Effect of cortisone or triiodothyronine administration to pregnant rats on lysosomal hydrolases in fetal forebrain and cerebellum[1]

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Summary. Triiodothyronine injected daily to pregnant rats for the last week of gestation (50 µg/100 g b.wt) increased the specific activities of 5 acid glycosidases in the fetal forebrain and cerebellum. Cortisone (50 mg/100 g b.wt) administered in the same period had no effect on cerebellum acid hydrolases, but decreased their activity in the forebrain.

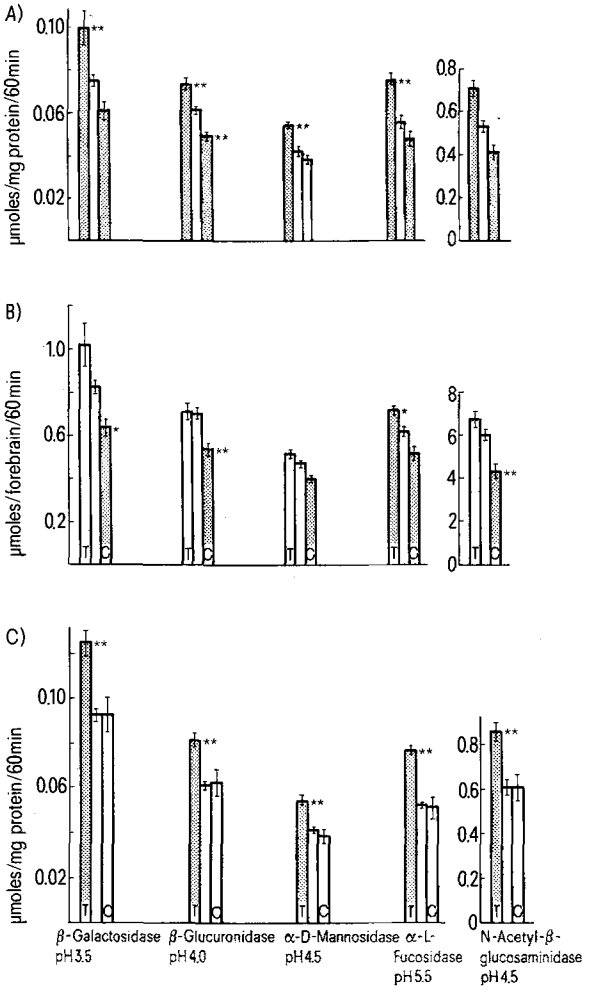
In the process of normal brain metabolism lysosomal hydrolases play an important role. Studies from various laboratories have shown that during perinatal development changes occur in the activity of various brain lysosomal enzymes. However, the postnatal period has attracted greater attention than the fetal period, which has had a few studies performed in man[2,3] and in rabbit.[4].

The question arose whether prenatal development of brain lysosomal enzymes could be influenced; effect of administration of cortisone and triiodothyronine to pregnant rats was considered first. Experiments where hormones were administered directly to the fetuses or to the pregnant mother have shown that corticoids influence the prenatal development of liver[5], lung[6,7] and small intestine[8,9]; thyroid hormones[9-12] had a similar effect. To the best of our knowledge, analogical studies on fetal brain have not been published.

Materials and methods. Pregnant rats of Charles River strain CD (Wilmington, Mass., USA) were shipped from the producer by air on day 14 of pregnancy. On day 16, rats were randomly assigned to either the control or hormone-treated groups. The animals were weighed and injected daily. The controls received either 0.005 N NaOH or 0.9% NaCl i.m. (0.2 ml/100 g b.wt). Cortisone acetate (Merck, Sharp & Dohme, Rahway, N.J., USA) was injected i.m. at a dosage of 50 mg/100 g b.wt). L-Triiodothyronine (free acid; Sigma Chemical Co., St. Louis, Mo., USA) dissolved in 0.005 N NaOH was injected s.c. at a dosage of 50 µg/100 g b.wt). All mothers were decapitated between 09.00 and 11.00 h on day 22 of pregnancy (this strain gives birth in the late afternoon/night on day 22). Fetuses were obtained by caesarian section. Fetuses present in each uterine horn were pooled and treated as 1 sample. Fetuses were counted, cleaned, dried, weighed, and then killed by decapitation. Cerebellum and forebrain were separated and frozen at −40 °C.

Activities of β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-D-mannosidase, and α-L-fucosidase were assayed in total homogenates of forebrain or cerebellum using p-nitrophenyl derivatives as substrates; pH of the assay mixtures is given in the legend of the figure (for other details see Lau et al.[13]). Enzyme activities are expressed as specific activity (µmoles of substrate liberated/h/mg protein). It is obvious, however, since specific activity is value of a fraction (enzyme activity/tissue protein), a change in this value could be caused not only by a change of the numerator, but also by a change in the denominator (tissue protein). The denominator can change not only quantitatively but also qualitatively, i.e. changes in tissue protein other than the structures carrying the hydrolases studied can occur. Therefore, to evaluate the data from this aspect, we have also calculated the changes in total activity (µmoles of substrate liberated/h/organ). Protein was determined as described by Lowry et al.[14] using bovine serum albumin as standards. Student's t-test was used to determine statistical significance of differences between means.

