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Developmental Exposure to the Pesticides Paraquat and Maneb and the Parkinson's Disease Phenotype

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Abstract

Idiopathic Parkinson's disease (PD) is associated with advanced age, but it is still unclear whether dopaminergic neuronal death results from events initiated during development, adulthood, or represents a cumulative effect across the span of life. This study hypothesized that paraquat (PQ) and maneb (MB) exposure during critical periods of development could permanently change the nigrostriatal dopamine (DA) system and enhance its vulnerability to subsequent neurotoxicant challenges. C57BL/6 mice were treated daily with saline, 0.3 mg/kg PQ, 1 mg/kg MB or PQ + MB from post-natal (PN) days 5 to 19. At 6 weeks, a 20% decrease in activity was evident only in the PQ + MB group, with a further decline (40%) observed at 6 months. A subset of mice were re-challenged as adults with saline, 10 mg/kg PQ, 30 mg/kg MB, or PQ + MB 2× a week for 3 weeks. Mice exposed developmentally to PQ + MB and re-challenged as adults were the most affected, showing a 70% reduction in motor activity 2 weeks following the last re-challenge dose. Striatal DA levels were reduced by 37% following developmental exposure to PQ + MB only, but following adult re-challenge levels were reduced by 62%. A similar pattern of nigral dopaminergic cell loss was observed, with the PQ + MB treated group exhibiting the greatest reduction, with this loss being amplified by adult re-challenge. Developmental exposure to PQ or MB alone produced minimal changes. However, following adult re-challenge, significant decreases in DA and nigral cell counts were observed, suggesting that exposure to either neurotoxicant alone produced a state of silent toxicity that was unmasked following adult re-exposure. Taken together, these findings indicate that exposure to pesticides during the PN period can produce permanent and progressive lesions of the nigrostriatal DA system, and enhanced adult susceptibility to these pesticides, suggesting that developmental exposure to neurotoxicants may be involved in the induction of neurodegenerative disorders and/or alter the normal aging process.

Keywords: Parkinson's disease; Development; Substantia nigra; Pesticides; Striatum

INTRODUCTION

Parkinson's disease (PD) is typically considered an aging-related neurodegenerative disorder given its typical onset after 60 years of age and subsequent progression. This pattern of manifestation of signs

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and symptoms later in life is not, of course, necessarily indicative of the timing of etiological factors. In fact, one possibility that can be posited is that PD could arise from events that occur early in development that have long-term but delayed adverse consequences for the nigrostriatal dopamine (DA) system. Possible developmental events could include exposures to environmental neurotoxicants. Under such scenarios, it is conceivable that the population of nigrostriatal DA cell bodies is reduced early in life, and that with normal aging-related loss, the DA system will eventually reach levels associated with PD. Alternatively, or in conjunction with such a model, is the possibility that nigrostriatal DA system damage early in development, whether or not it is associated with cell body loss, nevertheless renders the system more vulnerable to subsequent environmental risk factors associated with PD, effectively increasing their potency, resulting in PD that might not otherwise have occurred.

Environmental risk factors have, in fact, long been implicated in the etiology of Parkinson's disease (PD). Several epidemiological studies report an increased incidence of PD in association with pesticide exposures and associated conditions that include well water drinking, farming, and rural living (Gorell et al., 1998; Semchuk et al., 1992; Tanner, 1989; Tanner et al., 1987). The increased prevalence of PD in industrialized countries and its geographic heterogeneity have also been suggested to be the result of the greater use of environmental chemicals (Li et al., 1985; Morens et al., 1996; Schoenberg et al., 1985; Schoenberg et al., 1988). The potential for environmental risk factors to contribute to PD gained particular attention following the report of a study of 19,000 pairs of twins, the largest of its kind, by Tanner and colleagues (Tanner et al., 1999) citing no difference in PD rates between monozygotic and dizygotic twins with onset of PD after age 60.

Our laboratory recently established a model of environmental Parkinsonism in adult mice that resulted from repeated combined exposure to the herbicide/dessicant paraquat (PQ) with the ethylenebisdithiocarbamate fungicide maneb (MB), both of which are known to adversely impact DA systems. These exposures produced selective nigrostriatal DA system neurotoxicity, including loss of striatal DA and of cell bodies of DA neurons in the substantia nigra pars compacta (SNpc; Thiruchelvam et al., 2000a; Thiruchelvam et al., 2000b). Compared to the commonly used neurotoxicant model MPTP, combined PQ + MB produces permanent effects. While the mechanism of toxicity of MPTP is relatively well understood, those underlying the selective nigrostriatal DA neurotoxicity of PQ + MB remain to be determined. PQ is a free radical generator, due to its ability to redox cycle, and is commonly used experimentally as an oxidative stressor (Dey et al., 1990; Woolley et al., 1989). While previous studies report somewhat equivocal effects of systemically-administered PQ on the nigrostriatal system, new findings show that PQ alone, even at very low doses, can produce DA cell loss in the substantia nigra and increase expression of alpha-synuclein (Manning-Bog et al., 2002; Thiruchelvam et al., 2002). Less is known about the mechanism of neurotoxicity of MB, but it has been reported to inhibit glutamate transport and disrupt DA uptake and release (Vaccari et al., 1998; Vaccari et al., 1999; Vaccari et al., 1996).

