MINIREVIEW

Intracellular Localization of Corticosteroid Receptors in Brain: Potential Interactions with Signal Transduction Pathways (43503B)

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Adrenal corticosteroids have diverse actions in the central nervous system (CNS). Glucocorticoids, the major adrenal corticosteroids, regulate the actions of several classical neurotransmitters and peptides, neuronal and glial proliferation and differentiation, neuronal survival, cognitive function, and behavior (1). Mineralocorticoids, e.g., aldosterone, regulate salt appetite and blood pressure (2, 3). As with other members of the steroid receptor superfamily, most actions of adrenal corticosteroids are mediated by intracellular receptors (4). Activated corticosteroid receptors bind to a glucocorticoid response element and interact with other transcription factors to regulate the expression of various genes (5–8). Two types of corticosteroid receptors have been described (7–9). A Type I receptor, which is identical to the classical mineralocorticoid receptor of the kidney, salivary glands, and colon, has a high affinity (K_D = 0.5 nM) and low capacity for endogenous glucocorticoids in the brain. It binds preferentially to corticosterone in the limbic brain, and presumably mediates the effects of circadian levels of circulating corticosterone on CNS function. In the circumventricular regions and ventrolateral hypothalamus, the Type I receptor shows a preference for aldosterone, and may mediate the central effects of this mineralocorticoid on salt appetite and blood pressure. A lower affinity (K_D = 2.5–5 nM), high capacity Type II receptor, which is identical to the classical glucocorticoid receptor of the liver, has also been described in the CNS. It is thought to mediate the effects of stress levels of endogenous glucocorticoids and synthetic glucocorticoids, e.g., dexamethasone, on CNS function and central negative feedback regulation of plasma glucocorticoid levels.

Corticosteroid Receptor Immunoreactivity in the CNS

The availability of antibodies against Type I (10–12) and Type II (13–15) corticosteroid receptors has facilitated immunocytochemical studies on their distribution in various target tissues. Using MINREC antisera against peptides derived from the hinge and variable domains of the human renal Type I receptor cDNA (10, 11), and BUGR2 monoclonal antibody against rat liver Type II receptor (15), we have demonstrated the expression of both types of corticosteroid receptors in limbic, motor, sensory, and visceral neurons in the rat CNS (16–18). In most regions, the density of Type II-immunoreactive (ir) neurons is higher than Type I-ir neurons. In the locus ceruleus, neurons express only Type II-ir. However, in the pyramidal layer of field CA3 of Ammon's horn, globus pallidus, and entopeduncular and reticular thalamic nucleus, the density of Type I-ir neurons is more than that of Type II-ir neurons. Some large neurons in the caudate-putamen, and neurons in the hypoglossal nucleus express only Type I-ir.

Most glia express Type II-ir. Type I-ir is restricted to glia in a few fiber systems, e.g., the corpus callosum, spinal trigeminal tract, and funiculi of the spinal cord. Cells with Type I-ir are observed in the subfornical organ, but not in other circumventricular organs. In

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contrast, Type II-ir is distinctly absent in all circumventricular organs. The widespread overlap in corticosteroid receptor immunoreactivity in neurons is in agreement with the distribution of Type I and Type II receptor mRNA, but contrary to the patterns of binding of radiolabeled mineralocorticoids and glucocorticoids. Binding of [^{3}H]aldosterone, as a marker of Type I receptors, is reported to be restricted to the septohippocampus, parts of the hypothalamus, and circumventricular organs (1, 19). In contrast, binding by [^{3}H] dexamethasone and other specific glucocorticoids was demonstrated at all levels of the CNS (1, 19). We (16) have suggested that discrepancies in the location of the Type I receptor as revealed by immunocytochemistry, *in situ* hybridization, and binding to cognate steroids may arise from differences in the sensitivities of these methods. Alternatively, if one assumes that binding studies are sensitive enough to detect low levels of Type I receptors, a factor that could potentially restrict Type I receptor binding sites in spite of widespread expression of the Type I receptor gene is posttranslational modification of the receptor, e.g., phosphorylation, into a form capable of binding to aldosterone or other cognate steroids, e.g., corticosterone. Regional differences in the kinetics of posttranslational modification of the Type I receptor may determine its ability to bind to cognate steroids.

