Age Factors Loom in Parkinsonian Research

A drug model for Parkinson's disease becomes an even better representation of the natural condition when age is considered

By an odd and unpredictable quirk of fate, the sloppy manufacture of a "synthetic heroin" 4 years ago by a northern California amateur chemist transformed research in Parkinson's disease, changing what had for some years been a relatively quiescent and unproductive exercise into an exploding and fertile field of activity.

The drug, which reached a large number of users in the San Francisco Bay area, turned out to contain a contaminant—known as MPTP—that is a highly specific neurotoxin for the primary area in the brain that degenerates in natural Parkinson's disease. The immediate result was a rash of young patients who exhibited many of the classic symptoms—such as tremor and muscle rigidity—of parkinsonism. Researchers in many laboratories in this country and in Europe then very quickly began to give MPTP to laboratory animals in the hopes of generating, for the first time, a good animal model for Parkinson's disease.

During the past few months, those hopes seem to have come close to fruition. "Not only is there nothing better than the MPTP-induced condition as an animal model for Parkinson's disease," observes Donald E. Redmond of Yale University, "but there are extremely few animal models of human disorders that come as close to fulfilling the criteria for an animal model as this one does." Donald Price, of The Johns Hopkins University School of Medicine, agrees, and describes the MPTP system as "an extraordinarily good model for the motor deficits of Parkinson's disease."

Price also says that the MPTP-induced condition in animals permits a whole range of investigations that until now were virtually beyond experimental reach. These include trying to understand mechanisms by which the nerve cells are killed, exploring conditions for regeneration in the central nervous system, developing new approaches to stereotactic surgery for the relief of some of the movement symptoms and new strategies for drug therapy, and, most dramatic of all, testing techniques for grafting nerve cells into the parkinsonian brain. "I am a firm believer in the extraordinary usefulness of the MPTP model," says Price.

The MPTP parkinsonian model looked good from the beginning, but it had some obvious shortcomings. Most significant among these was the extent of brain damage

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inflicted by MPTP as compared with the natural disease condition. Specifically, although the key brain area—the substantia nigra—appeared to be targeted by MPTP in both humans and experimental animals, the toxin apparently did not touch at least two other brain areas that are frequently affected in natural Parkinson's disease. Now, in what must rate as one of the most important recent advances in this rapidly advancing field of research, that gap in the model has been more or less closed.

Two research groups have independently produced brain pathologies in MPTP-treated experimental animals—squirrel monkeys in one case and mice in the other—that mimic very closely what is seen in the natural human disease. The result of this extension of the model is twofold. First, researchers who are working with, for instance, potential brain graft systems can be much more confident about the fidelity of what they see. This is particularly important because a lot of investigators are jumping into this most dramatic aspect of the new phase of parkinsonian research.

Second, these new data seem to add considerable support to the increasingly popular notion that natural Parkinson's disease is caused by some kind of environmental toxin, perhaps by MPTP itself or something very similar to it. As Lysia Forno, William Langston, and their colleagues noted when they published their recent results on squirrel monkeys, "any agent capable of inducing all these changes (in the brain) would also deserve serious consideration as a possible etiological agent."

Wild as it might seem, this latter suggestion might just turn out to be correct. For instance, it has been known for some time that one activity that correlates negatively with the incidence of Parkinson's disease is cigarette smoking, which suggests that there might be something in cigarette smoke that protects the brain against environmental toxins. One such possibility is 4-phenylpyridine, or 4PP, which Ian Irwin, Louis DeLanney, and Langston of the Institute for Medical Research, San Jose, recently showed protects mice to some degree against MPTP. The protection appears to derive from the fact that 4PP blocks the enzymic conversion of MPTP to the substance known as MPP^+, which so far is most people's best candidate for the agent that actually kills neurons once MPTP gets into the brain.

A second, and at present extremely preliminary, piece of evidence along these lines comes from Sanford Markey and his colleagues in the National Institute of Mental Health, Bethesda. Using rabbits, they have raised antibodies against both MPTP and MPP^+, with the idea of screening parkinsonian patients for candidate toxins. Markey reported at the recent Neuroscience meeting* in Washington, D.C., that in one case he gets an antibody reaction that suggests the presence of MPP^+ in the brain of a parkinsonian individual. Moreover, the antibody reaction is found in the substantia nigra, but not in other parts of the brain. Markey is quick to point out that this is just one result, and a preliminary one at that. "But," he says, "it is very intriguing."

Although most authorities involved in the research would bet against MPTP being the culprit in natural Parkinson's disease, there is now a growing momentum to establish some thorough epidemiological studies. Once patterns of disease incidence are better outlined, analytical epidemiology might be able to home in on the causative agent or agents. Markey and his colleagues plan to use their antibodies in such an exercise.

It is worth noting that just as the idea for an environmental toxin is taking hold for Parkinson's disease, other neurodegenerative diseases are beginning to be viewed with the same idea in mind. For instance, Donald Calne of the University of British Columbia and three colleagues suggest in the current issue of *The Lancet* that in

*Society for Neuroscience, 16th Annual Meeting, 9–14 November.

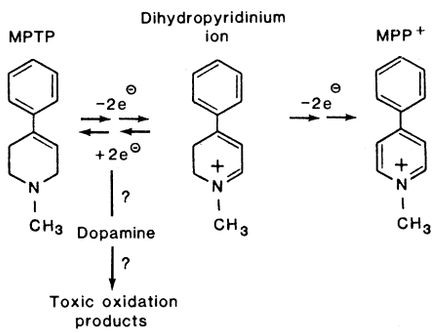
addition to the case for Parkinson's disease, environmental agents might be involved in the cause of Alzheimer's disease and motoneuron disease. They also cite aging as a common theme in these three conditions, and this has also been a key factor in the most recent developments in Parkinson's disease research.

