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Effects of Agmatine on Polyamine Metabolism and the Growth of Prostate Tumor Cells

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The effects of agmatine on the enzymes responsible for the biosynthesis of polyamines, the resultant levels of polyamines, and their effect on the growth of DU145 human prostate tumor cells were investigated. When agmatine was added to the medium, ornithine decarboxylase (ODC, EC 4.1.1.17) activity was substantially reduced, but S-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50) activity increased markedly. These changes in ODC and SAMDC activities were the result of an induction of ODC-antizyme and a decreased turnover rate of SAMDC in the presence of agmatine. Accordingly, there was a decrease in the intracellular levels of putrescine and spermidine but an increase in the intracellular level of spermine. Cell growth was markedly inhibited by agmatine treatment and this inhibition was not recovered by the addition of putrescine or spermidine. Our results suggest that agmatine alters the intracellular amounts of polyamine in the cells, closely related to the inhibition of cell growth.

Keywords: Agmatine, Ornithine decarboxylase, Polyamine, Prostate tumor, S-adenosylmethionine decarboxylase.

Introduction

Agmatine, 4-aminobutylguanidine, is produced by decarboxylation of L-arginine by the enzyme arginine decarboxylase (ADC). Agmatine was previously believed to be restricted to plants and bacteria where it serves as a biosynthetic precursor of polyamines (Tabor and Tabor, 1984), which are essential for cell growth and differentiation. Recently, agmatine and arginine decarboxylase were also found in mammalian organs (Li *et al.*, 1994; Morrissey *et al.*, 1995; Raasch *et al.*, 1995). Agmatine, a clonidine displacing substance, has been shown to be a novel endogenous ligand for imidazoline receptors (I receptor) (Reis *et al.*, 1995; Parini *et al.*, 1996; Regunathan *et al.*, 1996). Although the cellular mechanism by which agmatine exerts its biological actions is for the most part unknown, evidence indicates that agmatine is a biologically active substance in mammals. It stimulates the release of catecholamine from adrenal chromaffin cells (Li *et al.*, 1994), increases arterial blood pressure (Sun *et al.*, 1995), stimulates the release of insulin (Sener *et al.*, 1989), increases the release of luteinizing hormone-releasing hormone from the hypothalamus (Kalra *et al.*, 1995), enhances morphine analgesia, and is also a competitive inhibitor of all nitric oxide synthase isozymes (Galea *et al.*, 1996). Agmatine can also inhibit the growth and proliferation of vascular smooth muscle cells (Regunathan *et al.*, 1996; Regunathan and Reis, 1997).

The polyamines, putrescine, spermidine, and spermine, are essential for cell growth and proliferation (Tabor and Tabor, 1984; Pegg, 1988). Although the specific functions of these compounds still remain somewhat unclear, the polyamines have important roles in protein synthesis, cell division, protonation at physiological pH, and interaction with a variety of cellular targets. The polyamine biology have been extensively reviewed (Marton and Morris, 1987; Marton and Pegg, 1995; Williams, 1997). In contrast to these positive effects of polyamines, there are a few reports of cell growth inhibition caused by the addition of polyamines to culture medium (Brunton *et al.*, 1991; Monti *et al.*, 1996). Therefore, it appears that polyamines play both a positive and negative role in regulating cell growth. Our previous papers (Yang and Cho, 1991; Park and Cho,

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Abbreviations used: HPLC, high pressure liquid chromatography; ODC, ornithine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; TLC, Thin layer chromatography.

1992; Choi and Cho, 1994) showed that agmatine affected the key plant enzymes responsible for the biosynthesis of polyamines, suggesting that agmatine was an important regulator of polyamine metabolism in plants. However, information on the effect of agmatine on levels of polyamine in mammalian cells is very limited (Satriano *et al.*, 1998), and no satisfactory explanation of the means by which levels of polyamine are regulated is yet available. Although agmatine was found even in mammalian cells, determination of intracellular agmatine levels have been hampered: TLC Rf values and HPLC retention times of agmatine and putrescine are so close that it is difficult to determine the amount of intracellular agmatine.

At present, no information is available on the relationship between agmatine and S-adenosylmethionine decarboxylase (SAMDC), which is responsible for the biosynthesis of polyamines in mammalian cells. We utilized DU145 human prostate tumor cells to ascertain whether agmatine can affect enzymes such as SAMDC, the key enzyme for polyamine biosynthesis. The present investigation was designed to study the time course of changes in polyamine levels, enzymatic activities, and their correlation with cell growth in the presence of agmatine. We report here that a new useful method for determination of intracellular agmatine was developed. Most importantly, the data presented here shows that agmatine affects levels of polyamine, enzymatic activities via induction of ODC-antizyme, and causes a decrease in turnover rate of SAMDC and cell growth. We discuss the significant relationship among levels of polyamine, enzymatic activities, and inhibition of cell growth.

