EFFECTS ON PRECISE MOTOR RESPONDING OF BILATERAL 6-HYDROXYDOPAMINE INFUSION INTO THE SUBSTANTIA NIGRA

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INTRODUCTION

UNILATERAL injection of 6-hydroxydopamine (6-OHDA) into the substantia nigra (SN) produces turning ipsilateral to the lesioned side (UNGERSTEDT, 1971). Since the zona compacta of the SN has a high proportion of neuron somata containing dopamine, this "rotation" or "turning" model has been extensively used to assess the effects of pharmacological agents thought to affect predominately dopaminergic neurotransmission mechanisms (e.g. see UNGERSTEDT, 1971). Although this approach has yielded a considerable volume of interesting data, the question can legitimately be raised as to what the "rotation" model tells us about the fundamental functional significance for motor behaviour of the SN and neuroanatomically interconnected structures. Indeed, this model may only reflect the consequences of producing a functional imbalance in the extrapyramidal motor system. For example, ipsilateral turning is also observed after hemicerebellectomy (MANNI and DOW, 1963). In this paper we describe a behavioural technique, lever-positioning, which may permit a more precise evaluation of possible functional relationships between motor behaviour and decrements in nigral function produced by direct injection of 6-OHDA into the zona compacta of the SN.

METHODS

Behavioural procedures

Male Sprague–Dawley rats were reduced to 80 per cent of their *ad libitum* weights by means of restricted feeding. They were then trained to hold a constant force (2 g $\times$ gravity) lever within a displacement band 6° wide beginning at 17° and ending at 23° from the horizontal for 2·0 sec in order to obtain one 45-mg food pellet. The total possible displacement of the lever was 40°. Responses were recorded on a polygraph, on running time meters, and on digital counters. The rats were tested in daily consecutive sessions. A session was terminated (1) after 100 45-mg food pellets had been received and consumed or (2) after 1 hr, whichever came first. Quite stable motor performance baselines were obtained with this procedure, both within and between sessions. Following stabilization of the lever-positioning behaviour, each rat was injected bilaterally in the SN with 6-OHDA or its vehicle. Testing in the lever-positioning task commenced 24 hr after surgery and continued until the behavioural baseline again stabilized. Food intake and locomotor activity were also quantified for each experimental animal both before and after surgery.

Neurotoxic specificity of 6-OHDA and intranigral infusion procedures

Although initial experimental evidence suggested that 6-OHDA was a neurotoxic drug selective for catecholamines at low doses (e.g., 8 μg 6-OHDA in 4 μl vehicle; UNGERSTEDT, 1971), more recent studies (POIRIER *et al.*, 1972) have cast considerable doubt on this hypothesis. In our laboratory we have confirmed the basic conclusion of POIRIER *et al.* (1972) that intracerebrally administered 6-OHDA, using a vertical approach to the structure studied (cf. UNGERSTEDT, 1971), possesses considerable non-specific activity: (1) unilateral infusion into nucleus ruber of 8 μg 6-OHDA in 4 μl Ringer's–ascorbic acid solution produced within 48 hr a complete loss of neuron somata on the injected side. The zone of destruction was approximately 2 mm in diameter. Although this dose has been reported to produce selective degeneration of catecholamine neurons (UNGERSTEDT, 1971), the cell bodies of nucleus ruber contain neither dopamine nor noradrenaline (DAHLSTRÖM and FUXE, 1964). (2) Bilateral administration of 8 μg/4 μl 6-OHDA into the SN produced almost complete loss of neuron somata in the zona compacta correlated with the production of catalepsy and rigidity. Both the histopathology and consequent motor symptomatology could be duplicated by bilateral intranigral injection of 8 μg/4 μl copper sulfate, a relatively non-specific cytotoxic agent.

These data strongly suggest that "specificity" of neuron destruction can be obtained with 6-OHDA only to the extent that the drug is injected into brain regions which are neurochemically homogeneous. For this reason and because we were particularly interested in dopaminergic mechanisms of motor behaviour, we have attempted to produce 6-OHDA lesions confined as much as possible to the zona compacta of the SN. We have been able to achieve almost complete and relatively selective destruction of zona compacta neuron somata by injecting 6-OHDA through a cannula inserted at an angle of 53° from the horizontal. The cannula shaft lies between the row of zona compacta neurons and the medial lemniscus. This lateral entry obviates damage to the nucleus ruber and ventral noradrenaline bundle, both of which may be destroyed, either mechanically or by 6-OHDA, if the traditional vertical approach to the SN is employed. In the current experiments solutions of 6-OHDA were stereotaxically delivered to the SN through a stainless steel cannula (external diameter = 0·32 mm) at a rate of 1 μl/min and in amounts of either 4 μg/2 μl or 1 μg/2 μl. Solutions of 6-OHDA were prepared immediately before injection and were kept at 0°C until infusion. The Ringer's vehicle contained 0·2 mg ascorbic acid per ml to retard 6-OHDA auto-oxidation. The cannula was kept in place for 1 min after termination of the infusion period to allow for pressure equalization. The lateral approach was used for both the histological and behavioural studies described in the following section.

