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Alteration of glucose and glycogen in specific regions of mouse central nervous system by L-3,4-dihydroxyphenylalanine (levodopa)*

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THE IMPLICATION of discrete structures of the central nervous system (CNS) in the syndrome of Parkinsonism[1,2] and the known increase of dopamine in the CNS by L-3,4-dihydroxyphenylalanine (levodopa),[3] an agent effective in the treatment of Parkinsonism,[4] has led to a search for a regional impact of levodopa on the intermediary metabolism of the CNS. When used in combination with a monoamine oxidase inhibitor, levodopa increased blood and brain glucose and lowered brain glycogen.[5] In combination with reserpine, however, DL-3,4-dihydroxyphenylalanine (*dl*-DOPA) did not increase brain glucose.[6]

This report presents evidence that a high dosage of levodopa alone indeed alters levels of glucose and glycogen of mouse CNS. Moreover, the temporary lowering of glycogen resides at the lower levels of the neuraxis studied.

Groups of adult male mice (17–23 g) from Carworth Farms were fasted overnight and then injected intraperitoneally with 0·1 ml/10 g body wt. of a suspension of levodopa in 0·9% saline (400 mg/kg). Control animals received an equal volume of 0·9% saline. At various times, thereafter, whole animals were rapidly frozen in Freon 12 (CCl_2F_2) chilled to −150° with liquid N_2,[7] and stored at −80° until dissection.

Samples of cerebral cortex, caudate nucleus, brain stem (ponsmedulla) and spinal cord were dissected and weighed in a cold room at −15°. Approximately 15 mg of each region of four animals from either control or drug-treated groups, respectively, was combined for the preparation of precipitated

* A preliminary report of these results was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Palo Alto, Calif. (August 1970).

glycogen[8,9] and neutralized extracts,[9] which were stored at −80° until analysis. Blood was collected in heparinized tubes from separate groups of five to six decapitated animals, and the plasma was used directly for the determination of glucose. Enzymatic fluorometric methods were used for the duplicate analyses of glucose,[7] glycogen,[8] and lactate.[9] Enzymes were obtained from Boehringer & Sons, except for heart L-lactate; NAD oxidoreductase (EC 1.1.1.27) (Worthington Biochemical Corp.) and α-1,4-glucan: orthophosphate glucosyltransferase (EC 2.4.1.1) (Sigma Chemical Co.). The standard source of glycogen was from rabbit liver (Mann Chemical Co.). Levodopa was from different production lots made here. Rectal temperature was evaluated in separate groups of animals with an electric thermometer. Data from the respective groups were subjected to the Welch *t*-test.[10] Differences between control and drug-treated groups were not considered significant when $P > 0.05$.

At 30 min after injection when drug-treated animals displayed signs of stimulation, as evidenced by exopthalamos, hypersensitivity to touch, piloerection and Straub tail, glucose of plasma and the four regions of the CNS were elevated (Table 1). The differences between levels of CNS glucose from control and drug-treated groups were highest at 30 and 90 min. Afterwards, levels of glucose progressively fell until they were lower than control values at 6 hr. Levels of plasma glucose followed a similar course. At 90 min and later all animals were quiescent and indistinguishable. Rectal temperature at all times remained constant ±1°.

In contrast to the changes of glucose cited above, levels of glycogen at 30 min to 3 hr were altered almost exclusively in the brain stem (Table 2). Thirty min after levodopa, despite the marked elevations of glucose of plasma and brain, glycogen of brain stem and spinal cord was decreased. At all times thereafter brain stem glycogen increased over control values. The levels of glycogen of the other areas, however, were essentially unchanged until 6 hr when elevations occurred in all regions. It is interesting to note that while the increase in glycogen of caudate nucleus at 6 hr and the decrease of this energy reserve in spinal cord at 30 min were not statistically significant, these changes were in the direction of significant glycogen responses at these times. Alternative evaluation of these data by a paired *t*-test of the differences between control and test groups revealed that the above changes were very probable ($P = 0.01$). The 6-hr data suggest that levodopa given acutely to fasted mice elevates glycogen throughout the neuraxis.

