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ACCELERATED COMMUNICATION

Corticosterone Increases Protein Tyrosine Kinase Activity in the Locus Coeruleus and Other Monoaminergic Nuclei of Rat Brain

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SUMMARY

Protein tyrosine kinase was studied as a possible target of glucocorticoid action in discrete regions of rat brain. It was found that 7 days of corticosterone treatment produced a 20–25% increase in protein tyrosine kinase activity specifically in three monoaminergic nuclei known to be regulated by glucocorticoids *in vivo*. Thus, corticosterone increased enzyme activity in the locus coeruleus (LC), dorsal raphe, and ventral tegmentum, but not in a number of other brain regions studied. This phenomenon was characterized further in the LC. Bilateral adrenalectomy was shown to decrease protein tyrosine kinase levels in this brain region, an effect reversed completely by corticosterone replacement, indicating that this adrenal steroid plays a role in maintaining levels of the enzyme under physiological conditions *in vivo*. Chronic corticosterone was also found to increase levels of immunoreactivity of *c-src*, a well characterized protein tyrosine kinase, in the LC, results indicating that corticosterone increases protein tyrosine kinase activity in this brain region at least in part by increasing the total amount of this enzyme. Immunoblotting analysis of LC extracts with antibodies specific for phosphotyrosine indicated that the corticosterone-induced increased in protein tyrosine kinase activity in the LC is associated with a 60% increase in the incorporation of phosphotyrosine into endogenous proteins. The results demonstrate that protein tyrosine kinase activity is regulated by glucocorticoids in discrete regions of the central nervous system and raise the possibility that regulation of these brain regions by glucocorticoids is mediated in part through effects on this enzyme.

Accumulating evidence indicates that protein phosphorylation plays an important role in the regulation of neuronal function (1, 2). Phosphorylation of neuronal proteins is mediated by two major classes of protein kinase: the protein serine/threonine kinases and protein tyrosine kinases. Many types of protein serine/threonine kinases are second messenger-dependent enzymes and have been shown to be regulated by a variety of hormones and neurotransmitters in specific regions of the nervous system, where they have been implicated in the regulation of diverse aspects of neuronal function (see Refs. 1 and 2).

The situation is much less clear for the protein tyrosine kinases. This class of enzyme was first discovered in nonnervous tissues as the transforming gene products of certain oncogenic viruses, and normal cellular homologues of these oncoproteins have since been shown to be widely distributed in nontransformed cells (see Ref. 3). Some of these protein tyrosine kinases of normal cells are known to be domains of the receptor proteins for a number of growth factors, such as insulin and epidermal growth factor. Moreover, growth factor-induced activation of the receptor-associated protein tyrosine kinase activity has been shown to play an obligatory role in mediating at least some of the physiological actions of these growth factors in target cells (3). Based on this information, the enzymes have largely been thought to play a role in the regulation of cell growth and transformation.

However, several lines of evidence support the view that protein tyrosine kinases may be involved in the regulation of additional processes in the nervous system, notably the regulation of signal transduction (4, 5). First, adult brain contains very high levels of the enzymes (4, 6–11), despite the fact that the brain is a highly differentiated and nonproliferative tissue. Second, adult brain contains many species of protein tyrosine kinase (12–15), including one form that is expressed uniquely in neurons (12). Third, protein tyrosine kinase activity shows a marked regional distribution in brain (4) and is enriched in synaptic fractions of nervous tissue (4), where a number of substrates for the enzyme have been demonstrated (5). However, despite these indications of a role for the enzymes in the

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ABBREVIATIONS: LC, locus coeruleus; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; SDS, sodium dodecyl sulfate.

regulation of signal transduction processes, only a small fraction of the protein tyrosine kinase activity in brain is regulated by growth factors, with most of the enzyme activity unaccounted for with respect to its regulatory control (see Ref. 15). In particular, it has not yet been possible to demonstrate regulation of the enzyme by neurotransmitters or hormones in the nervous system.

