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Differences between tissues in response of *S*-adenosylmethionine decarboxylase to administration of polyamines

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1. Administration of spermidine or *sym*-norspermidine decreased the activity of AdoMet (*S*-adenosylmethionine) decarboxylase in extracts prepared from rat liver, kidney, psoas, diaphragm, soleus and small intestine, but not heart. The decline in psoas, diaphragm and soleus was much greater than that in liver and kidney. The difference in sensitivity to spermidine could not be explained by changes in the uptake and accumulation of the polyamine, because much higher contents were found in liver and kidney that in diaphragm and psoas. 2. Spermidine administration also led to a substantial increase in putrescine in all tissues examined. However, the rise in putrescine was not responsible for the decline in AdoMet decarboxylase activity, since norspermidine, which cannot form putrescine, also produced the decline. Also, administration of putrescine or 1,3-diaminopropane did not decrease AdoMet decarboxylase. 3. The decline in skeletal-muscle AdoMet decarboxylase activity in response to spermidine may be due to an increased rate of degradation of the enzyme protein. The $t_{\frac{1}{2}}$ (half-time) for the decline in activity after inhibition of protein synthesis by cycloheximide was almost halved in the psoas of spermidine-treated rats. Spermidine treatment did not change the $t_{\frac{1}{2}}$ in liver. 4. These results raise the possibility that there are at least two different forms of AdoMet decarboxylase and that the enzyme from psoas or diaphragm differs from that in liver. Additional support for this hypothesis was obtained by comparing the activation by putrescine of AdoMet decarboxylase from these tissues. The liver enzyme was stimulated 10-fold, but the muscle enzyme was stimulated 30-fold.

AdoMet decarboxylase (EC 4.1.1.50) is essential in the biosynthesis of the polyamines spermidine and spermine by mammalian cells, for the production of the aminopropyl donor, decarboxylated *S*-adenosylmethionine (Jänne *et al.*, 1978; Williams-Ashman & Canellakis, 1979; Williams-Ashman & Pegg, 1981). This enzyme has beeen purified to homogeneity from rat liver and prostate (Pegg, 1974, 1977, 1979; Demetriou *et al.*, 1978) and mouse mammary gland and liver (Sakai *et al.*, 1979) and yeast (Pösö *et al.*, 1975). The activity of the enzyme from these sources and from others (Pösö *et al.*, 1976) is substantially increased by putrescine, which provides a physiologically important regulatory mechanism by which polyamine synthesis is enhanced when putrescine concentrations increase (Pegg & Williams-Ashman, 1969; Pegg *et al.*, 1981*a*). Production of decarboxylated *S*-adenosylmethionine is also regulated by changes in the amount of AdoMet decarboxylase protein, which increases in response to a number of trophic stimuli and to the drug methylglyoxal bis(guanylhydrazone) {1,1'-[(methylethanediylidine)-dinitrilo]diguanidine} (Pegg, 1979; Sakai *et al.*, 1979; Pegg & Hibasami, 1980). Another regulatory factor for AdoMet decarboxylase has been discovered. Activities in cultured hepatoma cells and in the spleens of mice injected with L1210 cells were increased in response to α-difluoromethylornithine, a drug that depletes cellular spermidine content (Mamont *et al.*, 1978; Prakash *et al.*, 1980). More detailed study of this phenomenon in hepatoma and Ehrlich ascites cells has indicated that spermidine exerts a negative effect on AdoMet decarboxylase, so that the enzyme activity rises when the spermidine concentration falls (Alhonen-Hongisto, 1980; Mamont & Danzin,

Abbreviations used: AdoMet, *S*-adenosyl-L-methionine; AdoMet decarboxylase, *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50).

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1981; Mamont *et al.*, 1981). This effect could also be responsible for the decline in AdoMet decarboxylase activity in cultured mouse mammary gland in response to exogenous putrescine seen by Sakai *et al.* (1980), since the added putrescine may have been converted into spermidine in the cell. Also, Hopkins & Manchester (1980) observed a marked decline in AdoMet decarboxylase activity from rat diaphragm after injection of spermidine.

The distribution of spermidine in mammalian tissues varies substantially, with very high values (up to 6 μmol/g wet wt.) in ventral prostate and its associated secretions, intermediate values (0.5–2 μmol/g) in liver and kidney and significantly lower amounts (0.1–0.2 μmol/g) in skeletal muscle (Jänne *et al.*, 1978; McAnulty & Williams, 1977; Hopkins & Manchester, 1980). The organs with the higher content of spermidine actually have a higher amount of AdoMet decarboxylase than those with the lower spermidine content (Raina *et al.*, 1976), which was difficult to reconcile with the negative regulation of this enzyme by spermidine. Therefore the experiments described in the present paper were performed to evaluate the response of AdoMet decarboxylase in various tissues to spermidine and related compounds. The results indicate that muscle AdoMet decarboxylase is more sensitive than the liver enzyme to the decrease in activity brought about by spermidine. A preliminary abstract describing some of this work has been published (Pösö & Pegg, 1981).

