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*Steroid–Peptide Interactions in the Central Nervous System*

# CENTRAL PEPTIDERGIC NEURONS AS TARGETS FOR GLUCOCORTICOID ACTION. EVIDENCE FOR THE PRESENCE OF GLUCOCORTICOID RECEPTOR IMMUNOREACTIVITY IN VARIOUS TYPES OF CLASSES OF PEPTIDERGIC NEURONS

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**Summary**—By means of double immunolabeling procedures it has been possible to demonstrate glucocorticoid receptor (GR) immunoreactivity (IR) in large numbers of various peptidergic neurons of the brain including neurons containing gastrointestinal peptides, opioid peptides, and peptides with a hypothalamic hormone function. For each peptide system, however, marked heterogeneities exist among brain regions. Thus, in the neocortex and the hippocampal formation most of the brain peptide neurons lack GR IR, while the same types of peptide neurons in the arcuate and paraventricular nucleus [e.g. neuropeptide Y (NPY), somatostatin (SRIF) and the cholecystokinin (CCK) neurons] possess strong GR IR. Furthermore, in the arcuate, parvocellular part of the paraventricular nuclei and the central amygdaloid nucleus practically all the peptidergic neurons are strongly GR IR, while in the lateral hypothalamus, mainly the neurotensin (NT) and galanin (GAL) IR neurons are GR IR. These marked differences among areas probably reflect functional differences dependent upon their participation in stress regulated circuits. All the paraventricular NT, corticotropin-releasing factor (CRF), growth hormone-releasing factor (GRF), thyrotropin-releasing hormone (TRH) and SRIF IR neurons appear to contain GR IR, while the luteinizing hormone-releasing hormone (LHRH) IR neurons lack GR IR, underlying the importance of glucocorticoids (GC) in controlling endocrine function. Finally, the GC may influence pain and mood control mainly via effects on enkephalin (ENK) neurons especially in the basal ganglia (mood) and on all β-endorphin (β-END) neurons of the arcuate nucleus, while most of the dynorphin neurons are not directly controlled by GC.

## INTRODUCTION

Large numbers of nerve cell and glial cell populations in the central nervous system (CNS) of mammals have been shown to contain glucocorticoid receptor (GR) immunoreactivity (IR) located predominantly in the nuclei of the cells [1–5]. The transmitter identity of many of the GR IR nerve cell populations of the brain has been mapped out. A strong GR IR was shown to exist in large numbers of monoaminergic neurons and also within several types of peptidergic neurons [6–12]. These results indicate that large numbers of monoaminergic, especially noradrenaline (NA) and 5-hydroxytryptamine (5-HT) as well as several peptidergic neurons are important targets for the central actions of glucocorticoids (GC) [4, 5]. Recently, we have expanded our analysis of the colocalization of neuropeptides and GR in the brain using double immunolabeling procedures [7, 8]. The results will be presented and summarized in the present article. Several subclasses of central peptidergic neurons have been analyzed: neurons containing peptides of the hypothalamic hormone family; neurons containing peptides of the gastrointestinal family: and neurons containing opioid peptides. Finally, peptide neur-

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ons which do not belong to any special class of peptidergic neurons, such as galanin (GAL) and neuropeptide Y (NPY) IR neurons have been analyzed [4, 5, 12]. The results emphasize the important role of GC in the direct regulation of peptide neurons but also the regional heterogeneity of each type of neuron with respect to presence of GR IR.

## METHODOLOGY

Specific pathogen-free Sprague–Dawley rats (250 g, body wt; ALAB, Stockholm, Sweden) were used. The animals were kept under regular light conditions (lights on at 0600 h and off at 1800 h) receiving food pellets and tap water *ad libitum*. The operative procedures were conducted under chloral hydrate anesthesia (300 mg/kg body wt). The demonstration of the immunoreactivity for the peptides was facilitated by the intraventricular injection of colchicine (100 $\mu$g; 10 $\mu$l, saline), 24 h before killing. After this period the animals were perfused under barbiturate anesthesia via the ascending aorta with 50 ml warm saline followed by perfusion with 300 ml ice-cold fixative for 6 min. The fixative used [13] was 4% paraformaldehyde and 0.3% picric acid diluted in 0.1 M phosphate buffer, pH 6.9. The brains were rapidly removed and immersed in fresh fixative for 90 min. After washing in 5% buffered sucrose solutions overnight, coronal brain sections 20 $\mu$m thick were cut on a cryostat from bregma level −0.30 to bregma level −5.80 mm [14]. Since we aimed to study the colocalization of GR and peptides in 8 different peptide-containing populations, every eighth section was ascribed to each antibody against these neuropeptides. The two-color immunoperoxidase technique [15] was used. This technique has been described in detail in previous papers [7, 8, 12]. Briefly, the sections were incubated with a mouse monoclonal antibody against the rat liver GR [16] diluted 1/2000 in 0.1 M phosphate buffer (PBS) containing 0.3% Triton X-100 (Sigma, St Louis, MO, U.S.A.). The incubation was performed free-floating at room temperature overnight. The avidin–biotin–peroxidase method was employed (Vectastain kit, Vector, Burlingame, U.S.A.), using 3-3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma) as a chromogen, resulting in brownish staining of GR IR structures, mainly neuronal nuclei. After washing in PBS, the sections were transferred to buffered solutions of 5 and 20% methanol containing 0.5% $H_2O_2$ for 20 min. By this treatment the activity of the added biotinylated peroxidase is removed. After washing in PBS the sections were reincubated with rabbit antibodies against the following peptides: $\alpha$-MSH (Immuno Nuclear, Stillwater, MN, U.S.A.); rat corticotropin-releasing factor (CRF) (a gift from Dr W. Vale); rat growth hormone-releasing factor (GRF) (a gift from Dr W. Vale); GAL (Peninsula Lab., CA, U.S.A.); $\beta$-endorphin ($\beta$-END) (Amersham, Amersham, England); cholecystokinin (CCK-8) (a gift from Dr P. Frey [17], somatostatin (SRIF) (coded SS9, a gift from Dr R. Benoit); neurotensin (NT) (a gift from Dr G. Dockray); luteinizing hormone-releasing hormone (LHRH) (a gift from Dr Fasano); enkephalin (ENK) (a gift from Dr R. Elde [18]), porcine NPY (Peninsula Lab, CA, U.S.A.); thyrotropin-releasing hormone (TRH) [19]; vasoactive intestinal polypeptide (VIP) [20]; and dynorphin [21]. The antibodies were diluted 1/1000 and the incubation was performed at 4°C overnight. The avidin–biotin system was once again used with 4-chloro-1-naphtol as chromogen. After the staining the sections were mounted on gelatin-coated slides, coverslipped in glycerol–phosphate buffer (3/1) and studied by light microscopy (Nikon, Microphot FX). In other experiments a standard two-color immunofluorescence protocol was used involving a goat antimouse IgG conjugated with fluoresceine isothiocyanate (FITC) (Boehringer, Mannheim, Germany) (for GR) and goat antirabbit IgG conjugated with Texas Red (Amersham) (for peptides) [22].

