The 6-hydroxydopamine rotational model for the detection of dopamine agonist activity: reliability of effect from different locations of 6-hydroxydopamine

The ability of drugs to induce rotational patterns in rats after 6-hydroxydopamine (6-OHDA) lesion of the substantia nigra is widely used as a model for the detection of antiparkinson agents. It is hypothesized that the test will differentiate direct and indirectly acting dopaminergic agonists, direct agents causing contralateral turning by an action on presumed 'supersensitive' dopamine receptors in the striatum of the lesioned side, whilst the action of indirectly acting agents is limited to the intact side and causes ipsilateral turning (Ungerstedt, Avemo & others, 1973). However, in our hands, the intranigral application of 6-OHDA causes this contralateral/ipsilateral response in only a small proportion of animals (approximately 20%). Further, the intensity of circling is weak even though histological and biochemical assessments (which show a striatal dopamine concentration of $1{\cdot}07 \pm 0{\cdot}17$ μg g^{-1} wet tissue as compared to control values of $2{\cdot}77 \pm 0{\cdot}19$ μg, a difference of 61%, $P < 0{\cdot}001$) clearly indicate the correct placement of the 6-OHDA (Costall, Marsden & others, in preparation).

In recent studies, we have investigated the circling activity following 6-OHDA injections posterior and anterior to the substantia nigra and into the area of the nigrostriatal pathway in the lateral hypothalamus (Costall, Marsden & others, in preparation). Circling behaviour was not recorded for animals injected with 6-OHDA posterior to the substantia nigra (Ant. 1·2, Vert. $-3{\cdot}0$, Lat. $\pm 2{\cdot}25$; De Groot, 1959) although these lesions caused an approximate 40% reduction in striatal dopamine content. However, 50 to 70% of animals with 6-OHDA injected anterior to the substantia nigra (Ant. 3·0, Vert. $-2{\cdot}7$, Lat. $\pm 2{\cdot}0$) exhibited a contralateral/ipsilateral response and the lesion was shown to reduce striatal dopamine concentrations by approximately 70%. Furthermore, 100% of the animals with 6-OHDA lesions placed in the area of ascending dopamine pathways in the lateral hypothalamus (Ant. 4·6, Vert. $-2{\cdot}7$, Lat. $\pm 1{\cdot}9$) exhibited a contralateral/ipsilateral circling behaviour and, of these, over 90% of the animals gave a circling response of greater intensity than in animals receiving 6-OHDA anterior to the substantia nigra. The striatal dopamine depletions from the lateral hypothalamic injections were approximately 80%. Therefore, the present studies were designed to assess the reliability of the circling response of animals with 6-OHDA lesions of the medial forebrain bundle in the lateral hypothalamus to a wide variety of dopamine agonists.

The studies utilized male Sprague-Dawley rats, 250–300 g. Surgery was carried out using a Kopf stereotaxic instrument and chloral hydrate (300 mg kg^{-1}, i.p.) as anaesthetic. 6-OHDA, prepared in nitrogen bubbled distilled water, was delivered into the lateral hypothalamus via a stainless steel injection unit, 0·3 mm diameter, with its tip at Ant. 4·6, Vert. $-2{\cdot}7$, Lat. $\pm 1{\cdot}9$ (De Groot, 1959). A stainless steel guide cannula terminated 2·5–3·00 mm above the tip of the injection unit. 4 μl of a 2 μg in 1 μl solution of 6-OHDA was delivered from an Agla micrometer syringe at a rate of 1 μl min^{-1}. Rats immediately developed a spontaneous ipsilateral circling behaviour (up to 5 rev min^{-1}) which persisted throughout the entire experimental period (up to 56 days). From the first post-operative day apomorphine caused the rats to circle contralateral to the side of lesion whilst (+)-amphetamine enhanced the ipsilateral response. These effects increased in intensity to a maximum at day 8 and were maintained at this level throughout the experimental period. 100% of animals gave an ipsilateral/contralateral response and those responding with 15+ rev^{-1} min to 0·5 mg kg^{-1} apomorphine were selected to determine the circling effects of different dopamine agonists. These experiments commenced on the tenth postoperative day

