TITLE: **HALOTHANE ENHANCES TONIC $GABA_A$ MEDIATED NEURONAL INHIBITION BY ELEVATING INTRACELLULAR CALCIUM.**

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Objectives: Anesthetics may depress the central nervous system (CNS) by reducing neuronal or synaptic excitation or by enhancing cellular or synaptic inhibition. Recently, $GABA_A$ mediated miniature spontaneous inhibitory postsynaptic currents (sIPSCs) have been described throughout the brain. These sIPSCs represent a background inhibitory activity in the CNS and are independent of action potential activity since they persist in 1.0 µM tetrodotoxin (TTX). To investigate the effect of halothane on this tonic inhibition, the sIPSC decay time constants were measured in whole cell patch clamp recordings from *in vitro* hippocampal slice preparations. Studies have demonstrated that elevations in intracellular Ca^{2+} are involved in the effects of halothane on skeletal, cardiac, and vascular smooth muscle. We examined whether a similar mechanism could be responsible for halothane's ability to increase tonic neuronal inhibition in hippocampal neurons. Making use of intracellular dialysis, afforded by the whole cell patch clamp technique, we investigated the role of intraneuronal Ca^{2+} in sIPSC prolongation.

Methods: Coronal brain sections (400 µm) containing the hippocampus were prepared from adult (200-350 g) male Wistar rats and placed in a brain slice chamber perfused with oxygenated (95%O_2 and 5%CO_2) artificial cerebro-spinal fluid (ACSF). Patch-electrodes were made from thin walled glass capillaries on a Narashige PP83 electrode puller. For calcium experiments, intracellular solutions contained 11 mM 1,2-bis (2-aminophenoxy)ethane-N,N,N',N''-tetra-acetic acid (BAPTA) and 1 mM $CaCl_2$ or 15 µM dantrolene sodium. The volatile anesthetic halothane (Ayerst) was delivered from a calibrated vaporizer. Concentration in the vapor and perfusate of the slice chamber was continually monitored using a Puritan-Bennett (Model 222) gas analyzer. Recordings were made using an Axoclamp 2A or an Axopatch 1-D amplifier with >80% series resistance compensation.

Results: Halothane at 1 MAC prolonged the decay time constant of spontaneous $GABA_A$-mediated inhibitory postsynaptic currents by 275 % relative to control. Pentobarbital increased sIPSC decay time constants by 230% of control. When increases in intraneuronal Ca^{2+} were prevented by intracellular administration of the Ca^{2+} chelator BAPTA or the Ca^{2+} release inhibitor dantrolene, halothane's effect was reduced significantly ($p < 0.005$, ANOVA); in contrast, the pentobarbital effect was unchanged. The relative contribution of inhibition to the depression of field potentials by halothane was investigated by extracellular recordings. With inhibition intact or excitation increased by lowering extracellular Mg^{2+}, halothane depressed population spike amplitudes (80 -100%). When $GABA_A$-mediated inhibition was blocked by bicuculline, halothane reduced population spike amplitude by only 21%.

Conclusion: These findings indicate that the major depressant effect of halothane involves the enhancement of $GABA_A$-mediated inhibition through the release of intraneuronally stored Ca^{2+}.

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TITLE: **CNS CHOLINERGIC MECHANISMS IN ANESTHESIA**

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Introduction. During general anesthesia, the rate of discharge of cholinergic neurons is depressed in several brain regions by a wide variety of general anesthetic agents. Additionally, clinical experience with a frequent side-effect of anesthesia, the central anticholinergic syndrome, and perturbations of peripheral cholinergic mechanisms during anesthesia, have suggested that cholinergic mechanisms may be deranged during general anesthesia. Therefore, the effect on anesthetic requirement (MAC), by selective modulation of central cholinergic function, was undertaken to test the hypothesis that central cholinergic mechanisms might play a role in the mediation of general anesthesia.

Methods. Healthy male rats were anesthetized with 5% isoflurane in oxygen. They were then placed in a specially constructed apparatus with an oxygen delivery system (3L/min) and an isoflurane vaporizer in-line. The spontaneously breathing anesthetized rats were then exposed to 2.25% isoflurane concentration for 5 min. The concentration was then reduced in a stepwise fashion every 15 min until MAC was reached. Once baseline MAC had been determined, the isoflurane concentration was readjusted to 2.25%, and intracerebroventricular (icv) or intraperitoneal (ip) drugs administered. MAC was then redetermined as before. Gas in the apparatus was assayed for isoflurane concentration by calibrated gas chromatography. Arterial samples were analyzed for blood gas values. Icv injection sites were later verified. Values in the different treatment groups were compared with values in their control groups by one way analysis of variance (ANOVA). Within each treatment group, the baseline and post-treatment MAC were compared with the paired t-test (ptt).

Results. Comparison by ANOVA of animals (n=150) in the various groups revealed no difference in their baseline MAC which was 1.596% (SEM= 0.014%) for isoflurane in oxygen. Control animals (n=30), injected icv with saline (or that had no injection), did not change their MAC with repeat measurement. Icv injections of 10 µg and 20 µg clonidine decreased MAC by 33% (ptt and ANOVA $p<0.001$) and 53% (ptt $p<0.005$; ANOVA $p<0.001$) of baseline, respectively. Physostigmine 0.25 mg/kg injected ip prior to determining MAC increased baseline MAC by 14% (ANOVA $p<0.001$). Depression of central cholinergic activity with icv injection of 30 µg hemicholinium-3 reduced MAC by 18% of baseline (ptt and ANOVA $p<0.001$) while nicotinic blockade with icv injection of 20 µg, 10 µg and 5 µg pancuronium reduced MAC by 26%, 13% (ptt $p<0.005$; ANOVA $p<0.001$) and 8% (ptt $p<0.01$; ANOVA $p<0.005$) of baseline, respectively. Surprisingly, muscarinic blockade by icv injection of 30 µg atropine increased MAC by 3% (not significant) while scopolamine 0.025 mg/kg injected ip increased MAC by 9% (ANOVA $p<0.05$) of baseline. Conversely, icv injection of 20 µg oxotremorine decreased MAC by 29% (ptt $p<0.05$; ANOVA $p<0.001$). Icv injection of 5 µg nicotine increased MAC by 10% (ANOVA $p<0.01$). None of the treatments produced any significant effect on temperature or arterial blood gas analysis (pH, pCO_2, pO_2, or B.E.).

Discussion. It is not uncommon for the same neurotransmitter (in this case, acetylcholine) to have opposite effects on behavior, depending on which site is 'switched on' by it.[1,2] Global functional depression of cholinergic mechanisms, mainly of a nicotinic nature, mediates the anesthetized state. This masks the contribution of a muscarinic mechanism whose activation may mediate some quantitatively less important aspect of anesthesia, possibly antinociception.[1] This is distinct from a muscarinic cholinergic mechanism whose activation (or recovery from depression) facilitates recovery from general anesthesia.[2,3] The relative contributions of cholinergic and adrenergic mechanisms to the mediation of general anesthesia were not compared.

References.

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