Tetsuhiro Sakai MD,
Takashi Ichiyama MD,
Charles W. Whitten MD,
Adolph H. Giesecke MD,
James M. Lipton PhD

Laboratory Investigations

Ketamine suppresses endotoxin-induced NF- κ B expression

Purpose: Ketamine reduces endotoxin-induced production of proinflammatory cytokines, including tumour necrosis factor- α (TNF), in several types of inflammatory cells, including monocytes and macrophages. Transcription of the genes that encode production of these proinflammatory cytokines is regulated by nuclear factor-kappa B (NF- κ B). Cytoplasmic B protein is activated by endotoxin (LPS) as well as by TNF, allowing B protein to migrate into the cell nucleus to activate gene transcription for these inflammatory mediators. Because NF- κ B is likely involved in brain injury and inflammatory neurodegenerative disease, such as multiple sclerosis, we examined whether ketamine inhibits LPS-induced activation of NF- κ B in human glioma cells *in vitro* and intact mouse brain cells *in vivo*.

Methods: Endotoxin-induced NF- κ B expression in both the human glioma cells *in vitro* and the intact mouse brain cells *in vivo* was determined by electrophoretic mobility shift assays (EMSA) of nuclear extracts and measurement of NF- κ B expression by densitometry. Endotoxin was injected intracerebroventricularly *in vivo* and intact brain was harvested. Klenow fragment labeling was used to identify NF- κ B protein for both the *in vivo* and *in vitro* experiments.

Results: Endotoxin treatment increased NF- κ B expression ($P < 0.05$) both *in vivo* and *in vitro* compared with control (untreated) cells. Ketamine suppressed endotoxin-induced neuronal NF- κ B activation in a dose-dependent manner ($P < 0.05$, except for the 10^{-5} M concentration *in vitro*) both *in vivo* and *in vitro*.

Conclusion: Ketamine inhibits endotoxin-induced NF- κ B expression in brain cells *in vivo* and *in vitro* and it is suggested that this may have implications in the neuroprotective effects of ketamine reported by other investigators.

Objectif : La kétamine réduit la production de cytokines pro-inflammatoires induite par endotoxine, y compris le facteur nécrosant des tumeurs (TNF), dans certains types de cellules inflammatoires comprenant les monocytes et les macrophages. La transcription des gènes qui encodent la production de ces cytokines pro-inflammatoires est réglée par le facteur-kappa B nucléaire (NF-6B). La protéine cytoplasmique 6B est activée par l'endotoxine (LPS) et par le TNF et peut ainsi migrer dans le noyau cellulaire et activer la transcription génique pour ces médiateurs de l'inflammation. Comme le NF-6B participe probablement aux lésions cérébrales et aux maladies inflammatoires neurodégénératives, dont la sclérose en plaques, notre but était de savoir si la kétamine inhibe l'activation de NF-6B induit par LPS dans des cellules de gliome humain *in vitro* et dans des cellules cérébrales intactes de souris *in vivo*.

Méthode : L'expression du NF-6B induite par endotoxine dans les cellules humaines *in vitro* et dans les cellules de souris *in vivo* a été déterminée par une étude de retardement de la mobilité électrophorétique (ERME) d'extraits nucléaires et la mesure de l'expression du NF-6B a été faite par densitométrie. L'endotoxine a été injectée dans les ventricules cérébraux *in vivo* et du tissu cérébral intact a été prélevé. Le marquage de fragments de Klenow a été utilisé pour identifier la protéine du NF-6B des deux expériences *in vivo* et *in vitro*.

Résultats : Le traitement avec l'endotoxine a augmenté l'expression du NF-6B ($P < 0,05$) des cellules *in vivo* et *in vitro* comparées aux cellules témoin (non traitées). La kétamine a supprimé l'activation neuronale de NF-6B induite par endotoxine d'une façon dose-dépendante ($P < 0,05$, sauf pour une concentration de 10^{-5} M *in vitro*) des cellules *in vivo* et *in vitro*.

Conclusion : La kétamine inhibe l'expression de NF-6B induite par endotoxine dans des cellules cérébrales *in vivo* et *in vitro* et on croit que cela pourrait contribuer aux effets neuroprotecteurs de la kétamine dont parlent d'autres chercheurs.

From the Departments of Anesthesiology and Pain Management and Physiology, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75390-9068, USA.

