Response of tissue diamine oxidase activity to polyamine administration

Antonio PERIN,*† Angela SESSA† and M. Alfonsina DESIDERIO*

*Institute of General Pathology, University of Milan, and †Consiglio Nazionale delle Ricerche Center for Research in Cell Pathology, Milan, Italy

The administration to rats of putrescine (750 $\mu\text{mol/kg}$ body wt.) caused in liver, kidney and heart an increase in putrescine at 1 h and in diamine oxidase (EC 1.4.3.6) activity within 3–6 h. An increase in spermidine was observed at 9 h in liver and at 6 h in kidney, whereas in heart there was no change. The increase in diamine oxidase activity by exogenous putrescine was prevented by the administration of actinomycin D and cycloheximide, suggesting that syntheses of mRNA and protein are involved. Equimolar doses of 1,3-diaminopropane, 1,5-diaminopentane and monoacetylputrescine stimulated, similarly to putrescine, hepatic, renal and cardiac diamine oxidase activity. After the injection of a non-toxic dose of spermidine (750 $\mu\text{mol/kg}$ body wt.), the increase in diamine oxidase activity occurred at 9 h in all the tissues studied, when a substantial putrescine formation from spermidine occurred. *sym*-Norspermidine, which is unable to form putrescine, did not cause an increase in enzyme activity. The possibility that the tissue contents of putrescine might regulate diamine oxidase activity is discussed.

INTRODUCTION

The principal catabolic pathway for aliphatic diamines with three to six carbon atoms, their monoamino derivatives and histamine involves oxidation by diamine oxidase (EC 1.4.3.6). This enzyme, unevenly distributed in the organism, is involved in the terminal catabolism of polyamines, as are other copper-containing amine oxidases (Seiler *et al.*, 1985). Some observations suggest that diamine oxidase is responsible to a significant extent for putrescine catabolism *in vivo* (Sourkes & Missala, 1981), even though in the brain monoamine oxidase (EC 1.4.3.4) assumes physiological importance in degrading the monoacetyl derivative of putrescine (Seiler & Al-Therib, 1974).

Much of the early interest in diamine oxidase stemmed from the high enzyme activity found in physiological barriers (placenta, intestinal mucosa), where it protects against elevated concentrations of amines produced by the fetus and intestinal bacteria and originating in the diet (Guha & Jänne, 1976; Shaff & Beaven, 1976). Another remarkable feature of diamine oxidase is that it increases in response to drugs (Sessa *et al.*, 1984) and growth-promoting stimuli (Sessa *et al.*, 1981; Desiderio *et al.*, 1982; Perin *et al.*, 1983) in tissues with low enzyme activity. In growing tissues, the increase in diamine oxidase activity is associated with an enhanced content of putrescine and other polyamines, and the inhibition of enzyme activity by aminoguanidine causes an increase in putrescine. Thus we suggested that the enhanced diamine oxidase activity in growth conditions may represent a means by which the excess of putrescine can be removed and the amounts of polyamines controlled (Perin *et al.*, 1983, 1984).

The regulation of diamine oxidase is quite complex, and a transcriptional mechanism with increased synthesis of mRNA, followed by new synthesis of enzyme protein,

seems to be involved in tissues stimulated to grow or exposed to drugs (Desiderio *et al.*, 1982; Sessa *et al.*, 1982, 1984; Perin *et al.*, 1983). A role of the β_2 -adrenoceptor-adenylate cyclase system has been shown in normal and hypertrophic rat kidney by administration to rats of adrenergic agonists and antagonists, as well as of theophylline, an inhibitor of phosphodiesterase, or forskolin, an activator of adenylyl cyclase (Desiderio *et al.*, 1985). Changes in cellular polyamine contents might represent another regulatory factor for diamine oxidase expression. In this regard, primary amines, including polyamines themselves, are known to control in cultured cells and *in vivo* the activity of ornithine decarboxylase (EC 4.1.1.17) (Kay & Lindsay, 1973; Jänne & Hölttä, 1974; Clark & Fuller, 1975; Fong *et al.*, 1976; Heller *et al.*, 1976; Pösö & Jänne, 1976; Jefferson & Pegg, 1977; Kallio *et al.*, 1977; Pegg *et al.*, 1978) and *S*-adenosylmethionine decarboxylase (EC 4.1.1.50) (Pegg & Williams-Ashman, 1969; Pösö & Pegg, 1981), rate-limiting enzymes in polyamine biosynthesis, as well as spermidine *N*¹-acetyltransferase (Matsui *et al.*, 1982), the principal enzyme in polyamine interconversion.

