Vol. 148, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1987 Pages 9-14

Euthyroid Status Is Essential for the Perinatal Increase in Thermogenin mRNA in Brown Adipose Tissue of Rat Pups

Maria-Jesus Obregon[1,2], Rajan Pitamber[1], Anders Jacobsson[1], Jan Nedergaard[1], and Barbara Cannon[1]

[1]The Wenner-Gren Institute, University of Stockholm, Biologihus F3, S-106 91 Stockholm, Sweden

[2]Dpto. Endocrinología Experimental, Instituto de Investigaciones Biomédicas, Facultad Autónoma de Medicina (U.A.M.) Arzobispo Morcillo 4, 28029 Madrid, Spain

Received August 18, 1987

SUMMARY: The amount of mRNA coding for the brown-fat specific, uncoupling protein thermogenin was followed perinatally in fetuses and newborns from normal and hypothyroid rat dams. Although the growth of the fetuses and newborns was normal in the hypothyroid group, they had a lower amount of thermogenin mRNA already in-utero, and the dramatic postnatal increase in thermogenin mRNA was nearly completely abolished. It is concluded that the euthyroid state is essential for the regulation of the expression of the thermogenin gene.

Newborn hypothyroid rats are less resistent to cooling than are normal newborns (1), and it has been envisaged that this defect in thermoregulation may at least in part be due to a diminished heat production from the brown adipose tissue of these rats (for general review of this tissue in newborns, see (2)). This view is supported by the fact that administration of norepinephrine to brown adipose tissue slices from hypothyroid rat pups does not lead to the expected stimulation of lipolysis (3) which is seen in controls. Similarly, norepinephrine to a large extent loses its ability to increase respiration in brown fat cells isolated from hypothyroid fetal sheep (4). However, dibutyryl cyclic AMP is able, at least to a certain extent, to stimulate lipolysis and respiration in cells and tissue from hypothyroid newborns (3, 4). These observations have led to the view that the main effect of hypothyroidism is an impairment in the function of the adrenergic receptor system.

In the present investigation, we have studied the significance of the thyroid status for the perinatal recruitment of brown adipose tissue, i.e. for

0006-291X/87 $1.50

the increased expression of the brown-fat specific, uncoupling protein thermogenin in these mitochondria. The amount of this protein increases markedly in the first days after birth in the rat (5). We conclude that thyroid hormone is essential not only for mediation of the acute thermogenic signal (norepinephrine) but also for the proper perinatal recruitment of thermogenin, as the level of thermogenin gene expression in hypothyroid animals is much decreased.

MATERIALS AND METHODS

Animals

Pregnant Sprague-Dawley rats were obtained from Alab, Stockholm, with the day of appearance of the vaginal plug being taken as day zero of gestational age. The pregnant rats were housed at 22°C.

From day 14 of gestational age, some of the pregnant rats received 0.02% methimazole (= MMI) (Sigma) in the drinking water.

Groups of two or three pregnant rats were sacrificed under ether anesthesia on days 20, 21 and 22 of gestation, the uterus was dissected out, and fetuses were obtained, separated from the placenta, and weighed. Fetal brown adipose tissue was dissected out and immediately frozen on dry ice. The brown adipose tissue from five to six fetuses was pooled.

Brown adipose tissue from newborn pups was obtained on day zero (12-20 h after birth), and on days 1 and 2 after birth. On each day, four pups were taken from each dam, weighed, and brown adipose tissue dissected out as described for fetal samples. The tissue from two pups was pooled. At least 8 pups were kept in each litter.

Thermogenin mRNA estimation

Total RNA was prepared from the frozen tissues as already described (6). The amount of thermogenin mRNA was analyzed by slot blots as previously described with a nick-translated cDNA probe for thermogenin mRNA (7). After exposure, the intensity of the blots was evaluated in a laser densitometer.

RESULTS

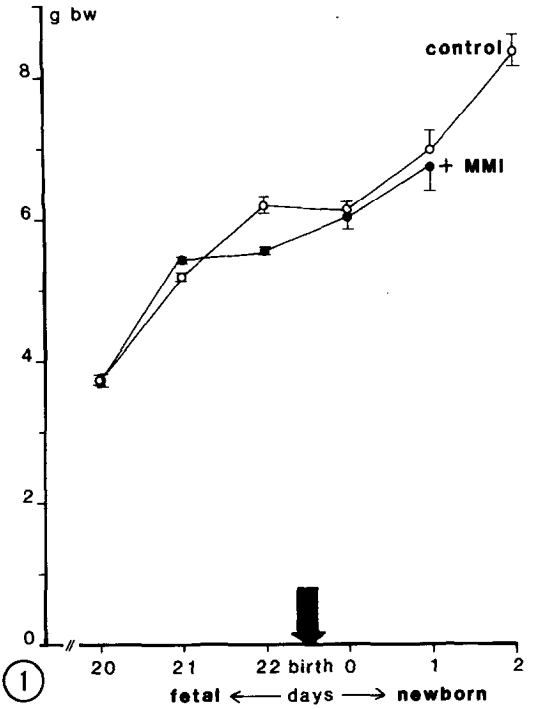
Fetal and neonatal development

To induce hypothyrodism in the rat fetuses and newborns, the dams were treated with methimazole in the drinking water. This treatment is known to cause maternal and fetal hypothyroidism, blocking fetal thyroid gland function as well as thyroid hormone production, and depleting most fetal tissues of thyroid hormones (8, 9). In the present study it was checked that fetal thyroids from methimazole-treated dams were enlarged and hyperemic.

