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Arginine decarboxylase inhibitors reduce the capacity of *Trypanosoma cruzi* to infect and multiply in mammalian host cells

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ABSTRACT **The capacity of blood (trypomastigote) forms of *Trypanosoma cruzi* to infect mouse peritoneal macrophages or rat heart myoblasts *in vitro* was inhibited by treatment of the trypomastigotes with DL-α-difluoromethylarginine (F_2MeArg), monofluoromethylagmatine, or (*E*)-α-monofluoromethyl-3-4-dehydroarginine—all irreversible inhibitors of arginine decarboxylase. Similar results were obtained when F_2MeArg-treated parasites were incubated with rat heart myoblasts. The inhibitory effects were characterized by marked reductions in both the proportion of infected cells and the number of parasites per 100 host cells. The concentrations of the arginine decarboxylase inhibitors that affected infectivity had no detectable effect on either the concentration or motility of the parasite and, therefore, could not have affected the collision frequency. F_2MeArg appeared to inhibit the ability of *T. cruzi* to penetrate the host cells since the drug had no significant effect on the extent of parasite binding to the surface of the host cells. The inhibitory effect of F_2MeArg was markedly reduced or abrogated in the presence of either agmatine or putrescine, as would have been expected if F_2MeArg acted by inhibiting arginine decarboxylase. Addition of F_2MeArg to macrophage or myoblast cultures immediately after infection or at a time when virtually all of the intracellular parasites had transformed into the multiplicative amastigote form, resulted in a markedly reduced parasite growth rate. This effect was also prevented by exogenous agmatine. These results indicate the importance of polyamines and polyamine biosynthesis in the following two important functions of *T. cruzi*: invasion of host cells and intracellular multiplication. Furthermore, concentrations of the inhibitors tested that affected the parasite did not alter the viability of the host cells, the cellular density of the cultures, or the ability of uninfected myoblasts to grow. Thus, arginine decarboxylase inhibitors may have a potential application in chemotherapy against *T. cruzi* infection.**

Trypanosoma cruzi is the protozoan causing Chagas disease or American trypanosomiasis—a major health problem in South and Central America (1). The infection is produced in most cases by trypomastigote forms borne by reduviid insects and, less frequently, by trypomastigotes contaminating blood used in transfusions (2, 3). Mammalian host cell invasion by *T. cruzi* is essential for the production of disease and the continuation of the life cycle of the parasite. Establishment of this parasite in mammalian hosts requires that the invasive trypomastigotes attain a cytoplasmic localization because it is in the host cell cytoplasm that they can transform into the multiplicative amastigote form. Amastigotes divide and many eventually transform into new trypomastigotes that are released from bursting infected cells and disseminate the infection to vital tissues, including those of the heart and nervous system. Although several laboratories, including our own, have examined some of the biochemical requirements for and reactions influencing the invasion of host cells by *T. cruzi* (4–12), our current understanding of this process—which could underlie the development of effective chemoprophylaxis and chemotherapy—is limited. Particularly deficient is knowledge about the role of polyamines and polyamine biosynthesis in *T. cruzi* infectivity. Polyamines are present in a number of trypanosomatids and ornithine decarboxylase (OrnDCase), which generates putrescine from ornithine, has been shown to play an important role in the proliferation of African trypanosomes (reviewed in refs. 13, 14, and 15). Although polyamines (e.g., putrescine, spermidine, and spermine) are present in *T. cruzi* trypomastigotes (P.P.M., A. J. Bitonti, F.K., and J.J.W., unpublished results), treatment with DL-α-difluoromethylornithine or other catalytic inhibitors of OrnDCase does not alter the capacity of these organisms to infect phagocytic or nonphagocytic host cells *in vitro* (unpublished data). It is conceivable, however, that *T. cruzi* relies on an alternative pathway for polyamine biosynthesis. This possibility has been explored in the present work in which we studied the effects of irreversible inhibitors of arginine decarboxylase (ArgDCase)—an enzyme heretofore identified in bacteria (16–19), plants (20–24), and, apparently, in the parasitic worm *Ascaris lumbricoides* (25)—on *T. cruzi* infection.

MATERIALS AND METHODS

Animals. The 6- to 9-week-old inbred CBA/J mice used to obtain resident peritoneal macrophages were purchased from The Jackson Laboratory. Four-week old Crl-CD-1(ICR)BR Swiss mice from Charles River Breeding Laboratories were used to maintain and produce blood forms of *T. cruzi*.

