Ketamine pharmacology revisited

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Chapter 6

Different role of nitric oxide in the psychedelic symptoms induced by racemic ketamine and esketamine in human volunteers.

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6.1 Introduction

Ketamine, a non-competitive antagonist of the *N*-methyl-D-aspartate receptor (NMDAR), was designed in the 1960s as a shorter acting alternative to phencyclidine.[1] While initially applied as anaesthetic in clinical human and veterinarian practice, it is currently also used off-label for the treatment of acute and chronic non-cancer pain, opioid-refractory cancer pain, migraine, post-traumatic stress disorder and major (therapy-resistant) depression.[1-7] Despite its efficacy for multiple indications, physicians are sometimes hesitant when considering ketamine treatment and patient compliance may be low because of symptoms that emerge during administration of the drug.[2,8] In rodents, NMDAR antagonists produce hyperlocomotion, stereotypical (psychotic-like) behaviour and ataxia;[9] in humans ketamine induces schizotypical or psychedelic effects (paranoia, hallucinations, derealisation, depersonalisation, anxiety) and an intense state of (drug) high.[2,8] As these symptoms mimic schizophrenic behavioural effects, ketamine is successfully used in volunteers as model of schizophrenia.[10,11] Although in patients psychedelic symptoms may be moderated with benzodiazepine or α_2-agonist treatment, complete disappearance occurs when treatment ends.[2,8]

Several mechanisms have been proposed to explain the psychedelic effects caused by ketamine and related NMDAR antagonists, such as decreased gamma-aminobutyric acid B receptor function or altered dopaminergic transmission.[12-14] One attractive theory relies on the model of NMDAR hypofunction. Ketamine binds to the phencyclidine site of the NMDAR, which blocks the inflow of Ca^{2+}-ions.[1,15] Normally, Ca^{2+}-ions that enter the cell in response to glutamatergic NMDAR activation bind to calmodulin that subsequently stimulates nitric oxide (NO) synthase to produce the gaseous neuromodulator NO from L-arginine.[16-18] NO interacts with guanylyl cyclase to generate cyclic guanosine monophosphate (cGMP) from guanosine triphosphate; cGMP subsequently interacts with cGMP-dependent protein kinase and has neuroplastic, neurotrophic and neuroprotective effects.[16-18] NMDAR antagonism-induced blockade of Ca^{2+}-ion inflow reduce intracellular NO synthesis.[19] Animal studies show that modulation of NO concentrations using mechanisms that bypass the NMDAR can reduce or even prevent NMDAR hypofunction-related psychotic behaviour. For example, in mice and rats, the NO donor sodium nitroprusside blocks phencyclidine- and racemic ketamine-induced psychotic behavior.[20,21] Additionally, sodium nitroprusside can attenuate racemic ketamine-induced memory defects and social withdrawal and has anxiolytic effects.[22] In a recent human study, improvement of schizophrenia symptoms was observed following nitroprusside treatment.[23]

To determine whether NMDAR antagonists-induced psychedelic symptoms are amendable by modulation of the nitrinergic pathway in humans, we tested the effect of sodium

nitroprusside intravenous in a healthy population during the infusion of increasing doses of ketamine. Psychedelic effects were measured using the Bowdle questionnaire, a validated list of thirteen questions developed for quantifying the psychedelic effects of ketamine in healthy volunteers.[24,25] We applied our paradigm to the two ketamine formulations that are currently commercially available for human use, racemic ketamine and esketamine. Racemic ketamine contains equal amounts of two optical isomers, the S(+)- and R(-)-enantiomers; esketamine exclusively contains the S(+)-isomer. Our approach will allow detection of nitroprusside effects specific to S(+)-ketamine, R(-)-ketamine or to both enantiomers. We hypothesize that nitroprusside will reduce racemic ketamine- and esketamine-induced drug high (our main end-point) and changes in internal and external perception (secondary end-points).

6.2 Methods

ETHICS

Participants were recruited after protocol approval was obtained from the Human Ethics Committee at Leiden University Medical Centre (Leiden, the Netherlands) and the Central Committee on Research involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek, The Hague, the Netherlands) by flyers posted on the Leiden University campus. All subjects gave oral and written informed consent before participation in the study. The study was registered in the Dutch trial register (www.trialregister.nl) under number NTR 5359. This study is part of a large project on: (1) the pharmacokinetics and pharmaco-dynamics of racemic ketamine *versus* esketamine and (2) the influence of sodium nitroprusside on ketamine's effect (psychedelic effects, mood, cardiovascular effects and pain relief). Here we report on the effect of sodium nitroprusside on the psychedelic effects of the two ketamine formulations currently commercially available.

