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Inhibition of Polyamine Accumulation and Deoxyribonucleic Acid Synthesis in Regenerating Rat Liver

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Repeated injections of 1,3-diaminopropane into rats after partial hepatectomy caused a repression-type inhibition of liver ornithine decarboxylase (EC 4.1.1.17) and totally prevented the marked increases in liver putrescine and spermidine concentrations that normally occur in response to partial hepatectomy. The inhibition of polyamine synthesis by diaminopropane was accompanied by a profound decrease (about 80%) in the synthesis of DNA in the regenerating rat liver without any changes in the synthesis of RNA and total liver protein.

Several lines of experimental evidence accumulated over the course of the past years have revealed that the decarboxylation of L-ornithine by ornithine decarboxylase (EC 4.1.1.17) is the rate-controlling reaction in the biosynthesis of natural polyamines, putrescine, spermidine and spermine, in animal tissues (Morris & Fillingame, 1974; Hölttä & Jänne, 1972; Hannonen *et al.*, 1972).

Ornithine decarboxylase exhibits dramatic stimulations, obviously partly due to its extremely short biological half-life (Russell & Snyder, 1969), under a great variety of conditions involving rapid growth, such as in rat liver after partial hepatectomy (Russell & Snyder, 1968; Jänne & Raina, 1968) or after treatment with growth hormone (Jänne & Raina, 1969). Virtually in every instance the stimulation of ornithine decarboxylase later results in an enhanced synthesis and accumulation of tissue spermidine (Raina *et al.*, 1966; Jänne *et al.*, 1968).

It is possible, even likely, that the activity of mammalian ornithine decarboxylase *in vivo* is regulated through a repression-type mechanism by putrescine and spermidine (Schrock *et al.*, 1970; Kay & Lindsay, 1973; Clark, 1974; Jänne & Hölttä, 1974). Whether this control occurs at the level of transcription or translation, as suggested by Jänne & Hölttä (1974) and Clark & Fuller (1975), is still debatable.

We found that not only natural polyamines (putrescine and spermidine) (Jänne & Hölttä, 1974), but also some more unphysiological but structurally closely related diamines, especially 1,3-diaminopropane, inhibited ornithine decarboxylase *in vivo* (Pösö & Jänne, 1976). Repeated injections of diaminopropane into partially hepatectomized rats not only decreased the activity of ornithine decarboxylase, but also completely prevented the increases in liver spermidine concentration normally occurring in response to partial hepatectomy (Pösö & Jänne, 1976).

In a further effort to unravel the physiological functions of natural polyamines we prevented the increased accumulation of putrescine and spermidine normally taking place in regenerating liver by repeated injections of diaminopropane in order to investigate the possible metabolic consequences resulting from the inhibition of the synthesis of natural polyamines.

Experimental

Partial hepatectomy was performed by the method of Higgins & Anderson (1931). [6-^{3}H]Thymidine (sp. radioactivity 23.3 Ci/mmol), [6-^{14}C]orotic acid (sp. radioactivity 57 mCi/mmol) and L-[U-^{14}C]leucine (sp. radioactivity 324 mCi/mmol) were from The Radiochemical Centre (Amersham, Bucks., U.K.) 1,3-Diaminopropane was the product of Fluka A.G. (Buchs SG, Switzerland).

The activity of ornithine decarboxylase was measured by the method of Jänne & Williams-Ashman (1971*a*), that of *S*-adenosylmethionine decarboxylase (EC 4.1.1.50) as described by Jänne & Williams-Ashman (1971*b*) and that of tyrosine aminotransferase (EC 2.6.1.5) by the method of Diamondstone (1966).

Putrescine, spermidine and spermine were measured in the trichloroacetic acid-soluble fraction after butanol extraction by the method of Raina & Cohen (1966). Putrescine was separated from diaminopropane by using 0.065 M-sulphosalicylic acid buffer (pH 3.1) in the final electrophoresis (Raina, 1963).

RNA and DNA were measured in the trichloroacetic acid-insoluble fraction after alkaline digestion (RNA) and acid hydrolysis (DNA) as described by Ashwell (1957).

