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Possible mechanism by which stress accelerates growth of virally derived tumors

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ABSTRACT **Stress accelerates the growth of certain types of tumors. Here we report a possible metabolic mechanism underlying this phenomenon. Some early features of transformation include increased number of glucose transporters and greatly enhanced rates of glucose uptake; this adaptation accommodates the vast energy demands needed for neoplastic growth. In contrast, glucocorticoids, a class of steroid hormones secreted during stress, inhibit glucose transport in various tissues; this is one route by which circulating glucose concentrations are raised during stress. We reasoned that should transformed cells become resistant to this inhibitory action of glucocorticoids, such cells would gain preferential access to these elevated concentrations of glucose. In agreement with this, we observed that Fujinami sarcoma virus-transformed fibroblasts became resistant to this glucocorticoid action both *in vitro* and in the rat. As a result, under conditions where glucocorticoids exerted catabolic effects upon nontransformed fibroblasts (inhibition of metabolism and ATP concentrations), the opposite occurred in the virally transformed cells. We observe that this glucocorticoid resistance upon transformation cannot be explained by depletion of glucocorticoid receptors; previous studies have suggested that transformation causes an alteration in trafficking of such receptors. Because of this resistance of transformed fibroblasts to the inhibitory effects of glucocorticoids upon glucose transport, glucose stores throughout the body are, in effect, preferentially shunted to such tumors during stress.**

A characteristic of a certain subset of tumors, particularly virally induced ones, is their ability to grow faster in animals undergoing either somatic or psychogenic stress (1–5). Stress can be immunosuppressive through a variety of humoral and neural mechanisms (6, 7), and it has often been theorized that stress-induced acceleration of tumor growth is a result of such immunosuppression (2, 4). However, the mechanistic links between such immunosuppression and accelerated tumor growth remain quite speculative. Here we demonstrate a possible mechanism by which stress accelerates growth in one type of virally induced tumor. Accelerated growth may derive from the metabolic consequences of stress in addition to any speculated immunological consequences.

One of the best characterized physiological reactions to stress is the release of glucocorticoids, a class of steroid hormones, from the adrenal. Glucocorticoids decrease glucose uptake in many peripheral tissues (8), both by sequestering glucose transporters to intracellular storage sites (9, 10) and by decreasing transporter transcription (11). The result of this and other glucocorticoid actions (e.g., enhanced gluconeogenesis) is increased circulating glucose.

In contrast, a characteristic change in transformed cells is a 5- to 7-fold increase in glucose uptake and utilization (12–14). This results from a 5- to 10-fold increase in transcription of the glucose transporter gene (15, 16), producing an equivalent increase in numbers of functional transporters at the cell surface (17). This genomic event is one of the earliest following transformation and is thought to accommodate the vast energy demands needed for neoplastic growth (15, 16).

Thus, virally transformed cells are modified in various and dramatic ways to ensure adequate energy supplies to support their hypermetabolic state. We theorized, in a similar vein, that transformation may also make transformed cells resistant to glucocorticoid-induced inhibition of glucose transport. If this were so, transformation would provide cells with an indirect metabolic advantage by allowing unique access to elevated circulating glucose mobilized from storage tissues during stress. We tested these ideas with Fujinami sarcoma virus (FSV)-infected fibroblasts, chosen because they fit many of the requirements of this study: glucocorticoids inhibit 2-deoxyglucose (dGlc) uptake in fibroblasts (10); glucose transport is increased by transformation in FSV-infected fibroblasts (16); and stress and glucocorticoids can accelerate growth of FSV-induced tumors in rats (5).

MATERIALS AND METHODS

Cell Transformation and Tumor Growth. Studies were performed on wild-type rat fibroblasts (3Y1, American Type Culture Collection) and on fibroblasts that were stably transformed with FSV or a temperature-sensitive FSV strain (ts225). Subcutaneous tumors were grown by injecting 1.5–2.0 $\times 10^7$ FSV-transformed fibroblasts into 300-g male Sprague–Dawley rats. After 2 weeks the cells formed unicentric nonproductive 5- to 10-g tumors at the site of injection.

