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Relevance of the rodent area postrema to dopamine-dependent behavioural effects

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The mechanisms by which dopamine agonist and antagonist drugs are able to modify behaviour in the rodent have been investigated with respect to the role of striatal, limbic and cortical dopamine projections. The tubero-hypophysial system has also received investigation, although emphasis has been placed primarily on neuroendocrine control. In contrast, any possible role of the other major dopamine containing area of the brain, the area postrema, has not been subject to study. Hence, we have developed a technique for lesion of the area postrema in the rodent, and report here on the relevance of its function to behavioural effects induced by dopamine agonists and antagonists.

Stereotaxic surgery was carried out in male Sprague-Dawley (C.F.E.) rats, 250 ± 30 g, using chloral hydrate anaesthesia (300 mg kg^{-1}). Electrolesions were placed in the region of the area postrema at a mid-line location, Posterior 7·0, 9·25 mm below the dura (zero according to Kopf stereotaxic instrument with incisor bar raised 5·0 mm). Lesions were caused by passing 0·5 mA for 10 s via a stainless steel electrode, 0·65 mm diameter and insulated except at the tip. Initial studies indicated that the cataleptic effect of haloperidol was markedly reduced or abolished in successfully lesioned rats (approximately 60%): this criterion was therefore used to select lesioned animals for use in further behavioural studies. Throughout the studies the responses of sham operated rats were found to be indistinguishable from those of normal animals, hence, control values for each experiment are from sham operated animals (prepared by lowering the electrode at the selected coordinates, but only 8·75 mm below the dura, without passage of current). Catalepsy was assessed by placing animals in Perspex cages (20 × 15 × 15 cm) fitted with a 10 cm high bar and, after a period of acclimatization, placing the animal's front limbs carefully over the bar. A normal animal recovered from this position within 6 s but a cataleptic animal maintained the abnormal imposed position for a period of time dependent on the degree of catalepsy. In order to account for animals maintaining the imposed position for an 'infinite' period of time, the following scoring system was adopted to express the intensity of catalepsy: 1 = 0·1–2·5 min, 2 = 2·6–5·0 min, 3 = 5·1–10·0 min, 4 = 10·1–20·0 min, 5 = 20·1 min–∞. Animals were tested frequently to assess onset of catalepsy and intensity was then assessed at 30 min intervals for 6 h. For measurement of stereotyped behaviour animals were placed in similar observation cages and the intensity of response assessed according to a simple system 0 = no stereotypy, 1 = periodic sniffing, 2 = continuous sniffing, 3 = periodic biting, 4 = continuous biting. After careful assessment of onset time, intensity was assessed at either 10 min (apomorphine) or 30 min ((+)-amphetamine) intervals for the duration of effect. All behavioural experiments were carried out between 08·00 and 18.00 h in a sound-proofed, diffusely illuminated room maintained at 21 ± 2 °C. Haloperidol (Janssen) was prepared in 1% lactic acid, sulpiride (SESIF) in HCl neutralized with sodium bicarbonate, apomorphine. HCl (Macfarlan Smith) in a 0·1% solution of sodium metabisulphite and (+)-amphetamine SO_4 in distilled water. In experiments designed to assess biochemical changes (homovanillic acid, HVA) animals were given 2·0 mg kg^{-1} haloperidol 90 min before cervical dislocation. Only those animals in which catalepsy was markedly reduced or abolished (tested at 60 min) were used in the HVA determinations. After exsanguination, brains were rapidly removed and the nucleus accumbens and caudate-putamen dissected over ice. HVA was determined fluorometrically according to Westerink & Korf (1975).

Lesions of the area postrema reduced the onset of apomorphine or (+)-amphetamine stereotypy, reduced the weak intensity catalepsy normally induced by sulpiride and reduced or abolished the marked cataleptic effect of haloperidol (data obtained from the 4th–32nd postoperative days was indistinguishable, that given is for the 14th postoperative day) (Fig. 1). However, the antistereotypic effects of haloperidol were not modified by the area postrema lesions. Thus, 0·1 mg kg^{-1} haloperidol (30 min pretreatment) prevented the stereotypy response to 10 mg kg^{-1} i.p. (+)-amphetamine and 0·5 mg kg^{-1} haloperidol prevented the development of stereotypy to 2·0 mg kg^{-1} s.c. apomorphine. At a time when haloperidol catalepsy was markedly reduced or abolished by the area postrema lesions, HVA levels in the caudate-putamen and nucleus accumbens were indistinguishable from those of sham operated or normal rats (HVA level, in $\mu g\ g^{-1}$ wet weight tissue, for the nucleus accumbens was 2·48 ± 0·31 in control rats, 2·22 ± 0·52 in the lesioned rats, and for the caudate-putamen was 2·50 ± 0·35 in control rats, 2·50 ± 0·46 in lesioned rats). 10 rats with lesions aimed at the area postrema and in which haloperidol catalepsy was markedly reduced or abolished, and 10 similarly treated rats in which catalepsy was not markedly affected, were

