DIFFERENTIAL EFFECTS OF INTRAFRONTOCORTICAL MICROINJECTIONS OF DOPAMINE AGONISTS AND ANTAGONISTS ON CIRCLING BEHAVIOR OF RATS

ROBERT J. STEWART, MICHEL A. MORENCY and RICHARD J. BENINGER

Department of Psychology, Queen's University, Kingston, Ont. (Canada)

Asymmetric posturing and circling behavior resulting from acute unilateral manipulation of central dopamine have been used to assess this neurotransmitter's contribution to motor control. Although providing extensive evidence for the involvement of mesolimbic and nigrostriatal dopamine in motor activity, this approach has not been used to study the mesocortical system. We now report circling behavior following acute manipulation of frontal cortical dopamine. Unilateral microinjections of the agonists, (+)-amphetamine (12 and 25 µg in 1.0 µl) and LY 141865 (12 µg in 1.0 µl) resulted in contraversive circling. Conversely, unilateral intrafrontocortical microinjections of the antagonist, metoclopramide (25 and 100 µg in 1.0 µl) resulted in ipsiversive circling in amphetamine (1.5 mg/kg, i.p.) pretreated rats. Lower central doses of each drug and vehicle injections had no significant effect. These results provide evidence for an excitatory influence of mesocortical dopamine on motor control. This finding may implicate frontal cortical dopamine in the extrapyramidal motoric side effects of chronic neuroleptic treatment which previously have been attributed to dopamine function in subcortical areas.

INTRODUCTION

The neurotransmitter dopamine (DA) is thought to influence locomotor activity and various forms of learning[4,18]. Drugs such as amphetamine, apomorphine and other similarly acting DA agonists produce an enhancement of locomotor activity[8,16,23]. Conversely, significant reductions in locomotor activity have been reported following administration of DA antagonists[2].

Much research has been directed towards the elucidation of the contribution of specific DA systems to the control of locomotor activity. One of these systems is the mesocortical DA projection to the medial prefrontal cortex[29]. Bilateral ablation of the frontal cortex has been shown to produce hyperactivity and an exaggerated amphet- amine response approximately 7 days later[1,13,15]. Electrolytic lesions of the ventral tegmental area and more selective 6-hydroxydopamine lesions of the frontal cortex have produced similar results[6,28]. These studies suggested a normally inhibitory influence of frontal cortical DA on locomotor behavior.

While lesioning cortical DA may have released its putative tonic inhibition of motor activity, it is also possible that hyperactivity resulted from an increase in the tone of subcortical DA systems. Biochemical data have demonstrated that following frontal cortical lesions, there is an increase in DA function and utilization in subcortical areas such as the nucleus accumbens and striatum[25,26] where DA-dependent hyperactivity and stereotypy are believed to be mediated[7]. This implies

Correspondence: R.J. Beninger, Department of Psychology, Queen's University, Kingston, Ont., Canada K7L 3N6.

that hyperactivity is dependent upon a sufficient time interval to respond to cortical DA loss and effect compensatory subcortical changes. An acute preparation, employing central microinjections of dopaminergic agents would allow an assessment of the contribution of frontal cortical DA to motor control independent of compensatory changes in the sensitivity of subcortical DA systems.

Circling behavior and asymmetric posturing have been described in numerous reports following unilateral manipulations of various brain sites[24]. A lateralized imbalance of DA mechanisms has been shown to induce dramatic circling behavior. Thus, animals characteristically circle away from (contralateral to) the side of higher DA activity[24]. Unilateral ablation of the frontal cortex of rats has been shown to induce ipsiversive circling for the first week following the lesion. By 30 days, contraversive circling was observed[3,14]. It is possible that ipsiversive circling was in response to frontal cortical DA loss, whereas the shift to contraversive circling reflected unilateral subcortical compensatory changes[14]. However, interest has focused on these subcortical changes rather than the immediate effects of loss of frontal cortical DA function. As a result, the ability of the frontal cortex to regulate subcortical function in response to denervation has been well documented but acute manipulations of frontal cortical neurotransmitter systems have not.

