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The impact of anesthetics and hyperoxia on cortical spreading depression

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Abstract

Cortical spreading depression (CSD), a transient neuronal and glial depolarization that propagates slowly across the cerebral cortex, is the putative electrophysiological event underlying migraine aura. It negatively impacts tissue injury during stroke, cerebral contusion and intracranial hemorrhage. Susceptibility to CSD has been assessed in several experimental animal models *in vivo*, such as after topical KCl application or cathodal stimulation. Various combinations of anesthetics and ambient conditions have been used by different laboratories making comparisons problematic and differences in data difficult to reconcile. We systematically studied CSD susceptibility comparing commonly used experimental anesthetics (isoflurane, α -chloralose, urethane) with or without N₂O or normobaric hyperoxia (100% O₂ inhalation). The frequency of evoked CSDs, and their propagation speed, duration, and amplitude were recorded during 2 hour topical KCl (1M) application. We found that N₂O reduced CSD frequency when combined with isoflurane or urethane, but not α -chloralose; N₂O also decreased CSD propagation speed and duration. Urethane anesthesia was associated with the highest CSD frequency that was comparable to pentobarbital. Inhalation of 100% O₂ did not alter CSD frequency, propagation speed or duration in combination with any of the anesthetics tested. Our data show anesthetic modulation of CSD susceptibility in an experimental model of human disease, underscoring the importance of proper study design for hypothesis testing as well as for comparing results between studies.

Keywords

isoflurane; alpha-chloralose; urethane; nitrous oxide; electrophysiology

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INTRODUCTION

Cortical spreading depression (CSD), discovered by Leão in 1944 (Leão, 1944), is a wave of neuronal and glial depolarization that slowly propagates throughout cortex irrespective of functional or vascular territories. The pivotal event in the generation and propagation of CSD is depolarization of a minimum critical mass of brain tissue associated with a massive increase in extracellular K^+ ($[K^+]_e$) and neurotransmitters. Several diverse stimuli can trigger CSD including direct cortical trauma, exposure to high concentrations of excitatory amino acids or K^+ , direct electrical stimulation, inhibition of Na^+/K^+ -ATPase, and energy failure.

Various experimental models have been employed to assess CSD susceptibility, such as recording the frequency of CSDs evoked during continuous topical KCl application (Ayata, et al., 2006, Kitahara, et al., 2001, Read, et al., 1997, Richter, et al., 2002, Wu, et al., 2003). This model showed good clinical correlation. For example, chronic but not acute treatment with migraine prophylactic drugs reduced the frequency of KCl-evoked CSDs (Ayata, et al., 2006), and knock-in mice carrying the human familial hemiplegic migraine type-1 mutation R192Q in $Ca_v2.1$ gene (P/Q-type Ca^{++} channel) developed higher frequency of KCl-evoked CSDs (Haerter, et al., 2006, van den Maagdenberg, et al., 2004). In stroke, the number of peri-infarct CSDs correlate with the extent of injury (Mies, et al., 1993). However, the impact of specific experimental conditions on CSD susceptibility has not been well explored.

General anesthetics modulate CSD susceptibility (Guedes and Barreto, 1992, Kitahara, et al., 2001, Piper and Lambert, 1996, Saito, et al., 1997, Saito, et al., 1995, Sonn and Mayevsky, 2006, Verhaegen, et al., 1992). Interestingly, nitrous oxide (N_2O), an inhalational anesthetic frequently used as an adjunct with other general anesthetics in experimental models, reportedly shows clinical efficacy in aborting acute migraine (Triner, et al., 1999), whereas normobaric hyperoxia (100% O_2) is ineffective (Myers and Myers, 1995). We, therefore, aimed to systematically study the impact of commonly used experimental anesthetics (isoflurane, α -chloralose, urethane, pentobarbital, N_2O), and 100% O_2 inhalation on the frequency and electrophysiological properties of KCl-induced CSDs in the rat.

