

Opiate Antagonist Nalmefene Improves Intracellular Free Mg^{2+} , Bioenergetic State, and Neurologic Outcome Following Traumatic Brain Injury in Rats

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Treatment of CNS trauma with the opiate antagonist naloxone improves outcome, though the mechanisms of action remain speculative. Nalmefene is another opiate-receptor antagonist, but it has substantially greater potency and duration of action than naloxone. It also has increased activity at κ opiate receptors and has recently been shown to limit histological changes and neurological dysfunction after traumatic spinal cord injury. The present study examined the effects of treatment with nalmefene on outcome after fluid-percussion-induced traumatic brain injury in rats, using magnetic resonance spectroscopy to monitor acute metabolic changes and behavioral tests to determine chronic neurological recovery. Single-dose treatment with nalmefene (100 μ g/kg, i.v.) at 30 min after trauma significantly improved ($p < 0.05$) neurological outcome (up to 4 weeks) as compared to saline-treated controls. Early changes in intracellular free-magnesium concentration, adenosine diphosphate concentration, and cytosolic phosphorylation potential were all significantly improved by nalmefene treatment, reflecting improved bioenergetic state. We suggest that the ability of nalmefene to improve cellular bioenergetics after trauma may in part account for the neuroprotective effects of this and related compounds.

Tissue damage following CNS trauma may reflect both primary and secondary injury. Primary injury encompasses the mechanical damage incurred at the time of trauma, such as neuronal shearing, compression, or axonal stretching (Povlishock, 1985). Secondary events are those that occur after the initial mechanical injury and include various biochemical alterations such as ionic imbalance, membrane changes, mitochondrial dysfunction, and accumulation or activation of "autodestructive" agents (see Cooper, 1985; Siesjo and Wieloch, 1985). These secondary biochemical alterations result in a compromised cellular bioenergetic state (Vink et al., 1988a) that may have deleterious effects on posttraumatic neurological outcome via a number of mechanisms. For example, a compromised energetic state would reduce the capacity for fatty acid reacylation after

membrane breakdown and promote production of oxygen free radicals and eicosanoids, would decrease the rates of protein synthesis and all ATPase reactions, would adversely affect neurotransmitter synthesis, release and uptake, and would disrupt transmembrane ionic equilibria (see Dagani and Erecinska, 1987; Erecinska and Silver, 1989). Because secondary injury occurs over a period of hours to days, it may be possible to prevent or attenuate such damage through use of pharmacological interventions. Among the therapies that have met with some success in experimental trauma are opiate-receptor antagonists (for review, see Faden, 1988).

Opiate antagonists have been examined in CNS trauma based upon the hypothesis that endogenous opioids contribute to the secondary pathophysiological response to CNS injury (Faden et al., 1981b). One such opiate antagonist, naloxone, has been shown to improve neurological outcome (Faden et al., 1981b; Flamm et al., 1982), electrophysiological activity (Young et al., 1981), and blood flow (Faden et al., 1981a; Young et al., 1981) following traumatic spinal cord injury. Similarly, following head injury, naloxone administration has been shown to improve cardiovascular function, respiratory function, cerebral perfusion pressure, and electrophysiological activity (Hayes et al., 1983). The relatively high doses required for these beneficial effects, combined with the stereospecificity of these actions, suggest that naloxone may be acting on non- μ opiate receptors such as the κ -receptor (Faden, 1988). Recent studies of both experimental spinal cord injury and brain injury have implicated the endogenous opioid dynorphin as a pathophysiological factor in secondary posttraumatic injury, with its actions mediated in part through κ -opiate receptors (Faden et al., 1987; McIntosh et al., 1987; Faden, 1990). Despite this, the mechanisms by which these beneficial effects are expressed remain unclear.

