Polyamine catabolism in rodent and human cells in culture

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*N*¹-Acetylspermidine (*N*¹AcSpd) accumulates in late exponential phase, or after certain stresses such as heat shock, in both human tumour (A549) and rodent (HTC, CHO) cells, grown in medium containing an inhibitor of the FAD-dependent polyamine oxidase (PAO). Inhibition of PAO has little effect on cell growth or on the cellular content of the major polyamines, putrescine, spermidine or spermine, found in proliferating cells in culture, but decreases cellular putrescine content in heat shocked cells. Putrescine and spermidine are generated when *N*¹AcSpd or *N*¹-acetylspermine (*N*¹AcSpm) respectively is added to either human or rodent cells depleted of the former amines by α -difluoromethylornithine. *N*¹AcSpm is formed in polyamine-depleted human A549 cells when *N*¹AcSpd is added to cultures treated with the PAO inhibitor. This reaction does not occur in either rodent line, suggesting that *N*¹AcSpd can be converted directly into *N*¹AcSpm in human, but not rodent, cells under specific conditions. The data presented demonstrate that a variety of human and rodent cells express PAO activity and catabolize polyamines by a mechanism which includes PAO. PAO activity is of little consequence to proliferating A549, HTC or CHO cells in culture, but does produce new putrescine in both late-exponential-phase and heat-shocked cells. These findings suggest that polyamine catabolism is part of a general response of both rodent and human cells to a variety of environmental and physiological stresses.

INTRODUCTION

The polyamines (putrescine, spermidine and spermine) are naturally occurring polycations that are essential for the normal growth and differentiation of eukaryotic cells (for general review, see Pegg, 1986). Ornithine decarboxylase (ODC) is the first enzyme in the polyamine-biosynthesis pathway. This enzyme is irreversibly inhibited by α -difluoromethylornithine (DFMO) and its activity has been correlated with growth responses in many systems. Intracellular polyamines are catabolized by a two-step mechanism, the first being the conversion of spermine or spermidine into the *N*¹-acetyl derivatives *N*¹AcSpm or *N*¹AcSpd respectively, by the enzyme spermidine/spermine *N*¹-acetyltransferase (*N*¹-SAT). The second step is the oxidation of the monoacetylpolyamine by the action of polyamine oxidase (PAO). Oxidation of *N*¹AcSpm and *N*¹AcSpd leads to the formation of the shorter-chain amines spermidine and putrescine respectively, H₂O₂ and 3-acetamidopropanal (Seiler *et al.*, 1985).

The physiological consequences of polyamine catabolism are not understood. Certain stresses, such as heat shock, stimulate polyamine catabolism in rodent cells in culture (Harari *et al.*, 1989a). *N*¹-SAT is induced by both physical and chemical stresses, such as the copper chelator diethyldithiocarbamate, in rodent and human cells (Harari *et al.*, 1989b; Fuller *et al.*, 1990). Seiler *et al.* (1985) have shown that *N*¹AcSpd and *N*¹AcSpm accumulate in HTC cells treated for 3 days with *N*¹-(2,3-butadienyl)-*N*²-methylbutane-1,4-diamine (MDL 72.521), an irreversible inhibitor of PAO, but noted that inhibition of this reaction did not affect the growth of these cells in culture. The activity of *N*¹-SAT increases and *N*¹AcSpd accumulates in colonic tumours of rats undergoing treatment with the carcinogen 1,2-dimethylhydrazine (Halline *et al.*, 1989). The size of these tumours is suppressed by

PAO inhibitors (Halline *et al.*, 1990), suggesting that PAO activity may be important for tumour growth *in vivo*.

It has been suggested that polyamine catabolism may be of less importance in human, compared with rodent, tumours, because human tumour cells apparently did not express PAO activity (Hirvonen *et al.*, 1989). The experiments described in the present paper were designed to determine if human cells catabolize polyamines in a manner similar to rodent cells. The results obtained show that cell lines derived from a number of normal and neoplastic human tissues contain a functional PAO activity and that at least one human tumour cell line has the unique ability to convert *N*¹AcSpd directly into *N*¹AcSpm.