This inability to demonstrate acute regulation of protein tyrosine kinase activity in the brain raises the possibility that regulation of this class of enzyme is associated with more long term processes by which neurons adapt to chronic perturbations. In the present study, we investigated the possibility that protein tyrosine kinase in brain is regulated by glucocorticoids. These adrenal steroids would appear to be good candidates for such long term regulation, because their diverse effects on neuronal function are achieved largely through alterations in the synthesis of specific tissue proteins (16, 17) and because intracellular regulatory pathways (such as second messenger and protein phosphorylation systems) are prominent among the known targets of glucocorticoid action in the nervous system (1, 16–23). For this study, we focused on a number of brain regions known to be regulated by glucocorticoids *in vivo*. We found that chronic treatment of rats with corticosterone increased levels of protein tyrosine kinase activity in the LC, dorsal raphe, and ventral tegmentum, three monoaminergic nuclei in brain that have been implicated in mediating some of the effects of glucocorticoids on brain function (24–26), but not in several other brain regions studied. The results represent the first demonstration that protein tyrosine kinases are under the regulatory control of hormones in the nervous system.

Methods

***In vivo* drug treatments.** Male Sprague-Dawley rats (initial weight, 175–225 g) were used in these studies. Rats were implanted subcutaneously with a single pellet containing 100 mg of corticosterone in a cholesterol base (Innovative Research, Toledo, OH), under light halothane anaesthesia. This corticosterone treatment has been shown to maintain a physiological serum concentration of corticosterone for at least 14 days (27). Control rats either underwent identical surgery with implantation of cholesterol pellets (Innovative Research) or underwent no treatment. These two types of controls did not differ in final determinations of protein tyrosine kinase activity. Unless specified otherwise, rats were used 7 days after pellet implantations.

In some experiments, animals underwent bilateral adrenalectomy under halothane anaesthesia. Some adrenalectomized rats were implanted with corticosterone pellets, as described above, immediately following adrenalectomy. Control animals were sham-operated; adrenal glands were identified but not excised. Animals were given free access to normal drinking water and 0.9% saline and were used 7 days after surgery.

Preparation of tissue extracts. Brains were removed rapidly from decapitated rats and maintained in ice-cold physiological saline (126 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM D-glucose, pH 7.4). Large brain regions were isolated by gross dissections. The LC, dorsal raphe, ventral tegmentum, and substantia nigra were isolated from 0.7-mm-thick coronal cross-sections of pons or midbrain by using a 15-gauge syringe needle to "punch out" individual nuclei as described (28).

Isolated brain regions were homogenized immediately (5 mg of tissue/ml) in homogenization buffer (20 mM Tris, pH 7.4, 10 mM EGTA, 5 mM EDTA, 10% glycerol, 10 mM β-mercaptoethanol, 20 μg/ml leupeptin, 20 kallikrein units/ml aprotinin) (4). In some experiments, total particulate fractions of the homogenates were obtained by centrifuging them at 150,000 × *g* in a Beckman airfuge for 10 min at 4°. The resulting pellets were resuspended in the original volume of assay buffer (final concentrations: 20 mM Tris, pH 7.4, 20 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM EGTA, 0.5 mM EDTA, 0.5 mM ouabain, 0.5 mM sodium orthovanadate) plus 1 mM dithiothreitol and 1% Triton X-100, whereas crude homogenates and supernatants were adjusted to contain the same buffer by addition of concentrated solutions. The tissue samples were kept at 4° and used immediately for assay of protein tyrosine kinase activity. The high concentrations of dithiothreitol and Triton X-100 included in this buffer were found to be necessary to maintain high levels of enzyme activity in the pontine and midbrain micronuclei (LC, dorsal raphe, ventral tegmentum, and substantia nigra), whereas they had little effect on enzyme activity in the other regions studied. For each brain region studied, >95% of the total protein tyrosine kinase activity was recovered in the particulate fraction of the tissue, as has been reported previously for a number of brain regions (4).

