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Mitochondrial Dehydrogenases in Different Taxa of Tetrahymena: Effect of Insulin

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Mitochondrial dehydrogenase activity was measured in seven taxa of Tetrahymena (T. pyriformis G1, T. hegewishi, T. malaccensis, T. pigmentosa, T. shapiro, T. thermophila CU-399, T. thermophila MS-1). Enzyme activity was different in the taxa investigated. Insulin reduced enzyme activity in six of the seven taxa studied. The duration of activity reduction was relatively long (5–10 min.) in most of the cases, and in T. hegewishi this lasted up to the end of the measurements (30 min.). There was no interrelation between the basic dehydrogenase activity of the taxon and the effect of insulin. There was also no correlation between the degree of relationship (of the taxa) and the dehydrogenase profile after insulin treatment.

KEY WORDS: Mitochondria; mt-dehydrogenase; evolution; insulin; hormonal effect.

INTRODUCTION

Unicellular organisms synthesize, store and secrete hormones characteristic to higher vertebrates (16–18) and can react to them via receptors (5–8). For example, insulin, immunobiologically and functionally similar to the mammalian one, is produced by Tetrahymena (16) and exogeneously given mammalian insulin can influence the sugar metabolism of this unicellular ciliate (9). Using antibodies to the α-subunit of the human insulin receptor, the presence of protein(s) reacting with these antibodies could be demonstrated on the cilia, in the nucleus and in/on the mitochondria. The amount of such immunoreactive material was influenced by exposure of the Tetrahymena to insulin (4). The presence of insulin receptors on the cilia is understandable, as these contain the receptorial structures needed for the recognition of the hormone and for transmission of its message to the body of the cell (5, 8). The presence of such type of receptors in the nuclear envelope is also known (2, 10, 13, 14). However, there was no evidence on the role of insulin (receptors) in (on) mitochondria.

In mammalian cells insulin can influence the activity of mitochondrial

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dehydrogenases. Pyruvate dehydrogenase activity was increased by 28% following a 10 min incubation of adipocytes with 400 microU/ml insulin (22). In contrast, pyruvate dehydrogenase activity was reduced in mitochondria of diabetic rats, compared with that of the control (12). The quantity of mitochondrial glycerol phosphate dehydrogenase of rats having non-insulin dependent diabetes mellitus is less than half of the normal value. This was normalized by insulin treatment (19). These data of the literature call attention to the correlation between the presence of insulin in mitochondria and dehydrogenase activity. In the present experiments the effect of insulin treatment on dehydrogenase activity in Tetrahymena was studied, considering the possible differences of mt-dehydrogenase activities in different taxa of Tetrahymena.

MATERIALS AND METHODS

Cells and Culturing

In the present experiment the following Tetrahymena taxa were tested: *T. pyriformis Gl, T. hegewishi, T. malaccensis, T. pigmentosa, T. Shapiro, T. thermophila CU-399, T. thermophila MS*-1.

The cells were cultured at 28°C in axenic conditions, in culture media containing 1% tryptone (Difco, Michigan, USA) and 0.1% yeast extract. Populations of different taxa were assayed in the logarithmic phase of growth, density of samples was 10^5 cell/ml.

Pretreatment of Cells

The groups of cells were pretreated with 10^{-6} M insulin (Actrapid MC; Novo, Copenhagen, Denmark). Length of treatment was 5, 10, 20 and 30 min. In each run there was a control group of the taxa with no pretreatment with the hormone.

Assay of mt-Dehydrogenase

The experiment had two parts. At first, the characteristic mt-dehydrogenase content was measured in the different taxa of Tetrahymena. Consecutively the effect of insulin pretreatment and its time course were tested in respect of mt-dehydrogenase content of the cells. MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide; Sigma Chemicals, St. Louis, MO, USA) was applied to detect the intracellular mt-dehydrogenase. The incubation time with 0.3 mg/ml MTT was 180 min. The products of the mt-dehydrogenase were

converted to water insoluble, blue MTT formazan crystals. These crystals were solubilized in acidic isopropanol. The samples were colorimetrically measured both at 540 and 620 nm (Labsystem Multiscan MS), and the mathematical subtraction of the two measurements are expressed as activity of MTT-reaction.

Statistical Evaluation

Each experiment was repeated eight times. Origin 2.8 was used for statistical evaluation of data. The p values, standard deviation and variances were obtained from ANOVA and Student's t-test.

