Acta Neurochir (Wien) (2004) 146: 819–830
DOI 10.1007/s00701-004-0281-9

Acta Neurochirurgica
Printed in Austria

Experimental Study

Differential effects of prolonged isoflurane anesthesia on plasma, extracellular, and CSF glutamate, neuronal activity, ^{125}I-Mk801 NMDA receptor binding, and brain edema in traumatic brain-injured rats

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Published online June 7, 2004
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Summary

Background. Volatile anesthetics reduce neuronal excitation and cerebral metabolism but can also increase intracellular water accumulation in normal and injured brains. While attenuation of neuronal excitation and glutamate release are beneficial under pathological conditions, any increase in edema formation should be avoided. In the present study we investigated duration-dependent effects of the commonly used isoflurane/nitrous oxide (N_2O) anesthesia on EEG activity, specific NMDA receptor binding, extracellular, CSF, and plasma glutamate, and cerebral water content in brain-injured rats subjected to short (30 minutes) or prolonged (4 hours) anesthesia.

Methods. Before controlled cortical impact injury (CCI), during prolonged (4–8 hours) or short anesthesia (7.5–8 hours after CCI), and before brain removal, changes in neuronal activity were determined by quantitative EEG analysis and glutamate was measured in arterial plasma. Brains were processed to determine acute and persisting changes in cerebral water content and ^{125}I-Mk801 NMDA receptor binding at 8 and 32 hours after CCI, i.e., immediately or 24 hours after short or prolonged anesthesia. During prolonged anesthesia glutamate was measured via microdialysis within the cortical contusion. CSF was sampled before brain removal.

Findings. Prolonged isoflurane (1.8 vol%) anesthesia significantly increased EEG activity, plasma, cortical extracellular, and CSF glutamate, cortical and hippocampal ^{125}I-Mk801 NMDA receptor binding, and cerebral water content in brain-injured rats. These changes were partially reversible within 24 hours after prolonged anesthesia. At 24 hours, CSF glutamate was significantly reduced following long isoflurane anesthesia compared to rats previously subjected to short anesthesia despite an earlier significant increase.

Conclusions. The partially reversible increases in EEG activity, ^{125}I-Mk801 NMDA receptor binding, cerebral water content, plasma and CSF glutamate appear important for physiological, pathophysiological, and pharmacological studies requiring prolonged anesthesia with isoflurane. Increases in extracellular cortical and plasma glutamate could contribute to acute aggravation of underlying tissue damage.

Keywords: Electroencephalography; excitotoxicity; in vitro receptor autoradiography; microdialysis; traumatic brain injury.

Introduction

Volatile anesthetics decrease synaptic transmission by decreasing glutamatergic neuronal excitation [3, 4, 25, 37] and increasing GABA-ergic neuronal inhibition [3]. Within the heterogenous group of glutamate receptors, normal excitatory transmission as well as pathological neuronal activation are mediated via integral membrane N-methyl-D-aspartate (NMDA) receptors [37]. Activation of NMDA receptors increases Na^+, K^+ and Ca^{2+} permeability which in turn, activate intracellular second and third messanger systems. The functional status of the ionotropic heteromeric NMDA receptors consisting of different subunits is tightly regulated by a multitude of complex extracellular and intracellular stimuli controlling transcriptional and translational processes, membrane expression, as well as closure and opening of the channel pore [51]. While allosteric regulation of the extracellular

glutamate- and glycine-binding sites determine the functional fate of the channel pore [3, 25, 37], phosphorylating kinases are responsible for the intracellular modulation of the activity of the NMDA receptor/channel complex [1].

Recent reports show that volatile anesthetics attenuate neuronal activity by allosteric regulation of transmitter-gated ion channels. Within this context, frequency of channel opening and mean channel opening time of cation-conducting glutamate receptors are decreased while the activity of anion-selective $GABA_A$ receptors is increased [3]. In addition to functionally modulating and stabilizing postsynaptic glutamate receptors [3, 30, 31, 34, 37], volatile anesthetics decrease presynaptic glutamate release [20, 25] and increase sodium-dependent glial glutamate uptake [32, 54].

Apart from attenuating glutamatergic neuronal excitation and reducing glutamate-mediated excitotoxicity [11], volatile anesthetics have also been reported to mediate less favorable effects. In this regard, increased responsiveness of cortical neurons during isoflurane-induced EEG bursts in rats [7], and disturbed electrolyte homeostasis resulting in increased brain edema formation in uninjured [41] and brain-injured dogs [44] have been reported. The edema-enhancing potential of volatile anesthetics is of particular concern for those studies investigating development, progression, and even attenuation of posttraumatic brain edema formation. Depending on the performed investigations, length of anesthesia will vary significantly, ranging from minutes to several hours and duration-dependent effects of isoflurane on e.g., cerebral polyamine metabolism in uninjured adult rat brains have been described [29]. In addition, volatile anesthetics significantly increase plasma levels of essential and non-essential amino acids [4, 14], including glutamate [46].

Thus, the present study was designed to investigate if specific NMDA receptor binding, neuronal activity, extracellular and plasma glutamate concentrations, and cerebral water content are significantly increased by prolonged (4 hours) compared to short (30 minutes) anesthesia with isoflurane in rats subjected to a focal traumatic brain injury (TBI).

Materials and methods

A total of 60 brain-injured rats were investigated in the present study which was approved by the local committee for animal research in Berlin.

Anesthesia

Spontaneously breathing rats were anesthetized with the commonly used combination of isoflurane in a mixture of nitrous oxide (N_2O) and oxygen (O_2) (2:1) [12, 18, 45]. Co-administration of N_2O reduces the concentration of isoflurane necessary to maintain an adequate level of anesthesia allowing to perform functional investigations in brain-injured rats [12, 18, 45]. Following induction of anesthesia (isoflurane 5 vol%, N_2O 1 l/min, O_2 0.5 l/min), isoflurane, N_2O, and O_2 were reduced and maintained at constant concentrations of 1.8 vol%, 0.3, and 0.15 l/min, respectively at all investigated time points. With these settings, mean arterial blood pressure (MABP) and arterial blood gases remained within physiological limits (Table 1), waiving the need for intubation and controlled ventilation.

Table 1. *Arterial blood gases and mean arterial blood pressure (MABP) determined before CCI and during 4 hours of isoflurane/nitrous oxide anesthesia in 10 spontaneously breathing brain-injured rats*

	Before CCI	Prolonged anesthesia		
		30 min	120 min	240 min
paO_2 [mmHg]	180 ± 10	160 ± 8	165 ± 10	160 ± 7
$paCO_2$ [mmHg]	44 ± 2.5	38 ± 1.1	36 ± 1.2	39 ± 1
PH	7.37 ± 0.02	7.4 ± 0.1	7.4 ± 0.1	7.39 ± 0.1
Base excess [mmol/l]	0.5 ± 0.6	−2.1 ± 0.4	−2.0 ± 0.5	−1.2 ± 0.2
Hematocrit [%]	39 ± 0.5	36 ± 2	35 ± 2	34 ± 2
MABP [mmHg]	87 ± 2	89 ± 2	88 ± 3	89 ± 2

Controlled cortical impact injury

A controlled cortical impact injury (CCI) to the left temporoparietal cortex was induced with a pneumatic driven cylinder as described previously [18, 47].

