Pharmacology Biochemistry & Behavior, Vol. 17, pp. 619–622, 1982. Printed in the U.S.A.

Rotational Behavior Produced by Unilateral Ventral Noradrenergic Bundle Lesions: Evidence for a Noradrenergic-Dopaminergic Interaction in the Brain

ADAM PŁAŹNIK, OLGIERD PUCIŁOWSKI, WOJCIECH KOSTOWSKI, ANDRZEJ BIDZIŃSKI* AND MIROSŁAWA HAUPTMANN*

*Department of Pharmacology and Physiology of the Nervous System and *Department of Biochemistry, Psychoneurological Institute 02-957 Warszawa, Al. Sobieskiego 1/9, Poland*

Received 31 January 1982

PŁAŹNIK, A., O. PUCIŁOWSKI, W. KOSTOWSKI, A BIDZIŃSKI AND M. HAUPTMANN. *Rotational behavior produced by unilateral ventral noradrenergic bundle lesions: Evidence for a noradrenergic-dopaminergic interaction in the brain.* PHARMAC. BIOCHEM. BEHAV. 17(4) 619–622, 1982.—In this study the effect of unilateral electrocoagulation of the ventral noradrenergic bundle (VB) on rotational behavior produced by dopaminergic agonists was investigated. Unilateral lesions to the VB produced a decrease in the concentration of noradrenaline but not dopamine, serotonin or 5-hydroxyindoleacetic acid (5-HIAA) in the ipsilateral part of the forebrain. These lesions produced also strong and dose-dependent preference for ipsilateral rotation after systemic injection of apomorphine or amphetamine. The results are discussed in terms of possible interaction between noradrenergic and dopaminergic systems and it is proposed that the VB plays a regulatory role in the function of brain nigro-striatal dopaminergic system.

Ventral noradrenergic bundle Unilateral lesion Dopaminergic agonists Rotational behavior

THE ventral noradrenergic bundle (VB) is the main ascending fiber system which originates within the ventral pontine tegmentum from several groups of neuronal cell bodies containing noradrenaline (NE) [1, 13, 21]. This system innervates a variety of forebrain subcortical areas including hypothalamus, septum and amygdala [13, 17, 21]. Apparently none of the fibres of the VB ends in the cortex or hippocampus [13,21].

The role of the VB in behavioral processes remains unclear. Our previous results showed that bilateral electrolytic lesion in this area resulted in locomotor hyperactivity and facilitation of avoidance acquisition in rats [4,9]. We suppose that these excitatory effects that occur after VB lesion could be explained on the basis of possible interaction between NE and dopaminergic (DA) neurons [12]. Some pharmacological evidences indicate indirectly that VB lesion leads to increased activity of DA nigro-striatal system. The most important finding supporting this hypothesis is that VB lesioned animals are less susceptible to cataleptogenic action of neuroleptics such as chloropromazine and haloperidol [4]. Since neuroleptic-induced catalepsy is commonly believed to be due to blockage of DA postsynaptic receptors in the striatum, the reduced cataleptogenic effect in VB-lesioned rats can be interpreted as resulting from increased activity of DA neurons [4,12].

More direct approach to study the involvement of DA nigro-striatal neurons in behavior was proposed by Ungerstedt [22,23]. This involves placement of unilateral lesion in the substantia nigra (SN) pars compacta which results in a degeneration of DA neurons in the nigro-striatal pathway. The resulting imbalance in pre- and postsynaptic DA mechanisms is manifested as circling behavior following administration of direct and indirect DA agonists as apomorphine and amphetamine respectively. Using this model MacG. Donaldson *et al.* [14–16] found that rats with unilateral lesions to the VB displayed contralateral rotatory movements after administration of apomorphine and amphetamine. The authors conclude that this effect is due to reduced activity of ipsilateral DA nigro-striatal system. In the other words, they assume that VB exerts a facilitatory action on the ipsilateral nigro-striatal DA pathway. Our results based on the bilateral VB lesions and response to neuroleptics, do not support this hypothesis and indicate rather inhibitory influence of VB upon ipsilateral nigro-striatal DA neurons [4, 11, 12].

Since these results were obtained using different methods from those used by MacG. Donaldson *et al.* [15,16], we tried

in this experiment to observe effects of amphetamine and apomorphine in rats with unilateral electrolytic lesions to the VB.

METHOD

Experiments were performed on male Wistar rats weighing 180/200 g at the beginning of the experiment. Animals were housed under standard laboratory conditions, with food and water ad lib.

