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TITLE: STEREOCHEMICAL ASPECTS OF HUMAN LIVER MICROSOMAL KETAMINE METABOLISM

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The importance of stereochemistry in drug action and disposition is becoming increasingly apparent. Ketamine is a racemic mixture of S(+) and R(-) enantiomers, which differ significantly in anesthetic efficacy and toxicity. The S(+) isomer is 4 times more potent than its R(-) isomer, while the latter causes a higher incidence of emergence reactions (1). Recovery following racemic ketamine anesthesia is slower than that from either isomer, engendering the speculation that S(+) metabolism exceeds that of R(-), and R(-) in the racemic mixture inhibits metabolism of S(+) ketamine, thereby delaying recovery from this more potent isomer (1). Ketamine is metabolized predominantly via N-demethylation to norketamine. Little is known however, regarding differences in the metabolism of ketamine isomers. The purpose of this investigation was to test the hypothesis that humans metabolize ketamine isomers at different rates, and that there is a metabolic enantiomeric interaction between the ketamine isomers.

Microsomes were prepared from livers of organ donors. Microsomal ketamine demethylation was determined from the formation of norketamine, measured by GC-mass spectrometry with selected ion monitoring. Deuterated S(+) ketamine was used for enantiomeric interaction experiments.

Rates of demethylation for S(+) ketamine exceeded those for R(-) ketamine at all substrate concentrations. At therapeutic ketamine concentrations (10 μM), S(+) metabolism was significantly greater than that of R(-). The rate for the racemic mixture was intermediate. S(+) ketamine demethylation was significantly diminished in the presence of equimolar R(-) ketamine. Eadie-Hofstee plots indicated the presence of two enzymes catalyzing the demethylation of each enantiomer, with one enzyme predominating at therapeutic concentrations.

These results suggest differences in the human hepatic metabolism of ketamine enantiomers. The enantiomeric interaction whereby R(-) inhibits S(+) ketamine metabolism, accounts for the decreased demethylation rate of the racemate relative to the isolated S(+) enantiomer. These results also support clinically observed differences in ketamine enantiomer pharmacokinetics and prolonged recovery from the racemate.

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REFERENCE

1. Br J Anaesth 57:197-203, 1985.

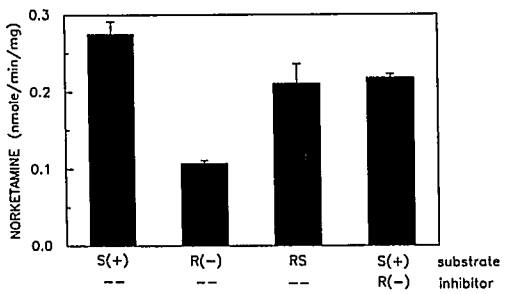

FIGURE: Human liver microsomal ketamine metabolism. All substrates and inhibitor were present at 10 μM. Norketamine formation rates shown are for substrate only.

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TITLE: MOLECULAR BASIS OF KETAMINE ACTION ON CLONED POTASSIUM CHANNELS USING VOLTAGE CLAMP TECHNIQUES.

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INTRODUCTION: Alterations in potassium (K^+) conductance are suggested to play an important role in the manifestation of general anesthesia[1]. Recently, a diverse group of potassium (K^+) channels from different species (e.g., fruit fly and rat brain) have been cloned and their amino acid (AA) sequences deduced. The general structure of K^+ protein consists of six identical membrane-spanning segments flanked by amino and carboxy (C) terminals facing the cytoplasmic side of the membrane. Using voltage clamp experiments, we investigated the effect of ketamine (ket) on these K^+ channel proteins expressed in the plasma membrane of Xenopus (frog) oocytes (XO). By comparing the actions of the anesthetic with the sequence, we hope to relate structures of the channels to their sensitivities to ket.

METHODS: mRNA was transcribed from cDNAs encoding K^+ channels from 3 families; *Shaker* (fruit fly: ShB1 and rat brain: RBK1), *shab* (rat brain: drk1, ΔC318) and *Isk* (I_{sk}-Human). ΔC318 is a deletion mutant of drk1 (lacking the last 318 AA of the C-terminus[2]). One to two days after micro-injection of the mRNA into XO, effects of ket on channel function were studied by voltage clamping. After establishing a stable baseline current, cells were exposed to graded concentrations of ket (25, 50, 75μM). pClamp system (Axon instruments) was used for the generation of voltage steps and data acquisition. Peak current amplitude (PA), decay time constant (tau), and voltage-dependency of activation were measured before, in the presence of, and after wash-out of ket (holding potential = -50mV; voltage steps, -30 to +50 in 20mV increments). The linear leakage and capacitative currents were subtracted from the data during analysis. Data are presented as mean ± SE and were analyzed by repeated measures of analysis of variance. Post-hoc comparisons were made with Neuman-Keuls test. $p<.05$ termed significant.

RESULTS: drk1 was found to be the most sensitive to ket, showing a "dose dependent" decrease in PA (figure) and tau values. RBK1, I_{sk}-H and ShB1 were significantly less sensitive to ket. Ket did not produce a shift in the voltage dependency of activation in any of the clones examined. PA and tau in ΔC318 were significantly less sensitive to ket than in drk1 (figure).

CONCLUSION: 1) Different K^+ channel clones show differential sensitivity to ket. 2) Ket inhibits drk1 channel conductance in a dose dependent manner. 3) Reduced sensitivity of the mutant clone (ΔC318) suggests an interaction between the C-terminus of the channel protein and ket.

REF: 1) Segal I.S., et al. ASA Abstracts A641, 1989
2) Antonius M.J., et al. Neuron, 5: 433-43, 1990

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