Biochemical Pharmacology, Vol. 38, No. 11, pp. 1787–1792, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

VESICULAR ACCUMULATION OF DOPAMINE FOLLOWING L-DOPA ADMINISTRATION

NGUYEN T. BUU*
Laboratory of the Autonomic Nervous System, Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada

(*Received* 21 *December* 1987; *accepted* 4 *October* 1988)

Abstract—In this study, the accumulations of dopamine (DA) and norepinephrine (NE) were measured in the brain tissues and in the synaptic vesicle fractions prepared from whole brain of control rats and rats injected with L-DOPA. In the normal rat brain, a 3-fold increase in DA following L-DOPA administration was followed by a small, but not significant increase in vesicular DA, indicating a restricted vesicular uptake of exogenous DA. At the same time, NE in the vesicular fraction and in the whole brain tissue did not change, suggesting a possible link between DA vesicular uptake of DA and brain NE. However, in rats pretreated with α-methyl-*p*-tyrosine, which significantly ($P < 0.05$) reduced DA and NE levels in brain tissues and in the synaptic vesicles, L-DOPA administration led to a significant increase in vesicular DA ($P < 0.05$), suggesting that catecholamine depletion may result in greater vesicular uptake of cytoplasmic DA. The increase in vesicular DA was accompanied by increases in tissue and vesicular NE, underscoring again the existence of a link between vesicular uptake of DA and brain NE following L-DOPA administration. The results also demonstrated a large increase in 3,4-dihydroxyphenylacetic acid (DOPAC) following L-DOPA, in the brain tissues but not in the synaptic vesicle, indicating that monoamine oxidase activity is confined to the cytoplasm.

Vesicular dopamine (DA) could play a major role in determining the physiological action of DA in the central nervous system for two reasons. First, vesicular DA, and not cytoplasmic DA, affects directly the concentration of norepinephrine (NE) because the conversion of DA to NE occurs almost exclusively within the synaptic vesicle where dopamine-β-hydroxylase (DβH) is located. Second, since the portion of DA most likely to interact with pre- and postsynaptic receptors is that released via exocytosis into the synaptic cleft, DA contained in the synaptic vesicle may reflect more directly the physiological effect of the neurotransmitter DA.

Surprisingly, despite numerous studies on the physiological function of DA, little attention has been paid to vesicular DA. It has not been determined whether vesicular DA reflects the concentrations of DA in the whole tissue. Of particular interest is the relationship between cytoplasmic DA and vesicular DA in the brains of animals treated with L-DOPA. Although it has been clearly demonstrated that L-DOPA treatment leads to increased DA levels in the brain of humans [1] and other animal species [2–6], there has not been, to our knowledge, any study which determines the content of DA in the synaptic vesicle following L-DOPA treatment. Such information may be useful not only for a better understanding of the action of DA but also to assess the relationship between NE and DA in the brain following L-DOPA administration. It has been shown repeatedly that the large increases of DA which follow L-DOPA injections are not accompanied by any change in NE [2–6] in the brain, but the reasons given for this lack of correlation between brain DA and NE differ. While some studies suggest that newly formed NE is displaced by the large DA increase [4, 5], others [2, 3, 6], including those from this laboratory, propose that the absence of change in brain NE is due to lack of accessibility of the exogenous DA to DβH.

Thus, in the following study the content of DA in the synaptic vesicle fraction of the brain was determined in control rats and in rats treated with L-DOPA. Vesicular DA was also measured in the DA- and NE-depleted brains of rats pretreated with α-methyl-*p*-tyrosine, an inhibitor of the rate-limiting tyrosine hydroxylase, to determine the possible effect of NE on vesicular accumulation of DA. The results revealed a surprising lack of correlation between vesicular DA and cytoplasmic DA and suggested that the vesicular accumulation of DA may be influenced by DA and NE concentrations in the brain.