Results and discussion. Since no significant differences were found between control rats receiving 0.005 N NaOH or 0.9% NaCl ($p > 0.05$), all data from these 2 groups were pooled and treated as 1 group. All pregnant rats gained weight steadily during the experimental period. Controls (13 rats) gained 63.7±2.7 g [mean±SEM], T_3-treated (6 rats) 20% less [50.0±4.2], cortisone-treated (6 rats) only 22.5±4.5 g. Treatments reduced mean fetal weights. The average body weights of rat term fetuses of L-triiodothyronine-[5.17±0.13 (12 specimen)] or cortisone-treated mothers [4.15±0.10 (12)] were significantly less than that of controls [6.71±0.09 (26)].



Activities of several acid hydrolases in brain of rat term fetuses. *A* and *B*: Specific and total activities in forebrain, *C* specific activities in cerebellum. Middle columns represent control values. T, mothers injected with L-triiodothyronine; C, mothers injected with cortisone. Vertical lines denote 2 SEM. Shaded columns denote significant differences between experimental and control groups by t-test ($p < 0.05$; * $p < 0.01$; ** $p < 0.001$).

Injection of L-triiodothyronine resulted in a significant increase in the specific activities of all the fetal lysosomal enzymes studied in both forebrain and cerebullum (figure). Comparing total activities in forebrain of control and T_3-treated groups showed small and statistically insignificant differences; only total activity of α-L-fucosidase was significantly increased. The differences in total activities of all enzymes studied in cerebellum between the control and T_3-treated rats were not significant. Administration of cortisone decreased both specific and total activities of β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, and α-L-fucosidase in forebrain (figure). In cerebellum, cortisone administration caused no significant changes in specific or total activity (differences between controls and cortisone-treated group were less than 5%).

Experiments have thus shown that administration of triiodothyronine or cortisone to pregnant rats can influence the activity of several fetal brain acid hydrolases. It is noteworthy that cortisone influenced glycosidases in the forebrain, but no effect was seen in cerebellum. Mechanisms involved in these effects are open for further studies.

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Evidence for the nonhydrophobic interaction of aromatic, nitrogen-containing compounds with the coenzyme binding site of a NAD-linked D-lactate dehydrogenase

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Summary. Quantitative structure activity relationships suggest that the binding of quinoline and phenanthroline analogs to D-lactate dehydrogenase from the barnacle, *Balanus nubilus* Darwin, does not involve primarily hydrophobic effects. This phenomenon appears to exist also for other lactate dehydrogenases.

A variety of polyaromatic compounds have been demonstrated to bind at the coenzyme binding sites of diphosphopyridine nucleotide-linked dehydrogenases. For example, salicylate[1], cibacron blue[2] and simple aromatic hydrocarbons such as benzene and naphthalene[3] are effective competitive inhibitors with respect to the coenzyme in a variety of dehydrogenases. Aromatic metal chelators (o-phenanthroline, 8-hydroxyquinoline) and their nonchelating analogs have been shown to catalytically inhibit yeast alcohol dehydrogenase[4], pig heart s-malate dehydrogenase[5], human liver aldehyde dehydrogenase[6] and beef liver glutamate dehydrogenase[7]. Anderson and Anderson[8] observed that the effectiveness of nicotinamide chlorides as inhibitors of yeast alcohol dehydrogenase was related to the length of hydrocarbon side chains attached to the nicotinamide ring. A linear relationship was observed between chain length and the inhibitory capacity of the compound, suggesting hydrophobic interactions were involved at the coenzyme binding site. Since aromatic chelators and their nonchelating analogs are hydrophobic in nature, a number of workers have invoked similar hydrophobic explanations for the mode of inhibition of these compounds on diphosphopyridine nucleotide-linked dehydrogenases[4-7]. Studies reported here using quantitative structure activity relationships suggest that the mode of binding of quinoline and phenanthroline analogs to a D-lactate dehydrogenase is not hydrophobic.

In kinetic studies it has been observed that the D-lactate dehydrogenase (E.C. 1.1.1.28) from the muscle of the giant barnacle, *Balanus nubilus* Darwin, is instantaneously inhibited by a wide range of aromatic metal chelators and their nonchelating analogs[9]. In all cases, the pattern of inhibition by quinoline and phenanthroline congeners is competitive with respect to the coenzyme, NADH, suggesting that these compounds bind at the coenzyme site on the enzyme molecule. In order to obtain a clearer picture as to whether hydrophobic (i.e. water desolvation) interactions are involved in the binding of these aromatic compounds to *B. nubilus* D-lactate dehydrogenase, we have analyzed our results using quantitative structure activity relationships (QSAR) as developed by Hansch and coworkers[10-12]. This type of analysis involves the correlation of the inhibitory capacities of compounds with certain of their physicochemical characteristics. We have directed our attention at 2 particular parameters, the logarithm of the octanol-water partition coefficient (log P) and the Lorentz-$_{\text{Lorenz}}$ molar refractivity (MR). The partition coefficient appears to correlate substituent interactions in the hydrophobic regions of enzymes. It presumably models the hydrophobic effect as determined by desolvation of substituent and enzyme. The molar refractivity is not well understood at the physical level but is thought to reflect the overall electronic configuration of the molecule[13]. This is the result of both the constitutive nature and number of atoms involved (leading to an additive parameter related to molecular volume or 'bulkiness' for organic molecules) and intra- and intermolecular electronic distortions (a polarizability parameter). Log P values have been determined experimentally and MR values were determined by calculation[10-12] for each of the inhibitory compounds. Linear regressions were calculated correlating log 1/apparent K_i with either log P or MR. The results of the QSAR's are listed in table 1. The correlation coefficient of equation 1 (table 1) for *B. nubilus* D-lactate dehydrogenase is very low ($r = 0.498$), whereas a relatively high correlation is observed (equation 2, table 1) for this enzyme when one looks at molar refractivity