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Glucocorticoids Exacerbate the Deleterious Effects of gp120 in Hippocampal and Cortical Explants

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Abstract: Glucocorticoids (GCs), the adrenal steroids secreted during stress, can compromise the ability of hippocampal neurons to survive numerous necrotic insults. We have previously observed that GCs worsen the deleterious effects of gp120, the glycoprotein of the acquired immune deficiency syndrome virus, which can indirectly damage neurons and which is thought to play a role in the neuropathological features of human immunodeficiency virus infection. Specifically, GCs augment gp120-induced calcium mobilization, ATP depletion, decline in mitochondrial potential, and neurotoxicity in fetal monolayer cultures from a number of brain regions. In the present report, we demonstrate a similar gp120/GC synergy in adult hippocampal and cortical explants. We generated explants from rats that were either adrenalectomized, adrenally intact, or intact and treated with corticosterone to produce levels seen in response to major stressors. Metabolic rates in explants were then indirectly assessed with silicon microphysiometry, and cytosolic calcium concentrations were assessed with fura-2 fluorescent microscopy. We observed that basal levels of GCs tonically augment the disruptive effects of gp120 on metabolism in the CA1 cell field of the hippocampus and in the cortex. Moreover, raising GC concentrations into the stress range exacerbated the ability of gp120 to mobilize cytosolic calcium in a number of hippocampal cell fields. Finally, we observed that the synthetic GC prednisone had similarly exacerbating effects on gp120. Thus, GCs can worsen the deleterious effects of gp120 in a system that is more physiologically relevant than the fetal monolayer culture and in a region-specific manner.
Key Words: Glucocorticoids—Glycoprotein gp120—Hippocampal explant—Cortical explant—Human immunodeficiency virus.
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About 20% of cases of human immunodeficiency virus (HIV) infection involve an array of neurological and neuropsychologic impairments, termed acquired immune deficiency syndrome (AIDS)-related dementia complex (ADC) (Masliah et al., 1992*a*,*b*; Gendelman et al., 1994). This appears to involve a complex multistep cascade in which HIV infection of macrophages and microglia results in release of a novel glutamatergic excitotoxin (whose identity remains somewhat controversial; see Giulian et al., 1996) or disruption of the removal of glutamate itself from the synapse, leading to NMDA receptor activation and damaging waves of calcium (Masliah et al., 1996; Lipton, 1998). The HIV envelope glycoprotein gp120 appears to play a role in these damaging effects, as both in vitro and in vivo studies as well as transgenic models demonstrate that gp120 can damage neurons and that it does so via this NMDA receptor- and calcium-dependent cascade (Dreyer et al., 1990; Toggas et al., 1994). The gp120 may also act directly on neurons via chemokine receptors (Lipton, 1998). Although ADC is likely to involve other factors, particularly cytokines and the inflammatory response, gp120 remains important in thinking about the deleterious effects of HIV within the brain.

We have been studying an endocrine modulation of these adverse effects of gp120. Glucocorticoids (GCs), the adrenal steroids released during stress, can worsen the toxicity of excitotoxic, hypoxic–ischemic, and energetic insults to the nervous system. As one possible explanation for the adverse effects of GCs, the steroids are known to decrease glucose uptake into hippocampal neurons and glia, and the resulting mild energy impairment compromises the ability of the hippocampus to contain glutamate, calcium, and oxygen radical levels during insults (for review, see Sapolsky, 1996). We have observed that GCs augment gp120-induced calcium mobilization, lipid peroxidation, and neurotoxicity in primary hippocampal, cortical, and striatal cultures (Brooke et al., 1997; Iyer et al., 1998; Howard et al., 1999). Moreover, GCs worsen the ability of gp120 to suppress ATP concentrations and mitochondrial potential in hip-

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Abbreviations used: ADC, acquired immune deficiency syndrome-related dementia complex; AIDS, acquired immune deficiency syndrome; CORT, corticosterone; DG, dentate gyrus; GC, glucocorticoid; HIV, human immunodeficiency virus.

pocampal cultures (Brooke et al., 1998). These findings raise the possibility that stress can exacerbate the adverse neurological effects of gp120; even more pertinent to HIV infection, this also raises the possibility that the use of synthetic GCs (to control other features of HIV infection, such as *Pneumocystis* pneumonia) can be endangering in the nervous system.

