British Journal of Anaesthesia **104 (3):** 351–8 (2010)
doi:10.1093/bja/aep395 Advance Access publication January 29, 2010

PAIN

Intranasal application of xenon: describing the pharmacokinetics in experimental animals and the increased pain tolerance within a placebo-controlled experimental human study

G. Froeba[1], M. Georgieff[1], E. M. Linder[1], K. J. Föhr[1], H. U. Weigt[2], T. F. Holsträter[3], M. A. Kölle[4] and O. Adolph[1]*

[1]Department of Anaesthesiology, University Hospital of Ulm, Steinhoevelstrasse 9, 89075 Ulm, Germany. [2]Department of Anaesthesiology, Klinik am Plattenwald, SLK Kliniken Heilbronn, Am Plattenwald 1, 74176 Bad Friedrichshall, Germany. [3]Department of Anaesthesiology, Federal Armed Forces Medical Centre Ulm, Oberer Eselsberg 40, 89070 Ulm, Germany. [4]Department of Psychiatry, University Hospital of Ulm, Leimgrubenweg 12-14, 89075 Ulm, Germany.

**Corresponding author. E-mail: oliver.adolph@uniklinik-ulm.de*

Background. Pain sensitizes the central nervous system via *N*-methyl-D-aspartate receptors (NMDARs) leading to an enhancement of pain perception. However, the enhanced responsiveness of pain-processing areas can be suppressed by subanaesthetic doses of the NMDAR antagonist xenon. To analyse the strength of the analgesic effect of low-dose xenon using new economical application methods, we tested xenon applied nasally in an experimental human pain setting.

Methods. We tested 10 healthy volunteers using a multimodal experimental pain testing in a randomized double-blind placebo-controlled repeated measures study. Xenon was administered using a novel low-pressure intranasal application device. Additionally, we measured xenon concentrations in blood samples obtained from intracranial veins of experimental animals to describe the pharmacokinetics of intranasally applied xenon in the cerebral compartment.

Results. Intranasal application of xenon at a rate of 1.0 litre h^{-1} for 30 min significantly increased pain tolerance of volunteers to ischaemic (+128%), cold (+58%), and mechanical (+40%) stimulation ($P<0.01$). However, 60 min after terminating the application of xenon, there was no significant alteration of pain tolerance compared with placebo. Cranial blood concentrations of xenon in pigs reached a steady state of ~450 nl ml^{-1} after 5 min.

Conclusions. In this placebo-controlled experimental human study, we described the increased pain tolerance induced by intranasally applied xenon. On the basis of our results, we conclude that intranasally administered xenon has analgesic properties and suggest that the novel application device presented here offers new possibilities for the administration of NMDAR antagonists within a multimodal analgesia approach.

Br J Anaesth 2010; **104**: 351–8

Keywords: anaesthetics gases; drug delivery, nasal; pain, experimental; pharmacokinetics, uptake

Accepted for publication: December 11, 2009

Intranasal application of xenon

The *N*-methyl-D-aspartate receptor (NMDAR) is an excitatory amino acid receptor that is involved in the modulation of prolonged pain states induced by sensitization processes.[1–3] Hence, one strategy for relieving postoperative pain is to use NMDAR antagonists to prevent or minimize the induction of central sensitization.[4–6] The preponderance of evidence suggests that xenon acts via non-competitive inhibition of NMDAR.[7–9] However, it cannot be ruled out that other targets of xenon mediate inhibitory effects of the noble gas.[10–12] Up to now, xenon's safety and efficacy profile appears to be

unequalled and only its relatively high costs and limited resources have precluded its widespread clinical use.[13]

Intranasal drug administration is a non-invasive method and allows therapeutic agents that do not cross the blood–brain barrier to be delivered to the central nervous system (CNS). This method eliminates the need for systemic delivery, thereby reducing unwanted systemic side-effects.[14–16] Lipid-soluble agents are absorbed rapidly and efficiently across the nasal membrane into the bloodstream via the transcellular pathway with a plasma profile resembling that of an i.v. injection. Once they reach the bloodstream, they can diffuse freely through the blood–brain barrier and reach the CNS. This diffusion is qualified by the degree of lipid solubility and molecular size, with small lipophilic atoms like xenon passing through the membrane more easily than larger and polar molecules.[14] Therefore, intranasal delivery may offer an economical new strategy for targeting xenon to the brain avoiding excessive loss by exhalation.

Rationale for the study

In a recently published study, we showed an enhanced responsiveness of pain-processing areas to repeated painful electrical stimulation using functional magnetic resonance imaging (fMRI). This enhancement was suppressed by the NMDAR antagonist xenon at subanaesthetic doses, providing evidence for an involvement of NMDAR in pain-evoked synaptic plasticity in the human brain.[3] Since there is no objective measure of subjects' feeling of pain, and there is no single experimental pain test that will be applicable to test all classes of anaesthetic/analgesic drugs, multimodal (e.g. mechanical, thermal, chemical pain) and multistructure (e.g. skin, muscle) pain induction techniques are essential. It is important to use stimuli that are longer lasting and cover large area, so that temporal and spatial nociceptive mechanisms can be activated.[17] Therefore, we tested the analgesic effect of intranasally applied xenon within a double-blind placebo-controlled repeated measures study design and chose *pain tolerance* as the end-point for the measurement of the drug effect. To gain a multimodal and multistructural setting, we used pressure, ice-water, and ischaemic pain tests. Since pain processing can also be impaired by pure hypnotic drugs,[18–20] vigilance was determined by using an alertness test. Time intervals of the study design were organized according to the pharmacokinetics of intranasally applied xenon, as *a priori* determined in cranial blood of experimental animals.

