INDUCTION OF SPERMIDINE N^1-ACETYLTRANSFERASE IN RAT KIDNEY BY TREATMENT WITH FOLIC ACID

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1. Introduction

The polyamine biosynthetic pathway in mammalian cells is now firmly established (reviews [1–3]). Putrescine is formed by the action of ornithine decarboxylase and is converted into spermidine by the addition of a propylamine group from decarboxylated *S*-adenosylmethionine in a reaction catalyzed by spermidine synthase. Spermine synthase then catalyzes an analogous reaction in which a second propylamine group is transferred from another molecule of decarboxylated *S*-adenosylmethionine to spermidine forming spermine. The spermidine synthase and spermine synthase reactions are essentially irreversible; however, when tracer amounts of labeled spermidine or spermine are administered there is conversion of the higher polyamines back into putrescine [4,5]. This reversal involves the sequential actions of two enzymes; spermidine/spermine N^1-acetyltransferase and polyamine oxidase [6–8]. The first of these enzymes produces the monoacetyl derivative from the polyamine and acetyl-CoA. With spermidine as substrate it forms exclusively the N^1-acetylspermidine isomer [6]. The N^1-acetylspermidine is then oxidized by polyamine oxidase to form putrescine and *N*-acetyl-3-aminopropionaldehyde [8]. Spermine is symmetrical and only one isomer can be formed by acetylation of the terminal amino group [6]. The N^1-acetylspermine is also an excellent substrate for polyamine oxidase and is converted to spermidine and *N*-acetyl-3-aminopropionaldehyde [8].

The activity of this acetylase/oxidase pathway for interconversion of the polyamines appears to be regulated in the rat liver by the amount of the acetylase present since polyamine oxidase is present in much greater amounts and does not change substantially in response to stimuli which affect the interconversion [3,6,7,9]. We have detected large and rapid changes when acetylase has been measured in liver extracts prepared from rats pretreated with carbon tetrachloride [10], thioacetamide [11], dialkylnitrosamines [12] and spermidine [12]. Smaller but significant changes were observed in response to growth hormone and partial hepatectomy [11]. In all of these cases, the enhanced acetylase activity correlated with increased conversion of spermidine into putrescine and it appears that the acetylase plays a critical role in regulation of intracellular polyamine levels. However, the induction of increased acetylase activity had only been observed in the liver and it was not known whether a similarly inducible enzyme occurs in other tissues or whether the acetylase/oxidase pathway for interconversion of polyamines was limited to liver. Administration of pharmacological amounts of folic acid to rats results in renal cell injury and subsequent hypertrophy and hyperplasia [13,14]. This provides a useful model system in which to investigate biochemical changes in the kidney occurring at early stages in this process. Here, we show that the spermidine N^1-acetyltransferase is induced in the kidney after treatment with folic acid and that this induction is responsible for an increase in putrescine content in this organ.

2. Methods

N^1-Acetylspermidine and N^8-acetylspermidine were kindly provided by Dr M. M. Abdel-Monem, Univer-

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sity of Minnesota (Minneapolis MN). α-Difluoromethylornithine was a generous gift from Merrell National Labs. (Cincinnati OH). [*acetyl*-1-^{14}C]Acetyl-CoA (49.8 mCi/mmol) and L-[1-^{14}C]ornithine (69.4 mCi/mmol) were purchased from New England Nuclear (Boston MA). Folic acid and all other biochemicals were obtained from Sigma Chemical Co. (St Louis MO). Male Sprague-Dawley rats (180–220 g body wt) were used in all experiments. Folic acid was dissolved in 0.3 M sodium bicarbonate just before use and i.p. injected at 250 mg/kg. α-Difluoromethylornithine was administered at 400 mg/kg by i.p. injection of a solution of 160 mg/ml in 0.9% NaCl. Control rats were injected with the vehicle alone.

For the determination of polyamines including N^1-acetylspermidine, kidneys were removed as rapidly as possible, frozen in liquid N_2 and stored at −70°C until assayed. The tissue was then homogenized in 10 vol. 0.2 M perchloric acid, the precipitate removed by centrifugation at 10 000 × *g* for 30 min and aliquots of the supernatant analyzed using an amino acid analyzer with fluorescence detection as in [10,11]. This method does not resolve N^1- from N^8-acetylspermidine but separate determinations using the paper chromatographic separation described below indicated that >85% of the acetylspermidine found in the kidney was the N^1-acetylspermidine isomer.