MATERIALS AND METHODS

Materials

The PAO inhibitor MDL 72.521 was generously provided by Marion Merrell Dow Laboratories (Cincinnati, OH, U.S.A.). All other reagents, except glycine (from Bio-Rad, Richmond, CA, U.S.A.), were supplied by Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Cell culture

All human tumour and rodent cell lines used in this study were propagated in McCoy's 5A (modified) medium containing 10 % (v/v) fetal-bovine serum and supplemented with penicillin (100 units/ml) and streptomycin (100 μ g/ml) (all from Gibco). The human normal and neoplastic Barrett's cells were grown in L-15 medium (Gibco) modified with cortisol (3.6 μ g/ml), insulin (10 μ g/ml), transferrin (10 μ g/ml), glutamine (292 μ g/ml), penicillin (100 units/ml), streptomycin (100 μ g/ml), sodium selenite (1.73 μ g/ml), GSH (15 μ g/ml), catalase (55 units/ml),

Abbreviations used: DFMO, α -difluoromethylornithine; MDL 72.521, polyamine oxidase inhibitor *N*¹-(2,3-butadienyl)-*N*²-methylbutane-1,4-diamine; ODC, ornithine decarboxylase; *N*¹SAT, spermidine/spermine *N*¹-acetyltransferase; PAO, FAD-dependent polyamine oxidase; *N*¹AcSpd, *N*¹-acetylspermidine; *N*¹AcSpm, *N*¹-acetylspermine.

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methylcellulose 15 CPS (2 µg/ml), polyvinylpyrrolidone-360 (1 µg/ml), 2-mercaptoethanol (0.8 µg/ml), orotic acid (15 µg/ml), DL-ornithine (15 µg/ml) and whole bovine pituitary extract (0.06 %). This medium was developed by Mr. A. Leibovitz of the Arizona Cancer Center Cell Culture facility for the maintenance of growth of the normal and precancerous cell lines used in this work. All cultures were maintained at 37 °C in a humidified atmosphere of air/CO₂ (19:1). Stock cultures were normally maintained in T-75 flasks and passaged twice per week. For cell growth experiments, cultures were seeded at 5×10^5 cells in 5 ml of normal growth medium per T-25 tissue-culture flask for all cell lines except HTC, which were initiated at 2×10^5 cells per ml in 400 ml suspension cultures. Cell doubling times under these conditions were: CHO ~ 14–16 h, HTC ~ 22–24 h, all human normal fibroblasts and epithelial cells ~ 20–22 h, the Barrett's oesophagus-derived cells ~ 30–36 h, and all human tumour lines ~ 17–26 h.

Polyamine analysis

Polyamines were separated and analysed by reverse-phase h.p.l.c. as previously described (Seiler & Knodgen, 1980). Protein was determined by the method of Bradford (1976).

PAO activity

Cells were harvested, washed twice in ice-cold phosphate-buffered saline (140 mM-NaCl/2 mM-KCl/8.1 mM-Na₂HPO₄/0.9 mM-K₂HPO₄) and counted with either a haemocytometer or an electronic particle counter. PAO activity was measured by two independent methods, one involving direct measurement of spermidine formation from *N*¹AcSpm by h.p.l.c., whereas the other used colorimetric measurement of H₂O₂ generated by the oxidation of *N*¹AcSpm. For the first method, cell pellets were resuspended in buffer containing 0.1 M-glycine/NaOH, pH 9.5, 5 mM-dithiothreitol and the monoamine oxidase inhibitors aminoguanidine (0.56 mM) and pargyline (36 µM), at a ratio of $(5\text{--}20) \times 10^6$ cells/ml in the absence or presence of 25 µM-MDL 72.521. The suspensions were then frozen at -80 °C for at least