A number of recent studies have used magnetic resonance spectroscopy (MRS) to examine secondary injury after brain trauma, particularly the mechanisms of action of various pharmacologic therapies with demonstrated beneficial effects on posttraumatic neurological outcome (McIntosh et al., 1988b; Vink et al., 1988b,c; Faden et al., 1990). This technique allows the continuous and noninvasive monitoring of a number of biochemical parameters, including high-energy phosphates such as ATP and phosphocreatine, as well as intracellular pH and free-magnesium concentration (Gupta et al., 1983; Petroff et al., 1985). It is the ability of the technique to measure intracellular free-magnesium concentration (Gupta et al., 1978) that has established the critical role that this cation plays in the patho-

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physiology of traumatic brain injury (Vink et al., 1988b). Moreover, therapies that have demonstrated beneficial effects on neurologic outcome following brain trauma have characteristic effects on magnesium metabolism and bioenergetic status as assessed by MRS (Vink et al., 1988c; Faden et al., 1990).

Nalmefene is an oxomethylene derivative of naltrexone (Key Pharmaceuticals, 1984), with increased activity at κ -opioid receptors (Michel et al., 1984). It has a much higher potency and longer duration of action than naloxone and has been shown to significantly improve functional neurological recovery after spinal cord trauma (Faden et al., 1988). The present experiments examined the effects of nalmefene treatment on neurological outcome following traumatic brain injury in rats. Furthermore, by using phosphorus MRS (^{31}P MRS), we noninvasively monitored several acute biochemical variables to determine the effects of nalmefene treatment on cell metabolism.

Materials and Methods

Fluid-percussion-injury model. Male Sprague-Dawley rats ($n = 30$, 350 ± 25 gm) were anesthetized with 60 mg/kg sodium pentobarbital and subjected to fluid-percussion head injury as described in detail previously (Vink et al., 1988b; McIntosh et al., 1989a). Briefly, a craniotomy was performed over the left parietal cortex, and a Leur-Loc fitting was implanted over the trauma site. The animal was connected to the fluid-percussion-injury device by interlocking the implanted Leur-Loc fitting to one at the end of the device. By releasing a pendulum weight from a predetermined height, a saline pressure pulse could be generated within the cylindrical saline reservoir of the device and transmitted to the cranial cavity of the rat via the Leur-Loc connection. This pressure pulse results in a brief deformation of brain tissue with resultant injury. The pressure impulse was recorded externally by a transducer and stored on an oscilloscope. Pressure pulses were in the range of 1.9–2.2 atmospheres. Arterial and venous catheters were implanted prior to injury for monitoring of blood pressure and for drug administration. Throughout the postinjury observation period in the MRS studies, animals were maintained on a constant infusion of sodium pentobarbital (15 mg/kg/hr).

Drug administration. Following injury, animals were randomly assigned to receive either 100 $\mu\text{g/kg}$ nalmefene ($n = 15$) or an equal volume of saline ($n = 15$). Drugs were administered as a single intravenous bolus injection 30 min posttrauma. The nalmefene dose was based on previous dose-response studies in spinal cord trauma (Faden et al., 1988).

MRS studies. Within each group of injured animals, 6 were randomly selected for monitoring by ^{31}P MRS. Spectra were obtained prior to and for 4 hr following injury using a GE CSI spectrometer operating at 2 tesla as previously described (Vink et al., 1987b, 1988a). Field homogeneity was optimized on the proton resonance of water. Phosphorus data was collected in 20-min blocks using a 700-msec repetition rate, 2048 data points, a sweep width of 4000, and a 90° pulse width centered at 2 mm cortical depth. A 9×5 -mm 2-turn elliptical surface coil was centered around the trauma site and used to transmit and receive signals. The accumulated free-induction decays were analyzed following Fourier transformation and convolution difference (25 Hz/400 Hz Gaussian multiplication).

Intracellular pH was determined from the chemical shift of inorganic phosphate (δPi) relative to phosphocreatine (PCr) according to the formula (Petroff et al., 1985):

$$\text{pH} = 6.77 + \log(\delta\text{Pi} - 3.29)/(5.68 - \delta\text{Pi}).$$

Intracellular free-magnesium concentration (Mg_i) was determined according to the method described in detail by Gupta and colleagues (Gupta et al., 1978). A binding constant for magnesium to ATP of 50 μM was used in all calculations (Gupta et al., 1983; Garfinkel and Garfinkel, 1984). This method has been used for free-magnesium determinations in a number of tissues and organs under a variety of conditions (Gupta et al., 1984; Kushmerick et al., 1986); including CNS trauma (Vink et al., 1987a, 1988a,b,c) and has been extensively reviewed (Gupta et al., 1983, 1984). ADP was calculated from the creatine kinase equation after adjusting the creatine kinase equilibrium constant for pH and Mg_i as previously described (Lawson and Veech, 1979; Vink et al., 1988a). Cytosolic phosphorylation potential (ΔG_p) was determined ac-