***In Vitro* dGlc Uptake.** Cells were grown in Dulbecco's modified Eagle's medium (5.5 mM D-glucose) containing 5% fetal bovine serum, L-glutamine (2 mM), streptomycin sulfate (100 μg/ml), and penicillin G (100 units/ml, GIBCO) in a humidified CO_2 incubator at 37°C. Some ts225 cells were maintained at the permissive temperature, 32°C. Cells were washed twice with Krebs–Ringer phosphate buffer containing amino acids and vitamins (GIBCO), L-glutamine (2 mM), D-glucose (5.5 mM), streptomycin, and penicillin at pH 7.4 (KRPB/Glc) and incubated with or without 100 nM dexamethasone for 5 hr at 37°C or 32°C. Cells were washed twice with glucose-free KRPB (37°C or 32°C), and after 10 min of equilibration, sugar uptake was initiated by adding 1 μCi of [^{14}C]dGlc (DuPont/NEN, 304 mCi/mmol, 1 Ci = 37 GBq) and nonradioactive dGlc (50 μM final concentration). After 3 min, uptake was terminated by pouring off the medium and dipping the cells five times in phosphate-buffered saline containing 100 μM phloretin at 0°C. Cells were solubilized in 1% sodium dodecyl sulfate and assayed for protein concentration (18) and for radioactivity (Beckman liquid scintillation

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Abbreviations: FSV, Fujinami sarcoma virus; dGlc, 2-deoxyglucose.
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counter). Uptake was linear for 10 min for all cells (data not shown) and was calculated as dpm/mg of protein.

Whole-Animal dGlc Uptake. Each rat was implanted with a chronic indwelling femoral venous cannula (19). One group of rats was adrenalectomized the next day and maintained on 0.9% NaCl. Another, nonadrenalectomized group was administered the species-typical glucocorticoid of rats, corticosterone (5 mg/day, given subcutaneously in 1 ml of peanut oil). Such a treatment produces sustained increases in circulating corticosterone concentrations to the range seen during major stressors (20). After 3 days of such treatment, rats were given a bolus injection of 2 μCi of [^{14}C]dGlc through the venous cannula. Rats were decapitated 45 min later, and tissue samples were quickly removed and placed in BTS tissue solubilizer (Beckman). Relative glucose uptake was determined by ^{14}C activity as measured in a liquid scintillation counter (Beckman) and normalized to wet tissue weight.

ATP Determinations. Neutralized acid extracts were analyzed for ATP content by the luciferin/luciferase assay procedure (Sigma; ref. 21). Tissues were prepared by growing tumors in rats (see above). Rats were adrenalectomized or treated with corticosterone. At experimentation, rats were anesthetized with urethane (20% solution, Sigma), and 2–3 g of each tissue was removed and quick-frozen in liquid N_2. The order of tissue removal was rotated to control for urethane effects. With a metal mortar and pestle maintained in liquid N_2, each tissue was ground to a powder and samples ($\approx$1.7 mg) of tissue were placed in Eppendorf tubes for extraction and analysis. Values for each tissue sample of each animal were derived from four separately prepared and analyzed tubes. Quantification of the luciferin/luciferase assay was made on an Analytical Luminescence Laboratory (San Diego) Monolight 2010.

