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Combination of escitalopram and a 5-HT_{1A} receptor antagonist selectively decreases the extracellular levels of dopamine in the nucleus accumbens relative to striatum through 5-HT_{2C} receptor stimulation; suggestive of antipsychotic potential ☆

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ABSTRACT

Serotonin 5-HT_{2C} receptors are widely distributed throughout the brain located on GABAergic interneurons and afferent neurons in the ventral tegmental area and substantia nigra. Consequently, activation of this receptor modulates the dopaminergic neurotransmission. The antipsychotic potential of the combined treatment with escitalopram, in therapeutic relevant doses, and the 5-HT_{1A} receptor antagonist, WAY-100635, has been evaluated by assessment of conditioned avoidance (CAR) behaviour and the use of microdialysis in freely moving rats. The combined treatment was found to decrease both CAR behaviour without affecting escape failures and the basal extracellular levels of dopamine (DA) in the nucleus accumbens (NAc) acutely without affecting DA levels in the striatum, suggesting an antipsychotic-like effect with mesolimbic selectivity. The escitalopram/WAY-100635-induced changes in CAR behaviour and DA were prevented by pretreatment with the 5-HT_{2C} receptor antagonist, SB242084, indicating that the effects are mediated by stimulation of the 5-HT_{2C} receptor. Thus, indirect activation of the 5-HT_{2C} receptor may induce antipsychotic-like effects. The observations on DA levels were in line with the findings made with the selective 5-HT_{2C} receptor agonist, vabicaserin, which was also shown to produce a mesolimbic selective decrease in DA levels in the present study. In addition, it was demonstrated that escitalopram, in combination with the partial 5-HT_{1A} agonist, (-)-pindolol, decreased basal DA levels in the NAc. A potential therapeutic effect could readily be assessed, since both escitalopram and (-)-pindolol are already on the market.

1. Introduction

Schizophrenia is a highly complex life-long disorder that varies in both manifestations and progression in the individual patient (Mueser and McGurk, 2004; Lang et al., 2007; Gray and Roth, 2007). Much attention has been focused on developing more effective antipsychotic drugs with novel mechanisms of action. However, all current antipsychotic drugs are dopamine (DA) D_2 receptor antagonists with varying degrees of potency (Carlsson et al., 2001; Leuner and Muller, 2006). Typical antipsychotic drugs, such as chlorpromazine and haloperiodol, show a high potency at the D_2 receptor, while atypical antipsychotic drugs, such as clozapine, show a lower potency at this receptor (Geddes et al., 2000; Kapur and Seeman, 2001; Remington, 2003). The effect on the limbic pathways is thought to mediate the antipsychotic effect, while extrapyramidal symptoms (EPS) are related to blockade of the nigrostriatal pathway controlling motor function (Carlsson, 1978; Arnt et al., 1997; Leuner and Muller, 2006). Besides affinity for the D_2 receptor, atypical antipsychotic drugs also show considerable affinity for other receptors, e.g. the serotonergic 5-HT_{2A} and 5-HT_{2C} receptors, the D_4 receptor, cholinergic and histaminergic receptors (Leysen et al., 1993). It has been suggested that the broader receptor profile of atypical antipsychotic drugs may produce improvements in negative and cognitive functions seen with these compounds, although the effects are marginal and remain controversial (Kapur and Seeman, 2001; Mueser and McGurk, 2004).

In recent years, there has been some research into the 5-HT_{2C} receptor as a potential target for obesity and schizophrenia (Wacker and Miller, 2008). The mRNA of the 5-HT_{2C} receptor is expressed in the ventral tegmental area (VTA), substantia nigra (SN) pars compacta, SN reticulate, and in the terminal regions of the nigrostriatal and mesolimbic dopaminergic pathways (Leysen, 2004; Alex and Pehek, 2007; Navailles et al., 2008). The majority of these receptors are believed to be located postsynaptically in relation to serotonergic neurons, although the receptor mRNA also is expressed in the raphe nuclei (Clemett et al., 2000). Serotonin 5-HT_{2C} receptors

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are primarily located on GABAergic neurons in the SN (Eberle-Wang et al., 1997; Leysen, 2004; Invernizzi et al., 2007), but are also expressed on GABAergic and dopaminergic neurons in the VTA (Bubar and Cunningham, 2007). The 5-HT_{2C} receptor is the only known G protein-coupled receptor that undergoes mRNA editing giving rise to polymorphisms (Hoyer et al., 2002; Berg et al., 2008), which has been linked to phenotypic expression of schizophrenia, and variable response to antipsychotic drug treatment (Reynolds et al., 2005; Zhang et al., 2006; Mulder et al., 2009).

It has recently been demonstrated that the addition of a low dose of the 5-HT_{2C} receptor agonist WAY-163909 to low doses of either haloperidol or clozapine improves the effect of these drugs in animal models of psychosis, which might result in a lower risk of side-effects in the clinic (Grauer et al., 2009). Data predictive of antipsychotic activity have been published on WAY-163909 alone. These results suggest that the effects of WAY-163909 are specific for the mesolimbic pathway, compared to the nigrostriatal pathway, thus indicating a potential treatment that lacks EPS liability (Dunlop et al., 2006a; Rosenzweig-Lipson et al., 2007; Marquis et al., 2007).

Eltayb et al. (2007), described that treatment with citalopram, or the 5-HT_{1A} antagonist, WAY-100635, did not show any effect when given alone, whereas the combined treatment significantly reduced the number of avoidances without affecting the number of escape failures in rats, which is predictive of an antipsychotic-like effect.

In this study, the effects of the combined treatment with escitalopram and WAY-100635 were investigated by assessing CAR behaviour and using microdialysis to determine the extracellular levels of DA in both the NAc and striatum in freely moving rats. The combination of escitalopram and the partial agonist 5-HT_{1A} receptor agonist, (-)-pindolol, was also investigated with respect to the extracellular levels of DA in the NAc. The selective 5-HT_{2C} receptor agonist, vabicaserin, (Dunlop et al., 2006b) was used as a reference compound in the microdialysis experiments.

