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The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: L-Glutamine inhibits the generation of L-arginine by cultured endothelial cells

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ABSTRACT **The mechanism by which L-glutamine (L-Gln) inhibits the release of endothelium-derived relaxing factor from bovine aortic cultured endothelial cells was investigated. The intracellular concentration of L-arginine (L-Arg) in Arg-depleted endothelial cells was inversely related to the level of L-Gln. Removal of L-Gln from the culture medium (usually containing L-Gln at 2 mM) abolished the inhibitory effect of the culture medium on L-Arg generation. L-Gln (0.2 and 2 mM) but not D-Gln inhibited the generation of L-Arg by both Arg-depleted and nondepleted endothelial cells. L-Gln did not interfere with the uptake of L-Arg or the metabolism of L-Arg-L-Phe to L-Arg but inhibited the formation of L-Arg from L-citrulline (L-Cit), L-Cit-L-Phe, and N^G-monomethyl-L-arginine. L-Gln also inhibited the conversion of L-[^{14}C]Cit to L-[^{14}C]Arg by Arg-depleted endothelial cells. However, L-Gln did not inhibit the conversion of L-argininosuccinic acid to L-Arg by endothelial cell homogenates. Thus, L-Gln interferes with the conversion of L-Cit to L-Arg probably by acting on argininosuccinate synthetase rather than argininosuccinate lyase. L-Gln also inhibited the generation of L-Arg by the monocyte–macrophage cell line J774 but had no effect on the conversion of L-Cit to L-Arg by these cells. As the release of endothelium-derived relaxing factor from cultured and non-cultured endothelial cells is limited by the availability of L-Arg, endogenous L-Gln may play a regulatory role in the biosynthesis of endothelium-derived relaxing factor.**

Vascular endothelial cells (1) and stimulated macrophages (2) generate nitric oxide (NO) or a chemically related molecule (3) through the oxidative metabolism of one of the guanidino nitrogens of the amino acid L-arginine (L-Arg). The release of NO derived from L-Arg accounts for the vasorelaxant properties of endothelium-derived relaxing factor (EDRF) and the cytotoxicity of activated macrophages (1, 4, 5). The availability of L-Arg analogues as NO synthase inhibitors and the unique pharmacologic profile of NO has enabled its biosynthesis from L-Arg to be demonstrated in many cell types including polymorphonuclear leukocytes (6), astrocytes (7), hepatocytes (8), mast cells (9), platelets (10), adrenal (11) and brain (12, 13) tissue(s), and possibly smooth muscle cells (14). Some information is available regarding the metabolism of L-Arg by intact cells or semipurified preparations from endothelial cells (15), cytotoxic macrophages (2), or adrenal (11) or brain (12) tissues, but little is known about the regulation and sources of intracellular L-Arg required for NO biosynthesis.

We have demonstrated (16) that cultured endothelial cells, deprived of extracellular L-Arg for 24 hr, when transferred to Krebs' solution, release increasing quantities of EDRF concomitant with the generation of L-Arg from an intracellular source, implying that the availability of intracellular L-Arg is rate-limiting for EDRF biosynthesis. While investigating the mechanisms by which endothelial cells generate L-Arg (17), we discovered a constituent of the culture medium inhibited both the generation of L-Arg and the release of EDRF (16, 18). This report describes the characterization of this factor, the amino acid L-glutamine (L-Gln), and suggests a biochemical mechanism for its inhibitory action on EDRF biosynthesis.

MATERIALS AND METHODS

Materials. The amino acids, indomethacin, adenosine diphosphate di(monocyclohexylammonium) salt (ADP), and superoxide dismutase from bovine erythrocytes were purchased from Sigma. L-[^{14}C]Arg and L-[^{14}C]citrulline (L-Cit) were obtained from Amersham. Peptides were purchased from Sigma or Bachem. Glyceryl trinitrate (Nitronal; nitroglycerin) was supplied by Lipha Pharmaceuticals Ltd. (West Drayton, U.K.). 9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U-46619) was a generous gift of J. Pike (Upjohn, Kalamazoo, MI). All other reagents and solvents were of the highest commercially available quality from Sigma or BDH.