Mouse Peritoneal Macrophages. The methods used to purify unelicited mouse peritoneal macrophages and for setting up cultures of these cells on 3-mm diameter glass wells cut in sterile, Teflon-coated microscope slides (Cel-Line, Newfield, NJ) have been described in detail (26). The monolayers consisted of >99% cells with typical macrophage morphology and positive staining for nonspecific esterase activity.

Rat Heart Myoblasts. Confluent monolayers of rat heart myoblasts (American Type Culture Collection, CRL 1446) were prepared on gelatin-coated microscope slide wells (see above) using Dulbecco's modified Eagle's medium (DMEM) containing penicillin at 100 units/ml and streptomycin at 100 μg/ml and 10% (vol/vol) heat-inactivated fetal bovine serum (GIBCO). Cell division was reduced to an insignificant level after the myoblast cultures attained confluence.

Parasites. Blood (trypomastigote) forms of Tulahuén strain *T. cruzi* were obtained from mice that had been infected 2 weeks previously with 2×10^5 parasites intraperitoneally.

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Abbreviations: ArgDCase, arginine decarboxylase; F_2MeArg, DL-α-difluoromethylarginine; FMeAgm, monofluoromethylagmatine; FMedHArg, (*E*)-α-monofluoromethyl-3,4-dehydroarginine; OrnDCase, ornithine decarboxylase.

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The trypomastigotes were purified by centrifugation over Isolymph (Gallard Schlesinger, Carle Place, NY) (27) followed by chromatography through a DEAE-cellulose column (28). The eluted parasites were concentrated by centrifugation (800 × *g*, 15 min, 20°C) and resuspended at 3–5 × 10^6 organisms per ml in the desired medium. The viability of blood-derived *T. cruzi* used in this work, determined by the criterion of motility, was >99.9%. The method for preparing *T. cruzi* amastigotes has been described in detail (29). In all cases, parasite concentrations were determined microscopically using a hemacytometer.

Reagents. The following inhibitors of arginine decarboxylase: DL-α-difluoromethylarginine (F_2MeArg) (19), monofluoromethylagmatine(FMeAgm)and(*E*)-α-monofluoromethyl-3,4-dehydroarginine (FMedHArg) (30) were synthesized at the Merrell Dow Research Institute. Putrescine was purchased from Sigma. Agmatine (Aldrich) was purified by ion-exchange chromatography on Dowex-50 using a gradient from 0 to 1.5 M HCl and determined to be free of other polyamines by HPLC and thin-layer electrophoresis. Solutions of these reagents were prepared in DMEM.

Treatment of Parasites with Inhibitors of Arginine Decarboxylase. Trypomastigotes were incubated at 37°C for 3–4 hr in DMEM alone or containing various concentrations of the tested inhibitors. These treatments were not extended beyond 4 hr so that the duration of the experiment, including the time of parasite coculture with host cells (1–2 hr), would not come too close to the time when isolated *T. cruzi* trypomastigotes start to show morphologic signs of transformation into other forms. The excess inhibitor was removed by washing with DMEM, and the parasite concentration was adjusted to 3–5 × 10^6 organisms per ml for use in experiments with macrophages or to 10^7 organisms per ml for use in experiments with myoblasts.

Assay of *T. cruzi* Interaction with Macrophages or Myoblasts. The conditions to determine *T. cruzi* association with macrophages or myoblasts have been described in detail (4, 5). Briefly, cultures of macrophages or myoblasts were incubated with drug-treated or mock-treated parasites at 37°C in a 5% CO_2/95% air incubator for 1 hr or 2 hr. The initial parasite/cell ratio was between 3:1 and 5:1 in the various experiments with macrophages and 10:1 when myoblasts were used. The interaction was interrupted by removal of the free extracellular parasites from the cultures by three washings with DMEM and immediate fixation with absolute methanol. After staining with Giemsa, the cultures were examined microscopically (×1000), screening not less than 200 cells in each culture. The numbers of screened cells, parasites associated with these cells, and cells associated with one or more parasites were recorded. Each assay was set up in triplicate, and the results were expressed as the mean percentage of cells with parasites ± 1 SD and the average number of parasites per 100 cells ± 1 SD.

