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Cortical Ablation and Drug-Induced Changes in Striatal Ascorbic Acid Oxidation and Behavior in the Rat

MADDALENA MIELE,[1] PAOLO ENRICO, GIOVANNI ESPOSITO, LUIGIA FRESU, ROSSANA MIGHELI, GUGLIELMO DE NATALE AND MARIA S. DESOLE

Institute of Pharmacology, University of Sassari, viale S.Pietro 43/B, O7100 Sassari, Italy

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MIELE, M., P. ENRICO, G. ESPOSITO, L. FRESU, R. MIGHELI, G. DE NATALE AND M. S. DESOLE. *Cortical ablation and drug-induced changes in striatal ascorbic acid oxidation and behavior in the rat.* PHARMACOL BIOCHEM BEHAV **50**(1) 1-7, 1995.—Rats whose frontoparietal cortex had been bilaterally ablated were allowed 21 days for recovery and then treated with apomorphine (APO), 1 mg/kg SC or scopolamine (SCOP), 0.6 mg/kg SC. Soon after a behavioral test, dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), ascorbic acid (AA), and dehydroascorbic acid (DHAA) levels were determined by HPLC/EC in striatal synaptosomes (left side) and whole striatum (right side). SCOP behavioural effects were attenuated by cortical ablation, while those of APO were affected to a lesser extent. In the striatum of unoperated and sham-operated rats DHAA contents and DHAA/AA ratio resulted increased after drugs administration. No change in AA oxidation was observed in the striatum of ablated rats. In the synaptosomes of unoperated and sham-operated rats both drugs led to a decrease in DHAA contents and DHAA/AA ratio. In unoperated and sham-operated rats APO and SCOP caused a decrease of the DOPAC/DA ratio in the whole striatum and striatal synaptosmes. In ablated rats APO caused a decrease of DOPAC/DA ratio in the whole striatum and synaptosomes, while SCOP effects on DA turnover resulted attenuated in the whole striatum and abolished in synaptosomes. We conclude that drug-induced AA oxidation is likely to occur in the extracellular space and requires intact corticostriatal glutamatergic pathways. The latter may play an enabling role in SCOP behavioral effects.

Corticostriatal glutamatergic pathways Striatal dopamine turnover Ascorbic acid oxidation Behavior Apomorphine Scopolamine Rat

ASCORBIC ACID (AA) is present in high concentrations in the mammalian brain (2,24). The concentrations vary between regions, and in the range between 1.1-3.4 μmol/g tissue (24) equivalent to an intracellular concentration between 1-3 mM. The extracellular concentrations is in the range of 200-400 μM (1) and is under homeostatic control (31).

It has been suggested that changes in AA lead to modulation of the dopaminergic system, because systemic (4,29), intracerebroventricular (31), or intrastriatally injected AA (34) antagonizes *d*-amphetamine behavioral effects, which are primarily mediated by DA (28). Moreover, changes in regional brain AA levels are correlated with motor activity (25).

When given systemically, a number of drugs, including apomorphine (APO) (37), scopolamine (SCOP) (20), *d*-amphetamine (1,21,35), and L-glutamate (26), raise extracellular AA concentrations in the rat striatum. When given intrastriatally, APO (37) and *d*-amphetamine (35) do not increase extracellular AA, but L-glutamate causes a great increase in AA extracellular concentrations (26). Neuronally released L-glutamate also increases extracellular levels of AA (26). Moreover, L-glutamate releases AA from the striatal synaptosomal fraction (11). These findings suggest that the rise in extracellular striatal AA levels is related to excitatory amino acid release (26), which may mediate the AA releasing effect of systemic APO and *d*-amphetamine. Indeed, the striatum receives a large glutamate projection from the cerebral cortex. An estimated 43% of striatal glutamate is derived from crossed corticostriatal fibers (8,9,12,32); unilateral cortical ablation decreases L-glutamate levels in striata both ipsilateral (14) and contralateral to the lesion (6,32). Moreover, bilateral cerebral

[1] To whom requests for reprints should be addressed.

cortex lesions reduce both the concentration of striatal AA (1,7,27) and the amount of AA released by systemic *d*-amphetamine (1). Therefore, it appears to be well established that corticostriatal glutamatergic pathways play a major role in regulating striatal extracellular levels of AA (1,26); in turn, endogenous AA is claimed to protect from NMDA-receptor mediated neurotoxicity (17).

On the other hand, striatal dopaminergic system activity is also modulated by glutamatergic pathways. Brain microdialysis studies showed that low concentrations of L-glutamate either activate striatal release of DA and efflux of DOPAC (16) or have no effect (19), while high concentrations either decrease (16) or increase (19) dopamine (DA) release. These findings are consistent with the alleged dual excitatory-inhibitory function of glutamatergic neurotransmission on striatal dopaminergic system activity (3,14,16).

Cortical ablation also affects the behavioral response to drugs. Haloperidol-induced catalepsy (32) and *d*-amphetamine-induced motor hyperactivity of the rat (7) are greatly attenuated or potentiated, respectively, by bilateral cortical ablation.

In a previous article (6), we showed that APO and SCOP, which both increase the extracellular level of striatal AA (20,37), actually increased AA oxidation in the rat striatum. The increase was correlated with a decrease in DA turnover.

In the research described below, we investigated in the rat the effect of bilateral cortical ablation on APO- and SCOP-induced behavioral changes; moreover, we determined post-mortem levels of AA, DHAA, DA, and DOPAC in striatal synaptosomes (taken as a model of neuronal terminals) and whole striatum, to get more informations on the role of corticostriatal glutamatergic pathways in the APO- and SCOP-induced increase in striatal AA oxidation and its relationship to dopaminergic system activity and behavior.

