Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion

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Abstract

Background: The commonality between chronic conditions that are treated with low-dose ketamine, such as specific chronic pain conditions, depression, and post-traumatic stress disorder, can be found in relation to the stress system, particularly the hypothalamus–pituitary–adrenal axis. In this study we assess the effect of ketamine on the stress system by measuring plasma and saliva cortisol production during and following exposure to low-dose ketamine.

Methods: In a double-blind, randomized, placebo-controlled study, the influence of subanaesthetic ketamine (0.29 mg kg$^{-1}$ h$^{-1}$ for 1 h, followed by 0.57 mg kg$^{-1}$ h$^{-1}$ for another hour) was studied with repeated plasma and saliva cortisol samples in 12 healthy male volunteers. A pharmacokinetic–pharmacodynamic model was used to describe the circadian rhythm–dependent ketamine-induced production of cortisol.

Results: The endogenous mean baseline cortisol production was 7.9 (±1.5) nM min$^{-1}$. Consistent with the circadian rhythm, cortisol production decayed by 1.25 nM min$^{-1}$ h$^{-1}$. Ketamine doubled the cortisol production at a concentration of 165 (±35) ng ml$^{-1}$. The salivary cortisol concentration closely mirrored the plasma concentration and was exponentially related to the plasma concentration with, at 100 ng ml$^{-1}$ ketamine, a saliva:plasma ratio of 0.036 (±0.006).

Conclusions: Ketamine has an appreciable effect on cortisol production. This may impact on critical physiological and psychological functions.

Clinical trial registration: This study was registered in the Dutch Trial Register under number NTR2717 at www.trialregister.nl.

Key words: cortisol; HPA axis; ketamine; pharmacodynamics; pharmacokinetics; stress

Since its introduction into clinical practice in the early 1960s, the use of ketamine has progressed from an anaesthetic induction agent to a more versatile drug commonly used in the treatment of acute and chronic pain, therapy-resistant major depression, migraine, and post-traumatic stress disorder (PTSD).1–5 Antagonism of the N-methyl-D-aspartate receptor (NMDA), an excitatory receptor ubiquitously present in the central nervous system, is believed to be ketamine’s major mechanism of action in the modulation of chronic pain and associated disorders.1 An interesting observation is that the commonality between chronic conditions that are treated with ketamine can be found in relation to the stress system, more specifically the hypothalamus–pituitary–adrenal (HPA) axis, which regulates the neuroendocrine system. HPA axis function is primarily characterized by the synthesis and release of the steroid hormone cortisol. Dysregulation of HPA axis function, marked by abnormal (increased or decreased) cortisol responses to stress, is demonstrated in various chronic pain conditions, as well as depression and PTSD.6–8

Although the relation between ketamine and HPA axis function has been studied in animals and humans with often ambiguous results (either finding an increased production or a blunted cortisol response),9–14 less is known about the effect of

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Ketamine on the stress system at the low doses used for treatment of depression, pain, and PTSD. We therefore developed pharmacokinetic–pharmacodynamic (PKPD) models to estimate the influence of low-dose ketamine on HPA axis function. Furthermore, since less invasive sampling of cortisol from saliva (compared with plasma cortisol) is a preferable method to test the stress system, it was considered important to extend the PKPD models using saliva cortisol sampling. This study is a first step in quantifying the complex interaction of ketamine and the increase in cortisol it causes on stress-related disorders (including chronic pain).

Methods

Twelve healthy male volunteers (ages 19–36 yr; BMI 21–27 kg m\(^{-2}\)) were recruited to participate in the study after approval of the protocol was obtained from the Leiden University Medical Centre ethics committee. Oral and written informed consent was obtained from all participants. Exclusion criteria for enrolment included medical disease (such as renal, liver, cardiac, or vascular disease, including hypertension), presence or history of a neurologic or psychiatric disease (e.g. increased cranial pressure, epilepsy, psychosis), glaucoma, obesity (BMI >30 kg m\(^{-2}\)), history of chronic alcohol or drug abuse, or use of any centrally acting medication. This study is part of a larger project on the effect of ketamine on brain function and was registered in the Dutch Trial Register (number NTR2717). In the current report we focus on the cortisol response to low-dose ketamine administration. The study was performed from May to July 2011.

