

# Cardiovascular stability and unchanged muscle sympathetic activity during xenon anaesthesia: role of norepinephrine uptake inhibition

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## Editor's key points

- The mechanism of the effect of xenon (Xe) on cardiovascular stability was studied.
- Human volunteers anaesthetized with Xe alone had increased arterial pressure.
- In cells, Xe decreased the uptake of norepinephrine (NE) by inhibiting the NE transporter, thereby increasing local NE availability.
- This may explain cardiovascular stability during Xe anaesthesia.

**Background.** Intraoperative hypotension is associated with increased risk of perioperative complications. The *N*-methyl-D-aspartate (NMDA) receptor (NMDA-R) antagonist xenon (Xe) induces general anaesthesia without impairment of cardiac output and vascular resistance. Mechanisms involved in cardiovascular stability have not been identified.

**Methods.** Muscle sympathetic activity (MSA) (microneurography), sympathetic baroreflex gain, norepinephrine (NE) plasma concentration (high-performance liquid chromatography), anaesthetic depth (Narcotrend® EEG monitoring), and vital parameters were analysed *in vivo* during Xe mono-anaesthesia in human volunteers ( $n=8$ ). *In vitro*, NE transporter (NET) expressing HEK293 cells and SH-SY5Y neuroblastoma cells were pre-treated with ketamine, MK-801, NMDA/glycine, or vehicle. Subsequently, cells were incubated with or without Xe (65%). NE uptake was measured by using a fluorescent NET substrate ( $n=4$ ) or [<sup>3</sup>H]NE ( $n=6$ ).

**Results.** *In vivo*, Xe anaesthesia increased mean (standard deviation) arterial pressure from 93 (4) to 107 (6) mm Hg and NE plasma concentration from 156 (55) to 292 (106) pg ml<sup>-1</sup>,  $P<0.01$ . MSA and baroreflex gain were unaltered. *In vitro*, ketamine decreased NET activity ( $P<0.01$ ) in NET-expressing HEK293 cells, while Xe, MK-801, and NMDA/glycine did not. Xe reduced uptake in SH-SY5Y cells expressing NET and NMDA-Rs ( $P<0.01$ ). MK-801 ( $P<0.01$ ) and ketamine ( $P<0.01$ ) also reduced NET activity, but NMDA/glycine blocked the effect of Xe on [<sup>3</sup>H]NE uptake.

**Conclusions.** *In vivo*, Xe anaesthesia does not alter sympathetic activity and baroreflex gain, despite increased mean arterial pressure. *In vitro*, Xe decreases the uptake of NE in neuronal cells by the inhibition of NET. This inhibition might be related to NMDA-R antagonism and explain increased NE concentrations at the synaptic cleft and in plasma, contributing to cardiovascular stability during Xe anaesthesia.

**Keywords:** adrenergic regulation; autonomic nervous system; norepinephrine; sympathetic activity; xenon anaesthesia

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Intraoperative hypotension is associated with increased risk of perioperative complications.<sup>1, 2</sup> Until now, it is unclear whether decreased arterial pressure causes increased postoperative mortality or it is an indicator of severity of the disease and therefore independently associated with adverse outcome.<sup>3</sup> Nevertheless, intraoperative hypotension is often unfavourable and usually requires immediate reversal with i.v. applied vasopressors, such as norepinephrine (NE). Most common anaesthetics interfere with sympathetic cardiovascular control and impair cardiac output and also vascular resistance, resulting in a decrease in mean arterial pressure.<sup>4</sup> In contrast, during xenon (Xe)-based anaesthesia, arterial pressure is maintained or even increased.<sup>5, 6</sup> However,

mechanisms of this important observation have not been identified. To assess the effects of Xe on the cardiovascular system, perioperatively administered drugs should be avoided, such that the effect of Xe alone can be identified.

The sympathetic nervous system is responsible for short-term arterial pressure regulation at rest and during cardiovascular challenges (e.g. hypovolaemia). Muscle sympathetic activity (MSA) correlates well with muscle vascular resistance. When a 50% increase in MSA was induced by lower body negative pressure, blood flow measured in the forearm and calf decreased significantly.<sup>7, 8</sup> Therefore, increased MSA may counteract arterial hypotension by increasing systemic vascular resistance.<sup>4</sup> Despite the idea

of individual regulation of sympathetic outflow to various organs, MSA correlates well with cardiac and renal sympathetic outflow.<sup>9 10</sup>

Microneurography is the only technique available to directly assess MSA in humans. Its advantage is the ability to detect rapid changes in sympathetic nerve traffic. Accordingly, it can be used to study both static and dynamic situations, for example, determination of the offset and the gain in situations of sympathetic activation induced by certain challenges. Thus, direct evaluation of MSA by microneurography may elucidate mechanisms underlying the cardiovascular stability during Xe anaesthesia in humans.<sup>4</sup>

We therefore tested the hypothesis that in healthy volunteers, administration of Xe increases NE plasma concentration by increased MSA and maintains sympathetic baroreflexes.

When NE plasma concentration was increased during Xe without sympathetic activation, we further speculated that Xe increases NE plasma concentration by the inhibition of NE transporters, irrespective of sympathetic outflow.

## Methods

### In vivo study

After IRB approval of the study protocol (IRB Medical Faculty, University of Düsseldorf, study ID MO-LKP-394, October 26, 2009), this open-label, single-group assignment phase I clinical trial was approved by the German Authorities (BfArm, EudraCT-No. 2009-012449-48) and registered at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) (NCT01043419). The clinical trial was performed in accordance with the Helsinki Declarations and GCP Regulations. Healthy volunteers, who had been recruited with the help of adverts in the medical school, were enrolled and gave written informed consent. Trial monitoring and data management were done by the clinical trials coordinating centre at the University Hospital Düsseldorf, Germany.