Subcellular Distribution of Corticosteroid Receptors

An advantage of immunocytochemical detection of corticosteroid receptors is the ability to resolve the intracellular location of the reaction product. The intracellular location of Type I-ir and Type II-ir in neurons of the rat varies according to circulating levels of corticosteroids (16, 18). Figures 1 and 2 illustrate the intracellular location of corticosteroid receptor immunoreactivity in neurons and classical mineralocorticoid and glucocorticoid targets.

Neuronal Type I-ir in adrenally intact rats is either predominantly nuclear (Fig. 1A) or diffusely nuclear and cytoplasmic (16). Scattered neurons in the brainstem reticular formation show mainly cytoplasmic Type I-ir (Fig. 1B). In most neurons, nuclear Type I-ir is unresolvable with light microscopy 4 weeks after adrenalectomy (ADX), but weak diffuse cytoplasmic Type I-ir is often present. A few neurons in the globus pallidus, motor trigeminal, and motoneurons in the spinal cord retain nuclear Type I-ir. Treatment with either aldosterone or corticosterone for 2 hr restores nuclear Type I-ir, but not to the level found in intact rats. There is a greater response of neuronal Type I-ir to treatment with corticosterone than to aldosterone. In comparison, Type I-ir in mineralocorticoid target tissues of adrenally intact rats, e.g., kidney, salivary glands, and colon, is also located in the nucleus and cytoplasm (10–12). In ductal epithelial cells of the parotid gland (Fig. 1C), colonic epithelial cells, and distal renal tubular cells, cytoplasmic Type I-ir is mostly localized to the apical (luminal) surface. As with Type I-ir in the CNS, Type I-ir in the parotid gland and colon disappears slowly (weeks) after ADX. Treatment with either aldosterone or corticosterone restores diffuse cytoplasmic Type I-ir within 1 hr, but has no significant effect on apical and nuclear Type I-ir (11). In contrast, in distal renal tubular cells, ADX has no significant effect on Type I-ir (10).

In intact adult males and females, the majority of neurons show a nuclear location of Type II-ir, although weak diffuse cytoplasmic immunoreactivity is often resolvable (17, 18, 20–22). At the ultrastructural level, the reaction product in cells with nuclear Type II-ir is organized into patches of variable size, which may be related to the pattern of distribution of chromatin (23, 24). For example, in the cerebellar cortex, granule cells have large patches of Type II receptor reaction product, whereas Purkinje cells have more numerous, smaller patches (24). In the adult female hippocampus, interneurons with intense diffuse or mainly cytoplasmic Type II-ir are present in the stratum oriens (25). During development, similar Type II-ir interneurons are observed transiently on postnatal days 25 and 30 (26). In contrast, in adult males, only a few immunoreactive glia are present in the stratum oriens (17, 18). In both sexes, there are two types of responses of nuclear Type II-ir to ADX (18). In the majority of neurons, nuclear Type II-ir disappears after ADX (Fig. 1, D and E). Depending on the antibody used, nuclear Type II-ir is unresolvable with light microscopy anywhere from a few days to weeks after ADX (8, 18, 20–22). Using BUGR2 monoclonal antibody, we (18) observed a rostrocaudal variation in the response of neurons to ADX. In the forebrain, nuclear Type II-ir was unresolvable with light microscopy 2 weeks after ADX. In contrast, nuclear Type II-ir persisted in some neurons in the hindbrain, e.g., the spinal cord and the motor trigeminal nucleus, 4 weeks after ADX. Contrary to previous reports describing an increase in cytoplasmic Type II-ir after ADX (8, 20–22), we (18) did not observe a similar increase with BUGR2; rather, both nuclear and cytoplasmic Type II-ir were unresolvable with light microscopy after long-term ADX. Treatment with corticosterone restored nuclear Type II-ir within minutes. Aldosterone treatment, despite increasing Type II-ir, was incapable of fully restoring it to levels observed in intact rats. Because the above pattern of response of nuclear Type II-ir to ADX and corticosterone treatment was similar to that described by other investigators using antibodies against different epitopes of the rat liver Type II receptor (8, 20–22), we (18) described it as the typical or Type A response.