MATERIALS AND METHODS

Materials

α-Methyl-*p*-tyrosine methyl ester hydrochloride (α-MPT) and L-3,4-dihydroxyphenylalanine (L-DOPA) were purchased from the Sigma Chemical Co. (St Louis, MO), and 5-[2-^{14}C]hydroxytryptamine binoxalate (sp. act. 56.7 mCi/mmol) and Aquasol were from New England Nuclear (Boston, MA). Acetonitrile was obtained from Anachemia Chemicals (Montreal, Quebec), heptanesulfonic acid from the Aldrich Chemical Co. (Milwaukee, WI), Sephadex G-10 from Pharmacia (Uppsala, Sweden), and Dowex AG 50W X8 (200–400 mesh H^+ form) from Bio-Rad Laboratories (Richmond, CA). All other compounds were purchased from Fisher Chemicals (Fairlawn, NJ).

* Correspondence: Nguyen T. Buu, Ph.D., Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec H2W 1R7, Canada.

Treatment with α-MPT and L-DOPA

Male Sprague–Dawley rats weighing 180–200 g were injected intraperitoneally (i.p.) with saline or α-MPT at a dose of 300 mg/kg, and received saline or L-DOPA (100 mg/kg, i.p.) after 3 hr. They were decapitated 60 min later when DA levels in the brain peaked [6]. Their brains were rapidly excised and dissected on ice along the demarcation lines described by Glowinski and Iversen [7]. The hypothalamus, striata and brainstem were removed, immediately frozen on dry ice, and stored at −80° until analyzed. The elapsed time between decapitation and freezing of tissues was approximately 1 min.

Reserpine treatment

In this experimental series, the rats were first injected i.p. with reserpine (5 mg/kg) 18 hr prior to saline or L-DOPA (100 mg/kg, i.p.) administration. They were killed 1 hr following the last treatment, and their whole brains were removed quickly for synaptic vesicle preparation, as described below.

Preparation of synaptic vesicles

Synaptic vesicle fractions were prepared from whole brains by the method of Ruth *et al.* [8]. Fresh brain tissue was homogenized in 5 vol. of cold buffer (100 mM potassium tartrate, 3 mM potassium bicarbonate, 1 mM potassium chloride, 1 mM magnesium chloride, 4 mM potassium phosphate, 10 μM ipronazid phosphate, 10 μM ascorbic acid and 1 μM EDTA) adjusted to pH 7.5 with potassium hydroxide solution, in a hand-held Dual Teflon/glass homogenizer. The suspension (S_1) was sequentially centrifuged at 4° at 3,000 *g* for 20 min, at 20,000 *g* for 30 min, and finally at 100,000 *g* for 45 min to yield a crude vesicle pellet (P_3). The latter was gently washed twice with 3 ml of cold buffer, and in the third wash was gently resuspended in 1 ml of buffer before centrifugation at 100,000 *g* for 30 min. The pellet was again resuspended in 1 ml of buffer, and aliquots were sampled for protein measurement. The remaining suspension was homogenized in a Brinkmann Polytron in 0.1 N $HClO_4$ and analyzed for catecholamines (CA) and CA metabolites.

To determine possible losses of DA and NE during the synaptic vesicle preparation, ^{3}H-labeled DA or NE was added to brain homogenates (S_1) (four samples for each CA) and processed exactly as described above to yield the pellet (P_3) and supernatant (S_3) fractions. Following alumina adsorption, the radioactive CA (DA or NE) in the S_1 and S_3 were compared to assess whether there was any loss of CA during the preparation. Radioactive CA in the synaptic vesicle fraction were also measured to determine whether uptake of CA occurred during the isolation of the synaptic fraction. The results showed that 98–99% of radioactive DA and NE introduced in the homogenates was recovered in the supernatant (S_3) fractions. There was negligible radioactivity detected in the pellet (P_3) fraction.