Surgical procedure of the VB lesions was described in detail in our previous papers [4,9]. In short, rats were anesthetized with chloral hydrate (400 mg/kg IP), and electrolytic lesions were made stereotaxically according to the coordinates of the König and Klippel atlas [5] modified after Ungerstedt [21]: A 1.3 mm, L 1.4 mm, V −2.0 mm. Parameters of electric current: 1.5 mA for 5–7 sec. Sham operated rats were prepared by inserting the electrode 2.0 mm dorsally to the VB but no current was passed. Rats were lesioned or sham operated unilaterally on the left or right side and 7 days later the animals were subjected to further testing without knowledge of the place of lesion.

On the test day rats were injected with apomorphine or amphetamine (SC) and the direction and number of rotations (360° turn around rats axis), in wire cages (21×21×24 cm), was visually counted. The observation period was 2 min, repeated 3 times: 15, 30 and 60 min after drug injection. The results were then pooled and mean value was calculated.

Drugs used in the experiment were: apomorphine (Vowe) 2.0 mg/kg SC, amphetamine (Polfa) 2.5–5.0 mg/kg SC.

At the completion of the experiment the rats were sacrificed by decapitation, their brains were quickly removed and dissected by precollicular section caudally to the hypothalamus for two parts: forebrain and brainstem. The brainstems were checked histologically after fixation in 5% Formalin, 40 μ sections were stained with hematoxyllin and eosin.

For biochemical assay the forebrain fragment of the brain was further divided on the left and right half. Biogenic amine levels were measured 7–8 days after the lesion in separate groups of untreated rats. The extraction and fluorimetric determination of brain amines were carried out according to Haubrich and Denzer [3], except that 5-HT was estimated after Korf and Sebens [6].

A two-way analysis of variance was used for statistical evaluation of results [2]. For biochemical data the Student's t-test for matched pairs was additionally applied.

RESULTS

Histological examination showed that lesions were mainly restricted to the VB and partially involved medial lemniscus and superior cerebellar peduncle. Rats with lesions not accurately positioned in the VB and involving other structures such as substantia nigra, the medial lemniscus and the mesencephalic reticular formation were excluded from the statistical analysis of the results (Fig. 1).

Two-factor analysis of variance showed significant effect of unilateral VB lesions on forebrain NE content, $F(1,20)=4.82$, $p<0.05$ (Table 1). Additional statistic with Student's t-test showed significant decrease of NE in the part of forebrain ipsilateral to the VB lesion, as compared with contralateral part of the forebrain, $t=2.39$, $p<0.05$, or ipsilateral part of the brain of sham operated animals, $t=2.47$, $p<0.05$. The forebrain concentration of DA, 5-HT and 5-HIAA were unchanged in lesioned rats (Table 1).

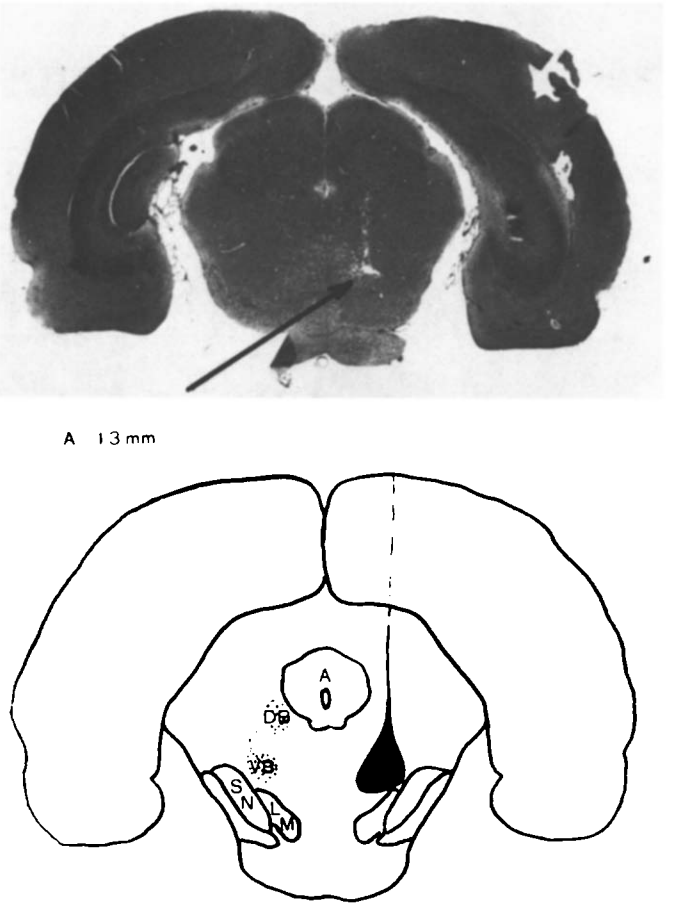

FIG. 1. Top: Photomicrograph of the frontal section of the brainstem showing typical unilateral lesion involving the VB region. Bottom: Schematic drawing showing the size and placement of the typical lesion involving the VB region. A=aqueduct, SN=substantia nigra, DB=dorsal noradrenergic bundle, VB=ventral noradrenergic bundle.