Materials and Methods

Materials L-[1-^{14}C] Ornithine (54 mCi/mmol), and S-[Carboxy-^{14}C]-adenosyl-L-methionine (58.9 mCi /mmol) were purchased from Amersham (Buckinghamshire, UK). Agmatine was purchased from Sigma Chemical Co. (St. Louis, USA). All other chemicals were of reagent grade and were obtained from Sigma unless otherwise stated.

Cell culture DU145 human prostate tumor cells were grown in medium 199 (GIBCO, Grand Island, USA) supplemented with 10% (v/v) calf serum and antibiotics (62.5 IU/ml penicillin, 0.005% streptomycin, and 0.5 μg/ml fungizone) at 37°C under a humidified 5% CO_2 atmosphere. Subculture was performed by 7–10 min incubation with 0.025% trypsin–1 mM EDTA in calcium-free and magnesium-free phosphate-buffered saline. Cells were conservatively subcultured at 1:2 to 1:10 dilutions.

Determination of intracellular agmatine amounts in cultured cells DU145 prostate tumor cells were harvested by low speed centrifugation at 200 × *g*, washed once with 2 ml physiological saline, and suspended in distilled water. The cells were disintegrated ultrasonically using a Branson Sonifier (3 × 5 s, half-maximum power). Prior to the determination of agmatine, cells were kept at 60°C for 2 h in order to inactivate any endogenous SAMDC. Any precipitate formed during heating was removed by high speed centrifugation at 13,000 × *g*.

Aliquots of heat-treated homogenates were added to the SAMDC assay system. A parallel series of standards containing known amounts of agmatine were run. The amount of agmatine in unknown samples was obtained from a standard curve plotted according to Seppänen *et al.* (1980), i.e., a plot of agmatine concentration versus the reciprocal of initial velocity.

The recovery of agmatine, prior to heat treatment, was virtually 90% in cell homogenates.

Determination of ODC and SAMDC activities 10^6 cells were harvested, washed, and suspended in ice-cold 0.1 M Tris·HCl, pH 7.5 containing 0.1 mM EDTA and 2.5 mM dithiothreitol. The cells were then disrupted by sonication. The homogenate was centrifuged for 20 min at 20,000 × *g* and 4°C. ODC and SAMDC activities were determined in aliquots of the supernatants by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine and S-[carboxy-^{14}C]adenosyl-L-methionine, respectively, as described previously (Yang and Cho, 1991; Kim and Cho, 1993; Choi and Cho, 1994).

Determination of ODC antizyme activity In order to dissociate the ODC-antizyme complex (Kitani and Fujisawa, 1984), the cell extracts were subjected to gel filtration on Sephadex G-75 in the buffer containing 0.5 M NaCl. The cell extracts were mixed with buffer containing 10 mM Tris pH 7.0, 1 mM DTT, 0.1 mM EDTA , 0.5 mM NaCl, and 0.01% Tween 80 in a final volume of 0.4 ml. The mixture was applied to a Sephadex G-75 superfine column (2.5 × 26 cm) previously equilibrated with 10 mM Tris buffer, pH 7.0, containing 1 mM DTT, 0.1 mM EDTA , 0.5 mM NaCl, and 0.01% Tween 80, and the column was eluted with the same buffer in 0.8 ml fractions. A 40 μl aliquot of each fraction was assayed for ODC activity in the presence of 0.05 unit of ODC. Active fractions of antizyme were pooled and assayed by varying the concentration of antizyme in the presence of a constant amount of ODC.

Determination of rates of synthesis of SAMDC The SAMDC synthesis rates were determined by measuring the incorporation of [^{35}S]methionine into the enzyme proteins. The cells were collected by centrifugation and reseeded in preheated (37°C) methionine-free medium. After 10 min preincubation at 37°C, the cells were supplemented with [^{35}S]methionine (20 μCi/ml) and then incubated for an additional 20 min. The incorporation of radioactivity into protein was stopped by the addition of two vol of ice-cold medium containing 5 mM methionine. The cells were collected by centrifugation at 1000 × *g* for 10 min at 4°C and sonicated in 0.1 M Tris·HCl, pH 7.5, containing 0.1 mM EDTA, 2.5 mM dithiothreitol, and 5 mM methionine. After centrifugation at 20,000 × *g* for 20 min at 4°C, aliquots of the supernatants containing equal amounts of radioactivity were incubated with an excess of anti-SAMDC antibody for 30 min at room temperature. The enzyme-antibody complex was precipitated by the addition of bacterial Protein A adsorbent (30 min at room temperature). The precipitate was washed four times with 10 mM Tris·HCl, pH 7.5, containing 0.1 mM EDTA, 2.5 mM dithiothreitol, 0.1% SDS, 0.1% Triton X-100, and 0.1% Tween-80. Precipitated SAMDC was fractionated by SDS/PAGE [12% (w/v) gel]. The radioactivity was detected by autoradiography after incubation of the gel in Amplify (Amersham, Richimond, USA).