As noted, these findings regarding gp120/GC interactions have been derived from primary cultures, which obviously greatly limit extrapolation of findings to the whole organism. Thus, in the present study, we extend these investigations to hippocampal and cortical slices. Specifically, we examine (1) whether gp120 causes calcium mobilization and disrupts metabolism in such slices, (2) whether there are regional differences within the hippocampus in vulnerability to any such gp120 actions, and (3) whether GCs exacerbate these endpoints. We find evidence for all of the above.

MATERIALS AND METHODS

Buffers/reagents

Earle's balanced salt solution without bicarbonate (GIBCO, Grand Island, NY, U.S.A.) was used for hippocampal dissection and microphysiometry. Most of its buffering capacity was reduced by omission of bicarbonate. Although some buffering capacity remained due to the presence of 1 m*M* sodium phosphate monobasic, sufficient detection of pH changes due to cellular acid excretion was possible (McConnell et al., 1992).

Glycoprotein gp120 (HIVSF2gp120; Austral Biological, Novato, CA, U.S.A.) was dissolved in a phosphate-buffered saline solution containing 1 m*M* EDTA and 1 m*M* EGTA. Aliquots of stock solution were kept at −80°C. Prior to use, stock solution was thawed and diluted to concentrations of either 200 p*M* or 10 n*M*, as indicated.

For slice studies, artificial CSF consisting of 119 m*M* NaCl, 2.5 m*M* KCl, 1.3 m*M* $MgSO_4$, 2.5 m*M* $CaCl_2$, 1.0 m*M* NaH_2PO_4, 26.2 m*M* $NaHCO_3$, and 22 m*M* glucose was used for slicing, fura-2AM incubation, and calcium imaging. Fura-2AM (Molecular Probes, Eugene, OR, U.S.A.) containing 0.01% Pluronic F-127 (Molecular Probes) and a control solution containing equivalent amounts of dimethyl sulfoxide and Pluronic were prepared in artificial CSF and used to incubate slices prior to imaging.

Corticosterone (CORT), the predominant rat GC, and prednisone, a synthetic GC, were obtained from Sigma (St. Louis, MO, U.S.A.). The latter was used as it is often used clinically in humans with HIV infections.

Animal procedure

Male Sprague–Dawley rats (Simonsen; 250–300 g) were divided into three groups: (1) Adrenalectomized (and maintained on 0.9% NaCl); this produces animals with circulating CORT levels below detection (i.e., <0.1 μg/dl). (2) Adrenally intact; the circulating CORT concentrations of these animals fluctuated with a circadian rhythm at <10 μg/dl (Sapolsky et al., 1995). (3) Intact and injected daily with 10 mg of CORT (s.c., in 1 ml of peanut oil); this produced sustained circulating CORT concentrations in the range seen for major stressors (i.e., 20–30 μg/dl; Sapolsky et al., 1995). In indicated experiments, equal amounts of prednisone were substituted for CORT. Three days later, rats were anesthetized (with 10 ml of urethane/kg of body weight) and decapitated. For microphysiometry studies, brains were rapidly removed, divided into cerebral hemispheres, and sliced into 400-μm-thick sections. The hippocampus and cortex were isolated from a single tissue slice, and 1-mm^2 explants were removed from the CA1, CA3, dentate gyrus (DG), and cortical regions (Fig. 1). Explants from the desired hippocampal region from two to three sequential coronal brain slices were often combined to obtain sufficient tissue. For calcium imaging studies, brains were rapidly removed, divided into cerebral hemispheres, and sliced into 200-μm-thick sections.

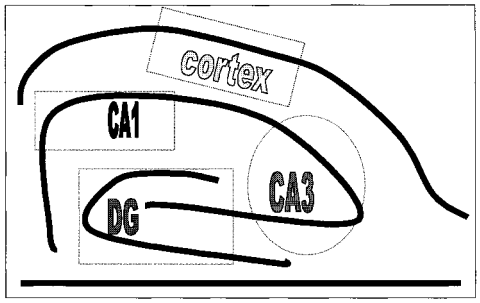

FIG. 1. Cross section of hippocampal tissue slice. Dotted lines indicate the approximate areas microdissected for use with the microphysiometer.

