAcc. Combining Cit with SB augmented the effect of Cit in the VTA. DA release in VTA and NAcc was only significantly increased after 2-days of treatment with Cit + SB. In conclusion, Cit + SB had synergistic effects on 5-HT and DA release after 2-days of treatment, probably related to a decreased tonic inhibition of DA release via 5-HT_{2C} receptors. Regional differences occur and future studies should elucidate if this augmentation strategy is beneficial at the behavioral level.

1. Introduction

Major depressive disorder (MDD) has a life-time prevalence of approximately 15% (Kessler and Bromet, 2013) and was the fourth leading cause of disability worldwide in the 1990s (Murray and Lopez, 1996). MDD may even become the second cause of disability by the year 2020. The pathophysiological mechanisms underlying MDD are not fully understood. The monoamine hypothesis of depression postulates that depressive episodes are related to a deficiency of the neurotransmitters serotonin, norepinephrine and dopamine in the human brain (Hirschfeld, 2000). Lack of norepinephrine may cause reduced alertness, energy, attention and interest in life, lack of serotonin anxiety, obsessive and compulsive behavior, and lack of dopamine may lead to diminished motivation, pleasure and reward. Although this hypothesis is probably a gross simplification, it has dominated research on depression in the past decades and has led to the development of many antidepressant drugs. Unfortunately, the current therapeutic approaches for MDD have major limitations. Selective serotonin reuptake inhibitors (SSRIs) are the first treatment option, but a large fraction of depressed patients (40–50%) do not respond satisfactorily to SSRIs (Gill and Hatcher, 1999). Moreover, the delayed onset of action of these

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antidepressant drugs causes prolongation of the impairments associated with depression, leaves depressed subjects vulnerable to an increased risk of suicide and increases the likelihood that some patients will prematurely discontinue therapy (Cipriani et al., 2009; Gumnick and Nemeroff, 2000; Machado-Vieira et al., 2008). Since many patients treated with SSRIs show partial responses, much room is left for improvement in anti-depressant treatment.

The main mechanism underlying the action of SSRIs is an increase of the levels of extracellular serotonin (5-HT). Other monoamines such as dopamine (DA) have also been linked to the pathophysiology of depression. For instance, two core symptoms of MDD: anhedonia and reduced motivation, are both believed to involve a diminished activity of the mesolimbic DA system (Nestler and Carlezon, 2006). Thus, it could be hypothesized that antidepressant drugs should not only increase release of the neurotransmitter 5-HT, but also that of DA and possibly even that of norepinephrine (NE) without causing severe side-effects (Chen and Skolnick, 2007).

Several studies have indicated that SSRIs may in fact reduce DA neuronal activity in the ventral tegmental area (VTA) and DA release in the striatum (Clark et al., 1996; Dewey et al., 1995; Dremencov et al., 2009). Inhibition of DA release could be a factor underlying their delayed onset of action, and could lead to partial response or lack of antidepressant response (Calcagno et al., 2009; Dremencov et al., 2004). The reduction of DA neuronal activity is probably related to a serotonergic–dopaminergic interaction in the mesolimbic system via 5-HT_{2C} receptors (5-HT_{2C}R) (De Deurwaerdere et al., 2004). The mesolimbic DA system consists of neurons projecting from the VTA to the nucleus accumbens (NAcc). Dopaminergic neurons in the VTA are under tonic inhibition by 5-HT through stimulation of 5-HT_{2C}R located on GABA-ergic or dopaminergic neurons (Bubar and Cunningham, 2007; Bubar et al., 2011; Guiard et al., 2008).

Previous studies have indicated that the effects of an SSRI on extracellular 5-HT in the hippocampus and pre-frontal cortex can be augmented by co-administration of a 5-HT_{2C}R antagonist (Cremers et al., 2004). This augmentation has been related to a reduction of GABA release following 5-HT_{2C}R antagonism (Boothman et al., 2006; Cremers et al., 2007; see Fig. 1 for a schematic overview of the hypothesized mechanism).