Determination of polyamine contents The cellular contents of putrescine, spermidine, and spermine were determined by reverse-phase HPLC by a modification of the method of Kabra and Lee (1986). 10^6 cells were harvested and extracted with 5% $HClO_4$. The sample was centrifuged at 20,000 × *g* for 30 min to remove precipitated proteins and then filtered through a 0.22 μm pore-size membrane. In a polypropylene tube, 50 μl of 1,6-diaminohexane for internal standard (200 pmole per 50 μl), 100 μl of saturated sodium carbonate, and 200 μl of dansylchloride (10 mg/ml) were added to 50 μl sample aliquots. The tube was capped and vortexed for 15 s, and then incubated overnight at room temperature. The contents were transferred to a Bond-Elut C18 column. After the column had been drained, it was washed with two column volumes of water. The dansylated polyamines were then eluted with 500 μl of methanol. The aliquot of eluate was injected into a reverse-phase column with bonded C18-aliphatic chains (5 μm, 15 cm × 3.9 mm column). We selected a mobile phase of acetonitrile and 10 mM phosphate buffer, pH 4.4. The gradients as described previously (Kabra and Lee, 1986) were found to be appropriate for the separation of polyamines and agmatine. The derivatives were analyzed using a fluorescence detector with excitation and emission wavelengths of 350 nm and 495 nm, respectively

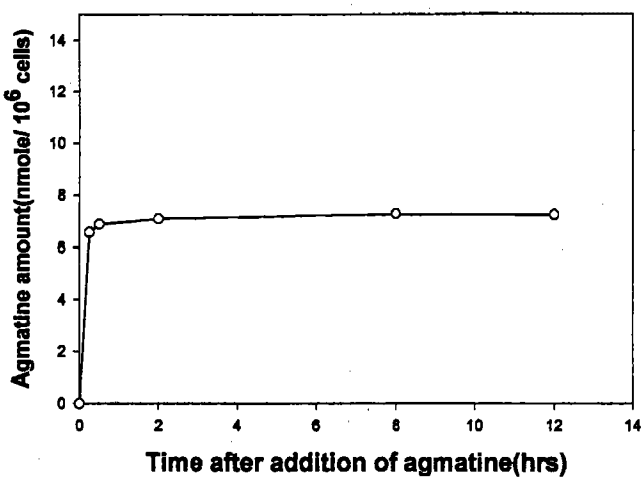

Fig. 1. Uptake of agmatine by DU145 prostate tumor cells at various times after addition of agmatine. The cells were grown in the presence of 1 mM agmatine at time 0. The concentration of agmatine was measured by SAMDC assays as described in Materials and Methods. The values are the means of four determinations.

Results

Enzymatic determination of agmatine in intracellular amounts Our previous report showed that agmatine sensitively inhibited activities of SAMDC and ADC in plants (Yang and Cho, 1991; Park and Cho, 1992; Choi and Cho, 1994). In this study, we also checked whether agmatine inhibits the enzyme in DU145 prostate tumor cells. Agmatine inhibited SAMDC enzyme activity substantially and competitively in a dose-dependent manner and the K_i value was 260 μM, which was somewhat higher than for soybean SAMDC (K_i = 40 μM). We have now developed a new enzymatic method for the rapid determination of agmatine in intracellular amounts, based upon the findings that agmatine is a potent inhibitor of putrescine-activated SAMDC. As shown in Fig. 1, when grown in the presence of 1 mM agmatine, the rapid concentration of intracellular agmatine observed in prostate tumor cells and the compound in the cells reached 7.5 ± 0.43 nmole/10^6 cells. The intracellular concentration of agmatine was not reduced until 12 h of incubation.

