Developmental Changes in the Activity of Lipoprotein Lipase (Clearing-Factor Lipase) in Rat Lung, Cardiac Muscle, Skeletal Muscle and Brown Adipose Tissue

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The lipoprotein lipase activity of the lung, skeletal muscle, heart muscle and brown adipose tissue of the rat was studied during the period from late foetal to adult life. The enzyme activity in all four tissues emerged substantially during the first 24 h after birth. Subsequently, heart and lung enzyme activity remained relatively constant per unit wet weight of tissue. The enzyme activity present in brown adipose tissue and skeletal muscle was elevated per unit weight of tissue during suckling compared with other periods of life. Delivery of near-term foetuses stimulated the emergence of enzyme activity in all four tissues with the same time course as that evoked by normal delivery. The significance of the presence of the enzyme in the tissues and the activity changes which occurred during development are discussed in relation to possible mechanisms of control.

Recently, the developmental changes in the total lipoprotein lipase activity of rat white adipose tissue have been reported (Hietanen & Greenwood, 1977; Cryer & Jones, 1978). The specific activity of the enzyme in this tissue emerged substantially with the neonatal cluster of enzymes as defined by Greengard (1971). The upsurge in the total activity present in the tissue was initiated during the first 6 h of life (Péquignot-Planche *et al.*, 1977), and like the subsequent developmental changes in enzyme activity was probably under the control of changes in circulating hormone, particularly insulin concentrations (Péquignot-Planche *et al.*, 1977; Cryer & Jones, 1978). Although the white adipose tissue of the rat represents an important site of plasma triacylglycerol fatty acid uptake (Cryer *et al.*, 1976) mediated by the action of lipoprotein lipase, other extrahepatic tissues are also active in this respect (Robinson, 1970).

It was the purpose of the present investigation to study the changes in lipoprotein lipase activity that occurred during development in extrahepatic tissues other than white adipose tissue in order to gain insight into the role of these tissues in the development of the regulation of plasma lipoprotein triacylglycerol concentrations.

Materials and Methods

Animals and tissues

Rats of the MRC hooded strain, from the colony of this department, were used in all the experiments. The animals were bred and the litters managed as

described previously (Cryer & Jones, 1978). The premature delivery of litters to be kept with the mother was induced by the intraperitoneal injection of 40 units of oxytocin/kg. Foetal animals for immediate study were removed from diethyl ether-anaesthetized females by caesarian section. Foetuses removed under these circumstances show no signs of anoxia. Only pups from litters containing from 8 to 12 individuals were studied. All the animals studied were killed between 09:00 and 10:00 h and were considered to be in the fed state since the stomachs were invariably full. Immediately after birth the animals were exsanguinated before the tissues to be studied were removed, rinsed in 0.15 M-NaCl solution, blotted dry and weighed. In addition to the heart and lungs, brown adipose tissue was obtained from the intrascapular site and skeletal muscle samples were obtained from the region of the thigh sartorius muscle. Care was taken to remove any white adipose tissue from the brown adipose tissue samples and both subcutaneous and intramuscular fat tissue was dissected away from the skeletal muscle samples. The tissues were pooled from young animals as previously described (Cryer & Jones, 1978).

Preparation of tissues for lipoprotein lipase assay

Samples of brown adipose tissue (35–100 mg wet wt.), heart (40–150 mg wet wt.), lung (120–500 mg wet wt.) and skeletal muscle (80–450 mg wet wt.) were homogenized in 5 ml of 2.5% (w/v) aqueous solution of casein (BDH Chemicals Ltd., Poole, Dorset, U.K.) before being dispersed in ice-cold acetone for the preparation of acetone/diethyl ether-

dried powders as described by Cryer *et al.* (1976). The powders were used in the assay of tissue total lipoprotein lipase activity (Riley & Robinson, 1974). Lipoprotein lipase activity was measured essentially by the method of Cunningham & Robinson (1969) as modified by Riley & Robinson (1974) and detailed by Cryer & Jones (1978). This assay utilized Intralipid (Vitrum, Stockholm, Sweden) as an artificial triacylglycerol substrate. The measured activity in all the preparations was characterized as lipoprotein lipase by 0.6M-NaCl inhibition studies and the requirement for serum in the assay for activity.

Results

Fig. 1 shows the changes in the weight of intrascapular brown adipose tissue, lung and heart as percentages of the total body weight during development of the rat. The body weight changes in the animal were as described by Cryer & Jones (1978). The weight of the intrascapular brown adipose tissue increased together with total body weight such that the tissue weight as a proportion of body weight remained constant during the period of study (i.e. up to 40 days of age). This tissue was not studied subsequently because it became increasingly infiltrated

with white adipose tissue, which rendered the preparation of exclusively brown adipose tissue samples impossible. By contrast, the heart, although it remained a constant proportion of body weight during the first 20 days of life, declined significantly ($P < 0.001$) during the subsequent period of study. The lung also declined substantially and significantly ($P < 0.001$) as a percentage of body weight after 17 days of age. The lung tissue represented 1.8% of total body weight during the early days after birth, but only 0.5% of body weight in adult animals.

