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BRIEF COMMUNICATION

No Change in Neostriatal D-2 Dopamine Receptors After NMDA Lesions of Rat Prefrontal Cortex

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BRIDGE, S., M. J. CHRISTIE AND P. M. BEART. *No change in neostriatal D-2 dopamine receptors after NMDA lesions of rat prefrontal cortex.* PHARMACOL BIOCHEM BEHAV 24(6) 1829–1832, 1986.—D-2 dopamine receptor function in rat nucleus accumbens and anterior corpus striatum was examined 6–8 days following N-methyl-D-aspartate lesions confined to medial prefrontal cortex. Lesions failed to alter the affinity or density of D-2 receptors labelled by [^{3}H]spiperone in membrane preparations of both subcortical areas. Locomotor activity and stereotyped behaviours induced by the D-2 agonist, LY-171555, were also not significantly altered in lesioned animals. These results suggest that D-2 dopamine receptors of nucleus accumbens and anterior corpus striatum are not localized on corticofugal afferents from medial prefrontal cortex.

Prefrontal cerebral cortex Glutamate transmitter Excitotoxin Nucleus accumbens Corpus striatum Dopamine D-2 receptors Locomotion Stereotypy

THE prefrontal cortex is thought to be essential for the synthesis of cognitive and motor acts into purposive sequences [7]. In particular, the medial subarea of the prefrontal cortex (MPFC) is of appreciable importance since it contains the terminals of mesocortical dopamine (DA) neurons [20] and because it is directly connected via an extensive network of corticofugal fibres to a number of DA-rich subcortical regions, including the nucleus accumbens septi (NAS) and the anterior corpus striatum (ACS) [1,25]. Appreciable evidence suggests that these corticofugal fibres utilize glutamate and/or aspartate as their transmitter [5] and that they are involved in the regulation of subcortical DA neurons [20–22]. The D-2 subtype of DA receptor is believed to play a key role in motor function, abnormal movement disorders and psychoses [12, 23, 24], and here D-2 receptor function in NAS and ACS has been analyzed using neurochemical and behavioural methods after the production of discrete lesions in the MPFC.

METHOD

Animals

Neurochemical experiments employed 80 Sprague-Dawley rats (250–310 g) of either sex and behavioural testing utilized 30 female Sprague-Dawley rats (200–250 g). Animals were housed in groups of 6–8 and maintained at 20–25°C under diurnal lighting conditions with lights on from 0800–2000 hr.

Surgery

Excitotoxin lesions directed at the MPFC, were performed by bilateral microinfusion of N-methyl-D-aspartate (NMDA) as previously described [2,3]. Sham-operated control animals received artificial CSF infusions into the MPFC. Neurochemical or behavioural analyses were carried out 6–8 days after surgery.

Neurochemical Procedures

The MPFC, NAS and ACS were dissected as previously described [3,20] at 4°C on a chilled dissecting plate. High affinity uptake of D-[^{3}H]aspartate (20 nM; 19 Ci/mmol, Amersham, U.K.) in synaptosome-containing preparations of MPFC was performed as given in detail elsewhere [3]. The ACS was defined as that part of the corpus striatum rostral to the optic chiasm (i.e., bregma zero) [19]. NAS and ACS were stored frozen at −80°C for 1–3 days and only the tissue from those animals showing an appreciable reduction of

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TABLE 1

MPFC LESIONS AND D-2 RECEPTOR BINDING PARAMETERS

	Control		Lesion	
	K_d	B_{max}	K_d	B_{max}
NAS	31 ± 6.0 (9)	8.2 ± 0.74 (9)	24 ± 4.2(10) (76%)	8.1 ± 0.49(10) (99%)
ACS	31 ± 3.2(17)	16 ± 0.66(17)	23 ± 1.8(18) (76%)	16 ± 0.73(18) (103%)

Parameters for the binding of [^{3}H]spiperone to D-2 receptors present in membrane preparations of NAS and ACS. Values are the mean ± S.E.M. (number of estimations in parentheses). K_d, pM; B_{max}, pmol/g wet weight. Percentiles in parentheses under Lesion data represent % of control values.

