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Structural Requirements of Analogues of Polyamines for Migration and Growth of IEC-6 Cells

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SUMMARY

Healing of gastrointestinal mucosal lesions occurs through two processes: an early one involving cell migration and a later one in which cell division replaces lost cells. Both processes require the presence of polyamines, but the mechanism of action of these compounds is unknown. In the present study, we examined the ability of analogues of spermidine and spermine to support migration and growth of IEC-6 cells that have been grown in α-difluoromethylornithine to inhibit polyamines. All analogues of spermidine with the general formula *x*-3 (referring to the numbers of carbon atoms on either side of the central nitrogen), where *x* = 2–12, competed with spermidine for entry into the cells. However, in addition to spermidine (*x* = 4), only compounds for which *x* = 2, 3, or 6 supported migration and only those for which *x* = 2 or 7 supported growth. Spermine analogues 3-*x*-3, for which *x* = 3, 6, 9, or 12, competed for entry into the cells, but only compounds for which *x* = 3 or 6 supported migration and only the compound for which *x* = 3, in addition to spermine (*x* = 4), supported growth. In addition, analogues 2-3-2, 3-2-3, and 2-(3)$_2$, a branched compound, supported both migration and growth but entered the cell via a mechanism different than that for spermidine and spermine. These data define some of the specific structural requirements for polyamines to produce their physiological effects.

The polyamines spermidine and spermine and their precursor, putrescine, are found in all eukaryotic cells and are essential for normal growth and differentiation (1, 2). Putrescine (1,4-diaminobutane) is formed by the decarboxylation of the amino acid ornithine, and the enzyme catalyzing the reaction, ODC [EC 4.1.1.(17)], is highly regulated. Spermidine and spermine are formed by the addition of aminopropyl groups to one or both of the amino functions of putrescine, respectively. Thus, spermidine contains three and spermine contains four amine groups, all of which are protonated at physiological pH. The role of polyamines in general appears to be related to growth or the functions of biological membranes (3). The mechanisms of action of polyamines at the molecular level, for the most part, are unknown but appear to depend on their ability to bind to negatively charged macromolecules such as proteins and nucleic acids.

Due to their multivalent nature and separation of charges by a flexible carbon backbone, the polyamines are able to bridge fixed distances in specific interactions (3). X-ray crystallography of the short, zig-zag skeleton of polyamines shows a three-dimensional structure that rotates around the C—C and C—N bonds, allowing stereospecific interactions with DNA (4). Spermidine and spermine molecules align in the minor groove of the DNA double helix. Bonding occurs between the -NH_3^+ groups of the polyamines and the -PO_4^{3-} groups of the DNA, forming interstrand bridges (4). Polyamine-deficient cells have altered DNA double-helical structures. The effects of polyamines on DNA combination have been the best studied actions of these compounds at the molecular level. Polyamines associate with specific base sequences of DNA (5), cause the condensation of DNA (6), and change the conformation of polynucleotides from the right-handed B-form to the left-handed Z-form (7). Spermine induces a bend in the helical axis, resulting in a change in the conformation (8). The shift from the right- to the left-handed form is important for nucleosome formation (9), chromatin condensation (10), and the expression of genes involved in growth (11). Inhibition of ODC with DFMO depletes cells of polyamines and inhibits their growth. The ability of polyamine analogues to rescue cells from this growth inhibition is correlated with their ability to induce the B-to-Z transition (12).

Polyamines are necessary for growth of the gastrointestinal mucosa (13) and the healing of mucosal lesions (14–16). Polyamines are required for mucosal growth in a diversity of

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ABBREVIATIONS: Putrescine, 1,4-diaminobutane; ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum.

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different experimental situations (see Ref. 13 for a review). The healing of mucosal erosions involves at least two processes. Beginning immediately after damage, an early process, termed mucosal restitution, involves the sloughing off of damaged epithelial cells, followed by the migration of undamaged cells from areas adjacent to, or just under, the damaged surface (17, 18). The migrating cells cover the denuded lamina propria sealing the mucosa. The process occurs too soon to be accounted for by cell division. At 12–16 hr after the damage, cell division begins to occur. This latter process, during which cells lost to injury become replaced, may take 1–2 days to complete (19). Polyamines are necessary for both the early process of cell migration and the latter process of cell division (15, 16).

