hydrolyzate was applied to a Shimadzu amino acid analyzing system with the use of *o*-phthalaldehyde postcolumn derivatization (*12*). The proportions of amino acids in the hydrolyzate of cPD1 were serine, 1.13; glycine, 1.57; valine, 1.00; methionine, 0.88; leucine, 1.92; and phenylalanine, 1.62, or roughly, 1:1:1:1:2:2, respectively. Although the molar ratio of glycine is high, we believe that the amino acid ratios are as indicated for the reasons described in (*13*). The amino acid sequence of cPD1 was determined by a manually operated direct Edman method. The phenylthiohydantoin amino acid derivative obtained at each degradation cycle was identified by high-performance liquid chromatography (HPLC) (*14*). The results reveal that the amino acid sequence of cPD1 was H-Phe-Leu-Val-Met-Phe-Leu-Ser-Gly-OH.

Fully protected cPD1 was synthesized in solution by a stepwise chain elongation from the carboxyl terminus. In each step, condensation was achieved by the HONB(*N*-hydroxy-5-norbornene-2,3-dicarboximide)–DCC(*N*,*N*′-dicyclohexylcarbodiimide) method (*15*). After removal of all the protective groups with hydrogen fluoride (*16*), the major product of synthesis was purified by reprecipitation from trifluoroacetic acid. Correct synthesis of cPD1 was confirmed by amino acid analysis and FAB mass spectrometry. The retention time on HPLC and the clumping-inducing activity of the synthetic octapeptide were fully identical with those of native cPD1.

It has been reported that the frequency of plasmid transfer can be increased by several orders of magnitude, by exposing donor cells to a cell-free filtrate of recipient cells for 20 to 50 minutes prior to mating (*6*, *7*). Enhancement of plasmid transfer was observed for both isolated and synthetic cPD1 adjusted to a concentration whose activity corresponded to that in a recipient cell-free filtrate (Table 2). The cPD1 did not affect mating between OG1S(pAD1::Tn*917*) and JH2-2, which involves a different conjugative plasmid (pAD1::Tn*917*) that responds to cAD1 (*17*). This was consistent with the observation that cPD1 failed to induce self-clumping of OG1S(pAD1::Tn*917*) at a concentration as high as 100 ng per 100 μl (approximately $1 \times 10^{-6} M$) in a microtiter dilution well. These results confirm the chemical specificity of characterized pheromone activity.

We believe that our findings are relevant to studies on the plasmid-determined pheromone-inactivation mechanism in donor cells. It is possible that the single serine residue is involved in the inactivation as a relationship between formation of a phosphodiester bond and pheromone-inactivaton has been reported (*8*).

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11. The isolated cPD1 (approximately 5 μg) was dissolved in 10 μl of 50 percent acetonitrile. A portion (2 μl) of sample solution was mixed with 2 μl of diethanolamine on a small stainless steel tip and then the FAB mass spectrum was measured with a JMS DX-300 mass spectrometer (JEOL) with xenon as the fast atom.
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Blockade of *N*-Methyl-D-Aspartate Receptors May Protect Against Ischemic Damage in the Brain

Abstract. *In rats ischemia of the forebrain induced by a 30-minute occlusion of the carotid artery, followed by 120 minutes of arterial reperfusion, produced ischemic lesions of selectively vulnerable pyramidal cells in both hippocampi. Focal microinfusion into the dorsal hippocampus of 2-amino-7-phosphonoheptanoic acid, an antagonist of excitation at the* N*-methyl-D-aspartate–preferring receptor, before ischemia was induced protected against the development of ischemic damage. It is proposed that excitatory neurotransmission plays an important role in selective neuronal loss due to cerebral ischemia.*

Cerebral ischemia and status epilepticus produce similar patterns of neuronal loss in the hippocampus (*1*), involving preferentially those pyramidal neurons (in regions CA1, CA3, and CA4) that most readily show burst firing (*2*). This led to the suggestion (*3*) that enhanced calcium entry during burst firing, in the course of either status epilepticus or the reperfusion phase after ischemia, accounts for selective neuronal vulnerability, with excessive intracellular calcium leading to cell death in a manner similar to that proposed for muscle and liver cells (*4*). We have shown that mitochondria in selectively vulnerable hippocampal neurons show massive overloading with calcium during status epilepticus and after 2 hours of reperfusion following cerebral ischemia (*5*).