The susceptibility of the developing nervous system to degeneration following exposure to environmental toxicants is well recognized. Exposure to neurotoxicants such as 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), polychlorinated biphenyls (PCBs), and pyrethroids produce permanent behavioral changes as well as neurochemical changes in the cholinergic system in the CNS of adult animals when administered even at low doses during critical periods of development (Ahlbom et al., 1994; Ahlbom et al., 1995; Eriksson et al., 1992; Eriksson and Fredriksson, 1991a; Fredriksson et al., 1993a). Developmental exposure to PQ or MPTP also produce permanent changes in striatal DA and behavior in the adult animal (Fredriksson et al., 1993b; Ochi et al., 1991; Perez-Otano et al., 1992; Weissman et al., 1989). Effects of developmental exposure to MB have been studied, but its effects on the DA system per se have not been examined (Bancroft and Prahlad, 1973; Chernoff et al., 1979; Sobotka et al., 1972).

The dopaminergic system develops both pre- and postnatally (PN), with receptor development and the brain growth spurt occurring predominantly in the PN period (Giorgi et al., 1987; Voorn et al., 1988). The hypotheses posed here were that developmental exposure to either PQ or MB alone, or in combination, would result in permanent nigrostriatal DA system neurotoxicity, and, secondly, would render the nigrostriatal DA system more susceptible to environmental chemical challenges later in life.

EXPERIMENTAL PROCEDURES

Animals and Drug Administration

C57BL/6 male mice were injected i.p. with either vehicle (saline), paraquat dichloride hydrate (PQ, Sigma, St. Louis, MO) at a dose of 0.3 mg/kg, MB

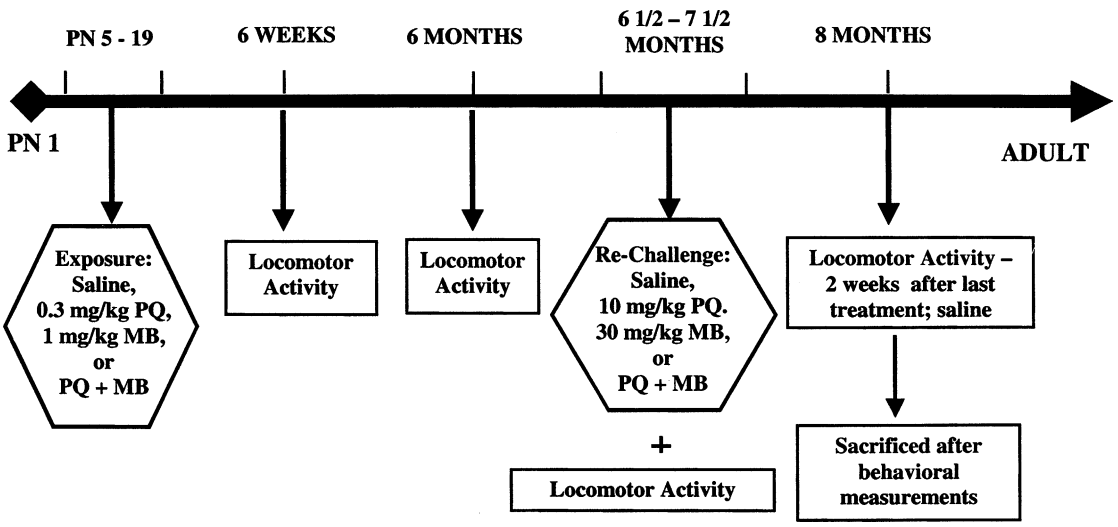

Fig. 1. Experimental time-line indicating post-natal exposure and adult re-challenge paradigms.

(manganese bisethylenedithiocarbamate; Dow Chemical) at a dose of 1 mg/kg, or with the combination from PN days 5 to 19. Both PQ and MB were dissolved in saline. For combined injections, two separate injections were administered. At 6.5 months of age, a subset of these animals were re-challenged with the original treatment using saline, 10 mg/kg PQ, 30 mg/kg MB or the combination of PQ + MB. A separate group of mice were treated only as adults to these same doses, yielding three exposure groups of mice: (a) PN only, (b) PN + adult, (c) adult only. PN exposure was carried out daily from days 5 to 19 (15 total doses), and subsequent adult re-exposure occurred twice a week for 3.5 weeks (total of seven treatments). Fig. 1 shows the experimental time line. Mice were housed in a room maintained under constant temperature (72–74 °F) and humidity conditions with a 12:12 light–dark cycle. During PN exposure, mice were housed with dams and littermates. Following weaning, mice to be used for behavioral studies were housed one per cage; all other mice were housed four per cage. To prevent litter-specific effects, a total of 30 litters were generated for each treatment group, with one mouse derived from each litter.

Food and water were available ad libitum. Body weights were obtained periodically over the course of the experiment. Animals were cared for and treated in accord with NIH and the University of Rochester Animal Care and Use Committee Guidelines.

Chemicals

Solvents for high performance liquid chromatography with electrochemical detection (HPLC-EC) were purchased from Sigma (St Louis, MO). All other chemicals, if not specified, were at least analytical grade and were purchased from Sigma (St. Louis, MO).

Locomotor Activity

Automated locomotor activity chambers equipped with infrared photobeams (Opto-Varimex Minor, Columbus Instruments International Corporation, Columbus, OH) were used to quantify locomotor activity. Photobeam breaks were recorded each minute for 45 min for horizontal, vertical, and ambulatory movements. Following PN exposure, motor activity was assessed at 6 weeks and again at 6 months of age. To assess the effects of developmental exposure to these toxicants at 6 weeks of age, all mice were run on three consecutive days and the data from the third locomotor session is reported here. At 6 months of age, mice were also habituated to the locomotor activity chambers in three 45 min sessions occurring on consecutive days, with all mice receiving i.p. vehicle injections prior to the session. After the third habituation session, treatments began either as re-challenge (PN + adult) or first challenge (adult only) or to vehicle only (PN), and effects on motor activity were assessed immediately and 24 h after each injection in 45 min test sessions with activity counts totaled in 3 min blocks across the session. Activity was also determined again 2 weeks after the final treatment to determine whether there were persistent effects of adult re-exposure. This behavioral session was preceded by a saline injection.