Assay of *T. cruzi* Binding to Macrophages or Myoblasts. To measure surface binding between trypomastigotes and host cells, the cocultures were set up as described in the preceding paragraph but were incubated at 4°C (31).

Measurement of Intracellular Parasite Load. After host cell infection and removal of free parasites as described above, cultures of macrophages and myoblasts were further incubated at 37°C and 5% CO_2/95% air for predetermined time intervals in fresh DMEM supplemented with 5% (vol/vol) fetal bovine serum that contained or lacked F_2MeArg. In some experiments, medium containing either F_2MeArg and 0.3 mM agmatine or agmatine alone was added to the infected cultures. During incubation, the solutions of the tested reagents were replaced every 12 hr with a fresh aliquot to compensate for any reagent consumption that may have occurred; control cultures received the same volume of medium alone at the same times. These cultures were terminated by washing with DMEM, fixed with absolute methanol, and examined microscopically as described above. All assays were set up in triplicate.

Assays for Arginine Decarboxylase and OrnDCase. Purified trypomastigotes and amastigotes suspended at concentrations ranging from 10^7 to 6 × 10^9 organisms per ml in 50 mM Tris·HCl (pH 7.5) containing 5 mM dithiothreitol and 1 mM EDTA were disrupted by sonication (three 1-min pulses at maximum setting; Biosonik III, Bronwill, NY). The presence of arginine decarboxylase or OrnDCase activity in these materials was tested by a modification (19) of the method of Wu and Morris (32) that measures $[^{14}C]O_2$ released from [1-^{14}C]arginine or [1-^{14}C]ornithine, respectively.

Presentation of Results and Statistics. Each set of results presented in the tables of this paper is typically representative of two to four repeated experiments. Differences between means are expressed in terms of the percentage change, calculated by the equation: % change = [(experimental activity − control activity)/control activity] × 100, and were considered to be significant if $P < 0.05$ as determined by the Mann–Whitney "U" test.

RESULTS

Effects of Arginine Decarboxylase Inhibitors on *T. cruzi* Interaction with Host Cells. Pretreatment of blood-derived trypomastigotes of *T. cruzi* with F_2MeArg, FMeAgm, or FMedHArg markedly decreased their capacity to infect untreated macrophages. This was shown by significant reductions in both the percentage of cells associated with parasites and the average number of organisms per 100 macrophages (Table 1). Although the extent of the observed reduction varied among repeat experiments (data not shown), the effect itself was highly reproducible and always statistically significant. In the various repeat experiments the minimal concentration of F_2MeArg causing significant inhibition was 10 mM (data not shown) to 12.5 mM (Table 1) whereas only 3 mM FMeAgm or FMedHArg was required to produce significant inhibition. Neither suspensions of trypomastigotes nor cultures of macrophages or myoblasts denoted any significant loss in cell viability after incubation with the tested concentrations of F_2MeArg, FMeAgm, and FMedHArg for 3–4 hr at 37°C. Furthermore, there was no detectable change in parasite motility or concentration after these treatments (data not shown). Since our supply of FMeAgm and FMedHArg was limited, we used F_2MeArg in all subsequent experiments.

The reduced level of parasite–macrophage interaction due to parasite treatment with arginine decarboxylase inhibitors might have resulted from impaired parasite binding to macrophages or a diminution of its membrane-penetrating capacity. To explore the first possibility, we carried out parallel assays at 37°C (a temperature at which both binding and penetration can occur) and at 4°C [only surface binding occurs at this temperature (31)]. As can be seen in Table 2, the extent of parasite–macrophage association was significantly reduced by F_2MeArg pretreatment of *T. cruzi* when the cocultures were incubated at 37°C; the extent of parasite–macrophage interaction was minimal at 4°C and was not visibly affected. The second possibility was examined in experiments in which F_2MeArg-treated *T. cruzi* were cocultured with myoblasts. Since these cells are not phagocytic, parasite interiorization results exclusively from membrane penetration. The results presented in Table 3 showed that concentrations of F_2MeArg similar to those effectively reducing parasite–macrophage interaction also affected the capacity of the trypanosomes to infect the myoblasts.