We used the IEC-6 cell line, originally derived from normal rat intestinal crypt cells (20), to examine mechanisms of polyamine involvement in both processes. We developed a model of gastrointestinal cell migration with wounded monolayers of these cells (21). Migration in this model mimics the early phase of cell restitution in that it is independent of DNA synthesis for at least 8 hr but is dependent on actin polymerization, protein synthesis, and polyamines (21). Depletion of polyamines by DFMO almost completely inhibits cell migration in this model, and the addition of exogenous polyamines to the DFMO-treated cells returns migration rates to normal (22). Cells treated with DFMO were also growth inhibited and had decreased expression of the proto-oncogenes c-*myc* and c-*jun*. Exogenous spermidine restored both normal growth and expression of proto-oncogenes (23). These findings suggest that polyamines are capable of interacting with cytoskeletal elements to alter migration and with DNA to change gene expression as it relates to growth.

In the present study, we attempted to gain some insight into the mechanism of action of the polyamines by examining the structural characteristics of the compounds required for the actions in question. We examined the ability of groups of analogues of spermidine (triamines) and spermine (tetraamines) to substitute for polyamines in cells treated with DFMO to deplete endogenous polyamines. There have been few systematic structure-function studies involving polyamines, and most of them have used bis-analogues and have not involved gastrointestinal tissues.

Experimental Procedures

Test compounds. Diethylenetriamine, triethylenetetraamine, and tris-(2-aminoethyl)amine were purchased from Aldrich (Milwaukee, WI). All other test polyamines were prepared as previously described (24, 25), except for the linear tetraamine 3-2-3 (preparation described later) and its branched chain isomer 2-$(3)_2$.[1]

Tetraamine 3-2-3. A solution of 1,3-propanediamine (250 g; 3.3 mol) in absolute ethanol (500 ml) was warmed to 72°. The heating source was removed, and a solution of 1,2-dibromoethane (125 g; 0.67 mol) in absolute ethanol (250 ml) was added by drops, with stirring, at a rate such that the internal temperature was maintained at 74–77°. After complete addition, the reaction was heated at reflux for 2 hr and then allowed to cool to ~40°. Potassium hydroxide pellets (83 g; 1.47 mol) were added slowly while the internal temperature was maintained at 40–50° and then the reaction mixture was allowed to cool and stand overnight at room temperature. Precipitated potassium bromide was removed the next day by suction filtration, and the ethanol was stripped off with the rotary evaporator. The residue was distilled under high vacuum to first remove unreacted 1,3-propanediamine and then to produce crude product (fraction distilling at 115–135°/0.01 torr). Redistillation of the crude material afforded pure free base product (bp 116–118°/0.01 torr), which hardened into small needles at room temperature (calculated for $C_8H_{22}N_4$: C, 55.13; H, 12.72; N, 32.15%; found: C, 55.31; H, 12.64; N, 32.11%). A 5-g aliquot of this material, dissolved in ethanol, was treated with anhydrous hydrogen chloride to yield the tetrahydrochloride salt as a white microcrystalline precipitate. The product was crystallized from 80% ethanol; mp 296–298° dec (calculated for $C_8H_{26}Cl_4N_4$: C, 30.01; H, 8:19; Cl, 44.30; N, 17.50%; found C, 30.03; H, 8.18; Cl, 44.61; N, 17.57%).

Reagents and materials. Cultureware were purchased from Corning Glass Works (Corning, NY). Media and other cell culture reagents were obtained from GIBCO-BRL (Grand Island, NY). FBS and dialyzed FBS (1000 molecular weight cutoff) were obtained from Sigma Chemical Co. (St. Louis, MO). Matrigel was obtained from Collaborative Research (Bedford, MA). DFMO was a gift from the Merrell Dow Research Institute (Cincinnati, OH). The IEC-6 cell line (ATCC CRL 1592) was obtained from the American Type Culture Collection (Rockville, MD) at passage 13.

Cell culture. IEC-6 cell stock was maintained in T-150 flasks in a humidified, 37° incubator in an atmosphere of 90% air/10% CO_2. The basic medium used for stock was DMEM/FBS [DMEM (432–2800; GIBCO) with 5% heat-inactivated FBS, 10 μg insulin/ml, 50 μg gentamicin sulfate/ml]. The stock was passaged weekly at 1:10 and fed three times a week. Passages 15–20 were used in the experiments; the cells were taken up with 0.05% trypsin plus 0.53 mM EDTA in Hanks' balanced salt solution without calcium and magnesium and counted by hemocytometer. Dialyzed FBS (dFBS) was used in the experiments.