D-[^{3}H]aspartate uptake was employed in receptor binding assays with [^{3}H]spiperone. Binding assays in NAS and ACS were performed on pooled tissue from 3 and 2 brains respectively. The procedures for preparation of membranes and binding assays have been described elsewhere [8]. Membrane preparations equivalent to 1–3 mg wet wt. of original tissue were incubated with [^{3}H]spiperone (0.025–1 nM; 19–23 Ci/mmole, Amersham, U.K., and N.E.N., USA) for 25 min at 37°C. Sulpiride (10 μM) was used to define non-specific binding. Binding data were analysed using iterative curve fitting programmes [14,18].

Behavioural Testing

Locomotor and stereotyped responses to trans-(−)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H (or 2H)-pyrazolo[3,4-g]quinoline monohydrochloride (LY-171555; Eli Lilly & Co., USA), a specific D-2 dopamine agonist (the active isomer of LY-141865) [8,24], were assessed following MPFC lesions or sham injections to examine alterations in D-2 mediated receptor function. DA systems of NAS and corpus striatum have been extensively implicated in the regulation of locomotor activity and stereotyped behaviour [4]. All behavioural testing was performed between 1000 and 1800 hr on previously habituated animals. On the day before the experiment rats were placed individually on an activity meter (Animex Activity Meter, type S; Farad Electronics, Sweden) inside a perspex cage (33×23×18 cm) for 30 minutes for habituation. On the next day lesioned and sham-operated control animals received the test-drug (LY-171555; 0.175 mg/kg, SC) or vehicle (saline; 1 ml/kg, SC). The dose of LY-171555 used in this experiment was determined from preliminary experiments in which the dose-response curves for both locomotor activity and stereotyped behaviour were determined. The dose selected was such that a positive or negative change in response to the test drug could be observed. The times for monitoring responses to LY-171555 were chosen from initial determinations of time-response curves for this drug, and were such that the hyperactive and stereotypic responses were stable and maximal. Locomotor activity was recorded for 30 minutes beginning 30 minutes after placing the animal in the activity cage. The intensity of stereotypy was assessed every 10 minutes for the same time period as locomotor activity using a modification of a previously described rating scale [11]: asleep or immobile=0, active=1, sniffing over wide cage area=2, sniffing in restricted cage area=3, occasional chewing=4 and continuous chewing=5. The activity cages were calibrated to the same sensitivity each day and fresh sawdust was placed on the bottom of the cage after the testing of each animal. After completion of the behavioural experiments each animal was processed for histology as previously described [2,3]. Frozen sections (40 μm) were stained with cresyl violet and examined for the extent of the lesion with reference to the atlas of Paxinos and Watson [19].

TABLE 2

MPFC LESIONS AND BEHAVIOURAL RESPONSES TO THE D-2 AGONIST LY-171555

	Control	Lesion	% Control
Locomotor activity	15 ± 2.9 (14)	10 ± 3.3 (11)	65
Stereotyped behaviour	2.5 ± 0.26(14)	2.1 ± 0.37(11)	80

The effect of lesions to the MPFC on locomotor and stereotyped responses to the selective D-2 agonist, LY-171555. Values represent the mean ± S.E.M. (number of animals in parentheses) of locomotor activity (counts/30 min) and stereotyped behaviour (mean score/30 min). Statistical analysis by two factor analysis of variance (factors: lesion (NMDA vs. Sham) and drug); $p<0.05$ chosen as criterion for significance. Locomotor activity scores were subjected to square root transformation ($X = \sqrt{(x + 1/2)}$) prior to statistical analysis.