In the present paper, we studied the relevance of tissue changes in polyamines on diamine oxidase activity in rats given putrescine, spermidine and their analogues.

EXPERIMENTAL

Chemicals

[1,4-¹⁴C]Putrescine (109 Ci/mol) was purchased from Amersham International, Amersham, Bucks., U.K.; 1,4-diaminobutane (putrescine), 1,5-diaminopentane (cadaverine) and spermidine, as hydrochloride salts, were from Fluka, Buchs, Switzerland; 1,3-diaminopropane dihydrochloride and monoacetylputrescine hydrochloride were from Sigma Chemical Co., St. Louis, MO,

† To whom reprint requests should be addressed.

U.S.A.; actinomycin D and cycloheximide were from Aldrich Chemical Co., Milwaukee, WI, U.S.A. The reversed-phase C₁₈ column (Hibar LiChrosorb RP-18) and 1,8-diamino-octane (base) were from Merck, Darmstadt, Germany. The dihydrochloride derivative of 1,8-diamino-octane was prepared by reaction with HCl and recrystallization from ethanol. *sym*-Norspermidine was kindly provided by Professor V. Zappia and Dr. G. Cacciapuoti, University of Naples. All other chemicals were analytical-grade products.

Animals

Male Wistar rats weighing 220–280 g were maintained on a 12 h-light/12 h-dark cycle and were given free access to food and water. All amines (750 $\mu\text{mol/kg}$ body wt.) were injected intraperitoneally between 09:00 and 10:00 h as a neutral solution in saline (0.9% NaCl) made up to a concentration so that the volume administered was 0.5 ml. Control animals received saline. Some putrescine-treated animals received contemporaneously an intraperitoneal injection of actinomycin D (2.5 mg/kg body wt.) or cycloheximide (2 mg/kg body wt.) in saline. Actinomycin D was dissolved by first warming in propylene glycol. All rats were killed by decapitation at various times after the treatments. Liver, kidney and heart were rapidly removed, weighed, frozen with solid CO₂ and stored at -80°C until assayed, since tissue diamine oxidase activity is stable for several weeks under these conditions.

Diamine oxidase activity assay

Tissue samples were homogenized (20%, w/v) with a Potter apparatus in ice-cold 0.25 M-sucrose containing 0.3 mM-EDTA and 1 mM-2-mercaptoethanol. Diamine oxidase activity was assayed in homogenates by the method of Okuyama & Kobayashi (1961), as described by Kusche *et al.* (1973), which measures Δ^1 -[¹⁴C]pyrroline formed from [¹⁴C]putrescine. The assay mixture contained, in a final volume of 0.75 ml, 150 μmol of sodium/potassium phosphate buffer, pH 7.4, homogenate (20 mg of tissue), saturating concentrations of labelled putrescine (sp. radioactivity 0.167 Ci/mol) as substrate (300 nmol for liver, 600 nmol for kidney and heart) and 7.5 μmol of acetaldehyde to exclude the interference of aldehyde-metabolizing enzymes with Δ^1 -pyrroline formation (Sessa *et al.*, 1981). Assay blanks were prepared by incubation of samples in the presence of 40 μM -aminoguanidine, a specific inhibitor of diamine oxidase activity (Okuyama & Kobayashi, 1961). For each determination, three test assays and three corresponding blanks were carried out. The time of incubation was 3 h in air. The results are expressed as pmol of Δ^1 -pyrroline formed/h per mg of protein. The protein content was determined by the biuret method, with bovine serum albumin as standard (Layne, 1957).