Study design

Acute and persisting effects of prolonged and short anesthesia on NMDA receptor binding, neuronal activity, extracellular glutamate concentrations, and cerebral water content were investigated. Short anesthesia (30 minutes) (n = 20) was begun at 7.5 hours after CCI while prolonged anesthesia (4 hours) (n = 20) was maintained between 4 and 8 hours. Acute effects were studied by killing rats immediately after anesthesia, i.e., at 8 hours after CCI. Persisting effects were assessed following short and prolonged anesthesia by removing brains 24 hours later, i.e., at 32 hours after CCI.

Arterial cannulation, blood and CSF collection

The right femoral artery was cannulated before CCI and recannulated at later time points as previously described [17, 46] to continuously measure MABP and withdraw blood in heparinzed syringes. In a cross-over study in 10 additonal rats we determined the increase in plasma glutamate during isoflurane anesthesia and its reversibility. For this, rats initially received chloral hydrate (360 mg/kg body weigt i.p.); 30 minutes later isoflurane (1.8 vol%) was administered. After 4 hours the brain-injured rats were either anesthetized with chloral hydrate or isoflurane and at 24 hours after CCI, rats that had previously received chloral hydrate were anesthetized with isoflurane and vice versa. An additional ten rats were killed immediately after short (n = 5) or long (n = 5) chloral hydrate administration to determine changes in CSF.

Before brain removal, CSF was collected by puncturing the atlanto-occipital membrane with a beveled needle as previously published [49].

Analysis of glutamate

Glutamate was analyzed by high performance liquid chromatography (HPLC) using ortho-phthaldialdehyde pre-column derivatisation and fluorescence detection as previously reported [48].

Electroencephalographic (EEG) activity

EEG activity was assessed by attaching two Ag/AgCl electrodes to the skull bone (left frontal and right parietal region along the margins of the craniotomy) and one to the tail as zero reference as described previously [18, 47]. EEG signals were amplified and digitized using a custom-build system. To avoid electrical interference a 50 Hz notch filter was applied. Wide-band filtered data was sampled at 1 kHz and recorded on a PC-computer system. Off-line signal analysis in terms of quantitative EEG was performed using an automated artifact rejection algorithm on 1-minute epochs. Spectral power was calculated from artifact-free epochs using a Hanning-window prior to the Fast Fourier Transformation.

Microdialysis

Microdialysis catheters (CMA 12/membrane length 2 mm) inserted in the cortex of 10 traumatized (within the contusion) and 3 non-traumatized rats at a depth of 2 mm (−8 mm from bregma and −3 mm from the saggital suture) were perfused with artificial cerebrospinal fluid at 2 μl/min. Microdialysis samples collected in 30 minute intervals were corrected by the in vitro recovery determined before and after each experiment as recently published [18, 47]. After prolonged anesthesia microdialysis catheters were removed. To avoid prolongation of anesthesia, microdialysis could not be performed in rats subjected to short anesthesia before brain removal as the duration of anesthesia was not to exceed 30 minutes.

Calculation of the extracellular and CSF to plasma glutamate ratio

To determine a potential passive increase in extracellular and CSF glutamate related to an underlying damaged blood brain barrier (BBB) [2] the extracellular and CSF to plasma glutamate ratios were calculated by dividing the extracellular and CSF concentrations with the corresponding plasma levels.

In vitro receptor autoradiography

Using quantitative in vitro receptor autoradiography, binding to postsynaptic ionotropic NMDA glutamate receptor was investigated with the specific ligand ^{125}I dizocilpine maleate (MK-801) [(+)-3-[^{125}I]Iodo-MK-801; NEN™ Life Science Products, Inc., Boston, USA]. For this, brains were frozen over liquid nitrogen and stored at −80 °C. Coronal sections (10 μm) from identical regions were mounted on gelatine coated slides and pre-incubated in Tris-acetate buffer (50 mM, pH 7.7) for 90 minutes at room temperature. Total binding of NMDA receptors and non-specific binding were determined by incubating tissue sections in Tris-acetate buffer containing 1.0 nM ^{125}I-MK-801 and 10 μM (+)-MK-801, respectively for 90 minutes at room temperature. Incubations were stopped by washing tissue slices in Tris-acetate buffer for 90 minutes at room temperature and dipping the slides in ice-cold water. Tissue slices were dried and coexposed with ^{125}I-MK-801 standards of known concentrations of radioactivity to [^{3}H]-sensitive film (Hyperfilm-^{3}H; Amersham, Braunschweig, Germany) for 24 hours. Autoradiograms were analyzed by transforming the grey values in the autoradiogram to concentrations of radioactivity expressed as fmol binding/mg protein with a non-linear conversion of optical density measures using the image analysis program MCID (Imaging Inc., St. Catharines, Canada). Subtraction of non-specific binding from total binding gives specific binding of ^{125}I-MK-801.

Determination of cerebral water content

Following brain removal the hemispheres were dissected along the interhemispherical line under a microscope. Both hemispheres were weighed to assess wet weight (WW) as previously described [18, 47]. Thereafter, hemispheres were dried for 24 hours at 104 °C to determine dry weight (DW). Based on hemispherical wet and dry weight water content of both, the traumatized and non-traumatized hemispheres were calculated: water content [%] = (WW − DW) WW × 100.

Statistical analysis

Results are presented as mean ± SEM. Parameters were compared for significant differences within and between the groups using one-way analysis of variances (ANOVA) for multiple comparisons followed by post-hoc Bonferroni correction. Differences were rated significant at $p < 0.05$.

Results

Physiologic parameters

MABP and blood gases remained within physiological limits at all time points (Table 1).

Plasma glutamate

Compared to the initial or previous administration of chloral hydrate, arterial plasma glutamate was significantly increased within 30 minutes of isoflurane administration and remained elevated during prolonged isoflurane anesthesia. These increases in plasma glutamate levels were reversible as they returned to baseline values during administration of chloral hydrate at 4 and 24 hours after CCI (Fig. 1).

Extracellular glutamate

Compared to non-traumatized rats, extracellular glutamate determined within the cortical contusion was significantly increased at 4 hours after CCI (19 ± 3 vs. 4.8 ± 1.1 μM; $p < 0.05$). During the first 2 hours of prolonged anesthesia, extracellular glutamate remained stable. By 4 hours, however, glutamate had significantly increased to 29 ± 5 μM ($p < 0.05$).

