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Effect of *p*-chlorophenylalanine and L-phenylalanine on deoxyribonucleic acid and protein content of developing rat cerebellum

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TREATMENT of laboratory animals with *p*-chlorophenylalanine (*p*-CPA), an irreversible phenylalanine and tryptophan hydroxylase inhibitor,[1,2] has been used as an experimental model of phenylketonuria.[3,4] However, supplemental L-phenylalanine is necessary to increase plasma and tissue phenylalanine levels significantly.[5] A consistent observation in neonatal animals undergoing this treatment has been an irreversible reduction in brain weight.[6,7] The cause of the reduced brain weight is unknown, but its irreversibility suggests a decrease in the number of brain cells. DNA concentration is constant within diploid cells in a given species[8] and has been used as a measure of cell number.[9] Protein to DNA ratios indicate cell size.[9] This paper reports the effect of *p*-CPA and phenylalanine treatment on changes of the cerebellum in developing rat pups and in adult rats rehabilitated after early postnatal treatment. Brain growth was examined by measuring weight changes and DNA and protein concentrations.

TABLE 1. EFFECT OF *p*-CHLOROPHENYLALANINE AND L-PHENYLALANINE TREATMENT ON BODY AND CEREBELLUM WEIGHTS OF DEVELOPING RAT*

Age (days)	Body wt (g)		Cerebellum wt (mg)	
	Control	Experimental	Control	Experimental
9	18·3 ± 3·0 (6)	16·1 ± 1·0 (6)	91 ± 15	68 ± 13†
15	27·3 ± 5·1 (14)	20·5 ± 4·1† (14)	152 ± 19	112 ± 17†
21	46·7 ± 4·1 (13)	31·8 ± 4·3† (15)	190 ± 13	130 ± 16†
154	254·0 ± 25·2 (6)	268·5 ± 33·6 (6)	297 ± 16	218 ± 23†

* For details of treatment see text. Numbers of animals are given in parentheses.
† $P < 0.01$.

Rat pups born to Sprague–Dawley females (Simonsen, Gilroy, Calif.) were intubated once a day from birth with DL-*p*-chlorophenylalanine and L-phenylalanine prepared in 0·2% agar.[7] The combined dosage was 100 mg each/kg given in a volume of 10 ml/kg body weight. Control animals received the agar vehicle. Treatment was terminated after 21 days. One group of rats was weaned and maintained without drug treatment for an additional 19 weeks.

At 9, 15, 21 and 154 days postpartum, animals were sacrificed by decapitation. Brains were removed rapidly, chilled and sectioned into four regions: cerebellum, brain stem, midbrain-hypothalamus and cerebral cortex.[10] After weighing, the brain tissue was homogenized in 5 vol. of 6% trichloroacetic acid and the homogenate centrifuged at 18,000 *g* and 4° for 40 min. DNA in the pellet was determined by a modification[11] of the method of Burton.[12] Protein was determined by the method of Lowry *et al.*[13] Data were analyzed statistically using Student's *t*-test.

At each age examined, the mean weights of all regions except brain stem were decreased significantly by drug treatment. The cerebellum showed the greatest sensitivity to treatment, and weights of this region are presented in Table 1. Though DNA and protein concentrations tended to be lower in midbrain and cerebral cortex of experimental animals when compared to controls, these differences were not statistically

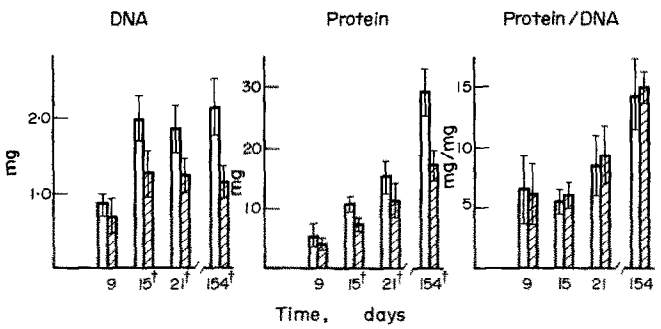

FIG. 1. DNA, protein and protein to DNA ratio in developing cerebellum from *p*-chlorophenylalanine- and L-phenylalanine-treated rats. Clear bars, control; cross-hatched bars, experimental. Mean ± S.D. For sample size at each time, see Table 1. Symbol (†), $P < 0{\cdot}005$.

significant. However, cerebellar DNA and protein concentrations were reduced significantly in the experimental group at 15, 21 and 154 days (Fig. 1). No significant differences were observed in protein to DNA ratios at any time period. Thus, the deficit in cerebellar weight can be attributed to a reduction in cell number rather than to smaller cell size.

