J. Biochem. 128, 477–480 (2000)

Manganese Administration Induces the Increased Production of Dopamine Sulfate and Depletion of Dopamine in Sprague-Dawley Rats[1]

J.G. Shirani Ranasinghe,* Ming-Cheh Liu,†,2 Yoichi Sakakibara,* and Masahito Suiko*

*Department of Biochemistry and Applied Biosciences, Miyazaki University, Miyazaki 889-21; and †Biomedical Research Center, The University of Texas Health Center, Tyler, TX 75708, USA

Received May 18, 2000; accepted June 23, 2000

Sprague-Dawley rats were used as an experimental model for investigating the effects of manganese poisoning on the serum levels of unsulfated and sulfated forms of dopamine and its biosynthetic precursors, L-Dopa and L-*p*-tyrosine. Groups of rats were treated daily with Mn^{2+} (20 mg or 40 mg; in the form of $MnSO_4$) or Na^+ (20 mg; in the form of Na_2SO_4). High performance liquid chromatography (HPLC) analysis of the serum samples taken after a 50-day experimental period revealed that the serum level of dopamine sulfate increased by more than 10 times compared with untreated control rats or rats treated with sodium sulfate. In contrast, there was a dramatic decrease (by as much as 4.8 times) in the serum level of unsulfated dopamine in manganese-treated rats. The serum levels of L-Dopa sulfate and L-*p*-tyrosine sulfate were also markedly elevated, although not as much as those of dopamine sulfate. Meanwhile, the serum levels of unsulfated L-Dopa and L-*p*-tyrosine showed no dramatic changes. Atomic absorption spectrophotometric analysis revealed in general an accumulation of manganese in the four organ samples taken from manganese-treated rats. Compared with liver, heart, and kidney, the highest degree of manganese accumulation in manganese-treated rats appeared to be in brain. These results together suggested a role for manganese in stimulating the dopamine-sulfating sulfotransferases in brain, thereby leading to the depletion of dopamine *in vivo*.

Key words: dopa, dopamine, dopamine sulfate, manganese, tyrosine.

Manganese is known to be widely distributed among both prokaryotes and eukaryotes (*1*, *2*). It has been shown to constitute an essential component of a number of enzymes and proteins such as photosynthesis system II and superoxide dismutase (*3–5*). Despite its important functional roles, however, manganese at excessively high concentrations may become neurotoxic for humans and other mammalian animals (*6*). For example, occupational exposure to manganese dust among miners has been shown to cause neurological symptoms resembling Parkinsonism (*7–9*). Animal studies using rats have demonstrated that mining wastes which contain manganese induced alteration of striatal dopaminergic parameters (*10*). As in Parkinson's disease, chronic manganese intoxication appeared to be associated with abnormal dopamine metabolism. Using squirrel monkeys, it was demonstrated that, upon prolonged manganese administration, there was a selective depletion of dopamine and serotonin *in vivo* (*11*). It was speculated that the lowering of dopamine might have been due to the blocking of either Dopa decarboxylase or L-tyro-sine hydroxylase. Moreover, manganese itself may directly affect the enzyme responsible for the conversion of dopamine to norepinephrine (*12*). The real target(s) in the perturbation of dopamine metabolism associated with manganese poisoning, nevertheless, remain(s) to be clarified.

Dopamine is known to function as a brain and peripheral sympathoadrenal medullary neurotransmitter in mammals (*13*). L-Dopa, deriving from the hydroxylation of L-*p*-tyrosine, serves as the immediate precursor for the biosynthesis of dopamine *in vivo* (*14*). In view of the functional importance of dopamine, L-Dopa, and L-*p*-tyrosine., it is likely that mechanisms may operate *in vivo* for their homeostatic regulation. That sulfate conjugation may be important for their homeostatic regulation was first indicated by the finding that approximately 99% of dopamine in human plasma circulates in sulfate-conjugated form (*15*). Subsequent studies revealed the so-called monoamine (M)-form phenol sulfotransferase (M-PST) to be the responsible enzyme for the sulfation of dopamine in humans (*16*). Our recent studies demonstrated further that human M-PST was capable of catalyzing the sulfation of not only dopamine, but also Dopa and tyrosine (*17*). Interestingly, we found that addition of 10 mM $MnCl_2$ exerted remarkable stimulatory effects on the Dopa/tyrosine-sulfating activities of M-PST (*17*). This led us to speculate on a possible link with the neurotoxic effects of manganese mentioned above. It is pos-