MATERIALS AND METHODS

Surgical preparation

Sprague-Dawley rats (256–710g, male) were anesthetized using isoflurane (3% for induction, 2% for maintenance in 30% O_2 +70% N_2O during surgical procedures). Bupivacaine (0.25%) was administered locally at the surgical sites for analgesia before skin incisions were made. Rats were intubated via a tracheostomy for mechanical ventilation (SAR-830; CWE, Ardmore, PA). Continuous measurement of mean arterial blood pressure (PowerLab; ADInstruments, Colorado Springs, MO) and arterial blood sampling were performed via a femoral artery catheter. Rats were then paralyzed using pancuronium (0.32–0.40 mg/kg/h) to facilitate mechanical ventilation. Arterial blood gases and pH were measured every 15–30 minutes and ventilation parameters were adjusted to maintain pCO_2 at 35–45 mmHg (Corning 178 blood gas/pH analyzer; Corning, NY). Rectal temperature was kept at 36.9 to 37.1° C using a thermostatically controlled heating pad (Frederick Heat Company, Bowdoinham, ME). Level of anesthesia was maintained throughout the procedure to abolish blood pressure and heart rate response to tail pinch. Adequate measures were taken to minimize pain or discomfort. Study protocol strictly followed institutional guidelines for animal care and use for research purposes, in accordance with international standards on animal welfare and local and national regulations.

In all treatment groups, mean arterial blood pressure, arterial pCO_2 and pH were within the normal physiological range for anesthetized and mechanically ventilated rats (Table 1). Urethane caused a small but statistically significant reduction in arterial blood pressure and

pH compared to other anesthetics. Inhalation of N₂O significantly reduced the urethane dose required to achieve surgical depth of anesthesia.

Electrophysiological recordings

Rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and three burr holes (1 mm diameter of exposed pial surface) were drilled under saline cooling over the right hemisphere at the following coordinates (mm from bregma): (1) posterior 5, lateral 2 (occipital cortex): KCl application; (2) posterior 3.5, lateral 2 (parietal cortex): recording site 1; (3) posterior 1, lateral 2 (frontal cortex): recording site 2. Dura overlying the occipital cortex was gently removed and care was taken to avoid cortical damage or bleeding. The steady (DC) potential and electrocorticogram were recorded with glass micropipettes filled with 150 mM NaCl, 300 μ m below the dural surface (FHC, Inc. Bowdoinham, ME). Ag/AgCl reference electrode was placed subcutaneously in the neck. After surgical preparation, the cortex was allowed to recover for 15 minutes under saline irrigation. The data were continuously recorded using a data acquisition system for off-line analysis.

CSD induction

A cotton ball (2 mm diameter) soaked with 1M KCl was placed on the pial surface and kept moist by placing 5 μ l of KCl solution every 15 minutes. The number of KCl-induced CSDs was counted for 2 hours. Propagation speed was calculated from the distance (mm) between the recording electrodes 1 and 2, divided by the latency (min) between the CSDs recorded at these sites. The amplitude of DC shift and its duration at half-maximal amplitude were also measured.

Experimental groups and protocols

A total of nine groups of rats were studied using a combination of one of three general anesthetics (isoflurane, α -chloralose or urethane; see Table 1 for anesthetic doses) and three different ventilation gas mixtures (I, 30% O₂+70% N₂; II, 30% O₂+70% N₂O; III, 100% O₂). In addition, a subgroup of rats was studied under pentobarbital anesthesia (50 $\pm$ 7 mg/kg/h) breathing 30% O₂+70% N₂. After completion of surgical procedures, test anesthetic was administered (α -chloralose, urethane or pentobarbital), test gas mixture started, and surgical anesthesia (isoflurane) weaned off over 15 minutes. Electrophysiological recordings and KCl application started an additional 15 minutes after isoflurane was completely off (i.e., 30 min after α -chloralose, urethane or pentobarbital injection). In our experience, these durations are sufficient to eliminate isoflurane and N₂O from the system. Both isoflurane and N₂O have low blood-gas solubility (and hence rapid induction and emergence), and isoflurane has a short context-sensitive half time (~5 min) (Bailey, 1997); however, we cannot rule out possible delayed effects or interactions with the test anesthetics. When isoflurane was the test anesthetic, it was simply dialed down to 1%, and recordings were started 15 min later.

