Induction of spermidine/spermine *N*¹-acetyltransferase in rat tissues by polyamines

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Treatment of rats with spermidine, spermine or *sym*-norspermidine led to a substantial induction of spermidine/spermine *N*¹-acetyltransferase activity in liver, kidney and lung. The increase in this enzyme, which was determined independently of other acetylases by using a specific antiserum, accounted for all of the increased acetylase activity in extracts from rats treated with these polyamines. Spermine was the most active inducer, and the greatest effect was seen in liver. Liver spermidine/spermine *N*¹-acetyltransferase activity was increased about 300-fold within 6 h of treatment with 0.3 mmol/kg doses of spermine; activity in kidney increased 30-fold and activity in the lung 15-fold under these conditions. The increased spermidine/spermine *N*¹-acetyltransferase activity led to a large increase in the liver putrescine content and a decline in spermidine. These changes are due to the oxidation by polyamine oxidase of the *N*¹-acetylspermidine formed by the acetyltransferase. Our results indicated that spermidine was the preferred substrate *in vivo* of the acetylase/oxidase pathway for the conversion of the higher polyamines into putrescine. The induction of the spermidine/spermine *N*¹-acetyltransferase by polyamines may provide a mechanism by which excess polyamines can be removed.

INTRODUCTION

The polyamines spermidine and spermine are formed from putrescine by the addition of aminopropyl groups from *S*-adenosylmethionine. The addition of a single aminopropyl group to putrescine forms spermidine, and the transfer of a second aminopropyl group to spermidine yields spermine (Tabor & Tabor, 1976; Jänne *et al.*, 1978; Williams-Ashman & Pegg, 1981; Pegg & McCann, 1982). Although this biosynthetic pathway is very highly regulated and the content of polyamines is closely linked to the rate of cell growth, the degradation of the polyamines is much less well understood. A variety of enzymes are known to be capable of the further metabolism of polyamines, but some of these have a limited distribution, and others are extracellular (Seiler, 1981; Seiler *et al.*, 1981a, 1982, 1983; Van den Berg *et al.*, 1984; Tabor & Tabor, 1984). Their relative roles in the physiological turnover of polyamines (which are ubiquitous intracellular components) are therefore not well defined, although studies using specific inhibitors of polyamine catabolism should help to clarify this situation (Seiler *et al.*, 1985). It is shown that degradation of spermine into spermidine and of spermidine into putrescine can occur in the cell, but that this interconversion does not take place by reversal of the aminopropyltransferase reactions responsible for synthesis (Pegg *et al.*, 1982). Instead, the conversion of the higher polyamines into putrescine is brought about by the combined actions of two enzymes, polyamine oxidase and SAT (Bolkenius & Seiler, 1981; Matsui *et al.*, 1981; Pegg *et al.*, 1981, 1982; Seiler *et al.*, 1981a). SAT catalyses the formation of the monoacetyl derivative of spermine, which is a substrate for cleavage by polyamine oxidase,

forming spermidine and acetylaminopropanal. Spermidine is also a substrate for SAT, and the *N*¹-acetylspermidine which is produced is then degraded by polyamine oxidase to form putrescine and another molecule of acetylaminopropanal (Della Ragione & Pegg, 1983, 1984; Bolkenius & Seiler, 1981). In general the SAT activity is much less than that of polyamine oxidase, and the content of acetylated polyamine derivatives is very small because they are rapidly degraded (Seiler *et al.*, 1981b; Matsui *et al.*, 1981; Pösö & Pegg, 1982).

SAT activity in most normal rat tissues which have been examined so far is very low, and the extent of putrescine production from the higher polyamines in these tissues may be quite limited. SAT is very highly inducible by hepatotoxins such as carbon tetrachloride, thioacetamide and dimethylnitrosamine, and smaller changes in SAT occur in response to stimuli such as somatotropin and partial hepatectomy (Matsui *et al.*, 1981, 1982; Seiler *et al.*, 1981b; Pegg *et al.*, 1981; Pösö & Pegg, 1982; Della Ragione & Pegg, 1984). However, the physiological importance of SAT and the acetylase/oxidase pathway in the maintenance of cellular polyamine concentrations is not yet fully understood. We have previously published experiments suggesting that the acetylase/oxidase pathway is activated in rats by administration of spermidine (Matsui *et al.*, 1982; Persson & Pegg, 1984). Here we present evidence that SAT is strongly induced in response to administration of exogenous polyamines and that spermine is the most potent inducer. The activated acetylase/oxidase pathway appears to be an important factor in bringing about the removal of excess polyamines by enhancing the conversion of spermidine into putrescine.

