Endocrine, *vol. 8, no. 2, 135–141, April 1998* 0969–711X/98/8:135–141/$9.75

Glucocorticoids Regulate Protein Synthesis in Hippocampal Slices Under Mild Heat Shock Conditions

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Glucocorticoid hormones potentiate the toxic effects of neuronal stressors. Alteration of gene expression by glucocorticoids could contribute to neuronal susceptibility by downregulating the synthesis of proteins necessary to adapt to challenge. Using heat shock of hippocampal slices as a model for cellular insult, protein synthesis has been examined in response to acute glucocorticoid administration to rats. Incubation of hippocampal slices at 39°C produces a heat-shock pattern of protein synthesis in that total incorporation of labeled amino acid is diminished, whereas synthesis of the major heat-shock proteins, HSP90 and HSP70, is increased. Prior administration of corticosterone to rats does not affect subsequent synthesis of HSP90 or HSP70 in slices. However, at 4 or 24 h following a single corticosterone injection, the synthesis of two acidic proteins is found to be altered: a 25-kDa protein is downregulated in the nuclear and synaptosomal-mitochondrial fraction of the hippocampus, and a 47-kDa protein is downregulated in all three fractions of the hippocampus, cortex, and cerebellum. These effects are mimicked by administration of RU-28362, a specific glucocorticoid (GR or Type II) receptor agonist. Since decreased synthesis of p25 and p47 is the only glucocorticoid-mediated response observed in slices under heat-shock conditions, these proteins may be related to the adaptation to heat shock.

Key Words: Glucocorticoids; corticosterone; heat shock; protein synthesis; hippocampus; aging.

Introduction

Corticosteroids are hormones that are secreted as part of the adaptive response to stress *(1)*. In the rat, basal secretion of glucocorticoids is necessary for the survival of hippocampal granule cells *(2)*, but cumulative exposure to corticosteroid excess, occurring concomitant with age or as a consequence of chronic stress, results in neurodegenerative pyramidal cell loss by placing cells in "endangerment," a state in which they become more susceptible to metabolic insult *(3)*. Consequently, a cellular insult, such as hyperthermia, ischemia, or seizure, may be more likely to result in the loss of cells when preceded by an increase in adrenal secretion.

Corticosteroids bind to high affinity, mineralocorticoid receptors (MR or Type I) or to low-affinity, glucocorticoid receptors (GR or Type II), which act as transcription factors at response elements, alone or in combination with other factors, to modulate gene expression in both positive and negative directions *(4)*. Mineralocorticoid receptors are constitutively occupied, whereas low-affinity, glucocorticoid receptors are predominantly occupied during periods of stress and, to a lesser extent, during the circadian peak in corticosteroid secretion *(5)*. The marked expression of both receptor types by neurons within the hippocampal formation causes this region of the brain to be sensitive to changes across the physiological range of glucocorticoid secretion *(6)*.

Although endangerment of neurons reflects a degree of energy depletion *(7)*, it may also correlate with corticosteroid-mediated alterations in gene expression *(8)*. In order to characterize the endangered state of glucocorticoid-sensitive neurons on a molecular level, the effects of steroid administration on the synthesis of hippocampal proteins that are potentially involved in the adaptive response to cellular stressors have been investigated. Corticosteroid-induced diminution of the synthesis of such proteins could interfere with the ability of neurons to survive the trauma of metabolic insult and could ultimately lead to the loss of neurons.

Heat-inducible proteins serve a protective function when cells are presented with thermal challenge. Because many of these proteins are known to be synthesized in response to other cellular stressors as well *(9–13)*, we have chosen to characterize more fully the glucocorticoid sensitivity of such proteins by using mild heat shock as a model for cellular insult. Since the hippocampus, when compared to brain regions, such as the cerebellum and cerebral cortex, is known to be particularly susceptible to increases in

Received September 19, 1997; Revised December 24, 1997; Accepted January 9, 1998.

