TABLE 4. LIVER GLYCOGEN AND PLASMA LACTATE AND PYRUVATE IN FASTED RATS*

Treatment	Liver glycogen (mg/g wet tissue)	Lactate (mg/100 ml)	Plasma Pyruvate (mg/100 ml)	Lactate/pyruvate ratio
Control	5·0 ± 1·0†	19 ± 1	0·47 ± 0·08	41 ± 7
T-9078	3·6 ± 0·6	43 ± 7‡	0·92 ± 0·20‡	45 ± 10

* Fasted rats received an intraperitoneal injection of saline or 0·75 m-mole/kg of T-9078 and were killed by decapitation 2 hr after the injection.

† Indicates mean ± S.D. of five rats.

‡ $P < 0.01$ by analysis of variance.

The development of tachyphylaxis in experimental animals precludes clinical trials for antidiabetics.

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Amphetamine-induced changes in striatal dopamine and acetylcholine levels and relationship to rotation (circling behavior) in rats

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UNILATERAL lesions of the nigro-striatal system cause rats to turn in circles toward the side of the lesion, soon after recovering from the surgery. The rotational behavior is potentiated by amphetamine and can also be induced by amphetamine long after the animals have recovered from their tendency to rotate spontaneously.[1,2] This behavior has been attributed[2] to a neurochemical imbalance between the sides ipsilateral and contralateral to the lesion in the nigro-striatal system. Amphetamine presumably stimulates the intact system (by releasing dopamine), further enhancing the bilateral imbalance. Recently, high doses (15–25 mg/kg of the *d*-isomer) of amphetamine have been found to induce rotation in normal rats resembling that induced by lower doses (1–5 mg/kg of the *d*-isomer) in lesioned rats. As in lesioned rats, the direction of rotation is consistent for normal rats: when tested on 3 different days, some rats consistently rotated to the left, while others consistently rotated to the right.[3] The former results suggest the presence of an intrinsic and normal bilateral imbalance in the dopamine content of left and right nigro-striatal systems which is accentuated by amphetamine. The work described here was designed to test this hypothesis and to study the possible relationships to rotation of dopamine, acetylcholine and norepinephrine.

TABLE 1. MEAN UNILATERAL DOPAMINE ACETYLCHOLINE AND NOREPINEPHRINE LEVELS IN REGIONS OF THE BRAIN OF RATS TREATED WITH *d*-AMPHETAMINE SULFATE (AMPHET.), 20 mg/kg, OR SALINE*

	Group	Left (μg/g)	Right (μg/g)	High (μg/g)	Low (μg/g)	Mean ratio H/L	Ipsi. (μg/g)	Contra. (μg/g)	Mean ratio C/I
Striatal	Saline	7·04	6·83	7·39	6·48	1·14	6·67	7·20	1·08
DA ± S.D.		± 1·29	± 1·14	± 1·06	± 1·08	± 0·10	± 1·15	± 1·17	± 0·09
	Amphet.	4·75†	4·99†	5·43†	4·31†	1·26‡	4·33†	5·41†	1·25‡
		± 0·68	± 0·88	± 0·43	± 0·30	± 0·16	± 0·32	± 0·46	± 0·16
Striatal	Saline	4·01	3·89	4·06	3·84	1·06	3·93	3·97	1·01
Ach ± S.D.		± 0·69	± 0·74	± 0·68	± 0·73	± 0·04	± 0·71	± 0·75	± 0·07
	Amphet.	4·61†	4·51†	4·74†	4·37†	1·09	4·46†	4·66†	1·05
		± 1·47	± 1·35	± 1·40	± 1·40	± 0·11	± 1·52	± 1·41	± 0·09
Tel-dienceph.	Saline	0·327	0·344	0·347	0·324	1·07	0·328	0·343	1·05
NE ± S.D.		± 0·48	± 0·056	± 0·055	± 0·050	± 0·06	± 0·052	± 0·051	± 0·07
	Amphet.	0·194†	0·199†	0·201†	0·192†	1·05	0·196†	0·197†	1·01
		± 0·039	± 0·039	± 0·036	± 0·031	± 0·03	± 0·030	± 0·041	± 0·06
Tel-dienceph.	Saline	2·06	2·06	2·12	2·00	1·06	2·06	2·06	1·00
Ach ± S.D.		± 0·25	± 0·15	± 0·18	± 0·21	± 0·04	± 0·16	± 0·12	± 0·08
	Amphet.	2·13	2·10	2·18	2·05	1·06	2·14	2·09	0·98
		± 0·39	± 0·30	± 0·40	± 0·28	± 0·05	± 0·39	± 0·35	± 0·05

* Mean dopamine (DA), acetylcholine (Ach) and norepinephrine (NE) levels were computed in three ways: left side vs right side; side containing highest level vs side containing lowest level; and side ipsilateral to the direction of rotation vs side contralateral to the direction of rotation. In addition, the mean high side/low side (H/L) and contralateral/ipsilateral (C/I) ratios were also computed. Each group had 12 rats.

† Levels significantly different from saline control (*t*-tests; $P < 0.05$ to 0·01).

‡ Ratios significantly different from saline control (*t*-tests; $P < 0.02$).

Twenty-four naive female Sprague–Dawley rats about 3 months of age and weighing approximately 250 g were placed individually in a rotometer[4] modified after that described by Ungerstedt and Arbuthnott.[5] The apparatus consisted of a white opaque Plexiglass sphere, 12 in. dia., within which the rat rotated. Fifteen min after being placed in the apparatus, the rat was injected i.p. with either *d*-amphetamine sulfate, 20 mg/kg (N = 12) or saline (N = 12). Rotations were automatically recorded on a printout counter during the 15 min before and 30 min after injection. Rotations to the left or right during the pre- and post-injection periods were separately totalled and the positive rotational difference (i.e. rotations in the dominant direction minus rotations in the opposite direction) was determined for each rat.