Table 1. Glucocorticoid receptors and arcuate peptide neurons

| Area | Density of GR IR neurons | Density of peptidergic neurons | | | | Proportion of GR IR peptidergic neurons | | | |
|---|---|---|---|---|---|---|---|---|---|
| Arcuate nucleus | High (strong immunoreactivity) | GRF/GAL medium | NPY medium–high | | β-endorphin medium | GRF/GAL all | NPY all | β-Endorphin all | |
| | | CCK low | NT medium | SRIF low | ENK medium | CCK all | NT all | SRIF all | ENK all |

The vast majority of the peptidergic neurons of the arcuate nucleus are targets of action of GC. Data based on Refs [6, 7, 9, 10, 12] and unpublished data.

Table 2. Glucocorticoid receptors in central amygdaloid and paraventricular peptide neurons

| Area | Density of GR IR neurons | Density of peptidergic neurons | | | Proportion of GR IR peptidergic neurons | | |
|---|---|---|---|---|---|---|---|
| Central amygdaloid nucleus | High (strong intensity) | SRIF low-medium | CCK very few | ENK scattered | SRIF all | CCK all | ENK very high |
| | | DYN scattered | CRF scattered | NT low–medium | DYN all | CRF all | NT very high |
| PaFp + PVI | High (strong intensity) | CRF high | TRH high | SRIF high | CRF all | TRH all | SRIF very high |
| | | NT medium | ENK medium | CCK medium | NT all | ENK all | CCK high |
| PaFm | Few | Vasopressin high | Oxytocin high | | Vasopressin none | Oxytocin none | |

Central amygdaloid and parvocellular paraventricular peptide neurons are major targets for glucocorticoid action. Data based on Refs [6–8, 10] and unpublished data.

Table 3. Glucocorticoid receptors and cortical peptide neurons

| Area | Density of GR IR neurons | Density of peptidergic neurons | | Proportion of GR IR peptidergic neurons | |
|---|---|---|---|---|---|
| Neocortex | High (moderate immunoreactivity) | SRIF scattered | CCK scattered | SRIF none | CCK low (layer VI) |
| | | VIP scattered | NPY scattered | VIP none | NPY none |
| | | αMSH (pyramidal cells) medium | | αMSH (pyramidal cells) high | |

The neocortical peptidergic interneurons are not targets of action of GC. Data based on Refs [5, 12] and unpublished data.

## RESULTS

### *Colocalization of GR and peptides in hypothalamic hormone containing neurons (see Tables 1–5)*

*CRF IR neurons.* Strong nuclear GR IR has been discovered in all the CRF IR neurons of the parvocellular part of the paraventricular hypothalamic nucleus (PaFp) projecting into the external layer of the median eminence [6, 7, 10] as well as of the central amygdaloid nucleus. In contrast, only about 50% of the CRF IR nerve cells of the bed nucleus of the striae terminalis and of the preoptic area show GR IR, in this case of medium to strong intensity [7, 10].

*TRH IR neurons.* All the TRH IR neurons of the medial and dorsal parvocellular part of the paraventricular hypothalamic nucleus are strongly GR IR, which is also the case for those in the other parts of the dorsal hypothalamus [6, 8, 10]. In contrast, the TRH IR neurons within the medial tuberal area, the perifornical nucleus, and the medial preoptic area are only weakly GR IR or lack GR IR. The TRH IR neurons projecting to the median eminence (from PaFp), however, appear all to be strongly GR IR.

*GRF IR neurons.* All the GRF IR nerve cell bodies within the lateral magnocellular part of the arcuate nucleus show moderate to strong nuclear GR IR. Thus, all the GRF IR neurons projecting into the median eminence can be directly controlled by GC [7].

