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CHAPTER 5

THE EFFECTS OF THE GLYCINE REUPTAKE INHIBITOR R213129 ON THE CENTRAL NERVOUS SYSTEM AND ON SCOPOLAMINE-INDUCED IMPAIRMENTS IN PSYCHOMOTOR AND COGNITIVE FUNCTION IN HEALTHY SUBJECTS

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Abstract
In this study the effects of R213129, a selective glycine transporter 1 inhibitor, on central nervous system function were investigated in healthy males in the absence and presence of scopolamine. This was a double-blind, placebo-controlled, 4-period crossover ascending dose study evaluating the following endpoints: body sway, saccadic and smooth pursuit eye movements, pupillometry, electroencephalography, visual analogue scales for alertness, mood, calmness and psychedelic effects, adaptive tracking, finger tapping, Visual and Verbal Learning Task, Stroop test, hormone levels and pharmacokinetics. R213129 dose levels were selected based on exposure levels that blocked the GlyT1 sites >50% in preclinical experiments. Forty-three of the 45 included subjects completed the study. Scopolamine significantly affected almost every central nervous system parameter measured in this study. R213129 alone compared with placebo did not elicit pharmacodynamic changes. R213129 had some small effects on scopolamine-induced central nervous system impairments. Scopolamine-induced finger tapping impairment was further enhanced by 3 mg R213129 with 2.0 taps/10 seconds (95% CI -4.0, -0.1), electroencephalography alpha power was increased by 10 mg R213129 with respectively 12.9% (0.7, 26.6%), scopolamine-induced impairment of the Stroop test was partly reversed by 10 mg R213129 with 59 milliseconds (-110, -7). Scopolamine produced robust and consistent effects in psychomotor and cognitive function in healthy volunteers. The most logical reason for the lack of R213129 effects seems to be that the central nervous system concentrations were too low. The effects of higher doses in healthy volunteers and the clinical efficacy in patients remain to be established.

Introduction
It has been proposed that hypofunction of the glutamatergic system, and specifically a hypofunction of N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission, contributes to the pathophysiology of schizophrenia (Javitt, 2006; Stone et al, 2007). If schizophrenic symptoms are the result of diminished functioning N-methyl-D-aspartate (NMDA) receptors, stimulation of the NMDA receptors might be a possible mechanism for new schizophrenia medication.

As direct agonists of NMDA receptors are neurotoxic (Stone et al, 2007), indirect ways to enhance receptor function have been investigated (Javitt, 2002). One way to indirectly augment NMDA receptor function is through facilitation by glycine, an obligatory co-agonist for glutamate at the NMDA receptor (Javitt, 2006). So far, explorative clinical trials using glycine enhancement strategies have shown (modest) improvements in positive and negative symptoms and cognitive function in schizophrenic patients (Goff et al, 1999; Goff and Coyle, 2001; Javitt, 2006; Lane et al, 2005). However, gram-level doses of glycine are needed to significantly elevate central nervous system (CNS) concentrations. Inhibition of presynaptic glycine reuptake may be a more efficient way to increase pharmacological glycine activity in the brain (Kemp and McKernan, 2002).

The action of glycine is essentially terminated by rapid reuptake, mediated by at least two glycine transporters, glycine transporter 1 (GlyT1) and glycine transporter 2 (GlyT2) (Aragon and Lopez-Corcuera, 2003). As the distribution of GlyT1 overlaps with that of NMDA receptors (Smith et al, 1992) and electrophysiological studies have shown that the responses of NMDA receptors are enhanced after inhibition of GlyT1 (Aragon and Lopez-Corcuera, 2005), it is suggested that GlyT1 is involved in NMDA activity. Glycine reuptake inhibitors have been effective in a variety of schizophrenia animal models (Chen et al, 2003; Depoortere et al, 2005; Harsing et al, 2003; Javitt et al, 1999; Le Pen et al, 2003) and several have recently entered early phase clinical trials in humans. To date, a few of the clinical trials studying the glycine reuptake inhibitor sarcosine in schizophrenia have been published and show effects on positive and negative symptoms (Lane et al, 2005; Tsai et al, 2004). The disadvantage of sarcosine is that it is a low potency antagonist that also requires gram-level dosing. Therefore other selective, more potent glycine reuptake inhibitors may be more efficient.
R213129 (see Figure 1 for the structural formula) is a selective inhibitor of the GlyT1 and it is hypothesized to have beneficial effects on positive and negative symptoms and cognitive performance of patients with schizophrenia (Goff et al, 1999; Goff and Coyle, 2001; Javitt, 2006; Lane et al, 2005).