METHOD

All experiments were conducted on male Wistar rats (290–330 g b.wt.), maintained under standard animal care conditions, on a 12 L : 12 D cycle, and given food and water ad lib. APO (HCl) and SCOP (sulphate) (Sigma) were dissolved in distilled water and injected at a volume of 2 ml/kg SC. Controls were given 2 ml/kg of saline SC. L-Glutamate (500 mg/kg, Sigma) was dissolved in distilled water and injected in a volume of 2 ml/kg IP.

Under ketamine anaesthesia the head was fixed in a stereotaxic instrument, the calvarium removed, and the dura reflected. A 2-mm strip of bone was left in place along the midline. Cortical tissue dorsal to the corpus callosum and lying between coordinates bregma and A 4.0, P 3.0, L 0.5, and 3.0 (28) was then removed on both sides (32), using gentle suction applied through a glass cannula connected to a vacuum pump. The cavity was filled with sterile gelfoam and the wound sutured. Figure 1 shows the extent of the lesion. The animals received 30.000 UI Penicillin G IM and were allowed 21 days to recover prior to drug injection.

Controls were age matched, sham-operated animals whose skull bone had been removed and replaced with gelfoam on both sides using the above coordinates.

Immediately after drug injection, the animals were placed for 45 min in single Warner cages without barrier; the crossing from one side to the other of the cage lowered the grid floor and the crossing was automatically monitored. Independent observers, unaware of the type of treatment, also recorded behavioral items for 3-min periods at regular 3-min intervals starting 3 min after drug injection.

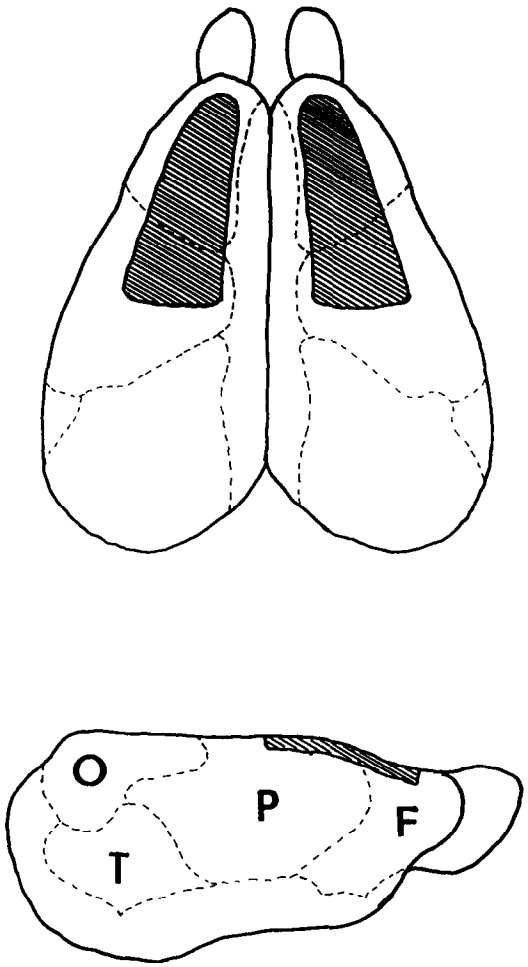

FIG. 1. Schematic drawing of dorsal and lateral views of rat brain. Hatchings indicates area of frontoparietal cortex removed by suction. Coordinates: bregma and A 4.0, P 3.0, L 0.5, and 3.0 (26). F, frontal; P, parietal; O, occipital; T, temporal.

Stereotypy items (rearing + sniffing, licking + biting) were measured according to the criteria of White et al. (30), modified as follow: a) intensity: O = not present; 1 = mild ; 2 = moderate; 3 = intense; 4 = extreme; b) duration: 1 = discontinuous; 2 = continuous. These ratings were multiplied (32) to yield a single value (e.g., the maximum score at each observation period is 2 × 4 for each items pair). The sum of scores for each animal was then computed. Total scores for animals within each group were averaged to generate a group mean score.

DA, 3,4-dihydroxyphenylacetic acid (DOPAC), AA, and dehydroascorbic acid (DHAA) determinations in striata on the right side were done by high-performance liquid chromatography with electrochemical detection (HPLC/EC), as previously described (6), using a high-pressure pump (Varian 9001 with a Rheodyne injector), column (15 cm × 46 mm i.d. TSK-ODS-80 TM), electrochemical detector (BAS LC-4B) and integrator (Spectra–Physics SP 4290). The mobile phase was composed of citric acid (0.1 M) and sodium acetate 0.1 M (pH = 3.0), methanol (4%), EDTA 1 mM, and sodium octylsulfate 55 mg/l; the flow was 1.2 ml/min, the sample injected, 20 μl.