Eight non-premedicated, healthy, normotensive volunteers [ASA classification I, mean age 25 (2) yr, male/female: 6/2, mean BMI: 23.5 (1.8) kg m<sup>-2</sup>] were included in this study. None of the subjects was taking any medication. After an overnight fast, all subjects were studied in the supine resting position in the morning.

### Muscle sympathetic activity

Multiunit postganglionic MSA was recorded by microneurography (Supplementary methods) in the peroneal nerve at the fibular head as previously described.<sup>11–14</sup> The nerve signal was amplified, filtered (bandpass, 0.5–2 kHz), and fed through a discriminator for further noise reduction and audio monitoring (662C-3 Nerve Traffic Analysis System, University of Iowa, Bioengineering, USA). An integrated mean voltage signal was obtained by passing the original signal through a resistance–capacitance circuit. MSA recording sites were accepted when burst amplitude was at least twice as great as baseline noise, bursts occurred 1.2–1.4 s after an R-wave of the ECG, and reproducible increases in MSA were obtained in response to a standardized challenge

(apnoea of >40 s). Subsequently, MSA bursts were counted and expressed as burst frequency (bursts min<sup>-1</sup>) during 3–5 min recording periods.

### Cardiovascular variables

Heart rate was determined from the surface ECG. After determination of resting arterial pressure by oscillometry at the right upper arm, a catheter (20 G) was inserted into the left radial artery under local anaesthesia and radial arterial pressure was continuously recorded by electromanometry.

### NE plasma concentrations

Arterial blood drawn from the radial arterial catheter was sampled at specific intervals into chilled tubes with EDTA, cooled to +4°C, and immediately centrifuged. Plasma was stored at –80°C until analysis using high-performance liquid chromatography with electrochemical detection in an authorized laboratory (Dr Limbach, Heidelberg, Germany). The lower detection limit was 10 pg ml<sup>-1</sup> with a normal reference range of 165–460 pg ml<sup>-1</sup>.

### Blood gas analyses

Arterial oxygen and carbon dioxide partial pressures and also pH and base excess were assessed by standard blood gas measurements (ABL 700 series, Radiometer, Willich, Germany).

### Data recording and processing

Analogue variables (MSA, ECG, radial arterial pressure) were fed into a personal computer and digitized (sampling frequency: 200 Hz, DT 3000, Data Translation Bietigheim-Bissingen, Germany). All analyses were performed with computer support (offline) using customized software (Professor Dr M. Elam and T. Karlsson, Göteborg, Sweden).

### Sympathetic baroreflex gain during spontaneous arterial pressure fluctuations

Sympathetic baroreflex gain during spontaneous arterial pressure fluctuations were determined as previously described (Supplementary methods).<sup>14</sup> During a 3–5 min observation period, all diastolic pressures and corresponding MSA bursts were determined compensating for a baroreflex delay of 1.2–1.4 s.

For the calculation of baroreflex gain during spontaneous pressure fluctuations, all diastolic pressures of individual heartbeats were grouped into intervals of 2 mm Hg. For each of these pressure categories, the percentage of cardiac cycles associated with a sympathetic burst (burst incidence) was plotted against the mean of the individual's diastolic pressures followed by a linear regression analysis. The slope of this regression line represents the individual's sympathetic baroreflex gain during spontaneous arterial pressure fluctuations. For graphical data presentation, the lowest diastolic arterial pressure during each observation period was inserted into the linear equation of the regression analysis. Accordingly, corresponding nerve activities could be

calculated for the lowest diastolic arterial pressure observed in each individual.<sup>14</sup>

### Treatment protocol

The final 5 min of a 30 min resting period was used to calculate baseline MSA and spontaneous baroreflex gain. Subsequently, oxygen ( $F_{I_{O_2}} > 0.9$ ) was administered via a closed facemask by a commercially available Xe anaesthesia machine (Tangens 2C mobile 12, Eku Elektronik, Leiningen, Germany), indicating breathing frequency, minute ventilation, gas measurement, and EEG monitoring of anaesthesia depth (Narcotrend®, Drs B. and A. Schulz, Hannover, Germany). After subjects' adaptation to the facemask and closed circuit breathing, the final 5 min of a 20 min resting period was used to calculate MSA and spontaneous baroreflex gain. Then, arterial blood samples were obtained for determination of blood gases and catecholamine plasma concentrations. Xe anaesthesia was induced with 70% Xe in oxygen (LENOXe, Air Liquide Santé, Paris, France). After achieving steady-state conditions with end-tidal Xe concentrations of at least 60%, MSA and spontaneous baroreflex gain were determined from 3 to 5 min recording periods. Again, arterial blood samples were obtained for determination of blood gases and catecholamine plasma concentrations. At the end of the study, Xe administration was discontinued and volunteers awoke from anaesthesia.

### In vitro study

#### Cell cultures

Human epithelial kidney cells (HEK239) stably expressing human NE transporters (hNET),<sup>15</sup> their parental wild-type cells (HEK293 wild-type), and human neuroblastoma cells (SH-SY5Y; ATCC® number CRL-2266) have been characterized before.<sup>16</sup> All cell lines were cultured under equal conditions including a humidified atmosphere containing 5% carbon dioxide at 37°C and were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal calf serum and 50 U ml<sup>-1</sup> penicillin and 50 µg ml<sup>-1</sup> streptomycin. Reverse transcriptase-polymerase chain reaction (RT-PCR) and western blot analysis to confirm expression of NET and *N*-methyl-D-aspartate-receptor (NMDA-R) were performed using standard protocols as described previously (Supplementary methods).<sup>17</sup> For western blot analysis, mouse monoclonal anti-NET antibody (cat no. MAB5620; Millipore, Billerica, MA, USA) and rabbit monoclonal anti-NMDA-R1 (D65B7) antibody (cat. no. 5704; Cell Signaling, Danvers, MA, USA) were used as primary antibodies. For RT-PCR, total RNA from HEK293 cells (wild-type and hNET) and SHSY5Y cells was extracted using Trizol Reagent (Ambion, Life Technologies) according to the manufacturer's protocol. RNA was reverse transcribed and amplified. The primer sequences were as follows: for hNET 5'-GGATTGATGCCGCA ACTCAGA-3', hNET-rev 5'-GGCCTCTGGATACAGGATGA-3' (306 bp, 35 cycles), for NMDA-R subunit 1 5'-AACCTGCAGAA CCGCAAG-3', NMDA-R subunit 1\_rev 5'-GCTTGATGAGCAGGT

CTATGC-3' (333 bp, 35 cycles), and for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) 5'-ACCACAGTCCATGCCA TCAC-3' and GAPDH\_rev 5'-TCCACCACCTGTGTGTGT-3' (451 bp, 25 cycles). PCR products were electrophoresed on 1.5% agarose gels and photographed under UV light with a digital camera (Photometrics, Tucson, AZ, USA).