Methods

Subjects

Ten healthy male and unpaid volunteers (mean age 34.4 yr) gave written informed consent before the study conforming with the Declaration of Helsinki and in accordance with the ethics board of the University of Ulm. All subjects were right-handed physicians working at the Ulm University Hospital. None had a history of neurological or psychiatric disorders or any sign of a nasopharyngeal disease. Allergies, any drug therapy, a history of adverse reactions to anaesthetics, and regular consumption of more than 20 g of alcohol per day[21] were the exclusion criteria.

Application of xenon

The xenon application system contained a low-pressure metallized gas reservoir, xenon-proofed tubes connected by multidirectional stopcocks and tube clamps (B/Braun, Melsungen, Germany), a pressure control unit (data recording; Greisinger GMH 3150, Regenstauf, Germany), two pressure-tight xenon-permeable latex application devices, and two drain tubes leading to exhaust (avoiding air contamination). Air and xenon were delivered into the latex application devices at a rate of 1.0 litre h^{-1} by an adjustable peristaltic pump (Bäder 75D, Ulm, Germany). A constant pressure of 3.0 kPa (30 mbar) was maintained by a second peristaltic pump regulating the flow within the drain tubes leading to exhaust. Therefore, concentrations of xenon within the devices could be kept constant over time and never decreased below 94% ($[Xe]_{exhaust}$=96.2 (SD 1.9)%; n=10). Since the nasopharyngeal passage is completely sealed by the latex balloons, nasal breathing during application is impossible and the drug delivery is therefore independent of respiration. To illustrate the delivery system, we provide Supplementary Fig. S4, showing MRI views of a volunteer and a schematic representation of the application system. Xenon 4.0 was obtained from Messer GmbH (Krefeld, Germany).

Haemodynamic and respiratory parameters

Haemodynamic and respiratory parameters upon intranasal application of xenon were studied in healthy volunteers using a randomized double-blind placebo-controlled repeated measures study design. In 22 sessions, at least 5 days apart, they were exposed to either xenon or placebo (air) during 45 min each while being monitored with a 5-lead ECG, non-invasive arterial pressure sampling, and pulse oximetry (Datex, Helsinki, Finland) at a sample rate of 2.5 min. The end-expiratory carbon dioxide concentration (CO_2) was measured by connecting the volunteers to a Cicero EM anaesthesia workstation (Dräger, Lübeck, Germany).

Blood–gas analysis

With the approval of the institutional animal care committee and performed in accordance with the legal regulations for use of laboratory animals, we investigated seven pigs (four females and three males) aged 13–16 weeks

weighing 42 kg (median; range 38.2–44.1 kg). In this study, we used a well-established pattern of drugs and monitoring devices.[22 23] The animals were pre-medicated with azaperone 4 mg kg^{-1} i.m. and atropine 0.05 mg i.m. 1 h before anaesthesia was induced with i.v. atropine 0.01 mg kg^{-1} (Sigma-Aldrich, Deisenhofen, Germany), ketamine 2 mg kg^{-1} (Pfizer, Lysaker, Norway), and pentobarbital 8–10 mg kg^{-1} (Sigma-Aldrich).

To facilitate tracheal intubation, the pigs were paralysed using alcuronium dichloride (0.25 mg kg^{-1}; Roche, Basel, Switzerland). Ventilation was performed with 30% O_2 in N_2 using a standard semi-closed ventilator (Cicero EM, Drägerwerk AG, Lübeck, Germany). Ventilatory settings throughout the whole experiments were tidal volume V_T=10–14 ml kg^{-1} (adjusted to maintain Pa_{CO_2} 5.0–5.6 kPaf), ventilator frequency f=12 min^{-1}, and a PEEP of 5 cm H_2O.[22] Body temperature was maintained between 37.5°C and 38.5°C with a heating blanket.

A femoral artery was exposed to insert a catheter for blood sampling and continuous monitoring of arterial pressure. Anaesthesia was maintained using sevoflurane (1.8–2.3% end-expiratory; Abbott, North Chicago, IL, USA) and a single bolus of buprenorphine 0.03 mg kg^{-1} (Reckitt Benckiser, London, UK). To ensure an adequate plane of anaesthesia, its depth was assessed by haemodynamic variables (heart rate, arterial pressure) and continuous EEG monitoring (Neurotrac, Interspec, Inc., Conshohocken, PA, USA). The 95% spectral edge frequency remaining below 15 Hz during the whole experiment. No relevant haemodynamic response (arterial pressure, heart rate) or changes of alertness (EEG) to intubation or craniotomy was observed. For sampling cerebral venous blood, a burr hole was drilled into the skull over the midline and a catheter was passed into the sagittal sinus.[24] Xenon concentrations of the blood samples were measured by static headspace gas chromatography mass spectrometry (headspace GCMS).[23]

Study design

Assessment of pain was performed within a randomized double-blind placebo-controlled study design. By use of a table of random numbers, volunteers were allocated into one of two groups (*xenon–placebo* or *placebo–xenon*). A study supervisor, who did not participate in the assessment, prepared a gas reservoir (low-pressure metallized gas bags) filled with either xenon or air as a placebo. The investigator who performed the pain experiments was unaware of the type of gas applied. Volunteers were also blinded to their group assignment and blinding was maintained throughout the study. Participants were informed that the intranasal application device would contain either xenon or placebo (air). The volunteers received a standardized oral and written instruction on the tests and on the definition of *pain tolerance threshold*. They were asked to abstain from alcohol and excessive coffee consumption (defined as five cups or 400 mg caffeine)[25] for 24 h and from drinking and eating for 6 h before pain testing.

