Adv Exp Med Biol - Clinical and Experimental Biomedicine (2019) 7: 89–95
https://doi.org/10.1007/5584_2019_425

Published online: 31 August 2019

Activity of Lysosomal Enzymes During Protein Malnutrition and Progesterone Supplementation in the Mouse

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Abstract

This study investigated the effects of protein malnutrition and progesterone supplementation on the activities of a spectrum of lysosomal enzymes in tissue fragments of mouse liver and kidney. The working hypothesis was that the known anti-stress action of progesterone could have to do with the inhibition of lysosomes which are engaged in apoptotic and oxidative stress-induced responses. The study investigated the effects of exogenous progesterone in chronically (3 weeks) protein-malnourished (10% protein) mice on the activities of lysosomal hydrolases in liver and kidney tissues. Progesterone was injected intraperitoneally in a dose of 2 μg/g body mass dissolved in a vehicle volume of 10 μL/g body mass during the final 3 days of exposure to either low 10% or standard 16% protein content in the chow. After euthanizing the animals, tissue fragments of liver and kidney assayed for the content of lysosomal enzymes. The results demonstrated the stimulating effect of protein malnutrition on lysosomal activities. We further found, contrary to our hypothesis, that progesterone supplementation during both standard and low-protein conditions enhanced lysosomal activities, particularly acting in concert with protein malnutrition in kidney tissue. The effects were selective concerning both lysosomal enzymes and tissues and of highly variable magnitude. Nonetheless, we believe we have shown that progesterone assists protein malnutrition in stimulation of lysosomal enzymes, which suggests the possibility of the hormone's engagement in cleansing the cellular milieu in disorders consisting of accumulation of toxic molecules.

Keywords

Lysosomal enzymes · Lysosomes · Mice · Progesterone · Protein restriction

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1 Introduction

Lysosomal enzymes are involved in the physiological degradation and recycling of cellular components, and thus in the maintenance of cellular homeostasis, which determines the adaptability of cells in response to changes in the surrounding environment. Beside the involvement in autophagy, these enzymes also are at play in the progression of apoptosis, notably in cellular responses to oxidative stress-induced apoptotic responses (Stanisławska et al. 2018; Brunk et al. 2001). Dysfunction of apoptosis may worsen the course of a spate of disorders, namely of atherosclerotic or neurodegenerative background, often having to do with enhanced oxidative stress. In such conditions enhancement of lysosomal hydrolytic activity might seem advantageous. However, there is an obvious dichotomy concerning the issue of lysosomal function, as in other pathologies, for instance, in diabetes where the enzymes are released from ruptured cell lysosomes, their degradative proteolytic effects may factor in microvascular diabetic complications (Witek et al. 2018).

Progesterone is produced by luteal cells of the corpus luteum in female ovaries and it enables the implantation of an embryo in the uterine mucosa and its maintenance during the beginning weeks of pregnancy. An adequate content of progesterone also is important for the proper course of a menstrual cycle and for the exfoliation of endometrium (Wu et al. 2018; Wessel et al. 2014). Progesterone also is a precursor of steroid hormones (Clark et al. 2018). Thus, progesterone's physiological action goes much beyond the preparation for pregnancy and its maintenance (De Ziegler et al. 2018). Progesterone is considered a hormone of adaptation and resistance to stress, including oxidative stress, and it has an anti-inflammatory function (Aksoy et al. 2014; Nadal et al. 2009). During stress, progesterone mitigates the production of adrenal cortisol and thus plays a protective role against the harmful effects of excess adrenocortical hormones (Miller 2018). Reproductive physiology in female mice is akin to that of other mammals concerning the peak secretion of progesterone in the estrus and post-estrus phase of the estrous cycle, except the cycle repeats itself every 5 days or so (Sato et al. 2016).