RESULTS

Each of the seven Tetrahymena taxa studied demonstrated mt-dehydrogenase activity. Low dehydrogenase activity was present in T.399, *T. hegewishi, T. thermophila* MS-1 and *T. pigmentosa.* Higher activity was observed in *T. pyriformis* G1 and *T. shapiro.* The highest activity was expressed by *T. malaccensis* (Fig. 1).

T. pyriformis G1, T. malaccensis and *T.CU-399* produced almost the same profile during insulin treatment. There was a significant decrease in dehydrogenase concentration at 5 and 10 min after the start of the treatment, with a return almost to control level at 20 and 30 min. The profile was similar in *T.*

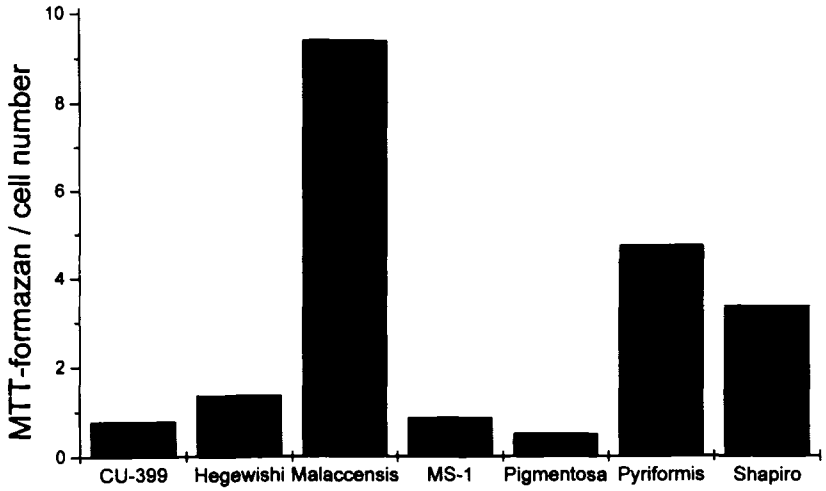

Fig. 1. Taxon dependence of mt-dehydrogenase content in seven taxa of Tetrahymena (with no pretreatment). In the ordinate relative values of absorbance differences measured at 620 and 540 nm (activity of reaction).

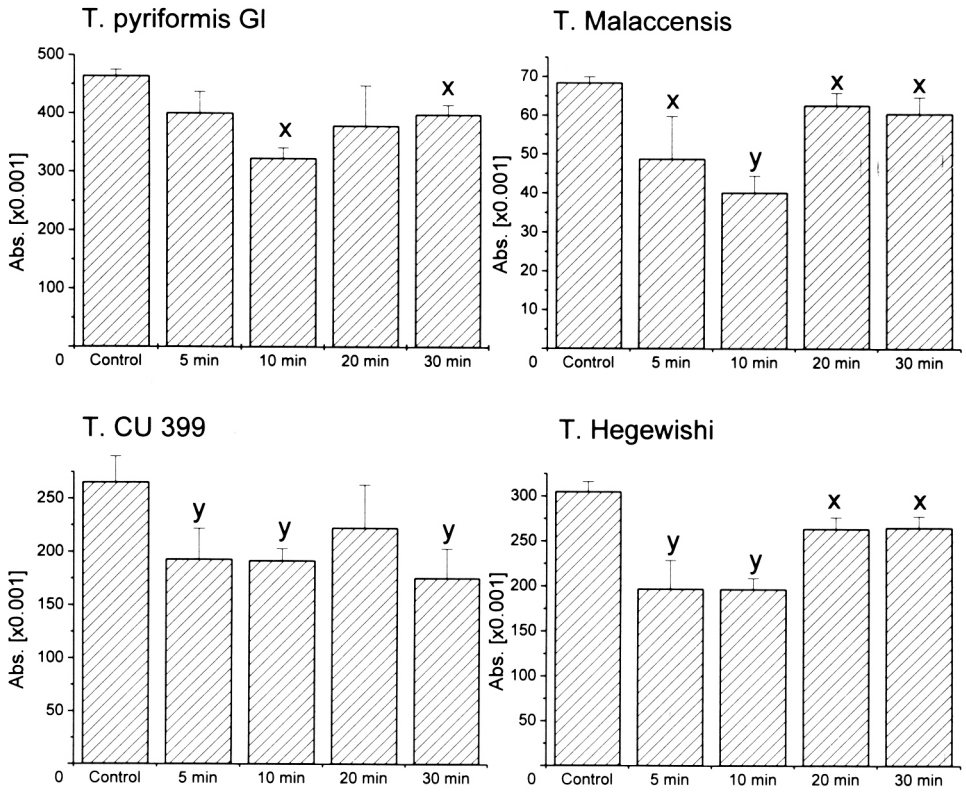

Fig. 2A. Time- and taxon-dependence of insulin pretreatment on mt-dehydrogenase content. Abs = absorbance difference of values measured at 620 and 540 nm. x = $p < 0.05$; y = $p < 0.01$. (a) taxa with long-term responsiveness; (b) taxa with short-term responsiveness to insulin treatment Abs = absorbance: difference of values measured at 620 and 540 nm (activity of reaction).

