

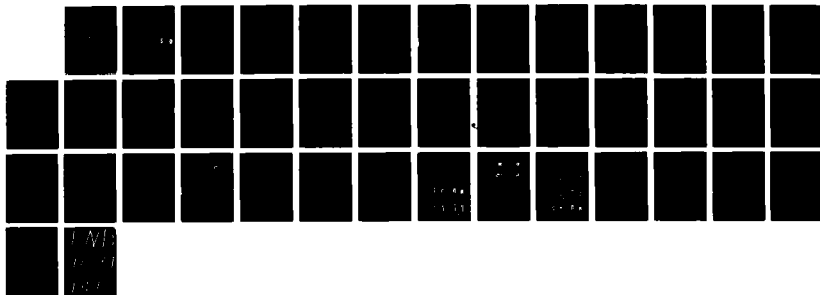
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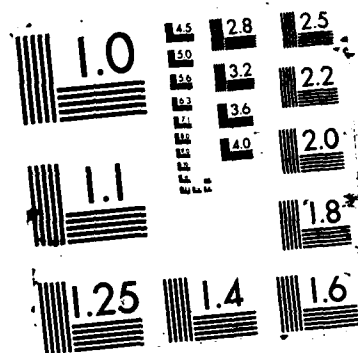
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Comparison of Naloxone and Thyrotropin-Releasing Hormone
in the Treatment of Experimental Spinal Injury

Annual and Final Report

G. Feverstein
Alan I. Faden

September 1, 1984

Supported by

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We have also evaluated the effects of longer acting and more potent TRH and opiate receptor analogues. The κ -selective opiate receptor antagonist WIN44,441-3 produced significant improvement in long-term neurological recovery following traumatic spinal cord injury in the cat. Similarly, the potent TRH analogue CG3509 proved significantly effective in the same injury model. This contrasts with the effects of another TRH analogue, MK-771, which proved ineffective. Taken together, these findings indicate that opiate receptor-selective antagonists and certain classes of TRH analogues may be beneficial in the treatment of traumatic CNS injury; by virtue of greater selectivity and far longer biological half-lives than either TRH or naloxone, these compounds may prove to be superior therapeutic agents in the treatment of human injuries to the CNS.

During the second half of the contract period, we developed a new model of spinal cord injury, a traumatic model in the thoracic region of the rat, in order to evaluate changes in endogenous opioids following spinal cord injury. Graded spinal cord injury was associated with progressive increases in dynorphin-like immunoreactivity but not enkephalin immunoreactivity at the injury region. The changes in dynorphin levels correlated in a highly significant way with the progressive neurological function after injury. Finally, we have shown that the dynorphin family of opioids, which have high selectivity for the κ -opiate receptor, are unique amongst opioids in producing dose-related hindlimb paralysis in the rat. Taken together, the findings in the rat studies strongly suggest that the pathophysiological effects of endogenous opioids after spinal cord injury are mediated by the dynorphin family of opioids and the κ -opiate receptor. Pharmacological

studies in the cat also support this hypothesis, with the demonstration that κ -selective opiate antagonists are effective in improving neurological recovery after injury.

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FOREWORD

Citations of trade names in this report do not constitute an official Department of the Army endorsement or approval of the use of such items.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 78-23, 1978).

TABLE OF CONTENTS

SUMMARY	.2
FOREWARD.	.5
PROBLEM	.7
BACKGROUND.	.8
MATERIALS AND METHODS	10
RESULTS	12
CONCLUSIONS	14
RECOMMENDATIONS	16
LITERATURE CITED.	17
FIGURES	20
TABLES.	24

This is the final report submitted under contract #USAMRDC 01120-82 and covers the period 1 August 1982 - 30 September 1984. The studies reported include: (1) comparison of the beneficial effects of TRH, naloxone and corticosteroids following experimental spinal cord injury in the cat; (2) effect of megadose corticosteroids (both dexamethasone and methylprednisolone) in this same injury model; (3) independent studies examining the dose-response effects of TRH on spinal cord injury in the cat, as well as the effects of late (24 h post-injury) treatment; (4) evaluation of the therapeutic effects of the TRH analogues CG3509 and MK-771, as well as those of the κ -selective opiate receptor antagonist WIN44,441-3 in cat spinal cord injury; (5) studies establishing a spinal trauma model in the rat; (6) intrathecal infusions of selective endogenous opioids and opiate agonists to assess the potential role of specific opioids and/or opiate receptors in the paralysis which follows traumatic injury; and (7) examination of changes in various endogenous opioids (e.g., dynorphin, enkephalins) in the spinal cord after injury.

Problem. It is clear that much of the neurological deficit which follows traumatic injury to the CNS results not from the immediate and direct effects of the trauma in severing neuronal connections, but rather from secondary responses initiated by the injury which result, in part, from reductions of blood flow due to release of endogenous factors. It has previously been established that endogenous opioid systems contribute to the secondary pathophysiological events by contributing to the reduction in spinal cord blood flow. Both the opiate antagonist naloxone and the physiologic opiate antagonist TRH have been shown to be effective in a model of traumatic spinal cord injury in the cat. The purposes of the present studies were to: (a) compare naloxone and TRH against one another and to other experimental therapies;

(b) to establish the dose-response characteristics and the effects of time of treatment of TRH in experimental spinal injury; (c) to evaluate the actions of more selective, potent and long-acting TRH analogues and opiate receptor antagonists; (d) to determine which endogenous opioids and which opiate receptor systems might play a role in the pathophysiologic response in spinal cord injury; and (e) to establish a less costly yet predictive spinal injury model in the rat.

BACKGROUND

Traumatic injuries to the spinal cord may cause neurologic impairment in two ways - by directly interrupting neuronal pathways and/or by initiating a series of pathophysiologic changes which lead to progressive ischemic damage to the spinal cord (1-3). There is experimental evidence that these ischemic changes are potentially reversible and result, at least in part, from a reduction of spinal cord blood flow (SCBF) (4-6). We have postulated that spinal trauma activates endorphin systems which contribute to post-traumatic reduction of SCBF and that treatment with opiate antagonists, by reversing the effects of endorphins, improves SCBF and neurological outcome. We have tested this hypothesis, using a cat spinal injury model (7,8). Experimental spinal cord injury did cause an elevation of plasma β -endorphin immunoreactivity (β -endorphin-ir) associated with a reduction in SCBF; naloxone treatment significantly improved SCBF as compared with saline controls (8). More critically, naloxone-treated animals showed significantly better neurological recovery over a three-week follow-up period: the average naloxone-treated animals walked well with minimal spasticity, whereas the average saline control

animals were unable to walk without support. In addition, there was a lower mortality rate in naloxone-treated animals than in saline controls. We later replicated and extended these findings in a more severe spinal injury model; again naloxone-treated animals showed significantly greater functional neurological recovery than saline controls as late as six weeks following the spinal injury (9). In the latter study naloxone administration was shown to improve neurological recovery even if treatment was not instituted until four hours following spinal injury.

Subsequently, we demonstrated that thyrotropin-releasing hormone (TRH), which appears to act in vivo as a partial "physiological opiate antagonist," also markedly improves functional neurological recovery after spinal injury (10). Moreover, we have shown that TRH is superior to naloxone in improving SCBF after traumatic injury in the rat (11). A potential advantage of TRH over naloxone is that TRH does not affect pain sensitivity, whereas naloxone, by blocking the analgesic effect of endogenous opioids, could potentially exacerbate post-traumatic pain states.

The purposes of the present studies, therefore, were: (1) to directly compare naloxone and TRH against one another and against treatment with high-dose corticosteroids, which is considered the treatment of choice for human spinal cord injury in many spinal centers; (2) to determine the effects of treatment dose and time of treatment on the therapeutic actions of TRH; (3) to determine whether megadose corticosteroids, currently proposed by investigators as superior to even high-dose corticosteroid treatment, compare to treatment with either naloxone or TRH; and (4) to determine whether more

selective, potent and long-acting TRH analogues and opiate antagonists may have a potential therapeutic role in CNS injury. In addition, because of the very high cost of the cat trauma model, which relies on pathogen-free animals, we have attempted to establish a similar traumatic spinal cord model in the rat. Finally, the aims of the present studies were to further address the issue of which endogenous opioids and which opiate receptor systems might play a role in the pathophysiological process which follow traumatic injury.