The i.v. anaesthetic and NMDA-R antagonist ketamine was used as a positive control as the inhibitory effect on NE uptake has been described before.<sup>18,19</sup> Additionally, the specific NMDA-R antagonist MK-801 was used to evaluate the role of specific NMDA-R antagonism on NE uptake and the highly potent NET inhibitor desipramine was used to determine the degree of unspecific NE uptake in the used cell culture model. Unless stated otherwise, reagents were purchased from Sigma Aldrich (St Louis, MO, USA).

#### Xe gas application

A pre-made gas mixture containing Xe (Xe 65%, O<sub>2</sub> 30%, CO<sub>2</sub> 5%) and a gas mixture without Xe (N<sub>2</sub> 65%, O<sub>2</sub> 30%, CO<sub>2</sub> 5%; negative control) were provided by Air Liquide Santé. To investigate the concentration-response relationship, additional gas mixtures containing 32.5% or 50% Xe were used. All experiments were performed in a specialized gas chamber under temperature control as described before (Supplementary methods).<sup>20</sup> Briefly, dishes containing cells were placed on a tray in the centre of the chamber. The respective gas mixtures were administered from below the culture dishes and distributed by a fan inside the chamber. Gas concentrations were monitored at the outlet of the chamber by a gas analyzer (Capnomatic Ultima; Datex, Helsinki, Finland). The temperature within the chamber was kept at 37°C by means of a heating plate installed at the bottom of the chamber. A temperature-controlling device (Model T48; Red Lion Controls, York, PA, USA) connected to a thermometer probe exactly regulated the heating plate and thus the temperature within the chamber.

#### Fluorescence-based uptake assay

For measurements of neurotransmitter uptake, cells were detached from tissue culture flasks, counted, and plated on poly-D-lysine (0.1 mg ml<sup>-1</sup>)-coated, black 96-well plates at a density of 1 × 10<sup>5</sup> cells per well. Subsequently, cells were allowed to adhere for at least 12 h. For pre-treatment with ketamine (1 mmol litre<sup>-1</sup>), desipramine (5 µmol litre<sup>-1</sup>), a combination of *N*-methyl-D-aspartic acid (25 µmol litre<sup>-1</sup>; NMDA) and glycine (10 µmol litre<sup>-1</sup>), MK-801 (2 µmol litre<sup>-1</sup>), or no additive (vehicle; negative control), the culture medium was replaced with substances diluted in Hank's buffered salt solution (HBSS; Gibco Invitrogen) supplemented with 0.1% bovine serum albumin. Treated cell culture plates were placed in the gas application chamber as described above and the Xe or control gas mixture was applied for 20 min. A fluorescent substrate for neurotransmitter transporters<sup>21</sup> was then added following the manufacturer's recommendations (Neurotransmitter Uptake Kit; Molecular Devices, Sunnyvale, CA, USA) followed by

incubation for 20 min during continued gas application. The fluorescent substrate is combined with a masking dye that prevents fluorescence unless the substrate has been transported into the cell. Therefore, the fluorescence intensity of samples at a wavelength of 520 nm was detected after excitation at 440 nm using a fluorescence plate reader (Synergy 2; BioTek Instruments, Winooski, VT, USA) as a measure for substrate uptake immediately after the end of incubation time.

### [<sup>3</sup>H]NE uptake assay

To determine intracellular [<sup>3</sup>H]NE content, cells were detached from tissue culture flasks, counted, and plated on poly-D-lysine (0.1 mg ml<sup>-1</sup>)-coated, clear 6-well plates at a density of 1 × 10<sup>5</sup> cells per well. After at least 12 h incubation for cell adherence, pre-treatment with ketamine (1 mmol litre<sup>-1</sup>), desipramine (5 μmol litre<sup>-1</sup>), NMDA (25 μmol litre<sup>-1</sup>), glycine (10 μmol litre<sup>-1</sup>), MK-801 (2 μmol litre<sup>-1</sup>), or vehicle (HBSS buffer; negative control) during gas application as described above was performed. After 20 min of gas application, [<sup>3</sup>H]NE (200 nmol litre<sup>-1</sup>) was added. After a further 5 min, [<sup>3</sup>H]NE uptake was stopped by washing all samples with ice-cold phosphate-buffered saline (PBS; Gibco Invitrogen). Cells were then lysed by 5 min exposure to a solution containing 300 mmol litre<sup>-1</sup> NaCl, 25 mmol litre<sup>-1</sup> Tris-HCl, and 0.1% Triton-100. The cell lysates were then resuspended with 1 ml PBS and transferred to analysis tubes containing 4 ml of scintillation fluid (Ultima Gold; PerkinElmer, Waltham, MA, USA). The amount of intracellular [<sup>3</sup>H]NE was measured by means of decay counts per minute using a liquid scintillation counter (Tricarb 2100 TR; Packard, Berkshire, UK).

### Data analysis and statistics

Values from fluorescence-based uptake measurements and decay counts per minute from scintillation counting were normalized to samples that were pre-treated with vehicle (negative controls), while subtracting the mean background fluorescence that was detected despite maximal transporter inhibition with desipramine (5 μmol litre<sup>-1</sup>).