Study design

In this single-blind, randomized, placebo-controlled crossover study, subjects were examined on two occasions at least 1 week apart. Subjects received S\(^+\)-ketamine (Ketanest-S; Eurocept, Ankeveen, The Netherlands) on one occasion and placebo (NaCl 0.9%) on the other. No details regarding treatment effects were given apart from the possibility of experiencing a drug ‘high’ during treatment. To control for circadian effects, all subjects were asked to arrive in the laboratory around 8:30 AM. Two intravenous lines were inserted, one for blood sampling and one for administration of ketamine. Drug infusion with either S\(^+\)-ketamine or placebo was started at t=0 (at 10 AM±10 min) at a low dose, 20 mg h\(^{-1}\) (70 kg)\(^{-1}\), for 1 h, followed by a high dose, 40 mg h\(^{-1}\) (70 kg)\(^{-1}\), for another hour. These doses are based on the average S\(^+\)-ketamine infusion rates required to produce long-term relief of chronic pain.  

Ketone and cortisol sampling

Venous blood was drawn to determine the plasma concentration of ketamine (and its metabolite norketamine) and cortisol. Cortisol was measured from both saliva (passive drool providing at least 1 ml of saliva) and plasma in order to examine the sensitivity of each variable to our experimental condition. Cortisol samples were obtained before, during, and after ketamine infusion. Plasma samples were obtained at t=0 (baseline), 15, 30, 60, 75, 90, 120, 130, 160, and 200 min following the start of ketamine infusion; saliva samples were obtained at t=−30 (baseline 1), 0 (baseline 2), 60, 90, 120, 170, and 200 min.

All blood samples were centrifuged at 3500 rpm for 10 min within 15 min of collection. Plasma was then stored at −25°C for later analysis. Saliva was collected in 2 ml SaliCaps (IBL, Hamburg, Germany) and centrifuged at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity, and was stored at −25°C for later analysis. Plasma ketamine was measured using high-performance liquid chromatography. The assay had a coefficient of variation of 5.66% and 2.49% at concentrations of 192 and 936 µM, respectively. Plasma cortisol was measured by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) using an Elecsys 2010 immunoanalyzer (Roche Diagnostics). The assay had a coefficient of variation of 1.3% and 1.1% at 208 and 1268 nM, respectively. Salivary concentrations were measured using a luminescence-enhanced enzyme immunoassay (IBL International, Hamburg, Germany). In the current report we focus on the cortisol response to low-dose ketamine administration. The study was performed from May to July 2011.

Data analysis

The PKPD model consisted of a ketamine PK component coupled to a second part that describes the production and elimination of cortisol (in which the endogenous production of cortisol is made dependent on time to describe the circadian effect on cortisol production) using the following indirect response model:

\[
\frac{d C_{p}(t)}{dt} = k_{IN} \cdot \left[1 - \alpha \cdot t + \frac{C_{KT}(t)}{C_{100}} \right] - k_{OUT} \cdot C_{p}(t),
\]

where \(C_{KT}\) is the ketamine concentration in plasma, \(C_{p}\) is the cortisol concentration in plasma, \(C_{100}\) is the plasma ketamine concentration that doubles \(C_{IN}\), \(k_{IN}\) is the endogenous plasma cortisol production rate at \(t=0\) (just before any ketamine/placebo infusion), and \(k_{OUT}\) is the cortisol elimination rate. Cortisol production in the body follows a periodic pattern consisting of ultradian oscillations (20–120 min periods) and a circadian oscillation (with a period of 24 h) that can be described with complex mathematical models. Such modelling approaches depend on a sufficient rate of sampling over a longer period of time. Our study took place over 3 h, with sparse sampling starting 1 h after the start of infusion followed by half-hour (or longer) intervals. According to diurnal circulation patterns, the highest level of cortisol is expected in the early morning, with a significant increase at the time of awakening and gradual decay starting a half-hour after awakening. Since we sampled cortisol for 3 h, we modelled the cortisol decay with a linear function (\(\alpha \cdot t\)).

The saliva cortisol concentration (\(C_{S}\)) was related to \(C_{p}\) using an exponential function:

\[C_{S} = C_{p} \cdot \exp(\lambda + \beta \cdot C_{p})\]

where \(\lambda\) and \(\beta\) are coefficients.

The population PK analysis was performed using the stochastic software package NONMEM (version 7.3.0; Icon Development Solutions, Hanover, MD, USA). Plasma ketamine, plasma cortisol, and salivary cortisol were modelled simultaneously. For ketamine, one- and two-compartment models were tested; for cortisol, a delay between plasma and salivary compartments was...
tested as well as a linear function to describe the transition from plasma to saliva. The concentration data were log transformed and an additive model was used for residual error. The final model structure was based on the minimum objective function value with statistical significance set at P-values <0.01. All NONMEM output values are typical value (SE).