2 h. Samples were then thawed and clarified by centrifugation. *N*¹AcSpm was added to equal a final concentration of 25 µM in a volume of 500 µl, and 100 µl samples were taken every 20 min for 1 h. These were then analysed for polyamines as described above. All rates were linear for the entire time course. Activity was determined by the difference in metabolism of *N*¹AcSpm between controls and those inhibited by the PAO inhibitor MDL 72.521. The second method employed was a modification of that of Hayashi *et al.* (1989). Cell pellets were resuspended in 50 mM-glycine/NaOH buffer, pH 9.5, and stored at -80 °C before assay. Samples of the thawed and clarified supernatant were adjusted to a final concentration of 25 µM-MDL 72.521 and incubated on ice for 30 min. These and control samples without the inhibitor were then incubated at 30 °C in a total volume of 500 µl of assay cocktail containing 50 mM-glycine/NaOH, pH 9.5, 0.82 mM-aminoantipyrine, 10.6 mM-phenol, 5 mM-*N*¹AcSpm, 25 mM-aminotriazole and 1 unit of horseradish peroxidase. H₂O₂ formation was estimated by the peroxidase-coupled formation of dye product. This was quantified spectrophotometrically by the *A*₅₀₀. To confirm the stoichiometry of this reaction, the reaction volume was made 0.2 M with HClO₄ and the formation of spermidine was measured directly by h.p.l.c.: one-to-one stoichiometry was observed for these reaction conditions (results not shown). For the very low activities of the human cell lines, it was necessary to use 4×10^6 cells per assay and an incubation time of 2 h. For CHO cells, however, linearity was observed only up to 45 min at 10^6 cells per assay. One unit of enzyme activity is defined here as 1 pmol of *N*¹AcSpm oxidized/min of reaction incubation.

RESULTS

The accumulation of *N*¹AcSpd was measured in one human and two rodent cell lines growing in the absence or presence of the PAO inhibitor MDL 72.521, during exponential and plateau phases of growth. Under these conditions of growth in culture, proliferation of the three lines studied was either not, or only

Fig. 1. Effect of PAO inhibition on the growth of rodent and human cells in culture

Cultures were seeded on day 0 in medium with (○) or without (●) 25 µM MDL 72.521. Cell densities were 5×10^5 cells in 5 ml of medium per 60 mm-diam. Petri dish (CHO and A549) or 2×10^5 cells per ml in 400 ml spinner cultures (HTC), as described in the Materials and methods section. Cell numbers were determined at daily intervals thereafter. Values shown represent means of triplicate plates (CHO and A549) or samples (HTC).

Fig. 2. Polyamine contents in HTC cells growing in the presence (○) or absence (●) of MDL 72.521

Cultures, whose growth kinetics are shown in Fig. 1, were harvested at daily intervals and polyamine contents were determined as described in the Materials and methods section. Values of polyamine contents shown in this and subsequent Figures are single determinations from a representative experiment, which has been replicated at least once.

Fig. 3. N¹AcSpd accumulation during the growth of rodent (CHO) and human (A549) cells in culture, in the presence (○) or absence (●) of the PAO inhibitor MDL 72.521 in the culture medium

Polyamine contents were determined from cultures whose growth kinetics are shown in Fig. 1.

minimally, affected by inhibition of PAO (Fig. 1). Polyamine contents were analysed in these same cultures throughout the growth period. Total polyamine distributions for one cell line (HTC) are shown in Fig. 2. Putrescine levels were unaffected by PAO inhibition, whereas N¹AcSpd (and to a much lesser extent N¹AcSpm; results not shown) content increased from