Preclinical studies have supported the potential therapeutic value of this compound (Johnson & Johnson, data on file). R213129 appeared more potent than sarcosine, evidenced by a lower IC50, and increased extracellular glycine levels in the prefrontal cortex in rats and glycine levels in the cerebrospinal fluid in dogs. R213129 also had efficacy in preclinical models for schizophrenia. It normalized the disturbed prepulse inhibition paradigm in dopamine transporter knockout mice and decreased amphetamine-induced hyperactivity in rats with neonatal lesions of the hippocampus. Furthermore, a reduction of the potentiation of amphetamine-induced dopamine release in the prefrontal cortex of rats receiving phencyclidine was detected. In animal models, effects on cognition were inconsistent.

Three doses of R213129 (3, 10 and 30 mg) were used in this study based on exposure levels that block the GlyT1 site for more than 50% (data on file). In previous clinical studies, R213129 was well tolerated in healthy men in single and multiple doses up to 50 mg. The main focus of these studies was to test the tolerability and pharmacokinetics of the compound, although some CNS effects were evaluated. No clear or consistent pharmacodynamic changes in the electroencephalography and cognitive function tests following single doses of 1, 3, 10, 30, and 50 mg and multiple dose administration of 10, 30 and 50 mg R213129 were measured in these studies.

As Tsai et al and Lane et al have shown that the glycine reuptake inhibitor sarcosine has effects on positive and negative symptoms and cognitive function in schizophrenia (Lane et al, 2005; Tsai et al, 2004), the hypothesis is that R213129 may also have effects on these symptoms. Since it is not possible to detect changes in positive and negative symptoms in healthy volunteers and to evaluate the positive effects on cognitive function in subjects already performing at their maximal capacity, CNS biomarkers have been studied in healthy subjects (in the absence and presence of the scopolamine model). By using a battery of quantitative CNS tests sensitive to several CNS-active drugs (De Visser et al, 2001b, 2003; Gijsman et al, 2002; Kemme et al, 2003; Van der Post et al, 2004), evidence for CNS brain penetration can be shown, and a global impression of the effects of R213129 could be obtained. In addition, to get an indication of potential pro-cognitive effects of R213129 in healthy men, scopolamine was used to induce a transient and reversible thought and memory disturbance. Although the scopolamine model does not capture the complexity of cognitive decline in human psychopathology, it has become the most frequently used model for studies of cognitive impairment in experimental animals and healthy volunteers (Broks et al, 1988; Hall et al, 1990; Riedel and Jolles, 1996; Vitiello et al, 1997).

A methodological disadvantage of the model is the sedation induced by scopolamine possibly contributing to the impairment of memory and cognition. However, in many studies measures of sedation of scopolamine were unrelated to the adverse effect on memory (Bartus et al, 1982; Caine et al, 1981; Curran et al, 1991; Drachman and Leavitt, 1974; Kopelman and Corn, 1988), and no cognitive impairment was produced by, for example, lorazepam in a dose inducing similar sedation as scopolamine (Sunderland et al, 1989). Moreover, stimulant drugs that were added to scopolamine were unable to reverse the scopolamine-induced cognitive impairment (Bartus, 1978; Drachman, 1977; Martinez et al, 1997), whereas cholinergic agents were (Ghoneim and Mewaldt, 1977).