Standardized visual predictive check (SVPC)
SVPCs were performed for ketamine, plasma cortisol, and saliva cortisol concentrations to evaluate the performance of the model. The SVPC gives the percentile of the observations of each subject in the marginal distribution of the corresponding data simulations (ketamine or cortisol concentrations) as a function of time.29

Results
All participants completed the study without major side effects. During exposure to ketamine, most subjects experienced feeling a ‘drug high’, which increased in severity during the course of the ketamine infusion. In Figure 1A the plasma ketamine concentration during and following infusion are given. The mean peak plasma ketamine concentrations at the end of the first and second infusion hour were 74.9 (SD 15.6) and 187.5 (SD 32.8) ng ml⁻¹, respectively. The mean plasma and saliva cortisol levels in placebo and ketamine sessions are given in Figure 1B and C. For both plasma and saliva cortisol concentrations, the change over time during and following placebo treatment is indicative of the circadian changes in cortisol production. The cortisol plasma concentration peaked at the end of the ketamine infusion (t=120 min), to 530 (SD 145) nM, a concentration twice as high as
Ketamine infusion
Hypothalamus
CRH
Anterior pituitary
ACTH
Adrenal cortex
plasma saliva
Cortisol

Fig 2. Pharmacokinetic–pharmacodynamic model describing the production of plasma and salivary cortisol by ketamine. $V_1$ is the volume of the single ketamine compartment, CL is the ketamine clearance, $k_{IN}$ is the endogenous production of cortisol, which is under the influence of the circadian rhythm (clock) and ketamine, $C_{Cp}$ and $C_{Cs}$ are the plasma and salivary cortisol concentrations, and $k_{OUT}$ is the cortisol elimination rate.

Fig 3. Individual plasma and salivary cortisol levels, individual (blue) and population data fits (pink) for (A) plasma cortisol/ketamine treatment, (B) plasma cortisol/placebo treatment, (C) salivary cortisol/ketamine treatment, and (D) salivary cortisol/placebo treatment.
that observed at the end of the placebo infusion [229 (SD 135) nM]. Saliva cortisol peaked at t=170 min, 50 min after ending the ketamine infusion, to 35 (SD 24) nM, about five times the concentration observed after placebo infusion at t=170 min.

The final PKPD model is presented in Figure 2 and consists of one ketamine compartment and two cortisol compartments (plasma and saliva), without any delay between the cortisol compartments. Individual cortisol measurements, individual data fits and population data fits are given in Figure 3 for both plasma and saliva cortisol concentrations during and following ketamine or placebo treatment; goodness of fit plots are shown in Figure 4A–C. The estimated model parameters are collected in Table 1. The endogenous cortisol production rate (i.e. without the effect of ketamine; $k_{IN}$) was estimated to be 7.88 nM min$^{-1}$ at t=0 (10 AM actual time) with an estimated baseline concentration of 329 nM ($k_{IN}/k_{OUT}$). Due to the circadian effect, the cortisol production decayed by 1.26 nM min$^{-1}$ h$^{-1}$ causing a decrease in cortisol production to 4.11 nM min$^{-1}$ at t=3 h (1 PM).

Ketamine doubled the cortisol production at an effect-site concentration of 165 ng ml$^{-1}$. At that ketamine concentration, the cortisol production increased to 15.8 nM min$^{-1}$ at 10 AM. Due to the circadian effect on cortisol release, the absolute ketamine effect will differ at other time points. For example, when the subject is exposed to 165 ng ml$^{-1}$ ketamine at 1 PM the cortisol production will increase from 4.1 to 8.2 nM min$^{-1}$.

The exponential function describing the relation between saliva and plasma cortisol levels yielded significantly better data fits compared with the tested linear function (P<0.01). Parameter estimates of the exponential function are given in Table 1. To get an indication of the cortisol saliva:plasma ratio, we performed a Monte Carlo simulation using equation 2 and the estimates of $\lambda$ and $\beta$ with their SEs. The ratio is 0.036 (SD 0.006) at 100 ng ml$^{-1}$ ketamine. This indicates that just a small but readily detectable amount of cortisol is present in saliva that closely follows the plasma concentration.