TABLE 1. EFFECT OF LEVODOPA, 400 mg/kg I.P., ON GLUCOSE OF THE CENTRAL NERVOUS SYSTEM AND PLASMA OF MICE*

Tissue	Time after injection			
	30 min	90 min	3 hr	6 hr
Cerebral cortex				
Control	1·63 ± 0·19	1·07 ± 0·06	0·90 ± 0·07	1·00 ± 0·04
Test	3·80 ± 0·31	2·81 ± 0·12	1·21 ± 0·05	0·69 ± 0·05
Caudate nucleus				
Control	1·52 ± 0·10	0·93 ± 0·05	0·73 ± 0·07	1·05 ± 0·11
Test	2·84 ± 0·30	2·55 ± 0·11	1·01 ± 0·07	0·61 ± 0·06
Brain stem				
Control	1·37 ± 0·17	0·93 ± 0·10	0·76 ± 0·06	0·86 ± 0·04
Test	2·96 ± 0·28	2·48 ± 0·17	1·06 ± 0·07	0·55 ± 0·05
Spinal cord				
Control	1·78 ± 0·16	1·19 ± 0·08	1·13 ± 0·10	1·19 ± 0·04
Test	3·14 ± 0·31	2·86 ± 0·14	1·42 ± 0·12†	0·87 ± 0·05
Plasma				
Control	6·03 ± 0·88	6·29 ± 0·58	5·78 ± 0·49	5·33 ± 0·33
Test	11·6 ± 0·81	12·9 ± 2·15	6·33 ± 0·16†	4·04 ± 0·23

* Values are expressed as mean millimoles per kilogram of wet weight ± S.E.M. from groups of four to six combined samples of each region of the CNS; plasma, mean millimoles per liter ± S.E.M. from groups of five to six animals.

† $P > 0.05$. In all other instances $P < 0.05 \geqslant 0.01$.

TABLE 2. EFFECT OF LEVODOPA, 400 mg/kg I.P., ON GLYCOGEN OF THE CENTRAL NERVOUS SYSTEM OF MICE*

Region of CNS	Time after injection			
	30 min	90 min	3 hr	6 hr
Cerebral cortex				
Control	2·12 ± 0·13	2·78 ± 0·12	2·79 ± 0·18	2·47 ± 0·09
Test	2·46 ± 0·25	2·49 ± 0·05	3·29 ± 0·32	2·99 ± 0·11†
Caudate nucleus				
Control	2·45 ± 0·16	2·62 ± 0·08	2·93 ± 0·15	2·41 ± 0·17
Test	2·63 ± 0·35	2·75 ± 0·11	3·11 ± 0·32	2·90 ± 0·17
Brain stem				
Control	2·83 ± 0·22	2·66 ± 0·21	3·09 ± 0·13	2·96 ± 0·08
Test	1·94 ± 0·17†	3·64 ± 0·28†	3·93 ± 0·22†	4·04 ± 0·22†
Spinal cord				
Control	4·17 ± 0·29	4·12 ± 0·26	4·35 ± 0·05	4·62 ± 0·28
Test	3·45 ± 0·28	4·55 ± 0·19	4·87 ± 0·21	5·51 ± 0·13†

* Values are expressed as mean millimoles per kilogram of wet weight ± S.E.M. from groups of four to six combined samples of each region of the CNS.
† $P < 0.05 \leqslant 0.01$.

At all times, levels of lactate were not altered in any of the regions examined.

