Title: Subarachnoid EDTA Induces Hindlimb Myoclonus in Rats

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Introduction: Recently, Sodium bisulfite, antioxidant in 2-chloroprocaine Nesacaine (CE), has been replaced by a chelator, disodium EDTA dihydrate. Literature review revealed that EDTA has multiple adverse effects including teratogenicity,[1] collagen fibril depletion,[2] intestinal villi shortening,[3] neural crest tumor formation and cell death in rats/mice,[4] hepatic necrosis in calves,[5] and contact allergy,[6] vasculitis, cardiac tamponade, hemolytic anemia and renal damage in man.[7,8] This study focused on the EDTA effects in the subarachnoid space (SAS) in rats.

Method: With institutional approval, under intraperitoneal ketamine anesthesia 75-100 mg/kg, subarachnoid catheters were implanted through lumbar laminectomies in 500-600 gram male Sprague Dawley rats. One week postsurgery, catheter position was verified by induction of spinal anesthesia with 0.5 ml of 1.5% lidocaine. One week after this test, animals were divided into 2 groups: G1 (n=6) received SAS normal saline (NS) 0.05 ml, pH 5, hourly for 3-4 hrs. G2 (n=8) received SAS, 0.05 ml of 0.1% EDTA pH 4.5 hourly for 3-7 hours. Some rats in G2 were injected with EDTA again at a later date. The number of injections varied between 4-15.

Results: Group 1 did not have any motor abnormality following the NS injection. One minute after the 3rd to 15th EDTA injection, 7 of 8 rats circled continuously for several minutes. This was followed by hindlimb clonus for 15-20 minutes. The myoclonic contractions were 100+ per minute. Two rats developed hindlimb paralysis (HLP) post EDTA injection: one after receiving the 3rd & 6th, and the other, following the 5th injection. One animal underwent euthanasia with IV pentobarbital for histological examination after sustaining HLP for 10 days secondarytothe 7th injection. Table 1 summarizes the results.

Tab.1. SUBARACHNOID EDTA IN RATS

#	Inj	EDTA	Clonus	HLP	Recovery	Death	Sacrifice
1	6	0.3	0	0	NA	0	NA
2	6	0.3	3rd (15')	3rd (10')	+		
			6th (10')	6th (10d)			+
3	3	0.15		3rd (18')	0	3rd	NA
4	9	0.45	3rd (16')		+		
			7th (18')		+	+	
5	4	0.2	4th (17')		+		
6	15	0.75	2nd (20')	5th (2d)	+	0	NA
			15th (14')		+	0	NA
7	11	0.55	3rd (15')	0	+	0	NA
8	11	0.55	4th (15')	0	+	0	NA
			5th (13')	0	+	0	NA
			7th (15')	0	+	0	NA

Legend: #=rat number, Inj=injection number, EDTA in mg, Clonus(hindlimb) preceded by # of Inj when clonus occurred, followed by (minutes)=duration of clonus. Recovery= recovery from HLP and clonus, NA=not applicable, Sacrifice= euthanasia for histological examination.

Histological examination disclosed active, moderate to severe Wallerian degeneration of some lumbar sacral roots.

Discussion: Data show that subarachnoid EDTA alters neurological function and may be toxic in rats. Myoclonic seizures have been reported in rats following a 10-fold analgesic dose of morphine.[9] Relationship of the mechanism of action between EDTA and morphine is unknown. Accidental dura puncture is always a risk with epidural anesthesia. Drugs intended for epidural use, could enter SAS inadvertently. Therefore, incorporating EDTA in epidural anesthetics is unwise before safety is established.

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TITLE: Antinociceptive Effect of Intrathecal Midazolam and Fentanyl in the Sheep

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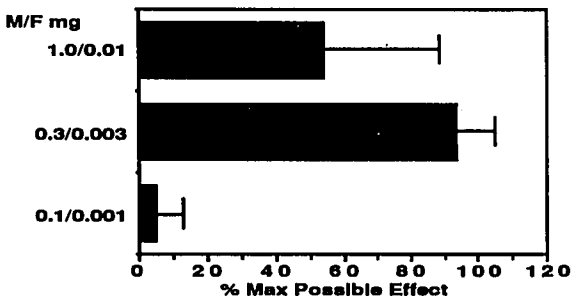
INTRODUCTION: Intrathecally administered midazolam(M) produces segmental analgesia by a non-opiate spinal mechanism in the rat.[1] To further understand this action, we examined the effect of intrathecal M and fentanyl(F) on electrical current threshold for pain in conscious, unrestrained sheep.

METHODS: Adult mixbreed western male sheep were studied in this IACUC approved study. Under local anesthesia both a 20g subarachnoid catheter, at the level of the last lumbar vertebra, and a femoral artery cannula were inserted. Following a 48h recovery, a pair of surface electrodes were placed on both the flank and shoulder with a fixed inter-electrode distance of 8cm and connected to a Grass S5 stimulator. Antinociception was measured at both the shoulder and flank by increasing stimulus intensity in 10 volt increments up to 80 volts or until an avoidance response was seen. Intrathecal dose response curves were determined by injecting saline, M (0.3, 1, 3mg), F (0.003, 0.01, 0.03mg) and M/F combinations (0.1/0.001, 0.3/0.003, 1.0/0.01mg) in a randomized, blinded manner with a minimum of 24h between injections. Measurements of HR, BP and antinociception were made at 0, 5, 10, 20, 30, 60, 90 and 120 min and ABG at 0, 30, 60, 90 and 120 min. The percent maximum possible effect (%MPE) was calculated for each sheep at each dose and time. Two-way analysis of variance for repeated measures was used to compare arterial blood gas and hemodynamic data within and between groups.

RESULTS: Six sheep were evaluated in this study. F, M, and M/F produced dose related segmental antinociceptive effects. Antinociception increased linearly with increasing doses of M and F. However, with M/F, antinociception was greater following the 0.3/0.003mg dose than following the larger 1.0/0.01mg dose (Fig.). No drug had any significant effect on blood gas or hemodynamic parameters.



DISCUSSION: Benzodiazepines are not normally considered to be analgesics. However, when administered intrathecally in the rat, M produces segmental antinociception to electrical current threshold for pain, reversible by the benzodiazepine antagonist flumazenil. We successfully demonstrated a segmental antinociceptive effect from intrathecal M in adult sheep. However, the interaction of M with F seen in this study is complex. A similar inhibitory trend with higher M doses has been reported between intrathecal M and morphine in the rat.[2] Additionally, systemic M antagonizes the analgesic effect of systemic morphine.[3] Perhaps systemic absorption of the largest dose of M antagonizes the spinally mediated analgesic effects of M/F.

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