Fig. 4. Measurement of N¹AcSpd oxidation in HTC cells

For this experiment, 2×10^5 HTC cells/ml were inoculated into 400 ml spinner flasks and cultured for 2 days in the presence of 5 mM-DFMO. At 2 h after addition of the PAO inhibitor (25 μ M-MDL 72.521) (b) or a saline control (a), the medium of each flask was adjusted to a final concentration of 10 μ M-N¹AcSpd. Flasks were harvested at the times indicated and the polyamine contents determined. N¹AcSpd (●) was judged to be oxidized by PAO if spermidine (■) contents increased in the absence, and remained suppressed in the presence, of the PAO inhibitor and if N¹AcSpd accumulated to a greater degree in cells treated with, compared with those not exposed to, the PAO inhibitor.

undetectable levels (less than 0.05 nmol/mg of protein) early in growth to nearly 4 nmol/mg of protein after 4 days in culture in the presence of the PAO inhibitor. No acetylated derivatives were detectable in cultures growing in the absence of MDL 72.521. Spermidine and spermine levels were either similar or slightly elevated compared with untreated controls.

N¹AcSpd also only accumulated in CHO cultures incubated with the PAO inhibitor (Fig. 3). In the human A549 cells, N¹AcSpd accumulation was greater in the presence of MDL 72.521 than in its absence, but measurable quantities of this polyamine accumulated even in the absence of the PAO inhibitor (Fig. 3). N¹AcSpd accumulated late in growth in both of these cell lines, similar to the finding for HTC cells (Fig. 2). As in the HTC cells, levels of the other intracellular polyamines, putrescine, spermidine and spermine, were largely unaffected by PAO inhibition in CHO and A549 cells (results not shown).

To investigate further the mechanism of polyamine catabolism in rodent and human cells, N¹AcSpd or N¹AcSpm were added to HTC and A549 cells treated with DFMO. The DFMO treatments were not toxic to these cells, and were used to suppress endogenous putrescine and spermidine pools so that any catabolism could be more readily assessed. When 10 μ M-N¹AcSpd was added to DFMO-treated HTC cells, spermidine accumulated in a linear manner over a 6 h period, while N¹AcSpd accumulated to a lesser degree (Fig. 4a). When MDL 72.521 was added to the cultures to inhibit PAO activity, spermidine accumulation was suppressed and N¹AcSpd accumulated in a linear fashion (Fig. 4b). Spermine levels did not change during this time, and no other polyamines accumulated to detectable levels in either treatment group (results not shown). Because we have found that A549 cells took up monoacetylpolyamines less readily than did HTC cells, we added 100 μ M-monoacetylpolyamines to these human tumour cells (Table 1). Cultures were incubated for either 3 or 6 h and acid-soluble polyamines were analysed. N¹AcSpm, added exogenously, accumulated endogenously, and spermidine

Table 1. Polyamine contents in human A549 tumour cells after addition of monoacetylpolyamines

Cultures were treated for 5 days with 5 mM-DFMO; monoacetylpolyamines were then added at concentrations of 100 μ M in the medium. The PAO inhibitor MDL 72.521 (25 μ M) was included in the final incubation medium for times as indicated in the Table. Values shown are from a single experiment, which has been replicated. Abbreviation: n.d., none detected (less than 0.05 nmol/mg of protein)

Polyamine added	Incubation time (h)	MDL 72.521	Polyamine contents (nmol/mg of protein)				
			Putrescine	Spermidine	Spermine	N ¹ AcSpd	N ¹ AcSpm
None	—	No	n.d.	1.51	2.05	n.d.	n.d.
N ¹ AcSpd	3	No	n.d.	3.18	2.04	0.29	n.d.
		Yes	n.d.	1.54	1.88	0.23	0.27
	6	No	0.26	3.10	1.37	0.58	n.d.
		Yes	n.d.	1.28	1.71	0.58	0.55
N ¹ AcSpm	3	No	n.d.	3.30	2.16	n.d.	0.17
		Yes	n.d.	1.67	2.24	n.d.	0.76
	6	No	n.d.	3.18	1.49	n.d.	0.16
		Yes	n.d.	1.58	2.18	n.d.	1.59

