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Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain

(immunocytochemistry)

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ABSTRACT **A monoclonal antibody against the rat liver glucocorticoid receptor was used in combination with rabbit antibodies against tyrosine hydroxylase, phenylethanolamine *N*-methyltransferase, and 5-hydroxytryptamine to demonstrate strong glucocorticoid receptor immunoreactivity in large numbers of central monoaminergic nerve cell bodies of the male rat. The receptor immunoreactivity was predominantly located in the nucleus, whereas the tyrosine hydroxylase, phenylethanolamine *N*-methyltransferase, and 5-hydroxytryptamine were detected mainly in the cytoplasm. The vast majority of the noradrenergic nerve cell bodies of groups A1–A7 and of the 5-hydroxytryptaminergic cell bodies of groups B1–B9 were found to contain strong glucocorticoid receptor immunoreactivity. The majority of the phenylethanolamine *N*-methyltransferase-immunoreactive nerve cells of the adrenergic cell groups C1–C3 and of the dorsal subnuclei of the nucleus tractus solitarius in the medulla oblongata were also strongly immunoreactive for glucocorticoid receptor. In the midbrain dopaminergic groups A8–A10, moderately (A8, A9) to strongly (A10) glucocorticoid receptor-immunoreactive cells were found, ranging from 40 to 75% of the total population. In the hypothalamic dopaminergic cell groups, all the cells of groups A12 and A14, as well as the majority of the dopaminergic cells of the zona incerta (A13), were found to contain moderate to strong glucocorticoid receptor immunoreactivity, but none of the large dopaminergic cells of the posterior hypothalamus (A11) showed such immunoreactivity.**

Using a monoclonal IgG2a antibody against a highly purified rat liver cytosolic glucocorticoid receptor preparation, we have in previous studies (1–3) localized receptor-immunoreactive neurons in the telencephalon, diencephalon, and lower brain stem of the rat. The central monoaminergic neurons are known to have important role(s) in stress responses, and their transmitter synthesis is affected by glucocorticoids (4–8). Therefore, we used two-color immunocytochemistry (9) to examine monoaminergic nerve cells for the presence of glucocorticoid receptors.

MATERIALS AND METHODS

Antibodies. Fifty specific-pathogen-free male Sprague–Dawley rats (150 g; ALAB, Stockholm, Sweden) were used. They were given free access to food pellets and water and were kept under standardized lighting conditions (lights on at 0600 and off at 2000). Rats were killed in the morning.

The glucocorticoid receptor was purified from rat liver cytosol as described (10, 11). The purity of receptor used in the absorption experiments was >80%, as judged by NaDodSO$_4$/PAGE. The preparation and characterization of the monoclonal antibody against the receptor have been described in detail (12). The monoclonal antibody was produced by growing the hybridoma cells in ascites fluid. The unpurified ascites fluid was used for this immunocytochemical analysis. Control ascites fluid was obtained by injecting the non-Ig-producing mouse myeloma cell line Sp2/0 (13), which was originally used as fusion partner in the hybridoma production, i.p. into a C57BL mouse. In the blocking experiments with purified receptor (8 μg/ml of diluted antiserum) the incubation with diluted (1:1000) primary antibody or a mixture of diluted primary antibody and purified receptor was carried out in 1 mM EDTA/20 mM Tris·HCl, pH 7.0.

Tyrosine 3-hydroxylase [TyrOHase; tyrosine 3-monooxygenase; L-tyrosine,tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] was purified from the rat pheochromocytoma cell line PC12 and phenylethanolamine *N*-methyltransferase (PNMeTase; *S*-adenosyl-L-methionine:phenylethanolamine *N*-methyltransferase, EC 2.1.1.28) was purified from rat adrenal medulla, and antisera were raised in rabbits (14). The antibodies against TyrOHase and PNMeTase have been characterized in detail (15). The antisera against TyrOHase and PNMeTase were used at dilutions of 1:750 and 1:1500, respectively. In the experiments on TyrOHase and PNMeTase immunoreactivity, normal rabbit serum was used as control serum (15, 16). Using normal rabbit serum, no immunoreactive nerve terminals or cell bodies could be detected.

