Title: **ENHANCEMENT OF GABA-ACTIVATED CHLORIDE CONDUCTANCE IN CULTURED RAT HIPPOCAMPAL NEURONS BY ENFLURANE**

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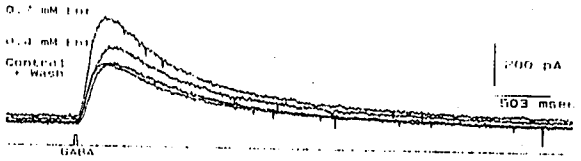
Relatively little is known of the interactions between volatile anesthetics and molecules important in regulating neuronal excitability in the mammalian brain. γ-Aminobutyric acid (GABA) is the major inhibitory transmitter in the mammalian brain; GABA acts on two classes of receptor, one of which, the $GABA_A$ receptors, is an important target for anesthetic agents such as the barbiturates. A recent report[1] described some observations on the modulation of $GABA_A$ receptors by halogenated anesthetics in sensory neurons. The present study investigated the effects of enflurane in hippocampal neurons and showed that transient current responses to GABA are increased in amplitude by enflurane in a concentration-dependent manner.

Hippocampal neurons from day 20 embryonic rats were dissociated and maintained in culture. Whole-cell recordings were made at 25°C with the patch-clamp technique, using an intracellular solution based on K gluconate. GABA was applied to individual neurons in brief (25-100 ms) pulses from a micropipette containing 50 μM GABA positioned within 5 μm of the cell body. Neurons were held at -40 mV under voltage-clamp; application of GABA elicited transient outward currents (I_{GABA}) that reversed in polarity at about -70 mV. The neurons were continuously perfused (2 ml/min) with a HEPES-buffered saline. Anesthetic solutions were applied via the perfusion line, and experimental concentrations of anesthetic were measured by withdrawing small samples of experimental saline from the chamber for gas chromatographic analysis.

Enflurane enhanced I_{GABA} in 12 of 13 experiments in which it was present at concentrations ≥ 0.5 mM (e.g., Fig. 1). Application of enflurane-containing solutions to the chamber resulted in a gradual increase in the peak amplitude of I_{GABA} up to 300% of control (Fig. 2). The percentage enhancement of I_{GABA} increased with increasing enflurane concentration (Fig. 3). We conclude that enflurane modulates the $GABA_A$ receptors on cultured rat hippocampal neurons to produce an increase in GABA-activated chloride conductance. This modification of $GABA_A$ receptor-operated Cl^- channel function occurs at anesthetic concentrations within the clinical range.

Reference: FASEB J 3:1850-1854, 1989

1



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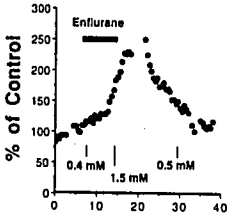

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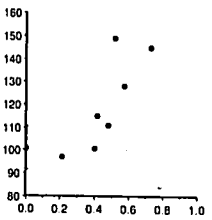

Title: A COMPARISON OF THE CAFFEINE HALOTHANE CONTRACTURE TEST, WITH MOLECULAR GENETICS DIAGNOSIS OF MALIGNANT HYPERTHERMIA (MH)

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The laboratory diagnosis of MH is currently made by the Caffeine Halothane Contracture Test (CHCT). Recently, genetic linkage between ryanodine receptor gene (RYR) polymorphisms and the MH phenotype determined by CHCT was demonstrated.[1] Our study compared genetic linkage analysis to CHCT results in a large kindred. After study approval and informed consent, genetic linkage analysis was performed on 16 members of a three generation family.[1,2] The RYR cDNA probe HRR1-1600 was used to detect the Ban I polymorphism (RFLP).[1] Eleven subjects had undergone quadriceps muscle biopsy in 1985-86, with CHCT performed according to the method of Rosenberg and Reed.[3] Abnormal CHCT responses were defined as: caffeine specific concentration (CSC) < 4 mM, halothane caffeine specific concentration (HCSC) < 1 mM, contracture > 0.2 g at 2 mM caffeine, and/or contracture > 0.2 g to 1% halothane.

The proband (IV-1) was an 8 year-old boy who developed trismus and mild fever after succinylcholine. Two first cousins of proband's mother (III-3) died under anesthesia; her 6 siblings and 5 other first cousins underwent muscle biopsy (Table). The RYR Ban I RFLP B allele cosegregated completely with a positive halothane contracture test, but not with an abnormal CSC result > 2 mM. Cosegregation was also seen with 2 mM caffeine > 0.5 g and HCSC < 0.3 mM. Using genetic linkage studies we may better interpret CHCT results and the expression of the MH phenotype.

Table - Results of CHCT and linkage studies

#	CSC	HCSC	2mM Caff	1% Halo	Diagnosis	RYR
III-1	2.5	0.51	0.0	0.0	MHS	A
III-2	0.9	0.26	3.8	1.1	MHS	A/B
III-3	Mother of proband				n/a	A/B
III-4	2.3	0.38	0.5	0.0	MHS	A
III-5	2.5	0.60	0.15	0.0	MHS	A
III-6	3.0	0.79	0.0	0.0	MHS	A
III-7	2.4	0.43	0.2	0.0	MHS	A
IV-1	Proband				n/a	B
III-10	2.9	0.58	0.0	0.0	MHS	A
III-11	0.7	0.13	6.1	3.6	MHS	A/B
III-12	1.2	0.16	2.7	0.7	MHS	A/B
III-13	4.1	0.66	0.0	0.0	MH(-)	A
III-14	1.1	0.14	4.5	5.0	MHS	A/B
II-1	Father of III-1 to 7				n/a	A
II-2	Mother of III-1 to 7				n/a	A/B
II-4	Mother of III-10 to 14				n/a	A

(III-1 to 7 are siblings; III-10 to 14 are siblings and are first cousins of III-3; MHS = MH-susceptible)

1. Nature 343:559,1990. 2. Am J Hum Genet 44:140,1989. 3. Anesth Analg 62:415,1983.