In this study we reasoned that the action of progesterone could have to do with the inhibition of lysosomes which are engaged in apoptosis and oxidative stress. We further hypothesized that any such action of progesterone could better come to light on the background of enhanced hydrolytic enzyme activity. Protein malnutrition is one condition that is reportedly reported to increase lysosomal enzyme activity, cellular catabolic rate, apoptosis, and oxidative stress (Mahadik et al. 2006; Iyengar and Vakil 1985). Therefore, we sought to define the effects of progesterone supplementation on the activities of a spectrum of lysosomal enzymes in the liver and renal tissues on the background of chronic protein-malnourished mice (10% protein), compared with standard protein nutrition (16% protein in chow). Contrary to our hypothesis, progesterone supplementation acted in concert with protein malnutrition to enhance the activities of lysosomal enzymes.

2 Methods

2.1 Animals and Study Protocol

The study was conducted in 60 Swiss female mice, weighing of 25.0 ± 1.3 g, aged 6 weeks. The animals came from the breeding farm of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzębiec, Poland. They were kept at 12-h light/dark cycle at a temperature of 20–22 °C with water ad libitum. The animals were divided into two groups of 30 each, differing in the content of protein provided in the chow: standard 16% protein content and low 10% protein content; the former providing 14.04 MJ/kg and the latter providing 13.47 MJ/kg of daily energy intake ($p > 0.05$). After 21 days, the two groups were further divided into the progesterone and control subgroups of 15 mice each. The former subgroup received i.p. injections of progesterone (Jelfa SA,

Jelenia Gora, Poland) in a dose of 2 μg/g body mass dissolved in a volume of 10 μL/g body mass of *oleum pro injectione*. The injections for the latter group consisted of *oleum pro injectione* alone, given in like manner. The scheme of injections was the same in both groups and consisted of a single injection daily, made at 8:00 am for three consecutive days.

At the time of weaning away from the mothers at the age of 6 weeks, the mice showed diestrous behavior. The quiescent period of the reproductive cycle was confirmed by the measurement of plasma progesterone at baseline before the commencement of its administration. The level of progesterone was 14.5 ± 2.7 ng/mL and 15.8 ± 3.4 ng/mL in the 16% protein and 10% protein groups, respectively; $p > 0.05$. The dose of exogenous progesterone was chosen on the inferential basis, supported by a couple of pilot trials, so that it would cause a 50–60% increase in blood progesterone content. The presumptive reasoning was that this kind of progesterone excess would be sufficient to induce changes in the activity of the lysosomal enzymes investigated; yet not interfering with the course of systemic physiological processes.

2.2 Lysosomal Enzymes

Six hours after the last injection of progesterone on Day 3 of the experimental paradigm, the mice of all groups were decapitated, and the kidneys and liver were immediately removed. The organs were superfused with chilled 0.9% NaCl and tissue fragments were homogenized in 0.1 mM phosphate buffer of pH 6.0 at a concentration of 0.5 g of solute in a final volume of 5 mL of buffer solution (10% m/v) at 200 RPM (Potter Elvehjem; Thomas Scientific; Swedesboro, NJ). Homogenates were further subjected to fractionated centrifugation, according to the method of Beaufay (1972), to obtain a lysosomal tissue fraction. Then, activities of the following lysosomal hydrolases were determined: acid phosphatase, β-N-acetyl-hexosaminidase, β-galactosidase, β-glucuronidase, lysosomal esterase, lysosomal lipase, alanine aminopeptidase, and leucine aminopeptidase. The exact method use for the determination of each lysosomal hydrolase, including the substrates used is depicted in Table 1.

Extinction measurements were made at 520 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific; Waltham, MA). The protein content was determined by a modified method of Kirschke and Wiederanders (1984). The activity of lysosomal enzymes was expressed in nmol/mg protein/h. All substrates were produced by SERVA Feinbiochemica (Heidelberg, Germany).

Data were presented as means ±SD. Differences in the mean values of enzyme activity were assessed using a paired or unpaired two-tailed *t*-test as required. A p-values <0.05 defined statistically significant differences.