A subgroup of neurons in the hippocampal pyramidal layer and stratum oriens (Fig. 1E), caudate-puta-

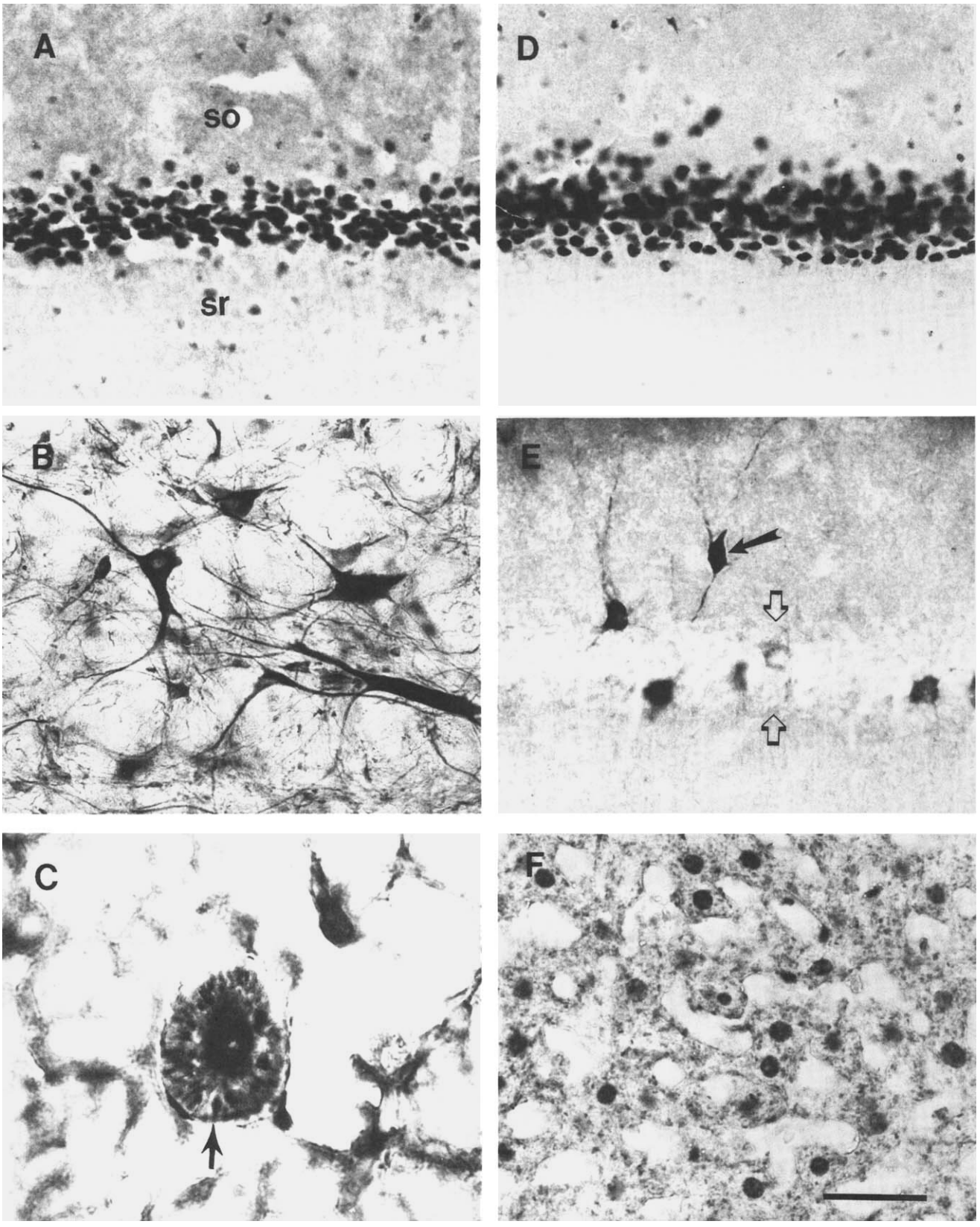

Figure 1. Photomicrographs illustrating corticosteroid receptor immunoreactivity in classical target tissues and the CNS. (A) Field CA1 of Ammon's horn showing a high density of neurons in the pyramidal layer with nuclear Type I-ir in an adrenally intact rat. Few labeled cells are present in the stratum oriens (so) and stratum radiatum (sr). (B) Gigantocellular reticular nucleus showing neurons with intense cytoplasmic Type I-ir of an adrenally intact rat. (C) Parotid gland showing Type I-ir in a striated duct. Note the intense immunoreactivity in the apical (luminal) region. Arrow marks cell with nuclear Type I-ir. (D) Field CA1 of hippocampus from high density of neurons with nuclear Type II-ir in the

