Antipsychotic-like effect by combined treatment with citalopram and WAY 100635: involvement of the 5-HT$_{2C}$ receptor

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Abstract

Catalepsy occurs following high dopamine (DA) D$_2$ blockade by typical antipsychotic drugs (APDs). We showed that a combination of a high dose of citalopram, a selective serotonin reuptake inhibitor (SSRI) and the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 produces significant catalepsy in rats, similar to APDs. Here, we investigated the potential antipsychotic activity of lower doses of citalopram + WAY 100635, using the conditioned avoidance response (CAR) test. Cataleptogenic liability of the combination was evaluated with the catalepsy test. Citalopram and WAY 100635 in combination, but not when given alone, produced a significant antipsychotic action in CAR without significant catalepsy, similar to the effect of a low dose of the typical APD haloperidol. Pretreatment with a selective 5-HT$_{2C}$ receptor antagonist, SB 242084, completely prevented the citalopram/WAY 100635-induced suppression of CAR indicating an involvement of the 5-HT$_{2C}$ receptor. In summary, treatment with an SSRI/5-HT$_{1A}$ antagonist combination might prove beneficial in psychiatric disorders with psychotic/depressive symptoms.

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Introduction

We previously found that, while ineffective when given alone, combined treatment with a high dose (40 mg/kg) of the selective serotonin re-uptake inhibitor (SSRI) citalopram and the selective serotonin (5-HT)$_{1A}$ receptor antagonist WAY 100635 produced significant catalepsy in rats (Eltayb et al., 2001). Catalepsy in rats is primarily seen following treatment with high doses of antipsychotic drugs producing a dopamine (DA) D$_2$ receptor occupancy > 80% (Wadenberg et al., 2001). Citalopram does not possess any appreciable affinity for DA receptors (Baumann, 1996), and although WAY 100635 has been reported to have some affinity for the DA D$_2$ receptor (Johansson et al., 1997), it is unlikely that the observed catalepsy in our study would be the result of the very modest DA D$_2$ blockade caused by this drug. SSRIs have, however, been found to produce Parkinsonism in susceptible individuals (Gerber and Lynd, 1998).

Moreover, 5-HT$_{1A}$ receptor agonists may reverse neuroleptic-induced catalepsy in rats, indicating that interactions between serotonin and DA modulate extrapyramidal motor functions (Prinssen et al., 1999). The fact that the combined treatment with a high dose of citalopram and the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 produced catalepsy in similarity with antipsychotic drugs suggested that this drug combination might also show antipsychotic-like effect. Thus, the objective of the present study was to test, in rats, the hypothesis that combined treatment with lower doses of citalopram (10–20 mg/kg) and WAY 100635 may indeed produce an antipsychotic-like effect with less cataleptogenic propensity. We here used the conditioned avoidance response (CAR) test for antipsychotic activity with high predictive validity (Arnt, 1982). Extrapyramidal side-effect (EPS) liability was evaluated by means of the catalepsy test.

Materials and methods

Animals

Adult male Wistar rats (BK Universal, Sollentuna, Sweden), with an average weight of 300 g upon arrival, were used. The animals were housed, three or
four per cage (Makrolon® IV; BK Universal AS, Nittedal, Norway) under standard laboratory conditions with a temperature of 21.0±0.4 °C and relative humidity of 55–65%. Food (R34, Ewos, Södertälje, Sweden) and tap water were available ad libitum. The animals were kept on a reversed light–dark cycle 12:12 h (lights off at 07:00 hours), and acclimatized for at least 1 wk before training started. On experimental days, animals were transferred from the animal quarters to the laboratory 1 h before the experiments started, and were housed in a ventilated cabinet between observations.

The studies were approved by the Local Animal Ethics Committee, Stockholm North (Norra Djurförsöksetiska Nämnden).

Drugs
Citalopram HBr was a generous gift from Lundbeck (Copenhagen, Denmark) and WAY 100635 N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-(2-pyridinyl) cyclohexane carboxamide, was also a generous gift from Wyeth-Ayerst (Princeton, NJ, USA). Citalopram and WAY 100635 were dissolved in physiological saline with pH adjusted to 6.0–6.4. Both drugs were administered subcutaneously (s.c.) in a volume of 2.0 ml/kg body weight. SB 242084 (6-Chloro-5-methyl-1-[2-[2-methylpyrid-3-yl]oxypyrid-5-yl] carbamoyl)indoline) 2 HCl from Sigma-Aldrich (St. Louis, MO, USA), was dissolved in 8% of hydroxypropyl-beta-cyclodextrin (Lundbeck, Copenhagen, Denmark) in 0.9% NaCl with pH adjusted to 6.0, and administered intraperitoneally (i.p.) in a volume of 2.0 ml/kg body weight.

