Volume 224, number 2, 353–356 November 1987

Physiological activation of brown adipose tissue destabilizes thermogenin mRNA

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Received 26 September 1987

The amount of mRNA coding for the brown fat specific uncoupling protein thermogenin was followed in the brown adipose tissue of adult mice. As expected, cold exposure or norepinephrine injection caused an increase in the amount of thermogenin mRNA. However, contrary to expectation, the half-life of thermogenin mRNA was dramatically reduced, from about 18 h to about 3 h, when the mice were cold exposed. This destabilization of thermogenin mRNA was not related to the activity of protein synthesis. It was concluded that in brown adipose tissue an unusual mechanism operates which leads to a destabilization of thermogenin mRNA under the same physiological conditions which increase thermogenin gene expression.

mRNA stability; Nonshivering thermogenesis; Uncoupling; Thermogenin; Norepinephrine; (Brown adipose tissue)

1. INTRODUCTION

It has been the accepted view that experimental conditions leading to an increased expression of a specific gene also increase the stability of that gene (if they affect it at all). This has, for example, been shown for casein [1] as well as for vitellogenin [2], histone H3 [3] and heat shock proteins [4], all examined in in vitro systems. We here present evidence that the mRNA for the brown fat specific uncoupling protein thermogenin demonstrates a paradoxical behaviour: it has a markedly reduced half-life in vivo under physiological conditions where the degree of expression of the gene is markedly increased.

The rate-limiting step for thermogenesis in brown adipose tissue is the amount of thermogenin in the mitochondria (review [5]). The total amount of thermogenin is markedly increased when an animal is acclimated to cold [5], and this increase is the molecular basis for cold acclimation in mammals. Recently, after the isolation of cDNA clones for thermogenin [6–8], it has become possible to investigate the regulation of the acclimation process at the molecular level.

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2. MATERIALS AND METHODS

2.1. *Animals*

Adult, male mice (8 weeks old, 30 g) of the NMRI strain were obtained from a local supplier (Eklunds, Stockholm). They were preacclimated to 28°C for 1 week before experiments.

2.2. *Determination of amount of thermogenin mRNA*

Total RNA was extracted from brown adipose tissue as in [6,9] and bound to filter paper for slot blotting. The blots were hybridized with a nick-translated thermogenin cDNA probe (10^8 cpm/μg). Exposed films were evaluated with a laser densitometer.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

3. RESULTS AND DISCUSSION

3.1. *Determination of thermogenin mRNA half-life*

In fig.1A, it is shown that in an animal exposed to cold, there was a very rapid increase in the amount of mRNA coding for thermogenin [6,9,10]. It can also be seen that in animals at 28°C, a single injection of norepinephrine (which is the neurotransmitter responsible for stimulation of thermogenesis) led to a clear increase in the amount of thermogenin mRNA [9,10]. It is noteworthy that the level of thermogenin mRNA remained elevated in these animals for more than 8 h, despite the fact that the thermogenic effect of a single norepinephrine injection is transient and has fully disappeared after 30 min.

In the experiments depicted in fig.1B we have examined the half-life of thermogenin mRNA. For these experiments, mice were cold-exposed for 36 h, at which time a maximal level of thermogenin mRNA is found in the tissue [6]. The mice were then injected with actinomycin D, to block further synthesis of thermogenin mRNA [9]. Some mice were then retransferred to warm environmental conditions, some remaining in the cold.

It is clear from fig.1B that the environmental temperature had a very marked effect on the stability of the mRNA. Thus, in the animals retransferred to 28°C, the mRNA was very stable and had a half-life which could be calculated to be about 18 h (with 13–27 h being the 95% confidence interval). However, surprisingly, in the