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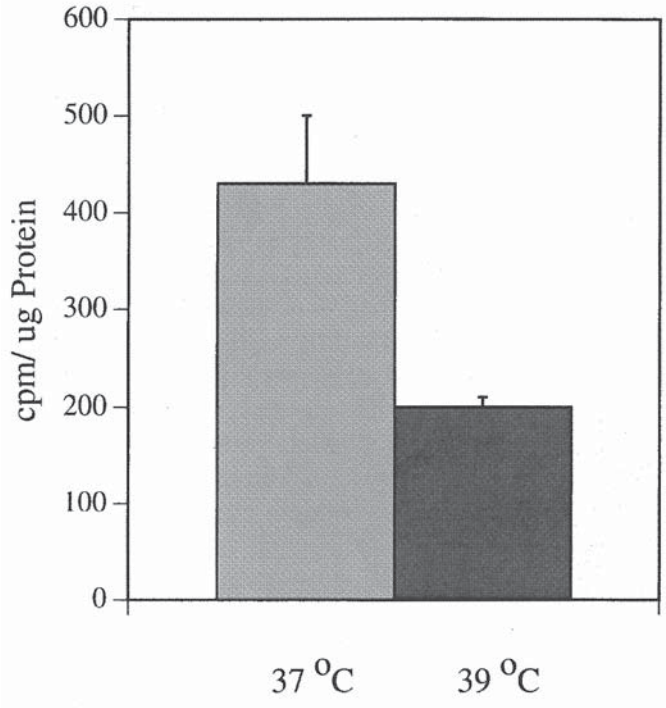
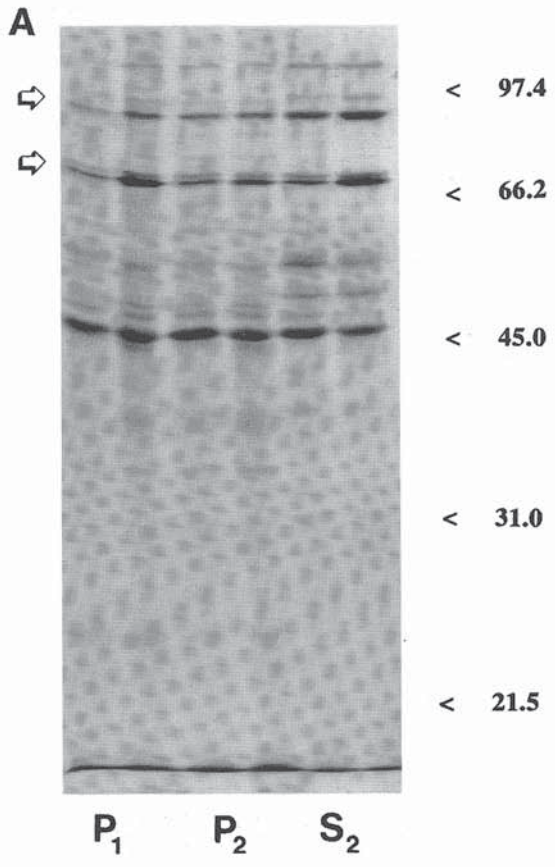


Fig. 1. Comparison of total protein synthesis in hippocampal slices at either 37°C or 39°C. Incorporation of [^{35}S]-methionine-cysteine into proteins synthesized in the P_1 fraction of hippocampal slices incubated at either 37 or 39°C was measured and normalized to the protein content of each fraction. The results shown are the mean ± SEM from 4 experiments.

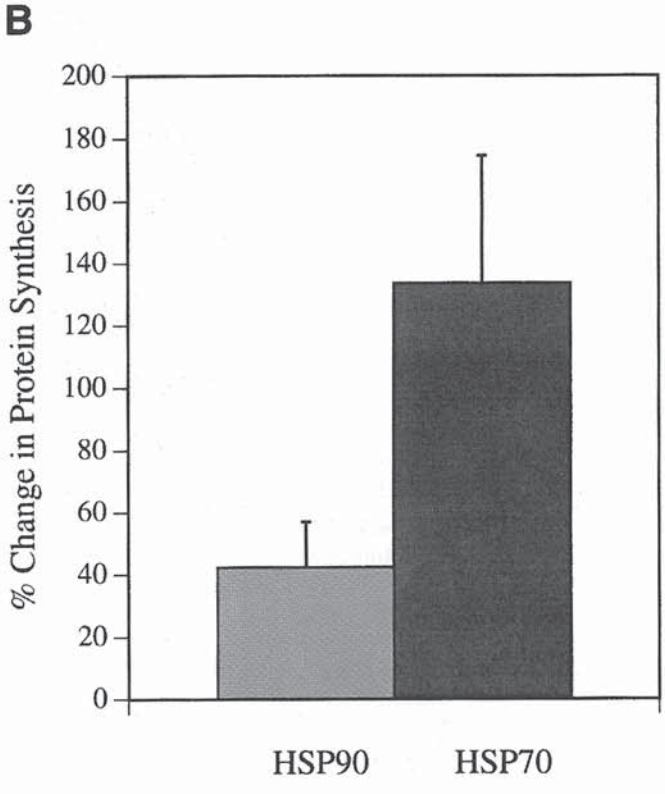


adrenal secretion *(14)*, the effects of corticosteroid administration on the synthesis of proteins under mild heat-shock conditions have been compared across these brain regions.

