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COMMUNICATIONS

The effect of diphenylhydantoin on central catecholamine containing neuronal systems

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Diphenylhydantoin (DPH) has been used for many years in the treatment of grand mal and focal epilepsy (Merritt & Putnam, 1939). Overdosage usually causes a syndrome of nystagmus, dysarthria, and ataxia (Glaser, 1972). However, recently reports of chorea and dystonia occurring in association with DPH intoxication have appeared (Chadwick, Reynolds & Marsden, 1976). Such extra-pyramidal syndromes have not, in general, been recorded with other anticonvulsants.

The dyskinesias produced by DPH resemble those produced by antipsychotic drugs (Chadwick & others, 1976), which are believed to result from an effect of these neuroleptic drugs on cerebral dopamine receptors (Marsden, 1973). Some evidence exists that DPH may possess similar pharmacological properties. Thus, DPH diminishes the therapeutic response to L-dopa in Parkinson's disease in man and blocks L-dopa induced turning in rodents with unilateral nigrostriatal lesions (Mendez, Cotzias & others, 1975). Such dopamine receptor blocking actions might be unexpected in an anticonvulsant drug, since the administration of dopamine antagonists results in an increased seizure susceptibility (Meldrum, Anlezark & Trimble, 1975). We have therefore examined the activity of DPH in a variety of behavioural and biochemical test systems purported to be susceptible to the actions of dopamine receptor blocking agents. At the same time the influence of DPH on central noradrenergic systems has also been investigated.

Inhibition of stereotypy induced by apomorphine HCl (0·5 mg kg^{-1}, s.c.) in male Wistar rats (225-275 g; Animal Suppliers Ltd) was assessed 15 min following apomorphine administration using the scoring system of Costall & Naylor (1973). Inhibition of apomorphine HCl (0·5 mg kg^{-1}, s.c.) induced locomotor activity in reserpine (10 mg kg^{-1}, i.p. 18–24 h previously) treated animals was assessed using batches of three Swiss S or P strain male mice (20–25 g) in Animex activity meters as previously described (Dolphin, Jenner & Marsden, 1976). Unilateral nigrostriatal lesions were produced in mice according to the method of Pycock, Tarsy & Marsden (1975). Inhibition of circling behaviour produced by apomorphine HCl (0·5 mg kg^{-1}, s.c.) or (+)-amphetamine sulphate (4 mg kg^{-1}, i.p.) was determined 15 or 30 min respectively following agonist administration by measurement of the number of turns completed in 1 min. Animals were subsequently killed, brains rapidly removed and divided mid-sagittally. Concentrations of dopamine, noradrenaline and 5-hydroxytryptamine were determined in individual half-brains according to the methods of Laverty & Sharman (1965), Maickel, Cox & others (1968) and Curzon & Green (1970) respectively. Animals used in these behavioural experiments were pretreated with DPH (Epanutin; Parke Davis Ltd) in doses of 5–120 mg kg^{-1} (i.p.) 1 h before behavioural assessment. In other experiments rats were implanted with bilateral striatal cannulae (Peringer, Jenner & others, 1976) and subsequently received unilateral injections of DPH (50 μg in 3 μl 0·9% saline) into each striatum on separate occasions. Circling behaviour following the subsequent administration of apomorphine HCl (0·5 mg kg^{-1}, s.c.) was then observed. Animals were later killed and brains removed for histological examination.

Dopamine turnover was assessed by the measurement of cerebral homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) concentrations, according to the method of Murphy, Robinson & Sharman (1969), and the measurement of dopamine concentrations 30 min after the administration of α-methyl-*p*-tyrosine methyl ester HCl (250 mg kg^{-1}, i.p.; Sigma Chemical Co.). Striatal and mesolimbic areas were dissected as previously described (Peringer, Jenner & Marsden, 1975). The mesolimbic tissue taken contained the corpora amygdala, the olfactory tubercle and the nucleus accumbens. Noradrenaline turnover was assessed in rats by measurement of whole brain 4-3-methoxy-4-hydroxyphenylglycol sulphate (MOPEG SO_4) according to the method of Meek & Neff (1972). Animals used in these biochemical experiments were pretreated with DPH in doses of 20–80 mg kg^{-1} (i.p.) 1, 2·5 or 4 h before death.