Experimental

Materials

L-[1-^{14}C]Ornithine (54.4 Ci/mol) and *S*-adenosyl[*carboxy*-^{14}C]methionine (54 Ci/mol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. [2,3-^{3}H]Putrescine (20 Ci/mmol) was a product of NEN, Boston, MA, U.S.A. Methylglyoxal bis(guanylhydrazone) and 1,3-diaminopropane were obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. *sym*-Norspermidine was purchased from Eastman Kodak Co., Rochester, NY, U.S.A. All other biochemical reagents came from Sigma Chemical Co., St. Louis, MO, U.S.A. α-Difluoromethylornithine was a generous gift from Merrell–Dow Research Center, Cincinnati, OH, U.S.A.

The di- and poly-amines used in this study were tested for contamination as previously described (Pegg *et al.*, 1981*b*) and found to contain less than 1% of any other amine. AdoMet was repurified before use by chromatography on Dowex 50 (H^+ form) and the purity was checked by paper electrophoresis (Pegg & Williams-Ashman, 1969).

Treatment of animals

Male Sprague–Dawley rats weighing 260–300 g, from Charles River Breeding Laboratories, Wilmington, MA, U.S.A., were used in all experiments except those described in Table 3, where smaller animals weighing about 225 g were used. All rats were maintained on a 12 h-light/12 h-dark controlled cycle and fed *ad libitum* with Purina rat chow. The amines and other drugs were administered by intraperitoneal injection of a neutral solution in 0.9% NaCl made up at a concentration so that the volume administered was 0.5 ml. Control animals received the 0.9% NaCl alone. Injections of the amines were started at 17:00 h for all experiments to avoid the possible complications of the diurnal periodicity of ornithine decarboxylase and AdoMet decarboxylase. Survival of animals treated with the di- and poly-amines was 100%. Partial hepatectomy was performed by the method of Higgins & Anderson (1931). Food consumption was not checked, and it is possible that the amines depressed appetite and had some inhibitory effect on hepatic decarboxylase activities in this way. However, this would merely increase the observed differences between liver and muscle. In some experiments, AdoMet decarboxylase activity was enhanced by treatment with methylglyoxal bis(guanylhydrazone) (80 mg/kg, 16 h before death) as described by Pegg (1973).

Assay of ornithine decarboxylase and AdoMet decarboxylase

Tissue extracts were prepared by homogenization in 2–6 vol. of 25 mM-Tris/HCl/2.5 mM-dithiothreitol/0.1 mM-EDTA (disodium salt), pH 7.5 at 4°C. The homogenates were centrifuged at 4°C for 30 min at 105000 *g* in a Beckman ultracentrifuge and the supernatants used for the enzyme assays. Supernatant extracts were stored frozen at −20°C before assay. Measurement of activity in some samples without freezing and then after freezing and thawing indicated that no changes in the relative activities were produced by freezing, which resulted in the loss of about 20% of each activity. Putrescine is known to stabilize AdoMet decarboxylase in purified enzyme preparations (Pegg, 1974; Raina & Jänne, 1975; Pösö *et al.*, 1975), but addition of 2.5 mM-putrescine to the homogenizing buffer did not change the observed activities in the crude tissue extracts used in the present experiments.

For the experiments in which the ability of putrescine to activate AdoMet decarboxylase was studied, crude enzyme preparations were isolated from 2 g of diaphragm, 10 g of psoas or 30 g of liver by fractionation with $(NH_4)_2SO_4$ (special enzyme grade; Schwarz/Mann, Orangeburg, NY, U.S.A.) at 0°C. Proteins precipitated between 35 and 60% saturation with $(NH_4)_2SO_4$ (Hannonen *et al.*, 1972) were collected, dissolved in a small volume of the homogenization buffer and dialysed overnight

against 500ml of the same buffer. The ability of the dialysis to remove putrescine was tested by adding 0.1μCi (5pmol) of [2,3-^{3}H]putrescine to the solution. Measurement of the amount of radioactivity present after dialysis indicated that the concentration of the putrescine added to the assay by virtue of that in the enzyme preparation was less than 0.01μM.

The activities of ornithine decarboxylase and of AdoMet decarboxylase were assayed by measuring the evolution of $^{14}CO_2$ from the appropriate substrate as described by Pegg & Williams-Ashman (1969). For ornithine decarboxylase assays, sufficient protein was added to release at least 800c.p.m. during the assay, and for AdoMet decarboxylase more than 600c.p.m. was released in all assays. The production of $^{14}CO_2$ in the AdoMet decarboxylase assay was completely inhibited by the addition of methylglyoxal bis(guanylhydrazone). Results were expressed in terms of the protein added to the enzyme assays, which was determined by the method of Bradford (1970), with crystalline bovine serum albumin as standard.