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

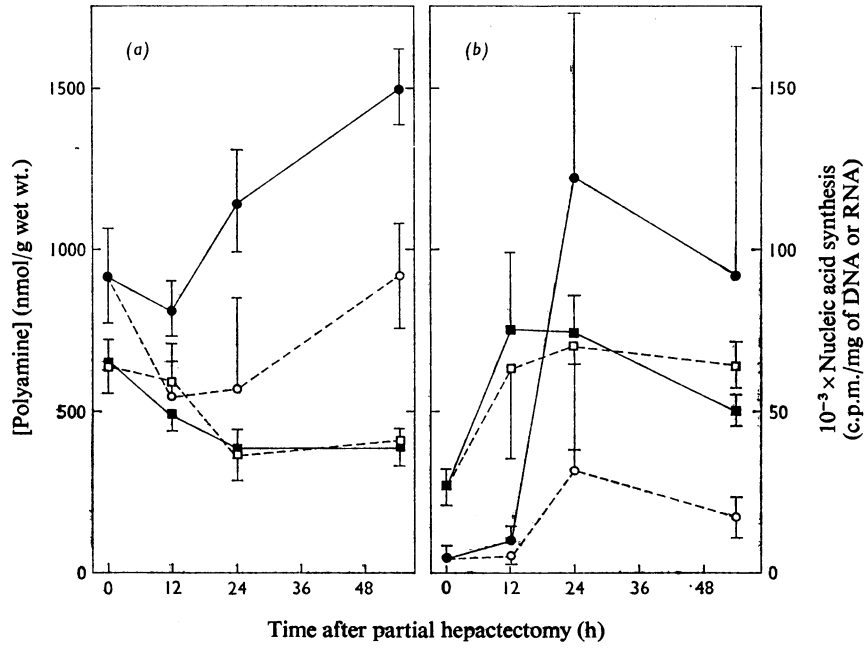

Fig. 1. *Effect of* 1,3-*diaminopropane on the accumulation of spermidine and spermine* (*a*) *and on the synthesis of DNA and RNA* (*b*) *in regenerating rat liver*

The treated animals (-----) received 75 μmol (per 100 g body wt.) of neutralized solution of 1,3-diaminopropane intraperitoneally every 3 h starting at the time of the operation. At 2 h before death all animals received 5 μCi of [6-^{14}C]orotic acid and 20 μCi of [6-^{3}H]thymidine as an intraperitoneal injection. Each group consists of three or four rats. The vertical bars represent the S.D. of the means. (*a*): ●, spermidine (control); ○, spermidine (+diaminopropane); ■, spermine (control); □, spermine (+diaminopropane). (*b*): ●, DNA (control); ○, DNA (+diaminopropane); ■, RNA (control); □, RNA (+diaminopropane).

Results and Discussion

As shown in Fig. 1(*a*), intraperitoneal administration of 1,3-diaminopropane (75 μmol/100 g body wt.) every 3 h into partially hepatectomized rats almost completely abolished the increase in spermidine concentration in the regenerating liver remnant. The concentration of spermidine increased about 1.5-fold at 54 h after partial hepatectomy in control animals, whereas it hardly reached the value found before the operation in the livers of rats receiving diaminopropane (Fig. 1*a*). Only minor changes occurred in liver spermine content.

As illustrated in Fig. 1(*b*), the synthesis of RNA (incorporation of [^{14}C]orotate into total liver RNA) in the regenerating liver continued virtually undisturbed in rats receiving diaminopropane, whereas the synthesis of DNA (incorporation of [^{3}H]thymidine) was profoundly inhibited (70–80%) in the diaminopropane-treated animals.

Another experiment, listed in Table 1, revealed that repeated injections of diaminopropane into partially hepatectomized rats during the first 30 h of regeneration virtually completely abolished the enhancement of ornithine decarboxylase activity and likewise prevented the increases in tissue putrescine and spermine concentrations that normally occur during early liver regeneration.

The activity of *S*-adenosyl-L-methionine decarboxylase, which also has a short biological half-life of only 35 min (Hannonen *et al.*, 1972), and that of tyrosine aminotransferase, similarly possessing a rapid molecular turnover rate (Kenney, 1967), either remained unchanged (adenosylmethionine decarboxylase) or markedly increased (tyrosine aminotransferase) in rats receiving diaminopropane. Liver protein synthesis (incorporation of [^{14}C]leucine into total protein) was slightly stimulated by the treatment with diaminopropane (Table 1). There was no significant difference in the synthesis of total RNA in rats injected with the compound as compared with the animals serving as controls. The weight gain of the liver was clearly, though not significantly, retarded

Table 1. *Effect of* 1,3-*diaminopropane on polyamine, protein and nucleic acid synthesis in regenerating rat liver*

Unoperated rats, partially hepatectomized (30h earlier) rats and rats partially hepatectomized and treated with 1,3-diaminopropane (as described in the legend for Fig. 1) 2h before death received 2.5μCi of [^{14}C]orotate, 10μCi of [^{3}H]-thymidine and 1μCi of [^{14}C]leucine as an intraperitoneal injection. The means (±S.D.) of the groups are given. The incubation time for ornithine decarboxylase and adenosylmethionine decarboxylase was 30min, and that for tyrosine aminotransferase 5min.