Measurements of Rates of ATP Hydrolysis with Silicon Microphysiometry. 3Y1 and FSV cells were maintained in Dulbecco's modified Eagle's medium (5.55 mM glucose; GIBCO) supplemented with 10% fetal bovine serum (GIBCO), and 1% (vol/vol) penicillin/streptomycin solution (GIBCO). Cells were plated 4–7 days prior to the experiment and reached confluence the day before the experiment. Cells were grown on glass coverslips with a conductive indium–tin oxide surface. This coating did not affect cell adherence or viability. Twenty-four hours before experimentation, cells were treated with 1 μM corticosterone. Metabolic rates were measured by silicon microphysiometry (22, 23). Briefly, coverslips containing cells were placed in a low-volume flow chamber, one side of which is a silicon-based light-addressable potentiometric sensor that measured small changes in extracellular medium pH. Such changes are proportional to other indices of cellular metabolism such as lactate production and oxygen consumption (22, 23). The other side of the chamber was the cell-bearing coverslip coated with indium–tin oxide. Cultures were perfused with a low-buffering-capacity (1 mM) medium at 15 μl/min for 150 sec, followed by a 100-sec period of halted flow (controlled by IBM PC interface). Metabolic rate was determined as the rate of acidification of the external medium during the 100-sec halt in perfusion. Medium acidification rates were measured at regions of the silicon/electrolyte interface by using a light-emitting diode. Metabolic rate is presented as μV/sec (22, 23).

Statistics. *In vivo* dGlc uptake data, *in vitro* dGlc uptake comparisons between values in nmol/mg per min, and all ATP data were analyzed by unpaired *t* test. *In vitro* dGlc uptake comparisons between percent changes were analyzed by *Z* test, which tests the amount of association between two ratios by constructing confidence limits on each ratio and assessing their overlap. It was used to determine whether the response to dexamethasone treatment differed in the FSV and 3Y1 cell types. The rate of acidification in the microphysiometer studies was determined as the slope of a linear least-squares fit to the relation of pH vs. time; rates were then compared by two-way ANOVA.

RESULTS

Transformation with FSV made cells resistant to the inhibitory effects of glucocorticoids on transport of the glucose analogue [^{14}C]dGlc *in vitro*. As reported previously (12–16), dGlc uptake was greatly enhanced in transformed cells (Fig. 1*A*). Dexamethasone (a synthetic glucocorticoid) decreased dGlc transport in both untransformed fibroblasts (3Y1 cells) and transformed fibroblasts (FSV cells; $P < 0.001$); however, the magnitude of the effect was significantly smaller in the FSV cells ($P < 0.001$; Fig. 1*C*). We also examined cells transformed with a temperature-sensitive FSV mutant, which show the transformed phenotype at 32°C. Transformation was again associated with enhanced dGlc uptake (Fig. 1*B*). Dexamethasone decreased dGlc uptake at the nonpermissive temperature ($P < 0.001$), but not at the permissive temperature (Fig. 1*D*).

This effect also occurred in the whole animal. [^{14}C]dGlc uptake in FSV-derived tumors was greatly enhanced in glucocorticoid-free rats relative to other tissues (Fig. 2*A*). Glucocorticoids decreased dGlc uptake 35–50% in nontransformed tissues but failed to inhibit dGlc uptake in the FSV-derived tumor (Fig. 2*B*).

Thus, glucose uptake rates in FSV tumors remain enhanced and unhindered (compared with nontransformed tissues) at times when circulating glucose concentrations are likely to be elevated [i.e., during stress (24)]. Therefore, during stress-induced hyperglycemia, such tumors may outcompete nontransformed tissue and gain preferential access to circulating glucose. This idea generated the prediction that under circumstances where glucocorticoids exert catabolic effects on nontransformed tissue, they should exert anabolic effects on FSV tumors.

Glucocorticoids decreased ATP concentrations 46% in nontransformed fibroblasts (rat ear tissue; $P < 0.05$ by unpaired *t* test) but caused a 3-fold increase in ATP concentrations in transformed fibroblasts (FSV tumors; $P < 0.05$,