Microphysiometry

The microphysiometer measures the acidification rate in cells in real time as an indicator of metabolic function. As the main products of cellular metabolism are lactic acid and carbon dioxide, the amount of metabolites extruded into the extracellular environment can be used as a measure of cellular metabolism (McConnell et al., 1992). The microphysiometer also provides an advantage over conventional pH-sensitive electrodes. Although capable of measuring pH, the micropipette pH electrode alone would not be sufficient for the reliable measurement of the acidification rate [d(pH)/dt]. The latter is more directly indicative of metabolic activity, as opposed to pH, which is more related to other homeostatic functions; adapting a pH meter with a microfluidics system to accurately measure the rate of change of pH, in effect, constitutes a microphysiometer.

As originally designed, the microphysiometer indirectly measured metabolic rate in monolayer cultures. We provided its initial characterization with neuronal monolayer cultures (Raley-Susman et al., 1992) and subsequently adapted it for use with brain tissue explants (Ajilore and Sapolsky, 1997), its present application. The explant of tissue, sandwiched between two porous membranes to maintain its position, is placed in a square depression at the bottom of a microphysiometer plunger specifically designed for tissue slices. A plastic spacer at the bottom of the plunger stabilizes the membranes to ensure that medium flows directly onto the tissue slice for adequate perfusion. The assembled plunger is placed in the sensor chamber of the microphysiometer where proton efflux is measured. A debubbler–degasser membrane maintains constant temperature and oxygen content within the chamber throughout the experiment, as even small changes in these parameters may affect the microphysiometer's sensitivity. Previous studies have provided a more detailed description of the microphysiometer (McConnell et al., 1992; Trafton et al., 1996; Ajilore and Sapolsky, 1997).

Medium is pumped from a fluid supply for 60 s into the sensor chamber containing the tissue. Medium flow is then

halted for 60 more s, during which time protons accumulate in the extracellular environment. Change in pH (in microvolts) over time (in seconds) is measured by recording the rate at which protons accumulated in the sensor chamber during this static period. As the process of ATP production is tightly coupled with cellular ATP consumption and therefore with the rate of proton extrusion from the cell, acidification rate measurements serve as indirect indicators of a tissue's metabolic rate. In the present study, baseline rates of metabolism were established by averaging the acidification rates during the 30 min prior to switching the fluid supply from the control medium to the sham or gp120 medium. The switch from the control medium to another test tube containing the same control medium was defined as a sham switch, whereas switching from the control to a fluid supply containing the Earle's balanced salt solution medium containing gp120 was considered the experimental exposure. Each tissue slice was exposed, in sequence, to 2 h of control, 1 h of sham, 1 h of control, and 2 h of the gp120-containing medium.

Calcium imaging

Fura-2AM dye imaging techniques allow for real-time determination of calcium concentrations in slices. As fura-2AM reversibly binds to calcium and changes its wavelength of excitation, calcium levels can be measured by comparing the ratio of fluorescent emissions between 340- and 380-nm excitation at any time point.

Immediately after slice production, each slice was placed on a piece of filter paper saturated with either artificial CSF control solution or the aforementioned fura-2AM solution. The container was saturated with 95% O_2/5% CO_2. After a minimum of 90-min incubation, the slices were mounted on the stage of an Olympus inverted microscope in a continuously flowing stream of oxygenated artificial CSF. Emissions from 340- and 380-nm excitation wavelengths were recorded using standard imaging techniques, FLUOR software, and an imaging board from Universal Imaging (West Chester, PA, U.S.A.). Studies were carried out with a 4× objective that allowed us to see over half of the sagittal slice at once, including parts of CA1, CA3, and DG.

As the NADH/NAD ratio, indicative of the redox potential of cells in the slice, has also been found to fluoresce after excitation at 340 and 380 nm, we took steps to eliminate this confounding autofluorescence from the slices by recording from slices that had not been treated with fura-2AM (Brooke et al., 1996). By subtracting signals from the nontreated slices from signals from the fura slices prior to data analysis, levels of free cytosolic calcium could be determined.

After 20 min of settling time, calcium levels were recorded from the slices for 5 min prior to the administration of either gp120 or a continued control solution, after which the imaging continued for 20 min. Images were taken every 20 s. Average intensities from each of the brain areas of interest, CA1, CA3, DG, and cortex, were compared between fura-2AM and control. The integrals of the calcium response were compared between gp120 treatments and brain areas.

Statistical analysis

Data are presented as comparisons of percent metabolic decrease between sham and gp120 exposures for each treatment (CORT treated, untreated, adrenalectomized) and each brain region studied. Data are presented as mean ± SEM values. Statistical significance was assessed using ANOVA and Bonferroni post hoc tests.

