Brain Enzyme Is the Target of Drug Toxin

A chemical known as MPTP causes a Parkinson-like state in humans and monkeys; biochemical and autoradiographical studies are closing in on the mechanism

Just over a year ago researchers at Stanford University School of Medicine identified a contaminant in a locally produced "synthetic heroin" that induced a Parkinson-like state in some of those unfortunate enough to use the preparation (*1*). The contaminant, known as MPTP, appears selectively to destroy the same small group of brain cells—those of the substantia nigra—that degenerate in naturally occurring Parkinson's disease. That discovery has stimulated a tremendous revitalization of inquiry into the disease, both at the basic level of its cause and into possible new therapeutic regimes (*2*).

Central to all this activity, the results of which have recently been published or are just about to appear in several different journals, is the identity of the toxin, the mechanism of the highly specific nerve cell damage, and the relation of MPTP toxicity to the cause of the natural disease. The latest information on the metabolism of MPTP was strongly featured at a symposium at a meeting of the American Society for Pharmacology and Experimental Therapeutics held in Indianapolis on 21 August.

What is now clear from a broad convergence of experiments is that MPTP is a substrate for the enzyme monoamine oxidase (type B), which yields two identifiable metabolites, both of which are potentially toxic. What is not clear is which of the two products is the culprit and how the damage is inflicted. There is, however, a set of hypotheses emerging. In addition, these discoveries are encouraging an evaluation of the therapeutic use of monoamine oxidase inhibitors in Parkinson's disease in the United States. (Several European countries already follow such a regime.)

When, early in 1983, William Langston and his Stanford University colleagues identified MPTP, or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, as the potential neurotoxic contaminant of "synthetic heroin" they initiated a large series of investigations, including an inquiry into the metabolism of the substance in squirrel monkeys. Langston, a neurologist, and Ian Irwin, a chemist, worked on the hypothesis that the pyridine ring would be oxidized, thus yielding the positively charged pyridinium ion, MPP^+. A similar reaction scheme had been described two years earlier for certain dihydropyridines by Nicholas Bodor and his colleagues at the University of Florida.

The prediction proved to be correct, but the Stanford team was not alone in its search. Sanford Markey and his colleagues at the National Institute of Mental Health, Bethesda, were deeply involved in similar research. They developed the first "monkey model" of Parkinson's disease using MPTP (*3*) and were able to make the first public announcement of the identification of MPP^+ as the in vivo metabolite of MPTP at a December 1983 meeting of the American College of Neuropsychopharmacology in Puerto Rico. Langston and his colleagues later published the same conclusion (*4*).

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Meanwhile, Neal Castagnoli and Anthony Trevor of the University of California, San Francisco (UCSF), had begun scrutinizing MPTP metabolism using in vitro systems. Initially, they chose liver homogenates as the principal reaction mixture, with brain homogenates included out of interest but with little expectation that they would yield much. Surprisingly, the brain homogenates did work, and experimental honing stripped the reaction mixture down to mitochondrial suspension from which an oxidized product was isolated.

Castagnoli and Trevor thought the product was the dihydropyridinium compound, which they had predicted. However, conversation with Markey, whose earlier work they were unfamiliar with at the time, convinced them that it was instead the pyridinium ion, MPP^+. In any case, here was in vitro confirmation of what Markey, and later Langston, had found in vivo: that MPTP is rapidly oxidized to the highly polar product, MPP^+, which is relatively stable.

Through the application of chemists' logic, and the inspired experiments of their graduate student Kan Chiba, Castagnoli and Trevor were soon able to report that the enzyme that was metabolizing MPTP was monoamine oxidase and that it was a type B oxidase, because its action was blocked by pargyline (*5*). In a preliminary experiment completed just days before the August Indianapolis meeting, Castagnoli and his colleagues seemed to nail down the metabolic niche of MPTP, by showing that the drug is a substrate for purified human monoamine oxidase B at a rate comparable with natural substrates, such as dopamine.

These demonstrations, which could not have been predicted by a biochemist familiar with the normal substrates of monoamine oxidases, set in train a series of important investigations.