Fig. 2 shows the changes in the total tissue lipoprotein lipase activity for lung and heart during development of the rat. The enzyme was of low specific activity in tissues taken from foetal animals, but during the first 24h of life a rapid and significant ($0.01 \geq P \geq 0.001$) increase in specific activity occurred. In both tissues the enzyme activity per unit wet weight remained unchanged during the subsequent period of study up to 145 days of age. The total tissue activity altered only in relation to changes in total tissue weight. Fig. 3 shows that the enzyme activity emerged significantly ($0.01 \geq P \geq 0.001$) from low foetal to relatively high neonatal levels during the first 24h of life in brown adipose tissue and skeletal muscle also. The pattern of

Fig. 1. Changes in the weights of brown adipose tissue, lung and heart as percentage of total body weight during development of the rat

The weights are expressed as a percentage of total body weight, the points indicate the means and the bars indicate the standard deviation for between 8 and 14 independent observations in all cases. NB on the age axis indicates newborn. The percentage weights of hearts (○), lungs (●) and brown adipose tissue (△) taken from both male and female animals have been pooled since no difference between the sexes was detected.

Fig. 2. Changes in the lipoprotein lipase activity in the lung and cardiac muscle of rats during development

Animals of both sexes were studied between 17 gestational days and 145 postnatal days of age. Tissues (○, lung; ●, heart) were treated and enzyme activity was estimated as described in the Materials and Methods section. Points indicate the means for between 8 and 15 independent observations including animals of both sexes since no sex differences were observed. The bars indicate s.d. NB on the age axis indicates newborn.

change in activities per unit weight in these two tissues was distinct thereafter and differed from the other tissues studied. The enzyme activity present in the brown adipose tissue exhibited two peaks of activity at 2 and 12 days of age. The enzyme activity declined between the two dates with a significant ($P < 0.001$) minimum at day 7. Following the peak at day 12 the activity declined progressively to relatively low levels, equivalent to those found in foetal tissue, by the time of weaning. Fig. 3 also shows the pattern of enzyme activity change in skeletal muscle during development. A significant ($0.01 \geq P \geq 0.001$) peak of lipoprotein lipase activity occurred in this tissue at mid-suckling. At weaning a relatively low specific activity of the enzyme was present in the tissue equivalent to that found in tissue taken from both foetal and adult animals. Fig. 4 shows the pattern of emergence of lipoprotein lipase activity in (a) heart, (b) lung, (c) skeletal muscle and (d) brown adipose tissue during the first 16 h of extrauterine life for animals delivered normally at term or prematurely 24 h before the estimated time

Fig. 3. Changes in the lipoprotein lipase activity in the brown adipose tissue and skeletal muscle of rats during development

Animals of both sexes were studied between 17 gestational days and either 30 days for the brown adipose tissue (○) or 145 days for the skeletal muscle (●). Points indicate the means for between 6 and 26 (skeletal muscle) or 6 and 12 (brown adipose tissue) independent observations including animals of both sexes since no sex differences were observed. The bars indicate s.d. NB on the age axis indicates newborn.

of birth. In all the tissues and animals studied the pattern of change over the 16 h of study was the same for the premature and the term-delivered pups. As for all the studies reported no sex difference in the response to premature delivery was noted. Oxytocin injection within 6 h of the estimated time of delivery also had no effect on the pattern of enzyme emergence (A. Cryer & H. M. Jones, unpublished work).

Discussion

As with the lipoprotein lipase activity of white adipose tissue (Péquignot-Planche *et al.*, 1977; Cryer & Jones, 1978) the enzyme present in the four tissues studied here emerged substantially during the first 24 h of life. The lipoprotein lipase activity of the non-adipose extrahepatic tissues of near-term foetuses also emerged with the same time course, which would suggest that the emergence is consequent to some as-yet unidentified change that occurred as a response to extrauterine life.

Changes in brown adipose tissue lipoprotein lipase activity have been previously studied (Hahn & Drahota, 1966; Hemon *et al.*, 1975) at limited time

Fig. 4. Changes in (a) heart, (b) lung, (c) skeletal muscle and (d) brown adipose tissue lipoprotein lipase activity after normal and premature delivery

Rats delivered naturally (●) or induced 24h before the estimated time of delivery (○), by administration of oxytocin to the mother, were studied. The points indicate the means for between 4 and 6 independent observations; the bars indicate s.d. Animals were studied during the first 16h of extrauterine life, which was spent with the mother.

periods that did not reveal the double peak of activity noted in the present study. Hemon *et al.* (1975) have reported data consistent with the view that normal thyroid hormone secretion and the hypoinsulinaemia characteristic of suckling rats (Blázquez *et al.*, 1970) both contribute to the maintenance of high total tissue enzyme activity during suckling. Although these hormonal influences may be involved in the control of brown adipose tissue total lipoprotein lipase activity during development, the simultaneous changes in enzyme activity in white adipose tissue (Cryer & Jones, 1978) would suggest that an exclusive involvement is unlikely since relatively high plasma insulin concentrations are required to maintain enzyme activity in the latter tissue.