RESULTS

In adrenally intact rats, 200 p*M* gp120 failed to significantly mobilize calcium concentrations above baseline in any of the three hippocampal cell fields (Fig. 2). In the cortex, there was a near significant mobilization ($p < 0.07$). Removal of endogenous GCs by adrenalectomy had no effect on gp120 actions in any of the brain regions. In contrast, administration of CORT in amounts that generated high-stress circulating levels of the GC in rats premortem caused a significant exacerbation of gp120 effects in all three hippocampal regions; the effect was particularly dramatic in CA1 and CA3.

We then examined gp120/GC influences on metabolism in explants. There were no significant differences in

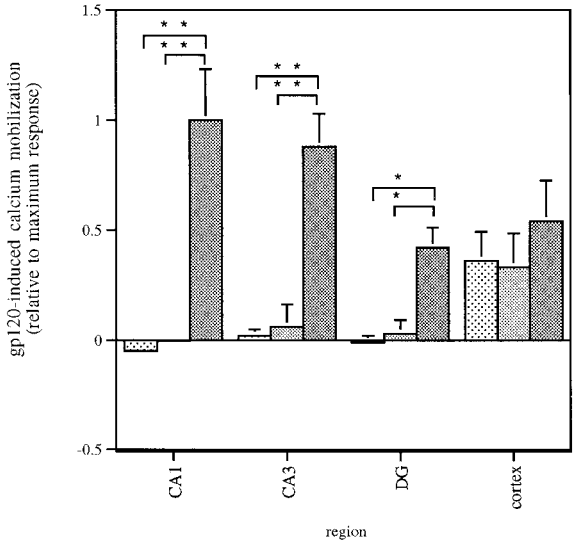

FIG. 2. gp120-induced calcium mobilization in cortical, DG, CA1, and CA3 explants taken from rats that were GC-free due to adrenalectomy (dotted columns; n = 7), adrenally intact (light-hatched columns; n = 6), or administered sufficient CORT to sustainedly raise circulating CORT concentrations into the upper stress range (dark-hatched columns; n = 7). The *y*-axis indicates extent of gp120-induced calcium mobilization above baseline. In the cortex, there was no significant effect of GC status (NS by one-way ANOVA): neither adrenalectomy nor intact values differed from baseline (by unpaired *t* test), whereas values in CORT-treated tissue did ($p < 0.02$). In DG, GC status significantly modulated calcium response ($p < 0.02$ by one-way ANOVA), with CORT-treated rats differing from the other two groups ($p < 0.05$ in both cases by Newman–Keuls post hoc test). In addition, only CORT-treated values differed from pre-gp120 baseline values ($p < 0.05$ by unpaired *t* test). In CA1 explants, GC status significantly modulated calcium response ($p < 0.001$ by one-way ANOVA), with CORT-treated rats differing from the other two groups ($p < 0.01$ in both cases by Newman–Keuls post hoc test). In addition, only CORT-treated values differed from pre-gp120 baseline values ($p < 0.01$ by unpaired *t* test). In CA3, GC status significantly modulated calcium response ($p < 0.001$ by one-way ANOVA), with CORT-treated rats differing from the other two groups ($p < 0.01$ in both cases by Newman–Keuls post hoc test). In addition, only CORT-treated values differed from pre-gp120 baseline values ($p < 0.001$ by unpaired *t* test). * $p < 0.05$, ** $p < 0.01$, by indicated statistical tests.

TABLE 1. *"Basal" metabolic rates in explants*

	GC status ($-\mu$V/s)		
Tissue	Adrenalectomized	Intact	CORT treated
Cortex	435 ± 122	489 ± 111	524 ± 95
DG	541 ± 136	293 ± 60	439 ± 49
CA1	392 ± 129	363 ± 117	421 ± 61
CA3	406 ± 86	531 ± 64	352 ± 188

"Basal" is defined as the average of the microphysiometric determinations from the final 30 min of stabilization prior to introduction of gp120. Neither tissue nor GC status significantly altered metabolic rate (NS, by two-way ANOVA). n = 5/group.

"basal" metabolism (i.e., metabolism during the final 15 min of stable readings prior to the introduction of gp120) as a function of GC status or among explants from different regions (Table 1).