*SRIF IR neurons.* The SRIF IR neurons projecting into the median eminence and releasing SRIF to inhibit growth hormone secretion have their cell bodies within the anterior periventricular hypothalamic nucleus. Around 50% of the large SRIF IR nerve cells of this region showed strong nuclear GR IR [6, 10], while the

Table 4. Glucocorticoid receptors and neostriatal peptide neurons

| Area | Density of GR IR neurons | Density of peptidergic neurons | | | | Proportion of GR IR peptidergic neurons | | | |
|---|---|---|---|---|---|---|---|---|---|
| Neostriatum | High (moderate GR IR) | ENK high | NPY scattered | SRIF scattered | NT low | ENK all | NPY none | SRIF none | NT all |

Neostriatal enkephalin IR neurons are a major target of actions of glucocorticoids. Data based on Ref. [12] and unpublished data.

Table 5. Glucocorticoid receptors and lateral hypothalamic peptide neurons

| Area | Density of GR IR neurons | Density of peptidergic neurons | | | Proportion of GR IR peptidergic neurons | | |
|---|---|---|---|---|---|---|---|
| Lateral hypothalamus + perifornical area | Medium (moderate–strong intensity) | MCH Medium | NT low | DYN low | MCH very low (weak IR) | NT all (strong IR) | DYN none |
| | | ENK low | GAL low | SRIF low | ENK none | GAL high | SRIF very low |

Neurotensin neurons are major targets for glucocorticoids in the lateral hypothalamus. Data based on Ref. [9] and unpublished data.

other 50% showed a weak GR IR in their nuclei. Thus, a large heterogeneity exists among the large SRIF IR neurons in this region with regard to their content of GR. In contrast, all the parvocellular SRIF IR nerve cells of this area showed strong nuclear GR IR, which was true also for the arcuate SRIF IR nerve cells (Fig. 1) A moderate to strong GR IR was also observed in all the SRIF IR cell bodies of the central amygdaloid nucleus. However, all the other areas analyzed namely suprachiasmatic nucleus, the piriform cortex, the neocortex, the

