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Pharmacological Evidence for Dopaminergic Pallido-Striatal Interaction

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WESTERMANN, K. H. AND K. F. FUNK. *Pharmacological evidence for dopaminergic pallido-striatal interaction.* PHARMAC. BIOCHEM. BEHAV. 8(6) 645–649, 1978. – In rats the contents in dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) of neostriatum (nucleus caudatoputamen, NCP) and paleostriatum (globus pallidus, GP) were measured after transection of the capsula interna (CI) or after injection of 6-hydroxydopamine (6-OHDA; 20 μg/2 μl) into the GP of one side. The circling behaviour of the lesioned animals following apomorphine was also studied. 6-OHDA as well as transection decreased the contents in DA and DOPAC in NCP and GP significantly. Following both treatments DA levels in neostriatum were lowest. Nigro-neostriatal pathway lesioned animals (transected or injected with 6-OHDA 16 μg/2 μl into substantia nigra, SN) rotated towards the side of lesion after apomorphine (5 mg/kg IP), whereas GP lesioned animals rotated towards the intact side. In animals with both GP and SN lesions at one side turnings of similar intensity towards both sides were seen. In intact rats DA injections (200 μg/2 μl) into SN or NCP exhibited contralateral, injections into GP exhibited ipsilateral rotations. The results strengthen the hypothesis on the participation of GP in the regulation of neostriatal content of DA and shows the interaction of the hypothetical dopaminergic pallido-striatal pathway with nigro-neostriatal pathways.

Neostriatum Globus pallidus Dopamine 3,4-dihydrophenylacetic acid 6-OHDA

INJECTIONS of 6-hydroxydopamine into the substantia nigra (SN) [2, 18, 35] as well as lesions in the course of the nigro-neostriatal pathway [6,21] are known to decrease the dopamine content of the striatum (nucleus caudatoputamen (NCP) plus globus pallidus (GP)). Detailed studies have shown that the dopamine content in the GP remains unchanged [35]. On the other hand Seitelberger *et al.* [25] observed a substantial loss in neostriatal dopamine following lesions of GP and Anden *et al.* [3] described a reduced amount of dopaminergic nerve cells in ventromedial parts of the neostriatum following such destructions. In these studies the simultaneous destruction of dopaminergic nerve fibres running in the internal capsul (CI) could not be excluded. On the basis of these results a participation of paleostriatum (GP) in the regulation of dopamine concentration in the neostriatum (NCP) should be expected.

The present study was initiated in order to compare the effects of nigrostriatal axotomy with the effects of pallidal lesions on striatal dopamine content and to find out the functional significance of simple and combined lesions for rotational behaviour (circling, turning) appearing in unilateral nigro-striatal lesioned rats following dopaminergic receptor stimulating agents [4, 10, 11, 20, 29, 31, 36, 37].

MATERIALS AND METHOD

Surgical Procedures

The animals used were female Wistar rats initially weighing 140–150 g. Unilateral partial transection (axotomy) of the CI was performed with a stereotaxic localized thin steel knife (coordinates according to König and Klippel [19]; ant. 3.0 mm; lat. 1.0–4.0 mm; vert. –3.0 mm). Seven days later we injected 20 μg 6-hydroxydopamine-HCL (6-OHDA, Fa. Ferak), dissolved in 2 μl saline containing ascorbic acid 0.2 mg/ml under light ether anesthesia into the GP of each side (coordinates: ant. 6.5 mm; lat. 2.5 mm; vert. –0.5 mm).

To destroy dopaminergic cells of the SN we injected 16 μg 6-OHDA in 2 μl into this brain region (coordinates: ant. 2.2 mm, lat. 2.2 mm, vert. –2.5 mm) six days before testing the rotational behaviour.

For intracerebral injection dopamine-HCl (DA; Fa. Schuchardt) was dissolved in saline (200 μg/2 μl) and injected with a microsyringe into SN, NCP or GP of untreated animals by a stereotaxic method [34]. For injections only thin steel needles (0.3 mm external dia.) were used. Haloperidol (Fa. Janssen) was dissolved in hydrochloric acid containing saline.

Recording of Rotational Behaviour

The unilaterally lesioned or injected animals were tested in a rotometer [29,31]. Mean rotational behaviour (turns/60 min) of 6–8 animals was calculated to evaluate the effects of apomorphine (5 mg/kg IP) in lesioned animals and of intracerebrally injected DA in normal rats.

Biochemical Procedures

Fourteen days after transection or seven days after

6-OHDA-application rats were killed, six brain regions (NCP, GP and the medial part (MP) of the left and right side) were prepared as described earlier [35] and DA and norepinephrine (NE) determined fluorimetrically by the method of Ansell and Beeson [5]. For one determination the brain areas of six rats have been pooled. 3,4-dihydroxyphenylacetic acid (DOPAC) was determined as described by Murphy *et al.* [22]. Wet weight/dry weight relation was determined according to Beythien [8].