Cultured Bovine Aortic Endothelial Cells. Bovine aortic endothelial cells (19) were grown on Cytodex 3 microcarrier beads (Pharmacia) in Dulbecco's modified Eagle's medium (DMEM; Flow Laboratories) containing 0.6 mM L-Arg, 4 mM L-Gln, and 10% (vol/vol) fetal calf serum (FCS). Endothelial cells were identified as such by their cobblestone-like appearance when seeded onto Petri dishes and by positive immunostaining for factor VIII. The confluent cells were either used directly or transferred to Eagle's minimum essential medium (MEM) containing 2 mM L-Gln without L-Arg or FCS for 24 hr.

Determination of EDRF Release by Bioassay. Approximately 6×10^7 endothelial cells were packed into a jacketed chromatography column and perfused at 5 ml/min with warmed (37°C) oxygenated (95% O_2/5% CO_2) Krebs' solution containing 5.6 μM indomethacin and superoxide dismutase at 10 units/ml. The Krebs' solution (pH 7.4) consisted of 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.17 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 5.6 mM glucose. The effluent from the column superfused a cascade (20) of four spirally cut rabbit aortic strips (RbAs), which were denuded of endothelium and preconstricted with 30 nM U-46619. The lengths of the RbAs were monitored with auxotonic levers and displayed on a Wantanabe WR3101 recorder. Glyceryl trinitrate was used to calibrate the response of the detector tissues that were electronically adjusted to give similar relaxations to a standard dose.

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Abbreviations: EDRF, endothelium-derived relaxing factor; Cit, citrulline; NO, nitric oxide; FCS, fetal calf serum; RbA, rabbit aortic strips; MeArg, N^G-monomethyl-L-arginine; Argsucc, argininosuccinate.

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HPLC/Fluorescence Detection Analysis of L-Arginine and L-Citrulline Levels in Endothelial Cells. HPLC analysis and quantification of the intracellular amino acid concentrations were performed as described in ref. 17. Briefly, confluent endothelial cells on beads were washed 5–10 times with 9 vol of warmed (37°C) oxygenated (95% O_2/5% CO_2) Krebs' bicarbonate buffer. A portion of the cells (100 μl corresponding to 3.5×10^6 endothelial cells) was directly extracted by adding 5 vol of methanol for determination of basal amino acid levels. The remaining cells (200 μl) were incubated in Krebs' buffer (total volume, 1 ml) in the absence or presence of various amino acids for 60 min at 37°C. Incubations were terminated by transferring 100 μl of the cells into ice-cold methanol (500 μl). The samples were left for 10 min at 0–4°C (to facilitate deproteinization) and centrifuged at 10,000 × *g* for 10 min, and the supernatants were stored at −20°C prior to HPLC analysis.

TLC Analysis of L-[^{14}C]Cit Conversion to L-[^{14}C]Arg by Endothelial Cells. L-[*carbamoyl*-^{14}C]Cit (18.4 μM; specific activity, 54.5 Ci/mol; 1 Ci = 37 GBq) was incubated with Arg-depleted endothelial cells (200 μl) in 1 ml of Krebs' buffer in the absence or presence of L-Gln (200 μM) for 60 min at 37°C. Incubations were terminated by placing 100 μl of the cells into 500 μl of ice-cold methanol. The samples were centrifuged (10,000 × *g* for 10 min) and 50 μl of the supernatant was spotted onto TLC plates (Whatman silica gel 150A). The plates were developed in the solvent system (22) chloroform/methanol/ammonium hydroxide/water, 0.5:4.5:2.0:1.0 (vol/vol), over a distance of 16 cm. After drying, the plates were scanned with an Isomess model IM3000 TLC linear analyzer. The R_f values for ^{14}C-labeled L-Arg, L-ornithine, and L-Cit (0.53, 0.77, and 0.92, respectively) were identical to those of authentic standards.

Preparation of Endothelial Cell Homogenates. Endothelial cells (3 ml) grown in Arg-free MEM for 24 hr were washed 5–10 times with Krebs' buffer (9 vol) and lysed by five cycles of freezing in liquid nitrogen and thawing and further homogenized with a glass–glass homogenizer. Ice-cold Krebs' buffer (1.5 ml) was added and the homogenate was centrifuged for 10 min at 1200 × *g* and 4°C. The supernatant was either used directly or frozen in liquid nitrogen and stored at −20°C.

To assay the conversion of various amino acids to L-Arg, 100 μl of the supernatant was added to Krebs' buffer (total volume, 250 μl) and either extracted directly (0-min sample) with methanol (5 vol) or incubated for 60 min at 37°C. Incubations were terminated by adding 1.25 ml of ice-cold methanol and samples were processed for HPLC analysis as described above.