Address correspondence to: Charles W. Whitten MD. Phone: 214-590-8508; Fax: 214-590-6945; E-mail: charles.whitten@email.swmed.edu

Accepted for Publication June 29, 2000.

NUCLEAR factor- κ B (NF- κ B) is an inducible transcription factor that is required for the transcription of several genes, including those that encode the proinflammatory cytokines tumour necrosis factor- α (TNF- α), and interleukin 6 and 8 (IL-6 and IL-8).¹ Latent NF- κ B proteins are present in the cytoplasm of most cells, including brain, as an inactive heterodimer of p50 and p65 proteins which coexists in combination with an inhibitory protein, termed I κ B.¹ Exposure of cells to TNF- α or lipopolysaccharide (LPS, endotoxin) results in degradation (phosphorylation) of I B, allowing free B to translocate into the cell nucleus and activate DNA strands which encode for specific genes, such as the TNF- α gene.¹ In turn, the increased cellular presence of the TNF- α gene causes transcription of TNF- α mRNA, resulting in the subsequent cellular production and release of TNF- α .

The intravenous anesthetic ketamine has considerable effects on several inflammatory processes, including dose-dependent reduction of LPS-induced whole blood inflammatory cell TNF- α production,^{2,3} reduction of LPS-induced TNF- α release from rat macrophages,⁴ and inhibition of TNF- α induced rat mesangial cell proliferation.⁵ In addition, ketamine attenuates the systemic release of the proinflammatory cytokine IL-6 in humans during and following cardiopulmonary bypass *in vivo*,⁶ a clinical condition characterized by systemic endotoxemia and proinflammatory cytokine production and release. Because NF- κ B activation initiates LPS-induced production of TNF- α by inflammatory cells, these reports suggest that ketamine may exert its antiinflammatory effect by inhibiting LPS-induced NF- κ B activation.

Nuclear factor- κ B activation contributes to the pathophysiology of brain injury syndromes by causing increased neuronal cell production and release of the proinflammatory cytokines.⁷⁻¹⁰ Ketamine has well described neuroprotective effects.^{11,12} Since ketamine inhibits LPS-induced proinflammatory cytokine production, this study evaluates if ketamine inhibits LPS-induced cellular NF- κ B activation *in vivo* and *in vitro* in order to define a possible cellular mechanism of the neuroprotective effects of ketamine.

Methods

Cells and culture (in vitro experiments)

The A-172 cell line, a well-characterized human glioma cell line,¹³ was used for the *in vitro* experiments. These cells show consistent production and release of TNF- α when stimulated with LPS.¹³ The cells were grown to confluence in Dulbecco's Modified Eagle's Medium (DMEM) containing glucose 4.5% and supplemented

with fetal bovine serum (FBS, 10%), 110 mg·L⁻¹ sodium pyruvate, 10 mM L-glutamine, 100 U·mL⁻¹ penicillin, and 100 units·mL⁻¹ streptomycin. The day before to each experiment, cells were seeded into six-well tissue culture dishes (Costar, Cambridge, MA) at a density of 1×10⁶ cells/well and cultured in DMEM overnight at 37°C.

Cell treatments

The A-172 cells were treated for two hours with LPS (*Escherichia Coli*, 0111:B4, Sigma Chemical Company, St. Louis, MO), 10 ng·mL⁻¹ (positive control) or media only (negative control), in the presence or absence of pretreatment for 30 min with ketamine HCl (Research Biochemicals International, Natick, MA), 10⁻³, 10⁻⁴ or 10⁻⁵M (1000, 100 or 10 mM, respectively).

Animals and Treatment (in vivo experiments)

The experiments were approved by the local Internal Review Board for animal research. Ten Male BALB/c mice (Simonsen Laboratories, Gilroy, CA) were divided into five equal groups: positive (LPS) and negative (no LPS) control groups and three groups given ketamine [2, 5, or 10 mg·kg⁻¹, *iv*, n=2, each group]. The LPS treated animals received an intracerebroventricular (ICV) injection of LPS (2.5 μ g in 20 μ l saline) as previously described^{14,15} 30 min following the administration of ketamine *iv* or an equal volume of saline (control). Ninety minutes after the LPS administration, the intact brain was removed, frozen in liquid nitrogen, and the brain cells prepared and isolated, and nuclear extracts were analyzed for NF- κ B activation as previously described.¹⁵⁻¹⁷

Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts from the A-172 glioma cells and mouse brain cerebral cortical cells were harvested using standard procedures.^{13,16,17} In brief, the cells from both sources were washed with 1 ml phosphate buffered saline (PBS) and incubated in PBS with EDTA 1% for 15 min at 4°C. Cells were harvested with a cell scraper, transferred to 1.5 ml tubes, and centrifuged at 5,400 g for 10 sec. The supernatant was aspirated and the cell pellet was resuspended in 400 μ l of buffer I (10 mM Tris-HCl, pH 7.8, 5 mM MgCl₂, 10 mM KCl, 0.3 mM EGTA, 0.5 mM dithiothreitol, 0.3 sucrose, 1 mM phenylmethylsulfonyl fluoride, 10 mM β -glycerol phosphate, and 1.0 mg·mL⁻¹ each of the following protease inhibitors: aprotinin, antipain, leupeptin, chymostatin, and pepstatin A) and incubated on ice for 15 min. Nonidet P-40 (Sigma Chemical Company, 25 μ l of 10% solution) was added, and the tubes vortexed and centrifuged at 7,200 g for 10 sec. Supernatants were aspirated and the resulting pellets containing nuclei were resuspended in 100 μ l of buffer

II (20 mM Tris-HCl, pH 7.8, 5 mM Mg Cl₂, 320 mM KCl, 0.2 mM EGTA, 0.5 mM dithiothreitol, and the mixture of protease inhibitors described above). Nuclear proteins were placed on ice for 15 min and then centrifuged at 13,500 g for 15 min. The protein concentration of the supernatant containing nuclear extracts was determined using Bio-Rad (Hercules, CA) protein concentration reagent. Nuclear extracts were stored at -80°C.

Double-stranded oligonucleotides for the consensus binding sites of NF- κ B (5'-AGTTGAGGGGACTTTC-CCAGGC-3'; Promega, Madison, WI) were labeled using Klenow fragment (Life Technologies, Gaithersburg, MD) in the presence of 100 μ Ci of [α -³²P] dCTP (3,000 Ci/mmol, NEN, Boston, MA) and unlabeled dATP, dGTP and dTTP at 100 μ M in a final volume of 50 μ l. Unincorporated nucleotides were removed using a G-25 (Pharmacia, Piscataway, NJ) spin column. Five micrograms of nuclear proteins were preincubated with reaction buffer (50 μ g·ml⁻¹ poly[dI-dC], glycerol 5%, 10 mM Tris-HCl, pH 7.8, 1 mM EDTA, 40 mM KCl, and 1 mM dithiothreitol) on ice for 20 min before addition of the radiolabeled oligonucleotide probe and incubation at room temperature for 20 min. Specificity of the binding reaction was determined in competition assays by co-incubating with 100-fold molar excess of unlabeled NF- κ B consensus oligonucleotide (Promega), and by supershift assay with polyclonal rabbit anti-NF- κ B-p50 and anti-NF- κ B-p65 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). A linear relationship between the NF- κ B complex and radioactivity was verified. Reaction products were separated in a polyacrylamide 4% gel. Gels were run at room temperature at 10 V·cm⁻¹ for 45 min and were dried in a gel dryer under vacuum at 80°C. The dried autoradiographs were then scanned by an ARCUS II scanner (AGFA) and analyzed by densitometry (FotoLook and Molecular Analyst Software Program, Bio-Rad Laboratories, Molecular Bioscience Group, Hercules, CA), resulting in a numerical readout (counts x mm²) and allowing for statistical analysis.

Data are expressed as means $\pm$ SEM. Analysis of variance was used to compare means between dosing levels for the treatment groups. Dunnett's two-tailed t test was used to determine if any dosing levels were significantly different from the control. *P* values of < 0.05 were regarded as statistically significant.

Results

Activation of NF- κ B by LPS and its inhibition by ketamine *in vitro*

The EMSA of nuclear extracts from A-172 human glioma cells following incubation with LPS for two

TABLE I Densitometer numerical read-out of NF- κ B bands of EMSA gels (counts x mm², Means $\pm$ SEM) of *in vitro* human glioma cells (A-172).

Condition	Counts x mm ²
Negative Control (No LPS)	33,950 $\pm$ 745*
Positive Control (LPS treated)	40,951 $\pm$ 975†
LPS + Ketamine (10 ⁻³ M)	33,136 $\pm$ 1,010*
LPS + Ketamine (10 ⁻⁴ M)	34,455 $\pm$ 995*
LPS + Ketamine (10 ⁻⁵ M)	39,100 $\pm$ 1,120†

**P* < 0.05, compared with Positive Control (LPS treated);

†*P* < 0.05, compared with Negative Control (No LPS) (n=6, each condition).