SUBJECTS

Male subjects, aged 18-34 years and with a body mass index of 30 kg m^{-2} or less, were recruited. Participation was possible after passing a physical examination and after reported absence of any health issues including presence or history of any psychiatric, medical or neurologic disorder, presence or a history of illicit drug use or excessive alcohol consumption (> 21 units per week), or known allergies to study medication. Additionally, subjects were excluded from participation if they had a positive drug screen on the day of screening or on any of the study days, had participated in another trial in the three months prior to enrolment, or used any medication on a regular basis (e.g. pain medication). The decision to enrol the subject into the study was made by the independent physician that performed the subjects' screening. Subjects were asked to refrain from food or drinks for the 8 hours before dosing, not to consume caffeinated food

or beverages in the 24 hours before dosing and not to consume any grapefruit-containing food or beverages during the 7 days preceding the study day.

On each occasion, upon arrival in the laboratory, two intravenous access lines were placed in the large veins of the underarm, one for administration of ketamine (racemic ketamine or esketamine) and the other for infusion of nitroprusside or placebo. An arterial line was placed in the left or right radial artery to continuously monitor blood pressure and allow arterial blood sampling. Blood samples were not used in the analysis of current study. Subjects were monitored by ECG, oxygen saturation was measured via a finger probe and blood pressure through an arterial line.

STUDY DESIGN

This was a placebo-controlled double blind randomized crossover proof-of-concept study with acronym SNIK. Subjects were studied on four occasions that were identical in study performance except for the medication that was administered. On occasions A and B, subjects received a dose-escalation of intravenous racemic ketamine (Ketalar^{TM}; Pfizer Pharma, Berlin, Germany) in combination with either intravenous nitroprusside (occasion A; Apotheek Haagse Ziekenhuizen, The Hague, The Netherlands) or placebo (occasion B; normal saline); on occasions C and D subjects received a dose-escalation of intravenous esketamine (Ketanest-S^{TM}; Eurocept BV, Ankeveen, The Netherlands) in combination with either intravenous nitroprusside (occasion C) or placebo (occasion D). The infusion of nitroprusside 0.5 µg kg^{-1} min^{-1} or placebo was started one hour before ketamine dosing and continued until the end of the ketamine administration. The racemic ketamine doses infused on occasions A and B were 0.28 mg kg^{-1} h^{-1} for 1 h, followed by 0.57 mg kg^{-1} h^{-1} for another hour and finally 1.14 mg kg^{-1} h^{-1} for the last hour of ketamine administration (Figure 1). The equivalent administered esketamine doses were 0.14 mg kg^{-1} h^{-1} for 1 h, followed by 0.28 mg kg^{-1} h^{-1} for another hour and finally 0.57 mg kg^{-1} h^{-1} for the last hour of ketamine administration (Figure 1). The two-fold difference in racemic and esketamine dose was based on data from the literature indicating that the S(+)-ketamine is twice as potent as the racemic mixture, [26] and a pilot study in which we infused 50 mg racemic ketamine or esketamine per hour in six subjects and measured psychedelic symptoms. The effects observed during 50 mg esketamine infusion were more intense by a factor of 2 than those observed during racemic ketamine infusion, as based on the area-under-the-curve (AUC) of the visual analogues scores of the different psychedelic end-points (data not shown). Reduction of the esketamine dose would reduce the chance that blinding would be broken due to differences in effect sizes.

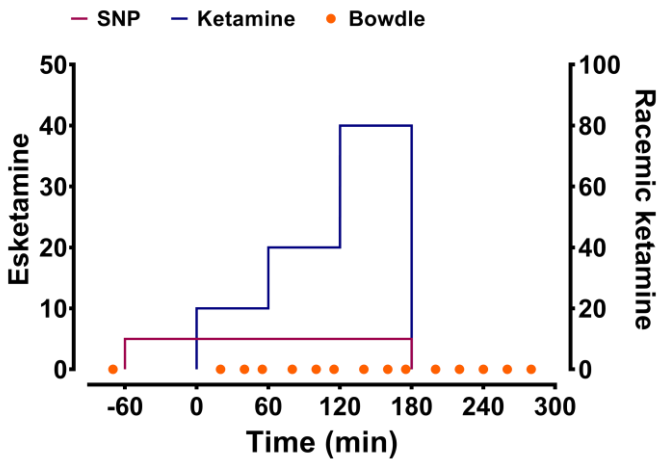

Figure 1. Study design. Racemic ketamine and esketamine infusion in mg 70 kg^{-1} h^{-1}. The sodium nitroprusside dose is 0.5 µg kg^{-1} min^{-1}. Bowdle is the 13 question Bowdle questionnaire.