Table 2. Polyamine contents in Chinese hamster (CHO) cells after addition of N¹AcSpd to DFMO-treated cultures

Cultures were plated into fresh medium containing 5 mM-DFMO and incubated for 8 h. At this time, the medium of all cultures was adjusted to 50 μ M-N¹AcSpd and 25 μ M-MDL 72.521, and cells were incubated for an additional 16 h. Culture medium was then removed, cells were washed and fresh medium containing DFMO and MDL 72.521 (as indicated above) was added for the incubation times indicated. The values shown for 0 h are means $\pm$ S.E.M. of values obtained from three independent cultures in a single experiment. The other values in this Table are single representative observations, which have been verified in an independent experiment. Abbreviation: n.d., none detected (less than 0.05 nmol/mg of protein).

Time after loading (h)	MDL 72.521 in medium	Polyamine contents (nmol/mg of protein)				
		Putrescine	Spermidine	Spermine	N ¹ AcSpd	N ¹ AcSpm
0	Yes	n.d.	0.59 $\pm$ 0.03	3.17 $\pm$ 0.11	21.19 $\pm$ 0.19	n.d.
3	No	0.73	1.23	4.14	19.93	n.d.
	Yes	n.d.	0.72	3.68	18.63	n.d.
6	No	2.19	1.48	3.67	11.61	n.d.
	Yes	n.d.	0.57	3.51	14.32	n.d.

levels were lower in cells treated with MDL 72.521 than in cultures not treated with the PAO inhibitor. When N¹AcSpd was added to the culture medium, levels of endogenous spermidine, and in some cases putrescine, increased in cells not treated with the PAO inhibitor. In the presence of the inhibitor, N¹AcSpm contents increased in cultures treated with DFMO, and for all incubation times with N¹AcSpd. Exogenous N¹AcSpm led to increased spermidine levels in the absence of the PAO inhibitor, compared with those when the inhibitor was present, indicating that these human cells can oxidize both N¹AcSpd and N¹AcSpm.

The observation that N¹AcSpm was formed as a consequence of N¹AcSpd addition by a mechanism not involving PAO in human A549 cells was surprising, in that this conversion was not observed in the rodent HTC cells. To determine if this reaction could occur in another rodent cell line, CHO cells were initially loaded with high concentrations of N¹AcSpd in the presence of MDL 72.521. They were then incubated for additional periods with or without the inhibitor (Table 2). As shown in Table 2, N¹AcSpd is converted into putrescine in the absence of MDL 72.521, but no N¹AcSpm is formed under any condition.

The above data confirm that at least one human cell line, A549, expresses a functional PAO that converts N¹AcSpd and N¹AcSpm into the shorter-chain amines spermidine and putrescine respectively. To determine if other human cell types expressed this activity, we measured PAO activity, using an assay *in vitro*, in both rodent cell lines and in a variety of normal and

neoplastic tissue-derived cells (Table 3). Three main findings are shown in Table 3. First, PAO activity was dramatically higher in the CHO cells compared with all other cell types investigated. Second, PAO activity was detected in most of the human cells. Third, this enzyme activity was not uniquely greater or smaller in tumour cells compared with the normal and precancerous cell types studied. Some cell lines did not express a PAO activity detectable by our assay. Whether these cells are able to express their activity under other conditions is not known.

To confirm that human cells can catabolize polyamines in response to stress, replicate cultures of exponential-phase A549 cells, treated with and without MDL 72.521 to inhibit PAO, were heat-shocked (45 °C for 20 min). Parallel cultures of CHO cells were handled similarly as positive controls. Fig. 5 shows the levels of putrescine and N¹AcSpd in CHO cells (Fig. 5a) and A549 cells (Fig. 5b) following this stress. N¹AcSpd accumulated in heat shocked CHO cells only in the presence of the PAO inhibitor. Putrescine levels increased in these rodent cells after heat-shock in the absence, and slightly declined in the presence, of MDL 72.521. The increase in putrescine levels was due to the conversion of N¹AcSpd into putrescine by the action of PAO. In human A549 cells, N¹AcSpd levels initially increased after a heat-shock in the absence and presence of the PAO inhibitor. At later times, the N¹AcSpd levels were elevated only in the presence of the PAO inhibitor, suggesting that some PAO activity was expressed in these heat-shocked A549 cells.