TABLE 1

EFFECTS OF BILATERAL CORTICAL ABLATION ON APO-INDUCED CHANGES IN LEVELS OF DA, DOPAC, AA, DHAA, AND IN THE DOPAC/DA AND DHAA/AA RATIOS IN THE RAT STRIATUM

	Unoperated		Sham-Operated		Ablated		Kruskal-Wallis	
	Saline	APO	Saline	APO	Saline	APO	TS	p
DA	466.3 ± 56.65	506.4 ± 56.98	475.9 ± 54.31	504.7 ± 65.36	469.8 ± 36.07	528.5 ± 70.63	3.35	>0.6
DOPAC	74.06 ± 15.37	49.91 ± 10.83*	85.76 ± 17.20	56.81 ± 17.52*	73.07 ± 9.96	47.91 ± 10.28†	19.62	<0.00
DOPAC/DA	0.153 ± 0.024	0.100 ± 0.025†	0.174 ± 0.030	0.113 ± 0.031†	0.156 ± 0.017	0.090 ± 0.009†	24.40	<0.00
AA	12.98 ± 0.675	12.28 ± 1.322	12.55 ± 1.067	12.26 ± 1.074	11.02 ± 1.113‡	12.16 ± 1.133	8.20	>0.1
DHAA	1.689 ± 0.213	2.413 ± 0.323†	1.739 ± 0.324	2.354 ± 0.230†	1.873 ± 0.155	1.735 ± 0.313	23.73	<0.00
DHAA/AA	0.131 ± 0.009	0.199 ± 0.043†	0.139 ± 0.033	0.190 ± 0.020†	0.154 ± 0.020	0.153 ± 0.022	19.27	<0.00

Values are mean ± SD. DA and DOPAC, pmol/mg protein; AA and DHAA, nmol/mg protein; n = 6/group. APO 1 mg/kg SC; pertinent control groups were given saline 2 ml/kg. p-Values: vs. saline: * < 0.05; † < 0.01; vs. sham-operated: ‡ < 0.5.

Rats were decapitated 1 h after drug injection, at the end of the behavioral test. Heads were cooled by rapid immersion in liquid nitrogen; thereafter, striata of both side were rapidly removed. The striata of the left side were immediately processed for synaptosome preparations, those on the right side frozen at −70°C. Thereafter, samples were weighed and then homogenized in metaH_3PO_4 1% containing EDTA 1 M. After centrifugation (17500 × g for 10 min at 4°C), the supernatant was divided into two aliquots: the first aliquot was filtered and immediately analyzed to determine DA, DOPAC, and AA concentrations. The second aliquot was adjusted to pH 7.0 with K_2HPO_4 45% containing DL-homocysteine (13) 1% was added to reduce DHAA to AA. The sample was incubated for 30 min at 25°C, then adjusted to pH 3.0 with metaH_3PO_4 30%, filtered and injected (20 µl) for total AA determination. DHAA concentration was calculated from the difference in AA content between the first and second aliquots.

Crude synaptosomes of striata on the left side were prepared according to a modification of the Gray and Whittaker (21) method. Striata were homogenized in 30 vol of ice-cold 0.32 M sucrose buffered at pH 7.4 with phosphate, using a Teflon-glass system. The homogenate was centrifuged at 4°C for 10 min at 1,500 × g to remove nuclei and debris, and then crude synaptosomes were isolated from the supernatant by centrifugation at 4°C at 22,000 × g for 20 min. To lyse synaptosomes, the pellet was resuspended by sonication in 0.9 ml ice cold metaphosphoric acid and an aliquot of 50 µl was taken for protein analysis. After centrifugation (17,200 rpm for 7 min), 20 µl aliquots were taken for AA, DHAA, DA, and DOPAC determinations. Additional synaptosome preparations in presence or absence of AA 1 mM allowed to exclude any influence of the preparation on AA oxidation.

All values were expressed in nmol or pmol/mg protein.

Biochemical data were analyzed with ANOVA or the Kruskal-Wallis test, and then with Student's two-tailed t-test.

All studies were carried out in accordance with the italian Decreto No. 116/1992 of the Ministry of the Public Health (Directive 86/609/EEC on experimental animals care and protection).

RESULTS

Effects of APO on AA Catabolism and DA Turnover

Results are given in Tables 1 and 2.

Whole striatum. APO significantly increased DHAA concentrations in unoperated (+43%) and sham-operated rats (+35%), compared to saline-treated controls, but failed to increase them in ablated rats. AA levels in saline-treated ablated rats were significantly lower (−12%) than in sham-operated rats. APO-induced changes were of minor importance. The resulting DHAA/AA ratios had increased significantly in unoperated (+52%) and in sham-operated rats (+35%), but not in ablated rats.

APO slightly increased DA levels and significantly de-

TABLE 2

EFFECTS OF BILATERAL CORTICAL ABLATION ON APO-INDUCED CHANGES IN LEVELS OF DA, DOPAC, AA, DHAA, AND IN THE DOPAC/DA AND DHAA/AA RATIOS IN THE STRIATAL SYNAPTOSOMES OF THE RAT

	Unoperated		Sham-operated		Ablated		Kruskal-Wallis	
	Saline	APO	Saline	APO	Saline	APO	TS value	p
DA	114.2 ± 31.65	126.0 ± 13.78	124.8 ± 13.79	136.4 ± 17.33	146.9 ± 30.30	171.8 ± 17.49	13.89	<0.02
DOPAC	70.13 ± 5.66	55.66 ± 3.99*	81.29 ± 11.31	62.87 ± 6.01*	69.16 ± 7.71	67.36 ± 13.40	19.19	<0.002
DOPAC/DA	0.701 ± 0.212	0.466 ± 0.050†	0.674 ± 0.132	0.465 ± 0.059*	0.480 ± 0.061	0.396 ± 0.064†	19.45	<0.002
AA	5.433 ± 0.834	5.657 ± 1.245	5.593 ± 0.391	5.634 ± 0.431	4.576 ± 1.003‡	4.519 ± 0.427	13.36	<0.02
DHAA	1.775 ± 0.317	1.409 ± 0.124*	1.861 ± 0.220	1.383 ± 0.245*	1.618 ± 0.647	1.454 ± 0.226	12.47	<0.05
DHAA/AA	0.337 ± 0.092	0.257 ± 0.038	0.336 ± 0.062	0.246 ± 0.043†	0.364 ± 0.142	0.323 ± 0.049	9.16	>0.1

Values are mean ± SD. DA and DOPAC, pmol/mg protein; AA and DHAA, nmol/mg protein; n = 6/group. APO 1 mg/kg SC; pertinent control groups were given saline 2 ml/kg. p-Values: vs. saline: * < 0.01; † < 0.05; vs. sham-operated: ‡ < 0.05.