The present experiments were undertaken to examine the effects on circling behavior of unilateral injections of 3 dopaminergic agents into the medial prefrontal cortex of rats. The drugs were: (+)-amphetamine, an indirectly acting DA agonist[27], LY 141865, a DA agonist specific for the D-2 receptor subtype[30] and metoclopramide, a D-2 specific antagonist[11,22]. If frontal cortical DA exerts an inhibitory influence on motor control, an animal would circle towards the side of higher DA activity as this side would be the locus of greater motor inhibition.

MATERIALS AND METHODS

For each of 3 experiments, 18 male albino Wistar rats weighing 200–250 g were obtained from Charles River, Canada. Animals were individually housed in a climatically controlled colony room on a 12 h light (06.00–18.00h)/dark cycle. Food and water were continuously available in the home cages.

All rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and stereotaxically implanted with chronic indwelling unilateral guide cannulae (23-gauge; stainless steel) aimed at the medial prefrontal cortex with coordinates: 4.5 mm anterior to bregma, 0.7 mm lateral to the midline and 2.0 mm ventral to the dura mater. The incisor bar was set 5.0 mm above the interaural line[21]. Cannulae were anchored to the skull with stainless steel screws and acrylic cement. Stainless steel obturator pins secured with silicone were used to seal the cannulae between injections. For 9 rats cannulae were implanted into the left frontal cortex and 9 into the right.

Three polyurethane-sealed circular wooden bases 30 cm in diameter were used as arenas for behavioral testing. A cylinder of wire mesh 36 cm high was placed around the base to contain the animal during testing. Each arena was fitted with a Plexiglas cover.

Testing began approximately 7 days after surgery. Six animals were tested each day in two sessions, allowing 3 days between central injections for each animal. Test sessions began with a central injection and placement into the circular arena.

In experiment 1, manual injections of (+)-amphetamine sulphate in distilled water at doses of 0 (0.9% saline), 6, 12 and 25 $\mu g/\mu l$ were administered in a volume of 1.0 μl through a 5-μl Hamilton microsyringe. Injection cannulae were constructed with 30-gauge stainless steel tubing cut to extend 0.5 mm beyond the tip of the guide cannulae and attached to the microsyringe by a length of polyethylene tubing. The injection was delivered in 15 s and the injection cannula was left in place for an additional 60 s to ensure sufficient diffusion of the drug.

All complete turns (360°), ipsiversive and contraversive to the side of the cannulae were counted during each observation period. Three animals were scored during each 60-min session, observation periods being at 0–5, 15–20, 30–35

69

and 45–50 min. For the time taken to administer the central injection (maximum of 2 min) the clock was stopped. Animals were started at staggered intervals of 5 min such that only one animal was being scored at any time. Thus, each animal was scored for a total of 20 min, in four 5-min blocks at approximately equal intervals throughout the session.

Each animal was tested 7 times as follows: (1) no central injection; (2) central injection of saline; (3) each of the 3 doses of (+)-amphetamine, with a counterbalanced order of administration over 3 test sessions; (4) replication of saline; (5) replication of no-injection.

Circling behavior was expressed as the ratio of ipsiversive turns to the total number of turns (ipsiversive + contraversive). Ratio values of 0.5 indicated equal turning in both directions. Values greater or less than 0.5 indicated a tendency for ipsiversive or contraversive circling respectively. The total number of turns per session (ipsiversive + contraversive) served as the second dependent measure.

Experiment 2 examined the effects of central administration of the D-2 agonist, LY 141865. The design was identical to experiment 1, the doses of LY 141865 being 0 (saline), 6, 12 and 24 μg/μl.

Experiment 3 examined the effects of the D-2 antagonist, metoclopramide hydrochloride in doses of 0 (saline), 6, 25 and 100 μg/μl. The design was similar to experiments 1 and 2 except that animals were not tested under the no-injection condition; also, all animals were pretreated with an injection of (+)-amphetamine (1.5 mg/kg, i.p.) 15 minutes prior to every test session to increase overall activity.

