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REPORT

The activity of lysosomal enzymes in blood serum, liver, kidney and skeletal muscle of rabbits divergently selected for locomotor activity in open-field test

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Twenty males were used from generation 8 of rabbits divergently selected for high (line H, n=10) *vs*. low (line L, n=10) locomotor activity in the open-field (OF-test). At the age of 98 days, blood samples were withdrawn from the ear vein and the rabbits were killed. Immediately, liver, kidney and the left femoral muscle samples were excised. In lysosomal fractions of blood serum and three tissues mentioned the activity of lysosomal enzymes AlaAP, LeuAP, ArgAP, Cat D and L, AcP, EL, LAL, BGRD, BGAL, BGLU, aGlu, MAN and HEX was determined. Significant interline differences were identified in activity of blood and tissue enzymes in question. The results suggest that the divergent selection for high *vs*. low locomotor activity in the open-field (OF-test) alters the lysosomal degradation processes in the organism.

KEY WORDS: lysosomal enzymes / open-field test / rabbits / selection

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The metabolic processes in live cells and tissues depend, among others, on specific rate of synthesis and degradation of basic energetic compounds. These processes are regulated by current activity of suitable enzymes [Nakano *et al*. 1995, Luzio *et al*. 2000, Witek 2000, Jóźwik *et al.* 2003, 2004], and they take place mainly in lysosomal system at low pH [Lange *et al*. 1998, Cabrita *et al.* 1999, Molano *et al*. 1999, Rider *et al*. 2000, Konecka *et al.* 2002].

It has been well established that the cell lysosomes are a natural and dynamic system that plays an important role in adaptative reactions of cells and of the whole organism as an entity. The lysosomal system restores the biochemical homeostasis that can be affected, for example, by stress factors [Kołątaj 1993, Dittmer *et al.* 1999, Itabe *et al.* 2000, Lankoff and Kołątaj 2000, Kołątaj *et al*. 2001, Śliwa-Jóźwik *et al.* 2002].

The mechanism that regulates functioning of the lysosomal enzymes in the cell is still not fully elucidated, especially in relation to animals of different genotypes, obtained by selection.

The open-field test (OF-test) is one of the oldest and most frequently used behavioural tests [Hall 1934, Prut and Belzung 2003]. There are different opinions whether it reflects the animal's needs for locomotion and/or for exploration, or rather fear reactions and behavioural inhibition of an animal in a novel surrounding [Stam *et al*. 1989, Ossenkopp *et al.* 1994, Prut and Belzung 2003, Ramos *et al.* 2003]. The OF-test has widely been used for a variety of purposes, e.g. for comparison of lines and strains of rats [Schmitt and Hiemke 1998] and rabbits [Zelnik *et al.* 1990, Ferrante *et al.* 1992], to evaluate crossbreeding effects and heterosis in rabbits [Brun *et al.* 2002], or to assess effects of handling [Denenberg *et al.* 1977]. Most often, however, the OF-test was used for examination the effects of anxiolytic drugs on behavioural changes in laboratory animals [Choleris *et al.* 2001, Ramos *et al.* 2003].

In the experiment arranged earlier by Daniewski and Jezierski [2003] on rabbits divergently selected for high *vs*. low reactivity in the open-field (OF-test), body weight of animals from two lines differed significantly in each generation ($P<0.001$). The lines differed also with respect to some correlated traits, namely at the age of 4-8 weeks the H rabbits were significantly lighter than those of the L line. Moreover, the H line females showed lower reproduction indicators. It seems highly probable that the H and L rabbits differed in their metabolic rate. Thus, the aim of the present study was to compare the rabbits from H and L lines with respect to the activity of some lysosomal enzymes in the blood, liver, kidney and skeletal muscle.

Material and methods

Twenty males were used from generation 8 of rabbits divergently selected for high (H line, n=10) or low (L line, n=10) locomotor activity in the OF-test. A detailed description of the OF-test and the selection procedure was given by Daniewski and Jezierski [2003]. The rabbits used for the present study were weaned at the age of

6 weeks and then kept individually in wire cages 60 × 55 × 30 cm, in a room with natural lighting, at temperature 5-8 °C in winter and 20-25 °C in summer. The rabbits were fed *ad libitum* with standard pelleted rabbit feed.

All procedures with rabbits were approved by the 3rd Local Commission for Ethics in Animal Experimentation, Warsaw, Poland (14.1/2007).