For instance, the NIMH researchers began dissecting the metabolism of MPTP in monkeys, which respond like humans to doses of the drug, and in rats, which do not, in relation to the distribution of monoamine oxidase in these animals' brains. Uptake of MPTP appears to be the same in both animals, but the metabolic product, MPP^+, persists in monkeys while it disappears fairly rapidly in rats. One possibility is that a difference between monkeys and rats in the cellular distribution of monoamine oxidase A, which does not metabolize MPTP, and monoamine oxidase B, which does, might account for these observations and hence for the differential toxicity between the animals. Markey and his colleagues are due to publish details of their work on metabolism, including ideas on the identity of the true toxin, in a forthcoming *Nature*.

When Richard Heikkila of Rutgers University heard from Castagnoli about the blocking of MPTP metabolism by the monoamine oxidase inhibitor pargyline, it prompted him and his colleagues to do "the obvious experiment." Earlier in the year he and his two colleagues Arthur Hess and Roger Duvoisin had shown that, somewhat to most people's incredulity, mouse brains appear to be simi-

larly susceptible to MPTP insult as human brains: levels of dopamine decrease in the neostriatum and cells of the substantia nigra are lost (*6*). The obvious experiment was to dose the animals with pargyline before administering MPTP, in the expectation that the neurons might be protected. They were. The result is soon to appear in *Nature*.

Langston heard about Castagnoli's pargyline work from Heikkila, and, after first consulting with the UCSF workers, proceeded with the obvious experiment in squirrel monkeys, results of which are published in this issue of *Science* (page 1480). As expected, by inhibiting the activity of monoamine oxidase B, pargyline blocks the conversion of MPTP to MPP^+ and prevents brain damage. The buildup of MPTP in potentially susceptible areas of the brain unequivocally demonstrates that MPTP itself is not the toxic agent and that monoamine oxidase B activity is required for toxicity to develop.

Heikkila and his colleagues have probed further on the nature of MPTP as a substrate for monoamine oxidase by measuring the reactivity of almost a dozen structural analogs of the drugs. None of the analogs—made by removal or modification of the methyl group or the benzene ring—is as metabolically fitted to the enzyme as MPTP itself, an observation that is confirmed by separate work at Stanford and other laboratories. It is one of those cruel turns of fate that MPTP, the accidental contaminant of "synthetic heroin", is the most potent substrate of this important brain enzyme.

In parallel with all this work on the metabolism of MPTP have been various investigations into the sites at which MPTP binds in the brains of rats, mice, monkeys, and humans. When Jonathan Javitch, George Uhl, and Solomon Snyder of Johns Hopkins University School of Medicine produced autoradiographs of rat and human brain slices treated with radioactive MPTP, they had in mind that distribution of the drug might shadow that of some known transmitter—a very natural expectation (*7*). In their original publication, Langston had speculated on the possibility that MPTP might be hitting the binding site of a normal brain chemical, an "endogenous MPTP".

First, Snyder and his colleagues noted that MPTP binds with high affinity to areas known to be destroyed in people who take the drug, which was anticipated. But there is also some binding in normally unaffected areas, which is something of a puzzle. Most puzzling

First Look at the Deepest Hole

The first foreign visitors ever to visit the site of the Soviet superdeep Kola borehole were mightily impressed. "It is a stupendous technological achievement having no equal outside the U.S.S.R.," says Wilfred Elders of the University of California at Riverside, one of five Americans in the international party of about 45 that visited the hole last month. The Americans at least also voiced one disappointment. "It's not apparent that they've got much science out of it yet," says one visitor. Disappointing or not, the emphasis for the time being seems to be on technology and drilling.

One of the more impressive Soviet achievements is that their drillers have reached a depth of 12,063 meters without a single major problem. The relatively benign drilling conditions and the extreme care exercised by Soviet drillers have helped ensure such a spotless record. Such care does not ensure great speed. The drill bit can make 2 to 3 meters of headway for every hour of drilling, and an exceptional system of drill rig automation allows complete retrieval and reinsertion of 12 kilometers of drill pipe in 18 hours ("which is really hopping," according to one visitor). But 14 years have passed since drilling began and, according to one report, only 65 meters have been added since last December. American drillers contemplating a proposed 10-kilometer hole in the southern Appalachians are allowing only 3 years of drilling, which assumes that all will go well (*Science*, 29 June, p. 1418).

