

TITLE: ENHANCED EXPRESSION OF PROTO-ONCOGENE C-FOS PROTEIN IN RATS GIVEN KETAMINE.

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Histological Expression of proto-oncogene *c-fos* has been reported after activation of certain brain regions.¹ Dragunow et al.² have used *c-fos* immunocytochemistry as a metabolic marker to trace polysynaptic pathways in the brain, such as those involved in the spread of seizures. The present study examined the expression of *c-fos* immunoreactive protein after ketamine administration to rats in order to indicate its site of action.

One group of Sprague Dawley rats received 0 (saline control), 45, 80, or 120 mg/kg ketamine i.p. and the resultant behavioral activity was graded at 10 min. intervals over the next two hours. The doses of ketamine selected produced a dose response dependent effect, with only the 120 mg/kg, dose producing surgical anesthesia. The two lower doses achieved ataxia and difficulty righting, but did not abolish their response to pain consistently.

A separate group of rats were given saline or ketamine (40, 80 or 120 mg/kg i.p.) 2 hours prior to

death by perfusion with 4% paraformaldehyde and 0.2% glutaraldehyde. Serial vibration sections were prepared at various levels of the brain to permit the detection of regional expression of *c-fos* immunoreactive protein using a polyclonal antisera (Cambridge Biologicals). Examination of many areas of the brain revealed variable expression of *c-fos* relative to control with the posterior cingulate cortex demonstrating the most intensely reactive material. The intensity of staining at this site was dose dependent, and prior administration of naloxone (10 mg/kg s.c.) did not abolish *c-fos* expression at that site.

Crosby et al.³ reported increased glucose utilization in the hippocampus and cingulate cortex, but *c-fos* expression in our experiments was most profound in our rats in the posterior cingulate cortex, with little expression in the hippocampus. Olney et al.⁴ have reported that ketamine in doses similar to the lowest doses used here produced vacuole formation in large neurones of the cingulate cortex. Thus, the cortical changes in *c-fos* noted in our studies could represent a metabolic correlate of cell activation or the early signs of cellular damage and repair.

References

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TITLE: STUDY OF PROTON PUMPS BY DOPH-IMPREGNATED MILLIPORE MEMBRANE: EFFECTS OF ALCOHOLS

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Phospholipid-impregnated Millipore membranes are often used as a model for biological membranes. DOPH (dioleoyl phosphate)-impregnated Millipore membrane has a unique character that it undergoes phase transition between hydrophilic and hydrophobic states according to the electrolyte concentrations. In the hydrophobic state, the membrane shows high membrane resistance. This membrane also shows high membrane resistance in the presence of calcium ion. In this study, we fused the bacteriorhodopsin vesicles to the DOPH impregnated Millipore membrane and examined the proton pump function of bacteriorhodopsin by measuring the light-induced voltage. A membrane filter (Millipore, 0.22 μ m) was immersed into DOPH-benzene solution and was stored in 100 mM KCl solution after drying. Bacteriorhodopsin (5 mg) and soybean phospholipid (100 mg) was suspended in 5 ml of 150 mM KCl solution. The mixture was sonicated to form vesicles. The DOPH membrane was sandwiched between two half-cells with 100 mM KCl and 50 mM CaCl₂. Bacteriorhodopsin vesicles were placed on one side and were fused to the filter membrane in the presence of calcium ion. The membrane was illuminated with a 500 W projector lamp from one side. The light-induced voltage generated by bacteriorhodopsin was measured by a Keithley 614 multimeter through the Ag/AgCl electrodes. After 60 min incubation with vesicle suspension, the light-induced voltage reached a stable value. Alcohols were added to the same side where vesicles were fused. The reversibility was checked by washing with 100 mM KCl and 50 mM CaCl₂ solution.

The figure shows the typical traces of the light-induced voltage. Ethanol reduced this light-induced voltage in a dose dependent manner. After washing out ethanol, the light-induced voltage was recovered near to the control value. Ethanol also induced conformational changes of bacteriorhodopsin in a purple membrane. The ethanol concentration which induced this change was higher than that reduced the light-induced voltage. Because the membrane is stable and easy to handle, this system appears to be a suitable model to study the effects of anesthetics. Supported by the VA Medical Research, and NIH grants GM25716 and GM27670.

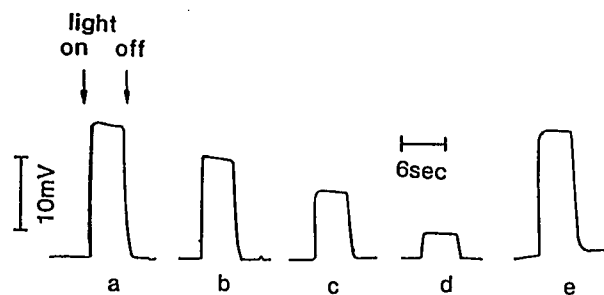


Figure: Light-induced voltages in the bacteriorhodopsin vesicles attached to DOPH-impregnated Millipore membrane. Bacteriorhodopsin vesicles (400 μ l) containing 8 mg phospholipid and 0.4 mg bacteriorhodopsin were placed on one side of DOPH-impregnated Millipore membrane in a medium containing 50 mM CaCl₂ and 100 mM KCl (final concentrations: 0.8 mg/ml of phospholipid, 0.04 mg/ml of bacteriorhodopsin). Control:(a), 423 mM ethanol:(b), 846 mM ethanol:(c), 1577 mM ethanol:(d), after washing out ethanol:(e).