Burst firing can be triggered in hippocampal, cortical, or striatal neurons by iontophoresis of aspartate or of other compounds acting on the *N*-methyl-D-aspartate (NMDA)–preferring receptor, including quinolinic acid (*6*). The excit-

atory effect of dicarboxylic amino acids at the postsynaptic receptor can be blocked by various analogs of glutamate and aspartate (*7*). Among these, 2-amino-7-phosphonoheptanoic acid (APH) is a highly potent antagonist that is selective for NMDA receptors (*8*). To investigate the possible role of excitatory amino acid neurotransmission in the development of ischemic brain damage, we assessed the effect of APH, focally injected into one hippocampus, on the local development of ischemic brain damage in a rodent model in which bilateral carotid occlusion is combined with systemic hypotension to reduce forebrain blood flow to less than 5 percent of control levels (*9*). Immediately before the induction of ischemia, 1 μl of buffer containing 20 μg of APH (*10*) was infused over 2 minutes through guide cannulas into one dorsal hippocampus; buffer alone was injected contralaterally. After 30 minutes of ischemia and 2 hours of reperfusion, the animals were killed by transaortic perfusion with fixative (*11*) and brain sections containing both hippocampi were serially sectioned and prepared for light microscopy. Figure 1 summarizes data on sections made through the dorsal hippocampus directly anterior and posterior to the injection site. On the buffer-injected side a variety of cellular changes indicated early ischemic damage (*12*). The interanimal variability was typical of that occurring in models of ischemia (*5, 13*).

In the hippocampus in which pharmacological blockade of excitatory neurotransmission had been induced, neuronal damage was markedly attenuated compared to the contralateral hippocampus. Often the protected side appeared normal (Fig. 2), while the contralateral side showed severe damage. The protection extended to all cell types—pyramidal, granule, and polymorphic (Fig. 1)—and included a sphere 1 to 2 mm in diameter surrounding the injection site, but also on occasion extending more anteriorly or posteriorly and ventrally in the hippocampal formation.

This protective action of APH implies that excitation at the NMDA receptor plays an important part in the development of ischemic cytopathology. It was previously shown that potent agonists at the NMDA receptor (such as ibotenate or quinolinate) produce neuronal degeneration when injected focally into the hippocampus and that such degenerative changes can be blocked by coadministration of APH. The endogenous agent acting at the NMDA receptor, during ischemia or in the postischemic phase when burst firing is occurring, is not definitively known. Aspartate and quinolinate both induce burst firing (by an action on the NMDA receptor) if applied by microiontophoresis (*6, 14*). They are present in millimolar and micromolar concentrations, respectively (*15*).

In animal models of epilepsy APH is a potent anticonvulsant (as potent as diazepam when administered intraventricularly and as potent as valproate when given intravenously) (*16*). Its protective action against ischemic neuronal changes may thus have two components, an immediate local effect blocking excitatory action on the NMDA receptor and an anticonvulsant action preventing the spread of burst discharges or epileptic activity. Both actions have the effect of diminishing intracellular calcium accumulation during the reperfusion phase (*5*). Thus our findings support the concept that excitatory synaptic activity and associated enhanced entry of calcium contribute to selective neuronal vulnerability to ischemia (*5, 17*).

Use of drugs in the prophylaxis and therapy of ischemic brain damage has been the object of extensive experimentation (*8*). The main finding to date has been a modest protective action of barbiturates in some animal models of focal cerebral ischemia (*18*). The mechanism of this protection remains hypothetical, but may involve changes in inhibitory and excitatory transmission (*19*). Our results indicate that pharmacological

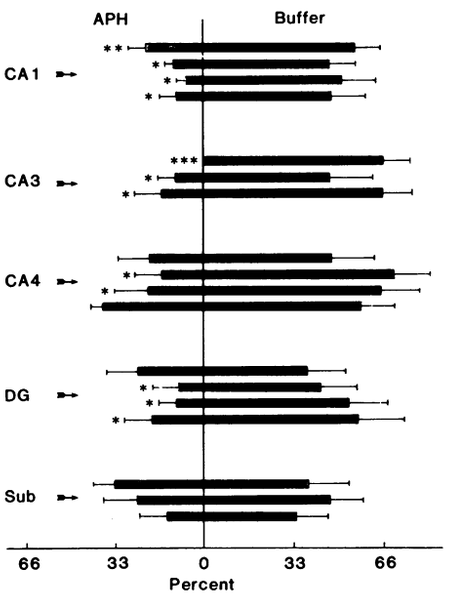

Fig. 1. Protection by APH against ischemic cell change in rats (n = 7). Before ischemia one hippocampus was infused with APH and the contralateral hippocampus with buffer alone. The bars represent mean percentages (+ standard errors) of neurons showing ischemic damage (scored on a three-point percentage scale) for the indicated type of cell (DG, dentate granule cells; Sub, pyramidal neurons of subiculum) (*20*). The arrows indicate the injection site (4.5 mm anterior to the intra-aural line, 2.0 mm lateral to the midline, and 3.5 mm below the cortical surface). Bars above arrows refer sequentially to histological sections 4.7 and 5.7 mm anterior and bars below arrows to sections 3.7 and 2.7 mm anterior. Statistically significant differences between APH- and buffer-infused hippocampi are indicated by asterisks: (*) $P < 0.5$, (**) $P < 0.01$, and (***) $P < 0.001$ (paired two-tailed t-test).

