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Dopaminergic Pathways in the Rat Central Nervous System and Rotational Behaviour[1]

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FUNK, K. F. AND K. H. WESTERMANN. *Dopaminergic pathways in the rat central nervous system and rotational behaviour.* PHARMAC. BIOCHEM. BEHAV. 11(2) 135–139, 1979.—Unilateral lesion of substantia nigra or ventral tegmental area causes ipsilateral rotational behaviour after receptor stimulation by apomorphine, whereas contralateral rotations were observed after lesion of globus pallidus. The alterations in dopamine and noradrenaline content of relating structures were determined by radiometric microassay. There is no strong correlation between transmitter depletion and motoric asymmetry. The site and extent of lesion seems to be more determinative in respect to motoric disturbances.

Rotational behaviour Dopaminergic pathways Lesion

DOPAMINERGIC transmitter imbalance between the brain hemipheres is related with asymmetric motoric behaviour which grows evident after receptor stimulation. The imbalance may be inborn [17] or produced by unilateral lesion of dopaminergic cell bodies or fibres. It can be quantified by measuring the rotational behaviour in a rotometer. Intensity and direction of rotation depend on extent and site of lesion and have been interpreted as a sign of receptor supersensitivity [14]. Besides the nigroneostriatal pathway other dopaminergic fibres or nuclei are to be taken into account for regulation of motoric behaviour, especially the mesolimbic pathways [5–7, 10]. Recently we presented evidence for significant effects of the globus pallidus (GP) in this respect [16]. In the present paper the influence of lesions of either system by 6-hydroxydopamine (6-OHDA) on rotational behaviour and catecholamine content has been investigated.

METHOD

Stereotaxic Injections

Female Wistar rats initially weighing 140–160 g were injected unilaterally under light ether anesthesia with 2 μl of a fresh prepared 6-OHDA solution (saline containing 0.2 mg/ml ascorbic acid).

Coordinates according to König and Klippel [9] were:

Substantia nigra (SN)	ant. 2.2	lat. 2.2	vert.–2.5 mm
Area ventralis tegmenti (AVT)	ant. 2.2	lat. 0.7	vert.–2.8 mm
Globus pallidus (GP)	ant. 6.5	lat. 2.5	vert.–0.5 mm

A Hamilton cannula with 0.3 mm external diameter was used.

Histology

After the experiments the exact site of injection was verified histologically on cryostat sections or sections from formaldehyde-fixed brains (Nissl-staining).

Recording of Rotational Behaviour

The animals were tested in an automatically counting spherical rotometer [15] for one hour after IP injection of 5 mg/kg apomorphine 1–2 weeks after lesioning.

Preparation of Brain Samples

Two weeks after lesion the animals were decapitated. The brain was frozen in cooled hexane and cut into frontal serial sections. The slices were adjusted at a punching device similar to the apparatus of Schlumpf *et al.* [12] and the desired tissue areas were punched out.

The data for the isolated tissue samples were: Nucleus accumbens (NAC): frontal section from ant. 9.7 to 8.9 mm; punching tube 2.0 mm internal diameter; lat. 1.1, vert.—0.6 mm. Nucleus caudatoputamen (NCP): frontal section from ant. 8.6 to 7.4 mm punching tube 1.5 mm int. diam.; lat. 2.2, vert. 1.0 mm. Globus pallidus (GP): frontal section from ant. 6.8 to 6.0 mm; punching tube 1.5 mm int. diam.; lat. 2.0, vert.—0.6 mm. The fresh weight of the tissue cylinders was determined at an analytical balance and then the samples were homogenized in 50 μl 0.1 M $HClO_4$ with 0.1 mg/ml EDTA by sonification.