The gp120 and CORT interacted in causing a decline in metabolism in CA1 explants. Low doses of gp120 (200 p*M*) did not decrease metabolism in slices from adrenalectomized animals but did in the presence of normal CORT levels (in intact animals) or high CORT concentrations (Fig. 3). High gp120 concentrations (10 n*M*) suppressed metabolism in all groups, but the effect was more significant in explants from intact or CORT-treated rats. Thus, in CA1 explants, the ability of gp120 to decrease metabolism was worsened by the presence of GCs.

In cortical explants (Fig. 4), low doses of gp120 had no effect on metabolism in explants from adrenalectomized or intact animals but significantly suppressed metabolism in the high-GC milieu of CORT-treated animals. High concentrations of gp120 failed to decrease metabolism in explants from adrenalectomized animals but did so in tissue from intact and CORT-treated animals (with the stronger effect in intact tissue).

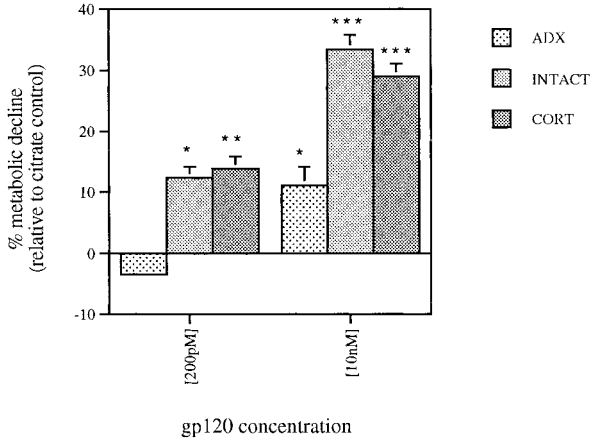

FIG. 3. Effects of two different doses (200 p*M* or 10 n*M*) of gp120 and of adrenal status [adrenalectomized (ADX), intact, or CORT treated] on metabolic rate in CA1 explants, as assessed indirectly by microphysiometry. The *y*-axis indicates the percent decline in metabolism during the 120 min following gp120 exposure. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, by unpaired *t* test comparing rates of decline during those 120 min with decline in cognate tissue from the same rat but exposed to citrate buffer instead. n = 5.

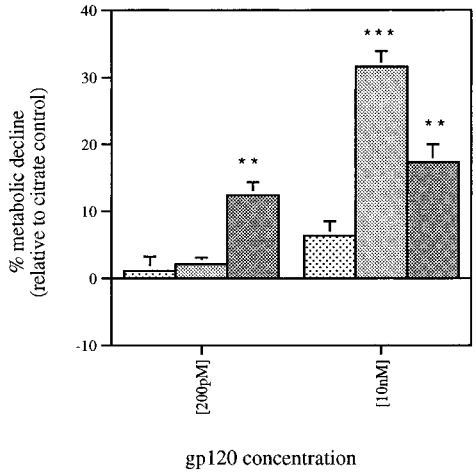

FIG. 4. Effects of two different doses (200 p*M* or 10 n*M*) of gp120 and of adrenal status (adrenalectomized, intact, or CORT treated) on metabolic rate in cortical explants, as assessed indirectly by microphysiometry. The *y*-axis indicates the percent decline in metabolism during the 120 min following gp120 exposure. ** $p < 0.01$, *** $p < 0.001$, by unpaired *t* test comparing rates of decline during those 120 min with decline in cognate tissue from the same rat but exposed to citrate buffer instead. n = 5.

In DG explants, there were no significant changes in metabolism in response to 200 p*M* gp120 under any hormone condition and significant declines in response to 10 n*M* gp120 that were unrelated to CORT exposure (Table 2). In CA3 explants, neither dose of gp120 had a significant effect on metabolism under any GC condition (Table 2).

We then tested whether prednisone also exacerbated the metabolic effects of gp120. This was examined in CA1, the region in which CORT had its strongest modulation of gp120 effects on metabolism. Prednisone, when administered in equimolar concentrations as was CORT in these experiments, significantly worsened the gp120 effect (Fig. 5); its potency was equivalent to that of CORT at the lower gp120 dose but was significantly more potent at 10 n*M* gp120.