The marked increases of glucose without associated decreases of lactate in all regions of the CNS examined suggest that levodopa at relatively high dosage does not seriously impair aerobic glycolysis in these tissues. In all probability most of the changes of CNS glucose are derived from the alterations of plasma glucose, the increase of which was also observed by Hakanson *et al.*[11] It might have been anticipated that increases of glycogen would result from the elevated plasma and thus brain glucose in view of the elevations in mouse[12] and rat[13] brain glycogen after peripheral glucose loading. The involvement of other factors, however, is evident from the findings that elevations of brain glucose do not cause initial increases of levels of glycogen in any of the areas studied but instead were associated with a fall in the levels of glycogen in the brain stem and the spinal cord. In addition, glycogen was found to be elevated after 6 hr when brain glucose had fallen below control values. The precise regulation of glycogenesis and glycogenolysis in areas of the CNS remains to be elucidated.

The initial changes in levels of glycogen in the brain stem and perhaps the spinal cord suggest a possible sensitivity of these lower levels of the neuraxis to levodopa. Interestingly, this regional specificity is to the exclusion of the anterior sample (caudate nucleus) of the nigro-striatal pathway traditionally invoked in the etiology and therapeusis of Parkinsonism. Whether or not the biphasic changes of glycogen, and perhaps glucose, are associated with the regulation of neuronal transmission in the spinal cord, brain stem and their associated neuronal pathways remains to be determined. Other workers[14] recently associated failure of neuronal transmission in the superior cervical ganglion of the rat with the disappearance of these energy reserves rather than the high energy phosphate ATP. The relationships between the results reported here and more refined pharmacological counterparts of the action of levodopa are in progress.*

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* During the preparation of this manuscript, a report on the decrease of mouse brain glycogen by levodopa alone appeared,[15] substantiating part of our results above.

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Mechanism of action of antipsychotic drugs on biological electron transport

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SEVERAL pharmacological agents are known to be potent inhibitors of the respiratory chain in bacteria, yeast and mammalian mitochondria. Chlorpromazine, other phenothiazine derivatives, and several of the barbiturates[1] have been shown to cause inhibition of mitochondrial electron transport and coupled phosphorylation.[2] Interestingly enough, it has been shown that there is some correlation between the tranquilizing activity of chlorpromazine and its inhibition of mitochondrial electron transfer reactions; it acts as an uncoupler of oxidative phosphorylation at low concentrations (0–80 μM) and as an inhibitor of electron transport at high concentrations (80–265 μM).[3] We have recently observed an interesting interaction between an antipsychotic drug, Clomacran phosphate (CLO), SK & F 14,336, (2-chloro-9-[3-dimethylaminopropyl] acridan, phosphate salt) and the monosodium salt of riboflavin-5-phosphate (FMN);[4] the latter was shown to catalyze the oxidation of CLO to its acridine derivative (2-chloro-9-[3-dimethylaminopropyl] acridine) in the presence of visible light under anaerobic conditions. As the FMN was not reduced during this reaction, the action of the flavin was attributed to a triplet–triplet energy transfer between light-excited FMN and CLO. These observations plus the fact that phenothiazines and dihydroacridines exhibit structural similarities prompted us to investigate the mechanism of action of CLO on the biological electron transport chain. The preliminary data indicate that CLO interferes with electron transfer in the NADH-dehydrogenase segment of the respiratory chain in *Pseudomonas saccharophila*, a facultative autotrophic bacterium.

The organism was grown aerobically in a liquid medium described by Doudoroff[5] with succinate as the carbon and energy source and NH_4Cl as the sole nitrogen source. After 16 hr of incubation at 30°, the cells were harvested, washed twice with 0·05 M tris-HCl buffer (pH 7·2). The cell paste containing 10 g of wet-packed cells were suspended in 10 ml of the sonication medium employed by Aleem[6] for the preparation of actively phosphorylating particles from *Nitrobacter agilis*. The medium contained: 0·3 M sucrose, 1·0 mM $MgCl_2$, 0·5 mM EDTA-Na_2, 0·5 mM reduced glutathione and 0·05 M tris-HCl (pH 7·2). The cells were disrupted by passing twice through an AMINCO-French