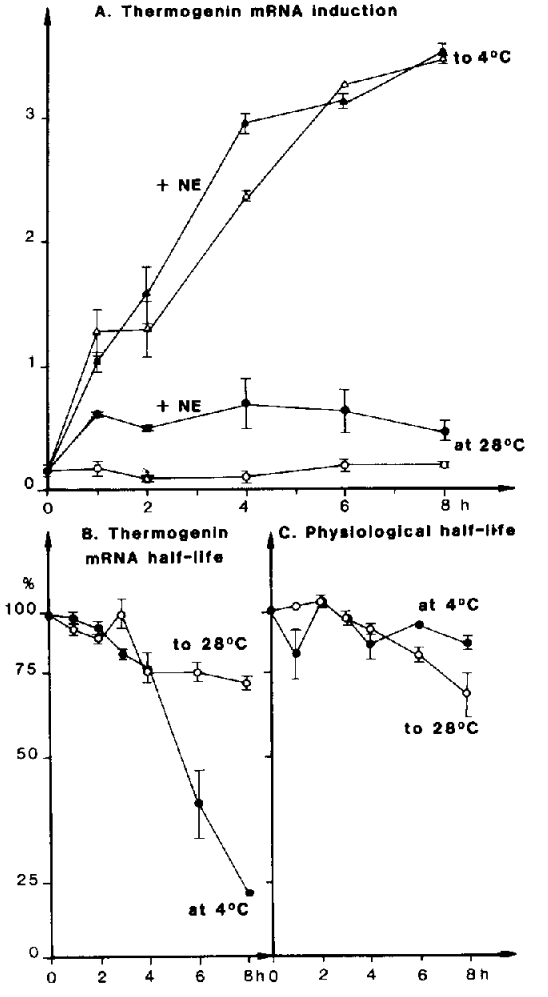

Fig.1. Amount of thermogenin mRNA in brown adipose tissue. (A) Adult male mice either remained under control conditions (28°C) or were exposed to cold (4°C) for the indicated time, or were injected intraperitoneally with norepinephrine (NE) [1 mg (3 μM) norepinephrine bitartrate (Sigma) per kg body wt] or with saline. Brown adipose tissue RNA was extracted and the amount of thermogenin mRNA estimated as described. Values are means and individual values of 2 animals from each time point and are expressed in arbitrary units. Two-way analysis of variance showed a very significant effect ($P < 0.001$) of norepinephrine at 28°C; in animals at 4°C the effect of norepinephrine was only significant at 4 h ($P < 0.05$). (B) Mice were exposed to cold for 36 h and then treated with 0.4 mg actinomycin D per kg body wt (time 0). The mice either remained at 4°C or were retransferred to 28°C and the amounts of thermogenin mRNA analysed as above. Two mice were analysed at each time point. Initial mean amounts of thermogenin mRNA were set to 100%. Linear regression analysis was performed with the logarithmically transformed values, including values for time points 0–8 h for 28°C (excluding the 3 h value); the correlation coefficient was 0.90. For 4°C, time points 2–8 h were included, yielding a correlation coefficient of 0.97. (C) Mice were exposed to cold for 36 h and then either retransferred to 28°C or remained in the cold, and the amount of thermogenin mRNA analysed as above. In the mice remaining in the cold, there was no significant effect of time on thermogenin mRNA. Regression analysis of time points 2–8 h of animals retransferred to 28°C yielded a correlation coefficient of 0.94.

animals remaining at 4°C, where the demand for thermogenin is high, the thermogenin mRNA half-life was dramatically reduced (to 3.2 h, with 2.7–4.0 h being the confidence interval).

In control experiments in which we examined the half-life of actin mRNA, we found no effect of environmental temperature on this mRNA species, which, as expected, was not cold-induced (not shown). However, we were also unable to observe such a temperature-dependent change in half-life of the mRNA coding for glycerol-3-phosphate dehydrogenase or of the mRNA corresponding to the cDNA clone CIN-7, both of which are cold-induced [6,11]. It is thus clear that there must exist a special molecular mechanism in the brown fat cell which is responsible for the selective increase in the degradation process of thermogenin mRNA.

In fig.1C we followed the amount of thermogenin mRNA in animals which were retransferred from 4 to 28°C without any pharmacological treatment. Again paradoxically, this treatment, which would be expected to lead to a rapid breakdown of thermogenin mRNA [12] which was now no longer necessary for thermogenin synthesis, did not induce an immediate disappearance of thermogenin mRNA. Rather, the decay of thermogenin mRNA was close to that seen after actinomycin administration and retransfer of the animals to the warm environment (the calculated half-life was now about 10 h with 7–14 h being the confidence interval). Thus, it would seem that the absence of the physiological stimulus of cold exposure leads to a rapid cessation of thermogenin mRNA synthesis and that, in parallel with this, the intracellular signal responsible for accelerating the breakdown of thermogenin mRNA in the cold is turned off.

3.2. *Effect of inhibition of protein synthesis*

In the experiments depicted in fig.2 we have tested the possibility that the increased degradation of thermogenin mRNA may in some way be related to the mRNA being avidly used for protein synthesis, perhaps with a detrimental effect on the mRNA. Mice were again preexposed to cold, but in this experiment some mice were treated with the protein synthesis inhibitor, cycloheximide, at a dose which we have demonstrated earlier leads to complete inhibition of protein synthesis in the tissue [13]. It was again observed that thermogenin

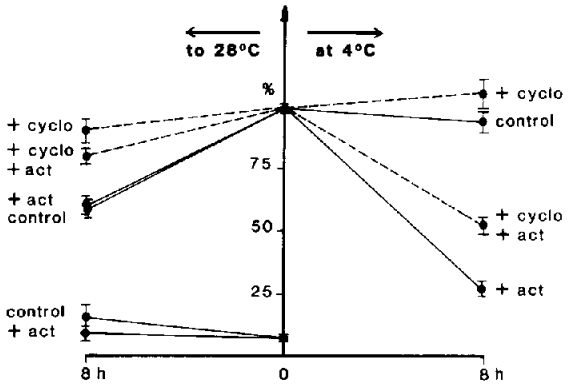

Fig.2. Effect of cycloheximide on thermogenin mRNA half-life. Mice were treated as described in the legend to fig.1B,C, but some mice were additionally injected with 2 mg cycloheximide per mg body wt. In each experimental series the initial value in the cold was set to 100%. Values are means ± SE of 2–16 animals. Significant effects of cycloheximide are indicated by * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; Student's *t*-test.

mRNA degradation was much more rapid in mice remaining in the cold. Cycloheximide had a general stabilizing effect on thermogenin mRNA under all conditions (probably analogous to that demonstrated in some other systems; e.g. [14]), but cycloheximide was equally potent as an mRNA stabilizer in warm- and cold-exposed animals. The cold-induced reduction in thermogenin mRNA does not therefore seem to be a direct effect of the mRNA being used for accelerated protein synthesis.

Rather, here we have an unexpected condition, in which the same physiological stimulus increases both the expression of a gene and the degradation of the mRNA coded by that gene. The intracellular mechanism underlying this phenomenon, as well as the identity of the mRNA signal which tags thermogenin mRNA to become rapidly degraded, have yet to be resolved.

ACKNOWLEDGEMENTS

This investigation was supported by a grant from the Swedish Natural Science Research Council. The authors thank Barbro Svensson for technical assistance, and Dr L. Kozak for access to the glycerol-3-phosphate dehydrogenase cDNA clone.

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