

ACTIVITY OF DEHYDROGENASES AND ENZYMES OF NITROGEN METABOLISM IN CARDIAC TISSUE AND SKELETAL MUSCLE OF STEERS FED MONENSIN

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Abstract

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Activities of lactate dehydrogenase (E.C. 1.1.1.27), malate dehydrogenase (E.C.1.1.1.40), 2-oxoglutarate dehydrogenase, (E.C.1.2.4.2) glutamate dehydrogenase (E.C.1.4.1.3), glutamin synthetase (E.C.6.3.1.2), arginase (E.C.3.5.3.1), ornithine carbamoyltransferase (E.C.2.1.3.3), aspartate aminotransferase (E.C.2.6.1.1) and alanine aminotransferase (E.C.2.6.1.2) were measured in cardiac and skeletal muscles of steers fed ration with or without monensin. Steers, 9 months old at the beginning, were fed concentrate, molasses, grass, lucerne and maize chaff. Five steers received monensin in amount of 0.5 mg per 1 kg of live body mass per day. Control ration, without monensin was fed to the other five steers. After 10 months steers were slaughtered and activity of enzymes assayed in the mitochondrial and cytoplasmic fraction of cells. Monensin increased activity of 2-oxoglutarate dehydrogenase and decreased activity of glutamate dehydrogenase in both fractions of the skeletal muscle tissue. Enzymatic activities found in heart mitochondria were higher in monensin-fed steers than in control steers. Steers given monensin gained 8.23 % more than control steers (263 vs 243 kg).

Monensin, enzyme, heart, muscle

Feed antibiotics, including monensin, have the ability to improve performance and feed efficiency in ruminants. It is known that monensin is absorbed from the alimentary tract (D a v i s o n 1983; D o n o h o 1984) and several authors demonstrated its influence on intermediary metabolism of ruminants (A r m s t r o n g and S p e a r s 1988; B e n z et al. 1989; M a r o u n e k et al. 1989). Little is known about the effect of monensin on activity of tissue enzymes in ruminants. K a l a č n j u k et al. (1993) measured activity of various enzymes in rumen mucosa and liver of steers fed monensin at recommended level. Authors found no consistent effect of monensin on enzymes of both tissues. In this paper we report data on activity of nine enzymes in cardiac and skeletal muscles of steers fed ration with or without monensin for ten months. Cardiac and skeletal muscles are primary target tissues at high intake of monensin (T o d d et al. 1984; V a n V l e e t et al. 1985) and, presumably, also during long-term supplementation of rations under normal feeding conditions.

Materials and Methods

Ten crossbred steers, 9 months old at the beginning of experiment, were divided into two groups, according to feed additive treatment. Steers were individually housed and kept on a diet consisting of concentrate (1 kg per 100 kg of the live weight), molasses (0.5 - 1.0 kg) and grass, lucerne and maize chaff ad libitum. In winter, the roughage portion of the diet consisted of maize silage and beet ad libitum. Concentrate contained ground barley (63%), dried poultry litter from broilers which did not receive a ionophore in their diet (20%), grass meal (15%) and zeolite (2%).

Five steers received monensin (Elanco, USA) in amount of 0.5 mg per 1 kg of live weight daily. Initial weight of steers was 209 kg and 206 kg in the control and treated group, respectively. After 10 months steers were slaughtered, samples of tissues taken (heart, musculus longissimus dorsi) and stored in liquid nitrogen until analyzed. Samples were pulverized and homogenized in the Potter-Elvehjem homogenizer. Mitochondrial and cytosolic fraction of cells were obtained by differential centrifugation according to H o g e b o o m (1955). Activities of nine enzymes were assayed at 37 °C in extracts, which were prepared by method of M o r t o n (1955). Lactate dehydrogenase (LDH), malate dehydrogenase (MDH), 2-oxoglutarate dehydrogenase (OGDH), glutamate dehydrogenase (GDH), glutamin synthetase (GS), arginase (A), and ornithine carbamoyltransferase (OCT) were assayed using established methods, which were compiled by C o l l o w i c k and K a p l a n (1955 a,b). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using Bio-la-tests (Lachema, Brno, Czech Rep.). Protein contents in samples was measured according to L o w r y et al. (1951). Student's *t*-test was used for the statistical evaluation of the significance of the differences.

