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L-DOPA INDUCED INCREASES IN BRAIN URIC ACID IN AN ANIMAL MODEL OF PARKINSON'S DISEASE: A RELATIONSHIP TO BEHAVIORAL ACTIVATION

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Summary

Rats with severe unilateral dopamine denervation (≥95% dopamine deficit), produced by intracerebral injection of 6-hydroxydopamine (6-OHDA) into the ventral tegmentum nigrostriatal dopamine neurons, were administered 25 mg/kg L-DOPA (3,4-dihydroxyphenylalanine) methyl ester/2 mg/kg carbidopa. The neurochemical effects of the L-DOPA treatment on uric acid in the cortex and striatum of the intact and 6-OHDA hemisphere were measured. In comparison to saline animals, uric acid concentrations in brain were increased in the L-DOPA treated animals. There were no interhemispheric differences in the uric acid concentrations either in the L-DOPA or in the saline treated animals. Interhemispheric differences were, however, observed in terms of the correlations obtained between L-DOPA and uric acid concentrations in the intact vs. the 6-OHDA hemisphere. Statistically significant correlation coefficients were found in the striatal and cortex samples obtained from the 6-OHDA hemisphere. Furthermore, high correlation coefficients were observed between contralateral rotation frequencies and uric acid concentrations in the cortex and striatum of the 6-OHDA hemisphere. In contrast, only low and statistically non significant correlations were observed in the tissue samples obtained from the intact hemisphere. These observations suggest that L-DOPA activation of the dopamine supersensitive receptors of the DA denervated hemisphere and the associated metabolism of purines with high energy phosphate bonds (e.g. ATP and GTP) increases uric acid as an end-product of purine metabolism. These findings are consistent with other findings indicating that uric acid in the brain can provide an index of metabolic activation in brain tissue.

Key Words: uric acid, 6-hydroxydopamine, rotation, L-DOPA, dopamine

A number of studies suggest that uric acid in the brain is the end-product of the breakdown of purines (e.g., ATP, GTP) and may provide an index of energy metabolism (1-3). More recently, studies using *in vivo* voltametry have reported increases in uric acid in brain tissue in response to

dopaminergic drug stimulation (4-7). Thus, uric acid in the brain appears to be substantially influenced by neuronal activity in dopaminergic systems. To further examine the relationship between dopamine and uric acid in the brain, the present study examined the impact of two manipulations of brain dopamine upon brain tissue uric acid concentrations. One procedure employed was to unilaterally denervate nigrostriatal dopamine neurons and then compare the uric acid levels in the dopamine denervated vs. the dopamine intact striatum. The second approach was to administer the antiparkinsonian treatment, L-DOPA (3,4-dihydroxyphenylalanine) and then determine the relationship between L-DOPA activation of the dopamine denervated striatum and uric acid concentration.

Methods

Adult naive male Sprague-Dawley rats (Taconic Farms, Germantown, NY) ranging from 400 - 500 g were housed in individual clear-plastic cages (55 × 30 ×20 cm) with continuous access to food and water upon receipt from the supplier. The cages were placed in a climate controlled (22 ±1°C) and light controlled room (12 hr light - dark cycles). All treatment and testing was carried out during the light cycle. The animal care and experimental treatment protocols employed were approved both by the VA Medical Center and by the SUNY Health Science Center at Syracuse.

L-DOPA methyl ester (Sigma) was dissolved in de-ionized sterile H_2O and administered by i.p. injection. Carbidopa (Sigma) was dissolved in de-ionized sterile H_2O and also administered i.p. To attenuate peripheral metabolism of L-DOPA, the L-DOPA/carbidopa treatments were in a 10:1 ratio (8).