	Normal liver	Regenerating liver (controls)	Regenerating liver (diaminopropane)
No. of expts. ...	5	5	4
Ornithine decarboxylase activity (nmol/mg of protein)	0.16±0.05	1.73±0.39	0.32±0.19
Putrescine concn. (nmol/g)	73.7±15.2	142±17	56.9±18.4
Spermidine concn. (nmol/g)	660±71	1135±58	699±59
Spermine concn. (nmol/g)	625±46	386±44	375±35
Adenosylmethionine decarboxylase activity (nmol/mg of protein)	0.11±0.03	0.19±0.01	0.17±0.07
Tyrosine aminotransferase activity (nmol/mg of protein)	26.4±8.1	47.7±13.2	83.0±14.1
10^{-3}×DNA synthesis (c.p.m./mg)	5.1±1.0	118±17	31±5
10^{-3}×RNA synthesis (c.p.m./mg)	12.8±2.7	29.1±4.6	31.0±3.7
Total protein synthesis (c.p.m./mg of protein)	95±14	196±38	244±25
DNA concn. (mg/g)	1.20±0.04	0.95±0.04	0.83±0.07
RNA concn. (mg/g)	9.6±1.2	9.8±0.9	8.7±0.5
Increase in liver weight (g)	—	1.1±0.2	0.6±0.5

and the concentrations of both RNA and DNA were somewhat lower in animals receiving diaminopropane.

The inhibition of DNA synthesis, which in this particular experiment was about 75% (Table 1), thus represents the sole clear abnormality recorded in the diaminopropane-treated animals in addition to the decreased polyamine synthesis.

In another experiment (not tabulated) we found that a single injection of 1,3-diaminopropane (75μmol/100g body wt.) given 3h before death of the animals did not influence the incorporation of [^{3}H]thymidine into liver DNA, nor did it have any effect on the concentration of liver spermidine.

It remains to be established whether a causal relationship exists between the inhibition of putrescine and spermidine accumulation by diaminopropane and the prevention of the stimulation of DNA synthesis normally occurring in response to partial hepatectomy. Some work might support the latter possibility. Inonue *et al.* (1975) reported that α-hydrazino-δ-aminovaleric acid (5-amino-2-hydrazinopentanoic acid; a derivative of ornithine) inhibited ornithine decarboxylase activity, putrescine accumulation and DNA synthesis in mouse parotid gland. Unsaturated derivatives of ornithine and putrescine have also been used as inhibitors of polyamine synthesis in chick-embryo muscle cultures (Relyea & Rando, 1975). One of these, dehydro-ornithine, has been shown to inhibit cell division and differentation when added to muscle cell cultures (Relyea & Rando, 1975). Further, 1,1′-[(methylethanediylidene)-dinitrilo]diguanidine [methylglyoxal bis(guanylhydrazone)], a potent and specific inhibitor of adenosylmethionine decarboxylase and synthesis of spermidine (Williams-Ashman & Schenone, 1972), has been reported to inhibit synthesis of DNA and protein during lymphocyte activation (Kay & Pegg, 1973).

The inhibition of polyamine synthesis by directly influencing the synthesis of ornithine decarboxylase offers certain distinct advantages in comparison with the use of various structural analogues of L-ornithine, for instance. It is likely that this type of inhibition is more specific without disturbing the neighbouring reactions of the polyamine-biosynthetic pathway. On the other hand, the use of diaminopropane or any related amine as an inhibitor of the synthesis of higher polyamines is complicated by the fact that the inhibitor, being itself an amine, could take over all or some of the biological functions of natural polyamines. However, the results of the present paper can be taken as partial evidence of certain importance of spermidine accumulation during liver regeneration.

We naturally realize that the use of whole animals for this kind of experiment is complicated by a vast number of uncontrollable factors that might make it impossible to obtain any unambiguous answer to the question of the physiological function of natural polyamines. Especially embarrassing is the fact that the toxicity of higher polyamines apparently complicates a simple reversion of the observed inhibition by exogenous spermidine.

However, we feel confident that this kind of repression-type inhibition of polyamine biosynthesis merits further investigation using other and hopefully more feasible systems.

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