Protein tyrosine kinase assays. Protein tyrosine kinase activity was quantitated by use of a filter paper assay (4). Duplicate aliquots of tissue samples (containing 5 μg of protein) were incubated in a final volume of 60 μl in assay buffer plus (final concentrations) 0.1 mM dithiothreitol, 0.1% Triton X-100, and 50 μM [γ^{32}P]ATP (500–1000 cpm/pmol; New England Nuclear, Boston, MA) in the absence or presence of 15 μg of poly(glu^{80},tyr^{20}) (Sigma Chemical Co., St. Louis, MO). The reactions were initiated by the addition of the ATP and were carried out for 15 min at room temperature. Reactions were terminated by addition of 10 μl of (final concentrations) 10 mM ATP/0.25 mM EGTA and the mixtures were blotted onto 2.3-cm filter paper discs (Whatman 3MM). The filters were washed five times for 5–10 min each at room temperature in 10% trichloroacetic acid/10 mM sodium pyrophosphate, washed once in 95% ethanol, rinsed in diethyl ether, and air dried. The filters were then counted by liquid scintillation. Protein tyrosine kinase activity was calculated as the difference in ^{32}P incorporation in the presence and absence of the synthetic substrate poly(glu^{80},tyr^{20}) (the level of ^{32}P incorporation was more than 5-fold higher in the presence of the synthetic substrate than in its absence) and was expressed as pmol of ^{32}P incorporated per min per mg of protein [protein content was determined by the method of Lowry *et al.* (29), using bovine serum albumin as standard].

Under the assay conditions used, protein tyrosine kinase activity was linear over a 5-fold range of tissue concentration and between 5 and 30 min of incubation time. The effect of corticosterone on enzyme activity in the LC was observed over these ranges of tissue concentrations and incubation times. The specificity of this assay for measuring protein tyrosine kinase activity was confirmed, as described (4), by analyzing some assay samples by SDS-polyacrylamide gel electrophoresis [which confirmed that the difference in ^{32}P incorporation in the absence and presence of poly(glu^{80},tyr^{20}) was due to the phosphorylation of the synthetic substrate] and by phosphoamino acid analysis (which confirmed that phosphorylation of the synthetic substrate occurred on tyrosine residues). The corticosterone-induced increase in ^{32}P incorporation in the LC was shown to be due to increased phosphorylation of the synthetic substrate by use of the above procedures. Corticosterone had no effect on levels of phosphorylation carried out in the absence of the synthetic substrate. The nature of the protein kinase activity observed in the absence of the synthetic substrate is unknown, but it was found to phosphorylate endogenous proteins detectably on serine and threonine residues only, and its activity was unaffected by specific inhibitors of cyclic AMP-dependent and calcium-dependent protein kinases.

The corticosterone-induced increase in poly(glu^{80},tyr^{20}) phosphorylation in the LC was shown to be due to an increase in protein tyrosine kinase activity, and not to a decrease in protein tyrosine phosphatase activity. First, protein tyrosine phosphatase activity was virtually completely inhibited under standard assay conditions. This was shown by phosphorylating poly(glu^{80},tyr^{20}) with protein tyrosine kinase activity present in LC extracts, as described above, in the presence of [γ^{32}P]ATP and in the presence of varying concentrations (0–2 mM) of sodium orthovanadate [included in the assays to inhibit protein tyrosine phos-

phatases (4)]. After 15 min of reaction, ATP (final concentration, 10 mM) was added and the mixture was incubated for 1 to 60 min more. The reactions were terminated by the addition of 10 mM EDTA and the solutions were blotted onto filter papers, which were then washed and counted as described above. It was found that, in the absence of sodium orthovanadate, there was ~70% dephosphorylation of poly(glu^{80},tyr^{20}) over the 60-min incubation period, whereas, in the presence of 0.5 mM sodium orthovanadate (the concentration used in the standard assays), the substrate was dephosphorylated by less than 5% after 60 min. Second, comparison of LC extracts isolated from control and corticosterone-treated animals revealed no difference in the rate or extent to which poly(glu^{80},tyr^{20}) was dephosphorylated over the range of sodium orthovanadate concentrations used.