DISCUSSION

The ability of gp120 to indirectly damage neurons has been considered to be relevant to ADC. Recent literature suggests that GCs can worsen the neurotoxic effects of gp120, a finding with potential clinical implications (Brooke et al., 1997, 1998; Limoges et al., 1997; Iyer et al., 1998; Howard et al., 1999). To date, the majority of such studies have been derived from work with fetal monolayer cultures. The present report extends those findings to adult cortical and hippocampal slices, making

TABLE 2. *Effects of gp120 and GCs on metabolic rate*

	gp120 concentration	
	200 p*M*	10 n*M*
DG		
Adrenalectomized (n = 4)	6.7 ± 1.1	15.4 ± 3.3[a]
Intact (n = 6)	7.3 ± 3.2	28.7 ± 2.1[b]
CORT treated (n = 5)	−3 ± 2.2	13.2 ± 1[a]
CA3		
Adrenalectomized (n = 5)	2.1 ± 0.6	5.4 ± 1
Intact (n = 5)	9.8 ± 1	7.3 ± 1.8
CORT treated (n = 6)	4.1 ± 1	5.2 ± 1.4

Experimental conditions and statistical analyses were as in Figs. 3 and 4. Values indicate the percentage decline in metabolism versus citrate controls from cognate tissue at the same time.

[a] $p < 0.05$, [b] $p < 0.01$, by unpaired *t* test comparing average metabolic rate during the 120 min following gp120 exposure with the rate in cognate tissue from the same rat but exposed to citrate buffer rather than gp120.

the gp120/GC interactions more physiologically relevant and providing anatomical specificity to the interactions unavailable in monolayer cultures. Specifically, we observe that (1) gp120 can mobilize cytosolic calcium and cause a significant decline in metabolic rate in brain tissue, (2) these effects did not occur uniformly throughout the hippocampus, and (3) these gp120 effects can be exacerbated by both endogenous and synthetic GCs. Before discussing these findings in more detail, it is necessary to consider some technical issues, because it is relatively rare for calcium imaging to be carried out in tissue slices (rather than monolayer cultures) and microphysiometry, in general, remains a fairly novel technique in neuroscience.

There are a number of confounds in the use of microphysiometry, related to the fact that metabolic rate is measured only indirectly. These concern circumstances in which there is a marked change in metabolic demand rather than in substrate availability, where there is a specific defect in the mechanism for proton extrusion, where there has been a dramatic shift from oxidative phosphorylation to glycolysis, or when the extracellular buffering conditions are insufficient to prevent a steep extracellular proton gradient from forming (McConnell et al., 1992; Raley-Susman et al., 1992; Brooke et al., 1996). None of these conditions appears relevant to the present study.

An additional confound must be discussed with respect to its use in tissue explants rather than monolayer cultures. Because the signal detected with the microphysiometer is an aggregate measure from the entire explant, one cannot be certain of the extent of glial contribution to the signal or of the extent to which there is unequal input from cells on the surface of the implant versus those deep inside. However, our prior studies suggest relatively little glial input in hippocampal explants and that the spatial confound can be controlled for so long as explants are roughly the same thickness (Ajilore and Sapolsky, 1997).

The imaging of cytosolic calcium concentrations with calcium-sensitive dyes is quite routine in monolayer cultures. Similar measurements in slices, however, are confounded by a significant and variable degree of autofluorescence at the wavelengths used for calcium determination. Such autofluorescence is due primarily to the NADH molecule; it and other pyridine nucleotides have emission and excitation wavelengths that overlap with the fura salt, and changes in this autofluorescent signal can arise from changes in the ratio of NAD to NADH. This can be controlled for, however, by running fura-free control slices under the same conditions and subtracting their signal (due entirely to nucleotide autofluorescence) from the total signal detected in fura-treated slices (Brooke et al., 1996). This was done in the present study. In addition, slices from different animals differ considerably in the amount of fura-2 that they absorb, leading to highly variable basal readings. As a result, the gain between the camera and the computer often has to be amplified to obtain a detectable basal signal. For that reason, it is not meaningful to present "basal" data on such slices, making it impossible to tell whether GCs alter basal values or if they differ significantly among various cell fields.