FIGURE 1 Representative gel shift of NF- κ B DNA binding activity in human glioma (A172) cells. Nuclear protein extracts were isolated in untreated (Lane 1), LPS only treated (Lane 2), and LPS plus ketamine (10⁻³, 10⁻⁴, and 10⁻⁵M) (Lanes 3, 4, and 5, respectively). NF- κ B activity was assayed as described in methods.

hours demonstrated NF- κ B activation; the intensity of the gel shift band increased (*P* < 0.05) when compared with signals from control (untreated) cells (Table I, Figure 1). When compared with nuclear extracts from A-172 cells stimulated with LPS only, ketamine pretreatment suppressed NF- κ B activation at both 10⁻³ and 10⁻⁴M concentrations, but not for the 10⁻⁵M concentration (*P* < 0.05) (Table I, Figure 1).

Endotoxin-induced NF- κ B activation in mouse brain cells and inhibition by ketamine pretreatment *in vivo*

Activation of NF- κ B in nuclear extracts from intact mouse brain cells increased after LPS stimulation (ICV injection) when compared with unstimulated (negative control) brain cells (Table II, Figure 2) (*P* < 0.05). Ketamine (*iv*) pretreatment inhibited NF- κ B activation in intact brain cells in a concentration dependent manner at all three (2, 5, and 10 mg·kg⁻¹ *iv*) dosing levels (*P* < 0.05, when compared with LPS only treated mice) (Table II, Figure 2).

TABLE II Densitometer numerical read out of NF- κ B bands of EMSA gells (counts $\times$ mm², Means $\pm$ SEM) of rat brain (cerebral cells) *in vivo*.

Condition	Counts $\times$ mm ²
Negative Control (No LPS)	21,650 $\pm$ 612*
Positive Control (LPS treated)	28,950 $\pm$ 935†
LPS + Ketamine (10 mg·kg ⁻¹)	21,100 $\pm$ 519*
LPS + Ketamine (5 mg·kg ⁻¹)	22,600 $\pm$ 720*
LPS + Ketamine (2 mg·kg ⁻¹)	23,150 $\pm$ 937*

* $P < 0.05$, compared with Positive Control (LPS treated);

† $P < 0.05$, compared with Negative Control (No LPS) ($n=2$, each condition).

FIGURE 2 Representative gel shift of NF- κ B DNA binding activity in mouse brain. Nuclear protein extracts were isolated from cortex of mice untreated (Lane 1), treated with LPS only (Lane 2), and LPS plus ketamine (10, 5, or 2 mg·kg⁻¹, *iv*) (Lanes 3, 4, and 5, respectively). NF- κ B activity was assayed as described in methods.

Discussion

Our results demonstrate that ketamine suppresses LPS-induced NF- κ B activation *in vivo* and *in vitro* in a concentration-dependent manner. This is the first evidence of inhibition of LPS-induced NF- κ B activation in human brain cells by ketamine. Since NF- κ B activation is a required event in the production of the proinflammatory cytokines,¹ this effect of ketamine suggests a mechanism for previous reports demonstrating that ketamine inhibits LPS-induced proinflammatory cytokine production and release from cultured whole blood and macrophages.²⁻⁴ In addition, Jimi *et al.*⁵ demonstrated that ketamine inhibits TNF- α induced [³H]thymidine incorporation into rat mesangial cells, demonstrating that ketamine also inhibits other well-described biologic effects of TNF- α .

Ketamine reduces the systemic production and release of the proinflammatory cytokine IL-6 during

and following cardiopulmonary bypass (CPB).⁶ Since CPB is a clinical condition characterized by systemic endotoxemia,⁶ these reports²⁻⁶ demonstrate that ketamine has antiinflammatory activity (reduces the LPS-induced release and/or biological effects of the proinflammatory cytokines). However, the mechanism of suppression by ketamine of the LPS-induced proinflammatory cytokine production and release has not previously been reported. Our results indicate that this mechanism involves inhibition of LPS-induced activation of NF- κ B protein. These data suggest that ketamine inhibits NF- κ B activation by preventing the LPS-induced degradation of the cytoplasmic inhibitory protein I κ B, preventing free I κ B protein from migrating into the cell nucleus to become NF- κ B, and thus preventing the transcription of genes responsible for the production of the proinflammatory cytokines.