In 20 sessions, at least 7 days apart, volunteers were exposed to either xenon or placebo for 30 min (five subjects, air–xenon; five subjects, xenon–air). Pain tolerance to cold, pressure, and ischaemic stimuli were obtained three times (*reference* at 0 min, *exposure* after 30 min of xenon or placebo administration, *post-exposure* 60 min after terminating intranasal gas exposure) within each session. Note that the *exposure* value was taken while the intranasal gas application was continued (Fig. 1). To perform pain tests within the *exposure* session, 17.8 (2.35) min were required under xenon and 17.2 (1.93) min were spent under placebo (P=0.54).

Pressure pain test

An electronic pressure algometer (TYPE2, Somedic AB, Stockholm, Sweden)[18 26 27] was used to determine the maximally tolerated pressure on the interdigital skin fold of the third and fourth fingers of the dominant hand (determined by using the *Edinburgh Handedness Inventory*). A probe with a surface area of 0.95 cm^2 was used, and the pressure was increased with a rate of 30 kPa s^{-1}. The volunteer stopped the procedure when the *pain tolerance threshold* (highest stimulation intensity tolerated)[17] was reached. To avoid tissue damage, the cut-off limit was 1500 kPa.[26]

Ice-water pain test

The dominant foot was immersed up to the lateral malleolus in ice-saturated water as described [0 (0.5)°C].[26 28] The volunteer stopped the procedure when the pain tolerance threshold was reached. For safety reasons, the cut-off limit was 180 s.

Ischaemic pain test

A maximal-effort tourniquet test was performed on the dominant arm. After compression of a power grip hand exerciser at a metronome-controlled rate of 1.0 Hz, an arterial pressure cuff was inflated to 250 or at 100 mm Hg above systolic pressure, whichever was the higher.[26] To avoid ischaemic tissue damage, the cut-off limit was 900 s. Exercises were continued until the *pain tolerance threshold* was reached.

Alertness tests

In 10 sessions, at least 5 days apart, five healthy volunteers (mean age 31.0 yr) were exposed to either xenon or placebo (air) for 30 min after a *reference* value of reaction times (RTs) (psycho-motor RTs, alertness) had been taken. Then, the RT tests (n=200 per session) were performed while intranasal gas application was continued (*exposure* value). Participants were positioned in front of a computer screen and were instructed to respond to the appearance of a white cross in the middle of the black

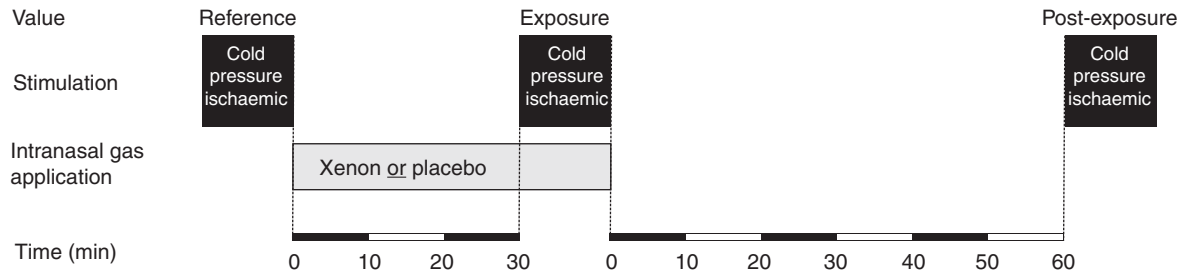

Fig 1 Study design. Pain tolerance values (cold, pressure, ischaemic) were obtained three times (*reference* at 0 min, *exposure* after 30 min of xenon or placebo administration, *post-exposure* 60 min after termination of intranasal gas exposure) within each session.

screen by pressing a response button. RTs were recorded continuously by the computer.

Two trial types were implemented in the paradigm. In standard trials, the white cross appeared without an acoustical warning, and in warning trials, the appearance of the white cross was preceded by a 1000 Hz warning tone.[29] Hence, tonic (general) and phasic alertness (increased responsiveness induced by a warning tone) could be tested within a single experimental design.[29–31]

Statistical analysis

Within-subjects placebo-controlled pain experiments allowed us to analyse the data using analysis of variance[26] over subjects, followed by the Bonferroni post-test analyses. Haemodynamic and respiratory parameters and the results of the RT tests were analysed using paired *t*-tests. Owing to novelty of the entire experimental setup and the lack of *a priori* information available on expectable condition or group differences, computation of power analyses was not performed. Therefore, we estimated the required number of subjects for a randomized double-blind placebo-controlled repeated measures study design analysing comparable experimental pain studies.[18 20 26–28] Results are reported as mean values (SD). A difference between results was considered significant when $P<0.05$.

Results

The haemodynamic and respiratory parameters of volunteers are summarized in Table 1. Statistical comparisons between sessions (xenon or air) were computed in a fixed-effects model with subjects not treated as a random variable. Therefore, parameters were averaged over corresponding sessions (air or xenon) and then entered into two-tailed paired sample *t*-tests. Except for systolic arterial pressure, there were no statistically significant differences between groups.

