Neuropharmacology 61 (2011) 699–706

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Regional differences in the effects of isoflurane on neurotransmitter release

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ARTICLE INFO

Article history:
Received 17 October 2010
Received in revised form
4 February 2011
Accepted 16 May 2011

Keywords:
Na^+ channels
Glutamate
GABA
Nerve terminal
Tetrodotoxin
Rat

ABSTRACT

Stimulus evoked neurotransmitter release requires that Na^+ channel-dependent nerve terminal depolarization be transduced into synaptic vesicle exocytosis. Inhaled anesthetics block presynaptic Na^+ channels and selectively inhibit glutamate over GABA release from isolated nerve terminals, indicating mechanistic differences between excitatory and inhibitory transmitter release. We compared the effects of isoflurane on depolarization-evoked [^{3}H]glutamate and [^{14}C]GABA release from isolated nerve terminals prepared from four regions of rat CNS evoked by 4-aminopyridine (4AP), veratridine (VTD), or elevated K^+. These mechanistically distinct secretegogues distinguished between Na^+ channel- and/or Ca^{2+} channel-mediated presynaptic effects. Isoflurane completely inhibited total 4AP-evoked glutamate release ($IC_{50} = 0.42 \pm 0.03$ mM) more potently than GABA release ($IC_{50} = 0.56 \pm 0.02$ mM) from cerebral cortex (1.3-fold greater potency), hippocampus and striatum, but inhibited glutamate and GABA release from spinal cord terminals equipotently. Na^+ channel-specific VTD-evoked glutamate release from cortex was also significantly more sensitive to inhibition by isoflurane than was GABA release. Na^+ channel-independent K^+-evoked release was insensitive to isoflurane at clinical concentrations in all four regions, consistent with a target upstream of Ca^{2+} entry. Isoflurane inhibited Na^+ channel-mediated (tetrodotoxin-sensitive) 4AP-evoked glutamate release ($IC_{50} = 0.30 \pm 0.03$ mM) more potently than GABA release ($IC_{50} = 0.67 \pm 0.04$ mM) from cortex (2.2-fold greater potency). The magnitude of inhibition of Na^+ channel-mediated 4AP-evoked release by a single clinical concentration of isoflurane (0.35 mM) varied by region and transmitter: Inhibition of glutamate release from spinal cord was greater than from the three brain regions and greater than GABA release for each CNS region. These findings indicate that isoflurane selectively inhibits glutamate release compared to GABA release via Na^+ channel-mediated transduction in the four CNS regions tested, and that differences in presynaptic Na^+ channel involvement determine differences in anesthetic pharmacology.

1. Introduction

The synaptic mechanisms of general anesthetics are not clearly understood (Hemmings, 2009). Inhaled anesthetics selectively inhibit release of the excitatory transmitter glutamate compared to the inhibitory transmitter GABA from cerebral cortex nerve terminals (Westphalen and Hemmings, 2006b). Most inhaled anesthetics potentiate inhibitory transmission by potentiating postsynaptic GABAa receptors (Hemmings et al., 2005a) and depress excitatory transmission by blocking postsynaptic glutamate receptors (Franks, 2006). Thus, selective presynaptic inhibition of excitatory compared with inhibitory neurotransmitter release could play a pivotal role in anesthetic depression of CNS activity. The relevant molecular sites of action by which anesthetics selectively inhibit neurotransmitter release have not been fully elucidated. Identification of these sites is essential in understanding the balance of anesthetic effects on excitatory and inhibitory synaptic transmission.

Neurotransmitter release requires the coordinated actions of voltage-gated Na^+, K^+ and Ca^{2+} channels, Ca^{2+}-sensors coupled to the synaptic vesicle release machinery, and vesicle exocytosis/endocytosis mechanisms (Dittman and Ryan, 2009; Südhof, 2004). Nerve terminal-specific differences in the presynaptic mechanisms regulating transmitter release could lead to transmitter-selective effects on release. Presynaptic specializations in ion channel subtype expression (Bowman et al., 1993; Mechaly et al., 2005; Reid et al., 1997), ion channel modulation (Hemmings, 1998; MacDermott et al., 1999), excitation–exocytosis coupling (Prakriya and Mennerick, 2000), and/

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doi:10.1016/j.neuropharm.2011.05.013

or synaptic vesicle fusion machinery (Herring et al., 2009; Südhof, 2004) could determine nerve terminal- and transmitter-specific pharmacological differences.