At the age of 98 days, blood samples were withdrawn from the ear vein and the rabbits were killed. Immediately, samples of liver, kidney and the left femoral muscle were excised and perfused in a cooled 0.9% NaCl solution. The lysosomal fractions were prepared according to Beaufay [1972]. In these fractions and in blood serum the activity of lysosomal enzymes was estimated using specific substrates.

The alanyl-aminopeptidase (AlaAP, EC 3.4.11.2), leucyl-aminopeptidase (LeuAP, EC 3.4.11.1), and arginyl-aminopeptidase (ArgAP, EC 3.4.11.6) activities were determined according McDonald and Barrett [1986].

The cathepsin D and L (Cat D., EC 3.4.23.5; Cat L., EC 3.4.22.15) activities were measured according to Langner *et al*. [1973].

The activities of acid phosphatase (AcP, EC 3.1.3.2), lysosomal esterase (EL, EC 3.1.1.2), lysosomal lipase (LAL, EC 3.1.1.13), β-D-glucuronidase (BGRD, EC 3.2.1.31), β-D-galactosidase (BGAL, EC 3.2.1.30), β-D-glucosidase (BGLU, EC 3.2.1.21), α-D-glucosidase (aGlu, EC 3.2.1.20), mannosidase (MAN, EC 3.2.1.25) and N-acetyl- β-D-hexosaminidase (HEX, EC 3.2.1.52) were measured according to Barrett and Heath [1972].

The activity of lysosomal enzymes was presented in nmol/mg protein/h. The protein was determined according to the modified method of Kirschke and Wiederanders [1984]. The spectrophotometric analyses were performed using the LambdaBio20 apparatus manufactured by PERKIN ELMER (USA).

For the statistical evaluation, the one-way ANOVA was used.

Results and discussion

In the serum (Fig. 1) the activities of the LAL, BGRD and BGAL were higher in H compared to L rabbits. Only activity of AlaAP was higher in animals of L line.

In the liver activities of all enzymes of line H, except BGLU, occurred significantly different from those found in liver of L line rabbits (Fig. 2). The activities of AlaAP, LeuAP, ArgAP, AcP, BGRD, BGAL, aGlu and MAN were significantly higher in H than in L line, while the activities of CatD,L, EL, LAL and HEX were significantly higher in line L.

In the kidney the activities of AlaAP, AcP, BGRD, BGAL, BGLU, aGlu, MAN and HEX were significantly higher in rabbits of the H line (Fig. 3). Cat D,L and EL demonstrated significantly higher activity in line L.

In the muscle (Fig. 4) the animals from H line showed higher activities of AcP, BGRD, BGLU, aGlu, MAN and HEX than those from L line. The AlaAP, LeuAP, ArgAP and LAL enzymes showed lower activities in H than in L line rabbits. The

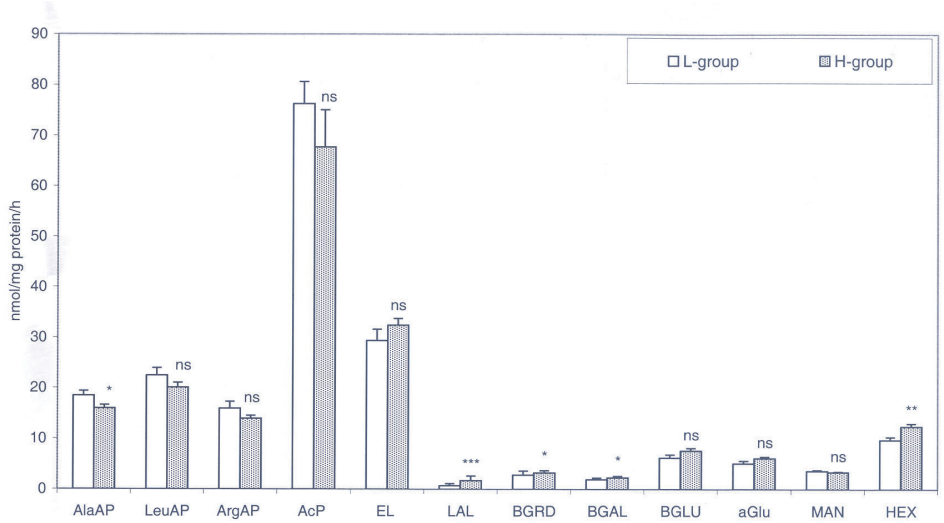

Fig. 1. The activity of estimated enzymes (nmol/mg of protein/hour) in serum.
Within each enzyme significant differences between lines at: *P≤0.05; **P≤0.01; ***P≤0.005; ns - non-significant.
The activity of LAL, BGLU and aGlu is expressed in 10* nmol/mg of protein/hour.