All data are expressed as mean [standard deviation (SD)]. Differences between means were tested by Student's *t*-test or one- or two-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test as appropriate. Graph Pad Prism Software version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). A *P*-value of <0.05 was considered significant.

## Results

### In vivo

Xe anaesthesia was successfully performed in all subjects reaching an end-tidal Xe concentration of 63 (6)%. All volunteers lost consciousness when the end-tidal Xe concentration reached 40–50%, and did not respond to verbal or touch stimuli. The EEG-index (Narcotrend<sup>®</sup>) decreased from 98 (1) to 46 (10) indicating anaesthesia. Subjects' spontaneous movements resulted in a loss of MSA recording sites in five

out of eight subjects during emergence from anaesthesia. Two subjects with a history of postoperative nausea and vomiting during previous anaesthesia experienced short-lasting nausea and vomiting immediately after awakening. Further adverse events were not observed.

MSA was similar during oxygen breathing and during breathing of room air without a mask 19 (9) and 20 (10) bursts min<sup>-1</sup>, respectively. Sympathetic baroreflex gain during spontaneous arterial pressure fluctuations significantly decreased during oxygen breathing from 4.6 (1.7) to 3.7 (1.5) bursts 100 heartbeats<sup>-1</sup> mm Hg<sup>-1</sup> (*P*=0.02), while arterial pressure and heart rate remained unchanged.

After induction of Xe anaesthesia (70%), MSA was not altered. Representative recordings of MSA during oxygen breathing and also during Xe anaesthesia of all subjects are shown in Figure 1A. Generally, Xe anaesthesia did not alter MSA (Fig. 1B) and sympathetic baroreflex gain during spontaneous arterial pressure fluctuations (Fig. 2). However, despite unchanged sympathetic outflow, NE plasma concentrations increased significantly from 156 (55) to 295 (106) pg ml<sup>-1</sup> (*P*<0.01; Fig. 3). This increase in NE plasma concentration was associated with a significant increase in mean arterial pressure from 93 (4) to 107 (6) mm Hg (*P*<0.05), while heart rate remained unchanged [awake 63 (10) beats min<sup>-1</sup>; Xe 70 (10) beats min<sup>-1</sup>; *P*=0.12].

Xe anaesthesia was also associated with an increase in spontaneous breathing frequency and minute ventilation (Table 1). Arterial carbon dioxide partial pressure and pH were not altered; oxygen partial pressure was decreased as after Xe anaesthesia (*F*<sub>I<sub>O</sub>2</sub> ≈ 0.35; Table 1).

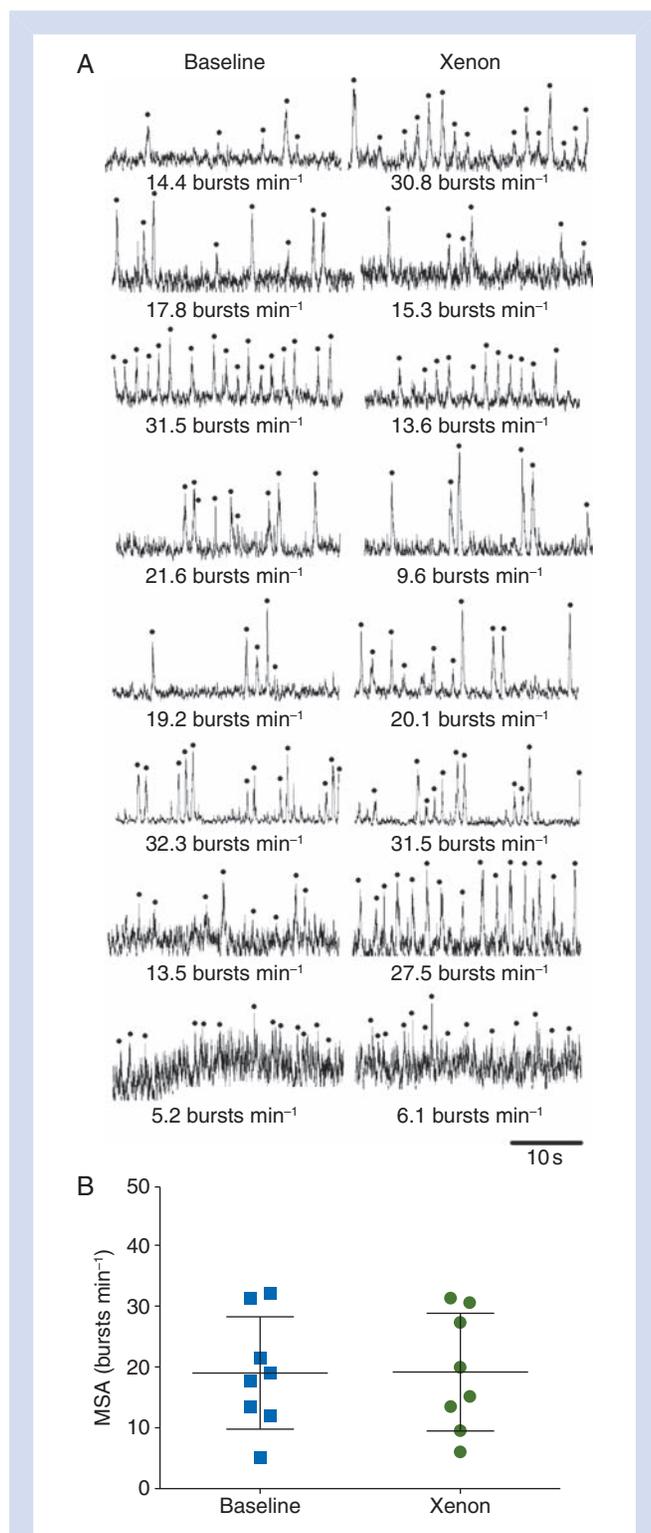
### In vitro

RT-PCR of hNET HEK293 and SH-SY5Y cells confirmed stable expression of hNET, while hNET expression was not detected in parental HEK293 wild-type cells (Supplementary Fig. S1). Additionally, stable gene expression of NMDA-R subunit 1 was detected only in SH-SY5Y cells, and was absent from HEK293 hNET and HEK293 wild-type cells (Supplementary Fig. S1). Western blot analysis of protein expression confirmed these findings (Supplementary Fig. S1).