CSF glutamate

Compared to uninjured rats, CSF glutamate was significantly elevated after CCI (Fig. 2). Compared to rats subjected to short isoflurane or long chloral hydrate administration, CSF glutamate was significantly increased by prolonged isoflurane anesthesia. At 24 hours after short or prolonged isoflurane anesthesia,

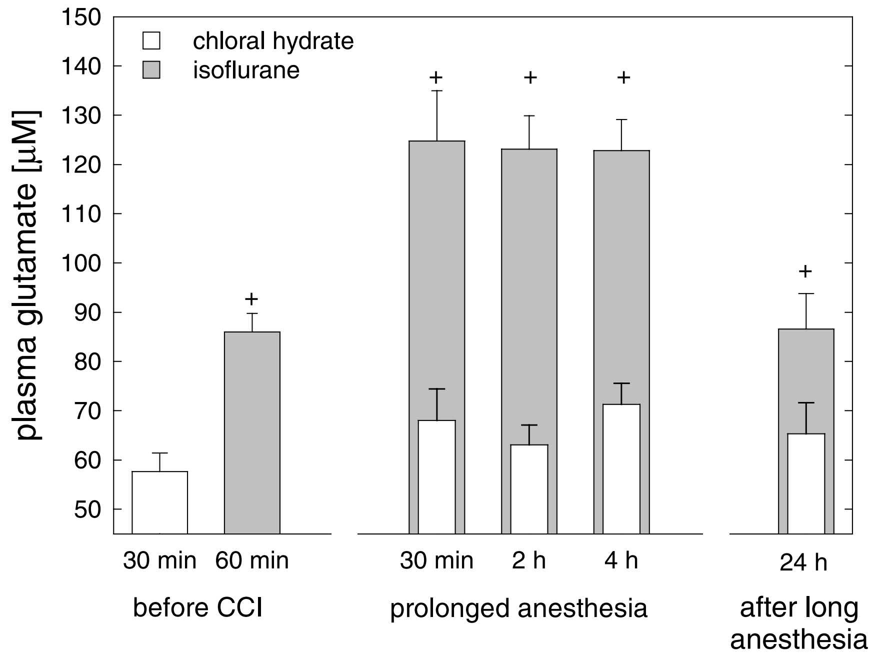

Fig. 1. Arterial plasma glutamate was significantly increased during isoflurane anesthesia compared to rats receiving chloral hydrate ($p < 0.005$). The cross-over study design revealed that these changes were reversible in chloral hydrate treated-rats, previously receiving isoflurane and that isoflurane significantly elevated plasma glutamate 24 hours following previous chloral hydrate administration ($p < 0.005$)

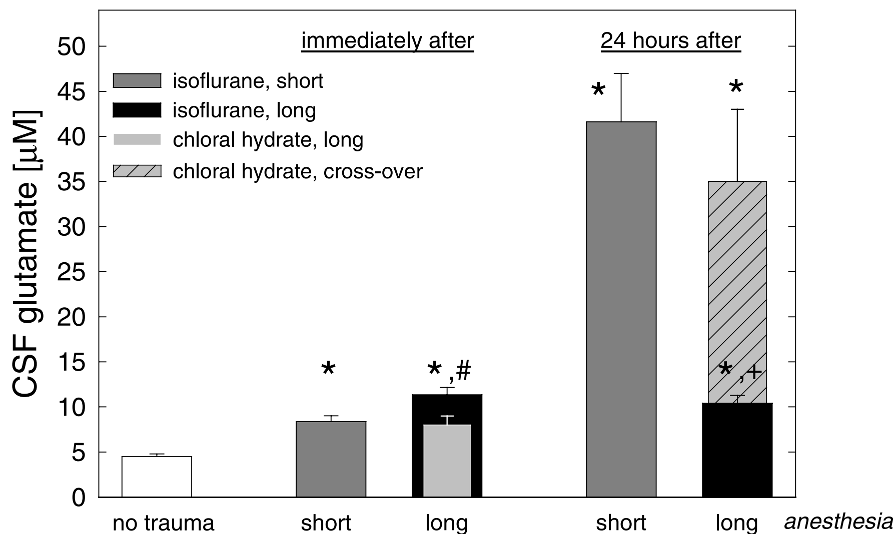

Fig. 2. Following CCI, CSF glutamate was significantly elevated compared to non-injured controls ($^{*}p < 0.001$). When determined immediately after anesthesia, CSF glutamate was significantly increased by prolonged isoflurane anesthesia compared to short isoflurane and long chloral hydrate anesthesia ($^{\#}p < 0.05$). Twenty four hours later, CSF glutamate was significantly decreased in those rats previously subjected to long isoflurane administration compared to short isoflurane anesthesia and rats previously receiving chloral hydrate (cross-over study) ($^{+}p < 0.005$)

CSF glutamate was significantly decreased in rats previously subjected to long isoflurane anesthesia compared to short isoflurane anesthesia and previous chloral hydrate administration.

Extracellular to plasma glutamate ratio

Compared to non-traumatized rats, the extracellular to plasma glutamate ratio was significantly increased at 4 hours after CCI (0.15 ± 0.03 vs. 0.04 ± 0.01) which was elevated even further by the end of the prolonged isoflurane anesthesia (0.24 ± 0.05) ($p < 0.05$).

CSF to plasma glutamate ratio

Compared to control rats, the CSF to plasma glutamate ratio was not increased following short anesthesia (0.08 ± 0.001 vs. 0.075 ± 0.003). Following prolonged

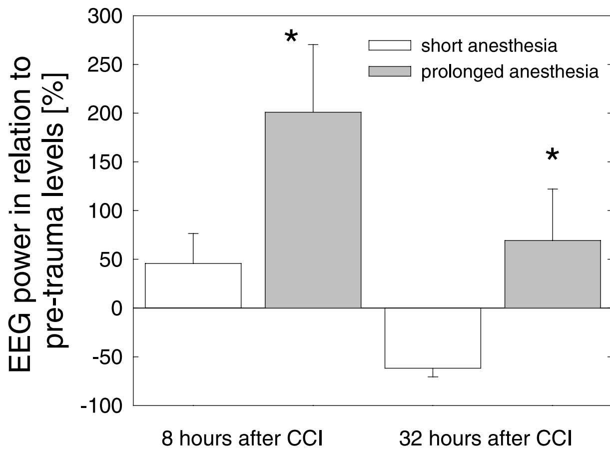

Fig. 3. Immediate and persisting changes in neuronal activity assessed by quantitative EEG analysis during short and prolonged isoflurane anesthesia. Neuronal activity was significantly increased during prolonged compared to short anesthesia. These changes persisted for 24 hours after prolonged anesthesia (*$p < 0.05$, *t*-test)

anesthesia, however, the calculated ratio was significantly elevated to 0.104 ± 0.001 ($p < 0.05$). At 24 hours after short anesthesia, CSF to plasma glutamate ratio reached highest values (0.42 ± 0.009) significantly exceeding values determined in rats previously subjected to prolonged anesthesia (0.12 ± 0.001).

EEG activity

Before CCI, isoflurane combined with N_2O, maintained at 1.8 vol% and 0.3 l/min induced a burst-suppression pattern of approximately 4–6 bursts per minute, resulting in a calculated EEG power of $12 \pm 5\,\mu V^2/Hz$. After CCI, the same anesthesia settings as used before CCI failed to induce the same burst-suppression pattern and EEG recordings revealed predominant continuous high amplitude, low frequency, i.e., delta activity. At 8 hours after CCI, EEG activity was significantly increased by approximately 50% in rats subjected to short anesthesia compared to pre-trauma levels which was significantly sustained over time in those rats subjected to prolonged anesthesia (Fig. 3). During 4 hours of anesthesia, neuronal activity steadily increased compared to baseline values determined within the first 30 minutes of the prolonged anesthesia period (Fig. 4) which persisted up to 24 hours. In those rats subjected to short anesthesia, EEG activity was significantly decreased 24 hours later (Fig. 3). These changes in neuronal activity were reflected by alterations in cortical glutamate concentrations during prolonged anesthesia as shown in one representative rat (Fig. 5).