EXPERIMENTAL

Materials

[1-¹⁴C]Acetyl-CoA (56.5 Ci/mol) was purchased from New England Nuclear, Boston, MA, U.S.A. *sym*-Norspermidine (3,3'-diaminopropylamine) was purchased from Eastman Kodak Co., Rochester, NY, U.S.A. All other biochemicals, including polyamines and acetylated polyamines, were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Determination of polyamines

Tissue polyamine concentrations were determined by using an amino acid analyser fitted with fluorescence detection as described by Matsui *et al.* (1982). This method does not provide complete separation of the *N*¹- and *N*⁸-isomers of monoacetylspermidine, but paper-chromatographic separations on selected samples as described by Erwin *et al.* (1984) indicated that all of the monoacetylspermidine formed was *N*¹-acetylspermidine.

Treatment of rats with polyamines

Polyamines were administered by intraperitoneal injection of solutions in 0.9% NaCl, which were neutralized with HCl to between pH 6 and 7 where necessary. The concentration of the solutions was adjusted so that a volume between 0.3 and 0.6 ml was given. Control rats received an equal volume of saline alone. The rats used were female Sprague-Dawley strain, weighing about 250 g. They were maintained under controlled 12 h-light/12 h-dark lighting conditions and allowed free access to water and a standard laboratory rat chow diet. All injections were given in the morning between 09:00 and 11:00 h.

Assay of SAT activity

Cytosolic extracts containing SAT were prepared as described by Matsui *et al.* (1981) and either assayed immediately or kept frozen at -20 °C for less than 1 week and thawed only once before assay. No loss of activity occurred under such storage unless the samples were repeatedly frozen and thawed. The samples were subjected to immunoprecipitation with either a control rabbit antiserum or a monospecific antiserum against SAT as described by Persson & Pegg (1984). The remaining activity capable of the acetylation of spermidine was then measured with 3 mM-spermidine and 8 μ M-[1-¹⁴C]acetyl-CoA as substrates and a 10 min incubation at 30 °C as described by Erwin *et al.* (1984). The results were expressed as units of acetylase activity, where 1 unit catalyses the production of 1 pmol of acetylated spermidine/min at 30 °C. The amount of SAT activity was determined for each sample by subtracting the residual activity after immunoprecipitation with the antiserum specific for SAT from the activity found after exposure to the control rabbit antiserum (Persson & Pegg, 1984). Protein was determined by the method of Bradford (1976), and the SAT activity is given as units/mg of protein.

RESULTS

Treatment of rats with spermidine leads to a substantial increase in the ability of liver extracts to catalyse the formation of monoacetylspermidine when incubated with spermidine and acetyl-CoA *in vitro*

Table 1. Dose-dependence of induction of spermidine/spermine *N*¹-acetyltransferase

Rats were treated with the dose of polyamine shown by intraperitoneal injection and the immunoprecipitable SAT activity was determined in extracts of the tissues shown. Results are given as means $\pm$ S.D. for at least four separate measurements on tissues from different animals.