RESULTS

Our experiments indicated the DA level to be highest in the NCP, lower in the GP and lowest in the MP, supporting earlier results [35]. 6-OHDA injection into the GP or transection of the CI caused significant decrease in striatal DA ($p<0.001$). Performing both treatments we found lowest DA levels. The differences between 6-OHDA injection alone and combined pretreatment were significant in the paired sample t-test [38] at 1% level.

Similar changes have been found in the DOPAC content (Table 1). The NE content of NCP was very small and showed no drastic changes after pretreatment. Contrary to changes of DA in the GP the level of DA in the median part remained constant following 6-OHDA injection or transection. In some cases the content in DA of GP after 6-OHDA was too low to be determined (Table 2). Except for a significant loss ($p<0.001$) in wet weight of the forebrain of transected right side (10.0 ± 1.7%) we did not find changes in the wet weight/dry weight relation 14 days after section of CI. Protein content per mg wet weight remained constant in both sides too.

The most strong rotational behaviour following apomorphine IP has been found in rats with transected CI, a less strong circling in rats with lesions of SN. In both groups the rotations were directed to the lesioned side. In the group with lesions of GP we observed some rotations into this direction and after that a marked circling to the intact side following injection of apomorphine IP. Having additionally injected 6-OHDA into SN to make the secondary lesion after lesion of GP we observed intensified rotations to the right, lesioned side and diminished rotations to the left intact side compared to unique lesion of GP (Table 3). Direction and intensity after double lesions are specifically for each animal, dependent on the degree of rotation after the first lesion. Injections of DA into several brain regions showed different results. Whereas microinjections into SN and NCP elicited clear circling towards the left untreated side, injections into the GP induced rotation towards the side of treatment. The stimulating action of DA is obvious in all target areas and can be inhibited by haloperidol. Saline induced only a few rotations in either direction (Table 4).

DISCUSSION

Our biochemical results strengthen the hypothesis on the participation of GP in the regulation of DA content in the NCP in addition to the regulatory influences of SN [1, 6, 26, 35]. As described earlier [7, 9, 35] the GP of different species has a remarkable content of dopamine and its metabolite homovanillic acid. Extrinsic dopaminergic influences on the paleostriatum, probably not originating in group A 9 of the SN [15,30] can be assumed because of the dopamine sensitivity of this brain structure [10, 14, 39] dealt with in these experiments too.

On the other hand effects of the brain stem on DA content in GP cannot be excluded since axotomy contrary to 6-OHDA injection into the SN induced a strong decrease of neo- and paleostriatal DA levels. These effects as well as the weight decrease in transected forebrain are results of a general degeneration process taking place following axotomy.

Application of 6-OHDA into the GP exhibited strongest

TABLE 1

MONOAMINE CONTENT OF THE NCP 14 DAYS AFTER AXOTOMY AND/OR INJECTION OF 6-OHDA INTO GP (μG/G WET WT. ± SEM)
NUMBER OF DETERMINATIONS IN PARENTHESIS AND % DECREASE

	Control	6-OHDA	CI-Transection	Trans.+6-OHDA
DA	6.50 ± 0.17 (13)	3.87 ± 0.31 (11) 40*	2.99 ± 0.40 (8) 54*	2.56 ± 0.28 (11) 61*
DOPAC	0.41 ± 0.03 (10)	0.27 ± 0.05 (7) 34‡	0.22 ± 0.02 (7) 46*	0.16 ± 0.02 (7) 61*
NE	0.28 ± 0.02 (7)	0.23 ± 0.01 (4) 18*	0.21 ± 0.02 (4) 24†	not determined

* $p<0.001$; ‡ $p<0.05$; † not significant; compared to the control group (t test)

TABLE 2

DA CONTENT IN GP AND MP AFTER AXOTOMY AND/OR INJECTION OF 6-OHDA INTO GP(μG/G WET WT. ± SEM)
NUMBER OF DETERMINATIONS IN PARENTHESIS AND % DECREASE

	Control	6-OHDA	CI-Transection	Trans. + 6-OHDA
GP	2.14 ± 0.14 (10)	0.79 ± 0.22 (8) 63*	0.88 ± 0.15 (6) 59*	0.65 ± 0.27 (8) 70*
MP	1.08 ± 0.10 (12)	1.06 ± 0.08 (9) 2†	0.95 ± 0.11 (8) 12†	0.91 ± 0.08 (9) 16†

* $p<0.001$; † not significant; compared to the control group (t test)