Subjective perception

In a post-session debriefing after the study, volunteers were asked for their subjective impressions during the application of either xenon or placebo. They were not able to distinguish between having received air or xenon and no side-effects (nausea, vomiting, reduction of vigilance, etc.) were reported. The two pain tests' sessions were successfully performed in all the 10 subjects with no adverse effects from the pain tests themselves.

Table 1 Haemodynamic and respiratory parameters. Mean (SD) haemodynamic and respiratory parameters upon intranasal application of either air or xenon (n=198 values/parameters). Data were first averaged across 18 sampling points and then over 11 sessions. None of the analyses yielded any significant differences (all significance levels $P>0.05$). $t(10)$: *t*-value referring to Student's *t*-distribution with 10 d.f.

	Placebo (air)	Xenon	$t(10)$	*P*-value
Systolic pressure (mm Hg)	125.8 (4.4)	122.3 (7.2)	1.81	0.10
Diastolic pressure (mm Hg)	70.3 (5.1)	69.8 (4.3)	0.34	0.74
Mean arterial pressure (mm Hg)	79.9 (7.1)	78.8 (3.5)	0.51	0.62
Heart rate (beats min^{-1})	54.7 (3.4)	54.1 (2.7)	0.41	0.69
Ventilatory frequency (bpm)	10.2 (3.4)	8.8 (3.3)	1.17	0.27
Minute volume (litre min^{-1})	9.1 (1.1)	9.3 (0.9)	−0.99	0.35
Carbon dioxide (kPa)	5.2 (0.2)	5.2 (0.2)	0.29	0.78
Oxygen saturation (%)	98.3 (0.8)	98.4 (0.7)	−0.36	0.73

Blood–gas analysis

Concentrations of xenon measured in the venous blood of the sagittal sinus of seven anaesthetized pigs reached a steady state of ∼450 nl ml^{-1} after 5 min of intranasal application (Fig. 2). Blood samples obtained from the femoral artery at the same time (n=14) never contained more than 20 nl ml^{-1} of xenon.

Reliability of experimental pain tests

To test for the reliability and intra- and interindividual variations of the applied pain stimuli, we compared the reference values of the xenon and the placebo sessions (Table 2).

Alertness tests

RTs were normalized to their baseline values ($RT_{exposure}/RT_{reference}$) and a paired *t*-test on individual difference values ($normRT_{xenon}-normRT_{air}$) was calculated. RT (psycho-motor RT, alertness) was not significantly affected by xenon ($P>0.05$; Table 3) and could therefore

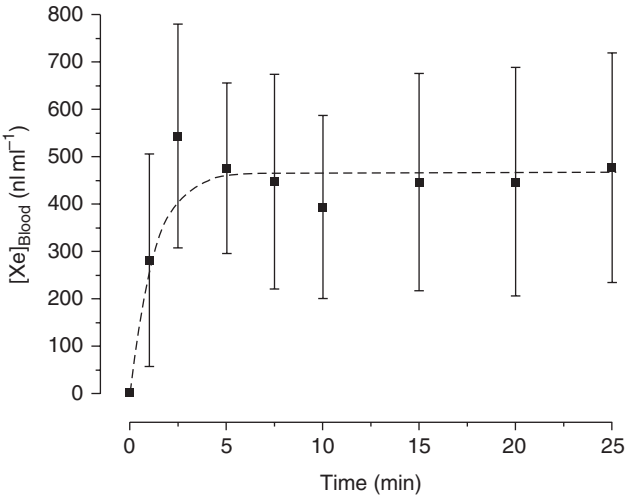

Fig 2 Mean (SD) concentrations of xenon measured in cranial blood. Concentrations of xenon measured in the venous blood of the sagittal sinus of seven pigs reflect xenon concentrations in the cerebral compartment. A steady state was reached within ~5 min providing evidence for a direct pathway from the nose to the brain.

Table 2 Reliability of the used pain tests. Comparison of the *reference* values (c.f. Fig. 1) taken at the beginning of placebo and xenon sessions. Inter-individual variations account placebo and xenon sessions (20 values). Values are means (SD). There were no significant differences between or within groups

	Placebo session	Xenon session	Intraindividual variations	Interindividual variations
Ice water (s)	74.7 (59.6)	80.6 (51.9)	0–61	16–180
Ischaemic pain (s)	289.4 (211)	276.9 (201)	42–203	52–701
Pressure pain (kPa)	620.6 (318)	770.4 (315)	94–635	238–1305

Table 3 Alertness tests. Individual RTs were normalized to their baseline values, recorded immediately before exposure to xenon or air. A paired *t*-test on individual difference values did not show any significant difference in RTs; neither short term, warning tone-induced alertness nor general, intrinsic alertness is affected by xenon. A xenon-induced impairment of alertness could therefore be excluded. RT, reaction time

	Mean RT (ms)	Normalized RT (SD)
Standard trials		
Xenon	229.3 (16.9)	1.05 (0.09)
Air	235.8 (30.3)	1.07 (0.07)
Difference		−0.018 (0.095) (P=0.696)
Warning tone trials		
Xenon	213.5 (21.11)	1.04 (0.1)
Air	219.4 (19.13)	1.04 (0.11)
Difference		0.0049 (0.107) (P=0.924)

be excluded as the reason for the increases in pain tolerance thresholds in the following pain tests.