[1] This work was supported in part by grants (#12836012 and #11760072) from the Ministry of Education, Science, Sports and Culture of Japan (to M.S. and Y.S.) and a grant from the Research Council, UTHCT (to MCL).

[2] To whom correspondence should be addressed. Tel: +1-903-877-2862, Fax: +1-903-877-2863, E-mail: liu@uthct.edu

Abbreviations: L-Dopa, L-3,4-dihydroxyphenylalanine; HPLC, high performance liquid chromatography; PITC, phenyl isothiocyanate.

© 2000 by The Japanese Biochemical Society.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

sible that, upon manganese poisoning, the dopamine/Dopa/tyrosine-sulfating sulfotransferase activities may be activated *in vivo*. Increasing amounts of dopamine, L-Dopa, and L-*p*-tyrosine may become sulfated and excreted. The lowering of the levels of these compounds eventually results in neurologic disorders. In line with these hypothetical events, manganese-poisoned individuals have been reported to display symptoms resembling those of Parkinson's disease (*7, 8*), and reduction or even elimination of some of these symptoms has been observed in those receiving treatment with L-Dopa (*18*). To test the validity of the above-mentioned hypothesis, we have recently carried out an animal study to investigate the effects of manganese on the urinary excretion of the sulfated form of *m*-tyrosine administered to rats (*19*). The results confirmed that sulfated *m*-tyrosine is indeed excreted by manganese-treated rats, but not untreated control rats.

We report in this communication the results from a follow-up study on the serum levels of unsulfated and sulfated forms of endogenous dopamine, L-Dopa, and L-*p*-tyrosine in untreated and manganese-treated rats. Furthermore, by employing atomic absorption spectrophotometry, manganese accumulation in major organs of the experimental rats was determined.

MATERIALS AND METHODS

Materials—Dopamine, L-Dopa, and L-*p*-tyrosine were products of Sigma Chemical. All HPLC grade reagents were purchased from Wako Pure Chemical Industries. Sulfated forms of dopamine, L-Dopa, and L-*p*-tyrosine were synthesized according to the method of Jevons (*20*). All the other chemicals and reagents were of the highest grades commercially available.

Animals—Twelve male Sprague Dawley rats weighing approximately 300 g each were divided into 4 groups, Group 1 (control) (n=2), Group 2 (n=2), Group 3 (n=4), and group 4 (n=4). The rats were placed in individual cages and kept in a room at 24°C with a 12-h light-dark cycle. Food and water were available ad lib. After a four-day adaptation period, food intake and weight of individual rats were monitored throughout the experimental period. The four groups of rats were treated as follows: Group 1, untreated control; Group 2, 20 mg Na^+ (administered orally as Na_2SO_4)/day; Group 3, 20 mg Mn^{2+} (administered orally as $MnSO_4$)/day; Group 4, 40 mg Mn^{2+} (administered orally as $MnSO_4$)/day. Rats were sacrificed by decapitation at the end of a 50-day experimental period. Blood samples were collected in plastic tubes, incubated at room temperature for 1.5 h, and centrifuged for 5 min at 5,000 ×g. Serum samples collected from the supernatant fractions were stored at −80°C until use. Four organs, liver, kidney, brain, and heart, from each rat were collected and immediately frozen until use.

