Effects of Pharmacological Agents upon a Transgenic Model of Parkinson’s Disease in Drosophila melanogaster

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ABSTRACT
The human gene that codes for the protein α-synuclein has been transferred into the Drosophila melanogaster genome. The transgenic flies recapitulate some of the essential features of Parkinson’s disease. These include the degeneration of certain dopaminergic neurons in the brain accompanied by the appearance of age-dependent abnormalities in locomotor activity. In the present study, we tested the locomotor response of these transgenic flies to prototypes of the major classes of drugs currently used to treat this disorder. A time course study was first conducted to determine when impaired locomotor activity appeared relative to normal “wild-type” flies. A climbing or negative geotaxis assay measuring the ability of the organisms to climb up the walls of a plastic vial was used. Based on the results obtained, normal and transgenic flies were treated with each of the drugs in their food for 13 days and then assayed. The activity of transgenic flies treated with L-DOPA was restored to normal. Similarly, the dopamine agonists pergolide, bromocriptine, and 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SK&F 38393) were substantially effective. Atropine, the prototypical muscarinic cholinergic receptor antagonist, was also effective but to a lesser extent than the other antiparkinson compounds. p-Chlorophenylalanine, an inhibitor of serotonin synthesis, was without beneficial effect as was α-methyl-p-tyrosine, an inhibitor of tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis. This behavioral study further demonstrates the utility of this model in studying Parkinson’s disease and reinforces the concept that inhibition of the action of α-synuclein may be useful in its treatment as may dopamine D₁ receptor agonists.

Parkinson’s disease is a common neurodegenerative syndrome in humans characterized anatomically by a loss of dopaminergic neurons in the substantia nigra and the appearance of intracellular Lewy bodies that contain large amounts of normal or mutated forms of the protein α-synuclein. Functionally, Parkinson’s disease is an extrapyramidal (involuntary) movement disorder involving muscle rigidity, bradykinesia, postural impairment, and resting tremors (Standaert and Young, 1996). Recently, plasmids containing the human gene that codes for human wild-type or mutant α-synuclein were injected into fly embryos by Feany and Bender (2000). These flies were subsequently crossed with ones containing a pan-neural activating system (elav-GAL4 driver) for the gene (Robinow and White, 1988; Brand and Dordam, 1995). Enhanced expression of wild-type or mutant α-synuclein in the progeny was associated with the progressive degeneration and loss of dorsomedial dopamine containing neurons in the fly central nervous system. Subsequently, the ability of flies to perform a movement-related task, specifically climbing (negative geotaxis), decreased with time (Feany and Bender, 2000).

In the study reported here, transgenic flies obtained from Feany and Bender and expressing the human wild-type genotype (UAS-wild-type α-synuclein) were tested for their climbing response after treatment with representatives of the major classes of drugs used to treat patients with Parkinson’s disease. In humans, these compounds act to restore the net balance between degenerating dopamine-producing neurons, which normally produce both excitatory and inhibitory effects, and excitatory neurons, which release acetylcholine onto muscarinic receptors. These synaptic events occur primarily in the subcortical cerebral nuclei (basal ganglia) of the extrapyramidal system (Standaert and Young, 1996).

Materials and Methods
Wild-type Canton S Drosophila melanogaster were maintained at 25°C on a 12-h light/dark cycle in bottles containing an agar, corn meal, corn syrup, water, and dried yeast medium. Propionic acid was added to prevent fungal growth. All drugs were added to the medium at a final concentration of 1 mM, and the mixture was heated to a boil

ABBREVIATIONS: GAL4, protein transcription factor with four DNA binding sites that normally activates galactose metabolism in yeast; UAS, upstream activating system; elav, neuronal locus; SK&F 38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine; aMT, α-methyl-p-tyrosine; PCPA, p-chlorophenylalanine.
with continuous stirring. Ascorbic acid (25 mg/100 ml) was added to flasks containing l-DOPA and SK&F 38393 to prevent drug oxidation. Control flasks for experiments containing these drugs also contained ascorbic acid. Transgenic α-synuclein flies obtained from Feany and Bender (2000) were maintained and tested under the same conditions. Canton S flies were used as the basis for comparison with the transgenic organisms because they are a genetically normal, constant breeding stock whose functional behavior in our standard locomotor assay described below was identical with the transgenic stock during the first 4 days of life (see Results).