The antibodies against 5-hydroxytryptamine coupled to bovine serum albumin were raised in rabbits and have been characterized extensively (17, 18). Before the 5-hydroxytryptamine antiserum was used, it was absorbed with bovine serum albumin at 100 μg/ml. To provide control serum, the antiserum was absorbed with 5-hydroxytryptamine (100 μg/ml) overnight at 4°C. The 5-hydroxytryptamine antiserum was used at a dilution of 1:750–1:1000.

All antibodies were diluted in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.3% (vol/vol) Triton X-100 (Sigma) and 1% (vol/vol) normal goat serum (Cappel Laboratories, Cochranville, PA).

Immunocytochemical Procedures. Rats were anesthetized with methohexital sodium [Brietal (Eli Lilly), 10 mg per rat, i.p.] and killed by transcardiac perfusion with 0.9% NaCl (50 ml, 37°C) followed by a 30-min perfusion with 500 ml of cold (4°C) 0.1 M sodium phosphate buffer (pH 7.4) containing 4%

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Abbreviations: TyrOHase, tyrosine hydroxylase; PNMeTase, phenylethanolamine *N*-methyltransferase.

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(wt/vol) paraformaldehyde (Merck). The brains were kept in the fixative for 12 hr at 4°C. Coronal sections (30 μm thick) in the Horsley–Clark plane (19) (medulla oblongata) or in the plane of König and Klippel (20) were made with a Lancer (St. Louis, MO) Vibratome series 1000 sectioning system at 4°C in 0.1 M sodium phosphate (pH 7.4). In all the immunocytochemical experiments the sections were first stained for glucocorticoid receptor immunoreactivity by the unlabeled peroxidase-antiperoxidase (PAP) method of Sternberger (21) in combination with 3,3′-diaminobenzidine hydrochloride (Sigma) (for details, see ref. 1). After the completion of the first staining procedure (see below), the same sections were incubated with antiserum to TyrOHase, PNMeTase, or 5-hydroxytryptamine and treated according to the PAP method in combination with α-chloronaphthol (Sigma). This procedure is a modification of the two-color immunocytochemical procedure of Oertel *et al.* (9). After the first staining, sections were incubated overnight in phosphate buffer containing 4% formaldehyde at 4°C to destroy the peroxidase activity of the first PAP. Then the sections were washed five times in 0.17 M NaCl/0.1 M phosphate, pH 7.4, and incubated in diaminobenzidine/H_2O_2 solution to test for remaining peroxidase activity. As seen in the figures, the glucocorticoid receptor immunoreactivity in the nerve cell nuclei gave a brownish color and the TyrOHase, PNMeTase, or 5-hydroxytryptamine immunoreactivity in the cytoplasm gave a bluish color (formed from α-chloronaphthol). In studies on the locus coeruleus, glucocorticoid receptor immunoreactivity was also demonstrated by the use of α-chloronaphthol as a chromagen for the peroxidase.

Three sections were analyzed from each monoaminergic nerve cell group in each rat. The location of the glucocorticoid receptor-immunoreactive structures in cytoarchitectonically defined areas was made possible by counterstaining the sections with cresyl violet. In control experiments, each of the six constituents of the two linkages was omitted one at a time, and one or both antisera were replaced by the control serum (4). The chromagen sequence was also tested by comparing glucocorticoid receptor/TyrOHase and TyrOHase/glucocorticoid receptor localization with either diaminobenzidine or α-chloronaphthol as the first chromagen. The two sequences always gave comparable results, and similar studies have been performed with glucocorticoid receptor/5-hydroxytryptamine and glucocorticoid receptor/PNMeTase localization. Occasional sections showed black staining due to the formation of brown reaction product in the blue product and were not considered in the analysis. The immunocytochemical specificity of the glucocorticoid receptor antibody was tested in experiments using anti-receptor antibody after absorption with partially purified receptor. After this procedure, no immunoreactivity was detected in the dopaminergic, noradrenergic, adrenergic, 5-hydroxytryptaminergic cell body groups. Cell counts of coexistence were performed in the dopaminergic cell groups, where the majority of the catecholaminergic cells were not double-labeled.