Preparation of Serum Samples for HPLC Analysis—Two ml of cold (−20°C) ethanol was added to 1 ml of serum sample, and the mixture was incubated at −20°C for 2 h, then centrifuged at 5,000 ×g for 5 min (*21*). The supernatant was collected, dried by evaporation, and the residues were redissolved in 100 μl of ethanol/water/triethyl amine (2:2:1; v/v) (Solution 1) and dried by evaporation. One hundred microliters of ethanol/water/triethyl amine/phenyl isothiocyanate (PITC), (7:1:1:1; v/v) (Solution 2) was added to the dried sample. The preparation was thoroughly mixed, kept for 20 min at room temperature, and dried by evaporation. The dried residues were redissolved in 1 ml of a Buffer A (see below), filtered through a Cosmonic filter (0.45 μm pore size; 4 mm diameter), and subjected to HPLC analysis as described below.

HPLC Analysis—A Hitachi L-6000 HPLC system fitted with an ODS-80 TM TSK-GEL column (84.6 mm i.d.; TOSOH, Tokyo) was employed. Buffer A and Buffer B were, respectively, 6% acetonitrile/60 mM sodium acetate (pH 6) and 60% acetonitrile/60 mM sodium acetate (pH 6). After injection of the sample, the column was eluted at 35°C with a linear gradient from 100% Buffer A to 40% Buffer A and 60% Buffer B over the first 30 min, followed by a linear gradient to 100% Buffer B from 30 to 31 min, and then a steady flow of 100% Buffer B through 36 min.

Analysis of Manganese Contents of Organ Samples—Manganese contents of the organ samples collected were determined using a Shimadzu Model AA-646 Atomic Absorption/Flame Emission Spectrophotometer.

RESULTS AND DISCUSSION

To follow-up on our previous findings on the stimulation of manganese on the urinary excretion of sulfated form of *m*-tyrosine administered to rats (*19*), the current study aimed to examine whether the levels of sulfation of endogenous

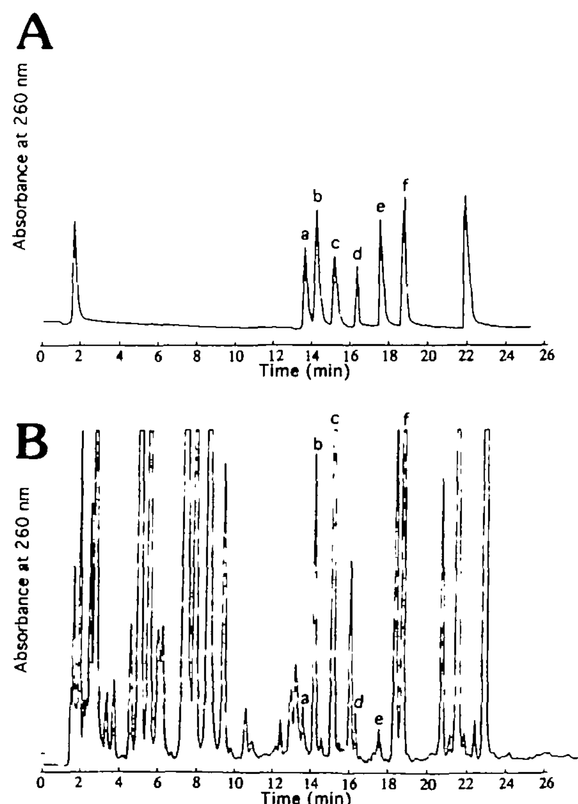

Fig. 1. **HPLC chromatograms of (A) dopamine, L-Dopa, L-*p*-tyrosine and their *O*-sulfate derivatives, and (B) serum sample of manganese-treated rat.** The corresponding compounds for individual peaks are: dopamine sulfate (peak a), L-Dopa sulfate (peak b), L-*p*-tyrosine sulfate (peak c), dopamine (peak d), L-Dopa (peak e), and L-*p*-tyrosine (peak f).

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

dopamine, L-Dopa, and L-*p*-tyrosine are also affected in manganese-treated rats.