Treatment	Immunoprecipitable SAT activity (units/mg) in tissue shown		
	Liver	Kidney	Lung
Untreated	0.4 $\pm$ 0.1	1.5 $\pm$ 0.5	1.8 $\pm$ 0.6
Spermidine			
0.2 mmol/kg	2.5 $\pm$ 0.5	7 $\pm$ 1	6 $\pm$ 1
0.4 mmol/kg	22 $\pm$ 4	13 $\pm$ 3	34 $\pm$ 8
0.8 mmol/kg	74 $\pm$ 8	37 $\pm$ 10	38 $\pm$ 12
Spermine			
0.15 mmol/kg	17 $\pm$ 2	13 $\pm$ 5	11 $\pm$ 2
0.3 mmol/kg	126 $\pm$ 14	57 $\pm$ 6	32 $\pm$ 5
<i>sym</i> -Norspermidine			
0.2 mmol/kg	2.4 $\pm$ 0.6	3 $\pm$ 0.7	3.6 $\pm$ 1.1
0.4 mmol/kg	4 $\pm$ 1	4 $\pm$ 1	7 $\pm$ 2
0.8 mmol/kg	46 $\pm$ 8	12 $\pm$ 5	11 $\pm$ 4

Table 2. Time course of induction of spermidine/spermine *N*¹-acetyltransferase by spermine (0.3 mmol/kg)

SAT activity was determined as in Table 1 in rats treated with 0.3 mmol/kg doses of spermine for the time shown. Results are given as means $\pm$ S.D. for at least four different determinations.

Time of treatment (h)	Activity of SAT (units/mg)		
	Liver	Kidney	Lung
0	0.4 $\pm$ 0.1	1.8 $\pm$ 0.6	1.8 $\pm$ 0.5
3	65 $\pm$ 11	13 $\pm$ 6	18 $\pm$ 5
6	122 $\pm$ 19	59 $\pm$ 8	29 $\pm$ 5
9	82 $\pm$ 8	61 $\pm$ 7	26 $\pm$ 7
12	89 $\pm$ 12	39 $\pm$ 6	28 $\pm$ 5

(Matsui *et al.*, 1982). The total production of monoacetylspermidine when crude tissue extracts are incubated with these substrates is due to the combined effects of several proteins, and possibly to some non-enzymic acetylation as well. A substantial part of the product when uninduced tissue extracts were used was *N*⁸-acetylspermidine, but the inducible SAT forms only *N*¹-acetylspermidine (Matsui *et al.*, 1981, 1982; Erwin *et al.*, 1984; Persson & Pegg, 1984). In order to estimate the extent to which the increase in activity in response to spermidine and other polyamines was due to the inducible SAT, tissue extracts were treated with a specific antiserum to this protein and the immunoprecipitable activity was measured. As shown in Table 1, SAT was increased substantially in liver, kidney and lung by treatment with spermidine. The total extent of induction is difficult to estimate precisely, because the immunoprecipitable SAT is such a small fraction (10% or less of

Table 3. Polyamine contents in livers of rats treated with polyamines

Rats were treated with the polyamine shown for the time indicated and the content of polyamines in the liver was measured. Results are shown as means $\pm$ s.d. for at least four separate determinations. *N*⁸-Acetylspermidine and *N*¹-acetylspermine were below the limit of detection (10 nmol/g) in all samples.

	Polyamine content (nmol/kg)			
	Putrescine	Spermidine	Spermine	<i>N</i> ¹ -Acetyl-spermidine
None	4 $\pm$ 2	843 $\pm$ 78	808 $\pm$ 40	< 10
Spermidine (0.8 mmol/kg), 6 h	1024 $\pm$ 187	1267 $\pm$ 121	960 $\pm$ 65	199 $\pm$ 58
Spermine (0.3 mmol/kg), 3 h	30 $\pm$ 9	681 $\pm$ 57	2459 $\pm$ 140	< 10
Spermine (0.3 mmol/kg), 6 h	327 $\pm$ 70	380 $\pm$ 75	2631 $\pm$ 127	76 $\pm$ 11
Spermine (0.3 mmol/kg), 9 h	320 $\pm$ 67	322 $\pm$ 59	2314 $\pm$ 184	58 $\pm$ 14
Spermine (0.3 mmol/kg), 12 h	380 $\pm$ 111	298 $\pm$ 39	2275 $\pm$ 236	62 $\pm$ 20

the total activity) in the untreated controls that its amount in these animals is hard to determine. However, SAT activity in liver increased by at least 150-fold within 6 h of treatment with 0.8 mmol of spermidine/kg, and by more than 20-fold in kidney and lung (Table 1). The increase in SAT accounted for virtually all of the increased capacity of tissue extracts to catalyse this acetylation, and SAT represented more than 90% of the total activity in the extracts from rats treated with spermidine (Persson & Pegg, 1984) or with the other polyamines (results not shown).