The recovery of rock samples from the entire length of the Soviet hole is another notable achievement. The effort to recover a complete core sample during drilling has returned samples of as much as 60 to 80 percent of the rock drilled, although recoveries during continuous coring at 12 kilometers are apparently about 20 percent. Some reports from visitors hold that 8 kilometers of rock core are now in hand. The effort to core continuously is impressive in itself, and the present rate of recovery in such hard rock would be respectable even at much shallower depths. Aside from providing bountiful material for crucial analyses, such as age determinations, the ambitious coring will simplify interpretation of results from instruments, called logging tools, that are lowered down the hole to record physical and chemical properties of the rock. American plans for deep-hole coring remain vague, but preliminary thinking has included coring during only about 10 or 20 percent of the drilling instead of 100 percent.

Those who heard about the Kola hole at the International Geological Congress in Moscow or traveled to the site on the postmeeting field trip came away with a high regard for Soviet technology and the Soviets' sheer determination to drill deeply, but they gathered disappointingly few details of the resulting scientific findings. "Everybody felt that we didn't get any details, any facts," says M. E. Bickford of the University of Kansas. That was despite the openness of their hosts and the obvious centerpiece position of the Kola hole at the meeting. Everyone had been intrigued by early reports of strange activity deep in the hole (*Science*, 29 June, p. 1420). But how were the great depths of reported fluid and gas influx determined? What geophysical techniques were used to survey the area? What are the interpretations of the 18 different logs used in the hole? All the uncertainty left many of the visitors assuming that much of the scientific work remains to be done as the technology and the drilling of the hole continue to be pushed ahead.

American scientists are eager to cooperate in the analysis of the cores. They made overtures at the meeting and will pursue the possibility through international channels, but the Soviets have not yet revealed their attitude toward cooperative analysis. If their ambitious plans are any indication, they may have their hands full in their own backyard. The second superdeep hole at Saatly near the Caspian Sea stands at 8260 meters following some drilling difficulties and, contrary to recent reports, will be deepened toward crystalline basement at about 11 kilometers. A web of geophysical profiles will connect 11 deep and superdeep boreholes as part of an effort to elucidate the geology of the U.S.S.R. and identify new mineral resources.

—RICHARD A. KERR

overall, however, was the observation that the binding pattern did not conform to the distribution of any known neurotransmitter receptor. Perhaps the binding site was the receptor of a yet to be discovered neurotransmitter?

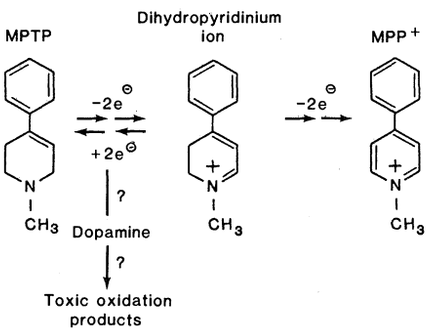
While reserving this as a possibility, the Johns Hopkins group also noted the observation passed onto them by Heikkila that monoamine oxidase inhibitors protected mice against MPTP toxicity. They already knew that membrane-bound enzymes can act as receptor sites and offered the possibility that this enzyme may be the target of MPTP in their brain slices. Like others at this time, Snyder and his co-workers began to think of MPTP as a possible inhibitor—not a substrate—of monoamine oxidase, which, if true, could possibly have explained the drug's disruptive effect on certain dopamine-utilizing cells.

This was certainly the approach being developed by Thomas Rainbow,† Bruce Parsons, and Caroline Wieczorek of the University of Pennsylvania. In two short papers they recorded the distribution of radioactive MPTP in rat brain sections and their recognition that this coincided with the distribution of monoamine oxidase (*8*). They concluded that MPTP inhibition of monoamine oxidase would lead to a potentially harmful buildup of brain monoamines. When they later heard about the blocking of MPTP transformation to MPP^+ by pargyline, they realized that the drug was indeed a substrate for the enzyme, not merely an inhibitor. Rainbow and his colleagues subsequently repeated their MPTP binding experiments with human brain tissue and were beginning to focus on the identity and mode of action of the elusive neurotoxin, which is the goal of much of this burgeoning research field.