RANDOMIZATION, BLINDING AND MONITORING

A computer-generated randomization list with a four-block design was generated from www.randomization.org by a study manager (not involved in the execution of the study) on June 11, 2015. The list was sent to the local pharmacy and after subject allocation, the medication was prepared according to Good Manufacturing Practice. Study medication was prepared on the study day and dispensed to the study team before dosing. The study was double blind for ketamine (racemic ketamine *vs.* esketamine) and nitroprusside (nitroprusside *vs.* placebo) treatments. All medications were prepared by the pharmacy in 50 ml syringes marked with (coded) subject and visit numbers. Nitroprusside and placebo (but not ketamine) syringes were covered with foil to prevent inactivation of nitroprusside by ambient light. The research team remained blinded to study medication until all data were collected (August 24, 2017). The study was independently monitored and data analysis was performed after the monitor had filed the final report (September 29, 2017) ensuring that all Good Clinical Practice requirements were met.

MEASUREMENTS

We queried the subjects at regular intervals regarding the occurrence of ketamine side effects before, during and after drug infusion using the Bowdle questionnaire (at baseline, i.e. before any drug administration, and at t = 20, 40, 55, 80, 100, 115, 140, 160, 175, 200, 220, 240, 260 and 280 min after start of racemic ketamine or esketamine administration; Figure 1). The questionnaire uses a visual analogue scale (VAS) to indicate the severity of effects or feelings reported by the subject.[24 25]

The Bowdle questionnaire evaluates three psychedelic ketamine effects, drug high and changes in internal and external perception, from 13 questions scored on a 100-mm VAS scale from zero (no effect) to 100 (maximum effect).[24 25] Drug high is derived from question 11 of the questionnaire (Supplementary Table S1). Internal perception reflects inner feelings that do not agree with reality; it is derived from questions on unrealistic voices or sounds, unrealistic thoughts and paranoid or anxious feelings (questions 4, 5, 8, 9, 10, 13). The external perception indicates a misperception of external stimuli or the surroundings; it is derived from questions on body parts, surroundings, the passing of time and the perception of colour and sound (questions 1, 2, 3, 6, 7). The list of Bowdle questions is given in Supplementary Table S1.

Sample size, data analysis and data validation As we had no indication from the literature what the magnitude of effect of nitroprusside would be on ketamine's psychedelic effects, we assumed that nitroprusside would reduce the drug high AUC by 20% (e.g., a reduction from 8000 to 6400 with standard deviation (SD) 1600), whereby we would need 17 subjects to detect an effect with power greater than 80% and alpha of 0.05. We reasoned that a 20% reduction in effect would be clinically relevant. A sample size of 20 was chosen to take subject withdrawal into account.

The area-under-the time-effect curves (AUC) for drug high (the main end-point of the study), internal and external perception were calculated. The effect of nitroprusside was calculated separately for racemic ketamine and esketamine. The effect of nitroprusside was tested by paired two-sided t-tests or non-parametric tests using GraphPad Prism version 7 for MAC OS X, GraphPad Software (La Jolla, CA USA); *P*-values < 0.05 were considered significant.

An internal validation of the data was obtained by performing 100 000 bootstrap simulations and calculating the 95% confidence intervals of the AUCs. The validation process was performed in R (The R Foundation for Statistical Computing, www.r-project.org, accessed October 27, 2017).

6.3 Results

Thirty-five subjects were recruited (see consort flow chart in Figure 2); fourteen withdrew from the study for various reasons [ketamine side effect too intense (n = 5), ketamine-induced tachycardia (n = 1), did not show up on the first visit (n = 4), withdrew consent after screening (n = 2), other reasons (n = 2)]. One subject could not participate due to the inability to place the arterial line. Twenty subjects completed the four experimental sessions. The questionnaire data from three subjects were discarded as they were deemed unreliable because of protocol violations (subjects did not score the psychedelic

effects according to instructions). As the remaining number of subjects was similar to our intended sample size, we did not recruit additional subjects. Hence, the data from seventeen male subjects were analysed. Their mean age [SD] (range) was 22.8 [2.0] (19-28) years, weight 82.3 [9.5] (60-98) kg, height 190 [6] (175-193) cm and body mass index 23.9 [2.3] (19.5-28.4) kg m^{-2}. All completed the study without major side effects. Nitroprusside had no effect on any of the measured psychedelic end-points as all scores obtained during infusion but before any ketamine administration were zero or close to zero (Figures 3-5). Additionally, there was no systematic occurrence of side effects related to just nitroprusside, such as flushing or headaches, before infusion of racemic ketamine or esketamine.

