Differential Reconstitution of Mitochondrial Respiratory Chain Activity and Plasma Redox State by Cysteine and Ornithine in a Model of Cancer Cachexia

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ABSTRACT

The mechanism of wasting, as it occurs in malignant diseases and various etiologically unrelated conditions, is still poorly understood. We have, therefore, studied putative cause/effect relationships in a murine model of cancer cachexia, C57BL/6 mice bearing the fibrosarcoma MCA-105. The plasma of these mice showed decreased albumin and increased glutamate levels, which are typically found in practically all catabolic conditions. Skeletal muscles from tumor-bearing mice were found to have an abnormally low mitochondrial respiratory chain activity (mito.RCA) and significantly decreased glutathione (GSH) levels. The increase in mito.RCA was correlated with an increase in the i.m. GSH disulfide/GSH ratio, the plasma cysteine/thiol ratio, and the GSH disulfide/GSH ratio in the bile. This is indicative of a generalized shift in the redox state extending through different body fluids. Treatment of tumor-bearing mice with ornithine, a precursor of the radical scavenger spermine, reversed both the decrease in mito.RCA and the change in the redox state, whereas treatment with cysteine, a GSH precursor, normalized only the redox state. Treatment of normal mice with difluoromethyl-ornithine, a specific inhibitor of ornithine decarboxylase and spermine biosynthesis, inhibited the mito.RCA in the skeletal muscle tissue, thus illustrating the importance of the putrescine/spermine pathway in the maintenance of mito.RCA. Ornithine, cysteine, and α-acetyl-cysteine (NAC) also reconstituted the abnormally low concentrations of the GSH precursor glutamate in the skeletal muscle tissue of tumor-bearing mice. Higher doses, however, enhanced tumor growth and increased the plasma glucose level in normal mice. In the latter, cysteine and NAC also decreased i.m. catalase and GSH peroxidase activities. Taken together, our studies on the effects of ornithine, cysteine, and NAC illuminate some of the mechanistic pathways involved in cachexia and suggest targets for therapeutic intervention.

INTRODUCTION

A common complication in malignant diseases is the massive loss of skeletal muscle mass (cachexia; 1, 2). A similar process is seen in old age (3, 4) and in patients with sepsis, trauma (1, 5, 6), or certain infectious diseases, including HIV infection (7). The mechanism of this phenomenon is still poorly understood. Indirect evidence suggests, however, that the mitochondrial oxidative energy metabolism may be severely compromised in the skeletal muscle tissue relatively early in the catabolic process. A high rate of glycolytic activity and lactate production is commonly seen in the skeletal muscle tissue in practically all catabolic conditions, including cancer (8, 9), burn injuries (10), and sepsis (11). Importantly, it was found even in well-nourished cancer patients, i.e., relatively early in the catabolic process (12). Because the glycolytic metabolism is normally suppressed by ATP generated by the mitochondrial oxidative energy metabolism, the high glycolytic activity suggests that the capacity of the mitochondrial energy metabolism is too weak to meet the cellular demand for ATP. An abnormally low ATP level has been demonstrated already in patients with sepsis (13), and a decreased phosphocreatine level has been found in the skeletal muscle of cachectic and precachectic SIV3-infected macaques (14).

The mitochondrion is known to be exquisitely sensitive against reactive oxygen intermediates (15, 16) but generates superoxide radicals and hydrogen peroxide, especially if its transmembrane potential (i.e., the energy state) is low (17–19). This generates a potentially vicious circle unless contained by protective mechanisms. In addition, mitochondria were found to produce NO (20, 21), which is also potentially damaging for mitochondria (21–24) and has been implicated in another animal model of cachexia (24). It, therefore, makes sense that mitochondria require normally adequate concentrations of antioxidants and radical scavengers, such as GSH (25, 26). Spermine is another important scavenger of ROIs (27) that was found to exert protective effects on mitochondria (28–30). Among other claims, it was claimed that spermine strongly inhibits the induction of mitochondrial nitric oxide synthase (31). Here, therefore, we investigated mito.RCA in the skeletal muscle tissue from tumor-bearing and normal mice and determined the effects of the GSH precursor cysteine, the spermine precursor ornithine, and the NO precursor arginine.