Fluorescence-based uptake experiments (*n*=4) revealed that fluorescence intensity was decreased in HEK293 hNET cells (not expressing NMDA-R) by ketamine (1 mmol litre<sup>-1</sup>; *P*<0.01; Fig. 4A). On the contrary, Xe (65%), MK-801 (2 μmol litre<sup>-1</sup>), and also NMDA (25 μmol litre<sup>-1</sup>) and glycine (10 μmol litre<sup>-1</sup>) in combination did not exhibit any effects (Fig. 4A). As expected, there was no specific increase in fluorescence intensity and therefore NET activity in HEK 293 wild-type cells (data not shown).

In contrast, Xe reduced fluorescence intensity in SH-SY5Y neuroblastoma cells expressing NMDA-R in addition to hNET (*P*<0.01, Fig. 4B). While MK-801 and ketamine also reduced NET activity, both *P*<0.01, the combination of these substances with Xe had no additive effect (Fig. 4B).

Radiometric results (*n*=6) confirmed the inhibition of NE uptake in SH-SY5Y cells by Xe (*P*<0.01; Fig. 5) and MK-801,



**Fig 1** MSA during Xe mono-anaesthesia. (A) Original recordings of MSA of each subject ( $n=8$ ) in the awake state (baseline) and during Xe anaesthesia. Bursts considered for analysis are indicated by a dot. In four individuals, there was no change in MSA, while two exhibited an increase and two a decrease. (B) Xe anaesthesia does not alter MSA compared with baseline values as determined from 3 to 5 min recording periods. Data presented as individual data points and mean (sd). Blue squares are baseline values and green circles are during Xe anaesthesia.

$P<0.01$ ). The combination of Xe and MK-801 did not exhibit additive effects compared with the application of MK-801 alone. The agonistic combination of NMDA and glycine reversed the inhibition of NE uptake by Xe almost completely (Fig. 5).

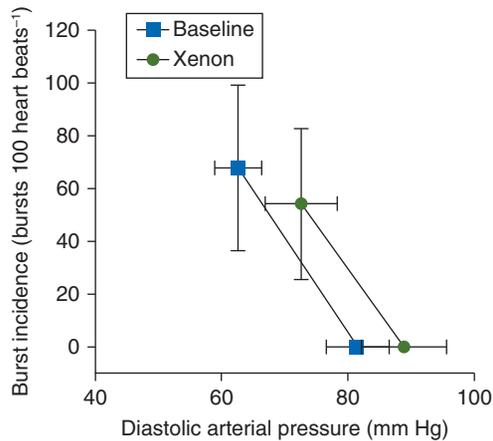
To investigate a possible concentration–response relationship, additional experiments were conducted comparing the effect of 0, 32.5, 50, and 65% Xe (Fig. 6). While 32.5% Xe did not lead to a significant effect, 50% Xe resulted in a reduction in NE uptake function ( $n=6$ ;  $P<0.05$ ). Increasing Xe concentration to above 65% had no further effect.

## Discussion

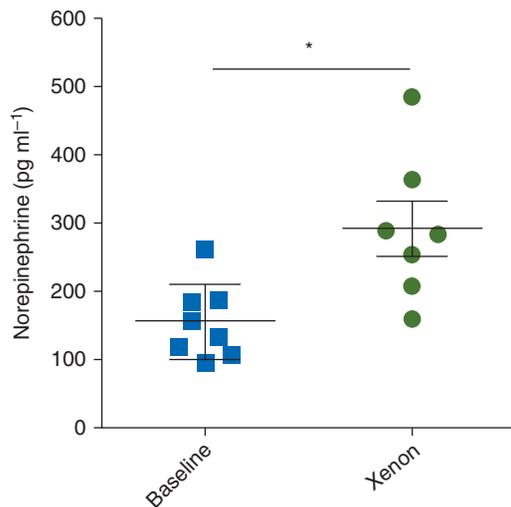
Despite unchanged sympathetic outflow to muscle, NE plasma concentrations almost doubled during Xe anaesthesia in healthy volunteers. As shown *in vitro*, clinically relevant concentrations of Xe decreased the uptake of NE in human neuroblastoma cells by an NMDA-R-dependent mechanism. Thus, our findings explain increased NE concentrations at the synaptic cleft and in plasma, contributing to the observed cardiovascular stability in patients during Xe.

We have demonstrated that NE plasma concentrations are increased during Xe mono-anaesthesia in healthy volunteers, despite unchanged sympathetic outflow to muscle. Since sympathetic outflow to muscle correlates well with cardiac and renal sympathetic activity, it is rather unlikely that sympathetic activation to other organ systems accounts for the observed increase in NE plasma concentrations.<sup>4 9 10 22</sup> Nevertheless, how can an increase in NE plasma concentration be explained in the face of unchanged sympathetic activity? NE reuptake transport restores about 90% of NE originating predominantly from sympathetic nerves, while only about 10% of released NE reaches the blood stream.<sup>23</sup> We found that Xe inhibited NE uptake by approximately one-third at a concentration that is commonly used for maintaining anaesthesia. Considering that a reduction in NET reuptake function could possibly lead to an increase in NE escaping from the synaptic cleft (extra-neuronal turnover) by up to four times, this effect may lead to increased systemic spillover and therefore contribute to the doubled NE plasma concentration and the observed sympathetic effects.<sup>24</sup> Nevertheless, other mechanisms such as reduced hepatic catecholamine clearance<sup>25</sup> or increased release of NE<sup>26</sup> during Xe anaesthesia cannot be excluded.

