SHORT COMMUNICATION

ALTERATIONS IN SOME DEHYDROGENASE PROFILES OF SCIATECTOMIZED TOAD GASTROCNEMIUS MUSCLE-METABOLIC MODIFICATIONS BY MALATE

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Summary : Sciatectomized toad gastrocnemius has shown a progressive loss in lactate (LDH), succinate (SDH) and malate (MDH) dehydrogenase activities and elevation of glutamate dehydrogenase (GDH) activity during post-neurectemic days. The possible role of malate in the restoration of metabolic homeostasis in denervated muscle is discussed.

Key words . sciatectomy, metabolic restoration, oxidative metabolism, ammoniagenesis

INTRODUCTION

Muscle metabolism is intriguingly regulated by the trophic influence of the nerve to a large extent (8). Denervation atrophy forms an excellent model for understanding the etiology of several neuro-muscular diseases (5). Marked depletion of energy rich substrates (1) and enzymes of oxidative cycle (2) and mitochondrial dysfunction in muscle (7) during early days of post sciatectomy, have already been investigated. Negative energy balance is a prominent feature of sciatectomized muscle (15). Malate, an energy substrate of oxidative cycle can easily penetrate muscle mitochondria by simultaneous counter movement of phosphate (11). In the present investigation, experiments were designed to study the possible role of malate in restoring metabolic homeostasis of denervated muscle.

MATERIAL AND METHODS

Two batches of medium sized *Bufo melanostictus* were sciatectomized under asceptic conditions as described earlier (12). The right neurectomized muscle was designated as denervated muscle and the left non-neurectomized muscle served as the

contralateral muscle. Animals of the second batch were intraperitoneally administered with 2 μM of malate in saline every day (after due standardization) for four consequtive days. The contralateral and denervated muscle of these animals were considered as contralateral experimental (CE) and denervated experimental (DE) muscles respectively, and those of the first batch of animals (Administered with isovolumetric quantities of saline were considered as contralateral control (CC) and denervated control (DC) muscles. As there was no significant difference observed between contralateral and normal muscles, the former was taken as the control.

Lactate (LDH, EC 1.1.1.27), succinate (SDH, EC 1.3.99.1) and malate (MDH, EC 1.1.1.37) dehydrogenase activities were estimated by the method of Nachlas *et al.* (14) and glutamate dehydrogenase (GDH, EC 1.4.1.3) was assayed by Lee and Lardy method (9). The protein content in the enzyme source was estimated using crystalline bovine serum albumin as standard (10).

RESULTS AND DISCUSSION

Lactate (LDH), succinate (SDH), and malate (MDH) dehydrogenase activities were significantly decreased and that of glutamate dehydrogenase (GDH) was elevated at one day period in denervated control (DC) as compared with that of the contralateral control (CC) and the same trend persisted up to 4 days period. Progressive decrease in NAD dependent LDH activity in DC muscle is suggestive of gradual fall in the rate of lactate oxidation. Since sciatectomic induced stimulation of ammonia genesis (6) depletes oxidative cycle intermediates (4) and affects the flux of pyruvate into the oxidative cycle, the decreased LDH activity may be ascribed to such a metabolic situation in denervated muscle. Administration of malate markedly enhanced the LDH activity in contralateral (CE) and denervated (DE) muscles. Greater elevation of the enzyme activity in denervated muscle than in contralateral, suggests replenishment of TCA cycle intermediates leading to elevated metabolism of lactate in denervated muscle.

The low SDH and MDH activities of sciatectomized muscle are reflective of reduced oxidative metabolism and energy budget. Malate administration increased the SDH and MDH activities in both denervated and contralateral muscles and the percent elevation was higher in denervated muscle. Glutamate dehydrogenase (GDH) activity contributes α-oxoglutarate to citric acid cycle (3) and it has shown a significant ($P<0.001$) elevation in denervated muscle on 1 day period and this increment was sustained up to 4 days period (Table) suggesting the increased flux of glutamate for oxidation and release of ammonia. Administration of malate significantly reduced the GDH activity in denervated muscle thereby decreasing the glutamate-based ammoniagenesis.