Monoamine oxidase (MAO) determination

Whole brains were homogenized in 10% (w/v) 0.08 M phosphate buffer (pH 7.2) and centrifuged at 900 *g* for 10 min at 4°. The supernatant fractions were measured for MAO activity by the method of Campbell *et al.* [9], using [^{14}C]5-hydroxytryptamine as substrate. Sonicated preparations were assayed in triplicate. Following a 30-min incubation at 37°, the samples were placed on ice and then transferred to Pasteur pipettes containing 0.5 × 2.5 cm of Dowex AG 50W X8 (200–400 mesh H^+ form) previously washed with 0.1 N HCl and water. The columns were eluted with 3 ml of deionized water, and the entire eluate was collected in vials containing Aquasol. The radioactive products were assayed by liquid scintillation spectrometry. Protein content was determined by the method of Lowry *et al.* [10].

Analysis of CA and metabolites

Tissue treatment. On the day of analysis, each tissue was weighed and homogenized in ten times its weight of 0.1 M perchloric acid. The homogenates were then centrifuged at 15,000 *g* for 20 min, and the clear supernatant fraction was used to determine free DA, NE, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) by high-performance liquid chromatography (HPLC) with electrochemical detection, according to the method of Westerink [11]. Essentially, 0.5 to 1 ml of the supernatant fractions was applied to Sephadex G-10 columns (5 × 70 mm) prepared in Pasteur pipettes. After the tissue extracts had passed through the columns, they were washed with 2 ml of 0.01 M formic acid. DA, NE and MHPG were eluted with 1.5 ml of formic acid, followed by 1 ml of 5 mM sodium phosphate dibasic containing 10 mM EDTA (pH 9.0). The columns were then washed with 0.5 ml of the same phosphate buffer, and DOPAC was eluted with 2 ml of 0.02 M ammonia (pH 10.7) into tubes containing 50 μl of formic acid (70%) (final pH is 2.0). Samples were measured with and without internal standards which included all the CA and metabolites to be measured in the assay. Recoveries of CA and the metabolites by this assay ranged from 90 to 100%. Eluates were analyzed by HPLC with electrochemical detection on the same day of extraction. Internal and external standards were used for correction of recovery and for calculations of concentrations.

Reverse-phase HPLC with electrochemical detection. The HPLC system consisted of a high-pressure pump (model M45, Waters Associates, Milford, MA) connected to a reverse-phase Bondapak C_{18} column (Chromatography Science, Mount Royal, Quebec). The column eluate was monitored by an amperometric detector (LC-4A, BAS, West Lafayette, IN) using a glassy carbon electrode. The oxidation current was registered by a data processor (C-RIB, Beckman, Irvine, CA). Samples were injected by an automatic sample injector (Wisp-710B, Waters Associates). All mobile phases were filtered through a membrane (pore size 0.45 μm) and degassed before use. The temperature was ambient during all analyses. The mobile phase differed slightly according to the compounds analyzed. For NE and DA, it consisted of 0.1 M sodium phosphate (pH 4.3) with 0.01% EDTA, 1 mM heptanesulfonate and 4% acetonitrile. For MHPG and DOPAC, a 0.1 M (pH 4.8)

Table 1. CA and CA metabolites in the hypothalamus, striatum and brainstem of control and α-MPT-pretreated (300 mg/kg) rats following saline or L-DOPA (100 mg/kg, i.p.) administration