NMDA receptor binding

Cortex

Within the contusion, cortical NMDA receptor binding was significantly reduced compared to pericontusional and contralateral non-traumatized cortex (Table 2). Over time, NMDA receptor binding continued to decrease within the contusion, corresponding to ongoing cell loss within the growing contusion.

Immediately after prolonged anesthesia specific NMDA receptor binding was significantly increased within the pericontusional and non-traumatized cortex compared to rats subjected to short anesthesia (Table 2). Twenty four hours later, specific NMDA receptor binding was still significantly increased in the non-trauma-

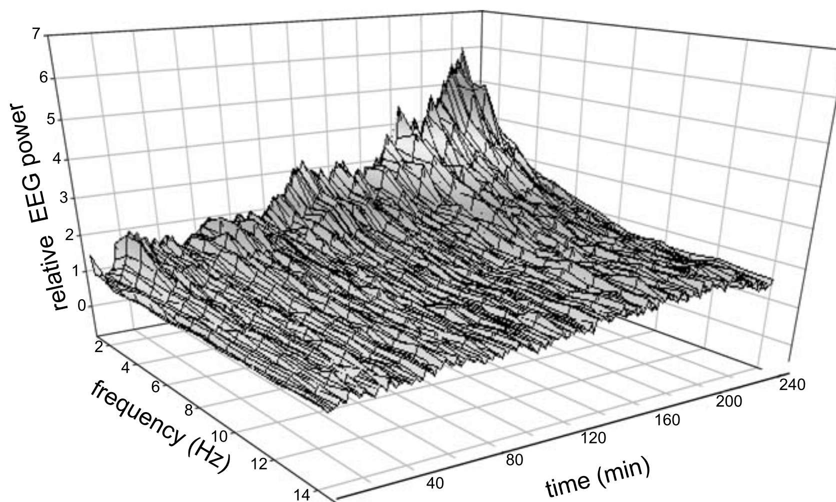

Fig. 4. Evolution of compressed spectral array of EEG activity assessed during prolonged isoflurane anesthesia between 4 and 8 hours following CCI in 10 rats. EEG power is expressed relative to the initial 10-minute-baseline values. Predominant changes occurred within the delta frequency band (0.1–3.5 Hz)

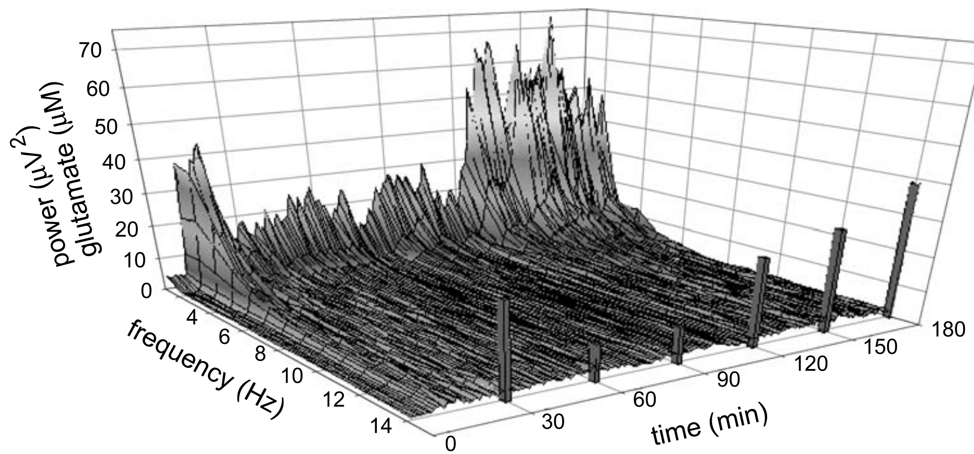

Fig. 5. Time-dependent changes in EEG power and extracellular glutamate levels (bars) during prolonged isoflurane anesthesia in one representative rat. Increased neuronal activity predominantly occurring within the delta frequency band (0.1–3.5 Hz) coincided with elevated glutamate and vice versa

Table 2. *Changes in ^{125}I-MK-801 [fmol/mg] binding in different brain regions in brain-injured rats following short (30 minutes) and prolonged (4 hours) anesthesia determined immediately or 24 hours later. Over time, NMDA receptor binding was significantly decreased within the contusion ($^{+}p<0.05$; ANOVA). Following prolonged anesthesia, NMDA receptor binding was significantly increased within cortical and hippocampal structures compared to short anesthesia. These changes persisted up to 24 hours after prolonged isoflurane anesthesia ($^{§}p<0.05$; ANOVA). Within the CA1 and CA3 region of the hippocampus, NMDA receptor binding was significantly increased in the traumatized compared to non-traumatized hemisphere ($^{*}p<0.05$; t-test)*

Time point of analysis	Immediately after anesthesia		24 Hours after anesthesia	
length of anesthesia	30 minutes (n = 5)	4 hours (n = 5)	30 minutes (n = 5)	4 hours (n = 5)
Specific NMDA receptor binding [fmol/mg] in cortex				
Contusion	8.0 ± 1.2	7.2 ± 1.7	3.3 ± 0.6^{+}	4.1 ± 0.4^{+}
Pericontusion	23.9 ± 1.1	30.6 ± 2.2§	24.8 ± 2.6	28.5 ± 0.8§
Contralateral	22.3 ± 1.5	31.1 ± 1.5§	25.2 ± 0.8	34.1 ± 1.8§
Specific NMDA receptor binding [fmol/mg] in hippocampus				
CA1				
traumatized side	47.1 ± 1.2*	54.0 ± 3.2§	49.0 ± 3.1*	59.0 ± 3.0§
non-traumatized side	41.2 ± 1.2	53.4 ± 2.9§	42.1 ± 3.3	58.0 ± 1.8§
CA3				
traumatized side	25.2 ± 0.9*	27.6 ± 1.6	26.4 ± 2.0	32.9 ± 1.8§
non-traumatized side	20.0 ± 1.2	28.6 ± 2.1§	24.2 ± 3.3	32.9 ± 1.1§
DG (dentate gyrus)				
traumatized side	38.3 ± 2.3	43.5 ± 2.1	40.5 ± 0.1	47.9 ± 1.9§
non-traumatized side	39.4 ± 0.9	45.3 ± 5.5	41.4 ± 2.1	53.2 ± 1.7§

tized cortex of rats following prolonged anesthesia (Table 2).

Hippocampus

Within the hippocampus formation, NMDA receptor binding was significantly increased in the CA1 and CA3 regions of the traumatized hemisphere compared to the non-injured hemisphere (Table 2). The increase in NMDA receptor binding was only sustained in the CA1 region in rats subjected to prolonged anesthesia (Table 2). Following prolonged anesthesia, NMDA receptor binding was persistently increased for 24 hours in the CA1 and CA3 regions in the traumatized and non-traumatized hemispheres compared to rats subjected to short anesthesia.