Results

Lactate dehydrogenase belongs to principal glycolytic enzymes. As expected, its activity was higher in cytosol than in mitochondria (Tables 1, 2). Malate dehydrogenase and 2-oxoglutarate dehydrogenase function in the citric cycle and their activities were, therefore, higher in mitochondria than in cytosol. Arginase and ornithine carbamoyltransferase are enzymes of the urea cycle. The former enzyme was more active in the cytosolic fraction, whereas the latter one had higher activity in mitochondria. Activity of aspartate aminotransferase was higher in mitochondria than in cytosolic fraction. Activity of alanine aminotransferase was almost uniformly distributed in both fractions of the cellular material.

Table 1

Enzymatic activities¹ in samples of musculus longissimus dorsi from control and monensin-fed steers

Enzyme ²	Mitochondria		Cytosolic fraction	
	Control	Monensin	Control	Monensin
LDH ³	22.7 ± 2.8	23.7 ± 2.0	2039 ± 40	2008 ± 218
MDH ⁴	2279 ± 267	2327 ± 428	367 ± 45	424 ± 15
OGDH ⁵	32.3 ± 2.3	44.3 ± 3.7*	22.0 ± 3.4	29.5 ± 2.0*
GDH ⁶	20.6 ± 3.0	16.5 ± 1.7*	6.4 ± 0.6	4.8 ± 0.6*
GS ⁷	2.1 ± 0.7	1.4 ± 0.3	0.7 ± 0.1	0.8 ± 0.1
A ⁸	92.7 ± 26.7	79.6 ± 6.3	332 ± 67	333 ± 79
OCT ⁹	13.8 ± 2.3	8.1 ± 0.8	6.9 ± 0.7	7.7 ± 1.2
AST ¹⁰	39.3 ± 2.0	37.9 ± 2.4	4.0 ± 0.3	4.0 ± 0.4
ALT ¹¹	5.1 ± 2.6	4.0 ± 0.6	4.5 ± 0.2	4.4 ± 0.4

¹ Per 1 mg of protein, ² see "Material and Methods" for explanation,

³ nmol NADH/min, ⁴ nmol NADP/min, ⁵ nmol NAD/min, ⁶ nmol NADPH/min,

⁷ nmol glutamylhydroxamate/h, ⁸ nmol urea/min, ⁹ nmol NH₃/min,

¹⁰ nmol oxaloacetate/min, ¹¹ nmol pyruvate/min

*Significantly different from control at P < 0.05

Table 2
Enzymatic activities¹ in samples of heart tissue from control and monensin-fed steers

Enzyme ²	Mitochondria		Cytosolic fraction	
	Control	Monensin	Control	Monensin
LDH ³	47.2 ± 5.5	68.0 ± 6.2*	129 ± 12	271 ± 35*
MDH ⁴	5255 ± 513	5491 ± 325	2615 ± 273	2928 ± 196
OGDH ⁵	34.0 ± 3.7	56.5 ± 4.4*	13.0 ± 0.7	13.1 ± 0.8
GDH ⁶	10.1 ± 1.9	10.7 ± 1.5 4.	0 ± 0.3	3.6 ± 0.3
GS ⁷	1.14 ± 0.34	1.32 ± 0.26	1.4 ± 0.1	1.4 ± 0.1
A ⁸	31.5 ± 4.7	51.4 ± 6.5*	245 ± 54	256 ± 24
OCT ⁹	61.4 ± 7.2	125.2 ± 8.9*	8.6 ± 1.0	7.8 ± 1.8
AST ¹⁰	5.2 ± 0.6	8.4 ± 0.7*	4.0 ± 0.2	3.8 ± 0.2
ALT ¹¹	5.3 ± 0.5	8.9 ± 1.5*	4.5 ± 0.2	4.2 ± 0.3

For 1)-11) see Table 1

* Significantly different from control at P < 0.005

Monensin increased activity of 2-oxoglutarate dehydrogenase and decreased activity of glutamate dehydrogenase in both fractions of the skeletal muscle tissue (Table 1). Enzymatic activities found in heart mitochondria were higher in monensin-fed steers (Table 2). In six out of nine enzymes the effect was statistically significant.