The observed reduction in *T. cruzi* infectivity caused by F_2MeArg might have been due to an accumulation of arginine owing to putative inhibition of arginine decarboxylase or to

Table 1. Effects of pretreatment of blood forms of *T. cruzi* with F_2MeArg, FMeAgm, or FMedHArg on their capacity for interaction with macrophages

Reagent	Concentration, mM	% macrophages with parasites	Parasites, no. per 100 macrophages
None		35.7 ± 1.6	48.5 ± 3.8
F_2MeArg	3	34.5 ± 2.4 (−3.4)	52.0 ± 5.6 (7.2)
	6	33.9 ± 3.4 (−5.0)	44.1 ± 8.0 (−9.1)
	12	31.4 ± 0.6 (−12.0)	36.4 ± 0.8 (−24.9)
	25	19.5 ± 1.3 (−45.4)	27.4 ± 3.9 (−43.5)
	50	18.9 ± 4.0 (−47.1)	23.0 ± 5.2 (−52.6)
FMeAgm	3	23.7 ± 1.3 (−33.6)	27.4 ± 1.8 (−43.5)
	6	18.5 ± 3.5 (−48.2)	26.7 ± 3.1 (−44.9)
	12	11.3 ± 3.2 (−68.3)	13.1 ± 4.4 (−73.0)
	25	7.2 ± 0.5 (−79.8)	7.5 ± 0.3 (−84.5)
	50	5.1 ± 0.8 (−85.7)	5.1 ± 0.8 (−89.5)
FMedHArg	3	26.8 ± 2.4 (−24.9)	33.2 ± 5.5 (−31.5)
	6	20.3 ± 1.2 (−43.1)	24.4 ± 2.8 (−49.7)
	12	21.1 ± 1.4 (−40.9)	27.0 ± 3.9 (−44.3)
	25	12.8 ± 4.6 (−64.1)	15.0 ± 4.1 (−69.1)
	50	8.6 ± 1.3 (−75.9)	8.7 ± 1.4 (−82.1)

The trypomastigotes were incubated at 37°C for 3 hr with DMEM alone or DMEM containing the indicated concentrations of F_2MeArg, FMeAgm, or FMedHArg, washed, and cocultured with untreated macrophages for 2 hr. All differences between the experimental values obtained with FMeAgm, FMedHArg, or with concentrations of F_2MeArg ≥12 mM and the corresponding control values were statistically significant ($P < 0.05$). Values in parentheses are the percent change.

the presence of F_2MeArg, an arginine analogue. To test this possibility we measured the level of interaction between *T. cruzi* and macrophages after pretreating the flagellates with DMEM containing or lacking concentrations of DL-arginine ranging from 0.5 mM to 50 mM. Both the percentages of infected macrophages and the average numbers of trypanosomes per 100 macrophages obtained with DL-arginine-treated and mock-treated parasites were comparable (data not shown).

If F_2MeArg reduced *T. cruzi* infectivity by inactivating arginine decarboxylase, the effect should be prevented by agmatine, the product of arginine decarboxylation. As can be seen in Table 4, this was indeed the case. Similar results were obtained when putrescine, the product of metabolic conversion of agmatine, was present during the F_2MeArg treatment.

Although the bond between F_2MeArg and arginine decarboxylase is covalent and causes irreversible inactivation of the enzyme (19), the inhibitory effects of F_2MeArg started to subside 1 hr after the treatment and were virtually undetectable after 2 hr (Table 5). In these assays, the experimental results were compared with those of parallel controls in which we used parasites that had been incubated with medium alone instead of the F_2MeArg solution and were then incubated in fresh medium for the same lengths of time. This was necessary to take into account the time-dependent increases in parasite infectivity that occur when recently isolated trypomastigotes are maintained in cell-free medium for a few hours (33).

Table 3. Effects of F_2MeArg pretreatment of blood forms of *T. cruzi* on their capacity to infect myoblasts

F_2MeArg, mM	% infected myoblasts	Parasites, no. per 100 myoblasts
0	40.3 ± 1.1	72.3 ± 4.7
10	21.3 ± 1.7 (−47.1)	38.9 ± 1.5 (−46.2)
50	20.9 ± 2.7 (−48.1)	28.8 ± 2.9 (−60.2)

In this experiment, trypomastigotes incubated at 37°C for 4 hr with DMEM alone or containing the indicated concentration of F_2MeArg were washed and cocultured with untreated myoblasts for 2 hr. All differences between the experimental values and the corresponding control values were statistically significant ($P < 0.05$). Values in parentheses are the percent change.