3 Results

Blood plasma content of progesterone increased after the 3 days of progesterone administration from 14.5 ± 2.7 to 23.4 ± 0.8 ng/mL and from 15.8 ± 3.4 to 26.6 ± 1.2 ng/mL in the standard

Table 1 Lysosomal enzymes investigated

Enzyme	Symbol and EC number	Method
Acid phosphatase	AcP (EC 3.1.3.2)	Barrett and Heath (1977)
β-N-acetyl-hexosaminidase	Hex (EC 3.2.1.52)	
β-galactosidase	β-Gal (EC 3.2.1.23)	
β-glucuronidase	β-GlcUr (EC 3.2.1)	
Lysosomal esterase	EL (EC 3.1.1.2)	
Lysosomal lipase	LL (EC 3.1.1.13)	
Alanine aminopeptidase	AlaAP (EC 3.4.11.2)	McDonald and Barrett (1986)
Leucine aminopeptidase	LeuAP (EC 3.4.11.1)	

Table 2 Activity of lysosomal enzymes (nmol/mg protein/h) in the mouse liver after 3-day-long progesterone administration on the background of standard 16% nutritional protein and protein restriction to 10%

	16% Protein		10% Protein	
Enzyme	Control	Progesterone	Control	Progesterone
AcP	4.65 ± 1.17	6.79 ± 1.99**	43.00 ± 4.07†††	41.70 ± 5.57
Hex	3.56 ± 1.01	5.72 ± 1.89***	24.70 ± 3.04†††	24.40 ± 3.53
β-Gal	9.01 ± 2.05	14.7 ± 5.32***	3.13 ± 0.48†††	4.16 ± 0.99*
β-GlcUr	0.38 ± 0.10	0.52 ± 0.01*	0.41 ± 0.06	0.52 ± 0.06*
EL	3.35 ± 0.80	2.97 ± 0.74	2.17 ± 0.20††	3.41 ± 0.42***
LL	3.49 ± 0.72	5.36 ± 1.03***	3.04 ± 0.46	4.19 ± 0.71*
AAP	4.98 ± 1.23	6.54 ± 1.04*	13.90 ± 1.07†††	23.10 ± 3.58***
LAP	3.22 ± 0.87	4.64 ± 1.09**	26.40 ± 2.91†††	30.80 ± 1.95

Data are means ±SD. *AcP* acid phosphatase, *Hex* β-N-acetyl-hexosaminidase, *β-Gal* β-galactosidase, *β-GlcUr,* β-glucuronidase, *EL* lysosomal esterase, *LL* lysosomal lipase, *AAP* alanine aminopeptidase, *LAP* leucine aminopeptidase, *Cath D* cathepsin D

*$p \leq 0.05$; **$p \leq 0.01$; and ***$p \leq 0.001$ for differences between progesterone and control in either protein content; $^{\dagger\dagger}p < 0.01$ and $^{\dagger\dagger\dagger}p < 0.001$ for differences in the control level, i.e., the effect of protein restriction alone

16% protein and 10% protein malnourished groups, respectively. The increases of plasma progesterone were significant ($p < 0.001$) in either group, but the differences between the two groups were not ($p > 0.05$).

Changes in the activities of lysosomal enzymes in both liver and renal tissues in response to chronic protein malnutrition and the effects of progesterone administration on these activities are presented in Tables 2 and 3. Protein restriction caused distinct several-fold increases in the content of acid phosphatase, hexosaminidase, alanine aminopeptidase, and leucine aminopeptidase and decreased β-galactosidase in both tissues, with the activity of the other enzymes being rather mildly affected.

Administration of progesterone on the background of the standard 16% protein content in the chow caused significant increases in all enzymes in both tissues, except lysosomal esterase in the liver and β-galactosidase, β-glucuronidase, and alanine aminopeptidase in the kidney, whose content fluctuated around the baseline level. Likewise, there were strong increases in the enzyme activities after progesterone administration on the background of nutritional protein restriction to 10%. The content of all enzymes significantly increased in both tissues, except acid phosphatase and β-N-acetyl-hexosaminidase in the liver and alanine aminopeptidase in the kidney. None of the enzymes in either liver or kidney and at either nutritional protein level was significantly inhibited by progesterone administration.