Quantitative Determination of Unsulfated and Sulfated Dopamine, L-Dopa, and L-p-Tyrosine in Serum Samples of Control and Manganese-Treated Rats—Serum samples taken from control and manganese-treated rats were analyzed for dopamine, L-Dopa, L-*p*-tyrosine, and their sulfated products. PITC derivatives of these compounds were analyzed by HPLC using a reverse-phase column as described in "MATERIALS AND METHODS." Figure 1 shows typical HPLC chromatograms obtained using this procedure. Peaks corresponding to unsulfated and sulfated dopamine, L-Dopa, and L-*p*-tyrosine (Part A) were all present in the serum sample as indicated by the elution peaks with identical retention times and their co-migration with added authentic sulfated compounds (Part B). When the serum sample was first treated with a cation exchange resin (Dowex 50-X8), the subsequent HPLC analysis revealed only peaks corresponding the sulfated, but not unsulfated, dopamine, L-Dopa, and L-*p*-tyrosine (data not shown). Furthermore, when sulfated dopamine, L-Dopa, and L-*p*-tyrosine collected were subjected to acid hydrolysis with 1 N HCl at 100°C for 15 min, unsulfated dopamine, L-Dopa, and L-*p*-tyrosine were generated as identified by the HPLC analysis.

Table I shows the data derived from the HPLC analysis of unsulfated and sulfated dopamine, L-Dopa, and L-*p*-tyrosine in serum samples collected from control and manganese-treated rats. For rats treated with manganese (Groups 3 and 4), the serum level of dopamine sulfate increased by more than 10 times compared with untreated control rats or rats treated with sodium sulfate (Groups 1 and 2). In contrast, there was a dramatic decrease in the serum level of unsulfated dopamine in manganese-treated rats. The serum levels of L-Dopa sulfate and L-*p*-tyrosine sulfate were also markedly elevated, although not as much as those of dopamine sulfate. Meanwhile, the serum levels of unsulfated L-Dopa and L-*p*-tyrosine showed only minor changes. In the case of L-Dopa, a greater than 65% increase was found for Group 4 rats. It is interesting to note that the combined amounts of unsulfated and sulfated dopamine for manganese-treated rats were more than three times the combined amount determined for control rats. Among other possibilities, the higher combined amounts of unsulfated and sulfated dopamine could have been due to the stimulatory effects of manganese on tyrosine hydroxylase (*22*), which catalyzes the rate-limiting step during the biosynthesis of dopamine in dopaminergic neurons (*23*). Previous studies using rats suggested that manganese may be involved in the activation of tyrosine hydroxylase *in vivo* (*24*). The relatively small, yet significant, increases in the serum levels of L-Dopa in manganese-treated rats seemed to provide some support for this postulation. That the majority of dopamine in manganese-treated rats was present in sulfated form, nevertheless, indicated clearly that manganese administration stimulated the generation of dopamine sulfate, thereby leading to the depletion of unsulfated dopamine. It will be of interest and importance to elucidate the target sulfotransferase enzyme(s) that catalyze the sulfation of dopamine and the mode of action of manganese in manganese-treated rats. It is notable that a decrease in dopamine concentration in brain has also been reported for rabbits subjected to manganese dioxide intoxication (*12*).

Accumulation of Manganese in Major Organs of Manganese-Treated Rats—An important question concerns the tissue/organ specificity of the accumulation of manganese in manganese-treated rats. As shown in Table II, rats treated with manganese generally accumulated manganese in the four organ samples analyzed. While liver showed the highest content of manganese in both control and manganese-treated rats, brain appeared to be the organ with the highest degree of manganese accumulation in manganese-treated rats. It will be interesting to find out whether the high degree of manganese accumulation in the brains of manganese-treated rats is related to the abnormal dopamine metabolism and, if so, whether the accumulated manganese exerts its effects through the activation of certain dopamine-sulfating sulfotransferase enzyme(s) in brain cells.

