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Anesthetic effects on susceptibility to cortical spreading depression

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ABSTRACT

Cortical spreading depression (CSD) is a transient neuronal and glial depolarization and disruption of membrane ionic gradients that propagates slowly across the cerebral cortex. Recent clinical and experimental evidence has implicated CSD in the pathophysiology of migraines and neuronal injury states. In the current study, we examined the influence of four different anesthetics (propofol, dexmedetomidine, isoflurane, pentobarbital) on CSD susceptibility in a KCl application animal model. We found that isoflurane and dexmedetomidine suppressed CSD frequency, and tended to reduce the CSD propagation speed. Our data suggest that these anesthetics may be therapeutically beneficial in preventing CSD in diverse neuronal injury states.

1. Introduction

Cortical spreading depression (CSD) is a wave of neuronal and glial depolarization accompanied by loss of membrane ionic gradients that slowly propagates throughout the cortex irrespective of functional or vascular territories (Kraig and Nicholson, 1978; Leao, 1944). The pivotal event in the generation and propagation of CSD is depolarization of a minimum critical mass of brain tissue, which is associated with a massive increase in extracellular K^+ 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.

This type of electrophysiological change in the cortex is thought to underlie the pathophysiology of neuronal injury states, such as stroke, subarachnoid hemorrhage (SAH), intracranial hemorrhage (ICH), and traumatic brain injury (TBI) in acute neurocritical care settings. It has been reported that injury depolarizations worsen tissue outcome through hemodynamic and metabolic mechanisms that exacerbate the energy supply-demand mismatch (Hashemi et al., 2009; Shin et al., 2006). Since suppression of injury depolarizations by drugs that inhibit CSD shows efficacy in animal models of stroke and in acute human brain injury (Hertle et al., 2012; Shin et al., 2006), testing the impact of drugs on CSD may be an important step in establishing new therapy for acute neuronal injury states.

General anesthetics modulate CSD susceptibility (Kitahara et al., 2001; Kudo et al., 2008). However, most previously tested anesthetics have not been common in clinical use and many new anesthetics have been developed recently. We therefore systematically studied the impact of four anesthetics widely used in clinical practice, propofol, dexmedetomidine, isoflurane, pentobarbital, on the frequency and electrophysiological properties of KCl-evoked CSDs in rats.

2. Materials and methods

2.1. Surgical preparations

All experiments were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals. The study protocol was reviewed and approved by the Institute of Experimental Animal Sciences at Osaka University Graduate School of Dentistry. Sprague–Dawley rats (228–500 g, male) (Charles River Laboratories, Kanagawa, Japan) were anesthetized using isoflurane (5% for induction, 2% for maintenance in 30% O_2 + 70% N_2O during surgical procedures). Rats were intubated via a tracheostomy for mechanical ventilation (SAR-830; CWE, Ardmore, PA, USA). Continuous measurements of mean arterial blood pressure (Powerlab; ADInstruments, Colorado Springs, MO, USA) and arterial blood sampling were performed via a femoral artery catheter. Rats were then paralyzed by continuous infusion of vecuronium bromide (0.076–0.12 mg/

Abbreviations: CSD, cortical spreading depression; GABA, γ-aminobutyric acid; ICH, intracranial hemorrhage; ICU, intensive care unit; MAC, minimal alveolar concentration; NMDA, *N*-methyl-D-aspartate; PHS, propofol hemisuccinate; SAH, subarachnoid hemorrhage; TBI, traumatic brain injury.

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kg/h) (Mascurate; Maruishi Pharmaceutical, Osaka, Japan) to facilitate mechanical ventilation. Arterial blood gas and pH were measured every 40 min and ventilation parameters were adjusted to maintain pCO_2 between 35 and 45 mmHg (Rapidlab 860; Bayer, Tokyo, Japan). Rectal temperature was kept at 37.0 ± 0.1 °C using a thermostatically controlled heating pad (ATB-1100; Nihon Kohden, Tokyo, Japan). Anesthesia levels were maintained throughout the procedure to abolish blood pressure and heart rate response to tail pinch. Adequate measures were taken to minimize pain and discomfort.

2.2. Electrophysiological recordings

Rats were placed in a stereotaxic frame (Narishige, Tokyo, Japan), and three burr holes were drilled under saline cooling over the right hemisphere at the following coordinates (mm from bregma): (1) posterior 6, lateral 2 (occipital cortex): KCl application; (2) posterior 3, 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 mmol/L NaCl, at 300 μm below the dural surface (FHC, Bowdoinham, ME, USA). An Ag/AgCl reference electrode was placed subcutaneously in the neck. After surgical preparation, the cortex was allowed to recover for 30 min under saline irrigation. Data were continuously recorded using a data acquisition system for off-line analysis (AD Instruments).