Dopamine and Metabolite Analyses by HPLC

Neurotransmitter concentrations were measured 2 weeks following the last injection of the assigned treatment. All groups (PN only, PN + adult, adult only exposure) were sacrificed at the same time point. Following rapid decapitation, striatal blocks were dissected and placed in 0.1 N perchloric acid. The tissues were sonicated and centrifuged for 15 min at 1000 × *g*. The supernatants were stored at −80 °C until analyzed for the concentrations of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and serotonin (5HT) by HPLC-EC. The pellets were digested in 1 ml of 0.5 N NaOH for measurements of protein concentration using the Bio-Rad assay. HPLC analysis was carried out as described previously (Thiruchelvam et al., 2000a). The concentrations of the neurotransmitters were expressed in units of ng/mg protein. DA turnover was expressed as the ratio (DOPAC + HVA)/DA.

Immunohistochemistry (IHC) for Tyrosine Hydroxylase and Cell Counting

Tissue Preparation

Paraformaldehyde (PFA) post-fixed brains were used for immunolabeling studies. Fixed brains were cut into 30 μm sections and collected in cryoprotectant. Sections were washed with 0.1 M phosphate buffer (PB), blocked for non-specific binding, and incubated with a 1° antibody (Ab) to TH (Chemicon International, Temecula, CA) for 48 h at a dilution of 1:4000. Sections were subsequently incubated with a 2° biotinylated anti-rabbit Ab (Vector Laboratories, Burlingame) at a dilution of 1:200. Sections were subsequently washed and incubated with avidin-biotin solution using the Vectastain Elite kit (Vector Laboratories, Burlingame) for 1 h at room temperature. Sections were developed in 3-3′-diaminobenzidine tetrachloride (DAB) for 2–3 min. Following several rinses, sections were mounted, counterstained with cresyl violet and cover slipped.

Stereological Analysis

After delineation of the SNpc at low magnification (4× objective), every fourth section from the entire region was sampled at higher magnification (100× objective) using the stereology module of the MCID imaging program (Imaging Research, St. Catherines, ON) with an Olympus Provis microscope. The optical fractionator method was used to count TH^+ and TH^-cells. The entire depth of field was sampled ignoring the upper and lower 1.5 μm to avoid counting cells that might be missing nuclei. The thickness of each section was measured. The total number of TH positive (TH^+ and cresyl violet positive neurons) and TH negative (cresyl violet positive only) neurons in the substantia nigra was estimated using the optical fractionator method.

Peripheral Organ Histopathology

Representative sections of lung, heart, kidney, and liver ($n = 6$ per treatment group) were prepared by formalin fixation, paraffin embedding, sectioning at 4 μm, and staining with hematoxylin and eosin. Sections were examined without knowledge of treatment group for evidence of alterations in microscopic pathology.

Statistical Analysis

Overall effects of treatment on horizontal locomotor activity were first analyzed with repeated measure analyses of variance (RMANOVA) using treatment and developmental group (i.e. PN, PN + adult, or adult only exposure) as between group factors and injections as a within group factor. This was followed by individual ANOVAs using treatment and developmental group as between group factors for each injection and subsequent Fisher's post hoc tests to compare treatment groups. To assess treatment-related changes within an activity session, RMANOVAs with treatment and developmental group as between group factors and time block as a within group factor were utilized; significant main effects of treatment or interactions were followed by ANOVAs at each time point. Changes in DA, DOPAC and turnover were first evaluated using treatment and developmental group as between group factors for ANOVA. Effects of all other endpoints were analyzed using one factor ANOVA with treatment and development group as the between group factors, followed by Fisher's tests in the event of significant main effects of treatment.

RESULTS

Body Weight and Pathology

No treatment-related changes in body weights were observed in any of the groups at any time point in the experiments. Lungs were graded for signs of alveolitis, bronchiolitis, bronchitis, lymphoid aggregation, bronchiectasis, and fibrosis and found to be histologically

normal. Similarly, no pathological changes were observed in heart, kidney or liver.

Locomotor Activity

Locomotor activity was evaluated at 6 weeks of age and again at 6 months of age after PN exposure to saline, 0.3 mg/kg PQ, 1 mg/kg MB or the combination of the two (Fig. 2). At 6 weeks of age, only the group that received combined PQ + MB showed a significant decrease (23%) in horizontal activity, as confirmed by a main effect of treatment in the statistical analysis ($F(3, 34) = 3.456$, $P = 0.027$) with subsequent post hoc tests indicating lower activity levels than groups treated with saline and with MB alone (both $P < 0.05$). The PQ only treated group showed a marginal decrease in activity levels at this early time point (14%). By 6 months of age, the PQ + MB treated group showed a further reduction in locomotor activity (38%) such that activity levels were significantly lower than those of the other three groups (all probabilities are $P < 0.05$), confirmed by a significant main effect of treatment ($F(3, 34) = 3.376$, $P = 0.03$) in the RMANOVA. This represented a further decrease of approximately 15% in the PQ + MB group between 6 weeks and 6 months (Fig. 2), demonstrating progressive effects of PQ + MB ($P < 0.0001$).