MATERIALS AND METHODS

Details regarding the materials and methods utilized in the present studies are provided in a number of accompanying manuscripts, either published, in press or in review (12-19). Briefly, three series of studies have been performed. The cat trauma model utilizes pathogen-free cats which are anesthetized with pentobarbital and paralyzed (12). A 20 g weight is dropped a distance of 30 cm through a guide tube onto the exposed spinal cord. At a fixed time following injury (from 1 - 24 h), treatment is begun as a bolus, i.v. injection followed by i.v. infusion over 4 h. Following removal of catheters, animals are returned to home cages where they are evaluated in blinded fashion by two investigators; neurological scores are rated on a 10-point ordinal scale, as previously described, based on motor function (12). Subsequently, animals are killed and the spinal cords processed and examined for histopathological changes. The rat trauma studies are very similar, except that animals are anesthetized with a combination of pentobarbital and ketamine, and the site of injury is at T-10 as compared with C-7 in the cat (19). Injury parameters in the rat vary from 25 g-cm to 100 g-cm, the latter

producing complete paraplegia in animals as long as one month following injury. Intrathecal infusion studies in the rat utilized a slight variation of the method by Yaksh and Rudy (20). This method is detailed separately (14) and permits intrathecal infusions through PE 10 tubing in the awake, freely moving rat. Equal volume infusions are administered utilizing highly selective opioids or opiate agonists.

Pharmacological studies in the cat utilized naloxone at a dose of 2 mg/kg bolus, followed by 2 mg/kg/h (12). TRH doses ranged from 0.02 mg/kg to 2 mg/kg, each given as i.v. bolus, followed by 4 h i.v. infusion (12). Dexamethasone was given in a similar manner at doses from 0.5 mg/kg up to 14 mg/kg; in contrast and in keeping with other studies, methylprednisolone was given intravenously (first dose), followed by t.i.d. declining doses over a 10 day post-operative period (15). TRH analogues were given in variable doses between 0.2 mg/kg and 5 mg/kg, administered as intermittent bolus, i.v. injections (21). The WIN compound was similarly given as an intermittent bolus injection (dose = 2 mg/kg) (17).

Immunoreactive dynorphin A-(1-17) (Dyn A-(1-17)), Dyn A-(1-8), leucine-(leu) and methionine-(met) enkephalin have been assayed utilizing highly specific sera as outlined in the attached papers (18,19). Immunoreactive Dyn A was determined through use of the "Lucia" antiserum which recognizes the larger precursors of Dyn A, as well as Dyn A-(1-13) and Dyn A-(1-12), but not shorter fragments: α -neo-endorphin, enkephalins, or β -endorphin. The "R-2" antiserum was utilized to determine Dyn A-(1-8) immunoreactivity (Dyn A-(1-8)-1r); this antiserum has minimal cross-reactivity for Dyn A, Dyn

A-(1-13), Dyn A-(1-9), Dyn A-(1-7), Dyn A-(1-6), α -neo-endorphin, β -neo-endorphin or leu-enkephalin. Leu-enkephalin-ir was determined utilizing the "Llugh" antiserum; this antiserum shows negligible cross-reactivity with met-enkephalin, Dyn A, Dyn A-(1-13), Dyn A-(1-18), α -neo-endorphin, β -neo-endorphin, β -endorphin and to extended met-enkephalin. Met-enkephalin was determined utilizing an antiserum developed by Gregory Mueller, which has negligible cross-reactivity with leu-enkephalin, dynorphins, or β -endorphin.

RESULTS

Cat Studies. In the cat spinal injury model, both naloxone and TRH therapies proved significantly superior to either high-dose corticosteroids or to saline controls (Fig. 1) (12). At six weeks post-injury the median naloxone animal showed normal function in forelimbs and only mild spasticity in hindlimbs; the median TRH animal appeared to be entirely normal, whereas animals treated with corticosteroids or saline were indistinguishable from one another and demonstrated severe spastic quadriplegia. In this model, megadose corticosteroids utilizing up to 30 mg/kg of methylprednisolone, and up to 14 mg/kg of dexamethasone, proved ineffective and, indeed, somewhat increased mortality rates (Fig. 2) (15). In independent studies, TRH proved statistically superior to saline controls in a dose-related manner (Fig. 3) (16). Although optimal effects were observed with the highest dose utilized (2 mg/kg), significant beneficial effects on motor recovery were observed with even relatively low doses (0.02 mg/kg). Remarkably, TRH, at high doses, proved effective even when administered as late as 24 h after injury (Fig. 3). Combination treatment with naloxone and TRH did not prove to be superior to

treatment with TRH alone (data not shown). The TRH analogue CG3509, which has an orotyl moiety substituted in place of the first amino acid (pyroglutamyl) of TRH (Fig. 4), proved approximately equieffective with TRH; this analogue proved significantly better than physiological saline (Table 1) (21). In contrast, the analogue MK-771, which has substitutions at both ends of the TRH molecule (Fig. 4), was ineffective in promoting neurological recovery after traumatic injury (Table 1). Finally, treatment with the κ -selective opiate antagonist WIN44,441-3 (Fig. 5) also proved to have significant therapeutic benefit in promoting neurological recovery after injury (Table 2), and had therapeutic effects which were comparable to those observed with TRH or naloxone.

Rat Studies. During this contract we have successfully established a new traumatic spinal cord injury in the rat, based on the same Allen method technology as used in the cat. Injury parameters of 25 g-cm, 50 g-cm, 75 g-cm and 100 g-cm applied to the T-10 region in laminectomized rats produced dose-related paraparesis at four weeks post-injury (see Table 3).

We utilized this traumatic injury model in the rat to evaluate changes in endogenous opioids after injury. Traumatic injury was associated with a time-dependent and highly significant increase in dynorphin A-like immunoreactivity (Fig. 6). These changes were well localized to the injury site (Fig. 7) and were highly correlated with neurological dysfunction after injury (Fig. 8). In contrast, there was a decline in immunoreactive enkephalins at the injury site following trauma (Fig. 6); such changes were found after moderate but not severe injury and were not well localized to the injury site (Fig. 9).

Finally, intrathecal infusion studies demonstrated that the dynorphin family of endogenous opioids, unique amongst opioids, produced dose-related and partially reversible hindlimb paralysis in the rat (Fig. 10). In contrast, a variety of synthetic enkephalins, active at the μ - and δ -opiate receptors, as well as β -endorphin, which is active at the ϵ -receptor, failed to produce any change in motor function, even at doses significantly higher than those used for dynorphin. The most potent effects in the dynorphin family were observed with the native ligand Dyn A-(1-17).

CONCLUSIONS

The present studies demonstrated that both TRH and naloxone significantly improve long-term motor recovery following traumatic spinal cord injury in the cat. Both therapies were superior to either high-dose or megadose corticosteroids, which have long been considered the treatment of choice in human spinal cord injury. In studies directly comparing the effects of naloxone and TRH, the latter proved significantly better than naloxone, with the average animal showing essentially complete recovery at six weeks following traumatic injury. The beneficial effects of TRH on motor recovery were clearly dose-related, with beneficial effects observed with doses as low as 0.02 mg/kg. Of great importance was the somewhat unexpected observation that TRH treatment, at high doses, was effective in promoting neurological recovery, even when treatment was delayed as long as 24 h following injury. Taken together, the present animal studies provide the basis for a therapeutic trial for TRH in human spinal cord injury.

The TRH analogue CG3509 proved to have significant beneficial effects in the same spinal cord injury model, in contrast to treatment with either physiological saline or another TRH analogue, MK-771. Since CG3509 is more potent than TRH, has fewer endocrine effects, and has a far longer biological half-life, this finding indicates that TRH analogues may have significant potential therapeutic actions, superior even to those of TRH. Parallel studies demonstrated that the κ -selective opiate antagonist WIN44,441-3 also proved significantly effective in spinal cord injury. This compound has similar advantages to those of CG3509, with a high degree of potency, substantial selectivity for only one class of opiate receptor, and a long biological half-life. Since this compound has also undergone phase studies, it may ultimately prove superior to naloxone in the treatment of CNS injury.

Experimental studies in the rat provided evidence to suggest that the endogenous opioid dynorphin, which appears to be the endogenous ligand for the κ -opiate receptor, may be the pathophysiological endorphin in spinal cord injury. Dynorphin-related peptides, unique amongst opioid peptides or synthetic enkephalins, caused dose-related hindlimb paralysis in the rat. This finding suggests that therapies which are directed at dynorphin or at the κ -opiate receptor may have even a higher degree of selectivity as therapeutic agents in traumatic spinal cord injury.

Finally, consistent with the hypothesis that dynorphin and the κ receptor may be involved in mediating secondary pathophysiological effects of CNS injury, we found that dynorphin A-like immunoreactivity was significantly

increased at the injury site following trauma in a time-dependent manner. Changes in dynorphin were highly correlated with the ultimate neurological dysfunction in contrast to changes in met- and leu-enkephalin. Taken together, these findings strongly indicate a potential pathophysiological role for dynorphin and/or the κ receptor in spinal cord injury.