In the present study using an animal model, we have investigated the effects of a specific 5-HT_{2C}R antagonist on changes of released, extracellular 5-HT and DA in VTA and NAcc after administration of an SSRI, using intracerebral microdialysis. Since the effects of an acute challenge may be different from those seen after prolonged treatment, we compared the impact of an acute challenge and 2-days of treatment with an SSRI and a 5-HT_{2C}R antagonist. We used the same treatment regimen as in the study of Dremencov et al. (2009), because this appeared to reduce neuronal activity in the VTA.

2. Methods

2.1. Animals

Adult male Wistar rats, age 10 weeks, body weight 332 ± 14 g (fresh from the breeding company Harlan, Zeist, The Netherlands, never used before), were housed in groups with *ad libitum* food and water and on a 12:12 h light: dark cycle, with lights on at 7.00 a.m. After surgery (placement of probes), rats were individually housed in Plexiglass cages (35 × 35 × 40), specifically suitable for microdialysis experiments. The animal experiments were performed by licensed investigators in compliance with the Law on Animal Experiments of The Netherlands. The protocol was approved by The Institutional Animal Care and Use Committee of the University of Groningen.

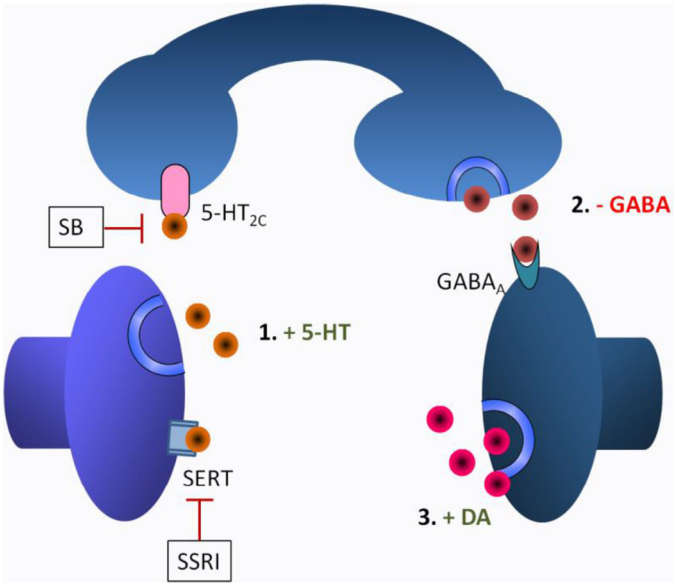

Fig. 1. Interaction between serotonin and dopamine through 5-HT_{2C} receptors. Under normal circumstances, 5-HT released by serotonergic neurons stimulates 5-HT_{2C} receptors on GABA-ergic neurons. The inhibitory neurotransmitter GABA then tonicly inhibits the release of dopamine (DA). Since an SSRI increases the extracellular levels of 5-HT, SSRI treatment may increase the release of GABA and may finally decrease the levels of extracellular DA. When the SSRI is combined with a 5-HT_{2C} antagonist like SB 242084 (SB), 5-HT is still increased by the SSRI (1) but SB decreases the release of GABA, by blocking the stimulation of 5-HT_{2C} receptors by extracellular 5-HT after treatment (2), thus preventing any decrease in DA, and possibly even increasing the levels of extracellular DA (3).

2.2. Treatment

Three different treatments were applied (at timepoint 0): citalopram (Cit) ($N = 5$), SB 242084 (SB) ($N = 5$), and Cit + SB ($N = 4$). The acute effects of treatment on neurotransmitter release were then assessed by microdialysis. In another series of experiments, animals were treated with: vehicle + saline ($N = 4$), Cit + saline ($N = 4$), vehicle + SB ($N = 5$), and Cit + SB ($N = 5$) for a period of 2 days and the impact of this longer period of treatment on neurotransmitter release was also assessed by microdialysis. During this last series of experiments, a challenge with SB (2 mg/kg) was applied at timepoint 0 in all treatment groups. The applied drug doses were based on data reported in the literature concerning augmentation of 5-HT release in the hippocampus (Cremers et al., 2004, 2007).