Challenges with saline, 10 mg/kg PQ, 30 mg/kg MB or the combination of the two were carried out in a subset of mice after the 6 months motor activity assessment. Additional naive mice were treated only as adults with the same doses and PN only exposed group was treated with saline. Locomotor activity was evaluated immediately after these treatments and again

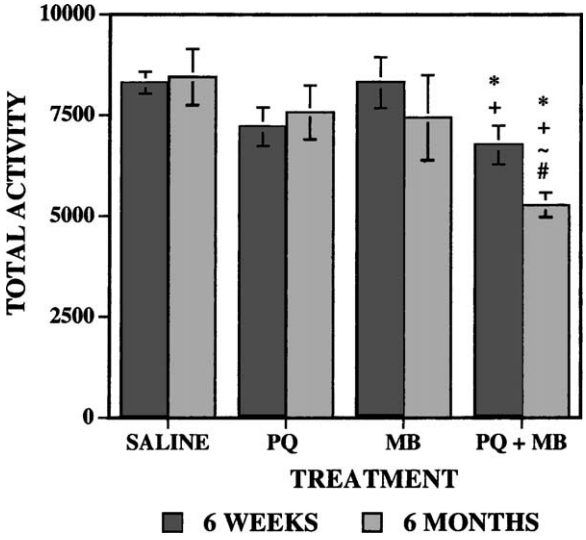

Fig. 2. Total horizontal locomotor activity 6 weeks and 6 months following post-natal exposure to saline, 0.3 mg/kg paraquat, 1 mg/kg maneb or the combination of the two. Data is shown as group mean ± S.E. ($n = 10$ per treatment group). Post hoc analysis revealed significance from: (∗), saline; (+), MB alone; (#), PQ alone; (∼), 6 weeks old mice treated with PQ + MB.

24 h later followed by an additional assessment 2 weeks later preceded by saline only injections. The corresponding locomotor activity levels are depicted in Fig. 3. As it indicates, mice that received PQ + MB post-natally and were subsequently re-challenged with PQ + MB as adults exhibited a marked decrease (70%) in locomotor activity. A significant main effect of treatment ($F(3, 106) = 6.36$, $P = 0.0005$) and group (PN, PN + adult, and adult only; $F(2, 106) = 3.00$, $P = 0.05$) were confirmed in the statistical analysis. The levels of activity in the PN + adult PQ + MB

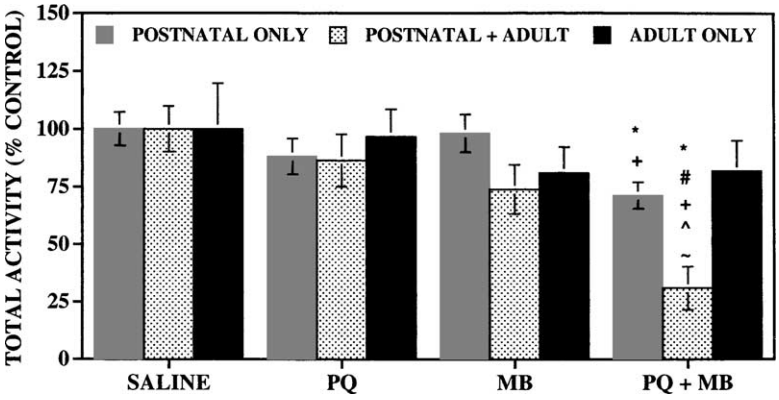

Fig. 3. Horizontal locomotor activity 2 weeks after last adult re-challenge. Data is shown as group mean ± S.E. ($n = 10$ per treatment group), normalized to corresponding saline controls for post-natal only (PN), post-natal + adult exposure (PN + adult) as well as adult only (adult) exposure. Adult re-challenge or adult only exposure to either saline, 10 mg/kg PQ, 30 mg/kg MB or the combination of the two was carried out at 6.5 months of age. Post-natal only treated mice were injected with saline prior to being placed in activity chambers. Post hoc analysis showed significant difference from: (∗), saline; (+), MB alone; (#), PQ alone; (∼), PQ + MB post-natal only treated mice; (∧), PQ + MB adult only treated mice.

group were lower than those of the PN only and adult only groups exposed to PQ + MB (both $P < 0.01$) and were also lower than levels in the PN + adult groups that received either PQ or MB alone (both $P < 0.01$). MB alone mice exposed both post-natally and as adults exhibited a slightly greater reduction in activity than the groups that were exposed either post-natally only or as adults only, although these decreases were not statistically significant.

Striatal Dopamine, Metabolites, Turnover and Serotonin

Striatal levels of DA, DOPAC, HVA, 5HT and DA turnover were evaluated 2 weeks after the last adult exposure, immediately after last locomotor session (Fig. 4). All groups of mice were sacrificed at the same time. Striatal DA levels (Fig. 4A) were significantly affected by treatment ($F(3, 105) = 24.8$, $P < 0.0001$),