Radiometric Assay of Catecholamines

Twenty μl of supernatant were taken for determination of dopamine (DA) and noradrenaline (NA) content. Twenty μl

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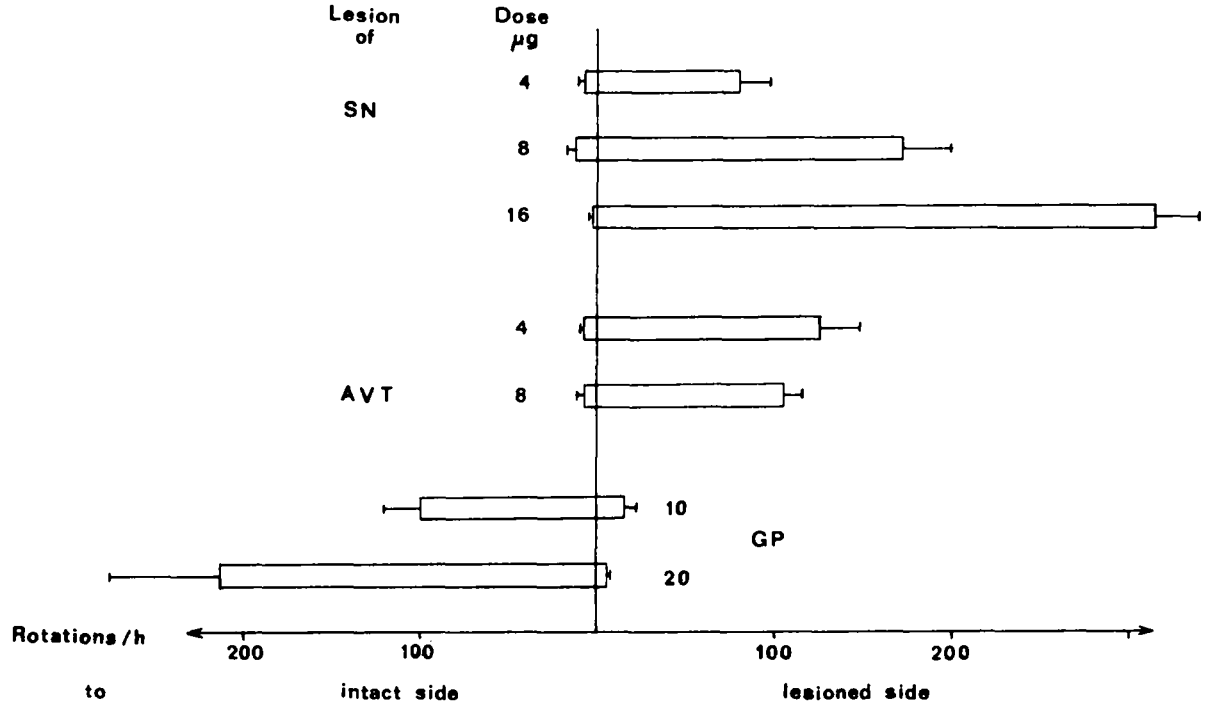

FIG. 1. Intensity and direction of rotation 1–2 weeks after lesion by 6-hydroxydopamine (mean ± SEM).

reaction mixture—consisting of 3 µl COMT-präparation (2), 0.05 µCi ^{14}C-adenosyl-methionine, 15 µl 1 M trishydroxymethylaminomethane, 1.5 µl 0.15 M $MgCl_2$, 30 µg dithioerythritol—were added. After 30 min incubation at 37°C the reaction was stopped by addition of 100 µl 0.5 M borate buffer pH 11 containing 7 µg 3-methoxytyramine and 3.5 µg normetanephrine as carrier. The reaction products were extracted into 6 ml water-saturated ethylacetate, the organic layer washed with 100 µl borate buffer and reextracted with 250 µl 0.1 M HCl. After acetylation with two times 10 µl acetic anhydride and solid sodium bicarbonate in excess, the water layer was extracted with 2 ml ethyl acetate and the extract taken to dryness. The residue was dissolved in acetone and spotted on a silicagel thin layer plate. The chromatogram was developed with toluene-isopropanol-chloroform (2:1:1) and the spots visualized by spraying with ethylendiamine and diazotized p-nitroaniline subsequently. The spots were scraped out and transfered directly into counting vials. The labelled amines were eluted by shaking slightly with 1 ml ethyl alcohol. For liquid scintillation spectrometry 10 ml of a toluene scintillation fluid were added.