hegewishi, but with a difference in the significance of the 20 and 30 min. values (Fig. 2A). In the taxa *T. Shapiro* and *T. pigmentosa* the decrease appeared at 5 min with a quick return to the control 10 min after treatment. The taxon *T. thermophila* MS-1 did not produce significant changes at all (Fig. 2B).

DISCUSSION

The method we used is suitable to study the activity of mitochondrial dehydrogenases (3, 11, 21, 23). Our present experiments demonstrated that dehydrogenases are present in the mitochondria of Tetrahymena. Though significant differences were demonstrated in the activity of these enzymes in different taxa, there was no taxon without demonstrable dehydrogenase. In six of the seven taxa studied, insulin influenced the quantity of formazane product (activity of dehydrogenases), however there was no correlation between the basal quantity of the enzyme and the effect of insulin on it.

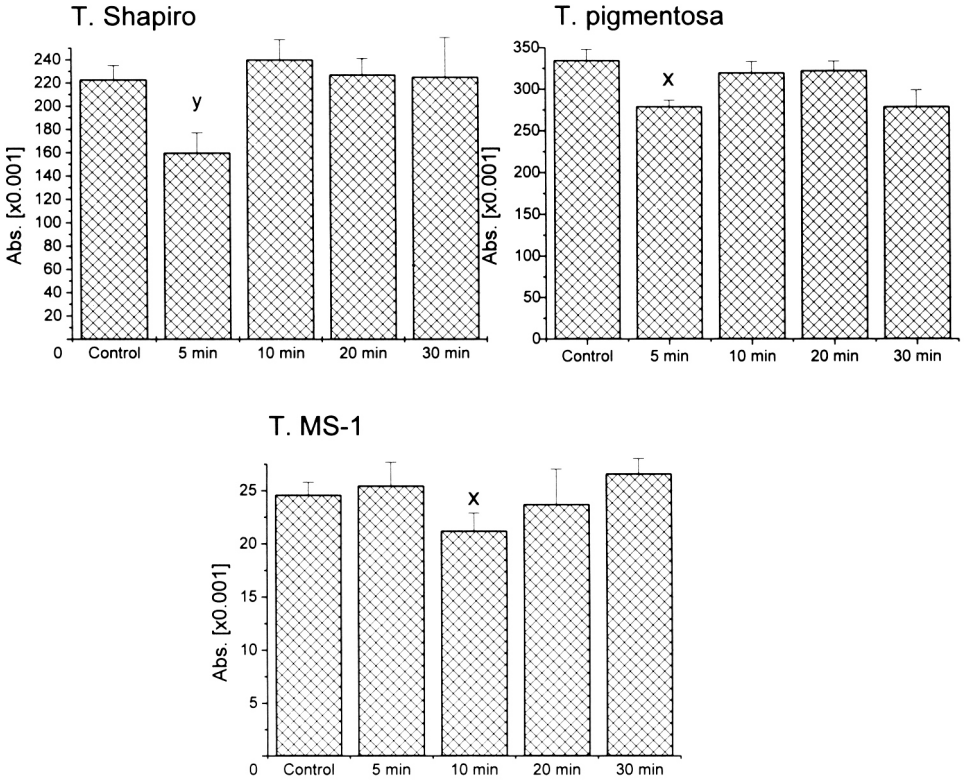

Fig. 2B.

The effect of insulin in the above mentioned six taxa always reduced the activity of mt-dehydrogenases. The reduction appeared at the first measurement after treatment (5 min); in four taxa it was demonstrated as well at the second measurement (10 min); in one taxon up to the end of the experiment (30 min). On the basis of these experiments it cannot be established whether the enzymes became refractory to the hormonal effect after a time, or insulin—as a protein—was engulfed and digested by the cells of groups finishing increased enzyme activity earlier. However this latter version seems to be more likely. We also don't know whether the hormonal effect was receptor-mediated using insulin stimulated second messenger systems (which are present and working in Tetrahymena –6,8) or whether it is a direct effect of insulin, which after treatment could be present in the mitochondria of Tetrahymena (4).