Cell migration. Cell migration experiments were carried out as previously described (21, 22). Briefly, approximately one third of a monolayer of day 4 cells growing on a thin Matrigel layer was removed with a razor blade, and the remainder was allowed to migrate for 6 hr. The number of cells migrating into the denuded area from a 1-mm segment of the scratch was captured and quantified by computer. Each experiment contained a set of controls consisting of untreated, DFMO-treated, and DFMO/spermidine-treated cells plus DFMO/analogue$_1$- and DFMO/analogue$_2$-treated cells. Spermidine and the polyamine analogues were used at concentrations of 5 and 10 μM. Results are reported from use of the most effective dose. Each experiment was carried out on six separate dishes. The controls were combined, and the data were converted to percentage of control (the untreated set).

Growth studies. The cells were plated at the same density as in the migration experiments (6.25×10^4 cells/cm^2) in T-25 flasks in DMEM/dFBS with or without 5 mM DFMO, 5 μM spermidine, and polyamine analogues. Each experiment contained five sets of flasks of control, DFMO-treated, DFMO/spermidine-treated, DFMO/analogue$_1$-, and DFMO/analogue$_2$-treated flasks. Analogues were used at a concentration of either 5 or 10 μM depending on which had best

[1] During the course of this work, it was discovered that the previously reported tetraamine product 3-2-3 (24) was incorrectly identified. This conclusion results from the observation of lack of identity of the product from the cyanoethylation/reduction route (Ref. 24; synthetic route A) with the material prepared by unambiguous synthesis, as described herein (synthetic route B). Thus, although the two free base products had essentially identical boiling points (116–188°/0.2 torr for the product from route A versus 116–118°/0.1 torr for the route B product) and, for the corresponding tetrahydrochloride salts, similar melting points (290–300° dec versus 296–298° dec, respectively) with no depression on admixture, the two hydrochloride salt samples showed differences in migration on thin layer chromatography [glass-backed silica gel plates, 250-μm layer; butanol/acetic acid/water (4:1:1, v/v/v)] and differences with respect to the nature and rate of reaction with *p*-toluenesulfonyl chloride and in the physical properties of the resulting pertosylated derivative (e.g., m.p. of tosyl product: route A, 221–226°; route B, 245–250°). These differences dictate that the two products cannot be the same, and because route B unambiguously results in the linear tetraamine 3-2-3, the route A product can be the only other structure possible given the reaction scheme, i.e., the branched chain isomer N^2-(2-aminoethyl)-dipropylenetriamine-[tetraamine2-$(3)_2$].

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supported cell migration. Experiments continued for 10 days; on alternate days, an entire set was taken up with trypsin/EDTA, and the number of cells in an aliquot was determined with a Coulter counter. The cells in the remaining flasks were then fed. Each experiment was carried out in six separate flasks. The controls were combined and expressed as a percentage of control.

Transport studies. Transport of analogues was assessed by measurement of their ability to compete for the spermidine/spermine carrier. Earlier studies with isolated rat enterocytes produced kinetic data indicating that spermidine and spermine shared a carrier that is distinct from the one for putrescine (26). Cells were plated into 35-mm plates at the same density used in the migration experiments. On day 2, the plates were washed with Hanks' balanced salt solution, and serum-free DMEM was added. On day 3, after the serum-free media were removed, DMEM/dFBS containing 100 pM [^{3}H]spermidine (DuPont-NEN, Boston, MA) with or without 10 μM spermidine or the analogues was added to the plates. After a 60-min incubation at 37°, the plates were washed three times with ice-cold DPBS containing 1 mM spermidine, and the cells were lysed with 1 M NaOH. A sample of 100 μl was added to 5 ml scintillation cocktail (ICN Biomedicals, Costa Mesa, CA), and radioactivity was determined with liquid scintillation spectroscopy. The protein content of each sample was measured according to the method of Bradford (27). All experiments were carried out in triplicate, and each analogue was tested at least three times. The concentration of labeled spermidine was calculated from the dpm, the specific activity of the radioactive spermidine, and the total cellular protein content.

Statistical Analysis. All data from the three different control groups (controls, DFMO, and DFMO plus spermidine) were combined (54 experiments); each analogue was used in 6 experiments. The mean percentage for each analogue was compared with the DFMO control by analysis of variance. The level of significance was determined with Dunnett's test (28).