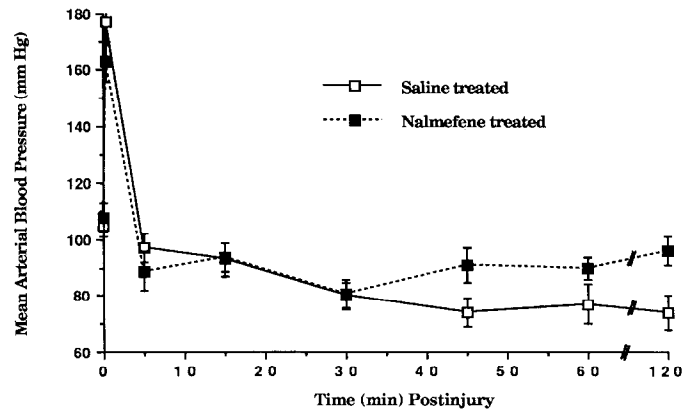


Figure 1. Mean arterial blood pressure prior to and following fluid-percussion brain injury in rats. At 30 min postinjury, animals were administered with nalmefene (100 $\mu\text{g/kg}$, i.v.) or an equal volume of saline.

cording to the equation

$$\Delta G_p = [\text{ATP}]/[\text{ADP}][\text{Pi}],$$

where [ATP] and [Pi] were measured from the ^{31}P MRS spectra (βATP integral and PCr:Pi ratio) assuming initial preinjury concentrations of [ATP] and [PCr] of 2.59 and 4.72 $\mu\text{mol/gm}$ wet weight, respectively (Lawson and Veech, 1979). A brain water content of 80% was assumed based on previous head-injury studies in our laboratory (Vink et al., 1987a). All calculations were performed taking into account the rules governing propagation of error.

Neurological scoring. Posttraumatic neurological deficit was determined in all non-MRS animals (9 per group) at 24 hr and weekly up to 4 weeks postinjury. Animals were blindly scored using a battery of tests developed to characterize specific neurological consequences of traumatic brain injury (McIntosh et al., 1989a). The neurologic damage induced by lateral brain injury is evaluated using a series of motor tests designed to detect differences in motor function controlled by the injured and noninjured hemispheres. Any unilateral deficit can be readily determined, particularly in those tests that incorporate resistance to lateral forces. The motor tests used in the current experiments included (1) ability to maintain horizontal position on an inclined angle board for 5 sec (2) latency to traverse a 2-cm-wide wooden beam, (3) contralateral forelimb flexion upon suspension by the tail, (4) resistance to lateral pulsion when attempting to roll the animal onto its back, and (5) circling behavior upon spontaneous ambulation. All animals were graded based on their performance in each task according to the following scale: 4, normal; 3, mild deficit; 2, moderate deficit; 1, severe deficit; and 0, afunctional. By combining the scores of all tests, a composite neuroscore was developed ranging from 0 to 20.

Data analysis. MRS data, mean arterial blood pressure (MABP), and angle-board neurological scores were analyzed utilizing analysis of variance (ANOVA) followed by individual Student-Newman-Keul's or Bonferroni t tests. Ordinal measurements, such as composite neurological scores, were analyzed utilizing the nonparametric Kruskal-Wallis ANOVA followed by individual nonparametric Mann-Whitney U tests. All values are expressed as mean \pm SE. A p value of < 0.05 was considered statistically significant.

Results

Fluid-percussion head injury produced a brief hypertensive response, followed by a sustained hypotension commencing at 30 min after trauma (Fig. 1). MABP remained significantly depressed in the saline-treated animals for the duration of the experiment. Treatment with nalmefene resulted in a tendency toward improved MABP; however, this improvement never achieved statistical significance when compared to saline-treated controls. Nalmefene did, however, improve MABP sufficiently such that by 2 hr postinjury, it was no longer significantly different from preinjury values.