TABLE 3

EFFECTS OF BILATERAL CORTICAL ABLATION ON SCOP-INDUCED CHANGES IN LEVELS OF DA, DOPAC, AA, DHAA, AND IN THE DOPAC/DA AND DHAA/AA RATIOS IN THE RAT STRIATUM

	Unoperated		Sham-Operated		Ablated		Kruskal-Wallis	
	Saline	SCOP	Saline	SCOP	Saline	SCOP	TS	p
DA	478.7 ± 19.65	491.6 ± 84.28	471.1 ± 56.81	491.5 ± 73.18	458.7 ± 37.09	477.2 ± 49c.58	0.78	>0.9
DOPAC	69.23 ± 14.22	57.04 ± 5.64	70.62 ± 13.91	54.99 ± 15.73	71.90 ± 11.98	67.11 ± 7.90	7.73	>0.1
DOPAC/DA	0.150 ± 0.032	0.117 ± 0.016*	0.156 ± 0.036	0.103 ± 0.020*	0.155 ± 0.017	0.139 ± 0.004*§	12.74	<0.03
AA	12.83 ± 0.47	12.43 ± 2.19	13.25 ± 1.64	11.75 ± 1.55	11.11 ± 1.02‡	12.33 ± 2.17	7.52	>0.1
DHAA	1.631 ± 0.368	3.142 ± 0.101†	1.741 ± 0.527	2.441 ± 0.502*	1.678 ± 0.291	1.990 ± 0.096	20.86	<.001
DHAA/AA	0.126 ± 0.029	0.262 ± 0.047†	0.133 ± 0.041	0.222 ± 0.076*	0.137 ± 0.0242	0.124 ± 0.052	20.46	<0.001

Values are mean ± SD. DA and DOPAC, pmol/mg protein; AA and DHAA, nmol/mg protein; n = 6/group. SCOP 0.6 mg/kg SC; pertinent control groups were given saline 2 ml/kg. p Values: vs. saline: *<0.05; †<0.01; vs. sham-operated: ‡<0.05vs. sham-operated and SCOP treated: §<0.05.

creased DOPAC levels in all groups. The resulting DOPAC/DA ratios had significantly decreased in unoperated (−35%), sham-operated (−35%), and ablated rats (−42%).

Synaptosomes. DHAA levels were significantly decreased by APO in unoperated rats (−21) and sham-operated rats (−26%). The decrease in ablated rats (−10%) did not reach statistical significance. AA levels in saline-treated ablated rats were significantly lower (−18%) than in sham-operated rats. APO-induced changes were of minor importance. The resulting DHAA/AA ratios were all lower, but statistical significance was reached only in sham-operated rats (−27%).

APO slightly increased DA levels and decreased DOPAC levels in all experimental groups. The resulting DOPAC/DA ratios were all significantly decreased.

Effects of SCOP on AA Catabolism and DA Turnover

Results are given in Tables 3 and 4.

Whole striatum. SCOP significantly increased DHAA concentrations in unoperated (+93%) and sham-operated rats (+40%), compared to saline-treated controls, but failed to increase them in ablated rats. AA levels in saline-treated ablated rats were significantly lower (−16%) than in sham operated rats. SCOP-induced changes were of minor importance. The resulting DHAA/AA ratios had increased significantly in unoperated (+74%,) and sham-operated rats (+67%), but not in ablated rats.

SCOP slightly increased DA levels and lowered DOPAC levels in all groups. The resulting DOPAC/DA ratios had significantly decreased in unoperated (−24%), sham-operated (−24%), and ablated rats (−12%).

Synaptosomes. DHAA levels were lowered by SCOP in unoperated (−17%), sham-operated rats (−17%), and ablated rats (−19%),compared to saline-treated group. AA levels in saline-treated ablated rats were significantly lower (−17%) than in sham-operated rats. SCOP-induced changes were of minor importance. The resulting DHAA/AA ratios were all lowered, but not to the point of statistical significance.

SCOP slightly increased DA levels and decreased DOPAC levels in all experimental groups. The resulting DOPAC/DA ratios were all significantly decreased, but in ablated rats, were it was even increased, although not to the point of statistical significance.