Polyamine determination

Tissue samples, with addition of 1,8-diamino-octane dihydrochloride as internal standard, were homogenized (20%, w/v) in 0.2 M-HClO₄, and supernatants, made alkaline (pH 9.5–10.5) with 1.5 M-Na₂CO₃ buffer, were dansylated (Igarashi *et al.*, 1974). The benzene extracts were evaporated and dissolved in methanol, and fluorescent polyamines were assayed by a Perkin-Elmer h.p.l.c. system (excitation at 368 nm and emission at 500 nm). The elution system consisted of a linear gradient

Fig. 1. Effect of putrescine administration on diamine oxidase activity and polyamine contents of some rat tissues

Putrescine (750 $\mu\text{mol/kg}$ body wt.) was injected intraperitoneally at various times before death. Diamine oxidase activity and polyamine contents were measured in tissue homogenates as reported in the text. The results are shown for liver (a), kidney (b) and heart (c), as means for six animals. The bars indicate s.e.m. and, where not shown, these lie within the symbol. ●, Diamine oxidase activity; □, putrescine; △, spermidine.

made up of acetonitrile (A) and 4.2 mM-Na₂HPO₄ (B), which went from 50% (A) to 90% (A) in 12 min, followed by an isocratic period of 2 min. The flow rate was 1.8 ml/min.

Statistical analysis

The significance of the differences between the means was evaluated by Dunnett's (1955) test. The values were considered significant when $P < 0.05$.

RESULTS

Fig. 1 shows the behaviour of diamine oxidase activity and putrescine and spermidine contents in liver, kidney and heart from rats treated with a single dose of putrescine (750 $\mu\text{mol/kg}$ body wt.). In all the tissues studied, diamine oxidase activity increased significantly at 3–6 h after the injection by about 2-fold and began to decline thereafter, returning to basal values at 12 h. Shortly after putrescine injection (within 1 h), a marked enhancement in its tissue content was observed, 25-, 34- and 11-fold respectively in liver, kidney and heart, in accordance with results of Pegg *et al.* (1978). Then

Table 1. Effect of actinomycin D or cycloheximide on putrescine-stimulated diamine oxidase activity of some rat tissues

Actinomycin D (2.5 mg/kg body wt.) or cycloheximide (2 mg/kg body wt.) was injected intraperitoneally contemporaneously with putrescine (750 μ mol/kg body wt.) 6 h before death. Results are shown as the means $\pm$ S.E.M. for five animals: * P < 0.01 compared with saline-treated animals by Dunnett's (1955) test.

Treatment	Diamine oxidase activity (pmol/h per mg of protein)		
	Liver	Kidney	Heart
Saline	16.84 $\pm$ 0.81	24.54 $\pm$ 2.21	28.77 $\pm$ 2.12
Putrescine	38.92 $\pm$ 4.66*	58.43 $\pm$ 9.11*	57.03 $\pm$ 2.88*
Putrescine + actinomycin D	14.77 $\pm$ 1.77	27.87 $\pm$ 3.32	29.02 $\pm$ 5.26
Putrescine + cycloheximide	15.45 $\pm$ 2.01	24.63 $\pm$ 3.25	25.97 $\pm$ 2.75

Table 2. Effect of diamine administration on diamine oxidase activity of some rat tissues

1,3-Diaminopropane, 1,5-diaminopentane or *N*-acetyl-1,4-diaminobutane was injected intraperitoneally at a dose of 750 μ mol/kg body wt., and the animals were killed at various times after diamine administration. Results are shown as the means $\pm$ S.E.M. for the numbers of animals in parentheses: * P < 0.05, ** P < 0.01 compared with saline-treated animals by Dunnett's (1955) test.