RESULTS

Lesion of SN or AVT causes rotations towards the lesioned side and lesion of GP towards the intact side. The intensity of rotational behaviour is correlated with the dose of 6-OHDA (Fig. 1). Transection of the median forebrain bundle (MFB) causes intensive rotation towards the lesioned side (Table 1). Dopamine levels are changed differently: Lesion of SN decreases DA mainly in NCP, lesion of AVT affects the DA content in NAC, NCP and GP and lesion of GP reduces DA levels in NCP and GP markedly (Fig. 2–4). All the changes are restricted to the lesioned side. In hemitransected animals both intensively diminished DA levels and unchanged ones are to be seen, both connected with an uniformly strong rotational behaviour (Table 1). All kinds of lesion diminish NA levels in GP but not in NAC. The content of NA in NCP was below the limit of determination.

DISCUSSION

The alterations in DA content after lesion of dopaminergic cell bodies in SN (A9) and AVT (A10) agree well with the course of the dopaminergic afferences [13]. So the lesion of A9 cell group acts on NCP and not on mesolimbic areas (Fig. 2). The reduction of DA content in the NAC after the extremely high doses of two times 16 µg 6-OHDA within one week may be caused by diffusion of some 6-OHDA into the A10 cell group. Lesion of A10 results in a marked decline of DA in NCP and NAC (Fig. 3). Similar results have been obtained with electrolytic lesion techniques by Koob *et al.* [8].

Injection of 6-OHDA into the GP decreases DA content in NCP and GP but not in NAC.

The DA content in the GP is much smaller than in the adjacent NCP and is not influenced by the nigrostriatal pathway. The decrease of DA content in GP after lesion of A10 should be contemplated with caution. A complete separation of the GP from the adjoint DA rich area of the NCP may be realized with difficulty, therefore a decrease in DA may reflect partially the situation in NCP. Even the direct destruction of the GP does not remove the DA completely in it. A more pronounced reaction is seen in NA, each kind of lesion diminishes NA level in GP. Whether or not that implies a direct participation of noradrenergic system in motoric behaviour remains to be investigated.

On the other hand the existence of dopaminergic receptors in the GP can not be neglected, since we observed well defined rotations towards the injected side after unilateral