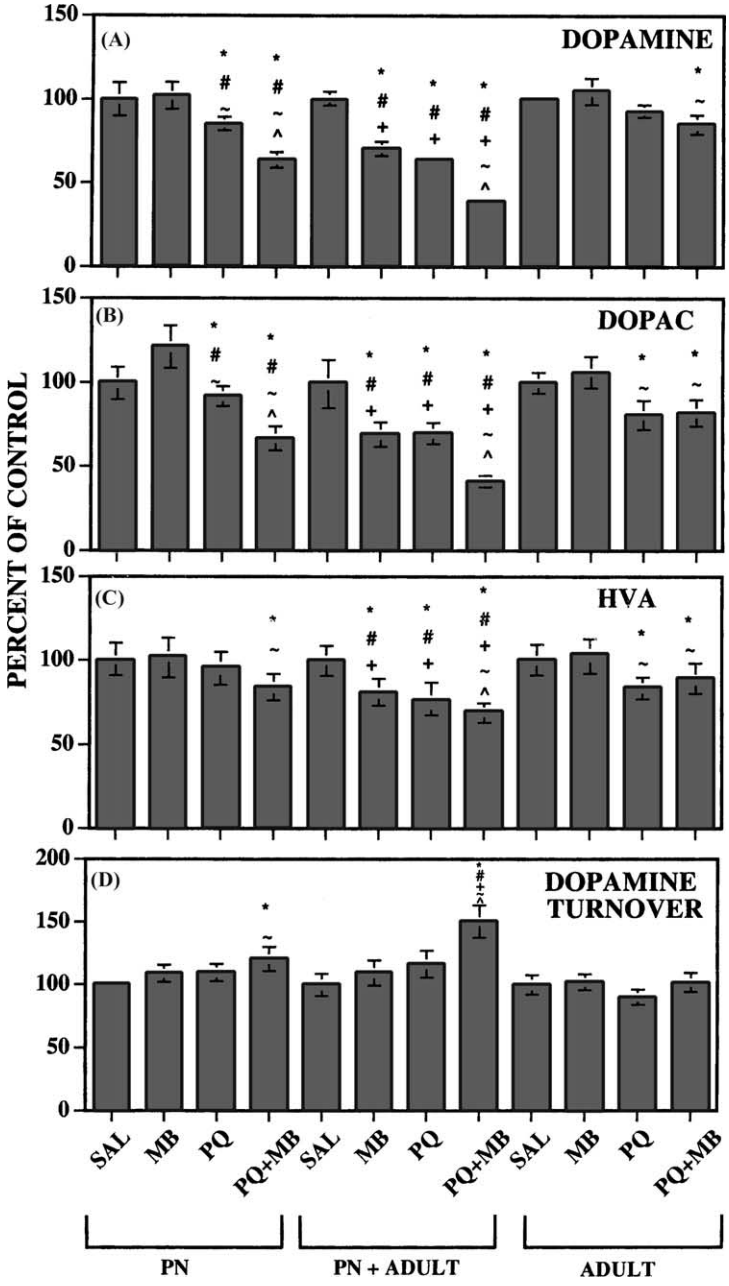

Fig. 4. Striatal DA (A), DOPAC (B), HVA (C), and DA turnover (DOPAC + HVA)/DA (D) levels 2 weeks after the last injection of saline or 10 mg/kg PQ, 30 mg/kg MB or the combination of the two ($n = 10$ for each treatment group). Post-natal exposure was carried out from post-natal days 5 to 19 (PND 5–19), with adult re-challenge or adult exposure only occurring at 6.5 months of age. Data are shown as group mean ± S.E. (percent of corresponding saline-treated group). Fisher's post hoc confirmed significant difference from: (∗), saline; (#), from adult only exposure; (+), PN only exposure; (∼), MB alone; (∧), PQ alone.

and differed by group as well (PN, PN + adult, and adult only: $F(2,106) = 20.3$, $P < 0.0001$; treatment by group interaction: $F(6,105) = 3.8$, $P = 0.0018$). PN only exposure to PQ + MB significantly decreased DA levels by about 36% relative to saline, PQ or MB alone ($P < 0.05$), while PQ alone marginally decreased DA. Adult only exposure to PQ + MB decreased DA levels by approximately 15% ($P < 0.05$), findings consistent with our previous observations (Thiruchelvam et al., 2000b).

With PN + adult exposure, PQ alone decreased DA levels (Fig. 4A) by 36%, MB alone by 30%, and PQ + MB by 62% as compared to the corresponding saline-treated group. This stands in contrast to the lack of effect on DA levels in response to PQ or MB alone following adult only exposure. Similarly, PQ + MB treated mice in the PN + adult exposed group showed enhanced reductions in DA levels compared to those exhibited by the corresponding PN only or adult only groups ($P < 0.001$).

Changes in DOPAC levels paralleled those seen in DA (Fig. 4B), with a significant effect of treatment ($F(3,105) = 11.7$, $P < 0.0001$), group ($F(2,105) = 9.8$, $P < 0.0001$), and an interaction between treatment and group ($F(6,105) = 2.31$, $P = 0.03$). For all three treatment protocols, exposure to PQ alone, or to PQ + MB decreased DOPAC levels, with this reduction being greatest in the PN + adult group ($P < 0.01$). In fact, the decrease in DOPAC levels in the PN + adult exposed PQ + MB appears additive of the effects observed in the PN only and adult only exposure groups. While MB alone treatment did not alter DOPAC following exposure either during development or in adulthood, PN + adult exposure did result in significant and notable decreases in DOPAC ($P < 0.05$). Effects on HVA levels (Fig. 4C) mirrored those seen with DOPAC, but were of smaller magnitude, with significant effects of treatment ($F(3,105) = 2.9$, $P = 0.037$) and group ($F(2,105) = 2.88$, $P = 0.05$) in the statistical analysis, but not an interaction between the two.

DA turnover (Fig. 4D), changes reflected the alterations in DA and metabolite levels (main effect of treatment ($F(3,105) = 4.6$, $P = 0.004$) and group ($F(2,105) = 0.002$). Specifically, PQ + MB treatment during PN days 5–19 significantly increased turnover by 20% compared to effects produced by either compound alone or by saline treatment. Adult only exposure to PQ + MB did not alter DA turnover in any of the groups. Exposure to PQ + MB both PN and as adults significantly increased DA turnover (50%) compared to the corresponding saline group. This increase was also significantly higher than that of the PN only treated PQ + MB group. Although, not statistically significant, both PQ and MB alone increased DA turnover (16 and 10%, respectively) in the PN + adult group. No significant changes in 5HT levels were observed in any of the treatment groups (data not shown), indicating the selectivity of these treatments for the dopaminergic system.