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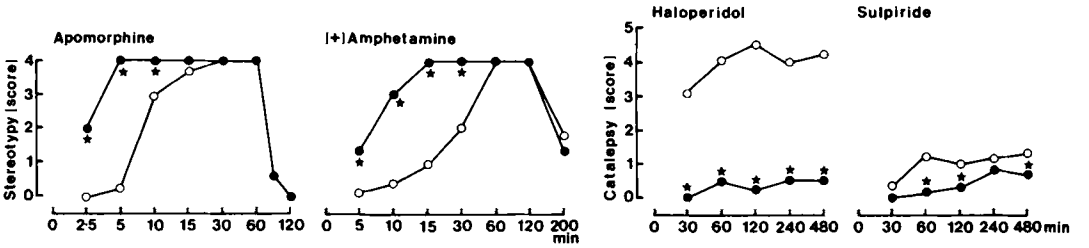

FIG. 1. Changes in the stereotypic responses to apomorphine and (+)-amphetamine and in the cataleptic responses to haloperidol and sulpiride caused by electrolesions of the area postrema of rat. ●——● responses of lesioned rats and ○——○ sham operated rats. Apomorphine and (+)-amphetamine caused dose-dependent stereotypies (scores 1 through to 4 according to Methods) in the respective dose ranges 0·25–2·0 mg kg^{-1} s.c. and 1·25–10 mg kg^{-1} i.p. At each dose, onset was more rapid in lesioned rats: the change in response to 1·0 mg kg^{-1} apomorphine and 5·0 mg kg^{-1} (+)-amphetamine are shown. 0·25–2·0 mg kg^{-1} i.p. haloperidol caused a dose-dependent catalepsy (scores 0·5 through to 5 according to Methods) although the weak cataleptic response to sulpiride, apparent at doses of 40+ mg kg^{-1} i.p. was independent of dose. At each dose the intensity of catalepsy was reduced in the lesioned rat: the changes in response to 2·0 mg kg^{-1} haloperidol and 80 mg kg^{-1} sulpiride are shown. n = 6–10. Responses of lesioned animals significantly different from control $*P < 0{\cdot}01$–$P < 0{\cdot}001$ (Student's t-test).

subject to histological examination. Damage in the former group was essentially confined to the region of the area postrema (Fig. 2) whilst in the latter group damage to the area postrema was minimal or nil but frequently extended to surrounding structures, nucleus gracilis, nucleus solitarius, tractus solitarius and fasciculus gracilis.

In higher animals dopamine stimulation in the area postrema is known to induce emesis whilst dopamine antagonism prevents the response (Borison 1974). The rodent is not able to vomit, and the role of the area postrema in dopamine agonist-antagonist interactions has received minimal attention, even though this brain area has a high dopamine content and catecholamine fluorescent fibres (Fuxe & Owman 1965; Valzelli & Garattini 1968). There is a preliminary report that the area postrema may be important for the action of α-melanotropin in modifying dopamine turnover (Lichtensteiger & Lienhart 1977), and one would predict that if this area is important for the action of other drugs which affect dopamine mechanisms, for example, the neuroleptics, that the functioning of the area may be relevant to normal motor control. However, in the present studies lesion of the area postrema of rat had no obvious effect on motor behaviour either acutely or chronically, and Borison (1974) has similarly reported lack of effect in the cat. Nevertheless, in the present study it was shown that damage to the area postrema could alter the motor behavioural response to drugs; thus, following lesion, the onset of the stereotypy responses to the dopamine agonists, apomorphine and (+)-amphetamine, was facilitated whilst the catalepsy responses to the dopamine antagonists, haloperidol and sulpiride, were reduced or abolished (although the antistereotypy effects of haloperidol were not modified). Furthermore, at a time when haloperidol catalepsy was markedly reduced or abolished, this neuroleptic agent was still effective in elevating HVA levels in the nucleus accumbens and caudate-putamen. Such an increase in HVA levels is usually taken as a biochemical index of a functional blockade of mesolimbic and striatal dopamine receptors, with catalepsy as the behavioural correlate. Clearly, a complex situation exists, and it would appear that functioning of the area postrema may normally facilitate the development of neuroleptic catalepsy and oppose drug induced stereotypy. The mechanisms via which the area postrema could achieve this remain obscure, either through dopamine agonist-antagonist action within the area postrema or through an influence of the area postrema on forebrain structures. Whatever the mechanism(s) involved, the possibility that a hind-brain region can influence behavioural effects resulting from drug action in telencephalic dopamine systems warrants further study.