Apomorphine HCl in all experiments was dissolved in distilled water containing sodium metabisulphite as antioxidant. DPH as the sodium salt was dissolved in 40% propyleneglycol, 10% ethanol and made up to volume with water for injection. This solution was diluted with normal saline (0·9%) according to dosage requirements. (+)-Amphetamine sulphate was dissolved in distilled water.

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All data was analysed by Student's *t*-test and considered significant when $2P < 0{\cdot}05$.

DPH produced a dose-dependent inhibition of both apomorphine and amphetamine-induced circling behaviour (Fig. 1) in agreement with previous findings on L-dopa-induced circling (Mendez & others, 1975). Biochemical assessment of the animals used showed a 52% reduction in cerebral dopamine concentrations on the lesioned side (lesioned side 670 ± 24 ng g^{-1} $n = 8$; unlesioned side 1410 ± 67 ngg^{-1} $n = 8$; $P < 0{\cdot}001$). There was no difference in the concentrations of noradrenaline (lesioned side 202 ± 22 ng g^{-1} $n = 8$; unlesioned side 261 ± 33 ng g^{-1} $n = 8$; $P > 0{\cdot}05$) or 5-HT (lesioned side 871 ± 51 ng g^{-1} $n = 8$; unlesioned side 874 ± 32 ng g^{-1} $n = 8$; $P > 0{\cdot}05$). The inhibitory action of DPH suggests this drug, like other compounds producing extrapyramidal disorders, produces a functional blockade of striatal dopamine activity. This suggestion is reinforced by the observation that unilateral intrastriatal administration of DPH to animals ($n = 7$) subsequently receiving a peripheral administration of apomorphine produced ipsiversive turning ($4{\cdot}0 \pm 0{\cdot}7$ turns min^{-1}) in 12 out of 14 striata tested. In these animals no turning was observed in response to an intrastriatal administration of saline (3 μl 0·9% saline) followed by peripheral apomorphine administration. The solution of DPH used for intrastriatal administration, however, was alkaline and was not neutralized since the solubility of DPH is then greatly reduced. However, vehicle injections and the use of other alkaline and acidic solutions in previous studies have shown non-specific effects to be unlikely. The most probable effect of the alkaline vehicle would be permanent damage to the striatum but this was not observed; all turning behaviour induced by DPH being temporary, lasting 3-4 h. Subsequent histological examination of the brains showed that injection sites were symmetrically located within the striatum at the level of the anterior commisure.

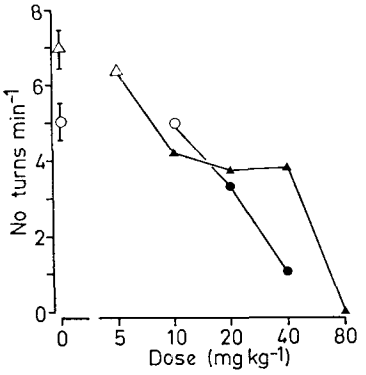

FIG. 1 The inhibition of circling behaviour induced by △, ▲ apomorphine (0·5 mg kg^{-1}), s.c. or ○, ● amphetamine (4 mg kg^{-1}, i.p.) in mice with unilateral nigrostriatal lesions following administration of diphenylhydantoin (5-80 mg kg^{-1}, i.p.). Closed symbols represent values significantly different from control values. Open symbols represent non-significant changes. Control values for turning were 7.0 ± 0.5 following apomorphine and 5.1 ± 0.6 following amphetamine. Each point represents the mean of at least 12 determinations.

However, DPH (5–80 mg kg^{-1}) was without effect on apomorphine-induced reversal of reserpine akinesia (apomorphine controls 5052 ± 262 counts/2 h; $n = 6$ apomorphine plus DPH 80 mg kg^{-1} 4250 ± 366 counts /2 h; $n = 6$ $P > 0{\cdot}05$). Also DPH (5–120 mg kg^{-1}) was without effect on apomorphine-induced stereotypy. Even at a dose of 120 mg kg^{-1} DPH had no effect on stereotyped behaviour (mean score control $n = 12$, $2{\cdot}8 \pm 0{\cdot}13$; DPH-treated groups $n = 12$, $2{\cdot}8 \pm 0{\cdot}13$; $P > 0{\cdot}05$); despite a profound catalepsy, which DPH induced before apomorphine administration. The production of stereotypy and locomotor activity are both currently thought to be mediated *via* cerebral dopamine neuronal systems and these findings would appear incompatible with the effects of DPH on turning behaviour.