Determination of polyamine concentrations

Tissue samples were homogenized with 6–10vol. of ice-cold 10% (w/v) trichloroacetic acid or 0.4M-$HClO_4$. After removal of the protein by centrifugation (7700$g_{max.}$ for 10min), the polyamine content was determined as previously described (Pegg *et al.*, 1981*b*). Comparison of the concentrations found with the two methods of homogenization indicated that there was no significant difference between them.

Results

Fig. 1 shows the activity of AdoMet decarboxylase from liver, kidney, heart, small intestine, diaphragm and psoas of rats treated with a single dose of 750μmol of spermidine/kg body wt. This dose had no obvious toxic effect on the animals, although considerably higher doses of 1250μmol/kg were toxic, as reported by Tabor & Tabor (1966). The activity of the enzyme in liver, kidney and small intestine reached a maximal decrease of 50% at 15h, after which activity started to recover. In diaphragm and psoas the maximal decrease was also at 15h, but was much greater, amounting to 90%. The activity in heart was not affected. The striking difference between tissues in the response of AdoMet decarboxylase to spermidine did not extend to ornithine decarboxylase (Fig. 2). The decrease in activity of this enzyme in response to exogenous di-

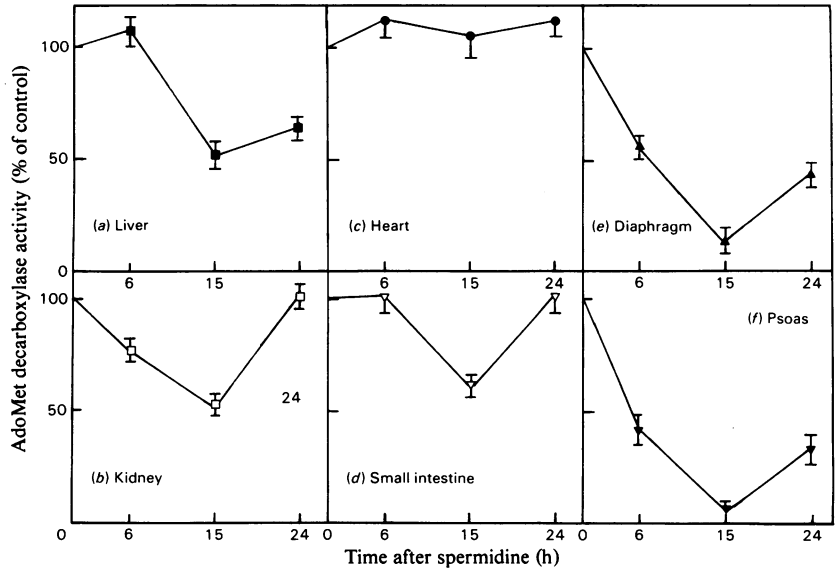

Fig. 1. *Effect of spermidine on the activity of S-adenosylmethionine decarboxylase in various rat tissues*
The rats received spermidine (750μmol/kg) as an intraperitoneal injection and were killed after the injection as indicated in the Figure. The remaining enzyme activities after spermidine treatment are expressed as percentages of the control value. There were three or four animals in each group. The vertical bars represent standard deviations. Results are shown for liver (*a*, ■), kidney (*b*, □), heart (*c*, ●), small intestine (*d*, ▽), diaphragm (*e*, ▲) and psoas (*f*, ▼). The control activities measured at 17:00h were 170 ± 30pmol/30min per mg for liver, 129 ± 5 for kidney, 45 ± 5 for heart, 75 ± 21 for small intestine, 52 ± 10 for diaphragm and 15 ± 3 for psoas.