Individual Correlation Between Drug-Induced Changes in DHAA/AA and DOPAC/DA Ratios

Individual values of the DHAA/AA ratio in the whole striatum of unoperated and sham-operated rats given saline or APO were inversely correlated with individual values of the DOPAC/DA ratio ($r(22) = -0.4$, $p < 0.05$, Fig. 2). Individual values of the DHAA/AA ratio in the whole striatum of unoperated and sham-operated rats given saline or SCOP

TABLE 4

EFFECTS OF BILATERAL CORTICAL ABLATION ON SCOP-INDUCED CHANGES IN LEVELS OF DA, DOPAC, AA, DHAA, AND IN THE DOPAC/DA AND DHAA/AA RATIOS IN THE STRIATAL SYNAPTOSOMES OF THE RAT

	Unoperated		Sham-Operated		Ablated		Kruskal-Wallis	
	Saline	SCOP	Saline	SCOP	Saline	SCOP	TS value	p
DA	126.9 ± 33.95	158.5 ± 22.84	132.0 ± 20.14	165.7 ± 35.65	133.3 ± 21.44	147.1 ± 15.78	10.27	>0.06
DOPAC	85.25 ± 10.53	73.43 ± 8.07	72.08 ± 6.69	61.11 ± 7.89*	66.00 ± 9.39	75.85 ± 6.61	14.85	<0.02
DOPAC/DA	0.674 ± 0.095	0.473 ± 0,063†	0.555 ± 0.090	0.381 ± 0.086†	0.499 ± 0.059	0.520 ± 0.082	17.96	<0.005
AA	5.322 ± 0.779	5.980 ± 0.687	5.576 ± 0.432	5.328 ± 0.372	4.617 ± 0.334‡	4.716 ± 0.498	12.02	<0.05
DHAA	1.814 ± 0.377	1.506 ± 0.184	1.928 ± 0.560	1.599 ± 0.273	1.821 ± 0.430	1.468 ± 0.213	7.61	>0.1
DHAA/AA	0.346 ± 0.082	0.255 ± 0.044	0.346 ± 0.096	0.299 ± 0.041	0.381 ± 0.112	0.328 ± 0.068	8.00	>0.1

Values are mean ± SD. DA and DOPAC, pmol/mg protein; AA and DHAA, nmol/mg protein; n = 6/group. SCOP 0.6 mg/kg SC; pertinent control groups were given saline 2 ml/kg. p-Values: vs. saline: * <0.05; † <0.01; vs. sham-operated: ‡ <0.01.

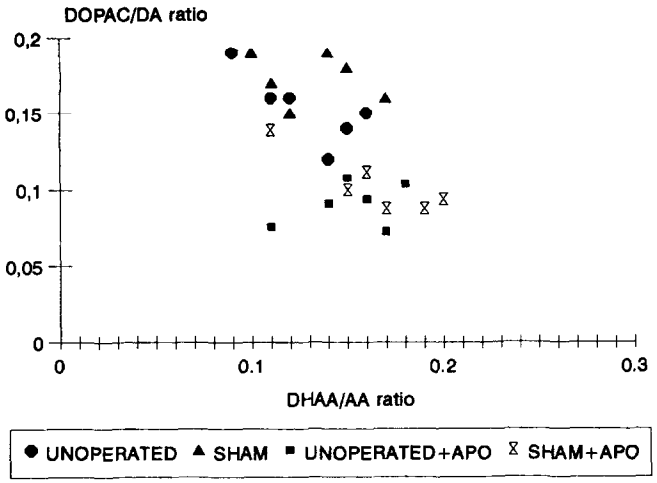

FIG. 2. Relationship between individual DOPAC/DA and DHAA/AA ratios in the striatum of rats unoperated, sham operated, unoperated, and APO treated, and sham operated and APO treated. Regression equation: $y = 0.210 - 0.539x$; significance of slope: $t(22) = 2.32$, $p < 0.05$. Pearson's correlation coefficient, $r = -435$, $p < 0.05$.

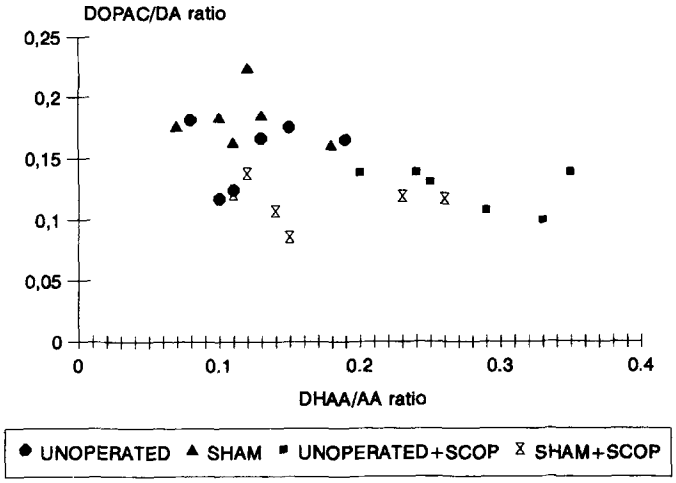

FIG. 3. Relationship between individual DOPAC/DA and DHAA/AA ratios in the striatum of rats unoperated, sham operated, unoperated, and SCOP treated, and sham operated, and SCOP treated. Regression equation: $y = 0.179 - 0.198x$; significance of slope: $t(22) = 2.29$, $p < 0.05$. Pearson's correlation coefficient, $r = -454$, $p < 0.05$.

were also inversely correlated with individual values of the DOPAC/DA ratio ($r(22) = -0.4$, $p < 0.05$, Fig. 3). In ablated rats given saline, APO, or SCOP, no correlation was found between individual DHAA/AA and DOPAC/DA ratio values.

Stereotypy and Motor Hyperactivity

Results are given in Table 5. SCOP significantly increased the number of crossings in unoperated (9.5 times), sham-operated (6.5 times), and ablated (2.5) rats, compared to pertinent saline-treated groups. The average number of crossings in the ablated and SCOP-treated group (26.2 ± 13.4) was significantly lower than that (56.3 ± 29.1) in the sham-operated and SCOP-treated group.

APO significantly increased motor activity to a lesser extent in unoperated (3.6 times), sham-operated (3.2 times), and ablated (2.5 times) rats. Such increase, however, did not reach statistical significance.