With MPTP itself eliminated as a direct cause of the nerve damage, one obvious suspect is the stable product of oxidation, MPP^+, which is a highly polar substance of considerable potential toxicity. Other possibilities include intermediate oxidation products generated during the conversion of MPTP to MPP^+.

Because of its high polarity, MPP^+ cannot cross the blood/brain barrier if it is administered to an animal. More direct approaches have to be taken to test its neurotoxic potential. For instance, in preliminary experiments Heikkila has introduced MPP^+ directly into the neostriatum of mice, which induces the same kind of dopamine reduction seen with MPTP dosage. The question of how selective this toxicity is remains to be established.

William Nicklas, a colleague of Heikkila's, finds that MPP^+ is rather toxic to mitochondria in suspension: respiration and therefore oxygen uptake are depressed. There is some hint in preliminary work that mouse mitochondria are more susceptible to this effect than are mitochondria from rats, which could explain the differential toxicity of MPTP between these animals. Nicklas also plans to test mitochondria from different areas of the mouse brain, which might display different vulnerabilities.



Metabolic profile of a neurotoxin

MPTP is a substrate for the brain enzyme monoamine oxidase (type B), which converts it to the dihydropyridinium ion. This product can be further oxidized to MPP^+ or revert to MPTP, thus forming a powerful oxidation center. This oxidation center might convert dopamine to toxic oxidation products, which might destroy certain brain cells.

Two other workers, Catherine Mytilineou and Gerald Cohen of Mount Sinai School of Medicine, New York, also point an accusing finger at MPP^+. They recently developed a tissue culture preparation of rat brain, which responded just like the intact brain when MPTP was added (*9*). Exploiting the direct access allowed by tissue culture, they are now testing the affect of MPP^+. They find again in preliminary experiments that they can get selective toxicity with MPP^+ at dosage levels considerably less than is required for MPTP. Addition of larger quantities of MPP^+ inflicts general, nonselective, tissue damage.

So, has the obvious suspect been pinned down? Langston thinks not, because MPP^+ is widely distributed in the body of an MPTP-dosed animal, whereas cell damage is highly localized. And Castagnoli thinks not, because, as chemist, he is intrigued by the very high reactivity of the intermediates in the MPTP conversion.

Castagnoli and his UCSF colleagues have recently characterized the major intermediate in the MPTP oxidation as the dihydropyridinium compound. Unlike MPP^+, this molecule has a half-life measured in minutes rather than days and readily takes part in electron exchange—redox—reactions. In addition to proceeding to the stable oxidation product, the dihydropyridinium compound can, under favorable conditions, revert to MPTP, thus forming a powerful oxidation center. One possible substrate for this oxidation reaction is dopamine, which is in high concentration in brain areas affected in drug-induced Parkinson's disease. Now, it happens that many of the oxidation products of dopamine are potential neurotoxins.

Therefore, in Castagnoli's hypothesis—so far untested—the toxic agent in MPTP-induced parkinsonism is a cocktail of dopamine oxidation derivatives, with the dihydropyridinium compound acting as a catalyst in their formation.

In any search for the potential parkisonian agent, the focus must always close in on the selectivity of destruction. None of the studies on distribution and metabolism of MPTP in themselves solve this puzzle, because there are many brain localities that assimilate the drug without lasting harm. Presumably, there is something peculiar to the substantia nigra, which makes it especially susceptible. Is it simply the very high level of dopamine produced there, which is not a unique feature of this tissue? Or is it this property in combination with the accumulation there of the dark pigment neuromelanin, which is a dopamine oxidation product?

If consideration of the esoteric chemistry of MPTP really does point a finger at one of the body's most improbable oxidation products—neuromelanin—then this would resurrect an idea only recently abandoned that this dark pigment is the key to the disease. Moreover, the research activity that has been focusing on monoamine oxidase and its metabolism of potential inimical substrates is prompting people to consider similar patterns in other degenerative neurological conditions, such as Alzheimer's disease—**ROGER LEWIN**

†Dr. Rainbow was killed in an automobile accident on 6 September.

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