ly in certain cells, or recognize different target cell types. The last-mentioned possibility is consistent with the findings that cloned human interferon proteins display varied activity in different animals and on cultured cells derived from various organs (*18*).

The interferon gene family appears to be genetically linked. The fibroblast interferon gene and all of the approximately ten LeIF genes detectable by DNA cross-hybridization have been localized to human chromosome 9 by blot hybridization to DNA derived from mouse-human hybrid cell lines (*19*). At least some of these genes are closely linked. The λHLeIF2 clone described here contains two LeIF genes separated by 12 kb, while the clone λHLeIF1 (*20*) contains two other LeIF genes 5.0 kb apart. Nagata *et al.* (*2*) have also reported two λ clones isolated from the same human genome library which apparently contain two linked LeIF genes each. It remains to be seen whether all members of the IF gene family are clustered. Continued studies of interferon genes and their products may help to clarify the nature of interferon gene organization and expression and of interferon protein activity.

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Corticosterone Increases the Amount of Protein 1, a Neuron-Specific Phosphoprotein, in Rat Hippocampus

Abstract. *Corticosterone increased the amount of the neuron-specific phosphoprotein protein 1 in the hippocampus, a brain region rich in corticosterone receptors, but not in several brain regions that contain relatively few corticosterone receptors.*

Steroid hormones influence a variety of functions in the nervous system (*1*). The molecular mechanism of action of steroid hormones in nervous as well as in nonnervous system tissue appears to involve an increase or decrease in the synthesis of specific tissue proteins (*2*). Identifying those proteins in the nervous system that are regulated by steroid hormones may help elucidate the mechanism by which steroid hormones regulate various nervous system functions, including behavior.

One protein that is specific to nervous tissue and found throughout the central and peripheral nervous systems is a phosphoprotein referred to as protein 1 (*3–6*). Protein 1, which is present only in neurons (*3–5*), is concentrated in most, and possibly all, presynaptic nerve terminals (*5, 7*), where it appears to be associated predominantly with neurotransmitter vesicles (*5, 8*). This association suggests that protein 1 plays an important role in the functioning of those vesicles.

Protein 1 is a prominent endogenous substrate in nervous tissue both for cyclic AMP (adenosine 3′,5′-monophosphate)–dependent (*3*) and for calcium-dependent (*9*) protein kinases. Phosphorylation of protein 1 is stimulated in intact cell preparations by a variety of agents that regulate the cyclic AMP and calcium systems of neurons. For example, depolarizing agents, which induce the movement of calcium into nerve endings, phosphodiesterase inhibitors, which increase cyclic AMP levels, and derivatives of cyclic AMP stimulate the phosphorylation of protein 1 in the rat cerebral cortex (*10*). Serotonin and dopamine, neurotransmitters that increase cyclic AMP concentrations, increase the state of phosphorylation of protein 1 in well-defined regions of the central (*11*) and peripheral (*12*) nervous systems.

Since steroid hormones appear to act at least in part by altering the amount of specific proteins in target tissues, and since protein 1 may play an important role in the physiology of the synapse, we studied the possibility that steroid hormones might regulate the total amount of protein 1 in regions of the nervous system that contain steroid receptors. We report that corticosterone increases protein 1 in the rat hippocampus.

The ability of steroid hormones to regulate protein 1 in the brain was tested by treating adrenalectomized and ovariectomized rats with either corticosterone or estradiol under conditions that are known to elicit various physiological and behavioral responses (*1*). The effect of such treatment on protein 1 in four brain regions, measured by radioimmunoassay (*13*), is shown in Table 1. Corticosterone significantly increased protein 1 in the hippocampus, a brain region known to concentrate ^{3}H-labeled corticosterone in vivo (*14*) and to contain the highest concentration of cytosolic and nuclear corticosterone receptors in the brain (*15*). In contrast, corticosterone did not alter the amount of protein 1 in the preoptic area, medial basal hypothalamus, or parietal cerebral cortex, brain regions that contain relatively few corticosterone receptors. Estradiol did not alter the amount of protein 1 either in the preoptic area and medial basal hypothalamus, brain regions rich in estradiol receptors (*1*), or in the hippocampus and parietal cerebral cortex, brain regions that contain relatively few estradiol receptors.

