Simulation of Phenylketonuria in Rats by Extended p-Chlorophenylalanine Treatment

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The brain damage which occurs in untreated phenylketonuria probably results from the high plasma phenylalanine concentration prevailing in this condition, as treatment with a low-phenylalanine diet mitigates the situation. The actual cause of the brain damage is not known, but investigations have been carried out with high phenylalanine loads to simulate phenylketonuria and so elucidate the nature of the ensuing abnormalities. Hypotheses involving phenylalanine or its toxic metabolites in the impairment of vital metabolic processes have been tested (Agrawal et al., 1970; Weber et al., 1970). The results obtained, however, under short-term acute loads probably do not reflect the situation prevailing in phenylketonuria; although the plasma phenylalanine reaches phenylketonuria values for short periods, tyrosine also rises appreciably.

A number of workers have used p-chlorophenylalanine as an inhibitor of phenylalanine hydroxylase in vivo (Lipton et al., 1967), hoping to produce a more sustained hyperphenylalaninaemia with a near normal tyrosine concentration, i.e. a more valid model of phenylketonuria. However, we found that a single pretreatment of young rats with p-chlorophenylalanine does not significantly alter the response to a phenylalanine load. We have extended this by carrying out phenylalanine tolerance tests on weanling rats after treatment with p-chlorophenylalanine for up to 20 days. Four groups of weanling rats were treated daily, by intraperitoneal injection, with (a) 300mg of p-chlorophenylalanine/kg body wt., or (b) 300mg of p-chlorophenylalanine/kg plus 200mg of L-phenylalanine/kg, or (c) 200mg of L-phenylalanine/kg, or (d) an equal volume of saline. Animals from each group were killed on different days and their liver phenylalanine hydroxylase activity was determined by using L-[U-14C]phenylalanine as substrate, separating the product chromatographically and counting the radioactivity in both substrate and product (Bender & Coulson, 1972). Liver phenylalanine hydroxylase activity reached a minimum between 5 and 7 days in groups (a) and (b) (about 15% of control values), whereas group (c) did not differ from control value. After 7 days of p-chlorophenylalanine treatment the activity gradually increased towards the control values and cessation of treatment resulted in a rapid return to control values (2 days).

Phenylalanine tolerance tests were performed by injecting rats with 500mg of L-phenylalanine/kg, and removing blood at 30min intervals. Plasma phenylalanine and p-chlorophenylalanine were determined by the method of McCaman & Robins (1962), after separation on t.l.c. (this eliminates the mutual interference between phenylalanine and p-chlorophenylalanine). Tyrosine was determined by the method of Wong et al. (1964). Figs. 1(a) and 1(b) show phenylalanine and phenylalanine/tyrosine ratios after phenylalanine loads in control animals and in rats after 1 and 7 days of p-chlorophenylalanine treatment. Both are of the same order as those observed in untreated phenylketonuria children. The basal phenylalanine concentration (before injection of the phenylalanine load) was subtracted from each value, after the peak, and the net elevated values plotted against time on semi-logarithmic paper. The slope of the line gives the first-order rate constant for the disappearance of phenylalanine load (k) (Woolf et al., 1967). Fig. 2 shows phenylalanine tolerance curves for animals treated with p-chlorophenylalanine for different periods and for controls. Phenylalanine loads produce a sustained hyperphenylalaninaemia over several hours in the 5–7-day-treated animals.

Further, there is a good correlation (r = 0.89; P < 0.001) between k and the liver phenylalanine hydroxylase activity. This supports the view that the main metabolic pathway of phenylalanine in these animals is by liver hydroxylation to tyrosine.

From these studies we concluded that extended treatment with p-chlorophenylalanine over 5–7 days causes a decrease in liver phenylalanine hydroxylase activity which consequently lowers phenylalanine tolerance so that loading doses of phenylalanine produce a simulated phenylketonuria, over a period of several hours.
Fig. 1. Plasma phenylalanine concentration (a) and phenylalanine/tyrosine ratios (b) in p-chlorophenylalanine-treated rats

p-Chlorophenylalanine-treated rats, 1 day (○), 7 days (□), and control rats (▲).

Fig. 2. Phenylalanine tolerance curves in rats after a phenylalanine load

Control rats (a), p-chlorophenylalanine-treated rats 1 day (b), 3 days (c), 5 days (d) and 7 days (e). Phe, and Phe₀ are molar concentrations of phenylalanine at time t and time zero.
The mode of action of the p-chlorophenylalanine in these animals is not clear. p-Chlorophenylalanine, in vitro, is not an appreciable inhibitor of phenylalanine hydroxylase, and this is apparently so in vivo also as the lowering of phenylalanine hydroxylase activity shows a delayed response to p-chlorophenylalanine treatment. However, the decrease in phenylalanine hydroxylase activity is not caused by the accumulation of p-chlorophenylalanine or an inhibitory metabolite of this compound because, when livers from p-chlorophenylalanine-treated rats are stored at -20°C until the enzyme activity has decayed, no inhibition of freshly prepared purified phenylalanine hydroxylase is observed. One possible explanation is that inhibition is due to a metabolite of p-chlorophenylalanine which is a tightly bound non-competitive inhibitor, excess of which is further metabolized. It is also possible that the decreased activity results from some incorporation of p-chlorophenylalanine into the enzyme protein, so producing an impaired protein, a suggestion already made by Gál et al. (1970).

Phenylalanine hydroxylase is an unstable enzyme and may therefore have a high turnover which would render it particularly susceptible to such an event. This may be so as we observed the enzyme in p-chlorophenylalanine-treated livers is apparently less stable to storage than enzyme from untreated livers. If this latter suggestion is correct it creates a problem with our model in that the treated animals have a raised plasma-p-chlorophenylalanine as well as a raised phenylalanine, which may have direct effects on protein synthesis. In projected experiments it will be necessary to compare the experimental animals (i.e. p-chlorophenylalanine-treated phenylalanine-loaded) with untreated control animals and p-chlorophenylalanine-treated control animals.


The Metabolism of Dexamethasone in the Rat

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Although dexamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione), a potent synthetic glucocorticoid, has been in clinical use for over a decade, very little is known about its metabolism and excretion in man or in other animal species. It is known that those structural modifications of the cortisol molecule which are needed to increase the biological potency also decrease the rate of metabolism (Florini & Buyske, 1959; Fotherby & James, 1972). This decrease in metabolism is reflected by an increased excretion of the unchanged drug, a slower removal from plasma, and hence a prolonged plasma half-life of the compound. In the dog, plasma half-life of dexamethasone is 60min compared with 34 and 44min for corticosterone and cortisol respectively (Florini et al., 1961). Haque et al. (1972) reported a half-life of 252min for dexamethasone in man. They also found that dexamethasone was converted into unidentified polar metabolites which were excreted unconjugated in the urine. We have investigated the metabolism of dexamethasone in the rat and the preliminary findings are reported here.

[1,2-3H]Dexamethasone (43.5μCi/μmol) was given intraperitoneally at a dose of 0.23μmol/kg to male 48-day-old Wistar albino rats. Urine and faeces were collected at 6 and 24h and thereafter every 24h for 4 days. Urinary radioactivity was fractionated into free and conjugated steroids according to Frantz et al. (1961) and Edwards et al.

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