Pressure pain test

Repeated pressure pain stimulation (*reference*, *exposure*, and *post-exposure*) did not lead to significant alteration of pain tolerance under placebo [n=10; P=0.73 (*exposure*) and P=0.79 (*post-exposure*); Fig. 3A (white)]. In contrast, 30 min of intranasal xenon application significantly increased pressure pain tolerance to 140.4 (17.3)% with respect to control [n=10; P<0.001; Fig. 3A (grey)]. Sixty minutes after termination of the xenon application, pressure pain tolerance decreased to 92.3 (15.2)% [n=10; P=0.13; Fig. 3A (grey, shaded)].

Ice-water pain test

Placebo failed to evoke significant alterations of cold pain tolerance [n=10; P=0.94 (*exposure*) and P=0.61 (*post-exposure*); Fig. 3B (white)]. In contrast, 30 min of intranasal xenon application significantly increased the ability of the volunteers to tolerate ice-water immersion to 157.6 (47.8)% in comparison with control [n=10; P=0.002; Fig. 3B (grey)]. Sixty minutes after termination of the xenon application, cold pain tolerance decreased to 103.4 (22.5)% [n=10; P=0.64; Fig. 3B (grey, shaded)].

Ischaemic pain test

Repeated exposure to ischaemic pain did not lead to significant alteration of pain tolerance under placebo [n=10; P=0.09 (*exposure*) and P=0.75 (*post-exposure*); Fig. 3C (white)], whereas 30 min of intranasal xenon application significantly increased ischaemic pain tolerance to 228.1 (94.7)% [n=10; P=0.001; Fig. 3C (grey)]. Sixty minutes after termination of the xenon application, ischaemic pain tolerance decreased to 171.4 (118.3)% [n=10; P=0.073; Fig. 3C (grey, shaded)].

Discussion

In this placebo-controlled experimental human study, we described the increased pain tolerance induced by intranasally applied xenon. Therefore, we conclude that intranasally administered xenon has analgesic properties and suggest that the novel application device presented here offers new possibilities for the administration of NMDAR antagonists within a multimodal analgesia approach.

Quality of the used pain tests

Human studies are commonly conducted with healthy volunteers to allow for maximally standardized conditions. Thereby, experimental pain models to measure analgesic drug effects should be non-invasive, non-noxious, standardized, and repeatedly applicable. Note that the sensitivity of the test to the drug effect may be reduced by concomitant placebo and time effects and by inter- and intraindividual variabilities of the endpoint (e.g. *pain tolerance* or *pain detection threshold*).[26]

Even low doses of inhalationally applied xenon ($F_{i\ Xe}$ ≥10% or more than ~10 μl xenon/ml blood)[20 23 28] cause clearly perceptible alterations of consciousness. These compromising effects alter responses to experimental pain