Table 1. Median neurological scores for individual motor tests following fluid-percussion brain injury in rats

	Angleboard		Contralateral forelimb flexion		Lateral pulsion		Circling		Beamwalk		Composite	
	Saline	Nalmefene	Saline	Nalmefene	Saline	Nalmefene	Saline	Nalmefene	Saline	Nalmefene	Saline	Nalmefene
24 h	1	2	2	2	2	3	1	2	1	2	7	10
1 week	1	3*	1	3	3	4	1	2	2	3	8	14*
2 weeks	2	4	2	3	3	4	2	3*	3	4	12	16*
3 weeks	2	4*	2	3	3	4	2	4*	3	4	12	18*
4 weeks	2	4*	2	4	4	4	3	4	3	4	14	19*

Animals were administered with nalmefene (100 μ g/kg, i.v.) or an equal volume of saline at 30 min postinjury.

*, $p < 0.05$ versus saline-treated controls.

Neurological scores over time following trauma are summarized in Table 1. Saline-treated animals at 24 hr displayed a severe to moderate deficit in all of the motor-function tests. Individual motor-function test scores improved slightly over the 4-week observation period; however, a significant motor deficit was still noticeable at 4 weeks. Animals treated with nalmefene demonstrated a slight to moderate deficit at 24 hr posttrauma, but improved markedly over time, showing essentially no observable motor deficits at 4 weeks. Of the individual motor-function tests, angleboard and circling were the most sensitive indicators of improved neurologic function with nalmefene treatment. While all individual motor tests may not, in themselves, have the sensitivity to detect small neurologic improvements after treatment, when each individual animal's scores are summed together and expressed as a composite score, small improvements in motor function with treatment are readily detected. Thus composite scores provide an additional, sensitive indicator of neurologic outcome. Although composite scores are the sum of individual animal's motor scores, they may not necessarily equate to the sum of all the animal's median scores. In terms of composite neuroscores, nalmefene-treated animals had a significantly improved outcome when compared to saline-treated controls for all time points after 24 hr posttrauma (Table 1).

Typical ³¹P MRS data prior to injury are shown in Figure 2. A number of peaks can be identified on the basis of previous work (Petroff et al., 1985; Vink et al., 1987b). These include PCr, Pi, phosphomonoesters, phosphodiester, and ATP. From the chemical shift of the Pi resonance, intracellular pH prior to injury was calculated to be 7.14 ± 0.03 and 7.18 ± 0.03 in the saline- and nalmefene-treatment groups, respectively (Table 2).

Following injury, there were small fluctuations in intracellular pH recorded in both groups similar to that described in detail previously for this level of injury (Vink et al., 1987b). There were no significant differences in intracellular pH profiles between groups (Table 2). Alterations in PCr and Pi following injury are summarized in Figure 3. Although small changes occurred with respect to the concentration of these metabolites in both treatment groups, there were no significant changes either within or between the groups. Similarly, there were no significant changes in ATP concentration following injury, as has been previously described in detail for this model of brain injury (Vink et al., 1987b). When expressed as ratios, these metabolite concentrations have been widely used as indicators of metabolic state (Gyulai et al., 1985; Chance et al., 1986). The PCr:ATP ratio was comparable in both groups, with no significant differences apparent for the duration of the experiment (Table 2). Similarly, PCr:Pi decreased following injury, but no significant differences were recorded between the treatment groups (Table 2).

Intracellular free-magnesium concentration prior to injury, as calculated from the chemical-shift difference between α - and β -ATP, was 0.79 ± 0.09 for the saline-treated group and 0.81 ± 0.07 for the nalmefene-treated group. These figures are in excellent agreement with those previously determined using a variety of techniques (Veloso et al., 1973; Ebel and Gunther, 1980). Following injury, Mg_i declined in both groups (Fig. 4). In the saline-treated group, there was no significant recovery over the 4-hr posttrauma observation period. Treatment with nalmefene at 30 min postinjury caused a significant increase in Mg_i as compared to saline-treated controls ($p < 0.05$). By 2 hr postinjury, the Mg_i in the nalmefene-treated group was no longer