Results

Hippocampal Protein Synthesis at 37 and 39°C

When compared to non-heat-shocked controls, the total synthesis of proteins was found to be diminished in hippocampal slices incubated at 39°C. As demonstrated by incorporation of [^{35}S]-methionine-cysteine into proteins of the nuclear (P_1) fraction (Fig. 1), heat-shock conditions decrease protein synthesis by 53% ($p < 0.05$; $N = 4$). Similar effects were observed in other subcellular fractions as well (data not shown). In spite of the decrease seen in total protein synthesis, the synthesis of specific proteins, most notably those that were confirmed by immunoblotting to correspond to the major heat shock proteins, HSP90 and HSP70, increased in subcellular fractions prepared from hippocampal slices that were incubated at 39°C. When proteins of subcellular fractions prepared from slices incubated at 37 or 39°C were standardized with regard to incorporation of labeled amino acid and resolved on SDS-polyacrylamide gels, HSP90 and HSP70 accounted for a larger percentage of the synthesized proteins (Fig. 2A).



Densitometric analysis was used to quantify the increase in labeling at 39°C, as compared to 37°C, of HSP90 and HSP70 in the S_2 (cytosolic) fraction, in which these proteins were most prominent (Fig. 2B). In four separate experiments, the synthesis of HSP90 was increased by an average of 42.4 ± 14.6% ($p < 0.05$), whereas the synthesis of HSP70 increased by 133.6 ± 41.0% ($p < 0.025$). These alterations in the pattern of protein labeling at 39°C indicate that only a slight elevation in incubation temperature is necessary for the introduction of a degree of thermal challenge in hippocampal slices.

Hippocampal Protein Synthesis Following Glucocorticoid Treatment

The synthesis of proteins with molecular masses of 25 kDa (p25) and 47 kDa (p47) was diminished in hippocampal slices prepared from animals treated with 5 mg of corticosterone at 4 h prior to sacrifice (Fig. 3 and Table 1). Downregulation of both proteins was maintained for up to 24 h following treatment. The negative effects of corticosterone on synthesis of p25 and p47 were mimicked by administration of 100 µg of the specific Type II receptor agonist, RU-28362, suggesting that regulation of the synthesis of these proteins occurs in response to GR activation. Reductions in the synthesis of p25 and p47 in hippocampal slices were also observed at 4 h following treatment with 4 U of adrenocorticotrophic hormone (ACTH). This dose of ACTH has previously been shown to produce plasma glucocorticoid titers in the range of 200 ng/mL at 4 h following injection *(15)*. The effect of ACTH indicates that corticosteroid-induced changes in protein synthesis occur in response not only to a pharmacological dose of corticosterone, but also to a physiological stimulus that elevates endogenous levels of corticosterone as well. In contrast to the effect of corticosterone administration on synthesis of p25 and p47, there was no effect of this steroid on synthesis of HSP90 and HSP70 in hippocampal slices incubated at 39°C 4 h after injection (Table 1).