In Figure 4D and E, the results of the SVPC are given. The accuracy of the PKPD model was acceptable with 95% of the ketamine (Fig. 4D), saliva cortisol, and plasma cortisol (Fig. 4E) data points within the 2.5 to 97.5 percentiles.

**Discussion**

Cortisol is a stress biomarker that probably affects every system in the human body (e.g. metabolism, immune response, brain function). Cortisol production displays a typical diurnal rhythm (consisting of ultradian 20–120 min oscillations and a circadian 24 h pattern), with a peak concentration in the morning hours (8–10 AM; with peak plasma values ranging from 170 to 500 nM and peak saliva values <24 nM) and a valley in the late afternoon/early evening (4–8 PM; with peak plasma values ranging from 60 to 300 nM and peak saliva values <12 nM). Our study protocol standardized circadian effects by setting the ketamine infusion time at 10 AM, giving the subjects 90 min to recover from early morning and potentially stress-related variations upon arrival at 8:15–8:30 AM. We observed cortisol values at baseline (t=–30 min) were in the range of 12–24 nM (saliva) and 315–407 nM (plasma), in close agreement with values reported in the literature.
A robust reduction of cortisol levels in the sham-placebo session was observed, which is consistent with the expectation of diurnal decay (Figs. 1 and 3). Because our study provides information over a short part of the cortisol cycle, we described the cortisol decay using a linear function (equation 1). Data analysis of cortisol diurnal patterns using more complex models (exponential, stochastic, sinusoidal) requires plasma or saliva sampling over longer time periods.\(^{21-26,31,32}\) As an alternative to the current approach, we modelled the cortisol decay using exponential and sinusoidal functions (data not shown). While the data were well fitted by the alternative models, the more simple approach yielded the best fit in terms of the minimal objective function. The linear model correctly predicted a drop in plasma and saliva cortisol release during the 3 h of our study (Fig. 3), which is in agreement with values reported in the literature. Note that prediction of cortisol concentrations from this model beyond the time frame of the study may not be appropriate.

In our study, low-dose ketamine (dose range 0.29–0.57 mg kg\(^{-1}\)) produced a dose-dependent increase in cortisol production, with a (relative) twofold increase at a concentration of 165 ng ml\(^{-1}\) (C100, Table 1). Since the ketamine effect was linked to \(k_{IN}\) in the PK model (equation 1), the absolute ketamine effect varies according to the diurnal cortisol release pattern (with a reduction in effect with a decaying cortisol production rate). While it is possible to link ketamine effect to other parameters of equation (1), such as \(k_{OUT}\), our approach seems more intuitive (e.g. ketamine influences cortisol secretion).\(^{20}\)

In recent years, salivary cortisol measurement has become an important biomarker in psychological and psychiatric assessments. By repeatedly measuring both plasma and saliva cortisol levels we demonstrated that these two variables were significantly correlated, with saliva concentrations ~4% of plasma concentrations (at 100 ng ml\(^{-1}\) ketamine). The relation between plasma and saliva cortisol was best described by an exponential function. Our PK model predicted that salivary cortisol closely mirrored plasma cortisol concentrations, with a simultaneous peak concentration (occurring in-between sample times; Fig. 3a and c).

Although closely related, plasma and salivary cortisol are not identical variables. In blood, >90% of cortisol is bound to proteins (corticoid-binding globulin and albumin) and erythrocytes, and a free unbound fraction that interacts its receptors.\(^{27,28}\) Salivary cortisol reflects the portion of the biologically active fraction of this steroid hormone (~5%), which is unbound. We were unable to observe a significant delay between salivary and cortisol plasma concentration in the PK model \((P > 0.01)\). However, visual comparison of Figure 1a and c suggests a delay in reaching the peak of plasma cortisol levels (appearing at min 120; 95% CI 245, 458 nM) and saliva (appearing at min 170; 95% CI 7.8, 35.7 nM). The discrepancy between the model and descriptive analyses may be related to the sparse sampling approach that we employed (with a true peak in both saliva and plasma cortisol between 120 and 170 min). Alternatively, it may be related to the greater variability in the saliva data. Comparing Figure 1a and c, it can be seen that the degree of between-subject variability is greater in salivary cortisol compared with plasma cortisol. Hypothetically, salivary cortisol may be related to a latent psychological modulation of the neuroendocrine response measured from saliva, compared with immediate endocrine effects that can be measured from plasma.\(^{34}\)