Upon completion of testing, animals were sacrificed for histological confirmation of cannulae placements. All rats were administered a lethal dose of sodium pentobarbital and exsanguinated with 0.9% saline followed by 10% Formalin. Brains were extracted, sectioned at 50 μm and stained with thionin[12].

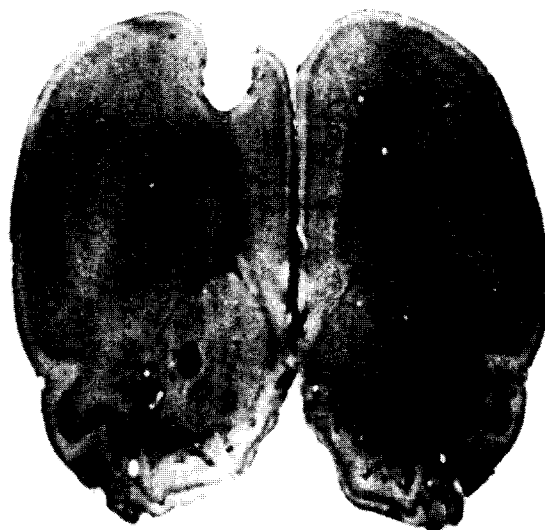RESULTS

The number of rats included in the statistical

Fig. 1. Representative coronal section of rat brain indicating a cannula tract in the right medial prefrontal cortex.

analyses for the 3 experiments were 13, 11 and 12, respectively (Fig. 1). The remaining rats were discarded due to blocked cannulae or inaccurate placements. It is important to note that those discarded animals with placements lateral to the medial prefrontal cortex (n = 3) failed to show significant effects.

For experiment 1, t-tests for correlated measures of the turning ratio data for the first and second no-injection scores as well as the first and second administration of saline revealed no significant differences. Therefore, each animal's scores from these two sessions were combined for subsequent analyses.

As shown in Fig. 2A, the no-injection, saline and 6 μg conditions produced turning ratios of approximately 0.5, indicating no directional bias. The 12 and 25 μg doses produced markedly lower turning ratios indicating a contraversive bias. An analysis of variance (ANOVA) confirmed a significant dose effect, $F_{4,48} = 28.25$, $P < 0.0001$. Post hoc pairwise comparisons between each condition and saline revealed that the 12 and 25 μg turning ratios were significantly lower than saline.

The mean number (± standard error) of total turns per session for each dose of (+)-amphetamine was: no-injection, 14.3 (± 1.5); saline,
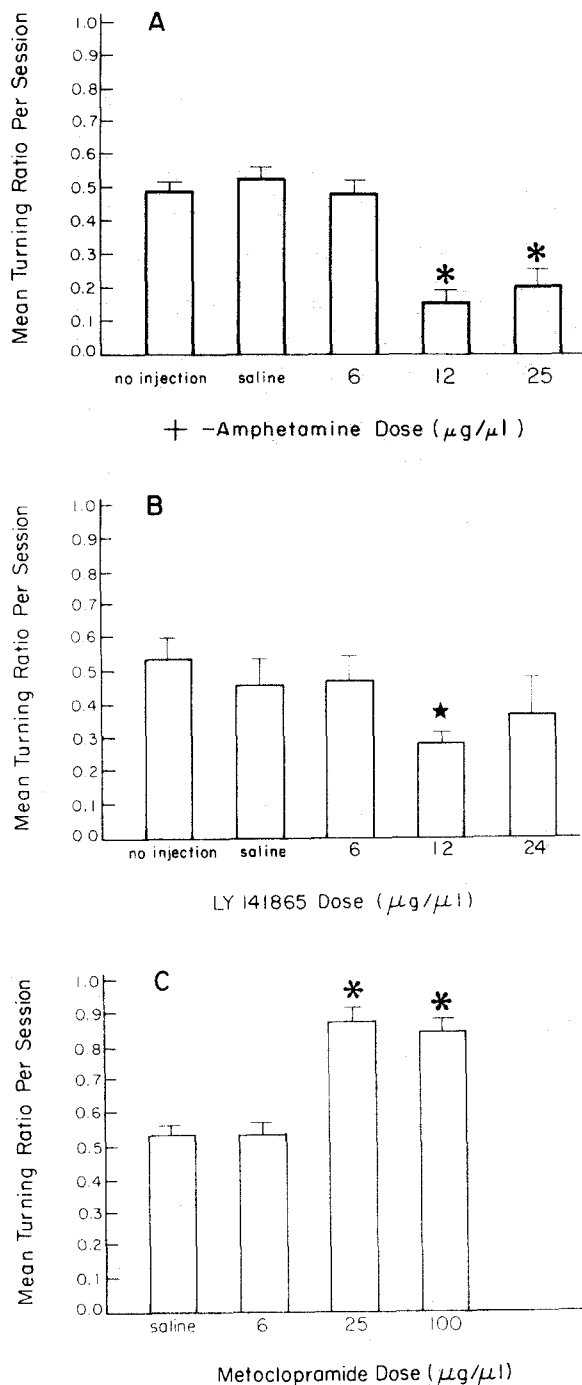
Fig. 2. Mean turning ratio per session (ipsiversive turns/ total turns) as a function of drug dose in the 3 experiments. In each figure, the symbols over the bars indicate significant differences from saline. Note that turning ratios greater or less than 0.5 indicate tendencies for ipsiversive and contraversive circling respectively. Pairwise comparisons were made using post hoc analyses of variance: ★ $P < 0.05$; * $P < 0.001$.