Citalopram.HBr (Trademax Pharmaceuticals & Chemicals Co., Shanghai, China) was dissolved in Ultra Pure water. In the acute administration experiment, 5 mg/kg was applied subcutaneously and in the 2-day treatment experiment, 20 mg/kg/day was applied subcutaneously through osmotic minipumps (volume 100 µL, speed 1 µL/h; Alzet, Cupertino, CA, USA). The 5-HT_{2C}R antagonist SB 242084.2HCl (Tocris Bioscience, Ellisville, MO, USA) was dissolved in 0.9% saline with 10% β-cyclodextrin. In both the acute administration and 2-day treatment experiment, 2 mg/kg SB was applied by subcutaneous injection. For acute administration, the combination of Cit (5 mg/kg) and SB (2 mg/kg) was dissolved in Ultra Pure water and was subcutaneously injected.

2.3. Microdialysis procedure

Microdialysis probes (9 mm, 1.5 mm exposed membrane; polyacrylonitril, MW cut-off 40–50 kDa; Brainlink, Groningen, The Netherlands) were implanted in the VTA (coordinates from Bregma and Dura: A/P –5.0, L/M 0.9, V/D –8.2; Paxinos and Watson, 2007)

and NAcc shell (coordinates: A/P 2.0, L/M 1.2, V/D –7.9; Paxinos and Watson, 2007) of isoflurane-anesthetized animals (induction 5%, maintenance 2–2.5%). After implantation the probes were fixed with dental acrylic. In the 2-day treatment experiment, the minipumps for continuous drug administration were subcutaneously implanted directly after insertion of the microdialysis probes. Bupivacaine and fynadine (2 mg/kg, s.c.) were applied as local- and postoperative analgetics, respectively.

After 24 hours of recovery in the acute administration experiment, and 12 hours after the last treatment-injection in the 2-day experiment, the probes were flushed with Ringer solution (140 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$, and 1 mM $MgCl_2$), at a flow rate of 1.5 µl/min. Microdialysis samples from VTA and NAcc were collected every 15 min by a fraction collector and were diluted with 7.5 µl formic acid, to prevent breakdown of monoamines, before storage at –80 °C until further analysis. The first five samples were collected at baseline. Then, different challenges were applied by s.c. drug injection in the neck region. After the experiment, animals were sacrificed by extirpation of the heart and brains were fixed in 4% paraformaldehyde to verify probe position.

Samples from the VTA were initially analyzed by HPLC with electrochemical detection, but it appeared necessary to switch to Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) to enable a more sensitive and reliable analysis. Thus, DA and 5-HT were analyzed by isotope dilution mass spectrometry using serotonin-d4 and dopamine-d4 as internal standards. Acute administration results for the VTA proved not reliable and are therefore not shown.

2.4. Data analysis

Data are presented as a percentage of the baseline 5-HT and DA concentrations (the average value of the first five samples). Statistical significance of changes in neurotransmitter release was examined by repeated measures ANOVA (two-way), with Student–Newman–Keuls as post-hoc test. Changes in neurotransmitter release after 2-days of treatment were examined by one-way ANOVA, with Student–Newman–Keuls as post-hoc test, using the program Sigma Plot. The value at $t = 0$ was used as the reference value to monitor treatment effects as a function of time. Thresholds of significance were set at $P < 0.05$.

3. Results

3.1. Acute experiment

Concentrations of the neurotransmitters 5-HT and DA at time zero in the acute experiment are reported in Table 1. In this experiment, there was a significant interaction between time, treatment and 5-HT release in the NAcc (Fig. 2A, $F_{(28,\ 110)} = 4.64$, $P < 0.001$). Post-hoc analysis revealed that 5-HT release in this brain region was significantly increased after administration of Cit and Cit + SB,