Nigral Dopaminergic Cell Counts

The number of dopaminergic cells in the SNpc was determined 2 weeks after the last adult treatment (Fig. 5), with all mice sacrificed at the same time point. TH^+ neurons (Fig. 5A) actually represent TH^+ and cresyl violet positive neurons indicating that the changes observed are a true loss of dopaminergic neurons rather than just a down regulation of the enzyme, with no change in TH^- neurons. PN only exposure to any of the treatments (PQ or MB alone or combined PQ + MB) all decreased the number of TH^+ neurons as compared to the corresponding saline-treated group significantly (all comparisons, $P < 0.0001$), with the PQ + MB group exhibiting the largest reduction, and differing from PQ and MB alone. Adult only exposure to PQ alone and to PQ + MB also reduced the number of dopaminergic cells (both comparisons, $P < 0.0001$). The PN + adult exposure regimen decreased numbers of TH^+ cells most dramatically ($P < 0.0001$), again with the PQ + MB group showing the largest decrease (67% decrease) relative to saline ($P < 0.0001$). PQ + MB also reduced TH^+ neurons to a significantly greater extent than PQ alone or MB alone. The decreases in the PN + adult groups were all potentiated relative to corresponding losses in either the PN or adult only groups for all the treatments. These effects were confirmed in the statistical analysis by a significant main effect of treatment ($F(3,36) = 275$, $P < 0.0001$), group ($F(2,36) = 110$, $P < 0.0001$), and an interaction of treatment and group ($F(6,36) = 31.1$, $P < 0.0001$). In contrast, there were no differences in TH^- neurons under any conditions (Fig. 5B), suggesting that these neurotoxicant treatments destroyed only dopaminergic neurons of the SNpc.

DISCUSSION

Although, PD is considered a neurodegenerative disorder, the possibility that it results from damage to the nigrostriatal system incurred developmentally but expressed only as normal aging processes unfold,

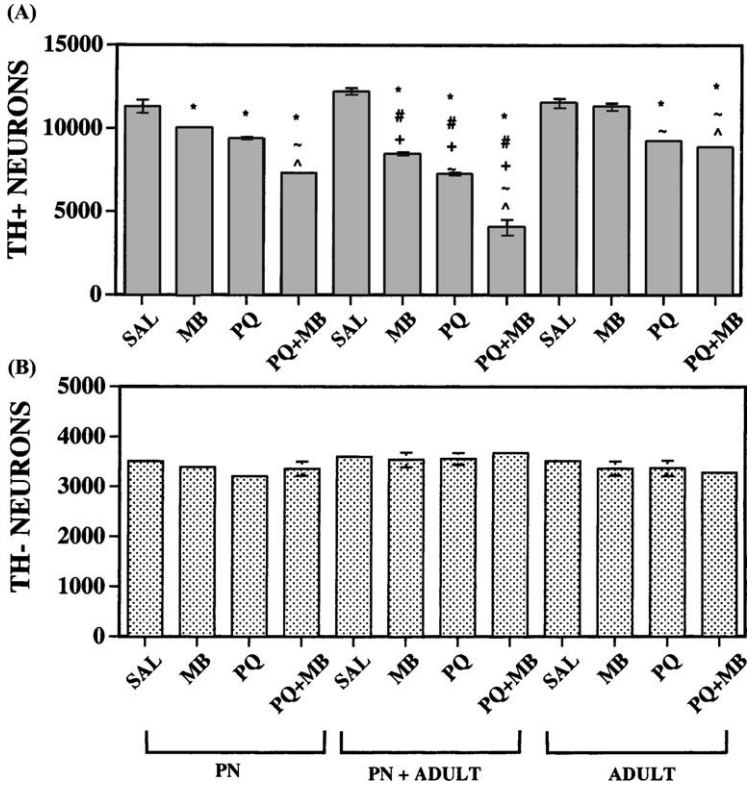

Fig. 5. Total number of TH^+ (A) and TH^- (B) neurons in the substantia nigra pars compacta 2 weeks after the last treatment of either saline or 10 mg/kg PQ, 30 mg/kg MB or the combination of the two ($n = 4$ for each treatment group). Post-natal exposure was carried out from days 5 to 19 (PN 5–19) were followed by adult re-challenge or adult only exposure corresponding compounds at 6.5 months of age, with mice being sacrificed 2 weeks after the last dose. Data represents total number of TH^+/cresyl violet positive (A) or cresyl violet only positive (B) neurons and is represented as group mean ± S.E. post hoc analysis further revealed significance from: (∗), saline; (#), from adult only exposure; (+), PN only exposure; (∼), MB alone; (∧), PQ alone.

while speculated, has yet to be examined. The aim of this study, were to examine the hypothesis that targeting the nigrostriatal dopaminergic system developmentally could result in permanent neurotoxicity to the DA system and, further increase its vulnerability to subsequent neurotoxic challenges occurring later in life. In concert with those assertions, developmental exposures to PQ or MB alone as well as their combination impart sustained alterations to the nigrostriatal DA system that are still evident at 6 months of age, i.e. 5.5 months after the last exposure, suggesting permanent DA system alterations. This was demonstrated by locomotor activity, especially in the PQ + MB treated group, where activity levels were reduced at 6 weeks of age but had fallen even further by 6 months of age, consistent with progressive neurotoxicity. The increased DA turnover in the PN treated PQ + MB group evident even 6 months after exposure suggests a continuous alteration of DA metabolism, an effect that could signal the sustained formation of free radicals as a mechanism for the progressive degeneration that appears to take place. Determining the increases in free radical generation and its by-products will allow us to examine this possibility.