As a final technical issue, gp120 effects in monolayer studies have typically been demonstrated with 200 p*M* glycoprotein. Whereas we observed some effects at this dose in slices, most of our results were obtained with 50-fold higher concentrations of gp120. Although it is difficult to assess what would be a "physiological" extracellular concentration of gp120 in the HIV-infected human brain (Lipton, 1998), far higher concentrations of compounds are typically needed to permeate slices than

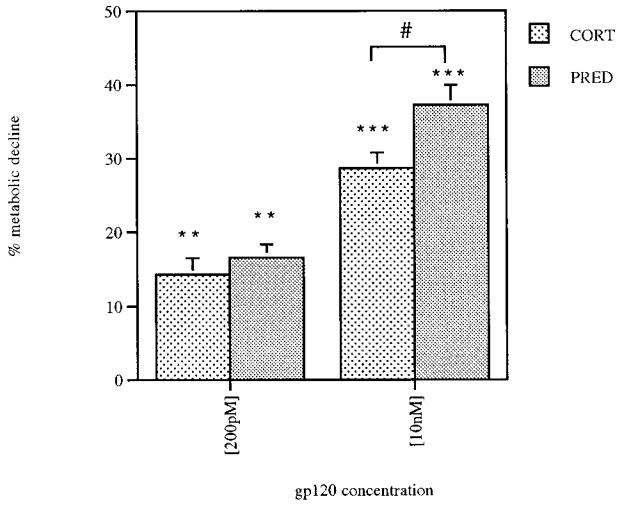

FIG. 5. Comparison of the effects of CORT and of prednisone (PRED) on gp120-induced declines in metabolism in CA1 explants. CORT data were identical to those in Fig. 3. ** $p < 0.05$, *** $p < 0.01$, by unpaired *t* test comparing average metabolic rate during the 120 min following gp120 exposure with the rate in cognate tissue from the same rat but exposed to citrate buffer rather than gp120. # $p < 0.05$ when comparing CORT and prednisone at 10 n*M* gp120 by unpaired *t* test. n = 4–6.

would be efficacious in monolayer studies. For example, a 1,000-fold higher concentration of kainic acid is needed to elicit a microphysiometric response in a hippocampal slice than in a hippocampal monolayer culture (Ajilore and Sapolsky, 1997).

We now can consider the findings in this study. The first concerns the effects of gp120 alone. Its ability to mobilize cytosolic calcium and to disrupt metabolism in slices supports current understanding of the cascade of neurodegeneration that it causes. As a novel finding, there was anatomical specificity to these effects. The glycoprotein at 200 p*M* failed to mobilize calcium in any region tested in adrenally intact animals, although there was near significant mobilization ($p < 0.07$) in the cortex. In addition, 10 n*M* gp120 disrupted metabolism in CA1, DG, and cortical slices, but not CA3 slices, from intact animals. No prior study has tested for selective vulnerability of specific hippocampal cell fields to gp120, but the sparse literature regarding selective neuronal vulnerability to immunosuppressive viruses has been inconclusive. Infection of rhesus monkeys with simian immunodeficiency virus results in neuronal atrophy to an equal extent throughout the hippocampus (Luthert et al., 1995). In contrast, neuron loss in HIV-infected human hippocampus occurs preferentially in CA3 interneurons (Masliah et al., 1992*b*). Such selectivity could arise from regional differences in numbers of NMDA receptors, of chemokine receptors, in efficacy of calcium sequestering, and so on (Lavi et al., 1998; Lipton, 1998).

The other finding of this report concerns the GC exacerbation of gp120's ability to mobilize cytosolic calcium and to disrupt metabolism. As noted, GCs increase the neurotoxicity of excitotoxic and energetic insults in the hippocampus, cortex, and, to a lesser extent, elsewhere in the brain. A key feature of this "endangerment" is that GCs decrease glucose uptake into cultured hippocampal neurons and glia (Horner et al., 1990; Virgin et al., 1991) and decrease glucose utilization throughout the brain (Kadekaro et al., 1988; Freo et al., 1992; Doyle et al., 1994). Although the magnitude of this effect is on the order of ~25% (versus, e.g., a 75% inhibition of glucose transport by GCs in fat cells), this appears to leave cells metabolically vulnerable. As evidence, during insults, GCs worsen the decline in ATP concentrations in hippocampal neurons and glia (Tombaugh and Sapolsky, 1992; Lawrence and Sapolsky, 1994). As a result, the neurons are less able to afford the costly task of containing the consequences of an excitotoxic or metabolic insult. Thus, during such insults, GCs exacerbate insult-induced accumulation of extracellular glutamate, of cytosolic calcium, and of calcium-dependent degenerative events; as evidence of the energetic roots of these GC actions, energy supplementation can reverse the exacerbative effects of GCs (for review, see Sapolsky, 1996).