Analysis of variance showed no significant differences between groups.

It should be stressed that all non-treated rats with unilateral VB lesion displayed, in the course of the whole experiment, no spontaneous body or movement asymmetry.

In VB lesioned rats both apomorphine and amphetamine produced strong and long lasting preference to rotate ipsilaterally to the place of the lesion. Two-factor analysis of variance indicated that after apomorphine or amphetamine treatment VB lesioned rats rotated significantly more intensively than sham operated, $F(1,116)=7.52$, $p<0.01$, amphetamine 5.0 mg/kg; $F(1,68)=4.22$, $p<0.05$, amphetamine 2.5 mg/kg; $F(1,116)=75.23$, $p<0.01$, apomorphine 2.0 mg/kg, and preferred to rotate ipsilaterally to the lesion, $F(1,116)=10.02$, $p<0.01$, amphetamine 5.0 mg/kg; $F(1,68)=21.88$, $p<0.01$, amphetamine 2.5 mg/kg; $F(1,116)=96.59$, $p<0.01$, apomorphine 2 mg/kg (Fig. 2). In addition a significant interaction between lesion and preference for ipsilateral rotation was found, $F(1,116)=18.46$, $p<0.01$, amphetamine 5 mg/kg; $F(1,68)=4.38$, $p<0.05$, amphetamine 2.5 mg/kg $F(1,116)=115.41$, $p<0.01$, apomorphine 2 mg/kg (Fig. 2).

TABLE 1

EFFECT OF UNILATERAL LESION TO THE VB ON CONCENTRATION OF NE, DA, 5-HT AND 5-HIAA IN THE IPSI AND CONTRALATERAL HALF OF THE FOREBRAIN

	SH		VB	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
NE	463.17 ± 35.52	484.50 ± 35.98	357.67 ± 23.48*	450.17 ± 30.17
DA	1320.00 ± 126.60	1442.83 ± 102.10	1242.00 ± 231.10	1647.67 ± 232.30
5-HT	500.00 ± 22.25	543.50 ± 79.11	561.83 ± 64.39	624.67 ± 79.60
5-HIAA	1518.83 ± 204.90	1506.00 ± 224.20	1375.33 ± 69.30	1372.17 ± 60.70

Data are expressed in ng/g of tissue and shown as mean ± standard error of mean.

SH=sham operated control rats; VB=rats lesioned unilaterally to the VB; number of rats in each group=6.

*$p<0.05$ differs from VB-contralateral and SH-ipsilateral half of the forebrain (Student t-test for matched pairs, two tailed).

DISCUSSION

The results of our experiment indicate that unilateral lesions to the VB produce ipsilateral rotational movements after injection of dopamine agonists: apomorphine and amphetamine. This phenomenon indicates on NE-DA interaction within brain nigro-striatal system.

According to Ungerstedt [22,23] ipsilateral direction of circling may be related to domination of postsynaptic DA mechanism within the contralateral nigro-striatal system. On the basis of our results we may suppose that unilateral VB lesion produce imbalance in the brain nigro-striatal systems that leads to relative prevalence of contralateral nigro-striatal DA neurons. It appears, therefore, that VB lesion produces either decreased activity of DA nigro-striatal neurons ipsilateral to the lesioned side or increased activity of contralateral DA nigro-striatal neurons.

Biochemical examinations showed decreased NE content in the ipsilateral part of the forebrain without any changes in concentrations of DA, 5-HT and 5-HIAA. This finding confirms our previous results concerning the effects of bilateral VB lesion on behavior and amine content in the forebrain of rats [4, 7, 10]. MacG. Donaldson *et al.* [14–16] reported that unilateral lesion of the VB caused 26% fall in NE in the ipsilateral hypothalamus with simultaneous increase in striatal DA concentration. More precise microbiochemical methods paired with punching technique showed that VB innervates bilaterally also other structures and nuclei of the telencephalon including nucleus caudatus and accumbens, tied with function of nigro-striatal and meso-limbic DA systems [17,19].