The effect of corticosterone on protein 1 in the hippocampus and cortex is shown as a function of the duration of exposure in Fig. 1. A significant increase in protein 1 in the hippocampus was first apparent after 1 day. The amount was maximal after 7 days, at which time the protein 1 content of the hippocampus of corticosterone-treated rats was about 30 percent greater than that of control rats. After 14 days of exposure to corticosterone, protein 1 decreased somewhat but

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was still significantly elevated over control levels. Protein 1 amounts in the parietal cerebral cortex were not altered when rats were exposed to corticosterone for 14 days.

The hippocampus contains at least two classes of glucocorticoid receptors, one predominantly neuronal and one predominantly glial, which can be distinguished by their ability to interact with various glucocorticoids (*16, 17*). The receptors in the neurons of the hippocampus appear to be specific for corticosterone relative to dexamethasone in that they preferentially retain ^{3}H-labeled corticosterone over ^{3}H-labeled dexamethasone in vivo. We found that protein 1 in the hippocampus was significantly elevated by corticosterone but not by dexamethasone (Table 2). The same doses of corticosterone and of dexamethasone as those used in our study were equally effective in inducing hippocampal glycerolphosphate dehydrogenase (*17*), a glial-specific enzyme (*18*). Protein 1 in the cortex was not altered by either corticosterone or dexamethasone. The ability of corticosterone, but not of dexamethasone, to elevate the amount of protein 1 in the hippocampus indicates that this effect is mediated through a direct action on neuronal steroid receptors rather than through an indirect action on glial cells.

The rat hippocampus contains anatomically well-defined areas that differ in the amount of ^{3}H-labeled corticosterone retained in vivo as determined by autoradiography (*14*). Corticosterone significantly elevated protein 1 in the subiculum, anterior hippocampus, dentate gyrus, CA1, and CA2 regions but not in the CA3 region (*19*). One possible explanation for this finding is that the CA3 region appears to concentrate less corticosterone in vivo than the other areas in the hippocampus (*14, 20*).

Our results demonstrate that corticosterone increases the amount of protein 1 specifically in the hippocampus. It is not clear, however, whether the increase in the amount of protein 1 observed in response to corticosterone reflects an increase in the number of presynaptic nerve terminals in the hippocampus, an increase in the number of neurotransmitter vesicles per presynaptic nerve terminal, an increase in the amount of protein 1 per neurotransmitter vesicle, or an increase in the turnover of protein 1. The increase in protein 1 elicited by corticosterone may represent an important molecular mechanism by which this steroid hormone regulates hippocampal function.

Corticosterone affects hippocampal function in several ways. Brief exposure to the steroid hormone decreases the spontaneous firing rate of hippocampal neurons (*21*) and stimulates the synthesis of various unidentified hippocampal proteins (*22*). More prolonged exposure to corticosterone modifies several rat behaviors, such as extinction behavior, initiation of locomotor activity, and paradoxical sleep, all of which involve the hippocampus (*23*). Moreover, prolonged corticosterone treatment of adrenalectomized rats decreases the uptake of γ-aminobutyric acid into hippocampal synaptosomes (*24*) and increases the amount of a putative neurotransmitter, vasoactive intestinal polypeptide, in the hippocampus (*25*).

Phosphorylation of protein 1 is regulated by various neurotransmitters (*11, 12*) and by neuronal membrane depolarization (*10–12*) through the activation of cyclic AMP–dependent and calcium-dependent protein kinases. The regulation of total protein 1 content by corticosterone indicates that not only the phosphorylation of protein 1, but also the total amount of protein 1, can be altered in the nervous system. Moreover, our results support the suggestion (*26*) that protein phosphorylation systems represent a final common pathway for the actions of a large number of diverse regulatory