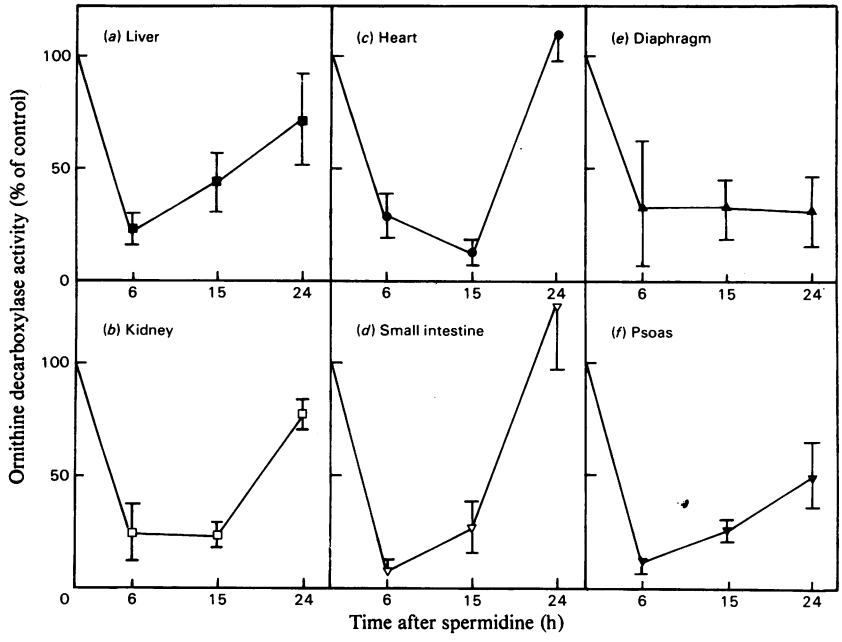

Fig. 2. *Effect of spermidine on the activity of ornithine decarboxylase in various rat tissues*
Treatment of rats, the panels and symbols were as described in Fig. 1. The control activities (expressed as 100%) were 86 ± 13 pmol/30 min per mg for liver, 95 ± 34 for kidney, 48 ± 16 for heart, 20 ± 8 for small intestine, 36 ± 7 for diaphragm and 8 ± 3 for psoas.

and poly-amines is well documented for many different tissues (Jänne *et al.*, 1978), and activity was decreased in the present experiments by 70–90% in all the organs examined over the period from 6 to 15 h after spermidine injection.

This finding suggests that the differential response of AdoMet decarboxylase to spermidine was not due to preferential uptake into those tissues most affected, and the results shown in Figs. 3 and 4 confirm this. These Figures show the impact of the treatment with spermidine on putrescine (Fig. 3) and spermidine (Fig. 4) contents of the rat liver, kidney, small intestine, heart, diaphragm and psoas. Fig. 3 shows that the injection of spermidine led to a dramatic increase in putrescine in all of these tissues. This cannot be due to synthesis of putrescine via ornithine decarboxylase, since this enzyme is greatly decreased by the treatment with spermidine (Fig. 2), but is due to the conversion of the spermidine into putrescine via the acetylation/oxidation pathway (I. Matsui, H. Pösö & A. E. Pegg, unpublished work) described by Matsui *et al.* (1981). Only small changes in spermine resulted from the injection of spermidine (results not shown). In kidney there was a small decrease in spermine, and in small intestine, diaphragm and psoas there was a small increase. Spermidine content was increased significantly in all of the organs at 6–15 h after injection, with the greatest increase occurring in the kidney (6-fold), a 2–3-fold increase in muscle, 70% increase in liver and small intestine and about 30% increase in heart (Fig. 4). Even when the AdoMet decarboxylase activity in diaphragm and psoas was almost completely lost at 15 h after spermidine injection, the content of spermidine in these tissues was less than that present in kidney or liver before the injection. These results suggest that the AdoMet decarboxylase of diaphragm and psoas muscle is more sensitive to repression by spermidine than that of liver or kidney.

From the results shown in Figs. 1–4, we cannot rule out the possibility that the changes in putrescine content after spermidine injection may be responsible for the alteration in AdoMet decarboxylase activity. However, as shown in Table 1, treatment with putrescine or 1,3-diaminopropane did not decrease AdoMet decarboxylase activity, whereas treatment with *sym*-norspermidine, the C_6 analogue of spermidine, which cannot form putrescine, was just as effective as spermidine in decreasing AdoMet decarboxylase (Table 2). Also shown in Table 1 are results for another skeletal muscle, soleus, which

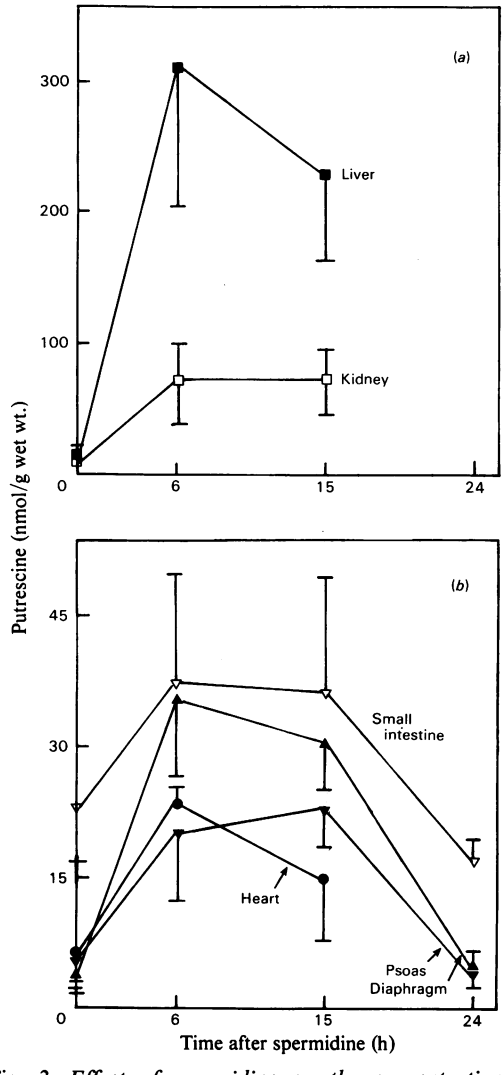

Fig. 3. *Effect of spermidine on the concentration of putrescine in various rat tissues*

