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Presynaptic inhibition of the release of multiple major central nervous system neurotransmitter types by the inhaled anaesthetic isoflurane

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Editor's key points

- The effects of isoflurane on neurotransmitter release were studied in isolated rat nerve terminals *in vitro*.
- Isoflurane inhibited presynaptic release of five major neurotransmitters.
- Such effects have implications for therapeutic and toxic effects of volatile anaesthetic agents involving altered synaptic transmission.

Background. Presynaptic effects of general anaesthetics are not well characterized. We tested the hypothesis that isoflurane exhibits transmitter-specific effects on neurotransmitter release from neurochemically and functionally distinct isolated mammalian nerve terminals.

Methods. Nerve terminals from adult male rat brain were prelabelled with [^{3}H]glutamate and [^{14}C]GABA (cerebral cortex), [^{3}H]norepinephrine (hippocampus), [^{14}C]dopamine (striatum), or [^{3}H]choline (precursor of [^{3}H]acetylcholine; striatum). Release evoked by depolarizing pulses of 4-aminopyridine (4AP) or elevated KCl was quantified using a closed superfusion system.

Results. Isoflurane at clinical concentrations ($<$0.7 mM; $\sim$2 times median anaesthetic concentration) inhibited Na^+ channel-dependent 4AP-evoked release of the five neurotransmitters tested in a concentration-dependent manner. Isoflurane was a more potent inhibitor [expressed as IC_{50} (SEM)] of glutamate release [0.37 (0.03) mM; $P<0.05$] compared with the release of GABA [0.52 (0.03) mM], norepinephrine [0.48 (0.03) mM], dopamine [0.48 (0.03) mM], or acetylcholine [0.49 (0.02) mM]. Inhibition of Na^+ channel-independent release evoked by elevated K^+ was not significant at clinical concentrations of isoflurane, with the exception of dopamine release [IC_{50}=0.59 (0.03) mM].

Conclusions. Isoflurane inhibited the release of the major central nervous system neurotransmitters with selectivity for glutamate release, consistent with both widespread inhibition and nerve terminal-specific presynaptic effects. Glutamate release was most sensitive to inhibition compared with GABA, acetylcholine, dopamine, and norepinephrine release due to presynaptic specializations in ion channel expression, regulation, and/or coupling to exocytosis. Reductions in neurotransmitter release by volatile anaesthetics could contribute to altered synaptic transmission, leading to therapeutic and toxic effects involving all major neurotransmitter systems.

Keywords: acetylcholine; γ-aminobutyric acid; anaesthetics; dopamine; exocytosis; glutamate; Na^+ channels; nerve terminal; neurotransmitter release; norepinephrine

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General anaesthetics exert profound effects on the central nervous system (CNS) primarily by altering synaptic transmission.[1] These effects involve both presynaptic, postsynaptic, and extrasynaptic actions that vary between anaesthetic agents and brain regions. These pharmacological actions lead to general depression of fast excitatory and/or enhancement of fast inhibitory synaptic transmission mediated by the principal excitatory and inhibitory neurotransmitters glutamate and γ-aminobutyric acid (GABA), respectively.[2] The relative importance and mechanisms of anaesthetic effects on the balance between excitatory and inhibitory synaptic transmission, mediated primarily by glutamate and GABA and also by other neurotransmitters, are unknown.

Volatile inhaled anaesthetics at clinical concentrations inhibit depolarization-evoked release of the major excitatory (glutamate) and inhibitory (GABA) neurotransmitters from isolated brain and spinal cord nerve terminals.[3–5] Inhibition of the release of the excitatory neurotransmitter glutamate occurs with greater potency than the release of the inhibitory neurotransmitter GABA, consistent with greater depression of excitatory relative to inhibitory signalling.[5 6] This presynaptic selectivity, in conjunction with potentiation of postsynaptic and extrasynaptic $GABA_A$ receptors, provides potentially synergistic pathways to CNS depression by volatile anaesthetics based on electrophysiological data obtained in isolated cellular and brain slice preparations.[1 7 8] The relative

potencies of anaesthetic effects on the release of other major CNS neurotransmitters have not been reported.