Results

In previous studies, we found that treatment of IEC-6 cells with 5 mM DFMO for 4 days reduced putrescine and spermidine to undetectable levels. In the same cultures, spermine levels were lowered to 38% of control. ODC activity at this time was virtually zero (22).

Triamines. Unlabeled spermidine (10 μM) inhibited the uptake of [^{3}H]spermidine by approximately 50%. For the purpose of data expression, this degree of inhibition was set at 100%. The effects of 10 μM concentrations of analogues on [^{3}H]spermidine uptake were determined and expressed as a percentage of the inhibition by cold spermidine (Fig. 1). In all experiments, the molar ratios of labeled and unlabeled were identical. If spermidine is designated as 4-3, referring to the numbers of carbon atoms on either side of the central nitrogen, then the analogues all fit the formula x-3 (Fig. 1). All analogues from 2-3 through 12-3 displayed some degree of competition for the spermidine carrier. Analogues 6-3 through 10-3 were as effective as spermidine (Fig. 1).

After 4 days of treatment, DFMO inhibited cell migration by approximately 66% (Fig. 2). The addition of spermidine to cells grown in the presence of DFMO returned migration to 93% of the control level. Analogues 2-3, 3-3, and 6-3 significantly restored migration in the presence of DFMO, although none were as effective as spermidine. Analogues in which $x >$ 6 were unable to increase migration significantly.

Cell growth plateaued after 8 days in both control and DFMO-treated cultures (Fig. 3). Counts at day 8 were used in all growth experiments. At that time, cultures grown in the presence of DFMO contained approximately 42% of the cells

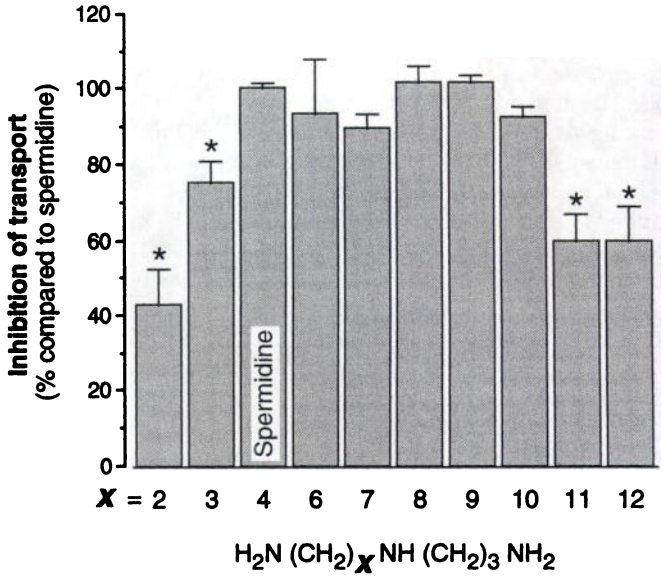

Fig. 1. Effect of spermidine and other triamines on [^{3}H]spermidine uptake by IEC-6 cells. Percent inhibition of [^{3}H]spermidine transport by 10 μM spermidine was designated as 100%, and the effects of equimolar concentrations of analogues are shown as percentages of the spermidine effect. Six experiments for each compound. Data are given as mean + standard error. *, $p < 0.05$ compared with spermidine.

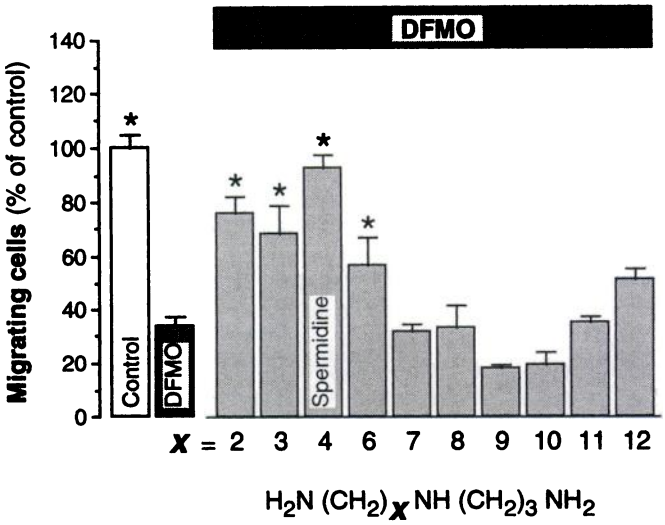

Fig. 2. Effect of 5 mM DFMO, DFMO plus 5 μM spermidine, and DFMO plus 5 μM or 10 μM of various spermidine analogues on the percentage of cells migrating across 1 mm of a scratch on a confluent culture of IEC-6 cells. Data are given as mean + standard error. Six experiments for each mean value. *, $p < 0.05$ compared with DFMO alone.