RECOMMENDATIONS

The present studies, combined with previous studies from our laboratory, provide the experimental basis for clinical trials of TRH and TRH analogues in human spinal cord injury. Phase I studies have already begun at the University of California, San Diego. If TRH proves safe at doses which are considered necessary in the treatment of spinal cord injury, multi-institutional Phase II and Phase III studies will be initiated.

Similarly, κ -selective opiate antagonists have significant beneficial activity in experimental spinal cord injury. WIN44,441-3 has already undergone phase testing by Sterling-Winthrop laboratories and may provide a superior alternative to naloxone, a drug currently being evaluated in controlled trials for human spinal cord injury.

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TABLE 1

COMPARISON OF NEUROLOGICAL SCORES AFTER TREATMENT IN
SPINAL CORD TRAUMATIZED CATS⁺

	Saline	MK771	CG3509	TRH 2.0 mg/kg	TRH 0.2 mg/kg
	(n=17)	(n=7)	(n=9)	(n=6)	(n=6)
Weeks After Injury					
1	3	4	6*	5	5
2	3	5	6*	6*	6*
3	5	5	6*	7*	7*
4	5	5	7*	8.5*	6
5	5	6	8*	9*	8.5*
6	6	6	8*	9*	8.5*

⁺Scores represent median values for the treatment groups.

*Indicates statistical significance as $p < 0.05$, Kruskal-Wallis ANOVA,
Mann-Whitney U tests.

TABLE 2

COMPARISON OF WIN(-) AND WIN(+) TREATMENTS AFTER
ISCHEMIC LUMBAR INJURY IN RABBITS

Neurological	WIN(-)	WIN(+)	Saline
Score	0.4 mg/kg	0.4 mg/kg	
3	XX	-	-
2	XXXXX	X	X
1	XXXX	XXXXXXXX XXX	XXXXXXXX XXXX
0	XXXX	XXXX	XXX

Hindlimb scores at 48 hours after injury, based on a 4-point ordinal scale
(see Methods).

TABLE 3

RAT MOTOR FUNCTION AT ONE WEEK FOLLOWING TRAUMATIC INJURY

Neurological Score	Impact Energy (g-cm)				
	10	25	50	75	100
0	---	---	---	xxx	xx
1	xxx	xx	xxxxxxxx	xxxxxxxxxx	xx
2	xx	x	x	---	---
3	xxxx	---	x	---	---

Neurological scores are as follows: 0 = no movement; 1 = spontaneous movement, unable to walk; 2 = able to walk with marked spasticity or ataxia; and 3 = normal walking. x = Individual animals scores.

FIGURES

Figure 1. Effect of TRH (T), naloxone (N), dexamethasone (D) and saline (S) treatments on neurologic recovery after traumatic cervical spinal cord injury in cats. Both TRH- and naloxone-treated animals showed significantly higher neurologic scores than saline controls over the six week follow-up period. Moreover, scores in TRH animals were significantly higher than either naloxone or dexamethasone animals at six weeks post-injury. Points represent the sum of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores.

Figure 2. Effects of methylprednisolone (n=6) or saline (n=8) treatment on neurologic recovery six weeks after cervical spinal cord injury. Points represent the sum of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores. No significant differences were observed between the groups.

Figure 3. Effects of thyrotropin-releasing hormone or saline treatment on neurologic recovery six weeks after cervical spinal cord injury in cats. TRH treatment significantly improved motor recovery in a dose-related manner. TRH also significantly reduced late paralysis, even when treatment was not administered until 24 h

after injury. Points represent the sum of forelimb and hindlimb neurologic scores for individual animals; histograms represent median scores.

Figure 4. Chemical structures of TRH and the two TRH analogues (CG3509 and MK-771) used in cat spinal injury studies. CG3509 has an orotyl (6-ring) substitution for the pyroglutamyl (5-ring) portion of TRH, resulting in a compound which resists enzymatic degradation and is more potent than TRH. MK-771 has alterations at both ends of TRH, resulting in a compound with greater behavioral activity and a longer biological half-life than TRH.

Figure 5. Chemical structures of naloxone, the opiate agonist (oxymorphone) from which it is derived, and the class of related metazocinealkanones (from which WIN44,441-3 is derived).

Figure 6. Percent change of peptide immunoreactivity in the rat thoracic spinal cord (injury level) at various times after traumatic injury. Medium injury produced a significant time-dependent decrease in both leu- and met-enkephalin-ir (regression ANOVA; $F = 6.46$, $p < 0.05$, and $F = 12.24$, $p < 0.01$, respectively). High injury produced a significant time-dependent increase in Dyn A-ir (regression ANOVA; $F = 16.19$, $p < 0.01$). Neither degree of injury produced significant alterations in Dyn A-(1-8)-ir.

Figure 7. Levels of Dyn A-ir and Dyn A-(1-8)-ir at different degrees of injury. Open bars represent laminectomy control animals, shaded bars represent medium degree of injury, and hatched bars represent high degree of injury. Graded injury was associated with a progressive and significant increase in Dyn A-ir, which was localized to the injury site and adjacent lumbar region. Asterisks indicate statistical significance at $p < 0.05$, regression ANOVA.

Figure 8. Comparison of peptide level and degree of motor dysfunction at 24 h and 2 weeks after injury. Significant increases in Dyn A-ir were associated with poor motor function (non-walkers, neurologic score = 0 or 1) at both time periods. A significant decrease in leu-enkephalin-ir was observed in non-walkers at 24 h but not 2 weeks. Asterisks represent $p < 0.05$ by unpaired t-tests.

Figure 9. Levels of leu- and met-enkephalin-ir at different degrees of spinal injury. Open bars represent laminectomy control animals, shaded bars represent medium degree of injury, and hatched bars represent high degree of injury. There were no progressive changes for either peptide with increasingly severe injury at 24 h or 2 weeks (regression ANOVA, $p < 0.05$).

Figure 10: (A) Dose-response effects of Dyn A-(1-13) on motor function in the rat following intrathecal administration. Dyn A-(1-13) produces dose-related, partially reversible paralysis of hindlimb function, with peak effects at approximately 50 nmol.

(B) Comparison of effects of Dyn A-(1-17), Dyn A-(1-13), Dyn A-(1-8) and α NE motor function in the rat. Each peptide produces hindlimb dysfunction, with the order of potency being Dyn A-(1-17) > Dyn A-(1-13) >> α NE $\simeq$ Dyn A-(1-8).

