L-3,4-Dihydroxyphenylalanine-induced hypersensitivity simulating features of denervation

(propensity for dyskinesia/hyperactivity/stereotypy/adenylate cyclase/dietary administration)

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ABSTRACT The manner in which dyskinesia and intermittency of neurological control had emerged late in the therapy of Parkinsonism with L-3,4-dihydroxyphenylalanine (levodopa) had suggested to us that this drug can imprint on the brain a chemical memory of its passage. The majority of authors ascribed these events to denervation hypersensitivity caused by the nigral and other lesions of the disease. By feeding levodopa to mice, however, we induced a state that simulated denervation hypersensitivity, including hyperreactivity to single injections of levodopa and increased dopamine-stimulated adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] activity in homogenates of caudate nuclei. These phenomena were not caused by actual denervation, because the hypersensitivity declined and disappeared some weeks after the dietary levodopa was stopped.

Long-term therapy of Parkinson's disease with L-3,4-dihydroxyphenylalanine (levodopa) can be marred by the gradual, late emergence of adventitious movements (dyskinesia) (1). Diminishing the drug diminishes the dyskinesia but can induce episodic re-emergence of the original symptoms ("on-off" phenomenon) (2). Most authors ascribed these events to "denervation hypersensitivity," presumably caused by irreversible nigral and other lesions of the brain (3-9). We, in contrast, interpreted the manner in which these events manifested themselves as suggesting that these "... patients have shown evidence of having stored a chemical memory of the drug's passage" (10). Circumstantial evidence, including the potentiation of the actions of levodopa by the growth hormone that it releases (11-13), was compatible with our suggestion. It became, therefore, probable that there exists a mechanism, activated by large amounts of levodopa consumed over lengthy periods, that can simulate denervation hypersensitivity. Demonstration of such a mechanism would reconcile divergent views while promoting therapeutic investigations precluded by actual, instead of simulated, denervation.

MATERIALS AND METHODS

Animals. Five groups of mice were used. These represented Swiss albino Hale Stoner (HS) and C3H/HeJ strains (14) so that the groups differed among each other in the intensity of dyskinetic, adventitious, and stereotyped movements evoked by single intraperitoneal injections of levodopa (15). All mice were females. At the beginning, the HS were 5 weeks and the C3H/HeJ 7 months old.

Pilot Experiments. In these we determined the doses of injected levodopa that produced weak but discernible dyskinesia in untreated mice of both strains. These experiments are not further detailed, except to state that for the HS, 0.8 mg/g of body weight, and for the C3H/HeJ females, 1.2 mg/g of body weight were sufficient doses of levodopa.

Main Experiments. The mice, receiving water ad libitum, were divided into groups on the basis of their diets. One or more of these groups received levodopa in their Purina Laboratory Chow while one consumed Purina without the drug. The HS mice received the amounts of dietary levodopa that had increased their lifespan (40 mg of drug per g of chow) (16, 17). The concentration given to the C3H/HeJ mice (100 mg/g of body weight) was approximating the maximal tolerated one.

Each group was subdivided into two subgroups. The first consisted of 18 animals. Six of these were used for scoring the dyskinesia, the adventitious movements, the stereotypy, and some other reactions evoked by the single standardized intraperitoneal injections of levodopa (15). Twelve mice were used for the determinations of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] in homogenates of their caudates. Six were used for measuring the basal activity, the other six for measuring the total activity, which consisted of the basal activity plus the dopamine-stimulated one (18). Thus, the data were also checked for possible increase of the total activity by the endogenous dopamine produced from the levodopa.

The dopamine concentrations added to activate the cyclase in the homogenates of the caudate nuclei were chosen on the basis of the experiments detailed under Observations, whereas the rest of the methodology was identical to that developed in Greengard's laboratory (19). The 3',5'-cyclic AMP was measured by the method of Gilman (20).

Supportive Experiments. These were designed to demonstrate whether levodopa-consuming mice would respond to small intraperitoneal doses of the drug to which the controls did not respond and to show whether the hyperreactivity induced by feeding levodopa was reversible. The reversibility was tested in groups of female Swiss albinos that had been consuming levodopa (40 mg/g of Purina) for 3 months. After they were switched to levodopa-free Purina, representative animals were injected at intervals of several days intraperitoneally with the metabolic inhibitor α-methyltyrosine hydrazine (0.15 mg/g of body weight) followed by levodopa (0.2 mg/g of body weight). Sets of three mice were placed in an Animex® automatic motor activity meter and their motor activities were measured at 15-min intervals for 2 hr. The inhibitor was used to maximize the reactions to levodopa and the Animex® activity meter to confirm these findings with automatic measurements. The activities of the animals that had been taken off levodopa were compared with those of animals that had never consumed the drug.

OBSERVATIONS

Neurological Scores. The general behavior of the animals that were consuming levodopa was similar to that of their...
controls. Specifically, they did not show licking, stereotyped movements, gnawing, headbobbing, or the other scorable items (15). The larger challenging doses of levodopa (0.8 and 1.2 mg/g of body weight for HS and C3H/HeJ mice, respectively) induced adventitious movements in the levodopa-consuming and in the control groups of both strains (Table 1). The scores obtained after 8 weeks on the experiment were significantly higher in the levodopa-consuming groups than in their controls. When the challenging doses of levodopa were lowered (0.6 and 1.0 mg/g for the respective strains) the control mice barely reacted, whereas those consuming levodopa showed obvious dyskinesia.