Brown adipose tissue in suckling rats has been shown to be important in a thermogenic capacity (Himms-Hagen, 1976). The high total and heparin-releasable (Hemon *et al.*, 1975) lipoprotein lipase activity present in the tissue during suckling may provide a mechanism whereby a preferential

utilization of circulating triacylglycerol fatty acid either as oxidizable fuel for thermogenesis or as a source of stored intracellular triacylglycerol by the tissue could be envisaged. The decline in brown adipose tissue lipoprotein lipase activity at mid-suckling remains unexplained. However, the increases in tissue enzyme activity stimulated by cold in suckling (Hemon *et al.*, 1975), early weaned (A. Cryer & H. M. Jones, unpublished work) and cold-adapted adult rats (Rodomski & Orme, 1971) would indicate that cold stimulation, which in normal suckling rats occurs at normal room temperature (22°C), could play an important part in the control of brown adipose tissue lipoprotein lipase activity during early development. This cold stimulation of enzyme activity could provide a means whereby behavioural changes (e.g. exploratory activity) might be implicated in the observed changes.

Skeletal muscle has also been suggested to be important in non-shivering thermogenesis (Himms-Hagen, 1976). Although insufficient evidence exists to implicate skeletal muscle lipoprotein lipase

activity in the provision of substrate for heat generation in this tissue it could be that similar hormonal influences, as yet only partially understood even in the case of skeletal muscle from the adult (Tan *et al.*, 1977), act similarly in both muscle and brown adipose tissue. The peak of skeletal muscle enzyme activity and the second peak of brown adipose tissue activity occurred half way through suckling, which was the time when white adipose tissue lipoprotein lipase activity was also high (Cryer & Jones, 1978). The activity per unit weight of tissue (and per whole tissue pool also) declined in all three tissues thereafter to relatively low levels at the commencement of weaning. Thus the normal reciprocity between enzyme activity changes in white adipose tissue and skeletal muscle observed in adult animals (Cryer *et al.*, 1976) was not observed for the relatively long-term developmental changes in enzyme activity. It seems likely therefore that different underlying mechanisms of enzyme activity change predominate in the two situations. Whether suckling animals are able to respond to short-term nutritional changes, by changes in tissue lipoprotein lipase activity, remains to be investigated.

The changes in the lipoprotein lipase activity of heart muscle and lung tissue, well characterized in the tissues of adult animals (Borensztajn *et al.*, 1972, 1973; Hamosh *et al.*, 1976), may also respond to different stimuli for change when compared with the enzyme activity changes observed during early development. However, the involvement of the enzyme in both tissues may be of major physiological importance during early life. Already Felts (1964) and Hamosh & Hamosh (1975) have suggested a role for lung lipoprotein lipase activity in the provision of precursor fatty acids for the formation of pulmonary surfactant in adults, and this could therefore be of even greater importance to the new-

born. Further studies will, however, be necessary in order to identify the aspects of birth that are involved in the emergence of lipoprotein lipase activity in tissues and the subsequent developmental changes in enzyme activity.

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References

- Blázquez, E., Montoya, E. & Quijada, C. L. (1970) *Endocrinology* **48**, 553-561
Borensztajn, J., Samols, D. R. & Rubenstein, A. H. (1972) *Am. J. Physiol.* **223**, 1271-1275
Borensztajn, J., Keig, P. & Rubenstein, A. H. (1973) *Biochem. Biophys. Res. Commun.* **53**, 603-608
Cryer, A. & Jones, H. M. (1978) *Biochem. J.* **172**, 319-325
Cryer, A., Riley, S. E., Williams, E. R. & Robinson, D. S. (1976) *Clin. Sci. Mol. Med.* **50**, 213-221
Cunningham, V. J. & Robinson, D. S. (1969) *Biochem. J.* **112**, 203-209
Felts, J. M. (1964) *Health Phys.* **10**, 973-979
Greengard, O. (1971) *Essays Biochem.* **7**, 159-205
Hahn, P. & Drahotka, Z. (1966) *Experientia* **22**, 706-707
Hamosh, M. & Hamosh, P. (1975) *Biochim. Biophys. Acta* **380**, 132-140
Hamosh, M., Yeager, H., Shechter, Y. & Hamosh, P. (1976) *Biochim. Biophys. Acta* **431**, 519-525
Hemon, P., Ricquier, D. & Mory, G. (1975) *Horm. Metab. Res.* **7**, 481-484
Hietanen, E. & Greenwood, M. R. C. (1977) *J. Lipid Res.* **18**, 480-490
Himms-Hagen, J. (1976) *Annu. Rev. Physiol.* **38**, 315-351
Péquignot-Planche, E., de Gasquet, P., Boulangé, A. & Tonnu, N. T. (1977) *Biochem. J.* **162**, 461-463
Riley, S. E. & Robinson, D. S. (1974) *Biochim. Biophys. Acta* **369**, 371-386
Robinson, D. S. (1970) *Compr. Biochem.* **18**, 51-116
Rodomski, M. M. & Orme, T. (1971) *Am. J. Physiol.* **220**, 1852-1856
Tan, M. H., Sata, T. & Havel, R. J. (1977) *J. Lipid Res.* **18**, 363-370