Fig. 1 shows the body weight of the fetal and newborn pups from control and methimazole-treated dams. It is seen that there was no growth retardation due to the methimazole treatment.

Amount of thermogenin mRNA

The analysis of the amount of thermogenin mRNA is shown in Fig. 2. It is seen that in control animals, the fetal levels of thermogenin mRNA are low, although clearly above background, at a level similar to that found in the



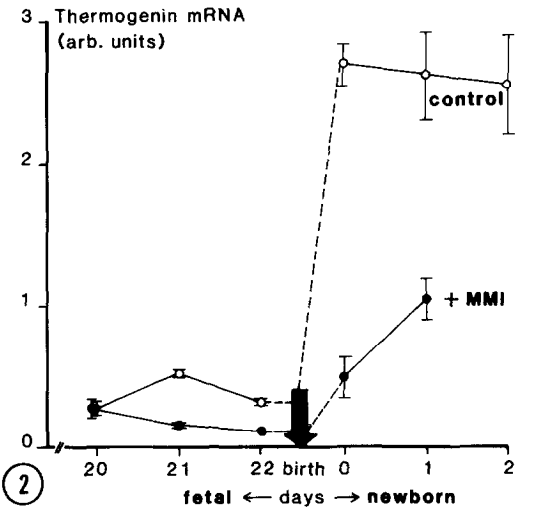


Fig. 1. Effect of hypothyroidism on perinatal rat growth.
Pregnant rats were treated with methimazole (MMI) as described in Materials and Methods. Values are the means and S.E. of the body weights of 20-37 fetuses or 7-15 newborns, from 2-4 litters.

Fig. 2. Effect of hypothyroidism on the level of thermogenin mRNA in developing rat pups.
RNA samples obtained from 6 rat fetuses or 2 newborn rats were analysed for the amount of thermogenin mRNA. Values are means ± S.E. from 4-6 samples.

adult rat at 28°C (i.e. the gene is being expressed even in-utero). After birth, there is a very sharp rise in the amount of thermogenin mRNA. The maximal level is observed already 12-20 h after birth, with a postpartum increase in levels of about 10-fold. The levels reached are similar to those obtained in brown adipose tissue from adult rats exposed overnight to 4°C. In preliminary experiments we have observed that the amount of thermogenin mRNA is still low during the first 3-4 h after birth (10); this is surprising as the level rises very quickly (in less than 1 h) if adult animals are exposed to cold (6, 7, 11, 12). Thus, the postnatal increase in thermogenin mRNA expression occurs as a single step in the time interval from 4 to 12 h after birth, and then remains at this elevated level for at least several days (a few animals tested at day 3 still showed the same level). This pattern is principally in agreement with a recent report where mRNA levels were estimated perinatally (13). The pattern is also in good agreement with what is known about the postnatal increase in the amount of thermogenin in brown adipose

tissue mitochondria, which continues to increase during the first 4-5 days after birth (5).

Effect of hypothyroidism on the amount of thermogenin mRNA

The effect of hypothyroidism on the thermogenin mRNA levels during the fetal and neonatal period is also shown on Fig. 2. Two important features are evident:

Firstly, it is clear that the marked postnatal increase in thermogenin mRNA levels is much blunted in the hypothyroid animals. In fact, 12-20 h after birth, when the maximal level has already been reached in control animals, the level of thermogenin mRNA in the hypothyroid animals is still only at the level found in-utero in normal rats. From this it can be concluded that the postnatal recruitment of the thermogenic machinery in the hypothyroid state is much diminished and retarded, and that the low thermogenic capacity of hypothyroid newborns results not only from an inability of the acute sympathetic signal to reach the cell interior and activate the mitochondria (lack of functionally coupled adrenergic receptors) but also from a much decreased amount of the rate-limiting factor for thermogenesis (14): the amount of thermogenin.

Secondly, it is seen that already at the late fetal stage, hypothyroidism leads to a decreased amount of thermogenin mRNA. This implies that the amount of thermogenin mRNA observed in-utero is not a constitutive level, representing the lowest level of thermogenin in differentiating brown fat cells. Rather, there are apparently, already in the fetus, hormonal or neuronal factors which regulate and induce the amount of thermogenin mRNA being produced in the tissue.

