CONTROL OF ACTIVITY OF *S*-ADENOSYLMETHIONINE DECARBOXYLASE IN MUSCLE BY SPERMIDINE

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Received 26 November 1979

1. Introduction

Synthesis of the polyamines spermidine and spermine from the amino acids ornithine and methionine involves two decarboxylases, ornithine decarboxylase (EC 4.1.1.17), converting ornithine to putrescine, and *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), converting *S*-adenosylmethionine to its decarboxylated form, from which the propylamine unit is transferred to putrescine to form spermidine. The two decarboxylases are often considered to be the rate-limiting enzymes in polyamine synthesis [1,2].

Ornithine decarboxylase is believed to possess a very short half-life and its activity is affected by a variety of stimuli, usually of a growth-promoting nature [3,4]. However its activity has frequently been observed to show a biphasic response, rising rapidly and equally rapidly falling [1,5–7]. Administration of exogenous putrescine appears to bring about a sharp decrease in extractable activity [6,8,9], and it is possible that a rapid elevation in putrescine concentration in vivo, as a result of enhanced ornithine decarboxylase activity, may in turn lead to suppression of further enzyme synthesis. Administration of spermidine also diminishes the rise in hepatic ornithine decarboxylase activity following partial hepatectomy [6].

Control of adenosylmethionine decarboxylase activity has been less studied. Incubation of lymphocytes with putrescine or spermidine was found to lower activity of both ornithine decarboxylase and adenosylmethionine decarboxylase [10], whereas injection of spermidine in the hen was found to produce a dramatic increase in hepatic adenosylmethionine decarboxylase [11], though putrescine had little effect. Putrescine and spermidine administered under conditions in which they suppressed hepatic ornithine decarboxylase activity in the rat were without significant effect on adenosylmethionine decarboxylase activity [6]. Larger doses of putrescine than used in [6,11] were found to depress activity of the decarboxylase in rat seminal vesicle [12]. In none of these reports was actual synthesis of polyamine measured.

We have recently investigated the metabolism of polyamines in diaphragm muscle during the transient hypertrophy that follows denervation and find that both spermidine concentration and adenosylmethionine decarboxylase activity rise transiently (D.H., K.L.M., unpublished). We report here that injection of rats with spermidine lowers the activity of adenosylmethionine decarboxylase and reduces the synthesis of spermidine from putrescine and *S*-adenosylmethionine in cytosol prepared from diaphragm muscle. It is suggested that in muscle activity of adenosylmethionine decarboxylase may be regulated by spermidine.

2. Methods

For measurement of synthesis of [^{14}C]spermidine from [^{14}C]putrescine hemidiaphragms from 5–6 rats were homogenised in 1 vol. ice cold 25 mM Na-phosphate buffer (pH 7.2) containing 5 mM DTT and 0.1 mM EDTA. The homogenate was centrifuged for 10 min at 5000 × *g* and the supernatant for 1 h at 105 000 × *g*. The resulting supernatant was passed through a column (1 × 4 cm) of Sephadex G-25 equilibrated with homogenising buffer. For formation of [^{14}C]spermidine from [^{14}C]putrescine and methionine the reaction mixture, based on that in [13],

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contained in 600 μl final vol. 100 mM Na-phosphate buffer (pH 7.2), 5 mM DTT, 3.3 mM ATP, 15 mM $MgCl_2$, 20 mM methionine, 0.18 μCi (3 nmol) [^{14}C]-putrescine, with or without 0.5 mM unlabelled putrescine, and 400 μl muscle supernatant which contained 3–5 mg protein. For formation of [^{14}C]spermidine from [^{14}C]putrescine and *S*-adenosylmethionine the reaction mixture contained in 600 μl final vol. 100 mM Na-phosphate buffer (pH 7.2), 0.2 mM *S*-adenosylmethionine, 2.5 mM methionine, 0.18 μCi (3 nmol) [^{14}C]putrescine, with or without 0.5 mM unlabelled putrescine, and 400 μl muscle supernatant contained 3–5 mg protein. Incubation was carried out at 37°C for 4 h in conical centrifuge tubes and stopped by the addition of 1 ml 10% trichloroacetic acid (w/v) containing 0.5 μmol carrier spermidine. The protein precipitate was removed by centrifugation and the supernatant applied to a small column (4 × 0.2 cm) of Dowex 50 H^+ (50–100 mesh). Putrescine was washed out with 100 ml 1 N HCl and spermidine was eluted with 10 ml 4 N HCl. This fraction was collected, evaporated to dryness on a hot sand bath and the residue dissolved in 1 ml water and scintillation counted.