Effect of agmatine on ODC activity Although agmatine is present even in animals, little is known about its effect on ODC activity (Satriano *et al.*, 1998). Hence, we examined first whether agmatine inhibits mammalian ODC activity, which catalyzes the regulatory step in the synthesis of putrescine. Agmatine had no effect on the ODC activity *in vitro* even when high concentrations (5 mM) were added to the assay system. However, when agmatine was added to the culture medium of DU145 prostate tumor cells, there was a concentration-dependent suppression of ODC activity after 12 h exposure (IC_{50}, 50 μM). Agmatine was not toxic even at 5 mM as determined by trypan blue exclusion. Reseeding DU145 human prostate tumor cells in agmatine-free medium containing calf serum resulted in a rapid increase in ODC activity. When the cells were exposed to 0.5 mM agmatine, the inhibitory effect on ODC activity was evident after 4 h exposure, reaching a maximum at 8 h (Fig. 2).

Effect of agmatine on ODC antizyme activity In order to obtain more information on the inhibition of ODC by agmatine, we examined the effect of agmatine on antizyme

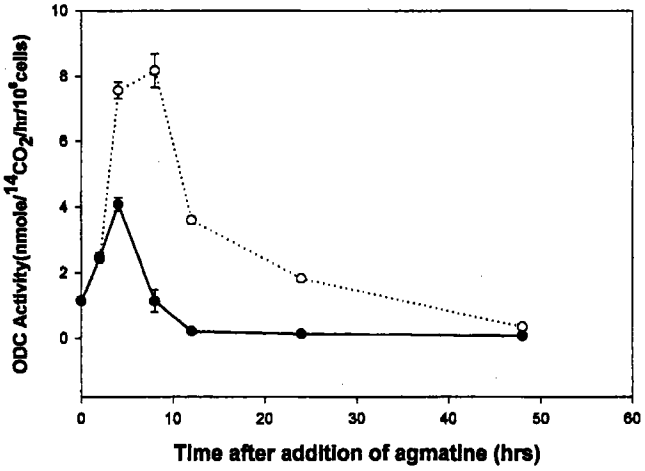

Fig. 2. Effect of agmatine on ODC activity in DU145 prostate tumor cells. Cells were seeded in the absence (○) or presence (●) of 0.5 mM agmatine. Cells were harvested at various times and analyzed for ODC activity.

activity. After the cells were incubated with 0.5 mM agmatine for 12 h, the cell extracts were subjected to gel filtration on Sephadex G-75 in the buffer containing 0.5 mM NaCl. The elution position of ODC was that corresponding to a molecular weight of about 55,000. The antizyme activity was recovered at an elution position corresponding to a molecular weight of about 22,000 with an apparent recovery of about 80%. Figure 3 shows the effects of agmatine on antizyme activity obtained by varying the concentration of antizyme in the presence of a constant amount of ODC. The partially-purified ODC activity was inhibited in the presence of the agmatine-treated antizyme fraction (Fig. 3). However, we obtained small amounts of antizyme in the control cells compared to that of agmatine-treated cells. The data indicate that some antizyme was also induced in agmatine-treated prostate tumor cells.

Effects of agmatine on the regulation of SAMDC activity We have examined the effect of agmatine on another regulatory enzyme, SAMDC, which is an essential enzyme in the biosynthesis of the polyamines, spermidine and spermine. As shown in Fig. 4, the activity of SAMDC in the presence of agmatine increased, reaching values 2.5-fold higher than those observed in the absence of agmatine.

In order to provide more information on the increase of SAMDC activity, we examined the effects of agmatine on the rates of synthesis of this enzyme by determining the incorporation of [^{35}S]methionine into the enzyme. The labeled protein with a molecular mass of approximately

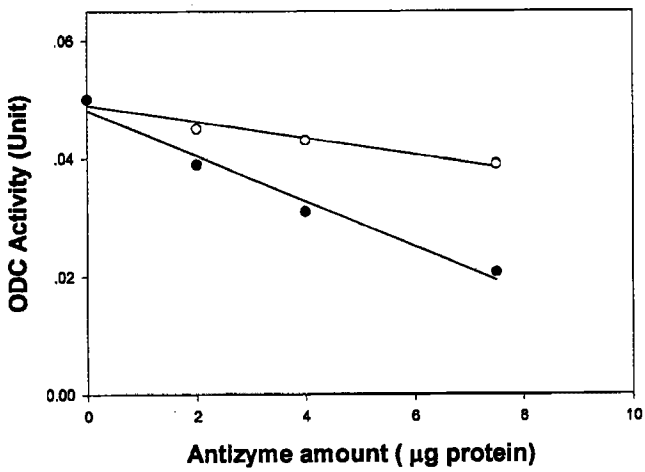

Fig. 3. Effect of agmatine on the antizyme activity of its inhibitory ODC activity. Increasing amounts of antizyme dissociated from ODC by Sephadex G-75 were preincubated with the activity of 0.08 unit of ODC on ice for more than 5 min and then the ODC activity was determined as described in Materials and Methods. The ODC activitiy was inhibited by active antizyme fraction isolated from the cells grown in the absence (○) or presence (●) of 0.5 mM agmatine for 12 h. The values are the means of four determinations.