14.4 ($\pm$ 1.6); 6 μg, 17.6 ($\pm$ 1.6); 12 μg, 21.2 ($\pm$ 2.2); 25 μg, 20.9 ($\pm$ 2.4). A one-factor, repeated measures ANOVA including the no-injection and saline conditions, revealed a significant main effect of (+)-amphetamine dose, $F_{4,48} = 3.65$, $P < 0.05$.

In experiment 2, the first and second no-injection as well as the first and second saline scores were combined due to the absence of any significant differences between them. A significant main effect of dose of LY 141865 on the turning ratio was found, $F_{4,40} = 9.58$, $P < 0.0001$. Post hoc comparisons revealed that only the 12 μg dose produced a turning ratio which was significantly different from saline (Fig. 2B). Thus, both (+)-amphetamine and LY 141865 produced contraversive circling.

The mean number ($\pm$ standard error) of total turns per session for each dose of LY 141865 was: no-injection, 12.2 ($\pm$ 1.2); saline, 9.6 ($\pm$ 0.9); 6 μg, 9.4 ($\pm$ 1.0); 12 μg, 8.6 ($\pm$ 1.1); 24 μg, 4.5 ($\pm$ 1.0). The effect of dose on total turns was found to be significant, $F_{4,40} = 6.40$, $P < 0.001$.

In experiment 3, the first and second saline scores were not significantly different. Consequently, they were combined for the ANOVA which revealed a significant main effect of dose, $F_{3,33} = 21.72$, $P < 0.0001$. Post hoc comparisons revealed that both the 25 and 100 μg doses produced significantly higher turning ratios than saline (Fig. 2C). Thus, metoclopramide with systemic (+)-amphetamine pre-treatment produced marked ipsiversive circling.

The mean number ($\pm$ standard error) of total turns per session for each group receiving systemic (+)-amphetamine followed by central metoclopramide was: saline, 76.7 ($\pm$ 7.0); 6 μg, 88.4 ($\pm$ 6.2); 25 μg, 102.0 ($\pm$ 8.8); 100 μg, 98.6 ($\pm$ 6.1). Again, there was a significant effect of dose, $F_{3,33} = 3.79$, $P < 0.05$.

For all experiments, an arcsine transformation was conducted on the ratio data to satisfy the assumptions of the analysis of variance. Raw data are presented in Fig. 2. Analysis of variance of these raw data yielded the same profile of significant results.