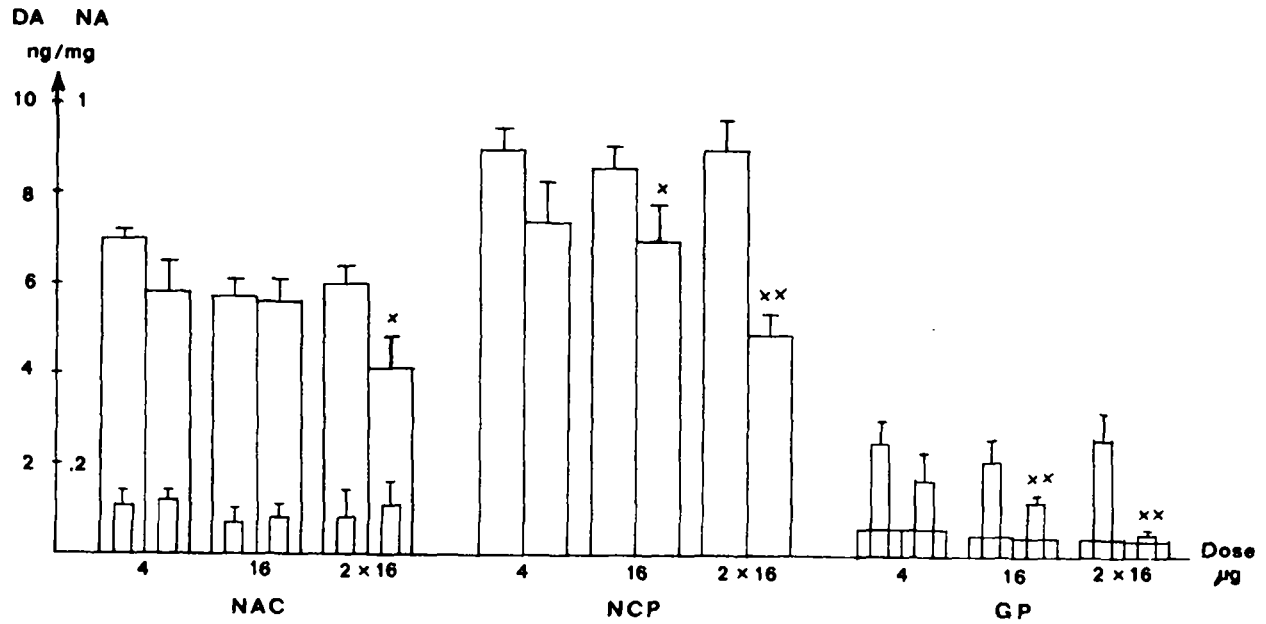

FIG. 2. Levels of dopamine and noradrenaline 2 weeks after lesion of subst. nigra by 6-hydroxydopamine (ng/mg wet weight; mean ± SEM). Wide columns: DA; narrow columns: NA; left columns: intact side; right columns: lesioned side; Significance: x $p<0.05$, xx $p<0.01$.

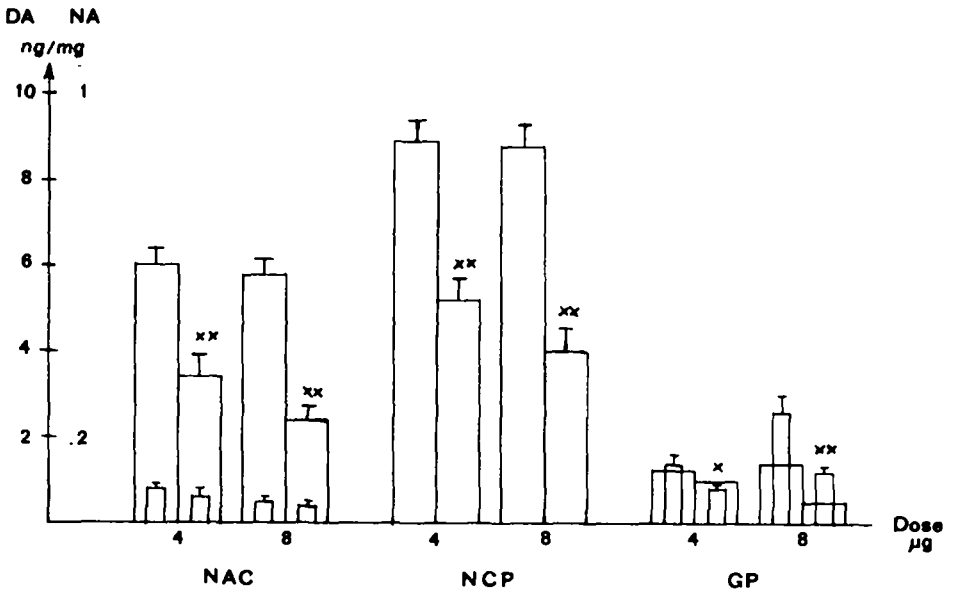

FIG. 3. Levels of dopamine and noradrenaline 2 weeks after lesion of area ventr. tegmenti. For details see Fig. 2.

injection of DA into the GP, whereas unilateral dopaminergic stimulation of the NCP induced contralateral rotations [16].

The asymmetric behaviour seems to be a more complex problem than a simple reflection of receptor or transmitter imbalance. The latter may be true for the naturally occurring asymmetry and the asymmetric behaviour after unilateral intracerebral injection of DA or dopaminergic agonists. But a uniform interpretation turns out to be plausible.