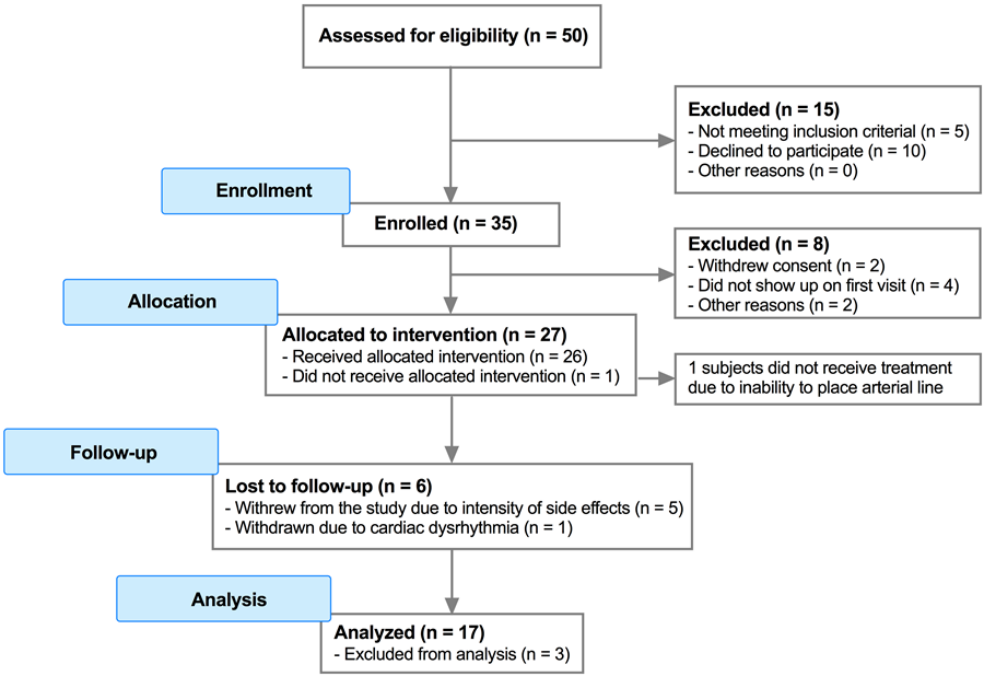

Figure 2. Consort flow chart.

DRUG HIGH

Compared to placebo, nitroprusside significantly reduced the sensation of high (Figure 3) during infusion of racemic ketamine (effect size 22%, P = 0.02, Table 1) but not during infusion of esketamine (effect size 8%, P = 0.65). The racemic ketamine AUC observed during treatment with nitroprusside reached values close to those of esketamine

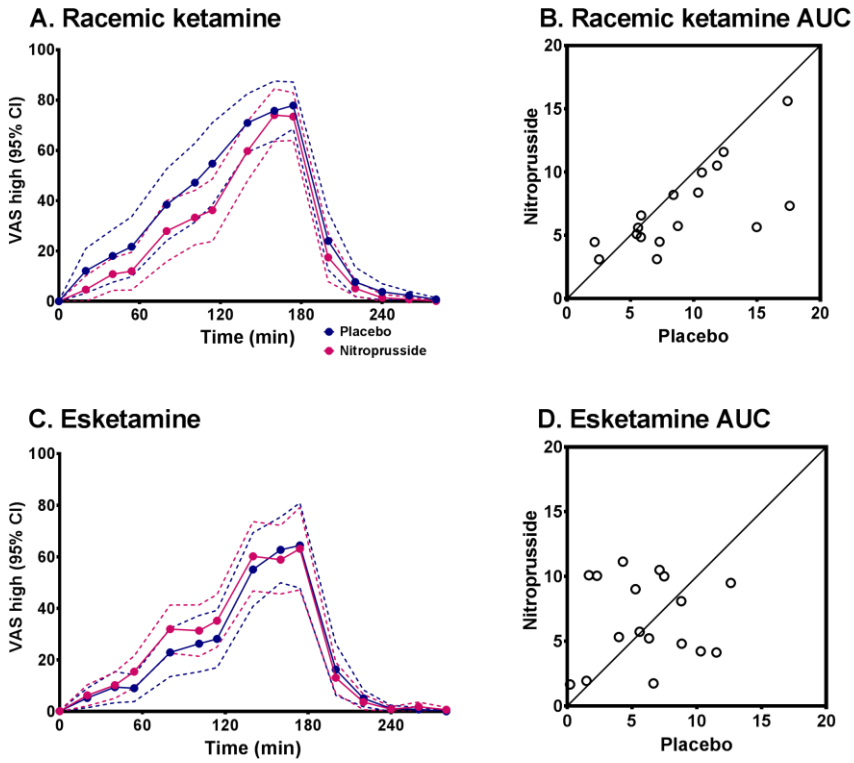

Figure 3 Effect of nitroprusside on ketamine-induced perception of high. **A**. Nitroprusside and placebo effects on racemic ketamine (RS-ketamine)-induced drug high. **B**. Scatter plot of the area-under-the curve (AUC) of nitroprusside *vs*. placebo treatment during racemic ketamine infusion. **C**. Nitroprusside and placebo effects on esketamine (S-ketamine)-induced drug high. **D**. Scatter plot of the AUC of nitroprusside *vs*. placebo treatment during esketamine infusion. **A** and **C**: Nitroprusside, pink symbols; placebo, blues symbols. Values are mean (95% confidence interval) (dotted lines). **B** and **D**: x-axis placebo AUC (x 1,000); y-axis nitroprusside AUC (x 1,000). CI, confidence interval; VAS, visual analogue scale.