The concentration of putrescine was measured after the injection of spermidine at time points indicated in the Figure. Results are shown in panel (*a*) for liver (■) and kidney (□) and in panel (*b*) for small intestine (▽), heart (●), diaphragm (▲) and psoas (▼). Results for all tissues except small intestine were significantly ($P < 0.05$) greater than the zero-time value at 6 and 15 h.

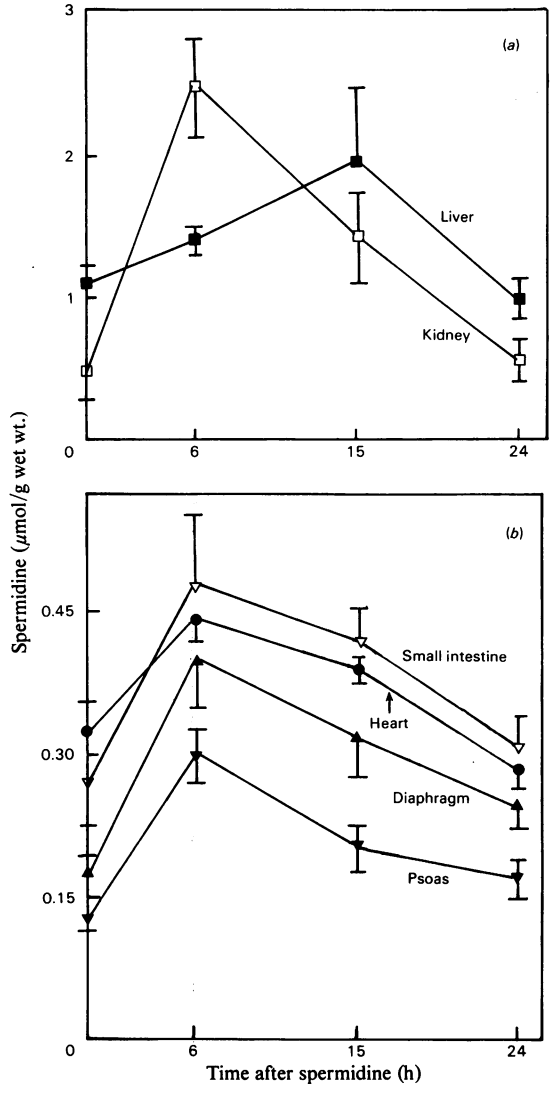

Fig. 4. *Effect of spermidine on the concentration of spermidine in various rat tissues*

Details and symbols are as in Fig. 3. Results for all tissues are significantly ($P < 0.01$) greater than the zero-time value at 6 and 15 h.

resembled diaphragm and psoas and differed from liver and heart in its response to spermidine.

Table 2 shows the accumulation of polyamines and the activities of ornithine decarboxylase and AdoMet decarboxylase in various tissues of rats treated with spermidine and norspermidine. As expected, norspermidine did not lead to the accumulation of putrescine in any of the tissues examined. Also, the uptake and intracellular concentration of norspermidine was greater in liver and heart (which showed only small or no decreases in AdoMet decarboxylase activity) than in psoas or diaphragm, in which AdoMet decarboxylase was affected greatly. Treatment with norspermidine decreased ornithine decarboxylase in all of the tissues.

A further experiment showing that AdoMet decarboxylase of diaphragm is more sensitive than

Table 1. *Effect of various diamines and polyamines on activities of S-adenosylmethionine decarboxylases in various tissues*
Amines were administered at a dose of 750 μmol/kg body wt. 15 h before measurement of the enzyme activities. Results are shown as means ± S.D. for four to six animals. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared with saline-treated animals by Student's *t* test.

		Activity of AdoMet decarboxylase (pmol/30 min per mg)				
Tissue	Treatment ...	Saline	1,3-Diaminopropane	Putrescine	Spermidine	Norspermidine
Liver		125 ± 17	144 ± 36	114 ± 24	69 ± 25^b	77 ± 29^a
Heart		60 ± 11	66 ± 20	53 ± 10	55 ± 13	80 ± 18
Diaphragm		50 ± 6	50 ± 13	40 ± 19	11 ± 2^c	20 ± 4^c
Psoas		18 ± 3	17 ± 2	15 ± 2	4 ± 1^c	5 ± 1^c
Soleus		15 ± 3	14 ± 2	15 ± 5	2 ± 0.5^c	4 ± 1^c

Table 2. *Comparison of the effects of spermidine and norspermidine on polyamine content and ornithine decarboxylase and S-adenosylmethionine decarboxylase activities in various tissues*
Treatment with spermidine or norspermidine was as for Table 1. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared with saline-treated animals. Abbreviation: N.D., not detected.