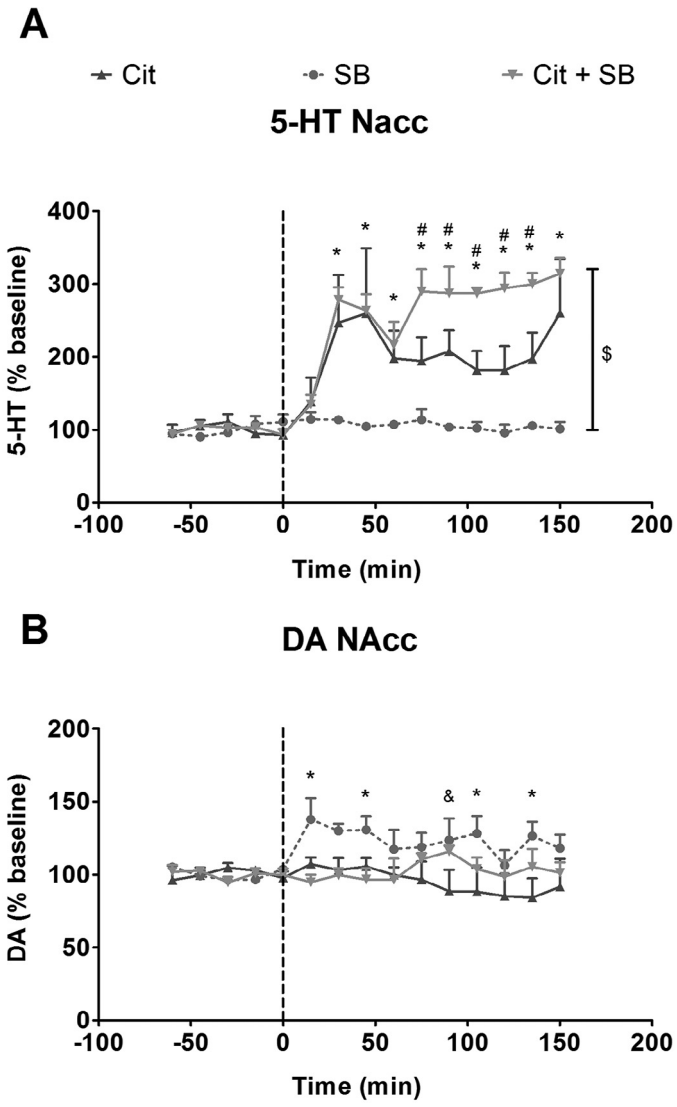

Fig. 2. Acute effects of drug treatment on levels of 5-HT and DA in NAcc. Challenges of citalopram (Cit), SB 242084 (SB), or the combination (Cit + SB) were applied s.c. at $t = 0$, and changes of extracellular serotonin (5-HT, A) and dopamine (DA, B) in NAcc were measured by microdialysis. *SB differs significantly ($P < 0.05$) from the other groups. #The Cit + SB combination differs significantly ($P < 0.05$) from Cit alone. &: Cit differs significantly ($P < 0.05$) from the other groups. $: Significant effect of time ($P < 0.05$). Data are presented as mean ± SEM.

Table 1
Neurotransmitter concentrations.

	5-HT VTA (nM)	5-HT Nacc (nM)	DA VTA (nM)	DA Nacc (nM)
Acute				
Cit	0.15 ± 0.07	0.06 ± 0.02		0.84 ± 0.80
SB	0.18 ± 0.09	0.06 ± 0.01		0.59 ± 0.44
Cit + SB	0.12 ± 0.05	0.08 ± 0.02		1.56 ± 0.67
2-Day treatment				
Vehicle-saline	0.08 ± 0.03	0.04 ± 0.02	0.08 ± 0.06	1.11 ± 0.65
Vehicle-SB	0.07 ± 0.02	0.06 ± 0.06	0.08 ± 0.04	1.67 ± 0.40
Citalopram-saline	0.18 ± 0.08**	0.19 ± 0.06***	0.09 ± 0.06	1.53 ± 1.25
Citalopram-SB	0.57 ± 0.16***	0.22 ± 0.07***	0.18 ± 0.05***	1.78 ± 0.61*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from vehicle-saline. Data are presented as a mean ± SD.

compared with SB alone (from $t = 30$ mn). The combination of Cit + SB increased 5-HT release between $t = 75$ and 135 min, compared with Cit alone. After a Cit or a Cit + SB challenge, almost all time points differed significantly from $t = 0$, except $t = 15$ min (see Fig. 2A).