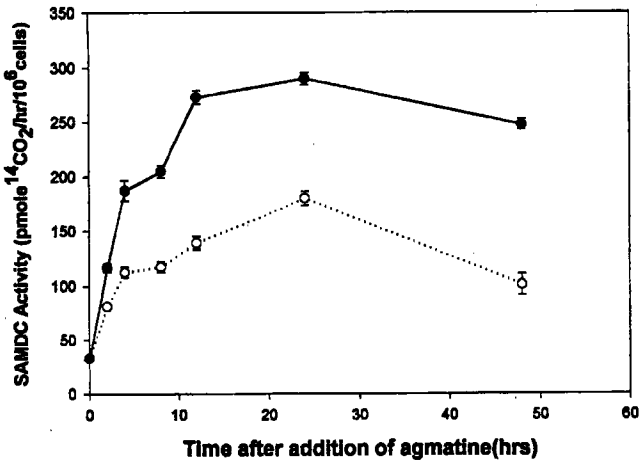

Fig. 4. Effect of agmatine on SAMDC activity in DU145 prostate tumor cells. Cells were seeded in the absence (○) or presence (●) of 0.5 mM agmatine. Cells were harvested at various times and analyzed for SAMDC activity.

31 kDa corresponded well to that of the larger subunit of SAMDC. However, a labeled proenzyme with a molecular mass of approximately 38 kDa was not detected, indicating a rapid conversion of the proenzyme form into its subunits. In spite of the fact that the increased activity of SAMDC was found in the cells seeded in the presence of agmatine for 12 h, the [^{35}S]methionine incorporation into SAMDC was independent of agmatine (Fig. 5). This result shows that the increased synthesis of SAMDC protein was not occurring in response to agmatine in spite of the increased activity of SAMDC.

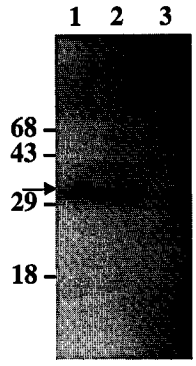

Fig. 5. Effects of agmatine on the synthesis of SAMDC in DU145 prostate tumor cells. Cells were grown for 12 h in the absence or presence of 0.5 mM agmatine before being analyzed for the rate of SAMDC synthesis by pulse-labeling with [^{35}S]methionine. Lane 1, SAMDC, untreated control cells; lane 2, SAMDC, agmatine treated cells; lane 3, pre-immune serum. The migration of purified rat prostate SAMDC labeled with S-[^{3}H]adenosyl-L-methionine (molecular mass 31 kDa) is indicated. ^{14}C-labeled methylated proteins were used as molecular mass markers; positions of markers in kDa are shown at the left.

However, the half-life of SAMDC activity increased significantly in cells grown in the presence of agmatine (Fig. 6). In cells grown in the absence of agmatine, SAMDC activity decayed with a half-life of approximately 55 min after treatment with cycloheximide, whereas in cells grown in the presence of agmatine the half-life of SAMDC activity was approx. 120 min. The half-life of SAMDC protein using radioimmunoassay was much longer than that of SAMDC activity and revealed to be between 2 and 3 h in cells grown in the absence of agmatine, whereas the protein was almost stable (approximately 20 h) in cells grown in the presence of agmatine (results not shown).

Effect of agmatine on the intracellular polyamine levels The inhibition of ODC activity and an increase in SAMDC activity by agmatine administration were reflected in changes in the cellular content of the polyamines, putrescine, spermidine, and spermine. Exposure of the cells to 0.5 mM agmatine reduced the putrescine and spermidine content (Fig. 7). The spermine content, in contrast, was substantially increased in cells seeded in the presence of agmatine (Fig. 7).

Effect of agmatine on cell growth Since the control of cellular polyamine contents has been a major component in cell growth regulation, we examined whether such changes would be related to the cell growth. As shown in Fig. 8, the cell entered exponential growth phase, at which period agmatine started reducing cell growth concentration-dependently. The IC_{50} (concentration causing 50% inhibition of cell growth) for agmatine in DU145 prostate tumor cells was about 0.5 mM. Concentrations higher than 5 mM caused a loss in cell viability, as measured by Trypan blue exclusion. Idazoxan, which also binds to I receptors, inhibited cell growth much more (IC_{50}, 50 μM).