For enzyme assays the rats were killed by cervical dislocation and the kidneys homogenized as described for liver extracts [10,11]. The centrifuged homogenates were used as source of enzyme. Ornithine decarboxylase was assayed by following the release of $^{14}CO_2$ from [1-^{14}C]ornithine [15] and spermidine N^1-acetyltransferase was determined by following the incorporation of [*acetyl*-1-^{14}C]acetyl-CoA into monoacetylspermidine [11]. Both enzymes were assayed under conditions in which the enzyme activity was strictly proportional to protein content and to the time of incubation. Incubations of 10 min at 30°C were used for the acetyltransferase and of 30 min at 37°C for the decarboxylase. Results were expressed as product formed/mg protein added which was determined as in [16] using reagent from Bio-Rad Labs. (Richmond CA) and bovine serum albumin as standard.

For the determination of product of the acetylase reaction, the assay mixture was scaled up to 0.4 ml total vol. containing 1.2 μmol spermidine, 40 μmol Tris–HCl (pH 7.8) and 3.2 nmol (0.16 μCi) [1-^{14}C]-acetyl-CoA. After incubation at 30°C for 10 min the reaction was stopped by the addition of 0.4 ml 0.4 N perchloric acid and then centrifuged to remove the precipitate. The supernatant was extracted into butanol and the acetylated spermidine derivatives separated by paper chromatography [6].

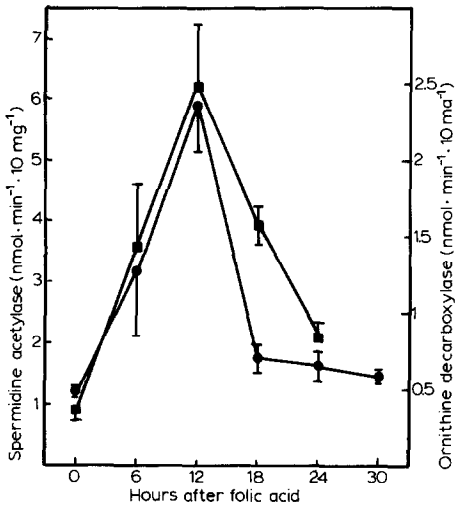

Fig.1. Effect of folic acid treatment on renal ornithine decarboxylase and spermidine N^1-acetyltransferase activities. Rats were given folic acid (250 mg/kg), killed at the time shown and ornithine decarboxylase (■) or spermidine N^1-acetyltransferase (●) activities measured. The activity of ornithine decarboxylase is expressed as nmol CO_2 produced in 30 min at 37°C. The activity of spermidine N^1-acetyltransferase is expressed as nmol monoacetylspermidine produced in 10 min at 30°C. The shorter time and lower temperature was used for the latter enzyme to ensure that both activities were measured under conditions where the activity was proportional to the amount of protein present [11].

3. Results

Both ornithine decarboxylase and spermidine N^1-acetyltransferase were increased substantially in the kidney within a few hours of treatment with folic acid (fig.1). Ornithine decarboxylase reached a peak value at 12 h with a 6-fold increase and then declined rapidly. Spermidine N^1-acetyltransferase activity also peaked at 12 h and declined even more quickly to levels only slightly above control by 18 h. Fig.1 underestimates the degree to which the inducible spermidine N^1-acetyltransferase was increased. In the control rat kidney extracts ≥50% of the acetylation of spermidine was carried out by non-specific acetylase which acts on histones and a variety of polyamines

Table 1
Polyamine content of rat kidney after treatment with folic acid

Time after folic acid	Polyamine content (nmol/g wet wt)			
	Putrescine	N^1-acetylspermidine	Spermidine	Spermine
0	17.6 ± 3.1	n.d.	520 ± 49	690 ± 62
6 min	38.5 ± 10.6	5.9 ± 2.9	520 ± 30	640 ± 65
12 min	72.6 ± 3.0	14.7 ± 0.5	622 ± 17	595 ± 29
18 min	47.6 ± 14.2	2.6 ± 1.4	574 ± 43	687 ± 81
24 min	29.2 ± 8.8	2.4 ± 1.1	567 ± 83	589 ± 32
30 min	26.4 ± 4.4	n.d.	660 ± 46	729 ± 52

Results are shown as mean ± SD for ⩾4 meas. in rats treated with 250 mg folic acid/kg body wt for the time shown; n.d., not detected (<1.5 nmol/g wet wt)