RESULTS

In those animals employed in neurochemical analyses, the presence of lesion was verified by the prior measurement of the high affinity uptake of D-[^{3}H]aspartate in synaptosome-containing preparations of MPFC. In agreement with our earlier reports [2,3], the NMDA lesions significantly reduced the uptake of D-[^{3}H]aspartate (50% of control, $p<0.0001$, unpaired two-tailed t-test). The mean uptake values in the synaptosomal preparations of the MPFC from lesioned and sham-operated control animals were 6.49±0.78 (n=40) pmol/g/min and 13.06±0.74 (n=40) pmol/g/min respectively. Saturation analysis of [^{3}H]spiperone binding in membrane preparations of the NAS and the ACS from animals of either sex yielded virtually identical data, which were pooled for ease of presentation (Table 1). Lesions of

the MPFC did not significantly alter (two tailed Student's t-test; $p>0.05$) either the dissociation constant (K_d) or the receptor density (B_{max}; Table 1).

Subcortical DA function was further assessed by monitoring the behavioural responses induced by the D-2 selective agonist, LY-171555. Histological examination revealed that the intracerebral infusion of NMDA resulted in a lesion that was confined to the MPFC, with minor damage to the most anterior aspect of the anterior cingulate cortex of most animals. Lesions were characterized by the presence of small glial cells and have been presented in detail elsewhere [2,3]. Animals with minor lesions extending into the septal region (n=5) were excluded from statistical analyses since there is evidence that the septum is directly associated with the NAS [13]. No significant differences occurred between the two groups although a trend to decrease was seen: locomotor and stereotyped responses of the lesioned animals were 65%, $F(1,23)=1.65$, $p=0.21$, and 80%, $F(1,25)=1.04$, $p=0.32$, respectively of control values.

DISCUSSION

The results presented here demonstrate that the bilateral infusion of NMDA into the MPFC produced necrosis confined largely to this region, and confirm our earlier evidence [2,3]. Our failure to observe alterations in D-2 receptor function in NAS and ACS after NMDA lesions of MPFC, and hence destruction of corticofugal fibres [3], would suggest that D-2 receptors of these subcortical regions are not localized on afferents from this cortical subarea. Receptors of the D-2 subtype, at least in the corpus striatum, are reported to be divided between corticostriatal and nigrostriatal afferents, and intrinsic striatal neurones [9]. Thus decortication has been reported to effect a 20–40% reduction of [^{3}H]spiperone binding consistent with the presence of D-2 receptors on presynaptic terminals of corticostriatal fibres [9,22]. Such lesions involve the extensive removal of frontal and parietal cortex, and hence provide no information about the relative importance of topographically-organized afferents to subcortical structures. Excitotoxin lesions provide a more discrete lesion than the classical approaches of ablation, transection or electrolytic lesion by destroying local somata, while sparing terminal fields and fibres of passage. Other studies in our laboratory are consistent with these axon-sparing actions in the NMDA leaves the cholinergic, dopaminergic and noradrenergic fields of MPFC still intact ([3]; in preparation). The MPFC represents the major source of corticoaccumbal fibres [1,3] and also projects to the medial ACS [1,25]. Thus corticofugal afferents arising in MPFC, in contrast to those from other parts of neocortex to corpus striatum, do not bear D-2 receptors: although they are apparently a site of localization of D-1 receptors [21]. Caution has been urged in the interpretation of the effects of lesions on the density of binding sites since adaptive changes may mask the true effect on a receptor population. Indeed, trans-synaptic degeneration occurs in the striatum after lesions of the corticostriatal pathway [10].

As far as we are aware there has been only one other report [15] of an attempt to employ the D-2 specific agonist, LY-171555, to analyse the functional role of this subtype of DA receptor. In our hands, LY-171555 produced increases in locomotion, sniffing and chewing, but licking and rearing as produced by the general DA agonist apomorphine [6], were never observed. This spectrum of activity found with LY-171555 differs somewhat from the behaviours promoted by SKF 38393, a selective D-1 agonist, which include locomotion, sniffing, rearing and grooming [16]. The use of such selective agonists to promote dopaminergic behaviours allows the clarification of the functional role of the putative receptor subtype under study. In our investigation, the behavioural results obtained with LY-171555 were consistent with the implications of our radioligand binding data. In summary, D-2 receptors of NAS and ACS appear not to be associated with corticofugal afferents from the MPFC. This finding supports the notion that the ventral striatum, and in particular the NAS, should be considered as a separate functional unit of differing neuronal (and receptor) organization to the corpus striatum [17].

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