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Effects of Aliphatic Diamines on Rat Liver Ornithine Decarboxylase Activity

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Rat liver ornithine decarboxylase activity was decreased by administration of putrescine (1,4-diaminobutane) or other diamines, including 1,3-diaminopropane, 1,5-diaminopentane and 1,6-diaminohexane. This effect was seen in control rats and in rats in which hepatic ornithine decarboxylase activity had been increased by administration of growth hormone (somatotropin) or thioacetamide. Loss of activity was not dependent on the conversion of putrescine into polyamines and was short-lived. Within 6h after intraperitoneal administration of 0.8mmol/kg body wt., ornithine decarboxylase activity had returned to normal values. This return correlated with the rapid loss of the diamines from the liver, and the decrease in activity could be slightly prolonged by treatment with aminoguanidine, a diamine oxidase inhibitor. A decrease in ornithine decarboxylase activity by these diamines was accompanied by the accumulation in the liver of a non-diffusible inhibitor that decreased the activity of a purified ornithine decarboxylase preparation. The possibility that administration of non-physiological diamines that are not converted into polyamines might be useful for the inhibition of polyamine synthesis is discussed.

Many studies have indicated that an enhanced rate of synthesis and accumulation of spermidine accompanies changes leading to increased cell growth (Cohen, 1971; Tabor & Tabor, 1972, 1976; Bachrach, 1973; Raina & Jänne, 1975). Transient but quantitatively larger increases in the concentration of putrescine, the precursor of spermidine, also occur in response to growth-promoting stimuli. However, the relevance of these changes in amine concentrations to cell division and transformation is not yet well understood. One approach to this question is to study the effects of inhibitors of polyamine synthesis on polyamine concentrations and cellular metabolism. The formation of spermidine in mammalian cells occurs by transfer of a propylamine group from decarboxylated *S*-adenosylmethionine to putrescine. Putrescine is derived from L-ornithine by the action of L-ornithine decarboxylase (Pegg, 1970; Morris & Fillingame, 1974; Raina & Jänne, 1975). Two potent inhibitors of mammalian *S*-adenosylmethionine decarboxylase, methylglyoxal bis(guanylhydrazone) {1,1′-[(methylethanediylidene)dinitrilo]-diguanidine} (Williams-Ashman & Schenone, 1972; Pegg, 1973) and 1,1′-[(methylethanediylidene)dinitrilo]bis-(3-aminoguanidine) (Pegg & Conover, 1976), have been described and both can prevent the synthesis of spermidine. However, putrescine concentrations are increased in the presence of these compounds, since this diamine can no longer be converted into spermidine, and in addition both compounds inhibit diamine oxidase activity (Pegg, 1973; Hölttä *et al.*, 1973; Pegg, 1978). Several inhibitors of ornithine decarboxylase have been described, but most are analogues of the amino acid and are weak competitive inhibitors (Inoue *et al.*, 1975; Relyea & Rando, 1975; Mamont *et al.*, 1976; Williams-Ashman *et al.*, 1976; Newton & Abdel-Monem, 1977). Therefore they may be effective only in cells that have low endogenous ornithine concentrations.

Another possible approach to inhibition of polyamine synthesis is provided by observations that polyamine synthesis is closely controlled by putrescine. Putrescine concentrations may regulate both the activity of *S*-adenosylmethionine decarboxylase (Pegg & Williams-Ashman, 1969) and possibly the activity of ornithine decarboxylase (Raina & Jänne, 1975; Jefferson & Pegg, 1977). A marked decline in ornithine decarboxylase activity in response to exogenous putrescine or spermidine has been noted in cultured cells (Kay & Lindsay, 1973; Clark & Fuller, 1975; Fong *et al.*, 1976; Heller *et al.*, 1976), in rat liver *in vivo* (Jänne & Hölttä, 1974; Pösö & Jänne, 1976*a*) and in isolated perfused rat liver (Jefferson & Pegg, 1977). The aims of the present study were to investigate: (*a*) whether this inhibition was due to the compounds themselves, or to their conversion into other molecules, (*b*) whether the decline in ornithine decarboxylase activity was a specific effect of the physiological polyamines or could be pro-

duced by other diamines and (*c*) the length of time during which a single treatment could significantly inhibit ornithine decarboxylase activity.

It was found that other non-physiological diamines, which are not converted into spermidine derivatives, were active in decreasing the activity of ornithine decarboxylase in mammalian cells. Such diamines or other derivatives may be of value in preventing polyamine formation by mammalian cells, While this work was in progress a similar report that 1,3-diaminopropane administration decreased ornithine decarboxylase activity in rat tissues was published (Pösö & Jänne, 1976*a*).

Materials and Methods

Materials

L-[1-^{14}C]Ornithine (12–30mCi/mmol) was purchased from New England Nuclear Corp., Boston, MA, U.S.A. 1,3-Diaminopropane, 1,6-diaminohexane, aminoguanidine bicarbonate, diaminoguanidine dihydrochloride and methylglyoxal bis(guanylhydrazone) were purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. All other biochemicals were products of Sigma Chemical Co., St. Louis, MO, U.S.A. Monoacetylputrescine was prepared by the method of Tabor *et al.* (1971). Bovine growth hormone (somatotropin; NIH-GH-B18) was provided by the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, MD, U.S.A. The diamines used in the experiments were found not to be contaminated with significant amounts of other diamines (less than 1%) by electrophoretic separation and staining with ninhydrin as described below.