Cerebral water content

Eight hours following CCI, cerebral water content was significantly increased in the traumatized compared to the non-traumatized hemisphere (Fig. 6). Four hours

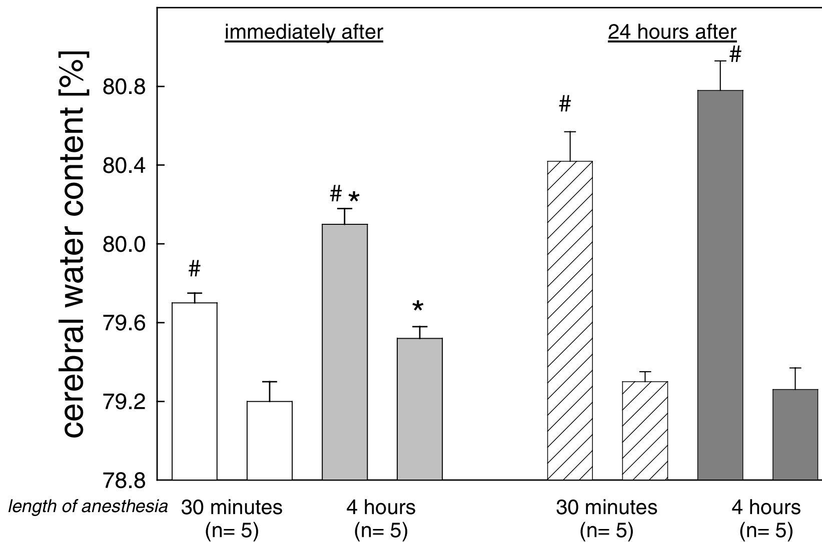

Fig. 6. Hemispheric water content following short (30 minutes) and prolonged (4 hours) anesthesia determined immediately and 24 hours after isoflurane anesthesia. Differences in hemispherical water contents between left (traumatized) and right (non-traumatized) hemisphere (#) and between the investigated groups (*) were significant ($p < 0.05$; ANOVA)

of anesthesia significantly increased water content of both the traumatized and non-traumatized hemispheres compared to rats following short anesthesia. Twenty four hours later, cerebral water content still showed a tendency to increased values in the traumatized hemisphere after prolonged anesthesia compared to short anesthesia without, however, reaching statistical significance. At 24 hours after anesthesia, water content in the non-traumatized hemisphere was similar in rats subjected to prolonged and short anesthesia.

Discussion

Prolonged anesthesia with isoflurane maintained at 1.8 vol% for 4 hours during the early posttraumatic phase (4 to 8 hours after CCI) significantly increased plasma, cortical extracellular, and CSF glutamate concentrations, EEG activity, specific cortical and hippocampal ^{125}I-MK801 NMDA receptor binding, and hemispheric cerebral water content. Some of these changes were partially reversible and CSF glutamate levels were even significantly decreased 24 hours after prolonged isoflurane anesthesia despite a significant increase during the previous long anesthesia.

One interpretation for the acute changes is that increased EEG activity and elevated extracellular glutamate reflect sustained NMDA receptor activity during prolonged anesthesia, thereby aggravating edema formation. Changes in EEG activity preceded alterations in cortical extracellular glutamate by approximately 60 minutes. However, certain technical limitations need to be considered. Extracellular glutamate was sampled with a low regional and temporal resolution (one microdialysis probe positioned within the cortical contusion, analysis every 30 minutes), and EEG activity was recorded with a low spatial but high temporal (1 kHz) resolution measuring the difference in summed field potential between the traumatized and non-traumatized hemisphere. In addition, the present results do not allow us to assess if the increased NMDA receptor binding preceeded or followed the elevated EEG activity and increased glutamate levels since the autoradiographic studies were performed only once at the end of each experiment.

Underlying tissue injury strongly influences the investigated parameters. While NMDA receptor binding and cerebral water content remained elevated up to 24 hours after long anesthesia in the traumatized hemisphere, cerebral water content had normalized in the non-traumatized hemisphere despite persistently elevated NMDA receptor binding, suggesting that sustained ^{125}I-MK801 NMDA binding in the uninjured hemisphere was not associated with irreversible tissue damage.

Sustained NMDA receptor binding

Contrary to the continuous decrease in NMDA receptor binding within the core of the cortical contusion reflecting neuronal cell loss as seen following fluid percussion brain injury in rats [28, 43], NMDA receptor binding remained preserved in the ipsilateral periontusional cortex and hippocampal areas and the corresponding contralateral regions void of neuronal cell degeneration as determined by H. & E. and Fluoro Jade staining (van Landeghem, personal communication). Prolonged isoflurane anesthesia induced a widespread

significant increase in NMDA receptor binding in the pericontusional cortex, bilateral CA1 and CA3 regions and contralateral cortex. Within the contusion, however, prolonged isoflurane anesthesia did not augment ^{125}I-Mk801 binding, suggesting that the sustained NMDA receptor binding in the surrounding areas reflects viable neurons. Detailed histological studies are needed to determine if prolonged isoflurane anesthesia started at 4 hours after TBI reduces cortical lesion and neuronal cell loss within the contusion. The long-term significant reduction in CSF glutamate despite a marked increase during prolonged isoflurane anesthesia maintained between 4 and 8 hours after CCI suggests that isoflurane is neuroprotective. It remains to be determined if this decrease in CSF glutamate will also relate to structural preservation.

Volatile anesthetics attenuate glutamate-mediated neuronal excitation by reversibly decreasing presynaptic glutamate release [23, 25] and allosterically inhibiting glutamate receptor transmission [26]. Chronic and acute pharmacological inhibition of NMDA receptors, in turn, can result in their compensatory up-regulation [27, 36, 51, 53], an effect which might explain the significantly increased NMDA receptor binding in the pericontusional and non-traumatized cortex as well as the ipsilateral and contralateral hippocampus during prolonged isoflurane anesthesia.

As outlined by Martin and colleagues (1995) [26] the entry of MK-801 to its binding site within the ion channel is sterically restricted as it depends on the activation of the NMDA receptor and requires conformational changes for the channel to open. Following CCI, glutamate is significantly increased in the extracellular space [38] and as seen in the present study continues to increase during the early phase following CCI, a time frame in which glial glutamate transporters (GLAST and GLT-1) are significantly decreased [49]. Thus, it is likely that the endogenous agonist glutamate might have activated the NMDA receptor/channel complex, thereby opening the channel pore and allowing sustained binding of ^{125}I-MK-801 during prolonged anesthesia.

In addition to glutamate-mediated activation of the NMDA receptor/channel complex we suspect that intracellular messenger systems involving protein kinase C and tyrosine kinase scr modulated the conformational state and activity of these receptor channel complexes by phosphorylating intracellular domains of the NMDA receptor [6, 54]. TBI pathologically increases intracellular calcium which, in turn, activates various downstream second messanger cascades involving protein kinases and tyrosine kinases. Protein kinase C is a crucial modulator of NMDA channel activity as it changes intrinsic channel properties and regulates receptor and channel trafficking within the cell membrane, resulting in slow and rapid exocytosis and lateral diffusion of the NMDA receptors from the synaptic to extrasynaptic portions of the neuronal membrane [6]. The underlying TBI-induced alterations and the possible superimposed anesthesia-mediated changes make it difficult to decipher mutual influences and interferences since many intracellular alterations known to participate in the TBI-induced cascades are also activated by volatile anesthetics [8, 16].