Mice with methylcholantrene-induced transplantable tumors have been used already in earlier studies of cancer cachexia (32, 33). The relevance of our experimental tumor model, the transplantable fibrosarcoma MCA-105 (34), is illustrated, among others, by the fact that even mice with relatively small tumors express characteristic biochemical changes that are typically found in practically all catabolic conditions tested thus far. These changes include, among others, a conspicuous increase in the plasma glutamate level (33), which is similarly found in cancer patients (1, 35, 36), HIV/SIV infection (37, 38), non-insulin-dependent diabetes mellitus (39), amyotrophic lateral sclerosis (40), and old age (39). Even in healthy human subjects, episodes with elevated plasma glutamate levels were significantly correlated with a decrease in body cell mass (41). A study on lung cancer patients revealed that plasma glutamate levels were significantly correlated with mortality (36). Whether the increase in plasma glutamate is merely an epiphenomenon in the wasting process or directly involved in the pathogenetic mechanism remains to be determined. In view of the competitive inhibition of the cysteine transport by the membrane transport system x−c, it has been suggested that elevated plasma glutamate levels may account for (some of the) immunological dysfunctions that are commonly seen in these conditions (35, 38). In addition, the elevated plasma glutamate levels have been shown to be associated with a decreased muscular uptake of glutamate and a corresponding decrease in i.m. glutamate and GSH levels (33, 39, 42).

The biochemical changes in our murine tumor model also include the decrease in the plasma albumin level (see below). This phenomenon is associated with practically all catabolic conditions and has been widely used as a quantitative measure of cachexia (43, 44). The decrease in albumin was found to be strongly correlated with a decrease in body cell mass and with the probability of survival.
The causative role of the albumin level in the mechanism of wasting is still uncertain. The albumin level has, however, an important function in the maintenance of the oncotic pressure and prevention of edema (43–45). A recent study on cancer cachexia and senescence revealed a linkage between the decrease in plasma albumin and the increase in the plasma cystine/acid soluble thiol ratio, an indicator of the redox state (45). Treatment of cancer patients with NAC caused not only a shift in the redox state to more reducing conditions but also a relative increase in plasma albumin and body cell mass (45). The mechanisms responsible for the shift in the plasma redox state in cachexia and senescence remained unknown. We, therefore, studied, among others, the hypothesis that the changes in the plasma redox state and in the plasma glutamate level may be mechanistically related to changes in the mito.RCA of the skeletal muscle tissue.

**MATERIALS AND METHODS**

**Materials.** L-Cysteine, sulfosalicylic acid, and NADPH were purchased from Boehringer Ingelheim (Heidelberg, Germany), DFMO was purchased from Bachem (Heidelberg, Germany), and KCN was purchased from Merck (Darmstadt, Germany). All other chemicals were purchased from Sigma (Munich, Germany).

**Mice.** Female C57BL/6 mice were obtained from the Central Animal Laboratories of the German Cancer Research Center (DKFZ, Heidelberg, Germany). The mice were fed ad libitum with a standard diet and were usually 10–16 weeks old at the start of the experiment. The figures typically show the means of three or more experiments, with four to eight mice per group. The experiments have been approved by the animal committee of the German Cancer Research Center and by the regulatory authorities of the state and were conducted in accordance with the rules and regulations of this country.

