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Pre-synaptic function explains age-dependent actions of general anesthetics in the rat hippocampal synaptic transmission

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ABSTRACT

Mechanisms by which age modifies general anesthetic requirements remain uncertain. In order to examine the age-related modification of general anesthetics in the central nervous system, we have studied the effects of thiopental and sevoflurane on hippocampal synaptic transmission in young and elderly rats. Field potentials of area CA1 were electrically elicited in hippocampal slices from young (4-month) and elderly (2-year) male Wistat rats. The effects of sevoflurane on both excitatory and inhibitory synaptic transmission were similar in the young and elderly preparations. In contrast, thiopental produced a greater effect on inhibitory synaptic transmission in young than elderly hippocampi, whereas the actions on excitatory synaptic transmission were negligible in both preparations. Corresponding experiments revealed (a) that the duration of recurrent inhibition was more prolonged by thiopental in young compared to elderly animals and (b) that thiopental enhanced the γ-amino-butyric acid (GABA) release from pre-synaptic terminals in an age-dependent manner. The thiopental actions on GABA discharge from pre-synaptic terminals appear to be responsible for the observed difference between young and elderly animals. The age-dependent reduction in neurotransmitter stores in pre-synaptic terminals may explain the age-related alterations in general anesthetic actions.

1. Introduction

Although anesthesiologists recognize that the aging process is associated with reduced anesthetic requirements (Avram et al., 1993; Eger, 2001), little is known about the neural mechanism(s) for the age-dependent modifications of general anesthetic actions. Since an age-dependent changes in the rate of metabolism and distribution may contribute to the alterations of anesthetic actions *in vivo*, it is considered worthwhile to examine the age-related modification of anesthetic effects in the central nervous system *in vitro*.

It is difficult to establish elderly animal models in basic biological research. Although senescence-accelerated animal models have been developed and show shorter life spans than control animals, investigators have raised the question whether early death of elderly model animals may be caused by systemic diseases (Miyaishi et al., 2000a, b). Since natural age-related biophysics might be uncertain in the senescence-accelerated model animals, we established an aging farm for Wistar rats to be used in the present study, and carefully reared animals for ~2 years under identical environments.

Previous studies from our laboratory have demonstrated the agent-specific actions of general anesthetics, indicating that intravenous anesthetics (thiopental, pentobarbital, propofol) mainly enhance inhibitory synaptic transmission, whereas volatile anesthetics (sevoflurane, isoflurane) act on both excitatory and inhibitory synaptic transmission in the hippocampus (Hirota et al., 2010; Asahi et al., 2006; Wakasugi et al., 1999). It has been reported that physiological aging is associated with impairment of inhibitory synaptic transmission (Papatheodoropoulos and Kostopoulos, 1996) and with increased excitability of hippocampal circuits (Barnes et al., 1987). Therefore we hypothesized that actions of general anesthetics could be differently modified with aging. In the present study, we studied effects of intravenous and volatile anesthetics on synaptic transmission in young and senile hippocampus.

2. Materials and methods

Ethical approval was obtained from the Animal Research Committee of the University of Toyama, Japan. Male Wistar rats were housed individually in the aging farm, raised in an air-conditioned room (20 °C) under a 12/12-h light/dark cycle, with free access to food and water. It has been reported that age-dependent alterations in synaptic activities mainly occur during the first 7–10 months and continue until 30 months in the hippocampus of Wistar rats (Papatheodoropoulos and Kostopoulos, 1996). Thus, we considered 4-month and 2-year Wistar rats as young and elderly subjects, respectively.