In primates, an interaction between cholinergic and glutamatergic systems on cognitive function has been demonstrated (Matsuoka and Aigner, 1996a, b; Sirvio et al, 1992). The exact mechanism behind this interaction has not been resolved, but suggestions have been made. One is that glycine would increase acetylcholine release in certain neuronal tissues (Fishkin et al, 1993; Matsuoka and Aigner, 1996b). Another is the presence of NMDA receptor sites on cell bodies of cholinergic neurons and a subsequent increased acetylcholine release by glycine (agonists) due to depolarization of the receptor (Fishkin et al, 1993; Matsuoka and Aigner, 1996b). Various other preclinical experiments have demonstrated
a reversal of scopolamine-induced impairments using agonists for the glycine site on the NMDA receptor (Andersen et al., 2002; Kishi et al., 1998; Zajaczkowski and Danysz, 1997). To the best of the authors’ knowledge there is only one study in healthy volunteers with the partial glycine site agonist, D-cycloserine, showing significant improvement of scopolamine-induced memory impairment (Jones et al., 1991). Therefore in this study, the objectives were to study the CNS profile of R213129 and its effects on scopolamine-induced impairments in healthy male subjects.

Methods

Subjects

A total of 45 male subjects aged 18-55 with body mass index (BMI) of 18-28.5 kg/m² were recruited by the Centre of Human Drug Research. After signing an informed consent, subjects were medically screened within three weeks prior to study participation. Exclusion criteria included the use of agents known to affect CNS performance (including nicotine, drugs or alcohol) and evidence of relevant clinical abnormalities. The use of medication and the above-mentioned agents were not allowed during the study period. The Ethics Review Board of the Leiden University Medical Centre approved the study protocol.

Study design

This study was a double-blind, placebo-controlled, four-period crossover ascending dose study. The periods were separated by a washout period of at least one week.

Drugs

Scopolamine 0.5 mg or placebo was given intravenously over a period of 15 minutes starting at T= 0 and 3, 10, or 30 mg of R213129 or placebo was orally administered at T = 0.5 hours. The four treatments were scopolamine+placebo, scopolamine+one dose of the novel compound, placebo+placebo and placebo+one dose of the novel compound, with washout periods of at least one week. Each treatment group consisted of 15 subjects.

Safety

Adverse events, electrocardiogram (ECG), body temperature, blood pressure and heart rate measurements were performed throughout the study. ECGs were assessed using Cardioperfect ECG recorder (Welch Allyn). Blood pressure and heart rate were measured using an automated device (Nihon Kohden, Life Scope EC, Tokyo, Japan).

Pharmacodynamics

Eleven blocks of pharmacodynamic (PD) measurements were performed: pre-dose (twice before scopolamine administration) and 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 6.5 and 8.5 hours post-dose. Average baseline values for each variable were obtained by calculation of the mean of two baseline assessments. Pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the same room per session. Tests were performed in the following order: body sway, saccadic eye movements, smooth pursuit measurement, pupillometry, pharmaco-electroencephalography (EEG), visual analogue scale (VAS) Bond & Lader, VAS Bowdle, adaptive tracking, finger tapping, Stroop test, and Visual and Verbal Learning Task (VVLT). Blood for hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin) was taken regularly from four minutes until 24 hours after scopolamine administration. Subjects had a standardized breakfast one hour before scopolamine administration. All subjects were thoroughly trained and familiarized with the psychometric tests within 14 days preceding study start to minimize learning effects during the study.
ADAPTIVE TRACKING
The adaptive tracking test has proved to be useful for measurement of CNS effects of alcohol, various psychoactive drugs and sleep deprivation (Van Steveninck et al, 1991, 1993). The adaptive tracking test was performed as originally described by Borland and Nicholson (Borland and Nicholson, 1984) using customized equipment and software. Adaptive tracking is a pursuit-tracking task with a circle moving randomly on a computer screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance and the standard deviation of scores over a 3.5-minute period were used for analysis, including a 0.5 minute run-in time, during which data were not recorded.

BODY SWAY
Changes in body sway have been seen for many different CNS active drugs, including GABA-ergic drugs (De Haas et al, 2007, 2008; De Visser et al, 2003) and THC (Zuurman et al, 2008). The body sway meter records body movements in a single plane, providing a measure of postural stability. Body sway was measured with an apparatus similar to the Wright ataxiameter (Wright, 1971). With a string attached to the waist, all body movements over two minutes were integrated and expressed as mm sway on a digital display. The contribution of vision to postural control was eliminated by asking subjects to close their eyes. Subjects were instructed to wear the same pair of comfortable, low-heeled shoes on each session. Before starting a measurement, subjects were asked to stand still, with their feet approximately 10 cm apart and their hands in a relaxed position alongside the body.