The increase in SAT activity in response to spermidine was dose-dependent, but the response was clearly not linear. A much greater effect was produced by the higher doses; 0.2 mmol/kg doses gave only a 5-fold increase, whereas 0.8 mmol/kg gave more than 150-fold enhancement (Table 1). This effect was also seen with other polyamines (spermine and *sym*-norspermidine), which were also potent inducers of SAT (Table 1).

Spermine was considerably more active as an inducer than spermidine, but owing to the toxicity associated with administration of spermine by intraperitoneal injection it could only be tested at doses of 0.3 mmol/kg or less. Such doses increased SAT by more than 300-fold in liver, by at least 30-fold in kidney and by 15-fold in lung (Table 1).

Spermine is a substrate for SAT with a higher affinity for the protein, as indicated by its K_m of 34 μ M, compared with 130 μ M for spermidine (Della Ragione & Pegg, 1983). However, the capability to induce this protein is not directly related to this affinity, since *sym*-norspermidine, which is a better substrate (K_m 9 μ M), was less active than spermidine or spermine in increasing SAT activity (Table 1). Although *sym*-norspermidine was the least active of the polyamines tested as inducers, it still gave a substantial increase in SAT content in all of the tissues examined (Table 1).

The time course of induction of SAT by spermine showed that a large increase was produced within 3 h in all of the tissues examined and that activity reached a maximum value at 6 h (Table 2). This rapid change in SAT content is consistent with the very short half-life of SAT in thioacetamide-stimulated rat liver (Persson & Pegg, 1984). SAT activity remained elevated for at least a further 6 h (Table 2).

The changes in polyamine content in liver of rats treated with doses of spermine and spermidine which induced the SAT were measured (Table 3). The content

of *N*¹-acetylspermine was always below the limit of detection (10 nmol/g), but *N*¹-acetylspermidine was present in relatively low amounts only in rats exposed to exogenous spermidine or spermine. There was a striking increase in the content of putrescine in rats treated with the higher polyamines (Table 3). This increase cannot arise from synthesis *de novo* via ornithine decarboxylase, since this enzyme is strongly repressed by treatment with exogenous spermidine or spermine (Canellakis *et al.*, 1979; McCann, 1980; Pegg *et al.*, 1982), and must come from the degradation of spermidine via the acetylase/oxidase pathway. High amounts of putrescine were present in rat liver at the times at which SAT activity was elevated (Table 3), but the content of putrescine was higher in rats treated with spermidine than with spermine even though the induction of SAT was highest with spermine (Table 1). Treatment with spermine led to a very pronounced fall in the hepatic spermidine content (Table 3).

DISCUSSION

The substantial induction of SAT produced by administration of exogenous polyamines suggests that this enzyme may play a physiological role in the regulation of polyamine content. The rapid increase in SAT leads to the degradation of the higher polyamines, particularly spermidine, to putrescine. This in itself may protect the cell against injury caused by the presence of high concentrations of the polyamines. Many biochemical processes are known to be affected by polyamines, and frequently excess polyamines have inhibitory actions (Jänne *et al.*, 1978; Tabor & Tabor, 1976, 1984). Putrescine is a diamine which is less basic than the higher polyamines, and it may be desirable to convert the cellular spermidine into it under conditions in which excess exogenous polyamines are available. It is also possible that the further degradation of putrescine by diamine oxidases may be the normal pathway of polyamine degradation. However, as discussed by Seiler *et al.* (1985), many of the copper-containing amine oxidases attack not only diamines but also the polyamines themselves. Several such oxidases have been described [reviewed by Tabor & Tabor (1976), Seiler (1981) and Seiler *et al.* (1985)], but their relative importance in polyamine catabolism is not well defined, and in some cases detailed kinetic comparisons of

different substrates have not been reported. If putrescine was the preferred substrate for whichever of these enzymes play a major physiological role in polyamine degradation, the degradation via the acetylase/oxidase pathway could represent the first step in this process.