present in control cultures (Fig. 4). However, the presence of spermidine in DFMO-containing cultures resulted in normal growth rates (Fig. 3), and at day 8 these plates had 100% of control cell numbers (Fig. 4). Analogues 2-3 and 7-3 also significantly reversed the effects of DFMO, but they were not as effective as spermidine. Interestingly, 3-3 and 6-3 analogues had deleterious effects, and these cultures contained fewer cells than those grown only in the presence of DFMO.

Tetraamines. Unlabeled spermine inhibited the uptake of [^{3}H]spermidine by approximately 50%. This was approximately equal to the inhibition produced by an equimolar concentration of spermidine. The inhibition produced by 10

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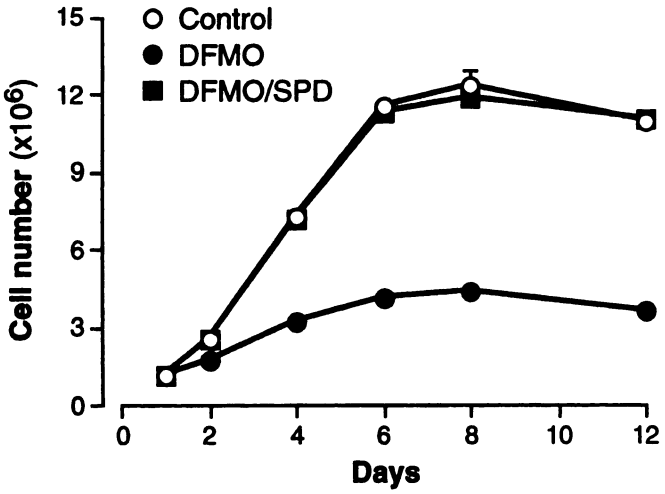

Fig. 3. Growth curves from days 2–10 after plating of control IEC-6 cells and those grown with 5 mM DFMO and with DFMO plus 5 μM spermidine (*SPD*). Data are given as mean ± standard error. Representative of six experiments.

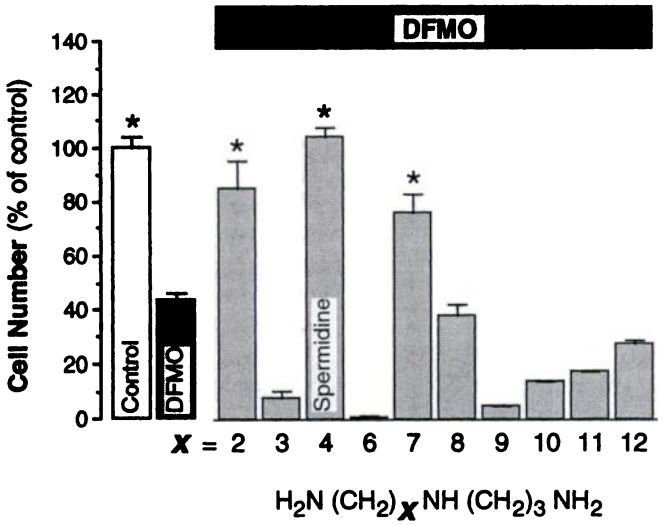

Fig. 4. Effect of 5 mM DFMO, DFMO plus 5 mM spermidine, and DFMO plus spermidine analogues on number of IEC-6 cells 8 days after plating. Data are given as percentages of control and as mean + standard error of at least six plates. *, $p < 0.05$ compared with DFMO alone.

μM spermine was set at 100%, and the effects of equimolar concentrations of the analogues were compared with it (Fig. 5). The general formula for spermine and the analogues is shown in Fig. 5. Except for one branched compound, all of the tetraamine analogues have the formula *y-x-y*, where *x* and *y* refer to the number of carbon atoms separated by the two central nitrogen atoms. Thus, spermine can be designated 3-4-3. The branched compound has the formula $H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}N(CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2)_2$. Analogues for which $x \geq 3$ and $y = 3$ competed equally with spermine for uptake. Neither the branched compound nor 3-2-3, 2-2-2, and 2-3-2 analogues inhibited spermidine uptake.