FINGER TAPPING
The finger tapping test was adapted from the Halstead Reitan Test Battery (Andrew, 1977). The test evaluates motor activation and fluency. Speed of finger tapping was measured for the index finger of the dominant hand and a session contained five performances of 10 seconds. The volunteer was instructed to tap as quickly as possible on the space bar of a computer. The mean tapping rate and the standard deviations were used for statistical analysis.

SACCADIC AND SMOOTH PURSUIT EYE MOVEMENTS
Saccadic and smooth pursuit eye movements have shown dose-and concentration-related effects on many different CNS-active drugs, including GABA-ergic (De Haas et al, 2007, 2008; De Visser et al, 2003), serotonergic (Gjismann et al, 2002), noradrenergic (De Visser et al, 2001b; Kemme et al, 2003; Van der Post et al, 2004), and dopaminergic drugs (CHDR, data on file) which were recorded as described previously (Van Steveninck, 1994; Van Steveninck et al, 1993, 1996, 1997, 1999). Average values of saccadic peak velocity (SPV), latency (= reaction time) and inaccuracy were calculated for all artefact-free saccades. The average percentage of smooth pursuit for all stimulus frequencies was used as response parameter.

PHARMACO-ELECTROENCEPHALOGRAPHY
Pharmaco-EEG was performed as a general measure of CNS activity (Cohen et al, 1985). The literature suggests that antipsychotics show distinct profiles of EEG changes (Sobczak et al, 2003; Wright, 1971). Pharmaco-EEG was measured as previously described (De Haas et al, 2008). Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta-(0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha-(7.5-11.5 Hz) and beta-(11.5-30 Hz) frequency ranges. The square root of the total power (in µV) was analysed.

VISUAL AND VERBAL LEARNING TASK
At CHDR the VVLT test has shown the CNS effects of various compounds such as benzodiazepines (De Visser et al, 2003), antidopaminergic (De Visser et al, 2001a), and cannabinoid drugs (Zuurman et al, 2008). Memory function (impairment) includes different aspects of learning behaviour, i.e. acquisition, consolidation, storage and retrieval. The VVLT (Schmitt et al, 2000) contains three different subtests that cover most
of these memory components, i.e. immediate and delayed word recall and a delayed word recognition. This test is an adapted version of the Auditory Verbal Learning Test (Rey, 1964) with an increased number of items to reduce ceiling effects. Thirty words are presented in the same sequence in three trials on a computer screen. Each trial ends with a free recall of the words (immediate recall). Thirty minutes after the first trial, the subject is requested to recall as many words as possible (delayed recall). This is followed by a recognition test, consisting of 15 previously presented words and 15 other but comparable words, in which the subject has to respond ‘Yes/No’ as quickly as possible to indicate recognition of the word (delayed recognition). Outcome variables for the immediate and delayed word recall were the average and the maximum number of correct responses. For the delayed word recognition, the number of correct items and mean response time for correct responses were analysed.

STROOP TEST
The Stroop effect is helpful in understanding attention, perception and reading as well as the cognitive and neural mechanism of mental inhibition, interference and controlled versus automatic processing (Stroop, 1935). Individual differences in the degree of Stroop interference are considered to be among the most reliable or stable measures (Laeng et al, 2005). Two single-trial computerized versions of the classic colour-word Stroop tasks are presented to the test subjects. In the first trial, 20 coloured items are presented at random. The subjects are asked to respond as quickly and as accurately as possible by pressing the keys 1, 2 or 3 on the number pad with the index finger, middle finger and ring finger of the dominant hand, corresponding with the correct answer. In the second trial, directly after the first trial, 20 colour and word pairs are presented randomly to the subject, forming either congruent or incongruent matches. The subjects are again asked to respond as fast as possible by pressing the keys 1, 2 or 3 on the number pad, corresponding with the correct answer. Three colours are shown, which are green, red and blue. The coloured items are presented in a random order. The words that are used are ‘Rood’ (red), ‘Groen’ (green) and ‘Blauw’ (blue). The outcome parameters are the number of correct answers and the reaction time in both the basic and conflict situation.