**Tumor Growth and Inoculation.** The MCA-105 fibrosarcoma was originally induced in a C57BL/6 mouse by i.m. injection of methylcholantrene (34). Mice were inoculated as described (33) and sacrificed by cervical dislocation 3 weeks after tumor inoculation, when the tumor had a diameter of 1.5 cm. The “tumor volume” (see Fig. 5) was computed by the formula:

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\text{tumor volume} = \frac{0.57 \times (\text{width})^2 \times \text{length}}{3}
\]

**Determination of Amino Acids and Acid-soluble Thiol in Blood Plasma.** The mice were bled by a small cut into the tail vein after a warm-up period under infrared light. Blood was collected in heparinized tubes, and the amino acid concentrations in the plasma were determined with an amino acid analyzer, as described (33). The term “acid-soluble thiol” refers to the amount of...
sulfhydryl groups in the plasma that reacts with 5,5-dithiobis-2-nitrobenzoate after removal of the proteins by precipitation. It was determined by a photometric assay, as described previously (46).

**Processing of Bile.** The bile duct was cannulated with a small steel cannula. To prevent autooxidation of GSH, the bile (10 μl) was immediately added to 490 μl of ice-cold 2.5% sulfosalicylic acid. After precipitation of the protein (10 min; 4°C) and centrifugation (10 min; 12,000 × g; 4°C), the supernatant was assayed for GSH and GSSG, and the corresponding pellet was subjected to protein determination as described (33).

**Determination of Intracellular GSH and Amino Acids in Skeletal Muscle Tissue.** Gastrocnemius and vastus lateralis muscles were prepared separately and dissected into a red section (muscle fiber type 2A) and a white section (type 2B). The red sections were discarded and the white section was stored at −80°C and pulverized in liquid nitrogen. Samples of muscle powder were transferred into 0.4 ml of 2.5% sulfosalicylic acid, subjected to three sonications for 5 min each with the Branson Sonifier W-250, (Branson Inc., Danbury, CT 06813-1961), and kept on ice for at least 20 min. After centrifugation for 10 min at 12,000 × g, the supernatants were used for the determination of amino acids, total GSH, and GSSG, and the corresponding pellet was used to determine the protein content as described (33).

**Enzyme Assays.** Enzyme activities have been determined in pulverized tissue preparations from the white sections of the gastrocnemius and vastus lateralis muscles. Catalase activity was determined as described (47) by measuring the decomposition of hydrogen peroxide at 240 nm. Enzyme activities are expressed as μmol · min⁻¹ · (mg protein)⁻¹. The GSH peroxidase activity was measured as described (48) by oxidation of reduced GSH and subsequent reduction by GSH reductase. The consumption of NADPH was monitored at 340 nm. The assay was performed with a Beckman spectrophotometer at 25°C. Enzyme activities are expressed as nmol · min⁻¹ · (mg protein)⁻¹.

**Isolation of Skeletal Muscle Mitochondria.** Mitochondria were isolated as described (49) with minor modifications. The entire skeletal muscles from hind legs of C57Bl/6 mice were excised and minced with scissors in 7.5 ml of isolation medium (100 mM sucrose, 100 mM Tris-HCl, and 46 mM KCl) supplemented with 10 mM EDTA, 3 mg/ml fatty acid-free BSA, and 0.2 mg/ml protease type XXVII (Sigma, Deisenhofen, Germany). After incubation on ice for 3 min, the samples were homogenized in a Potter homogenizer (seven strokes at 800 rpm), mixed with 7.5 ml of isolation medium, and subjected to centrifugation at 2000 × g for 3 min. The resulting supernatant was again centrifuged at 12,000 × g for 8 min, the supernatant and the upper fluffy layer of the resulting pellet were removed, and the remaining brown tight mitochondrial pellet was resuspended and washed by centrifugation for 3 min at 10,000 × g. The pellet was again resuspended in 0.05 ml of isolation medium, and the concentration of mitochondrial protein was measured by the Bio-Rad protein assay (Bio-Rad Laboratories GmbH, München, Germany). The samples were kept on ice for 4–10 h before assessment of the mito.RCA.