DFMO reduced cell migration to 34% of normal, and spermine restored it to 93% of the original level (Fig. 6). All analogues except 3-9-3 and 3-12-3 significantly increased migration over that occurring in the presence of DFMO alone. No analogue, however, was as effective as spermine,

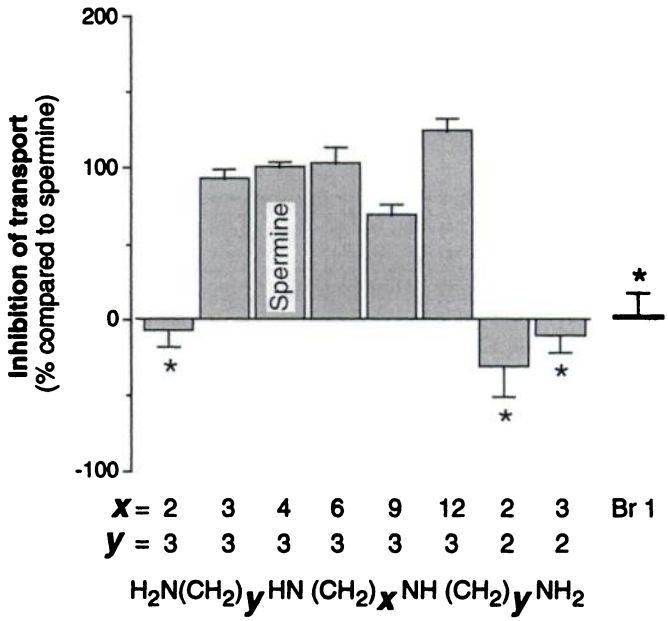

Fig. 5. Effects of spermine and other tetraamines on [^{3}H]spermidine uptake by IEC-6 cells. The effect of 10 μM spermine on [^{3}H]spermidine uptake was designated as 100%, and the effects of equimolar concentrations of analogues are given as percentages of the spermine effect. Six experiments for each compound. Data are given as mean + standard error. *, $p < 0.05$ compared with spermine. *Br 1*, Branched analogue with the formula $H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}N{\cdot}(CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2)_2$.

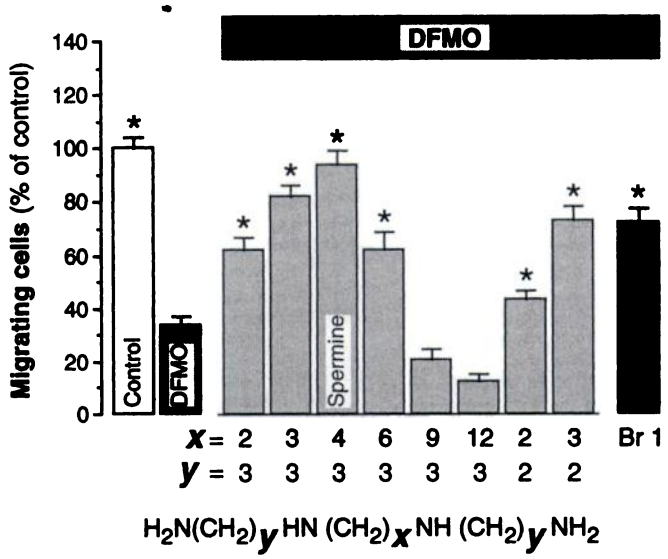

Fig. 6. Effects of 5 mM DFMO, DFMO plus 5 μM spermine, and DFMO and spermine analogues on the percentage of cells migrating across 1 mm of a scratch on a confluent culture of IEC-6 cells. Data are given as mean + standard error. More than six experiments for each mean value. *, $p < 0.05$ compared with DFMO alone. *Br 1*, Branched analogue with the formula $H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}N{\cdot}(CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2)_2$.

and the 2-2-2 compound produced an effect that was <50% of control.

Cultures grown in the presence of DFMO had 42% of the cells present in the control cultures. The addition of spermine fully restored the cell number to 100% of control (Fig. 7). In addition, analogues 3-2-3, 3-3-3, and 2-3-2 and the branched compound significantly increased cell growth in the presence of DFMO. Each of these compounds increased cell numbers to approximately 72% of control.