VISUAL ANALOGUE SCALES
The Bond and Lader VAS was performed to measure subjective alertness, mood and calmness and the Bowdle VAS of psychedelic effects to evaluate psychedelic effects. These were performed as previously described (De Haas et al, 2008). The psychedelic effects measured by the VAS Bowdle cluster into two distinct total sum scores: internal perception (reflects inner feelings that do not correspond with reality, including mistrustful feelings); and external perception (reflects a misperception of an external stimulus or a change in the awareness of the subject’s surroundings) (Zuurman et al, 2008). The Bowdle VAS was expanded with an additional subscore for ‘feeling high’.

PUPILLOMETERS
Estimation of pupil size by digital photography is more repeatable and accurate than estimates by common clinical techniques over a wide range of illumination (Twa et al, 2004). Pupil diameter was determined using a digital camera (Canon PowerShot A620). After at least five minutes adaptation in ambient lighting, the subject was instructed to look into the lens. A sharp picture of the eyes was taken using a single flash. The program Qpupil (designed by the ‘Division of Image Processing (LKEB)’, Leiden University Medical Center, the Netherlands) automatically analyses the ratio between the diameter of the iris and the pupil of both eyes.

HORMONES
Blood samples for LH, FSH and prolactin were collected and kept at room temperature for at least 15 and maximally 60 minutes before storage. The hormones were analysed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center) using an electrochemiluminescence-immunoassay (ECLIA) for prolactin, and a fluoroimmunoassay for LH and FSH.
Pharmacokinetics

R213129

Blood samples (5 ml) for plasma concentrations of R213129 were drawn pre-dose, and at 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 8.5, 10 and 24 h post dose. Samples were protected from light at all times to prevent degradation of the compound. Plasma samples were analysed to determine R213129 concentrations using a validated, selective and sensitive liquid LC-MS/MS method (Lower Limit Of Quantification (LLOQ) = 1 ng/ml). R213129 was performed by the Department of Bioanalysis, J&J PRD, Belgium.

SCOPOLAMINE

Blood samples (4 ml) for plasma concentrations of scopolamine were drawn at 0.5, 0.75, 1.0, 2.5, 6.5 h after the scopolamine infusion was stopped. Samples were protected from light at all times. Plasma samples were analysed to determine scopolamine concentrations using a validated, selective and sensitive liquid LC-MS/MS method (LLOQ = 10 pg/ml). Scopolamine bioanalysis was performed by Pharma Bio-Research Group B.V., Zuidlaren, the Netherlands.

Statistical analysis

Pharmacodynamics

PD parameters were analysed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline is defined as the average of the available values obtained prior to dosing. This resulted in LS means estimates that indicate the change from baseline where baseline in the graph is set at 0 for t = 0 min. Treatment effects were reported as the contrasts between placebo and scopolamine, where the average of the measurements up to and including 8.5 hours (22 hours for neuroendocrine parameters) were calculated within the statistical model. Contrasts were reported along with 95% confidence intervals and analyses were two-sided with a significance level of 0.05.

PHARMACOKINETICS

Summary statistics of the plasma concentration data and estimated pharmacokinetic parameters for R213129 and scopolamine were calculated. The following pharmacokinetic parameters for R213129 and scopolamine were determined using Pharsight’s WinNonlin pharmacokinetic analysis software (version 4.0.1): C_{MAX}, \tau_{0.5h} (scopolamine), T_{MAX}, AUC_{last}, AUC_{\infty}, t_{1/2} term., CL/F (R213129), and CL (scopolamine).

Results

Subject characteristics

Forty-three of the 45 included healthy male subjects completed the study. Two subjects decided to stop participation of the study. For one subject the reason for withdrawal was non-compliance. The other subject experienced a vasodepressive reaction after both scopolamine administrations. Subjects were on average 29.0 years old (range: 18-55).

Clinical effects

Scopolamine induced well-characterized anticholinergic effects (increased pupil size, dry mouth, drowsiness, and impaired eye focusing) in 42 of the 44 subjects. Another reported effect was nausea in six of the 44 subjects. R213129 at all doses of 3, 10, and 30 mg was comparable with placebo in its adverse effects profile.