It should be stressed that our results do not confirm MacG. Donaldson *et al.* data [15,16] which suggested facilitatory effect of the VB on the activity of ipsilateral nigro-striatal pathway. These authors reported, on the contrary to our observation, strong preference for contralateral turning induced by systemic injection of DA agonists, after unilateral electrocoagulation of VB or nucleus of the locus coeruleus (LC), but not the dorsal noradrenergic bundle (DB). They suggest that this direction of circling is the result of destruction of NE fibres travelling from the LC via VB to nigro-striatal system, normally enhancing nerve impulse flow between the SN and striatum. Therefore destruction of the VB should result in hypofunction of ipsilateral nigro-striatal pathway with development of postsynaptic receptor hypersensitivity, manifested by contralateral turning after DA agonist injection.

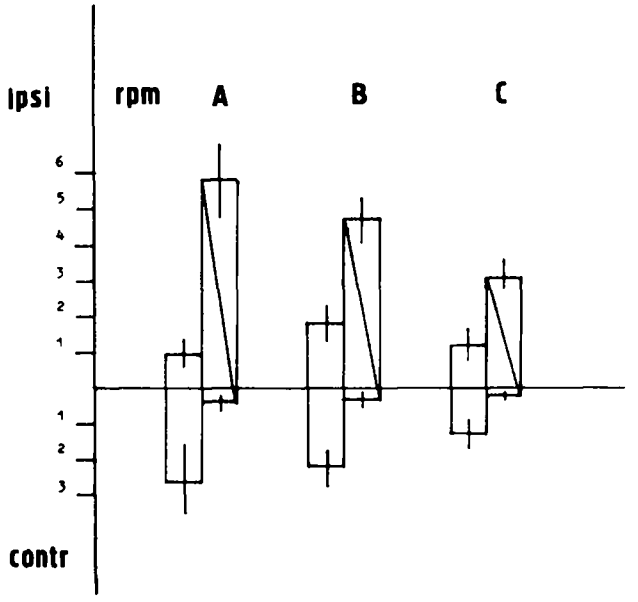

FIG. 2. The effect of the unilateral VB lesion on dopamine agonist-induced rotational behavior. Data are expressed as mean+SEM. rpm=rotation per minute, contralateral or ipsilateral to the side of lesion; ⌊=sham operated, control rats (SH), n=10, ⌈=unilaterally VB lesioned rats (VB), n=10; A–amphetamine treated rats (5.0 mg/kg, SC); B=amphetamine treated rats (2.5 mg/kg, SC); C=apomorphine treated rats (2.0 mg/kg, SC).

Our data, together with our earlier results, point at an opposite mechanism, namely inhibitory effect of NE released via VB on nigro-striatal neurons. It was shown by us previously that bilateral electrocoagulation of the VB produced symptoms of behavioral excitation, antagonism of neuroleptic-induced catalepsy and the fall of NE level within mesencephalon but not in the cortex [4]. On the contrary, lesions to the LC decreased the behavioral activity, enhanced action of neuroleptics and produced decrease in NE

and increase in 5-HT levels in the forebrain [7,11]. These and other data suggest that VB has an inhibitory effect on brain nigro-striatal system but that it facilitates the 5-HT activity [12]. An opposite role for LC-originating NE pathway was proposed: suppression of 5-HT and facilitation of DA systems.

If one accepts this line of arguments proposed by MacG. Donaldson's method of deduction, our results can be interpreted as suggesting that release of nigro-striatal system from inhibition after unilateral VB transection leads to hyperactivity of ipsilateral nigro-striatal pathway. This can be followed by development of postsynaptic DA receptors hyposensitivity, manifested by stronger effect of DA agonists on the opposite side and ipsilateral turning.

Available up to now data do not allow to discuss the reasons for these fundamental differences between MacG. Donaldson's and our results, especially because from the methodological point of view both papers are very similar (parameters of lesions, rats, doses of drugs, etc.).