[6,17,18]. This enzyme forms predominantly the N^8-acetylspermidine isomer whereas the inducible enzyme is entirely specific in producing only N^1-acetylspermidine [6]. When the products of the reaction by control kidney extracts were analyzed here by paper chromatography, only 49% of the product was N^1-acetylspermidine and 51% was N^8-acetylspermidine. When extracts were assayed from rats treated with folic acid 12 h before death the product of the reaction was 93% N^1-acetylspermidine and only 7% N^8-acetylspermidine showing that only the spermidine N^1-acetyltransferase activity was increased by the treatment.

Treatment with folic acid produced a significant increase in the content of N^1-acetylspermidine in the kidney (table 1). In control rats this product was below the level of detection (~1.5 nmol/g wet wt) and this metabolite increased to ~15 nmol/g at a time corresponding to the highest acetylase activity. An even greater increase was produced in putrescine which reached values of 73 nmol/g wet wt and also peaked at the time at which ornithine decarboxylase and spermidine N^1-acetyltransferase were maximal. Spermine declined slightly at this time and both spermidine and spermine increased slightly 30 h after treatment (table 1).

These results are consistent with our hypothesis, based on experiments with rat liver after hepatotoxins, that the early rise in putrescine in such livers is due to oxidation of N^1-acetylspermidine [3,6,10,11]. However, in these experiments the acetylase activity increased before that of ornithine decarboxylase. Here, with kidney extracts, the decarboxylase was also elevated and could be contributing to the rise in putrescine. To test this possibility, the rats were treated with α-difluoromethylornithine a specific inhibitor of ornithine decarboxylase [19]. As shown in table 2, this inhibitor totally abolished the rise in

Table 2
Effect of α-difluoromethylornithine on polyamine content, spermidine N^1-acetyltransferase activity and ornithine decarboxylase activity in rat kidney after treatment with folic acid

Treatment	Polyamine content (nmol/g wet wt)				Spermidine N^1-acetyltransferase (nmol . mg^{-1} . 10 min^{-1})	Ornithine decarboxylase (nmol . mg^{-1} . 30 min^{-1})
	Putrescine	N^1-acetylspermidine	Spermidine	Spermine		
Control	22 ± 8	<2	622 ± 68	815 ± 72	0.08 ± 0.01	0.37 ± 0.07
Folic acid	79 ± 7	14 ± 3	719 ± 26	660 ± 60	0.42 ± 0.06	3.08 ± 0.28
Folic acid + α-difluoromethylornithine	62 ± 8	12 ± 1	665 ± 38	693 ± 40	0.38 ± 0.05	0.08 ± 0.01

Rats received α-difluoromethylornithine (400 mg/kg) 1 h before and 6 h after i.p. injection of folic acid (250 mg/kg). Rats were killed 12 h after the injection of folic acid. Results are shown as the mean ± SD for at least 4 est.

ornithine decarboxylase activity without affecting the increase in the acetylase. Even when ornithine decarboxylase was inhibited, putrescine rose by 280% compared to a rise of 360% with folic acid alone. Therefore in this system also, the major part of the early rise in putrescine in response to a toxic stimulus is due to the acetylase/oxidase pathway.

4. Discussion

These results show that spermidine N^1-acetyltransferase is not confined to liver but is also present in rat kidney. The renal enzyme like that in liver is rapidly inducible and appears to be the rate-limiting step in the conversion of spermidine back into putrescine. As indicated by the rapid fall in the acetylase activity 6–12 h after treatment it appears that in kidney as in liver [20] this enzyme has a very rapid rate of turnover which permits substantial changes in activity within a short time.

The acetylase appears to provide the bulk of the rise in putrescine although ornithine decarboxylase activity was also increased in parallel to the change in acetylase. It is apparent from these data and [3,6,10–12,21–23] that a full understanding of the regulation of polyamine levels in all mammalian tissues requires measurement of the acetylase/oxidase system as well as assays of the biosynthetic enzymes. The purpose of the increased acetylase activity remains unclear and it is possible that the N^1-acetylspermidine itself has some important role in the cell apart from its use as a precursor of putrescine. The early rise in putrescine may also protect against tissue damage. The availability of specific inhibitors of the acetylase may help to answer this question and attempts to produce such inhibitors and to purify the enzyme to homogeneity are in progress.

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