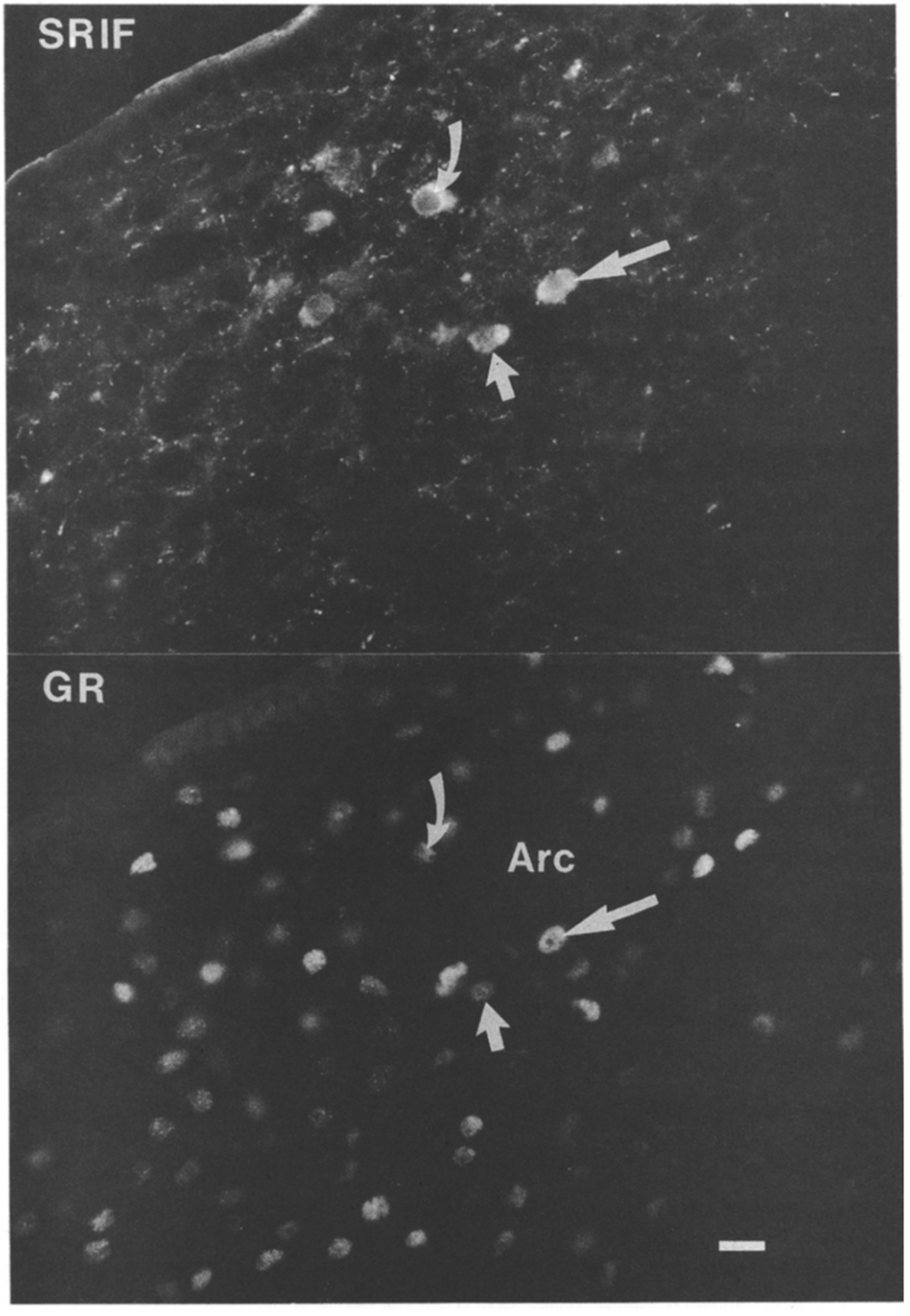


Fig. 1. Somatostatin (SRIF) and glucocorticoid receptor (GR) immunoreactivities (IR) are shown by the double immunofluorescence technique in a 14 $\mu$m thick coronal section of the rat arcuate nucleus. The rat was pretreated with colchicine (100 $\mu$g/rat; i.v.t.; 24 h). SRIF IR is visualized by a secondary antirabbit antibody conjugated with Texas Red and the GR IR by a secondary antimouse antibody conjugated with fluoresceine isothiocyanate (FITC). All the SRIF neurons of the arcuate nucleus (Arc) show GR IR (arrows). Bar 50 $\mu$m. Bregma level $-2.30$ mm.

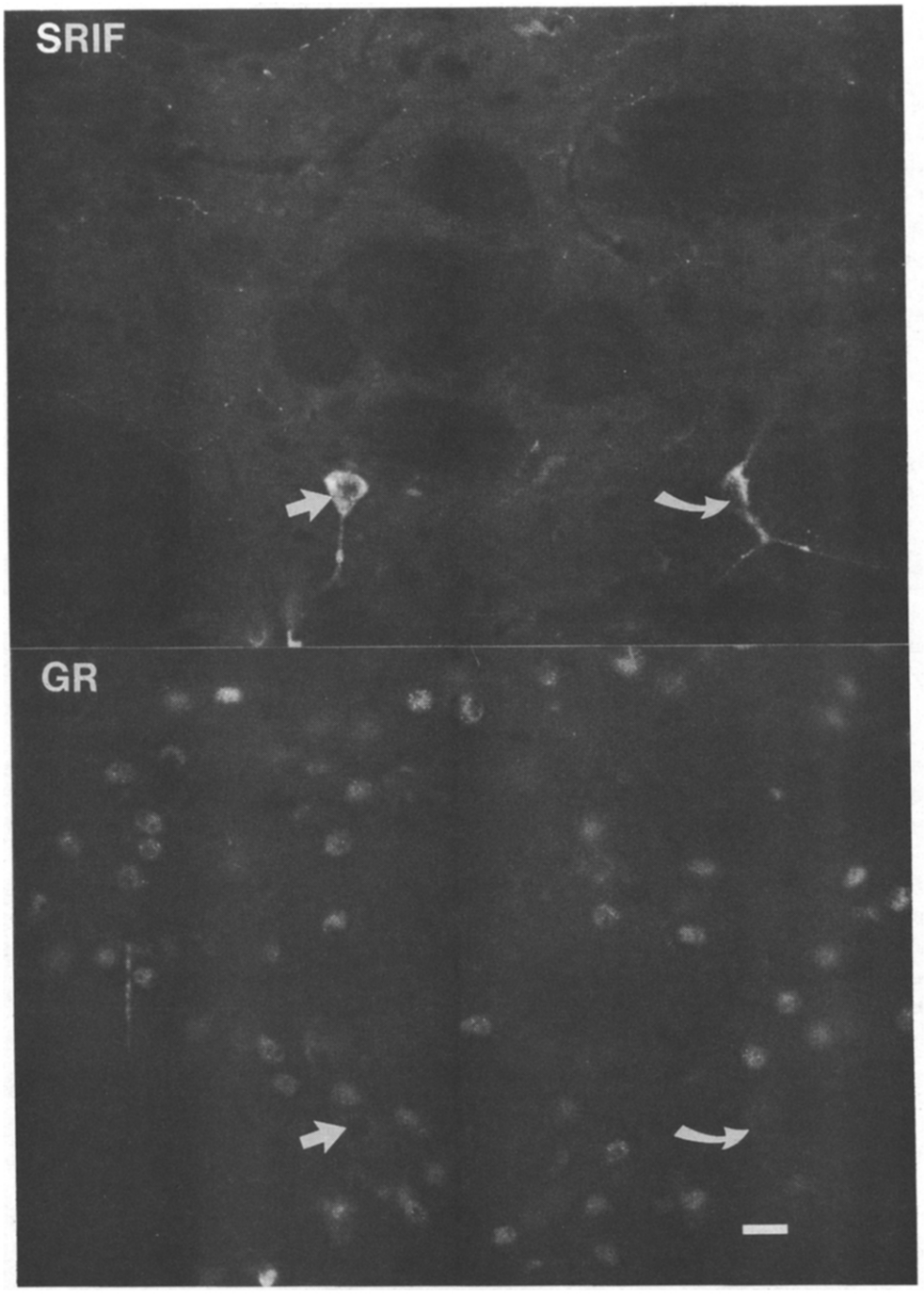


Fig. 2. Neostriatal somatostatin (SRIF) and glucocorticoid receptor (GR) immunoreactivities (IR) are demonstrated in a 14 $\mu$m thick coronal section of the rat brain at the Bregma level −0.20 mm. The SRIF IR neurons of the putamen lack GR IR. Bar 50 $\mu$m. For details, see legend to Fig. 1 and text.

neostriatum (Fig. 2), and the olfactory tubercle contained SRIF IR nerve cell bodies which all lacked nuclear GR IR [6, 10].