(AUC racemic ketamine/nitroprusside *vs*. AUC esketamine/placebo: $P = 0.27$; AUC racemic ketamine/nitroprusside *vs*. AUC esketamine/nitroprusside: $P = 0.67$). The scatter plots show the difference in effect of nitroprusside with 13 subjects (76%) that showed a decrease in racemic drug high AUC while the effect on esketamine AUC was equivocal with eight of 17 subjects (47%) showing a decrease in drug high with nitroprusside treatment.

INTERNAL PERCEPTION

We observed a 43% reduction by nitroprusside relative to placebo of the internal perception AUC during racemic ketamine infusion (Figure 4; $P< 0.01$). During infusion of

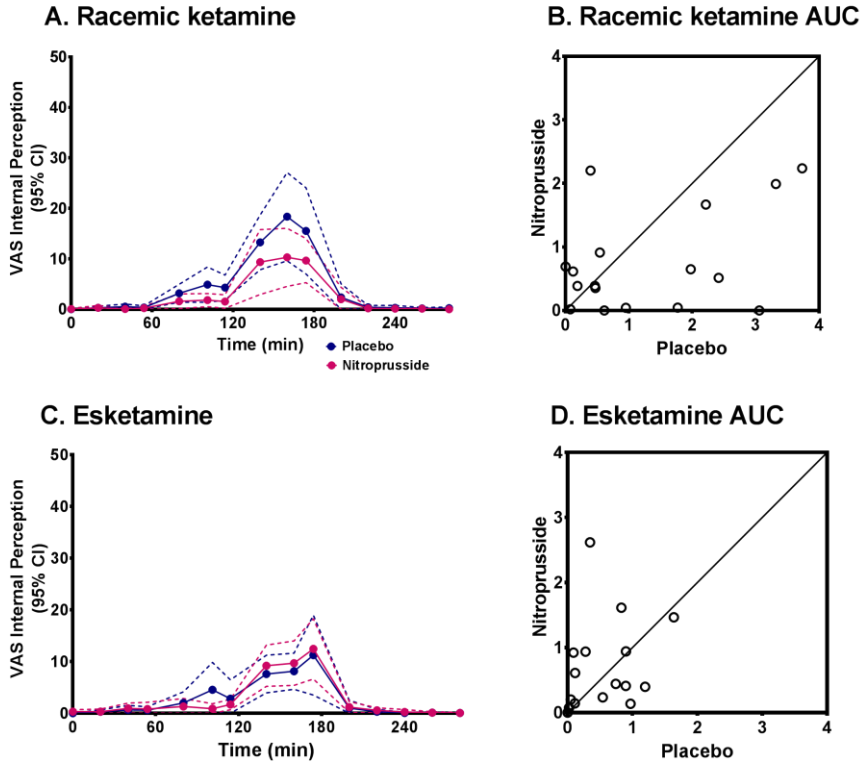

Figure 4. Effect of nitroprusside on ketamine-induced changes in internal perception. **A**. Nitroprusside and placebo effects on racemic ketamine (RS-ketamine)-induced changes in internal perception. **B**. Scatter plot of the area-under-the curve (AUC) of nitroprusside *vs*. placebo treatment during racemic ketamine infusion. **C**. Nitroprusside and placebo effects on esketamine (S-ketamine)-induced changes in internal perception. **D**. Scatter plot of the AUC of nitroprusside *vs*. placebo treatment during esketamine infusion. **A** and **C**: Nitroprusside, pink symbols; placebo, blues symbols. Values are mean (95% confidence interval) (dotted lines). **B** and **D**: x-axis placebo AUC (x 1,000); y-axis nitroprusside AUC (x 1,000). CI, confidence interval; VAS, visual analogue scale.

esketamine, nitroprusside did not affect the AUC (P = 0.26). Twelve subjects showed a reduction in psychedelic effects from racemic ketamine against six subjects from esketamine (Figure 4B).

EXTERNAL PERCEPTION

Compared with placebo, nitroprusside significantly reduced external perception AUC (Figure 5) during infusion of racemic ketamine (effect size 30%, P = 0.02, Table 1) but not during infusion of esketamine (effect size -6%, P = 0.83). Thirteen subjects had an nitroprusside-induced AUC reduction during racemic ketamine *vs*. eight during esketamine infusion.