Table 2. Alterations in intracellular pH and PCr:ATP and PCr:Pi ratios following fluid-percussion brain injury in the rat and treatment at 30 min postinjury with either nalmefene (100 μ g/kg, i.v.) or an equal volume of saline

	pH		PCr:ATP		PCr:Pi	
	Saline	Nalmefene	Saline	Nalmefene	Saline	Nalmefene
Preinjury	7.14 ± 0.03	7.18 ± 0.03	2.1 ± 0.2	2.4 ± 0.3	3.7 ± 0.4	3.6 ± 0.3
1 hr	7.17 ± 0.03	7.07 ± 0.02	1.8 ± 0.1	2.2 ± 0.2	2.9 ± 0.4	2.4 ± 0.2
2 hr	7.22 ± 0.05	7.07 ± 0.07	2.0 ± 0.2	2.1 ± 0.3	2.6 ± 0.3	2.8 ± 0.3
3 hr	7.22 ± 0.05	7.19 ± 0.06	2.0 ± 0.2	1.9 ± 0.3	2.8 ± 0.3	3.0 ± 0.6
4 hr	7.21 ± 0.04	7.12 ± 0.04	2.0 ± 0.3	1.9 ± 0.2	2.7 ± 0.4	2.6 ± 0.4

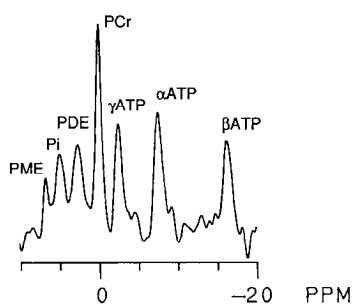


Figure 2. Typical phosphorus MRS spectrum obtained from the left hemisphere of an anesthetized rat prior to fluid-percussion brain injury. PME, phosphomonoesters; Pi, inorganic phosphate; PDE, phosphodiester; PCr, phosphocreatine; ATP, adenosine triphosphate.

significantly different from preinjury controls, whereas in the saline-treated group, Mg_i was still reduced more than 60% from preinjury levels (Fig. 4). The levels in both groups showed no further significant changes between 2 and 4 hr.

The calculated ADP concentrations prior to and following injury are shown in Figure 5. Prior to injury, ADP concentration in the saline- and nalmefene-treated groups was 26.6 ± 1.2 and 28.7 ± 1.3 nmol/gm wet weight, respectively. These values are similar to values previously published for the rat brain (Veech et al., 1979; Vink et al., 1988a) and are thought to represent free-cytosolic concentrations (Veech et al., 1979; Erecinska and Silver, 1989). Following injury, but before treatment, there was a significant increase in ADP concentration in both groups. After saline treatment, the ADP concentration continued to rise significantly, whereas nalmefene treatment resulted in a fall in ADP concentration. Thirty min after treatment, there was a significant difference between the 2 treatment groups ($p < 0.05$). There were no significant changes in ADP concentration within each group after 30 min posttreatment.

Alterations in phosphates following injury and treatment had marked effects on cytosolic phosphorylation potential (ΔG_p). Brain injury resulted in an approximate 45% decline in ΔG_p by 30 min postinjury (Fig. 6). Treatment with saline did not prevent a further decline in ΔG_p to only 37% of preinjury values by 1 hr posttrauma. In contrast, treatment with nalmefene at 30 min postinjury resulted in ΔG_p recovering to 71% of preinjury values by 2 hr posttrauma. This ΔG_p was significantly greater than that in saline-treated controls at this time point ($p < 0.05$). After 2 hr postinjury, there were no further significant changes in either treatment group.

Discussion

Fluid-percussion brain injury in rats has been well characterized with regard to physiological, neurochemical, histological, and behavioral changes (Hayes et al., 1983; Vink et al., 1987b, 1988a; McIntosh et al., 1989a) and has been previously used for magnetic resonance and pharmacological studies (Vink et al., 1987b, 1988b,c; McIntosh et al., 1988b; Faden et al., 1990). The fact that the tissue injury and biochemical changes are consistent with the degree of injury severity as assessed by neurological outcome makes this model ideal for evaluation of pharmacotherapies. The present studies examined the efficacy of the opiate antagonist nalmefene as a therapeutic agent after traumatic brain injury using ^{31}P MRS to monitor bioenergetic changes. Nalmefene treatment significantly improved long-term neurological