Immunoblot analysis of *c-src*. Brain extracts were analyzed for *c-src* content by use of immunoblotting procedures. Briefly, aliquots of crude homogenates (containing 40 μg of protein in 2% SDS) were subjected to SDS-polyacrylamide gel electrophoresis (see Ref. 28) with 7.5% acrylamide/0.30% bisacrylamide in the resolving gels. Proteins in resulting gels were transferred electrophoretically to nitrocellulose paper, which was then immunolabeled for *c-src* exactly as described (30). Mouse monoclonal anti-*c-src* antibody (No. 327, 1:250; kindly provided by Dr. J. Brugge of SUNY, Stony Brook) and ^{125}I-labeled goat anti-mouse IgG (500 cpm/μl) were used for these experiments. Resulting immunoblots were autoradiographed and revealed only single bands of M_r 58,000, close to published values of 60,000 for *c-src* (4, 6-10, 12). Individual *c-src* bands were cut out of blots and counted in a γ-counter. Under the immunolabeling conditions used, levels of *c-src* immunoreactivity were linear over at least a 3-fold range of tissue concentration.

Immunolabeling of phosphotyrosine. The effect of corticosterone on levels of phosphorylation of endogenous proteins on tyrosine residues was studied by an immuno-dot-blotting procedure similar to that used previously for other proteins (31). Briefly, aliquots of brain homogenates (containing 5 μg of protein in 10 μl of 2% SDS) were pipetted directly onto nitrocellulose strips, which were then fixed by incubation for 1 hr at room temperature in 10% acetic acid/25% 2-propanol. The strips were then immunolabeled exactly as described (30) except that the immunoblotting buffer contained 5% non-fat dry milk. Antiphosphotyrosine antiserum (5 μg/ml of affinity-purified, rabbit polyclonal antiserum; kindly provided by Drs. D. T. Pang and P. Greengard, The Rockefeller University) and ^{125}I-labeled goat anti-rabbit IgG (800–1000 cpm/μl; New England Nuclear) were used in these studies. Resulting strips were air dried and autoradiographed, and individual dots were cut out from the strips and counted in a γ-counter. Under the conditions used, levels of phosphotyrosine immunoreactivity were linear over a 4-fold range of tissue concentration, and the corticosterone-induced increase in immunoreactivity in the LC was observed over this entire range of tissue concentration. No labeling of dot-blots was observed when nonimmune antiserum was used, and incubation of antiphosphotyrosine antiserum for 4 hr at 4° with 1 mM phosphotyrosine (Sigma), but not with phosphoserine or phosphothreonine (Sigma), reduced labeling to nondetectable levels.

Attempts were made to identify the individual phosphoproteins in the LC that are phosphorylated on tyrosine residues by use of the above antisera and standard SDS-polyacrylamide gel electrophoresis and immunoblotting procedures. Unfortunately, it was not possible to obtain sufficient labeling of specific proteins with the antiphosphotyrosine antisera available.

Results

Regulation of protein tyrosine kinase activity by corticosterone. The ability of glucocorticoids to regulate protein tyrosine kinase activity in brain was tested by treating rats with a single subcutaneous corticosterone pellet for 1 week, conditions known to elicit a number of specific biochemical, physiological, and behavioral responses to the hormone (16, 17). Protein tyrosine kinase activity in extracts of various isolated brain regions was then measured by a standard filter paper assay that used a synthetic protein as substrate, as described in Methods. It was found, as shown in Table 1, that corticosterone treatment significantly increased protein tyrosine kinase activity by about 20–25% in three monoaminergic nuclei, the LC, dorsal raphe, and ventral tegmentum, but had no effect on enzyme activity in the other brain regions studied, which included the substantia nigra, frontal cortex, hippocampus, amygdala, cerebellum, neostriatum, and whole pons.

The increase in enzyme activity in the three monoaminergic nuclei showed anatomical specificity; no change in the enzyme

TABLE 1

Effect of corticosterone on protein tyrosine kinase activity in rat brain

Rats were treated with corticosterone by subcutaneous pellet implantation and were used 7 days later. Brain regions were isolated rapidly from treated and control rats, and crude homogenates were assayed for protein tyrosine kinase activity as described in Methods. Data are expressed as percent of control specific activity ± standard error (n = number of rats). The specific activities of the enzyme (in pmol/min/mg of protein) in brain regions from control rats ± standard error (n = 4–8) were as follows: LC, 276 ± 14; dorsal raphe, 231 ± 18; ventral tegmentum, 265 ± 30; substantia nigra, 421 ± 25; frontal cortex, 493 ± 45; hippocampus, 572 ± 27; amygdala, 474 ± 27; cerebellum, 528 ± 19; neostriatum (caudate/putamen), 421 ± 36; and pons, 223 ± 16. Levels of enzyme activity in the larger brain regions correspond well to those reported previously (4).