These data are insufficient to determine whether the reduction in cerebellar cell number is neuronal or glial in nature. That the disturbance might be neuronal is suggested by the observation of neuronal lesions in the cerebellum after the administration of excess phenylalanine to neonatal rats.[14]

Weight and DNA concentration of the developing cerebellum are also more sensitive to severe postnatal undernutrition than are other brain regions.[15] While it is not possible to demonstrate unequivocally that undernutrition plays no part in the effects attributed here to *p*-CPA and phenylalanine treatment several lines of evidence do bear on this question. Barnes and Altman[16] have shown that mild lactational undernutrition which resulted in a body weight deficit of 25 per cent at 20 days of age (comparable to the 32 per cent deficit found in this study) did not produce a deficit in cerebellar weight. Further, behavioral observations show rehabilitated *p*-CPA- and phenylalanine-treated rats more active than controls,[6] whereas rehabilitated undernourished rats are less active.[17] These observations argue against the suggestion that undernourishment can entirely explain the effects of *p*-CPA and phenylalanine treatment.[3]

In conclusion, the observed cerebellar weight and DNA changes indicate an irreversible interference in normal cellular development in the cerebellum caused by *p*-CPA and phenylalanine treatment during the period from birth to weaning.

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Glypentide: A new hypoglycaemic agent

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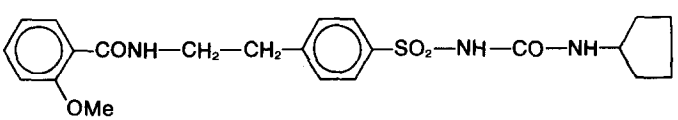
GLYPENTIDE is a new oral hypoglycaemic agent, known by the research code name UR-661.[1,2] Its chemical structure is *N*-[4-beta-(*o*-anisamidethyl)-benzenesulphonyl]-*N'*-cyclopentylcarbamide:



The activity and toxicity of glypentide was studied in rabbits, dogs and man.

Albino rabbits (1·5–2·0 kg) and beagle dogs (10–12 kg) were used. Glypentide was administered either by gastric intubation as a suspension in carboxymethylcellulose (0·5% w/v), in doses from 0·01 to 10 mg/kg body wt, or intravenously as a solution in distilled water at doses from 0·02 to 0·25 mg/kg. Control animals received an equal volume of the vehicle.

The animals were fasted overnight, water being allowed *ad lib.* Blood was collected from the vein (rabbits: ear marginal; dogs: saphenous) before and at various intervals after the administration of glypentide. Blood sugar was estimated according to the *o*-toluidine method.*

Glypentide lowered the blood sugar in albino rabbits and dogs (Figs. 1 and 2). In the rabbit, the maximum hypoglycaemic action was reached 4–5 hr after oral, and 3–4 hr after intravenous administration of glypentide. Twenty-four hours after the administration, the blood sugar levels had returned to normal. In all cases there was a dose–response relationship. In the dog, a single oral dose of 0·05 mg/kg glypentide reduced the mean blood sugar level by 11 per cent, 5 hr after administration. Higher doses induced greater hypoglycaemic responses, giving a dose–response relationship. Oral doses of 2 mg/kg, or higher, produced hypoglycaemic effects that lasted more than 24 hr.

According to these data, glypentide is approximately 1000 times more potent than tolbutamide in rabbits and 200 times more potent in dogs.[3]

Administration of a single oral dose of 5 mg glypentide to twelve healthy human subjects resulted in an intense and prolonged decrease of blood sugar levels. The maximum decrease (to 47 per cent of the control) was obtained two hours after administration and lasted 16 to 18 hr.[4]

* Merkotest Art. 3353.