men, globus pallidus, entopeduncular nucleus, reticular thalamic nucleus, habenula, and brainstem reticular formation, classified as Type B, showed intense diffuse or predominantly cytoplasmic Type II-ir after ADX (18). Immunoreactivity in these neurons was abolished within 5 min by corticosterone and to a lesser extent by aldosterone treatment. In the cerebellum, the majority of neurons showed a Type A response to ADX and corticosteroid treatment. However, in some vermal Purkinje cells, classified as Type C, cytoplasmic Type II-ir persisted 4 weeks after ADX (18, 23). Unlike the Type B cells described earlier, treatment with corticosterone increased the number of Type C Purkinje cells, whereas aldosterone had no effect. Cytoplasmic Type II-ir in Type C Purkinje cells extended into apical dendrites (Fig. 2A) and occasionally into axons. At the ultrastructural level, the reaction product for Type II receptors in the cytoplasm of Type C Purkinje cells was not associated with organelles; rather, it was located in the cytoplasmic matrix (23) (Fig. 2B). We could not, however, exclude a possible association between Type II receptor reaction product and free ribosomes and soluble cytoplasmic factors. This was in contrast to a previous report by Liposits *et al.* (23), who reported that in corticotropin-releasing hormone-producing neurons of the parvocellular paraventricular hypothalamic nucleus, cytoplasmic Type II-ir was localized over ribosomes and some vesicles.

In the liver (Fig. 1F) and most other glucocorticoid targets outside the CNS (27, 28), Type II-ir is located mainly in the nucleus in the presence of glucocorticoids, although weak cytoplasmic immunoreactivity is also present. However, in a few cases, e.g., epineprine-producing cells of the adrenal medulla, Type II-ir is mainly cytoplasmic (29). As in the CNS, ADX or depletion of culture media of glucocorticoids results in an abolition of nuclear immunoreactivity, whereas treatment with glucocorticoids restores it (20, 27, 28).

Implications of Intracellular Location of Corticosteroid Receptors

According to the classical model of corticosteroid receptor activation, the unbound receptor is located in the cytoplasm as a heterocomplex with cytoplasmic proteins, e.g., heat shock proteins (8, 30). After binding by cognate steroids, e.g., corticosterone and synthetic glucocorticoids in the case of the Type II receptor, the receptor is translocated into the nucleus (8, 30). Two nuclear-localizing signals, NL1 and NL2, have been described in a region close to the DNA-binding and the hormone-binding domains, respectively, of the rat Type II receptor (31). Although NL1 can translocate an unrelated protein into the nucleus in the absence of corticosteroids, its function appears to be repressed in the Type II receptor. NL2 requires corticosteroids to translocate the transformed Type II receptor into the nucleus, presumably by a process that involves microtubules (30).

Like most other members of the steroid receptor superfamily, corticosteroid receptors are phosphorylated (32–34). Type II receptors are phosphorylated mainly on serine residues in the variable (N-terminal) domain. Phosphorylation may regulate cycling of Type II receptors between nuclear and cytoplasmic compartments, thus regulating transcription by activated Type II receptors (35, 36).

In previous studies, the increase in the intensity of nuclear Type II-ir in response to glucocorticoid treatment, and loss of nuclear Type II-ir following ADX was thought to mimic the classical model of receptor activation (8, 22). However, apparent compartmentalization of corticosteroid receptor immunoreactivity may also depend upon the permeability of sections to antibody (8, 22, 28). For example, using the monoclonal antibody IgG 2a nr7, Wikstrom *et al.* (28) demonstrated that in a hepatoma cell line, high concentrations of Triton X-100, a detergent, increased the intensity of nuclear Type II-ir. However, in neurons, such a relationship has not been established (22). A low concentration of Triton X-100, 0.02%, has been shown to be sufficient for demonstrating both nuclear and cytoplasmic Type I-ir and Type II-ir (16–18, 20–23).

