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Lesion of cholinergic neurons in nucleus basalis enhances response to general anesthetics

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ABSTRACT

Acetylcholine in the brain has been associated with consciousness and general anesthesia effects. We tested the hypothesis that the integrity of the nucleus basalis magnocellularis (NBM) affects the response to general anesthetics. Cholinergic neurons in NBM were selectively lesioned by bilateral infusion of 192IgG-saporin in adult, male Long–Evans rats, and control rats were infused with saline. Depletion of choline-acetyltransferase (ChAT)-immunoreactive cells in the NBM and decrease in optical density of acetylcholinesterase (AChE) staining in the frontal and visual cortices confirmed a significant decrease in NBM cholinergic neurons in lesioned as compared to control rats. AChE staining in the hippocampus and ChAT-positive neurons in the medial septum–vertical limb of the diagonal band were not different between lesioned and control rats. When a general anesthetic was administered, lesioned compared to control rats showed significantly longer duration of loss of righting reflex (LORR) after propofol (5 or 10 mg/kg i.v.), pentobarbital (20 or 40 mg/kg i.p.) but not halothane (2%). However, the behavioral excitation, as indicated by horizontal movements, induced by halothane was reduced in lesioned as compared to control rats. Reversible inactivation of NBM with $GABA_A$ receptor agonist muscimol increased slow waves in the neocortex during awake immobility, and prolonged the duration of LORR and loss of tail-pinch response after propofol, pentobarbital and halothane. In summary, lesion of NBM cholinergic neurons or inactivation of the NBM prolonged the LORR response to general anesthetic drugs.

Introduction

Acetylcholine (Ach) in the brain has long been associated with consciousness (Perry et al., 1999). Ach release was found to increase during waking and rapid-eye movement sleep as compared to slow-wave sleep (Vazquez and Baghdoyan, 2001; Phyllis, 2005). Cholinergic function is considered to be vital in attention, memory and other cognitive processing (Everitt and Robbins, 1997; Sarter et al., 2005). Cholinergic neuronal pathology, as found in Alzheimer's disease, is accompanied by cognitive and behavioral deficits (Whitehouse et al., 1981; Guela and Mesulam, 1994; Bartus, 2000; Auld et al., 2002).

Cholinergic inputs to the cerebral cortex originate from neurons in the basal forebrain, among which the nucleus basalis magnocellularis (NBM) projects to the neocortical mantle, and medial septum–diagonal band nuclei project to the hippocampus (Bigl et al., 1982; Mesulam et al., 1983; Mckinney et al., 1983; Saper, 1984; Wenk, 1997; Semba, 2000; Jones, 2004). The basal forebrain also contains GABAergic and glutamatergic neurons that project to the cortex (Gritti et al., 1997; Manns et al., 2003; Hassani et al., 2009). The basal forebrain (Wenk, 1997; Detari et al., 1999; Cape and Jones, 2000; Szymusiak et al., 2000; Lee et al., 2005), together with the thalamus (Steriade, 2000; Dringenberg and Olmstead, 2003), is important for cortical activation, manifested as a decrease in slow waves and an increase in high-frequency oscillations.

General anesthesia is a reversible state of unconsciousness, during which surgical operations may be performed, and the desirable components are loss of response to pain, general awareness, movement and memory without compromising vital functions (Franks, 2008). Despite advances made on the molecular action of various general anesthetics, such as their action on K^+ channels, $GABA_A$, nicotinic and glutamate receptors (Franks, 2008), brain structures underlying general anesthesia remain unclear. Anesthetic-induced loss of consciousness was enhanced if arousal related brain structures were ablated or inactivated (Devor and Zalkind, 2001; Nelson et al., 2002; Flint et al., 2010; Franks, 2008; Kelz et al., 2008; Lu et al., 2008; Luo and Leung, 2009), and sleep deprivation increased the potency of propofol and isoflurane (Tung et al., 2002).

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Relations between Ach and general anesthesia have emerged. An anticholinesterase that increased central Ach levels was shown to reduce the depth of anesthesia induced by propofol or isoflurane (Meuret et al., 2000; Hudetz et al., 2003). Pain et al. (2000) reported that central Ach depletion by intracerebroventricular (icv) administration of 192IgG-saporin reduced the sedative potency of low-dose (30 mg/kg i.p.) propofol, although a subsequent study (Laalou et al., 2008) reported that the same depletion increased the anesthesia potency of propofol (>100 mg/kg i.p.). Laalou et al (2008) concluded that the main effect was mediated by immunotoxin lesion in the medial septum/vertical diagonal band of Broca.

Consistent with the view that cholinergic neurons in the NBM contribute to the maintenance of waking, we hypothesize that lesion of NBM cholinergic neurons, or inactivation of the NBM, will enhance the behavioral effects of a general anesthetic. Specific lesion of cholinergic neurons in NBM was made by local infusion of 192IgG-saporin, and inactivation of the NBM was made by local infusion of muscimol, a $GABA_A$-receptor agonist. Three general anesthetics, halothane, pentobarbital and propofol were used, and the loss of righting reflex (LORR) and the loss of tail-pinch pain responses, together with neocortical EEG changes were measured. LORR in animals after a general anesthetic drug is considered to be a measure that corresponds to a loss of consciousness in humans (Franks, 2008).

Methods

Animals

Experiments were carried out on adult male Long Evans rats (250–280 g; Charles River Canada). All animals were given water and regular rat chow ad libitum and housed under climate-controlled conditions with a 12 h light/dark cycle (lights on at 7:00 h). All procedures were approved by the local Animal Use Committee and conducted according to the guidelines of the Canadian Council for Animal Care.