For assay of adenosylmethionine decarboxylase diaphragm homogenates were prepared as above. The reaction mixture contained in 600 μl total vol. 100 mM Na-phosphate buffer (pH 7.2), 2.5 mM putrescine, 2.5 mM methionine, 0.2 mM *S*-adenosylmethionine containing 0.125 μCi *S*-adenosyl [*carboxyl*-^{14}C]methionine and 400 μl muscle extract containing 3–5 mg protein. The reaction was carried out in sealed glass tubes, equipped with a small glass centre-well attached to the rubber cap, which were incubated for 2 h at 37°C in a shaking waterbath. The reaction was stopped by injecting 0.5 ml 50% trichloroacetic acid (w/v) through the cap and released CO_2 was trapped in 0.1 ml hyamine hydroxide which was injected into the centre well. The tubes were shaken for another 30 min at 37°C. The hyamine hydroxide was removed from the well, which was washed twice with 0.3 ml methanol. The combined hyamine hydroxide and methanol was transferred to a scintillation vial and counted.

The concentration of unlabelled putrescine added in the assays of spermidine synthesis was lower than with adenosylmethionine decarboxylase assays to avoid excessive dilution of the specific activity of the labelled putrescine. A 4 h incubation was used to increase the measured counts. Spermidine formation over this period was not strictly linear. Although initial rates are greater than the figures quoted, we believe these figures fairly represent the comparative rates for different samples.

3. Results and discussion

Spermidine synthesis from putrescine and either methionine or *S*-adenosylmethionine has been measured in the cytosol fraction of diaphragm muscle. The amount of spermidine formed (table 1) is greatly affected by the concentration of putrescine added, presumably because, as shown with other tissues [1, 13,14], adenosylmethionine decarboxylase is strongly activated by the diamine. In the presence of *S*-adenosylmethionine and 0.5 mM putrescine the amount of spermidine synthesis is close to the activity of the decarboxylase as assayed (50–75 pmol . mg protein^{-1} . h^{-1}). Adenosylmethionine decarboxylase and its messenger probably have fairly short half-lives since in rats 24 h pre-treated with actinomycin-D, activity falls 80% and synthesis of spermidine by the cytosol is reduced to ~45% (table 2).

Adenosylmethionine decarboxylase of muscle, as in other tissues [15–17], is inhibited by MGBG (methylglyoxal-bis(guanylhydrazone)) and synthesis of spermidine is likewise reduced (table 3). Conversely administration of MGBG in vivo markedly enhances assayable decarboxylase activity and spermidine synthesis by cytosol is correspondingly increased. These data indicate that adenosylmethionine decarboxylase participates in spermidine synthesis in muscle and

Table 1
Synthesis of [^{14}C]spermidine from [^{14}C]putrescine and either methionine or *S*-adenosylmethionine

Incubation mixture contained	[^{14}C]Spermidine formation
Methionine 5 μM putrescine	0.25
Methionine 500 μM putrescine	25
S-Adenosylmethionine 5 μM putrescine	5
S-Adenosylmethionine 500 μM putrescine	50

Activity is expressed as pmol product formed . mg cytosol protein^{-1} . h^{-1}

Table 2
Influence of actinomycin-D administration on activity of adenosylmethionine decarboxylase and formation of spermidine

Treatment	Adenosylmethionine decarboxylase activity	Spermidine synthesis at [putrescine]	
		5 μM	500 μM
Saline	87 ± 14	3.0 ± 0.3	71 ± 9
Actinomycin	14 ± 7	1.1 ± 0.4	34 ± 1
	$P < 0.001$	$P < 0.01$	$P < 0.01$

Actinomycin (1 μg/g body wt in saline) was i.p. injected 24 h before slaughter. Figures (pmol . mg protein^{-1} . h^{-1}) are the mean ± SEM of 4 obs.

that the rate of spermidine synthesis is responsive to modulation of decarboxylase activity either as a result of putrescine activation or regulation of the concentration of enzyme protein.

