ability of the synthetic peptide will greatly enhance our ability to investigate the biochemical processes involved in hormonal control of sex pheromone production in moths. The knowledge thus gained could lead to the development of new methods for the control of insect pests through the interruption of pheromone biosynthesis and reproduction.

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6. Brain-subesophageal ganglion complexes dissected from about 2500 insects and stored at −80°C in 5% formic acid v/v, 15% trifluoroacetic acid (TFA) v/v, 1% NaCl w/v, 1*N* HCl [H. P. J. Bennett, C. A. Brown, S. Solomon, *Anal. Biochem.* **128**, 121 (1983)]. The tissues were homogenized as described previously (*7*). The defatted extract was subjected to an HPLC purification procedure based on a sequence of three chromatographic steps: (i) Supelcosil LC-18 DB with Pelliguard guard column (Supelco) eluted with a concave gradient (Waters Curve no. 7) of 10 to 60% acetonitrile containing 0.1% v/v TFA in 0.1% aqueous TFA over 1 hour at ambient temperature and 1 ml/min on a Model 840 HPLC with autosampler (Waters). The eluant was monitored at 214 nm. (ii) Four Waters Protein-Pak 125 HP-SEC columns plumbed in series were eluted isocratically with a 40% aqueous acetonitrile (0.1% TFA) at ambient temperature and 1 ml/min on the same instrument as in the first step. (iii) Vydac 218 TP 54 C-18 column (Separations Group) was eluted with a linear gradient of 10 to 50% acetonitrile containing 0.1% TFA in 0.1% aqueous TFA, over 1 hour at 28°C at 0.4 ml/min on a Model 1090M HPLC with photodiode array detector and Chemstation (Hewlett-Packard).
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10. The hydrochloric acid used for hydrolysis contained 0.1% sodium sulfite, a reducing agent that may have partially converted the methionine sulfoxide to methionine. The sulfoxide was not determined under the chromatographic conditions used.
11. The sample was applied in the form of a 1% TFA solution containing about 30 pmol of peptide to a nitrocellulose-covered [G. P. Jonsson *et al.*, *Anal. Chem.* **58**, 1084 (1986)] aluminized Mylar foil. The sample was run at 15-kV accelerating volts with a 30-cm flight path, and the signal was allowed to accumulate for 19 hours.
12. Solid-phase synthesis of the peptide amide was done with benzhydrylamine resin, on a Biosearch 9600 peptide synthesizer. All amino acids were protected with *N-tert*-butyloxycarbonyl group. Side chain–protecting groups were: Arg, 4-toluenesulfonyl; Asp, cyclohexyl ester; Glu, benzyl ester; Lys, 2-chlorobenzyloxycarbonyl; Met, sulfoxide; Ser and Thr, benzyl; and Tyr, 2-bromobenzyloxycarbonyl. The dried peptide-resin was treated with the low-high HF method of cleavage [J. P. Tam, W. F. Heath, R. B. Merrifield, *J. Am. Chem. Soc.* **105**, 6442 (1983)]. The crude peptide was extracted from the resin with aqueous acetic acid (20%) and then initially purified by HPLC (Ultrasphere column, 4.6 by 250 mm) with a gradient of 0 to 60% acetonitrile (0.1% TFA) at a flow rate of 1.5 ml/min.
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The Role of Excitatory Amino Acids and NMDA Receptors in Traumatic Brain Injury

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Brain injury induced by fluid percussion in rats caused a marked elevation in extracellular glutamate and aspartate adjacent to the trauma site. This increase in excitatory amino acids was related to the severity of the injury and was associated with a reduction in cellular bioenergetic state and intracellular free magnesium. Treatment with the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist dextrorphan or the competitive antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid limited the resultant neurological dysfunction; dextrorphan treatment also improved the bioenergetic state after trauma and increased the intracellular free magnesium. Thus, excitatory amino acids contribute to delayed tissue damage after brain trauma; NMDA antagonists may be of benefit in treating acute head injury.

EXCITATORY AMINO ACIDS (EAAs) have been implicated in delayed brain tissue injury after cerebral ischemia (*1*) and hypoglycemia (*2*) through actions mediated by NMDA receptors. Traumatic brain injury (TBI) also causes secondary neurochemical changes that are associated with delayed tissue damage, many of which are similar to those occurring after cerebral ischemia (*3*). However, it is not known whether EAAs play a role in TBI. We have used ^{31}P magnetic resonance spectroscopy (MRS), microdialysis, and behavioral measures to examine the hypothesis that EAAs are released after TBI and that they contribute to secondary tissue damage through effects at NMDA receptors.