DISCUSSION

The finding that the D-2 specific agonist, LY 141865 and (+)-amphetamine similarly produced contraversive circling suggests that the effects of (+)-amphetamine may have been mediated by DA. Further support for this possibility arises from the finding that the D-2 specific blocker, metoclopramide produced ipsiversive circling in rats pretreated with (+)-amphetamine. These results provide evidence for an excitatory contribution of frontal cortical DA to motor control.

The failure to find significant differences between the first and second administration of the no-injection and saline injection conditions indicates that subsequent to the drug sessions, rats returned to baseline turning ratios. This finding illustrates the acute effects of the central drug injections. Furthermore, as the saline scores were not significantly different from no-injection, it is clear that unilateral administration of 1.0 μl of saline did not induce a directional bias. It has been shown that an intracerebral injection volume of 1.0 μl diffuses into a sphere of approximately 1.0 mm[19]. This would make the possibility of diffusion of the drugs from the frontal cortex to the striatum (a distance of 3–4 mm) seem remote.

Increasing doses of central (+)-amphetamine resulted in an increase in total turns per session, whereas increasing doses of LY 141865 resulted in a decrease. Stereotyped movements, possibly interfering with circling, were noted in the rats receiving LY 141865. No systematic observations were made however, and the explanation of this discrepancy awaits further study. Increasing doses of metoclopramide resulted in an increase in total turns. This finding may be a result of a tighter turning posture with a consequent reduction in partial turns and a resultant increase in total turns. Thus, the total number of turns may be elevated in this instance due to a more dramatic directional bias. Therefore, the respective increase and decrease in total turns in the metoclopramide and LY 141865 experiments may be unrelated.

Hyperactivity previously observed 7–10 days after frontal cortical DA lesions[6,28] may reflect the demonstrated compensatory increase in function of subcortical DA systems[25,26]. These compensatory changes may be occurring quite apart from the normal excitatory influence of cortical DA on motor activity in the intact animal. Thus, bilateral frontal cortical injections of a DA antagonist would be expected to produce hypoactivity.

Abnormal functioning of DA systems has been implicated in schizophrenia and Parkinson's disease[5,9,10,20]. Many researchers have attempted to determine the contribution of specific DA systems to the symptomatology of these conditions. As we now provide evidence for an excitatory influence of mesocortical DA on motor control, this area may subsequently be implicated in disorders previously attributed to DA function in subcortical areas. This finding also raises the possibility that extrapyramidal motoric side effects, often observed following chronic neuroleptic treatment[17] may in part be influenced by frontal cortical DA.

REFERENCES

1 Adler, M.W., Changes in sensitivity to amphetamine in rats with chronic brain lesions, *J. Pharmacol. Exp. Ther.*, 134 (1961) 214–221.

2 Anden, N.E., Butcher, S., Corrodi, H., Fuxe, K. and Ungerstedt, U., Receptor activity and turnover of dopamine and noradrenaline after neuroleptics, *Eur. J. Pharmacol.*, 11 (1970) 303–314.

3 Avemo, A., Antelman, S. and Ungerstedt, U., Rotational behavior after unilateral frontal cortex lesions in the rat, *Acta Physiol. Scand.*, Suppl. 396, 84 (1973) 77.

4 Beninger, R.J., The role of dopamine in locomotor activity and learning, *Brain Res. Rev.*, 6 (1983) 173–196.

5 Bernheimer, H., Birkmayer, W., Hornykiewicz, O. and Jellinger, K., Humans: brain dopamine and Parkinson and Huntington syndromes, *J. Neurol. Sci.*, 20 (1973) 415–455.

6 Carter, C.J. and Pycock, C.J., Behavioral and biochemical effects of dopamine and noradrenalin depletion within the medial prefrontal cortex of the rat, *Brain Res.*, 192 (1980) 163–176.

7 Costall, B., Marsden, C.D., Naylor, R.J. and Pycock, C.J., Stereotyped behavior patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei, Brain Res., 123 (1977) 89–111.

8 Costall, B. and Naylor, R.J., The behavioral effect of dopamine applied intracerebrally to areas of the mesolimbic system, Eur. J. Pharmacol., 32 (1975) 87–92.