Biochemical evidence for a functional blockade of cerebral dopamine receptors is also contradictory. In epileptic patients receiving conventional anticonvulsant therapy, including DPH, a tendency for an elevation of cerebrospinal fluid HVA concentration has been observed but this failed to reach significance (Chadwick, Jenner & Reynolds, 1975). Similarly, in the present animal experiments DPH (40 mg kg^{-1}) had no effect on HVA or DOPAC concentrations in striatal or mesolimbic areas (Table 1) when given to mice 1 or 2·5 h before death. Also, administration of DPH 40 mg kg^{-1} 4 h before death or 20 or 80 mg kg^{-1} 2·5 h before death failed to alter the AMPT-induced decrease in cerebral dopamine concentration (Table 1). An effect on cerebral dopamine was only observed when DPH 40 mg kg^{-1} was

Table 1. *The effect of DPH given 2·5 h before death on brain concentrations of HVA, DOPAC and MOPEG-SO$_4$, and on cerebral concentrations of dopamine following AMPT pretreatment* (200 mg kg^{-1}; 30 min before death).

Treatment	HVA ng g^{-1}* Striatum	HVA ng g^{-1}* Mesolimbic	DOPAC Striatum	DOPAC Mesolimbic
Saline	519 ± 69	121 ± 17	805 ± 128	148 ± 64
DPH 40 mg kg^{-1}	473 ± 65	110 ± 5	650 ± 98	146 ± 10

	Whole brain MOPEG-SO$_4$
Saline	109 ± 4
DPH 40 mg kg^{-1}	134 ± 4**
DPH 80 mg kg^{-1}	114 ± 4

	Whole brain dopamine
Saline	1630 ± 110
AMPT	940 ± 60**
AMPT + DPH 20 mg kg^{-1}	810 ± 90**
AMPT + DPH 40 mg kg^{-1}	1570 ± 60
AMPT + DPH 80 mg kg^{-1}	930 ± 120**

* Mean values ± 1 s.e.m. Each value is based on at least 8 observations.

** $P < 0{\cdot}001$ compared to saline treated groups (other values not significant)

administered 2·5 h before death; this prevented the AMPT-induced fall in dopamine (Table 1). DPH 80 mg kg^{-1} 2·5 h before death did not alter whole brain concentrations of the noradrenaline metabolite MOPEG SO_4 although a dose of 40 mg kg^{-1} 2·5 h before death elevated the concentration of this metabolite (Table 1).

The mechanisms by which DPH influences cerebral dopamine systems remains uncertain. It is interesting that the two behavioural tests in which DPH showed clear evidence for a blockade of cerebral dopamine receptors involve specifically the striatal area. The production of locomotor activity on the other hand appears to be mediated primarily by the nucleus accumbens (Jackson, Andén & Dahlström, 1975; Pinjenburg, Honig & others, 1976) while stereotypy and catalepsy may have both striatal and mesolimbic components (Costall & Naylor, 1974a, b). It might be conjectured, therefore, that DPH specifically acts on striatal dopamine systems and it is *via* this action that its extrapyramidal side effects manifest themselves in man. Such a blockade would be expected to cause a local elevation in striatum of the dopamine metabolites HVA and DOPAC, due to a compensatory feedback mechanism (Andén, Roos & Werdinius, 1964; Andén, Butcher & others, 1970), but this was not observed. The only positive biochemical effect suggested that DPH decreases dopamine turnover and this only occurred with one dose (40 mg kg^{-1}) and one time interval (2·5 h). The failure to observe a decrease in dopamine turnover at other times and at a higher dosage, however, suggests that DPH does not significantly alter dopamine turnover in either striatum or mesolimbic areas.

In conclusion DPH does appear to have some effect on cerebral dopamine systems, but the nature of this interaction is complex. In this respect the current interest in the existence of two or more types of cerebral dopamine receptors (Cools & van Rossum, 1976; Costall & Naylor, 1975) may be of importance since a preferential action of DPH on one type of dopamine receptor may be responsible for the production of the extrapyramidal manifestations. Alternatively, DPH may act on some other neuronal systems so as to mimic some of the effects of dopamine receptor blockade.

In this context the modulation of striatal function by GABA-, 5-HT-, noradrenaline- and acetylcholine-containing neuronal systems must be considered.

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