	Concentration (ng/g tissue)			
	NE	MHPG	DA	DOPAC
Hypothalamus				
Control + saline (6)	1,100 ± 60	ND*	430 ± 20	86 ± 3
Control + L-DOPA (6)	1,100 ± 80	420 ± 30	1,500 ± 100†	9,100 ± 100†
α-MPT (9)	500 ± 60†	ND	100 ± 20†	ND
α-MPT + L-DOPA (9)	830 ± 10‡	360 ± 50	1,100 ± 100‡	8,000 ± 800
Striatum				
Control + saline (6)	250 ± 15	ND	3,400 ± 200	500 ± 60
Control + L-DOPA (6)	290 ± 30	69 ± 6	7,000 ± 500†	12,000 ± 1,000†
α-MPT (9)	100 ± 14†	ND	1,400 ± 200†	200 ± 20†
α-MPT + L-DOPA (9)	220 ± 20‡	56 ± 7	4,800 ± 300‡	9,100 ± 900
Brainstem				
Control + saline (6)	430 ± 30	ND	130 ± 10	20 ± 2
Control + L-DOPA (6)	400 ± 30	170 ± 20	500 ± 35†	8,600 ± 700†
α-MPT (9)	180 ± 20†	ND	90 ± 20	ND
α-MPT + L-DOPA (9)	350 ± 40‡	130 ± 10	530 ± 60‡	7,100 ± 500
Whole brain				
Control + saline (10)	157 ± 17	ND	440 ± 30	250 ± 4
Control + L-DOPA (8)	180 ± 7	137 ± 8	890 ± 30	5,600 ± 370†
α-MPT (6)	76 ± 5†	ND	133 ± 9†	42 ± 3†
α-MPT + L-DOPA (6)	163 ± 8‡	122 ± 8	820 ± 15‡	4,500 ± 450‡

Values are means ± SE; figures in parentheses represent the number of rats. Normetanephrine was not detectable in tissues before or after L-DOPA.

* Not detectable by the present method.

† Statistically significant ($P < 0.05$) vs control saline group.

‡ Statistically significant ($P < 0.05$) vs the α-MPT group.

sodium phosphate buffer (adjusted with acetic acid), containing 3% acetonitrile and 0.01% EDTA, was used. The oxidation potential was set in both cases at 0.75 V vs an Ag/HgCl reference electrode.

Statistical analysis

Statistical comparison between two groups was performed with the unpaired Student's *t*-test (two-tailed). When comparisons were made between several groups the Newman–Keul's test was employed. In all cases, a $P < 0.05$ value was considered statistically significant.

RESULTS

Table 1 lists the concentrations of DA, NE, and their principal metabolites, MHPG and DOPAC, in the hypothalamus, brainstem, striatum and whole brain of controls and rats pretreated with a high dose of α-MPT following an injection of exogenous L-DOPA. The response to L-DOPA was different between the control and α-MPT-pretreated groups. In control rats, L-DOPA injection led to a significant increase of DA and MHPG concentrations with no change in NE, confirming the findings of previous studies [2–6]. In contrast, in α-MPT-pretreated animals, in which DA and NE were decreased significantly ($P < 0.05$) due to the inhibition of tyrosine hydroxylase activity [12], L-DOPA administration resulted in a significant elevation of NE along with a marked rise in DA and MHPG. Although the MHPG increase appeared to be lower in α-MPT-pretreated rats than in the controls, the difference was not statistically significant. This pattern of response of both groups to L-DOPA administration was also observed in the whole brain, indicating that the reaction of α-MPT-pretreated rats to L-DOPA was not confined to specific areas of the brain rich in NE or DA, but was generalized over other brain tissues.

Following L-DOPA injection, there was a striking increase in DOPAC concentrations in the brains of the control and α-MPT-pretreated rats (Table 1), attesting to the importance of the MAO pathway in the inactivation of exogenous DA in the brain [13]. However, in the α-MPT-pretreated group, the levels of DOPAC in the hypothalamus, brainstem and whole brain following L-DOPA injection were significantly ($P < 0.05$) lower than those in the controls. This was not due to any decrease in MAO activity, which remained unchanged after either L-DOPA administration or α-MPT-pretreatment (Table 2).