9 Creese, I., Burt, D.R. and Snyder, S.H., Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs, Science, 192 (1976) 481–483.

10 Denny-Brown, D., The Basal Ganglia, Oxford University Press, Oxford, 1962.

11 Dolphin, A., Jenner, P., Marsden, C.D., Pycock, C.J. and Tarsy, D., Pharmacological evidence for cerebral dopamine receptor blockade by metoclopramide in rodents, Psychopharmacology, 41 (1975) 133–138.

12 Donovick, P.J., A metachromatic stain for neural tissue, Stain Technol., 49 (1974) 49–51.

13 Glick, S.D., Changes in sensitivity to D-amphetamine in frontal rats as a function of time: shifting of the dose–response curve, Psychonom. Sci., 19 (1970) 51–54.

14 Glick, S.D. and Greenstein, S., Possible modulating influence of frontal cortex on nigro-striatal function, Br. J. Pharmacol., 49 (1973) 316–321.

15 Iversen, S.D., Wilkinson, S. and Simpson, B., Enhanced amphetamine responses after frontal cortex lesions in the rat, Eur. J. Pharmacol., 13 (1971) 387–390.

16 Jackson, D.M., Anden, N.E. and Dahlstrom, A., A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain, Psychopharmacologia, 45 (1975) 139–149.

17 Jenner, P. and Marsden, C.D., Neuroleptics and tardive dyskinesia. In J.T. Coyle and S.J. Enna (Eds.), Neuroleptics: Neurochemical, Behavioral and Clinical Perspectives, Raven, New York, 1983, pp. 223–253.

18 Joyce, J.N., Multiple dopamine receptors and behavior, Neurosci. Biobeh. Rev., 7 (1983) 227–256.

19 Myers, R., Handbook of Drug and Chemical Stimulation of the Brain: Behavioral, Pharmacological and Physiological Aspects, Van Nostrand, New York, 1974.

20 Olsen, R.W., Reisine, T.D. and Yamamura, H.I., Neurotransmitter receptors: biochemistry and alterations in neuropsychiatric disorders, Life Sci., 27 (1980) 801–808.

21 Pellegrino, L.J., Pellegrino, A.S. and Cushman, J.A., A Stereotaxic Atlas of the Rat Brain, Plenum, New York, 1979.

22 Peringer, E., Jenner, P., Donaldson, I.M., Marsden, C.D. and Miller, R., Metoclopramide and dopamine receptor blockade, Neuropharmacology, 15 (1976) 463–469.

23 Pijnenberg, A.J., Honig, W.M., Van der Heyden, J.A. and Van Rossum, J.M., Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity, Eur. J. Pharmacol., 35 (1976) 45–58.

24 Pycock, C.J., Turning behaviour in animals, Neuroscience, 5 (1980) 461–514.

25 Pycock, C.J., Carter, C.J. and Kerwin, R.W., Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat, J. Neurochem., 34 (1980) 91–99.

26 Pycock, C.J., Kerwin, R.W. and Carter, C.J., Effects of lesions of cortical dopamine terminals on subcortical dopamine receptors in rats, Nature (Lond.), 286 (1980) 74–77.

27 Scheel-Kruger, J., Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of catecholamines in the brain, Eur. J. Pharmacol., 14 (1971) 47–59.

28 Tassin, J.P., Stinus, L., Simon, H., Blanc, G., Thierry, A.M., LeMoal, M., Cardo, B. and Glowinski, J., Relationship between the locomotor hyperactivity induced by A10 lesions and the destruction of the frontocortical dopaminergic innervation in the rat, Brain Res., 141 (1978) 267–281.

29 Thierry, A.M., Blanc, G., Sobel, A., Stinus, L. and Glowinski, J., Dopaminergic terminals in the rat cortex, Science, 182 (1973) 499–501.

30 Tsuruta, K., Frey, E.A., Grewe, C.W., Cote, T.E., Eskay, R.L. and Kebabian, J.W., Evidence that LY 141865 specifically stimulates the D-2 dopamine receptor, Nature (London), 292 (1981) 463–465.