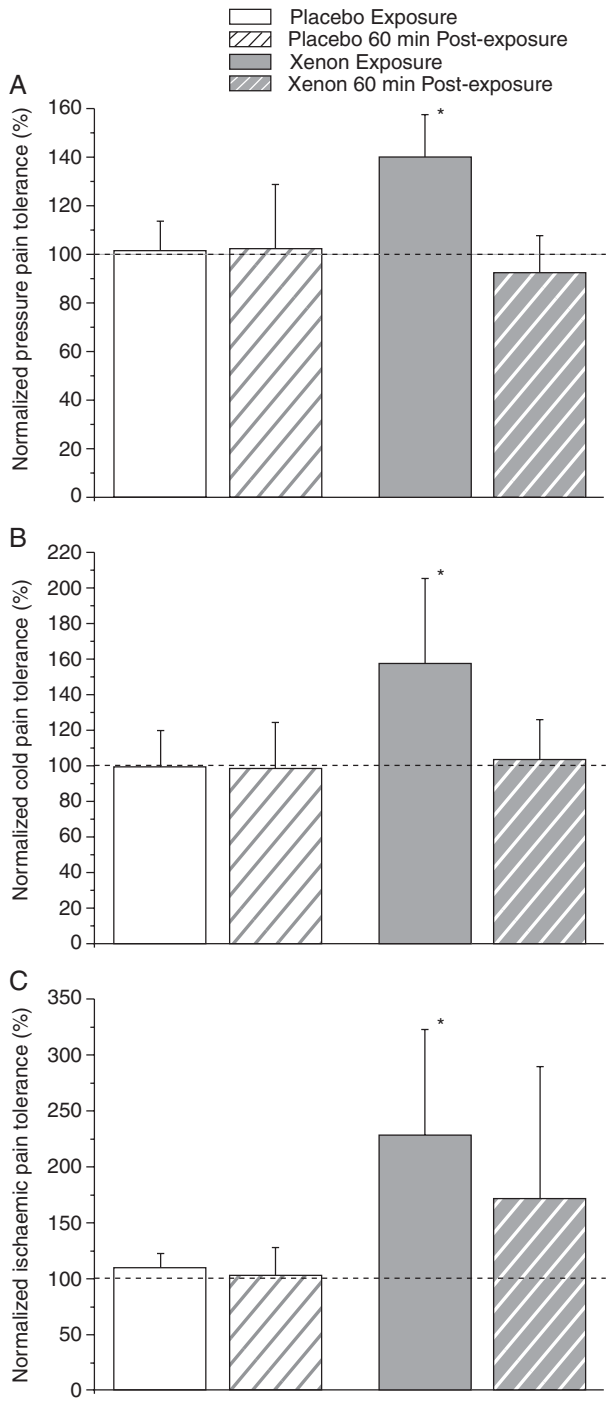

Fig 3 Intranasal application of xenon increases pain tolerance. (A) Pressure pain: 30 min of intranasal xenon application significantly increased pressure pain tolerance to 140.4 (17.3)%, whereas placebo (air) failed to alter pressure pain tolerance significantly. Sixty minutes after termination of the xenon application, the effect was not significantly different to control. Values of each session (xenon, air) are normalized to a reference value taken at the beginning of the experiment and presented as mean (SD). *$P<0.05$. (B) Cold pain: intranasally applied xenon increases the ability of the volunteers to tolerate ice-water immersion to 157.6 (47.8)%. Sixty minutes after termination of the xenon application, the analgesic effect was no longer detectable. *$P<0.05$. (C) Ischaemic pain: 30 min of intranasal xenon application significantly increased pressure pain tolerance to 228.1 (94.7)%. Sixty minutes after termination of the xenon application, the effect was not significantly different to control. *$P<0.05$.

by sedation[19] and lead to placebo effects.[17] In contrast, monitoring of haemodynamic and respiratory parameters and post-session debriefing of subjects receiving intranasal xenon did not reveal any effects (heart rate changes, nausea, vomiting, CO_2 changes, etc.)[28 32] that could have biased the double-blind placebo-controlled study design (objectivity). Owing to the fact that xenon does not affect RTs (psycho-motor RTs, alertness),[20 29] we ensure that the noble gas did not increase thresholds in pain tolerance by sedation.[19] These facts enabled us to perform within-subjects placebo-controlled experiments avoiding time and placebo effects.[19] Moreover, interindividual variability of drug effects, pain perception, or both was minimized. *Exposure* and *post-exposure* values of the placebo session led to comparable results representing a good reliability of the pain tests.

We used a multimodal (pressure, ice-water, ischaemic) and multistructured (skin, subdermal tissue, muscle) experimental pain test in humans as it is essential to describe the very complex and multifactorial aspects of clinical pain. Therefore, we used a combination of experimental tests which closely resembles clinical conditions improving the validity of our study.[17] In this study, we used *pain tolerance threshold* (defined as the highest stimulation intensity tolerated)[17] as the endpoint for the measurement of the drug effect, since this value is known to be more reliable in detecting true analgesic effects than the *pain detection threshold*.[33]

Comparison of different pain tests

The pressure pain test is a very localized and relatively brief (maximum duration 50 s) stimulus. It is one of the most commonly used experimental pain tests[17] that is characterized by a good reliability. This characteristic is reflected by the lowest intra- and interindividual variances when comparing the three used tests (Table 2). In this study, the well-known time–effect of pressure pain[17] is excluded by placebo control. In accordance with this study, investigations using other NMDAR antagonists like ketamine[18 19] showed an increase in pressure pain tolerance. Moreover, inhalatively applied xenon (10–20%) significantly increased the pressure pain tolerance threshold in a single-blind and non-placebo-controlled experimental pain test setting.[28]

Ischaemic pain tests cause a deep and slow increasing pain, last up to 15 min and cover a large tissue volume. Repeated stimuli elicit the desired temporal and spatial summation mechanisms leading to an improvement of the validity of the pain test.[17] Note that particularly temporal summation effects are subject to suppression by NMDAR antagonists.[34 35] In accordance with the results presented here, the NMDAR antagonist ketamine led to an increase in ischaemic pain in several experimental pain studies.[36 37] Even xenon, when applied inhalatively (10–20%), significantly increased the ischaemic pain tolerance of volunteers.[28]

Comparable with ischaemic pain tests, cold pain tests cause a slow increasing pain that first affects the skin and gradually involves deep tissue areas. Cold pain is also known to elicit temporal and spatial summation mechanisms.[17] Consistent with a study of Petersen-Felix and colleagues[28] who used inhalatively applied xenon (10–20%), we found an increase in the duration of immersion in ice-saturated water when xenon was applied intranasally.

Pharmacodynamical and safety aspects

It is suggested in the literature that a drug administered nasally is able to reach the CNS by neural pathways (olfactory and trigeminal) or the bloodstream.[14 15] However, the apolar and highly lipophilic nature of the chemically inert and structureless xenon is well known[12 38] and lipid-soluble agents are absorbed predominantly across the nasal membrane into the bloodstream with a bioavailability of up to 100%. Once in the bloodstream, they can diffuse freely through the blood–brain barrier and reach the CNS.[14]

Although data from experimental animals can never be transferred one-to-one to humans, animal models (e.g. rabbits or dogs) are thought to be reliable proof systems for pharmacokinetic studies when drugs are applied intranasally.[15] For polar molecules, such as peptides or peptidomimetics, that exhibit slow absorption rates relative to nasal clearance, anaesthesia can play a major role in determining nasal bioavailability. In contrast, anaesthesia is thought to have no effect on nasal bioavailability for drugs with fast absorption rates, such as small lipophilic molecules or atoms like xenon.[16] Therefore, we conclude that data of anaesthetized pigs are appropriate to estimate pharmacokinetic effects of nasally applied xenon in humans. In this study, we demonstrated a fast wash-in kinetics of xenon completed within ~5 min suggesting an extraneural route. We assume that after 10–15 min, an intracranial equilibrium state was reached, and based on these results, we developed our study design.[26]

Experimental studies using selected healthy volunteers are inappropriate to evaluate rare side-effects or possibly dangerous interactions. However, xenon has proven to be a potent and safe inhalation drug.[39] When applied inhalatively as an anaesthetic, blood concentrations of xenon reach up to ~70 μl ml^{-1}.[23] Note that this concentration is more than 100-fold higher than the values can be reached by intranasal administration. Even though severe side-effects of intranasally applied xenon cannot be excluded entirely, they are therefore rather unlikely.

