

## Effect of Hydrocortisone on the Activity of some Lysosomal Enzymes in Mice

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### Abstract

**OBJECTIVES:** Changes in the activity of cathepsin D and L, alanine aminopeptidase, leucine aminopeptidase, N-acetyl- $\beta$ -glucosaminidase, lysosomal arylesterase and lysosomal lipase in the liver and kidney of unselected and selected mice, subjected to 7.5 mg/kg b.w. of hydrocortisone injection for 4 and 8 days.

**METHODS:** The homogenates of the liver and kidney were subjected to differentiated centrifuging and determination of studied enzymes.

**RESULTS:** Injection of hydrocortisone caused an increase in the activity of all investigated lysosomal enzymes in the liver and kidney of mice.

**CONCLUSION:** The reactions of selected mice were stronger in comparison with unselected ones. The highest increase in the activity investigated enzymes was observed after 8 days of hydrocortisone injection.

## Introduction

Glucocorticosteroids, and first of all hydrocortisone (cortisol), have influence on the metabolism of proteins, lipids and carbohydrates, and only in physiological concentrations plays an important role in regulatory processes, such as for instance in maintaining intracellular homeostasis [1–4]. The metabolic effects of hydrocortisone action arises from the type of the target tissue. For example, in muscles, adipose and lymphatic tissues it reveals catabolic activity, but in liver it stimulates synthesis and glycogen storage [5–7]. In physiological conditions adrenal cortex secretes about 20 mg of hydrocortisone within twenty-four hours, but during stressful reaction its secretion can increase even 10–times [8].

Environmentally unfavourable effects on animals cause, among much else, stress responses. One of the cell arrangements which take part in those responses is the lysosomal compartment. The physiological importance of the lysosomal structure has been described by numerous authors, which agree that it is the principal site of intracellular degradation processes [9–15]. It also a terminal compartment for the intracellular transportation of newly synthesised lysosomal enzymes [16].

In our studies we observed the activity of some lysosomal enzymes of mice subjected to hydrocortisone injections as model factors affecting homeostasis of an organism. The aim of our experiment was to determine the influence of the time of administration of exogenous hydrocortisone on the activity some proteolytical enzymes, glycosidases and lipases in the liver and kidney of mice selected on economic feed consumption and unselected ones, whose parents were matched at random.

## Material and methods

The experiment was carried out on 30 56-day-old males mice from a line selected for 12 generations for economic feed consumption and 30 unselected mice, whose parents were chosen from a random match. The animals were bred in the Institute of Genetics and Animal Breeding, the Polish Academy of Sciences in Jastrzebiec. They were constantly maintained in a ventilated room at 21 °C with 12 h daylight and 12 h darkness. Mice were fed with standard “Murigran” feed –16% of protein, (Animal Food Company, Lomna near Warsaw, Poland), with constant access to water. All animals received good veterinary care.

Mice were divided into groups (I–III selected mice, and IV–VI unselected mice;  $n = 10$  in each group) and injected intraperitoneally daily (8:00 a.m.) according to the following scheme:

- I control 250  $\mu$ l 0.9% NaCl
- II hydrocortisone 7.5 mg/kg b.w. 4 days
- III hydrocortisone 7.5 mg/kg b.w. 8 days
- IV control 250  $\mu$ l 0.9% NaCl
- V hydrocortisone 7.5 mg/kg b.w. 4 days

VI hydrocortisone 7.5 mg/kg b.w. 8 days

The mice of experimental groups (II, III, V, VI) received daily 7.5 mg/kg b.w. of exogenous hydrocortisone (Hydrocortisonum hemisuccinatum, Pharmaceutical Company Jelfa, SA, Poland).

The mice were killed by breaking the spinal cord, and the slices of the liver and kidney were perfused with 0.9% NaCl solution cooled to +5 °C. The liver and kidney slices were suspended in 0.1 M phosphate buffer cooled to +5 °C at pH 7.0 (500 mg tissue/5ml buffer), and homogenized in a Potter homogenizer with a teflon piston at 200 rot./min. The liver and kidney homogenates were subjected to differentiated centrifuging according to [17].