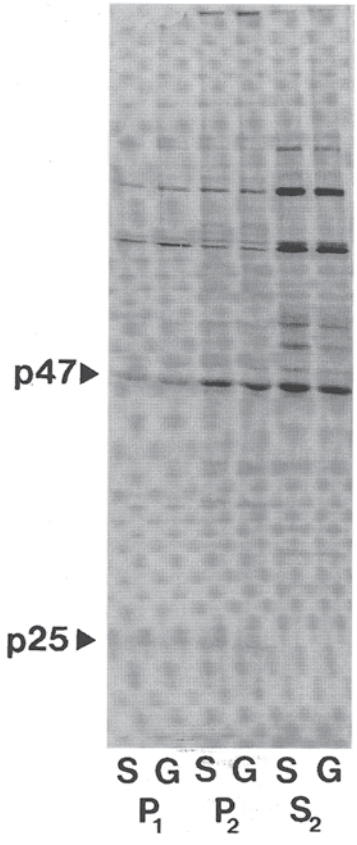


Fig. 3. Protein synthesis in rat hippocampal slices following treatment of rats with corticosterone. Hippocampal slices were prepared from animals that had received a sham (S) or corticosterone (glucocorticoid, G) injection 4 h prior to sacrifice. To each sample, 100 µCi of [^{35}S]-methionine-cysteine were added, and after a 3-h incubation at 39°C, subcellular fractionation was performed. The nuclear (P_1), synaptosomal-mitochondrial (P_2), and cytosolic (S_2) fractions were standardized with respect to incorporation of ^{35}S, as determined by TCA precipitation, and samples were separated by SDS-PAGE. Newly synthesized proteins were identified by exposing the gel to X-ray film after fluorography. The positions of p25 and p47 are designated.

Fig. 2. *(previous page)* Protein synthesis in hippocampal slices at 37 or 39°C. **(A)** Hippocampal slices were prepared from animals, and 100 µCi of [^{35}S]-methionine-cysteine were added. After a 3-h incubation at either 37°C (lanes 1, 3, 5) or 39°C (lanes 2, 4, 6), subcellular fractionation was performed. Portions of the nuclear (P_1), synaptosomal-mitochondrial (P_2), and cytosolic (S_2) fractions were standardized with respect to incorporation of ^{35}S as determined by TCA precipitation, and samples were separated by SDS-PAGE. Newly synthesized proteins were visualized by exposing the gel to X-ray film after fluorography. Open arrows designate the positions of proteins of 90 and 70 kDa, identified by immunoblotting to be the major heat-shock proteins, HSP90 and HSP70. The positions of protein standards, in kDa, are shown to the right. **(B)** Synthesis of HSP90 and HSP70. Hippocampal slices were incubated at 37 or 39°C with [^{35}S]-methionine-cysteine, and the S_2 fractions were prepared. Samples were standardized with regard to incorporation of ^{35}S, and proteins were resolved with SDS-PAGE. The synthesis of HSP90 and HSP70 was quantified with densitometry of protein bands on X-ray films after fluorography of the gels. Results shown are the percent changes in protein synthesis when comparing the density of the protein bands synthesized at 39°C as compared to 37°C and are the mean ± SEM from 4 experiments.

Characterization of p25 and p47

Subcellular fractionation after incubation of hippocampal slices at 39°C demonstrated p47 to be distributed in all subcellular fractions examined (P_1, nuclear; P_2, synaptosomal-mitochondrial; and S_2, cytosolic), whereas p25 was present in only the P_1 and P_2 fractions (Fig. 4). A comparison of protein synthesis in response to mild heat shock at 39°C of slices prepared from the hippocampus, cerebral cortex, and cerebellum demonstrated glucocorticoid-sensitive synthesis of p47 to be present in all brain regions studied. The synthesis of p25 was apparent in the hippocampus and, to a lesser extent, in the cerebral cortex (labeling of p25 was 53.2 ± 2.9% less in the cortex than in the

Table 1
Alterations in Protein Synthesis Following Steroid or ACTH Treatment[a]

Protein	Steroid	Treatment	% Change	*N*	*p*
p25	Corticosterone	4 h	–34.3 ± 5	9	<0.005
	Corticosterone	24 h	–29.9 ± 4	14	<0.005
	RU-28362	4 h	–43.3 ± 3	3	<0.005
	ACTH	4 h	–24.1 ± 1	3	<0.005
p47	Corticosterone	4 h	–22.4 ± 4	8	<0.005
	Corticosterone	24 h	–21.6 ± 3	9	<0.005
	RU-28362	4 h	–22.3 ± 4	3	<0.005
	ACTH	4 h	–13.1 ± 2	3	<0.01
HSP90	Corticosterone	4 h	–7.8 ± 5.2	5	n.s.
HSP70	Corticosterone	4 h	–10.5 ± 6.3	5	n.s.

[a]Densitometric analysis of protein synthesis showing a comparison of the effects of administration of steroid (5 mg of corticosterone; 100 µg of RU-28362) or 4 U of ACTH on the synthesis of p25 and p47 in the P_2 fraction and HSP90 and HSP70 in the S2 fraction of rat hippocampal slices incubated at 39°C. The % change is the difference in density of each protein band on X-ray films after SDS-PAGE and fluorography of samples from treated as compared to sham-injected rats for the indicated number of experiments *(N)*.