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2.1. Slice preparation and electrophysiological recordings

Methods for the preparation of rat hippocampal slices and electrophysiological protocols have been previously described (Hirota et al., 2010). In brief, young (4-month) and elderly (2-year) rats were deeply anesthetized with sevoflurane and then decapitated. Because of lower blood/gas partition coefficient of sevoflurane (0.63), the anesthesia for decapitation could not interfere with the later procedure of preparation or electrophysiological recordings. The brain was rapidly removed, and 400 μm transverse slices were prepared from the dissected hippocampus in cold, oxygenated artificial cerebrospinal fluid (ACSF) using a Rotorslicer DTY-7700 (DSK, Osaka, Japan). The slices were placed on a nylon mesh screen at the interface of ACSF liquid (90 mL/h) and humidified 95%O_2/5%CO_2 gas (1 L/min) phases in a recording chamber. In order to accelerate the rate of drug equilibration and to obtain stable recordings of field potentials, we have developed the liquid/gas interface brain slice chamber with minimal perfusate volume (0.8 mL). Slices were warmed to 37 °C slowly and then allowed to equilibrate for 90–120 min without electrical stimulation.

Two glass extracellular recording microelectrodes (3–5 MΩ filled with 2 mol/L of NaCl) were positioned in the region of cell bodies and dendrites of the CA1 pyramidal neurons to record field population spikes (PSs) and field excitatory post-synaptic potentials (EPSPs). A bipolar stimulating electrode (tungsten steel, coated with epoxy resin, Unique Medical, Tokyo, Japan) was placed in the region of the Schaffer-collateral–commissural fibers (Sch) to stimulate the input to CA1 neurons, and a second electrode was located in the region of the alveus hippocampi (Alv) to activate inhibitory interneurons of the CA1 (Fig. 1A) (Asahi et al., 2006; Hirota et al., 2010). PS amplitudes were determined from peak positive to peak negative of the waveform. EPSP slopes were calculated by fitting digitized data points between onset and peak negativity to a linear function (dV/dt).

The hippocampal network model used in this study has the advantage that both excitatory and inhibitory synaptic transmissions are simultaneously recorded. EPSP slopes were measured to evaluate the glutaminergic excitatory synaptic transmission, and the recurrent inhibitory-enhanced (RIE) circuit was employed to evaluate inhibitory synaptic transmission. In order to establish the RIE circuit, the Alv was stimulated with a pre-pulse to activate the γ-amino-butyric acid (GABA) mediated recurrent inhibition. Electrical stimulation of the Sch was then applied (10 ms after the pre-pulse) as a test-pulse to elicit PS. When the recurrent inhibition was enhanced *via* presynaptic mechanisms, the reduction of PS was detected.

Representative recordings of RIE experiments were shown in Fig. 1B. An enhancement of recurrent inhibition with the pre-pulse had minimal effect on the PS amplitude in the absence of anesthetic (ACSF, left panel). An application of thiopental (10^{-5} mol/L) remarkably reduced the PS amplitude with a pre-pulse (right, lower panel). Note that thiopental failed to affect PS *without* a pre-pulse, indicating that anesthetic produces the inhibitory actions only when the recurrent inhibition is enhanced, as previously reported from our laboratory (Hirota et al., 1998, 2010; Wakasugi et al., 1999; Asahi et al., 2006).

In some experiments, the intervals between pre- and test-pulses were changed from 10 ms to a range of 20–60 ms. A number of pre-pulses (n = 0–320) were applied as a train (200 Hz) in order to access the GABA release from pre-synaptic terminals (pre-pulse train protocol).7 DL-2-amino-5-phosphonovaleric acid (AP5, 10 − 4 mol/L) was used to prevent NMDA (N-methyl-D-aspartate) receptor-related synaptic plasticity (Winder and Sweatt, 2001).