Cultured J774 Cells. The mouse monocyte–macrophage cell line J774.2 (ECACC No. 85011428) was obtained from the European Collection of Animal Cell Cultures (Salisbury, U.K.), certified to be mycoplasma-free at the time of purchase. The cells were cultured as a semisuspension in DMEM containing 4 mM L-Gln and 10% FCS. After reaching 1–2 × 10^6 cells per ml, the cells were cultured in Arg-free MEM without FCS for 24 hr. They were then washed five times with Krebs' buffer (25 ml) and 1 × 10^6 cells in 100 μl were either directly extracted with 500 μl of methanol or incubated in Krebs' solution (total volume, 1 ml) for 60 min at 37°C. The cells were centrifuged for 2 min at 800 × *g* and resuspended in 100 μl of Krebs' buffer to which methanol (500 μl) was added. Samples were processed for HPLC or TLC analysis as described above.

Statistics. All data are presented as mean ± SEM. The stimulatory or inhibitory effects of added amino acids on intracellular L-Arg generation are expressed as a percentage stimulation or inhibition, relative to the L-Arg levels in 60-min control samples. Data were analyzed for statistical significances by an unpaired Student's *t* test with $P < 0.05$ taken as significant.

RESULTS

L-Gln Inhibits the Release of EDRF from Cultured Endothelial Cells. Fig. 1 depicts the inhibitory effect of L-Gln on the release of EDRF from endothelial cells grown in the presence of L-Arg, as determined by the cascade bioassay technique. Infusions of L-Gln (0.1–2.0 mM) over the assay tissues (OT) did not interfere with the detection of EDRF. However, when infused through the column of endothelial cells (TC), L-Gln significantly reduced the release of EDRF induced by ADP. The inhibition produced by L-Gln was maximal at 0.2 mM (46.5 ± 5.5%; $n = 8$), for L-Gln had no additional inhibitory effect at 2 mM (48.1 ± 7.2%, $n = 3$). L-Gln also caused a graded increase in the tone of the detector tissues, indicative of inhibition of basal EDRF release. The inhibitory effects of L-Gln (0.2 mM) on stimulated and basal EDRF release were fully reversed by coinfusions of L-Arg (0.1 mM; $n = 6$) but not D-Arg (0.1 mM; $n = 3$). L-Gln (0.02–2 mM) exerted a similar inhibitory effect on the release of EDRF from Arg-depleted endothelial cells (18). D-Gln (2 mM) had no effect on EDRF release ($n = 4$). Similarly, L-glutamic acid (L-Glu; 2 mM; $n = 4$) or ammonium chloride (1 mM; $n = 5$), products of the metabolism of L-Gln by endothelial cells (23), did not affect EDRF release.

Amino Acid Analysis. The intracellular concentrations of L-Glu, L-Cit, and L-Arg in Arg-depleted endothelial cells were 1312 ± 408 μM, 14 ± 3 μM, and 14 ± 4 μM, respectively ($n = 3$, Fig. 2). Incubation of the cells in Krebs' buffer for 10, 30, and 60 min initiated a rapid time-dependent decline in L-Gln to 52 ± 24 μM after 60 min. The reduction in L-Gln was associated with a time-dependent increase in L-Arg, which reached maximal levels (163 ± 55 μM) after 30 min. L-Cit levels did not significantly change over the 60-min incubation period.

L-Gln Inhibits the Generation of L-Arg by Arg-Depleted Endothelial Cells. The ability of Arg-depleted endothelial cells to generate L-Arg once placed into physiologic buffers (16) suggests the presence of an inhibitory factor in the culture medium. As seen in Fig. 3, the addition of MEM or FCS (10%) to Arg-depleted endothelial cells incubated in Krebs' buffer inhibited the increase in L-Arg by 97.0 ± 5.3% ($n = 3$) and 94.5 ± 2.6% ($n = 3$). Incubation of Arg-depleted endothelial cells with L-Gln (0.2 and 2 mM), a major constituent of both serum and MEM, inhibited the generation of Arg by 81.5 ± 6.3% ($n = 5$) and 79.7 ± 10.0% ($n = 6$). In contrast, incubations in MEM without L-Gln or in Krebs' buffer containing D-Gln (0.2 and 2 mM) did not decrease L-Arg levels. Indeed, D-Gln and L-asparagine (L-Asn) potentiated the generation of L-Arg by 104.1 ± 33.7% for 0.2 mM D-Gln ($n = 3$), 238.0 ± 73.6% for 2 mM D-Gln ($n = 3$), and 190.2 ± 43.9% for 0.2 mM L-Asn ($n = 3$). L-Glu (2 mM) and ammonium chloride (1 mM) did not inhibit L-Arg synthesis (Fig. 3).