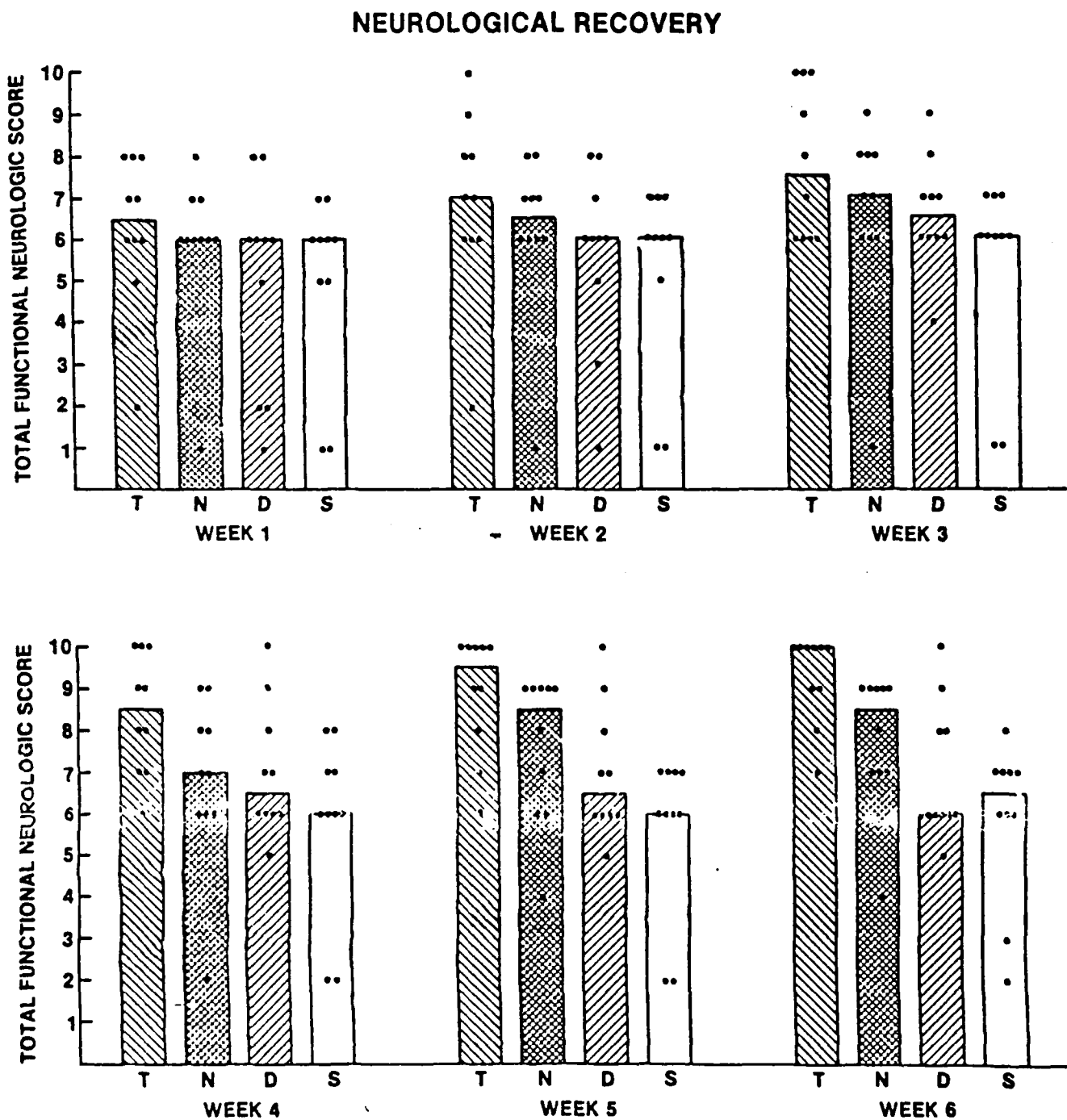


FIGURE 1

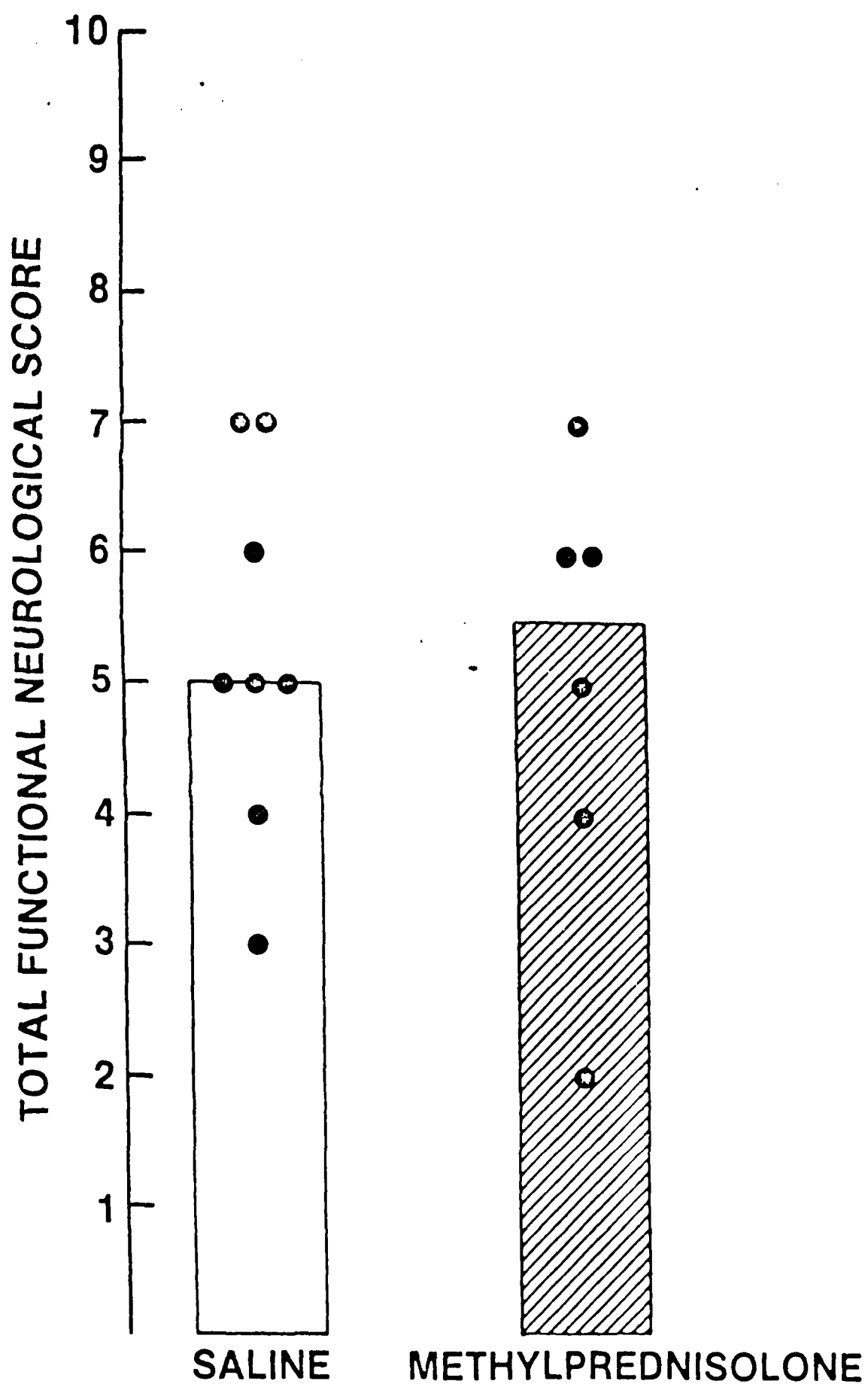


FIGURE 2

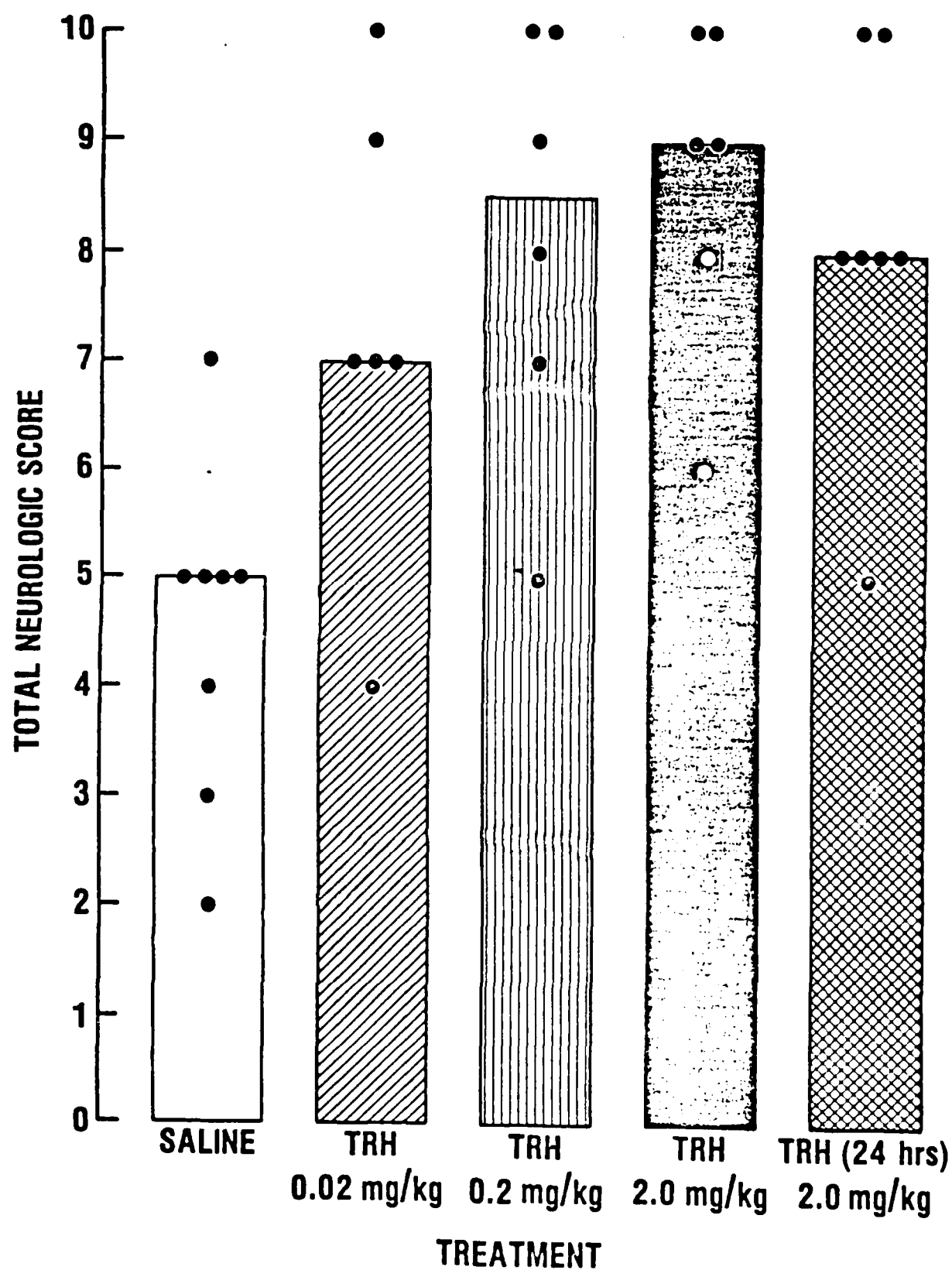
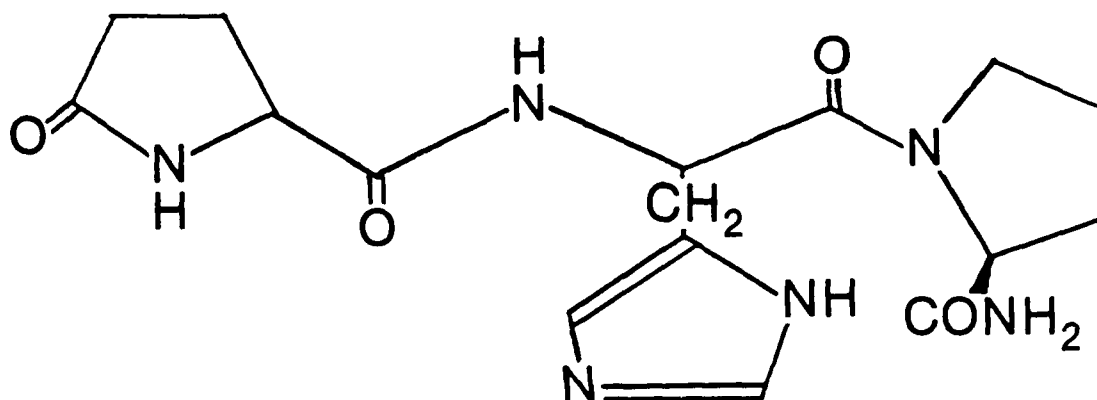