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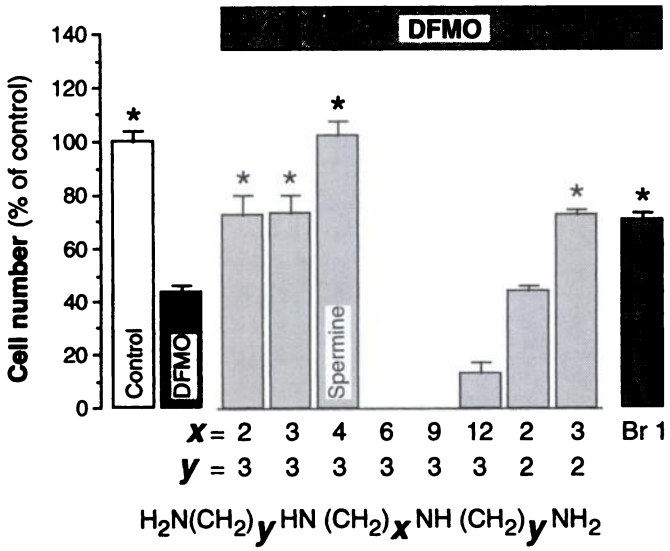

Fig. 7. Effects of 5 mM DFMO, DFMO plus 5 mM spermine, and DFMO and spermine analogues on the number of IEC-6 cells 8 days after plating. All data are given as percentages of control and as mean + standard error of at least six plates. *, $p < 0.05$ compared with DFMO alone. *Br 1*, Branched analogue with the formula $H_2N\text{-}CH_2\text{-}CH_2\text{-}N\text{-}(CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2)_2$.

Discussion

Endogenous polyamines are synthesized in eukaryotic cells primarily through the decarboxylation of ornithine to form putrescine. The addition of an aminopropyl unit to one of the nitrogen atoms of putrescine produces spermidine, and the addition of a second aminopropyl group to the other nitrogen of putrescine converts spermidine into spermine. All of the spermidine analogues that were used in the present study were synthesized by varying the number of carbon atoms that would normally be donated by putrescine, and thus they have the general formula *x*-3 (Fig. 1). Many, but not all, of the spermine analogues were synthesized by also varying the number of carbon atoms in the "putrescine" moiety, and they have the general formula 3-*x*-3 (Fig. 5). Restricting the discussion to these compounds, all of the compounds for which $x > 2$ competed with the spermidine/spermine carrier for uptake (Figs. 1 and 5). In addition, the spermidine 2-3 analogue was a weak uptake competitor. These data indicate that each of these analogues gains access to the interior of the cell via the spermidine/spermine carrier.

It should be pointed out that the effects of analogues on spermidine transport are relevant only to the exogenous spermidine concentration examined (100 pM). The purpose of this study was not to describe spermidine transport but rather only to determine whether the analogues gained entry to the cells. The actual percent inhibition caused by any analogue will vary according to the exogenous spermidine concentration.

Even though all of these analogues enter the cell, only those compounds for which $x = 2$, 3, 4, or 6 supported cell migration in the presence of DFMO. This suggests that separation of the charged nitrogen atoms by more than six carbon atoms produces a compound that cannot effectively interact with the cytoskeleton. The inability of analogues to substitute for spermidine or spermine in migration, even though they compete for entry, suggests that the effects of polyamines on the cytoskeleton are due to structure-specific interactions rather than to nonspecific ionic effects. The spermine analogue 3-2-3 supported migration without competing for entry. Although these findings might appear to be anomalous, they probably indicate the existence of a separate transport mechanism for this compound.

The only effective spermidine analogues for growth were 2-3 and 7-3 compounds, in addition to spermidine. Although the 3-3 and 6-3 analogues supported migration, they did not support cell growth. Similarly, the spermine analogue 3-6-3 failed to support growth, whereas it did support migration. Thus, several compounds supported migration that did not support cell growth, but there were no compounds that supported growth and did not also support migration. In addition, it is interesting to note that analogues 3-3, 6-3, and 3-6-3 not only failed to support growth but also inhibited it to a level less than the inhibition caused by DFMO. Inhibition was also seen with 9-3, 3-9-3, and 3-12-3. In each of these compounds, *x* equals a multiple of 3. It appears that these compounds are capable of binding to the same sites as the endogenous polyamines but are unable to produce the desired change. Thus, they may act as competitive inhibitors.