Table 3. PAO activity in rodent cells and in human cell lines derived from both normal and neoplastic tissues

The enzyme activities shown are means $\pm$ S.E.M. of triplicate determinations from a single representative experiment, which has been replicated. The number of replicates is at least three in all cases. Abbreviation: n.d., none detected.

Species	Classification	Cell line	PAO activity (pmol/min per 10^6 cells)
Rodent		Chinese hamster (CHO)	323.0 ± 91.0
		Rat hepatoma (HTC)	2.7 ± 0.3
Human	Normal		
	Foreskin fibroblasts	HF	4.1 ± 1.2
		NFSL18	4.2 ± 0.5
		NFSL80	1.8 ± 0.2
		NFSL83	2.9 ± 1.9
	Epithelial cells*	ES	1.6 ± 0.6
	Precancer		
	Barrett's oesophagus	AZCC596RF	2.7 ± 0.7
		AZCC600	n.d.
	Cancer		
	Glioblastoma	D54	5.1 ± 0.4
		D247	22.5 ± 1.5
	Lung carcinoma	A549	1.2 ± 0.2
	Melanoma	C8146C	1.9 ± 0.2
	Breast carcinoma	MCF7	n.d.
	Cervical carcinoma	HeLa	n.d.

* Derived from squamous oesophageal tissue.

DISCUSSION

The results presented here demonstrate that human cells, like those of rodent origin, catabolize longer-chain polyamines to shorter-chain amines. Similar physiological conditions, such as growth into high densities and heat-shock, stimulate this process in both species, and the mechanism of catabolism involves both N^1 -SAT and PAO activities. A major difference between human and rodent cells is that, under conditions of PAO inhibition, the human A549 cells form N^1 AcSpm as a consequence of N^1 AcSpd addition. Neither the HTC nor the CHO cells carry out this reaction under similar conditions. It is not yet known whether other human cells can carry out this reaction, or if this process is unique to A549 cells. This result may mean that N^1 AcSpd is converted directly into N^1 AcSpm in human, but not in rodent, cells, further suggesting that the substrate specificity of spermine synthase may be different in these two species. Verification of these possibilities requires future studies to compare substrate specificities of the purified enzymes.

Most human cells, like rodent cells, express PAO activity. Similar to the report of Hirvonen *et al.* (1989), some of the human cells evaluated here expressed undetectable levels of this enzyme. However, this is not a general property of human cells, nor is it specific to tumour cells. Whether these cells can oxidize N^1 -acetyl-derivatives of spermidine or spermine under other conditions than those used here is unknown. When HeLa cells are grown to high densities in medium containing MDL 72.521, we cannot detect accumulation of any acetyl polyamines (results not shown), although this same treatment does lead to acetyl polyamine accumulation in A549, HTC and CHO cells.

Fig. 5. Effect of heat-shock on polyamine catabolism in rodent (CHO) (a) and human (A549) (b) cells, in the presence (●) or absence (■) of the PAO inhibitor MDL 72.521, as measured by changes in putrescine and N^1 AcSpd levels

All cultures were initiated as in Figs. 1 and 2. Cells were treated with $25 \mu\text{M}$ -MDL 72.521, or with a saline control, for 2 h before heat-shock. Polyamine contents were then determined at 2 h intervals in CHO cells (a) after a $43^\circ\text{C}/90 \text{ min}$ heat-shock and in A549 cells (b) after a $45^\circ\text{C}/20 \text{ min}$ heat-shock.