Other Effects of L-Gln. L-Gln (200 μM) also inhibited the increase in L-Arg observed in nondepleted endothelial cells (95.9 ± 2.1% inhibition; $n = 4$; $P < 0.05$), when these cells were transferred to Krebs' solution. There was no change in intracellular ammonia levels (103.8 ± 7.2% compared with a 60-min control; $n = 3$) in the presence of L-Gln (200 μM), but there was a significant increase (156.2 ± 8.8%; $n = 3$; $P < 0.01$) in the release of ammonia, indicating a metabolism of L-Gln to L-glutamic acid and ammonia. Similar changes in ammonia (determined as described in ref. 17) were also observed with Arg-depleted endothelial cells ($n = 3$). L-Gln (200 μM) did not affect the uptake of L-Arg (50 μM) into Arg-depleted cells (93.0% of control L-Arg uptake in endothelial cells incubated with L-Arg plus L-Gln; $n = 2$).

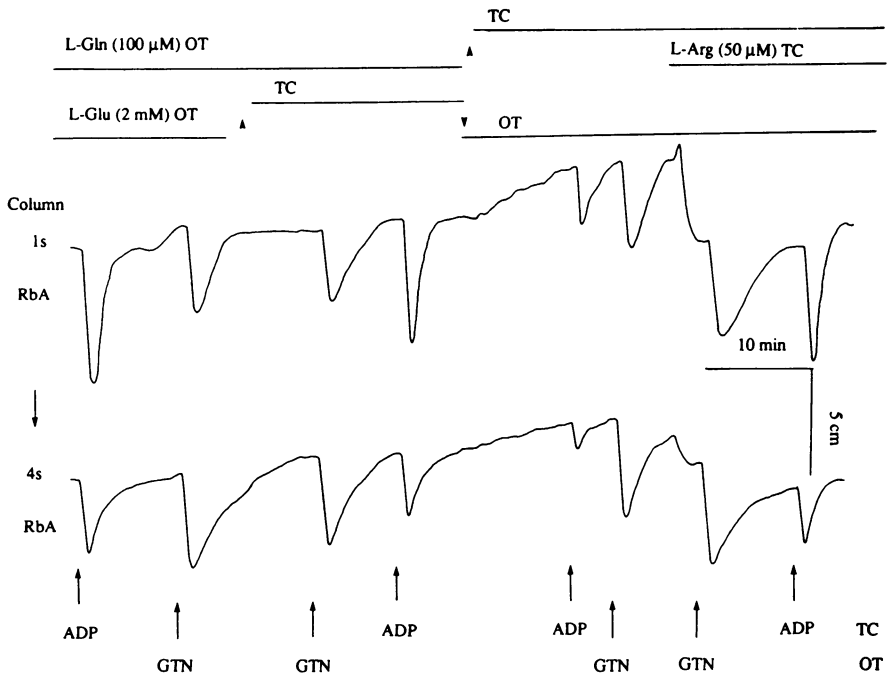

FIG. 1. L-Gln, but not L-Glu, inhibits the release of EDRF from endothelial cells cultured in the presence of L-Arg. The figure shows the responses of a cascade of preconstricted spirally cut RbAs denuded of endothelium. The first two of four RbAs are shown. Injections of ADP (6 nmol) infused through the column of endothelial cells (TC) caused a release of EDRF that relaxed the first RbAs more than glyceryl trinitrate (GTN). Infusions of L-Glu (2 mM) had no effect on EDRF release, whereas infusions of L-Gln (100 μM) caused a graded increase in tone of the RbAs and inhibited the release of EDRF induced by ADP. Infusions of L-Arg (50 μM) returned the tone of the RbAs to basal levels and reversed the inhibition of ADP-induced EDRF release. Similar results were obtained with L-Gln (0.02–2 mM) in 16 experiments. OT, infusions over the assay tissues.