Pharmacokinetic results

The mean concentration-versus-time profile of R213129 for 3 mg is presented in Figure 2. For all doses of R213129, peak concentrations of around 200, 500, and 1400 ng/ml for the respective doses were reached between two and four hours after dosing, and the terminal elimination half-life was between 12 and 15 hours. Clearance was between 1.2 and 1.5 l/h. Pharmacokinetics were dose-linear for the tested doses. When combined with scopolamine, the pharmacokinetic profile was slightly
altered for some parameters due to an anticholinergic delay of stomach emptying. After combination with scopolamine the area under curve (AUC) of 3 mg R213129 changed from approximately 3400 to 3800, of 10 mg from 9300 to 11000, and of 30 mg from 26500 to 32000 ng h/ml.

The scopolamine concentration 0.5 hours after the end of dosing (with and without R213129) varied between 1200 and 1300 pg/ml and the terminal elimination half-life between 1.4 and 1.6 hours. Clearance was around 190 l/h. Scopolamine was not affected by the addition of R213129.

**Pharmacodynamic results**

**SCOPOLAMINE**

Scopolamine resulted in a considerable number of CNS effects and affected almost every parameter measured in this study (Table 1 shows a selection of results, as the complete report is shown in another article describing the PK-PD of scopolamine). Scopolamine deteriorated adaptive tracking, body sway and finger tapping rate performance compared with placebo. Both saccadic peak velocity and smooth pursuit performance decreased after scopolamine compared with placebo.

Alpha and beta power (Fz-Cz and Pz-Oz) decreased after scopolamine compared with placebo. Delta power (Fz-Cz and Pz-Oz) after scopolamine was statistically significantly higher than after placebo. Theta power (Fz-Cz and Pz-Oz) after scopolamine did not differ significantly from placebo.

Scopolamine deteriorated all parameters of the VVLT: delayed word recall (number correct), immediate word recall (number correct), and delayed word recognition (both number correct and reaction time). Similarly, all parameters of the Stroop test were deteriorated compared with placebo (number correct or reaction time and basic situation or conflict situation).

VAS alertness was significantly lower after scopolamine than after placebo, VAS calmness was higher, and VAS mood was not changed by scopolamine. VAS results of internal perception, external perception and feeling high were all higher after scopolamine.

All measured hormone levels increased after scopolamine compared with placebo, as did pupil size.

R213129 alone compared with placebo did not show pharmacodynamic changes on any of the parameters in this study.

R213129 AND SCOPOLAMINE

R213129 had some small effects on scopolamine-induced CNS impairment (see Table 1). Scopolamine-induced finger tapping impairment was further impaired by 3 mg R213129 with 2.0 taps/10 seconds (95% CI -4.0, -0.1). EEG alpha power (Fz-Cz and Pz-Oz) statistically significantly increased with, respectively, 12.9% and 16.0% after the combination of 10 mg R213129 and scopolamine compared with scopolamine alone (respectively, 95% CI 0.7, 26.6% and 0.3, 34.2%). The scopolamine-induced impairment of the number of correct Stroop responses (in the conflict situation) was reversed by 3 mg R213129 with 0.6 items (95% CI 0.0, 1.1). The scopolamine-induced impairment of the reaction time (in the conflict situation) was partly reversed by 10 mg R213129 with 59 milliseconds (95% CI -110, -7). R213129 had no significant impact on the scopolamine effects at a dose of 30 mg.

**Discussion**

In this study the CNS effects of the new glycine reuptake inhibitor R213129 were investigated using a battery of quantitative CNS tests, sensitive to classic neuroleptic agents and other CNS-active drugs. As this study was performed in healthy volunteers, a scopolamine model was used to examine the potential reversal of cognitive impairments by R213129. Based on experiments with the partial glycine agonist D-cycloserine in animals (Andersen et al., 2002; Kishi et al., 1998; Zajaczkowski and Danysz, 1997) and healthy subjects (Jones et al., 1991), it was hypothesized that scopolamine-induced (cognitive) deficits would be reversed by R213129.

