Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 1985, Vol. 9, pp. 735–738
Printed in Great Britain.

0278–5846/85 $0.00 + .50

EFFECTS OF UNILATERAL MICROINJECTIONS OF SULPIRIDE INTO THE MEDIAL PREFRONTAL CORTEX ON CIRCLING BEHAVIOR OF RATS

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(Final form, July, 1985)

Abstract

Morency, Michel A., Robert J. Stewart and Richard J. Beninger: Effects of unilateral microinjections of sulpiride into the medial prefrontal cortex on circling behavior of rats. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 1985, 9 (5/6): 735-738.

1. Bilateral 6-OHDA lesions of rats' medial prefrontal cortex increased locomotor activity after 7-10 days suggesting that cortical DA may normally inhibit motor behaviour. However, hyperactivity may have resulted from enhanced subcortical DA function.
2. Acute manipulation of frontal cortical DA neurotransmission in the present experiment avoided lesion-induced subcortical changes.
3. Sulpiride (0, 6, 12, 24 ug in 1 ul) was injected unilaterally into the medial prefrontal cortex of rats pretreated with (+)-amphetamine (1.5 mg/kg, i.p.).
4. Circling behavior was scored during four 5-min intervals of a 60-min test session which began with injections and placement in a flat, circular arena.
5. SUL resulted in ipsiversive circling whereas its vehicle did not. These results were consistent with those seen with other DA drugs and suggest an excitatory influence of frontal cortical DA on locomotor activity.

Keywords: central injection, circling, dopamine, medial frontal cortex, sulpiride, rats

Abbreviations: dopamine (DA), sulpiride (SUL), 6-hydroxydopamine (6-OHDA)

Introduction

DA plays a key role in the control of motor activity (Beninger, 1983); reductions produce hypoactivity (Anden et al., 1970) and enhancement produces hyperactivity (Pijnenberg et al., 1976).

Circling is commonly used to assess the role of DA in motor control. Circling may be induced by unilateral manipulations of DA function, the direction characteristically being towards the side of lower DA activity. This approach has implicated the nigrostriatal and mesolimbic DA systems in motor control (Pycock, 1980). However, the involvement of mesocortical DA remains poorly understood.

The medial prefrontal cortex was ascribed an inhibitory role in motor control as a result of studies demonstrating hyperactivity 7-10 days after frontal cortical DA depletion (Joyce et al., 1983). However, biochemichal data demonstrated an increase in DA function in subcortical areas (Pycock et al., 1980). Therefore, lesioning cortical DA may have released tonic inhibition of motor activity or hyperactivity may have been the consequence of time-dependent, subcortical changes.

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The present study employed acute manipulations of frontal cortical DA. In our laboratory, contraversive circling has been induced by unilateral intracortical microinjections of the DA agonists, LY 141865 , (+)-amphetamine (Stewart et al., in press) and cocaine (in preparation). Conversely, metoclopramide, a DA antagonist, produced ipsiversive circling in amphetamine pretreated rats (Stewart et al., in press). These results suggest an excitatory influence of frontal cortical DA on motor control. If this DA system had an inhibitory influence, rats would have circled towards the side of higher DA activity. The purpose of this experiment was to test the hypothesis that unilateral microinjections of the DA antagonist, SUL into the medial prefrontal cortex induce ipsiversive circling behavior.

METHODS

Animals and Surgery. Eighteen male albino Wistar rats (Charles River Canada) were anesthetized (sodium pentobarbital 60 mg/kg, i.p.) and secured in a stereotaxic instrument, with the incisor bar set 5.0 mm above the interaural line. A stainless steel guide cannula (23 guage) was implanted into the medial prefrontal cortex at the coordinates: 4.5 mm anterior to bregma, 0.7 mm lateral to the midline and 1.5 mm ventral to the dura matter. Cannulae were implanted into the left frontal cortex of half the animals and into the right of the others.

Drugs. (+)-Amphetamine sulphate (Smith, Kline and French) was dissolved in distilled water. SUL (Delagrange) was dissolved in a minimal amount of 0.5 N HCl, neutralized with 0.5 N NaOH (pH 7.0-7.5) and made up to volume with distilled water.

Apparatus. Three polyurethane-sealed circular wooden bases (30 cm diam), enclosed within a cylinder of wire mesh (36 cm high), were each fitted with a Plexiglas cover.

Injection Procedure. Animals were pretreated with (+)-amphetamine (1.5 mg/kg, i.p.) 15 min prior to sessions. Manual injections of 1.0 ul SUL at doses of 0 (vehicle), 6, 12 and 24 ug/rat were delivered by a 5 ul Hamilton microsyringe. The 30 gauge injection cannula extended 1 mm beyond the tip of the guide cannulae. Injections were delivered in 15 sec and the cannula was left in place for an additional 60 sec.