Yoshida and colleagues<sup>26</sup> recently reported that Xe increases the release of NE in the cerebral cortex of rats as measured using a microdialysis technique. Although they clearly demonstrated that Xe at a clinically relevant concentration induced a considerable increase in dialysed NE and therefore extracellular NE concentration, their results do not allow discrimination between increased NE release and inhibited reuptake as the responsible mechanism. Our data clearly support their findings and suggest that inhibited reuptake might play an important role for increased NE during Xe application. Increased central noradrenergic activity yields increased sympathetic outflow that in turn is



**Fig 2** Sympathetic baroreflex gain during Xe mono-anaesthesia. Sympathetic baroreflex gain during spontaneous arterial pressure fluctuations in the awake state ( $F_{I_{O_2}} > 0.9$ ) and during Xe anaesthesia. Although the overall range of observed diastolic arterial pressures is slightly shifted to higher pressures by Xe, the slope of the regression line indicating the sympathetic response to spontaneous arterial pressure variations is not altered ( $n=8$ ). Data presented as mean (SD). Blue squares are baseline values and green circles are during Xe anaesthesia.



**Fig 3** Effect of Xe mono-anaesthesia on NE plasma concentrations. NE plasma concentration was significantly increased during Xe anaesthesia compared with awake state ( $n=7$ ). Data are presented as individual data points and mean (SD). Blue squares are baseline values and green circles are during Xe anaesthesia.  $*P < 0.01$  ( $n=7$ ; baseline vs Xe treatment, paired Student's *t*-test).

immediately decreased by baroreflex inhibition. Thus, MSA was not altered, despite increased arterial pressure, indicating an altered setpoint of the baroreflex.<sup>27</sup>

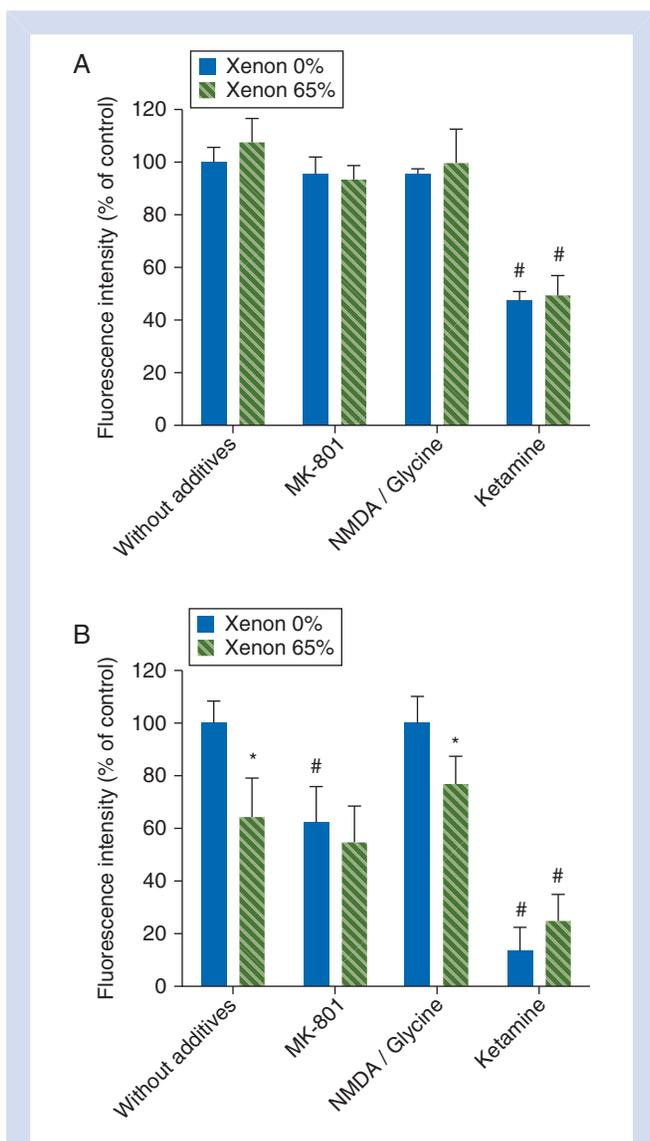
**Table 1** Results of blood gas analysis and respiratory parameters. Data are presented as means (SD).  $*P < 0.01$

|   | Awake ( $F_{I_{O_2}} > 0.9$ ) | Xenon anaesthesia (end-tidal concentration 63%) |
|---|-------------------------------|---|
| $P_{O_2}$ (mm Hg)                             | 471 (29)                      | 173 (19)*                                       |
| pH  | 7.41 (0.02)                   | 7.43 (0.04)                                     |
| $P_{CO_2}$ (mm Hg)                            | 40 (3)                        | 45 (6)  |
| Base excess (mmol litre <sup>-1</sup> )       | 1.0 (1.1)                     | 1.3 (1.6)                                       |
| Respiratory rate (bpm)                        | 12.1 (2.4)                    | 23.1 (7.6)*                                     |
| Minute ventilation (litre min <sup>-1</sup> ) | 6.9 (2.0)                     | 10.9 (2.1)*                                     |