Organ	Treatment	Ornithine decarboxylase (pmol/30 min per mg)	AdoMet decarboxylase (pmol/30 min per mg)	Putrescine (nmol/g wet wt.)	Spermidine (nmol/g wet wt.)	Spermine (nmol/g wet wt.)	Norspermidine (nmol/g wet wt.)
Liver	Saline	66 ± 24	101 ± 28	10 ± 4	771 ± 80	651 ± 36	N.D.
Liver	Spermidine	21 ± 9^b	67 ± 12^a	280 ± 148^c	1116 ± 165^b	754 ± 143	N.D.
Liver	Norspermidine	32 ± 14^a	69 ± 21	7 ± 3	689 ± 107	648 ± 38	803 ± 220
Diaphragm	Saline	25 ± 4	49 ± 2	3 ± 1	180 ± 14	171 ± 7	N.D.
Diaphragm	Spermidine	10 ± 6^b	17 ± 8^c	72 ± 8^c	445 ± 47^c	214 ± 15^c	N.D.
Diaphragm	Norspermidine	6 ± 2^c	10 ± 4^c	5 ± 2	190 ± 55	157 ± 91	100 ± 30
Psoas	Saline	8 ± 3	20 ± 4	2 ± 1	132 ± 21	213 ± 30	N.D.
Psoas	Spermidine	2 ± 1^a	3 ± 1^c	28 ± 4^c	268 ± 12^c	248 ± 16	N.D.
Psoas	Norspermidine	1 ± 0.5^a	4 ± 1^c	5 ± 2	132 ± 48	238 ± 42	74 ± 40
Heart	Saline	63 ± 11	45 ± 3	7 ± 1	326 ± 18	334 ± 26	N.D.
Heart	Spermidine	6 ± 2^c	51 ± 5	63 ± 44^a	542 ± 96^b	342 ± 33	N.D.
Heart	Norspermidine	20 ± 10^c	57 ± 23	9 ± 3	333 ± 22	282 ± 40	261 ± 50

Table 3. *Effect of α-difluoromethylornithine and putrescine treatment on polyamine concentration and S-adenosylmethionine decarboxylase activity in rat diaphragm and liver*
Rats were subjected to two-thirds partial hepatectomy and treated with α-difluoromethylornithine (400 mg/kg every 4 h) and putrescine (300 μmol/kg every 4 h) by intraperitoneal injection commencing 1 h after operation. The rats weighed 226 ± 16 g. Measurements were made 52 h after partial hepatectomy and results are shown as means ± S.D. for four to six animals. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared with saline-treated animals.

Tissue	Treatment	AdoMet decarboxylase (pmol/30 min per mg)	Putrescine (nmol/g)	Spermidine (nmol/g)	Spermine (nmol/g)
Liver	Saline	267 ± 42	53 ± 23	2230 ± 240	740 ± 70
Liver	α-Difluoromethylornithine	222 ± 31	2 ± 1^c	1690 ± 72^c	780 ± 30
Liver	Putrescine + α-difluoromethylornithine	281 ± 45	23 ± 9	2470 ± 12	790 ± 40
Diaphragm	Saline	40 ± 1	6 ± 1	248 ± 28	169 ± 40
Diaphragm	α-Difluoromethylornithine	61 ± 7^a	3 ± 1	187 ± 12^b	263 ± 41^c
Diaphragm	Putrescine + α-difluoromethylornithine	22 ± 9^b	13 ± 2^b	286 ± 12	280 ± 14^c

that of liver to suppression by spermidine is shown in Table 3. In this experiment, rats were subjected to partial hepatectomy to increase the liver AdoMet decarboxylase activity and then treated with α-difluoromethylornithine (a specific ornithine decarboxylase inhibitor) either alone or with putrescine. It was hoped that by using this procedure a substantial decrease in spermidine could be achieved as compared with the putrescine-treated controls. The α-difluoromethylornithine-treated rats had lower