Behavioral Testing. Approximately 7 days after surgery, 6 animals were tested each day in two sessions, thereby allowing three days between central injections. Complete turns (360^{o}), ipsiversive and contraversive to the side of the cannula were counted. Three animals were scored during each 60-min session, observation periods being at 0-5, 15-20, 30-35 and 45-50 min. Animals were started at staggered intervals of 5 min such that only one animal was being scored at any time. Each animal was tested 7 times as follows: (1) no central injection; (2) central injection of the vehicle; (3) each of the doses of SUL, with order counterbalanced across rats; (4) replication of vehicle; (5) replication of no central injection. Circling behavior wa expressed as the ratio of ipsiversive turns to the total number of turns (ipsiversive + contraversive). Ratio values greater than or less than 0.5 indicated a tendency for ipsiversive or contraversive circling, respectively. The total number of turns per session served as the second dependent measure. At the conclusion of behavioral testing, animals were administered a lethal dose of sodium pentobarbital, exsanquinated and perfused intracardially with 10% Formalin. Frozen sections (50 um) were mounted and stained with thionin.

RESULTS

Five rats were discarded due to blocked cannulae while histology revealed that the cannula of one animal was medial to the target area. A representative placement is indicated in Figure 1.

Two *t*-tests for correlated measures were performed, one on the turning ratios for the first and second no-central injection and one for the vehicle injection conditions. No significant differences were found in either case, t (11) = 1.01 and 0.74, $p>0.10$, respectively, and each of these conditions was combined. SUL produced circling ipsilateral to the side of the guide cannula (Table 1). A significant main effect was confirmed by a one-factor, repeated measures analysis of variance, F (4,44) = 10.99,

$p<0.0001$. Planned orthogonal comparisons revealed no significant differences between the no-injection and vehicle conditions. The combined noinjection and vehicle conditions were different from the combined ratios of the three drug dose conditions, F (1,11) = 32.28, p <0.0005, which did not differ significantly from one another. To satisfy the assumptions of the analysis of variance, an arcsine transformation was conducted on the ratio data. Analysis of variance of the raw data yielded the same profile of significant results. The mean (±SEM) total turns for each dose of SUL (Table 1) differed significantly, F (4,44) = 3.63, $p<.05$.

Table 1

Number of turns (±SEM) and turning ratios following intrafrontocortical microinjections of SUL

	No-injection	Vehicle	6 ug	12 ug	24 ug
Ipsiversive	22.00±5.41	21.50±5.10	39.00±7.02	44.42±8.16	38.33±9.91
Contraversive	23.46±1.96	33.50±4.55	24.83±3.88	21.00±2.92	25.75±4.77
Total turns	45.46±6.30	55.00±8.09	63.83±7.08	65.42±6.76	64.08±9.98
Turning ratio	.412±.049	.377±.048	.589±.061*	.637±.066*	.523±.087**

* p <.001 : ** p <.005

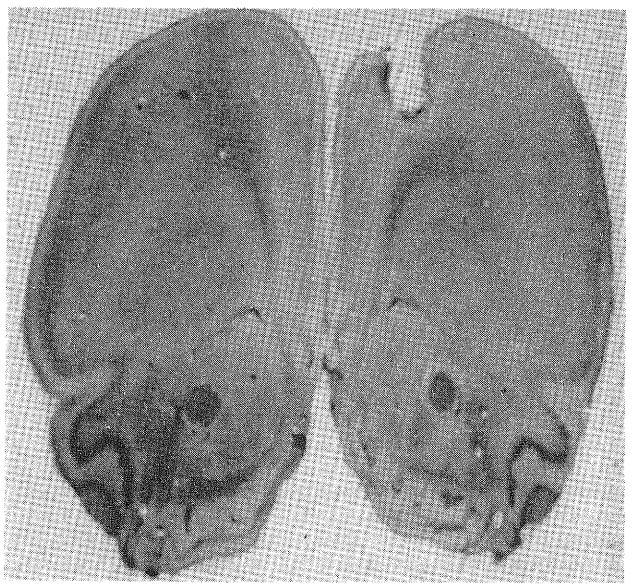

Figure 1. Representative section showing a cannula tract in the left frontal cortex.

The failure to find significant differences between the first and second no-injection and vehicle injection conditions indicates that the rats returned to baseline turning ratios following drug sessions. This demonstrates the acute nature of the injections. The possibility of diffusion of SUL from the frontal cortex to the striatum seems unlikely; the distance separating these two brain areas is approximately 3-4 mm and previous reports have indicated that a 1.0 ul injection volume diffuses into a sphere of approximately 1 mm (Myers, 1974).

The increase in total turns did not necessarily reflect an increase in locomotor activity but rather a directional bias. It was this bias which indicated the role of the frontal cortical DA system in motor control. If frontal DA had an inhibitory influence on motor control rats would have circled towards the side of higher DA activity. Instead, rats circled toward the side of lower DA activity. These findings are in agreement with the observations of contraversive turning following injection of (+)-amphetamine, LY141865 (Stewart et al., in press) and cocaine (in preparation) and ipsiversive circling following metoclopramide (Stewart et al., in press).

Conclusion

Circling behaviour resulting from acute unilateral manipulation of central DA has been used to assess this transmitter's contribution to motor control. SUL (6, 12 and 24 ug), when injected unilaterally into the medial prefrontal cortex of rats pretreated with (+)-amphetamine (1.5 mg/kg, i.p.), produced a significant increase in ipsiversive circling. These results provide further evidence for an excitatory influence of mesocortical DA on motor control.

Acknowledgements

The excellent technical assistance of Andrea T.A. Korda and F.H. Gouzoules is gratefully acknowledged. This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Health to R.J.B.

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