Treatment of animals

All amines, thioacetamide and other chemicals were administered to rats by intraperitoneal injection of a neutral solution in 0.9% NaCl. The concentration of the solution was adjusted so that the volume injected was between 0.3 and 0.8ml. Solutions were adjusted to neutral pH by the addition of 6M-HCl. Growth hormone was dissolved at a concentration of 4mg/ml in 0.9% NaCl solution adjusted to pH8.0 with NaOH and administered by subcutaneous injection at a dose of 4mg/kg body wt.

Male Sprague–Dawley rats weighing 225–250g were purchased from Charles River Breeding Laboratories, Wilmington, MA, U.S.A. For all experiments, the rats were maintained on a 12h light/12h dark cycle and fed *ad libitum* on Purina Rat Chow. Ornithine decarboxylase was induced in the livers of these rats by administration of 150mg of thioacetamide/kg body wt. 19–26h before they were killed (Fausto, 1970).

Ornithine decarboxylase preparation and assays

Ornithine decarboxylase was purified by the method of Hölttä (1975) and in the final preparation the specific activity was 80nmol of $^{14}CO_2$ released/min per mg of protein at 37°C. Ornithine decarboxylase activity was assayed as previously described by measuring the release of $^{14}CO_2$ from [1-^{14}C]ornithine (Pegg & Williams-Ashman, 1968). The assay mixture contained 100mM-Tris/HCl, pH7.5, 2.5mM-dithiothreitol, 0.1mM-pyridoxal phosphate, 0.5mM-L-[1-^{14}C]ornithine (2μCi/μmol) and tissue extract in a total volume of 0.25ml. When purified ornithine decarboxylase was added, sufficient enzyme was used so that at least 10000c.p.m. was released in those samples that did not have added inhibitors.

Crude tissue extracts for the measurement of ornithine decarboxylase activity were prepared by homogenization of the tissue at 4°C in 20mM-Tris/HCl/2.5mM-dithiothreitol/0.1mM-EDTA (disodium salt), pH7.5. The homogenates were centrifuged at 4°C for 30min at 15000rev./min in a Sorvall RC5 refrigerated centrifuge and the supernatant was used for the enzyme assay. In some experiments, the tissue extract was dialysed overnight against 100vol. of the solution used for homogenization. Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard. Results are expressed as pmol of CO_2 released/30min per mg of protein added, but none of the treatments that altered ornithine decarboxylase activity had any significant effect on the yield of protein in the centrifuged supernatant fractions. The same relative values would therefore be observed if the activity were expressed per g of tissue.

Separation of ornithine decarboxylase activity by gel filtration

About 5ml of liver extract containing ornithine decarboxylase activity prepared as described above was applied to a column (2.6cm×30cm) of Bio-Gel A-1.5m (100–200 mesh; purchased from Calbiochem, San Diego, CA, U.S.A.). The column was equilibrated with 20mM-Tris/HCl (pH7.5)/2.5mM-dithiothreitol and was eluted with the same solution. Fractions (10ml) were collected and assayed for protein and ornithine decarboxylase activity as described above. The fractions were also assayed for the presence of an inhibitor of ornithine decarboxylase by addition of 0.1ml of the extract to an ornithine decarboxylase assay containing purified enzyme. All of the assays of ornithine decarboxylase activity were carried out in assay media of the same ionic strength and addition of the column eluate fractions did not alter this significantly. It is unlikely therefore that changes in the salt content of the column eluates could account for the observed changes.

Diamine determination

The diamine concentration of tissue samples was determined by extraction of the amines into butanol and subsequent paper electrophoresis and staining with ninhydrin. Tissue samples were frozen in liquid N_2 and stored at −70°C until required. About 1g of tissue was then homogenized in 0.01M-HCl and the diamines present were extracted into butanol (Pegg *et al.*, 1970). The diamines were then separated by electrophoresis at 5000V for 60min on Whatman 3MM paper with 65mM-sulphosalicylic acid, pH3.4 (Raina, 1963), in a Savant FP30B electrophoresis apparatus (Savant Instruments, Hicksville, NY, U.S.A.). The paper was dried and the amines were determined by measurement of the colour produced by reaction with ninhydrin (Pegg *et al.*, 1970).

The results were corrected for losses due to incomplete recovery at the butanol-extraction phase. The recovery at this step was 65% for 1,3-diaminopropane, 91% for 1,4-diaminobutane, 72% for 1,5-diaminopentane and 42% for 1,6-diaminohexane. Recovery was measured by addition of the diamines to the tissues from untreated rats immediately after homogenization and was independent of the tissue used. Although the tissue samples were rapidly rinsed to remove blood before freezing, it is possible that a small amount of the measured diamine was in fact present in the extracellular space rather than within the cell. This may lead to a slight overestimation of the intracellular concentration, particularly at times shortly after injection of the diamine. However, the diamines were rapidly removed from the plasma, and by 1h after injection hepatic concentrations greatly exceeded those present in the blood (A. E. Pegg, unpublished work).