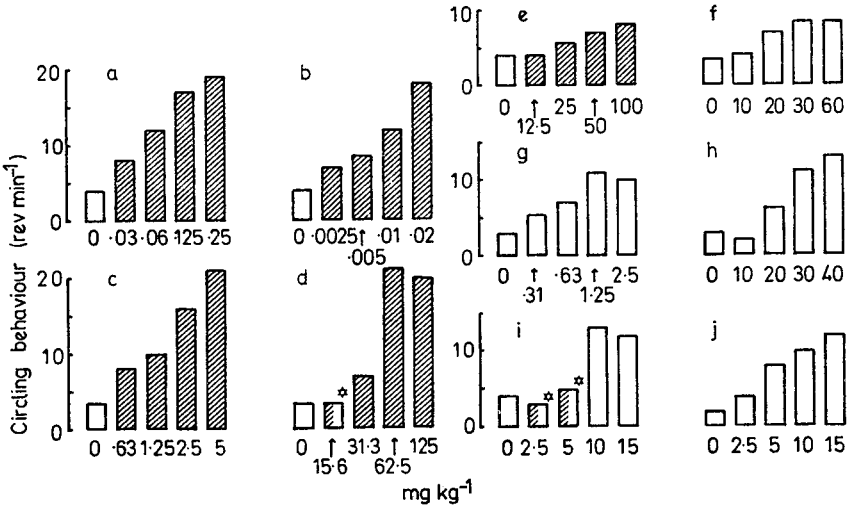

FIG. 1. Circling behaviour induced by dopaminergic agonists after 6-OHDA lesion of the medial forebrain bundle in the lateral hypothalamus. (a) Apomorphine; (b) (−)-NPA; (c) bromocriptine; (d) L-dopa; (e) piribedil; (f) amantadine; (g) (+)-amphetamine; (h) D145; (i) nomifensine; (j) M_2-metabolite. Hatched columns—circling behaviour contralateral in direction to the side of lesion location or Open columns—ipsilateral. *Definite circling movements in both directions. Circling behaviour is expressed as the maximum number of revolutions achieved in a 1 min period during the action of a drug. Animals were tested every 15–30 min throughout the duration of a drug effect. Each value is the mean of responses from 8–12 animals. Standard errors are all less than 18% of the means.

and animals were used every 4–7 days to completion of the studies. Apomorphine hydrochloride (Macfarlan Smith) and (−)-*N*-n-propylnorapomorphine hydrochloride-[(−)-NPA] (Neumeyer) were dissolved in 0·1% sodium metabisulphite, (+)-amphetamine sulphate (Sigma), amantadine hydrochloride (Ciba-Geigy), D145 (1,3-dimethyl-5-aminoadamantan) (Merz), the M_2-metabolite of nomifensine (8-amino-2-methyl-4-(4-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline) hydrogen maleate (Hoechst) and piribedil monomethanesulphonate (Servier) were dissolved in distilled water. Bromocriptine (Sandoz) was prepared in a minimum quantity of tartaric acid and parent nomifensine (Hoechst) in a minimum quantity of hydrochloric acid, each made up to volume with distilled water. L-Dopa (Roche) was prepared as an aqueous suspension in 2% carboxymethylcellulose. Nomifensine, its M_2-metabolite, (−)-NPA and apomorphine were administered by the subcutaneous route and all other agents intraperitoneally.

The directions and maximum intensities of circling behaviour induced by the dopaminergic agonists tested are shown in Fig. 1. On completion of the studies brains were subjected to the biochemical techniques previously described (Costall, Fortune & others, 1975). The lateral hypothalamic lesions were shown to cause a marked reduction in striatal dopamine content (striatal dopamine concentration after lesion was 0·48 ± 0·22 μg g^{-1} wet tissue as compared with control values of 2·60 ± 0·23 μg g^{-1}, a decrease of 82%, $P < 0.001$) and a fall in mesolimbic dopamine (dopamine content lesioned side, 0·19 ± 0·02 μg g^{-1}, on control side, 0·51 ± 0·04 μg g^{-1}, a difference of 63%, $P < 0.001$) although there were no significant changes in cortical, striatal or mesolimbic 5-hydroxytryptamine content. However, the lesions did cause a reduction in noradrenaline content of the cortex (noradrenaline content of lesioned side was 197·6 ± 26·6 ng g^{-1} wet tissue and 315·6 ± 14·7 ng g^{-1} on non-lesioned side, a difference of 37%, $P < 0.005$) and limbic areas (277·7 ± 29·8 ng g^{-1} on lesioned side compared with 433·6 ± 38·7 on control side, a fall of 36%, $P < 0.01$).