Our study has some limitations. Our results were obtained from a group of healthy young male participants and thus are not generalizable to women, the elderly, and children, or to any clinical population. Our study was done over a short time interval and the intersampling intervals were short (>30 min), therefore we have been unable to model the circadian or ultradian cycles. Considering that the stress response has a latency of 15–20 min,\(^{27}\) we may have missed response fluctuations between the low and the higher dose range of our ketamine infusion. Also, our study was timed strictly, thus we were unable to model ketamine–cortisol interactions based on diurnal phases. Ketamine (but not placebo) infusion comes with psychomimetic side effects (most importantly ‘drug high’) that may have unblinded the study.\(^{1}\) These psychomimetic effects may also have influenced the subjective stress response of the participants, but we did

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**Table 1** Pharmacokinetic–pharmacodynamic model parameter estimates. \(\sigma^2\) is the between-subjects variability (in the log domain); %CV is the coefficient of variation; \(V1\) is volume of the ketamine compartment; \(C100\) is the concentration of ketamine causing a twofold increase in \(k_{IN}\); \(k_{OUT}\) is the endogenous plasma cortisol production rate at \(t=0\) (just before any ketamine/placebo infusion); \(k_{OUT}\) is the cortisol elimination rate; \(\alpha\) is slope of the linear function that describes the circadian release of cortisol over the short duration of the study; \(\lambda\) and \(\beta\) are coefficients of the exponential relationship between plasma and salivary cortisol; \(\sigma^2\) is the variance of the residual error (in the log domain); IOC is the interoccasion variability (i.e. between ketamine and placebo treatments), and \(CCp\) and \(CCs\) are the cortisol plasma and salivary concentrations, respectively. *Not included in the statistical model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value (±st)</th>
<th>(\sigma^2) (±st) [%CV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V1) (litre)</td>
<td>165 (35)</td>
<td>0.037 (0.006)</td>
</tr>
<tr>
<td>Clearance (litre min(^{-1}))</td>
<td>2.2 (0.1)</td>
<td>0.02 (0.01) [12]</td>
</tr>
<tr>
<td>(C100) (ng ml(^{-1}))</td>
<td>165 (35)</td>
<td>0.46 (0.22) [68]</td>
</tr>
<tr>
<td>(\sigma^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(k_{IN}) (nM min(^{-1}))</td>
<td>7.9 (1.5)</td>
<td>0.17 (0.07) [40]</td>
</tr>
<tr>
<td>(k_{OUT}) (min(^{-1}))</td>
<td>0.024 (0.003)</td>
<td>0.09 (0.06) [31]</td>
</tr>
<tr>
<td>(\alpha) (h(^{-1}))</td>
<td>0.16 (0.02)</td>
<td>0.06 (0.03)</td>
</tr>
<tr>
<td>IOC variability on baseline (CCp)</td>
<td>0.04 (0.005)</td>
<td>0.14 (0.07) [38]</td>
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<tr>
<td>(CCp)~(CCs)</td>
<td>-3.4 (0.16)</td>
<td>0.0012 (0.003)</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>0.12 (0.01)</td>
<td></td>
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<tr>
<td>(\beta)</td>
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<td></td>
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<tr>
<td>(\sigma^2)</td>
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not collect enough psychometric data to incorporate such factors in the PK model, and were unable to fully dissociate the pharmacological and psychological effects of ketamine on cortisol. Venepuncture often entails HPA axis activity and may have influenced the study. However, the i.v. lines were inserted at least 90 min prior to the first cortisol sample, so interference was unlikely. The experimental context and the a priori states of anticipatory stress may have had significant impact on the cortisol response. We did not control for such effects in our study, but assume that placebo and ketamine studies were similarly affected. Finally, ketamine clearance of 2 litre min⁻¹ is higher than previously observed and higher than the expected liver blood flow. We relate this to the characterization of the ketamine disposition with a one-compartment model rather than two- or three-compartment models, as well as to the short time period of the study with a relatively sparse sampling design.

In conclusion, we successfully performed a PKPD analysis to describe the endogenous and time-varying production of cortisol in plasma and saliva and quantified the effect of ketamine on cortisol production.

Authors’ contributions
N.K.-M. was involved in the experimental design, performed parts of the experiments, and wrote parts of the manuscript; C.M. performed the experiments; E.O. performed the data analysis; A.D. wrote the protocol and the manuscript; M.N. had the initial idea for the study, wrote the protocol, performed all experiments, and wrote parts of the manuscript; C.M. N.K.-M. was involved in the experimental design, performed part of the experiments, and wrote the protocol.

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Declaration of interest
None declared.

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