Statistical analysis

Data were analyzed using two- or three-way ANOVA, except when otherwise indicated. For two-way ANOVA, anesthetic (isoflurane, α -chloralose, urethane) and ventilation gas (groups I–III) were the independent variables, while CSD frequency over 2 hours, and systemic physiological parameters were the dependent variables. For three-way ANOVA, the additional independent variable of 1st vs. 2nd CSD was introduced to analyze CSD speed and duration. Post-hoc analyses were conducted using Bonferroni all-pairwise multiple comparison test. Data obtained in pentobarbital-anesthetized rats breathing 30% O₂+70% N₂ were compared only to urethane-anesthetized rats breathing 30% O₂+70% N₂, using t-test. Data were expressed as mean $\pm$ standard deviation (SD). P<0.05 was considered significant.

RESULTS

CSD frequency

Topical application of 1M KCl evoked repetitive CSDs in all groups studied (Figure 1A). In rats breathing 70%N₂+30%O₂ (Group I), the highest CSD frequencies were recorded under urethane anesthesia (Figure 1), and were comparable to pentobarbital (32±10 CSDs/2h, n=5). CSDs were fewer and did not differ between isoflurane (0.7 MAC) and α-chloralose anesthesia. Nitrous oxide (70%N₂O+30%O₂, Group II) significantly reduced CSD frequency when combined with isoflurane or urethane but not when added to α-chloralose anesthesia; the lowest CSD frequencies were recorded in isoflurane-anesthetized rats breathing 70%N₂O+30%O₂, and were approximately 50% of urethane group I. Normobaric hyperoxia did not significantly alter CSD frequency in combination with any of the three anesthetics when compared to normoxia (Figure 1). The CSD frequency did not differ between the first and second hours of recording in the isoflurane, α-chloralose or pentobarbital groups; under urethane anesthesia, CSDs were significantly more frequent during the second hour compared to the first (p<0.05; Figure 1B).

CSD propagation speed and duration

The ventilation gas mixture but not the anesthetic type altered the propagation speed and the duration of CSD (Tables 2, 3). In rats breathing 70%N₂O+30%O₂, propagation speeds were slower and DC shift durations shorter than control rats receiving 70%N₂+30%O₂; the inhibitory effect of N₂O was most marked within the isoflurane group. When compared to the 1st CSD after KCl application, all subsequent CSDs were shorter in duration and propagated slower in all groups. The addition of N₂O further decreased the duration of subsequent CSDs, and tended to reduce their speed. CSD amplitudes were 23 to 25 mV in all groups, and did not differ among groups receiving different anesthetics and inhalation gas mixtures. As with CSD frequency, normobaric hyperoxia did not impact CSD duration or propagation speed (Tables 2, 3). Pentobarbital anesthesia (70%N₂+30%O₂) did not significantly alter CSD duration or propagation speed compared to urethane (30±3 and 27±11 sec, 3.4±0.4 and 2.5±0.4 mm/min, 1st and 2nd CSD, respectively; p>0.05, one-way ANOVA).

DISCUSSION

We provide a systematic comparison of the frequency of KCl-evoked CSDs using four experimental anesthetics that belong to different pharmacological classes. We demonstrate that anesthetic choice impacts CSD frequency. Urethane anesthesia did not differ from pentobarbital, and was associated with higher CSD frequencies compared to isoflurane and α-chloralose, while N₂O significantly suppresses CSD susceptibility when combined with isoflurane or urethane, but not with α-chloralose. The CSD propagation speed and duration were also reduced by N₂O, but otherwise was not altered by anesthetic type, suggesting that sensitivity to or mechanisms for anesthetic suppression of CSD frequency differ from CSD speed and duration. Reduced propagation speed and duration upon repetitive CSDs, as previously described (Brand, et al., 1998, Gorelova and Bures, 1983, Weimer and Hanke, 2005), were not influenced by any of the anesthetic and ventilation gas mixtures in our study.