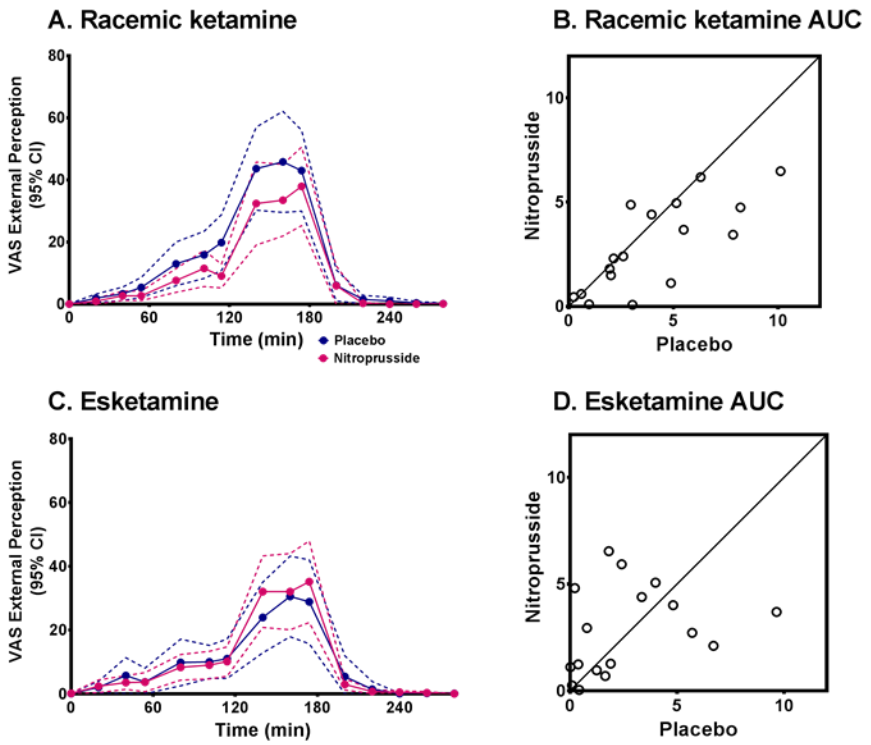

Figure 5. Effect of nitroprusside on ketamine-induced changes in external perception. A. Nitroprusside and placebo effects on racemic ketamine (RS-ketamine)-induced changes in external perception. B. Scatter plot of the area-under-the curve (AUC) of nitroprusside vs. placebo treatment during racemic ketamine infusion. C. Nitroprusside and placebo effects on esketamine (S-ketamine)-induced changes in external perception. D. Scatter plot of the AUC of nitroprusside vs. placebo treatment during esketamine infusion. A and C: Nitroprusside, pink symbols; placebo, blues symbols. Values are mean (95% confidence interval) (dotted lines). B and D: x-axis placebo AUC (x 1,000); y-axis nitroprusside AUC (x 1,000). CI, confidence interval; VAS, visual analogue scale.

The results of the internal validation are given in Supplementary Table S2. These analyses confirm that nitroprusside had a significant effect on psychedelic effects induced by racemic ketamine, but not on effects induced by esketamine.

6.4 Discussion

Earlier rodent studies showed that NO donor nitroprusside effectively eliminates the psychedelic effects observed during ketamine and phencyclidine administration.[19,20] Other manifestations of NMDAR blockade, such as memory impairment, are reversed by modulation of the nitrinergic pathway, either by NO donation or by administration of 8-bromo-cGMP.[27,28] We tested the hypothesis that modulation of the nitrinergic pathway through NO donation would also reduce psychedelic symptoms produced by blockade of

the NMDAR in humans. We studied the effect of relatively low-dose nitroprusside infusion during treatment with the two commercially available ketamine formulations, racemic ketamine (containing equal amounts of the two isomers) and esketamine, the S(+)-ketamine isomer. We observed that both formulations produced dose-dependent psychedelic symptoms that quickly resolved upon termination of the infusion. However, concomitant treatment with nitroprusside had a differential effect on the two isomers, with 20-40% less intense psychedelic effects experienced during racemic ketamine infusion but not during esketamine infusion. This indicates that nitroprusside has a divergent effect on S(+)- and R(-)-ketamine-isomers. Our findings are in agreement with preclinical studies that used racemic ketamine to examine the mechanism of ketamine-induced psychosis.[20,21]

Table 1. Effect of nitroprusside on psychedelic effects during racemic ketamine and esketamine. Values are mean [SD] (95% confidence interval of the mean)

	Treatment		
	Nitroprusside	**Placebo**	
Racemic ketamine			
Drug high	7,098 [3,317] (5,393–8,804)	9,072 [4,631] (6,691–11,453)	P= 0.02 (t= 2.46, df= 16)
Internal perception	748 [786] (344–1,152)	1,312 [1,250] (669–1,955)	P< 0.01 (t= 2.99, df= 16)
External perception	2,890 [2,122] (1,799–3,981)	4,018 [2,843] (2,556–5,479)	P= 0.02(t= 2.53, df= 16)
Esketamine			
Drug high	6,658 [3,325] (4,948–8,368)	6,144 [3,593] (4,297–7,992)	P= 0.60 (t= 0.54, df= 16)
Internal perception	745 [713] (379–1,112)	821 [1,232] (187–1,454)	P= 0.79 (t= 0.26, df= 16)
External perception	2,809 [2,059] (1,751–3,868)	2,658 [2,727] (1,256–4,060)	P= 0.83 (t= 0.22, df= 16)