Stereotaxic procedures were carried out as described in previous reports (9) following completion of an initial 10-day treatment phase of handling (7 days) and saline injection (3 days). Animals were deeply anesthetized with an injection of (0.1 ml/100 g bodyweight) of a ketamine HCl (100 mg/ml) and acetopromazine (10 mg/ml) solution. The 6-OHDA injections were aimed stereotaxically immediately dorsal to the A9 and A10 areas of the midbrain tegmentum using the following coordinates: 4.0 mm posterior to bregma, 7.5 mm below dura and 2.0 mm lateral to midline; and, also at 4.5 mm posterior to bregma, 8.0 mm below dura and 1.0 mm lateral to midline. The incisor bar was placed 3.2 mm above the interaural plane. To attenuate norepinephrine neurotoxicity, the animals were pretreated with desipramine HCl (25 mg/kg) 30 min prior to the 6-OHDA injection. At the 2.0 L site, 2.0 μl of 2 μg/μl (calculated as a free base) of 6-OHDA-HBr (Sigma) was injected at the rate of 0.5 μl/min. At the 1.0 L site, 1.0 μl was injected at the same rate. The 6-OHDA was dissolved in a vehicle solution of sterile 0.15 M NaCl containing 0.2 mg/ml of ascorbic acid. The injections were started 1 min after the cannula was fixed in position and the cannula was removed 3 min after completion of injection. Forty-one animals out of the 57 animals injected unilaterally with 6-OHDA sustained unilateral dopamine loses such that the dopamine concentration was $\leq$5% of the intact striatum. This degree of dopamine deficiency in the denervated striatum is associated with permanent behavioral asymmetries and dopamine receptor supersensitivity (9,10).

Postoperatively, the animals received appropriate supportive care and bodyweight was monitored daily. Following the acute postoperative phase, the animals had an uneventful recovery and most animals exhibited the typical ipsilateral response bias characteristic for severe unilateral denervation of dopamine neurons. The animals were handled daily and within the first two weeks the rats received saline and L-DOPA/carbidopa injections (25 mg/kg L-DOPA methyl ester/2mg/kg carbidopa). After two weeks without any injections and at four weeks postoperative, all animals were injected with saline and then 30 min later, tested for 10 min in the video-chamber. On the next day, one set of animals (n = 10) again received a saline injection and were tested 30 min later in the video-chamber. The other set of animals (n = 47) were given an injection of L-DOPA (25 mg/kg L-DOPA

methyl ester plus 2 mg/kg carbidopa) and tested 30 min later in the video-chamber for 10 min. A post-mortem selection criterion for severe unilateral DA denervation was used (DA concentration in the striatum of the 6-OHDA hemisphere had to be $\leq$5% of the DA concentration of the striatum of the intact hemisphere). Based upon this lesion criterion, 6 rats were in the saline group and 35 in the L-DOPA group. The data analyses were conducted on the results of those animals that met this criterion level of severe unilateral DA denervation.

The behavioral measurements were conducted in a square open-field chamber of 60 × 60 × 45 cm interior dimensions. The walls of the chamber were black and the floor was covered by a removable black paper which was replaced after every trial. A closed-circuit video-camera (RCA TC7911U) was mounted 50 cm above the open-field box. All video signals were analyzed by a video-tracking system, Videomex-V (Columbus Instruments, Columbus, OH) and converted to digitized behavioral scores which were collected, recorded into a spread-sheet through a software program and made ready for subsequent statistical analyses. Additionally, the video-tracking system collected analog data and transduced these signals into an exact tracing of the animal's locomotor movement in the open-field of the test chamber. All behavioral testing was conduced under conditions of dim red light illumination to enhance the contrast between the animal and the dark background of the test chamber. During each 10 min session, data were collected at every 2.5 min interval and stored into a computer for subsequent analyses. The path traced by the animal during each 2.5 min interval of the test session was recorded by a dot matrix printer (Epson FX-286e) placed outside of the test room. The video-tape analysis and the path tracings were used to confirm instances of high rates of contralateral rotation (e.g., 50 per session). In addition, the tight turn characteristic of DA-agonist induced rotation were verified.

Immediately following completion of the last day of behavioral testing in each experimental group, animals were placed in a plastic restraining cone (Braintree Products, Inc.) and sacrificed by decapitation. The brain was rapidly removed and dissected on a chilled glass plate. Under magnification, frontal cortex samples (2 × 2 mm unilateral sections of the medial frontal pole from each hemisphere) was dissected as well as a unilateral neostriatum samples dorsal to the nucleus accumbens from each hemisphere. Histological evaluation of the injection sites was made from paraffin sections of the ventral tegmentum. Following dissection, the samples of brain tissue were weighed, placed in tubes containing 0.5 ml of 0.1 M perchloric acid and 4.5 μl of 10 μg/ml dihydroxybenzylamine (DHBA) as an internal standard, and then homogenized and centrifuged. The resulting supernatant is filtered through 0. 2 μm pore filters and the extracts are stored at -70° C until the HPLC - EC analysis, which is completed within 24 - 72 h. The tissue samples are analyzed for dopamine, DA (3-hydroxytyramine), the dopamine metabolites, DOPAC (3,4-dihydroxyphenyl-acetic acid), HVA (homovanillic acid), NE (arterenol bitartrate) and UA (uric acid). An Alltech Adsorbosphere catecholamine column (C18 reverse phase, 100 × 4.6 mm, 3 μm) was used. The mobile phase was a 0.2 M sodium phosphate monobasic buffer was used at pH 2.5 with 0.25 mM SOS and 0.07 mM EDTA and the pH was adjusted with 0.2 M phosphoric acid. The mobile phase flow rate was 0.5 ml/minute and a BAS LC 4C dual electrode EC detector was used with the electrodes arranged in parallel and the potential set at 0.8 V.