Cellular expression of NF- κ B increases in brain tissue in response to traumatic brain injury.¹⁰ A possible mechanism may involve trauma-induced glutamate release, since this neuroexcitatory transmitter activates neuronal NF- κ B expression.⁸ Neuronal NF- κ B activation produces further proinflammatory cytokine production and release, these newly produced cytokines in turn cause further NF- κ B activation,⁹ inducing a progressive cascade of increasing neuronal injury and death. Following both brain ischemia-reperfusion injury¹⁰ and traumatic spinal cord injury,⁷ an increase in neuronal cellular NF- κ B expression has been demonstrated. These central nervous system (CNS) injury models demonstrate that CNS injury, whether ischemic or traumatic, results in an increase in brain cell neuronal NF- κ B expression, which causes increased tissue concentrations of the proinflammatory cytokines, including IL-1 and TNF- α resulting in neuronal inflammation, injury, and cell death.^{7-10,16} Proinflammatory cytokines cause neuronal injury by several mechanisms, including local inflammatory cell recruitment and activation, production of reactive oxygen intermediates, causing myelin loss, and the increased endogenous production and release of nitric oxide (NO).^{18,19} The results reported in this study therefore suggest a mechanism for the reported neuroprotective effects of ketamine following ischemic or traumatic brain injury,^{11,20,21} since activation of neuronal cell NF- κ B is currently considered to play a primary role in these inflammatory-based nervous system injury syndromes.

Nuclear factor- κ B activation also encodes production of inducible nitric oxide synthase (iNOS) mRNA.²² Increased cellular iNOS expression results in a sustained increase in endogenous NO production, elevated endogenous NO concentrations cause brain

injury and cerebral cell death (apoptosis).^{12,23,24} Ketamine decreases NOS activity in rat brain²⁵ and reduces LPS-induced NO production by rat macrophages.²⁶ Our demonstration that ketamine inhibits NF- κ B activation in brain cells is consistent with and defines a mechanism for these findings.^{23,25,26} It is interesting that thiopental (a commonly used intravenous anesthetic for neurologic surgery) augments proinflammatory cytokine-induced iNOS expression and subsequent cellular NO production and release.²⁷

The common clinical dosages for ketamine in humans range from 0.5 to 10 mg·kg⁻¹ iv,²⁸ with resultant plasma concentrations of 60–80 μ M.^{28,29} Therefore, the concentrations of ketamine used for the *in vitro* and *vivo* portions of these experiments are similar to the concentrations used clinically.

In summary, this study demonstrates that ketamine inhibits LPS-induced NF- κ B expression in a human glioma cell line *in vitro* and in whole mouse brain *in vivo*. This action of ketamine is consistent with, and provides a mechanism for, the demonstration by other investigators that ketamine inhibits the production and release of the proinflammatory cytokines. Since these cytokines are implicated in traumatic and ischemic brain injury, the demonstration that ketamine inhibits LPS-induced NF- κ B activation suggests a mechanism for the reported neuroprotective effects of ketamine.