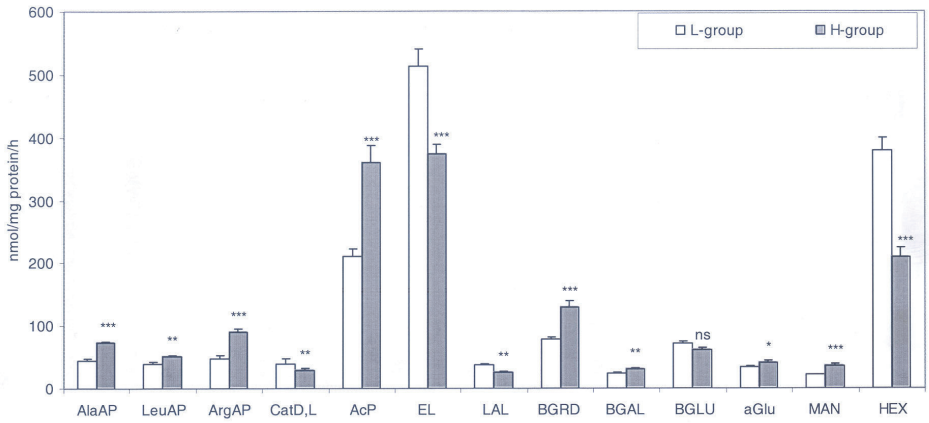

Fig. 2. The activity of estimated enzymes (nmol/mg of protein/hour) in liver.
Within each enzyme significant differences between lines at: *P≤0.05; **P≤0.01; ***P≤0.005. ns - nonsignificant.
The activity of Cat D and Cat L is 10* nmol/mg of protein/hour.

activity of CatD, L, EL, LAL and BGAL in the muscle did not differ significantly between lines.

The genetic selection for high locomotor activity in open field (OF-test) produced animals (H line) with higher motivation for exploration of the new surroundings and/

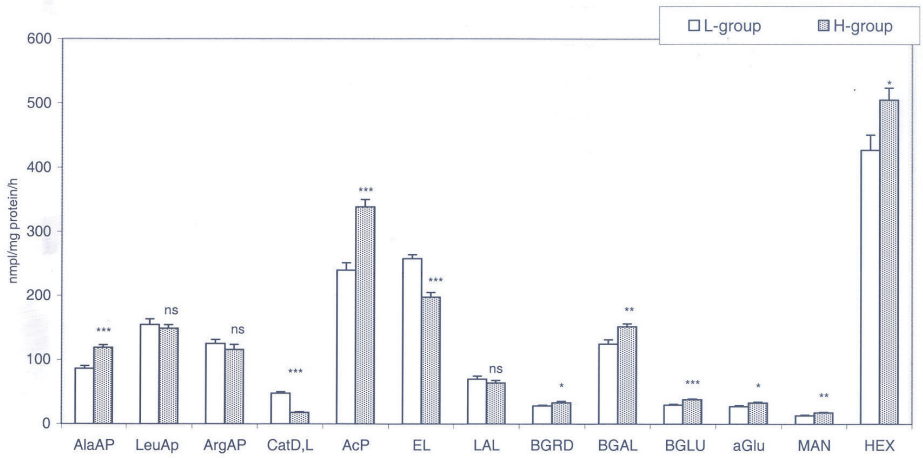

Fig. 3. The activity of estimated enzymes (nmol/mg of protein/hour) in kidney.
Within each enzyme significant differences between lines at: *P≤0.05; **P≤0.01; ***P≤0.005; ns - non-significant.
The activity of Cat D, Cat L and BGRD is expressed in 10* nmol/mg of protein/hour.