Results

Fig. 1 presents the uric acid and dopamine concentrations in the striatum of the dopamine denervated (6-OHDA) and intact hemispheres in rats treated with saline and L-DOPA. The upper half of figure 1 presents the UA concentrations in the striatum of the intact vs. 6-OHDA hemispheres of the saline and L-DOPA treated groups. The 6-OHDA treatment had no effect on UA concentration in that for both the saline and L-DOPA groups interhemisphere differences were not statistically

significant (saline; t=0.73, p>0.05 and L-DOPA; t=0.87, p>0.05, respectively). The L-DOPA treatment, however, induced a substantial bilateral increase in UA. Statistical comparison between the saline vs. L-DOPA groups indicated that UA concentrations were higher in the L-DOPA treatment groups for both the intact (t=2.52, p<0.05) and the 6-OHDA (t=4.32, p<0.001) hemispheres. The lower half of figure 1 presents the DA concentrations in the striatum of the intact vs. 6-OHDA hemispheres in the saline and L-DOPA groups. Fro both groups, the 6-OHDA treatment produced a severe loss of DA to levels of less than 5% of the intact hemisphere. The L-DOPA treatment produced a statistically significant increase in DA in the intact (t=2.98, p<0.01) but not in the 6-OHDA hemisphere (t=1.61, p>0.05). To further evaluate the relationship between L-DOPA in brain tissue and uric acid concentrations, a set of scatterplots and Pearson's *r* correlation coefficients were determined from the results with striatal and cortical tissue samples. As shown in Fig. 2, statistically significant correlations were observed only in the denervated hemisphere. One possible explanation for this L-DOPA and uric acid relationship in the denervated hemisphere may be due to the occurrence of dopamine receptor supersensitivity following the 6-OHDA denervation procedure so that the dopamine derived from L-DOPA produces a greater activation of the postsynaptic dopamine cells.

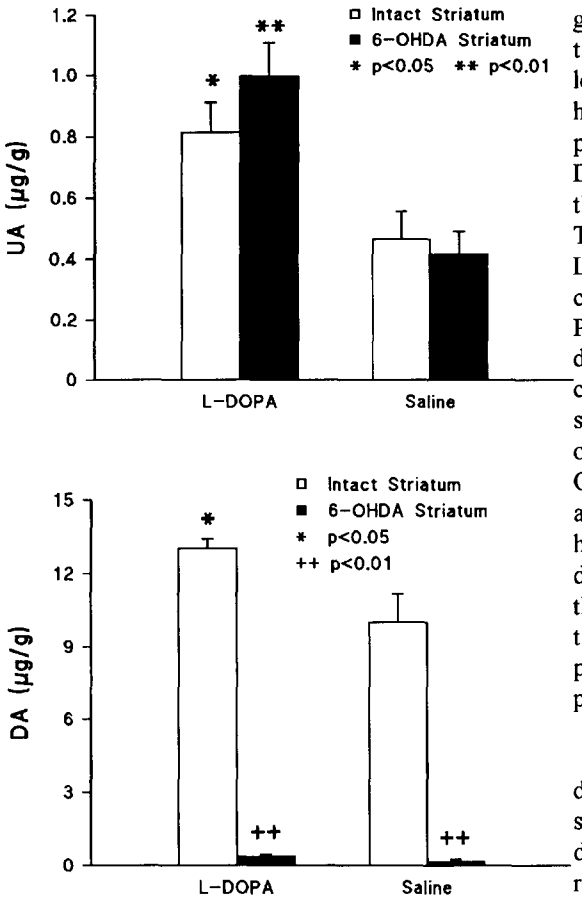

Fig. 1. Means and SEMs of DA (dopamine) and UA (uric acid) concentration levels in µg/g wet tissue for intact and 6-OHDA striatal tissue. ++ indicates statistically significant comparision between hemespheres (p<0.01). * indicates statistically significant differences between the saline vs. L-DOPA treatment groups (* denotes p<0.05 and ** denotes p<0.01).