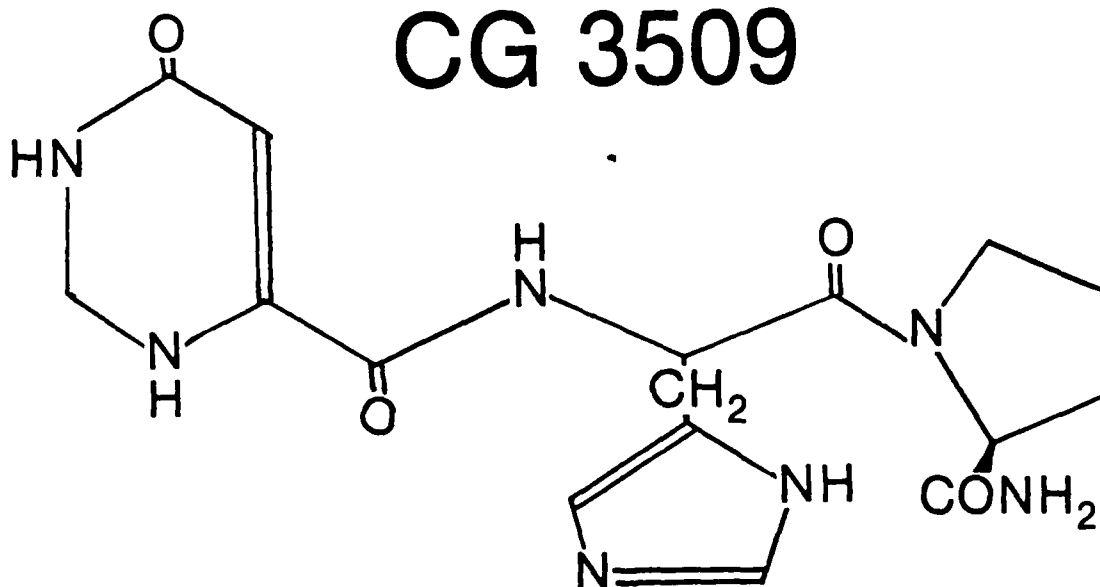


FIGURE 3

TRH



CG 3509



MK-771

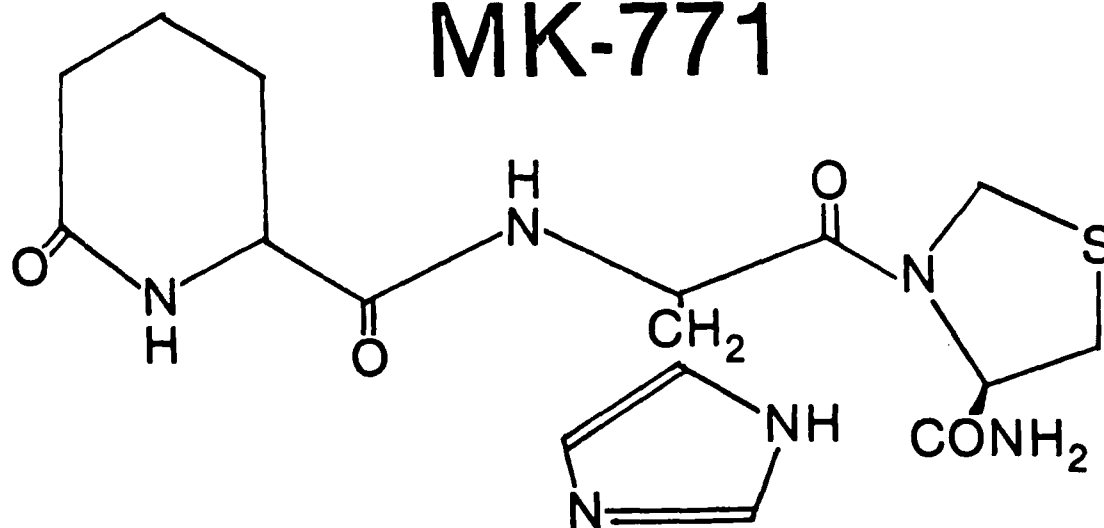
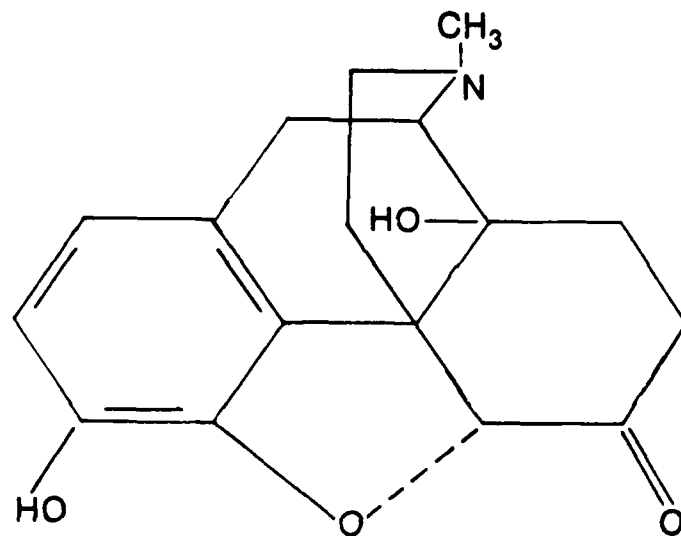
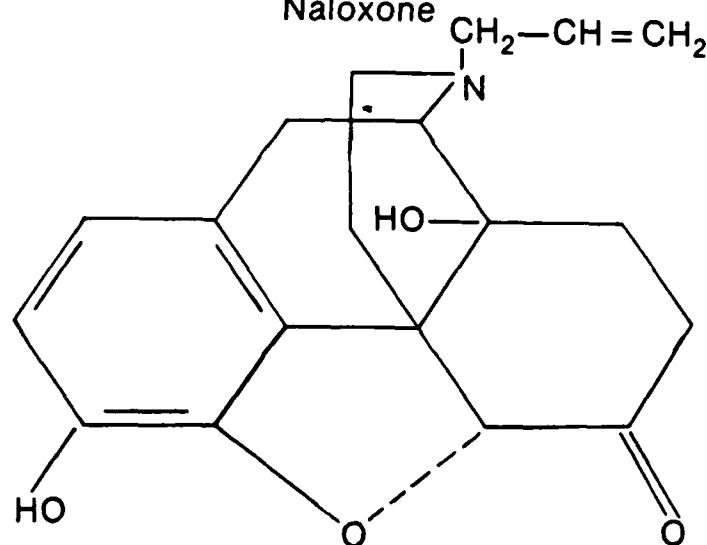