This study found that scopolamine produced very robust and consistent effects in healthy volunteers, across a wide range of CNS functions. Most of the CNS impairments of scopolamine were expected, and are in line
with those observed in previous studies (Ebert and Kirch, 1998; Ebert et al., 1998, 2001; Renner et al., 2005). However, we are unaware of previous reports of increases in the gonadotrophic hormones LH and FSH after anticholinergic agents. We also found an increase in prolactin, which has previously been found by Benkert et al. (1981) using the anticholinergic agent biperiden. Most effects disappeared after eight hours, except for pupil size increases, which were still detectable at the end of each scopolamine occasion.

R213129 did not have any CNS effects on its own in this study. Since we have been unable to find reports on other glycine reuptake inhibitors in healthy volunteers, it is difficult to interpret whether this lack of effects is due to insufficient brain penetration, low pharmacological activity, or absence of physiological changes during glycine reuptake inhibition in healthy humans. There may also be methodological reasons that precluded us from detection of small CNS effects, although in our experience almost all CNS-active drugs have some impact on the tests that we used in this study (De Haas et al., 2007; De Visser et al., 2001b; Kemme et al., 2003; Van der Post et al., 2004; Van Steveninck et al., 1991; Zuurman et al., 2008).

Our study showed some modifications of the effects of scopolamine, but these were small and did not seem consistent. Three tests showed statistically significant changes, some of which could be qualified as improvements and others as deteriorations. R213129 slightly reversed some aspects of scopolamine-induced cognitive deterioration on the Stroop test and the scopolamine-induced reduction in alpha EEG power, which might be indicative for improved alertness, but motor impairment (finger tapping rate) was slightly worsened. In addition, the effects were not consistently dose-related in the 3-30 mg dose range.

Several reasons for these marginal effects should be considered. The most likely explanation could be that the doses of R213129 did not adequately cover the dose-response curve. Three different doses of R213129 were investigated in this study. If R213129 had a bell-shaped dose-response curve, which was also suggested by other studies with D-cycloserine (Andersen et al., 2002; Jones et al., 1991), the selected dose levels could in part have been too high. However, the small and non-dose-related effects that were observed in this study could also suggest an indication of very early CNS changes that were too small and hence too variable to consistently exceed the detection limit. R213129 showed a limited blood-brain barrier penetration, which could increase the variability of its CNS effects in the low 3-30 mg dose range. In this case, (considerably) higher doses may lead to both larger and more consistent effects, provided that the brain penetration increases at a higher dose range.

Another possible explanation for the lack of consistent effects is that the scopolamine model in healthy subjects may have been unsuitable, or the impact of scopolamine may have been too strong to pick up possible cognition-enhancing properties of R213129. Considering the clear effect of scopolamine on all parameters of this study, the 0.5 mg dose could possibly have been too high and may have overshadowed any possible R213129 effects, particularly if these were small. The 0.5 mg dose of scopolamine was selected based on an earlier study of Ebert et al., showing that an intravenous dose of 0.5 mg scopolamine was suitable for PK-PD modelling using EEG, and still had an acceptable side effect profile (Ebert et al., 2001). Support for reversal of the scopolamine model by glycine reuptake inhibitors primarily comes from several preclinical studies that show reversal of scopolamine-induced impairments by D-cycloserine in various animals (Andersen et al., 2002; Fishkin et al., 1993; Ohno and Watanabe, 1996; Pitkanen et al., 1995; Sirvio et al., 1992). The evidence in healthy humans, however, is much more limited. A study similar to ours in scope and objectives showed reversal of scopolamine-induced memory deficits by D-cycloserine (Jones et al., 1991). However, this was only a short communication without more comprehensive publication or replication (as far as we could find), and it only showed effects of the 15 mg dose, but not at 5 or 50 mg.

A third factor which might explain the absence of results of R213129 is the composition of the CNS battery. The selected tests might not have been sensitive to the effects of a glycine reuptake inhibitor. In our experience false-negative results are quite rare with these tests, which are chosen for their sensitivity to a wide range of CNS-active compounds.
(De Haas et al, 2007; De Visser et al, 2001b; Kemme et al, 2003; Van der Post et al, 2004; Van Steveninck et al, 1991). This is confirmed by the scopolamine effects in this study, which were very clear and consistent. The previous study with D-cycloserine described reversal of memory effects that were also assessed in our study (immediate and delayed word recall and delayed word recognition) (Jones et al, 1991). It cannot be excluded, however, that the effects of R213129 in healthy volunteers would have been detectable with other cognitive tests or impairment models.