L-Gln Inhibits the Conversion of L-Cit to L-Arg. To examine the possible mechanism(s) by which L-Gln inhibits the generation of L-Arg, we incubated Arg-depleted endothelial cells, in the absence or presence of L-Gln (200 μM), with L-Cit (50 μM) or the dipeptides L-Cit-L-Phe (50 μM) or L-Arg-L-Phe (50 μM). As seen in Fig. 4, L-Cit and L-Cit-L-Phe were converted to L-Arg, increasing the control concentration of L-Arg at 60 min by 189.9 ± 17.1% (n = 5) and 173.3 ± 16.8% (n = 3), respectively. L-Arg-L-Phe was also cleaved to liberate L-Arg (608.9 ± 184.1% of 60 min control; n = 3). Incubation of the cells with L-Gln inhibited the increase in L-Arg observed with L-Cit or L-Cit-L-Phe by 56% (n = 5) and 50% (n = 3), respectively, but did not significantly interfere with the liberation of L-Arg from L-Arg-L-Phe (<7% inhibition; n = 3). The metabolism of N^G-monomethyl-L-arginine (MeArg), an inhibitor of EDRF biosynthesis, which is converted to L-Cit and subsequently L-Arg by endothelial cells (21), was also inhibited by L-Gln (41% inhibition from 308.8 ± 90.9% to 182.6 ± 58.3% of 60 min control; n = 3).

To examine whether L-Gln was interfering with the overall synthesis of L-Arg (Fig. 1) or simply with the conversion of L-Cit to L-Arg, Arg-depleted endothelial cells were incubated, in the absence and presence of L-Gln (200 μM), with L-[^{14}C]Cit for 60 min and the formation of L-[^{14}C]Arg was determined by TLC analysis. Arg-depleted endothelial cells

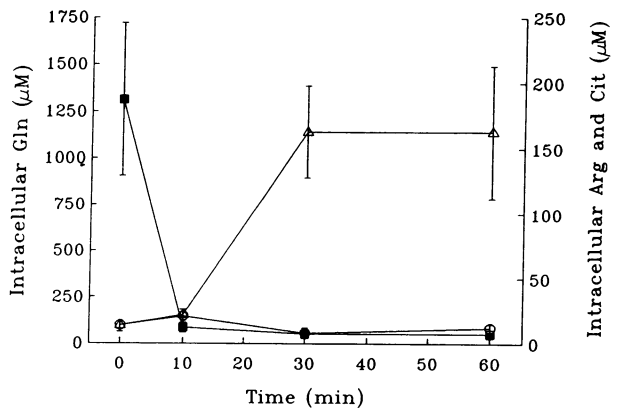

FIG. 2. Time-dependent changes in intracellular L-Arg (open triangles), L-Cit (open circles), and L-Gln (closed squares) in Arg-depleted endothelial cells incubated in Krebs' buffer at 37°C. The figure depicts the mean ± SEM of three experiments with different batches of cultured endothelial cells.

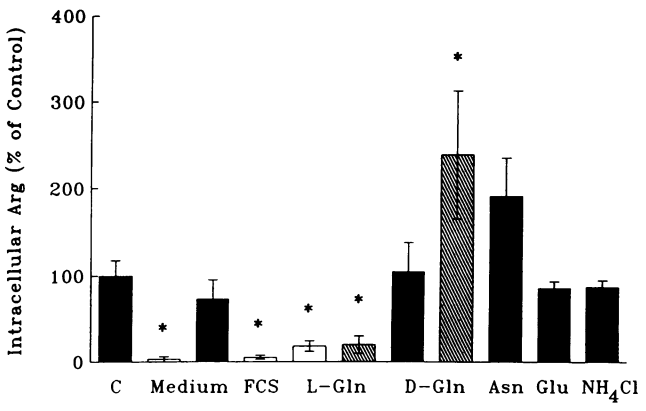

FIG. 3. L-Gln inhibits the generation of L-Arg by Arg-depleted endothelial cells. The figure shows the mean ± SEM of at least three experiments with different batches of cultured endothelial cells. The solid bars depict control experiments (C) or incubations with compounds having no inhibitory effect on L-Arg generation—i.e., MEM (bars Medium) without L-Gln, D-Gln (200 μM), Asn (200 μM), Glu (2 mM), or ammonium chloride (NH_4Cl, 1 mM). The open bars indicate inhibition of L-Arg generation with MEM containing L-Gln, FCS, or L-Gln (200 μM). The hatched bars represent incubations in the presence of 2 mM L-Gln or D-Gln. Values significantly different (P < 0.05) from control (*) are indicated.