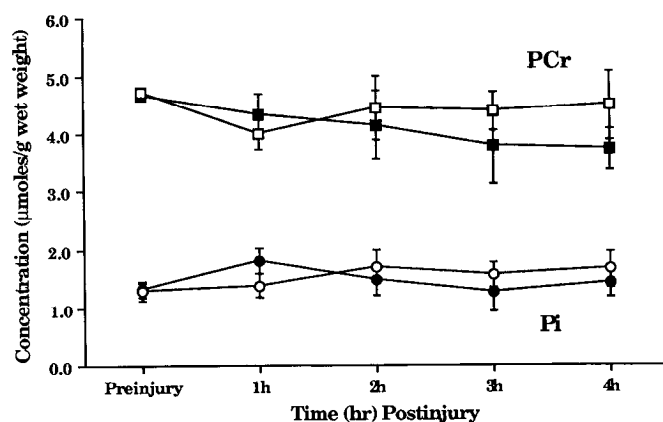


Figure 3. Alterations in phosphocreatine (squares) and inorganic phosphate concentration (circles) prior to and following traumatic brain injury in rats. At 30 min postinjury, animals were intravenously administered with either 100 μ g/kg nalmefene (solid symbols) or an equal volume of saline (open symbols).

outcome. This improvement was evident as early as 1 week after injury and persisted throughout the 4-week observation period. These beneficial effects of nalmefene on neurological outcome are similar to those previously observed with this compound following spinal cord trauma in rats (Faden et al., 1988) and with the opiate antagonist WIN 44,441-3 after fluid-percussion-induced brain trauma in cats (McIntosh et al., 1987). Although both the present study and the earlier cat study using WIN 44,441-3 demonstrated less posttraumatic hypotension in treated versus control animals, the blood-pressure effect is unlikely to contribute to the therapeutic actions of these compounds for several reasons. In the earlier report, dopamine-treated controls with identical blood-pressure responses to those treated with WIN 44,441-3 did not show any improvement in outcome as compared to saline-treated controls. Furthermore, in the present nalmefene experiments, blood-pressure differences between treated and saline control animals did not reach statistical significance.

The fact that structurally different opiate-receptor antagonists improve outcome after traumatic brain injury, combined with

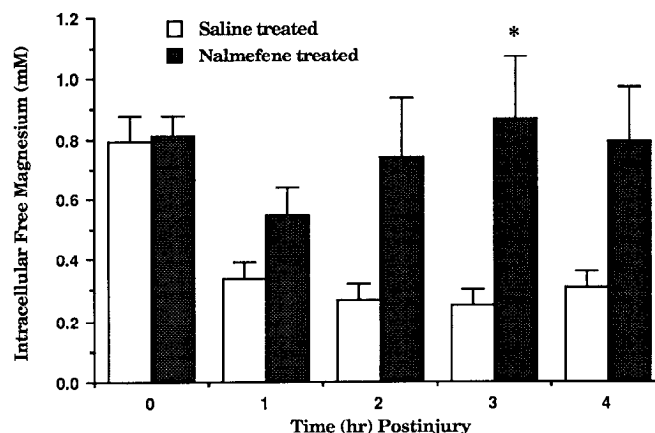


Figure 4. Intracellular free-magnesium concentration prior to and following fluid-percussion brain injury in rats. Intravenous treatment with 100 μ g/kg nalmefene at 30 min postinjury resulted in a significant improvement in Mg_i compared with saline-treated controls.

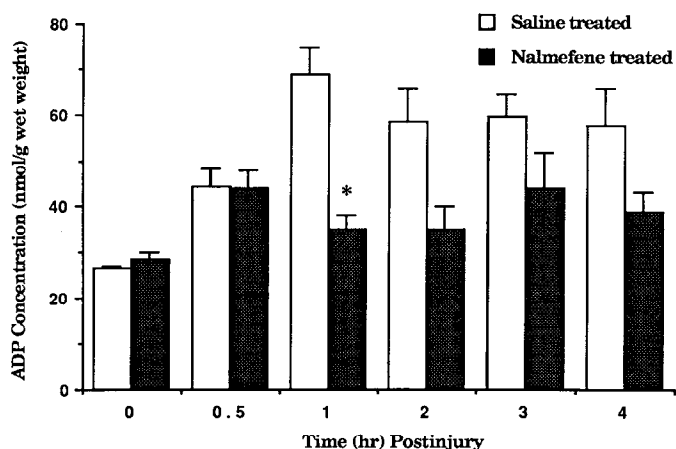


Figure 5. ADP concentration following traumatic brain injury in the rat. After treatment, nalmefene-treated animals had significantly lower ADP levels than saline-treated controls.