The gp120/GC interactions reported previously and in the present article readily fit into this framework. Insofar as gp120 indirectly leads to NMDA receptor activation, cytosolic calcium mobilization, and calcium-dependent degenerative events such as oxidative damage, it is working through the same excitotoxic pathway that is GC sensitive. Furthermore, such a pathway is likely both to deplete neurons of energy and to be in turn augmented by energy depletion (Beal, 1992; Turski and Turski, 1993) [and, as evidence, gp120 disrupts glucose utilization (Kimes et al., 1991) and augments the neurotoxicity of hypoglycemia (Barks and Liu, 1997)]. As such, it is not surprising that GCs should augment gp120's inhibition of ATP concentrations and mitochondrial potential in monolayer cultures (Brooke et al., 1998) and of metabolism in brain slices.

There was also anatomical specificity to gp120/GC interactions. Whereas GCs worsened gp120-induced calcium mobilization throughout the hippocampus, the effect was particularly pronounced in CA1 and CA3 explants. In addition, the GC modulation of gp120 effects on metabolism did not occur in CA3 explants. The basis for this is not understood, as all regions of the hippocampus have ample quantities of corticosteroid receptors (McEwen et al., 1986) and GCs have been shown to have the potential to worsen the toxicity of insults in all hippocampal cell fields (Sapolsky, 1996).

The use of slices, rather than fetal monolayers, made it possible to manipulate GC levels in adult animals prior to generating slices. We observed that the transition from a GC-free milieu (i.e., adrenalectomized rats) to levels in the basal, nonstressed range (i.e., intact rats) worsened the effects of high-dose gp120 on metabolism in CA1 and cortex. The transition from nonstressed GC values to stressed (i.e., CORT-treated rats) worsened gp120's mobilization of calcium in all regions. However, that transition did not worsen the effects on metabolism; in fact, in both DG and cortical explants, intact levels of CORT had a more exacerbative effect on gp120-induced metabolic declines than did stress levels of CORT. The reason for that reversal is not clear. However, the fact that the exacerbative effects of CORT occurred basally for the metabolic endpoints, but at stress levels for the calcium mobilization, suggests a greater vulnerability of the metabolic endpoints to the adverse effects of these steroids.

Of even greater clinical relevance are the effects of synthetic GCs. We previously observed that both prednisone and dexamethasone worsen gp120-induced calcium mobilization and neurotoxicity in cultured neurons (Brooke et al., 1997) and now observe that prednisone also worsened the metabolic effects of gp120 in CA1 explants, and to an even greater extent than did CORT at the higher gp120 dose. As a likely explanation for this, there are plentiful amounts of two corticosteroid receptors in the hippocampus (mineralocorticoid receptors, predominately occupied under basal circumstances, and GC receptors, predominately occupied during stress), with the latter mediating the adverse effects of GCs and the former mediating some protective effects (McEwen et al., 1986). CORT binds to both receptors, whereas the synthetic GCs under discussion bind only to GC receptors. One of the most serious consequences of HIV

infection comprises the frequent cases of *Pneumocystis carinii* pneumonia, and the current treatment of choice for severe cases involves administration of megadoses of synthetic GCs such as those used in this study (e.g., Gagnon et al., 1990). The potently endangering effects of what are probably considerably lower concentrations of prednisone in this study suggest that this route of exposure to elevated GC concentrations might have adverse neurological consequences for AIDS patients.

In conclusion, both synthetic and endogenous GCs worsen a number of the adverse effects of gp120 in both cortical and hippocampal slices. Although this represents a more physiological preparation than the monolayer culture, it must be reemphasized that extrapolation must be done cautiously from a brain slice to the brain and from the adverse effects of gp120 itself to the adverse effects of HIV. This is particularly the case because we have used a gp120 form/strain derived from a non-macrophage-tropic isolate that is not normally found in the nervous system. Of note, however, it has recently been reported that GCs worsen the neuron loss seen after inoculation of immunodeficient mice with HIV-infected human monocytes (Limoges et al., 1997), thus demonstrating that this phenomenon is relevant to both HIV and the whole brain. This suggests that further study is warranted to examine both the mechanisms underlying the endangering effects of GCs as well as whether this is relevant to the progression of the neuropathologic features of HIV infection in the human.

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