We tested the hypothesis that inhaled anesthetics affect the release of specific neurotransmitters through nerve terminal-specific presynaptic mechanisms by comparing the effects of the model inhaled anesthetic isoflurane on glutamate and GABA release from nerve terminals isolated from four neurochemically and functionally distinct regions of rat CNS. These neurochemical differences could underlie region-selective anesthetic effects on neuronal activity (Rudolph and Antkowiak, 2004; Veselis et al., 2002; White and Alkire, 2003).

2. Methods

2.1. Materials

Isoflurane was from Abbott Laboratories (North Chicago, IL). 4-Aminopyridine (4AP), veratridine (VTD) and buffer constituents were from Sigma–Aldrich Chemical Co. (St. Louis, MO). L-[^{3}H]Glutamate (42 Ci/mmol) was from Amersham Radiochemical Centre (Buckinghamshire, UK), and [^{14}C]GABA (0.24 Ci/mmol) was from PerkinElmer Inc. (Boston, MA) or American Radiolabel Chemicals Inc. (St. Louis, MO).

2.2. Nerve terminal preparation and neurotransmitter loading

Experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals as approved by the Weill Cornell Medical College Institutional Animal Care and Use Committee. Synaptosomes were prepared from adult (200–300 g) male Sprague-Dawley rat (Charles River Laboratories, Troy, NY) cerebral cortex, hippocampus, striatum or spinal cord (Westphalen and Hemmings, 2006a; Westphalen et al., 2010). Demyelinated synaptosomes were incubated with 5–10 nM L-[^{3}H]glutamate and 400–500 nM [^{14}C]GABA for 15 min at 30 °C in 10 ml Krebs–HEPES buffer (KHB, composition in mM: NaCl 140, KCl 5, HEPES 20, $MgCl_2$ 1, Na_2HPO_4 1.2, $NaHCO_3$ 5, EGTA 0.1, and D-glucose 10, pH 7.4 with NaOH). Synaptosomes were collected by centrifugation for 10 min at 20,000 × *g* at 4 °C, resuspended in ice-cold 0.32 M sucrose, and loaded into release chambers.

2.3. Glutamate and GABA release

Simultaneous release of glutamate and GABA was assayed as described (Westphalen and Hemmings, 2006a) by superfusion at 0.5 ml/min with KHB plus 0.1 mM EGTA at 37 °C using a customized Brandel SF12 superfusion apparatus (Gaithersburg, MD) set to collect 1 min fractions. Stock solutions of isoflurane (~2 mM) were prepared in KHB, diluted to aqueous concentrations equivalent to 0.1–8 times minimum alveolar concentration (MAC = 0.35 mM for isoflurane in rat at 37 °C, Taheri et al., 1991). Release experiments were performed in the absence or presence of 1.9 mM free extracellular Ca^{2+} (by the addition of 2 mM $CaCl_2$). To avoid basal effects of TTX and/or high concentrations of isoflurane, anesthetic solutions ± 1 μM tetrodotoxin (TTX) were perfused for 12 min prior to, as well as during and following depolarizing pulses of secretegogues. After 2 min pulses of 1 mM 4AP, 10 μM VTD, 15 mM KCl or 30 mM KCl (with KCl replacing equimolar NaCl in KHB) and the return to baseline release, experiments were terminated by synaptosomal lysis by perfusion with 0.2 M perchloric acid. Radioactivity in each 1 min fraction was quantified by liquid scintillation spectrometry with dual isotope quench correction (Beckman Coulter LS 6000IC, Fullerton, CA) using BioSafe II scintillation cocktail (RPI, Mt. Prospect, IL). Solutions were sampled upon exit from the release chambers into a gas-tight glass microsyringe at the end of each experiment for isoflurane quantification by gas chromatography (Ratnakumari and Hemmings, 1998).

2.4. Data analysis

Release of transmitter in each 1 min fraction of perfusate was expressed as a fraction of synaptosomal content of labeled transmitter prior to that fraction (fractional release [FR], Westphalen and Hemmings, 2003) The magnitude of evoked release was determined by subtracting baseline release (average of basal FR before and after stimulation) from cumulative FR values for each evoked release pulse (sum ΔFR). Sum ΔFR data from each experiment were normalized by the ratio of within assay control to overall mean control release in the presence of Ca^{2+} prior to curve fitting and/or statistical analysis. Data for inhibition of evoked release were fitted to concentration-effect curves by least-squares analysis to estimate I_{max} and IC_{50} (Prism 5.0; GraphPad Software, San Diego CA). Curve fits were tested for differences between the lower plateau of the curve (I_{max}) and zero sum ΔFR, and between the upper plateau of the curve (I_0) and control sum ΔFR. If significant differences were not found, curve fits were performed with I_{max} constrained to zero sum ΔFR and I_0 constrained to control, respectively.