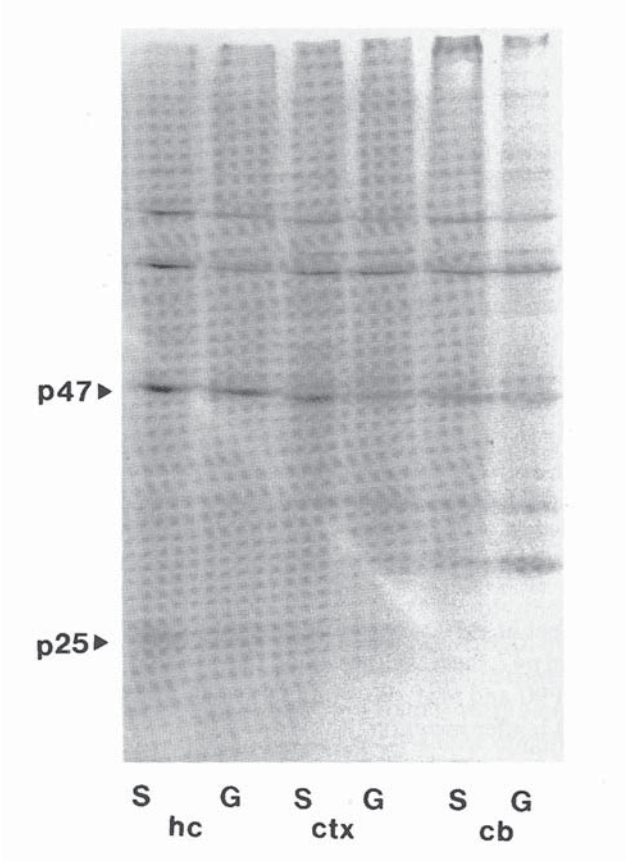


Fig. 4. Comparison of the effect of corticosterone on protein synthesis in the hippocampus, cortex, and cerebellum. Slices were prepared from the hippocampus (hc), cortex (ctx) and cerebellum (cb) of animals that had received a sham (S) or corticosterone (glucocorticoid, G) injection 4 h prior to sacrifice. To each sample, 100 µCi of [^{35}S]-methionine-cysteine were added, and after a 3-h incubation at 39°C, subcellular fractionation was performed. The P_2 fractions from the three tissues were standardized with respect to incorporation of ^{35}S, as determined by TCA precipitation, and samples were separated by SDS-PAGE. Newly synthesized proteins were identified by exposing the gel to X-ray film after fluorography.

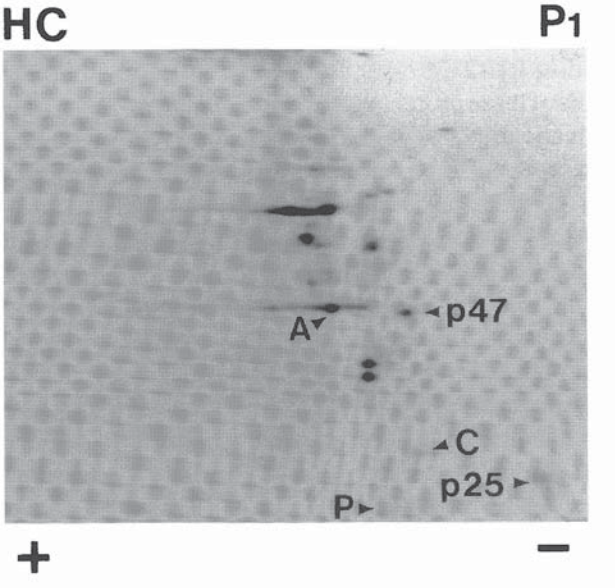


Fig. 5. Two-dimensional gel electrophoresis of proteins synthesized in vitro at 39°C. Hippocampal slices were prepared, and 100 µCi of [^{35}S]-methionine-cysteine were added. Subcellular fractionation was performed following a 3-h incubation at 39°C. Proteins from the P_1 fraction (approx 200 µg) were then separated by 2-DGE. Newly synthesized proteins were identified by exposing the gel to X-ray film after fluorography. The positions of the glucocorticoid-sensitive proteins, p25 and p47 as well as those of actin (A), p23 (P), and calbindinD-28 (C), as determined by immunoblotting, have been designated.

hippocampus, $N = 3$, $p \leq 0.05$), but was not observed in the cerebellum.