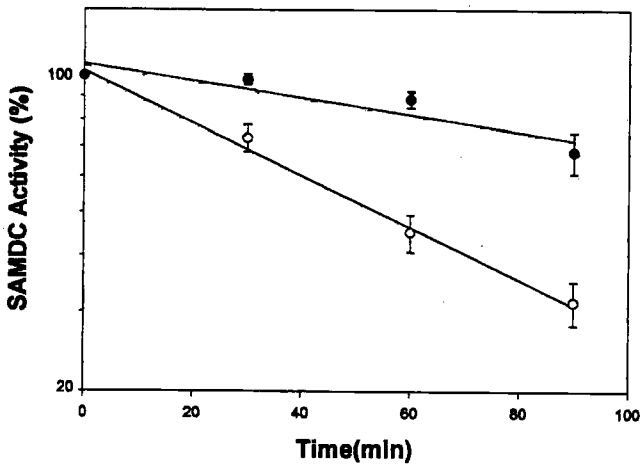

Fig. 6. Effect of agmatine on the turnover of SAMDC in DU145 prostate tumor cells. Cells were grown for 12 h in the absence (○) or presence (●) of 0.5 mM agmatine. The turnover of SAMDC in the cells was determined by following the decay of SAMDC activity using a SAMDC assay after the addition of cycloheximide (50 μg/ml). The regression line represents the mean turnover of SAMDC in the cells grown in the absence or presence of agmatine.

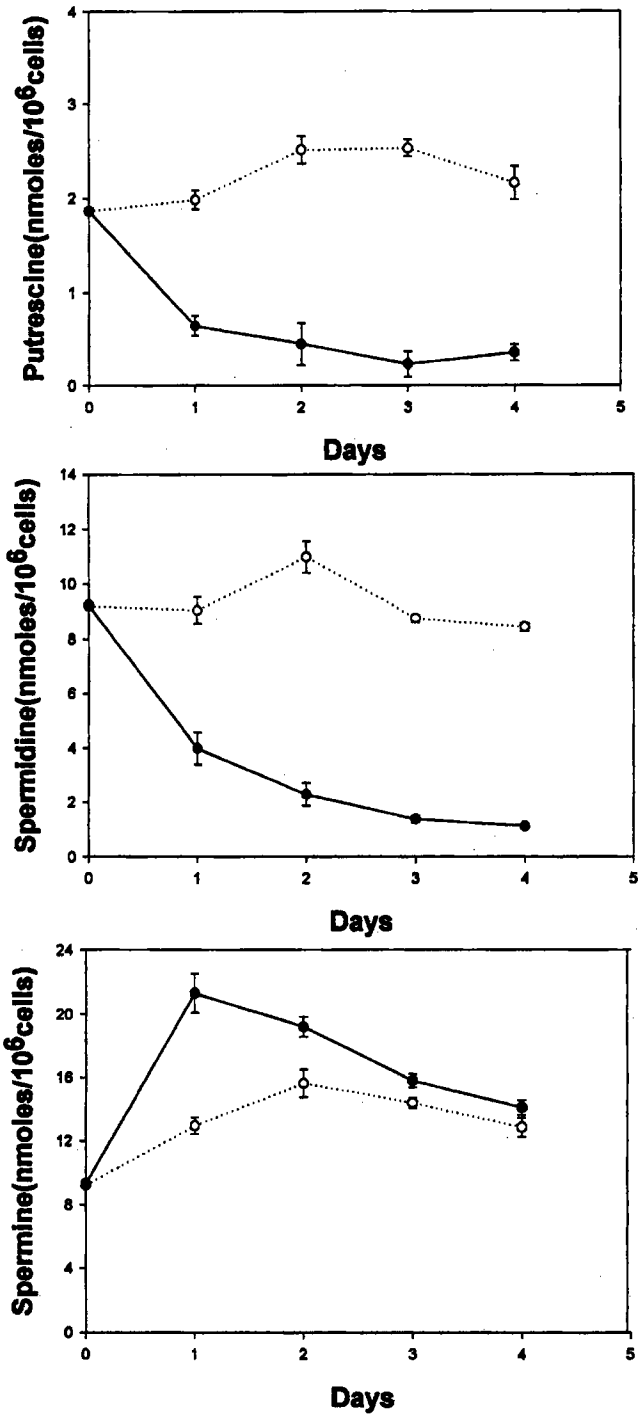

Fig. 7. Effect of agmatine on the polyamine content in DU145 prostate tumor cells. Cells were seeded in the absence (○) or presence (●) of 0.5 mM agmatine. Cells were harvested daily and analyzed for polyamine content.