Effects of F_2MeArg on Intracellular Growth of *T. cruzi*. We also tested whether F_2MeArg would interfere with the intracellular replication of *T. cruzi*. This was done by adding the agent to cultures of macrophages and myoblasts that had already been infected by the trypomastigotes. The parasite loads of the host cells were determined at 24-hr intervals for a 2-day (myoblasts) or 4-day (macrophages) period, starting immediately after the addition of F_2MeArg. As can be seen in Fig. 1, parasite counts were significantly lower in the F_2MeArg-treated cells than in the mock-treated controls. Similar inhibition was seen when 30 or 50 mM F_2MeArg was added to the cultures 12 hr (myoblasts) and 24 hr (macrophages) after removal of the free trypomastigotes, i.e., after all intracellular organisms had transformed into amastigotes, and parasite loads were determined 24 hr later (data not shown). In this type of experiment we also tested the effect of 0.3 mM agmatine during the F_2MeArg treatment and found the inhibitory effect to be either not detectable or significantly reduced (data not shown).

We used confluent myoblast monolayers in our experiments because cell division is brought to a virtual halt under these conditions and could not artifactually reduce the number of parasites per 100 cells over time. However, we wanted to know if the F_2MeArg treatment would have a toxic action affecting myoblast growth in younger cultures. There was no difference in the rate of growth of myoblasts treated with up to 50 mM F_2MeArg compared to that of cells incubated in medium alone (data not shown).

Arginine decarboxylase and OrnDCase activity could not be detected in samples containing up to 2.4×10^9 trypomastigotes and 2×10^7 amastigotes by measuring the release of $[^{14}C]O_2$ from [1-^{14}C]arginine (for arginine decarboxylase activity) and [1-^{14}C]ornithine (for OrnDCase activity) (19, 30, 32) (data not shown). These methods readily detect arginine decarboxylase activity in *Escherichia coli, Pseudomonas aeruginosa* (19), and plants (34) and OrnDCase activity in *E. coli* (30), African trypanosomes (15), and plants (34).

Table 2. Comparison of the effects of F_2MeArg pretreatment of blood forms of *T. cruzi* on their capacity to interact with macrophages at 37°C and 4°C

Treatment	Coculture temperature	% macrophages with parasites	Parasites, no. per 100 macrophages
None	37°C	53.2 ± 1.0	73.6 ± 3.9
50 mM F_2MeArg	37°C	27.4 ± 2.4 (−48.5)	32.9 ± 7.6 (−55.3)
None	4°C	2.7 ± 1.0	2.7 ± 1.0
50 mM F_2MeArg	4°C	2.1 ± 2.0 (−22.2)	2.3 ± 1.8 (−14.8)

The trypomastigotes were pretreated with medium alone or containing F_2MeArg, washed, and then cocultured with untreated macrophages at 37°C or 4°C for 1 hr. Only the differences between experimental values and control values observed after coculture at 37°C were statistically significant ($P < 0.05$). Values in parentheses are the percent change.

Table 4. Effects of the presence of exogenous agmatine or putrescine during F_2MeArg treatment of *T. cruzi*

Parasite pretreatment	% infected macrophages	Parasites, no. per 100 macrophages
DMEM	30.2 ± 4.0	34.1 ± 4.8
0.15 mM agmatine	31.9 ± 3.2 (5.6)	36.1 ± 5.4 (5.9)
0.15 mM putrescine	33.5 ± 1.6 (10.9)	41.5 ± 4.4 (21.7)
30 mM F_2MeArg	12.1 ± 1.5 (−59.9)	12.6 ± 1.5 (−63.0)
30 mM F_2MeArg/0.15 mM agmatine	32.9 ± 3.1 (8.9)	39.7 ± 3.0 (16.4)
30 mM F_2MeArg/0.15 mM putrescine	35.4 ± 0.7 (17.2)	44.0 ± 2.0 (29.0)
50 mM F_2MeArg	11.8 ± 0.9 (−60.9)	12.5 ± 1.0 (−63.3)
50 mM F_2MeArg/0.15 mM agmatine	30.1 ± 1.0 (0.3)	43.7 ± 2.3 (28.2)
50 mM F_2MeArg/0.15 mM putrescine	35.6 ± 1.9 (17.9)	44.3 ± 3.2 (29.9)

The trypomastigotes were pretreated as indicated (at 37°C for 3 hr), washed, and then cocultured with untreated macrophages for 1 hr. Only the differences between the values obtained with parasites treated with F_2MeArg alone and the corresponding control values (DMEM) were statistically significant ($P < 0.05$). Values in parentheses are the percent change.