Two-dimensional gel electrophoresis (2-DGE) of proteins present in the membrane fractions from heat-shocked hippocampal slices demonstrated there to be only one labeled protein with an apparent molecular weight of 25 kDa ($N = 12$). The isoelectric point of this protein was estimated to be 4.1 (Fig. 5).

Immunoblotting with antibodies to calbindin and p23, proteins that have characteristics of p25 *(16,17)*, clearly resolved these proteins on two-dimensional gels. Furthermore, 2-DGE resolved proteins with apparent molecular weights of 47 kDa into two major components, the more basic of which was confirmed by immunoblotting to be actin. The isoelectric point of the more acidic, glucocorticoid-sensitive p47 was approx 4.9 (Fig. 5). When this protein was resolved from actin with 2-DGE, densitometric analysis showed that prior administration of corticosterone inhibited its synthesis by –42.63 + 14% ($N = 4$, $p \leq 0.05$).

Discussion

The study of glucocorticoid-induced damage to neurons within the hippocampal formation has revealed several levels on which corticosteroids contribute to the placement of cells in a state of endangerment. Exposure of hippocampal neurons to excesses of glucocorticoids is known to decrease intracellular glucose levels by 10–20%, which produces a decrease in energy availability, sensitizing them to cellular insults *(7)*. Glucocorticoids also potentiate damage to neurons by increasing both the extracellular accumulation of excitatory amino acids and the magnitude and duration of the rise in intracellular calcium levels *(3)*. Consistent with the known ability of steroid hormones to modulate gene expression, it is also possible that glucocorticoids may interfere with the response of hippocampal neurons to insult by altering the synthesis of proteins that adapt the neuron to survive the insult. The experiments in this study were designed to test this hypothesis by examining the effect of prior steroid administration to rats on the synthesis of proteins in hippocampal slices prepared from these animals and incubated using heat shock as a cellular insult.

Much work in recent years has been devoted to the study of "stress" or heat-shock proteins, a class of proteins involved in adaptation to cellular stressors. The expression of heat-shock proteins increases in response to a diversity of insults, including hyperthermia, ischemia, trauma, epilepsy, and amphetamine administration *(9–13)*. Alterations in their expression are associated with various neurodegenerative states as well *(18,19)*. Prior exposure of neurons in culture to elevated temperature protects them from apoptotic cell death *(20)*. In addition, transient in vivo hyperthermia protects against damage resulting from subsequent forebrain ischemia in the rat *(21)*. There is, therefore, an implied generality of function for these proteins with regard to cellular adaptation.

We have found that the introduction of even a modest in vitro thermal challenge (39°C) is sufficient to produce alterations in the pattern of protein synthesis in rat hippocampal slices, most notably inhibition of total protein synthesis with concomitant elevation of the synthesis of the major 90- and 70-kDa heat-shock proteins. Although these two proteins account for a higher percentage of the proteins synthesized at 39°C as compared to 37°C, there was no effect of prior steroid administration on the synthesis of these proteins in hippocampal slices. Similar to our data, elevated glucocorticoid levels associated with acute or chronic immobilization stress had no effect on steady-state levels of HSP90 or HSP70 in the rat hippocampus *(22)*. Direct administration of a high dose of corticosterone to rats does produce a trend toward increase of HSP90 mRNA in the rat hippocampus *(23)*, but a lack of correlation between HSP90 mRNA and protein levels *(24)* in combination with the other studies indicates that hippocampal expression of HSP90 and HSP70 is not subject to regulation by elevation of glucocorticoid levels in the rat.

An acute injection with corticosterone does diminish subsequent synthesis in heat-shocked slices of two proteins with apparent molecular masses of 25 and 47 kDa (p25 and p47). Since synthesis of these proteins is maintained in slices during a period of heat shock, they may play a role in adaptation to metabolic insults. Although the percent changes in synthesis of p25 and p47 are relatively small, they are similar in magnitude to glucocorticoid-mediated changes in glucose uptake *(3,7)*, which are sufficient to result in eventual pyramidal cell loss. In addition, the inhibitory effect of glucocorticoids on the synthesis of these proteins persists for at least 24 h after a single injection. Similar stress-driven elevations of corticosterone titers in vivo could, therefore significantly reduce the ability of the hippocampus to adapt to insult. The regulation of p25 and p47 synthesis by corticosteroids appears to be mediated by the GR, as demonstrated by the alterations observed after administration of the specific GR agonist, RU-28362 *(25)*. Since glucocorticoid-induced damage to the hippocampal formation is proposed to be mediated primarily by this receptor type *(3,26)*, activated during periods of elevated secretion of glucocorticoids, the GR responsiveness of these proteins indicates them to be potential molecular correlates of the endangered state.