4 Discussion

The findings of this study were that the plasma content of the majority of lysosomal enzymes investigated increased in the mouse tissue fragments of both liver and kidney during protein malnutrition. However, stimulation of hydrolytic activity was rather selective and of highly variable magnitude. Notably, there were many-fold increases in acid phosphatase, β-N-acetyl-hexosaminidase, and leucine aminopeptidase activities; the major proteolytic enzymes responsible for breaking down toxic molecules and participating in peptide recycling. On the other side, β-galactosidase was inhibited by about 75% and 50% in the liver and kidney tissues, respectively. There was an apparent tissue selectivity of the enzyme responses as β-glucuronidase and lysosomal lipase tended changed inversely in the liver and renal tissues.

Administration of progesterone caused further significant increases in the activities of enzymes on the background of protein restriction-induced stimulation. The increases remained enzyme and tissue selective and highly variable, amounting to about 22% for alanine aminopeptidase in both tissues, 63% and 182% for β-galactosidase in

the liver and kidney, or about 50% and 100% for lysosomal esterases and lipases in the liver and kidney, respectively. Progesterone administration also increased lysosomal enzyme activities during the standard protein nutrition. The findings give a consistent impression that protein restriction with progesterone on top of it potentiated the increases in lysosomal enzymes, particularly concerning the kidney. However, a substantial spread of the enzyme data in the protein conditions used in this study makes a meaningful direct comparison unfeasible. Nonetheless, it seems a reasonable assumption that enhanced activity of lysosomal enzymes noticed would be an expression of intensified engagement in cleansing the liver and renal cellular milieu from biological debris to revert to normal homeostatic function.

The present findings are in line with previous studies showing an activation of lysosomal enzymes during protein malnutrition. Iyengar and Vakil (1985) have observed stimulation of selective lysosomal enzymes during restriction of protein intake in the rat. Aside from the stimulation, the authors report an inhibition of β-glucuronidase activity by 60% in 4% protein-fed rats. In the present study, we noticed about 33% inhibition of the enzyme's activity in 10% protein-fed mice in the kidney, but not the liver. Likewise, Tutel'ian et al. (1987) have shown an increase in lysosomal enzyme activities in the rat liver during restriction of protein intake. Glew et al. (1982) have shown a threefold increases in serum hexosaminidase and 0.5-fold increase in beta-glucuronidase activities on 3% casein diet for 2–4 weeks in the rat kidney, but decreases in acid phosphatase. The authors ascribe such changes to profoundly altered tissue blood perfusion during protein malnutrition, leading to secondary release of lysosomal enzymes. Muñoz-Martínez et al. (1982) have found a strong increase in beta-glucuronidase in the rat liver during protein intake restriction to 1%. In contrast, acid phosphatase remained unmodified. Muñoz et al. (1981) have also found increases in beta-glucuronidase and acid phosphatase in the rat spleen and thymus after 4 weeks of protein intake restriction to 4%, although there were decreases in some other hydrolytic enzymes. Yet, the effects of protein intake restriction on lysosomal enzyme activity remains a contentious issue. Other studies show an inhibition of lysosomal enzyme during protein restriction (Volgarev et al. 1984). It appears that the mechanisms regulating catabolic activity of lysosomes are unclear. Divergent literature data are likely due to different protocols of protein malnutrition, varying from 1% through 4%, 8%, and 14% protein content in the chow, different periods of exposure, different animal species, and different tissues being investigated, let alone the methodological differences of assays used in the past studies. The lack of experimental standardization makes it hard to draw definite conclusions on the issue.

In this study, interestingly, the majority of lysosomal hydrolases increased their activity under the influence of exogenous progesterone. That raises a suggestion that progesterone, one of the main female fertility regulating hormones, acts to intensify the catabolic rate of liver and kidney cells. These effects seem intensified by protein intake restriction. The observed increases in the activity of glycosidases (β-Gal and β-GlcUr) could be associated with increased glucose production and a breakdown of own proteins in the framework of renal cell response to decreased protein intake (Kilicalp et al. 2005). How exactly progesterone would cause an increase in the activities of lysosomal enzymes is however open to conjecture.