Results

As shown in Table 1 and previously reported by others (Morris & Fillingame, 1974; Raina & Jänne, 1975), growth-hormone administration to adult rats led to a 25-fold increase in ornithine decarboxylase activity within 4h. Administration of a large dose (150mg/kg body wt.) of putrescine (1,4-diaminobutane) at the same time as the hormone had little effect on this increase (results not shown), but when the putrescine was given 150min before death the magnitude of the growth-hormone-induced stimulation was decreased by at least 50%. This effect was not due to the conversion of some of the administered putrescine into spermidine, since it was not prevented by prior injection of a large dose of methylglyoxal bis(guanylhydrazone). This drug completely prevents the conversion of putrescine into spermidine under these circumstances (Pegg, 1973). These results indicate a difference between the rat liver system studied here and the inhibition of ornithine decarboxylase activity in lectin-stimulated human lymphocytes by putrescine, which is prevented by inhibition of the conversion of putrescine into spermidine (Kay & Lindsay, 1973).

Table 1. *Effect of diamines on stimulation of liver ornithine decarboxylase by growth hormone*

Growth hormone was given at a dose of 4 units/kg body wt. (0.8 unit/mg of protein) 4h before death. Diamines were given by intraperitoneal injection of a solution containing a dose of 150mg of the dichloride salt per kg body wt. 150min before death. MGBG [methylglyoxal bis(guanylhydrazone)] was administered by intraperitoneal injection of 60mg/kg body wt. at the same time as the growth hormone. Results are shown as the means±S.E.M. for four or five observations.

Growth-hormone treatment	Diamine treatment	Ornithine decarboxylase activity (nmol of $^{14}CO_2$/30min per mg of protein)
−	None	0.027±0.010
+	None	0.652±0.043
+	1,3-Diaminopropane	0.013±0.008
+	1,4-Diaminobutane	0.225±0.031
+	1,4-Diaminobutane +MGBG	0.195±0.009
+	MGBG	0.592±0.037
+	1,5-Diaminopentane	0.140±0.028
+	1,6-Diaminohexane	0.035±0.006

As shown in Table 1, other diamines were also able to inhibit the stimulation of hepatic ornithine decarboxylase by growth hormone. All of the diamines tested were active when given at a dose of 150mg/kg body wt. 90min after the hormone. 1,5-Diaminopentane (cadaverine) was only slightly more active than putrescine, but 1,3-diaminopropane and 1,6-diaminohexane were much more effective in diminishing ornithine decarboxylase activity. After treatment with these diamines, the activity was decreased to control values or below.

To show that the effects described above were not simply caused by interference of the diamines with the hormone action on the liver, their effects on hepatic ornithine decarboxylase activity after stimulation by thioacetamide (Fausto, 1970) were examined (Table 2). Thioacetamide is able to induce a large increase in hepatic ornithine decarboxylase activity in both normal and hypophysectomized rats and therefore cannot be exerting this effect by stimulating growth hormone release (Ono *et al.*, 1973). In this experiment, the thioacetamide was given 22h before the amines. As shown in Fig. 1, thioacetamide produced a large increase in ornithine decarboxylase activity, reaching a peak at 20–28h after administration. Thus the amines were administered and the rats killed over a time period throughout which the ornithine decarboxylase activity (although greatly stimulated over that

Table 2. *Inhibition of hepatic ornithine decarboxylase activity by diamines*

Hepatic ornithine decarboxylase activity was increased by administration of 150mg of thioacetamide/kg body wt. 24.5h before death. The diamines indicated were administered 2.5h before death as indicated in the legend to Table 1. Results for the ornithine decarboxylase activity are shown as the means±S.E.M. of seven or eight measurements.

Diamine administered	Ornithine decarboxylase activity (nmol of $^{14}CO_2$/30min per mg of protein)
None	1.229±0.080
1,3-Diaminopropane	0.107±0.024
1,4-Diaminobutane	0.461±0.085
1,5-Diaminopentane	0.581±0.122
1,6-Diaminohexane	0.180±0.022

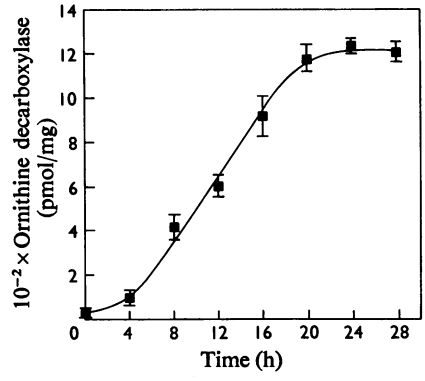

Fig. 1. *Time course of stimulation of hepatic ornithine decarboxylase by thioacetamide*

Rats were given a single dose of 150mg of thioacetamide/kg body wt. at 10:00h and killed at the times indicated.

in rats not treated with thioacetamide) was at a stable value. Within 150min after administration of the diamines there was a marked decrease in ornithine decarboxylase activity. Again, 1,3-diaminopropane and 1,6-diaminohexane, which diminished activity by 91 and 85% respectively, were more effective than 1,4-diaminobutane and 1,5-diaminopentane.