There was also a significant interaction between time, treatment and DA release in the NAcc (Fig. 2B, $F_{(28, 145)} = 1.57$, $P = 0.046$). Post-hoc analyses revealed that SB increased DA release compared to Cit ($t = 15$, 90, 105, 135 min) and Cit + SB ($t = 15$, 45 min). Thus, the significant increase in DA release observed after administration of SB was abolished by co-administration of Cit. A Cit challenge decreased DA release compared with the other groups at $t = 90$ min. After an SB challenge, DA release at $t = 15$ min was significantly increased compared with baseline (see Fig. 2B).

3.2. 2-Day treatment experiment

Concentrations of the neurotransmitters 5-HT and DA at time zero in the 2-day-treatment experiment are reported in Table 1. When 5-HT release in the 2-day treatment experiment was compared, significant differences between groups were noted in the NAcc (Fig. 3A, $F_{(3, 85)} = 52.9$, $P < 0.0001$). Post-hoc analysis indicated an increase in 5-HT release after administration of Cit (437%) and Cit + SB (524%), compared with vehicle + saline. Similar differences between the treatment groups were observed in the VTA (Fig. 3B, $F_{(3, 81)} = 136.5$, $P < 0.0001$). Post-hoc analysis indicated an increase in 5-HT release after administration of Cit (274%) and Cit + SB (849%), compared with vehicle + saline. When Cit and SB were combined, the effect of SB alone or Cit alone was significantly augmented (Fig. 3B).

Increases of DA release after 2-days of treatment were also noted, both in NAcc (Fig. 3C, $F_{(3, 86)} = 2.8$, $P = 0.04$) and VTA (Fig. 3D, $F_{(3,77)} = 16.9$, $P < 0.0001$). Post-hoc analyses showed that Cit + SB increased DA release in NAcc (162%) compared with vehicle + saline. Other treatments (SB alone, Cit alone) also tended to increase DA release in NAcc, but statistical significance was not reached in these cases (Fig. 3C). The combination of Cit and SB significantly increased DA release in the VTA (221%), compared with the vehicle + saline, the vehicle + SB or the vehicle + Cit groups (Fig. 3D).

A 2-day drug treatment did not change the effects of an SB challenge on 5-HT release in the NAcc (Fig. 4A, $F_{(3,14)} = 1.70$, $P = 0.21$), although an interaction between time and treatment was observed ($F_{(42, 188)} = 1.47$, $P = 0.044$). Post-hoc analysis indicated that 5-HT release in the Cit group was significantly increased at $t = 45$ min and $t = 75$ min (Fig. 4A). A 2-day treatment did also not change the effect of an SB challenge on 5-HT release in the VTA (Fig. 4B, $F_{(3, 14)} = 1.99$, $P = 0.16$). There was an effect of time ($F_{(14, 186)} = 7.17$, $P < 0.001$), and a trend toward an interaction between time and treatment ($F_{(42, 186)} = 1.42$, $P = 0.061$), but this did not reach statistical significance. Apparently, SB caused a similar increase in 5-HT release in all groups (Fig. 4B).

The observed changes of DA release in the NAcc after an SB challenge were not significantly different in the various treatment groups (Fig. 4C, $F_{(3, 14)} = 0.94$, $P = 0.45$). Changes of DA release in the VTA after an SB challenge were also similar in all groups (Fig. 4D, $F_{(3, 14)} = 2.38$, $P = 0.11$). These observations suggest that SB increases DA release in the NAcc and VTA in a similar way in all groups, independent of the kind of pre-treatment (Fig. 4C and D).