DISCUSSION

The reduced infective capacity of *T. cruzi* trypomastigotes after pretreatment with three different catalytic inhibitors of arginine decarboxylase suggested the presence of this enzyme and a role for it in host cell invasion. The lack of effect of parasite pretreatment with DL-arginine rendered unlikely the possibility that F_2MeArg acted by increasing the intracellular level of arginine or that F_2MeArg was itself acting by some other "arginine-analog" effect, and pointed to arginine decarboxylase inhibition as a more probable mechanism. The fact that exogenous agmatine prevented the inhibitory effect of F_2MeArg when it was present during parasite drug pretreatment further supported this possibility since agmatine can be derived metabolically only from arginine decarboxylation (17). Exogenous putrescine also prevented the F_2MeArg effect, suggesting that, in *T. cruzi*, this polyamine has agmatine as a precursor, as is the case in bacteria and plants (17).

Parasite pretreatment with F_2MeArg also inhibited their interaction with myoblasts (to which the parasite can gain access only by membrane penetration). This infers that arginine decarboxylase inhibitors affected mostly the capacity of the parasite to penetrate host cells and suggests that infection of both cell types relies on a common biochemical mechanism involving polyamine biosynthesis.

The F_2MeArg effect was seen when parasites and macrophages were cocultured at 37°C whereas at 4°C the interaction was minimal and was not visibly affected by F_2MeArg pretreatment of the parasites. Thus, it would appear that at least the membrane-penetrating capacity of the flagellate was seriously compromised by F_2MeArg. The low degree of parasite–macrophage interaction observed at 4°C suggested that surface binding was transient and unproductive in terms of infection.

The comparable levels of parasite–macrophage association seen 2 hr after removal of F_2MeArg (Table 5) indicated that the F_2MeArg effect was reversible. Given the covalent nature of F_2MeArg binding to arginine decarboxylase (19), this recovery could be explained by a relatively rapid turnover of arginine decarboxylase in the trypomastigotes. This would be

Table 5. Reversibility of the effect of F_2MeArg on *T. cruzi*

Treatment	Time, min	% infected macrophages	Parasites, no. per 100 macrophages
None	0	18.3 ± 1.6	26.8 ± 2.9
F_2MeArg	0	8.5 ± 2.0 (−53.6)	12.7 ± 3.6 (−52.6)
None	60	23.9 ± 2.6	34.9 ± 3.7
F_2MeArg	60	17.6 ± 3.2 (−26.4)	21.9 ± 5.8 (−37.2)
None	120	27.5 ± 6.1	35.3 ± 4.3
F_2MeArg	120	26.2 ± 4.2 (−4.7)	39.7 ± 7.0 (12.5)

In this experiment, trypomastigotes incubated at 37°C for 3 hr with DMEM alone or containing 30 mM F_2MeArg were washed and further incubated in fresh medium for the indicated periods of time before being washed again and incubated with untreated macrophages for 1 hr. Only the differences between the values obtained with F_2MeArg at times 0 and 60 min and the corresponding control values were statistically significant ($P < 0.05$). Values in parentheses are the percent change.

