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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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Table 1. Effect of graded doses of PLG on DOPA accumulation in selected brain regions.

Dose (mg kg^{-1})	Brain region: Striatum	Olfactory tubercle	Median eminence	Posterior pituitary
0	12·4 ± 1·1	12·2 ± 0·8	8·0 ± 0·8	0·8 ± 0·1
10	14·1 ± 0·6	12·0 ± 0·4	7·9 ± 0·9	0·9 ± 0·1
30	14·8 ± 0·9	11·8 ± 1·0	10·0 ± 1·0	0·9 ± 0·1
100	11·4 ± 0·7	10·7 ± 0·4	10·5 ± 1·0	0·9 ± 0·1

Each value represents DOPA accumulation (ng mg^{-1} protein/30 min) expressed as the mean ± 1 s.e. as determined from 6–8 rats. PLG or vehicle (0 dose) was injected s.c. 60 min, before death and NSD 1015 (100 mg kg^{-1}) was injected i.p. 30 min before death.

Table 2. Time course of the effects of PLG on DOPA accumulation in selected brain regions.

Time after PLG (min)	Brain region: Striatum	Olfactory tubercle	Median eminence	Posterior pituitary
0	11·2 ± 1·1	12·0 ± 1·2	14·4 ± 1·2	1·5 ± 0·1
30	12·2 ± 0·7	16·2 ± 1·5	13·6 ± 0·9	1·6 ± 0·2
60	11·7 ± 0·5	17·5 ± 1·4	12·1 ± 0·9	1·9 ± 0·1
120	12·2 ± 0·8	15·1 ± 1·6	15·0 ± 1·2	1·7 ± 0·2

Each value represents dopa accumulation (ng mg^{-1} protein/30 min) expressed as the mean ± 1 s.e. as determined from 4–8 rats. PLG (100 mg kg^{-1}) was injected s.c. at various times before death. All animals received NSD1015 (100 mg kg^{-1}, i.p.) 30 min before death.

thereafter. Thirty minutes before death each rat received an i.p. injection of 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015, 100 mg kg^{-1}; Sigma Chemical Co., St Louis, MO) to inhibit dopa decarboxylase.

After death the brains and pituitary glands were quickly placed on a cold plate. The striata, olfactory tubercles, median eminence and posterior pituitary were dissected and homogenized in 0·2 M perchloric acid as described by Alper et al (1980). The homogenates were centrifuged for 20 s in a Beckman Microfuge. Ten μl aliquots of the supernatants were assayed for dopa using a radioenzymatic method (Demarest & Moore 1980), and the protein content of the pellet was determined by the method Lowry et al (1951). Dopa concentrations are reported on the basis of the protein content of the brain homogenates. Data were analysed by a one-way analysis of variance with the level of significance set at $P < 0·05$ (Steele & Torrie 1960).

Values for dopa accumulation in four brain regions 60 min after systemic administration of three different doses of PLG are summarized in Table 1. Despite the fact that the doses were equivalent to some of the highest used in previous biochemical and behavioural studies, the drug had no effect on dopa accumulation in any of the brain regions examined. The effects of the highest dose of PLG were then evaluated at various times after injection. Again, dopa accumulation was not altered in any of the brain regions at the times examined (Table 2). The values for dopa accumulation in the median eminence and posterior pituitary were generally lower in the dose response (Table 1) than in the time course experiment. Nevertheless, the values are consistent within each experiment and are within the range of values reported previously (Demarest & Moore 1979, 1980; Demarest et al 1979).

The lack of effect of PLG on nigrostriatal DA neuronal activity is consistent with the results obtained using the AMPT-induced decline of DA (Kostrzewa et al 1975) and the concentrations of DOPAC and HVA (Kostrzewa et al 1979) as biochemical indices of neuronal activity. This suggests that the reported beneficial effect of PLG in ameliorating the symptoms of Parkinson's disease must be due to actions of this tripeptide on systems other than the nigrostriatal DA neurons. The present results also suggest that the reported behavioural effects of PLG (see Kastin et al 1973) are not the result of the ability this compound to alter the activity of mesolimbic DA neurons. Furthermore the tuberoinfundibular DA neuronal system is apparently not influenced by systemically administered PLG.