DISCUSSION

We have here shown that maintenance of the euthyroid state is essential for normal postnatal recruitment of brown adipose tissue to occur, i.e. for induction of the expression of the gene coding for the brown-fat specific, uncoupling protein thermogenin. This decrease in induction undoubtedly leads to a reduction in the amount of thermogenin protein found in the mitochondria, in agreement with recent results from thyroidectomised adult rats exposed to cold (15).

The mechanism by which thyroid hormones exert this effect on gene expression is currently unclear. There are thyroid receptors in the tissue (16, 17), and the effect of thyroid hormone may of course be mediated via these and be directly on the expression of the gene. However, it appears that thyroid hormones - at least in adult animals - are essential for mainte-

nance of β-adrenergic receptor density (18). In this respect, it can be pointed out that there is evidence that adrenergic stimulation is involved in the control of the expression of thermogenin (7, 11-13, 19). If this is the case, hypothyroidism may lead to the consequences presented here via inducing a chronic absence of the intracellular signal for thermogenin mRNA synthesis, normally mediated via the β-adrenergic receptor.

The observation presented here of a positively regulated level of thermogenin mRNA already in-utero in the rat may also have implications for the presently unsolved question of how the recruitment process is regulated in so-called precocial newborns (2), i.e. newborns that are born with (or at least very rapidly postnatally obtain) a very high amount of thermogenin protein (such as the guinea-pig (20)) and that also have high thermogenin mRNA in the fetal state (such as the rabbit (21)). Based on the data presented here, it would seem that even in the altricial rat, in which brown-fat recruitment appears to be induced after birth, the tissue must be considered to be under stimulatory influence already in-utero. Thus, what is seen in the precocial newborns may be an augmentation of this process.

ACKNOWLEDGEMENTS: This work was supported by a grant from the Swedish Natural Science Research Council. M-J. O. was the recipient of an exchange scholarship from the Spanish Science Research Council/Swedish Natural Science Research Council.

REFERENCES

1. Steele, R. E., and Wekstein, D. R. (1972) Am. J. Physiol. 222, 1528-1533.
2. Nedergaard, J., Connolly, E., and Cannon, B. (1986) In Brown Adipose Tissue (P. Trayhurn, and D. G. Nicholls, Ed.), pp. 152-213. Edward Arnold Ltd., London.
3. Hemon, P. (1976) Biol. Neonate 28, 241-255.
4. Klein, A. H., Reviczky, A., and Padbury, J. F. (1984) Endocrinology 114, 1065-1069.
5. Sundin, U., and Cannon, B. (1980) Comp. Biochem. Physiol. 65B, 463-471.
6. Jacobsson, A., Stadler, U., Glotzer, M. A., and Kozak, L. P. (1985) J. Biol. Chem. 260, 16250-16254.
7. Jacobsson, A., Nedergaard, J., and Cannon, B. (1986) Biosci. Rep. 6, 621-631.
8. Marchant, B., Brownlie, B. E. W., McKay Hart, D., Horton, P. W., and Alexander, W. D. (1977) J. Clin. Endocrinol. Metab. 45, 1187
9. Morreale de Escobar, G., Obregon, M. J., Ruiz-Ona, C., and Escobar del Rey, E. (1987) Subm. f. publ.
10. Cannon, B., Connolly, E., Obregon, M. J., and Nedergaard, J. (1987) In The Endocrine Control of the Fetus (W. Kunzel, and A. Jensen, Ed.), pp. In press. Springer Verlag, Berlin
11. Ricquier, D., Mory, G., Bouillaud, F., Thibault, J., and Weissenbach, J. (1984) FEBS Lett. 178, 240-244.
12. Jacobsson, A., Cannon, B., and Nedergaard, J. (1987) Subm. f. publ.
13. Ricquier, D., Bouillaud, F., Toumelin, P., Mory, G., Bazin, R., Arch, J., and Penicaud, L. (1986) J. Biol. Chem. 261, 13905-13910.

14. Cannon, B., Nedergaard, J., and Sundin, U. (1981) In Survival in Cold (X. J. Musacchia, and L. Jansky, Ed.), pp. 99-120. Elsevier-North, Holland, Amsterdam.
15. Bianco, A. C., and Silva, J. E. (1987) J. Clin. Invest. 79, 295-300.
16. Burgi, U., and Burgi-Saville, E. (1986) Am. J. Physiol. 251, E503-E508.
17. Bianco, A. C., and Silva, J. E. (1987) Endocrinology 120, 55-62.
18. Seydoux, J., Giacobino, J. P., and Girardier, L. (1982) Mol. Cell. Endocrinol. 25, 213-226.
19. Bouillaud, F., Ricquier, D., Mory, G., and Thibault, J. (1984) J. Biol. Chem. 259, 11583-11586.
20. Rafael, J., and Heldt, H. W. (1976) FEBS Lett. 63, 304-308.
21. Freeman, K. B., and Patel, H. V. (1984) Can. J. Biochem. 62, 479-485.