In addition to analogues with the general formula 3-*x*-3, several others were examined. These were compounds with the formulas 2-2-2 and 2-3-2 and a branched compound, $2\text{-}(3)_2$. None of these compounds competed for the spermine carrier for cellular uptake. Both the 2-3-2 and the branched analogue, however, supported both cell migration and growth, indicating they gain entrance to the cell through carrier mechanisms distinct from those for spermidine and spermine. The 2-2-2 compound was relatively ineffective, slightly supporting migration and having no effect on cell growth. In general, all compounds with nitrogen atoms separated by some combination of 2 and 3 carbon atoms were effective. This includes the spermidine analogue 2-3 and the spermine analogues 2-3-2, 3-2-3, and $2\text{-}(3)_2$. The finding that the 2-2-2 compound was ineffective indicates that two of the charged nitrogen atoms must be separated by at least three carbon atoms.

Some of the results of the present study are similar to those of a recent study involving homologues of putrescine (29). In that study, all diamines with 4–10 carbon atoms competed with putrescine for entry into the cell. Only putrescine, however, significantly supported migration of cells grown in DFMO. Only putrescine and cadaverine (5 carbon atoms) supported growth. The 3-carbon analogue 1,3-propanediamine supported growth even though it did not compete for the putrescine carrier, indicating again that it entered the cells by a different pathway.

Basu *et al.* (12) examined the ability of several spermine analogues to rescue 9L rat brain tumor cells from growth inhibition induced by DFMO and subsequent polyamine depletion. They found, as we did in the present study, that 3-3-3 and 3-2-3 analogues were nearly as effective as spermine. In general, bis-ethyl analogues were considerably less effective or ineffective. The same bis-compounds, however, caused intracellular polyamine depletion by inhibiting the enzymes responsible for polyamine synthesis. Thus, these analogues were able to enter the cells. In general, the ability of analogues to rescue growth-inhibited cells was correlated with their ability to induce B-to-Z changes in DNA conformation (12).

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Although the effect of polyamines on growth appears to relate to their abilities to bind to DNA (12) and regulate proto-oncogene expression (23), the mechanism by which they influence migration is unknown, but it no doubt relates to the cytoskeleton. Polyamine starvation in polyamine-auxotrophic Chinese hamster ovary cells leads to the disappearance of actin filaments and microtubules (30). Recently, we demonstrated that polyamine depletion in IEC-6 cells caused by DFMO results in a reorganization of the actin cytoskeleton (31). In polyamine-depleted cells, F-actin and tropomyosin were redistributed from stress fibers to the cell cortex. This was associated with a reduction in lamellipodia and a marked impairment of migration. The addition of exogenous polyamines to the DFMO-treated cells reversed all of the effects on the cytoskeleton, as well as on migration. Schuber (3) proposed that polyamines may bridge proteins and lipids in the plasma membrane, leading to increased protein/protein interactions in the cytoskeleton bridging membrane and cytoskeletal proteins. Because cell motility depends on the actin cytoskeleton, this is a likely explanation for the effect of polyamines on migration.

In conclusion, all spermidine analogues of the general formula x-3 where x = 2–12 competed for entry into the cell. Only those compounds for which x = 2, 3, 4, or 6 supported migration, and those for which x = 2, 4, or 7 supported growth of polyamine-depleted cells. Spermine analogues with the general formula 3-x-3 where x = 3, 4, 6, 9, or 12 completed for entry into the cells, but only those compounds for which x = 3, 4, or 6 supported migration and only those for which x = 3 or 4 supported growth. Another group of spermine analogues with a combination of two and three carbon atoms (3-2-3, 2-3-2, and a branched compound 2-(3)$_2$) gained entry into the cell by a mechanism independent of the spermidine/spermine transporter and supported growth and migration. These results indicate that analogues in which the nitrogen atoms are separated by more than six or seven carbon atoms are unable to substitute for endogenous polyamines. All of the analogues in which the nitrogen atoms are separated by combinations of two and three carbon atoms are effective and do not compete for uptake with the polyamines. In addition, analogues in which the nitrogen atoms are separated by multiples of three carbon atoms (3-3, 6-3, 3-6-3, 9-3, 3-9-3, and 3-12-3) with 3-3-3 being the only exception, cause inhibition of growth to a level less than that produced by DFMO. This may indicate that these compounds bind to the same structures as endogenous polyamines but are unable to produce the normal effect and, therefore, act as inhibitors. The results of the present study define some of the structural characteristics of the polyamines required for their physiological effects on growth and cell motility. They may provide keys to understanding their actions at the molecular level.

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