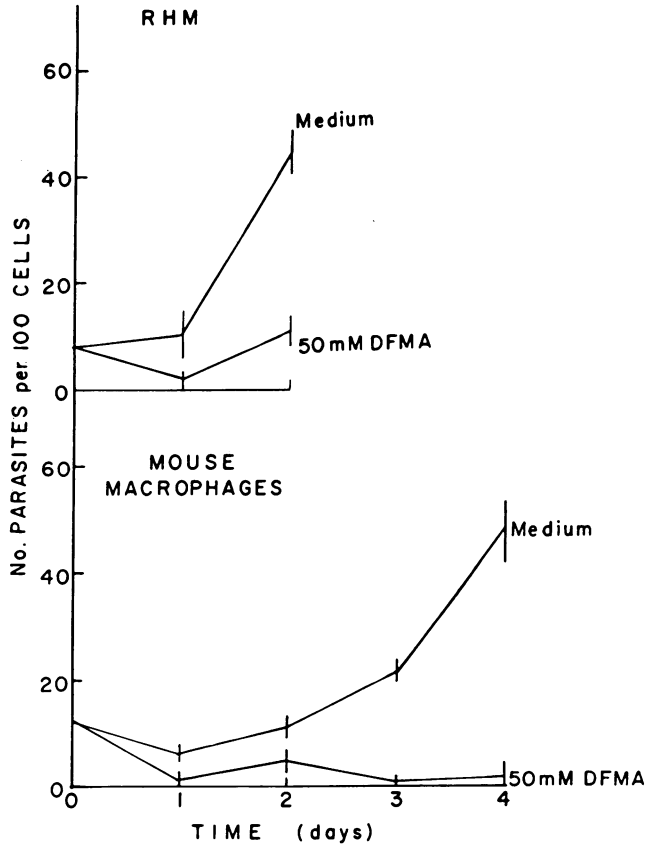

FIG. 1. Effect of F_2MeArg on the capacity of *T. cruzi* to proliferate intracellularly in rat heart myoblasts (RHM) and mouse macrophages. Untreated trypomastigotes were cocultured with the host cells at 37°C for 1 hr (macrophages) or 2 hr (myoblasts), and the free organisms were removed by three washings with DMEM. Fresh DMEM alone or containing F_2MeArg (DFMA) was then added to replicate cultures that were further incubated for the indicated periods of time. The fluid phase was replaced with fresh DMEM without and with F_2MeArg, as appropriate, every 12 hr until termination of the experiment.

reminiscent of the rapid turnover of OrnDCase in other trypanosomatids where this enzyme plays a key role in polyamine production (14).

Treatment of *T. cruzi* trypomastigotes with OrnDCase inhibitors under conditions similar to those used in the present study did not affect significantly their capacity to infect murine macrophages (unpublished data). In contrast, all three arginine decarboxylase inhibitors used in the present study curtailed infection, and such effect by F_2MeArg was prevented by agmatine. This suggested that the parasite utilizes arginine decarboxylase to synthesize polyamines and that ongoing polyamine biosynthesis plays an important role in infectivity. This would be a distinctive feature of *T. cruzi* because, outside of bacteria (16–19) and higher plants (24, 34), arginine decarboxylase has apparently only been found in the intestinal parasitic worm *A. lumbricoides* (25). However, by the method of Wu and Morris (32), we have been unable to detect active arginine decarboxylase in trypomastigotes and amastigotes of *T. cruzi* despite using relatively large numbers of these parasites. Nevertheless, the enzyme might be present in the parasite in an inactive state and become activated after the organism engages a host cell or might only be genetically expressed after contacting the host cell surface. Alternatively, arginine decarboxylase may become inactivated during parasite sonication. At present, we cannot rule out that the tested inhibitors might have affected an as yet undisclosed mechanism not involving arginine decarboxylase. However, the prevention of the F_2MeArg effects by putrescine and particularly by agmatine would seem to argue against the latter possibility.

Since polyamines play an important role in cellular proliferation (35, 36), impairment of intracellular parasite growth by F_2MeArg is likely to be accompanied by inhibited polyamine biosynthesis. This impairment was also prevented by exogenous agmatine, pointing to arginine decarboxylase inhibition as the relevant mechanism. However, in the absence of direct evidence for the presence of arginine decarboxylase in *T. cruzi*, an alternative mechanism cannot be ruled out.

T. cruzi, as well as the African trypanosome *Trypanosoma brucei brucei* and *Leishmania mexicana*, has also been reported to contain trypanothione, a spermidine-containing glutathione reductase cofactor (37). Thus, reduction of spermidine biosynthesis by restriction of putrescine via inhibition of arginine decarboxylase most likely would lower the amount of this required cofactor in the parasite. Such a mechanism could affect vital functions of *T. cruzi*, including infectivity and intracellular multiplication.

The absence of arginine decarboxylase in mammalian cells would present this enzyme as a selective target for chemotherapy for *T. cruzi* infection. Whether or not future research supports this hope, the identification of a biochemical event affecting two of the most important biological functions of *T. cruzi* is in itself an exciting new development deserving further attention.