Regulation of the intracellular location of corticosteroid receptor immunoreactivity does not always correlate with the classical model of activation. For example, in neurons, the intensity of cytoplasmic Type I-ir and Type II-ir does not always increase after ADX, as would have been expected with the classical model (16, 18). Second, nuclear Type I-ir in distal tubular cells of the kidney, a classical mineralocorticoid target, is not abolished after ADX (10). Although nuclear Type I-ir in colonic and ductal epithelial cells of the parotid gland is abolished after ADX, there is no increase in nuclear Type I-ir in response to treatment with corticosteroids, as would have been expected in the classical model of activation; rather, there is an increase in diffuse cytoplasmic Type I-ir (11). Third, restoration of neuronal Type II-ir by glucocorticoids occurs so rapidly that it is unlikely that transport of pre-existing or newly synthesized cytoplasmic receptor into the nucleus could

pyramidal layer of an adrenally intact rat. (E) Field CA1 of hippocampus showing a 4-week-adrenalectomized rat. Note the absence of cells with nuclear Type II-ir in the pyramidal layer (i.e., Type A response). Open arrows mark dorsolateral extent of the pyramidal layer. Cells with intense cytoplasmic Type II-ir (closed arrow) are present in the stratum oriens and pyramidal layer (i.e., Type B response). (F) Liver showing hepatocytes with nuclear Type II-ir. Scale bar, 25 μm in A and 50 μm in others.