RESULTS AND DISCUSSION

Histology

Animals ($N = 10$) injected unilaterally with 4 μl/2 μl 6-OHDA and sacrificed 48 hr later showed almost complete loss of zona compacta neuron somata with the exception of a few cell bodies in the lateral portion of the compacta (Luxol fast blue-basic fuchsin and also thionin stained sections). Infusion of 1 μg/2 μl 6-OHDA produced approximately 30 per cent loss of cell somata in the zona compacta on the

injected side ($N = 10$ rats). Most of the degenerated cell bodies lay in the medial portion of the compacta. With both doses of 6-OHDA there was no apparent loss of neuron somata in pars reticulata and only slight, if any, damage to pars lateralis. Furthermore, there did not appear to be any loss of myelinated fibres in the region of the SN.

Behaviour

Rats ($N = 6$) receiving 4 μg/2 μl 6-OHDA in each SN showed lever-positioning behaviour characterized by long holding times (Fig. 1). This motor pattern reflected the relatively cataleptic posture that the rats typically assumed. Locomotor activity was markedly reduced over a 6-day period such that statistically significant motility decrements to 51 per cent ($P < 0{\cdot}05$) and 26 per cent ($P < 0{\cdot}01$) of control were observed on days 1 and 6 after surgery, respectively. Similarly, food intake was decreased to 8 per cent ($P < 0{\cdot}01$) of control on the first post-operative day and to 5 per cent ($P < 0{\cdot}01$) on the sixth. Passive movement of the limbs revealed that they were markedly rigid. The hindlimbs were extended and appeared more rigid than the forelimbs.

The lever-positioning records of animals ($N = 6$) bilaterally injected with 1 μg/2 μl 6-OHDA showed oscillatory, tremor-like movements in addition to long holding times (Fig. 1). The tremor had both a regular amplitude and periodicity ($\simeq$ 5 Hz).

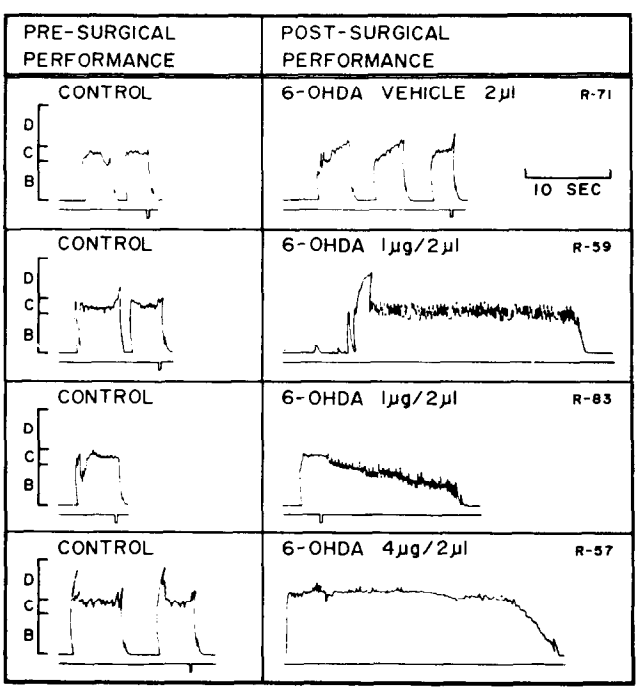

FIG. 1.—Effects on lever-positioning behaviour of bilateral 6-hydroxydopamine infusion into the substantia nigra. The post-operative performance is shown for rat R-71 on the second day after surgery, for rat R-59 on day 6, for rat R-83 on day 3, and for rat R-57 on day 6. The downward deflections on the lower trace in each pair of records represents the delivery of a food pellet. B = the lever displacement zone from 0 to 17°, C = the reinforced zone from 17 to 23°, and D = the zone from 23 to 40°.

These rats also displayed rigidity and hypokinesia but less severe than animals given 4 μg/2 μl 6-OHDA. Food intake was 7 per cent ($P < 0{\cdot}01$) of control on the first post-surgical day but improved to 41 per cent ($P < 0{\cdot}05$) of control by the sixth daily testing session. The initial decrease in motility to 49 per cent ($P < 0{\cdot}05$) of control 24 hr after surgery was replaced by an increase to 71 per cent ($P =$ N.S.) of control on the sixth post-operative day. Nonetheless, marked effects on lever-positioning were still observed 6 days after the operation (Fig. 1) indicating that the technique of lever positioning was capable of detecting motor deficits which were not readily apparent using more traditional behavioural observation methods. No motor abnormalities (Fig. 1) or deficiencies in food intake and locomotor activity were observed in animals ($N = 4$) injected with 2 μl of the vehicle alone.

Conclusions

Our data demonstrate that, depending on the dose of 6-OHDA bilaterally injected into the SN and also on the behavioural measuring technique, a preparation can be obtained which displays motor symptomatology similar to the syndrome observed in human Parkinsonism, taking into account obvious differences in motor expression due to species differences. Furthermore, our results suggest that the motor pathologies of rigidity, tremor, and hypokinesia may have a common neurochemical basis, possibly a deficiency of dopamine in the nigro-striatal system.

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