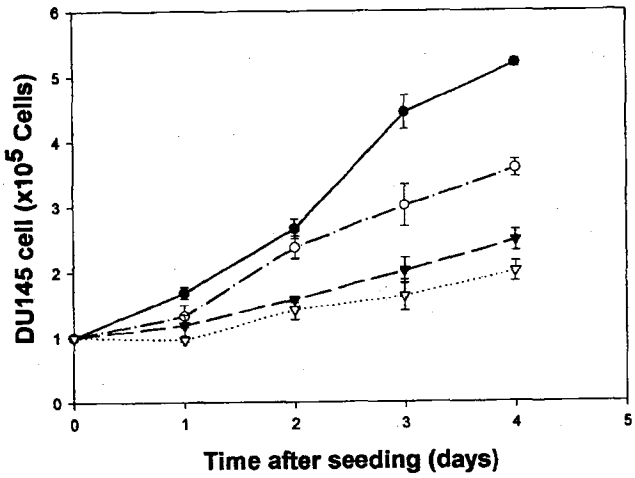

Fig. 8. Effect of agmatine concentrations on DU145 prostate tumor cell growth. Cells were seeded at an initial density of 10^4/ml and cultured in the presence of various concentrations of agmatine and were counted daily. Symbols: ●, control cell; ○, 0.1 mM agmatine; ▼, 0.5 mM agmatine; ▽ , 1 mM agmatine.

Similar results were observed in vascular smooth muscle cells (Regunathan *et al.*, 1996) but IC_{50} for agmatine was somewhat low (IC_{50}, 0.28 mM).

Discussion

The polyamine-biosynthesis pathway is a useful target for the design of chemotherapeutic agents. Alteration in polyamine levels has been shown to lead to decreases in cellular growth and alterations in cellular differentiation (Shirahata and Pegg, 1986; Autelli *et al.*, 1991; Stjernborg *et al.*, 1993; Svensson *et al.*, 1997). Hence, the use of inhibitors or polyamine analogs that alter normal polyamine levels by acting as inhibitors or activators of ODC and SAMDC provides another approach to chemotherapy (Lee and Cho, 1997). Furthermore, the information on the mechanism by which polyamine levels regulate SAMDC is needed to determine an effective way to design and implement such therapeutic strategies. Since the prostate has relatively high SAMDC activity in comparison to other tissues such as brain, liver, and spleen and appears to exist in imidazoline receptors bound by agmatine (Regunathan *et al.*, 1996), we performed these experiments in DU145 prostate tumor cells. To exclude the possibility that agmatine might be metabolized to putrescine by agmatinase or to other products by serum oxidase, we examined the conversion of agmatine to polyamine by HPLC determination and the oxidation of agmatine by colorimetric assay for amine oxidase in medium or cell extracts. However, we could detect no enzyme activity for metabolizing agmatine during the culture period.

We examined the intracellular agmatine levels easily by using the SAMDC assay based on the fact that agmatine inhibited SAMDC activity substantially and competitively in a concentration-dependent manner. Although a few analytical procedures such as HPLC and immunological methods have been devised for agmatine measurements, these methods are problematic owing to the instability of the derivatives of agmatine, the cross-reactivity of other amines, and it being very time consuming. The present method has proven to be precise and convenient for relatively high concentrations of agmatine in mammalian cells.

ODC and SAMDC are two key enzymes in polyamine biosynthesis. These enzyme activities are relatively low in nonproliferating tissues. However, activities of these enzymes are raised in proliferating tissues (Shirahata *et al.*, 1986; Autelli *et al.*, 1991; Stjernborg *et al.*, 1993; Svensson *et al.*, 1997). Thus, alteration of biosynthesis under both clinical and experimental conditions has usually been achieved by the use of ODC inhibitors or SAMDC inhibitors. In this report, we demonstrated that treatment of the cells with agmatine inhibited ODC activity by the induction of ODC-antizyme and increased SAMDC activity as the result of a decreased turnover of the enzyme. These effects appeared in the early stage of incubation with agmatine. We suggest that the effect of agmatine on these enzyme activities occurs concurrently but the effects are not due to an indirect effect on one of these enzyme activities.

The most recent report demonstrated that agmatine suppresses ODC activity by the induction of antizyme (Satriano *et al.*, 1998). Hence, we isolated the antizyme fraction from cell extracts using the Sephadex G-75 column and also observed the induction of antizyme in agmatine exposed DU145 prostate tumor cells. However, agmatine had no effect on the ODC activity *in vitro* even at high concentrations.