It is known that putrescine is a weak competitive inhibitor of ornithine decarboxylase activity (Pegg & Williams-Ashman, 1968). A complete study of the potency of other diamines in this regard has not yet been made. However, the decreased ornithine decarboxylase activity produced by administration of these diamines was not due to the presence of the compounds in the assay medium, since direct addition of 1mM concentrations to the assays produced little inhibition of the activity of purified

Table 3. *Addition of diamines and of extracts from livers of rats treated with diamines to purified ornithine decarboxylase*

All assays contained purified ornithine decarboxylase (about 5μg of protein). The first column shows the effects of adding 1mM of the diamine indicated on the activity of this enzyme. Activity in the absence of added diamines was set at 100%. The second column shows the effect on the activity of the addition of 3mg of protein prepared from the livers of rats treated with the diamines shown 1h previously, as in Fig. 3. For this column, 100% activity represents the expected sum of the activity present in the added liver extract and that of the purified ornithine decarboxylase. The activity of the purified enzyme was such that about 10nmol of $^{14}CO_2$ was released. This increased to about 14nmol when the crude liver extract from an untreated rat was added.

Treatment of rat, or diamine added	Ornithine decarboxylase activity (% of control): In the presence of 1mM-diamine	In the presence of 3mg of protein
None	100	97
1,3-Diaminopropane	106	30
1,4-Diaminobutane	91	62
1,5-Diaminopentane	99	78
1,6-Diaminohexane	85	32

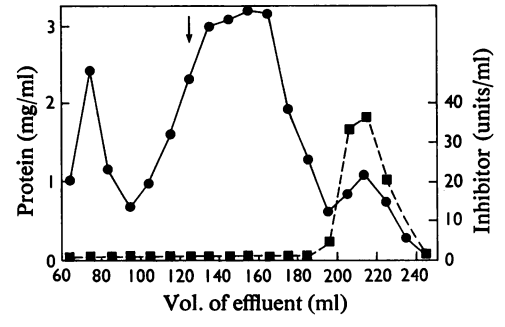

Fig. 2. *Fractionation of ornithine decarboxylase-inhibitory activity by passage through Bio-Gel A-1.5m*

An extract (5ml) from the liver of a rat injected with 1,6-diaminohexane (0.8mmol/kg body wt.) 4h before death was applied to the column. Fractions (10ml) were collected and 0.1ml samples assayed for ornithine decarboxylase inhibitor activity (■) and protein (●). The arrow indicates the elution position of ornithine decarboxylase. One unit of ornithine decarboxylase inhibitor was defined as causing 50% inhibition of the activity of 1 unit (releasing 1pmol of CO_2/30min) of the purified ornithine decarboxylase under the assay conditions described in the text.

ornithine decarboxylase (Table 3). This finding agrees with the lack of inhibition of ovarian ornithine decarboxylase by these diamines added directly

to the assay medium (Guha & Jänne, 1977). Also, extensive dialysis of the extracts from the diamine-treated rats did not increase the activity.

However, when extracts from the livers of diamine-treated rats were added to a purified ornithine decarboxylase preparation the activity was decreased (Table 3). This inhibition was produced by dialysed liver supernatant fractions, and the presence of this inhibitory factor may therefore account for some or all of the decrease in the endogenous liver ornithine decarboxylase activity in response to the diamines. These data support the report that high exogenous putrescine concentrations lead to the appearance of an inhibitor of ornithine decarboxylase in cells in culture and in rat liver (Fong *et al.*, 1976; Heller *et al.*, 1976). It appears that other diamines containing three to six carbon atoms can also lead to the production of this inhibitor.

As shown in Fig. 2, the inhibitory activity could be isolated after passage through a column of Bio-Gel A-1.5m. The inhibitory activity was eluted from the column at a later position than ornithine decarboxylase, suggesting that the activity was associated with a molecule of mol.wt. approx. 25000, whereas the monomeric form of the enzyme has mol.wt. approx. 70000 (Morris & Fillingame, 1974; Raina & Jänne, 1975). Fig. 2 therefore indicates that an inhibitory factor similar to that characterized from cells cultured in the presence of putrescine (Fong *et al.*, 1976; Heller *et al.*, 1976) is also produced in rat liver in response to 1,6-diaminohexane. Similar material was found after chromatography of extracts from rat livers of rats treated with 1,3-diaminopropane, 1,4-diaminobutane and 1,5-diaminopentane (results not shown). Heller *et al.* (1976) have reported that exposure of the complex of ornithine decarboxylase and the putrescine-induced inhibitor to 10% -satd. $(NH_4)_2SO_4$ leads to its dissociation, and active enzyme could be recovered from this salt-treated complex. However, we were unable to obtain any increase in ornithine decarboxylase activity after extracts from the livers of rats treated with diamines were made 10% satd. with respect to $(NH_4)_2SO_4$ and then passed through the Bio-Gel column to separate enzyme and inhibitor. None of the inhibited extracts treated in this way showed a greater amount of ornithine decarboxylase

