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HALOTHANE ENHANCES GABA-GATED CHLORIDE FLUX IN MICE SELECTIVELY BRED FOR SENSITIVITY TO DIAZEPAM

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Introduction: Pharmacogenetics provides a powerful approach to identify the molecular target of general anesthetics (GAs). For example, strains of mice have been bred that diverge in their sensitivity to ethanol [1] or N_2O [2], but attempts to identify the biochemical basis of the divergence have been frustrated by the lack of a defined site of action for the selecting agents. The recent development of mouse lines that are differentially sensitive to diazepam (DZP), [3] termed DS (DZP-sensitive) and DR (DZP-resistant), presents an opportunity to successfully exploit the pharmacogenetic approach, because the site of action of the selecting agent, DZP, is known; it is the $GABA_A$ receptor-chloride channel macromolecule ($GABA_A$-R). This altered sensitivity in vivo is paralleled in vitro by altered gating of the $GABA_A$-R channel by DZP.[4] We have previously demonstrated that DS mice are more sensitive than DR to obtundation by GAs.[5] Taken together, these studies lead to the prediction that halothane should differentially enhance $GABA_A$-R channel gating in the brains of these mice. If true, this would support the concept that the $GABA_A$-R serves as a molecular target of GAs.

Methods: Brain membrane microvesicles were prepared from DS and DR mice by differential centrifugation.[4] Fresh membrane suspensions (200 μl, in physiologic buffer, 6-10 mg protein/ml) were incubated in glass test tubes in a water bath at 34°C. $^{36}Cl^-$ (770 μCi/mmol, Amersham, diluted in buffer to 2 μCi/ml), ± buffer saturated with halothane, ± GABA 5 μM (total volume of 200 μl) was added. At the standard flux assay incubation time of 3 seconds,[4] reaction mixtures were quenched with 4 ml of ice-cold buffer containing 100 μM picrotoxin (a specific chloride-channel blocker), and then rapidly vacuum filtered over glass-fiber filters in a Hoeffer manifold. Filters were washed with 8 ml of cold buffer containing picrotoxin, then dissolved in scintillation cocktail, and counted (100% efficiency) over the energy spectrum of $^{36}Cl^-$. Specific enhancement of GABA-gated flux by halothane was calculated by subtracting the flux values obtained in the absence of halothane. Concentrations of halothane present in otherwise identical reaction mixtures at the moment of quenching were confirmed by gas chromatography. Data were obtained from 10 DR mice and 12 DS mice. Each mouse was used for control, 0.4 mM, and 1 mM halothane determinations. Data from the DS and DR groups were pooled, then statistically compared by two-tailed t-test.

Results: In the absence of halothane, 5 μM GABA produced equivalent flux responses in the DS and DR mice (14.0±0.9 and 14.7±0.7 nmol/mg protein/3 sec, respectively, p = N.S.). A therapeutic concentration of halothane (0.4 mM) enhanced GABA-gated flux significantly greater in DS than in DR mice (0.78±0.16 vs. 0.14±0.22 nmol/mg/3 sec, respectively, $P < 0.05$). A higher concentration of halothane (1 mM) further enhanced GABA-gated flux in both groups, but the difference between them was no longer statistically significant (1.60±0.20 for DS vs. 1.15±0.34 nmol/mg/3 sec for DR).

Discussion: Differential enhancement of GABA-gated chloride flux by halothane between the DS and DR lines neatly parallels our previous findings with these same animals in vivo.[5] Differential sensitivity to the effects of both DZP and halothane, in vivo and in vitro, strongly supports the notion of a common underlying mechanism involving the $GABA_A$-R as an important molecular target for GAs. The loss of differential sensitivity at high concentration may be explained by saturation of halothane's flux-enhancing effects, but complete concentration-response data will be required to establish the mechanism.

References: 1. Behav Genet 3:409, 1973. 2. Anesthesiology 52: 401, 1980. 3. Psychopharmacology 93: 25, 1987. 4. Brain Res 452:118, 1988. 5. Anesthesiology 73:A708, 1990.

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Title: **METHOHEXITAL AND VOLATILE ANESTHETICS REDUCE DEPOLARIZATION-INDUCED CALCIUM ENTRY INTO CULTURED RAT HIPPOCAMPAL NEURONS**

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Calcium ions play an important role in regulating neuronal activity. The intracellular free calcium concentration ($[Ca^{2+}]_i$) is tightly controlled. We have studied some of the mechanisms that regulate $[Ca^{2+}]_i$ in central neurons, using fura-2 based microfluorimetry (neurons loaded via the acetoxymethyl ester) and a combination of electrophysiology and microfluorimetry (neurons loaded with the potassium salt of fura-2 via a patch pipette).

Methods: Hippocampal neurons from day 17-18 embryonic rats were dissociated and maintained in culture for up to 6 weeks. Whole-cell recordings were made at 25°C with the patch-clamp technique,[1] using an intracellular solution based on K gluconate containing 100 µM fura-2 and 5 mM ATP. Neurons were continuously perfused with a HEPES-buffered physiological saline. Neurons were held at -60 mV in current clamp and stimulated by injection of brief current pulses (1-2 nA, 4 ms). In experiments not involving electrophysiology, neurons were stimulated with 30 s applications of 50 mM K^+ (high K). Anesthetic solutions were applied via the perfusion line, and experimental concentrations were measured by gas chromatography. Data are presented as mean ± SEM.

Results: Basal $[Ca^{2+}]_i$ was 69 ± 9 nM. Ca^{2+} accumulation in response to high K^+ was reduced by 38% by nimodipine (1 µM), indicating the presence of "L-type" Ca^{2+} channels. $[Ca^{2+}]_i$ returns rapidly to basal levels following trains of APs ($\tau \sim$ 3-5 s); the *net* increase in $[Ca^{2+}]_i$ in response to APs was highly frequency-dependent, rising over the range 6-50 Hz.

Ca^{2+} accumulation in response to high K^+ was reduced by 56 ± 9% in 20 µM methohexital and by 69% in 50 µM methohexital. The volatile anesthetics enflurane and halothane (1-2 MAC) reduced Ca accumulation by ~ 30%. None of the anesthetics tested in these experiments had significant effects on basal $[Ca^{2+}]_i$, although mitochondrial uncouplers elevated $[Ca^{2+}]_i$, and 10 mM caffeine caused transient increases in $[Ca^{2+}]_i$ from 65 to 128 nM in the absence of extracellular Ca^{2+} that were prevented by 10 µM ryanodine.

Conclusion: We conclude that Ca^{2+} accumulation in central neurons may be highly dependent on frequency of activation and that the anesthetics methohexital, halothane, and enflurane all reduce Ca^{2+} entry in response to depolarization, possibly by inhibition of voltage-sensitive Ca^{2+} channels.

Reference: J Physiol (Lond) 425:85-115, 1990

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