Lesion of NBM

A rat was anesthetized with sodium pentobarbital (60 mg/kg i.p.) and placed in a stereotaxic apparatus with bregma and lambda in a horizontal plane. NBM will refer to basal forebrain neurons that project to the neocortex. Cholinergic neurons in the NBM lesion was made by bilateral infusion of 192IgG-saporin (0.15 μg/0.5 μl/side or 0.5 μl of 1.44 μM solution in saline), via a 30-gage cannula, into substantia innominata (SI) at posterior (P) 1.4 mm and lateral (L) ± 2.7 mm of bregma, and 7.8 mm ventral (V) from the skull surface (Fig. 1; Paxinos and Watson, 1998). Sham-lesion rats received bilateral saline infusion (0.5 μl/side) into SI. The volume (0.5 μl) used was identical (Berntson et al., 2002; Kaur et al., 2008), or similar (0.4–0.8 μl used by Heckers et al., 1994; Laalou et al., 2008), to that used in other intra-NBM lesion studies. Saline with or without 192IgG-saporin was delivered by an infusion pump (Harvard Apparatus, South Natick, MA) over 10 min, after which the cannula was kept in place for another 10 min to allow for diffusion before retraction. Recording electrodes were inserted bilaterally into the frontal cortex (FC; at anterior (A) 1.4, L2, V1.5, units in mm), visual cortex (VC; P7, L3, V1.5), and hippocampus (bipolar electrodes at P3.8, L2.7, V3 and V2). Each electrode was a stainless steel wire of 125 μm diameter insulated with Teflon except at the cut end. The electrodes in the FC and VC were targeted at layer V of the cortex, while the hippocampal electrodes were targeted to straddle the CA1 cell layer. Two screws placed over the cerebellum and frontal cortex served as reference and ground electrodes respectively. Each electrode was connected to an amphenol socket, which was then embedded with the skull screws in dental cement on top of the rat's skull.

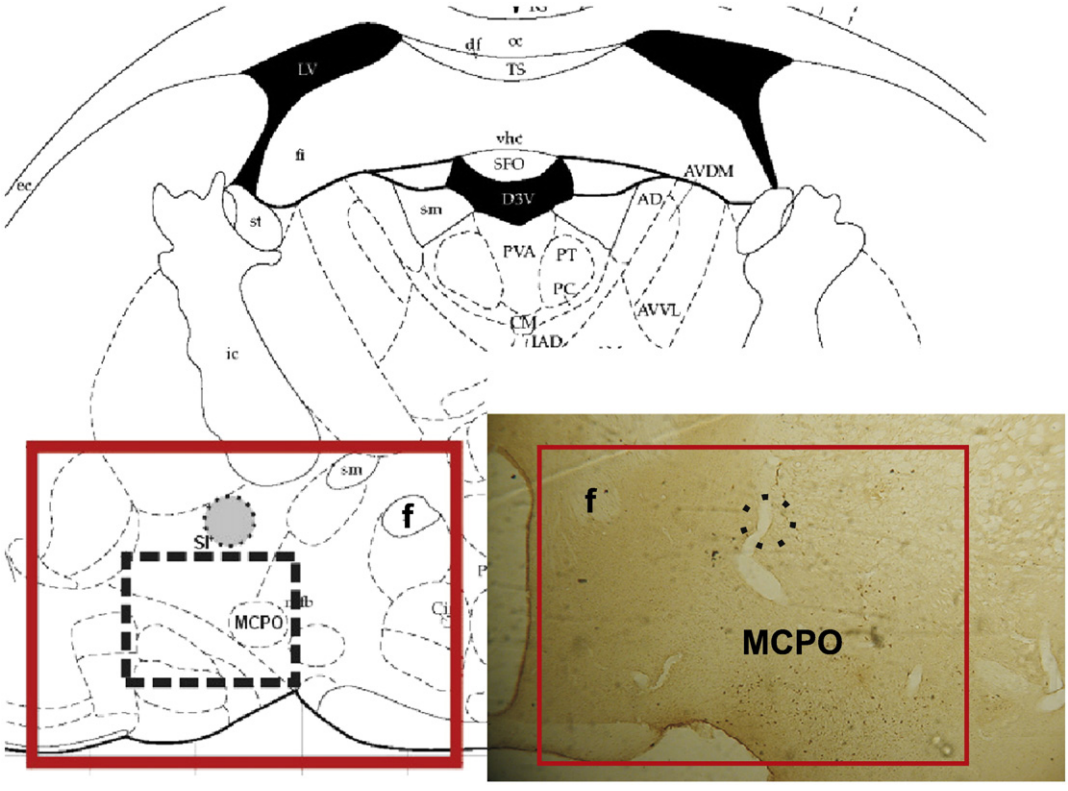

Fig. 1. Schematic diagram of coronal section 1.4 mm posterior to bregma (Paxinos and Watson, 1998) with superimposed choline acetyl transferase (ChAT) immunostained section (right side) from a control, sham-lesion rat. Left side shows intended target of cannula delivering 192IgG saporin bilaterally (gray dotted circle), large (solid line) rectangle delimits area for counting ChAT-immunopositive neurons, small (dashed line) rectangle for counting parvalbumin-immunopositive neurons. Right side shows putative cannula track in sham-lesion rat, and ChAT-immunopositive neurons in magnocellular preoptic area (MCPO). MCPO, SI (substantia innominata), and f (fornix) on the left side as labeled in Paxinos and Watson (1998); right side, corresponding structures in histological section.