A strong decrease of DA content in NCP to less than 50% may be followed by development of receptor supersensitivity realizing in contralateral turning after apomorphine [14]. In our experiments we attained only in GP lesioned and in MFB transected animals DA levels lower than 50%. The GP lesioned animals indeed rotated to the intact side but the transected ones did not in spite of a larger decrease of DA. This and other results [4] call in question the direct depend-

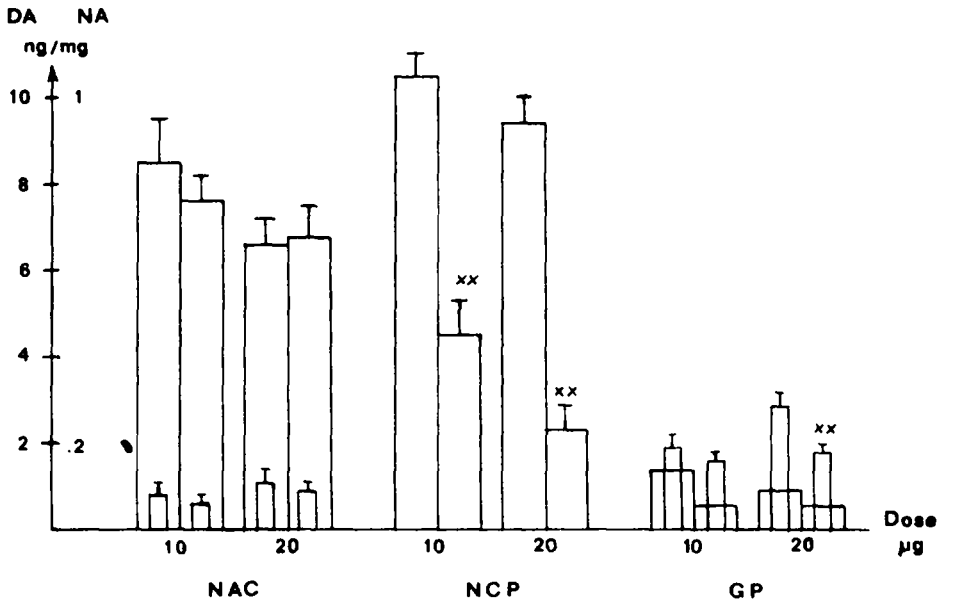

FIG. 4. Levels of dopamine and noradrenaline 2 weeks after lesion of globus pallidus. For details see Fig. 2.

TABLE 1

ROTATION AND DA CONTENT IN NAC AND NCP IN TRANSECTED ANIMALS

	rotations		µg/g DA in NAC		µg/g DA in NCP	
	c	i	c	i	c	i
1	0	263	8.8	4.2	12.0	1.25
2	0	255	7.0	3.3	10.4	10.50
3	4	95	5.3	0.5	6.0	0.40
4	1	425	6.0	6.1	10.9	10.10
5	1	361	6.7	8.8	12.6	11.80
6	1	376	5.6	5.0	10.8	1.40
7	5	548	7.8	5.9	12.7	11.30
8	6	468	6.7	6.3	11.7	0.90
unlesioned	82	78	7.3	7.3	10.0	11.60
"	3	7	6.5	7.1	11.1	10.00

c=contralateral i=ipsilateral side

ence of behaviour asymmetry on transmitter imbalance in NCP but puts the site of lesion into the field of view, especially the participation of the GP.

Rotation after lesion of the MFB most probably depends on the damage of the descending fibres, whereas transmitter depletion occurs only after damaging the ascending fibres [1]. This explains the dissociation of DA values and rotational behaviour in hemitransected animals (Table 1). Either result points at a direct relation between asymmetric behaviour and degree and site of damage of the dopaminergic system whereas the apparent correlation to the transmitter content is illusory. Both asymmetry and DA levels can be influenced by chemical or surgical damaging but that does not imply a direct relationship between them. In a similar way one may look upon the rotation inducing lesions of noradrenergic nuclei or pathways which are attended or not with alterations in DA content [3,11]

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