—SHORT COMMUNICATIONS

INFLUENCE OF LOCAL ADMINISTRATION OF
SEROTONIN ON MITOCHONDRIAL ENZYME
ACTIVITY OF RAT SKELETAL MUSCLE

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Influence of local administration of serotonin on mitochondrial enzyme activities of rat skeletal muscle was studied. We previously observed that local administration of dextran into the rat skeletal muscle provoked an evident increase in succinic dehydrogenase (SDH) activity in the injected muscle (1). Serotonin has been used as a phlogistic as well as dextran and carrageenin in this species (2, 3). The present experiment was performed to determine whether or not an intramuscular administration of serotonin also increases such a change in SDH activity in the affected muscle.

Two hundred μ g of serotonin (Wako Pure Chemicals Co. Ltd) in 0.1 ml of saline solution (pH 7) was injected into the left gastrocnemius muscle of Wistar strain albino male rats weighing about 200 g. The same amount of saline solution was injected into the right muscle as control. The muscles were removed 6 hr after injection and homogenized with 0.1 M Tris-HCl buffer containing 0.25 M sucrose (pH 7.4), after which the homogenates were centrifuged at $600\times g$ for 10 min (0°C). A part of the supernatant ($600\times g$ sup) was further centrifuged at $12,000\times g$ for 15 min (0°C), and the precipitate was suspended in the buffer. Six hundred $\times g$ sup, the suspension of the precipitate at $12,000\times g$ ($12,000\times g$ ppt), and the supernatant at $12,000\times g$ ($12,000\times g$ sup) were used as enzyme sources. Activities of SDH (4), malic dehydrogenase (MDH) (5), ascorbate oxidase (AO) (6), and lactic dehydrogenase (LDH) (5) were measured by the oxygen electrode method. ATPase activity was measured by the method of Tanaka and Strickland (7), and hexokinase activity was measured by the method of Darrow and Colowick (8). These enzyme activities were determined in the hypotonic medium according to the original report (4–8) and the isotonic medium adjusted with sucrose.

Increase in muscle weight reached a maximum 6 hr after serotonin injection, value being about 116% that of the control. SDH activity in $600\times g$ sup measured in isotonic medium was markedly increased by serotonin injection. The value was about 328% of the control as shown in Table 1. But, when SDH activity in $600\times g$ sup was measured in hypotonic medium, the enzyme activity was not changed by serotonin. SDH activity in $12,000\times g$ ppt was not changed by serotonin when the activity was measured in isotonic and hypotonic medium. SDH activity in $12,000\times g$ sup was significantly increased. On the

TABLE 1. Influence of local administration of serotonin on succinic dehydrogenase activity in rat skeletal muscle

	600 × g sup		12,000 × g ppt		12,000 × g sup
	Isotonic (6)	Hypotonic (4)	Isotonic (4)	Hypotonic (6)	Isotonic (10)
Control	1.14 ± 0.24	2.03 ± 0.46	12.30 ± 3.81	13.18 ± 1.41	0.97 ± 0.11
Serotonin injected	3.74 ± 1.20*	2.21 ± 0.55	12.60 ± 4.21	12.43 ± 3.05	1.18 ± 0.01**
% of Control	327.6	108.9	102.4	94.3	122.0

Value was expressed as nmole O₂/mg protein/min.

Number of experiments is indicated in parentheses.

*; P < 0.05, **; P < 0.01, Significantly different from control.

TABLE 2. Influence of local administration of serotonin on enzyme activities in rat skeletal muscle

		Control	Serotonin injected	% of Control
600 × g sup				
Isotonic				
ATPase	(5)	0.26 ± 0.01	0.28 ± 0.01	107.8
MDH	(6)	25.80 ± 2.81	24.08 ± 1.46	93.3
AO	(6)	3.44 ± 0.66	3.27 ± 0.30	94.9
LDH	(6)	28.39 ± 3.02	32.29 ± 3.60	113.7
Hexokinase	(5)	0.023 ± 0.002	0.024 ± 0.002	103.4
Hypotonic				
ATPase	(5)	0.38 ± 0.08	0.48 ± 0.03	127.4
MDH	(4)	29.98 ± 4.34	31.62 ± 5.19	105.5
AO	(4)	4.35 ± 0.75	4.63 ± 0.80	106.6
LDH	(4)	44.44 ± 4.43	44.06 ± 4.06	99.1
Hexokinase	(5)	0.025 ± 0.001	0.027 ± 0.001	109.2
12,000 × g ppt				
Isotonic				
ATPase	(5)	1.39 ± 0.17	1.02 ± 0.25	73.5
MDH	(3)	151.62 ± 35.75	116.00 ± 19.98	76.5
AO	(3)	55.24 ± 10.79	39.20 ± 6.20	71.0
LDH	(4)	0	0	
Hexokinase	(5)	0	0	
Hypotonic				
ATPase	(5)	4.36 ± 1.13	3.85 ± 0.81	88.3
MDH	(3)	184.54 ± 35.41	172.55 ± 19.98	93.5
AO	(3)	46.39 ± 3.22	40.69 ± 3.01	87.7
LDH	(4)	0	0	
Hexokinase	(5)	0	0	

ATPase activity: $\mu\text{gP/mg protein/min.}$

MDH, AO, and LDH activity: nmole O₂/mg protein/min.

Hexokinase activity: O.D./mg protein/min.

Number of experiments is indicated in parentheses.

other hand, ATPase, LDH, MDH, AO, and hexokinase activities in 600 × g sup and 12,000 × g ppt were not changed significantly by serotonin when measured in both isotonic and hypotonic medium as shown in Table 2.

De Grandis (9) and Nagatomo and Aizawa (10) reported that SDH activity in inflamed tissues is increased. It is also known that the size of mitochondria in rat normal tissues varies (11). SDH activity in $600 \times g$ sup which contains all sizes of mitochondria was markedly increased by serotonin, but the activity in $12,000 \times g$ ppt was not changed. It was expected that SDH activity of unprecipitated mitochondria at $12,000 \times g$ (light mitochondria) would be changed by serotonin. But the increase of SDH activity by serotonin was not observed when it was measured in hypotonic medium which may swell and rupture mitochondria (12, 13). Therefore, the amount of SDH in mitochondria may be unchanged by serotonin (13–15). These results suggest that the mitochondrial membrane permeability for substrate, especially succinate, might change with local administration of serotonin. In the present experiment, it was demonstrated that intramuscular injection of serotonin increased SDH activity in the injected muscle just as was observed when dextran was used as the phlogistic (1). These data collectively indicate that an increase in SDH activity induced by serotonin injection is not specific for serotonin but may be related to the nature of "inflammatory response" in the rat.

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DIFFERENT PATTERNS OF INHIBITION OF DOPAMINE- β -HYDROXYLASE BY CYSTEINE

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It has previously been reported (1) that dopamine- β -hydroxylase [DBH: 3,4-dihydroxyphenethylamine, ascorbate- O_2 oxidoreductase (hydroxylating), EC 1.14.2.1] was inhibited