2. Methods

2.1. Animals

The animals arrived at the animal facility at least five days before being used in experiments and were transported to the laboratory one day prior to the experiments.

Male Wistar rats (Harlan, The Netherlands) weighing approximately 200 g at the beginning of the training sessions and 350–400 g at the time of study were used for assessment of CAR behaviour. Male Sprague–Dawley rats weighing 200–225 g upon arrival (Charles River, Germany) and maximally 350 g at the time of use were utilized for microdialysis experiments. The animals were housed in pairs in woodchip-lined Makrolon® IV cages and kept under controlled conditions of temperature (21 ± 2 °C), relative humidity (55 ± 5%) and a 12-h light–dark cycle (lights on at 6:00 am) with one plastic house for enrichment. Food and tap water were freely available in the home cage. All experimental work was performed during the light phase. Approval of all animal procedures was granted by The Danish National Committee for Ethics in Animal Experimentation.

2.2. Drugs

Escitalopram ((+)-1-(3-(dimethylamino)propyl)-1-(4′-fluorophenyl)-1,3-dihydroiso-benzofuran-5-carbonitrile, oxalate), vabicaserin ((-)-4,5,6,7,9,9a,10,11,11,12,12a-de-cahydrocyclopenta[c]-[1,4]diazepino[6,7,I-ij]quinoline, hydrochloride), WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-piperazin-1-yl]ethyl]-*N*-pyridin-2-ylcyclohexa-necarboxamide, oxalate) and SB242084 (6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-car-boxyamide, dihydrochloride) were all synthesized at H. Lundbeck A/S (Valby, Denmark). (-)-Pindolol ((2S)-1-(1H-indol-4-yloxy)-3-(propan-2-ylamino)propan-2-ol) was purchased from Tocris (Bristol, UK) and dexamphetamine sulphate was purchased from Unikem (Copenhagen, Denmark). Drug doses are expressed in terms of salt. Escitalopram and dexamphetamine were dissolved in physiological saline, while SB242084 and vabicaserin were dissolved in 10% hydroxy-propyl-beta-cyclodextrin. (-)-Pindolol and WAY-100635 were dissolved in a few drops of 0.1 M citric acid and 0.1 M sodium hydroxide, respectively, and diluted with physiological saline. Escitalopram and WAY-100635 were given as a single injection. All drugs were administered subcutaneously (s.c.) in a volume of 5 ml/kg body weight.

2.3. Conditioned avoidance response (CAR) behaviour

CAR behaviour was assessed using a two-compartment shuttle-box equipped with a grid floor, which could provide an electric shock of 0.5 mA with a maximal duration of 10 s, and a stimulus light and a tone generator of 2900 Hz in each compartment. The position and movement of the rat were detected by photocell sensors. The shock, tone and sensors were controlled by a computer using an interface package (MED Associates). The shuttle-box was placed in a sound attenuated cubicle. The data were automatically collected using a software package (BigMother Software Package; H. Lundbeck A/S) where the following behavioural variables were recorded: avoidance (response to conditioned stimuli within 10 s), escape (response to the unconditioned stimuli within 10 s) and escape failures (failure to respond to the unconditioned stimuli within 10 s). The rats were habituated to the shuttle-box 3 min before the training session started. Each training session consisted of 30 trials with inter-trial intervals varied at random between 20 and 30 s. The rats were trained to move into the adjacent compartment within 10 s upon presentation of the conditioned stimuli (tone and light) in order to avoid the appearance of the unconditioned stimulus (electric shock). Training was carried out 5 days a week until the animals showed an avoidance response in at least 80% (24 trials) of the trials on 3 consecutive days.

Test sessions consisted of a 3 min habituation period followed by 10 trials randomly distributed between 20 s and 30 s. A compound test was always preceded by a pre-test and only animals displaying a stable performance with an avoidance of at least 80% (8 trials) were included in the compound test the following day. A 1-week wash-out between compound test and the next pre-test was allowed. SB242084 was administered 60 min prior to the test, while escitalopram and WAY-100635 were administered 30 min prior to the test.

Data were analysed as percentage inhibition of avoidance by comparing avoidance in the compound test with avoidance in the pre-test with each animal serving as its own control.

2.4. Microdialysis

Rats were anaesthetised with a 2:1 mixture of Hypnorm® (fentanyl citrate 0.32 mg/ml, fluanisone 10 mg/ml; H. Lundbeck A/S), and Dormicum® (midazolam 5 mg/ml; Roche) in a volume of 2.3 ml/kg s.c. and mounted in a stereotaxic frame. An intracerebral microdialysis guide cannula (CMA 12; CMA Microdialysis) was implanted in either the ventral striatum [AP: +0.7, ML: +2.8, DV: −4.0] or the NAc shell region [AP: +1.7, ML: +0.8, DV: −6.0], respectively (coordinates taken according to (Paxinos and Watson, 2007)). Dialysis experiments were conducted two to four days after surgery, where a probe (CMA 12; CMA Microdialysis) was inserted. Dialysis occurred through the semi-permeable membrane that extended the guide cannula, positioning the probe tip 6.0 mm ventral to dura mater for striatum and 8.0 mm for the NAc, respectively. The probe was perfused with Ringer's solution (145 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$, pH 7.4; H. Lundbeck A/S) applied with a flow rate of 1.0 μl/min and a 3-h habituation period following probe insertion was allowed before the microdialysis sampling was initiated to secure stable DA baseline levels. A 20 min sampling regime with a total of 12 fractions was used

throughout all experiments. Four baseline fractions were taken prior to injection of any drug. The content of DA in the dialysate samples was quantified using high performance liquid chromatography (HPLC) coupled to electrochemical detection (Coulochem III; ESA Biosciences). The lower limit of detection was 1.5 fmol/20 μL. The probe placement was histologically verified by staining the tissue with Tryphan Blue (Sigma-Aldrich) and subsequently slicing the brain using a cryostat.