In the lysosomal fractions of liver and kidney the activity (nmol/mg of protein/hour) of cathepsin D and L (Cath. D, EC 3.4.23.5 and Cath. L, EC 3.4.22.15) according to [18]; alanine aminopeptidase (AAP, EC 3.4.11.2) according to [19]; leucine aminopeptidase (LAP, EC 3.4.11.1) according to [20]; N-acetyl- $\beta$ -glucosaminidase (NAG, EC 3.2.1.30) according to [21]; lysosomal arylesterase (EL, EC 3.1.1.2) and lysosomal lipase (LL, EC 3.1.1.3) according to [22].

Protein was also determined in the lysosomal fractions [23]. All substrates were from Serva Feinbiochemica GmbH & Co., Heidelberg, Germany. The results obtained were analyzed statistically according to Student's  $t$  test.

The experiment was approved by the Ethics Commission for Animals Research of the Swietokrzyska Academy in Kielce.

## Results

As can be seen from Tables 1–4, hydrocortisone injection for 4 and 8 days caused statistically confirmed an increase in the activity of all the investigated lysosomal enzymes in liver and kidney of selected and unselected mice. The highest increase in the activity was observed in the liver and kidney of selected mice after 8 days of hydrocortisone injection.

## Discussion

The mechanism of hydrocortisone action on the course of inflammatory, immune and neoplastic processes has not been explained yet; however, anti-inflammatory properties of this hormone deserve a special attention in relationship to its participation in the inhibition of the immune reactions of organism connected with the grafts rejection [24–27]. In physiological concentrations, hydrocortisone contributes to the maintenance of the homeostasis of the metabolism of carbohydrates, proteins and lipids. Many data show that it plays the part of a stabilizer of cellular membranes – especially lysosome ones [28]. In such a situation, usually active lysosomal enzymes remain imprisoned within the lysosomal membranes, the effect of which can be, among others, the inhibition of the exudative phase in the course of allergic reactions

and acute and chronic inflammatory states [29–30].

Use of pharmacological doses of hydrocortisone, which exceed the physiological secretion of adrenal cortex may lead to disturbances of intracellular homeostasis and, in effect, to changes in the activity of the investigated lysosomal hydrolases [31]. A prolonged hydrocortisone administration in doses exceeding physiological ones leads to hyperglycaemia and adrenogenous diabetes because it lowers the use of glucose by cells, its transport through cellular membranes, and contributes to the decrease of glycolysis in peripheral tissue [32–34]. Hydrocortisone may also cause essential changes in the metabolism of proteins, increasing their catabolism and mobilization of amino acids mostly in muscles and bone tissue, as well as intensifying transformations of amino acids in hepatocytes [35].

The results obtained show that the injection of hydrocortisone caused a significant increase in the activity of all the investigated lysosomal enzymes. Our investigation has noted an increase in the activity of both cathepsins (Cath. D and L), aminopeptidases (AAP and LAP), lysosomal lipases and also lysosomal glycosidase (NAG), hydrolyzing glycoproteins and glycolipids [36]. Increase in the activities of lysosomal lipases is connected with the properties of hydrocortisone, which determines, among others, the course lipolysis and gluconeogenesis. The observed increase in the activity of both lysosomal lipases in the liver and kidney of mice subjected to the activity of this hormone is most likely connected with the mobilization of lipids and their intensive degradation. Administration of hydrocortisone caused an increase in the range and rate of the synthesis of lysosomal lipase and lysosomal arylesterase in the liver and kidney of selected mice [37–39].

The observed changes of the activity of the investigated lysosomal enzymes after hydrocortisone injection suggest that, lysosomal compartment reacts as one of the first cytoplasmic systems by activating the resistance mechanisms in situations constituting a threat to the maintenance of the existing homeostasis, they are also connected with the labilization of lysosomal membranes and the increase of their permeability and a release of proteases to the cytosol. In effect, this disturbs the functioning of the lysosomal system and leads to an irreversible destruction of cells [40–42].

**Table 1.** The activity of lysosomal enzymes ( $\bar{x} \pm SD$ ) in the liver of selected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

Enzyme	Control	Hydrocortisone			
		four days	%	eight days	%
<b>Cath.D and L</b>	0.076 ± 0.014	0.092 ± 0.017	<b>121</b>	0.112 ± 0.028	<b>147</b>
<b>AAP</b>	0.590 ± 0.201	0.646 ± 0.165	<b>109</b>	0.799 ± 0.235	<b>135</b>
<b>LAP</b>	1.25 ± 0.277	1.98 ± 0.770	<b>158</b>	2.75 ± 0.864	<b>220</b>
<b>NAG</b>	1.14 ± 0.193	1.19 ± 0.215	<b>104</b>	1.34 ± 0.396	<b>117</b>
<b>EL</b>	1.06 ± 0.215	1.57 ± 0.197	<b>148</b>	1.93 ± 0.421	<b>182</b>
<b>LL</b>	1.49 ± 0.320	1.51 ± 0.088	<b>101</b>	1.56 ± 0.109	<b>105</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – statistically confirmed differences;