Table 4 shows that spermidine synthesis in vitro is diminished in cytosol of muscle from rats 24 h pre-injected with spermidine. Activity of adenosylmethionine decarboxylase is likewise substantially reduced. Direct addition of spermidine to the assay system had no effect on either decarboxylase activity or [^{14}C]-spermidine formation (results not shown). The inhibition of adenosylmethionine decarboxylase by spermidine in vivo is observed by 6 h following treatment and results in >80% reduction in activity from 12–24 h after administration. The dose of spermidine used here (75 μmol/100 g body wt) was used in [6] and is thought to be within the range of physiological concentrations. Administration of the same dose of putrescine [6] caused a transient elevation in hepatic concentration of ⩽1 mM. A smaller injection of spermidine (12 μmol/100 g body wt) was found to have no significant effect on adenosylmethionine decarboxylase activity.

Rates of polyamine synthesis and activities of adenosylmethionine decarboxylase have not been published for diaphragm muscle and only briefly for heart and skeletal muscle [18,19]. Activity of the decarboxylase is only slightly lower than recorded for liver [18], but several-fold less than in ventral prostate and seminal vesicle [12]. The rate of spermidine synthesis in our cell-free system moves in parallel with the activity of adenosylmethionine decarboxylase and under optimal conditions displays activity comparable with that of the decarboxylase. The muscle enzyme appears similar to that from other tissues with respect to activation by putrescine, inhibition by MGBG in vitro and enhanced activity following administration of the drug.

Steady concentrations of polyamines in vivo are presumably the result of controlled metabolism essentially contingent on the activities of two enzymes.

Table 3
Influence of MGBG in vitro and in vivo on activity of adenosylmethionine decarboxylase and spermidine formation

Addition to assay system	Adenosylmethionine decarboxylase activity	Spermidine synthesis
No addition	109 ± 12 (9)	99 ± 13 (6)
MGBG (2 μM)	29 ± 6 (9)	42 ± 2 (3)
	$P < 0.001$	$P < 0.01$
Administered in vivo		
Saline	83 ± 15 (3)	96 ± 19 (4)
MGBG	1005 ± 131 (3)	315 ± 64 (4)
	$P < 0.01$	$P < 0.02$

Rat injected with MGBG received 8 mg/100 g body wt in 0.14 M NaCl 2 days before slaughter. Figures (pmol . mg protein^{-1} . h^{-1}) are the mean ± SEM of the no. obs. in parentheses

Table 4
Effect of spermidine in vivo on the activity of adenosylmethionine decarboxylase and spermidine synthesis

Treatment	Adenosylmethionine decarboxylase activity	Spermidine formation
1. Control	135 ± 6 (7)	83 ± 11 (4)
Spermidine[a]	24 ± 5 (5)	36 ± 8 (6)
24 h	$P < 0.001$	$P < 0.01$
2. Control	163 ± 18 (8)	
Spermidine[a]		
12 h	20 ± 4 (4) $P < 0.001$	
6 h	76 ± 10 (4) $P < 0.01$	
Spermidine[b]		
12 h	175 ± 15 (4)	
6 h	123 ± 4 (4)	

Rats injected with spermidine received [a]75 μmol or [b]12 μmol/100 g body wt in 0.14 M NaCl i.p. injected at the stated period before slaughter. Figures (pmol . mg protein^{-1} . h^{-1}) are the mean ± SEM of no. obs. in parentheses. Values of P for significant differences with respect to controls are indicated

Ornithine decarboxylase, is necessary for the synthesis of putrescine from ornithine and is subject to feedback inhibition by its immediate product putrescine.

Adenosylmethionine decarboxylase, is also required for the synthesis of spermidine (and spermine) from putrescine and is activated by the diamine.

Our results suggest that in addition to this positive regulation by putrescine spermidine exerts a negative regulatory effect on muscle adenosylmethionine decarboxylase. It is likely that spermidine affects the synthesis of enzyme protein rather than catalytic competence since the polyamine did not alter activity in vitro. That this effect operates intracellularly is suggested by the finding (D. H., K. L. M., unpublished) that during denervation-induced hypertrophy of the diaphragm there is an elevation in spermidine content in the tissue. This arises initially from enhanced activity of adenosylmethionine decarboxylase, but when the spermidine concentration reaches its peak decarboxylase activity declines to subnormal levels.

Acknowledgements

We are grateful to the South African Atomic Energy Board, the Council for Scientific and Industrial Research and the Muscular Dystrophy Associations of America for support for this work.

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