The DA concentration of the last 3 of the 4 baseline fractions was averaged and this value denoted as a 100% baseline DA activity. All subsequent sample values were expressed as a percentage of this pre-injection value.

2.5. Statistics

The data are expressed as group mean ± the standard error of mean (SEM).

For the assessment of CAR behaviour within each trial, the statistical evaluation of the effect of treatment (defined by the different combinations of escitalopram and WAY-100635) was carried out by one-way ANOVA followed by Tukey's Multiple Comparison Test (GraphPad Prism 5). A two-way ANOVA (SB242084 × treatment) was used to assess a possible interaction between SB242084 and treatment. The similarity of the two vehicle groups had been tested in advance by means of an unpaired t-test (SigmaStat 3.0). For the assessment of microdialysis, a two-way ANOVA (treatment × time) with repeated measures, followed by pairwise comparisons using the Bonferroni method to correct for multiplicity (SigmaStat 3.0) was performed. A p value of less than 0.05 was regarded statistically significant.

3. Results

3.1. Escitalopram in combination with the selective 5-HT$_{1A}$ receptor antagonist, WAY-100635, suppresses CAR behaviour

Neither escitalopram (1.25 or 5.0 mg/kg s.c.) nor WAY-100635 (0.2 mg/kg s.c.) produced any effect on CAR behaviour 30 min after administration when compared to the vehicle group (Fig. 1a). However, when the two drugs were administered in combination a statistically significant suppression of CAR behaviour was observed with both doses of escitalopram [$F(5,42) = 15.94$, $p < 0.0001$] without affecting the number of escape failures significantly. One escape failure was observed in the escitalopram (5.0 mg/kg s.c.) and WAY-100635 (0.2 mg/kg s.c.) group.

3.2. Pretreatment with the selective 5-HT$_{2C}$ receptor antagonist, SB242084, prevents the suppression of CAR behaviour in rats induced by combined treatment with escitalopram and WAY-100635

A significant interaction between SB242084 (0.5 mg/kg s.c.) and treatment was observed [$F(1,4) = 9.288$, $p < 0.001$]. SB242084 (0.5 mg/kg s.c.) alone did not alter CAR behaviour, but treatment with SB242084 60 min prior to the test prevented the suppression of CAR behaviour demonstrated with the combination of escitalopram and WAY-100635 administered 30 min prior to the test (Fig. 1a, b) [$F(5,42) = 1.429$, $p = 0.2339$]. No escape failures were observed in this experiment.

3.3. Basal extracellular levels of DA

The mean basal extracellular levels of DA, without correcting for probe recovery, were approximately 17.5 ± 1.08 fmol/20 μL (n = 30) and 10.7 ± 0.57 fmol/20 μL (n = 91) for the striatum and NAc, respectively.

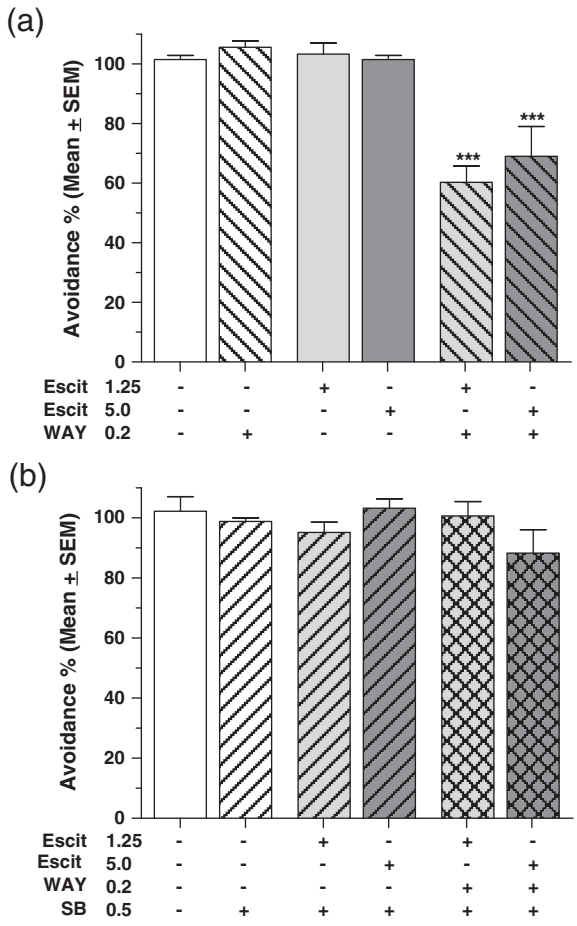

Fig. 1. a) The effects of escitalopram (Escit; 1.25 or 5.0 mg/kg s.c.) or WAY-100635 (WAY; 0.2 mg/kg s.c.) alone or in combination on conditioned avoidance behaviour. b) The effects of SB242084 (SB; 0.5 mg/kg s.c.) alone or in combination with escitalopram (Escit; 1.25 or 5.0 mg/kg s.c.) alone or together with WAY-100635 (WAY; 0.2 mg/kg s.c.). Escitalopram and WAY-100635 were administered 30 min prior to the test, while SB242084 was given 60 min prior to the test. Bars represent the mean (avoidance %) ± SEM based on the observation of the same eight animals serving as their own control in a change over design. ***$p < 0.001$ compared to vehicle-treated animals (two-way ANOVA followed by Tukey's Multiple Comparisons test).