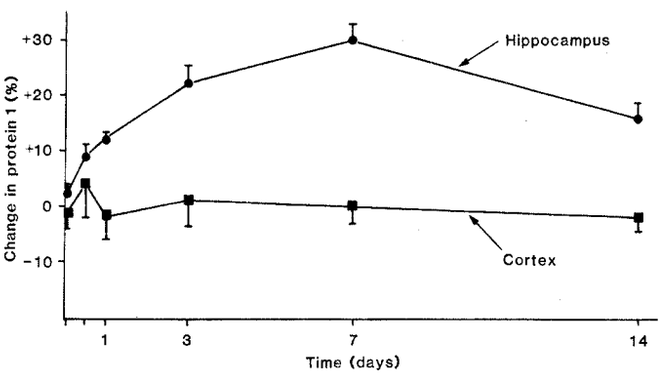

Fig. 1. Time-dependence of the effect of corticosterone on protein 1 in the hippocampus and parietal cerebral cortex determined by radioimmunoassay. Data are expressed as the percent of change from control amounts ± the standard error of the mean. The number of determinations varied from 5 to 12. The control level in the hippocampus was 56.8 ± 0.9 (18) and in the cortex, 57.4 ± 1.4 (16) pmole of protein 1 per milligram of protein.

Table 1. Effect of corticosterone and of estradiol on protein 1 determined by radioimmunoassay 7 days after implantation of the corticosterone pellets or 2 days after the second estradiol injection (*13*). Data are expressed as mean ± standard error of the mean. Numbers in parentheses represent the number of determinations.

Brain region	Predominant neuronal steroid receptor	Protein 1 (picomoles per milligram of protein)		
		Control	Corticosterone	Estradiol
Hippocampus	Corticosterone	58.4 ± 1.5 (8)	72.8 ± 2.8 (8)*	54.7 ± 1.6 (7)
Preoptic area	Estradiol	40.3 ± 1.0 (8)	42.5 ± 1.0 (8)	39.1 ± 2.8 (7)
Medial basal hypothalamus	Estradiol	32.1 ± 1.8 (8)	35.2 ± 1.6 (8)	33.5 ± 1.3 (7)
Parietal cerebral cortex	Both low	53.4 ± 1.3 (6)	52.3 ± 0.9 (7)	51.3 ± 0.9 (6)

*Significantly different from control ($P < .05$) by Student's t-test.

Table 2. Comparison of effects of corticosterone and dexamethasone on protein 1 in the hippocampus and cortex. Data are expressed as the percent of control ± the standard error of the mean. Numbers in parentheses represent the number of determinations. The control level of protein 1 in the hippocampus was 56.8 ± 0.9 (18) and in the cortex, 57.4 ± 1.4 (16) pmole of protein 1 per milligram of protein.

Brain region	Protein 1 (percent of control)	
	Corticosterone	Dexamethasone
Hippocampus	131 ± 4 (12)*	99 ± 3 (6)
Parietal cerebral cortex	100 ± 3 (11)	99 ± 2 (6)

*Significantly different from control ($P < .05$) by chi-square test.

agents, including steroid hormones. Steroid hormones alter the state of phosphorylation of the regulatory subunit (R_2) of the type 2 cyclic AMP–dependent protein kinase specifically in nervous and nonnervous target tissues (*27*). Thus, it appears that steroid hormones may modulate protein phosphorylation systems both at the level of protein kinases and at the level of specific substrate proteins for these protein kinases.