RACEMIC KETAMINE *VERSUS* ESKETAMINE

The differential effect of nitroprusside on the symptoms induced by the two ketamine formulations was unexpected. The insensitivity of esketamine to nitroprusside suggests that our observations are restricted to an interaction of nitroprusside with R(-)-ketamine-induced NMDAR hypofunction. Both isomers interact with the NMDR but with different affinities: S(+)-ketamine has a fourfold higher affinity for the NMDAR than R(-)-ketamine.[29,30] Higher nitroprusside doses might be required to overcome the downstream consequences of NMDAR hypofunction induced by an isomer with a higher NMDAR affinity relative to R(-)-ketamine. We based the nitroprusside dose on a previous study

that showed that 0.5 µg kg^{-1} min^{-1} nitroprusside given for 4 h is safe and effective in reducing acute symptoms in schizophrenia in humans.[23] We plan future studies on the effect of increasing nitroprusside doses on esketamine and racemic ketamine pharmacodynamics. This will establish whether the nitroprusside mechanism of action at (and beyond) the NMDAR is divergent for the two isomers or whether our current observation is best explained by nitroprusside underdosing. With respect to the former theory, animal studies show that the S(+)- and R(-)-ketamine isomers produce their antidepressant effects through different cellular pathways.[31,32] We cannot exclude that different cellular pathways contributed to our observations. The lack of a trend in the effects of nitroprusside on esketamine-induced side effects suggests that the S(+)-ketamine isomer induces its psychedelic effects through a NO-independent pathway.

So far, no separate mechanism of the psychedelic symptoms produced by the two ketamine isomers have been postulated. This may be related to the fact that all experimental work so far has been done with the racemic ketamine formulation.[20,22]

Although our study was not designed for a one-on-one comparison between isomers, our study protocol enables the estimation of the potency difference between the two isomers by comparing their placebo AUC values. We assume that the AUC of the R(-)-isomer = AUC racemic ketamine – AUC esketamine. The calculated R(-)-ketamine AUC was about half of the measured esketamine AUC, which implies a two-fold greater potency of the S(+)-isomer compared to the R(-)-isomer for induction of psychedelic symptoms. This observation is in agreement with a two-fold potency difference in the anesthetic effects of the S(+)- and R(-)-ketamine isomers.[26]

NITROPRUSSIDE-INDUCED MODULATION OF RACEMIC KETAMINE PSYCHEDELIC SYMPTOMS

Nitroprusside infusion reduced psychedelic effects induced by racemic ketamine, more specifically the R(-)-isomer, most likely through interaction within the NMDAR-Ca^{2+}-calmodulin-NO synthase-NO-cGMP pathway. In line with animal studies, we hold nitroprusside-induced neuronal increase in NO concentrations and consequently the NO-cGMP sequence reactivation responsible for the reduced intensity of behavioural effects that we observed in volunteers treated with racemic ketamine and nitroprusside. However, other mechanisms could play a role in the actions of nitroprusside, such as an inhibitory effect of NO on dopaminergic hyperfunction (independent of cGMP activity),[33] or direct nitroprusside effects. Examples of the latter are observations that nitroprusside itself may restore NMDAR hypofunction or improve function through neuroprotective and anti-inflammation by interaction with nuclear factor-κB, a nuclear factor that controls DNA transcription.[34-36] Irrespective of the postulated mechanisms, the exact process through which NO modulates ketamine-induced psychedelic effects in humans cannot be deduced

from our experiments and remains unknown. It is important to realise that in some animal models, reduced NO synthase activity is associated with reduced psychosis-like symptoms from phencyclidine or ketamine.[37,38] This highlights the complexity of the NMDAR-NO interaction that is still insufficiently understood.

CLINICAL IMPLICATIONS

The psychedelic symptoms associated with the use of ketamine led to reduced patient and doctor compliance in its use as analgesic.[2,8] We observed a 20-40% decrease in psychedelic effect by nitroprusside during racemic ketamine infusion. This is a moderate effect that might have a limited clinical importance. It is too early to consider nitroprusside a viable and clinically relevant addition to racemic ketamine treatment, in the sense that ketamine treatment might be better accepted and higher doses may be given to produce adequate and long term analgesic effects.[2,8] Further studies are needed (i) to corroborate our findings, (ii) to assess whether the reduction of psychedelic side effects can be increased above the current observation with higher nitroprusside doses, and (iii) to determine whether the ketamine-induced increases in blood pressure are reduced by nitroprusside. It is important to realise that in the treatment of depression, there is the suggestion that the dissociative effects of ketamine mediate its antidepressant effects.[39] Modulation of these effects by nitroprusside will then be counterproductive. Further (animal) studies are needed to determine the modulatory role of nitroprusside in ketamine treatment in depression. For now, as mood improvement is sometimes also desirable in the treatment of chronic pain patients,[40] the clinical use of nitroprusside in modulating the side effects of ketamine during pain treatment in chronic pain patients with depressive traits should only be practiced when other approaches fail (such as treatment with benzodiazepines or α_2-agonist).