The present results confirm previous findings with hepatotoxins (Matsui *et al.*, 1981; Pegg *et al.*, 1981; Pösö & Pegg, 1982; Persson & Pegg, 1984) that SAT is a very highly inducible enzyme which responds very rapidly to inducing stimuli. The 300-fold increase in this SAT protein which occurs within 6 h of treatment with spermine (Table 1) is a good example of this. Such changes are in many ways comparable with the variations in the content of ornithine decarboxylase activity which occur in response to a wide variety of stimuli (Jänne *et al.*, 1978; Pegg *et al.*, 1982; Pegg & McCann, 1982; Tabor & Tabor, 1984; Seely & Pegg, 1983). Both enzymes form putrescine (ornithine decarboxylase directly and SAT via the oxidation of N^1 -acetylspermidine), but their response to exogenous polyamines is completely different. Ornithine decarboxylase activity is greatly decreased in response to exogenous polyamines (Canellakis *et al.*, 1979; McCann, 1980; Seely & Pegg, 1983), whereas SAT is greatly increased, as shown here. This may indicate that compensatory mechanisms come into play to maintain putrescine concentrations, but it should be noted that decreasing the cellular putrescine content by treatment with an ornithine decarboxylase inhibitor did not by itself induce SAT activity (A. E. Pegg & B. E. Erwin, unpublished work).

There has been much recent interest in the possibility that inhibitors of polyamine biosynthesis may prove to be valuable therapeutic agents (Sjoerdsma, 1981; Pegg & McCann, 1982; Sjoerdsma & Schecter, 1984). Substantial depletion of cellular polyamines occurs in response to exposure of cells to ornithine decarboxylase inhibitors such as 2-difluoromethylornithine (Pegg & McCann, 1982; Tabor & Tabor, 1984). However, a portion of the cellular polyamine, particularly spermine, is very difficult to deplete with such inhibitors. It is possible that analogues of the naturally occurring polyamines can be found which are not themselves able to replace polyamines in their growth-promoting activity, and are not substrates for SAT but are able to induce SAT activity. Such gratuitous inducers might be used to deplete cellular polyamines via the induction of the acetylase/oxidase pathway and thus enhance the effectiveness of the ornithine decarboxylase inhibitors.

It is noteworthy that the content of spermidine declined greatly in rat liver after induction of SAT by treatment with spermine (Table 3). This decline suggests that acetylation of spermidine and its degradation to putrescine occurs more rapidly than the replacement of the spermidine by acetylation of spermine followed by oxidation of the N^1 -acetylspermine to give spermidine. However, studies *in vitro* of the enzymes involved suggest that the opposite should be the case, since the K_m for spermine is lower than the K_m for spermidine for SAT (Della Ragione & Pegg, 1983), and N^1 -acetylspermine is a better substrate for polyamine oxidase than N^1 -acetylspermidine (Bolkenius & Seiler, 1981). Other data indicating that spermidine is preferentially degraded by this pathway have been reported by Bolkenius *et al.* (1985), based on experiments in rats treated with a potent polyamine oxidase inhibitor. They found that N^1 -acetylspermidine accumulated in such animals to a much

greater extent than did N^1 -acetylspermine (Bolkenius *et al.*, 1985) and suggested that the cellular spermine is in some way compartmentalized so that it is less available to the cytosolic SAT than is spermidine. This possibility could also explain our results, but another explanation is that an alternative pathway exists for the degradation of N^1 -acetylspermine. This could be de-acetylation, since an acetylpolyamine deacetylase has been described (Libby, 1978, 1983; Blankenship, 1978, 1979; Santacroce & Blankenship, 1982), although its relative specificity towards N^1 - and N^8 -acetylspermidine and N^1 -acetylspermine is controversial (Blankenship, 1979; Santacroce & Blankenship, 1982; Libby, 1983; Bolkenius *et al.*, 1985).