In animals with severe unilateral dopamine denervation, as in the present study, dopamine receptor supersensitivity develops and animals exhibit contralateral rotation behavior when administered a direct acting dopamine agonist (e.g., apomorphine). While the L-DOPA dose level used in the present study can evoke contralateral rotation, the 25 mg/kg dose of L-DOPA methyl ester/2 mg/kg carbidopa used in the present study is a threshold dose and only a modest number of animals exhibit contralateral rotation based on a criterion of ≥50 rotations during a 10 min observation period. Consequently, there was only a subset of 10 animals which met this criterion. Given that the contralateral rotation response is a behavioral index of activation of the dopamine denervated hemisphere by L-DOPA, it might be expected that there would be a significant correlation between level of contralateral rotation responding and uric acid concentration in the denervated hemisphere. As can be seen in Fig. 3, uric acid concentrations in the cortex and striatum of the dopamine denervated hemisphere were highly correlated with contralateral

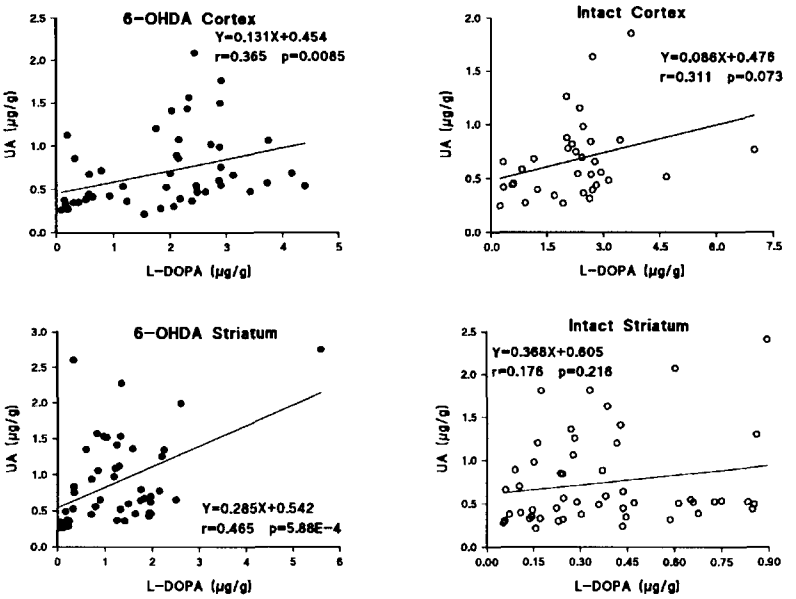

Fig. 2. Correlational analysis showing L-DOPA concentration levels as related to UA (uric acid) levels in μg/g wet tissue for the 6-OHDA cortex (upper left panel) and intact cortex (upper right panel), 6-OHDA striatum (bottom left panel) and intact striatum (bottom right panel). Regression equations (Y), correlations (r) and alpha level of significance (p) are shown in each panel for each analysis.