Figure 1 presents the different patterns of DA and NE accumulations in the synaptic vesicle of control rats and α-MPT-treated rats following L-DOPA injection. DOPAC and MHPG were not detectable in this fraction despite their large concentrations in the cytoplasm, indicating that MAO action is confined to cytoplasmic, non-vesicular sites. There was a distinct difference as far as DA was concerned

Table 2. MAO activity in the brain of control and α-MPT-treated rats

	MAO activity (nmol/mg protein/hr)
Saline	19.6 ± 1.8
L-DOPA	21.1 ± 2.4
α-MPT + saline	26.1 ± 1.6
α-MPT + L-DOPA	24.5 ± 3.1

MAO activity was measured in whole brains 3 hr after saline or α-MPT injection. Each group contained four rats. Values are means ± SEM.

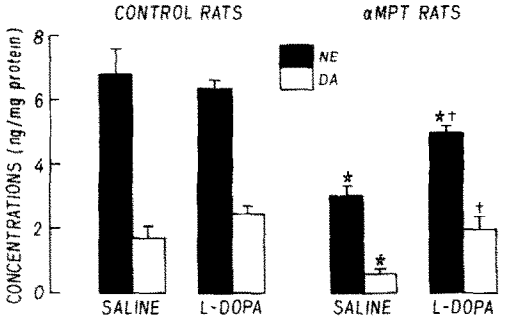

Fig. 1. DA and NE concentrations in the vesicular fraction prepared from whole brains of control and α-MPT-pretreated rats following L-DOPA administration (100 mg/kg, i.p.). α-MPT (300 mg/kg, i.p.) was given 3 hr before L-DOPA, and the rats were killed by decapitation. The whole brains were removed and homogenized, as described in the text. Aliquots were separated for analysis of CA and metabolites. The remaining homogenate was used for the preparation of synaptic vesicle fractions. Key: (*) statistically significant ($P < 0.05$) compared to control rats (not treated with α-MPT); (†) statistically significant ($P < 0.05$) compared to the controls within the same group (saline or α-MPT).

in the response to L-DOPA at the vesicular level between the normal brain and the CA-depleted brain. Thus, despite similar DA increases in both brains after L-DOPA injection, only a slight elevation was evident in the vesicular fraction of normal rat brain whereas in the CA-depleted brain of α-MPT-treated rats there was a significant increase in vesicular DA. The NE response to L-DOPA in vesicles paralleled that observed in brain tissues in that there was no change in the controls whereas a significant increase was observed in α-MPT-pretreated rats. As illustrated in this figure, the concentrations of DA and NE in the vesicular fractions of α-MPT-pretreated rats showed a marked decrease relative to the controls, suggesting that the effect of α-MPT reached the vesicular level at a rapid rate.

The concentrations of DA in the vesicular fraction differed sharply from those found in the whole tissue (Table 3). Thus, vesicular DA concentrations were ten times lower than tissue DA concentrations, whereas vesicular NE concentrations were two times higher than tissue NE. The lower DA concentrations in the vesicular fraction are unlikely the result of a loss due to its partial destruction during the preparation of the vesicle since such a loss of DA would be accompanied by a similar loss of NE, which was not observed. Furthermore, as previously stated in Materials and Methods, there was almost 100% recovery of radioactive DA or NE introduced into the homogenates, indicating that there was no loss of DA and NE during the vesicular fraction preparation. On the other hand, the increase in NE concentrations in the vesicular fraction (with respect to tissue concentrations) may suggest that the vesicular fraction was enriched with NE vesicles, or that NE is contained mainly in synaptic vesicle, whereas DA is largely cytoplasmic. That the CA concentrations found in the vesicular fractions reflected the CA in the vesicles is supported by the results presented in Table 4, showing that DA and NE were decreased markedly in vesicular fractions prepared from rats pretreated with reserpine, and did not change when these animals were injected with L-DOPA.