Because of normal laboratory practice, most of the animals that have been used in producing the MPTP models have been relatively young. And it was in these experiments that the "incomplete" neuropathology would be generated. From time to time, however, older animals just happened to be included in experimental series, and when they were there sometimes was a difference in their response to drug treatment. For instance, older animals were occasionally noticed to suffer a more severe neuropathology than younger animals and were more likely to die from acute toxicity. It wasn't until just a year ago, however, that any systematic analysis was done on the effect of age on sensitivity to MPTP. It proved to be crucial, both in generating a more faithful animal model of the human disease and in understanding more completely the disease itself.

Madi Gupta and her colleagues at the University of Rochester Medical School recently reported that older mice are behaviorally affected more severely than young mice when treated with the same dose of MPTP. They also see greater signs of damage in the substantia nigra and other related areas of the brain in older mice. Actual cell loss in these regions outside the nigra has not yet been demonstrated, however.

Langston and his colleagues have similar results on mice to those of the Rochester group, in addition to their data on aged squirrel monkeys. One of the most striking aspects of the work with monkeys is the appearance in the older MPTP-treated animals of prominently staining inclusions in cells of the substantia nigra and related areas. They are potentially significant because one of the hallmarks of neuropathology of true Parkinson's disease is the presence of so-called Lewy bodies in certain key populations of cells. Nothing like Lewy bodies has been seen before in the MPTP-induced animal models of the disease, and for many neuropathologists the presence of the particles in Langston's parkinsonian squirrel monkeys is a real clincher. "It is very similar to the human condition," observes Asao Hirano of the Montefiore Hospital, New York. "This is very important."

If, as now seems certain, older neurons are more vulnerable to exposure to MPTP, the question of what causes the greater sensitivity must be addressed. There is in addition the issue of what this implies for the natural disease condition. For instance, if the disease is caused by an environmental toxin, is there continuous exposure throughout life, which produces a gradual attrition of the substantia nigra and related regions? Or do the nigral cells become more sensitive as they age, and thus succumb more readily when exposure to the toxin occurs?



Metabolic profile of a neurotoxin. *MPTP is enzymically converted to the dihydropyridinium ion by monoamine oxidase B. This intermediate can then be further oxidized to MPP^+ or revert back to MPTP, thus generating a powerful oxidation center. MPP^+ has been shown to be avidly taken up by mitochondria, where it very effectively blocks respiration. Although it is not clear how this system might lead to the selective toxicity in the substantia nigra and related brain areas, MPP^+ is most observers' best candidate for the ultimate toxin.*

The question of what underlies increased vulnerability to MPTP is turning out to be quite complex, but there are patterns emerging. For instance, it seems that, as Langston and his colleagues have recently shown, older neurons in mice lose some ability to recover from exposure to MPTP, which is consistent with the idea that the cells become more sensitive with increased age. However, it is now also clear that older brains have an enhanced efficiency for converting MPTP to MPP^+. For any particular dose of the toxin, older animals will therefore experience a greater exposure to the candidate toxin, MPP^+. This almost certainly is involved in the greater neuropathology in older animals, but may not be the whole answer.

Another potential factor, as Solomon Snyder and his colleagues at The Johns Hopkins University School of Medicine emphasize, is the steady accumulation of the pigment neuromelanin in aging nigral and associated cells. Neuromelanin avidly binds MPP^+, and therefore may cause the cells containing the pigment to self-destruct. Langston describes the issue as "still an open question."

Next to the work on age-related sensitivity, exploration of brain graft possibilities rates as a hot topic these days. However, what the subject clearly enjoys in terms of dramatic appeal it seems as yet to lack in hard scientific evaluation, as several presentations at the recent Neuroscience meeting demonstrated.

At least half a dozen research groups in this country are exploring transplantation as a potential therapy. They are following in the footsteps of Swedish researchers who recently attempted without much beneficial effect to implant adrenal tissue, which is a source of dopamine, into the brains of parkinsonian patients. Most of the experimental work on animals, however, is employing fetal brain tissue for transplantation. "Scientifically it is a better bet to use fetal brain tissue," says Redmond, "but it has clinical problems that adrenal autografts would not have, including ethical problems."

Redmond and his colleagues at Yale and at the University of Rochester reported on brain grafts in three African Green monkeys at the Neuroscience meeting. By accident, says Redmond, they obtained something of a dose-response curve. One animal, which appeared to have a well-placed and well-developed graft, recovered its level of dopamine metabolites to within 5% of the pre-MPTP-treatment baseline. Its behavior was said to be almost normal. In a second animal two of the three transplant plugs had become dislodged, and the dopamine metabolites and behavioral levels were similarly deficient. A third animal lost its graft completely and showed no recovery at all.

Although on the face of it these results seem promising, they are very preliminary. Curt Freed, of the University of Colorado, cautions against inferring anything very definite about any of the transplant results available so far, including his own on bonnet monkeys and Redmond's. The key problems for researchers are to be certain about the viability of the graft and to ensure that they know how much natural recovery from MPTP exposure an animal is likely to have before conclusions are drawn about putative benefits of transplants.

"I would have to agree with anyone who said that the study was not adequately controlled," concedes Redmond. "The only way to answer this question properly is to have animals with matched MPTP deficits, half of which would be transplanted with fetal brain tissue while the rest would have a transplant of some inappropriate tissue," says Redmond. Such an experimental series is already established in Redmond's group.

If controlled experimental transplantation does bring consistently favorable results, it could lead to dramatic therapy. A number of groups are eager for a chance to try. ■

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