In addition to demonstrating permanent and progressive effects from developmental only exposures, two other major findings were notable. First, developmental exposures markedly enhanced vulnerability to subsequent pesticide treatments, and, secondly, developmental only exposures were associated with ''silent neurotoxicity'' that was only unmasked by later challenges to the DA system. The effects of adult re-challenge following developmental exposure to either PQ or MB alone or PQ + MB produced markedly greater reductions in locomotor activity, striatal DA and metabolites as well as nigral dopaminergic neurons compared to either PN only or adult only exposures to these neurotoxicants. With respect to the second finding, the effects of MB alone during development were found to be modest and without substantial behavioral impairments. However, following adult re-exposure to MB, a marked decrease in nigrostriatal dopaminergic function was observed. Developmental exposure to MB alone therefore, produced a ''silent toxicity'' such that the

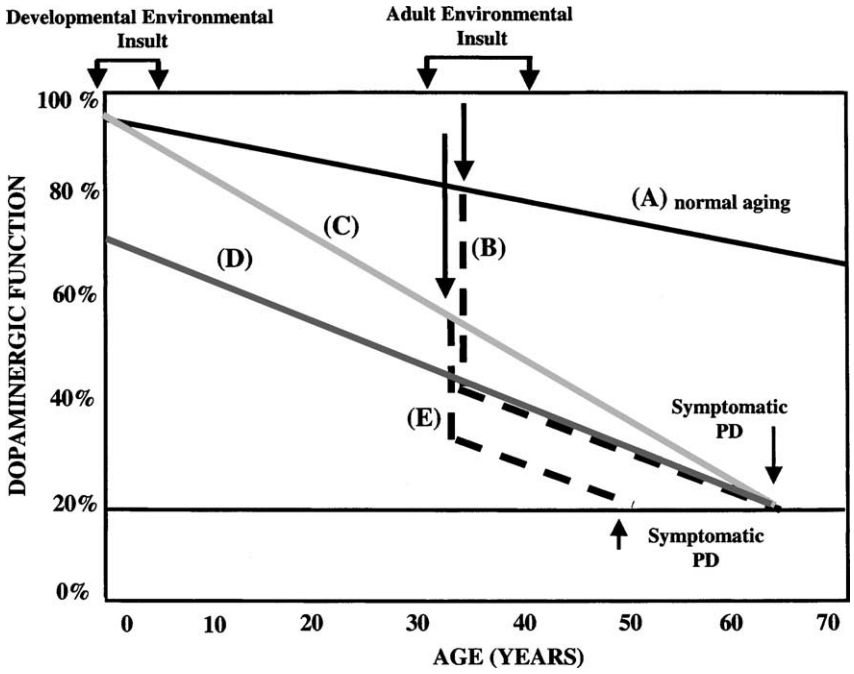

Fig. 6. Schematic representation of three different scenarios leading to the Parkinson's disease phenotype, with the developmental exposure hypothesis relatively unexplored. The first (line A) shows how an environmental insult somewhere in the third or fourth decade of life (line B) might produce Parkinsonism around the age of 60, assuming that dopaminergic (DA) function continues to decline with aging and an 80% or greater depletion of dopaminergic function is required for symptoms to develop. Contrary, exposure to DA neurotoxicants during critical periods of development can result in loss of DA function at the intercept of exposure (line D) or accelerate the age-related decline of dopaminergic function (line C), both leading to the disease phenotype sooner. However, an adult re-exposure to such an insult (line E) could lead to a Parkinsonian phenotype in the fifth or sixth decade of life.

system was already vulnerable, and re-exposure thereafter to the neurotoxicant produces dramatic effects.

Calne and Langston (1983) posited a decline in dopaminergic function with age that could be accelerated by an environmental insult in early or middle life that eventually reduces DA function below that necessary to maintain normal function as a model for PD (Fig. 6, lines A and B). Alternatives or additions to this hypothesis are posed in Fig. 6 (lines C and D). The progressive decline in locomotor activity with age following developmental exposure may be an indication of progressive loss of dopaminergic function across the life span. To document this, of course, would require a longer, time-course experiment. However, if one makes that assumption, several hypotheses can be explored. As depicted in Fig. 6, developmental insult might result in a loss of dopaminergic neurons at the onset of exposure, thus depleting the total pool of neurons, and this process may continue with age resulting in the disease phenotype appearing earlier in time compared to what might be predicted with normal aging alone (Fig. 6, line D). In another proposed scheme, neuronal number may not be altered with developmental exposure at the beginning of life, but rather the developmental insult could enhance the rate of cell death across the life span, again, leading to an earlier onset of the disease phenotype (Fig. 6C). Under both scenarios, a subsequent environmental insult during adulthood can shift these curves even further downward, as schematized (Fig. 6E). This study clearly shows that developmental exposures to these toxicants can render a system more vulnerable to subsequent exposures. Furthermore, it raises the possibility that individuals that may not have evidenced susceptibility through genetic background alone could, by virtue of early damage to the system, become vulnerable to the PD phenotype. It is important to point out however, that to date, only behavioral data has been presented to support the possibility of progressive effects following developmental exposure alone, making it premature to conclude that developmental exposure alone results in progressive damage to the nigrostriatal system. More experiments looking at the time course of these effects will have to be carried out to ascertain this possibility.