Activity levels of selected dehydrogenases (LDH, SDH, MDH and GDH) in sciatectomized and contralateral gastrocnemii of *Bufo melanostictus* and upon the i.p. administration of malate following progressive days after denervation.

Period/Parameters	*Lactate dehydrogenase (LDH)*		*Succinate dehydrogenase (SDH)*		*Malate dehydrogenase (MDH)*		*Glutamate dehydrogenase (GDH)*	
ZERO DAYS								
CC	4.123	±0.02	4.050	±0.35	0.620	±0.07	0.202	±0.001
DC	4.100	±0.13***	3.970	±0.46***	0.611	±0.05***	0.225	±0.005*
% Change over CC	-0.56		-1.97		-1.45		+10.2	
1 DAY								
CC	4.260	±0.05	3.960	±0.36	0.590	±0.02	0.215	±0.010
DC	3.834	±0.03*	3.192	±0.29*	0.516	±0.003*	0.235	±0.002*
% Change over CC	-10		-19.4		-12.5		+9.3	
CE	4.822	±0.09*	4.34	±0.06**	0.711	±0.009*	0.223	±0.009***
% Change over CC	+13.2		+9.6		+20.5		+3.72	
DE	4.827	±0.01*	3.715	±0.03*	0.736	±0.05*	0.226	±0.005*
% Change over DC	+25.9		+16.4		+42.6		-3.83	
2 DAYS								
CC	4.30	±0.05	3.80	±0.09	0.575	±0.03	0.225	±0.006
DC	3.60	±0.09*	2.92	±0.003*	0.356	±0.01*	0.239	±0.002*
% Change over CC	-16.3		-23.1		-38.0		+6.22	
CE	4.98	±0.01*	4.04	±0.05*	0.645	±0.06**	0.216	±0.01***
% Change over CC	+15.8		+6.3		+12.2		-4.0	
DE	4.75	±0.03*	4.08	±0.006*	0.535	±0.07*	0.216	±0.002*
% Change over DC	+32		+39.7		+50.3		-9.6	
3 DAYS								
CC	4.20	±0.26	3.901	±0.06	0.58	±0.009	0.195	±0.003
DC	3.35	±0.28*	3.05	±0.002*	0.342	±0.002*	0.212	±0.001*
% Change over CC	-20.2		-21.8		-41.0		+8.72	
CE	4.93	±0.04*	5.82	±0.16***	0.638	±0.01	0.195	±0.02
% Change over CC	+17.4		-2.07		+10.0			
DE	3.99	±0.39**	3.85	±0.13*	0.469	±0.01*	0.186	±0.006*
% Change over DC	+19.1		+26.2		+37.13		-12.3	
4 DAYS								
CC	4.201	±0.11	3.802	±0.01	0.58	±0.04	0.225	±0.002
DC	3.56	±0.09*	2.78	±0.009*	0.345	±0.06*	0.247	±0.013**
% Change over CC	-15.26		-26.9		-40.5		+9.8	
CE	4.45	±0.04*	4.18	±0.03*	0.663	±0.001*	0.221	±0.001*
% Change over CC	+5.9		+9.94		+14.3		-1.78	
DE	4.36	±0.09*	3.42	±0.034*	0.516	±0.003*	0.221	±0.003**
% Change over DC	+22.5		+23.02		+49.6		-10.5	

The activity levels are mean and S.D. of six experiments and are represented in μmoles of formazan formed/*mg* protein/hour

CC=Contralateral control; DC=Denervated control;

CE=Contralateral experimental; DE=Denervated experimental.

*$P<0.001$ **$P<0.01$ ***Not significant

To sum up, it can be inferred that energy producing systems become less efficient due to the depletion of energy substrates leading to declined lactate, succinate and malate oxidations and elevated glutamate oxidation during post-neurectemic days and the normalcy may be restored by the i.p. administration of malate. The effect of malate on denervated muscle is two dimentionally oriented: (i) to replenish TCA cycle intermediates for cellular oxidations, (ii) and to prevent excessive production of toxic ammonia from glutamate. Malate has more effectively restored the oxidative metabolism of denervated muscle than equimolar loads of succinate (13) or glutamate (16) or lactate (17). The biochemical superiority of malate in the restoration of metabolic homeostasis in denervated muscle, however, remains to be established.

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