*LHRH IR neurons.* The LHRH cell bodies, which project to the median eminence, are all located in the rat within the septal region and the preoptic area. All of them were found to lack GR IR [10].

*Colocalization of GR and peptides in neurons containing opioid peptides (see Tables 1–5)*

*β-END IR neurons.* Strong nuclear GR IR has been demonstrated within all the β-END and α-MSH IR neurons of the arcuate and periarcuate nuclei [9, 10]. The β-END IR cell bodies are exclusively found within the arcuate

nucleus and thus all β-END cell bodies in the brain can be strongly and directly regulated by GC. β-END IR neurons project to autonomic areas such as the hypothalamus and the preoptic area as well as to the limbic regions. They appear to have an important role in mood and pain control as well as in the control of autonomic functions such as neuroendocrine function and cardiovascular functions [10]. It should be noticed that GC appear capable of counteracting prolactin (PRL) release induced by opioid agonists as well as the analgesia produced by morphine and morphinomimetic drugs [23, 24]. Proopiomelanocortin mRNA levels are also increased after adrenalectomy within the arcuate nucleus [25]. All these results suggest that GC can reduce transmission mediated via opioid peptides which, at least with regard to the actions in the arcuate nucleus, appear to involve a direct regulation of β-END synthesis. The actions of GC on β-END neurons can also be of importance for the effects of GC on mood.

*Dynorphin IR neurons.* A clear majority of dynorphin IR nerve cell bodies demonstrated within various hypothalamic areas such as the periventricular nucleus, the paraventricular hypothalamic nucleus, and the lateral hypothalamus lack GR IR. However, within the central amygdaloid nucleus practically all the dynorphin IR cell bodies demonstrate a strong GR IR. Furthermore, weakly GR IR small dynorphin cell bodies have been identified within the bed nucleus of the striae terminalis and within the medial part of the preoptic area, and 50% of the dynorphin cells of the arcuate nucleus are GR IR. These results are in contrast to those obtained on the β-END neurons which all appear to be strongly and directly regulated by GC. In addition, it should be emphasized that moderate to strongly GR IR dynorphin-positive cell bodies were demonstrated within the most medial part of the ventromedial hypothalamic nucleus. Ventromedial hypothalamic nerve cell bodies are of special interest since they also contain a moderate cytoplasmatic GR IR, while the vast majority of other GR IR nerve cells contain only a weak cytoplasmatic GR IR or lack detectable cytoplasmatic GR IR. Finally, there exists a dense dynorphin innervation of the ventral and lateral part of the anterior hypothalamic and preoptic area, which contains large clusters of strongly GR IR nerve cells, in which a substantial degree of cytoplasmatic GR IR can be demonstrated.

*Enkephalin IR neurons.* The most striking finding was the observation that the vast majority of the small ENK IR nerve cell bodies within the neostriatum showed a weak to moderate nuclear GR IR. ENK IR nerve cell bodies with moderate GR IR have also been demonstrated within the lateral and ventral part of the preoptic area (innervated by dynorphin cells, see above) and strongly GR IR nerve cells containing ENK IR have been demonstrated in the central amygdaloid nucleus and within the PaFp and the arcuate nucleus (Fig. 3). In the PaFp the ENK IR may be present in the CRF IR neurons. However, the large ENK IR neurons within the perifornical area and the lateral hypothalamus as well as those within the hippocampal formation lacked GR IR, which was true also for the small ENK IR nerve cells of the dorsomedial and ventromedial hypothalamic nucleus. These results emphasize a differential regulation of ENK IR nerve cells and the existence of possible direct regulation by glucorticoids of enkephalinergic mechanisms within the neostriatum, PaFp and arcuate nucleus. Thus, a major action of GC on the basal ganglia may be exerted via effects on enkephalinergic neurons. It should be emphasized, however, that this direct regulation by GC of the enkephalinergic neurons of the basal ganglia also may importantly contribute to the ability of GC to participate in mood regulation, together with their effects on β-END neurons, since enkephalinergic neurons, especially within the nucleus accumbens, participate in the networks subserving reward mechanisms.

*Colocalization of GR and peptides in neurons containing neuropeptides of the gastrointestinal type (see Tables 1–5)*

*CCK IR neurons.* Large numbers of nerve cells containing moderate GR IR have been demonstrated within several thalamic nuclei. For the first time it has now become possible to show the transmitter identity of some of the thalamic nerve cells having GR IR. Thus, many of the moderately GR IR nerve cells of the thalamic midline nuclei, such as the mediodorsal thalamic nucleus, the centromedial thalamic nucleus and nucleus reuniens showed CCK IR. Some strongly GR IR/CCK IR nerve cell bodies could be demonstrated within the dorsal part of the parvocellular part of the paraventricular hypothalamic nucleus and the arcuate nucleus but few within the periventricular hypothalamic region. A weak to moderate GR IR was demonstrated within the CCK IR