**Determination of Oxidative Phosphorylation.** The mito.RCA was measured as described (50), with minor modifications. Mitochondria corresponding to 125 μg of mitochondrial protein were suspended in 0.75 ml of respiratory medium containing 20 mM Hepes, 250 mM sucrose, 10 mM MgCl₂, 1 mM ADP, and 2 mM KH₂PO₄ (pH 7.1). The oxygen consumption was measured at 37°C using a Clark-type oxygen electrode fitted to a 2-ml water-jacketed closed chamber (Helmut Saur-Laborbedarf, Reutlingen, Germany). After addition of

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Fig. 3. Effect of ornithine and cysteine treatment on mito.RCA and cystine/thiol ratio in tumor-bearing mice. Two weeks after inoculation of the tumor cells, the mice received injections of 0.4 ml of HBSS, containing 0.2 mg of ornithine (+ Orn), 0.125 mg of cysteine (+ Cys), or no additive (W/O) i.p. daily for a total period of 1 week. The mice were then sacrificed and tested for mito.RCA (A), plasma acid-soluble thiol (B), and the cystine/thiol ratio (C). · · · · · · mean levels of non-tumor-bearing mice.
5 mM glutamate, the oxygen consumption was again recorded to determine the complex I activity. To evaluate the activity of complexes II–III, we inhibited the NADH dehydrogenase with 0.1 mM rotenone, and respiration was started again with 5 mM succinate. After inhibition of complex III with 100 nM of antimycin A, 10 mM ascorbate, and 0.2 mM N,N,N,N-tetramethyl-p-phenylenediamine were added to drive electrons directly to cytochrome c (complex IV). The activity of complex IV was then blocked with 0.1 mM KCN to evaluate the KCN-resistant oxygen consumption. The activities were expressed as (nmol O₂)·min⁻¹·(mg protein)⁻¹.

Statistical Analysis. The data from different groups were compared statistically by the Student’s t test for independent samples. Correlations between parameters were described graphically by scatter plots and linear regression lines and were assessed by Pearson’s product correlation coefficient r. A P < 0.05 was regarded as statistically significant.

RESULTS

Decrease in mito.RCA and Changes in Cysteine and GSH Redox States in Tumor-bearing Mice. The analysis of the mito.RCA of skeletal muscle tissue from tumor-bearing mice showed that the activity of complex I was moderately and that of complex II/III and complex IV significantly impaired in comparison with normal mice (Fig. 1A). The decrease in mito.RCA was associated with

Fig. 4. Effect of ornithine and cysteine treatment on intracellular glutamate and GSH levels. A, tumor-bearing mice were treated with ornithine or cysteine as described in legend to Fig. 3. Intracellular glutamate and total GSH levels were determined in the white sections of gastrocnemius and vastus lateralis. The data from these two muscles were similar and have, therefore, been pooled. ••••, mean levels of non-tumor-bearing mice. B, effect of different doses of cysteine. C, effect of NAC. Statistically significant differences between treated and untreated groups are indicated: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Fig. 5. Effect of cysteine, NAC or ornithine on tumor size. Tumor-bearing mice were treated with the indicated daily doses of cysteine, NAC, or ornithine according to the time schedule in the legend to Fig. 3. After 3 weeks, the mice were sacrificed and the tumor volume was determined as described in “Materials and Methods.” Statistically significant differences between the treated and untreated groups are indicated: *, P < 0.05.
a significant decrease in the intracellular total GSH level and a marked increase in the i.m. GSSG/GSH ratio, the plasma cystine/thiol ratio, and the GSSG/GSH ratio of the bile (Fig. 1). In this study, the i.m. GSH and amino acid levels and GSSG/GSH ratios were only determined in white sections (type 2B) of vastus lateralis and gastrocnemius (Fig. 1A). A previous study with the same tumor model showed that total GSH decreases similarly in the red sections of vastus lateralis and gastrocnemius muscles (type 2A) but not in the soleus muscle (type 1) from tumor-bearing mice (33). The plasma cystine/thiol ratio of the individual mouse, an indicator of the individual plasma redox state, was significantly correlated with the intracellular redox state of the GSH redox couple and inversely correlated with the muscular mito.RCA (Fig. 2). In line with earlier findings in cancer patients and elderly healthy subjects (43), the increase in the plasma cystine/thiol ratio in the tumor-bearing mice was also associated with a decrease in the plasma albumin level, which is the major redox buffer in the plasma (398 ± 6 versus 463 ± 4 μM; \( P < 10^{-6} \)). The i.m. concentration of glutamate, one of the three amino acid constituents of GSH, was also significantly decreased in tumor-bearing mice (5.06 ± 0.36 (n = 50) versus 8.63 ± 0.49 (n = 50) nmol/mg protein; \( P < 10^{-6} \)).