CRITIQUE OF METHODS

As stated above, the lack of effect of nitroprusside during esketamine treatment could reflect the relatively low nitroprusside dose (0.5 µg kg^{-1} min^{-1}) that we used in this study. In future studies, we will examine the effect of higher nitroprusside doses on the intensity of esketamine-induced psychedelic symptoms. However, one needs to realise that high-dose nitroprusside (> 2 µg kg^{-1} min^{-1}), administered for longer periods of time, is associated with toxicity. Through interaction with oxyhaemoglobin, nitroprusside can form methaemoglobin and cyanide that can interfere with oxygen transport and cytochrome activity, respectively.[41,42]

We enrolled 35 subjects into the study and allocated treatment to 27 of them. We were able to analyse the data from just 17 subjects due to a variety of reasons. We used a relatively high dose of esketamine (0.3 mg kg^{-1} h^{-1}) and racemic ketamine (0.6 mg kg^{-1} h^{-1}), which is higher than we routinely use in clinical practice. This was done to maximise the

drug high and other psychedelic symptoms. Because of the occurrence of side effects, five subjects prematurely ended their participation in the study. One subject experienced an episode of tachyarrhythmia (heart frequency > 140 min^{-1}) that required the cessation of ketamine infusion. Additionally, three subjects either refused to score the intensity of psychedelic symptoms as requested or failed to understand the scoring system. The latter was probably related to cognitive impairment during ketamine administration. We accept the high number of dropouts, but also believe that improved screening and coaching will reduce failure of successful study completion. As we continued recruitment until we had 17 reliable and complete data sets, we assumed that the high subject loss would not influence our study outcomes. This was indeed confirmed by the internal validation of the data analyses.

6.5 Conclusions

In healthy male volunteers, the NO donor sodium nitroprusside significantly reduced psychedelic symptoms drug high and internal and external perception caused by racemic ketamine. No effects from nitroprusside were observed in volunteers treated with esketamine, the S(+)-ketamine isomer, suggesting that the nitroprusside effect was directed at the NMDAR actions of the R(-)-isomer but not the S(+)-isomer. Possibly higher nitroprusside doses are needed to relieve the psychedelic effects from S(+)-ketamine which has a four-fold higher affinity for the NMDARs than R(-)-ketamine. However, we cannot exclude that the two ketamine isomers produce their psychedelic symptoms through divergent cellular pathways. Finally, our proof-of-concept study requires further clinical proof in larger samples with higher nitroprusside doses (to produce greater effects than the current 20-40% reduction of psychedelic effects during racemic ketamine infusion) before we can implement nitroprusside treatment to counteract racemic ketamine-related psychedelic symptoms.

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Appendix A. Supplementary data

Table S1. The Bowdle scales

A. My body parts seemed to change their shape or position (BODY)
B. My surroundings seemed to change in size, depth, or shape (SURROUNDINGS)
C. The passing of time was altered (TIME)
D. I had feelings of unreality (REALITY)
E. It was difficult to control my thoughts (THOUGHTS)
F. The intensity of colors change (COLORS)
G. The intensity of sound changes (SOUND)
H. I heard voices and sounds that were not real (VOICES)
I. I had the idea that events, objects, or other people had particular meaning that was specific for me (MEANING)
J. I had suspicious ideas or the belief that others were against me (SUSPICIOUS)
K. I felt high (HIGH)
L. I felt drowsy (DROWSY)
M. I felt anxious (ANXIOUS)

Table S2. Internal validation of the effect of nitroprusside on psychedelic effects. Values are mean (95% Confidence interval of the mean) * 95% confidence interval of the difference

	Treatment		
	Nitroprusside	**Placebo**	
Racemic ketamine			
Drug high	7,057 (5,788–8,839)	8,892 (6,976–11,147)	703–3792*, P= 0.01
Internal perception	743 (437–1,169)	1,297 (776–1,949)	213–933*, P= 0.02
External perception	2,880 (1,942–3,880)	3,986 (2,805–5,455)	339–2,016*, P< 0.01
Esketamine			
Drug high	6,595 (5,502–8,061)	5,992 (4,411–7,581)	-2,341–1,053*, P= 0.49
Internal perception	739 (469–1,139)	768 (449–1,595)	-393–561*, P= 0.91
External perception	2,784 (1,907–3,767)	2,582 (1,582–3,991)	-1,405–1,094*, P= 0.76