A special problem is caused by the taxon T. thermophila MS-1, where there was a small decrease in the mt-dehydrogenase activity at 10 min, however, this was not significant and the other activities measured were at the control level. It is possible that the differences between the pyriformis and thermophila taxa can explain this behavior. T. thermophila also has a micronucleus and is therefore capable of sexual reproduction. In earlier experiments (15) it behaved opposite to

the *T. pyroformis,* considering hormone binding, hormonal imprinting and Ca^{2+} mediation. However, the taxon CU-399 is also a *T. thermophila* (1) with very similar behavior to *T. hegewishi* (which belongs to the *T. pyriformis* complex) in respect of mt-dehydrogenases. Likewise, *T. hegewishi* and *T. pigmentosa* are genetically related taxa, and T. malaccensis is genetically farther (15, 20). Nevertheless, studying the mt-dehydrogenase profile, *T. hegewishi* and *T. malaccens* are very related and *T. pigmentosa* is far from them. *T. pyriformis G1* has an intermediate position genetically, however nearer to *T. hegewishi* and *malaccensis,* than to *T. pigmentosa.* At the same time, *T. pyriformis* has a similar mt-dehydrogenase profile to *T. hegewishi* and *T. malaccensis.* This could support the importance of genetic relatedness. However, considering all of the data we believe, that the reactivity or unreactivity to insulin treatment by the mitochondrial dehydrogenases is dependent on the taxon itself and independent of the taxon complex.

The experiments demonstrate that insulin can influence the mitochondrial dehydrogenase activity of Tetrahymena. This influence-reduction of activity—is in contrast with the effect of insulin in mammals, where it stimulates the dehydrogenase activity (12, 19, 22). The reason of this difference is not known at present.

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REFERENCES

1. Banno, Y., Okano, Y., Furukawa, K., Tiedtke, A., Kobata, A. and Nozawa, Y. (1993) *J. Eukaryot. Microbiol.* **40:**515–520.
2. Burwen, S. J. and Jones, A. L. (1987) *TIBS* **12:**159–162.
3. Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D. and Mitchell, J. B. (1987) *Cancer Res.* **47:**943–946.
4. Christopher, G. K. and Sundermann, C. A. (1996) *Tissue and Cell* **28:**427–437.
5. Caba, G. (1980) *Biol. Rev.* **55:**47–63.
6. Csaba, G. (1985) *Int. Rev. Cytol.* **95:**327–377.
7. Csaba, G. (1986) *Experientia* **42:**715–719.
8. Csaba, G. (1994) *Int. Rev. Cytol* **155:**1–48.
9. Csaba, G. and Lantos, T. (1975) *Experientia* **31:**107–109.
10. Csaba, G. and Hegyesi, H. (1994) *Biosci. Rep.* **14:**25–31.
11. Denizot, F. and Lang, R. (1986) *J. Immunol. Methods.* **89:**271–277.
12. Flarsheim, C. E., Grupp, I. L. and Matlib, M. A. (1996) *Am. J. Physiol.* **271:**H192–202.
13. Horvath, A. (1978) *J. Cell. Physiol.* **97:**37–40.
14. Horvath, A. and Katsoyannis, P. G. (1975) *Biochim. Biophys. Acta* **382:**609–620.
15. Kovács, P. and Csaba, G. (1989) *Comp. Biochem. Physiol.* **94A:**627–631.
16. LeRoith, D., Schiloach, J., Roth, J. and Lesniak, M. A. (1980) *Proc. Natl. Acad. Sci. USA* **77:**6184–6186.
17. LeRoith, D., *et al.* (1982) *Proc. Natl. Acad. Sci. USA* **79:**2086–2090.
18. LeRoith, D. (1983) *Fed. Proc.* **42:**2602–2607.
19. MacDonald, M. J., Efendic, S. and Ostenson, C. G. (1996) *Diabetes* **45:**886–890.

20. Morin, G. B. and Cech, T. R. (1988) *Cell* **52:**367–374.
21. Mossman, T. (1983) *J. Immunol. Methods* **65:**55–59.
22. Moore, K. H., Tsatsos, P., Staudacher, D. M. and Kiechle, F. L. (1996) *Int. J. Biochem. Cell. Biol.* **28:**183–191.
23. Park, J. G., *et al.* (1987) *Cancer Res.* **47:**5875–5879.