Treatment	Time after diamine administration (h)	Diamine oxidase activity (pmol/h per mg of protein)		
		Liver	Kidney	Heart
Saline (5)	—	17.39 $\pm$ 1.38	24.65 $\pm$ 2.78	27.86 $\pm$ 2.88
1,3-Diaminopropane (5)	3	22.79 $\pm$ 2.96	39.67 $\pm$ 2.15**	36.21 $\pm$ 4.78
	6	26.10 $\pm$ 2.63*	45.17 $\pm$ 3.07**	48.44 $\pm$ 9.92**
1,5-Diaminopentane (5)	3	28.23 $\pm$ 3.82**	39.03 $\pm$ 1.73**	44.67 $\pm$ 4.25**
	6	35.79 $\pm$ 2.80**	47.16 $\pm$ 3.12**	50.81 $\pm$ 4.05**
<i>N</i> -Acetyl-1,4-diaminobutane (6)	3	46.40 $\pm$ 3.05**	42.60 $\pm$ 3.86**	32.62 $\pm$ 3.22
	4.5	36.41 $\pm$ 2.12**	38.33 $\pm$ 2.25**	55.72 $\pm$ 5.01**
	6	35.63 $\pm$ 1.94**	38.39 $\pm$ 5.40*	34.12 $\pm$ 4.36

putrescine decreased rapidly, especially in the kidney. The increase in putrescine concentrations was followed by a significant increase (1.4-fold) in spermidine, at 9 h in liver and at 6 h in kidney, whereas in the heart spermidine contents were unchanged. Spermine concentrations are not shown because they remained constant throughout the observations.

As reported in Table 1, the inhibition of synthesis of mRNA and protein by actinomycin D or cycloheximide completely prevented the increase in diamine oxidase activity after putrescine administration. It is noteworthy that actinomycin D and cycloheximide did not significantly affect the basal diamine oxidase activity (A. Perin, A. Sessa & M. A. Desiderio, unpublished work).

In further experiments, we tested the effect of structural analogues and derivatives of putrescine, given as a dose equimolar to putrescine, on hepatic, renal and myocardial diamine oxidase activity. The results reported in Table 2 show that 1,3-diaminopropane was active in stimulating enzyme activity at 3 h in kidney and at 6 h also in liver and heart, though to a lesser extent than putrescine. 1,5-Diaminopentane produced a lower diamine oxidase activity enhancement at 3 h and a similar one at 6 h than did putrescine. Monoacetylputrescine (*N*-acetyl-1,4-diaminobutane) caused the maximum increase in diamine oxidase activity at 3 h in liver and kidney, and at 4.5 h

in heart, with values similar to those obtained with putrescine.

Fig. 2 shows the accumulation of polyamines and the activity of diamine oxidase in liver, kidney and heart of rats treated with a fairly large but non-toxic dose of spermidine (750 μ mol/kg body wt.) (Pösö & Pegg, 1981). After 3 h, there was an increase in spermidine that led to an enhancement in putrescine content, with a maximum at 6 h, in accordance with Pösö & Pegg (1981) and Matsui *et al.* (1982). Putrescine increased about 11-fold in liver and 3-fold in heart, and the percentage increases were greater than those of spermidine, which only doubled at 3 h. In the kidney, putrescine enhancement was smaller (3-fold) than that of spermidine (8-fold), probably because this polyamine is concentrated in renal tissue before it is excreted into the urine (Matsui *et al.*, 1982). The peak values for hepatic, renal and cardiac diamine oxidase activity, which occurred at 9 h, were nearly 2-fold the basal values. Spermidine, putrescine and enzyme activity returned to normal values at 24 h. Spermine was not increased by the administration of spermidine and actually appeared to decrease slightly (results not shown).

The results reported in Table 3 show that the treatment with *sym*-norspermidine, the C₆ analogue of spermidine which cannot form putrescine, did not modify diamine oxidase activity in any of the studied tissues.