Male Sprague-Dawley rats (400 ± 25 g) were anesthetized with sodium pentobarbital (60 mg per kilogram of body weight, intraperitoneally). Although pentobarbital may have weak nonselective antagonism for EAA receptors (*4*), barbiturates have commonly been utilized in electrophysiological studies that examine the activity of EAA antagonists (*5*). Animals were subjected to a lateral (parasagittal) fluid percussion–induced injury (2.0 or 2.85 atm) through a 2-mm craniotomy site centered over the left parietal cortex (*6*). This fluid percussion method causes transient brain deformation with resultant tissue damage (*6, 7*), leading to moderate (2.0 atm) or severe (2.85 atm) behavioral and histological abnormalities. Microdialysis techniques (*8*) were used to determine whether EAAs are released in response to TBI and whether changes are related to the severity of the injury. Microdialysis probes were implanted stereotaxically ipsilateral to the trauma site, so as to position the probe just below the site of fluid percussion injury; these regions, including the CA2 and CA3 areas of the hippocampus, show delayed cell death after trauma (*9*). Samples were collected at a flow rate of 2 μl/min before and after trauma. Amino acid levels were assayed with high-performance liquid chromatography (*10*). Animals that experienced severe injury ($n = 6$) exhibited a rapid and significant increase in extracellular aspartate and glutamate (Fig. 1), as compared to uninjured, control animals ($n = 4$). Changes were related to injury severity with moderately injured animals ($n = 4$) showing peak increases in glutamate and aspartate of 282% and 273%, respectively, as compared to severely injured animals, which showed increases of 940% and 1849%. Peak effects occurred within the first 10 min, with elevations sustained for more than 1 hour in the severely injured group.

Earlier studies of TBI have demonstrated that the ^{31}P MRS–determined ratio of phosphocreatine to inorganic phosphate (PCr/

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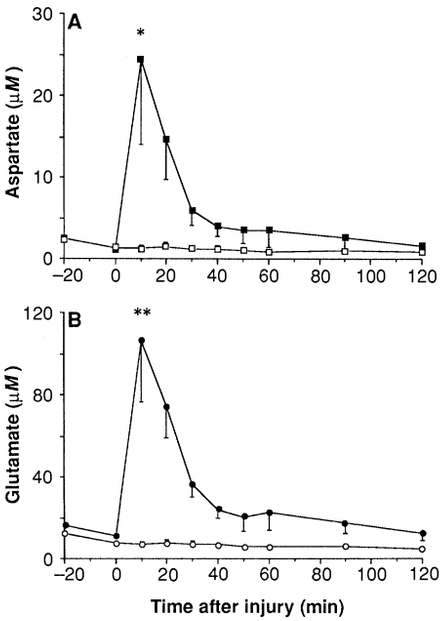

Fig. 1. Changes in the extracellular levels of the excitatory amino acids (**A**) aspartate and (**B**) glutamate before and after severe fluid percussion–induced TBI as measured by microdialysis techniques. Perfusions were made with a CMA/100 microinjection pump (Carnagie Medicin AB); the probe (Bioanalytical Systems) had an outside diameter of 0.5 mm with a molecular weight exclusion limit of approximately 20 kD. All probes were tested before and after dialysis against a standard solution of amino acids. Data represent averages for six injured animals (filled symbols) and four controls (open symbols) and are expressed as the concentration of amino acid in the dialyzate. At 10 min after injury, the concentrations of aspartate (A) and glutamate (B) in the dialyzate increased significantly ($P < 0.05$ and $P < 0.01$, respectively; repeated measurement analysis of variance); elevated levels of amino acids persisted for more than 60 min. Control animals were subjected to anesthetic and surgical procedures along with probe placement but were not injured.

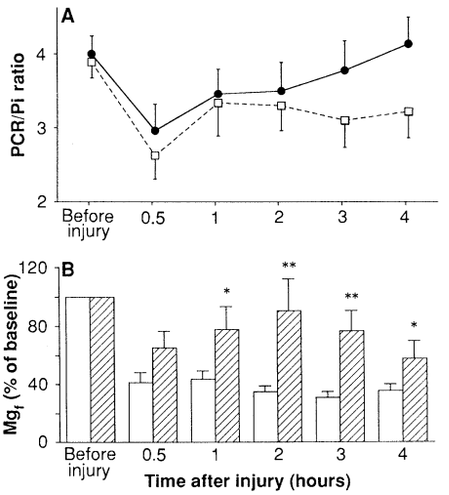

Fig. 2. (**A**) Changes in PCr/Pi ratio as measured by ^{31}P MRS, in animals intravenously administered the noncompetitive NMDA antagonist dextrorphan (circles) or an equal volume of physiological saline (squares) 30 min after moderate (2.0 atm) trauma (mean ± SEM). The PCr/Pi ratio was significantly increased at 4 hours in dextrorphan-treated animals as compared to values before treatment (*t* test with Bonferroni correction, $P < 0.05$). However, differences in the PCr/Pi ratio between dextrorphan- and saline-treated controls did not reach significance ($0.05 < P < 0.10$). (**B**) Changes in Mg_f as measured by ^{31}P MRS in animals treated intravenously with dextrorphan (hatched bars) or an equal volume of physiological saline (open bars) after moderate trauma. Mg_f, determined by the position of the β peak of ATP (*11*), declined after trauma. Mg_f levels increased significantly in dextrorphan-treated animals as compared to controls; *, $P < 0.05$; **, $P < 0.01$.