Both APO and SCOP greatly increased stereotypy scores in all experimental groups. However, the score average in the ablated and SCOP-treated group (45.0 ± 8.9) was significantly lower than that (64.3 ± 11.4) in the sham-operated and SCOP-treated group.

Effect of Systemic L-Glutamate on SCOP Behavioral Effects in Ablated Rats

Additional experiments were performed in ablated rats to get more information on the role of glutamatergic input on SCOP-induced behavior. The results are given in Table 6. L-Glutamate 500 mg/kg IP, given 24 h and 1 h before SCOP administration, significantly increased both stereotypy scores and motor activity. The L-glutamate treatment schedule was chosen according to the previous results (6) on strialal levels of glutamate after systemic injection of L-glutamate.

DISCUSSION

APO (36), SCOP (18) *d*-amphetamine (1,20,35), and L-glutamate (26), are able to increase striatal extracellular AA

TABLE 5

APO- AND SCOP-INDUCED MOTOR HYPERACTIVITY AND STEREOTYPY OF UNOPERATED, SHAM-OPERATED, AND CORTICALLY ABLATED RATS

	Unoperated		Sham-Operated		Ablated		ANOVA	
	Saline	APO	Saline	APO	Saline	APO	*F*	*p*
Crossings	3.8 ± 1.9	13.7 ± 5.2*	8.7 ± 5.3	27.7 ± 19.7*	7.8 ± 3.3	19.2 ± 16.9	7.8	>0.1
Stereotypy scores	14.0 ± 5.7	85.8 ± 10.2†	17.7 ± 7.1	78.0 ± 19.5†	10.0 ± 4.4	72.5 ± 11.3†	24.4	<0.0001
Crossings	4.7 ± 3.4	44.0 ± 20.4†	8.7 ± 3.3	58.8 ± 24.1†	10.2 ± 4.4	26.2 ± 13.4*‡	23.7	<0.0001
Stereotypy scores	14.5 ± 5.6	69.3 ± 10.5†	17.0 ± 6.3	64.3 ± 11.4†	7.8 ± 1.3	45.0 ± 8.9†§	22.6	<0.0001

Values are mean crossings or stereotypy scores ± SD in the 45 min following drug injection; $n = 6$/group. APO 1 mg/kg SC; SCOP 0.6 mg/kg SC; pertinent control groups were given saline 2 ml/kg.

p-Values: vs. saline: * <0.05; † <0.01; vs. sham-operated + SCOP: ‡ <0.05; § <0.01.

TABLE 6

EFFECT OF SYSTEMIC L-GLUTAMATE ON SCOP-INDUCED MOTOR HYPERACTIVITY AND STEREOTYPY OF CORTICALLY ABLATED RATS

	Saline	Saline + L-Glutamate	SCOP	SCOP + L-Glutamate	ANOVA	
					F	p
Crossings	7.3 ± 5.3	13.7 ± 5.0*	29.5 ± 12.3†	63.0 ± 11.0‡	48.3	<0.00001
Stereotypy scores	12.7 ± 4.4	17.7 ± 8.5	54.8 ± 12.4†	90.5 ± 34.1‡	22.5	<0.00001

Values are mean crossings or stereotypy scores ± SD in the 45 min following saline or SCOP (0.6 mg/kg SC) injection. L-Glutamate 500 mg/kg IP (24 h and 1 h before saline or SCOP injection); n = 6/group.
p-Values: vs. saline: * <0.05; † <0.01; vs. SCOP: ‡ <0.01.

concentrations in vivo when given systemically. Local infusion of APO in the striatum causes no change in extracellular striatal AA (37), while the infusion of L-glutamate did just this (26). These findings strongly suggests that APO must be acting at sites outside the striatum and that the corticostriatal glutamatergic pathways may mediate the effects. In early works (11,24), it has been suggested that striatal AA release is linked to glutamate release. This hypothesis has received recently experimental support (18): these is evidence that in freely moving rats AA release is triggered by the uptake of neuronally released glutamate. These findings suggest that striatal AA undergoes oxidation after its release.

In a previous study (6), we showed that APO, SCOP, but not L-glutamate, lose their ability to increase striatal AA oxidation in rats whose frontoparietal cortex had been unilaterally ablated. The results of the present study indicate that, in intact and sham-operated rats, the striatal AA oxidation induced by APO or SCOP is likely to occur in the extracellular space, because APO and SCOP, which both increased DHAA levels and the DHAA/AA ratio in the whole striatum, actually decreased them in the striatal synaptosomes (taken as a model of neuronal terminals). Because we demonstrated that an increase in AA oxidation can be selectively induced in striatal synaptosomes (5), the above hypothesis of an extracellular site of APO- and SCOP-induced AA oxidation appears to be logical. However, the DHAA/AA ratio is not an index of AA turnover, but gives only an index of oxidation state. Moreover, the DHAA/AA ratio in synaptosome preparation cannot be taken as a dynamic index of in vivo intraneuronal AA oxidation state. Unfortunately, the only method available to achieve that is voltammetry with carbon paste or carbon fibers electrodes, which can detect AA only in the extracellular compartment by oxidating it.

The mechanism by which APO activates the corticostriatal glutamatergic system is likely to be linked to both D_1- and D_2-dopamine receptor activation (21,23,37). That of SCOP cannot be explained by the results of this study and requires further investigations.