The release of neurotransmitters of different classes involves distinct cellular and molecular mechanisms between fast transmitters, catecholamines, and neuropeptides.[9–11] In order to gain further insight into the specificity of the presynaptic actions of anaesthetics, we compared the effects of the model volatile anaesthetic isoflurane on the release of three classical fast neurotransmitters packaged in small synaptic vesicles (glutamate, GABA, acetylcholine), and of two catecholamine neurotransmitters packaged in small dense-core vesicles (dopamine and norepinephrine). Release was evoked by 4-aminopyridine (4AP), a stimulus that mimics action-potential-evoked neurotransmitter release in requiring sequential activation of nerve terminal voltage-gated Na^+ and Ca^{2+} channels, or by elevated K^+ that only requires activation of Ca^{2+} channels, independent of Na^+ channel activation.[12] We tested the hypothesis that isoflurane differentially inhibits the release of specific neurotransmitters from neurochemically and functionally distinct nerve terminals prepared from three different regions of adult rat brain. This approach provides the most accessible approach to studying stimulus–secretion coupling of multiple transmitter types from different brain regions in isolation of interfering postsynaptic effects.[13]

Methods

Materials

Isoflurane was from Abbott Laboratories (North Chicago, IL, USA). 4AP, choline oxidase, buffer constituents, and other drugs were from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). L-[^{3}H]Glutamate (60 Ci mmol^{-1}), [^{14}C]GABA (55 mCi mmol^{-1}), and [^{14}C]dopamine (55 mCi mmol^{-1}) were from American Radiolabel Chemicals Inc. (St Louis, MO, USA), and [^{3}H]norepinephrine (15 Ci mmol^{-1}) and [^{3}H]choline (86 Ci mmol^{-1}) were from PerkinElmer Inc. (Boston, MA, USA).

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 and conformed to ARRIVE guidelines. Efforts were made to minimize the numbers and suffering of animals used; animals were allowed food and water *ad libidum*, and were housed in an approved facility with environmental controls and 12 h light/dark cycling. Synaptosomes were prepared as previously described[14 15] from the cerebral cortex, hippocampus, or stratum from adult (200–300 g) male Sprague–Dawley rats (Charles River Laboratories, Troy, NY, USA) killed by inhalation of 80% CO_2 in oxygen followed by decapitation. Demyelinated synaptosomes from regions rich in nerve terminals containing specific neurotransmitters were suspended in 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) and loaded with radiolabelled neurotransmitters as follows: cerebrocortical synaptosomes with both 10 nM L-[^{3}H]glutamate and 500 nM [^{14}C]GABA for 15 min at 30°C; hippocampal synaptosomes with 200 nM [^{3}H]norepinephrine for 45 min at 35°C; and striatal synaptosomes with either 5 μM [^{14}C]dopamine for 45 min at 35°C, or with 20 nM [^{3}H]choline for 30 min at 35°C. Internalized [^{3}H]choline is rapidly converted to [^{3}H]acetylcholine by choline acetyltransferase.[16] Experiments involving dopamine and norepinephrine included 10 μM pargyline to minimize metabolism, and either 100 nM desipramine or 10 nM GBR-12935, respectively, to prevent non-specific uptake, as demonstrated in control experiments (data not shown). After loading, 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.

Transmitter release assays

Release of all neurotransmitter was assayed as we have previously described[14] by continuous superfusion at 0.5 ml min^{-1} with KHB plus 0.1 mM EGTA at 37°C using a customized Brandel SF12 superfusion apparatus (Gaithersburg, MD, USA) set to collect 1 min fractions. Stock solutions of isoflurane (~12 mM) were prepared in KHB, diluted to aqueous concentrations equivalent to 0.1–8 times median alveolar concentration (MAC=0.35 mM for isoflurane in rat at 37°C),[17] and stored in gas-tight glass syringes connected to the superfusion apparatus. Isoflurane solutions, quantified by gas chromatography, were perfused for 12 min before, during, and after, 2 min depolarizing pulses of either 100 μM 4AP, 1 mM 4AP, or 15 mM KCl (with additional KCl replacing equimolar NaCl in KHB) in the absence or presence of 1.9 mM free extracellular Ca^{2+}. Experiments were terminated by synaptosomal lysis after superfusion with 0.2 M perchloric acid. Radioactivity in each 1 min fraction was quantified by liquid scintillation spectrometry with single or dual isotope quench correction. Released acetylcholine was separated from the precursor [^{3}H]choline in each fraction by enzymatic conversion of choline to betaine lysed synaptosome supernatant adjusted to pH 7.4–8.0 with NaOH using choline oxidase (150 mU) at 37°C for 30 min. [^{3}H]Acetylcholine was extracted into 500 μl of tetraphenylborate/butyronitrile (10:1 w/v) and radioactivity quantified in 200 μl samples by liquid scintillation spectrometry. Extracted tritium radioactivity was confirmed as >95% acetylcholine by cellulose chromatography (data not shown).

Secretogogues

Depolarization-evoked neurotransmitter release was stimulated with either 100 μM 4AP, 1 mM 4AP, or 15 mM KCl. 4AP-evoked release requires activation of both voltage-gated Na^+ (Na_v) and Ca^{2+} (Ca_v) channels by inducing repetitive spontaneous nerve terminal depolarizations.[12] The use of 1 mM 4AP is often employed to maximize release, but is less dependent on Na_v function than release evoked by 100 μM 4AP, but is included to facilitate comparisons between