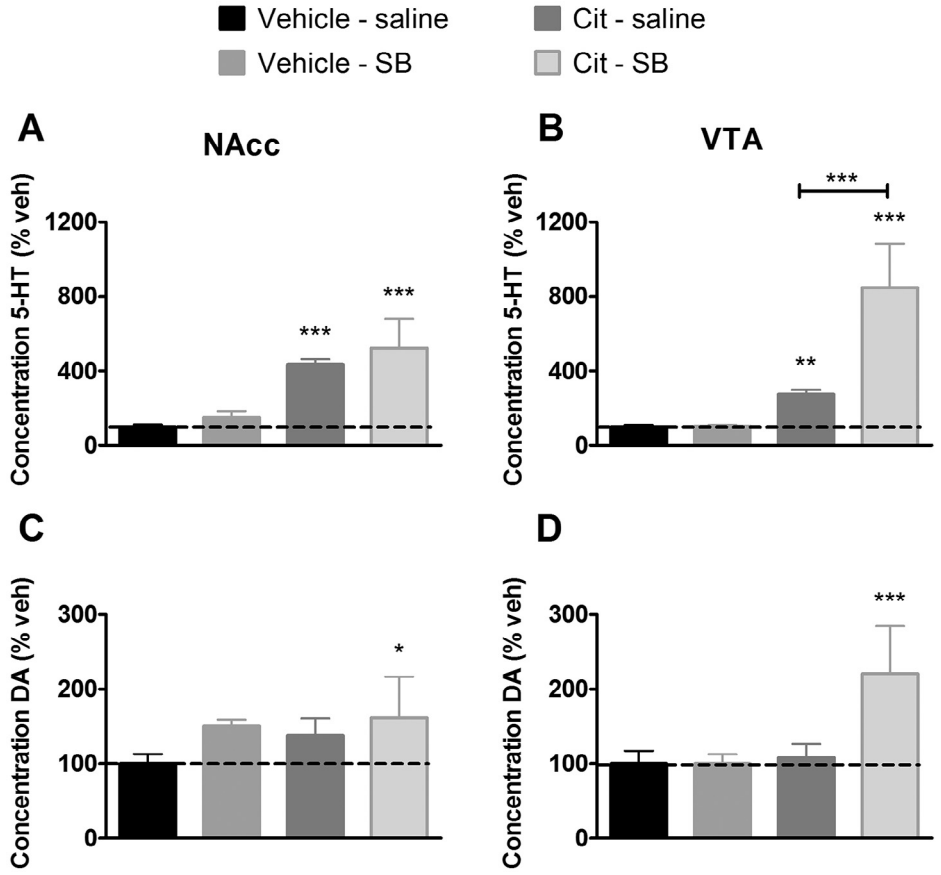

Fig. 3. Baseline levels of 5-HT and DA after 2 days of drug treatment. Concentrations of extracellular serotonin (5-HT) and dopamine (DA) in NAcc and VTA after 2 days of s.c. drug treatment are expressed as a percentage of the vehicle-saline (control) group. Both citalopram (Cit) and the Cit + SB 242084 (SB) combination increased 5-HT in the NAcc (A) and VTA (B). Only the Cit + SB combination increased DA in the NAcc (C) and VTA (D). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are presented as a mean ± SEM.