3.4. Escitalopram in combination with the selective 5-HT$_{1A}$ receptor antagonist, WAY-100635, reduces the basal extracellular levels of DA in the NAc

Neither escitalopram (5.0 mg/kg s.c.) (Fig. 2a) alone nor WAY-100635 (0.2 mg/kg s.c.) (Fig. 2b) alone altered the extracellular levels of DA significantly, [$F(1,16) = 0.000148$, $p = 0.99$] and [$F(1,11) = 0.353$, $p = 0.97$], respectively. However, simultaneous blockade of the 5-HT$_{1A}$ receptor with WAY-100635 (0.2 mg/kg s.c.) produced a decrease in the basal extracellular levels of DA in the NAc with both escitalopram (5.0 mg/kg s.c.) [$F(1,17) = 6.13$, $p = 0.024$] (Fig. 2a) and escitalopram (1.25 mg/kg s.c.) [$F(1,11) = 1.95$, $p = 0.040$] (Fig. 2b), respectively. The most pronounced decrease in the extracellular levels of DA was observed at 160 min with DA levels at 40.2% ± 16.7 of baseline following escitalopram (5.0 mg/kg s.c.) and WAY-100635 (0.2 mg/kg s.c.) treatment.

3.5. Vabicaserin decreases the basal extracellular levels of DA in the NAc

Vabicaserin (20 mg/kg s.c.) significantly reduced the basal DA levels in the NAc [$F(1,15) = 17.06$, $p < 0.001$] (Fig. 2c). The decrease in basal DA levels with vabicaserin was most pronounced at 160 min with the DA levels of 42.2% ± 5.8 of baseline.

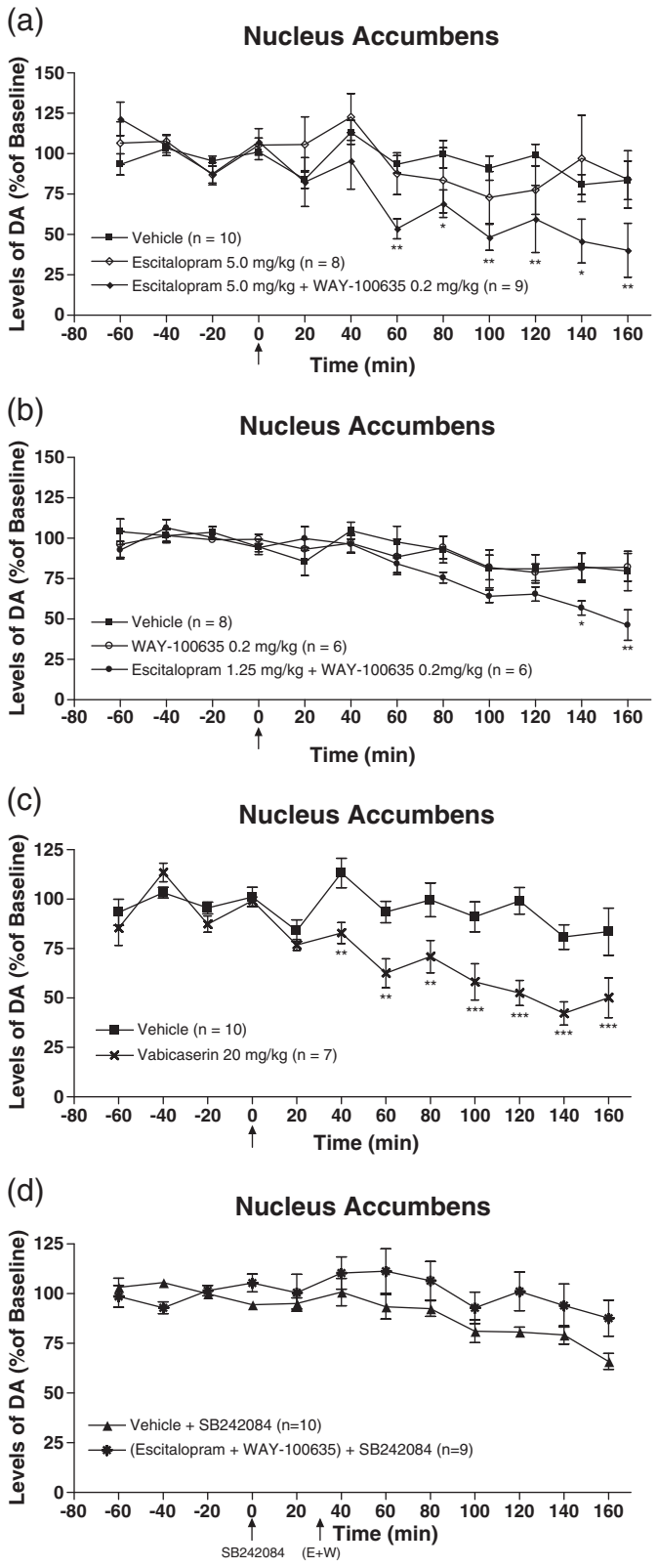

Fig. 2. Effects of either escitalopram (5.0 mg/kg s.c.) alone, escitalopram (5.0 mg/kg s.c.) and WAY-100635 (0.2 mg/kg s.c.) in combination (a), WAY-100635 (0.2 mg/kg s.c.) alone, escitalopram (1.25 mg/kg s.c.) and WAY-100635 (0.2 mg/kg s.c.) in combination (b), or vabicaserin (20 mg/kg s.c.) (c) on the basal extracellular levels of dopamine in the nucleus accumbens. Administration of SB242084 (0.5 mg/kg s.c.) 30 min prior to the administration of escitalopram and WAY-100635 prevented any alteration of the dopamine levels (d). Escitalopram, escitalopram and WAY-100635 and vabicaserin were all administered at time point zero, whereas SB242084 was administered 30 min before. Data are expressed as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, or $^{***}p<0.001$ compared to vehicle-treated animals (two-way ANOVA followed by Bonferroni correction).