the stereospecificity of this response (McIntosh et al., 1987), strongly suggests a role for opiate receptors in the pathophysiology of the secondary injury response. Both WIN 44,441-3 and nalmefene show markedly increased potency as compared to naloxone, and both compounds may have increased activity at κ -opiate receptors (Michel et al., 1984; Faden et al., 1988; Faden, 1990). However, neither compound shows a high degree of selectivity for the κ -receptor. Nonetheless, in pilot studies, we have recently found that the opiate-receptor antagonist nor-binaltorphimine, which is highly selective at κ -opiate receptors (Portoghese et al., 1987), also significantly reduces posttraumatic neurological deficits in the rat lateral-fluid-percussion model (R. Vink and A. I. Faden, unpublished observations). We have also shown that the opioid dynorphin, a proposed endogenous ligand for the κ -opiate receptor (Herman and Goldstein, 1985) accumulates after fluid-percussion trauma in anatomical sites that show the most severe histopathological changes as well as post-traumatic ischemia. Finally, intracerebroventricular administration of dynorphin A(1-17), but not dynorphin A(2-17), which is inactive at opiate receptors, worsens outcome after traumatic brain injury in rats (T. K. McIntosh et al., unpublished observations). Thus, the κ -opiate receptor is specifically implicated in the pathophysiologic response following traumatic brain injury. Such observations are consistent with findings in traumatic spinal cord injury models that show dynorphin accumulation at injury sites, protective effects with κ -active and κ -selective opiate antagonists, and limitation of posttraumatic injury with specific dynorphin antisera (but not antiserum to related peptides; Faden, 1990).

Unlike previous studies of opiate antagonists in the treatment of CNS trauma, the present experiments utilized MRS to monitor certain acute biochemical events following injury. Previous studies have shown that traumatic brain injury causes a profound decrease in brain intracellular free and total-tissue magnesium concentration (Vink et al., 1987a, 1988b). The degree of magnesium depletion following trauma was linearly correlated to the severity of injury, and the resultant neurological outcome (Vink et al., 1988b; McIntosh et al., 1989b). Moreover, improvement in magnesium status following brain injury has been shown to be associated with an improved neurological outcome, whereas magnesium deprivation has been shown to

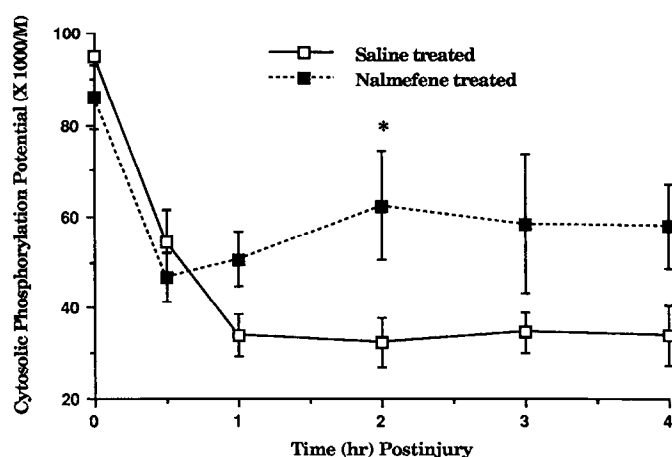


Figure 6. Brain cytosolic phosphorylation potential following fluid-percussion injury in rats. Treatment with nalmefene at 30 min postinjury resulted in a significant improvement in ΔG_p compared with saline-treated controls.

exacerbate injury (McIntosh et al., 1988a; 1989b; Vink et al., 1988b,c). The present studies have again shown that improved neurological outcome is associated with improved free magnesium. A similar recovery of free magnesium has been previously noted in studies examining thyrotropin-releasing hormone analogues and excitatory amino acid antagonists as treatments following traumatic brain injury (Vink et al., 1988c; Faden et al., 1989). Although the mechanisms by which nalmefene affects magnesium status are unclear, an association between magnesium and opiate-receptor binding has been previously reported (Sadée et al., 1982).