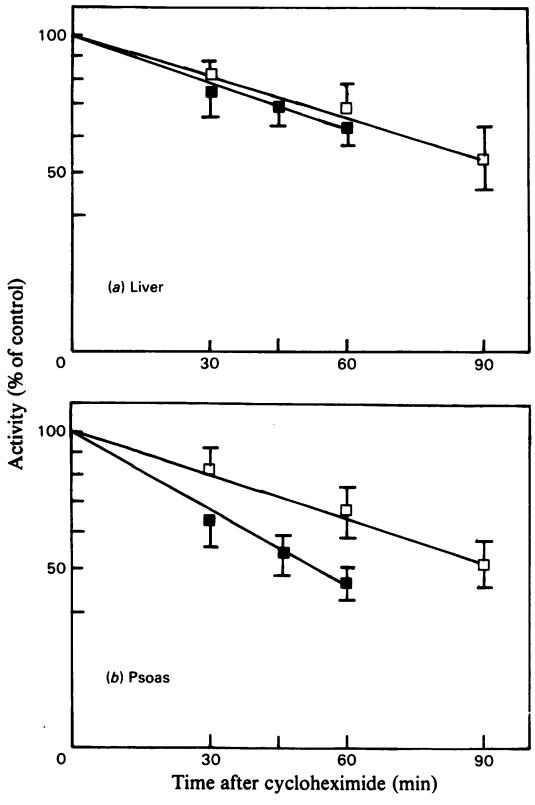

Fig. 5. *Effect of spermidine injection on the half-life of S-adenosylmethionine decarboxylase in liver and psoas when measured after cycloheximide treatment*

The animals were injected with spermidine 15h before death, as in Fig. 1. Cycloheximide (15mg/kg) was injected at time points indicated before death. Results are shown in panel (*a*) for liver in rats treated with spermidine (■, $t_{\frac{1}{2}}$ 92min) or saline (□, $t_{\frac{1}{2}}$ 96min) and in panel (*b*) for psoas in rats treated with spermidine (■, $t_{\frac{1}{2}}$ 55min) or saline (□, $t_{\frac{1}{2}}$ 90min). The remaining enzyme activities after cycloheximide treatment are expressed as percentage of the controls. There were five to seven animals in each group. The vertical bars represent standard deviations. Lines were computed by method of the least squares.

Table 4. *Stimulation by putrescine of S-adenosylmethionine decarboxylase from liver, psoas and diaphragm*

The source of the enzyme was a 35–60%-satd.-$(NH_4)_2SO_4$ precipitate which was dialysed overnight to remove putrescine. Results are expressed relative to activity in assays containing no putrescine.

Concn. putrescine added (μM)	AdoMet decarboxylase		
	Liver	Psoas	Diaphragm
0	1	1	1
1	1.2	1.8	2.0
5	1.6	5.5	5.6
10	1.9	10.0	8.5
25	4.9	14.8	12.3
50	6.6	25.0	22.0
250	9.0	31.4	26.8
3000	9.7	33.1	28.1

concentrations of putrescine and spermidine in both tissues. However, in diaphragm the AdoMet decarboxylase activity was elevated, whereas in liver it was unchanged (Table 3). Putrescine treatment completely reversed the decline in spermidine and decreased AdoMet decarboxylase in diaphragm, but had no effect on the enzyme in the liver. In regenerating rat liver spermidine does not decrease AdoMet decarboxylase 1h after administration, whereas 1,3-diaminopropane has an immediate inhibitory effect (Pösö, 1976; Pösö *et al.*, 1977). The latter may be due to the inhibition of protein synthesis by 1,3-diaminopropane (Kay & Benzie, 1980). The time needed for the decrease in AdoMet decarboxylase activity in response to spermidine may indicate that protein synthesis is required for the effect.

The effect of spermidine administration on AdoMet decarboxylase activity described above is not due to a direct interference by spermidine in the enzyme assays. Spermidine had only a small inhibitory effect on the enzyme activity from liver or psoas whether assayed at a saturating concentration of putrescine (3mM) or in the presence of a low putrescine concentration (5μM). The amount of spermidine present in the assays of the tissue extracts from spermidine-treated rats would be less than 1mM in all cases. There was no evidence for the presence of an inhibitor of the enzyme in the psoas extracts. When activity was assayed in mixtures of liver and psoas extracts the results were exactly as expected from the individual activities.

It is known that AdoMet decarboxylase from liver, kidney and prostate turns over rapidly, with a half-life of 1–2h (Pegg *et al.*, 1973; Pegg, 1979; Jänne *et al.*, 1978). One possible explanation for the loss of activity after spermidine treatment is that the spermidine concentration could affect the rate of degradation. This possibility is supported by the experiment shown in Fig. 5, in which cycloheximide was administered to control and spermidine-treated rats and the decline of AdoMet decarboxylase activity followed in liver and psoas. There was a clear decrease in the half-life of the enzyme in the psoas in response to spermidine treatment, whereas that of the enzyme in liver was not altered.

Finally, the results described above suggest that there may be significant differences between liver

and muscle AdoMet decarboxylases. We therefore examined these enzymes with respect to this important regulatory property of being activated by putrescine. As shown in Table 4, there was a striking difference in activation. When dialysed tissue extracts were tested, the liver enzyme was enhanced about 10-fold by addition of putrescine, whereas the enzymes from psoas and diaphragm were activated about 30-fold by putrescine.

