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Abolition of Nomifensine-Induced Stereotypy after 6-Hydroxydopamine Lesions of Ascending Dopaminergic Projections

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PRICE, M. T. C. AND H. C. FIBIGER. *Abolition of nomifensine-induced stereotypy after 6-hydroxydopamine lesions of ascending dopaminergic projections.* PHARMAC. BIOCHEM. BEHAV. 5(2) 107–109, 1976. – The effects of bilateral focal injections of 6-hydroxydopamine into the zona compacta of the substantia nigra (SNC) on nomifensine-induced stereotypy were examined in the rat. These lesions reduced neostriatal dopamine levels to less than 1 percent of control levels. They also abolished nomifensine-induced stereotyped behavior. It is suggested that nomifensine-induced stereotypy is mediated via a presynaptic action on dopamine uptake and release.

6-OHDA Nomifensine Dopamine inhibitor Dopamine Stereotyped behavior Zona compacta Substantia nigra Dopamine receptors

THERE is considerable evidence suggesting that drug-induced behavioral stereotypies are associated with increased stimulation of dopamine (DA) receptors in the forebrain. For example, injections of amphetamine can increase nerve impulse-induced release of neostriatal DA [15] and produce stereotypy. The latter effect is blocked in animals given bilateral 6-hydroxydopamine (6-OHDA) lesions to the head of the caudate nucleus [1] or to the zona compacta of the substantia nigra (SNC) [11]. Inhibition of catecholamine synthesis by α-methyl-p-tyrosine (α-MT) blocks amphetamine-induced stereotyped behavior but not a similar behavioral syndrome induced by apomorphine [3]. Also, apomorphine-induced stereotypy is potentiated rather than blocked by 6-OHDA lesions to the SNC [11]. These data have been taken as evidence that apomorphine, unlike amphetamine, may directly stimulate DA receptors. It has recently been shown that nomifensine, a potent inhibitor of DA uptake into synaptosomes of rat brain corpus striatum [7] produces stereotypy in the rat [2]. Costall *et al.* [2] reported, in addition, that either α-MT injections or electrolytic lesions of the SNC reduced but did not abolish nomifensine-induced stereotypy and concluded that this compound had some direct post-synaptic activity similar to that of apomorphine. Since electrolytic and 6-OHDA lesions may differentially affect drug-induced stereotypies [11] we used 6-OHDA lesions to the SNC to investigate further the role of ascending dopaminergic systems in nomifensine-induced stereotypy.

METHOD

Animals and Surgery

Under Nembutal (50 mg/kg) anaesthesia, male Wistar rats (300–320 g) received bilateral stereotaxic injections of 6-OHDA into the SNC. The animals (n = 11) were injected with 8 μg of 6-OHDA hydrobromide (dosage expressed as the base) in 4 μl of 0.15 M NaCl containing ascorbic acid (0.2 mg/ml). The injection rate was 0.2 μl/min. The coordinates, taken from stereotaxic zero, were A + 3.1 mm; L ± 2.1 mm; and DV + 2.1 mm [9]. The rats received IP injections of desipramine HCl (25 mg/kg) 30 min before the stereotaxic injections. In previous experiments it has been observed that in addition to reducing striatal DA levels by more than 90%, similar injections also produce large decreases in forebrain NA unless animals are first pretreated with desipramine [5,12]. Control animals (n = 10) underwent sham operations, but did not receive intracerebral injections. In previous unpublished experiments nigral injections of the vehicle solution have been found not to affect striatal DA levels nor amphetamine-induced stereotypy. The rats were housed individually on ad lib food and water. The bilateral SNC lesions produced aphagia and adipsia which necessitated intragastric feeding twice daily [6].

Procedure

Five weeks postoperatively, the animals were injected IP with nomifensine hydrogen maleate (20 mg/kg expressed as the salt) which was dissolved in a minimum quantity of HCl made up to volume with distilled water. The animals were then returned to their home cages. Stereotyped behavior was measured as previously described [4]: 0 = normal behavior; 1 = exploratory behavior, discontinuous sniffing; 2 = continuous sniffing; 3 = small compulsive head movements; 4 = licking or biting the wires of the cage.