Effect of Ornithine and Cysteine Treatment on the mito.RCA and Plasma Cystine/Thiol Ratio. Treatment of tumor-bearing mice with 0.2 mg of ornithine in 0.4 ml of HBSS i.p. daily for a total period of 1 week caused a significant increase in all respiratory chain activities under examination, i.e., complex I, complex II/III, and complex IV (Fig. 3A). This effect was accompanied by a significant increase in the plasma acid-soluble thiol level (Fig. 3B) and a corresponding decrease in the plasma cystine/thiol ratio close to the level of non-tumor-bearing control mice (Fig. 3C). Daily injections of 0.125 mg of cysteine, in contrast, mediated only an increase in plasma thiol and a corresponding decrease in the plasma cystine/thiol ratio of the tumor-bearing mice but had no significant effect on the mito.RCA (Fig. 3).

Effect of Ornithine, Cysteine, and NAC Treatment on Intracellular GSH and Glutamate Levels of Tumor-bearing Mice. Treatment of tumor-bearing mice with ornithine (0.2 mg/day) or cysteine (0.2 mg/day) significantly increased the GSH level in tumor-bearing mice (Fig. 6A). The effect of NAC was less pronounced, and a significant increase in the GSH level was only observed in the red sections of vastus lateralis and gastrocnemius muscles. The i.m. glutamate level was also significantly increased in tumor-bearing mice treated with ornithine or cysteine, whereas the plasma glutamate concentration was increased in tumor-bearing mice treated with ornithine or cysteine but not in mice treated with NAC.

Effect of DFMO on mito.RCA in non-tumor-bearing mice. Normal C57BL/6 mice were treated with 0.4 ml HBSS with or without (W/O) DFMO (20 mg) i.p. daily for a total period of 1 week. The mice were then sacrificed, and the mito.RCA was determined in mitochondria from total muscle tissue from the hind legs.
(0.125 mg/day) was found to not only reverse the intracellular GSH depletion but also reconstitute the intracellular glutamate concentration to practically normal levels (Fig. 4A). The dose requirements for cysteine varied to some extent as illustrated by the different experiments in Fig. 4. NAC showed dose-response curves similar to cysteine (Fig. 4C).

Enhancement of Tumor Growth by High Doses of Ornithine. The dose of 0.2 mg of ornithine per day, which was sufficient to reconstitute the mito.RCA, had only a marginal effect on the tumor growth (Fig. 5). A 100-fold higher dose of ornithine, however, enhanced the tumor size significantly ($P < 0.05$). High doses of cysteine or NAC caused a moderate increase (not significant), and 0.1 mg of NAC per day caused a decrease ($P < 0.05$) in tumor size (Fig. 5). The latter effect may deserve further attention.