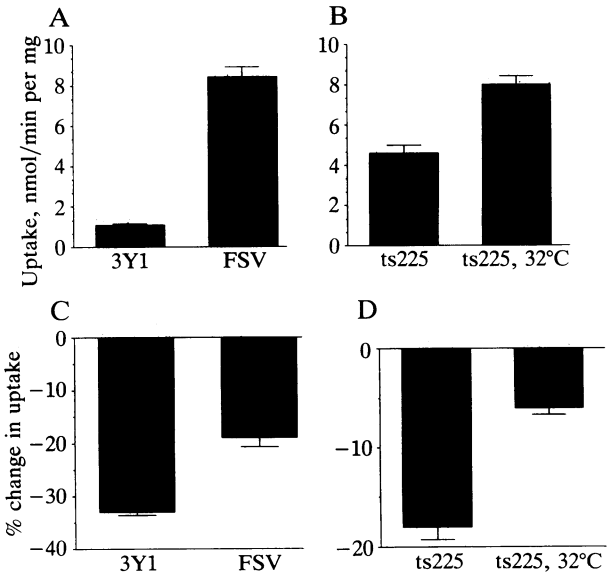

FIG. 1. Glucocorticoids decreased dGlc uptake in cultured wild-type fibroblasts but not in cultured FSV-transformed fibroblasts. Uptake in the various cell lines in the absence of glucocorticoids is shown in *A* and *B* and the percent decrease in uptake in response to dexamethasone treatment is shown in *C* and *D*. $n = 15$ for 3Y1, $n = 6$ for FSV, $n = 12$ for ts225, and $n = 15$ for ts225 at 32°C.

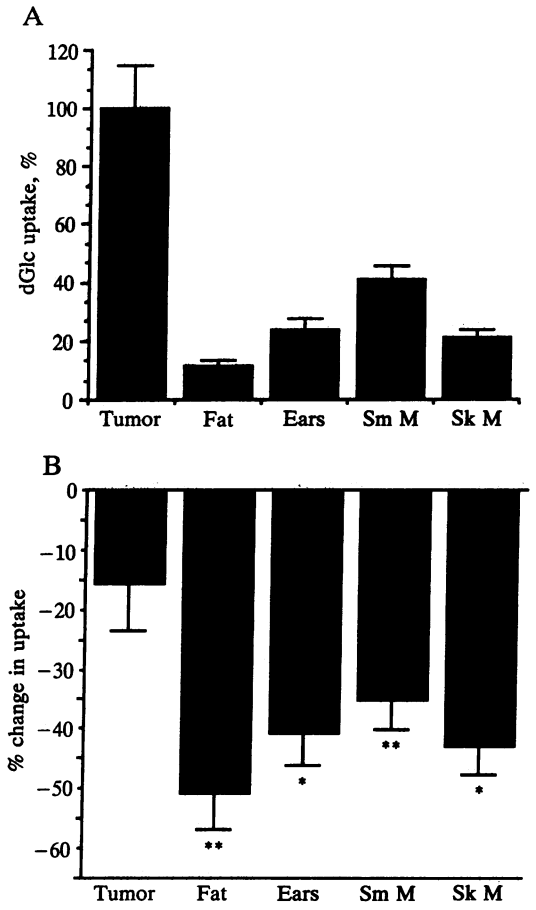

FIG. 2. (*A*) dGlc uptake was greatly enhanced in tumor tissues relative to nontransformed tissues in the adrenalectomized rats. Uptake by tumor was taken as 100%. (*B*) Corticosterone did not affect dGlc uptake by the FSV tumor but inhibited uptake in all other tissues. Fat was taken from a fat pad inside the abdominal cavity. Ears provided a nontransformed source of fibroblasts. Smooth muscle (Sm M) was taken from the stomach wall, and skeletal muscle (Sk M) came from the leg. *, $P < 0.05$; **, $P < 0.02$ for comparisons of corticosterone-treated animals ($n = 10$) with adrenalectomized controls ($n = 13$) for each tissue type.

Fig. 3). In addition, glucocorticoids differentially affected metabolic rate in nontransformed and transformed fibroblasts. Silicon microphysiometry demonstrated that metabolic rate increased with increasing glucose availability in both 3Y1 cells and FSV-transformed cells ($P < 0.001$ and 0.005, respectively) (Fig. 4). Moreover, transformed cells had markedly higher metabolic rates than nontransformed cells (e.g., a >4.5-fold higher rate at 5 mM glucose). This is consonant with the enhanced glucose uptake and utilization seen in transformed cells (Figs. 1 and 2). In addition, higher metabolic demands are a likely explanation for their decreased stores of ATP (Fig. 3). Corticosterone exposure decreased metabolism in 3Y1 cells ($P < 0.001$), but not in FSV-transformed cells ($P < 0.44$, Fig. 4).