Both the transgenic and Canton S flies have a normal life span of approximately 60 days (Ganetzky and Flanagan, 1978; Feany and Bender, 2000). A geotaxis time course study was conducted to determine the rate of functional degeneration of both genotypes. This was necessary to determine when the genotypically different flies expressed significantly different behavior. Flasks containing the desired stocks were emptied leaving pupa to emerge (eclose) as adults. The following day, and at 3- to 4-day intervals thereafter, the adults were tested in the geotaxis assay. Assays continued until day 19. The geotaxis assay was used to examine locomotion through climbing activity. Groups of 10 flies were transferred into empty 95 × 27-mm vials, around which a horizontal line 8 cm above the bottom of the vial was drawn. After the flies had acclimated for 10 min at room temperature, both control and transgenic groups were assayed at random, to a total of 10 trials for each. The procedure involved gently shaking the flies down to the bottom of the vial; after 10 s the number of flies that crossed the 8-cm mark (escaped) was recorded. The trial numbers were then averaged, and the resulting mean was used as the overall value for each single group of flies on a particular day. These values were then averaged, and a group mean and standard error were obtained. Where appropriate, the mean values of various fly groups were statistically compared using an unpaired or group Student’s t-test (Snedecor and Cochran, 1989). All behavioral studies were performed in an isolation room at 25°C under standard lighting conditions. Preliminary studies indicated no difference whether the studies reported below were conducted in normal light or in red light (dark) conditions.

After an appropriate time course for the development of impaired geotactic responses was determined, newly eclosed flies of both the transgenic and Canton S strains were placed in flasks that contained drug-treated food at 1 mM for 13 days. Control organisms were treated similarly except that the medium was drug free. The drugs used included l-DOPA, pergolide, bromocriptine, SK&F 38393, atropine, α-methyl-p-tyrosine (αMT), and p-chlorophenylalanine (PCPA).

**Results**

The climbing response of wild-type flies remained essentially unchanged over 19 days in a time course evaluation (Fig. 1) similar to that reported by Feany and Bender (2000). The climbing response of the transgenic flies was identical to that of the wild-type group on days 1 and 4 of the study, which strongly argues that the Canton S flies used as normal reference groups were appropriate (see Materials and Methods). From day 7 on, however, the responses of the transgenic group were progressively lower than those of the Canton S flies. Based on these results, we selected 13 days as the standard duration of treatment for our subsequent antiparkinson drug studies.

Each drug studied in this experiment was placed in the food of the test groups of Canton S and transgenic flies at a concentration of 1 mM (Figs. 2–6). This level was selected on the basis of previous studies indicating that it was an effective but submaximal and nonlethal dose for reserpine and αMT in decreasing fly locomotor function (Pendleton et al., 2000). Also, although this concentration is generally considered high for an in vitro assay, the effective concentration of...
any of these drugs at their site of action in the fly is not known but is presumed to be much lower (Pendleton et al., 1996).

L-DOPA completely reversed the deteriorating motor activity of the transgenic flies in this assay (Fig. 2). L-DOPA did not, however, affect climbing activity in the controls, indicating that it is not a general central nervous system stimulant. Similar effects were obtained with the direct dopamine receptor agonists pergolide and bromocriptine (Fig. 3), which act preferentially on the D2 receptor family in mammals (Standaert and Young, 1996), and SK&F 38393 (Fig. 4), the prototypical D1 receptor agonist (Pendleton et al., 1978), which is active in rodent models of Parkinson’s disease containing unilateral 6-hydroxidopamine lesions in the substantia nigra (Setler et al., 1978). As with L-DOPA, no effects were seen with these agents in control normal flies. Belladonna alkaloids, which contain mainly atropine (Brown and Taylor, 1996), and other muscarinic receptor antagonists have been long known to be effective antiparkinson drugs, although their efficacy is lower than that of dopaminergic agents (Standaert and Young, 1996). Results obtained with atropine in our transgenic fly model (Fig. 5) indicate partial efficacy in restoring activity in the transgenic flies without enhancing motor activity in the wild-type flies.

We have previously shown that αMT, an inhibitor of the rate-limiting enzyme in dopamine biosynthesis, decreases locomotor activity in wild-type D. melanogaster as it does in mammals and that this effect may be prevented with concomitant L-DOPA treatment (Pendleton et al., 2000). The depressant effect of αMT on locomotor activity was also observed in this study, both in control and transgenic animals (Fig. 6). In addition to dopamine, serotonin is a major biogenic amine in the Drosophila brain (Budnik and White, 1987). PCPA is an inhibitor of tryptophan hydroxylase, the rate-limiting enzyme in serotonin biosynthesis (Sharma et

![Fig. 2. Effects of L-DOPA on the geotactic response. Adult flies were treated as shown on the abscissa for 13 days and then assayed for climbing activity. Bars indicate the means and standard errors for each test. Asterisks show significant differences between Canton S (wild-type) and transgenic flies within each treatment group using a Student’s t test (p < 0.05). Also, the L-DOPA-treated transgenic fly response was significantly greater (p < 0.05) than in the corresponding control group. ■, Canton S; □, UAS-wild-type α-synuclein.](image)

![Fig. 3. Effects of bromocriptine and pergolide on the geotactic response. Adult flies were treated as shown on the abscissa for 13 days and then assayed for climbing activity. Bars indicate the means and standard errors for each test. Asterisks show significant differences between Canton S (wild-type) and transgenic flies within each treatment group using a Student’s t test (p < 0.05). Also, both drug-treated transgenic fly responses were significantly greater (p < 0.05) than in the corresponding control group. ■, Canton S; □, UAS-wild-type α-synuclein.](image)
al., 2000). It also reduced the performance of both the transgenic and wild-type groups of flies in this study (Fig. 6).