Elevated extracellular and CSF to plasma glutamate ratio

The calculated extracellular and CSF to plasma glutamate ratios suggest a passive entry of glutamate via the disrupted BBB which is maximal within the first 8 hours after CCI [2]. Further detailed studies injecting radioactively labeled glutamate and inserting several microdialysis catheters at different locations are required to determine if and to what extent elevated extracellular glutamate is influenced by the elevated plasma levels and if these changes also occur in areas without BBB damage. The significant increase in plasma glutamate is an unspecific effect induced within 30 minutes of isoflurane anesthesia. Decreased hepatic uptake, sustained release from muscle and erythrocytes, increased protein degradation, or attenuated renal elimination could account for the reversible increase in plasma levels [4, 14].

Increased EEG activity and elevated extracellular glutamate concentrations

In non-traumatized rats, isoflurane dose-dependently reduces neuronal activity determined by EEG recordings [13, 35], gradually progressing from a continuous predominant alpha and beta (low amplitude, high frequency) to delta activity (high amplitude, low frequency) and then to a burst suppression pattern (bursts of high amplitude/low frequency activity interspersed with low amplitude/low frequency to absent activity) [13]. The predominance of delta activity during isoflurane/N_2O anesthesia reflects cortical neuronal activity. In the present study, rats were anesthetized with isoflurane and N_2O, maintained at 1.8 vol% and 0.3 l/min at all time points. Before CCI, these settings

induced a characteristic burst suppression pattern. Following CCI, however, this pattern disappeared and was substituted by continuous delta activity, suggesting that higher amounts of isoflurane/N_2O are required to reinstate a pre-injury burst suppression pattern after CCI. This is in line with results published by Shapira and colleagues (1997) [42] showing that after TBI rats require higher amounts of isoflurane to prevent responsiveness to exteroceptive painful stimuli.

The induced cortical contusion significantly increased EEG activity by approximately 50% compared to pre-injury values, which was elevated even further during prolonged anesthesia. The present results suggest that prolonged anesthesia actively contributed to the subsequent increase in EEG activity since the EEG activity determined at 4 hours, i.e., beginning of prolonged anesthesia, was similar to changes measured between 7.5 and 8 hours after CCI in the short anesthesia group but was significantly increased by the end of the prolonged anesthesia period at 8 hours after CCI.

The hypothesis that underlying neuronal excitation persists during isoflurane/N_2O anesthesia is strengthened by several findings. Isoflurane given in similar concentrations neither reduces brain metabolism [17] nor abolishes responsiveness to noxious stimuli despite depressed EEG activity [40] and fails to prevent spontaneous increases in neuronal activity during prolonged isoflurane anesthesia as presently observed. Furthermore, additional blocking of glutamate receptors significantly decreases cerebral oxygen consumption during isoflurane anesthesia [24] and reduces inhaled minimum alveolar anesthetic concentration of isoflurane [19].

At present, it remains unclear whether sustained extracellular glutamate mediates or simply reflects significantly elevated EEG activity. In vitro, isoflurane increased glial glutamate uptake [55], and reduced presynaptic neuronal glutamate release [10, 20], an effect which has been shown to persist under pathological conditions in energy-deprived rat hippocampal slice cultures [21]. In vivo, however, isoflurane does not seem to reduce extracellular glutamate concentrations as easily, possibly depending on the investigated model (focal vs. global cerebral ischemia) [33, 39].

Glutamate transport is driven by a complex differentially regulated inward cotransport of 2 Na^+ ions, countertransport of 1 K^+ ion, and cotransport of 1 H^+ or countertransport of 1 OH^- ion [50]. Under pathological conditions disturbed energetic balance and altered Na^+ and K^+ gradients induce a reversal of neuronal and glial glutamate transporters resulting in an outward transport of glutamate. Since volatile anesthetics interfere with Na^+ homeostasis by inhibiting voltage-gated sodium channels [8] this mechanism might contribute to underlying reversal of glutamate transport, thus explaining the presently observed increase in extracellular glutamate.

Elevated cerebral water content

In the present study water content was reversibly increased in both hemispheres during prolonged isoflurane anesthesia. Local cerebral effects could account for this global increase in brain water content. A similar increase in bi-hemispherical water content was also observed in non-traumatized rats subjected to 4 hours of isoflurane/N_2O anesthesia compared to rats receiving chloral hydrate (Stover, unpublished data). Apart from neuroprotective effects [11, 15] volatile anesthetics have also been shown to increase cerebral water content in brain-injured [44] and non-traumatized dogs [41] related to conformational changes of proteins within the lipid bi-layer cell membrane, thereby influencing receptor function and ionic homeostasis. The potential edema-enhancing effect of isoflurane depends on concentration, time point and length of administration. Contrary to our present findings, Statler and colleagues did not see any increase in posttraumatic brain edema [45] in rats continuously anesthetized with a lower concentration of isoflurane (1 vol%) for 4 hours started immediately upon CCI. In our study we had allowed the brain-injured rats to recover from the initial anesthesia required for surgery and induction of CCI before subjecting these animals to an additional short or prolonged anesthesia using a higher isoflurane concentration (1.8 vol%) than Statler and colleagues. Maintaining isoflurane anesthesia during the acute phase immediately after TBI could reduce the evolving tissue damage while re-induction of anesthesia after an interval of several hours might aggravate underlying edema formation as seen in the present study. Future studies comparing various isoflurane concentrations are required to identify the least deleterious effects on injured brains.

Volatile anesthetics dose-dependently disturb autoregulation and induce vasodilation, resulting in increased cerebral perfusion and intracranial blood volume [22] which, in turn, could add to the elevated cerebral water content. This, however, seems less likely since all rats were completely bleed before gravimetrical analysis of cerebral water content. In addition, as recently published cortical perfusion determined by laser Doppler flowmetry remained unchanged during prolonged isoflurane

anesthesia using similar concentrations [18]. Furthermore, isoflurane did not increase cortical perfusion values of the traumatized and non-traumatized hemispheres compared to rats receiving chloral hydrate which does not cause cerebral vasodilation (Stover, unpublished data).

Conclusions

Based on these results, length of anesthesia needs to be chosen carefully when investigating brain edema formation, NMDA receptor binding, plasma, extracellular and CSF glutamate levels. While short isoflurane/N_2O anesthesia (30 minutes) does not influence cerebral water content and NMDA receptor binding, longer periods (up to four hours) interfere with these investigations. Increases in cortical and plasma glutamate could contribute to further tissue damage during the acute posttraumatic phase.

Acknowledgments

The authors thank Mrs. S. Rupprecht (German Primate Center) and Mrs. Seidlitz for their excellent technical assistance performing the in vitro receptor autoradiography and microdialysis analysis, respectively. This work was supported in parts by center grants (89531006/01 and 89531053/01 to JFS) from the Charité, Humboldt University, Berlin and the Research Award 2001 of the German Society for Neurotraumatology and Clinical Neuropsychology (to JFS).