3.6. Pretreatment with the selective 5-HT$_{2C}$ receptor antagonist, SB242084, prevents the decrease in the basal extracellular levels of DA demonstrated with the combination of escitalopram and WAY-100635 in the NAc

Treatment with SB242084 (0.5 mg/kg s.c.) 30 min prior to the treatment with escitalopram (5.0 mg/kg s.c.) and WAY-100635 (0.2 mg/kg s.c) fully prevented the decrease in the basal DA levels demonstrated with the combination of escitalopram and WAY-100635 [$F(1,17)=0.186$, $p=0.671$] (Fig. 2d).

3.7. Neither escitalopram alone or in combination with WAY-100635, nor vabicaserin modifies the basal extracellular levels of DA in the striatum

Neither escitalopram (5.0 mg/kg s.c.) alone [$F(1,12)=0.215$, $p=0.651$], nor in combination with WAY-100635 (0.2 mg/kg s.c.) [$F(1,12)=0.523$, $p=0.483$], nor vabicaserin (20 mg/kg s.c.) [$F(1,12)=0.525$, $p=0.481$], produced any changes in the basal extracellular levels of DA in the striatum (Fig. 3).

3.8. Escitalopram in combination with the partial 5-HT$_{1A}$ receptor antagonist, (-)-pindolol, decreases the basal extracellular levels of DA in the NAc

Escitalopram (5 mg/kg s.c.) administered 20 min after (-)-pindolol (10 mg/kg s.c.), did not affect the basal extracellular levels of DA in the NAc significantly [$F(1,18)=2.162$, $p<0.159$] (Fig. 4). However, a significant reduction in the basal extracellular levels of DA was observed with the combination of escitalopram (5 mg/kg s.c.) and (-)-pindolol (15 mg/kg s.c.) [$F(1,15)=9.670$, $p<0.007$].

4. Discussion

Assessment of CAR behaviour has been used extensively to evaluate the effect of potential antipsychotic compounds in animals (Arnt, 1982; Wadenberg and Hicks, 1999; Geyer and Ellenbroek, 2003). Reduction in avoidance response without affecting escape failure is highly predictive of a potential antipsychotic effect and thus all clinically effective antipsychotic drugs are active in this model (Arnt, 1982; Geyer and Ellenbroek, 2003). In the present study, we demonstrate that escitalopram in combination with the 5-HT$_{1A}$ receptor antagonist, WAY-100635, in contrast to escitalopram alone reduces the avoidance response without affecting escape failures significantly. Eltayb et al. (2007) showed that citalopram in combination with WAY-100635 produced a decrease in avoidance response, but this was demonstrated with high doses of citalopram.

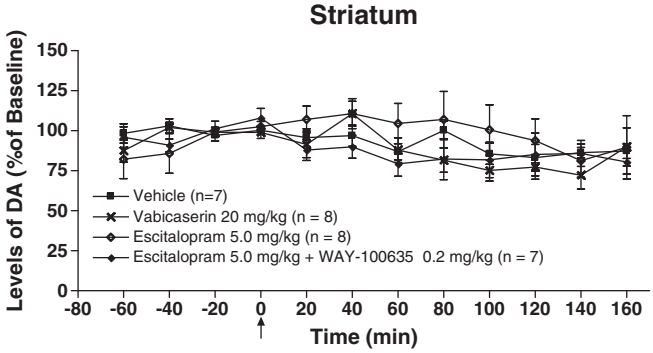

Fig. 3. Effects of either vabicaserin (20 mg/kg s.c.), escitalopram (5.0 mg/kg s.c.) alone, escitalopram (5.0 mg/kg s.c.) and WAY-100635 (0.2 mg/kg s.c.) in combination on basal dopamine levels in the striatum. Compounds were all administered at timepoint zero. Data are expressed as mean ± SEM. (two-way ANOVA followed by Bonferroni correction).

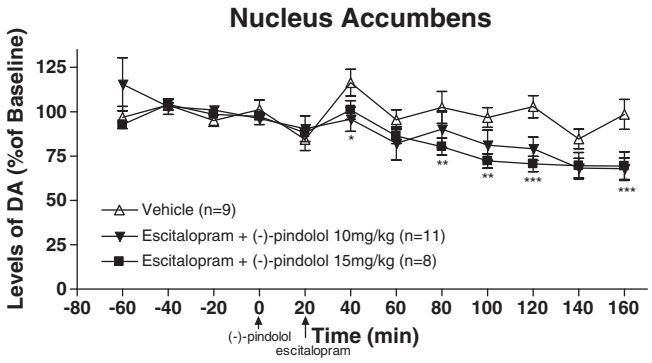

Fig. 4. Effects of escitalopram (5.0 mg/kg s.c.) in combination with (-)-pindolol (10 mg/kg or 15 mg/kg s.c.) on basal dopamine levels in the nucleus accumbens. (-)-Pindolol was administered at timepoint zero, whereas escitalopram was administered at 20 min. Data are expressed as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, or $^{***}p<0.001$ compared to vehicle-treated animals (two-way ANOVA followed by Bonferroni correction).

The present finding demonstrates an effect of the combined treatment with escitalopram in doses that yield clinically relevant plasma levels (Bundgaard et al., 2006; Rao, 2007).

The combined treatment with citalopram and WAY-100635 has been shown to produce a larger increase in the levels of extracellular 5-HT compared to treatment with citalopram alone (Cremers et al., 2000). Theoretically, this excessive amount of extracellular 5-HT activates 5-HT_{2C} receptors on the GABAergic interneurons in the VTA, thereby decreasing the dopaminergic transmission. Indeed, the observed effects of escitalopram and WAY-100635 were prevented by pretreatment with the 5-HT_{2C} receptor antagonist, SB242084.