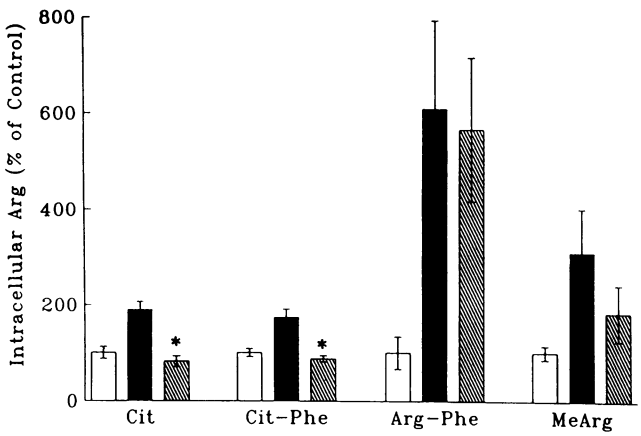

FIG. 4. L-Gln inhibits the conversion of L-Cit, L-Cit-L-Phe, and MeArg but not L-Arg-L-Phe to L-Arg. Arg-depleted endothelial cells were incubated for 60 min at 37°C, in the absence (closed bars) or in the presence (hatched bars) of L-Gln (200 μM), with Cit (50 μM, n = 5), Cit-Phe (50 μM, n = 3), Arg-Phe (50 μM, n = 3), and MeArg (50 μM, n = 3). Data are expressed as % of 60 min control values—i.e., the endogenous generation of L-Arg (open bars). Values significantly different ($P < 0.05$) between incubations with and without L-Gln (*) are indicated.

produced 8.4 ± 2.1% L-[^{14}C]Arg (n = 3) in the absence and 3.4 ± 0.3% L-[^{14}C]Arg (n = 3) in the presence of L-Gln (200 μM), representing a 60% inhibitory effect of L-Gln on the conversion of L-Cit to L-Arg (Fig. 5).

The generation of L-Arg from L-Cit may arise by the formation of L-argininosuccinate (L-Argsucc) as an intermediate (17). Therefore, the effect of L-Gln (200 μM) on the conversion of L-Argsucc (100 μM) to L-Arg was investigated by using endothelial cell homogenates, for L-Argsucc appears to be unable to cross the cell membrane (17). Indeed, L-Argsucc was converted to L-Arg by homogenates (4.6 ± 1.8% conversion after 60 min; n = 5), but this reaction was not inhibited by L-Gln (4.2 ± 1.6% conversion; n = 5).

L-Gln Inhibits the Generation of L-Arg by J774 Cells but not the Conversion of L-Cit to L-Arg. Unstimulated J774 cells also generate L-Arg (2.5 ± 0.6-fold increase over 60 min; n = 3) and this process is inhibited by L-Gln in a dose-dependent manner [50.2 ± 20.6% inhibition (n = 4) and 100.0 ± 2.0% inhibition (n = 3) at 0.2 and 2 mM, respectively]. However, L-Gln did not inhibit the conversion of L-Cit to L-Arg, as the increase in L-Arg in the presence of 50 μM L-Cit was the same with [174.0 ± 36.6% (n = 3) and 177.2 ± 51.9% (n = 3) at 0.2 and 2 mM] or without L-Gln (188.6 ± 6.9%; n = 3). This finding was confirmed by TLC analysis using L-[^{14}C]Cit (18.4 μM). In control incubations, J774 cells formed 14.8 ± 5.7% L-[^{14}C]Arg from [^{14}C]L-Cit (n = 3), a conversion rate that was not significantly different in the presence of L-Gln at 0.2 mM (17.0 ± 6.5%; n = 3) or 2 mM (13.4 ± 7.2%; n = 3). Moreover, J774 cells did not metabolize L-Arg to L-Cit, as analyzed by either HPLC or TLC analysis (n = 3 for each).