Previous studies of traumatic brain injury in rats have shown that decreased intracellular free-magnesium concentration is associated with a decline in ΔG_p (Vink et al., 1988a). Indeed, these studies demonstrated that the relationship between the 2 parameters was linear. While not linearly correlated with injury severity, decline in ΔG_p was associated with the development of irreversible tissue damage following brain trauma (Vink et al., 1988a). In the present studies, nalmefene treatment resulted in a significant recovery of ΔG_p and a significant improvement in cytosolic ADP concentration. It follows that nalmefene is therefore having an inhibitory effect on the rate of ATP hydrolysis or a stimulatory effect upon the rate of ADP phosphorylation. Although there were no trends suggesting any alterations in ATP concentration with nalmefene treatment, Figure 3 suggests that there was a trend toward increased PCr hydrolysis. Because PCr may act as an ATP buffer, this would suggest that ATP hydrolytic rate has increased rather than decreased. However, ADP levels after nalmefene treatment fall significantly, while Pi levels also show a tendency toward decreasing rather than increasing. One can therefore speculate that nalmefene's beneficial effects on ΔG_p are mediated through an increased rate of ADP rephosphorylation. This is consistent with the fact that increased magnesium concentration in nalmefene-treated animals would favor ADP phosphorylation, in contrast to saline-treated controls, where the decreased magnesium levels would inhibit ADP phosphorylation. Magnesium's effect on ADP levels could be mediated by the creatine kinase, adenylate kinase, and the ATPase reactions (Lawson and Veech, 1979; Vink et al., 1988a), though the relatively more pronounced regulation of the creatine kinase and adenylate kinase equilibrium constants by free-mag-

nesium concentration would suggest that these 2 reactions have a dominant effect. The net effect of nalmefene treatment therefore seems to be of a stimulatory nature analogous to increasing metabolic rate.

Irrespective of how nalmefene restores free-magnesium concentration, ADP levels, and ΔG_p , any improvement in these parameters would signify an improved bioenergetic capacity that would translate to beneficial effects on a number of proposed secondary-injury factors. For example, improved bioenergetic state would indicate a normalization of functions such as mitochondrial metabolism, glycolytic rate, and Krebs cycle activity (Erecinska and Silver, 1989), thus reflecting an increased efficiency of energy production and utilization. This would presumably allow for recovery processes to take place, such as reacylation of free fatty acids, phospholipid synthesis, RNA aggregation, and protein synthesis, all of which are energy-dependent processes. The associated improvement in membrane stability would decrease permeability to ions such as sodium and calcium and reestablish transmembrane ionic equilibria. The maintenance of ionic gradients alone is responsible for up to 60% of all ATP hydrolysis (Erecinska and Silver, 1989). Any associated reduction in membrane fluidity may also be beneficial in terms of membrane-associated enzyme activity, such as the Na^+/K^+ ATPase activity. Finally, neurotransmitter synthesis, packaging, and transport is also under bioenergetic control (Dagani and Erecinska, 1987). These beneficial effects of nalmefene on bioenergetic function and secondary-injury factors have also been observed in cerebral ischemia (Faden et al., 1990). In these studies, nalmefene improved postischemic recovery of PCr, Pi, ATP, and lactate, as well as limited the increase in free fatty acids and decline in excitatory amino acids.

In conclusion, nalmefene significantly improved intracellular free-magnesium status and cellular bioenergetics after injury, which may have beneficial effects on a number of other proposed secondary-injury factors such as excitatory amino acid release (Nowak et al., 1984), calcium fluxes, cerebral blood flow (Altura and Altura, 1982), edema (Ebel and Gunther, 1980), and membrane breakdown (see also Siesjö and Wieloch, 1985; Vink et al., 1988a,b,c). Because nalmefene is considered to be a relatively pure opiate-receptor antagonist, the present study provides further support for the hypothesis that opioid peptides may play a significant role in secondary injury. Furthermore, we suggest that the neuroprotective actions of opiate antagonists such as nalmefene following traumatic brain injury may relate in part to their early effects on bioenergetics.

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