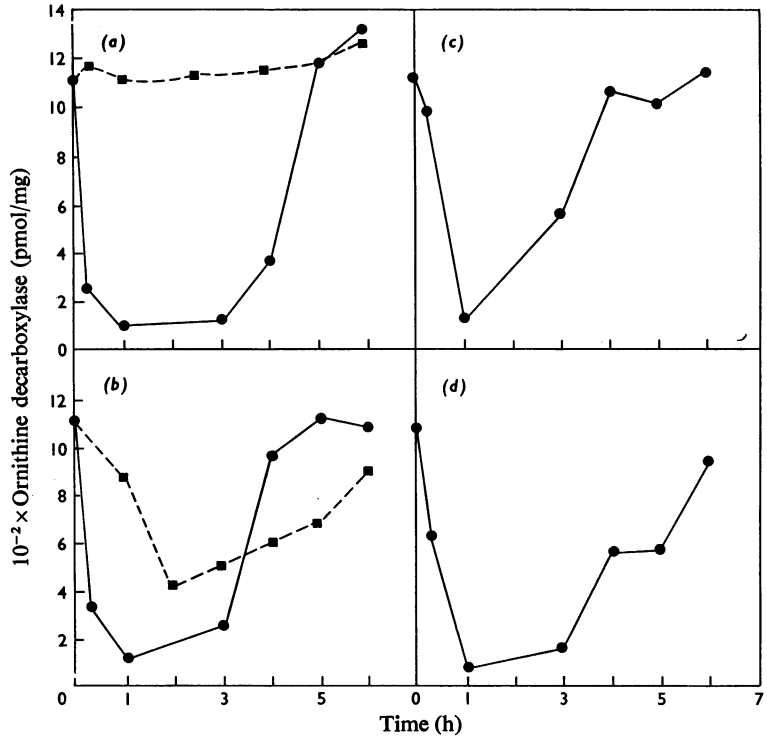

Fig. 3. *Time course of inhibition of hepatic ornithine decarboxylase by diamines*
Rats that had received thioacetamide 20h earlier as described in Fig. 1 were given a single injection of 0.9% NaCl alone (*a*, ■), or 0.8mmol of diamine/kg body wt.: 1,3-diaminopropane (*a*, ●), 1,4-diaminobutane (*b*, ●), *N*-acetyl-1,4-diaminobutane (*b*, ■), 1,5-diaminopentane (*c*, ●) or 1,6-diaminohexane (*d*, ●).

activity in the appropriate region of the column eluate than was present before salt treatment.

The length of time for which ornithine decarboxylase activity in the liver remained decreased after treatment with the diamines was investigated, in each case after a single dose of 0.8 mmol/kg body wt. As shown in Fig. 3, there was a rapid loss of enzyme activity after intraperitoneal injection of each diamine, and within 1 h activity was almost completely lost. Activity remained depressed for only a short time and was restored to the control value within 6h. There were differences between the diamines tested in both the rapidity with which loss of ornithine decarboxylase activity was achieved (1,3-diaminopropane and putrescine produced a much greater decrease in activity within 15 min than did the longer-chain diamines) and the period over which a maximum decrease was produced. Greater than 70% loss of activity was produced by 1,3-diaminopropane for 4h, whereas by 4h the effects of putrescine and 1,5-diaminopentane had almost completely worn off. 1,6-Diaminohexane also had a longer-lasting effect than putrescine or 1,5-diaminopentane. Thus the experiments depicted in Tables 1 and 2 indicating that the 1,3-diamino and 1,6-diamino compounds appeared to be more potent than the diamines with four and five carbon atoms in decreasing ornithine decarboxylase activity can be explained by the fact that activity was measured 2.5h after treatment, at which time the effects of the former compounds were still in force but those of the latter were wearing off.

Monoacetylputrescine (*N*-acetyl-1,4-diaminobutane) administered at an equimolar dose was slower and less active than putrescine in promoting the fall in ornithine decarboxylase activity (Fig. 3). Maximum inhibition was seen after 2h, at which time activity was about 38% of control. There was a continued slight decrease in activity for a further 4h. The effect was therefore somewhat more long-lasting than that of putrescine (Fig. 3). These data are consistent with the interpretation that the monoacetylputrescine is deacylated to putrescine, which is responsible for the effects on ornithine decarboxylase. Putrescine concentrations measured in the liver agree with this interpretation, as they rose more slowly after monoacetylputrescine treatment than after injection of putrescine itself (see below), reaching a peak of only 0.4 μmol/g wet wt. by 1.5–2h after injection and then