SAMDC is regulated at a multitude of levels, including translational and post-translational, and has a very rapid turnover rate with a half-life of often less than 1 h. Increases in SAMDC activity in response to an inhibition is well known (Autelli *et al.*, 1991; Stjernborg *et al.*, 1993; Svensson *et al.*, 1997). An even greater increase in SAMDC protein is seen when irreversible inactivators of SAMDC are used to deplete cellular polyamines. Hence, any change in the synthesis or degradation of this enzyme will rapidly affect the cellular amount of the enzyme and thus the rate of polyamine synthesis. However, the mechanism by which these changes are brought about and the relative roles of spermidine and spermine in the regulation of SAMDC are not well understood. From our results, we demonstrated that the increase of SAMDC activity by agmatine was a result of a decreased turnover rate of this enzyme.

In spite of the fact that methylglyoxal bis (guanylhydrazone) (MGBG) and aminoguanidine are SAMDC inhibitors, these compounds give rise to a

paradoxical increase in SAMDC activity (Pegg *et al.*, 1973; Stjernborg and Persson, 1993). These increases of SAMDC activity have been demonstrated to be due to a decreased turnover rate of SAMDC. Thus, the binding of these compounds to SAMDC probably evokes a structural change in SAMDC, resulting in the reduction of proteolytic degradation (Pegg *et al.*, 1973; Stjernborg and Persson, 1993). Since the turnover of SAMDC is normally very fast, a change of turnover rate of SAMDC gives rise to an increase in the cellular level of the enzyme. Therefore, it is conceivable that agmatine, although with much less affinity than MGBG and higher affinity than aminoguanidine, can bind to SAMDC and cause a similar effect on the turnover of the enzyme. However, other compounds enhancing the activity of SAMDC have shown to increase the synthesis rate of the enzyme (Autelli *et al.*, 1991; Stjernborg *et al.*, 1993; Svensson *et al.*, 1997).

Such cumulative results on enzymes suggest the following: (1) The decrease in the level of putrescine is related to a decrease in ODC, which also has an effect on the decrease in the level of spermidine in agmatine-treated cells, and (2) the accumulation of high spermine levels is due to an increase in the cellular content of SAMDC, which supplies more decarboxylated S-adenosylmethionine (dcSAM) to be utilized to convert more spermidine to spermine, thus reducing the level of spermidine in agmatine-treated cells. It has been reported that ODC activity is subject to negative-feedback regulation by high levels of polyamines (Hölttä and Pohjanpelto, 1986). Accordingly, the intracellular accumulation of high spermine levels by agmatine could suppress ODC activity. Although we could not exclude the possibility of intracellular conversion of spermine to spermidine by polyamine oxidase (Hölttä, 1983), there was no detectable activity in the cells. Besides, our preliminary results show that agmatine has no effect on the activities of spermidine and spermine synthases.

Polyamines are essential for cell growth, especially in rapidly proliferating tissues. In contrast to this positive effect, polyamine overaccumulation has been shown to be toxic in both whole organisms and in *in vitro* systems (Brunton *et al.*, 1991). Although the mechanism is not clear, there are several reports of cell-growth inhibition caused by the addition of millimolar levels of polyamine to the culture medium (Smith *et al.*, 1995; Monti *et al.*, 1996). In mammalian cells, excess spermidine and spermine accumulation can be toxic even under conditions where the toxic polyamine oxidation products have been removed, suggesting that the amines themselves can cause cell death (Brunton *et al.*, 1991). Therefore, a decrease in putrescine and spermidine levels and an increase in spermine may cause the growth inhibition of cells in the presence of agmatine. If lower levels of putrescine and spermidine are responsible for growth inhibition in the presence of agmatine, the polyamine-deprived cells may recover from their growth inhibition by the addition of putrescine or spermidine. However, addition of putrescine or spermidine ranging from 1 to 10 μM had no effect on the growth of cells in the presence of agmatine (data not shown). In recent reports, agmatine also suppressed polyamine transport in a MCT kidney proximal tubule cell line (Satriano *et al.*, 1998). Hence, we could not exclude the possibility that our result is attributable to the inhibition of polyamine transport by agmatine. On the other hand, since spermine accumulated in the cells in the presence of agmatine, spermine may be the factor causing growth inhibition.

In conclusion, our results demonstrated that agmatine could affect the regulatory control of key enzymes responsible for polyamine biosynthesis, resulting in depletion of putrescine and spermine and accumulation of spermine. Because the intracellular mechanism by which agmatine exerts its biological actions is for the most part not established, it is not certain whether inhibition of cell growth by agmatine was due to the intracellular reduction in spermidine and putrescine or to the accumulation of spermine. We suggest that concomitant reduction of putrescine and spermidine and accumulation of spermine caused by the changing activities of key enzymes in the presence of agmatine are closely associated with the inhibition of the growth of DU145 prostate tumor cells.

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