The 5-HT_{2C} receptor agonists, WAY-163909 (Marquis et al., 2007) and vabicaserin (Marquis K, personal communication), have also been demonstrated to produce a dose-dependent decrease in avoidance response in rats, with only little or no effect on the number of escape failures. Similarly, the combined 5-$HT_{2A/2C}$ receptor agonist, CP-809,101, has been shown to dose-dependently suppress the avoidance response. This was assumed to be due to agonism at the 5-HT_{2C} receptor, since a 5-HT_{2A} receptor agonist did not show any effect on CAR behaviour (Siuciak et al., 2007). It has been reported that both CP-809,101 and WAY-163909 decrease the avoidance response to a similar degree as clozapine and haloperidol (Dunlop et al., 2006; Siuciak et al., 2007).

We demonstrate that escitalopram in combination with WAY-100635 decreases the basal extracellular levels of DA in the NAc to approximately 40% compared to vehicle-treated animals, which is similar to what we observed with the 5-HT_{2C} receptor agonist, vabicaserin. In contrast, WAY-100635 alone did not induce any alterations on either CAR behaviour or the basal extracellular levels of DA in the NAc, which is in line with previous findings demonstrating that 5-HT_{1A} receptor antagonism alone did not alter 5-HT levels (Muller et al., 2002). In contrast, single administration of both typical and atypical antipsychotic drugs has been shown to result in an increase in the extracellular DA levels in the NAc following acute administration (Kuroki et al., 1999; Mørk et al., 2009). Current antipsychotic drugs cause an initial increase in DA levels in the NAc, which only after chronic treatment returns to baseline or decreases (Blaha and Lane, 1987; Ichikawa and Meltzer, 1991; Hertel et al., 1996). The finding that an initial decrease in the basal DA levels was observed with the combined treatment of escitalopram and WAY-100635 may be indicative of a fast onset of effect in patients. It is believed that the controversial delayed onset of both typical and atypical antipsychotic drugs arises because repeated administration is needed to produce a full depolarization-induced blockade of neuronal firing of the DA neurons in the VTA, while this was seen with acute administration of WAY-163909 (Marquis et al., 2007). It has previously been demonstrated that the non-selective 5-HT_{2C} agonist, mCPP, reduces the basal firing rate and bursting activity of DA neurons in the VTA (Prisco et al., 1994; di Giovanni et al., 2000), whereas the selective 5-HT_{2C} receptor antagonist, SB242084, increases the basal firing rate and bursting activity of DA neurons (di Giovanni et al., 2000). Invernizzi et al. (2007) found that the selective 5-HT_{2C} receptor antagonist, SB243213, did not alter the basal firing rate and pattern of the presumed GABAergic neurons in the SN pars reticulata, suggesting that the 5-HT_{2C} receptor does not exert neither inhibitory nor excitatory tonic control upon these neurons.

A selective effect on the basal DA levels in the NAc compared to the striatum was observed with both escitalopram in combination with WAY-100635 and vabicaserin, thus suggesting that these compounds exert a mesolimbic selective effect. This may be explained by the finding that WAY-163909 selectively decreased the number of spontaneously active DA neurons in the VTA without affecting the number of spontaneously active DA neurons in the SN pars reticulata (Marquis et al., 2007). In line with this, di Giovanni et al. (2000) demonstrated a higher maximal excitation with mCPP in the non-dopaminergic neurons, presumably GABAergic interneurons, in the VTA compared to the non-dopaminergic neurons in the SN pars reticulata. This effect was ascribed to the agonistic activity of mCPP at the 5-HT_{2C} receptor, since SB242084 blocked the excitation caused by mCPP. These findings might explain the differential response to mCPP and other 5-HT_{2C} receptor agonists, preferentially inhibiting the mesolimbic compared to the nigrostriatal dopaminergic function (di Giovanni et al., 2000). This apparent preferential reduction of DA neurotransmission in the mesolimbic pathway compared to the nigrostriatal pathway of 5-HT_{2C} receptor agonists may potentially result in avoidance of EPS. The present finding suggests that the combined treatment with escitalopram and WAY-100635 shows antipsychotic-like effects in a similar manner as the highly selective 5-HT_{2C} antagonists, vabicaserin and WAY-163909. Nevertheless, it should be noted that the 5-HT_{2C} receptor may rapidly undergo desensitization (Porter et al., 1999). However, Marquis et al. (2007) showed electrophysiologically that a sustained effect of WAY-163909 was observed during chronic treatment. Dremencov et al. (2009), showed that escitalopram produced a decrease in the DA neuronal firing rate and burst activity in the VTA only after repeated dosing, and these were reversed by co-administration of SB242084. The decrease in the firing rate and burst activity of DA neurons in the VTA following escitalopram administration were sustained after 14 days at a time when autoreceptors are known to be desensitized (Dremencov et al., 2009).

The combination of an SSRI and (-)-pindolol has been widely investigated with respect to achieving a faster onset of antidepressant effect in patients (Blier and Bergeron, 1995; Artigas et al., 1996; Ballesteros and Callado, 2004). Escitalopram in combination with (-)-pindolol reduces the DA levels in the NAc, although this treatment does not reduce the DA levels to the same degree as the treatments with escitalopram in combination with WAY-100635 or vabicaserin. Since pindolol in doses of 8 mg/kg s.c. have been shown to potentiate the 5-HT levels following SSRI treatment (Cremers et al., 2001) it is not likely that the lower effect with the (-)-pindolol combination is due to insufficient levels of (-)-pindolol. Instead, the lower effect may be due to the fact that (-)-pindolol is a partial agonist (Newman-Tancredi et al., 1998) and would be expected to possess some intrinsic activity at 5-HT_{1A} receptors. It is of interest that escitalopram in combination with (-)-pindolol can decrease basal DA levels in the NAc, since both compounds are being used in the clinic, so much is known about their pharmacokinetics, safety, and tolerability in humans.