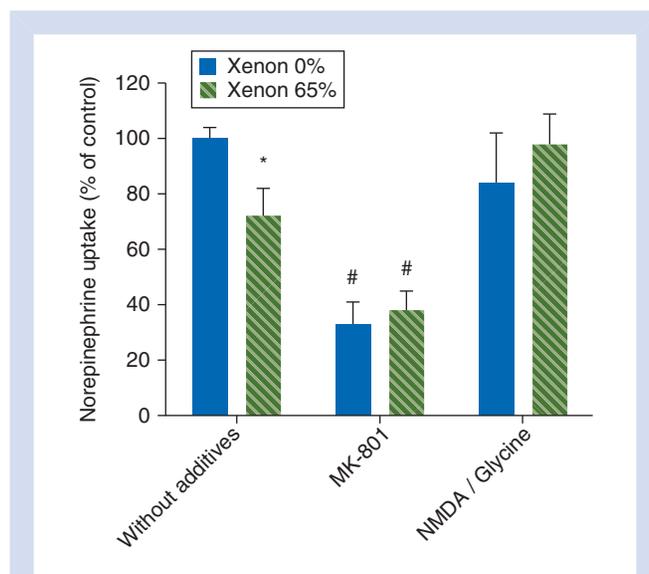
Despite an increase in arterial pressure and unimpaired baroreflexes, heart rate surprisingly did not decrease. Regulation of heart rate is even more complex than sympathetic baroreflexes to the vasculature because it is immediately modulated by parasympathetic innervation as well. However, we did not study parasympathetic outflow to the heart in our volunteers. Nevertheless, a similar line of arguments may apply also to heart rate control during Xe compared with muscle sympathetic outflow to the vasculature. First, sympathetic baroreflex gain is not altered in our volunteers during Xe, despite increased arterial pressure. We believe that this observation is caused by the inhibition of NE uptake in the brain and therefore increasing sympathetic outflow. Thus, the setpoint of sympathetic baroreflexes may be altered to higher arterial pressures. Central sympathetic innervation of the heart may be modulated in a similar manner.

Xe is known to exert its anaesthetic and analgesic properties at least in part by the inhibition of NMDA-Rs<sup>28–30</sup> similar to ketamine.<sup>31</sup> Racemic ketamine increased arterial pressure and NE plasma concentrations, while MSA was actually decreased due to baroreflex inhibition.<sup>13</sup> Instead of direct effects on sympathetic outflow, ketamine causes an inhibition of NET function leading to impaired reuptake in part depending on NMDA-R expression as reported earlier and confirmed by our data.<sup>18–32</sup> Accordingly, a greater fraction of NE released from neurones reaches the systemic circulation, leading to an increase in NE plasma concentration. While Xe inhibits NE uptake via an NMDA-R-dependent mechanism, ketamine inhibits NET function even in the absence of NMDA-R. This phenomenon warrants further investigation.

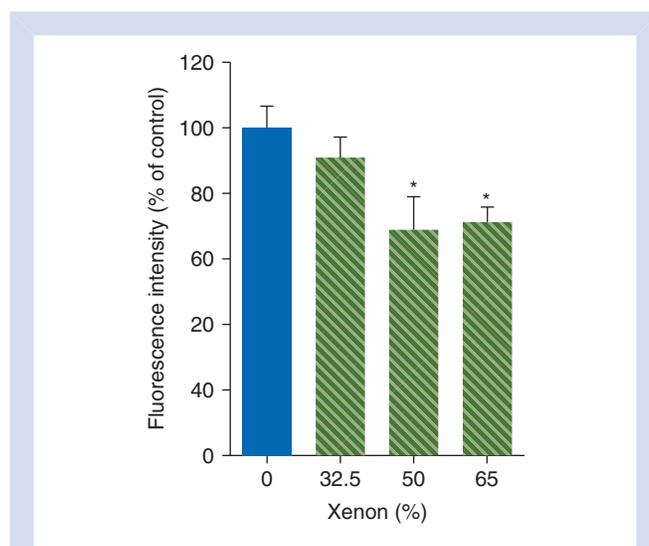
We would like to point out that the inhibition of NET function despite unchanged MSA is a reasonable mechanism for even increased cardiac output and systemic vascular resistance in patients undergoing Xe-based anaesthesia.<sup>33</sup> Even in patients with markedly impaired left ventricular function undergoing cardioverter defibrillator implantation, arterial pressure and cardiac function were not depressed. Furthermore, our results explain maintained left ventricular



**Fig 4** Effect of Xe on neurotransmitter transporter function. (A) In HEK293 cells overexpressing only hNETs in the absence of NMDA receptors, fluorescence intensity as a measure for neurotransmitter uptake was not changed by incubation with 65% Xe compared with control conditions. Ketamine (1 mmol litre<sup>-1</sup>) inhibited neurotransmitter uptake. Coapplication of Xe had no additive effect. (B) In human neuroblastoma cells (SH-SY5Y) endogenously expressing NETs and NMDA receptors, Xe inhibited neurotransmitter uptake significantly. Specific NMDA inhibition by MK-801 (2 µmol litre<sup>-1</sup>) mimicked this effect, while additional agonistic stimulation with NMDA (25 µmol litre<sup>-1</sup>) and glycine (10 µmol litre<sup>-1</sup>) restored neurotransmitter uptake in the presence of Xe to the level of controls. Data are presented as mean (sd). Blue bars are values after 0% Xe treatment, and green striped bars are values after treatment with 65% Xe. \**P*<0.01 vs Xe 0%; #*P*<0.01 vs without additives (*n*=4; two-way ANOVA and *post hoc* Bonferroni test).



**Fig 5** Effect of Xe on uptake of [<sup>3</sup>H]NE. Confirming the results of fluorescence-based measurements, Xe (65%) inhibited the uptake of [<sup>3</sup>H]NE. Specific NMDA inhibition by MK-801 (2 µmol litre<sup>-1</sup>) mimicked this effect, while the agonistic stimulation with NMDA (25 µmol litre<sup>-1</sup>) and glycine (10 µmol litre<sup>-1</sup>) reversed it. Data are presented as mean (sd). Blue bars are values after 0% Xe treatment, and green striped bars are values after treatment with 65% Xe. \**P*<0.01 vs Xe 0%; #*P*<0.01 vs without additives (*n*=6; two-way ANOVA and *post hoc* Bonferroni test).