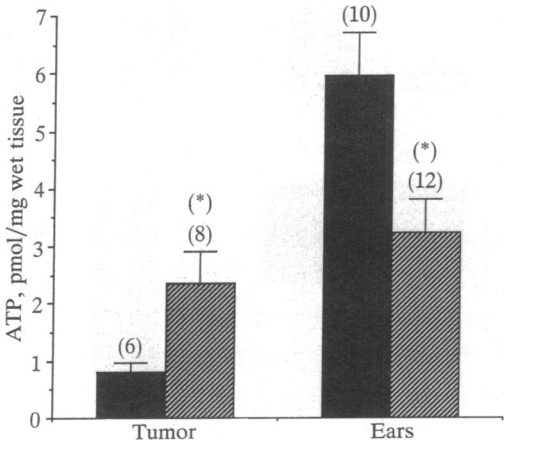

FIG. 3. Glucocorticoids decrease ATP content in rat ears but increase ATP content in FSV tumors. Rats were either adrenalectomized (black bars) or given corticosterone (hatched bars). *n* for each group is shown in parentheses. *, $P < 0.05$ compared with adrenalectomized controls.

DISCUSSION

Stress can accelerate the growth of certain tumors, particularly those that are virally derived (1–5). Speculations concerning the mediating mechanisms for such accelerated growth have concentrated on the inhibition of the immune system by glucocorticoids during stress because (*i*) exogenous glucocorticoids, like stress, can accelerate growth of some tumors (2, 5); (*ii*) glucocorticoids, as part of their general inhibitory effect upon immunity, inhibit immune constituents known to be tumoricidal, including natural killer cells, antibody-dependent killer cells, macrophages, and polymorphonuclear lymphocytes (6, 7); (*iii*) profound immunosuppression can be associated with the enhanced establishment and accelerated growth of tumors (4); and (*iv*) virally derived tumors, the type whose growth is most accelerated

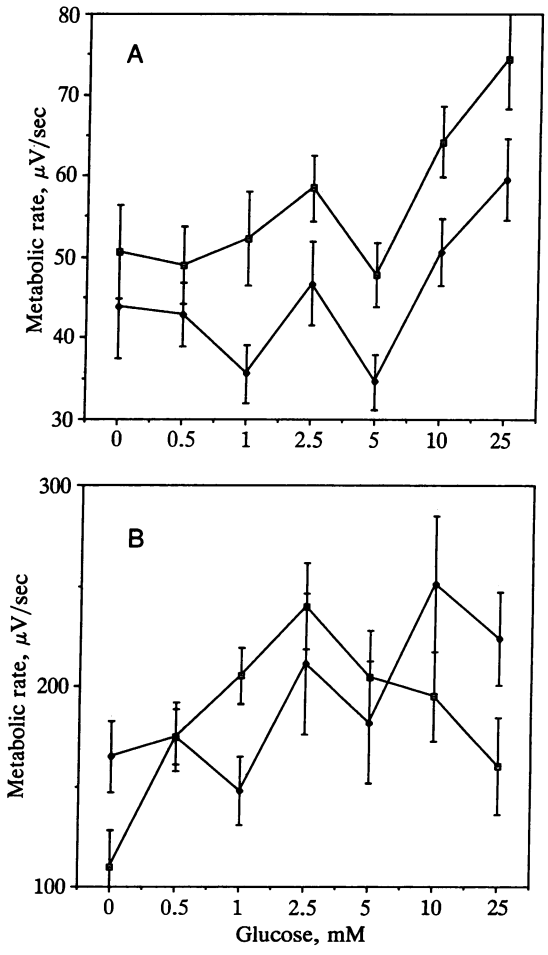

FIG. 4. Glucocorticoids suppress metabolic rate as measured by microphysiometry in 3Y1 fibroblasts (*A*) but not in FSV-transformed fibroblasts (*B*). $n > 10$ per glucose concentration. □, Control; ⊡, corticosterone-treated.

by stress, are also the tumor types most sensitive to immune status (2, 4).