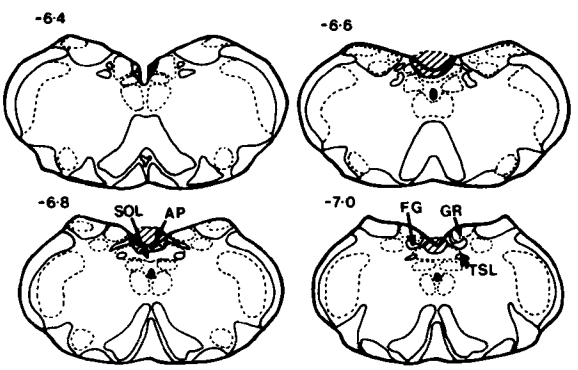

FIG. 2. Diagrammatic representation of the site of lesion location in the region of the area postrema constructed from data obtained from 10 rats. The shaded area represents lesion damage common to all rats. AP—area postrema, FG—fasciculus gracilis, GR—nucleus gracilis, SOL—nucleus solitarius, TSL—tractus solitarius. Areas were identified with reference to the studies of Palkovits & Jacobowitz (1974) and Pellegrino et al (1979).

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Systemically administered prolyl-leucyl-glycinamide fails to alter dopaminergic neuronal activity in the rat brain

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L-Prolyl-L-leucyl-glycinamide (PLG), the C terminal tripeptide of oxytocin, has been isolated from the hypothalamus and shown to inhibit the release of α-melanocyte-stimulating hormone (MSH) from the intermediate lobe of the pituitary (Nair et al 1971; Vivas & Celis 1977). Accordingly, this tripeptide has been referred to as melanocyte-stimulating hormone inhibitory factor (MIF). Other investigators, however, have been unable to confirm the inhibition of MSH release by PLG (Bower et al 1971; Tilders & Smelik 1977).

While the role of PLG as a MIF may be controversial, results of pharmacological studies have suggested that this tripeptide possesses extrapituitary actions. Most interesting of these were the reports (Kastin & Barbeau 1972; Chase et al 1974) that PLG produced clinical improvement in patients suffering from Parkinson's disease. It was suggested that this clinical effect could result from an interaction of PLG with dopamine (DA) mechanisms in the brain.

There has been some support for this proposal. For example, systemic administration of PLG has been reported to potentiate the effects of L-dopa in hypophysectomized mice (Plotnikoff et al 1971, 1974) and to produce stereotyped behaviours in cats (North et al 1973). On the other hand, other investigators have failed to obtain behavioural evidence for an interaction of PLG with DA neuronal systems. The tripeptide has no effect on general motor activity (Kastin et al 1973), does not cause stereotyped behaviours or enhance stereotyped behaviours produced by DA agonists, and does not reverse neuroleptic-induced behavioural effects in rats (Cox et al 1976).

There are also controversies about the effects of systemically-administered PLG on neurochemical measures of DA neuronal activity. Friedman et al (1973) reported that PLG increased the synthesis of [^{3}H]-dopamine from [^{3}H]tyrosine in striatal slices, and Pugsley & Lippmann (1977) noted that PLG increased the α-methyltyrosine (AMPT)-induced decline of DA in whole brain. Using this latter technique Kostrzewa et al (1975) failed to find an effect of intraperitoneally administered PLG on the decline of DA in the striatum. Subsequently they reported that this tripeptide did not alter the striatal concentrations of dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) (Kostrzewa et al 1979). Increases in the concentrations of these DA metabolites in the striatum generally reflect increased activity of nigrostriatal DA neurons (Roth et al 1976).

In the present study we have examined the effects of PLG on central DA neurons using another biochemical estimate of their activity. In contrast to previous investigators who focused on nigrostriatal DA neurons, we have examined the effects of PLG on several different DA neuronal systems by measuring the rate of accumulation of dopa after the administration of a dopa decarboxylase inhibitor. Under normal circumstances the concentrations of dopa in brain regions containing DA nerve terminals is essentially zero, but following the inhibition of dopa decarboxylase the concentration of this precursor of DA increases linearly with time for at least 30 min (Demarest & Moore 1980). The rate at which dopa accumulates is an in vivo measure of tyrosine hydroxylation, and thus a biochemical index of the activity of DA neurons which terminate in the brain regions being examined (Carlsson et al 1972; Demarest et al 1979). At various times and after different doses of PLG we have quantified the accumulation of dopa in the striatum, olfactory tubercle, median eminence and posterior pituitary, regions containing the terminals of nigrostriatal, mesolimbic, tuberoinfundibular and tuberohypophyseal DA nerves, respectively.

Male Sprague-Dawley rats (Spartan Research Farms, Haslett, MI) received subcutaneous injections of PLG (Sigma Chemical Co., St Louis, MO) or its vehicle (0·1 M acetic acid) and were decapitated at various times

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