

Title: DOES CHRONIC INTRACEREBROVENTRICULAR INFUSION OF THIORPHAN PRODUCE TOLERANCE AND DEPENDENCE IN RAT?

Authors: M.L.Eustache, M.D., A.Michael-Titus, Ph.D., R.Bousselmame, M.S., J.Costentin, Ph.D.

Affiliation: Unité de Neuropharmacologie expérimentale and Département d'Anesthésie-Réanimation Chirurgicale, Hôpital Charles Nicolle, 76000 ROUEN - FRANCE

Introduction: Acute administration of thiorphan (T.), a centrally active enkephalinase inhibitor, or of its lipophilic peripherally active derivative acetorphan (A.), induce opioid-like effects which are antagonized by naloxone [1]. The aim of this study was to evaluate the tolerance and dependence to intracerebroventricular T. when given chronically.

Methods: After Animal Care Committee approval, 64 male Sprague-Dawley rats were anaesthetized and implanted stereotactically in the left lateral ventricle with a 23-ga cannula connected to an osmotic minipump (Alzet® model 2004, flow 5µl/h) inserted subcutaneously between the scapulae. Pumps and cannulae were filled with T. (5µg/µl) or normal saline and placed in 37°C isotonic saline for 24 hours prior to implantation. Locomotor activity (LA) was assessed using a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, Ohio). Antinociceptive effects were measured using the hot plate test. During the infusion, LA was evaluated on the 2nd, 4th, and 7th days. Tolerance was investigated by i.v. administration of A. (50µg/kg) or normal saline on the 8th day (LA) and the 11th day (hot plate test). Pumps were removed on the 14th day. Dependence was evaluated (LA, appearance of withdrawal signs) for the 48 following hours. Enkephalinase activity (nmol of hydrolysed substrate per mg of tissue) was assayed on the 1st

and 14th days and 2 days after the removal of the pump, using a method based upon the hydrolysis of a fluorogenic substrate [2]. Results were given as mean ± SEM and were compared using Student's t-test (p<0.05 significant).

Results: Chronic infusion of T. induced an increase in LA (Table I) on the 2nd and 4th days. Tolerance was suggested by the return of LA to control values on the 7th day.

Table I: LA (total distance, inches) during chronic T. infusion

Day	Control	Thiorphan	
2	346 ± 92	1592 ± 188	p<0.01
4	978 ± 57	1307 ± 140	p<0.05
7	1094 ± 111	1143 ± 168	n.s.

On the 8th day, A. administration induced a significant increase in LA in normal saline infused rats (+178%, p<0.001), but not in T. infused rats (+33%, n.s.). On the 11th day, A. induced a significant increase in jump latencies (hot plate test) in normal saline infused animals (+150%, p<0.001) but not in T. infused rats (+17%, n.s.). After pumps were removed, a withdrawal behavior was not observed. Enkephalinase activities are shown on Table II.

Table II: Enkephalinase activity and chronic T. infusion

Time	Control	Thiorphan	Inhibition
Day 1	16.0 ± 1.9	12.0 ± 1.7	+30 %
Day 14	16.0 ± 1.4	17.8 ± 2.5	-11 %
Withdrawal	12.9 ± 3.0	12.3 ± 3.4	+5 %

Discussion: This study showed that a chronic treatment by T. induces tolerance to A. antinociceptive and locomotor effects. However it did not induce dependence as withdrawal signs were not observed after T. administration was stopped. Changes in enkephalinase activity might account partly for tolerance.

References: 1. Lecomte J.M. et al., J.Pharmacol.Exp.Ther. 1986;237:937-44; 2. Spillanti M.G. et al., Neuropeptides 1986;8:111-7

Title: THE PATTERN OF BRAIN TISSUE CONCENTRATIONS OF GLUTAMATE, ASPARTATE, GLYCINE AND DOPAMINE WITH VARYING DURATIONS OF GLOBAL CEREBRAL ISCHEMIA IN THE RABBIT

Authors: A.J. Baker, M.D., M.H. Zornow, M.D., M.S. Scheller, M.D., T.L. Yaksh Ph.D.

Affiliation: Department of Anesthesia, University of California, San Diego, CA 92093

Much experimental evidence supports a role of excitatory amino acids (EAA) in the pathogenesis of ischemic neuronal injury. However the use of an EAA antagonist (MK-801) in models of transient global cerebral ischemia (TGCI) has not consistently demonstrated protection.(1) Recent studies have suggested that additional neurotransmitters (e.g. glycine and dopamine) may mediate ischemic injury. Accordingly this study was undertaken to determine the pattern of extracellular glutamate, aspartate and glycine in the hippocampus and dopamine in the caudate nucleus with varying durations of TGCI in the rabbit, in order to evaluate their possible pathogenetic role.

Methods: After approval of the Animal Care Committee, New Zealand White rabbits were anesthetized and ventilated with 1% halothane in oxygen. Monitored variables were mean arterial pressure (MAP), central venous pressure, heart rate, arterial blood gases, hematocrit, blood glucose and the EEG. A pneumatic tourniquet was secured loosely around the neck of the animal. Microdialysis probes 4 mm in length were stereotactically positioned in the left caudate nucleus and the right dorsal hippocampus and perfused with artificial CSF. In order to induce global ischemia, the MAP was lowered to < 50 mmHg using trimethaphan boluses and positive end expiratory pressure. The tourniquet was inflated to a pressure of 20 psi for 5, 10 or 15 min. Immediately upon deflation of the tourniquet a bolus and then an infusion of phenylephrine was administered to maintain the MAP > 75 mmHg. Dialysate was collected prior to, during, and after the ischemic period. Using high performance liquid chromatography the dialysate from the hippocampus was analyzed for glutamate, aspartate, glycine and the dialysate from the caudate nucleus for dopamine. The animals were killed and the brains removed for verification of probe position.

Results: Three patterns of neurotransmitter concentrations were observed. Glutamate and aspartate concentrations in the dialysate increased from baseline

by 1-, 5- and 10-fold and by 3-, 10- and 29-fold respectively for the three ischemic durations. The concentrations returned to baseline rapidly after reperfusion. Dopamine concentrations increased by a mean of 700-fold for each ischemic duration and also returned to baseline within 10 min of reperfusion. Glycine in contrast, increased during ischemia by a mean of 4-fold, but demonstrated sustained elevations throughout the 80 min period of reperfusion. Statistical significance (p < 0.05) was demonstrated between peak concentrations for glutamate and aspartate (Kruskal-Wallis test) and between the final and baseline concentrations for glycine (p < 0.001) using a paired t-test.

Discussion: The finding that glutamate and aspartate concentrations in the hippocampus co-vary with the duration of global ischemia is taken as supportive evidence of their pathogenetic role in ischemic neuronal injury. The observation that dopamine concentrations increased independently of ischemic duration suggests a maximal release with only 5 min of ischemia and that its role in the incremental injury seen with increasing ischemic duration is limited to prolonged high concentrations rather than increasing peak levels as seen with glutamate and aspartate. Delayed neuronal injury can be attenuated by EAA antagonists.(2) However, the observation that glutamate and aspartate concentrations returned to baseline soon after reperfusion raises the question of whether EAAs are indeed involved in this post-ischemic injury. Glycine has previously been shown to facilitate EAAs' activity.(3) The sustained elevation of glycine concentrations after ischemia therefore, may explain the ability of EAAs to have ongoing toxicity in the reperfusion phase despite their prompt return to baseline concentrations. **References:** 1. J Cereb Blood Flow Metab 9:795-804, 1989. 2. Neurosci, 22:471-480 1987. 3. Nature 325:529-531 1987.