DISCUSSION

This study demonstrated that, although the administration of L-DOPA invariably leads to a large increase of DA in the brain, this DA rise does not extend necessarily to the vesicular level. Thus, in the normal rat, a 3-fold elevation of DA in brain tissue following L-DOPA injection did not result in a significant DA augmentation in synaptic vesicle fractions prepared from the same tissue. On the other hand, in rats pretreated with a high dose of α-MPT, which blocks the rate-limiting step of CA synthesis [14] and significantly lowers DA and NE levels in brain tissues and synaptic vesicles, the DA increase in brain following L-DOPA injection was accompanied by a significant DA increase in the vesicular fraction (Fig. 1).

The mechanism accounting for the lack of any significant increase in the normal rat brain following L-DOPA administration is not clear. It has been shown previously [15–18] that the vast majority of the DA formed from L-DOPA is generated in sites other than catecholaminergic terminals, suggesting that only a small fraction can reach the nerve endings. However, such a view does not explain the rise in vesicular DA following L-DOPA administration in the α-MPT-pretreated rats. That this DA elevation is mediated through a vesicular uptake process is indicated by the finding that prior treatment with reserpine, a vesicular uptake inhibitor [19], drastically lowered vesicular CA and completely abolished any increase following L-DOPA administration.

The apparent lack of transport of DA into the synaptic vesicle following L-DOPA treatment in the normal rat brain is not likely due to saturation of the vesicle since it can be expanded [20, 21]. However, it is possible that in the normal rat brain the transport of DA into the synaptic vesicle is operating at or near saturation and increase in cytoplasmic DA, such as that following L-DOPA administration, would not change vesicular DA content. Supporting such a view is the finding that in the DA-depleted brain of the

Table 3. Comparison of DA and NE concentrations between the cytoplasmic and vesicular fractions in control and α-MPT-treated rats following saline or L-DOPA administration

	Concentrations (ng/mg protein)			
	Cytoplasmic		Vesicular	
	NE	DA	NE	DA
Control + saline (8)	4.1 ± 0.2	11.4 ± 0.9	6.9 ± 0.7	1.8 ± 0.2
Control + L-DOPA (7)	4.6 ± 0.3	24.3 ± 2.3*	6.3 ± 0.3	2.5 ± 0.4
α-MPT (6)	1.7 ± 0.1*	3.5 ± 0.3*	3.0 ± 0.3*	0.6 ± 0.1*
α-MPT + L-DOPA (6)	4.6 ± 0.2†	19.9 ± 0.9†	5.0 ± 0.2†	2.1 ± 0.4†

Values are means ± SE; figures in parentheses represent the number of rats.
* Statistically significant ($P < 0.05$) vs control saline group.
† Statistically significant ($P < 0.05$) vs the α-MPT group.

Table 4. NE and DA in the vesicular brain fraction of untreated controls and reserpine-treated rats

	NE	DA
	(ng/mg protein)	
Intact (4)	6.9 ± 0.9	0.59 ± 0.17
Reserpine (4)	0.24 ± 0.03*	0.13 ± 0.03*
Reserpine + L-DOPA (4)	0.31 ± 0.04*	0.19 ± 0.04*

Reserpine (5 mg/kg) was injected i.p. 18 hr prior to L-DOPA administration (100 mg/kg, i.p.) with the rats being killed 1 hr after L-DOPA treatment. Values are means ± SE; the figures in parentheses indicate the number of animals.
* Statistically significant ($P < 0.05$) difference from the control groups.

α-MPT-treated rat there was indeed a significant elevation of vesicular DA following L-DOPA. An earlier report by Spector *et al.* [22] showing that the rate of [^{3}H]DOPA incorporation into brain NE is increased 4-fold when tyrosine hydroxylase is inhibited is also consistent with this view.