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REFERENCES

- Blankenship, J. (1978) Arch. Biochem. Biophys. **189**, 20–27
- Blankenship, J. (1979) Proc. West. Pharmacol. Soc. **22**, 115–118
- Bolkenius, F. N. & Seiler, N. (1981) Int. J. Biochem. **13**, 287–292
- Bolkenius, F. N., Bey, P. & Seiler, N. (1985) Biochim. Biophys. Acta **838**, 69–76
- Bradford, M. (1976) Anal. Biochem. **72**, 248–254
- Canellakis, E. S., Viceps-Madore, D., Kyriakidis, D. A. & Heller, J. S. (1979) Curr. Top. Cell. Regul. **15**, 155–202
- Della Ragione, F. & Pegg, A. E. (1983) Biochem. J. **213**, 701–706
- Della Ragione, F. & Pegg, A. E. (1984) in Advances in Polyamines in Biomedical Science (Caldarera, C. M. & Bachrach, U., eds.), pp. 97–104, CLUEB, Bologna
- Erwin, B. G., Persson, L. & Pegg, A. E. (1984) Biochemistry **23**, 4250–4255
- Jänne, J., Pösö, H. & Raina, A. (1978) Biochim. Biophys. Acta **473**, 241–293
- Libby, P. R. (1978) Arch. Biochem. Biophys. **188**, 360–363
- Libby, P. R. (1983) Methods Enzymol. **94**, 329–331
- Matsui, I., Wiegand, L. & Pegg, A. E. (1981) J. Biol. Chem. **256**, 2454–2459
- Matsui, I., Pösö, H. & Pegg, A. E. (1982) Biochim. Biophys. Acta **719**, 199–207
- McCann, P. P. (1980) in Polyamines in Biomedical Research (Gaugas, J. M., ed.), pp. 109–124, John Wiley and Sons, New York
- Pegg, A. E. & McCann, P. P. (1982) Am. J. Physiol. **243**, C212–C221
- Pegg, A. E., Matsui, I., Seely, J. E., Pritchard, M. L. & Pösö, H. (1981) Med. Biol. **59**, 327–333
- Pegg, A. E., Seely, J. E., Pösö, H., Della Ragione, F. & Zagon, I. S. (1982) Fed. Proc. Fed. Am. Soc. Exp. Biol. **41**, 3065–3072
- Persson, L. & Pegg, A. E. (1984) J. Biol. Chem. **259**, 12364–12367
- Pösö, H. & Pegg, A. E. (1982) Arch. Biochem. Biophys. **217**, 730–737
- Santacroce, M. J. & Blankenship, J. (1982) Proc. West. Pharmacol. Soc. **25**, 113–118
- Seely, J. E. & Pegg, A. E. (1983) Biochem. J. **216**, 701–707
- Seiler, N. (1981) in Polyamines in Biology and Medicine (Morris, D. R. & Marton, L. J., eds.), pp. 169–180, Marcel Dekker, New York
- Seiler, N., Bolkenius, F. N. & Rennert, O. M. (1981a) Med. Biol. **59**, 334–346
- Seiler, N., Bolkenius, F. N., Knödgen, B. & Haegle, K. (1981b) Biochim. Biophys. Acta **676**, 1–7

- Seiler, N., Knödgen, B. & Haegele, K. (1982) *Biochem. J.* **208**, 189–197
- Seiler, N., Knödgen, B., Bink, G., Sarhan, S. & Bolkenius, F. (1983) *Adv. Polyamine Res.* **4**, 135–154
- Seiler, N., Bolkenius, F. N. & Knödgen, B. (1985) *Biochem. J.* **225**, 219–226
- Sjoerdsma, A. (1981) *Clin. Pharmacol. Ther.* **30**, 3–22
- Sjoerdsma, A. & Schecter, P. J. (1984) *Clin. Pharmacol. Ther.* **35**, 287–300
- Tabor, C. W. & Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285–306
- Tabor, C. W. & Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–790
- Van den Berg, G. A., Elzinga, H., Nagel, G. T., Kingma, A. W. & Muskiet, F. A. J. (1984) *Biochim. Biophys. Acta* **802**, 175–187
- Williams-Ashman, H. G. & Pegg, A. E. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 44–73, Marcel Dekker, New York

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