Other neurotoxicants have been shown to have a greater effect on the developing central nervous system compared to the adult brain (Ahlbom et al., 1994; Eriksson et al., 1992; Eriksson et al., 1991b; Moser et al., 1998; Moser et al., 2001; Spyker and Avery,

1977). Developmental exposure to MPTP, the commonly used neurotoxicant model of PD, produces permanent effects on the nigrostriatal system both in mice and non-human primates following developmental treatment but the changes observed are unlike those following adult exposure (Ali et al., 1993; Ochi et al., 1991; Perez-Otano et al., 1995; Perez-Otano et al., 1992; Weissman et al., 1989). A previous study reported that exposure to PQ on PND 10–11 produced permanent changes in striatal DA as well as behavioral changes (Fredriksson et al., 1993b). These findings are not completely consistent with those reported here, which could reflect differences in route of exposure (oral versus i.p. in our studies) and/or the timing of the exposure. Exposure to the insecticide DDT during PND 10–11 enhanced the effects produced by adult exposure to bioallethrin (Eriksson and Talts, 2000), as assessed using behavioral endpoints and cholinergic receptor pharmacology. However, the current study is, to the best of our knowledge, the first demonstration of a developmental effect on the nigrostriatal system that is progressive, and also leads to enhanced nigrostriatal system vulnerability to subsequent exposures, having obvious implications for the etiology of PD.

The mechanism(s) by which developmental exposure to PQ and MB alone or in combination enhance the toxic impact of subsequent exposures requires further investigation. PQ itself has been repeatedly shown to adversely affect the nigrostriatal DA system (Barbeau et al., 1985; Brooks et al., 1999). While the extent to which PQ crosses the blood brain barrier (BBB) has been questioned, it can indeed enter the brain and actually appears to accumulate differentially in certain regions following systemic injections of ^{14}C-PQ (Bagetta et al., 1992; Corasaniti and Nistico, 1993; Corasaniti et al., 1990; Corasaniti et al., 1992; Lindquist et al., 1988; Rose et al., 1976). Furthermore, systemically-administered PQ uptake in brain depends upon the age of the animal, with higher brain concentrations achieved in very young and older animals (Corasaniti et al., 1991; Widdowson et al., 1996).

Based on the neurochemical and behavioral changes, and it's apparently selective disruption of the nigrostriatal system, it is assumed that MB is able to cross the BBB. The development of the BBB proceeds from late gestation and continues through the PN period and may be a time of increased permeability. Thus, exposure to an environmental toxicant during this period could be associated with increased uptake and also disruption of the normal development and maturation of this crucial barrier (Kneisel et al., 1996; Rodier, 1994; Rodier, 1995; Saunders and Mollagard, 1991). An incomplete or immature BBB in the developing central nervous system could result in greater uptake of neurotoxic compounds into the central nervous system, which then exert greater effects on the dopaminergic system. Such a possibility could account for the more pronounced effects associated with developmental only as compared to adult only exposures in these experiments. The PN period is often one of greater vulnerability to toxic insults as compared to the adult stage of the life cycle because excretion processes are functionally inefficient at this point and many drug metabolizing systems have not yet completely developed (Gange and Brodeur, 1972). This would potentially result in a protracted time course of toxicant effects. While the basis for the potentiated neurotoxicity of combined PQ and MB has yet to be resolved, preliminary findings from our laboratory suggest that MB may increase the accumulation of PQ in the brain as compared to PQ administration alone (unpublished observation).

Thus, exposure to PQ and MB during critical periods of development may be disrupting the normal development of the BBB. Developmental exposure to several pesticides has been shown to alter BBB development and its functionality in the adult (Banks et al., 1996; Gupta et al., 1999; Srinivas et al., 1993). Consequently, a ''leaky'' BBB as a result of incomplete or aberrant maturation could increase subsequent permeability to toxicants, a scenario that might underlie the heightened susceptibility of adult animals treated PN and re-challenged as adults. Similar impacts could be envisaged if defense mechanisms, xenobiotic metabolizing enzyme systems, and/or neurotrophic factor networks were incompletely or incorrectly developed due to disruption during the critical periods by the effects of these exposures. Developmental expression of neurotrophic factors is important for numerous processes and it's expression is critically dependent on timing, duration and also context (Barone, 1999). Furthermore, pertubation of any of these systems may also have profound effects on downstream signaling processes that play an important role in the maintenance of cellular homeostasis.

Apoptotic cell death is also an important developmental process and occurs during both pre- and PN development. Exposure to toxicants during critical periods may perturb this process by altering the systems that regulate apoptotic signals, resulting in undesirable increases in apoptosis and consequent decreases in cell numbers. This altered cell number may lead to neurological dysfunction as seen in several neurological disorders including PD (Burke and Kholodilov, 1998).

The findings described here using this environmental exposure model have clear implications for the risk assessment processes used to evaluate pesticide safety for human populations. Numerous studies have now begun to document the placental transfer of various pesticides to the fetus (Siddiqui et al., 1981; Waliszewski et al., 2001). The environmental reality, of course, is that exposures occur to mixtures of chemicals rather than to single agents, as might be predicted from the fact that environmental pesticides are widely used in overlapping geographical locations in areas in the US and some even on the same crops (USGS, 1998). Recently published studies now beginning to examine human exposures to pesticides, primarily organophosphates, confirm the presence of metabolites of multiple pesticides in urine of children. In some cases these levels are not seasonal as might have been expected, and are present in higher concentrations than predicted by recent non-probability based samples (Adgate et al., 2001; Lu et al., 2001). Clearly, a further understanding of the mixtures of pesticides to which humans are exposed and the levels of these exposures is needed to permit a precise determination of the risks of such exposures both for various developmental disabilities as well as their potential contributions as risk factors in the etiology of neurodegenerative diseases.

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