Steers given monensin gained 8.23 % more than control steers (263 kg vs 243 kg). No health problems were encountered in the duration of experiment.

Discussion

The ionophores are defined as compounds which form lipid soluble cation complexes able to transport ions across biological membranes. Effect of ionophores on eukaryotic cells appears to involve the disruption of endomembrane function, particularly membranes associated with the Golgi apparatus (W e e t e et al. 1989). The resultant changes in ion gradients and electrical potentials often influence cellular functions. Calcium ionophores inhibited the glucose-stimulated release of insulin from pancreatic islets of mice (H e l l m a n 1975). Monensin stimulated the release of glucuronidase, hexosaminidase and galactosidase by mouse peritoneal macrophages (T a n a k o et al. 1984), stimulated catecholamine secretion (P e r l m a n et al. 1980) and inhibited the secretion of procollagen and fibronectin (U c h i d a et al. 1979).

In this study monensin fed to steers for 10 months increased activity of enzymes in heart mitochondria. Effect of monensin on musculus longissimus dorsi cells was much less pronounced. The heart tissue is particularly sensitive to toxic doses of monensin (G a l i t z e r et al. 1983). On the other hand, low doses of carboxylic ionophores stimulate cardiac contractility, coronary flow, and these agents may be desirable drugs for treating the low cardiac output syndrome (P r e s s m a n and F a h i m 1982). Cell

activation, which is probably calcium-mediated, requires monensin concentrations between 10^{-8} and 10^{-6} M.

Mitochondrial membranes were probably damaged after freezing and crushing of tissue samples. Mitochondrial enzymes thus could contaminate cytosolic fraction to some extent. However, when in a separate experiment the deep freezing was omitted in GDH assay, proportion of GDH activity present in mitochondrial and cytosolic fraction of cells of the rat cardiac tissue was not very different from those shown in Table 1 and 2 (45.5 vs 11.1 nmol NADPH/min.mg). Our results thus document that long-term supplementation of monensin to ruminants can influence cardiac metabolism at subcellular level.

Aktivita dehydrogenáz a enzymů metabolismu dusíku v srdečním a kosterním svalu býků krmených dávkou s přídatkem monensinu

Zjišťovali jsme účinek monensinu na aktivitu intracelulárních enzymů v tkáni srdce a kosterním svalu (musculus longissimus dorsi) býků krmených senem, siláží a koncentrátem. Věk býků na začátku pokusu byl 9 měsíců. Pět býků dostávalo po 10 měsících monensin v množství 0,5 mg/kg živé hmotnosti denně. Pět býků bylo kontrolních. Po porážce jsme stanovili aktivitu devíti enzymů v mitochondriální a cytoplasmatické frakci buněk : laktátdehydrogenázy (E.C.1.1.1.27), malátdehydrogenázy (E.C.1.1.1.40), 2-oxoglutarátdehydrogenázy (E.C.1.2.4.2), glutamátdehydrogenázy (E.C.1.4.1.3), glutaminsyntetázy (E.C.6.3.1.2), arginázy (E.C.3.5.3.1), ornitinkarbamoyltransferázy (E.C.2.1.3.3), aspartátaminotransferázy (E.C.2.6.1.1) a alaninaminotransferázy (E.C.2.6.1.2). Monensin zvýšil aktivitu 2-oxoglutarátdehydrogenázy a snížil aktivitu glutamátdehydrogenázy v obou frakcích buněk kosterního svalu. Aktivity mitochondriálních enzymů buněk srdečního svalu byly u býků krmených dávkou s monensinem vyšší než u býků kontrolních. Hmotnostní přírůstky býků krmených dávkou s monensinem byly o 8,23 % vyšší než u kontroly (263 vs 243 kg).

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