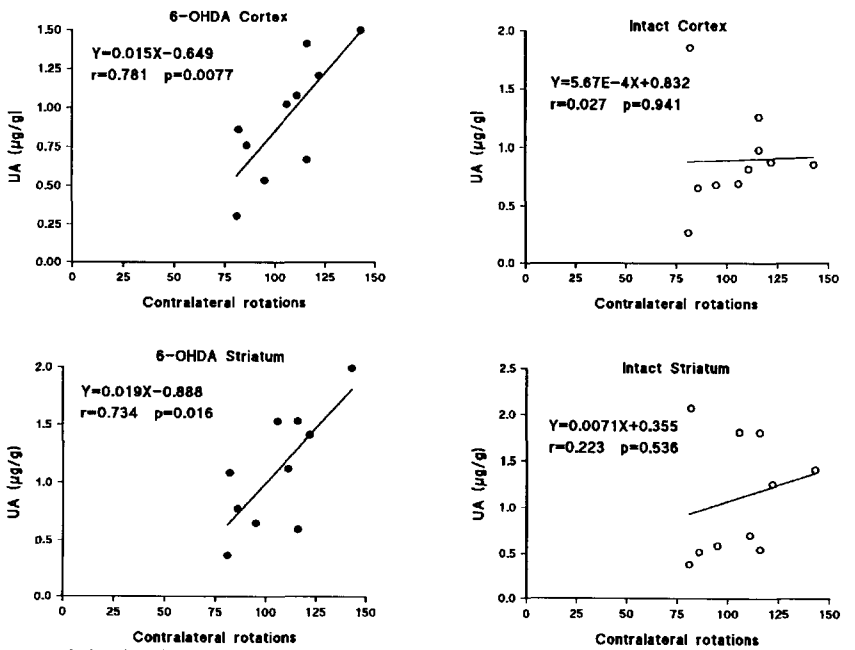

Fig. 3. Correlational analysis showing level of behavioral activation as expressed in frequency of contralateral rotations as related to UA (uric acid) levels in μg/g wet tissue for the 6-OHDA cortex (upper left panel) and intact cortex (upper right panel), 6-OHDA striatum (bottom left panel) and intact striatum (bottom right panel). Regression equations (Y), correlations (r) and alpha level of significance (p) are shown in each panel for each analysis.

rotation, whereas, the uric acid concentration in the intact hemisphere was not reliably correlated with contralateral rotation behavior.

Discussion

The L-DOPA findings are in broad agreement with a number of reports using *in vivo* voltametry in which increases in extracellular uric acid were observed following dopaminergic agonist treatments. Under non drug conditions, however, severe unilateral dopamine denervation as in the present study does not result in a reliable change in uric acid in either the striatum or in the cortex. Possibly a key factor in the interpretation of these observations is the substantial correlation observed in the present study between uric acid in brain tissue and rotational behavior. This finding is consistent with reports of increases in uric acid in conjunction with increases in spontaneous behavior (11). In the case of Parkinson's disease, the dopamine neurotransmitter loss in the brain is bilateral and behavior is impoverished so that, given a bilateral dopamine denervation, a decrease in uric acid levels might be observed under these circumstances. In the present study, the neurotransmitter deficit is unilateral and after four weeks postoperative, there remains a motoric asymmetry but overall locomotion is not diminished in a brief test such as that of the present study (9). Thus, the critical factor in the relationship between DA and uric acid is not with DA concentration *per-se* but whether the DA manipulation impacts upon behavior (i.e., induces activation or inactivation).

In addition to changes due to dopaminergic drug treatment, uric acid measured by HPLC and obtained from *in vivo* microdialysis or from *ex-vivo* tissue has also been shown to change markedly following ischemia or electro-convulsive shock (ECS) treatment (12-14). These findings point to a uric acid linkage with general metabolic processes in which purines with high energy phosphate bonds are metabolized. Uric acid, therefore, would appear relevant to dopaminergic neurotransmission inasmuch as activity in this system impacts upon overall brain activation. The absence of uniqueness in the relationship between dopamine and uric acid does not imply that the relationship is irrelevant. In fact, the suggested relationship between rotational behavior and uric acid in the striatum and cortex of the denervated hemisphere indicates that metabolic activation of the denervated hemisphere is linked to L-DOPA stimulation of the supersensitive dopamine receptors. Since the uric acid relationship to rotational behavior was the same for the striatal and cortical tissue samples, this finding indicates a generalized activation of the dopamine denervated hemisphere, a finding which parallels the animal's asymmetric behavior. This finding support earlier observations using *in vivo* voltametry (7) which suggest a linkage between the intensity of the contralateral rotation behavior and uric acid concentration in the denervated hemisphere. Thus, the present findings point to the utility and feasibility of combining uric acid determinations in conjunction with HPLC analyses of brain tissue samples to simultaneously assess neurotransmitter changes in combination with an index of general energy metabolism in brain tissue. Importantly, long term L-DOPA treatment has been linked to possible neurodegenerative effects (15) and the chronic production of increased uric acid levels may be relevant to this issue as well as to the untoward behavioral effects of chronic L-DOPA therapy (16,17). Additionally, in the long term treatment of Parkinson's disease with L-DOPA, the possible increase in uric acid formation in the brain could impact upon the free radical formation (18) and, thereby, have clinical significance (7,12-14,19).

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