The glucocorticoid sensitivity of proteins synthesized at 39°C in hippocampal slices differs from studies in which slices are incubated at 37°C *(8)*. Previous work in this laboratory has demonstrated the in vitro synthesis of a 35-kDa cytosolic protein, which was identified as glycerol phosphate dehydrogenase (GPDH), to be enhanced following acute treatment with exogenous corticosterone *(15,27-29)*. Repeated incubations at 39°C have failed to produce a similar result. In addition, examination of in vitro translation products of hippocampal mRNA at 37°C has shown that proteins with molecular masses of neither 25 nor 47 kDa are regulated by prior administration of exogenous glucocorticoids, although, as in the slice experiments, the synthesis of GPDH was increased *(30,31)*. Therefore, it is possible that both the basal synthesis and the glucocorticoid sensitivity of these proteins are altered when cells are faced with a thermal challenge.

The molecular weight, isoelectric point, heat inducibility, and/or glucocorticoid sensitivity of p25 is consistent with that of several proteins that might serve an adaptive function in protecting hippocampal neurons from cellular trauma, most notably, bcl-2, a protein involved in the protection of cells from apoptosis *(32)* and the small-mol-wt heat-shock protein HSP27 *(13)*. However, the predicted isoelectric point for bcl-2 is less acidic than that which we report for p25, while HSP27 is not synthesized in hippocampal slices at 39°C (Barr and Dokas, manuscript in preparation). Although the molecular weight, subcellular distribution, and isoelectric point of p25 match those previously reported for both calbindin and p23 *(16,17)*, they have been eliminated by immunoblotting as candidate proteins. Likewise, actin, which, in addition to having been reported to be glucocorticoid-sensitive has a similar molecular weight and isoelectric point to p47 *(33)*, can be distinguished from the latter on the basis of immunoreactivity. There were no labeled proteins on two-dimensional gels that corresponded to SNAP-25 or to GAP-43, two additional proteins that were considered as candidates for p25 and p47 *(34,35)*, respectively. The glucose transporter protein, which is synthesized in brain tissue in response to heat shock, has a molecular weight and isoelectric point similar to those of p47 *(36)*. Since endangerment of neurons results from diminished intracellular glucose concentrations, the glucose transporter protein is a candidate protein whose corticosteroid-mediated synthetic control would be an appropriate correlate of endangerment in the hippocampus. However, immunoblotting experiments have eliminated coidentity between the type 1 and type 3 isoforms of the glucose transporter and p47.

Although it is our hypothesis that diminished levels of the glucocorticoid-sensitive proteins, p25 and p47, are markers of endangerment, the possibility that inhibition of their synthesis by glucocorticoids could potentially serve an adaptive function must also be considered. At times of neuronal trauma, certain proteins may be expressed that either participate in apoptosis or serve as signals to induce apoptotic cell death *(37,38)*. If p25 and/or p47 serve such functions, then, by inhibiting their synthesis, glucocorticoids would mediate a protective, rather than a damaging, response. Although such a possibility would not be consistent with the demonstrated ability of glucocorticoid hormones to induce apoptosis in some cell types *(39)*, it should be noted that in the rat hippocampus, removal of glucocorticoids by adrenalectomy induces apoptosis in dentate granule cells *(2)*. Alternately, by inhibiting synthesis of p25 and p47, glucocorticoid hormones may be repressing normal functions that are not needed as part of an adaptive response. In any case, the glucocorticoid sensitivity of p25 and p47 synthesis, seen only in conjunction with heat shock, argues that the function of these proteins is related directly or inversely with the adaptation to this cellular stressor.

Materials and Methods

Treatment of Animals

Animals were treated as previously described *(15,27)* in order to compare the pattern of glucocorticoid-sensitive protein synthesis at 39°C to that observed in other experiments performed in this laboratory, in which slices were incubated at 37°C. Male Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA) weighing 150–250 g received sc injections of corticosterone (Sigma Chemical Co., St. Louis, MO; 5 mg suspended in 1 mL of sesame oil), RU-28362 (Dupont NEN Research Products, Boston, MA; 100 µg suspended in 1 mL of sesame oil), ACTH (Rhône-Poulenc Rorer Pharmaceuticals, Collegeville, PA; 4 U in 1 mL of distilled H_2O), or vehicle alone (sham-injection). Rats were sacrificed by decapitation under anesthesia (5 mg sodium pentobarbital/100 g body wt injected intraperitoneally) at either 4 or 24 h following a single corticosterone or sham injection. Animals receiving RU-28362 or ACTH injections were sacrificed 4 h following a single treatment. Protocols for use of experimental animals have been approved by the Institutional Animal Care and Use Committee of the Medical College of Ohio.