The intermediate lobe of the pituitary contains a dense network of DA nerve terminals belonging to the tuberohypophyseal system (Björklund et al 1973) which may play a role in the central regulation of MSH secretion from the pituitary gland (Tilders & Smelik 1977). The present study was not designed to explore this possible relationship, but the results do indicate that the acute administration of PLG, which may inhibit MSH release, does not appear to influence the activity of the tuberohypophyseal DA neurons and thereby alter the release of this hormone from the posterior pituitary. This study focused only on the neurochemical effects of systemically administered PLG; intracerebrally administered PLG may exert some action on central DA neuronal systems but, at least on the nigrostriatal system, this tripeptide appears to be without effect (Kostrzewa et al 1979).

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Acetylator phenotype determination in the rabbit: sulphamethazine or sulphadiazine, the choice of a substrate

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The use of diverse phenotyping methods has become a current clinical practice in order to predict and/or avoid some drug related diseases (Ellard 1976). To study drug acetylation, the closest animal model to man is the rabbit, as this animal presents a polymorphic pattern of acetylation (Frymoyer & Jacox 1963a,b). Several substrates have been proposed to segregate fast and slow acetylator rabbits, sulphadiazine (SDZ) being the most accepted. However, SDZ kinetics are non-linear (Souich et al 1978a,b) and as a consequence the classification of an animal according to its acetylator phenotype may in certain cases be difficult. On the other hand, the frequency of slow acetylator rabbits approximates 12% and simple phenotyping methods, using SDZ as substrate, will not avoid misclassifications (Souich et al 1978c) thus, it is relevant to dispose of accurate phenotyping methods. The aim of this study is to present the kinetics of sulphamethazine (SMZ) in the rabbit and to discuss the possible advantages as a phenotyping substrate.

Methods. Male and female New Zealand white rabbits (Canadian Breeding Farm and Laboratories Ltd), 2·6 to 3·5 kg, were maintained on Purina pellets with free access to water in individual well ventilated metabolic cages. Screens were placed below the animal living spaces to avoid faecal or debris contamination of urine specimens. Animals were kept at least 10 days before any experiment was undertaken. All animals (n = 26) were characterized in terms of SMZ plasma kinetics at a dose level of 20 mg kg^{-1} i.v. Blood samples (1 ml) were drawn at 0, 2, 4, 6, 8, 12, 16, 25, 40, 60, 90, 120, 150 and 180 min after drug administration, while urine was collected for 48 h after dose. Three rabbits received additional SMZ doses of 10 and 40 mg kg^{-1} at 10 day intervals. To assess the goodness of rabbit phenotyping using SMZ as a substrate, 5 animals received an additional i.v. 20 mg kg^{-1} dose of SDZ. SMZ or SDZ was assayed in plasma and urine by the Bratton-Marshall technique (Bratton & Marshall 1939). *N*-Acetyl sulphamethazine (NSMZ) or *N*-acetyl-sulphadiazine (NSDZ) were estimated by subtraction of free from total SMZ or SDZ after hydrolysis (6 M HCl at 80 °C for 5 min).

Graphical analysis of a plot of SMZ or SDZ plasma concentrations as a function of time depicted that SMZ or SDZ confer upon the body the characteristics of a two-compartment model. SMZ or SDZ concentration-time curves were adequately described by a biexponential equation, i.e.

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

As the metabolite (NSMZ or NSDZ) is more polar than the parent compound and no data concerning its body distribution are available, we have assumed a single compartment distribution. SMZ or SDZ pharmacokinetic parameters have been calculated by least squares non-linear regression analysis using a NONLIN program (Metzler 1969), fitting simultaneously parent compound and metabolite values in plasma and urine. Parameters calculated included: area under SMZ or SDZ plasma concentration time curve (AUC), peripheral and central volumes of distribution (Vp and Vc respectively), volume of distribution at steady state (Vss), volume of distribution ($V\beta$), total body clearance (Cl_{TB}), renal clearance (Cl_R), metabolic clearance

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