Clinical relevance of NMDAR

Although several other molecular targets have been discussed on which xenon may exert its effects under certain *in vitro* conditions,[8 10 12 38] the NMDAR type is thought to be the prime molecular effect site for xenon's analgesic properties *in vivo*.[1 8 9] Central sensitization results mainly from the activation of glutamate receptors in the CNS triggered by nociceptive afferent input from the periphery.[2] In a recently published study, we showed an enhanced responsiveness of pain-processing areas to repeated painful stimulation using fMRI experiments. This enhancement could be suppressed by xenon at subanaesthetic doses providing evidence for an involvement of NMDAR in pain-evoked long-term potentiation-related synaptic plasticity in the human brain.[3]

In this multimodal and multistructured placebo-controlled experimental human study, we described the increased pain tolerance induced by intranasally applied xenon. Intranasal administration of the NMDAR antagonist is simple, economical, and does not lead to anaesthetic states that necessarily require clinical monitoring of subjects. Moreover, subjects were not able to report any subjective perceptible alterations that could have biased the double-blind characteristic of the study design. Even though a distinct prediction of the strength of the analgesic effect of this unique anaesthetic agent is difficult, our results legitimize the accomplishment of future studies to determine the clinical relevance of intranasal application as an add-on treatment in pain management. Additionally, we envisage the present approach to be a promising vehicle to investigate xenon's impact on NMDAR-related acute (e.g. stroke or CNS trauma[40 41]) and chronic (e.g. Alzheimer's disease or mood disorders[41 42]) CNS alterations.

Supplementary material

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

Acknowledgements

We thank Stefan Bäder and Ulrich Wachter (Department of Anaesthesiology, University of Ulm, Germany) for their expert advice and excellent technical assistance regarding the application device and GCMS analysis. Additionally, we thank Georg Grön (Department of Psychiatry, University of Ulm, Germany) for MRI measurements and his extensive help with statistical analysis.

Funding

All expenditures on the study were financed by the Department of Anaesthesiology, University of Ulm.

References

1 Benrath J, Kempf C, Georgieff M, Sandkühler J. Xenon blocks the induction of synaptic long-term potentiation in pain pathways in the rat spinal cord *in vivo*. *Anesth Analg* 2007; **104**: 106–11

2 Sandkühler J. Understanding LTP in pain pathways. *Mol Pain* 2007; **3**: 9

3 Adolph O, Köster S, Georgieff M, *et al*. Xenon-induced changes in CNS sensitization to pain. *Neuroimage* 2010; **49**: 720–30

4 Subramaniam K, Subramaniam B, Steinbrook RA. Ketamine as adjuvant analgesic to opioids: a quantitative and qualitative systematic review. *Anesth Analg* 2004; **99**: 482–95

5 Bell RF, Dahl JB, Moore RA, Kalso E. Peri-operative ketamine for acute post-operative pain: a quantitative and qualitative systematic review (Cochrane review). *Acta Anaesthesiol Scand* 2005; **49**: 1405–28

6 Kissin I. Preemptive analgesia at the crossroad. *Anesth Analg* 2005; **100**: 754–6

7 Franks NP, Dickinson R, de Sousa SL, Hall AC, Lieb WR. How does xenon produce anaesthesia? *Nature* 1998; **396**: 324

8 Preckel B, Weber NC, Sanders RD, Maze M, Schlack W. Molecular mechanisms transducing the anesthetic, analgesic, and organ-protective actions of xenon. *Anesthesiology* 2006; **105**: 187–97

9 Salmi E, Laitio RM, Aalto S, *et al*. Xenon does not affect gamma-aminobutyric acid type A receptor binding in humans. *Anesth Analg* 2008; **106**: 129–34

10 Gruss M, Bushell TJ, Bright DP, *et al*. Two-pore-domain K+ channels are a novel target for the anesthetic gases xenon, nitrous oxide, and cyclopropane. *Mol Pharmacol* 2004; **65**: 443–52

11 Bantel C, Maze M, Trapp S. Neuronal preconditioning by inhalational anesthetics: evidence for the role of plasmalemmal adenosine triphosphate-sensitive potassium channels. *Anesthesiology* 2009; **110**: 986–95

12 Dinse A, Föhr KJ, Georgieff M, Beyer C, Bulling A, Weigt HU. Xenon reduces glutamate-, AMPA-, and kainate-induced membrane currents in cortical neurones. *Br J Anaesth* 2005; **94**: 479–85

13 Derwall M, Coburn M, Rex S, *et al*. Xenon: recent developments and future perspectives. *Minerva Anestesiol* 2009; **75**: 37–45

14 Illum L. Is nose-to-brain transport of drugs in man a reality? *J Pharm Pharmacol* 2004; **56**: 3–17

15 Mathias NR, Hussain MA. Non-invasive systemic drug delivery: developability considerations for alternate routes of administration. *J Pharm Sci* 2010; **99**: 1–20

16 Hussain MA, Aungst BJ, Kapil R, Mousa SA. Intranasal absorption of the platelet glycoprotein IIb/IIIa receptor antagonist, DMP 755, and the effect of anesthesia on nasal bioavailability. *J Pharm Sci* 1997; **86**: 1358–60