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1. Pan American Health Organization (1982) *Epidemiol. Bull. Pan Am. Health Organ.* **3**, 1–5.
2. Brener, Z. (1973) *Annu. Rev. Microbiol.* **27**, 347–382.
3. Brener, Z. (1979) *Pharmacol. Ther.* **7**, 71–90.
4. Lima, M. F. & Kierszenbaum, F. (1982) *J. Protozool.* **16**, 566–570.
5. Wirth, J. J. & Kierszenbaum, F. (1983) *J. Immunol.* **131**, 3028–3031.
6. Villalta, F. & Kierszenbaum, F. (1985) *Mol. Biochem. Parasitol.* **16**, 1–10.
7. Connelly, M. C. & Kierszenbaum, F. (1985) *Mol. Biochem. Parasitol.* **17**, 191–202.
8. Villalta, F. & Kierszenbaum, F. (1985) *Biochim. Biophys. Acta* **845**, 216–222.
9. Henriquez, D., Piras, R. & Piras, M. M. (1981) *Mol. Biochem. Parasitol.* **2**, 359–366.
10. Crane, M. St. J. & Dvorak, J. A. (1982) *Mol. Biochem. Parasitol.* **5**, 333–341.
11. Osuna, A., Ortega, G., Gamarro, F., Castanys, S. & Mascaro, M. C. (1984) *Int. J. Parasitol.* **14**, 253–257.
12. Colli, W. (1984) *Mem. Inst. Oswaldo Cruz* Suppl., **79**, 45–50.
13. McCann, P. P., Bacchi, C. J., Nathan, H. C. & Sjoerdsma, A. (1983) in *Mechanisms of Drug Action*, eds. Singer, T. P. & Ondarza, R. N. (Academic, New York), pp. 159–173.
14. Bacchi, C. J. (1981) *J. Protozool.* **28**, 20–27.
15. Bitonti, A. J., Bacchi, C. J., McCann, P. P. & Sjoerdsma, A. (1985) *Biochem. Pharmacol.* **34**, 1773–1777.
16. Tabor, C. W. & Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285–306.
17. Pegg, A. E. & Williams-Ashman, H. G. (1981) in *Polyamines in Biology and Medicine*, eds. Morris, D. R. & Marton, L. J. (Dekker, New York), pp. 3–42.
18. Morris, D. R. & Fillingame, R. H. (1974) *Annu. Rev. Biochem.* **43**, 303–325.
19. Kallio, A., McCann, P. P. & Bey, P. (1981) *Biochemistry* **20**, 3163–3166.
20. Ramakrishna, S. & Adiga, P. R. (1975) *Eur. J. Biochem.* **59**, 377–386.
21. Galston, A. W., Dai, Y.-R., Flores, H. E. & Young, N. D. (1983) in *Advances in Polyamine Research*, eds. Bachrach, U., Kaye, A. & Chayen, R. (Raven, New York), Vol. 4, pp. 381–393.
22. Choudhuri, M. M. & Ghosh, B. (1982) *Agric. Biol. Chem.* **46**, 739–743.
23. Smith, T. A. (1983) *Methods Enzymol.* **94**, 176–180.
24. Tabor, C. W. & Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–790.
25. López-Gorgé, J. & Monteoliva, M. (1969) *Rev. Ibér. Parasitol.* **29**, 3–39.
26. Wirth, J. J. & Kierszenbaum, F. (1984) *J. Immunol.* **133**, 460–464.
27. Budzko, D. B. & Kierszenbaum, F. (1974) *J. Parasitol.* **60**, 1037–1038.
28. Mercado, T. I. & Katusha, K. (1979) *Prep. Biochem.* **9**, 97–106.
29. Villalta, F. & Kierszenbaum, F. (1982) *J. Protozool.* **29**, 570–576.
30. Bitonti, A. J., Casara, P. L., McCann, P. P. & Bey, P. (1987) *Biochem. J.* part I, **242**, 69–74.
31. Andrews, N. & Colli, W. (1982) *J. Protozool.* **29**, 264–269.
32. Wu, W. H. & Morris, D. R. (1973) *J. Biol. Chem.* **248**, 1687–1695.
33. Piras, R., Piras, M. M., Henriquez, D. & Negri, S. (1982) *Mol. Biochem. Parasitol.* **6**, 83–92.
34. Birecka, H., Bitonti, A. J. & McCann, P. P. (1985) *Plant. Physiol.* **79**, 509–514.
35. Pegg, A. E. & McCann, P. P. (1982) *Am. J. Physiol.* **243**, C212–C221.
36. Pegg, A. E. (1986) *Biochem. J.* **234**, 249–262.
37. Fairlamb, A. H., Blackburn, P., Ulrich, P., Chait, B. T. & Cerami, A. (1985) *Science* **227**, 1485–1487.