Brain region	Protein tyrosine kinase activity (n)
	% of control
LC	126 ± 5 (10)*
Dorsal raphe	118 ± 4 (6)*
Ventral tegmentum	120 ± 2 (6)*
Substantia nigra	105 ± 3 (6)
Frontal cortex	99 ± 4 (9)
Hippocampus	96 ± 3 (9)
Amygdala	100 ± 4 (6)
Cerebellum	95 ± 7 (6)
Neostriatum	101 ± 6 (4)
Pons	103 ± 2 (4)

*$p < 0.025$ by χ^2 test.

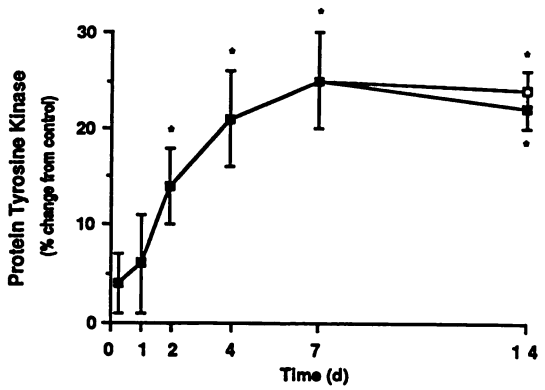

Fig. 1. Time dependence of the effect of corticosterone on protein tyrosine kinase activity in the rat LC. Rats were treated with corticosterone by subcutaneous pellet implantation and were used from 6 hr to 14 days later (■). In some cases, rats received a second corticosterone pellet on day 7 and were used on day 14 (□). Total particulate fractions of LC isolated from treated and control rats were then assayed for protein tyrosine kinase activity as described in Methods. [Total particulate fractions were used, rather than crude homogenates, because they were found to contain >95% of the protein tyrosine kinase activity in this brain region (see Methods). Similar results were obtained when crude homogenates were assayed.] Data are expressed as percent change from control specific activity ± standard error and represent the results from 4 to 10 animals at each time point. Enzyme specific activity in LC from control samples was 299 ± 9 (10) pmol/min/mg protein. *$p < 0.05$ by χ^2 test.

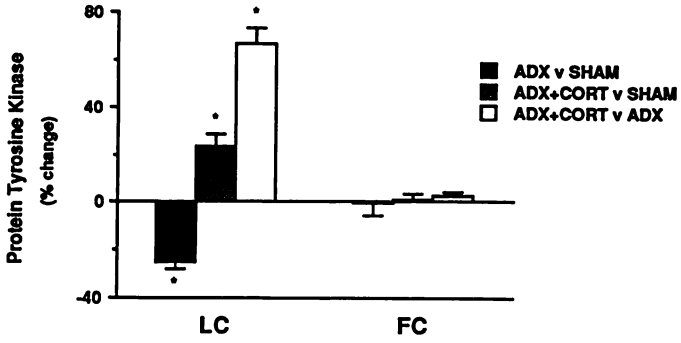

Fig. 2. Effect of adrenalectomy and corticosterone replacement on protein tyrosine kinase activity in the rat LC and frontal cortex. Rats were treated in three ways: *ADX*, adrenalectomized bilaterally; *SHAM*, sham-operated; or *ADX + CORT*, adrenalectomized and implanted immediately with a single corticosterone pellet. Rats were used 7 days after surgery. Total particulate fractions of LC and frontal cortex from the three groups were then assayed for protein tyrosine kinase activity as described in Methods (similar results were obtained when crude homogenates were assayed). Data are expressed as percent change ± standard error and represent the results of four to eight animals in each group. Enzyme specific activity (in pmol/min/mg of protein) in samples from sham-operated animals was LC, 319 ± 32 (4) and frontal cortex (*FC*), 534 ± 13 (4). $^*p < 0.05$ by χ^2 test.

was observed in pons or midbrain cross-sections from which the nuclei had been removed or in brain samples obtained just laterally or medially to the individual nuclei. Moreover, the increase in enzyme activity in these monoaminergic nuclei was not due to exogenous corticosterone being retained in tissue fractions, inasmuch as addition of corticosterone directly to protein kinase assays did not influence enzyme activity in these brain regions (data not shown).