17 Petersen-Felix S, Arendt-Nielsen L. From pain research to pain treatment: the role of human experimental pain models. *Best Pract Res Clin Anaesthesiol* 2002; **16**: 667–80

18 Petersen-Felix S, Arendt-Nielsen L, Bak P, *et al*. Analgesic effect in humans of subanaesthetic isoflurane concentrations evaluated by experimentally induced pain. *Br J Anaesth* 1995; **75**: 55–60

19 Petersen-Felix S, Arendt-Nielsen L, Bak P, Fischer M, Zbinden AM. Psychophysical and electrophysiological responses to experimental pain may be influenced by sedation: comparison of the effects of a hypnotic (propofol) and an analgesic (alfentanil). *Br J Anaesth* 1996; **77**: 165–71

20 Yagi M, Mashimo T, Kawaguchi T, Yoshiya I. Analgesic and hypnotic effects of subanaesthetic concentrations of xenon in human volunteers: comparison with nitrous oxide. *Br J Anaesth* 1995; **74**: 670–3

21 Lemmens HJ, Bovill JG, Hennis PJ, Gladines MP, Burm AG. Alcohol consumption alters the pharmacodynamics of alfentanil. *Anesthesiology* 1989; **71**: 669–74

22 Froeba G, Marx T, Pazhur J, *et al*. Xenon does not trigger malignant hyperthermia in susceptible swine. *Anesthesiology* 1999; **91**: 1047–52

23 Nalos M, Wachter U, Pittner A, *et al*. Arterial and mixed venous xenon blood concentrations in pigs during wash-in of inhalational anaesthesia. *Br J Anaesth* 2001; **87**: 497–8

24 Wenzel V, Linder KH, Augenstein S, Prengel AW, Strohmenger HU. Vasopressin combined with epinephrine decreases cerebral perfusion compared with vasopressin alone during cardiopulmonary resuscitation in pigs. *Stroke* 1998; **29**: 1462–7

25 Currie SR, Wilson KG, Gauthier ST. Caffeine and chronic low back pain. *Clin J Pain* 1995; **11**: 214–9

26 Luginbuhl M, Schnider TW, Petersen-Felix S, Arendt-Nielsen L, Zbinden AM. Comparison of five experimental pain tests to measure analgesic effects of alfentanil. *Anesthesiology* 2001; **95**: 22–9

27 Arendt-Nielsen L, Petersen-Felix S, Fischer M, *et al*. The effect of N-methyl-D-aspartate antagonist (ketamine) on single and repeated nociceptive stimuli: a placebo-controlled experimental human study. *Anesth Analg* 1995; **81**: 63–8

28 Petersen-Felix S, Luginbuhl M, Schnider TW, *et al*. Comparison of the analgesic potency of xenon and nitrous oxide in humans evaluated by experimental pain. *Br J Anaesth* 1998; **81**: 742–7

29 Grön G, Bittner D, Schmitz B, Wunderlich AP, Riepe MW. Subjective memory complaints: objective neural markers in patients with Alzheimer's disease and major depressive disorder. *Ann Neurol* 2002; **51**: 491–8

30 Zimmermann P, Fimm A. *Testbatterie zur Aufmerksamkeitsprüfung. TAP (V. 1.02)*. Würselen: Psytest, 1994

31 Posner MI. Measuring alertness. *Ann N Y Acad Sci* 2008; **1129**: 193–9

32 Coburn M, Kunitz O, Apfel CC, *et al*. Incidence of postoperative nausea and emetic episodes after xenon anaesthesia compared with propofol-based anaesthesia. *Br J Anaesth* 2008; **100**: 787–91

33 Arendt-Nielsen L, Nielsen J, Petersen-Felix S, Schnider TW, Zbinden AM. Effect of racemic mixture and the (S+)-isomer of ketamine on temporal and spatial summation of pain. *Br J Anaesth* 1996; **77**: 625–31

34 Sonner JM, Antognini JF, Dutton RC, *et al*. Inhaled anesthetics and immobility: mechanisms, mysteries, and minimum alveolar anesthetic concentration. *Anesth Analg* 2003; **97**: 718–40

35 Graven-Nielsen T, Aspegren Kendall S, Henriksson KG, *et al*. Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. *Pain* 2000; **85**: 483–91

36 Maurset A, Skoglund LA, Hustveit O, Oye I. Comparison of ketamine and pethidine in experimental and postoperative pain. *Pain* 1989; **36**: 37–41

37 Segerdahl M, Ekblom A, Sollevi A. The influence of adenosine, ketamine, and morphine on experimentally induced ischemic pain in healthy volunteers. *Anesth Analg* 1994; **79**: 787–91

38 Weigt HU, Georgieff M, Beyer C, Wachter U, Föhr KJ. Xenon incorporated in a lipid emulsion inhibits NMDA receptor channels. *Acta Anaesthesiol Scand* 2003; **47**: 1119–24

39 Lachmann B, Armbruster S, Schairer W, *et al*. Safety and efficacy of xenon in routine use as an inhalational anaesthetic. *Lancet* 1990; **335**: 1413–5

40 Danbolt NC. Glutamate uptake. *Prog Neurobiol* 2001; **65**: 1–105

41 Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr* 2000; **130**: 1007S–1015S

42 Maeng S, Zarate CA, Jr. The role of glutamate in mood disorders: results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. *Curr Psychiatry Rep* 2007; **9**: 467–74