In summary, we have demonstrated in the present study the dramatic increase in serum levels of sulfated dopamine, L-Dopa, and L-*p*-tyrosine, and the concomitant decrease in

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

TABLE I. **Levels of unsulfated and sulfated dopamine, L-Dopa, and L-*p*-tyrosine in serum samples of control and manganese-treated rats.**[a]

Substance	Substance concentration (nmol/ml serum)			
	Group 1 (None)	Group 2 (20 mg Na^+)[b]	Group 3 (20 mg Mn^{2+})[b]	Group 4 (40 mg Mn^{2+})[b]
Dopamine	0.041 ± 0.018	0.022 ± 0.010	0.014 ± 0.004	0.008 ± 0.005
Dopamine sulfate	0.014 ± 0.009	0.016 ± 0.010	0.179 ± 0.048	0.158 ± 0.062
L-Dopa	0.191 ± 0.058	0.227 ± 0.101	0.239 ± 0.033	0.316 ± 0.152
L-Dopa sulfate	0.013 ± 0.006	0.020 ± 0.003	0.117 ± 0.009	0.082 ± 0.034
L-*p*-Tyrosine	47.99 ± 7.63	42.67 ± 13.55	61.35 ± 14.91	48.89 ± 19.56
L-*p*-Tyrosine sulfate	0.142 ± 0.040	0.135 ± 0.023	0.173 ± 0.015	0.248 ± 0.097

[a]Data shown represent mean ± SD derived from five experiments. [b]As the sulfate.

TABLE II. **Effects of manganese administration on the accumulation of manganese in different rat organs.**[a]

Organ	Manganese content (μg/g dry organ sample)			
	Group 1 (None)	Group 2 (20 mg Na^+)[b]	Group 3 (20 mg Mn^{2+})[b]	Group 4 (40 mg Mn^{2+})[b]
Liver	6.20 ± 0.01	5.83 ± 0.18	10.45 ± 0.24	13.11 ± 0.01
Brain	1.06 ± 0.01	2.52 ± 0.63	3.04 ± 0.91	5.58 ± 0.17
Heart	1.89 ± 0.63	1.61 ± 0.18	2.79 ± 1.36	6.46 ± 3.31
Kidney	1.59 ± 0.01	2.09 ± 0.79	3.35 ± 0.57	7.65 ± 0.31

[a]Data shown represent mean ± SD derived from five experiments. [b]As the sulfate.

the serum level of dopamine in rats treated with manganese. These findings imply strongly that dopamine/Dopa/tyrosine-sulfating sulfotransferase enzyme(s) may represent major target(s) for mediating the neurological effects of manganese. It should be pointed out, however, that there may still be other mechanisms through which manganese may exert its adverse effects. For example, previous studies have demonstrated that manganese may accelerate dopamine auto-oxidation and produce semiquinones and orthoquinones, superoxide anions, hydrogen peroxide and hydroxyl radicals, thus overwhelming the protective mechanisms that regulate the redox process within dopaminergic neurons (*25*). More studies are needed in order to fully elucidate the cellular target(s) and the mechanism(s) of action of manganese.

We wish to thank Dr. Yoshitaka Nagatomo, Dr. Nobuhiro Fukuda, and Ms. Yumiko Takeshita of Miyazaki University for helpful suggestions and technical assistance in this study.