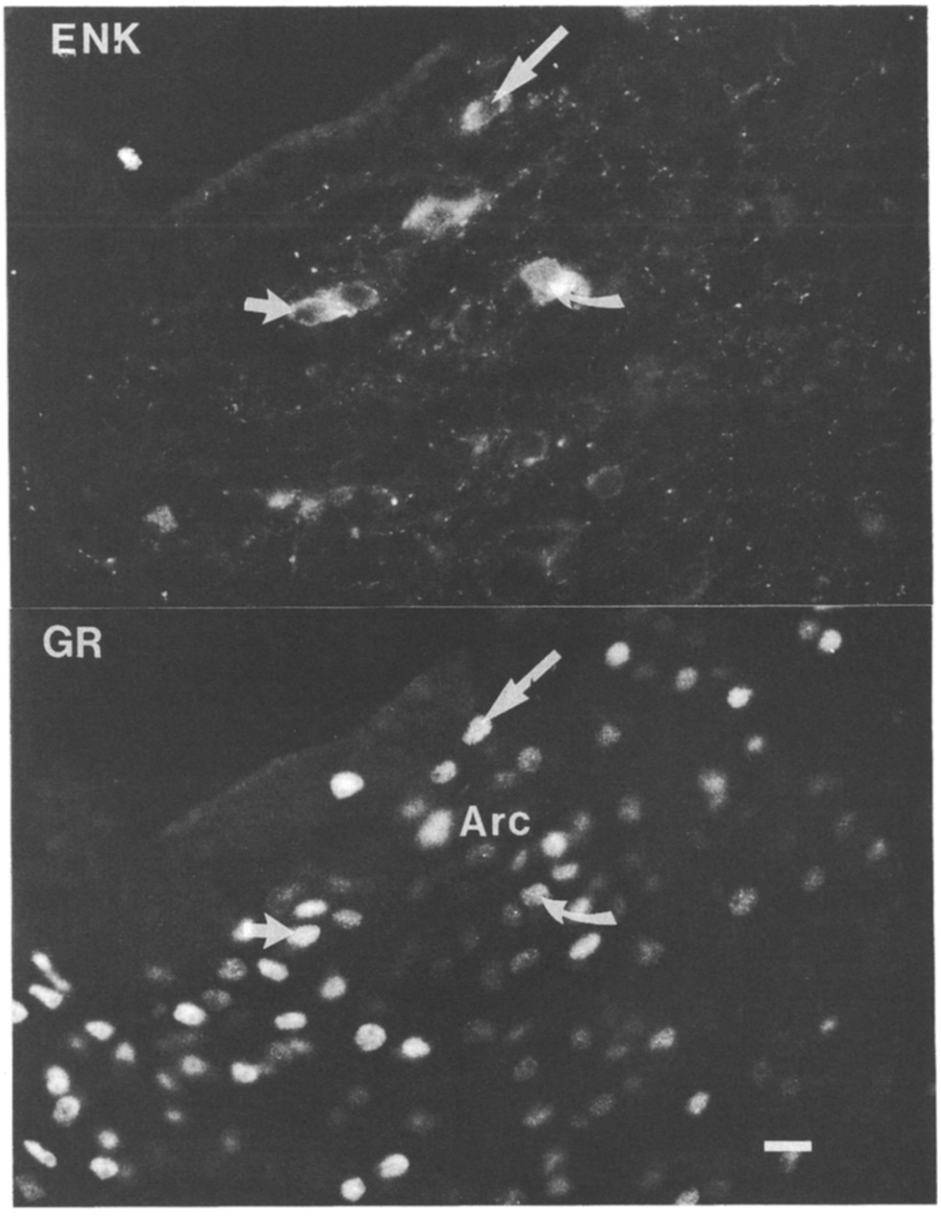


Fig. 3. Coronal section of the rat arcuate nucleus (14 $\mu$m thick) after pretreatment with colchicine. The enkephalin (ENK) IR and GR IR are displayed by using fluorescent antiantibodies conjugated with Texas Red and FITC, respectively. All the ENK IR neurons of the arcuate nucleus show GR IR. Bar 50 $\mu$m. Bregma level −2.80 mm. For details, see legend to Fig. 1 and text.

nerve cells of the ventromedial tegmental area and within the zona compacta of the substantia nigra. These nerve cells probably mainly represent DA/CCK costoring neurons [26]. The latter results are in line with the previous demonstration by Härfstrand *et al.* [11] that many of the DA neurons of the ventromedial tegmental area and of the substantia nigra show weak to moderate nuclear GR IR. In contrast, the large and strongly CCK IR cell bodies of the nucleus linearis rostralis showed no or only a very weak nuclear GR IR. Also the vast majority of the CCK IR nerve cells of the outer and inner layers of the neocortex and within the hippocampal formation (Fig. 4) lacked GR IR with the exception of some CCK IR cells in layer VI which showed a weak to moderate GR IR. An impressive finding was the dense CCK innervation of layer VI of the neocortex, especially within the ventral part of the neocortex and piriform cortex, a layer which is built up of huge numbers of moderately to strongly GR IR nerve cells. These results in layer VI suggest that GC may directly control CCK receptor mechanisms.