Fig. 2. Effect of spermidine administration on diamine oxidase activity and polyamine contents of some rat tissues

Spermidine (750 $\mu\text{mol/kg}$ body wt.) was injected intraperitoneally at various times before death. Diamine oxidase activity and polyamine contents were measured in tissue homogenates as reported in the text. The results are shown for liver (a), kidney (b) and heart (c), as means for six animals. The bars indicate S.E.M., and, where not shown, these lie within the symbol. ●, Diamine oxidase activity; □, putrescine; △, spermidine.

DISCUSSION

The data obtained in this study show that liver, kidney and heart from putrescine-treated animals displayed an increase in diamine oxidase activity shortly after the enhancement in putrescine contents, indicating a possible interaction of these two events. Since putrescine is an

immediate precursor of higher polyamines and in these experiments hepatic and renal spermidine was also enhanced, we emphasized the role of diamines by giving to rats some putrescine analogues, which are poor substrates for the aminopropyl transferase that forms spermidine, and thus are converted into polyamines to a very limited extent, if at all (Raina & Jänne, 1975). 1,5-Diaminopentane, a naturally occurring diamine (Tabor & Tabor, 1972), was virtually as effective as putrescine in stimulating enzyme activity, whereas 1,3-diaminopropane, which is not physiological but is structurally closely related to putrescine, increased diamine oxidase activity to a minor extent. Additional information is necessary to elucidate whether the effects of putrescine analogues on diamine oxidase activity are direct or might be secondary to their action on the concentration of putrescine. Monoacetylputrescine, which is formed and degraded in hepatic and renal tissues (Seiler & Al-Therib, 1974), enhanced, like putrescine, enzyme activity at 3 h in liver and kidney, but later in the heart, probably reflecting the different tissue rate of deacetylation to putrescine.

Experiments with exogenous higher polyamines (spermidine and *sym*-norspermidine) support more convincingly the idea that putrescine is responsible for the enhancement in diamine oxidase activity. In spermidine-treated animals, the increase in enzyme activity took place when a substantial production of putrescine occurred, via conversion from spermidine (Matsui *et al.*, 1982). As could be expected, treatment with the unphysiological, but structurally closely related, *sym*-norspermidine, which is unable to form putrescine (Pösö & Pegg, 1981), did not increase diamine oxidase activity. The failure of spermidine itself to control diamine oxidase was also supported by the evidence that in the heart of putrescine-treated rats the activity of this enzyme increased even if spermidine contents were unchanged.

In conclusion, polyamines, known to regulate the principal enzymes of their biosynthesis and conversion (Pegg & Williams-Ashman, 1969; Kay & Lindsay, 1973; Jänne & Hölttä, 1974; Clark & Fuller, 1975; Fong *et al.*, 1976; Heller *et al.*, 1976; Pösö & Jänne, 1976; Jefferson & Pegg, 1977; Kallio *et al.*, 1977; Pegg *et al.*, 1978; Cameron *et al.*, 1979; Pösö & Pegg, 1981; Matsui *et al.*, 1982) seem also to control diamine oxidase activity, an enzyme of their terminal catabolism, through the tissue contents of putrescine. This putrescine-mediated control involves new syntheses of mRNA and protein, probably with an enhanced expression of diamine oxidase or of a regulatory protein, which in turn may influence this enzyme. In the absence of cDNA probes and antibodies

Table 3. Effect of *sym*-norspermidine administration on diamine oxidase activity of some rat tissues

sym-Norspermidine (750 $\mu\text{mol/kg}$ body wt.) was injected intraperitoneally 9 h before death. Results are shown as means $\pm$ S.E.M. for six animals.

Treatment	Diamine oxidase activity (pmol/h per mg of protein)		
	Liver	Kidney	Heart
Saline	17.85 $\pm$ 1.18	23.73 $\pm$ 2.00	34.20 $\pm$ 1.92
<i>sym</i> -Norspermidine	19.62 $\pm$ 2.01	23.17 $\pm$ 2.33	29.60 $\pm$ 2.79

for quantitative measurement of mRNA and protein, we cannot clarify the mechanism(s) involved in diamine oxidase stimulation by putrescine.

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