When grown in the absence of the PAO inhibitor, rodent cells fail to accumulate N^1 AcSpd or N^1 AcSpm in response to stresses, such as heat-shock or growth into high densities. This occurs, apparently, because PAO activity is constitutively expressed at high enough levels in these cells to oxidize completely available pools of these monoacetyl polyamines. In the human A549 cells, however, monoacetyl polyamines do accumulate under certain conditions. This accumulation does not appear to be due either to greater levels of N^1 -SAT expression (Fuller *et al.*, 1990), or changes in PAO activity as a function of growth phase. As shown in Table 1, A549 cultures convert N^1 -acetyl polyamines into shorter-chain amines. This accumulation of N^1 AcSpd, in the absence of PAO inhibition, in human cells late in growth or after heat-shock could result, for example, from unique intracellular compartmentalization of enzyme or substrate, some other species-specific regulatory process that limits the ability of PAO to oxidize the monoacetyl polyamines, or some combination of these parameters.

The levels of polyamine oxidase activity that we measured are consistent with reported values. Our measured activities of 2–5 units/ 10^6 cells for N^1 AcSpm oxidation for these rat hepatoma cells are somewhat lower than those reported by Seiler *et al.* (1980), but they used an assay based on N^1 -acetylspermidine formation from N^1N^{12} -diacetylspermine. Our values are of the same order as those measured in crude extracts of rat liver (Bey *et al.*, 1985) and in a variety of mouse cell lines (Hirvonen *et al.*, 1989). PAO activities were found, by Romano & Bonelli (1986), to be elevated in normal human breast tissue (~ 240 units/mg of protein), compared with cancerous breast tissue (~ 10 units/mg of protein), as assayed by conversion of [^{14}C]spermidine into putrescine (which does not distinguish between PAO and copper-

dependent amine oxidase activities). Our values for PAO activity in human tumour cells are of the order of that reported for cancerous breast tissue, and are about 10-fold less than that for normal breast tissue (plateau-phase A549 cells contain ~ 0.35 mg of protein/ 10^6 cells; this can be used to convert the values reported here in units/ 10^6 cells into units/mg of protein). We do not find that PAO activity is generally higher in human normal cells than in neoplastic cell lines in culture, although we have not directly compared tumour cells with the normal cells from which they are derived. Whether the normal/tumour tissue differences reported by Romano & Bonelli (1986) are unique to tissues *in vivo* or to breast tissue specifically is yet to be established. Consistent with the report by Romano & Bonelli (1986), we find that PAO activity is very low in a human breast-cancer-cell line (MCF7). Several other groups have reported differences in the expression of amine oxidase activities in normal and neoplastic cells/tissues. Chanda & Ganguly (1987) found that histamine and diamine oxidase activities were elevated in some cancers. Quash *et al.* (1979) described growth-phase-dependent changes in diamine and polyamine oxidase activities in normal and transformed rodent cells in culture, and found that both enzyme activities were decreased in rat mammary and human oesophageal cancers, compared with similar activities found in adjacent normal tissues.

In summary, we have shown that many human cells of both normal and neoplastic origin express PAO activity, and that human cells catabolize polyamines by a similar mechanism and under similar conditions to rodent cells. At least one human cell line, A549, possesses the unique ability to convert N^1 AcSpd directly into N^1 AcSpm in the absence of PAO activity. This observation describes a novel aspect of polyamine metabolism and suggests that the human spermine synthase has a different substrate specificity from that of the rodent enzyme, since two rodent cell lines lack this function. Finally, we confirmed that polyamine catabolism is stimulated in response to stresses, but that PAO inhibition has little effect on cell proliferation of either rodent or human cells growing under optimal conditions in culture. Since Halline *et al.* (1990) report that PAO inhibition does suppress tumour growth during chemically induced colon carcinogenesis in the rat, it will be of interest to determine if the

polyamine catabolism is uniquely involved in cellular stress responses, including those which occur during the conversion of normal into malignant cells *in vivo*.

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