REFERENCES

1. Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine containing neurons in the central nervous system. Part I. Demonstration of monoamines in the cell-bodies of brain stem neurons. *Acta physiol. scand.* **232:** 1–55 1964.
2. Guilfort, J. *Fundamental Statistics in Psychology and Education.* New York: McGraw-Hill, 1956, pp. 276–293.
3. Haubrich, D. and J. Denzer. Simultaneous extraction and fluorymetric measurement of brain serotonin, catecholamines, 5-hdyroxy-indoleacetic acid and homovanilic acid. *Analyt. Biochem.* **55:** 306–312, 1973.
4. Jerlicz, M., W. Kostowski, A. Bidziński and M. Hauptmann. Effects of lesions in the ventral noradrenergic bundle on behavior and response to psychotropic drugs in rats. *Pharmac. Biochem. Behav.* **9:** 721–724, 1978.
5. König, J. and R. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Part of the Brainstem.* Baltimore: Williams and Wilkins, 1963.
6. Korf, J. and J. Sebens. Determination of o-conjugated of 5-hydroxytryptamine in human urine. *Clinical chim. Acta* **27:** 149–153, 1970.
7. Kostowski, W., M. Jerlicz, A. Bidziński and M. Hauptmann. Behavioral effects of neuroleptics, apomorphine and amphetamine after bilateral lesion to the locus coeruleus in rats. *Pharmac. Biochem. Behav.* **7:** 289–293, 1977.
8. Kostowski, W. and M. Jerlicz. Effects of lesions of the locus coeruleus and the ventral noradrenergic bundle on the antinociceptive action of clonidine in rats. *Pol. J. Pharmac. Pharm.* **30:** 647–651, 1978.
9. Kostowski, W. and A. Płaźnik. Effects of lesions of the ventral noradrenergic bundle on the two-way avoidance behavior in rats. *Acta physiol. pol.* **29:** 509–514, 1978.
10. Kostowski, W., M. Jerlicz, A. Bidziński and M. Hauptmann. Studies on the effects of lesion of the ventral noradrenergic tract on the antinociceptive action of morphine. *Psychopharmacology* **57:** 189–192, 1978.
11. Kostowski, W., M. Jerlicz, A. Bidziński and M. Hauptmann. Evidence for existence of two opposite noradrenergic systems controlling behavior. *Psychopharmacology* **59:** 311–312, 1978.
12. Kostowski, W. Two noradrenergic systems in the brain and their interaction with other monoaminergic neurons. *Pol. J. Pharmac. Pharm.* **31:** 425–436, 1979.
13. Lindvall, O. and A. Björklund. Organization of catecholamine neurons in the rat central nervous system. In: *Handbook of Psychopharmacology*, vol. 9, *Chemical Pathways in the Brain*, edited by L. Iversen, S. Iversen and S. Snyder. New York: Plenum Press, 1978, pp. 139–222.
14. MacG. Donaldson, I., A. Dolphin, P. Jenner, C. Marsden and C. Pycock. The involvement of noradrenaline in motor activity as shown by rotational behavior after unilateral lesion of the locus coeruleus. *Brain* **99:** 427–446, 1976.
15. MacG. Donaldson, I., A. Dolphin, P. Jenner, C. Marsden and C. Pycock. Contraversive circling behavior produced by unilateral electrolytic lesions of the ventral noradrenergic bundle mimicking the changes seen with unilateral electrolytic lesions of the locus coeruleus. *J. Pharm. Pharmac.* **28:** 329–330, 1976.
16. MacG. Donaldson, I., A. Dolphin, P. Jenner, C. Pycock and C. Marsden. Rotational behavior produced by unilateral electrolytic lesions of the ascending noradrenergic bundles. *Brain Res.* **138:** 487–509, 1978.
17. O'Donohue, T., W. Crowley and D. Jacobowitz. Biochemical mapping of the noradrenergic ventral bundle projections sites: evidence for a noradrenergic-dopaminergic interactions. *Brain Res.* **172:** 87–100, 1979.
18. Płaźnik, A. and W. Kostowski. Locus coeruleus lesions and avoidance behavior in rats. *Acta Neurobiol. exp.* **40:** 217–225, 1980.
19. Speciale, S., W. Crowley, T. O'Donohue and D. Jacobowitz. Forebrain catecholamine projections of the A-5 cell group. *Brain Res.* **154:** 128–133, 1978.
20. Starke, K. and T. Endo. Minireview: Presynaptic α-adrenoceptors. *Gen. Pharmac.* **7:** 307–312, 1976.
21. Ungerstedt, U. Stereotaxic mapping of monoamine pathways in the rat brain. *Acta physiol. scand.* **82:** Suppl. 367, 1–48, 1971.
22. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal system. *Acta physiol. scand.* **82:** Suppl. 367, 69–93, 1971.
23. Ungerstedt, U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behavior. *Acta physiol. scand.* **82:** Suppl. 367, 49–68, 1971.