Negative Side Effects of Ornithine, Cysteine, and NAC on Normal Mice. Because the insulin receptor tyrosine kinase activation is enhanced by oxidative conditions and, particularly, by hydrogen peroxide (51–53), we studied also the effects of ornithine, cysteine, and NAC on the plasma glucose level. The tumor-bearing mice have, on average, abnormally high plasma glucose levels, indicative of insulin resistance (Fig. 1). Treatment of normal non-tumor-bearing mice with ornithine, cysteine, or NAC for a period of 1 week caused a strong dose-dependent increase in the plasma glucose concentration, almost to the level of the tumor-bearing mice (Fig. 6). The mean plasma glucose level was also significantly increased by small amounts of arginine (0.2 mg/day) in comparison with untreated mice ($13.3 \pm 0.3$ versus $11.7 \pm 0.27$ mM; $P < 0.01$). In addition, treatment with cysteine or NAC was found to decrease the activities of the antioxidative enzymes catalase and GSH peroxidase in the skeletal muscle tissue of normal mice close to the abnormally low levels that are typically found in the tumor-bearing mice (Fig. 7). The catalase activity was also decreased by intermediate doses of ornithine.

Impairment of mito.RCA of Non-Tumor-bearing Mice by DFMO and by Arginine and High Doses of Ornithine. To ensure that ornithine contributes to the maintenance of mito.RCA in the skeletal muscle through the putrescine/spermine pathway, we determined the effect of DFMO, a specific inhibitor of ornithine decarboxylase (see legend to Fig. 8). The results showed that treatment of healthy non-tumor-bearing mice with DFMO caused a significant

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**Fig. 9.** Effect of ornithine and arginine treatment on mito.RCA in non-tumor-bearing mice. Normal C57/BL6 mice received 0.4 ml of HBSS with or without (W/O) the indicated doses of ornithine (Orn) or arginine (Arg) i.p. daily for a total period of 1 week. The mice were then sacrificed and tested for mito.RCA in the skeletal muscle tissue of the hind legs. Statistically significant differences between treated and untreated groups are indicated: *, $P < 0.05$; **, $P < 0.01$.

**Fig. 10.** Pathogenetic mechanisms in catabolic conditions and targets of intervention: a hypothetical model. Solid arrows, transport of metabolites. Dashed arrows, precursor/product relationships or cause/effect relationships. Dotted arrows, possible therapeutic interventions. For other details see text.
decrease of complex II/III activity and a marginal decrease in complex IV activity (Fig. 8).

Daily doses of 0.2 mg of ornithine, which significantly improved the mito.RCA in the skeletal muscle tissue of tumor-bearing mice (Fig. 3A), had no significant effect on the respiratory chain activity of non-tumor-bearing mice (Fig. 9), indicating that the ornithine/polyamine pathway is normally not limiting in healthy mice. High doses of ornithine (20 mg/day), in contrast, decreased the activities of complex II/III and complex IV. A similar effect was seen with relatively low doses of arginine (0.2 mg/day; Fig. 9).

**DISCUSSION**

Earlier studies on wasting have yielded an impressive mosaic of phenomenological information but relatively little information about cause/effect relationships or about targets of therapeutic intervention. Our studies on the effects of ornithine and cysteine unravel some of the causative mechanisms and suggest a link between recent findings from basic research on mitochondria and the mechanisms of wasting. Our key findings are schematically summarized in Fig. 10. The conspicuous changes in the blood plasma of the tumor-bearing mice, including the increase in the glutamate level, the increase in the cysteine/thiol ratio, and the decrease in the albumin level, are similarly seen in practically all catabolic conditions that have been tested thus far (see “Introduction”). This underscores the relevance of our experimental model and suggests that the events in Fig. 10 (shaded area) may be a common scenario in catabolic conditions.