WAY-163909 has been shown to produce a minor elevation of the basal extracellular levels of both acetylcholine and DA in the PFC (Marquis et al., 2007), which may be associated with an enhanced cognitive activity, since an enhancement of acetylcholine and DA in the PFC is reported to be responsible for the improved cognitive

effects of atypical antipsychotic drugs (Kapur and Remington, 1996; Marquis et al., 2007). The 5-HT_{2C} receptor agonist, CP-809,101, has been shown to be effective in the novel object recognition test (Siuciak et al., 2007), but by contrast it has been demonstrated that stimulation of the 5-HT_{2C} receptor by RO 60-0175 had no effect on basal DA levels in the PFC, but inhibited stress-induced increases in DA release (Pozzi et al., 2002). These findings suggest that an inhibitory influence of the 5-HT_{2C} receptor on dopaminergic neurotransmission in the PFC may primarily be during stress-conditions, but may not influence dopaminergic neurotransmission under basal conditions.

5. Conclusion

The present findings suggest that the combined treatment with escitalopram and the 5-HT_{1A} receptor antagonist, WAY-100635, has antipsychotic-like effects since this combination was shown to decrease avoidance response without affecting the escape failures and to reduce the basal extracellular levels of DA in the NAc. The 5-HT_{2C} receptor agonist, vabicaserin was shown to produce similar effects as the combined treatment with escitalopram and WAY-100635 on DA levels. Pretreatment with the 5-HT_{2C} receptor antagonist, SB242084, prevented the demonstrated effects of the combined treatment with escitalopram and WAY-100635. These findings suggest that the antipsychotic-like effects of escitalopram and WAY-100635 are mediated through stimulation of the 5-HT_{2C} receptor. In addition, it was demonstrated that escitalopram in combination with the partial 5-HT_{1A} receptor agonist, (-)-pindolol, reduced the DA levels in the NAc. The present findings demonstrated that the combined treatment with escitalopram and WAY-100635 did not alter the DA levels in the striatum, which suggests that this treatment also has mesolimbic selectivity.

Statement of interest

None.

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References

Alex KD, Pehek EA. Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol Ther 2007;113:296–320.
Arnt J. Pharmacological specificity of conditioned avoidance response inhibition in rats: inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta Pharmacol Toxicol 1982;51:321–9 (Copenh).
Arnt J, Skarsfeldt T, Hyttel J. Differentiation of classical and novel antipsychotics using animal models. Int Clin Psychopharmacol 1997;12(Suppl 1):S9-S17.
Artigas F, Romero L, de Montigny C, Blier P. Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. Trends Neurosci 1996;19:378–83.
Ballesteros J, Callado LF. Effectiveness of pindolol plus serotonin uptake inhibitors in depression: a meta-analysis of early and late outcomes from randomised controlled trials. J Affect Disord 2004;79:137–47.
Berg KA, Clarke WP, Cunningham KA, Spampinato U. Fine-tuning serotonin2c receptor function in the brain: molecular and functional implications. Neuropharmacology 2008;55:969–76.
Blaha CD, Lane RF. Chronic treatment with classical and atypical antipsychotic drugs differentially decreases dopamine release in striatum and nucleus accumbens in vivo. Neurosci Lett 1987;78:199–204.
Blier P, Bergeron R. Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. J Clin Psychopharmacol 1995;15:217–22.
Bubar MJ, Cunningham KA. Distribution of serotonin 5-HT2C receptors in the ventral tegmental area. Neuroscience 2007;146:286–97.
Bundgaard C, Larsen F, Jørgensen M, Gabrielsson J. Mechanistic model of acute autoinhibitory feedback action after administration of SSRIs in rats: application to escitalopram-induced effects on brain serotonin levels. Eur J Pharm Sci 2006;29:394–404.
Carlsson A. Antipsychotic drugs, neurotransmitters, and schizophrenia. Am J Psychiatry 1978;135:165–73.
Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. Annu Rev Pharmacol Toxicol 2001;41:237–60.
Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC. Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. Neuropharmacology 2000;39:123–32.
Cremers TI, de Boer P, Liao Y, Bosker FJ, den Boer JA, Westerink BH, et al. Augmentation with a 5-HT(1A), but not a 5-HT(1B) receptor antagonist critically depends on the dose of citalopram. Eur J Pharmacol 2000;397:63–74.
Cremers TI, Wiersma LJ, Bosker FJ, den Boer JA, Westerink BH, Wikstrom HV. Is the beneficial antidepressant effect of coadministration of pindolol really due to somatodendritic autoreceptor antagonism? Biol Psychiatry 2001;50:13–21.
di Giovanni G, di Matteo V, di Mascio M, Esposito E. Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin(2 C/2B) receptor agonists: a combined in vivo electrophysiological and microdialysis study. Synapse 2000;35:53–61.
Dremencov E, El Mansari M, Blier P. Effects of sustained serotonin reuptake inhibition on the firing of dopamine neurons in the rat ventral tegmental area. J Psychiatry Neurosci 2009;34:223–9.
Dunlop J, Marquis KL, Lim HK, Leung L, Kao J, Cheesman C, et al. Pharmacological profile of the 5-HT(2 C) receptor agonist WAY-163909; therapeutic potential in multiple indications. CNS Drug Rev 2006;12:167–77.
Dunlop J, Zhang J, Watts S, Ramamoorthy S, Harrison B, Barret JE, et al. In vitro pharmacological profile of SCA-136, a novel 5-HT2C receptor selective agonist. 36th Annual Meeting for the Society of Neuroscience. Atlanta, GA. Washington, D.C.: Society for Neuroscience; Oct 14–18 2006
Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF. Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. J Comp Neurol 1997;384:233–47.
Eltayb A, Wadenberg ML, Schilström B, Svensson TH. Antipsychotic-like effect by combined treatment with citalopram and WAY 100635: involvement of the 5-HT2C receptor. Int J Neuropsychopharmacol 2007;10:405–10.
Geddes J, Freemantle N, Harrison P, Bebbington P. Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. BMJ 2000;321:1371–6 (Clinical research ed.).
Geyer MA, Ellenbroek B. Animal behavior models of the mechanisms underlying antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1071–9.
Grauer SM, Graf R, Navarra R, Sung A, Logue SF, Stack G, et al. WAY-163909, a 5-HT2C agonist, enhances the preclinical potency of current antipsychotics. Psychopharmacology (Berl) 2009;204:37–48.
Gray JA, Roth BL. The pipeline and future of drug development in schizophrenia. Mol Psychiatry 2007;12:904–22.
Hertel P, Nomikos GG, Iurlo M, Svensson TH. Risperidone: regional effects in vivo on release and metabolism of dopamine and serotonin in the rat brain. Psychopharmacology (Berl) 1996;124:74–86.
Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 2002;71:533–54.
Ichikawa J, Meltzer HY. Differential effects of repeated treatment with haloperidol and clozapine on dopamine release and metabolism in the striatum and the nucleus accumbens. J Pharmacol Exp Ther 1991;256:348–57.
Invernizzi RW, Pierucci M, Calcagno E, di Giovanni G, di Matteo V, Benigno A, et al. Selective activation of 5-HT(2 C) receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. Neuroscience 2007;144:1523–35.
Kapur S, Remington G. Serotonin–dopamine interaction and its relevance to schizophrenia. Am J Psychiatry 1996;153:466–76.
Kapur S, Seeman P. Does fast dissociation from the dopamine d(2) receptor explain the action of atypical antipsychotics? A new hypothesis. Am J Psychiatry 2001;158:360–9.
Kuroki T, Meltzer HY, Ichikawa J. Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. J Pharmacol Exp Ther 1999;288:774–81.
Lang UE, Puls I, Muller DJ, Strutz-Seebohm N, Gallinat J. Molecular mechanisms of schizophrenia. Cell Physiol Biochem 2007;20:687–702.
Leuner K, Muller WE. The complexity of the dopaminergic synapses and their modulation by antipsychotics. Pharmacopsychiatry 2006;39(Suppl 1):S15–20.
Leysen JE. 5-HT2 receptors. Curr Drug Targets CNS Neurol Disord 2004;3:11–26.
Leysen JE, Janssen PM, Schotte A, Luyten WH, Megens AA. Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT2 receptors. Psychopharmacology (Berl) 1993;112:S40–54.
Marquis KL, Sabb AL, Logue SF, Brennan JA, Piesla MJ, Comery TA, et al. WAY-163909 [(7bR, 10aR)-1, 2, 3, 4, 8, 9, 10, 10a-octahydro-7bH-cyclopenta-[b][1, 4]diazepino[6, 7, 1hi]indole]: a novel 5-hydroxytryptamine 2C receptor-selective agonist with preclinical antipsychotic-like activity. J Pharmacol Exp Ther 2007;320:486–96.
Mørk A, Witten LM, Arnt J. Effect of sertindole on extracellular dopamine, acetylcholine, and glutamate in the medial prefrontal cortex of conscious rats: a comparison with risperidone and exploration of mechanisms involved. Psychopharmacology (Berl) 2009;206:39–49.
Mueser KT, McGurk SR. Schizophrenia. Lancet 2004;363:2063–72.
Mulder H, Cohen D, Scheffer H, Gispen-de WC, Arends J, Wilmink FW, et al. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: a replication study. J Clin Pychopharmacol 2009;29:16–20.
Muller CP, Carey RJ, Jocham G, Huston JP. Cocaine increases serotonergic activity in the hippocampus and nucleus accumbens in vivo: 5-HT1a-receptor antagonism blocks behavioral but potentiates serotonergic activation. Synapse 2002;45:67–77.