FIGURE 4
20

Oxymorphone



Naloxone



Metazocinealkanones

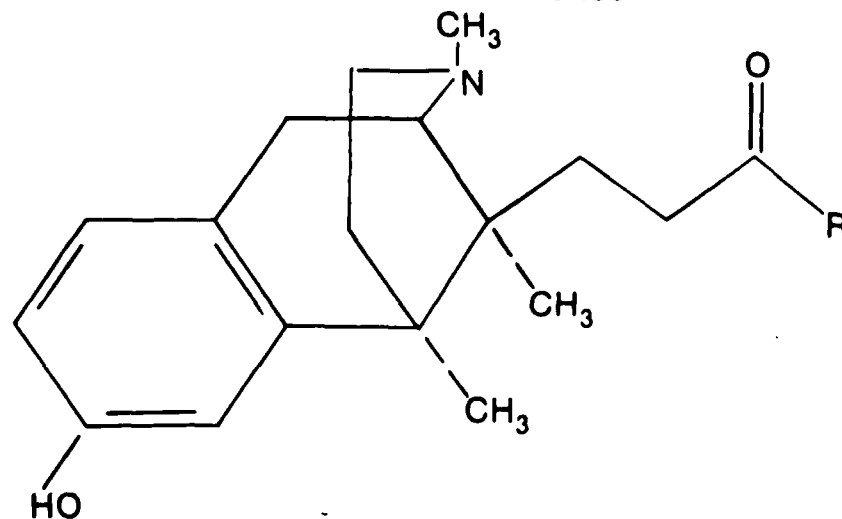


FIGURE 5
- 31 -

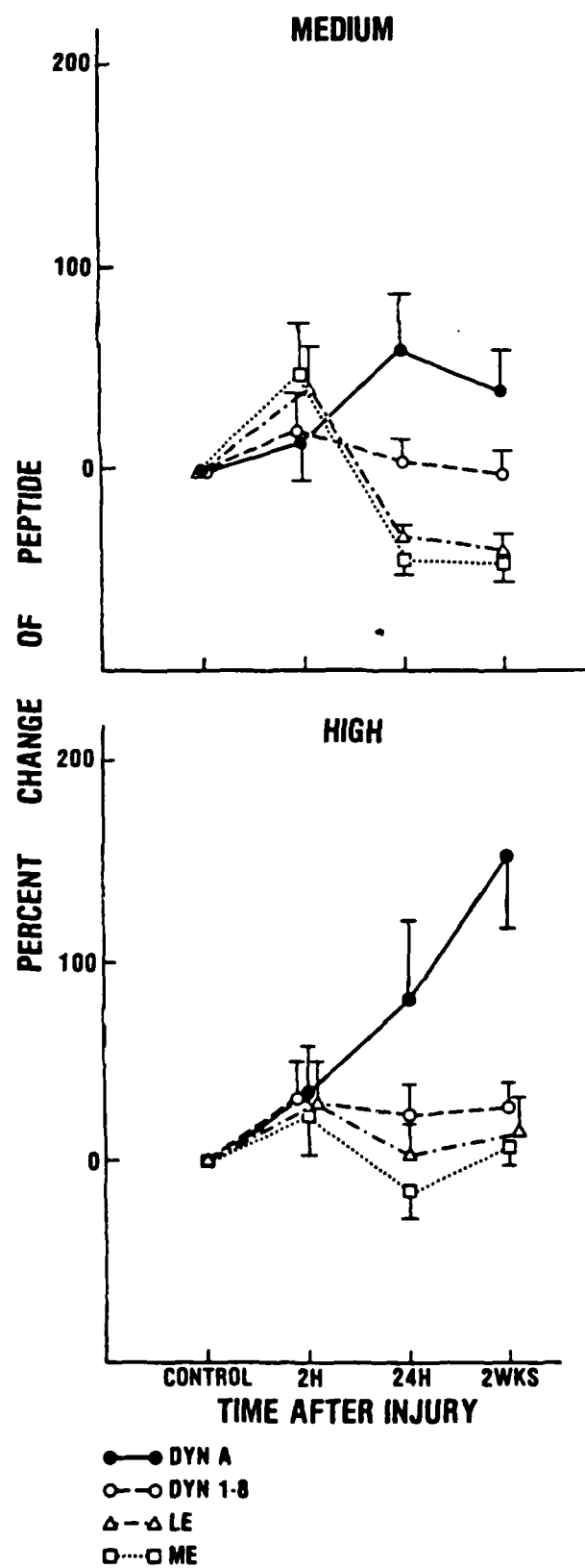


FIGURE 6

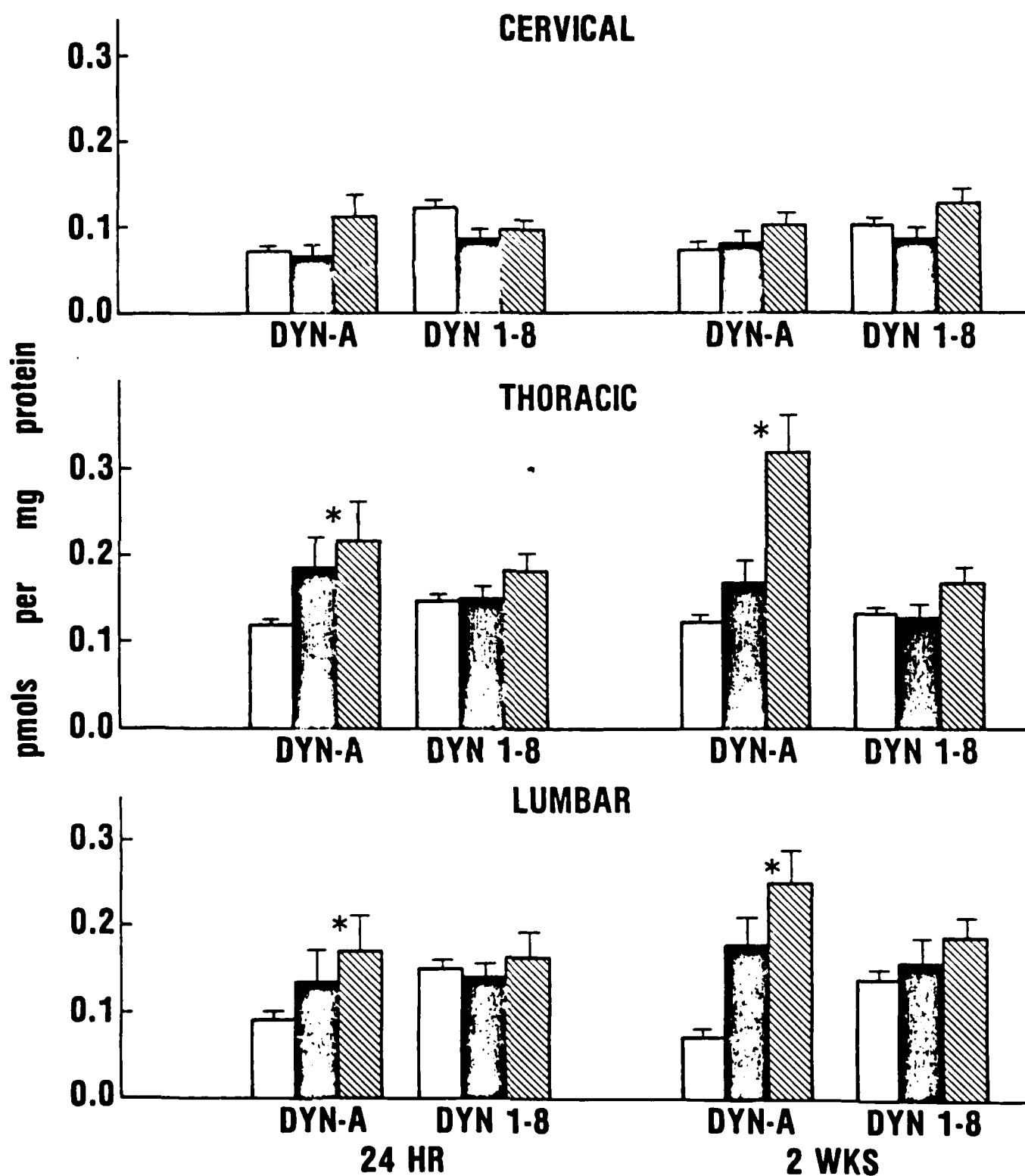
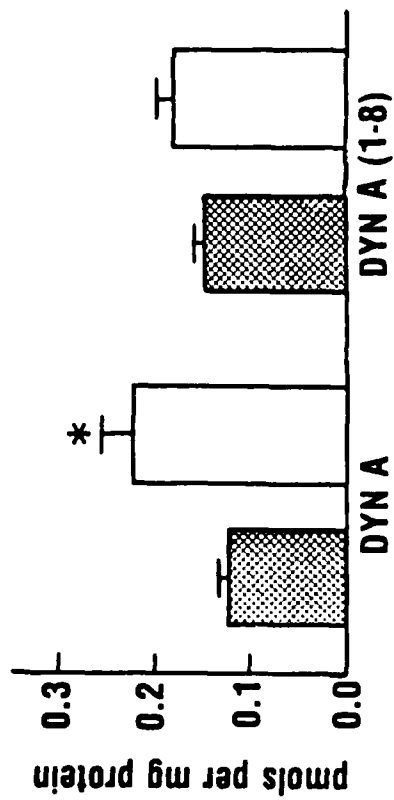


