

Title: Subarachnoid EDTA Induces Hindlimb Myoclonus in Rats
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Introduction: Recently, Sodium bisulfite, antioxidant in 2-chloro-
procaine Nesacaine (CE), has been replaced by a chelator,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 secondary to the 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') 6th (10')	3rd (10') 6th (10d)	+		+
3	3	0.15					
4	9	0.45	3rd (16') 7th (18')		+		
5	4	0.2	4th (17')		+	+	
6	15	0.75	2nd (20') 15th (14')	5th (2d)	+	0	NA
7.	11	0.55	3rd (15')	0	+	0	NA
8	11	0.55	4th (15') 5th (13') 7th (15')	0 0 0	+	0 0 0	NA NA 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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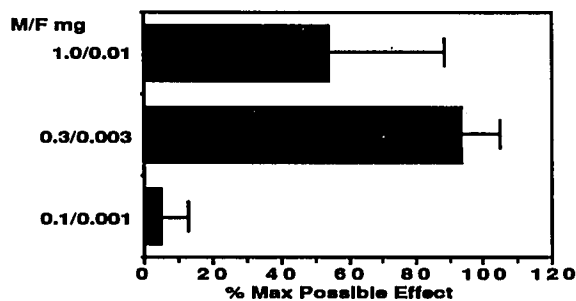
TITLE: Antinociceptive Effect of Intrathecal Midazolam
and Fentanyl in the Sheep
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INTRODUCTION: Intrathecally administered midazolam(M)
produces segmental analgesia by a non-opiate spinal mechanism in
the rat.¹ To further understand this action, we examined the effect
of intrathecal M and fentanyl(F) on electrical current threshold for
pain in conscious, unrestrained sheep.

METHODS: Adult mixedbreed 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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