The directions of circling behaviour recorded after 6-OHDA lesions in the lateral hypothalamus agree with those reported after substantia nigra lesions: apomorphine, (−)-NPA, L-dopa, piribedil and bromocriptine caused contralateral circling whilst (+)-amphetamine, nomifensine and its metabolite, D145 and amantadine induced ipsilateral circling (Corrodi, Fuxe & others, 1973; Ungerstedt & others, 1973; Fuxe, Agnati & others, 1975). Further, the circling responses after 6-OHDA lesions in the lateral hypothalamus were of high intensity in at least 90% of animals used. This greater reproducibility and effectiveness of the lateral hypothalamic model as compared to the substantia nigra model may reflect one or more of a number of factors. Firstly, the dopamine depletion after the lateral hypothalamic injections of 6-OHDA is greater than after similar injections into the nigra and is correspondingly associated with the development of a greater degree of 'denervation supersensitivity'. The greater potency of 6-OHDA injections into the lateral hypothalamus than in the region of the substantia nigra may indicate that the dopamine cell bodies are more resistant to the action of 6-OHDA than the axons, but probably also results from the direct injection of a neurotoxic compound into a dense fibre pathway rather than into a larger region of more diffuse cell bodies.

However, a further factor which must be considered in the circling response is a mesolimbic involvement. Whilst 6-OHDA placed at the centre of the substantia nigra caused only a modest fall in mesolimbic dopamine content ($0{\cdot}47 \pm 0{\cdot}05$ $\mu g\ g^{-1}$ wet tissue on lesioned side, $0{\cdot}67 \pm 0{\cdot}04$ $\mu g\ g^{-1}$ on control side, a fall of 30%, $P < 0{\cdot}01$), the lateral hypothalamic lesions caused marked reductions in mesolimbic dopamine. It has been suggested that circling behaviour may be a measure of two behavioural effects, the induction of asymmetries and the stimulation of locomotor activity (Costall & Naylor, 1974a). Although the evidence clearly indicates an important role for striatal dopamine in the induction of asymmetries (Costall & Naylor, 1974b), whilst asymmetric behaviour has not been recorded after unilateral lesions in the mesolimbic areas (Costall, Marsden & others, in preparation), mesolimbic dopamine mechanisms, in particular those of the nucleus accumbens (Pijnenburg & van Rossum, 1973; Costall & Naylor, 1975a; Elkhawad & Woodruff, 1975) and tuberculum olfactorium (Costall & Naylor, 1975a), have been implicated in the control of locomotor activity. Thus, it must be considered that the changes in mesolimbic dopamine content may contribute to the circling responses observed after the lateral hypothalamic lesions.

In addition to dopamine, other neurotransmitter mechanisms have been implicated in the control of circling behaviour, in particular 5-hydroxytryptamine (Costall & Naylor, 1974a) and noradrenaline (Glick & Greenstein, 1973; Pycock, Donaldson & Marsden, 1975). Although the lateral hypothalamic lesions, similar to those of the substantia nigra, failed to significantly alter 5-hydroxytryptamine concentration, the lateral hypothalamic lesion was associated with reduced noradrenaline content of both the cortex and limbic regions. Changes in noradrenaline contents have been shown to be less marked after placing 6-OHDA into the centre of the substantia nigra (no significant, $P > 0{\cdot}05$, change in limbic noradenaline, but cortical noradrenaline reduced by 27%, $P < 0{\cdot}05$) (Costall, Marsden & others, in preparation) and this difference may be reflected in the different circling activities for the two lesion locations.

Finally, it should be considered that the non-specific damage induced by 6-OHDA applied to the nigra may tend to simulate the effects of an electrolesion of the area which cause *ipsilateral* circling to *all* dopaminergic agonists (Costall & Naylor, 1975b) and thus reduce any contralateral effect.

Whatever the biochemical basis for the greater reliability and intensity of the contralateral/ipsilateral circling effect following 6-OHDA injections into the lateral hypothalamus, the interpretational difficulties of the model are the same as for sub-

stantia nigra lesions. Thus, the differentiation of direct/indirect dopamine agonist activity from contralateral/ipsilateral circling is open to criticism since piribedil causes contralateral circling although its dopamine agonist action has been shown to include a presynaptic component (Costall & Naylor, 1973; Fuxe & others, 1975), and nomifensine and its metabolite induce ipsilateral circling although both have been shown to possess some postsynaptic activity (Gerhards, Carenzi & Costa, 1974; Costall, Kelly & Naylor, 1975). Nevertheless, if the 6-OHDA rotational model is to be used to detect dopamine agonist activity it is suggested that a more intense and reliable effect may be obtained by injecting 6-OHDA into the area of the medial forebrain bundle in the lateral hypothalamus instead of into or around the substantia nigra.

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Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, West Yorkshire, U.K.

B. COSTALL
R. J. NAYLOR

Department of Neurology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, U.K.

C. PYCOCK

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