References

1. Ali DW, Salter MW (2001) NMDA receptor regulation by Scr kinase signaling in excitatory synaptic transmission and plasticity. Curr Opin Neurobiol 11: 336–342
2. Baskaya MK, Rao AM, Dogan A, Donaldson D, Dempsey RJ (1997) The biphasic opening of the blood-brain barrier in the cortex and hippocampus after traumatic brain injury in rats. Neurosci Lett 226: 33–36
3. Belelli D, Pistis M, Peters JA, Lambert JJ (1999) General anaesthetic action at transmitter-gated inhibitory amino acid receptors. TiPS 20: 496–499
4. Berg-Johnsen J, Langmoen IA (1992) The effect of isoflurane on excitatory synaptic transmission in the rat hippocampus. Acta Anaesthesiol Scand 36: 350–355
5. Carli F, Ronzoni G, Webster J, Khan K, Elia M (1993) The independent metabolic effects of halothane and isoflurane anesthesia. Acta Anesthes Scand 37: 672–678
6. Carroll RC, Zukin RS (2002) NMDA-recepor trafficking and targeting: implications for synaptic transmission and plasticity. TiNS 25: 571–577
7. Detsch O, Kochs E, Siemers M, Bromm B, Vahle-Hinz C (2002) Increased responsiveness of cortical neurons in contrast to thalamic neurons during isoflurane- induced EEG bursts in rats. Neurosci Lett 317: 9–12
8. Do SH, Kamatchi GL, Washington JM, Zuo Z (2002) Effects of volatile anesthetics on glutamate transporter, excitatory amino acid transporter type 3: the role of protein kinase C. Anesthesiology 96: 1492–1497
9. Duch DS, Rehberg B, Vysotskaya TN (1998) Volatile anesthetics significantly suppress central and peripheral mammalian sodium channels. Toxicol Lett 100–101: 255–263
10. Eilers H, Kindler CH, Bickler PE (1999) Different effects of volatile anesthetics and polyhalogenated alkanes on depolarization-evoked glutamate release in rat cortical brain slices. Anesth Analg 88: 1168–1174
11. Harada H, Kelly P, Cole D, Drummond JC, Patel P (1999) Isoflurane reduces N-methyl-D-aspartate in vivo in the rat cerebral cortex. Anesth Analg 89: 1442–1443
12. Hendrich KS, Kochanek PM, Melick JA *et al* (2001) Cerebral perfusion during anesthesia with fentanyl, isoflurane, or pentobarbital in normal rats studied by arterial spin-labeled MRI. Magn Reson Med 46: 202–206
13. Hudetz AG (2002) Effect of volatile anesthetics on interhemispheric EEG cross-approximate entropy in the rat. Brain Res 954: 123–131
14. Horber FF, Krayer S, Rehder K, Haymond MW (1988) Anesthesia with halothane and nitrous oxide alters protein and amino acid metabolism in dogs. Anesthesiology 69: 319–326
15. Kapinya KJ, Lowl D, Futterer C *et al* (2002) Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. Stroke 33: 1889–1898
16. Kindler CH, Eilers H, Donogoe P, Ozoer S, Bickler PE (1999) Volatile anesthetics increase intracellular calcium in cerebrocortical and hippocampal neurons. Anesthesiology 90: 1137–1145
17. Kochs E, Hoffman WE, Werner C, Albrecht RF, Schulte am Esch J (1993) Cerebral blood flow velocity in relation to cerebral blood flow, cerebral metabolic rate for oxygen, and electroencephalogram activity during isoflurane anesthesia. Anesth Analg 76: 1222–1226
18. Kroppenstedt SN, Sakowitz OW, Thomale UW, Unterberg AW, Stover JF (2002) Influence of norepinephrine and dopamine on cortical perfusion, EEG activity, extracellular glutamate and brain edema in rats following controlled cortical impact injury. J Neurotrauma 19: 1421–1432
19. Kuroda Y, Strebel S, Rafferty C, Bullock R (1993) Neuroprotective doses of N-methyl-D-aspartate receptor antagonists profoundly reduce the minimum alveolar anesthetic concentration (MAC) for isoflurane in rats. Anesth Analg 77: 795–800
20. Larsen M, Valo ET, Berg-Johnsen J, Langmoen IA (1998) Isoflurane reduces synaptic glutamate release without changing cytosolic free calcium in isolated nerve terminals. Eur J Anaesthesiol 15: 224–229
21. Larsen M, Haugstad TS, Berg-Johnsen J, Langmoen IA (1998) The effect of isoflurane on brain amino acid release and tissue content induced by energy deprivation. J Neurosurg Anesthesiol 10: 166–170
22. Lenz C, Frietsch T, Futterer C *et al* (1999) Local coupling of cerebral blood flow to cerebral glucose metabolism during inhalational anesthesia in rats: desflurane versus isoflurane. Anesthesiology 91: 1720–1723
23. Liachenko S, Tang P, Somogyi GT, Xu Y (1999) Concentration-dependent isoflurane effects on depolarization-evoked glutamate and GABA outflows from mouse brain slices. Br J Pharmacol 127: 131–138
24. Lu X, Sinha AK, Weiss HR (1997) Effects of excitatory amino acids on cerebral oxygen consumption and blood flow in rat. Neurochem Res 22: 705–711
25. MacIver MB, Mikulec AA, Amagasu SM, Monroe AM (1996) Volatile anesthetics depress glutamate transmission via presynaptic actions. Anesthesiology 85: 823–834