Previous studies testing the impact of anesthesia used variable methods to evoke CSD. When evoked by direct cathodal stimulation in rats, the threshold for CSD did not differ between halothane and isoflurane, or between 0.7 and 1.6 MAC of these anesthetics (Verhaegen, et al., 1992). In contrast, Saito et al. found a complete suppression of KCl-evoked CSDs in cats under 0.7 MAC halothane when compared to α-chloralose, although both groups received 70% N₂O, which may have potentiated the CSD suppression by halothane as observed in our study (Saito, et al., 1995). In another study, halothane (1–1.5 MAC) was more effective in

suppressing pinprick-induced CSDs compared to isoflurane (1.5 MAC); at this concentration, however, isoflurane still suppressed CSDs compared to α -chloralose (Piper and Lambert, 1996). Using a continuous topical 3M KCl application similar to ours, the frequency of evoked CSDs was suppressed in a concentration-dependent manner by halothane, isoflurane and sevoflurane; the lowest isoflurane concentration that suppressed CSDs was 1 MAC, while CSD frequency under 0.5 MAC isoflurane did not differ from pentobarbital (Kitahara, et al., 2001). These data, taken together with ours, suggest that low concentrations of isoflurane (<1 MAC) does not significantly suppress CSD compared to α -chloralose when tested using the KCl model of CSD susceptibility. Our data also show that when breathing room air (i.e., in the absence of N₂O), urethane anesthesia yields the highest CSD frequencies among the three anesthetics tested, that were comparable to pentobarbital. Because these experiments could not be carried out in unanesthetized rats in our laboratory, we cannot state whether urethane exerts any CSD suppression compared to awake rats. A combination of urethane and α -chloralose reportedly reduced CSD propagation speed compared to awake rats; however, CSD susceptibility per se was not tested (Guedes and Barreto, 1992). The consistently higher CSD frequencies during the second hour of recording in urethane-anesthetized rats may be due to a lower inhibitory potency of its active metabolites on CSD.

Importantly, N₂O suppressed CSDs in isoflurane- or urethane-anesthetized rats, but was ineffective under α -chloralose anesthesia. The effect of N₂O on CSD susceptibility was tested in only one other study, where it appeared to reduce the isoflurane concentration required to suppress CSDs. As N₂O alone does not provide surgical depth of anesthesia at 1 atmospheric pressure, its impact as the sole agent on CSD susceptibility cannot be tested. However, lack of CSD suppression by N₂O in α -chloralose-anesthetized rats in our study argues against a simple additive effect.