*NT IR neurons.* The most striking finding has been the first demonstration of the transmit-

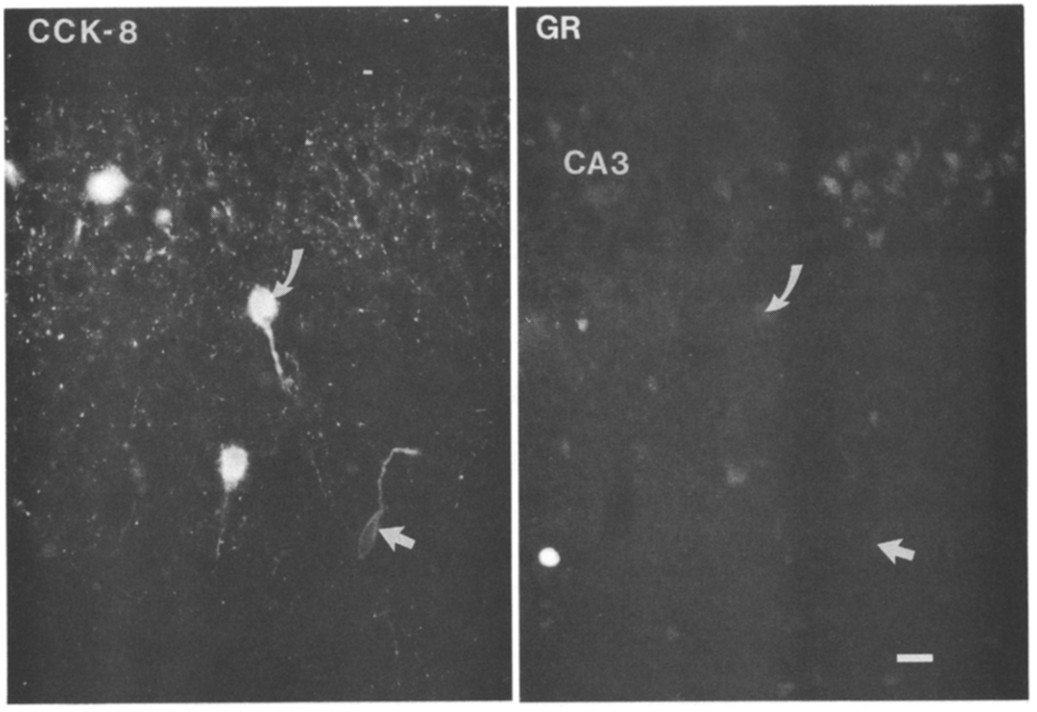


Fig. 4. Coronal section of the dorsal hippocampus (14 μm thick) stained for GR and CCK IR by using FITC and Texas Red conjugated antiantibodies, respectively. CCK IR neurons of the stratum radiatum lack GR IR. Notice that the pyramidal cells of the area CA3 are devoid of GR IR. Colchicine-treated rat. Bar 50 μm. Bregma level −2.80 mm. For details, see legend to Fig. 1 and text.

ter identity of the strongly GR IR nerve cells of the lateral hypothalamus of the perifornical area. Thus, the vast majority of the NT IR cells of these regions were demonstrated to be strongly GR IR. Also within the PaFp and the arcuate nucleus practically all the NT IR cells showed a strong nuclear GR IR; a few showed a weak GR IR. A weak to moderate GR IR was demonstrated in the vast majority of NT IR cells within the lateral preoptic region, within the area of the ventral pallidum, within the periventricular part of the preoptic area and within the neostriatum. Furthermore, the NT IR nerve cells of the ventral amygdaloid nucleus showed a moderate nuclear GR IR. It will be of substantial interest to evaluate how the GC can influence the NT neurons of the brain since they all are directly regulated by GC and large numbers contain strong GR IR.

*VIP IR nerve cells.* All the VIP IR bipolar neurons of the neocortex have been found to lack GR IR.

*Coexistence of GR and peptides in other types of peptidergic neurons (see Tables 1–5)*

*GAL IR neurons.* The vast majority of the GAL-positive nerve cell bodies have been demonstrated to be weakly to strongly GR IR within the dorsolateral hypothalamus, the perifornical region and the arcuate nucleus. The dorsomedial hypothalamic nucleus, the medial amygdaloid cortex, the paraventricular hypothalamic nucleus and the preoptic area mainly contain GAL IR neurons that lack GR IR. This is true also for the acetylcholine/GAL costoring neurons of the basal forebrain. Thus, in view of the fact that the majority of the 5-HT nerve cell bodies of the midbrain are strongly GR IR and also contain GAL IR [11, 27], it seems likely, based on the present findings, that the 5-HT/GAL costoring neurons and certain hypothalamic neurons are differentially affected by GC.

*NPY IR neurons.* Strong GR IR has been demonstrated within the catecholamine (CA) and NPY costoring neurons of the medulla oblongata as well as within all of the NPY IR nerve cell bodies found within the parvocellular part of the arcuate nucleus [12]. However, the NPY IR neurons of the neocortex, the hippocampal formation and the neostriatum appear to lack GR IR. Thus, a differential regulation of central NPY neurons exists.

*Melanin-concentrating hormone IR neurons.* The melanin-concentrating hormone (MCH) IR nerve cells of the dorsolateral hypothalamus

and the zona incerta [28] contain epitopes in the MCH precursor which are recognized by antisera raised against α-MSH and also rat CRF and certain human GRF fragments. They can therefore be mapped out using antisera against α-MSH [29, 30]. Using α-MSH IR as a marker for MCH containing nerve cells we have been able to demonstrate that the vast majority of the MCH IR nerve cells lack or contain only a weak GR IR. Thus, the large MCH containing neurons of the lateral hypothalamus and zona incerta which project especially to the cortical areas and which are involved in controlling arousal are not directly controlled by GC. Thus, the striking finding is the frequent absence of GR IR in this huge cortical projection system of the lateral hypothalamus, probably producing MCH [9].