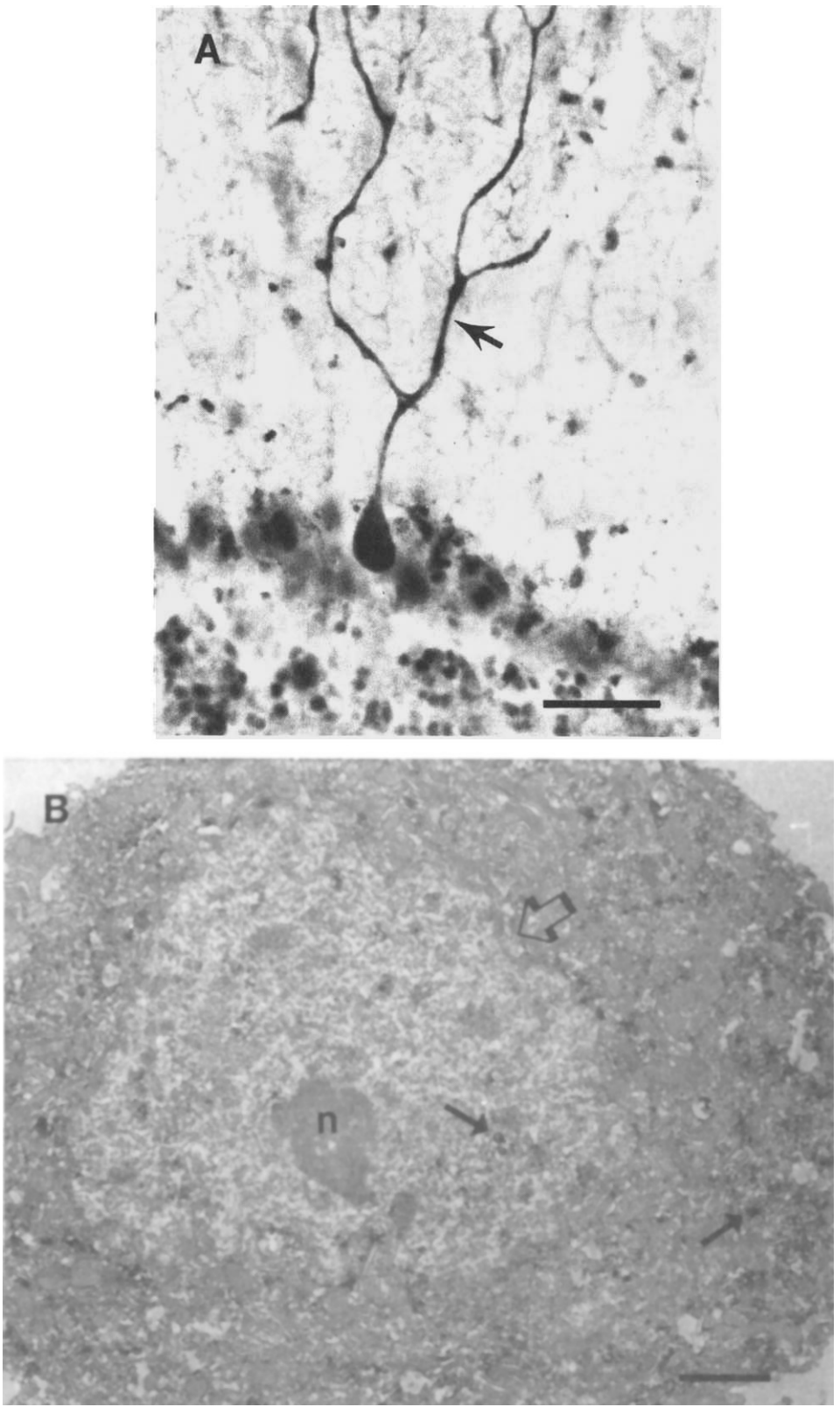

Figure 2. (A) Photomicrograph of a sagittal section of the cerebellum showing Type C Purkinje cell in vermal lobule 2 of a 4-week-adrenalectomized rat treated for 2 hr with corticosterone. Note the diffuse Type II-ir in the cell body and extension of immunoreactivity into apical dendrites (arrow). (B) Electron micrograph of a Type C Purkinje cell showing Type II-ir reaction product (closed arrows) in the nucleus and cytoplasm. Note the absence of immunoreactivity in the nucleolus (n). Open arrow marks the nuclear membrane. Scale bar, 50 μm in A, 1 μm in B.

account for the increase in nuclear Type II-ir (18). Although we cannot exclude the possibility that transport of corticosteroid receptors may account, at least in part, for changes in compartmentalization of corticosteroid receptor immunoreactivity after corticosteroid treatment, we (16, 18) have suggested that modulation of epitopes by corticosteroid-dependent factors in the cytoplasm and nucleus accounts in large measure for the ability to localize corticosteroid receptor immunoreactivity.

Nuclear Corticosteroid Receptor Immunoreactivity

The intracellular location of steroid receptors may be related to their sites of action in the cell. Receptors for estrogen, progesterone, and androgens have been localized almost exclusively in the nucleus in the presence of cognate steroids (37–40), a finding consistent with the mainly genomic role ascribed to them (4). Although corticosteroid receptors are located mainly in the nucleus, cytoplasmic Type I-ir and Type II-ir have been localized persistently in the presence of corticosteroids. Thus, while most studies have demonstrated a genomic role for corticosteroid receptors (1, 5–8) and have suggested that cytoplasmic corticosteroid receptor immunoreactivity represents inactive receptors, we speculate that some of the posttranscriptional actions of glucocorticoids (41, 42) may be mediated by cytoplasmic corticosteroid receptors. This concept is addressed further below.

The widespread distribution of both Type I and II receptors in the CNS suggests that they may mediate directly most of the diverse effects of adrenal corticosteroids on neuronal and glial functions (1). Nuclear Type II-ir has been localized in most catecholaminergic and serotonergic neurons, and several peptidergic neurons, in the rat brain (8, 22). Glucocorticoids regulate the synthesis and transport of several of these products. Because some of the genes involved in the synthesis of neurotransmitters/modulators, e.g., preproenkephalin (43), have glucocorticoid response elements in their regulatory regions, it has been suggested that glucocorticoids may regulate their expression directly via activation of corticosteroid receptors (43). The location of corticosteroid receptor immunoreactivity in the nuclei of neurons expressing these products would be consistent with this model.

Unlike binding of radiolabeled ligands to cytosolic corticosteroid receptors, immunocytochemistry does not quantify the levels or functional status of corticosteroid receptors. However, it is significant that nuclear Type I-ir and Type II-ir are restored to a greater extent by corticosterone, a glucocorticoid, and to a lesser extent by aldosterone, a mineralocorticoid. This suggests that in most regions of the CNS, both Type I and II receptors mediate the actions of glucocorticoids on gene expression, in agreement with previous binding studies (1). Activated Type I and II receptors bind to the same DNA response element, the glucocorticoid response element (44). It has been suggested that apart from mediating specific actions of corticosteroids in cells where they are expressed, coexpression of Type I and II receptors in neurons may serve to expand the range of response of genes with the glucocorticoid response element to varying levels of glucocorticoids (45, 46). At the same locus, the high-affinity low-capacity Type I receptor would mediate the effect of circadian levels of corticosterone on basal gene expression, while the low-affinity high-capacity Type II receptor mediates the actions of stress levels of corticosterone and pharmacological actions of glucocorticoids on gene expression. Our previous maps on the expression of Type I-ir and Type II-ir in the rat CNS suggest that since there is a regional variation in the degree of overlap in expression of corticosteroid receptors, this two-tier mechanism would confer considerable functional variability to glucocorticoid targets in the CNS.