In summary, scopolamine proved to be a robust and consistent model of CNS impairment in healthy volunteers. Most of the scopolamine-induced changes were in agreement with the findings of previous studies. R213129 had no CNS effect by itself, and produced marginal changes of scopolamine-induced CNS impairment, without a consistent dose-response relationship. The most logical explanation for this lack of clear effects in this study seems to be that the R213129 brain concentrations were too low and variable to detect any effects in this healthy volunteer study. Studies with higher doses would be required to show pharmacological activity of this compound in healthy volunteers. This could provide support for the dose selection for the patient studies that are required to investigate the clinical efficacy of this glycine reuptake inhibitor, which still remains to be established.

### Table 1: Pharmacodynamic effects for placebo, scopolamine, and the combination of scopolamine and R213129

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>LSmc PLAC</th>
<th>LSmc SCOP</th>
<th>Scopolamine effect</th>
<th>LSmc SCOP + R213129E</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Difference a</td>
<td>95% CI</td>
<td>Pvalue</td>
</tr>
<tr>
<td>Adaptive tracking (%)</td>
<td>22.16</td>
<td>12.82</td>
<td>9.33</td>
<td>6.42, 10.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body sway (mm)</td>
<td>288</td>
<td>450</td>
<td>50.2%</td>
<td>44.3, 66.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Finger tapping rate (taps/10 sec)</td>
<td>55.3</td>
<td>62.0</td>
<td>5.5</td>
<td>1.9, 9.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Saccadic Peak Velocity (deg/sec)</td>
<td>484.1</td>
<td>454.8</td>
<td>10.2</td>
<td>21.2, 13.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smooth pursuit (%)</td>
<td>46.60</td>
<td>41.52</td>
<td>5.07</td>
<td>2.03, 7.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EEG alpha Fz-Cz (µV)</td>
<td>2.99</td>
<td>2.04</td>
<td>-11.8%</td>
<td>-16.8, -26.4%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EEG alpha Pz-Oz (µV)</td>
<td>5.87</td>
<td>3.20</td>
<td>-45.4%</td>
<td>-50.4, -39.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Delayed word recall (# correct)</td>
<td>11.8</td>
<td>6.9</td>
<td>4.9</td>
<td>3.6, 6.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Immediate word recall (# correct)</td>
<td>12.0</td>
<td>8.0</td>
<td>4.0</td>
<td>3.1, 4.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Delayed word recognition (# correct)</td>
<td>25.4</td>
<td>32.7</td>
<td>7.3</td>
<td>1.0, 4.3</td>
<td>0.0018</td>
</tr>
<tr>
<td>Stroop (conflict, # correct)</td>
<td>19.5</td>
<td>18.7</td>
<td>0.8</td>
<td>0.4, 1.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>Stroop (conflict, rt, msec)</td>
<td>648</td>
<td>771</td>
<td>123</td>
<td>-157, -89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>3.75</td>
<td>3.97</td>
<td>5.8%</td>
<td>5.1, 6.6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LH (U/L)</td>
<td>4.05</td>
<td>5.41</td>
<td>16.4%</td>
<td>10.4, 22.7%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prolactin (µg/L)</td>
<td>5.16</td>
<td>4.57</td>
<td>13.3%</td>
<td>7.1, 19.8%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

A if 0 is included in the 95% Confidence Interval (95% CI) the difference is not conventionally different at the 5% level; a rt = Reaction time; lsmc is the Least Squares Means estimate; a the interaction effects are only indicated in case of a significant effect; a dose (mg) of R213129 inducing a change in scopolamine
human pharmacology of current and new treatments for schizophrenia

5-effects of glyt1 reuptake inhibitor on CNS & scopolamine-induced impairments in healthy men

Figure 1  Structural formula of R213129

Figure 2  Mean (±SD) plasma concentration profile of 3 mg R213129

REFERENCES