References

- 1 Baldwin AS Jr. The NF- κ B and I κ B proteins: new discoveries and insights. *Ann Rev Immunol* 1996; 14: 649–81.
- 2 Larsen B, Hoff G, Wilhelm W, Buchinger H, Wanner GA, Bauer M. Effect of intravenous anesthetics on spontaneous and endotoxin-stimulated cytokine response in cultured human whole blood. *Anesthesiology* 1998; 89: 1218–27.
- 3 Kawasaki T, Ogata M, Kawasaki C, Ogata J, Inoue Y, Shigematsu A. Ketamine suppresses proinflammatory cytokine production in human whole blood *in vitro*. *Anesth Analg* 1999; 89: 665–9.
- 4 Takenaka I, Ogata M, Koga K, Matsumoto T, Shigematsu A. Ketamine suppresses endotoxin-induced tumor necrosis factor alpha production in mice. *Anesthesiology* 1994; 80: 402–8.
- 5 Jimi N, Segawa K, Minami K, Sata T, Shigematsu A. Inhibitory effect of the intravenous anesthetic, ketamine, on rat mesangial cell proliferation. *Anesth Analg* 1997; 84: 190–5.
- 6 Roytblat L, Talmor D, Rachinsky M, et al. Ketamine attenuates the interleukin-6 response after cardiopulmonary bypass. *Anesth Analg* 1998; 87: 266–71.
- 7 Bethea JR, Castro M, Keane RW, Lee TT, Dietrich WD, Yezielski RP. Traumatic spinal cord injury induces nuclear factor-B activation. *J Neuroscience* 1998; 18: 3251–60.
- 8 Guerrini L, Blasi F, Denis-Donini S. Synaptic activation of NF- κ B by glutamate in cerebellar granule neurons *in vitro*. *Proc Natl Acad Sci USA* 1995; 92: 9077–81.
- 9 Moynagh PN, Williams DC, O'Neill LA. Interleukin-1 activates transcription factor NF- κ B in glial cells. *Biochem J* 1993; 294: 343–7.
- 10 O'Neill LAJ, Kaltschmidt C. NF- κ B: a crucial transcription factor for glial and neuronal function. *Trends Neurosci* 1997; 20: 252–8.
- 11 Himmelseher S, Pfenninger E, Georgieff M. The effects of ketamine-isomers on neuronal injury and regeneration in rat hippocampal neurons. *Anesth Analg* 1996; 83: 505–12.
- 12 Kahn RA, Panah M, Weinberger J. Modulation of ischemic excitatory neurotransmitter and -aminobutyric acid release during global temporary cerebral ischemia by selective neuronal nitric oxide synthase inhibition. *Anesth Analg* 1997; 84: 997–1003.
- 13 Ichiyama T, Zhao H, Catania A, Furukawa A, Lipton JM. -melanocyte-stimulating hormone inhibits NF- κ B activation and I κ B degradation in human astrocytes and in experimental brain inflammation. *Exp Neurol* 1999; 157: 359–65.
- 14 Haley TJ, McCormick WG. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br J Pharmacol* 1957; 12: 12–5.
- 15 Lipton JM, Macaluso A, Hiltz ME, Catania A. Central administration of the peptide -MSH inhibits inflammation in the skin. *Peptides* 1991; 12: 795–8.
- 16 Deryckere F, Gannon F. A one-hour miniprep technique for extraction of DNA-binding proteins from animal tissues. *Biotechniques* 1994; 16: 405.
- 17 Unlap T, Jope RS. Dexamethasone attenuates kainate-induced AP-1 activation in rat brain. *Mol Brain Res* 1994; 24: 275–82.
- 18 Rothwell NJ, Hopkins SJ. Cytokines and the nervous system II: actions and mechanisms of action. *Trends Neurosci* 1995; 18: 130–6.
- 19 Woodroffe MN. Cytokine production in the central nervous system. *Neurology* 1995; 45(Suppl 6): S6–10.
- 20 Kohrs R, Durieux ME. Ketamine: teaching an old drug new tricks. *Anesth Analg* 1998; 87: 1186–93.
- 21 Shapira Y, Artru AA, Lam AM. Ketamine decreases cerebral infarct volume and improves neurological outcome following experimental head trauma in rats. *J Neurosurg Anesth* 1992; 4: 231–40.
- 22 Oddis CV, Finkel MS. NF-B and GTP cyclohydrolase regulate cytokine-induced nitric oxide production by cardiac myocytes. *Am J Physiol* 1996; 270: H1864–8.

- 23 Dawson VL, Dawson TM, London ED, Bredt DS, Snyder SH. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci USA* 1991; 88: 6368–71.
- 24 Tseng EE, Brock MV, Lange MS, et al. Nitric oxide mediates neurologic injury after hypothermic circulatory arrest. *Ann Thorac Surg* 1999; 67: 65–71.
- 25 Galley HF, Webster NR Brain nitric oxide synthase activity is decreased by intravenous anesthetics. *Anesth Analg* 1996; 83: 591–4.
- 26 Shimaoka M, Iida T, Ohara A, et al. Ketamine inhibits nitric oxide production in mouse-activated macrophage-like cells. *Br J Anaesth* 1996; 77: 238–42.
- 27 Kessler P, Kronemann N, Hecker M, Busse R, Schinikow KB. Effects of barbiturates on the expression of the inducible nitric synthase in vascular smooth muscle. *J Cardiovasc Pharmacol* 1997; 30: 802–10.
- 28 Wieber J, Gugler R, Hengstmann JH, Dengler HJ. Pharmacokinetics of ketamine in man. *Anaesthesist* 1975; 24: 260–3.
- 29 Idvall J, Ahlgren I, Aronsen KF, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. *Br J Anaesth* 1979; 51: 1167–72.