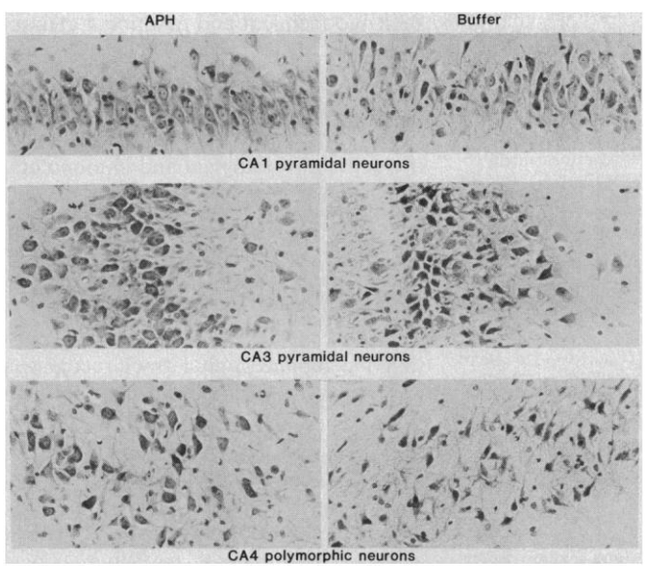

Fig. 2. Representative cytological changes in dorsal hippocampus 5.7 mm anterior to the intra-aural line (cresyl violet, ×250). The left hippocampus was injected with APH and the right with buffer 5 minutes before 30 minutes of ischemia and 2 hours of reperfusion. The injection site was 1.2 mm posterior to the section from which these photomicrographs were taken. Nearly all cells treated with APH were normal. Ischemic changes (condensed hyperchromatic cyto- and karyoplasm) were seen in cells from buffer-injected hippocampi.

blockade of a subclass of excitatory receptors may be a valuable approach to therapy in brain ischemia.

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10. APH was dissolved in 0.1 ml of 1*M* NaOH and brought to *p*H 7.4 with 0.1*M* sodium phosphate buffer (total volume, 1 ml).
11. Perfusion fixation was achieved with a modified Karnovsky solution containing 2 percent glutaraldehyde and 2.8 percent paraformaldehyde. Perfusion was done at a pressure of 120 torr for 10 minutes. The brain was removed after 24 hours and processed for paraffin embedding. Serial sections (6 μ*M*) were cut, with every fifth section being stained with hematoxylin and eosin or cresyl violet. The occurrence of ischemic damage in neurons in each zone of the hippocampus was rated on a percentage scale by a single observer (T.G.) under blind conditions. Anteroposterior variation in severity of neuropathology was minimal within a 3- to 4-mm block, so the sections studied (at 1-mm intervals) were representative of the dorsal hippocampus.
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Transglutaminase-Mediated Modifications of the Rat Sperm Surface in Vitro

Abstract. *Two transglutaminase-mediated modifications of the rat epididymal spermatozoon surface were demonstrated in vitro. Transglutaminase was effective in promoting the binding of spermidine to the sperm. Moreover, the enzyme, by reacting with one of the major proteins secreted by the rat seminal vesicle epithelium, produced a modified form of the protein with a higher molecular weight and the capability of binding to the sperm cells. A specific physiological role for the enzyme, bringing about modifications of the rat sperm surface in the seminal fluid environment, is suggested.*

The mammalian male gamete acquires its basic differentiated phenotype in the testis; however, numerous subsequent adjustments in its molecular architecture are required for complete maturation. The various microenvironments encountered by spermatozoa during their journey to the uterine tube appear to play an important role in modifying many of their biochemical and functional characteristics (*1*). Chemicals (enzymes, proteins, salts, and small organic molecules) in the epididymal fluid (*2*), by interacting with the sperm surface, markedly change the cell's physiological and immunological properties (*3*). Epididymal sperm cells acquire both the potential ability to move (*1*) and definite antigenic surface characteristics (*4*). At this point a biological problem arises: the antigenic properties of the epididymal sperm are such that the female genital tract recognizes them as nonself, and the male gamete faces the risk of immunological rejection (*3*).

The chemical environment surrounding the epididymal sperm cells changes again after ejaculation (*1*). In a matter of seconds they become suspended in a new medium resulting from the mixing of secretions from the accessory glands of the male reproductive tract. Many of the chemicals present in this medium are believed to have trophic and protective effects on the sperm cells (*1, 2*). The exact function of many of these factors is not known. At this stage the sperm, although capable of successfully facing the immunocompetence of the female genital tract, are still incompetent to fertilize a mature oocyte (*1*). The factors responsible for capacitation probably act by binding to the sperm plasma membranes. Recent data (*4*) suggest that a rat seminal vesicle secretory protein (molecular weight, 50,000) acts as a sperm-coating antigen. The immunosuppressive properties of rabbit seminal fluid have been referred (*3*) to transglutaminase (TG)–mediated binding of uteroglobin, a secretory protein of prostate origin. This points to Ca^{2+}-dependent TG activity, present in great amounts in mammalian prostate secretions, as an important factor in the molecular mechanism of sperm surface maturation (*5–7*). It is already known that this enzyme plays a role in the formation of the copulatory plug in rodents (*8*).

In rats one of the major proteins se-