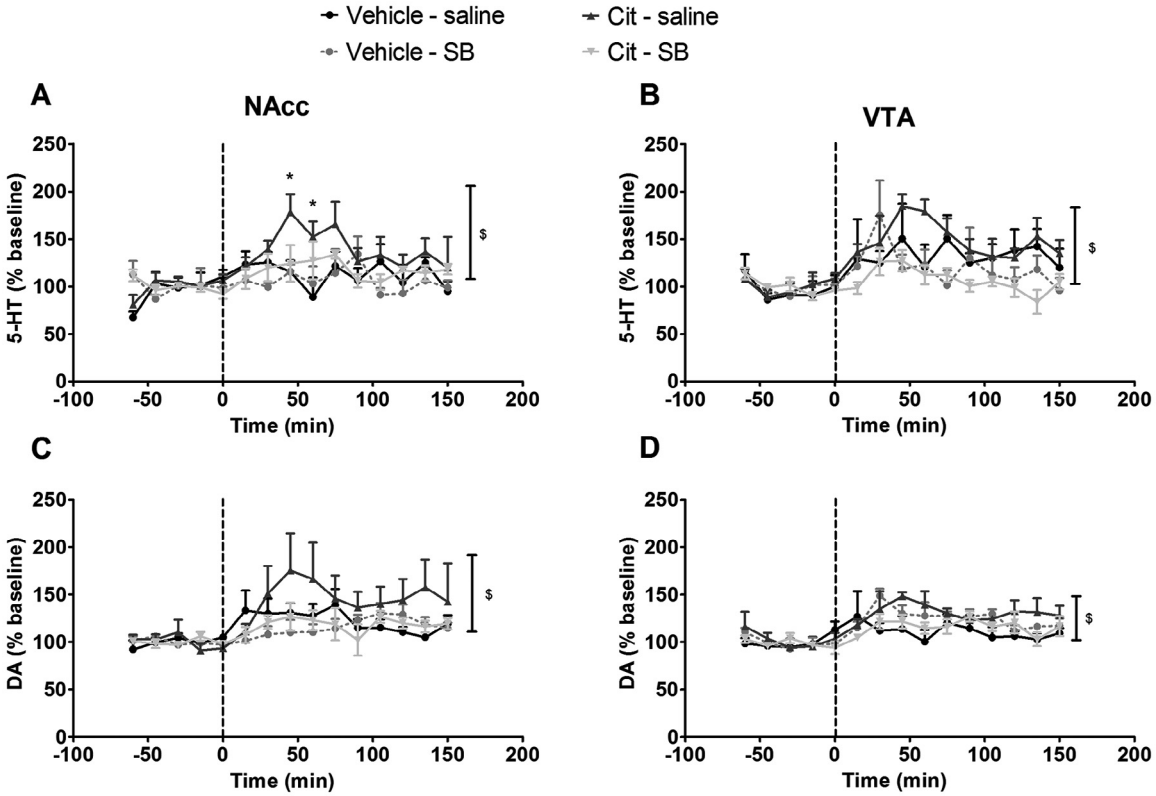

Fig. 4. Changes in 5-HT and DA release after 2-days of drug treatment and a challenge of SB 242084. A challenge of SB 242084 (s.c.) was applied at $t = 0$, after 2-days of treatment with various drugs. Effects of the challenge on levels of extracellular serotonin (5-HT) in NAcc (A) and VTA (B) were measured with microdialysis. Effects on dopamine (DA) release in NAcc (C) and VTA (D) were also measured. *Citalopram (Cit) differs significantly ($P < 0.05$) from vehicle. $: Significant ($P < 0.05$) effect of time. Data are presented as a mean ± SEM.

4. Discussion

The monoamine hypothesis postulates that there are deficits of 5-HT and DA in MDD, thus the extracellular levels of both neurotransmitters should be increased by antidepressant treatment. In this study we investigated if this aim may be achieved by combining an SSRI with a 5-HT_{2C}R antagonist.

Most of the microdialysis data which we acquired after acute administration of these drugs, either individually or in combination, suggest that the proposed augmentation strategy may indeed result in increases of both 5-HT and DA whereas the SSRI when administered alone only increases 5-HT and will not increase and may even depress, the release of DA. First, acute administration of the SSRI Cit combined with the 5-HT_{2C}R antagonist SB has greater effects on extracellular 5-HT levels in the NAcc than administration of each compound alone (Fig. 2A). This finding is in agreement with a previous study reporting a similar augmentation of 5-HT release in rat hippocampus and frontal cortex (Cremers et al., 2004). In addition, an acute challenge with SB increases DA release in the NAcc, and this effect is abolished by Cit, suggesting that Cit when administered alone has an inhibitory effect on DA release (Fig. 2B).

However, administration of Cit alone did not significantly affect DA release in the NAcc (Fig. 2B). This observation is at variance with a previous study which reported a significant decrease of DA release after exposure of animals to an SSRI (Dewey et al., 1995). The discrepancy between our results and those of the previous study may be caused by the fact that we studied the acute effect of a moderate dose of the SSRI, whereas the previous study concerned the effect of repeated administration of a high dose.

Dremencov and colleagues have shown that 2 days of treatment with SSRIs reduces the activity of dopaminergic neurons in the VTA (Dremencov et al., 2009). For this reason, we also explored the effects of the SSRI and 5-HT_{2C} antagonist on extracellular neurotransmitter levels after this treatment period. Most microdialysis data that were acquired after 2 days of treatment with the test drugs also suggest that the proposed augmentation strategy may actually work, viz. a co-administered 5-HT_{2C} antagonist may counteract the negative effects of an SSRI on dopaminergic neurotransmission. Depressed patients are treated with SSRIs for extended periods of time and the interval between onset of treatment and onset of efficacy is usually much longer than 2 days. It is thus quite encouraging that the beneficial effects of the tested augmentation strategy were already noticed within 2 days in the present study.