**Table 2.** The activity of lysosomal enzymes ( $\bar{x} \pm SD$ ) in the liver of unselected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

Enzyme	Control	Hydrocortisone			
		four days	%	eight days	%
<b>Cath.D and L</b>	0.067 ± 0.023	0.075 ± 0.015	<b>112</b>	0.091 ± 0.023	<b>136</b>
<b>AAP</b>	0.390 ± 0.056	0.395 ± 0.158	<b>101</b>	0.437 ± 0.101	<b>112</b>
<b>LAP</b>	1.09 ± 0.457	1.19 ± 1.03	<b>109</b>	1.24 ± 0.499	<b>114</b>
<b>NAG</b>	0.690 ± 0.098	0.713 ± 0.196	<b>103</b>	0.987 ± 0.200	<b>143</b>
<b>EL</b>	0.910 ± 0.076	1.03 ± 0.896	<b>113</b>	1.09 ± 0.872	<b>119</b>
<b>LL</b>	2.33 ± 1.03	2.71 ± 0.977	<b>116</b>	2.96 ± 1.22	<b>127</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – statistically confirmed differences;

**Table 3.** The activity of lysosomal enzymes ( $\bar{x} \pm SD$ ) in the kidney of selected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

Enzyme	Control	Hydrocortisone			
		four days	%	eight days	%
<b>Cath.D and L</b>	0.222 ± 0.050	0.380 ± 0.110	<b>171</b>	0.410 ± 0.150	<b>185</b>
<b>AAP</b>	2.66 ± 0.546	3.15 ± 1.13	<b>118</b>	3.25 ± 0.998	<b>122</b>
<b>LAP</b>	2.97 ± 0.410	3.49 ± 0.978	<b>117</b>	3.58 ± 0.876	<b>120</b>
<b>NAG</b>	0.648 ± 0.146	0.836 ± 0.099	<b>129</b>	0.901 ± 0.130	<b>139</b>
<b>EL</b>	1.52 ± 0.453	1.99 ± 0.900	<b>131</b>	2.15 ± 1.22	<b>141</b>
<b>LL</b>	0.208 ± 0.101	0.290 ± 0.034	<b>139</b>	0.299 ± 0.055	<b>144</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – the differences statistically confirmed;

**Table 4.** The activity of lysosomal enzymes ( $\bar{x} \pm SD$ ) in the kidney of unselected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

Enzyme	Control	Hydrocortisone			
		four days	%	eight days	%
<b>Cath.D and L</b>	0.810 ± 0.067	0.886 ± 0.103	<b>109</b>	0.900 ± 0.897	<b>111</b>
<b>AAP</b>	1.77 ± 0.870	1.80 ± 0.874	<b>102</b>	2.03 ± 1.02	<b>115</b>
<b>LAP</b>	1.98 ± 0.911	2.23 ± 1.02	<b>113</b>	2.13 ± 1.00	<b>107</b>
<b>NAG</b>	0.950 ± 0.220	1.13 ± 0.794	<b>119</b>	1.12 ± 0.996	<b>118</b>
<b>EL</b>	0.590 ± 0.090	0.688 ± 0.099	<b>117</b>	0.743 ± 0.197	<b>126</b>
<b>LL</b>	1.15 ± 0.796	1.39 ± 0.076	<b>121</b>	1.43 ± 0.764	<b>124</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – the differences statistically confirmed;

Our investigation has shown that mice selected for economic feed consumption reacted more intensively than unselected individuals, and they proved to be more susceptible to the hydrocortisone action. Changes in the activity of lysosomal enzymes in this group of mice former were higher in comparison with unselected mice.

The mechanisms regulating the activity of the lysosomal system and the secretion of its enzymes still evoke considerable interest in numerous biochemical

laboratories as indicated by the increasing number of papers on this topic found in the literature [43–46].

As until now the mechanisms which control the activity of lysosomal compartment have not been explained, the investigation performed induces one to widen the range of investigation using the changes of the activity of lysosomal enzymes to the observation of the course of adaptation reaction in humans and of animals.

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