Pi) and the intracellular free magnesium concentration (Mg_f) are significantly correlated with injury severity, as determined by histopathologic damage after trauma and chronic neurological outcome (*7, 11*). To determine whether these acute metabolic markers of irreversible injury could be modified by treatment with an NMDA antagonist, we used ^{31}P MRS to monitor biochemical changes after TBI and treatment with dextrorphan. Dextrorphan is a noncompetitive NMDA antagonist that attenuates glutamate neurotoxicity in cortical cell cultures and reduces ischemic neuronal damage in vivo (*12*). In the ^{31}P MRS measurements we used a GE CS-I spectrometer equipped with a 2.0-T, 26-cm horizontal superconducting magnet. A surface coil (5 mm by 9 mm) was positioned over the left hemisphere around the trauma site and was used to transmit and receive signal (*6, 7*). Trauma was produced outside of the magnet, and the animal was repositioned in the center of the magnet bore within 10 min. We determined the relative metabolite concentrations of each spectrum, using the GE computer "line-fitting" program GEMCAP to simulate the experimental spectrum and to integrate the area of individual peaks (*7*).

Moderate TBI was associated with a significant decline in the PCr/Pi ratio by 30 min after trauma (Fig. 2A), reflecting a decrease in cellular bioenergetic state (*13*). Only transient changes in *p*H and no changes in adenosine triphosphate (ATP) concentration were observed with this degree of injury, as previously described (*6, 7*). Administration of dextrorphan (10 mg/kg, intravenously, 30 min after trauma, $n = 8$) significantly improved the PCr/Pi ratio by 4 hours after injury, at which time the ratio reached baseline levels (before injury) (Fig. 2A). In contrast, control animals ($n = 8$) treated with an equal volume of vehicle (physiological saline) had PCr/Pi ratios 4 hours later that remained approximately 20% below the baseline levels and did not differ significantly from the values before treatment and after trauma. Both Mg_f, as determined by MRS, and total tissue Mg^{2+} decreases after TBI (*11, 14*). A similar decline in Mg_f after trauma was observed in the present study; dextrorphan treatment was associated with a significant recovery of Mg_f after trauma (Fig. 2B). The relative decline in Mg_f in dextrorphan-treated animals at 3 and 4 hours, although statistically insignificant, may reflect decreased dextrorphan levels because the drug, which may have a relatively short plasma half-life in rats, was administered as a single bolus injection.

After MRS measurements, animals recovered from anesthesia and were followed for 2 weeks, during which time they were blindly scored with regard to neurological recovery (*7*). Behavioral tests included the ability to maintain position on an inclined plane (vertical, right horizontal, and left horizontal positions), left and right forelimb flexion after suspension by the tail, and degree of resistance to right and left lateral pulsion. The seven individual scores, each graded from 0 (severely impaired) to 5 (normal function), were added to yield a composite neurological score ranging from 0 to 35. Animals treated with dextrorphan showed significantly better neurological recovery than saline-treated controls (Fig. 3A).

Because it is possible that the protective effects of dextrorphan were mediated through actions unrelated to NMDA antagonism, we also examined the effects of treatment with another NMDA antagonist, the competitive NMDA antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) (*15*), on outcome after TBI. Unlike dextrorphan, which has good central nervous system (CNS) penetration after systemic administration, CPP has only limited CNS activity after systemic administration. Therefore, CPP was administered centrally (intracerebroventricularly) through a stereotaxically placed catheter in the left lateral ventricle. From pilot studies in spinal cord injury, we established the drug dose (100 μg) and determined that animals given CPP should be subjected to a higher level of injury if we were to better discriminate the potential effects of treatment. Furthermore, because of technical difficulty associated with administering drugs intracerebroventricularly after trauma, it was necessary to administer CPP before injury. Likewise, control animals received an equal volume of vehicle [10 μl of artificial cerebrospinal fluid CSF)] immediately before injury. Animals treated with CPP ($n = 9$) showed significantly better neurological recovery at 2 weeks after trauma (2.85 atm) than controls ($n = 11$) (Fig. 3B).