Time dependence of corticosterone-regulation of protein tyrosine kinase activity in the LC. The effect of corticosterone on protein tyrosine kinase activity was characterized further in the LC, the brain region that showed the greatest effect of the hormone. Fig. 1 shows the regulation of the enzyme in the LC as a function of the duration of corticosterone exposure. A significant increase in protein tyrosine kinase activity was first apparent after 2 days of treatment and appeared maximal after 4 to 7 days. Enzyme activity remained near maximally elevated levels after 14 days and was not increased further at 14 days when rats received a second corticosterone pellet on day 7. This time course resembles the time courses by which some other proteins are regulated by corticosterone in the nervous system (16–23). In contrast to the LC, no effect on protein tyrosine kinase activity was seen in the frontal cortex at any of the time points studied (data not shown).

Effect of adrenalectomy on protein tyrosine kinase activity in the LC. The ability of corticosterone to increase protein kinase activity in the LC (and the other monoaminergic nuclei) indicates that chronic exposure to exogenous glucocorticoids alters enzyme activity. To determine whether the enzyme is also regulated by endogenous glucocorticoids, the effect of adrenalectomy on levels of protein tyrosine kinase activity was studied. As shown in Fig. 2, adrenalectomy significantly reduced protein tyrosine kinase activity in the LC, by about 25%. Corticosterone replacement completely reversed this effect, resulting in enzyme levels about 20% greater than those in sham-operated animals and about 65% greater than those in adrenalectomized animals. These results indicate that levels of protein tyrosine kinase activity in the LC are regulated by glucocorticoid hormones under physiological conditions *in vivo*. In contrast, no effect of these various treatments on enzyme activity was observed in frontal cortex (Fig. 2).

Regulation by corticosterone of *c-src* immunoreactivity in the LC. One of the best characterized protein tyrosine kinases in brain is the *c-src* protooncogene (see Refs. 3 and 15). It was, therefore, of interest to determine whether the corticosterone-induced increase in protein tyrosine kinase activity in the LC was associated with an increase in levels of *c-src* in this brain region. As shown in Fig. 3, chronic (7 day) corticosterone treatment was found to increase levels of *c-src* immunoreactivity by about 30% in the LC, an effect not observed in the frontal cortex.