Isoflurane effects on 4AP-evoked release in the presence of TTX were analyzed to determine the sum ΔFR value of TTX-insensitive 4AP-evoked release compared to isoflurane effects on 4AP-evoked release in the absence of TTX at equal isoflurane concentrations. Isoflurane effects on TTX-sensitive 4AP-evoked release were derived by subtraction of TTX-insensitive release values from 4AP-evoked release in the absence of TTX. Sum ΔFR values for isoflurane effects on TTX-sensitive 4AP-evoked release were fitted as above to estimate IC_{50}.

Significant differences between IC_{50} values were determined by *F*-test comparisons between best-fit values derived from separate curve fits to values derived from global fits with the parameter shared. Control evoked release and anesthetic effects on K^+-evoked glutamate and GABA release were analyzed by one-way ANOVA with Tukey *post hoc* test for multiple comparisons. Inhibition by isoflurane of TTX-sensitive glutamate and GABA release from the same preparations of nerve terminals were compared by paired *t*-tests.

3. Results

Depolarization by 4AP, VTD, or elevated K^+ were used to distinguish between Na^+ channel- and/or Ca^{2+} channel-mediated pharmacological effects on transmitter release from isolated nerve terminals (Farrag et al., 2008; Tibbs et al., 1989).

3.1. Release evoked by 4-aminopyridine

The K^+ channel blocker 4AP evokes transmitter release that requires activation of both voltage-gated Na^+ (Na_v) and Ca^{2+} (Ca_v) channels following transient depolarizations based on its sensitivity to specific channel blockers (Tibbs et al., 1989). Stimulation of nerve terminals isolated from rat cerebral cortex, hippocampus, striatum or spinal cord with a 2 min pulse of 4AP in the presence of extracellular Ca^{2+} evoked release of both glutamate and GABA (Fig. 1). Fractional GABA release (sum ΔFR) was greater than glutamate release for all four CNS regions ($P < 0.001$). Glutamate release from cortical ($n = 32$), hippocampal ($n = 21$), or striatal ($n = 24$) nerve terminals was similar, and was greater than that from spinal cord terminals ($n = 21$; $P < 0.001$ vs. cortex). GABA release from cortical and hippocampal nerve terminals was similar, and was greater than that from striatum and spinal cord terminals ($P < 0.001$ vs. cortex).

Isoflurane completely inhibited both glutamate and GABA release evoked by 4AP from nerve terminals prepared from all four regions of the CNS (Fig. 1). These effects occurred at concentrations relevant to clinical anesthesia (Figs. 1A and 2A). The potency of isoflurane for inhibition of glutamate release was greater for spinal cord than for cortex ($P = 0.0009$) and other brain regions (Fig. 2A). Isoflurane inhibited GABA release from striatal and spinal cord terminals more potently than from cortical and hippocampal terminals. Isoflurane inhibited glutamate release more potently than GABA release from cortical, hippocampal, and striatal nerve terminals, but inhibited glutamate and GABA release from spinal cord terminals equipotently (Fig. 2A).

3.2. Release evoked by veratridine

Veratridine (VTD) evokes transmitter release from isolated nerve terminals by direct interaction with TTX-sensitive Na_v to impair channel inactivation resulting in sustained depolarization (Farrag et al., 2008; Ulbricht, 1998). Stimulation of cortical nerve terminals with a 2 min pulse of VTD in the presence of Ca^{2+} led to equivalent release (sum ΔFR) of glutamate (0.20 ± 0.01) and GABA (0.24 ± 0.01) ($n = 30$). Isoflurane completely inhibited VTD-evoked release of both glutamate and GABA from cortical nerve terminals (data not shown) at clinically relevant concentrations (Fig. 2B); its potency was similar to that for inhibition of 4AP-evoked release (Fig. 2A). Isoflurane inhibited VTD-evoked glutamate release more potently (1.2-fold) than GABA release (Fig. 2B).