**Discussion**

The primary anatomical abnormality reported by Feany and Bender (2000) in the transgenic flies used in their experiments was a time-dependent loss of dopaminergic neurons in the dorsomedial group in addition to the presence of intracellular aggregates of -synuclein, resembling Lewy bodies. These changes were accompanied by functional losses in climbing ability similar to that found in the present study. The loss of dopaminergic neurons in the -synuclein transgenic flies and our previous results indicating decreased locomotor activity in flies with impaired catecholamine formation or storage suggest a point of commonality in the two approaches.

We have previously shown that treatment of wild-type *D. melanogaster* flies with either MT, an inhibitor of tyrosine hydroxylase, or reserpine, which inhibits catecholamine re-uptake into neuronal storage granules, decreases locomotor activity in a dose-dependent manner after 7 days of placing the inhibitor in the food (Pendleton et al., 2000). Similar results were obtained when temperature-sensitive homozygous or hemizygous conditional mutants of *pale*, the single-copy gene coding for tyrosine hydroxylase in *D. melanogaster*, were tested at their restrictive (28°C) temperature (Pendleton et al., 2002).

Normal young *D. melanogaster* display a strong negative geotactic response. When tapped to the bottom of the vial, they rapidly climb to the top (Ford et al., 1989). Flies older than 30 days progressively lose this response and instead make short abortive climbs and fall back to the bottom of the vial (Ganetzky and Flanagan, 1978; Le Bourg and Lints, 1992). In our time course study, transgenic flies initially climbed as well as control flies. However, over 19 days they declined in performance, in marked contrast with control flies.
organisms that were unaffected by age over this time period. The time course of locomotor dysfunction is in general agreement with the rate of degeneration of dopaminergic neurons and the appearance of α-synuclein inclusions reported by Feany and Bender (2000). Similar results have been reported in transgenic mice in which expression of the normal human α-synuclein gene was associated with accumulations of α-synuclein in the neocortex, hippocampus, and substantia nigra, a loss of dopaminergic neuronal terminals in the cerebral nuclei, and impaired rotorod performance (Masliah et al., 2000).

The principal aim of our study was to determine whether drugs found useful in treating Parkinson’s disease enhance negative geotaxis in D. melanogaster, either by restoring the activity of dopaminergic neurons or by reducing the activity of acetylcholine (anticholinergic drugs). The dopamine precursor L-DOPA, dopamine receptor agonists (pergolide, bromocriptine, and SK&F 38393), and the anticholinergic compound, atropine, all resulted with varying success in increasing geotactic activity. L-DOPA restored transgenic fly motor activity completely, presumably because it leads to the biosynthesis of dopamine in degenerating nerve terminals. Similar results were obtained with the direct dopamine agonists pergolide, bromocriptine, and SK&F 38393. They appeared to be somewhat less efficacious than L-DOPA except for SK&F 38393, which was certainly as good. Atropine, in spite of producing improvements in the transgenic flies, was the least effective of the drugs tested at this dose level. It should be stressed, however, that absolute efficacy or potency cannot be ascertained on the basis of single-dose studies.

The positive data obtained in these assays approximate results obtained with these drugs in Parkinson’s disease or, in the case of SK&F 38393, a well characterized rodent model of this disease. The drugs aMT and PCPA were used as negative controls and failed to produce beneficial responses. PCPA is an inhibitor of serotonin synthesis and aMT blocks catecholamine (dopamine) formation. Each of these results was expected since reductions in catecholamine biosynthesis should accelerate the loss of brain catecholamines, and serotonin neurons can also degenerate in Parkinson’s disease (Jellinger, 1991).

Our results suggest that this transgenic fly model mimics the motor impairments associated with Parkinson’s disease. As such, it may be a reliable assay for antiparkinson drugs and take advantage of the genetic screens that are possible based on the extensive knowledge of the fly genome. The data also suggest that compounds that inhibit the expression or action of α-synuclein as well as dopamine D1 receptor agonists may be useful in the treatment of this debilitating disorder (Goldberg and Lansbury, 2000; Masliah et al., 2000).

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References


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