CAR
Rats were trained and tested in a conventional shuttle-box (530 x 250 x 225 mm), divided into two equal compartments by a partition with an opening (75 x 75 mm). Upon presentation of 80 dB white noise (white Noise Generator, Lafayette, IN, USA), which is used as conditioned stimulus (CS), the rat had 10 s to avoid the unconditioned stimulus (UCS), an intermittent electric shock in the grid floor of ~0.2 mA (inter-shock interval 2.5 s, shock duration 0.5 s), by moving into the opposite compartment. White noise is a (hissing-like) type of noise that is produced by combining equal intensity sounds of all different frequencies to form a broadband spectrum type of sound. The position of the rat in relation to the opening between compartments was automatically registered by means of photocells located along the sides of the two compartments. The information from the photocells was automatically transferred onto a computer and the following behavioural variables were recorded: (1) avoidance (response to CS within 10 s); (2) escape (response to CS+UCS); (3) escape failure (if the rat was unable to respond to the shock by moving into the opposite compartment within 50 s the trial was terminated); (4) inter-trial crosses, i.e. movement between compartments between trials. The inter-trial interval (end of trial to start of new trial) varied at random between 20 and 40 s. The animals were trained in one training session which lasted for 15 min per animal per day for 5 consecutive days. Only animals reliably performing at a level of >90% avoidance were included in the study. The same animals were tested repeatedly serving as their own controls in a change-over design (Li, 1964). Experimental days were always separated by at least two non-experimental days. For further information on the CAR test procedure see Salmi et al. (1994).

Catalepsy measurements
Catalepsy was observed in a dimly lit room by placing the animals on an inclined grid (60°) for a maximum of 2.5 min. The animals were allowed 30 s of adaptation on the grid before observations started. Observations were performed 30, 60 and 120 min after citalopram administration. The catalepsy was scored from 0 to 5, according to the time (square root transformation) the rat remained immobile (min): 0 = 0.00–0.08; 1 = 0.09–0.35; 2 = 0.36–0.80; 3 = 0.81–1.42; 4 = 1.43–2.24; 5 = >2.25, e.g. if the rat remained immobile for 0.08 min, it was scored as 0, etc. (see Ahlenius and Hillegaart, 1986).

Statistics
Statistical evaluation was performed by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks test (CAR) or the Kruskal–Wallis one-way ANOVA, followed by the Mann–Whitney U test (catalepsy) (Siegel and Castellan, 1988).

Results
Effects of citalopram alone, or in combination with WAY 100635, on CAR behaviour
Citalopram (10, 20 or 40 mg/kg s.c.) when given alone had no effect on CAR. However, combined treatment with citalopram (20–40 mg/kg s.c.) and WAY 100635 (0.2 mg/kg s.c.) produced a statistically significant (p<0.01) suppression of CAR, at 20 and 90 min after
citalopram administration, compared with animals treated with citalopram alone (Figure 1a, b). All effects were gone at 240 min after citalopram (data not shown). WAY 100635 (0.2 mg/kg s.c.), when given alone in a separate set of six animals, had no effect on CAR compared with the corresponding controls (Figure 1c). Interestingly, combined treatment with citalopram (40 mg/kg) and WAY 100635 did not produce any further effects on CAR compared with the effects of combined citalopram (20 mg/kg)/WAY 100635 treatment. In fact, the citalopram (40 mg/kg)/WAY 100635 combination, appeared to be slightly less effective in suppressing CAR.

Effects of the selective 5-HT2C receptor antagonist SB 242084 on the suppression of CAR induced by combined citalopram/WAY 100635 treatment

In another set of animals, we investigated the effect of the selective 5-HT2C receptor antagonist SB 242084 (0.5 mg/kg i.p.) on combined citalopram (20 mg/kg s.c.)/WAY 100635 (0.2 mg/kg s.c.)-induced suppression of CAR. The citalopram/WAY 100635 combination produced a significant suppression of CAR 20 min after citalopram administration. Pretreatment with SB 242084 completely prevented (p < 0.05) the citalopram/WAY 100635-induced suppression of CAR (Figure 2). SB 242084 by itself had no effect on CAR. All effects were gone at 90 and 240 min (data not shown).

No escape failures were recorded under any treatment condition or time (i.e. a decrease in avoidance responses was always accompanied by a corresponding increase in escape responses).

Effects of WAY 100635, alone or in combination with citalopram in the catalepsy test

Administration of WAY 100635 (0.2 mg/kg s.c.) alone, or in combination with citalopram (20 mg/kg s.c.), did not produce significant catalepsy at any observation time (Table 1).
Our major finding is that combined treatment with the SSRI citalopram and the selective 5-HT$_{1A}$ receptor antagonist WAY 100635, while ineffective when given alone, produced a significant antipsychotic-like effect in rats. The magnitude of this effect was similar to that seen following doses in the range of 0.025–0.05 mg/kg of the typical antipsychotic haloperidol. Furthermore, pretreatment with the selective 5-HT$_{2C}$ receptor antagonist SB 242084 completely prevented the citalopram/WAY 100635-induced effects on CAR, indicating a specific involvement of the 5-HT$_{2C}$ receptor. No significant catalepsy was recorded.