Discussion

Our results, indicating that AdoMet decarboxylase activity from rat tissues declines in response to an increase in spermidine or norspermidine, but not to diamines, are in agreement with studies in cultured cells (Mamont & Danzin, 1981; Mamont *et al.*, 1981; Alhonen-Hongisto, 1980). Our data would support the hypothesis of Mamont *et al.* (1981) that it is the total content of spermidine which is important in this regulation rather than that of spermidine and spermine (Alhonen-Hongisto, 1980), but we were not able to test this directly by injection of spermine because it had pronounced toxic effects, as reported by Tabor & Tabor (1966). No toxicity was associated with the treatments with amines used in the present work, and the organ-specificity of the response could not be explained in this way. The greater sensitivity of AdoMet decarboxylase from psoas, soleus and diaphragm to spermidine may be of importance in the regulation of polyamine synthesis in muscle. There have been relatively few studies on polyamines in muscle compared with the vast literature on factors affecting polyamine synthesis in liver (see Jänne *et al.*, 1978), but several groups have observed changes in polyamine metabolism in response to diet, denervation, hormones and exercise (McAnulty & Williams, 1977; Kremzner *et al.*, 1978; Hopkins & Manchester, 1981; Conover *et al.*, 1980; Lutaya & Griffiths, 1981).

There are many possible explanations for the organ-specificity of response of AdoMet decarboxylase to exogenous spermidine or norspermidine. It is conceivable that this response is mediated via a receptor molecule which mediates the effect and which could be present in different amounts. However, at present there is no evidence for such a receptor molecule, and the accumulation of norspermidine shows clearly that the effect is not merely proportional to the uptake of the polyamine. Another possibility is that the enzymes differ in some way. Some suggestion that this might be the case is given in the experiment of Table 4, which shows that the muscle enzyme is activated by putrescine to a greater extent than the enzyme from liver. Further testing of this hypothesis will require the purification of the enzyme from muscle, which has not yet been reported and is a difficult task because of the low specific activity of the starting material. There is at present no evidence for specific isoenzymes of AdoMet decarboxylase, but as discussed in a review (Williams-Ashman & Pegg, 1981) this possibility has not been examined intensively. It is important to stress that the results described in the present work are not an artifact produced by the presence in the liver of an enzyme system described as a 'latent *S*-adenosylmethionine decarboxylase', which could be released by detergent treatment (Sturman, 1976). This system degrades *S*-adenosyl[*carboxy*-^{14}C]methionine in reactions which release $^{14}CO_2$, but do not produce decarboxylated *S*-adenosylmethionine (Eloranta & Raina, 1978; Wilson *et al.*, 1979). The latent enzyme is not inhibited by methylglyoxal bis(guanylhydrazone) (Wilson *et al.*, 1979), whereas the activity measured in our experiments was completely sensitive to this drug, so that its presence in the liver extracts along with the putrescine-activated enzyme could not account for the lower extent of activation and response to spermidine observed in our experiments. Also, the assay and extraction conditions used in our present work were such that less than 5% of the $^{14}CO_2$ release could result from the degradation of AdoMet by the spurious decarboxylase (Wilson *et al.*, 1979).

There is now experimental evidence in three separate systems that the spermidine concentration in the cell may regulate the rate of degradation of AdoMet decarboxylase protein (Fig. 5; Alhonen-Hongisto, 1980; Mamont *et al.*, 1981). These experiments followed only the loss of activity after inhibition of protein synthesis by cycloheximide. However, when the amount of enzyme protein was increased in rat liver an excellent correlation between activity and immunoreactive protein was observed (Pegg, 1979; Pegg *et al.*, 1981*a*). A possible mechanism for the effect of spermidine would be that the enzyme exists in two forms, where form A is resistant to degradation and form B, which is produced by the binding of spermidine, is sensitive.

$$\text{Spermidine} + \text{Enzyme}_A \rightleftharpoons \text{Enzyme}_B\text{–Spermidine}$$

Although there is no direct evidence for this, it is fully documented that the drug methylglyoxal bis(guanylhydrazone) greatly increases the half-life of the enzyme (Pegg *et al.*, 1973; Fillingame & Morris, 1973; Hölttä *et al.*, 1973; Pegg, 1979). This drug is a highly basic compound, which can be thought of as a spermidine analogue and could easily displace spermidine from the site needed to convert form A into form B.

There is a substantial body of evidence showing that ornithine decarboxylase activity is decreased in response to exogenous diamines and polyamines (Jänne *et al.*, 1978), but the mechanism differs from

that for AdoMet decarboxylase in several important respects. The loss of ornithine decarboxylase is much less specific, in that it occurs in response to virtually all α,ω-diamines and other polyamines tested (Jänne *et al.*, 1978), and the loss of ornithine decarboxylase activity is in some cases associated with the release or synthesis of an inhibitory macromolecule (Canellakis *et al.*, 1979; Heller *et al.*, 1976). No evidence for such an inhibitory molecule for AdoMet decarboxylase was obtained in the present experiments.

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