The relationship between DA and NE in the rat brain following an L-DOPA injection has been the subject of several studies. Although it has generally been agreed that increased DA in the rat brain does not alter NE levels [2–6], the explanations given are not uniform. Some studies [5, 23] suggested that the lack of an increase in NE following L-DOPA administration is due to displacement of newly-synthesized NE from storage vesicles by the expanded DA pool. In support of this explanation, the finding of a dose-dependent rise in MHPG following L-DOPA injection suggests that the displaced NE is metabolized by MAO and the elevated MHPG could mask any increase in NE. However, the present data argue against this explanation by, first, showing that there was no decrease of NE in the vesicles of rats treated with L-DOPA, although MHPG was augmented significantly. Secondly, in the α-MPT-pretreated rat brain where there was a significant increase in NE following L-DOPA administration, there was also an MHPG increase, comparable in magnitude to that in the normal rat brain, indicating that the NE and MHPG elevations could be parallel and not mutually exclusive. In contrast to MHPG, there was no apparent change in normetanephrine in either control rats or rats treated with α-MPT following L-DOPA administration, indicating that NE was not metabolized by an alternate pathway. The dose-dependent increases of MHPG with L-DOPA [4, 24] amid the lack of any change in vesicular and brain NE suggest that there is a fraction of extravesicular NE in brain, bound to protein and therefore protected against MAO action, which could be displaced from their binding by exogenous DA. That NE can be bound to protein is suggested by previous findings [25, 26] showing that up to 50% of a physiologic concentration of NE is bound to protein in the plasma.

On the other hand, the absence of any change in vesicular and tissue NE following significant DA increases could be due, as suggested by other studies [2, 3, 6], to lack of access of the expanded DA pool to DβH. The present data are in complete agreement with such an hypothesis, showing that in the control rat brain where no change in NE was observed following L-DOPA administration, vesicular DA was not significantly different from control rats not injected with L-DOPA, whereas in the α-MPT treated rats, which exhibited an NE increase following L-DOPA, vesicular DA also showed a significant increase (Fig. 1).

That vesicular accumulation of DA could influence NE concentrations is not unexpected in view of the fact that DβH, which is responsible for the conversion of DA to NE, is located almost exclusively within the storage vesicle. The concept that DA transport into the synaptic vesicle may play a role in the synthesis of NE is not new. There have been several studies [27–30] suggesting that restriction of DA uptake into the synaptic vesicle could limit NE synthesis.

It may be possible that depletion of NE in the synaptic vesicle may increase vesicular uptake of DA, although the mechanism for such a regulation cannot be drawn from the present data. CA depletion in the brain has been shown to induce changes in the general metabolism of CA due to a feedback mechanism [31]. Whether this feedback mechanism contributes to a greater vesicular DA increase in the α-MPT-treated rats remains to be studied further.

There was also a persistent decrease of DOPAC accumulation following L-DOPA in the brain of α-

MPT-treated rats, although the meaning of this decrease is not apparent. It is compatible, however, with the hypothesis that in these rats a greater proportion of DA formed from L-DOPA could enter into the synaptic vesicle where it is protected against MAO action. In a recent communication, Dwoskin *et al.* [31] reported that α-MPT treatment does not decrease the amount of endogenous DA collected in the superfusates of rat striatal slices following electrical stimulation despite a marked decrease in striatal DA content. The data suggest that, in the α-MPT-treated rats, a vast proportion of the newly synthesized DA, formed during the stimulation, was channeled into the (vesicular) releasable pool. This also suggests that, in the striatal tissue of normal, non-treated rat, the DA released following electrical stimulation was mostly newly synthesized DA, in agreement with the accepted notion that the new CA are the most readily released [33]. These authors observed, at the same time, a marked (82%) DOPAC decrease in the perfusate of striatal slices from α-MPT-treated rats. The diminution of DOPAC and the lack of any decrease of the releasable DA in their α-MPT-treated rats are compatible with the observation of a greater entry of exogenous DA into the NE-depleted vesicular fraction of the α-MPT-treated rats in this study.

Acknowledgements—The author thanks Dr T. A. Slotkin for discussions leading to this study, Ms Monique Angers for her technical assistance, and Mrs Sylvie Ouellet-Collon for her secretarial help. This work was supported by grants from the Medical Research Council of Canada, the Parkinson Foundation of Canada, and the Canadian Heart Foundation (Quebec).