26. Martin DC, Plagenhoef M, Abraham J, Dennison RL, Aronstam RS (1995) Volatile anesthetics and glutamate activation of N-methyl-D-aspartate receptors. Biochem Pharmacol 49: 809–817
27. McDonald JW, Silverstein FS, Johnston MV (1990) MK-801 pretreatment enhances N-methyl-D-aspartate-mediated brain injury and increases brain N-methyl-D-aspartate recognition site binding in rats. Neuroscience 38: 103–113
28. Miller LP, Lyeth BG, Jenkins LW *et al* (1990) Excitatory amino acid receptor subtype binding following traumatic brain injury. Brain Res 526: 103–107
29. Mills CD, Robertson CS, Contant CF, Henley CM (1997) Effects of anesthesia on polyamine metabolism and water content in the rat brain. J Neurotrauma 14: 943–949
30. Ming Z, Knapp DJ, Mueller RA, Breese GR, Criswell HE (2001) Differential modulation of GABA- and NMDA-gated currents by ethanol and isoflurane in cultured rat cerebral cortical neurons. Brain Res 920: 117–124
31. Ming Z, Griffith BL, Breese GR, Mueller RA, Criswell HE (2002) Changes in the effect of isoflurane on N-methyl-D-aspartic acid-gated currents in cultured cerebral cortical neurons with time in culture: evidence for subunit specificity. Anesthesiology 97: 856–867
32. Miyazaki H, Nakamura Y, Aral T, Kataoka K (1997) Increase of glutamate uptake in astrocytes: a possible mechanism of action of volatile anesthetics. Anesthesiology 86: 1359–1366
33. Nakashima K, Todd MM (1996) Effects of hypothermia, pentobarbital, and isoflurane on postdepolarization amino acid release during complete global cerebral ischemia. Anesthesiology 85: 161–168
34. Nishikawa K, MacIver MB (2000) Excitatory synaptic transmission mediated by NMDA receptors is more sensitive to isoflurane than are non-NMDA receptor-mediated responses. Anesthesiology 92: 228–236
35. Nishimaki H, Fukuda S, Ishimoto M *et al* (2002) Isoflurane ameliorates the posthypoxic deoxygenation of the rat brain- the role of cell adhesion molecules of polymorphonuclear leukocytes. Resuscitation 54: 207–214
36. Oh S, Hoshi K, Ho IK (1997) Role of NMDA receptors in pentobarbital tolerance/dependence. Neurochem Res 22: 767–774
37. Paas Y (1998) The macro- and microarchitectures of the ligand-binding domain of glutamate receptors. TINS 21: 117–125
38. Palmer AM, Marion DW, Botscheller ML, Swedlow PE, Styren SD, DeKosky ST (1993) Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. J Neurochem 61: 2015–2024
39. Patel PM, Drummond JC, Cole DJ, Goskowicz RL (1995) Isoflurane reduces ischemia-induced glutamate release in rats subjected to forebrain ischemia. Anesthesiology 82: 996–1003
40. Rampil IJ, Laster MJ (1992) No correlation between quantitative electroencephalographic measurements and movement response to noxious stimuli during isoflurane anesthesia in rats. Anesthesiology 77: 920–925
41. Schettini A, Furniss WW (1979) Brain water and electrolyte distribution during the inhalation of halothane. Br J Anaesth 51: 1117–1124
42. Shapira Y, Gurevich B, Artru AA *et al* (1997) Influence of closed head injury on isoflurane MAC in the rat. J Neurosurg Anesthesiol 9: 51–57
43. Sihver S, Marklund N, Hillered L, Langstrom B, Watanabe Y, Bergstrom M (2001) changes in mach, NMDA and $GABA_A$ receptor binding after lateral fluid-percussion injury: in vitro autoradiography of rat brain frozen sections. J Neurochem 78: 417–423
44. Smith AL, Marque JJ (1976) Anesthetics and cerebral edema, Anesthesiology 45: 64–72
45. Statler KD, Kochanek PM, Dixon CE *et al* (2000) Isoflurane improves long-term neurologic outcome versus fentanyl after traumatic brain injury in rats. J Neurotrauma 17: 1179–1189
46. Stover JF, Kroppenstedt SN, Thomale UW, Kempski OS, Unterberg AW (2000) Isoflurane doubles plasma glutamate and increases posttraumatic brain edema. Acta Neurochir (Wien) [Suppl] 76: 375–378
47. Stover JF, Sakowitz OW, Beyer TF *et al* (2003) Effects of LY379268, a selective group II metabotropic glutamate receptor agonist on EEG activity, cortical perfusion, tissue damage, and cortical glutamate, glucose, and lactate levels in brain-injured rats. J Neurotrauma 20: 315–326
48. Stover JF, Pleines UE, Morganti-Kossmann MC, Kossmann T, Lowitzsch K, Kempski OS (1997) Neurotransmitters in cerebrospinal fluid reflect pathological activity. Eur J Clin Invest 27: 1038–1043
49. van Landeghem FK, Stover JF, Bechmann I *et al* (2001) Early expression of glutamate transporter proteins in ramified microglia after controlled cortical impact injury in the rat. Glia 35: 167–179
50. Takahashi M, Billups B, Rossi D, Sarantis M, Hamann M, Attwell D (1997) The role of glutamate transporters in glutamate homeostasis in the brain. J Exp Biol 200: 401–409
51. Weiss HR, Sinha AK, Lu X (1996) Effect of up-regulation of NMDA receptors on cerebral O_2 consumption and blood flow in rat. Brain Res 730: 193–198
52. Wenthold RJ, Prybylowski K, Standley S, Sans N, Petralia RS (2003) Trafficking of NMDA receptors. Annu Rev Pharmacol Toxico l43: 335–358
53. Wilson MA, Kinsman SL, Johnston MV (1998) Expression of NMDA receptor subunit mRNA after MK-801 treatment in neonatal rats. Dev Brain Res 109: 211–220
54. Yu X-M, Salter WM (1999) Src, a molecular switch governing gain control of synaptic transmission mediated by N-methyl-D-aspartate receptors. Proc Natl Acad Sci USA 96: 1707–7704
55. Zuo Z (2001) Isoflurane enhances glutamate uptake via glutamate transporters in rat glial cells. Neuroreport 12: 1077–1080

Comments

Using a rodent model of controlled critical impact injury, the authors have investigated the effects of 30 minutes vs. 4 hours of general anaesthesia with isoflurane/nitrous oxide compared to chloral hydrate on CSF, extracellular, and plasma glutamate levels and on NMDA receptor binding in various locations of the injured and uninjured brain. They have also studied the effects of short and prolonged anaesthesia on EEG activity and on brain water content. At a concentration of 1.8 volumes/percent, they have demonstrated quite convincingly that isoflurane can cause increased NMDA receptor binding, increased brain edema, and increased plasma and CSF glutamate levels. This study has importance for clinicians caring for patients with severe traumatic brain injury in whom elevated glutamate levels and brain swelling are confounding factors potentially leading to secondary brain injury.

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In the present study it was found that prolonged isoflurane anaesthesia in the early phase following cortical impact injury significantly increased plasma, cortical extracellular, and CSF glutamate concentrations, EEG activity, specific cortical and hippocampal 125I-MK801 NMDA receptor binding, and hemispheric cerebral water content. Some of the changes were partially reversible and CSF glutamate levels were decreased 24 hours after prolonged isoflurane anaesthesia despite a significant increase during the previous long anaesthesia.

As a matter of fact AMP A receptors mediate fast excitatory neurotransmission in most of the synapses in the CNS. AMP A receptor channels had been considered to be permeable only to Na^+ and K^+, and almost impermeable to Ca^{2+} in central neurons. More lately the AMPA II receptor has been found to display a substantial permeability to Ca^{2+} (Neurosci Lett 155: 187–190).

The NMDA receptor is also found throughout the brain but predominantly within the forebrain. It mediates excitatory neurotransmission in the CNS in different ways from AMP A receptors; it has slow gating kinetics and characteristic ion permeation properties. Na^+, K^+ and Cs^+ ions, permeate through the channel with a low selectivity, but major differences from non-NMDA receptor channels exist in the permeation properties for Ca^{2+} and Mi^+. Ca^{2+} is highly permeant, whereas Mg^{2+} is a potent blocker (Nature 309: 261–263, J Physiol 399: 247–266). These unique properties have attracted the interest of neuroscientists, since they provide the NMDA receptor with a molecular basis for synaptic plasticity.

The effect on isoflurane on the brain is very complex. It reduces synaptic release of some transmitters, but also increases glutamate uptake into presynaptic terminals (Br J Anaesth 199778: 55–59) as well as glial cells (as the authors have noted). Even if isoflurane does not seem to reduce extracellular glutamate concentrations as easily in vivo, it must be remembered that this is a much more difficult setting to measure glutamate in.

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