Bilateral frontoparietal ablation significantly decreased striatal AA levels both in striatal synaptosomes and whole striatum. These results are consistent with the finding of a decrease in striatal extracellular AA levels following bilateral cortical ablation (1). Both SCOP and APO restored striatal AA levels in ablated rats. Such a finding may be explained by assuming that SCOP and APO both activate, as does *d*-amphetamine (1,5), a neuronal compensatory mechanism supplying AA to striatum.

It has been suggested that L-glutamate presynaptically controls, with a dual action (excitatory-inhibitory), striatal dopaminergic activity, by acting on NMDA-receptors located on dopaminergic terminals (3,14,15). In the present study, bilateral cortical ablation, which induces a 40% loss of striatal L-glutamate (1), did not affect APO-induced changes in DA turnover both in the whole striatum and in synaptosomes. On the contrary, SCOP effects on DA turnover were attenuated in the whole striatum and abolished in synaptosomes. These findings suggests that the glutamatergic input play an enabling role on SCOP effects on DA turnover.

The involvement of corticostriatal afferents in the striatal and behavioral response to systemic drugs is, however, more complex. Bilateral removal of the frontoparietal cortex (32) decreased spontaneous multiple-unit activity recorded in the striatum of freely moving rats and changed the striatal neuronal response to systemic *d*-amphetamine from the prevalently excitatory in control animals to the prevalently inhibitory in ablated animals; moreover, the ablation did not affect morphine behavioral effects, but attenuated haloperidol-induced catalepsy. Conversely, bilateral cortical ablation potentiated *d*-amphetamine-induced motor hyperactivity, stereotypy and inhibition of striatal DA turnover (7). In this study, bilateral cortical ablation significantly attenuated both SCOP-induced motor hyperactivity and stereotypy, while APO-induced behavioral changes were affected to a lesser extent. Because systemic administration of L-glutamate restored and even potentiated the SCOP-induced behavioral effects, it is logical to admit that the glutamatergic input play a critical enabling role in SCOP behavioral effects. The effect of cortical ablation on drug-induced changes in behavior and dopaminergic system activity is the object of continuing studies.

In conclusion, in individual unoperated and sham-operated rats the APO- and SCOP-induced increase in AA striatal oxidation is correlated with the decrease in DOPAC/DA ratio. Furthermore, drug-induced AA oxidation is likely to occur in the extracellular space and requires integrity of the corticostriatal glutamatergic pathways. The latter plays an enabling role in SCOP behavioral effects.

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REFERENCES

1. Basse-Tomusk, A.; Rebec, C. Corticostriatal and thalamic regulation of amphetamine-induced ascorbate release in neostriatum. Pharmacol. Biochem. Behav. 35:55-60; 1990.
2. Chinoy, N. J. Ascorbic acid levels in mammalian tissue and its metabolic significance. Comp. Biochem. Physiol. 42A:945-952; 1972.