REFERENCES

1. O. Hornykiewicz, *Life Sci.* **15**, 1249 (1974).
2. G. M. Tyce and C. A. Owen, *Biochem. Pharmac.* **28**, 3271 (1979).
3. C. R. Freed and R. C. Murphy, *J. Pharmac. exp. Ther.* **205**, 702 (1978).
4. P. Doshi and D. J. Edwards, *J. Chromat.* **210**, 505 (1981).
5. J. W. Commissiong, *J. Neurochem.* **42**, 1574 (1984).
6. N. T. Buu, J. Duhaime and O. Kuchel, *J. Neurochem.* **44**, 787 (1985).
7. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
8. J. A. Ruth, M. J. Gerhsten and J. K. Disbrow, *Experientia* **40**, 692 (1984).
9. I. C. Campbell, D. S. Robinson, W. Lowenberg and D. L. Murphy, *J. Neurochem.* **32**, 49 (1979).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. B. H. C. Westerink, *J. Neurochem.* **42**, 934 (1984).
12. F. Widerlov, *J. neural Transm.* **44**, 145 (1979).
13. B. H. C. Westerink and J. Korf, *Eur. J. Pharmac.* **40**, 131 (1976).
14. M. Levitt, S. Spector, A. Sjoerdsma and S. Udenfriend, *J. Pharmac. exp. Ther.* **148**, 1 (1965).
15. L. K. Y. Ng, T. N. Chase, R. W. Colburn and I. J. Kopin, *Neurology* **22**, 688 (1972).
16. L. D. Lytle, O. Hurko, J. A. Romero, K. Cottman, D. Leehey and R. J. Wurtman, *J. neural Transm.* **33**, 63 (1972).
17. N. T. Buu, *J. Neurochem.* **45**, 470 (1985).
18. G. L. Snyder and M. J. Zigmond, *Soc. Neurosci. Abstr.* **13**, 1642 (1987).
19. A. Carlsson, N. A. Hillarp and B. Waldeck, *Acta physiol. scand.* **59**, 1 (1963).
20. M. Fillenz and S. C. Stanford, *Br. J. Pharmac.* **73**, 401 (1981).
21. N. T. Buu, *Biochem. Pharmac.* **38**, 1685 (1989).
22. S. Spector, A. Sjoerdsma and S. Udenfriend, *J. Pharmac. exp. Ther.* **147**, 86 (1965).
23. D. J. Edwards and M. Rizk, *J. Neurochem.* **36**, 1641 (1981).
24. N. T. Buu, J. Duhaime and O. Kuchel, *J. neural Transm.* **70**, 39 (1987).
25. A. Danon and J. D. Sapira, *J. Pharmac. exp. Ther.* **182**, 295 (1972).
26. G. Powis, *Biochem. Pharmac.* **24**, 707 (1975).
27. L. Landsberg, J. De Champlain and J. Axelrod, *J. Pharmac. exp. Ther.* **165**, 105 (1969).
28. I. J. Kopin and V. K. Weise, *Biochem. Pharmac.* **17**, 1461 (1968).
29. W. J. Louis, A. E. Doyle and R. G. Sampson, *Clin. exp. Pharmac. Physiol.* **1**, 341 (1974).
30. M. J. Sole, G. G. Helke and D. M. Jacobowitz, *Am. J. Cardiol.* **49**, 1682 (1982).
31. A. Alousi and N. Weiner, *Proc. natn. Acad. Sci. U.S.A.* **56**, 1491 (1966).
32. L. P. Dwoskin, G. A. Gerhardt and N. R. Zahmiser, *Soc. Neurosci. Abstr.* **12**, 805 (1986).
33. N. B. Thoa, in *Frontiers in Catecholamine Research* (Eds. E. Usdin and S. H. Snyder), p. 613. Pergamon Press, New York (1973).