2.3. CSD induction

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

2.4. Experimental groups and protocols

A total of 43 rats were studied using one of four anesthetics; propofol ($n = 11$), dexmedetomidine ($n = 12$), isoflurane ($n = 10$) and pentobarbital ($n = 10$). Isoflurane (Escain; Mylan, Tokyo, Japan) dose was adjusted to 0.7 minimal alveolar concentration (MAC) (Kitahara et al., 2001). Propofol (Mylan) dose was adjusted to 0.7 MAC equivalent anesthetic dose achieved by loading of 14 mg/kg for 5 min, followed by continuous infusion of 28 mg/kg/h (Todd and Weeks, 1996). Pentobarbital (Somnopentyl; Kyoritsu Seiyaku Corporation, Tokyo, Japan) dose was adjusted to approximately 0.7 MAC equivalent anesthetic dose achieved by loading 21 mg/kg for 5 min, followed by continuous infusion of 10.7 mg/kg/h (Todd and Weeks, 1996). Dexmedetomidine (Precedex; Maruishi Pharmaceutical) dose was chosen according to a previously published paper (Bol et al., 1999). After completion of surgical procedures, N_2O was switched to N_2, and a test anesthetic (propofol, dexmedetomidine or pentobarbital) was continuously infused. Surgical anesthesia (isoflurane) was weaned off over 15 min. At each depth of anesthesia, an equilibration period of 1 h was allowed. Electrophysiological recordings and KCl application started 1 h after isoflurane was completely weaned off. When isoflurane was the test anesthetic, it was simply dialed down to 1.0%, and recordings started 1 h later.

2.5. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Sheffe's Multiple Comparisons test. Data were expressed as mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Physiological parameters

Systemic physiological parameters for each rat were calculated by averaging all measurements during CSD recordings. Although blood pressure under isoflurane was significantly lower than dexmedetomidine ($P < 0.01$), systemic physiological parameters were within the normal range in all treatment groups (Table 1).

Table 1
Systemic physiological parameters.

Experimental groups	N	Systemic physiology			
		pH	pCO_2 (mmHg)	pO_2 (mmHg)	BP (mmHg)
Propofol	11	7.43 ± 0.03	39 ± 3	136 ± 14	121 ± 16
Dexmedetomidine	12	7.44 ± 0.06	36 ± 6	121 ± 7	131 ± 20
Pentobarbital	10	7.45 ± 0.03	38 ± 4	133 ± 10	123 ± 10
Isoflurane	10	7.45 ± 0.04	38 ± 5	135 ± 23	105 ± 13[a]

[a] $P < 0.01$ versus dexmedetomidine.

3.2. Dexmedetomidine and isoflurane reduce CSD frequency induced by topical KCl

Repetitive CSDs evoked by topical application of KCl in all groups were recorded for 2 h (Fig. 1). Among four anesthetics, dexmedetomidine and isoflurane significantly reduced the frequency of CSDs compared to propofol and pentobarbital (Fig. 2). CSD frequency recorded under isoflurane was significantly less than propofol and pentobarbital in the first and second hour, respectively. On the other hand, CSD frequency under dexmedetomidine did not differ from propofol during the first hour, but was significantly less than propofol during the second hour.

3.3. The effects of anesthetics on CSD propagation speed

CSD propagation speed was also compared among four anesthetics. Although it was not significant, there was a tendency that the speeds under dexmedetomidine and isoflurane (5.0 ± 0.9 and 4.9 ± 1.2 mm/min, respectively) were lower than propofol and pentobarbital (5.4 ± 1.1 and 5.4 ± 0.9 mm/min, respectively) (Table 2).

3.4. Other electrical properties

These four anesthetics did not significantly alter other electrophysiological properties of individual CSDs, such as amplitude and duration (Table 2).

4. Discussion

Our data provide a systematic comparison of four anesthetics that are widely used in current clinical practice in a model of CSD susceptibility induced by KCl. Our data indicate that the choice of anesthetic impacts the frequency and propagation of CSDs. CSD frequency was significantly suppressed by dexmedetomidine and isoflurane in comparison to propofol and pentobarbital. In addition, CSD propagation speed tended to be slower by dexmedetomidine and isoflurane. Other electrophysiological parameters were not altered by either anesthetic.

We chose pentobarbital and isoflurane as negative and positive controls, respectively, based on previous reports (Kitahara et al., 2001; Kudo et al., 2008).

In this study, we chose the concentration of 1.0% for isoflurane (0.7 MAC) as this was enough to anesthetize animals and also suppress CSD (Kudo et al., 2008). The administered doses of propofol and pentobarbital were chosen to be equivalent to 0.7 MAC (Todd and Weeks, 1996). Since previous studies did not report the MAC of dexmedetomidine, we could not adjust the dose to 0.7 MAC. Rather, the dose of dexmedetomidine was based on previous papers that examined the correlation between plasma concentration and stimulus-responses, continuous electroencephalographic, and cardiovascular effects (Bol et al., 1999).

Dexmedetomidine is an α_2-adrenergic full agonist that has recently been in use as a sedative drug. Richter et al. have reported that clonidine, a partial agonist at the α_2-adrenergic receptor, inhibits CSD initiation and migration upon topical cortical application (Richter et al., 2005). Our data show that dexmedetomidine modulates CSD susceptibility upon systemic administration, clinically more relevant than topical application. We observed that dexmedetomidine significantly inhibited CSD frequency and