Square-wave stimuli (5–10 V, 50 μs), generated with a SEN-3301 stimulator (Nihon Kohden, Tokyo, Japan), were delivered to both pathways (Sch and Alv) simultaneously. The minimal stimulus intensity that elicited the maximal amplitude (maximal stimulus) was normally used. Stimulus frequency was fixed at 0.03 Hz, since the input frequency can modify anesthetic actions (Hirota et al., 2010). Field potentials were amplified with a MEZ-8301 amplifier (Nihon Kohden, Tokyo, Japan) and filtered 1 Hz–10 kHz. Analog-digital conversions of data were made at a rate of 100 kHz using an InstruNet (GW, Somerville, MA). The results were stored on the hard drive of a Macintosh computer (Apple, Cupertino, CA), and PS amplitudes and EPSP slopes were analyzed using SuperScope software (GW, Somerville, MA).

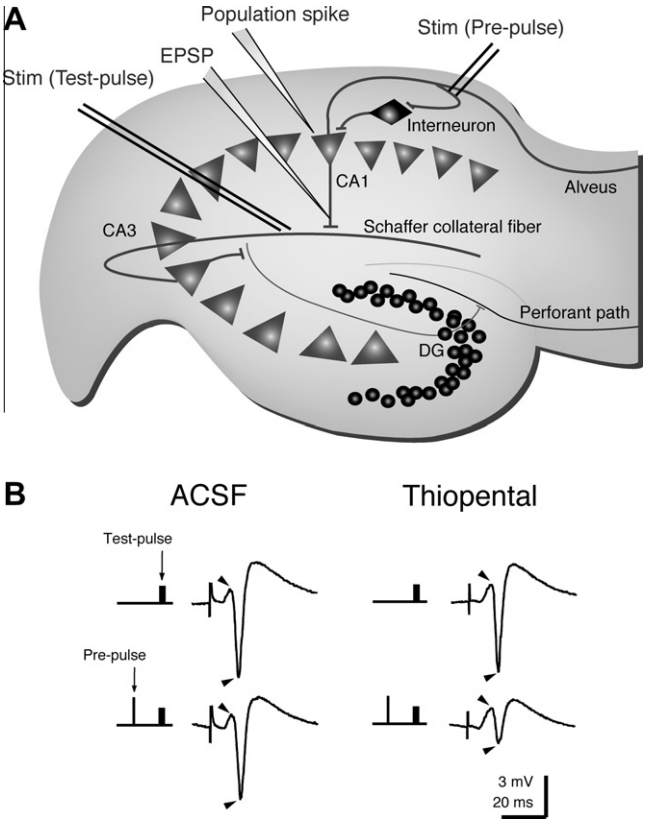

Fig. 1. (A) Two glass extracellular recording microelectrodes for recording population spike and excitatory post-synaptic potential were placed in the region of cell body and dendrites of CA1 pyramidal neurons. A bipolar stimulating electrode (for "Test-pulse") was located in the region of Schaffer-collateral–commissural fibers to stimulate the input to CA1 neurons, and a second electrode (for "Pre-pulse") was placed in the region of alveus hippocampi in order to activate inhibitory interneurons of the CA1. EPSP: excitatory post-synaptic potential. DG: Dentate gyrus. Stim: Stimulus electrode. (B) Effects of thiopental on field population spikes in the recurrent inhibition enhanced (RIE) circuit. Left panel: Artificial cerebrospinal fluid (no anesthetic). Right panel: Effects of 10^{-5} mol/L of thiopental.

2.2. Drug application and data acquisition

All preparations used in the present study showed control variability <5% during the initial data acquisition period and following washout of anesthetic drugs. Recovery responses were recorded at least 30 min after washout of anesthetic-equilibrated ACSF from the chamber. Sevoflurane was applied as vapors in the prewarmed carrier gas (95%O_2/5%CO_2) above the slices using a calibrated commercial vaporizer (Tec 3, Omeda, Steeton, West Yorkshire, UK). Concentrations, expressed as volume percent (vol%) refer to the dial settings on the vaporizer. Concentrations of sevoflurane in the perfusate of the recording chamber were determined using a portable volatile gas analyzer (OSP, Saitama, Japan): a linear relationship (6.5×10^{-4} mol/L per 1.0 vol%) up to 5.0 vol%.