FIGURE 7

A. 24 HOURS



B. 2 WEEKS

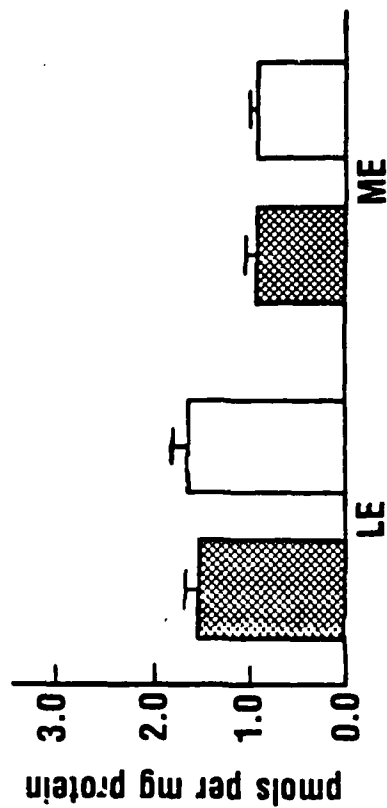
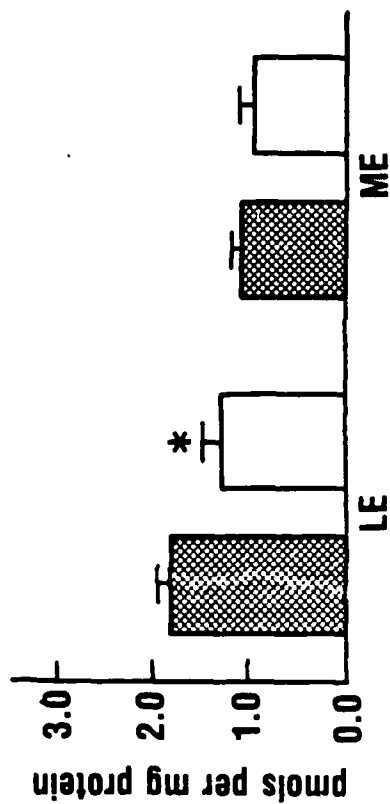
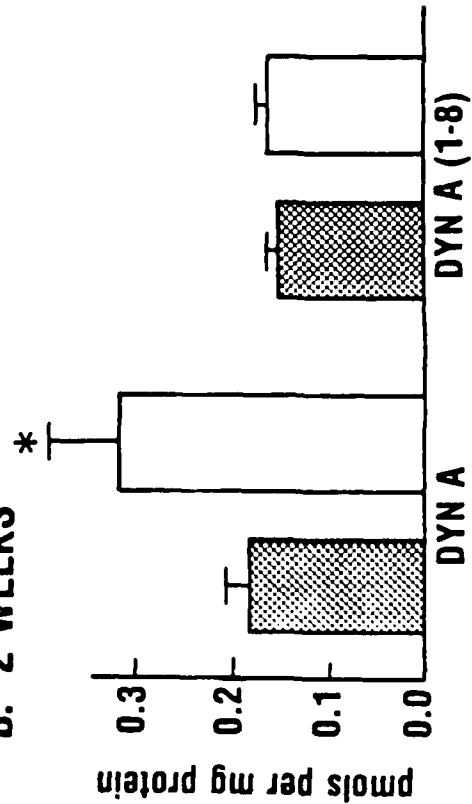


FIGURE 8

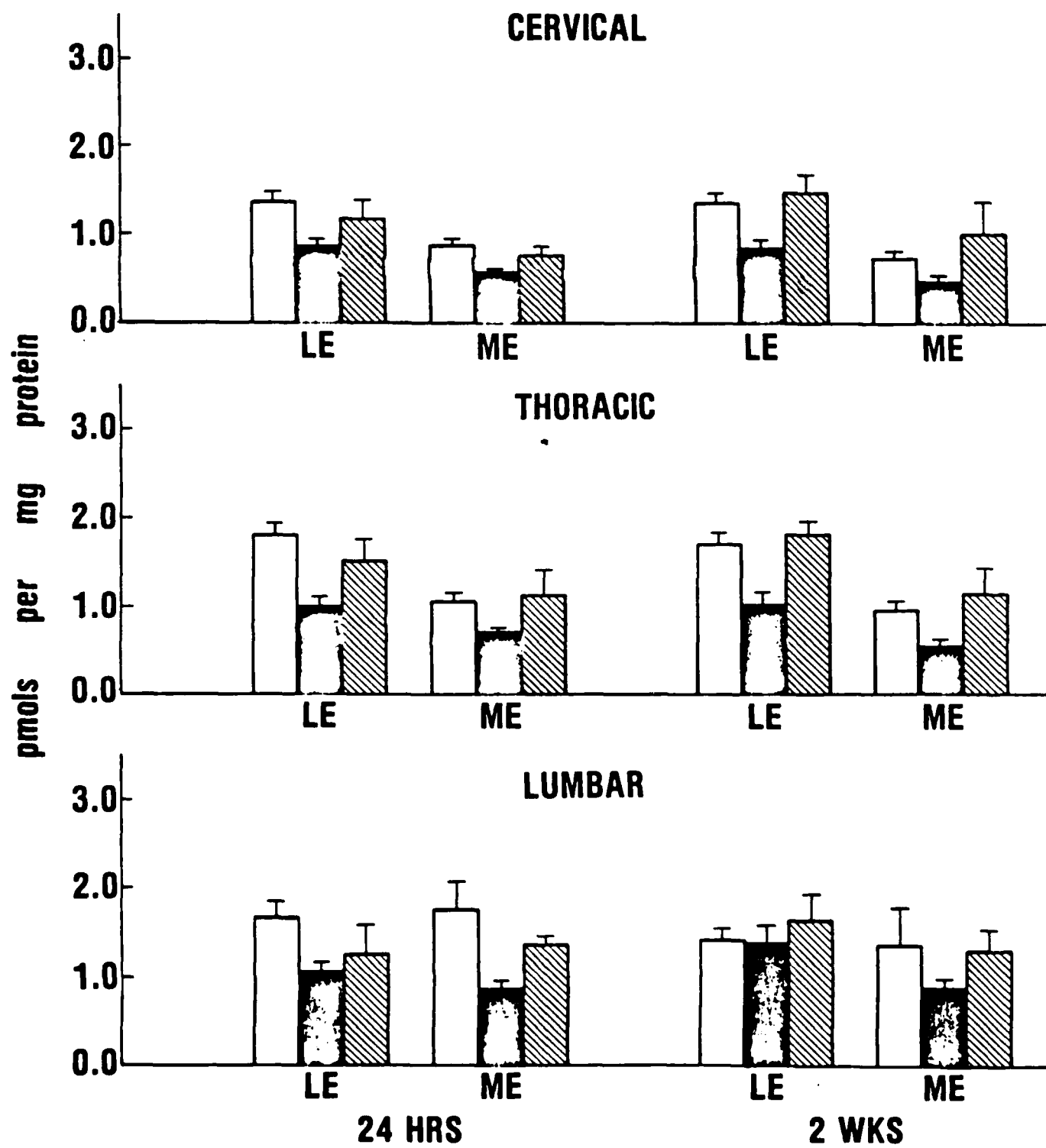
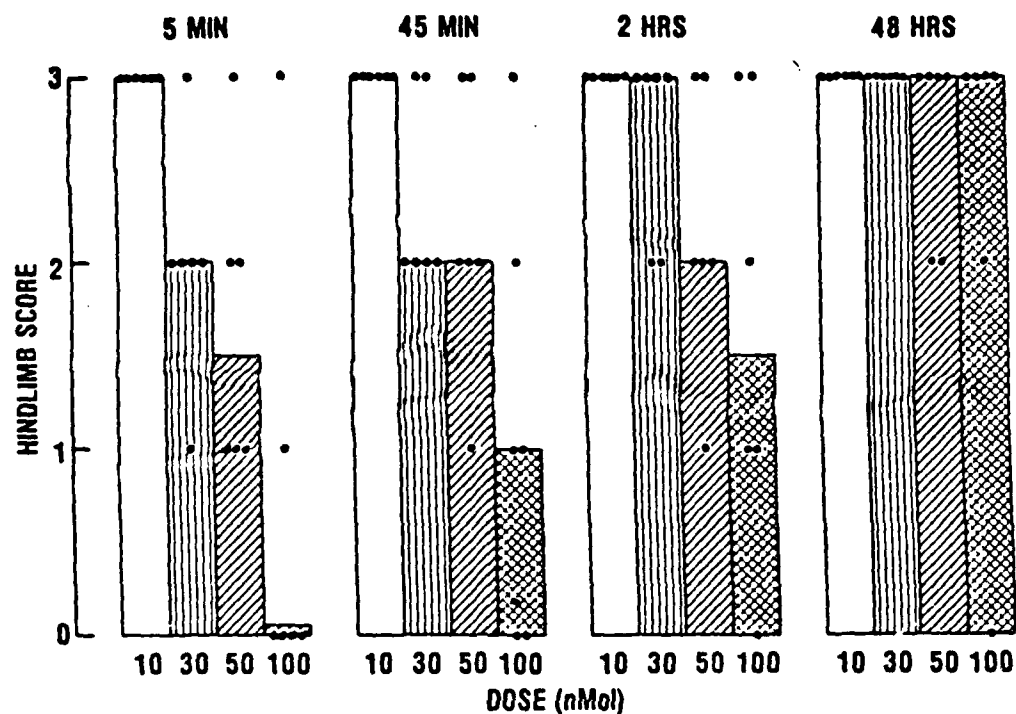