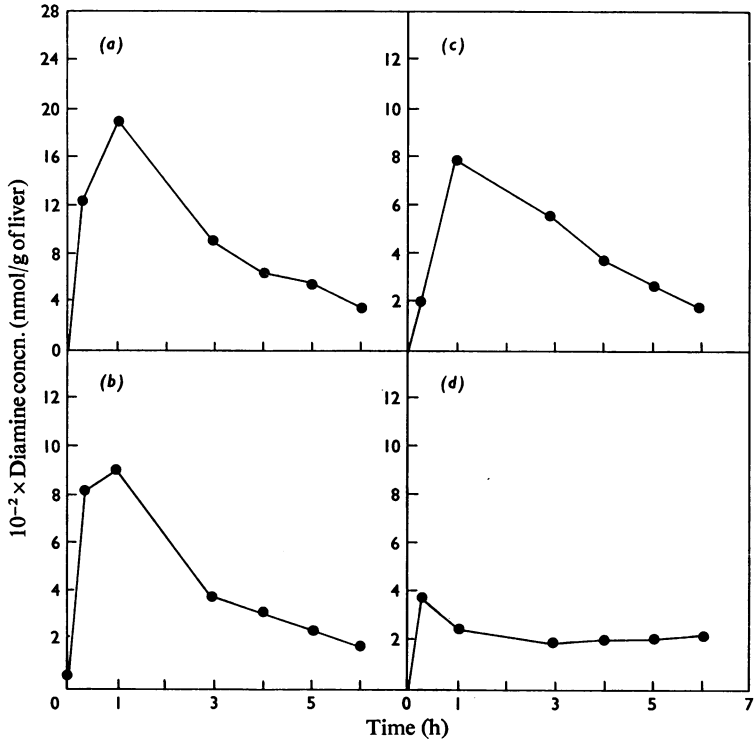

Fig. 4. *Diamine concentrations in the liver after administration by intraperitoneal injection*
Rats were treated as described in Fig. 3. Results are shown for 1,3-diaminopropane (*a*), 1,4-diaminobutane (*b*), 1,5-diaminopentane (*c*) and 1,6-diaminohexane (*d*), in each case after the injection of 0.8mmol/kg body wt.

declining. Whether the monoacetylputrescine is deacetylated at an extrahepatic site and then transported into the liver, or is taken up and then deacylated, is not yet known. Enzymic formation and degradation of monoacetylputrescine has been reported to occur with liver, kidney and brain extracts (Seiler & Al-Therib, 1974).

The actual concentrations of diamines present in the livers of rats after administration by intraperitoneal injection as in the above experiments are shown in Fig. 4. There was a substantial uptake of the administered diamines into the liver, reaching a peak at 1h after injection. Reflecting the rapidity of loss in ornithine decarboxylase activity, the uptake of the 1,3- and 1,4-diamino compounds appeared to be faster than that of the longer-chain diamines. Further, it appeared that the peak concentration of diamine found in the liver was greater for 1,3-diaminopropane (almost 2 μmol/g wet wt.) than for 1,4-diaminobutane and 1,5-diaminopentane (0.8–0.9 μmol/g wet wt.) and much greater than for 1,6-diaminohexane (0.2–0.3 μmol/g wet wt.). It should be noted, however, that the values obtained for the 1,6-diaminohexane may be more unreliable than those measured for the other diamines, since the efficiency of extraction of this compound into butanol was poor (see the Methods and Materials section). There was a fair correlation between the concentrations of the diamines present in the livers and the loss of ornithine decarboxylase activity, for the activity appeared to be restored as the concentrations of the diamines decreased (except for the 1,6-diaminohexane). However, even when the decrease of ornithine decarboxylase activity had completely passed, measurable concentrations of the diamines remained present in the liver. These amounts (200–400 nmol/g of tissue) were still substantially in excess of the normal putrescine concentration in the liver.

These data suggested that, if the very rapid decline in tissue diamine concentrations could be prevented, the decrease in ornithine decarboxylase activity might be prolonged. An experiment that supports this possibility is shown in Table 4. Rats were treated with aminoguanidine or with iproniazid (1-isonicotinoyl-2-isopropylhydrazine) shortly before administration of 1,3-diaminopropane. Prior administration of aminoguanidine alone did not have an appreciable effect on ornithine decarboxylase activity, and this drug had only a very slight potentiating effect on the decrease of ornithine decarboxylase activity by 1,3-diaminopropane at 3h after injection. By 6h after injection of 1,3-diaminopropane the effects of the diamine alone had worn off, as described above, and as shown (Fig. 3) ornithine decarboxylase activity was at slightly greater than control values. However, in those rats previously treated with aminoguanidine as well as 1,3-diaminopropane, ornithine decarboxylase activity was still substantially decreased (Table 4). Aminoguanidine is a potent inhibitor of diamine oxidase (Kapeller-Adler, 1970) and led to a substantial increase in the measured tissue concentration of 1,3-diaminopropane 3h after injection (about 1.6 μmol/g wet wt. of tissue compared with about 0.9 μmol/g in rats not treated with the aminoguanidine), although there was less difference in the values 6h after injection (0.8 and 0.5 μmol/g wet wt. of tissue respectively). Iproniazid, which is a potent inhibitor of monoamine oxidase but is much less inhibitory to diamine oxidase (Shindler & Bardsley, 1976), did not significantly prolong the inhibition of ornithine decarboxylase by 1,3-diaminopropane (Table 4).