**Fig 6** Concentration-dependent inhibition of neurotransmitter transporter function. The effect of 32.5% was not significantly different from control conditions, but 50% Xe inhibited neurotransmitter uptake. Increasing the concentration to 65% Xe did not enhance inhibition. Data are presented as mean (sd). Blue bars are values after 0% Xe treatment, and green striped bars are values after treatment with Xe. \**P*<0.05 (*n*=6; all values vs controls without Xe, one-way ANOVA and *post hoc* Bonferroni test).

contractility during Xe anaesthesia, either in patients without cardiovascular disease or in those awaiting coronary artery bypass surgery.<sup>5</sup> In contrast to most other anaesthetics,

not only resting sympathetic outflow but also sympathetic baroreflexes are not impaired even at slightly increased arterial pressure.<sup>4</sup> Thus, the cardiovascular system is still able to respond to challenges, for example, perioperative hypovolaemia or haemorrhage, despite general anaesthesia.

In many animal and human studies, Xe has been administered in combination with opioids so that the described cardiovascular effects could not be attributed to Xe alone. Since NE plasma concentrations were reported to be decreased in patients anaesthetized with a combination of Xe and remifentanyl<sup>34</sup> and increased in dogs,<sup>35</sup> the underlying mechanism for the observed haemodynamic stability had not been pinpointed to the sympathetic nervous system. Since opioids decrease MSA at rest and sympathetic baroreflex gain,<sup>12</sup> the combination of Xe and opioids is clinically very favourable but does not allow an evaluation of the intrinsic effect of Xe.

Whether maintained arterial pressure during Xe-based anaesthesia translates into a decrease in perioperative morbidity and mortality is currently being evaluated in larger randomized controlled multicentre trials ([www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT01120405 and NCT00919126).

In addition to the potential effects of the described mechanism on haemodynamics, enhanced agonistic effects of NE on  $\alpha$ -2-adrenoceptors may have widespread implications for clinical anaesthesia.<sup>36</sup> Furthermore, NMDA-R antagonism and NE are known to be key mediators in pain modulation.<sup>37</sup> Therefore, the findings of this study encourage further investigation of spinal inhibitory mechanisms of Xe-mediated antinociception.

Our findings suggest that the effect of Xe on NE uptake depends on the presence of NMDA-R. Furthermore, specific inhibition of NMDA-R by MK-801 mimics the effect of XE on NET activity. This finding is supported by another study demonstrating that MK-801 significantly increases NE release from rat prefrontal cortex.<sup>38</sup> Nevertheless, the exact mechanism by which NMDA-R inhibition by Xe induces a reduction in NET activity remains to be elucidated.

### Limitations

It was the goal of our study to assess the effects of Xe anaesthesia on muscle sympathetic outflow. Accordingly, we did not administer other additional anaesthetics despite the comparatively low anaesthetic potency of Xe (MAC<sub>50</sub> 50–70 vol%). Cardiovascular variables were recorded in unconscious participants while Narcotrend® EEG monitoring (values of 40–60) confirmed general anaesthesia. Participants were spontaneously breathing via a facemask so that tracheal intubation and mechanical ventilation were avoided. Nevertheless, ~1 MAC<sub>50</sub> of Xe was achieved without significant respiratory depression. In contrast, even a doubling of minute ventilation was observed while end-tidal P<sub>CO2</sub> remained unchanged. Since oxygenation was not impaired, we may exclude pulmonary atelectasis but rather assume increased impact of dead space ventilation cardiac output to be the major cause of increased minute ventilation

during Xe anaesthesia. Furthermore, metabolism and CO<sub>2</sub> production are ultimately linked to cardiac output. The inhibition of NE uptake during unchanged sympathetic outflow may increase cardiac output and metabolism. As observed in a canine model, i.v. infusion of NE increases cardiac output and CO<sub>2</sub> production in a linear fashion.<sup>39</sup> Accordingly, we speculate that increased NE concentration at the level of adrenergic receptors may have caused the observed increase in P<sub>CO2</sub> and ventilation. As heart rate did not change significantly during Xe anaesthesia, increased cardiac inotropy may be speculated to be the cause. However, a slight decrease in heart rate without changes in arterial pressure under Xe anaesthesia after induction with propofol has been reported in volunteers previously.<sup>40</sup> These, at first glance, contradictory results to our observation can be explained: subjects in our study were not receiving any other medication while in the study by Rex and colleagues, propofol was administered for induction of anaesthesia. Accordingly, residual propofol at calculated plasma concentrations even below 1  $\mu\text{g ml}^{-1}$  may have caused a reduction in efferent sympathetic activity<sup>41 42</sup> counteracting the sympathetic effects of Xe.

MSA may be influenced by respiration and at least in males, breathing rate correlates positively with sympathetic activity and total peripheral resistance.<sup>43</sup> However, while all volatile anaesthetics increase spontaneous breathing rate up to several hundred per cent, isoflurane, sevoflurane, and desflurane markedly decrease MSA.<sup>4</sup> Thus, it seems rather unlikely that MSA during Xe anaesthesia is maintained solely by increased breathing rate.

We would have preferred to evaluate the effect of Xe on the baroreflex setpoint more extensively reported previously.<sup>13</sup> Unfortunately, because of spontaneous movements during Xe anaesthesia, it was not possible to normalize arterial pressure by titrating nitroprusside before losing the MSA recording site.

One limitation of the *in vitro* observations is that the fluorescence-based assay does not only measure the uptake function of NET but also the function of dopamine and serotonin transporters.<sup>21</sup> Therefore, after achieving positive results by the fluorescent screening method, a specific uptake assay using radiolabelled NE was used to confirm that Xe reduces NE uptake.

In conclusion, NE plasma concentrations and arterial pressure increase during Xe anaesthesia, despite MSA and sympathetic baroreflex gain remaining unaltered. Xe inhibits NE uptake *in vitro* in an NMDA-R-dependent manner. This mechanism may be responsible for increased concentrations of NE at the synaptic cleft and in plasma and therefore contribute to the haemodynamic stability of patients during Xe anaesthesia.

### Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

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## Declaration of interest

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