The central finding is the significant impairment of the mito.RCA in the skeletal muscle tissue of the tumor-bearing mice and its attenuation by ornithine. A similar impairment of mitochondrial integrity was found in a tumor necrosis factor-α-induced model of cachexia (24), and a decreased mito.RCA may also explain the abnormally high glycolytic activity and muscular lactate production in catabolic conditions (8–12). The hypothesis of a cause/effect relationship between the decrease in mito.RCA and the change in the GSSG/GSH ratio and the plasma redox state (cysteine/thiol ratio; Fig. 10) is supported by the strong correlation (Fig. 2) and by the fact that the redox state returned to practically normal levels after reconstitution of the mito.RCA by ornithine (Fig. 3C). In addition, this causal relationship is predicted by the facts that mitochondria produce increased amounts of ROI if deenergized (17–19) and that hydrogen peroxide can affect the GSSG/GSH ratio through the GSH peroxidase reaction. A shift to higher plasma cysteine/thiol ratios was previously observed, not only in catabolic conditions such as malignant diseases and old age but also in healthy subjects during episodes of anaerobic physical exercise, i.e., a condition in which the muscular energy demand exceeds mito.RCA and the mitochondrial energy charge is accordingly low (41, 45).

The effect of ornithine on the mito.RCA (Fig. 3A) is reminiscent of earlier reports about anabolic effects of ornithine derivatives in experimental animals and patients with sepsis, burn injury, surgical trauma, and cancer of the gastrointestinal tract (54–57). The mechanism of this effect is not entirely clear but may be explained tentatively by the facts that ROI compromise the mitochondrial integrity and function (15, 16) and that the ornithine derivative spermine exerts protective effects on mitochondria (28–30). This interpretation is also supported by the effect of the ornithine decarboxylase inhibitor DFMO on the mito.RCA in healthy mice (Fig. 8). It should be noted that, in the absence of ornithine treatment, the i.m. ornithine levels from our tumor-bearing mice (n = 50) and healthy control mice (n = 50) showed remarkably little variation (0.98 ± 0.01 and 1.00 ± 0.13 nmol/mg protein, respectively). Recent experiments with another experimental tumor model indicated, however, that the ornithine decarboxylase activity may be inhibited at least partly by an ill-defined nuclear component from tumor cells (58). It has also been reported that spermine is an effective inhibitor of the induction of NO synthase (31), and that NO, in turn, is a strong inhibitor of mitochondrial functions (22, 23). A role of NO in cachexia was suggested recently by studies on a tumor necrosis factor-α-induced murine model of cachexia (24). In this model, skeletal muscle wasting was prevented by treatment with antioxidants or with the NO synthase inhibitor nitro-L-arginine (24).

The intracellular GSH level is strongly dependent on the glutamate level, because GSH competes with glutamate for the glutamate binding site of the γ-glutamyl-cysteine synthetase, the first and rate-limiting step of GSH biosynthesis (59). Substantially decreased i.m. glutamate levels have been found not only in our murine model of cachexia but also in patients with sepsis or trauma (60, 61) and in cachectic and precachectic SIV-infected macaques (42). In the SIV study as well as in our tumor-bearing mice, the decreased glutamate levels were shown to be associated with decreased GSH levels (see Fig. 4A). Studies on different models of cachexia suggest that the decrease in the i.m. glutamate level may result from a decrease in the net glutamate transport across the plasma membrane and is typically associated with a strong increase in the plasma glutamate level (33, 39, 42). Our experiments now showed that treatment with either ornithine or cysteine or the cysteine derivative NAC reconstituted the i.m. glutamate and GSH concentrations to almost normal levels (Fig. 4, A and B). These findings are reminiscent of the recent report that another transport system, i.e., the intestinal d-glucose active transporter, is modulated by changes of the redox status of GSH (62).

Finally, our experiments may also help to identify some negative side effects of ornithine and cysteine. The increase in the plasma glucose level (Fig. 6) is taken as an indication of insulin resistance. This is best explained by the fact that the insulin receptor tyrosine kinase is optimally activated under oxidative conditions and inhibited by radical scavengers (51, 53). The decrease in plasma glucose levels at low doses of NAC may be explained, accordingly, by the decrease in catalase and GPX activity. Whether the effects of ornithine on the tumor growth (Fig. 5) may be relevant for its clinical application remains to be determined.

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