Upon being taken out of the rotometer, each rat was immediately killed by decapitation, its brain was removed (the cerebellum and brain stem were discarded) and then dissected into four parts: left and right striatum, left and right teldiencephalon (excluding striatum). Left-sided structures were always dissected out and weighed before right-sided structures. Each part was initially homogenized by a Tissumizer in 4·0 ml acetonitrile containing 0·2 to 1·0 μg propionylcholine as an internal standard for analysis of acetylcholine. The homogenizer was washed with an additional 3·0 ml acetonitrile. Homogenates were combined and centrifuged at 2500 rev/min for 10 min. The acetonitrile phase was removed and assayed for acetylcholine by pyrolysis–gas chromatography.[6] The pellet was resuspended in 5·0 ml of 0·4 N perchloric acid (10 ml for tel-diencephalon) containing 0·1 ml of 10% EDTA, homogenized in a Teflon–glass homogenizer and then centrifuged at 15,000 rev/min for 20 min in a refrigerated centrifuge. Striatal dopamine and tel-diencephalic norepinephrine were determined in the extract by the spectrofluorometric method.[7]

Amphetamine elicited significant rotation (five rats rotated to the left and seven to the right): the mean rotational difference for 30 min ± S.D. was 45·6 ± 29·6 for the amphetamine-treated rats compared with 2·38 ± 0·78 for the controls; $P < 0.001$ by the *t*-test. Table 1 shows that amphetamine produced a 30 per cent decrease of striatal dopamine levels, a 41 per cent decrease of tel-diencephalic norepinephrine levels and a 15 per cent increase in striatal acetylcholine levels; no significant change in tel-diencephalic acetylcholine levels occurred. Bilateral differences in neurochemical levels were analyzed three ways: (1) Levels obtained from left-sided structures were compared to levels obtained from right-sided structures; any significant left–right differences would likely indicate a dissection or other methodological artifact. (2) Highest levels, regardless of whether they were obtained from left- or right-sided structures, were compared to lowest levels; any significant high–low difference would indicate a bilateral imbalance not necessarily related to rotation. (3) Levels obtained from structures ipsilateral to the direction of rotation were compared to levels obtained from structures contralateral to the direction of rotation; any significant ipsilateral–contralateral difference would indicate a bilateral imbalance related to the direction of rotation. As a further indication of a bilateral imbalance, high/low and contralateral/ipsilateral ratios were calculated for all levels for each rat. A series of regression correlations and *t*-tests yielded the following results: (1)

There were no consistent left–right differences for any of the chemical levels measured. (2) There were significant high–low differences for dopamine in both the amphetamine ($P < 0.001$) and saline ($P < 0.05$) groups. (3) When determined with respect to the direction of rotation, striatal dopamine levels in the amphetamine group alone were significantly ($P < 0.001$) lower in the side ipsilateral to the direction of rotation than in the side contralateral to the direction of rotation; there were significantly greater contralateral to ipsilateral and high to low ratios of striatal dopamine levels in the amphetamine group than in the saline group. (4) There were no significant differences between high and low or contralateral and ipsilateral levels of any other chemical measure, either in the saline or amphetamine group. Furthermore, high to low striatal dopamine ratios were significantly greater, both in the saline and amphetamine groups, then the respective ratios of each of the other three chemical measures ($P < 0.05$ to 0.01); this was also true ($P < 0.01$) for contralateral to ipsilateral ratios, but only in the amphetamine group. (5) In the amphetamine group alone, contralateral to ipsilateral striatal dopamine ratios were directly correlated with the magnitude of the rotational difference ($r = +0.66$; $P < 0.05$); rotation was also positively correlated ($r = +0.56$; $P < 0.05$) with contralateral dopamine levels and negatively correlated ($r = -0.64$; $P < 0.05$) with ipsilateral dopamine levels. There were no significant correlations between any of the other chemical ratios and rotation. (6) Lastly, in the amphetamine group alone, mean (average of left and right) striatal dopamine levels per animal and mean striatal acetylcholine levels per animal were each inversely related to the contralateral/ipsilateral striatal dopamine ratio ($r = -0.66$ and -0.61 respectively; $P < 0.05$). There were no other significant relationships among the various chemical measurements.

The results of these experiments indicate that there is a normal imbalance in the content of dopamine in the left and right striati and that the potentiation of this imbalance by amphetamine is associated with rotation. A normal dopamine imbalance is indicated by the significant high–low difference in the saline group and by the significantly greater high/low ratio for dopamine than for the other three measures in the saline group. The amphetamine-potentiated imbalance seems to be a function of the same mechanism responsible for the amphetamine-induced depletion of striatal dopamine, since the imbalance (contralateral/ipsilateral) was correlated with the depletion. Amphetamine may release more dopamine from the side of the nigro-striatal system that is more active.[8] The dopamine asymmetry in the amphetamine group would not appear to be a result of an unequal distribution of amphetamine to the two sides of the brain, since there was a bilateral difference in only one of the three amine levels affected by amphetamine.

Relationships between central cholinergic and dopaminergic mechanisms have been frequently postulated.[9] The amphetamine-induced increase in striatal acetylcholine levels (Table 1) could reflect a modulatory or inhibitory influence of cholinergic mechanisms on the striatal dopamine imbalance, since the greater the increase in acetylcholine levels, the lesser was the dopamine imbalance. Studies[10] suggest that the cholinergic effect of amphetamine may be induced indirectly by release of catecholamines, cholinergic feedback then serving to inhibit the drug action that is mediated by catecholamines.

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