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13. Female CD 1 rats (180 to 250 g; Charles River) were adrenalectomized and ovariectomized at least 1 week before treatment. Estradiol-treated rats received a subcutaneous injection of 10 μg of estradiol benzoate in 50 μl of sesame oil on two successive days. Corticosterone-treated rats received a 100-mg solid pellet of corticosterone, implanted subcutaneously for periods from 1 hour to 14 days. The pellets maintain a physiological serum concentration of corticosterone for at least 14 days (*17*). Dexamethasone-treated rats received a subcutaneous pellet of 25 percent dexamethasone in cholesterol, which remained there for 7 days. Control rats received no treatment, injections of sesame oil, or pellets containing cholesterol. Two days after the second estradiol injection, various periods of time after the implantation of corticosterone pellets, and 7 days after the implantation of dexamethasone pellets, the preoptic area, medial basal hypothalamus, hippocampus, and portions of the parietal cerebral cortex were removed from the rat brains as previously described [V. N. Luine, R. J. Khylchevskaya, B. S. McEwen, *J. Neurochem.* **23**, 925 (1974)]. The regions, quickly frozen on dry ice, were analyzed within 1 day after being homogenized in 1 ml of 1 percent sodium dodecyl sulfate (SDS) (4°C) per 5 to 10 mg of tissue. The SDS homogenates were then diluted 40-fold in a buffer containing (final concentrations) 200 m*M* NaCl, 10 m*M* EDTA, 10 m*M* NaH_2PO_4 (*p*H 7.4), 0.5 percent NP-40, 0.1 percent SDS. The concentration of protein 1 in each homogenate was determined in triplicate by radioimmunoassay [S. E. Goelz, E. J. Nestler, B. Chehrazi, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2130 (1981)]. This assay is detergent-based, competitive, and nonsolid phase in which SDS and NP-40 solubilize protein 1 and minimize its nonspecific interactions. The assay was performed in three steps: (i) rabbit antiserum to highly purified protein 1 from bovine brain was added to homogenates or to standard purified protein 1 from bovine or rat brain (protein 1 from these two species was immunochemically indistinguishable) and incubated for 20 minutes; (ii) ^{125}I-labeled purified protein 1 from bovine brain was then added and incubated for 1 to 8 hours; and (iii) protein A–bearing *Staphylococcus aureus* cells were added to precipitate the bound ^{125}I-labeled protein 1 and incubated for 25 minutes. The assay was linear over at least a 25-fold concentration range of standard protein 1 or homogenate and as little as 2 fmole of protein 1 could be accurately measured. The total protein concentration in each SDS homogenate was determined according to the method of O. H. Lowry *et al.* [*J. Biol. Chem.* **193**, 265 (1951)] with bovine serum albumin used as the standard. Freezing and storing the homogenates for up to 3 days did not significantly affect either the protein 1 or total protein determinations. No difference was observed in protein 1 content of the brain regions examined in the three types of control animals. Therefore, the data obtained for the various control groups in each experiment were combined.
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Optical Recording of Calcium Action Potentials from Growth Cones of Cultured Neurons with a Laser Microbeam

Abstract. *Simultaneous recordings of calcium action potentials directly from growth cones and from somata of neuroblastoma cells indicated that they could be generated in the neurites at or near growth cones. Growth cone responses were measured with a fluorescent voltage-sensitive dye and a 5-milliwatt helium-neon laser microbeam as a monitoring light source.*

Cell bodies and neurites often differ in electrical properties. Examples are found for both invertebrate (*1*) and vertebrate (*2–5*) preparations. Knowledge of the electrical properties of processes and growth cones may be important in understanding the role of a particular ion in regulating the growth of neurites (*5*), the regrowth of regenerating neurites (*6*), the formation and maintenance of intercellular connections (where usually only the postsynaptic properties are studied) (*7*), and the integrative characteristics of the basic computation elements, the dendrites (*5, 8*).

Even in vitro, studies of the electrical properties of processes or growth cones are difficult with current techniques. Extracellular recording from processes of cultured neurons has been reported (*9*). However, the extracellular technique is inadequate when studying graded potentials or regional variations in the form of action potentials. By using intracellular recording from the relatively large soma and extracellular stimulation of the neurite, it may be possible to study the electrical properties of neurites (*2, 4*); however, only propagated events can be detected, and frequently the results are susceptible to ambiguous interpretations.

Optical methods (*10, 11*) for monitoring membrane potential offer an alternative tool. These methods are simple in principle: voltage-sensitive dyes bind to external sites on the membrane, where they serve as molecular transducers, transforming changes in membrane potential into optical signals caused by the change in the optical properties of the stained membrane. Optical recordings have the same time course as intracellular electrical recording, but the magnitude of the potential change is not readily determined in the optical measurements. To study the electrical properties of neurites and growth cones, we devel-

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