Navailles S, Moison D, Cunningham KA, Spampinato U. Differential regulation of the mesoaccumbens dopamine circuit by serotonin2C receptors in the ventral tegmental area and the nucleus accumbens: an in vivo microdialysis study with cocaine. Neuropsychopharmacology 2008;33:237–46.
Newman-Tancredi A, Chaput C, Gavaudan S, Verriele L, Millan MJ. Agonist and antagonist actions of (-)pindolol at recombinant, human serotonin1A (5-HT1A) receptors. Neuropsychopharmacology 1998;18:395–8.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 2007.
Porter RH, Benwell KR, Lamb H, Malcolm CS, Allen NH, Revell DF, et al. Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. Br J Pharmacol 1999;128:13–20.
Pozzi L, Acconcia S, Ceglia I, Invernizzi RW, Samanin R. Stimulation of 5-hydroxytryptamine (5-HT(2C)) receptors in the ventrotegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. J Neurochem 2002;82:93-100.
Prisco S, Pagannone S, Esposito E. Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. J Pharmacol Exp Ther 1994;271:83–90.
Rao N. The clinical pharmacokinetics of escitalopram. Clin Pharmacokinet 2007;46: 281–90.
Remington G. Understanding antipsychotic “atypicality”: a clinical and pharmacological moving target. J Psychiatry Neurosci 2003;28:275–84.
Reynolds GP, Templeman LA, Zhang ZJ. The role of 5-HT2C receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:1021–8.
Rosenzweig-Lipson S, Dunlop J, Marquis KL. 5-HT2C receptor agonists as an innovative approach for psychiatric disorders. Drug News Perspect 2007;20:565–71.
Siuciak JA, Chapin DS, McCarthy SA, Guanowsky V, Brown J, Chiang P, et al. CP-809, 101, a selective 5-HT2C agonist, shows activity in animal models of antipsychotic activity. Neuropharmacology 2007;52:279–90.
Wacker DA, Miller KJ. Agonists of the serotonin 5-HT2C receptor: preclinical and clinical progression in multiple diseases. Curr Opin Drug Discovery Dev 2008;11:438–45.
Wadenberg ML, Hicks PB. The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neurosci Biobehav Rev 1999;23:851–62.
Zhang JY, Kowal DM, Nawoschik SP, Lou Z, Dunlop J. Distinct functional profiles of aripiprazole and olanzapine at RNA edited human 5-HT2C receptor isoforms. Biochem Pharmacol 2006;71:521–9.