Table 4. *Effects of aminoguanidine and iproniazid on inhibition of ornithine decarboxylase by* 1,3-*diaminopropane*

Rats were pretreated with thioacetamide 26h before death as described in Table 2. 1,3-Diaminopropane (80 μmol/kg body wt.) was administered at the time before death indicated. Aminoguanidine or iproniazid was administered by intraperitoneal injection of 100 mg/kg body wt. 30 min before the diamine. When aminoguanidine alone was given the rats were killed 6h later. Mean values ± S.E.M. are given for five to seven rats.

Treatment	Time after diamine administration (h)	Ornithine decarboxylase activity (nmol of $^{14}CO_2$/30min per mg of protein)
Control	—	1.17 ± 0.08
1,3-Diaminopropane	3	0.17 ± 0.04
1,3-Diaminopropane + aminoguanidine	3	0.13 ± 0.01
1,3-Diaminopropane	6	1.28 ± 0.13
1,3-Diaminopropane + aminoguanidine	6	0.64 ± 0.15
1,3-Diaminopropane + iproniazid	6	1.16 ± 0.14
Aminoguanidine	—	1.14 ± 0.05

Discussion

Although the loss of ornithine decarboxylase activity in response to administration of putrescine has been reported to occur in lymphocytes (Kay & Lindsay, 1973), mouse 3T3 cells (Clark & Fuller, 1975), H-35 rat hepatoma cells (Fong *et al.*, 1976), mouse neuroblastoma and L1210 cells (Heller *et al.*, 1976), in rat liver *in vivo* (Jänne & Hölttä, 1974) and in the isolated perfused rat liver (Jefferson & Pegg, 1977), in many of these experiments a similar diminution was produced by spermidine or spermine. Since putrescine is an immediate precursor of these polyamines it was possible that the effects of putrescine were mediated by the production of the polyamines. The present experiments argue strongly that the decrease is not produced in this way, for pretreatment of the rats with methylglyoxal bis(guanyl-

hydrazone) which prevents spermidine synthesis by inhibition of *S*-adenosylmethionine decarboxylase activity (Pegg, 1973; Williams-Ashman *et al.*, 1976), did not prevent the putrescine-induced decrease in rat liver ornithine decarboxylase activity. Further, some non-physiological diamine analogues of putrescine, which are poor substrates for the propylamine transferase forming spermidine, and are thus converted into polyamine analogues to a very limited extent, if at all (Raina & Jänne, 1975; H. Hibasami & A. E. Pegg, unpublished work), were also able to decrease ornithine decarboxylase activity. Thus the decrease appears to be due to the diamine itself rather than a polyamine metabolite. The loss of ornithine decarboxylase activity in response to polyamines could be due to the conversion of spermine or spermidine into putrescine (Pegg, 1970). This reaction is catalysed by an enzyme present in peroxisomes (Hölttä, 1977), but the extent to which it takes place *in vivo* is not yet established. One cannot rule out the possibility that the effect is due to other metabolites of the diamines rather than the amines themselves, but the close parallel between the loss of activity and the diamine concentrations argues against this. Since monoacetylputrescine was less active than putrescine it appears that this derivative, which it is known can be formed in the liver (Seiler & Al-Therib, 1974), is not responsible for the inhibitory action.

The decrease in ornithine decarboxylase activity and subsequent prevention of putrescine synthesis by administration of non-physiological diamines provides a means by which polyamine synthesis may be inhibited. Such a means might be a useful tool for investigating the function of polyamines. While these experiments were in progress, the inhibition of ornithine decarboxylase by 1,3-diaminopropane was reported by Pösö & Jänne (1976*a*). These investigators were able to inhibit polyamine accumulation in liver regenerating after partial hepatectomy by injections of this diamine every 3h, and this treatment prevented the rise in DNA synthesis 24h after the operation (Pösö & Jänne, 1976*b*). The interpretation of their data is complicated by the unknown pharmacological effects of the very high concentrations of metabolites of 1,3-diaminopropane that could accumulate over a 24h period in which a total dose of more than 5mmol/kg body wt. was given. We have found that a single dose of 1,3-diaminopropane combined with 1,1′-[(methylethanediylidene)dinitrilo]bis-(3-aminoguanidine), an irreversible inhibitor of *S*-adenosylmethionine decarboxylase (Pegg & Conover, 1976), can prevent DNA synthesis in regenerating liver (Wiegand & Pegg, 1978). This inhibition of DNA synthesis probably results from the prevention by these drugs of the normal accumulation of spermidine.