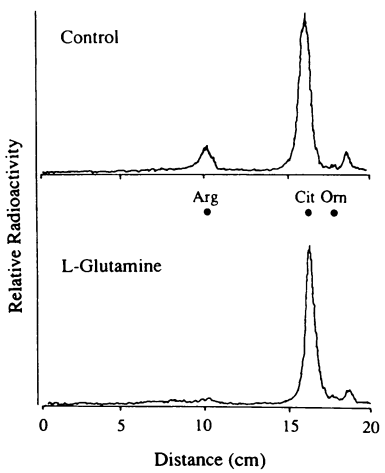

FIG. 5. Conversion of L-[^{14}C]Cit to L-[^{14}C]Arg by Arg-depleted endothelial cells. Samples were applied 3 cm above the bottom of the plate and migrated over a distance of 16 cm. The figure shows a typical TLC analysis of three experiments with different batches of endothelial cells. Control incubation with L-[^{14}C]Cit and the incubation in the presence of L-Gln (200 μM) is shown. The R_f values of authentic ^{14}C-labeled L-Arg, L-Cit, and L-ornithine (Orn) are indicated.

DISCUSSION

This study confirms our hypothesis (16, 18) that L-Gln is the inhibitory principle present in culture medium that prevents the intracellular generation of L-Arg and inhibits the release of EDRF from cultured endothelial cells. This concept is supported by the following findings: (*i*) the intracellular concentration of L-Arg in Arg-depleted endothelial cells is inversely related to the level of L-Gln, (*ii*) culture medium without L-Gln does not inhibit the generation of L-Arg, and (*iii*) incubation of endothelial cells with L-Gln, but not D-Gln, inhibits both the generation of L-Arg and the release of EDRF. Since the release of EDRF is limited by the levels of intracellular L-Arg in cultured (1, 16) and noncultured endothelial cells (24–26), L-Gln may play a regulatory role in EDRF biosynthesis.

Cultured endothelial cells have an unusually high rate of glutaminolysis, thereby generating L-glutamate and ammonia as by-products of L-Gln metabolism (23). The present study supports such a pathway, for endothelial cells treated with L-Gln excrete more ammonia than nontreated cells. However, incubation of endothelial cells with L-glutamate or ammonium chloride did not influence the generation of L-Arg, arguing against these products being inhibitory principles derived from L-Gln. The findings that D-Gln and L-Asn potentiate the time-dependent increase in L-Arg suggests that these structurally related molecules may antagonize the actions of endogenous L-Gln or exert a positive cooperative effect on the pathway(s) responsible for L-Arg generation.

The precise mechanism by which L-Gln inhibits the generation of L-Arg and the release of EDRF is not clear. However, we have found (17) that cultured endothelial cells can generate L-Arg from at least two sources, a protease-sensitive pool and by the metabolism of L-Cit. L-Gln substantially attenuates the time-dependent generation of L-Arg and the conversion of L-Cit, L-Cit-L-Phe, or MeArg to L-Arg but does not interfere with the conversion of L-Arg-L-Phe or L-Argsucc to L-Arg or with the uptake of L-Arg into endothelial cells. The inhibitory action of L-Gln on the conversion of L-Cit to L-Arg is most likely related to a direct allosteric interaction of L-Gln, or a metabolite, with argininosuccinate synthetase (27) or by competitively interfering with the availability of L-Cit, because of their structural similarity. However, the inhibitory effect of L-Gln (200 μM) on the conversion of ^{14}C-labeled L-Cit to L-Arg was only 60%, indicative of an additional inhibitory mechanism of the amino acid on L-Cit metabolism and perhaps on other pathways of L-Arg generation.

This alternative pathway of inhibition by L-Gln is apparent in the monocyte–macrophage cell line J774, for L-Gln inhibits the time-dependent generation of L-Arg but not the conversion of L-Cit to L-Arg. Thus, J774 cells may metabolize L-Cit to L-Arg by a pathway that is either unrelated to that present in endothelial cells or controlled on a different metabolic level. Another possibility is that an isoenzyme of argininosuccinate synthetase exists in J774 cells that is not susceptible

to inhibition by L-Gln. The selective effect of L-Gln on the Arg–Cit cycle (17) in endothelial cells may, therefore, reflect a specific control mechanism for the generation of L-Arg and, hence, the biosynthesis of EDRF. However, the inability of L-Gln to inhibit the conversion of L-Cit to L-Arg by nonstimulated J774 cells may simply reflect the low capacity of J774 cells to generate NO under these conditions (28).

We conclude that in cultured endothelial cells L-Gln inhibits the generation of L-Arg and the release of EDRF, in part by interfering with the recycling of L-Cit to L-Arg during EDRF biosynthesis (17). Moreover, the inhibition of both of these events by L-Gln emphasizes the possibility that the availability of L-Arg is under strict metabolic control and, therefore, rate limiting for the biosynthesis of EDRF.

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