TABLE 1

EFFECT OF BILATERAL 6-OHDA LESIONS OF THE SNC ON NOMIFENSINE-INDUCED STEREOTYPY

	Nomifensine (20 mg/kg)					
	0.5 hr	1 hr	1.5 hr	2 hr	2.5 hr	3 hr
Controls (n=10)	2.90 ± 0.17	3.00 ± 0.21	3.10 ± 0.10	2.90 ± 0.17	2.70 ± 0.21	2.20 ± 0.24
Substantia nigra 6-OHDA (n=11)	0.72 ± 0.13*	0.63 ± 0.14*	0.63 ± 0.14*	0.36 ± 0.14*	0.45 ± 0.15*	0.36 ± 0.14*

*Significantly different from controls $p<0.001$.
[Data represent means (± SEM)].

Measurements were taken 6 times, at half hr intervals beginning 30 min after the nomifensine injections. One week later, the lesioned animals were injected with an equal volume of solvent and behavior was again measured according to the stereotypy scoring system. Measurements were taken 30 and 60 min after these injections. Two weeks after this final test the animals were killed by cervical fracture and striatal DA and hypothalamic noradrenaline (NA) were measured [10].

RESULTS

Nomifensine (20 mg/kg) produced stereotyped behavior in the control animals at each of the test intervals, whereas stereotypy was never exhibited by the nigral-lesioned animals (Table 1). All of the control animals obtained scores of at least 3 (small compulsive head movements) at several of the test intervals. Two of the controls achieved scores of 4 (licking or biting the wires of the cage). Nigral lesioned animals were never given a score greater than 1 (exploratory behavior, discontinuous sniffing). The scores obtained by nigral-lesioned rats after nomifensine did not differ significantly from those obtained following injections of solvent indicating that the lesions resulted in an abolition, and not only a reduction, in stereotyped behavior. 6-OHDA lesions of the SNC reduced neostriatal DA to less than 1% of control values (controls 10.53 ± 0.54; SNC-lesioned 0.06 ± 0.03 μg/g) but did not significantly affect hypothalamic NA (controls 2.12 ± 0.08; SNC-lesioned 1.87 ± 0.11 μg/g). Errors are standard errors of the mean.

DISCUSSION

In previous work we observed that stereotypy produced by indirectly-acting sympathomimetics such as d-amphetamine was blocked by bilateral 6-OHDA lesions of the ascending DA systems while the effects of directly acting agonists such as apomorphine were potentiated [11]. This latter effect was attributed to the development of post-junctional supersensitivity following denervation. Kelly, Seviour and Iversen [8] have recently suggested that the most intense aspects of stereotypy (e.g., the biting or gnawing response) are mediated via the nigro-striatal DA projection while the mesolimbic DA projection is the substrate for behaviors such as continuous sniffing and repetitive head movements. Lesions identical to those used in the present experiments have, in previous work, been found to destroy both the mesolimbic and the nigro-striatal DA projections (Fibiger, unpublished observations). The finding that both the moderate and intense aspects of nomifensine-induced stereotypy were abolished by the 6-OHDA lesions suggests that both of these ascending DA systems were also destroyed in the present experiments.

The present observations with nomifensine indicate that the stereotypy induced by this compound can be attributed to a presynaptic action on dopamine uptake and release. If nomifensine did have some direct DA agonist activity then certain components of the stereotyped response should have remained intact or even been exaggerated after the 6-OHDA lesions [11]. These results are not in accord with the conclusions of Costall *et al.* [2] who suggested that in addition to a presynaptic effect, nomifensine also had a direct apomorphine-like postsynaptic action. The basis of this discrepancy is most likely the different lesioning procedures utilized in the 2 studies. Costall *et al.* [2] used electrolytic lesions which, unlike 6-OHDA, produce only moderate and unreliable reductions of neostriatal DA [11]. Furthermore, electrolytic lesions probably destroy striatal efferents as well as ascending DA systems. Unfortunately, Costall *et al.* [2] failed to measure DA levels in their experiments. The present data suggest therefore that nomifensine produces stereotypy by increasing the release and/or blocking the re-uptake of DA from terminals of ascending dopaminergic neurons. However, the fact that α-MT and reserpine pretreatment, but not α-MT by itself, blocks these effects of nomifensine suggest that this compound is more similar to methylphenidate than to d-amphetamine in its mechanism of action [13,14].

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