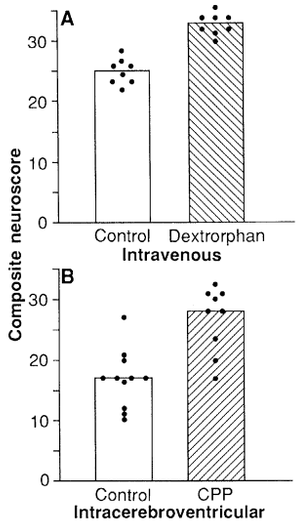

Fig. 3. (**A**) The effects of treatment with intravenous dextrorphan or an equal volume of physiological saline on behavioral outcome 2 weeks after moderate fluid percussion injury. Composite neurological scores reflect the summation of seven functional scores (each rated 0 to 5). Dextrorphan-treated animals showed significantly improved behavioral recovery after trauma as compared to controls (Mann-Whitney U test, $P < 0.01$). These are the same animals that were evaluated by MRS as shown in Fig. 2. Dots represent individual animal scores, and histograms represent median values. (**B**) Effects of treatment with the competitive NMDA antagonist CPP or an equal volume of vehicle (artificial CSF), administered by intracerebroventricular injection before injury, on behavioral outcome after severe (2.85 atm) fluid percussion–induced TBI. Rats treated with CPP showed significantly better behavioral recovery 2 weeks after trauma than controls (Mann-Whitney U test, $P < 0.005$).

Our studies demonstrate that levels of EAAs increase in the extracellular space after CNS trauma and that NMDA antagonists have beneficial effects in TBI, as reflected by metabolic and behavioral markers of injury. Taken together, our data indicate that TBI is associated with the release of EAAs, which contribute to neurological dysfunction after trauma through actions at NMDA receptors. Treatment with a noncompetitive NMDA antagonist improved the cellular bioenergetic state in the acute period after trauma and limited neurological dysfunction. Beneficial behavioral effects were also found with a competitive NMDA antagonist.

The mechanisms by which EAAs contribute to secondary tissue injury remain speculative, although increases in intracellular Na^+ and Ca^{2+} have been implicated in this delayed injury process (*16*). However, TBI causes a marked decrease in intracellular free Mg^{2+} as well as a decline in total tissue Mg^{2+}, the amount of which is correlated with injury severity (*11, 14*). Dextrorphan treatment reversed the decrease in Mg_f found after trauma, which is consistent with recent findings showing that treatment with the noncompetitive NMDA antagonist MK-801 partially restores total tissue Mg^{2+} levels after traumatic spinal cord injury (*17*). The ability of NMDA antagonists to restore Mg^{2+} levels after injury may be related to the improvement in cellular bioenergetic state observed after dextrorphan treatment and may, in part, underlie the protective effects of NMDA antagonists in brain trauma.

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Regulation of Calcium Release Is Gated by Calcium Current, Not Gating Charge, in Cardiac Myocytes

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In skeletal muscle, intramembrane charge movement initiates the processes that lead to the release of calcium from the sarcoplasmic reticulum. In cardiac muscle, in contrast, the similarity of the voltage dependence of developed tension and intracellular calcium transients to that of calcium current suggests that the calcium current may gate the release of calcium. Nevertheless, a mechanism similar to that of skeletal muscle continues to be postulated for cardiac muscle. By using rapid exchange (20 to 50 milliseconds) of the extracellular solutions in rat ventricular myocytes in which the intracellular calcium transients or cell shortening were measured, it has now been shown that the influx of calcium through the calcium channel is a mandatory link in the processes that couple membrane depolarization to the release of calcium. Thus, intramembrane charge movement does not contribute to the release of calcium in heart muscle.

THE EXCITATION-CONTRACTION COUPLING mechanisms may be qualitatively different in skeletal and cardiac muscle (*1*). In skeletal muscle, the signal for the release of calcium is associated with charge movement at the junction between the transverse tubule (T) and the sarcoplasmic reticulum (SR). Thus, the intramembrane charge movement (*2*), the intracellular Ca^{2+} transient (*3*), the development of tension (*4*), and the intrinsic birefringence signal associated with Ca^{2+} release (*5*) all show sigmoid voltage dependence. By contrast, in mammalian myocardium, the voltage dependence of intracellular Ca^{2+} transients (*6*), development of tension (*7*) and cell shortening (*8*), and the intrinsic birefringence signal (*9*) are bell-shaped and reflect the voltage dependence of the Ca^{2+} current (i_{Ca}) rather than the voltage dependence of the charge movement (*10*). Furthermore, Ca^{2+} may be released by repolarization from positive potentials (*6*), a finding inconsistent with release triggered by intramembrane charge movement. Recently, however, Cannell *et al.* (*11*) suggested that the release of Ca^{2+} from the intracellular pools in rat ventricular myocytes may be in part regulated by a charge-coupled release mechanism.

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