Larger augmenting effects were observed after 2 days of drug treatment rather than an acute challenge (Fig. 3). The combination of Cit and SB increased the release of 5-HT and DA in both VTA and NAcc, compared with the control group. In the VTA, Cit + SB (849%) increased 5-HT levels more than Cit alone (275%). Moreover, significant increases of DA in VTA and NAcc were only detected in the group receiving Cit + SB, suggesting that this combination increases DA as well as 5-HT levels in VTA and NAcc. However, drug effects on DA levels in the NAcc should be interpreted with caution as there is much variation in these data and the observed differences are not as big as those of DA in the VTA or those of 5-HT.

In order to investigate whether a 2-day period of treatment affects 5-HT_{2C}R sensitivity, an SB challenge was administered in each group. Significant differences between the groups were not observed, but the post-challenge levels of 5-HT in the NAcc were significantly different from time zero in the Cit group, while this was not the case in the other treatment groups (Fig. 4). This finding suggests a gradual sensitization of 5-HT_{2C}R in the NAcc after Cit treatment with a cor-

responding impact on 5-HT release, although the effect is rather small and the data show much variation. Additional experiments are necessary to explore the effect of Cit on 5-HT_{2C}R sensitivity.

We observed regional differences in the effect of 5-HT_{2C}R antagonism. For example, there is no tonic inhibition of DA release in the VTA, while there is in NAcc. Another study has shown that there is tonic inhibition of DA and NE release, but not of 5-HT release in the prefrontal cortex (Millan et al., 1998). 5-HT_{2C} receptors are present not only on GABA-ergic neurons (Fig. 1), but also on dopaminergic neurons (Bubar et al., 2011) and probably on other neurons as well. Since the effects of 5-HT_{2C}R stimulation in many target neurons are excitatory (Barnes and Sharp, 1999), stimulation of 5-HT_{2C}R in GABA-ergic and other neurons could have opposite effects. Therefore, the net release of DA could depend on the balance between excitatory and inhibitory effects of 5-HT_{2C}R stimulation. By administration of an SSRI, this balance may be shifted towards decreased DA release in the mesolimbic system. Co-administration of a 5-HT_{2C}R antagonist could alleviate the negative effects of the SSRI on DA release and might even result in increased levels of extracellular DA. Increased efficacy of an SSRI after co-administration of a 5-HT_{2C}R antagonist has been demonstrated in the forced swim test, a behavioral test that measures depressive-like behavior. Mice which did not respond to Cit in this test responded after co-administration of Cit with a 5-HT_{2C} antagonist (Calcagno et al., 2009). Thus there is both neurochemical and behavioral evidence that combining an SSRI with a 5-HT_{2C}R antagonist may increase antidepressant efficacy.

The augmentation strategy proposed here could easily be tested in patients, since there are already antidepressants available with 5-HT_{2C}R antagonistic properties. Agomelatin, for example, is an antagonist for 5-HT_{2C}R and an agonist for melatonin receptors. The increases of DA and NE in frontal cortex which are observed upon treatment with this compound can be attributed to its action on 5-HT_{2C}R, rather than melatonin receptors (Millan et al., 2003). Mirtazapine and fluoxetine are other examples of antidepressants with antagonist actions at 5-HT_{2C}R (Chanrion et al., 2008; Ni and Miledi, 1997).

5. Conclusions

Since we observed increases of extracellular DA and 5-HT in NAcc and VTA after 2 days of treatment (Fig. 3), combination therapy with an SSRI and a 5-HT_{2C} antagonist may hold promise in targeting both the dopamine- and serotonin-related symptoms of depression, *viz.* anhedonia and a depressed mood. Such a combination may also positively influence treatment response and the onset of action of SSRIs. Future research in this area should investigate the behavioral and long-term effects of an SSRI in combination with 5-HT_{2C} antagonist.

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Conflicts of interest

There are no conflicts of interest concerning this manuscript which should be reported.

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