## GENERAL DISCUSSION

The present findings give clearcut evidence that all the families of neuropeptide neurons in the CNS can contain nuclear GR IR and that marked heterogeneities frequently exist in each of the peptidergic neuronal systems depending upon the area analyzed. Thus, the conclusion is that the function of the area mainly determines the pattern of GR IR in the various peptide neurons in that area (see Tables 1–3). Within the arcuate nucleus, the PaFp and the central amygdaloid nucleus all the different peptide neurons are strongly GR IR, while the same peptide neurons show no GR IR in neocortex and hippocampal formation with the exception of the CCK IR nerve cells of layer VI. Thus, GC appear to play a crucial role in the information handling in certain limbic and hypothalamic nuclei. This global influence of GC in certain nuclei may allow the GC to alter the set point of neuronal activity in that nucleus as well as the set point for peptide synthesis.

Marked differences in GR IR have been demonstrated within members of the same family of peptidergic neurons such as those producing hypothalamic hormones. Thus, the evidence indicates that strong GR IR exist within practically all CRF, GRF, SRIF and TRH IR neurons projecting to the median eminence and releasing CRF, GRF, SRIF and TRH into the portal vessels, while all the LHRH IR nerve cells projecting to the median eminence appear to lack GR IR. Since the GC may exert a direct long latency, slow feedback inhibition of CRF, GRF, SRIF and TRH synthesis, a failure to demonstrate GR IR in LHRH IR neurons may reflect the need for a maintained LHRH synthesis in stress, so that reproductive function can be maintained. It must also be emphasized when using the present antibodies against the GR [14] it has never been possible to demonstrate GR IR within the oxytoxin and vasopressin IR nerve cells of the magnocellular part of the paraventricular hypothalamic nucleus (PaFm) projecting to the neural lobe. Nor has GR mRNA levels been found within the PaFm [10]. Thus, vasopressin and oxytocin neurons of the magnocellular paraventricular hypothalamic nucleus may lack a direct regulation by GC in contrast to the CRF, SRIF and TRH IR neurons of the parvocellular part.

A marked heterogeneity with regard to GR IR is well documented in the case of the central NPY IR neurons [12] and the central GAL IR neurons. Thus, e.g. the 5-HT/GAL IR neurons of the raphe nuclei contain strong GR IR, while the acetylcholine/GAL IR systems of the basal forebrain all completely lack GR IR, and the presence of GR IR among the GAL IR nerve cells demonstrated within hypothalamic and preoptic areas varies.

One of the most striking findings of the present paper are also the marked differences among the opioid peptide neurons with regard to their contents of nuclear GR IR. β-END IR neurons are strongly regulated by GC by a direct action, while the majority of the DYN IR cell bodies of the hypothalamus demonstrated so far lack GR IR. Therefore, the β-END but not the dynorphin neurons (except those of the central amygdaloid nucleus and many of the arcuate nucleus) are primary targets for glucocorticoid action. Heterogeneities exist among ENK IR neurons with regard to GR IR. Thus, the small ENK neurons of the basal ganglia are all moderately GR IR (Table 4) and those of the arcuate nucleus are all strongly GR IR, while those of the lateral hypothalamus lack GR IR. These results, thus, emphasize that the glucocorticoid modulation of opioid peptide neurotransmission at the presynaptic level may mainly involve actions on β-END and on some enkephalinergic neurons, including all those of the basal ganglia which may represent substrates for the ability of GC to control sensory motor integration (ENK), mood (ENK, β-END) and pain mechanisms (ENK, β-END).

We have in the present paper also obtained evidence that all the NT IR neurons of the brain

represent targets for direct GC actions. The NT neurons of the lateral hypothalamus and the perifornical area are of special interest, since all of these NT neurons contain strong GR IR (Table 5). It now becomes of substantial interest to evaluate the action of GC on brain NT mechanisms and to *inter alia* determine the projections of the lateral hypothalamic NT nerve cells. In contrast, the MCH IR neurons of the dorsolateral hypothalamus having *inter alia* cortical projections contain no or only a very weak GR IR. Thus, marked heterogeneities exist among peptidergic nerve cells of the lateral hypothalamus with regard to their contents of GR IR (Table 5). Furthermore, the acetylcholine neurons of the basal forebrain projecting to the cortical regions and increasing arousal are not directly affected by GC. In contrast, the NAergic and 5-HTergic projections to the cerebral cortex which control sleep-wakefulness mechanisms are directly and strongly influenced by GC in view of the existence of strong GR IR in all NA nerve cell bodies and 5-HT nerve cell bodies [11].

Finally, within the thalamus, neuropeptides may also be involved in mediating the actions of GC in view of the demonstration of CCK IR neurons within the midline thalamic nuclei containing moderate GR IR.

In conclusion, peptidergic neurons represent major targets of action for GC. The demonstrated heterogeneities among individual peptide neurons with regard to their contents of GR make possible a further subclassification of the various types of peptidergic neurons in the brain. The function of the area analyzed and not the type of peptide appears to be the major factor determining the degree of GR IR demonstrated in the various peptide neurons of that area. Each area possesses its own pattern of GR IR among the peptide neurons. Our evidence suggests that the GC can regulate many peptidergic neurons via a presynaptic genomic action to produce alterations in peptide synthesis and release. In view of the many examples of a dense peptide innervation of large numbers of GR IR nerve cells in many areas, GC probably also regulate peptide receptor synthesis and efficacy, which may involve effects also on G proteins, regulating receptor function. In this way combined pre- and postsynaptic actions of GC may make possible a concerted action on peptide neurotransmission resulting in an overall reduction or increase of transmission.

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