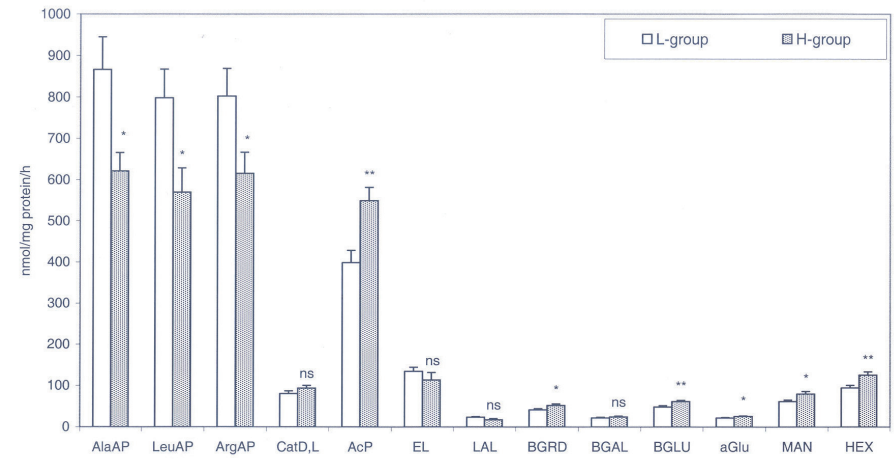

Fig. 4. The activity of estimated enzymes (nmol/mg of protein/hour) in muscle.
Within each enzyme significant differences between lines at: *P≤0.05; **P≤0.01; ***P≤0.005; ns - non-significant.
The activity of Cat D, Cat L, LAL, BGRD, BGLU and MAN is expressed in 10* nmol/mg of protein/hour.

or lower level of fear of novelty. Conversely, the rabbits of the L line (selected for low locomotor activity) demonstrated from the 4th selected generation, virtually no locomotor activity during the OF-tests. At the age of 4-8 weeks the L rabbits were

significantly heavier and the L females demonstrated a better fertility than the females H [Daniewski and Jezierski 2003]. It could be supposed that the metabolic rate in the L group was lower than in group H.

The activities of individual enzymes investigated in the blood serum, liver, kidney and skeletal muscle of line H rabbits were, in majority of cases, significantly different from those found in rabbits of line L. However, the trend of the differences did not follow the same pattern. Out of 13 enzymes studied, the BGRD of serum and all tissues was always more active in H than in L rabbits, while BGAL only in liver, kidney and serum.

The significantly higher activities of aminopeptidases, glicosidases (with except of BGLU) and AcP in the liver of H rabbits suggest the catabolic processes to prevail. The trend of the interline differences in the activity of the enzymes mentioned was similar in the serum, kidney, and muscle, being however opposite to that found in the liver.

The acid phosphatase is a main representative of lysosomal enzymes and a rise in its activity indicates an increase in the rate of energetic processes in the cell, which are related to the phosphate turnover. The activity of acid phosphatase was significantly higher in the liver, kidney and muscle of H than of L animals. It may reflect the rate of dephosphorilation of the products of proteolytic and lipolytic processes. A lower activity of EL in the liver and kidney of H rabbits may be related to a lower rate of lysosomal hydrolysis of some glycerol esters. The lower activity of lysosomal lipase (LAL) in the liver and muscle reveals a lower lipolytic activity in the H rabbits. Miszczuk-Jamska *et al.* [1993] found that changes in lysosomal lipase in human tissues are closely related to the level of lipids and cholesterol in their serum and lymphocytes.

Since lysosomal lipase takes part in the control of lipid level in cells and protects the liver, spleen and kidney cells from excessive burden by lipids, the ascertained decrease in the activity of LAL in the liver of H rabbits may indicate a lower turnover of lipid substances. This may also be related to a more intensive utilization of lipids for building of cell membranes or to the accumulation of lipids [Konecka *et al.* 2002, Jóźwik *et al.* 2003].

In conclusion, the divergent selection of rabbits for open-field locomotor activity changed the activity of lysosomal enzymes in some tissues as result of a correlated response to selection.

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Aktywność enzymów lizosomowych w wybranych tkankach i surowicy krwi królików selekcjonowanych rozbieżnie na aktywność ruchową w otwartym polu (test OF)

S t r e s z c z e n i e

Badania przeprowadzono na 20 samcach należących do ósmego pokolenia królików rozbieżnie selekcjonowanych na wysoką (H, n= 10) lub niską (L, n= 10) aktywność ruchową w otwartym polu (test OF). Krew z żyły usznej pobrano w 98 dniu życia królików, zwierzęta zdekapitowano i natychmiast pozyskano skrawki wątroby, nerki i mięśnia lewego uda, które poddano homogenizacji. We frakcjach lizosomowych czterech wymienionych materiałów oznaczono aktywność następujących enzymów: AlaAP, LeuAP, ArgAP, Cat D i L, AcP, EL, LAL, BGRD, BGAL, BGLU, aGlu, MAN i HEX.

Między dwiema selekcjonowanymi liniami królików stwierdzono istotne różnice w aktywności badanych enzymów. Uzyskane wyniki sugerują, że rozbieżna selekcja na wysoką i niską aktywność ruchową z zastosowaniem testu otwartego pola wpływa na przebieg lizosomowych procesów degradacyjnych w organizmie.