All four anesthetic agents tested in this study possess unique chemical and pharmacologic profiles. Isoflurane is a halogenated-ether widely used as a volatile anesthetic in the experimental setting; α -chloralose is a hypnotic agent the active metabolite of which, trichloroethanol, is also the active metabolite of chloral hydrate; and urethane (ethyl carbamate) is an injectable anesthetic favored in experimental anesthesia due to its unusually long duration of action due to active metabolites, and minimal cardiovascular and respiratory depression. Many targets for general anesthetic agents and N₂O have been identified, with effects ranging from suppression of membrane excitability to inhibition of presynaptic release and postsynaptic receptor. The GABA_A receptor is now accepted as a common target for many general anesthetics (Campagna, et al., 2003, Garrett and Gan, 1998, Hara and Harris, 2002), but enhanced GABAergic transmission is unlikely to modulate CSD susceptibility since barbiturates do not suppress CSD (Brand, et al., 1998, Kitahara, et al., 2001, Van Harreveld and Stamm, 1953). Relevant for CSD suppression, however, are the NMDA subtype of glutamate receptors, inhibition of which potently reduces CSD speed and duration, and blocks CSD (Gorelova, et al., 1987, Hernandez-Caceres, et al., 1987, Marrannes, et al., 1988, Obrenovitch and Zilkha, 1996). Interaction with NMDA receptors has thus far been convincingly demonstrated for isoflurane and N₂O. Isoflurane causes modest inhibition of NMDA receptors, likely via the noncompetitive glycine site (Carla and Moroni, 1992, Dickinson, et al., 2007, Puil and el-Beheiry, 1990, Puil, et al., 1990, Solt, et al., 2006). N₂O exerts a more potent and selective inhibitory effect on NMDA receptors at concentrations tested in our study (Jevtovic-Todorovic, et al., 1998, Yamakura and Harris, 2000). In addition to isoflurane and N₂O, chloral hydrate, which shares the same active metabolite with α -chloralose, was shown to non-competitively inhibit NMDA-induced depolarizations in mouse cortex (Carla and Moroni, 1992). In contrast, urethane does not significantly inhibit NMDA receptor channels at clinically relevant concentrations (Hara and Harris, 2002), and did not suppress CSD compared to pentobarbital in our study. Therefore, the efficacy of general anesthetics on CSD susceptibility appears to correspond to their reported efficacy on NMDA receptors. In

addition, isoflurane may suppress CSD by augmenting glutamate uptake in astrocytes (Miyazaki, et al., 1997), or reducing glutamate release (el-Beheiry and Puil, 1989, Kullmann, et al., 1989, Wu, et al., 2004). Another potential mechanism for CSD inhibition is membrane hyperpolarization via opening of K⁺ channels (Wu, et al., 2003). Isoflurane, chloral hydrate, and N₂O have been shown to activate TREK channels (Franks and Honore, 2004), which are background K⁺ channels that clamp the membrane potential at resting levels thus resisting depolarization.

Consistent with its clinical efficacy to abort migraine (Triner, et al., 1999), N₂O suppressed CSD susceptibility in this topical KCl model. In contrast, 100% O₂, reportedly ineffective in migraine (Myers and Myers, 1995), did not suppress CSD susceptibility, underscoring the predictive value of this model for migraine susceptibility. Interestingly, 100% O₂ inhalation did not reduce the DC shift duration, a finding contrary to a recent report in mice (Takano, et al., 2007). The data also suggest that suppression of peri-infarct spreading depressions by 100% O₂ in focal cerebral ischemia (Shin, et al., 2007) is not due to a non-specific reduction in susceptibility of cortex to CSD. Rather, it may act by specifically reducing CSD susceptibility in ischemic penumbra.

In summary, our data show that anesthetic regimen modulates CSD susceptibility, underscoring the importance of anesthetic choice to preserve the predictive value of experimental studies of CSD as a model for migraine susceptibility.

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Figure 1A

Figure 1B

Figure 1. The impact of anesthetics and inspired gas mixture on CSD susceptibility

Representative 30 minute DC potential recordings from parietal cortex (A), and average CSD frequencies (B) during 2h topical KCl application (1M) to occipital cortex. Data are mean $\pm$ SD. Calibration scales indicate 10 min and 20 mV. Error bars refer to first and second hour data combined.

*: $p < 0.05$, urethane vs. isoflurane (groups I–III combined).

†: $p < 0.05$, group II vs. I and III within isoflurane.

‡: $p < 0.05$, isoflurane vs. urethane and α -chloralose within II.

§: $p < 0.05$, urethane vs. isoflurane and α -chloralose within I.

#: $p = 0.05$, group II vs. I within urethane.