There is considerable overlap in the regional distribution of neurons expressing corticosteroid receptors, other steroid receptors, and transcription factors (16, 17, 45, 47–49). Hormone-activated corticosteroid, progesterone, and androgen receptors bind to the same consensus response element, the glucocorticoid response element, as dimers (44). The estrogen receptor binds as a dimer to an estrogen-response element (44). In neurons where these receptors are coexpressed, they may interact to regulate the expression of target genes. For example, activated Type II corticosteroid, estrogen, and progesterone receptors, while binding to their respective response elements, interact to differentially regulate the expression of target genes (50, 51). Protein-protein interaction also occurs between Type II corticosteroid receptors and components of the transcription complex AP1 (c-*fos* and c-*jun*) at composite glucocorticoid response elements in several genes, resulting in antagonism of transcription (52–54). Interaction between Type II receptors and c-*fos* may explain the opposite effects of glucocorticoid treatment on Type II-ir and *fos*-ir in the paraventricular hypothalamic nucleus (55). ADX results in a loss of nuclear Type II-ir in parvocellular cells and a concommitant increase in *fos*-ir in the same cells, whereas treatment with corticosterone reverses this pattern. Increased *Fos*-ir may represent an up-regulation of *Fos* synthesis in the absence of glucocorticoids. On the other hand, interaction between activated Type II receptors and *Fos* at the same locus may mask immunogenic sites on Type II receptor while unmasking epitopes on *Fos*. Interestingly, increased *Fos*-ir in response to ADX occurs in all corticotropin-releasing hormone-ir neurons and few vasopressin-ir neurons. The functional implications of this relationship are unclear; however, it is possible that

Fos may play a role in negative feedback regulation of these peptides by circulating glucocorticoids.

Cytoplasmic Corticosteroid Receptor Immunoreactivity

As discussed earlier, the presence of cytoplasmic corticosteroid receptor immunoreactivity in neurons varies according to the type of receptor, the region of the CNS, age, sex, and levels of circulating corticosteroids. In most cells, only weak cytoplasmic corticosteroid receptor immunoreactivity is present in the presence of corticosteroids. In the classical model of steroid receptor activation, cytoplasmic immunoreactivity may represent inactive receptor (8). Heat shock proteins, notably hsp90, have been proposed to be part of a heterocomplex of unactivated cytoplasmic Type II receptors (8, 30), and are thought to inhibit receptor transformation (30). Hsp90 dissociates from Type II receptors after activation by glucocorticoids (8, 30). Apart from heat shock proteins, cytoplasmic Type II-ir forms complexes with other proteins (56) and microtubules (30, 57, 58). Tubulin-ir has been associated with hsp90 (30) and Type II-ir (57, 58). Because the latter products segregate according to the pattern of microtubules (30, 58), it has been suggested that microtubules may be involved in the transport of activated Type II receptors from the cytoplasm into the nucleus.

The presence of mainly cytoplasmic corticosteroid receptor immunoreactivity in some neurons, and especially in mineralocorticoid target tissues of intact and corticosteroid-treated rats, suggests that cytoplasmic corticosteroid receptors may play a more active role in mediating some actions of corticosterone and aldosterone.

In ductal epithelial cells of the parotid gland, colonic mucosal cells and distal tubular cells of the kidney, there is intense Type I-ir in the apical regions and diffuse Type I-ir in the rest of the cytoplasm. Aldosterone stimulates sodium-potassium fluxes across apical membranes of epithelial cells of classical mineralocorticoid targets (59). Unlike antidiuretic hormone, aldosterone does not stimulate the synthesis of sodium channels in the apical membrane of the toad bladder epithelium (60, 61). Rather, aldosterone is thought to stimulate the synthesis of apical binding proteins, e.g. GP70, a group of glycoproteins that may modulate apical sodium channels (62, 63). Based on the heavy concentration of Type I-ir in the apical membrane, we speculate that activated Type I receptors may also modulate ionic channels at the apical membrane, and account for some of the effects of aldosterone on sodium fluxes. It is possible that cytoplasmic Type I-ir in some neurons, especially in regions of the CNS that respond selectively to aldosterone, e.g., circumventricular organs and the anteroventral hypothalamus, may play a similar role.

Apart from regulating transcription (5, 6), glucocorticoids have posttranscriptional actions. For example, it has been suggested that glucocorticoids regulate Type II corticosteroid receptor mRNA levels by a posttranscriptional effect on the stability of the transcripts (41, 42). It is interesting that activated Type II receptors have been shown to bind to tRNA (64) and small nuclear RNA (65), and may regulate gene expression by posttranscriptional mechanisms (65). It is possible that stabilization of specific mRNA by glucocorticoid treatment (41, 42) also involves binding of activated Type II receptors to the mRNA-ribosomal protein complex. The distribution of cytoplasmic Type II-ir may denote intracellular sites of binding of Type II receptors to components of the translational machinery.