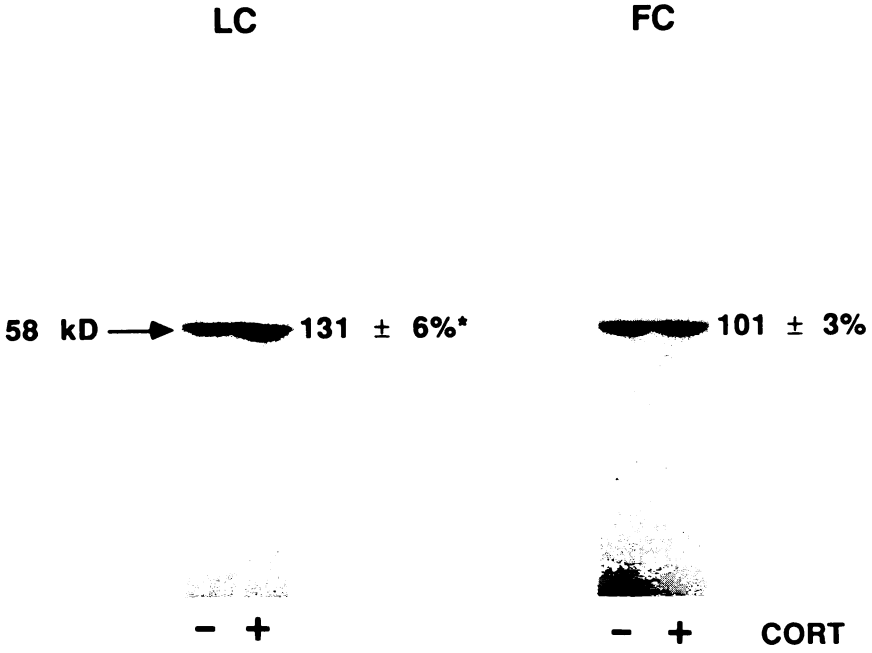

Fig. 3. Autoradiograms showing the effect of corticosterone on *c-src* immunoreactivity in the LC and frontal cortex. Rats were treated with corticosterone (*CORT*) for 1 week, after which time LC and frontal cortex (*FC*) homogenates were subjected to SDS-polyacrylamide gel electrophoresis and to immunolabeling for *c-src* using anti-*c-src* antibody and ^{125}I-labeled goat anti-mouse IgG, as described in Methods. Resulting immunoblots were autoradiographed and individual *c-src* bands were cut out of the blots and counted in a γ-counter. Data are expressed as percent of control ± standard error and represent analysis of six control and corticosterone-treated rats. $^*p < 0.025$ by χ^2 test.

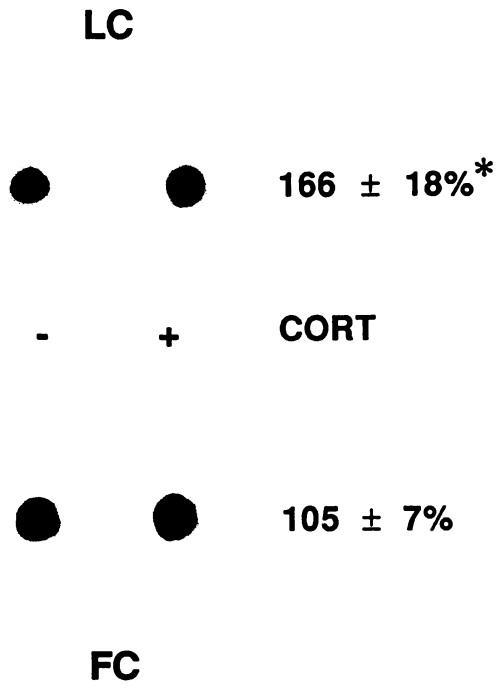

Fig. 4. Autoradiograms showing the effect of corticosterone on phosphotyrosine immunoreactivity in the LC and frontal cortex. Rats were treated with corticosterone (*CORT*) for 1 week, after which time LC and frontal cortex (*FC*) homogenates were blotted onto nitrocellulose strips. The strips were then immunolabeled with antiphosphotyrosine antiserum and ^{125}I-labeled goat anti-rabbit IgG, as described in Methods. Resulting strips were autoradiographed and individual dot-blots were cut out of the strips and counted in a γ-counter. Data are expressed as percent of control ± standard error and represent analysis of six (LC) or three (frontal cortex) control and corticosterone-treated rats. $^*p < 0.025$ by χ^2 test.

Regulation by corticosterone of protein tyrosine phosphorylation in the LC. In order to determine whether the corticosterone-induced increase in protein tyrosine kinase activity in the LC is associated with an increase in the phosphorylation of endogenous proteins on tyrosine residues, levels of phosphotyrosine were quantitated in extracts of LC from control and corticosterone-treated rats by standard immuno-dot-blotting procedures, as described in Methods. It was found that 7 days of corticosterone exposure increased phosphotyrosine levels in the LC by about two thirds, whereas no effect of corticosterone was observed in the frontal cortex (Fig. 4).

Discussion

The results of the present study demonstrate that glucocorticoids increase protein tyrosine kinase activity in discrete regions of rat brain and suggest that levels of this class of protein kinase are maintained by adrenal corticosteroid hormones *in vivo*. A striking finding of the study is the regional specificity of glucocorticoid action. Thus, enzyme activity was increased in three monoaminergic nuclei, the LC, dorsal raphe, and ventral tegmentum, but not in several larger brain regions studied. One possible explanation for this regional specificity is the relative homogeneity of the monoaminergic nuclei, compared with most of the other regions examined; the heterogeneity of these larger regions could make it difficult to detect alterations in enzyme activity that occur in certain subpopulations of neurons. Alternatively, the regional specificity of protein tyrosine kinase regulation could be due to unique characteristics of the monoaminergic nuclei. In either case, the results demonstrate physiological regulation of protein tyrosine kinase activity in brain for the first time and suggest that regulation of the enzyme in the nervous system by other hormones and neurotransmitters might also be revealed if anatomically well defined, relatively homogeneous brain regions were analyzed.