The CAR test for antipsychotic activity reliably detects drugs with antipsychotic properties. Antipsychotic drugs selectively suppress CAR in doses that also correlate well with clinically effective doses (Arnt, 1982; Seeman, 1992). The suppression of CAR by combined citalopram/WAY 100635 treatment in the present study was similar in magnitude to the effect of a low dose of haloperidol equivalent to the effect of a striatal DA D$_2$ receptor occupancy around 55–65% (Wadenberg et al., 2001). Generally, striatal D$_2$ occupancy of around 65–70% is needed for a sufficient therapeutic response in acutely psychotic schizophrenics (Kapur et al., 2000). However, patients on maintenance therapy, and other categories of patients with mild psychotic symptoms, may also do well on D$_2$ occupancy at the lower end (65%) of this spectrum, compatible with the magnitude of around 60% of effect suppression on CAR seen by combined citalopram/WAY 100635 in the present study.

The underlying mechanism by which this drug treatment suppresses CAR, however, is less clear. Neuroanatomical studies indicate that both substantia nigra (SN) and the ventral tegmental area (VTA), where the major brain DA cell populations are found, receive afferent projections from 5-HT-containing axon terminals originating in the raphe nuclei (Azmitia and Segal, 1978; see Steinbusch, 1981), and an interaction between these neurotransmitters may occur at several levels. The combined treatment with citalopram/WAY 100635 would be expected to cause an increase in synaptic levels of 5-HT that will be able to stimulate various available serotonergic receptor subtypes which may inhibit the mesolimbic DA system and thereby suppress CAR.

In the present study, pretreatment with the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (Kennett et al., 1997) completely prevented the suppression of CAR by combined citalopram/WAY 100635 treatment, which strongly suggests a specific mediation via 5-HT$_{2C}$ receptor stimulation. An involvement of, for example, the 5-HT$_{1A}$ or the 5-HT$_{1B/1D}$ receptors is unlikely, since the selectivity of SB 242084 for the 5-HT$_{2C}$ receptor reportedly is at least 100-fold over these receptor subtypes (Kennett et al., 1997). In addition, the 5-HT$_{1A}$ receptor agonist DOI, for example, has no effect on CAR (Wadenberg et al., unpublished observations).

5-HT$_{2C}$ receptor agonists such as m-chlorophenylpiperazine (mCPP), RO 60–0175 and the newly introduced WAY 163909, on the other hand, have been reported to show antipsychotic-like effects in animal models such as the CAR and the prepulse inhibition (PPI) tests (Browning et al., 1999; Grauer et al., 2004). Although, citalopram, as well as fluoxetine, by themselves seem to have some affinity for the 5-HT$_{2C}$ receptor (Dekeyne et al., 1999; Jenck et al., 1998; Pälvinäki et al., 1996), SSRIs are not as effective as antipsychotics and, accordingly, they do not, as shown in this study, produce antipsychotic-like suppression of CAR when given alone. Our present findings suggest, however, that adjunct treatment with a 5-HT$_{1A}$
receptor blocking compound to a SSRI can indeed generate antipsychotic properties. Adjunctive treatment with a 5-HT₁A receptor antagonist to a SSRI is in fact a relatively common regimen to accelerate the onset of action of SSRIs in depression. Within this context, it is interesting to note that 5-HT₂C agonists also have been proposed as potentially effective drugs in depression, obsessive–compulsive disorders and panic disorder (see e.g. Jenck et al., 1998). However, awaiting data from clinical trials with selective 5-HT₂C antagonists, a combined treatment with a SSRI and a 5-HT₁A receptor antagonist may offer an effective and useful, dual action treatment option not only for schizophrenic patients needing maintenance therapy, but also for individuals suffering from psychotic illness and/or with concomitant depressive features.

In our previous study, combined treatment with citalopram (40 mg/kg) and WAY 100635 produced significant catalepsy (Eltayb et al., 2001). Here we found that citalopram in a lower dose (20 mg/kg) in combination with WAY 100635 significantly suppressed CAR but did not produce any significant catalepsy. Thus, based on our previous findings (Eltayb et al., 2001) the therapeutic window regarding EPS liability might be somewhat restricted. Furthermore, our present study suggests that there would be no additional gain in antipsychotic efficacy by increasing the dose of the SSRI. Finally, weight gain is a common side-effect of atypical antipsychotic drugs. Polymorphisms of the 5-HT₁C receptor gene are associated with antipsychotic-induced weight gain (Reynolds et al., 2002), and 5-HT₁C receptor stimulation has been found to reduce weight gain in obese human subjects (Sargent et al., 1997). Moreover, citalopram-induced hypophagia in rats is enhanced by WAY 100635, an effect which seems to be mediated through 5-HT₁C receptors (Grignaschi et al., 1998). These data suggest that the citalopram/WAY 100635 combination may have a beneficial profile regarding weight gain.

In conclusion, combined treatment with the SSRI citalopram and the selective 5-HT₁A receptor antagonist WAY 100635 produced an antipsychotic-like effect in rats without significant catalepsy. The antipsychotic-like effect was completely prevented by pretreatment with the selective 5-HT₂C receptor antagonist SB 242084, indicating a specific involvement of the 5-HT₂C receptor. The effect, similar in magnitude to a low dose of haloperidol, is suggestive of an effective dual action treatment for individuals with mild psychotic illness and/or with concomitant depressive features.

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Statement of Interest

None.

References