An incorrectly balanced level of protein intake causes negative consequences in the area of cellular protein degradation. Alleman et al. (2000) have reported that a downward shift in protein intake, from 16% to 10%, in chickens increases the content of triiodothyronine, thyroxine, and insulin-like growth factor-1 in the blood, which, in turn, increases the amino acid transport to muscles and the intensity of ongoing proteolytic processes. As a consequence, alanine aminopeptidase activity may increase as well. In the present study, alanine aminopeptidase increased by about 180% and 55% in the mouse liver and kidney, respectively, in response to protein intake restriction to 10%. The increases could be a natural adaptive cellular reaction since protein

Table 3 Activity of lysosomal enzymes (nmol/mg protein/h) in the mouse kidney after 3-day-long progesterone administration on the background of standard 16% nutritional protein and protein restriction to 10%

	16% Protein		10% Protein	
Enzyme	Control	Progesterone	Control	Progesterone
AcP	14.40 ± 2.59	19.70 ± 4.46*	30.50 ± 2.98†††	53.70 ± 5.03***
Hex	2.83 ± 0.41	3.96 ± 0.94*	28.60 ± 2.58†††	43.10 ± 3.71***
β-Gal	7.30 ± 0.81	7.97 ± 1.62	3.58 ± 0.29†††	10.10 ± 0.69***
β-GlcUr	0.40 ± 0.10	0.35 ± 0.07	0.27 ± 0.04†††	0.62 ± 0.06***
EL	3.72 ± 0.93	5.02 ± 1.32*	2.69 ± 0.30†	5.29 ± 1.05***
LL	0.48 ± 0.05	0.62 ± 0.09*	0.53 ± 0.03	1.09 ± 0.06***
AAP	120.60 ± 13.3	107.10 ± 9.29	187.70 ± 13.30†††	229.10 ± 25.20
LAP	42.00 ± 6.92	64.90 ± 16.50***	126.60 ± 7.71†††	171.90 ± 14.10*

Data are means ±SD. *$p \leq 0.05$ and ***$p \leq 0.001$ for differences between progesterone and control in either protein condition; $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$, and $^{\dagger\dagger\dagger}p < 0.001$ for differences in the control level, i.e., the effect of protein restriction alone. See Table 2 for the acronyms of lysosomal enzymes

malnourished animals show a positive nitrogen balance for a period of time, while lowering the level of urea.

Lysosomal esterase and lipase were little altered with protein restriction in this study. However, a combination of progesterone with either standard or lowered protein content strongly enhanced the activity of both enzymes (Tables 2 and 3), which are mostly engaged in the degradation of fatty acid esters and cholesterol. These findings are in line with those of Yoshizawa et al. (1997), who have reported that protein malnutrition causes increases in free fatty acids and glycerol in the blood and in the percentage of fat in the liver and kidneys, which may increase the need for the action of lipases. An enhancement of the activity of both enzymes under the influence of progesterone implies that the hormone accelerates tissue lipid turnover.

In clinical diagnostics and treatment, lysosomal enzymes are increasingly used to assess the extent of genetic lysosomal storage disorders, such as Fabry's or Gaucher's diseases, and others in which there is an insufficient degradative processing of sphingolipid molecules that accumulated in cells, mostly in the brain (Giugliani et al. 2018). The enzyme replacement therapy faces hurdles since recombinant enzymes cannot sufficiently penetrate through the blood-brain barrier. The findings of the present study and other past studies showing the enhancement of lysosomal enzymes during low-protein nutrition offer a simple approach to enzyme activation. Likewise, exogenous supplementation of progesterone seems a similar effective route to increase the activity of lysosomal enzymes. Whether this would also be an effective measure to increase lysosomal enzyme activity in brain tissue is unknown. The low-protein-progesterone supplementation method would require trial investigations and tailoring to the specific clinical entities and individual patients, as well as the explorations addressing individual enzymes, all of which requires alternative study designs.

In conclusion, the findings of this study hint on the potential interaction of low protein nutrition and modest supplementation of progesterone in enhancing lysosomal enzyme activity. To the extent that lysosomes serve as the cell's detoxifying and molecular waste recycling centers, the enhancement of their function might have a bearing in disorders that consist of excessive intra-cellular accumulation of pathological molecules.

Conflicts of Interest The authors report no conflicts of interest in relation to this article.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study protocol was approved by the Bioethics Commission of the Swiętokrzyska Medical Chamber (permit 47/2016).

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