Despite these links, there is little consensus that the effects of stress upon tumor growth are entirely attributable to this route. Other ideas cited include glucocorticoid enhancement of angiogenesis (5), direct glucocorticoid induction of proviral transcription (3), stress-induced opioid inhibition of immunity (3), and the effects of stress upon diet (4). The present study presents evidence for a metabolic mechanism by which stress could accelerate tumor growth. By becoming resistant to the typically inhibitory effects of glucocorticoids on glucose transport, FSV-transformed cells are able to continue to take up glucose at enhanced rates at times when circulating glucose concentrations are elevated. Thus, glucose stores throughout the body are, in effect, preferentially shunted to such tumors during stress.

The mechanisms underlying the resistance of transformed cells to glucocorticoid action are unclear. Glucocorticoids inhibit glucose transport in normal tissues through at least two mechanisms. Within hours, glucocorticoids induce the synthesis of a protein which sequesters glucose transporters from the cell surface to intracellular storage sites (9, 10) and, over days, directly inhibit transcription of the glucose transporter gene (11). Transformation could disrupt either of these pathways at any of a variety of steps, including modification of glucose transporters, masking of genomic glucocorticoid-responsive elements, or depletion of glucocorticoid receptors. There is evidence that transformation disrupts the trafficking of glucocorticoid receptors. Although FSV-derived tumors contain receptors with normal binding affinities [maximal [^{3}H]dexamethasone binding capacity in tumors was 573 ± 49 fmol/mg of protein and binding affinity was 3.55 ± 1.5 nM as measured 12–15 hr after rats were adrenalectomized and assayed by the method of Reul and de Kloet (25)], steroid/receptor complexes in other types of transformed cells translocate to cell nuclei more transiently than normal (26) and are more readily redistributed to the cytosolic compartment in an inactivated form (26, 27). As a result, gene transcription in virally transformed cells becomes relatively glucocorticoid insensitive (28, 29). Glucocorticoid receptor trafficking, however, has not been studied in FSV-transformed cells.

A number of caveats should be voiced with respect to the observations in this study. (*a*) It is not clear whether our observations can be generalized beyond this particular tumor type. Glucocorticoids do not accelerate the growth of all tumors and are even utilized clinically to shrink some types [e.g., carcinoma of the breast (30)]. Should this prove a general metabolic feature of virally transformed cells, it may explain the ability of various stressors to accelerate their growth. (*b*) It is not clear how this particular cellular mechanism fits into the larger physiologic framework of repeated intermittent stressors; depending on the patterning and timing of such stressors, the size of the glucocorticoid stress response can either be enhanced, be diminished, or remain the same (31, 32). Moreover, with repeated stressors, the pattern of secretion of regulatory and counterregulatory metabolic hormones (insulin, glucagon, growth hormone, and catecholamines) will change as well, which will greatly influence the trafficking of energy substrates. Perhaps reflecting this complexity, sustained stress does not uniformly enhance tumor growth (3, 4). (*c*) Finally, our model does not assume that glucocorticoid excess is the sole means of accelerating tumor growth during stress. For example, one notable study showed that uncontrollable, but not controllable, stress accelerated tumor growth, yet the two stressors generally provoke equivalent glucocorticoid stress responses (1) [it should be noted that the tumor used in that study was not viral in origin (33)].

In conclusion, by becoming insensitive to a glucocorticoid signal, FSV-transformed cells can uniquely take advantage of the increased blood glucose levels created by glucocorticoids during stress. Should these observations prove to be generalizable to the effects of stress upon at least some other tumor types *in vivo*, it would suggest a method for intervention: if glucocorticoids preferentially shunt glucose to some transformed cells, then glucocorticoids might be utilized to preferentially shunt glucose-derived toxins, such as dGlc, to such cells as well.

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