3. Clow, W.; Jhamandas, K. Characterization of L-glutamate action on the release of endogenous dopamine from the rat caudate-putamen. J. Pharmacol. Exp. Ther. 248:722–728; 1989.
4. Desole, M. S.; Anania, A.; Esposito, G.; Carboni, F.; Senini, A; Miele, E. Neurochemical and behavioral changes induced by ascorbic acid and *d*-amphetamine in the rat. Pharmacol. Res. Commun. 19:441–450; 1987.
5. Desole, M. S.; Esposito, G.; Fresu, L.; Migheli, R.; Enrico, P.; Miele, M.; De Natale, G.; Miele, E. Correlation between 1-methyl-4-phenylpyridinium ion (MPP+) levels, ascorbic acid oxidation and glutathione levels in the striatal synaptosomes of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- treated rat. Neurosci. Lett. 161:121–123; 1993.
6. Desole, M. S.; Miele, M.; Enrico, P.; Esposito, G.; Fresu, G.; De Natale, G.; Miele, E. Effects of cortical ablation on apomorphine- and scopolamine-induced changes in the dopamine turnover and ascorbic acid catabolism in the rat striatum. Eur. J. Pharmacol. 219:67–74; 1992.
7. Desole, M. S.; Miele, M.; Enrico, P.; Fresu, G.; Esposito, G.; De Natale, G.; Miele,E. The effects of cortical ablation on *d*-amphetamine-induced changes in striatal dopamine turnover and ascorbic acid catabolism in the rat. Neurosci. Lett. 133:29–33; 1992.
8. Divac, I.; Fonnum, F.; Storm-Mathisen, J. High affinity uptake of glutamate in terminals of cortico-striatal axons. Nature 266: 377–378; 1977.
9. Fischer, R. S.; Shiota, C.; Levine, M. S.; Hull, C. D.; Buchwald, N. A. Interhemispheric organization of cortico-caudate projection in the cat: A retrograde double-labelling study. Neurosci. Lett. 48:369–373; 1984.
10. Gray, E. G.; Whittaker, V. P. The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat. 96:79–88; 1992.
11. Grünewald, R. A.; Fillenz, M. Release of ascorbate from a synaptosomal fraction of rat brain. Neurochem. Int. 6:491–500; 1984.
12. Hassler, R.; Haug, P.; Nitch, C.; Kim, J.-S.; Paik, K,-S. Effect of motor and premotor cortex ablation on concentrations of amino acids, monoamines and acetylcholine and on the ultrastructure in rat striatum. A confirmation of glutamate as the specific corticostriatal transmitter. J. Neurochem. 38:1087–1098; 1982.
13. Hughes, R. E. The use of homocysteine in the estimation of dehydroascorbate. Biochem. J. 64:203–208; 1956.
14. Keefe, K. A.; Zigmond, M. J.; Abercrombie, E. D. Extracellular dopamine in striatum: Influence of nerve impulse activity in medial forebrain bundle and local glutamatergic input. Neuroscience 47:525–532; 1992.
15. Kim, J.-S.; Hassler, R.; Haug P.; Paik, K.-S. Effect of frontal cortex ablation on striatal glutamic acid level in rat. Brain Res. 132:370–374; 1977.
16. Leviel, V.; Gobert A.; Guibert, V. The glutamate-mediated release of dopamine in the rat striatum: Further characterization of the dual excitatory-inhibitory action. Neuroscience 39:305–312; 1990.
17. Majewska, M. D.; Bell, J. A.; London, E. D. Regulation of the NMDA receptor by redox phenomena: Inhibitory role of ascorbate. Brain Res. 537:328–332; 1990.
18. Miele, M.; Boutelle, M. G.; Fillenz, M. The physiologically induced release of ascorbate in rat brain is dependent on impulse traffic, calcium influx and glutamate uptake. Neuroscience 68: 87–91; 1994.
19. Moghaddam, R.; Gruen, R. J.; Roth, R. H.; Bunney, B. S.; Adams, R. N. Effect of L-glutamate on the release of striatal dopamine: In vivo dialysis and electrochemical studies. Brain Res. 518:55–60; 1990.
20. Mueller, K. Voltammetric evidence in vivo of cholinergic modulation of extracellular ascorbic acid and uric acid in rat striatum. Brain Res. 408:313–316; 1987.
21. Mueller, K.; Haskett, C. Effects of haloperidol on amphetamine-induced increase in ascorbic acid and uric acid as determined by voltammetry in vivo. Pharmacol. Biochem. Behav. 27:231–234; 1987.
22. Mueller, K.; Kunko, P. M. The effects of amphetamine and pilocarpine on the release of ascorbic acid and uric acid in several rat brain areas. Pharmacol. Biochem. Behav. 35:871–876; 1990.
23. Oh, C.; Gardiner, T. V.; Rebec, G. V. Blockade of both D_1 and D_2-dopamine receptors inhibits amphetamine-induced ascorbate release in the neostriatum. Brain Res. 480:184–189; 1989.
24. Oke, A. F.; May, L.; Adams, R. N. Ascorbic acid distribution patterns in human brain. A comparison with non human mammalian species. Ann. NY Acad. Sci. 498:1–12; 1987.
25. O'Neill, R. D.; Fillenz, M. Circadian changes in extracellular ascorbate in rat cortex, accumbens, striatum and hippocampus: Correlations with motor activity. Neurosci. Lett. 60:331–336; 1985.
26. O'Neill, R. D.; Fillenz, M.; Sundstrom, L.; Rawlins, J. N. P. Voltammetrically monitored brain ascorbate as an index of excitatory amino acid release in the unrestrained rat. Neurosci. Lett. 52:227–233; 1984.
27. O'Neill, R. D.; Grünewald, R. A.; Fillenz, M.; Albery, W. J. The effect of unilateral cortical lesions on the circadian changes in rat striatal ascorbate and homovanillic acid levels measured in vivo using voltammetry. Neurosci. Lett. 42:105–110; 1983.
28. Pellegrino, L. J.; Cushman, A. J. A stereotaxic atlas of the rat brain. In: Rand, R. M.; Lindzay, G.; MacGorguodale, K., eds. Century psychology series. New York: Appleton-Century-Crofts; 1967.
29. Rebec, G. V.; Centore, J. M.; White, K. L.; Alloway, K. D. Ascorbic acid and the behavioral response to haloperidol: Implications for the action of antipsychotic drugs. Science 227:438–440; 1985.
30. Robinson, T. E.; Becker, J. B. Enduring changes in brain and behavior induced by chronic amphetamine administration: A review and evaluation of animal model of amphetamine psychosis. Brain Res. Rev. 11:1067–1079; 1986.
31. Shenk, J. O.; E. Miller, E.; Gaddis, E.; Adams, R. N. Homeostatic control of ascorbate concentration in CNS extracellular fluid. Brain Res. 253:353–356; 1982.
32. Warenycia, M. W.; McKenzie, G. M.; Murphy, M.; Szerb, J. C. The effects of cortical ablation on multiple unit activity in the striatum following dexamphetamine. Neuropharmacology 26: 1107–1114; 1987.
33. White, L. K.; Carpenter, M.; Block, M.; Basse-Tomusk, A.; Gardiner, T. W.; Rebec, G. V. Ascorbate antagonizes the behavioral effects of amphetamine by a central mechanism. Psychopharmacology (Berlin) 294:284–287; 1988.
34. White, L. K.; Maurer, M. A.; Sidell, E. A.; Kraft, M. E.; Oh, C.; Rebec, G. V. Ascorbate infusions into the neostriatum modulate components of the behavioral response to amphetamine and haloperidol. Soc. Neurosci. Abstr. 14:968; 1988.
35. Wilson, R. L.; Kamata, K.; Wightman, R. M.; Rebec, G. V. Unilateral infusions of amphetamine produce differential, bilateral changes in unit activity and extracellular levels of ascorbate in the neostriatum of the rat. Brain Res. 304:342–347; 1986.
36. Wilson, R. L.; Wightman, R. M. Systemic and nigral application of amphetamine both cause an increase in extracellular concentrations of ascorbate in the caudate nucleus of the rat. Brain Res. 370:219–226; 1985.
37. Zetterström, T.; Fillenz, M. Changes in striatal dopamine, ascorbic acid and motor activity in rats after administration of dopamine receptor agonists. Br. J. Pharmacol. 93:149P; 1988.