Regulation of protein tyrosine kinase activity in the monoaminergic nuclei could be due to a direct action of corticosterone on these neurons, inasmuch as they have been shown to contain very high levels of glucocorticoid receptors by *in vitro* autoradiographic procedures (32). Consistent with this interpretation is the observation that another monoaminergic nucleus, the substantia nigra, which contains many fewer glucocorticoid receptors (32), failed to show regulation of protein tyrosine kinase by corticosterone. The inability of corticosterone to regulate the enzyme in the hippocampus and amygdala, brain regions rich in glucocorticoid receptors (16, 17), raises the possibility that not all neurons that contain such receptors exhibit this phenomenon. However, it is also possible that failure to observe the effect in hippocampus and amygdala is due to the fact that only certain neurons in certain hippocampal subfields or amygdaloid nuclei are rich in glucocorticoid receptors (16, 17), such that a protein kinase response in this subpopulation of neurons is not detectable when the brain regions as a whole are analyzed by biochemical procedures. Alternatively, absence of a protein kinase response in the hippocampus and amygdala may be due to the fact that these brain regions appear to contain a different subtype of glucocorticoid receptor from that present in the monoaminergic nuclei (see Refs. 16 and 32). If this latter interpretation were the case, the results would suggest that the two receptor subtypes mediate some different actions of glucocorticoids *in vivo*.

Glucocorticoids are known to produce profound effects on noradrenergic, serotonergic, and dopaminergic systems in brain, and many of such effects appear to be mediated at the level of the cell bodies of these neuronal systems, located, respectively, in the LC, dorsal raphe, and ventral tegmentum (16, 17, 22, 24–26). For example, glucocorticoids are known to alter the physiological states of these neurons, including their rates of neurotransmitter turnover. Regulation by glucocorticoids of these various monoaminergic systems is thought to play a central role in the neural mechanisms underlying stress (16, 17, 24–26). Control of protein tyrosine kinase activity by glucocorticoids in these specific monoaminergic nuclei raises the possibility that some of the effects of glucocorticoids on neuronal function in these brain regions are achieved through alterations in the phosphorylation of target proteins on tyrosine residues. Thus, the findings of the present study support the view (4, 5) that this class of protein kinase plays a role in signal transduction processes in the nervous system.

Brain is known to contain many species of protein tyrosine kinase (3, 4, 12–15). One of the best characterized protein tyrosine kinases is *c-src*, the normal cellular homologue of the rous sarcoma virus oncogene product (3, 15), which is present at high levels in brain (4, 6–10, 12). In the present study, we have demonstrated that chronic corticosterone increases levels of *c-src* immunoreactivity in the LC. Presumably, this increase in *c-src* accounts for at least some of the increase in protein tyrosine kinase activity observed in response to corticosterone in this brain region. However, because *c-src* appears to represent ~40% of protein tyrosine kinase activity throughout the

brain (4), the observed 30% increase in *c-src* is unlikely to account for the entire 25% increase in total protein tyrosine kinase activity in the LC. It will be important in future studies to identify any additional types of protein tyrosine kinase(s) that are also affected by glucocorticoids in the LC and to determine whether the effects of glucocorticoids on these other enzymes are achieved through increases in enzyme protein, as with *c-src*, or increases in the functional activity of a constant amount of enzyme protein.

It will also be important to identify the specific substrate proteins in the LC that are responsible for the increased levels of phosphotyrosine observed in response to glucocorticoid exposure. Such increases in phosphotyrosine presumably reflect the increased levels of protein tyrosine kinase activity induced by glucocorticoids in this brain region but could, in addition, reflect increases in the total amounts of certain individual substrate proteins also induced by glucocorticoids. Through the characterization of glucocorticoid regulation of protein tyrosine kinase activity and its substrate proteins, much can be learned about the precise molecular mechanisms through which glucocorticoids regulate the function of the monoaminergic neuronal systems.

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