Although glucocorticoids are known to regulate the release of several neurotransmitters and peptides (1), the anatomical substrates involved have not been worked out. Presumably many of the transport mechanisms involved are regulated at the level of the genome by glucocorticoid regulation of transport protein synthesis. For example, levels of synapsin I, a synaptic phosphoprotein in the hippocampus, are increased by corticosterone treatment (66). We have demonstrated colocalization of GABA and Type II-ir in somata, dendrites, and axons in a subpopulation of vermal Purkinje cells (Type C) of ADX rats treated acutely with corticosterone (23). Although we have not established the anatomical relationship between activated cytoplasmic Type II receptors and GABA in these cells, we speculate that in Type C Purkinje cells, Type II receptors may exist in close relationship with the synthetic machinery for GABA in the cytoplasm, and may even serve as a GABA transporter protein. Interestingly, other regions of the CNS that express predominantly cytoplasmic Type II-ir, e.g., interneurons in the stratum oriens of intact females and postnatal rats, and Type B cells of the globus pallidus, entopeduncular nucleus, and reticular thalamic nucleus also express high levels of GABA (67, 68). It would be interesting to find out whether cytoplasmic Type II-ir in these neurons is also colocalized with GABA.

Glucocorticoids differentially modulate the actions of neurotransmitters and peptides on cAMP levels (1). This has been well demonstrated in the rat forebrain and limbic brain (1, 69, 70). For example, in hippocampal slices, isoproterenol, an adrenergic agent, vasoactive intestinal peptide, and histamine all stimulate increases in cAMP levels. Adrenalectomy increases further the levels of isoproterenol and vasoactive intestinal peptide-induced cAMP. In contrast, in adrenalectomized rats, the levels of histamine-induced cAMP are reduced. The differential modulation of cAMP levels by glucocorticoids is not rapid and is likely to involve a genomic action, e.g., on genes for cAMP-coupled receptors or proteins that couple receptors to adenylate cyclase (1).

Cytoplasmic Type II corticosteroid receptors are closely associated with protein kinases (32). In addition to being a good substrate for various kinases (32, 71), cytoplasmic Type II receptor is thought to have an intrinsic kinase activity capable of catalyzing phosphorylation of other proteins and autophosphorylation by a glucocorticoid-dependent mechanism (72, 73). However, more recent studies have suggested that protein kinases are contaminants of Type II receptor purification, and that Type II receptor has no intrinsic kinase activity (74). Nonetheless, the close association between cytosolic Type II receptors and protein kinases raises the possibility that some of the well-known effects of glucocorticoids on second messenger systems, e.g., the adenylate cyclase system in the CNS, may be mediated via interaction between various components of second messenger systems and cytoplasmic Type II receptors.

Although this concept is entirely speculative, some evidence does exist for interaction between the Type II receptor and the protein kinase C (PKC) signaling pathway. For example, it has been shown that either diacylglycerol, the endogenous lipid activator of PKC (75), or the PKC-activating tumor promoter, phorbol ester (76), induces an amplification of glucocorticoid-activated transcription of the genes encoding tyrosine aminotransferase and ornithine decarboxylase. That this effect was mediated by an interaction between PKC and glucocorticoid receptor is suggested by the lack of effect on expression of these genes by activation of PKC in the absence of glucocorticoid (75, 76). Further evidence for such an interaction is provided by more recent studies, in which synthetic (77) or endogenous (78) inhibitors of PKC were shown to inhibit glucocorticoid-induced expression of tyrosine aminotransferase. The synthetic inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7), also inhibited glucocorticoid-induced nuclear translocation of the Type II receptor in rat hepatocytes (77).

In conclusion, there is considerable heterogeneity in the intracellular location of corticosteroid receptor immunoreactivity in the CNS. Although the predominantly nuclear location of corticosteroid receptors in the presence of corticosteroids has encouraged the search for genomic mechanisms of action of these steroids, little attention has been paid to potential active roles for cytoplasmic corticosteroid receptors. By highlighting the regional distribution of cells with predominant cytoplasmic corticocosteroid receptor immunoreactivity, we hope to provide an anatomical basis for functional studies in this area.

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