Figure 2. The impact of anesthetics and inspired gas mixture on CSD duration and propagation speed

Representative DC potential shifts simultaneously recorded from parietal and frontal cortex using two microelectrodes (ME1 and ME2, respectively). Vertical lines mark the onset of each DC shift. The first 2–3 CSDs are shown after initial topical KCl (1M) application (arrowhead) to demonstrate the decrease in the duration and propagation speed (i.e., longer DC shift latency between ME1 and ME2) of the second and third CSDs, observed regardless of the type of anesthetic or ventilation gas. The variability in the latency between ME1 and ME2 among rats is due to variation in the distance between ME1 and ME2 in each experiment; this distance was measured in each experiment and propagation speeds calculated accordingly. Calibration scales indicate 2 min and 20 mV.

Table 1

Systemic physiological parameters.

Group	N ₂	O ₂	N ₂ O	Anesthetic	n	Dose	BW (g)	pH*	pCO ₂ (mmHg)	pO ₂ (mmHg)	BP* (mmHg)
I	70%	30%	-	Isoflurane	7	1%	327±37	7.42±0.03	37±3	127±5	110±19
				α -chloralose	8	87±31	403±38	7.40±0.04	35±4	129±11	107±20
II	-	30%	70%	Urethane	7	1.7±0.2	361±56	7.40±0.02	36±3	132±13	90±12
				Isoflurane	17	1%	443±117	7.43±0.02	37±2	133±14	102±9
III	-	100%	-	α -chloralose	7	84±57	408±89	7.41±0.03	36±3	132±8	118±15
				Urethane	7	1.2±0.3	372±54	7.37±0.03	37±2	134±7	103±9
				Isoflurane	10	1%	473±81	7.43±0.01	37±2	414±57	117±11
				α -chloralose	7	112±37	364±63	7.40±0.03	36±3	417±69	118±14
				Urethane	7	1.6±0.4	361±30	7.36±0.03	38±3	427±49	98±14

Arterial blood gas data are the average of 4–8 samples per experiment. Arterial BP data are average of 2h recordings. Isoflurane 1% corresponds to 0.7 MAC (minimum alveolar concentration) for rats (White, et al., 1974). Urethane and α -chloralose doses are given in g/kg and mg/kg/h, respectively. Arterial pH was higher in isoflurane and lower in urethane groups compared to α -chloralose, although all values were within normal range for anesthetized rodents (*: p<0.05, among all three anesthetics in groups I, II and III). Arterial blood pressure was lower in urethane groups compared to both isoflurane and α -chloralose (#: p<0.05, urethane vs. other anesthetics in groups I, II and III). N₂O significantly reduced urethane dose required for sufficient anesthesia (p<0.05, group II vs. I and III). BP, blood pressure; BW, body weight.

Table 2

CSD propagation speed (mm/min)

Experimental groups		N ₂ O	Isoflurane		α -chloralose		Urethane	
N ₂	O ₂		1 st CSD	2 nd CSD [†]	1 st CSD	2 nd CSD [†]	1 st CSD	2 nd CSD [†]
I	70%	-	4.1±0.4	3.1±0.5	4.1±0.8	3.4±0.9	3.8±0.4	2.9±0.6
II*	30%	70%	3.3±0.5	2.8±0.7	3.4±0.6	2.9±0.8	3.5±0.8	2.6±0.3
III	100%	-	3.8±0.5	3.1±0.6	3.8±0.3	3.3±0.3	3.3±0.9	2.9±0.6

* p<0.05, group II vs. I and III.
† p<0.05, 2nd CSD vs. 1st CSD.

Table 3

CSD duration (sec)

	Experimental group		N ₂ O	Isoflurane		α -chloralose		Urethane	
	N ₂	O ₂		1 st CSD	2 nd CSD [†]	1 st CSD	2 nd CSD [†]	1 st CSD	2 nd CSD [†]
I	70%	30%	-	30±5	19±4	28±4	20±3	29±6	19±4
II*	-	30%	70%	22±5	18±4	25±7	18±4	22±8	14±5
III	-	100%	-	23±5	19±3	29±5	22±5	24±4	19±7

* p<0.05, group II vs. I and III.
† p<0.05, 2nd CSD vs. 1st CSD.