Pulse Labeling of Brain Slices and Preparation of Subcellular Fractions

After decapitation, approx 100 mg of hippocampal, cortical, or cerebellar tissue from sham-injected and treated rats was placed in oxygenated Krebs Ringer Bicarbonate (KRB) buffer (116 m*M* NaCl, 24 m*M* $NaHCO_3$, 5 m*M* KCl, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, 2.6 m*M* $CaCl_2$, 10.8 m*M* glucose, pH 7.4). Slices (500 µm) were prepared using a McIlwain tissue slicer and were placed in 5 mL of oxygenated KRB buffer for a 15-min preincubation at 39°C. They were then transferred to fresh buffer containing 100 µCi of [^{35}S]-methionine-cysteine (Dupont NEN Research Products, Boston, MA; SA of 1175 Ci/ mmol) and were incubated for 3 h at 39°C in a 95% O_2–5% CO_2 atmosphere. In experiments to determine the effect of incubation temperature on protein synthesis, slices from normal animals were incubated with this protocol, but at 37 or 39°C. Tissue slices were homogenized in sucrose phosphate buffer (10 m*M* sodium phosphate buffer, pH 6.5, containing 0.32 *M* sucrose). The homogenate was separated by centrifugation into nuclear (P_1), cytosolic (S_2), and synaptosomal-mitochondrial (P_2) fractions as previously described *(28)*. The P_1 and P_2 pellets were then resuspended in sucrose phosphate buffer (1 mL and 500 µL, respectively). Protein synthesis was measured by trichloroacetic acid (TCA) precipitation of [^{35}S]-labeled proteins *(27)*.

Protein Electrophoresis

Tissue samples were standardized with respect to incorporation of ^{35}S and proteins were then separated (approx 15,000 cpm/lane) by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) *(40)*. In order to

determine the isoelectric points of the glucocorticoid- sensitive proteins, samples were analyzed with 2-DGE *(41)*. The ampholine range used was from pH 3.5 to 11.0, and the voltage for the isoelectric focusing gels was increased from 200 to 450 V for the last hour of the run. Buffer chambers contained 10% phosphoric acid (anode) and 0.1 *N* sodium hydroxide (cathode). A 1-cm lane was excised from the isoelectric focusing gel, and proteins within it were separated by SDS-PAGE in the second dimension. A parallel lane was also excised and cut into 1-cm pieces, which were extracted with 10 m*M* KCl for measurement of the pH. Gels were dried after fluorography and exposed to Kodak XAR-5 X-ray film. The apparent molecular masses of proteins of interest were determined by comparison to SDS-PAGE mol-wt standards (Bio-Rad Laboratories, Hercules, CA; 97.4, 66.2, 45.0, 31.0, 21.5, and 14.4 kDa).

Immunoblotting

Immunoblotting for GAP-43 was performed as described previously *(42)*. Immunoblotting for the remaining candidate proteins was performed in accordance with the procedures indicated by the respective manufacturers of antibodies to these proteins (mouse monoclonal anti-SNAP-25 from Sternberger Monoclonals, Inc., Baltimore, MD; mouse monoclonal anticalbindin D-28K from Swant, Bellinzona, Switzerland; rabbit polyclonal anti-bcl-2 from Santa Cruz Biotechnology, Inc., Santa Cruz, CA; rabbit polyclonal anti-actin from ICN Biomedicals, Inc, Costa Mesa, CA).

Analysis of Data and Statistics

Following SDS-PAGE and fluorography, the densities of protein bands of interest on X-ray films were measured. Comparisons between proteins synthesized in slices prepared from sham-injected and treated animals or from slices of normal rats incubated at 37 or 39°C were made only for parallel samples from the same experiment. The means of the percent change in labeling of proteins, as determined by densitometry for each indicated number of experimental repeats, were analyzed by the two-tailed Student's *t*-test. A p value of 0.05 or less was considered statistically significant.

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