REFERENCES

1. Searcy, K.B. and Searcy, D.G. (1981) Superoxide dismutase from the Archaebacterium *Thermoplasma acidophilum*. *Biochim. Biophys. Acta* **670**, 39–46
2. Kuhn, N.J., Wards, S., and Leong, W.S. (1991) Manganese in Golgi vesicle enzymes. *Eur. J. Biochem.* **195**, 243–250
3. Dekker, J.P. and van Gorkum, H.J. (1987) Electron transfer in the water-oxidizing complex of photosystem II. *J. Bioenerg. Biomembr.* **19**, 125–142
4. Bannister, J.V., Bannister, W.H., and Rotillo, G. (1987) Aspects of the structure, function, and applications of superoxide dismutase. *CRC Crit. Rev. Biochem* **22**, 111–118
5. Frausto da Silva, J.J.R. and Williams, R.J.P. (1991) *The Biological Chemistry of the Elements*, Oxford University Press, New York
6. Barbeau, A. (1984) Manganese and extrapyramidal disorders. *Neurotoxicology* **5**, 13–36
7. Montgomery, E.B. (1995) Heavy metals and the etiology of Parkinson's disease and other movement disorders. *Toxicology* **97**, 3–9
8. Iregran, A. (1994) Using psychological tests for the early detection of neurotoxic effects of low level manganese exposure. *Neurotoxicology* **15**, 671–677
9. Pakkenberg, H. and Brody, H. (1965) The number of nerve cells in the substantia nigra in paralysis agitans. *Pak Acta Neuropath.* **5**, 320–324
10. Rodriguez, V.M., Dufour, L., Carrizales, L., Diaz-Barrig, F., and Capdeville, M.E.J. (1998) Effects of oral exposure to mining waste on *in vivo* dopamine release from rat striatum. *Environ. Health Perspect.* **106**, 487–491
11. Neff, N.H., Barrett, R.E , and Costa, E. (1969) Selective depletion of caudate nucleus dopamine and serotonin during chronic manganese dioxide administration to squirrel monkeys. *Experimentia* **25**, 1140–1141
12. Mustafa, S.J. and Chandra, S.V. (1971) Levels of 5-hydroxytryptamine, dopamine and norepinephrine in whole brain of rabbits in chronic manganese toxicity. *J. Neurochem* **18**, 931–933
13. Nagatsu, T. and Ichinose, H. (1999) Molecular biology of catecholamines-related enzymes in relation to Parkinson's disease. *Cell. Mol. Neurobiol.* **19**, 57–66
14. Blaschko, H. and Chrusciel, T.L. (1960) The decarboxylation of amino acids related to tyrosine and their awakening action in reserpine-treated mice. *J. Physiol.* **151**, 272–284
15. Kuchel, O., Buu, N.N., Unger, T., Lis, M., and Genest, J. (1979) Free and conjugated plasma and urinary dopamine in human hypertension. *J. Clin. Endocrinol.* **48**, 425–429
16. Weinshilboum, R.M. (1986) Phenol sulfotransferase in humans: properties, regulation and function. *Fed. Proc.* **45**, 2223–2228
17. Sakakibara, Y., Katafuchi, J., Takami, Y., Nakayama, T., Suiko, M., Nakajima, H., and Liu, M.-C. (1997) Manganese-dependent dopa/tyrosine sulfation in HepG2 human hepatoma cells: novel dopa/tyrosine sulfotransferase activities associated with the human monoamine-form phenol sulfotransferase. *Biochim. Biophys. Acta* **1355**, 102–106
18. Mena, I. (1977) Manganese poisoning in *Handbook of Clinical Neurology* (Vinkeh, P.J. and Broyh, G.W., eds.) pp 217–327, Elsevier, New York
19. Ranasinghe, J. G.S., Liu, M.-C., Sakakibara, Y., Takeshita, Y., and Suiko, M. (2000) Stereoselective and manganese-dependent sulfation and urinary excretion of D-form and L-form *meta*-tyrosine *O*-sulfate by Sprague-Dawley rats. *J. Mol. Catalysis B: Enzymatic.* in press
20. Jevons, F.R. (1963) Tyrosine *O*-sulfate in fibrinogen and fibrin. *Biochem. J.* **89**, 621–624
21. Sotter, G., Verweij, J., and Schellens, J.H. (1996) Comparison of ethanol plasma-protein precipitation with plasma ultrafiltration and trichloroacetic acid protein precipitation for the measurement of unbound platinum concentration. *Cancer Chemother. Pharmacol.* **38**, 391–394
22. McGeer, E.G., Gibson, S., and McGeer, P.L. (1967) Some characteristics of brain tyrosine hydroxylase. *Can. J. Biochem.* **45**, 1557–1563
23. Levitt, M.S., Spector, S., Sjoerdsma, A., and Udenfiend, S. (1965) Elucidation of the rate-limiting step in N.E in the perfused guinea pig heart. *J. Pharm. Exp. Ther.* **148**, 1–8
24. Girja, S. and Chandra, S.V. (1981) Striatal dopamine turnover and L-dopa treatment after short-term exposure of rats to manganese. *Arch. Toxicol.* **47**, 191–196
25. Flauto, C.M., Paratt, E., Vescovi, A., and Groppetti, A. (1986) Manganese neurotoxicity: Effects of L-dopa and parglyline treatments. *Brain Res.* **367**, 8–13

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012