FIGURE 9

A. TIME AFTER INJECTION



B. 15 MINUTES AFTER INJECTION

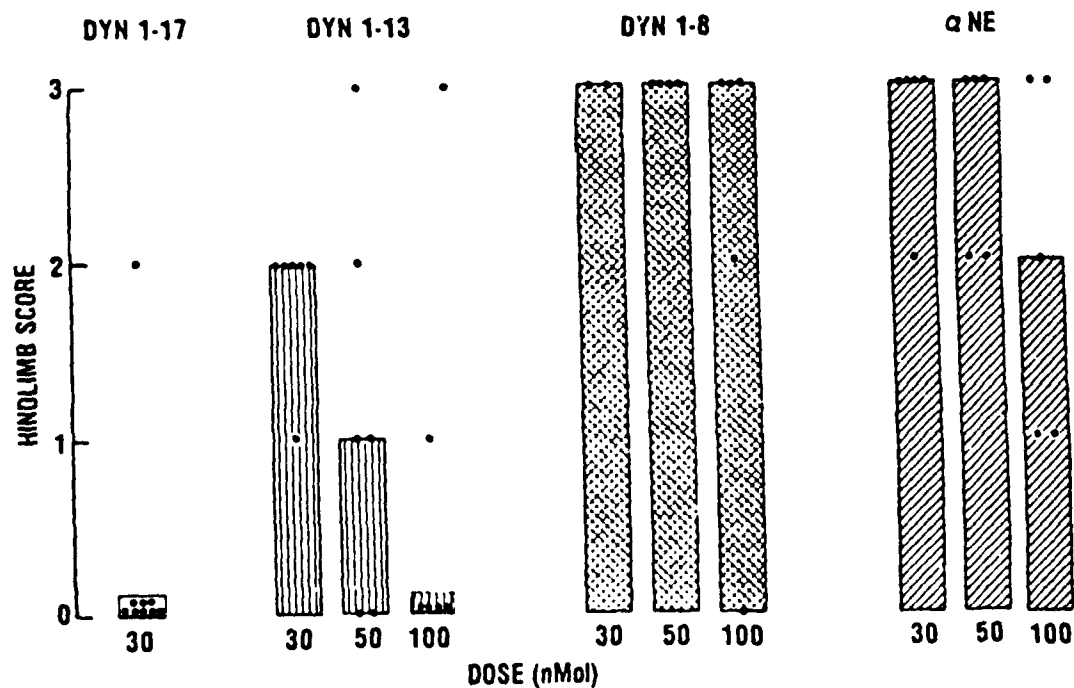


FIGURE 10

PERSONNEL RECEIVING CONTRACT SUPPORT

1. Susan Knoblach
2. George Smith

BIBLIOGRAPHY OF ALL PUBLICATIONS WRITTEN SUPPORTED BY CONTRACT

1. Faden AI, Jacobs TP: Dynorphin induces partially reversible paraplegia in the rat. Eur J Pharmacol 91:321-324, 1983.
2. Faden AI, Jacobs TP: Dynorphin-related peptides cause motor dysfunction in the rat through a non-opiate action. Br J Pharmacol 81:271-276, 1984.
3. Faden AI, Jacobs TP, Patrick DH, Smith MT: Megadose corticosteroid therapy following experimental traumatic spinal injury. J Neurosurg 60:712-717, 1984.
4. Faden AI, Jacobs TP, Smith MT: Thyrotropin-releasing hormone in experimental spinal injury: Dose response and late treatment. Neurology. (In Press)
5. Faden AI, Jacobs TP, Smith MT, Holaday JW: Comparison of thyrotropin-releasing hormone (TRH), naloxone, and dexamethasone treatments in experimental spinal injury. Neurology 33:673-678, 1983.
6. Faden AI, Knoblach S, Mays C, Jacobs TP: Motor dysfunction after spinal cord injury is mediated by opiate receptors. Peptides. (In Press)
7. Faden AI, Molineaux CJ, Rosenberger JG, Jacobs TP, Cox BM: Endogenous opioid immunoreactivity in rat spinal cord following traumatic injury. Ann Neurol. (Submitted)
8. Faden AI, Molineaux CJ, Rosenberger JG, Jacobs TP, Cox BM: Increased dynorphin immunoreactivity in spinal cord after traumatic injury. Nature. (Submitted)

NARRATIVE SUMMARY

Traumatic injuries to the central nervous system (CNS: including spinal cord and brain) cause neurologic impairment not only by directly interrupting neuronal pathways but by initiating a series of pathophysiologic changes which lead to progressive ischemic damage. We have provided evidence that the secondary ischemic changes resulting from experimental spinal trauma are potentially reversible and result, in part, from a reduction of spinal cord blood flow related to the release of endogenous opioids. Previously, we have shown that the opiate receptor antagonist naloxone improves both spinal cord blood flow and neurological outcome following experimental traumatic spinal cord injury in the cat. Subsequently, we found that thyrotropin-releasing hormone (TRH), which acts in part as a physiologic antagonist of endogenous opioid systems, also significantly improves blood flow and neurological recovery after experimental spinal injury. During this contract we have compared the effects of TRH and naloxone against corticosteroids and saline-treated controls. Both naloxone and TRH provided significantly superior to either saline or high-dose corticosteroids in improving long-term, functional neurological recovery in the cat. Moreover, TRH proved significantly better than naloxone in this regard. In separate studies we found that corticosteroids (including either dexamethasone or methylprednisolone), even in the megadose range, failed to improve neurological recovery in this traumatic cat model. Subsequently, we completed independent studies showing that the therapeutic effects of TRH were clearly dose-related, with beneficial actions observed at doses as low as 0.02 mg/kg. Of particular importance, TRH treatment significantly improved neurological recovery even when the drug was not administered until fully 24 h after traumatic injury.

We have also evaluated the effects of longer acting and more potent TRH and opiate receptor analogues. The κ -selective opiate receptor antagonist WIN44,441-3 produced significant improvement in long-term neurological recovery following traumatic spinal cord injury in the cat. Similarly, the potent TRH analogue CG3509 proved significantly effective in the same injury model. This contrasts with the effects of another TRH analogue, MK-771, which proved ineffective. Taken together, these findings indicate that opiate receptor-selective antagonists and certain classes of TRH analogues may be beneficial in the treatment of traumatic CNS injury; by virtue of greater selectivity and far longer biological half-lives than either TRH or naloxone, these compounds may prove to be superior therapeutic agents in the treatment of human injuries to the CNS.

During the second half of the contract period, we developed a new model of spinal cord injury, a traumatic model in the thoracic region of the rat, in order to evaluate changes in endogenous opioids following spinal cord injury. Graded spinal cord injury was associated with progressive increases in dynorphin-like immunoreactivity but not enkephalin immunoreactivity at the injury region. The changes in dynorphin levels correlated in a highly significant way with the progressive neurological function after injury. Finally, we have shown that the dynorphin family of opioids, which have high selectivity for the κ -opiate receptor, are unique amongst opioids in producing dose-related hindlimb paralysis in the rat. Taken together, the findings in the rat studies strongly suggest that the pathophysiological effects of endogenous

opioids after spinal cord injury are mediated by the dynorphin family of opioids and the κ -opiate receptor. Pharmacological studies in the cat also support this hypothesis, with the demonstration that κ -selective opiate antagonists are effective in improving neurological recovery after injury.

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