Experiments representing those performed during the first and second weeks after the interruption of the levodopa in the diets are shown in Fig. 1. The animals that had been consuming levodopa showed, during the first week, invariably much more intense reactions than their controls. This difference remained marked during the second week, and declined with time elapsing from the interruption of the levodopa in the diet (Fig. 1). The rate of this reversal could not be measured accurately because of considerable variance among individual animals. Nonetheless, 25 days after discontinuation of the drug-containing diet, there had not remained discernible differences between control mice and mice that had consumed levodopa in their responses to challenges with injected levodopa.

Adenylate Cyclase. The enzyme was determined in the homogenates of the caudates with concentrations of dopamine that induced activation of the enzyme, as shown in Figs. 2 and 3. Fig. 2 shows that full activation of the adenylate cyclase required higher concentrations of dopamine in the homogenates from female HS than in those of males (14). This difference was in keeping with the fact that adventitious movements were induced in the male mice by half the dose of injected levodopa that induced the movements in the females (14).

Fig. 3 compares the activation of the enzyme from HS females with that from C3H/HeJ females, illustrating that the cyclase from the strain that had responded to the smaller doses of injected levodopa (Table 1) was activated by the smaller concentrations of dopamine. Thereafter, the enzyme from female HS was routinely activated with 100 μM of dopamine and that from the C3H/HeJ female mice with 200 μM, regardless of whether the animals had been consuming levodopa-containing or control diets.

A significant increase in the dopamine-stimulated activity of the adenylate cyclase of animals receiving levodopa in their diets is shown in Table 1. This increase was highest in the animals consuming the highest intakes of the drug. The basal activity remained unaffected by the dietary intake of levodopa (14), proving that endogenous dopamine was not measurably involved in the activation of the cyclase in vitro by the added dopamine. The behavioral scores (Table 1) were again com-

Table 1. Effects of dietary levodopa on dopamine-stimulated adenylate cyclase and on behavioral scores evoked by injection of levodopa

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Net dopamine-stimulated adenylate cyclase activity</th>
<th>Behavioral scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>P</td>
</tr>
<tr>
<td>Strain HS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purina alone</td>
<td>84.8 ± 13.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Levodopa in diet† (40 mg/g)</td>
<td>148.8 ± 13.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Strain C3H/HeJ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purina alone</td>
<td>61.8 ± 7.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Levodopa in diet† (40 mg/g)</td>
<td>93.2 ± 27.7</td>
<td>0.05</td>
</tr>
<tr>
<td>(100 mg/g)</td>
<td>127 ± 16.5</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Note that feeding of levodopa increased significantly both the activity of the dopamine-stimulated adenylate cyclase and the behavioral scores evoked by injecting the larger of the doses of levodopa discussed in the text.

* pmol of cyclic AMP.
† Duration of levodopa in diet was 8 weeks.

FIG. 1. Motor activity measurements in an Animex® automatic motor activity meter. All animals were injected with α-methyl dopamine hydrazine and levodopa at 0 time (see text). (●) Levodopa-consuming animals 1 week after levodopa was stopped; (□) controls. (□) Levodopa-consuming animals 2 weeks after levodopa was stopped; (●) controls.

FIG. 2. Activation of dopamine-stimulated adenylate cyclase by dopamine-HCl in homogenates of caudate nuclei from Swiss albino HS strain male mice (●) and from their female controls (●).
mensurate with the net dopamine-stimulated enzymatic activities (14).

**DISCUSSION**

The female mice chosen for the present experiments had shown relative insensitivity to injected levodopa and high tolerance for dietary levodopa (14, 17), making them preferable to the males for experiments in which reactivity to levodopa was to be increased. When this increase emerged, it was manifested primarily by enhanced propensity for adventitious stereotyped movements and increased motor activity after standardized challenging doses of levodopa. This state of hypersensitivity included increases in activity of the dopamine-stimulated adenylate cyclase of the caudate nuclei from the corresponding animals. Both phenomena were enhanced by the higher dietary concentration of levodopa.

The motor and the enzymatic consequences of chronic feeding of levodopa have simulated some of the consequences of experimental derenervation of the brain (21, 22). Rats, for example, whose caudates had been denervated by means of nigral lesions, have responded to injected levodopa with unilateral adventitious movements causing a rotary pattern of behavior (23). Their denervated caudates have shown markedly increased dopamine-stimulated adenylate cyclase activity with all concentrations of dopamine tried (21). The actual derenervation of the brain, however, has remained irreversible (23), whereas its presently reported imitation was eventually fully reversed after the dietary levodopa was stopped.

The behavior of these mice parallels also some clinical phenomena encountered during the treatment of Parkinson’s disease with levodopa. The development of a propensity for drug-dependent involuntary movements in human beings has occurred after a relatively long time spent consuming maximal tolerated doses of the drug (24, 25). Discontinuation of levodopa after its chronic administration was followed by a hyperresponsiveness to challenging doses of levodopa (25). The hypersensitivity gradually declined in human beings, as it has here.

The above strengthened our earlier clinical impression that levodopa imprints a chemical memory of its passage (10). Our chemical analyses have been restricted to the caudate because the caudate, being a large structure, can be sampled reproducibly. It must be emphasized, however, that the dopaminergic apparatus innervates several additional cerebral structures (26–28). Since some of the dietary doses of levodopa tested here had earlier increased the mean life span of mice (16, 17), it is probable that the activity of the enzyme located in other structures (28) had also been changed by chronic feeding of levodopa.

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