TABLE 3

EFFECTS OF DIFFERENT PRETREATMENTS (LESIONS OF THE RIGHT SITED BRAIN STRUCTURES) ON ROTATIONAL BEHAVIOUR FOLLOWING APOMORPHINE (5MG/KG IP) ROTATIONAL BEHAVIOUR WAS TESTED 6 DAYS AFTER PRETREATMENT. FOR COMBINED LESION (4) THE INJECTIONS HAVE BEEN CARRIED OUT IN INTERVALS OF 6 DAYS. NUMBER OF TESTED ANIMALS IN PARENTHESIS. −: TURNS TOWARDS THE RIGHT, PRETREATED SIDE: +: TURNS TOWARDS THE LEFT, INTACT SIDE

Number	Pretreatment	Rotational behaviour (mean of turns/60 min±SEM)	Significance level (values of p*)
1	Transection of CI	−410±44 (28)	
2	6-OHDA into SN	−171±16 (35)	1/2<0.001*
3	6-OHDA into GP	− 8± 3 (12) +142±33	
4	6-OHDA into GP +6-OHDA into SN	− 52±19 (12) + 73±29	3/4<0.05‡ 3/4<0.02‡

* Calculated by students t test (†) or by paired t test (‡)

TABLE 4

ROTATIONAL BEHAVIOUR AFTER INJECTION OF DOPAMINE (200 μG/2μL) OR SALINE (2μL) INTO DIFFERENT BRAIN REGIONS (RIGHT SIDE), DETAILS SEE TABLE 3; HALOPERIDOL WAS APPLIED 30 MIN BEFORE DA

Number	brain region	pretreatment (Haloperidol IP)	Rotations following dopamine	Rotations following saline
1	NCP	—	+ 61 ± 21 (8)‡	−6±2 (11) +7±4
2	SN	—	+ 92 ± 26 (11)	−4±1 (6) +8±4
3	GP	—	− 23± 7 (7)*	−3±1 (8) +2±1
4	SN SN	0.5 mg/kg 1 mg/kg	+112±50 (6)‡ + 19±10 (9)†	— —

* $p<0.01$; † $p<0.05$; ‡ not significant; compared to group 2 (t test)

effects on DA content in this brain region but less marked effects on neostriatal DA. It was without effect on DA level in the MP (containing septal and preoptical regions) and on wet weight of the ipsilateral forebrain. Cutting of CI exerted a further diminishing effect on DA in both striatal structures (NCP and GP) after preceding pallidal 6-OHDA application. Changes in DOPAC content were parallel to those in DA whilst changes in NE failed to appear.

These data emphasize specificity of neostriatal DA changes and the importance of GP in the regulation of these levels likely on the basis of a dopaminergic pallido-neostriatal or striatopallidal connection [7,25].

Investigating the effect of GP stimulation on behaviour Hassler and Dieckmann [17] observed contraversive turnings of the head and body in cats, whereas ipsiversive movements were observed as a result of putaminal stimulation. Such opposite directions of the turning movements produced by stimulation of both structures have not been confirmed by others [10,20]. Both the opposite direction of rotational behaviour following dopaminergic stimulation of GP and NCP (Table 4) and the additional effect of CI-transection after 6-OHDA application into the GP on the decrease of DA content in NCP (Table 1) are compatible with the assumption of an intact nigro-neostriatal pathway following pallidal lesions by 6-OHDA. The highly different effects of apomorphine in nigral and pallidal lesioned animals (Table 3) speak in this favour too. As shown in Table 3 the effects of SN lesions are limited or reversed by 6-OHDA injection into GP depending on size and localization of lesion.

Expanding earlier results [11, 12, 14] we found dopamine sensitivity in the course of the nigro-neostriatal pathway including NCP and SN as well as in the paleostriatum. The stimulating effect of DA on striatal brain regions is relatively specific since DA injection into structures surrounding NCP [10] or SN [37] did not elicit circling behaviour. Although a contralateral asymmetric behaviour was observed upon stimulation of the nigrostriatal projection by dopamine other activities may become apparent following stimulation of the pallidum thereby comprising a rather limited compensatory system. Our results represent a functional basis for the observation that DA has different effects on the GP neurons than on those in the NCP. A marked increase in sensitivity of dopaminergic receptors was suggested by pretreatment with neuroleptic agents [11, 16, 33, 41] or unilateral damage of

the nigro-neostriatal system [28,32]. As in our earlier studies [36,37] haloperiodol was found to abolish the stimulatory effects of dopamine in a dose dependent manner (Table 4); signs of supersensitivity following such pretreatment have been observed.

Considering the results of Precht and Yoshida [23] and Yoshida *et al.* [40] that γ-aminobutyric acid (GABA) serves as transmitter in caudato-nigral pathway as well as in caudato-pallidal transmission system and taking into consideration the remarkable content in GABA and L-glutamic acid decarboxylase (GAD) activities in these target areas [13,24] we can assume a GABA-DA interaction in the GP. Accepting the dopaminergic pallido-neostriatal pathway and the hypothesis of GABAergic influences on dopaminergic nerve cells – till now described for extrapyramidal and mesolimbic dopaminergic pathways [27] – this interaction would be a rather general principle.

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