The very short-lived inhibitory action of the diamines on ornithine decarboxylase is presumably due to the rapid decrease in the cellular concentration. This phenomenon may limit their usefulness in inhibition of polyamine synthesis unless a means can be found to prevent the decline. The metabolism of exogenous diamines in the rat is not well understood. Up to 40% of the ^{14}C from [1,4-^{14}C]putrescine is known to be exhaled in rats (Jänne, 1967; McGill & Pegg, 1978) and mice (Seiler & Eichentopf, 1975) as $^{14}CO_2$ within a few hours of administration, and this $^{14}CO_2$ production is blocked by inhibitors of diamine oxidase such as aminoguanidine (Seiler & Eichentopf, 1975; McGill & Pegg, 1978). Diamine oxidase attacks 1,3-diaminopropane and 1,6-diaminohexane, although putrescine and 1,5-diaminopentane are better substrates (Bardsley *et al.*, 1974). This specificity could account for the longer-lasting diminution in ornithine decarboxylase by administration of the former amines. However, the importance of diamine oxidase in decreasing the liver concentration of putrescine is in doubt, for inhibition of diamine oxidase had only a small effect in increasing hepatic concentrations in the rat (McGill & Pegg, 1978) or in the mouse (Seiler & Eichentopf, 1975). Similarly, in the experiment described in Table 4, aminoguanidine only had a modest effect in prolonging the high concentrations of 1,3-diaminopropane in the rat liver. This was sufficient to maintain the decrease of ornithine decarboxylase for slightly longer than in untreated rats. A similar effect may contribute to the synergistic effects of combined doses of 1,3-diaminopropane and 1,1′-[(methylethanediylidene)-dinitrilo]bis-(3-aminoguanidine) described above, since this drug also inhibits diamine oxidase (McGill & Pegg, 1978).

Most of the oxidation of exogenous diamines by diamine oxidase probably occurs in the gastrointestinal tract, which has very large amounts of this enzyme (Seiler & Al-Therib, 1974; Shindler & Bardsley, 1976; Kapeller-Adler, 1970). Whether putrescine taken up by the liver is transported out of the liver at a sufficient rate to be available for oxidation elsewhere is not known. Another pathway for putrescine metabolism involving the formation of monoacetylputrescine and its subsequent oxidation by monoamine oxidase has been described (Seiler & Al-Therib, 1974; Seiler & Eichentopf, 1975), but since iproniazid, a potent monoamine oxidase inhibitor, had no effect in the present experiments on prolonging the inhibition of ornithine decarboxylase by 1,3-diaminopropane, or on hepatic concentrations of this diamine, this pathway cannot be important in these experimental conditions. It is therefore apparent that the diamine concentration in the liver after administration of the diamines by intraperitoneal injection is affected to only a small extent by diamine oxidase and to an even lesser extent by monoamine oxidase. When a greater understanding of the factors controlling diamine concentrations

in the liver under these conditions is available, it may be possible to devise conditions where the high concentration of diamine required to inhibit ornithine decarboxylase activity can be maintained.

The fact that such high concentrations of putrescine (and the other diamines) appear to be required for inhibition of ornithine decarboxylase activity and for production of the putative 'antizyme' (Heller *et al.*, 1976) casts some doubt on the physiological relevance of this inhibition. In general, putrescine concentrations in the liver are of the order of 15–30nmol/g wet wt. (Tabor & Tabor, 1972, 1976; Jefferson & Pegg, 1977) and values of greater than 150nmol/g wet wt. are seen only after abnormal physiological or pharmacological manipulations such as partial hepatectomy or treatment with thioacetamide or carbon tetrachloride (Raina & Jänne, 1975). In cultured hepatoma cells significant inhibition of ornithine decarboxylase activity was obtained by exposure to extracellular concentrations of 10μM-putrescine (Fong *et al.*, 1976; McCann *et al.*, 1977), although the extent to which the diamine might be concentrated within these cells is not known. The results obtained in the present experiments support recent findings that an inhibitor of ornithine decarboxylase is detectable in cells exposed to putrescine and a similar inhibitor was present in liver homogenates of rats treated with high concentrations of other diamines. Recently other workers have also reported that 1,3-diaminopropane led to production of such an inhibitor in hepatoma cells (McCann *et al.*, 1977) and regenerating rat liver (Kallio *et al.*, 1977). However, at present it would be premature to conclude that this inhibitor is in fact responsible for the very rapid fall in enzyme activity seen in response to diamines. The inhibitor appears to be manifest only under conditions where all ornithine decarboxylase activity has been lost and it has not yet been shown that increased accumulation of the inhibitor parallels the rapid fall in enzyme activity on exposure to diamines. Indeed, evidence that 1,3-diaminopropane or putrescine administration to rats decreases ornithine decarboxylase activity in regenerating liver by a mechanism not involving this inhibitor has been reported by Kallio *et al.* (1977). The inhibitor was not found in mouse 3T3 fibroblasts, although substantial falls in ornithine decarboxylase activity were produced by the addition of putrescine (Clark & Fuller, 1976). We have found that treatment of rats with diamines as described here also caused decreases in ornithine decarboxylase activity in other tissues including kidney, prostate and heart (A. E. Pegg & S. McGill, unpublished work). As in the liver, inhibition appeared to reflect the diamine concentrations in these tissues, and these were very high in the kidney shortly after injection and then fell more rapidly than in the liver. Inhibition of renal ornithine decarboxylase by this treatment was therefore also very short-lived and at no time were we able to detect any inhibitor of ornithine decarboxylase in kidney extracts even where no enzymic activity could be found. We suggest therefore that caution should be exercised in the use of the proposed term 'antizyme' for the inhibitor of ornithine decarboxylase (Heller *et al.*, 1976; Fong *et al.*, 1976) until the physiological role of this material is more firmly established.

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