RESULTS

Strong nuclear glucocorticoid receptor immunoreactivity was detected in large populations of monoaminergic nerve cell bodies of the brain. The dopaminergic cell bodies of the arcuate nucleus (A12, according to ref. 22) (see Fig. 1*A*) and the periarcuate nucleus (A12, ventrolateral part) showed distinct nuclear glucocorticoid receptor immunoreactivity. A large number of receptor-immunoreactive nerve cell nuclei of non-dopaminergic cells were present in the parvocellular part of the arcuate nucleus (Fig. 1*A*). In the same sections it was found that the majority of the dopaminergic cells of the zona incerta (A13) and the dorsomedial hypothalamic region contained glucocorticoid receptor immunoreactivity, whereas those in the posterior hypothalamus (A11) did not. All the dopaminergic cells of the peri- and paraventricular dopaminergic systems (A14) possessed glucocorticoid-receptor immunoreactive nuclei. None of the dopaminergic nerve cells in the glomerular layer of the olfactory bulb (A15) showed glucocorticoid receptor immunoreactivity.

In the mesencephalon, moderately (A8, A9) to strongly (A10) glucocorticoid receptor-immunoreactive nuclei were observed in the TyrOHase-immunoreactive cells in the ventral tegmental area (A10), especially in its medial parts (nucleus interfascicularis and nucleus linearis caudalis) along its entire rostrocaudal extent [61 ± 9% of all TyrOHase-immunoreactive cells (mean ± SEM, n = 3 rats)]. Furthermore, glucocorticoid receptor immunoreactivity was detected in the TyrOHase-immunoreactive nerve cell bodies of the ventromedial, central, and lateral substantia nigra (A9), in the following proportions: 63 ± 7%, 43 ± 9%, and 69 ± 17% (means ± SEM, n = 3 rats). In the lateroventral reticular formation (A8), 75 ± 8% of the TyrOHase-immunoreactive cells (n = 3 rats) exhibited glucocorticoid receptor immunoreactivity.

All the TyrOHase-immunoreactive nerve cell bodies of the medial subnucleus of the nucleus tractus solitarius (noradrenergic nerve cells; refs. 22–25) possessed strongly glucocorticoid receptor-immunoreactive nuclei (Fig. 1*B*). Reactivity with TyrOHase and glucocorticoid receptor antisera was also observed in nerve cells of the dorsal subnuclei of the nucleus tractus solitarius (mainly adrenergic nerve cells; refs. 23 and 25); the nuclei of some TyrOHase-positive nerve cell bodies were glucocorticoid receptor-positive, but others were not (Fig. 1*C*). The vast majority of the TyrOHase-immunoreactive nerve cell bodies of the locus coeruleus possessed glucocorticoid receptor-immunoreactive nuclei (Fig. 1*D* and *E*). Further, the vast majority of the TyrOHase-immunoreactive nerve cells of groups A1 (see Fig. 1*F*), A2, A4, A5, A6, and A7, as well as the noradrenergic cells of the subcoeruleus (Fig. 2*A*), showed strong glucocorticoid receptor immunoreactivity.

The majority of the PNMeTase-immunoreactive nerve cell groups (epinephrine-containing neurons) C1, C2, and C3 were found to contain glucocorticoid receptor immunoreactivity (16). These results are illustrated in Figs. 2 *B* and *C*. In the dorsal subnuclei of the nucleus tractus solitarius, 50% of the PNMeTase-immunoreactive cell population was glucocorticoid receptor-immunoreactive. All 5-hydroxytryptamine-containing nerve cell groups in the lower brain stem (B1–B9; ref. 22) were analyzed, and the vast majority of the 5-hydroxytryptamine-immunoreactive nerve cells of these groups were found to be strongly glucocorticoid receptor-immunoreactive (Fig. 2 *D–F*).

DISCUSSION

By means of two-color immunocytochemistry (9) using rabbit antisera against TyrOHase, PNMeTase, and 5-hydroxytryptamine and a monoclonal antibody monospecific for glucocorticoid receptor, the present study revealed that large numbers of dopamine-, norepinephrine-, epinephrine-, and 5-hydroxytryptamine-containing nerve cells of the brain stem of the male rat contain strong nuclear glucocorticoid receptor immunoreactivity. As shown in previous studies (1–3), the receptor immunoreactivity was located predominantly in the nerve cell nuclei, leaving the cytoplasm mainly unstained. The vast majority of the noradrenergic, adrenergic, and 5-hydroxytryptaminergic nerve cells of the lower brain stem showed strong nuclear glucocorticoid receptor immunoreactivity, whereas a marked heterogeneity existed among the dopaminergic cell groups of the brain with regard to content of glucocorticoid receptor immunoreactivity. Thus, most if

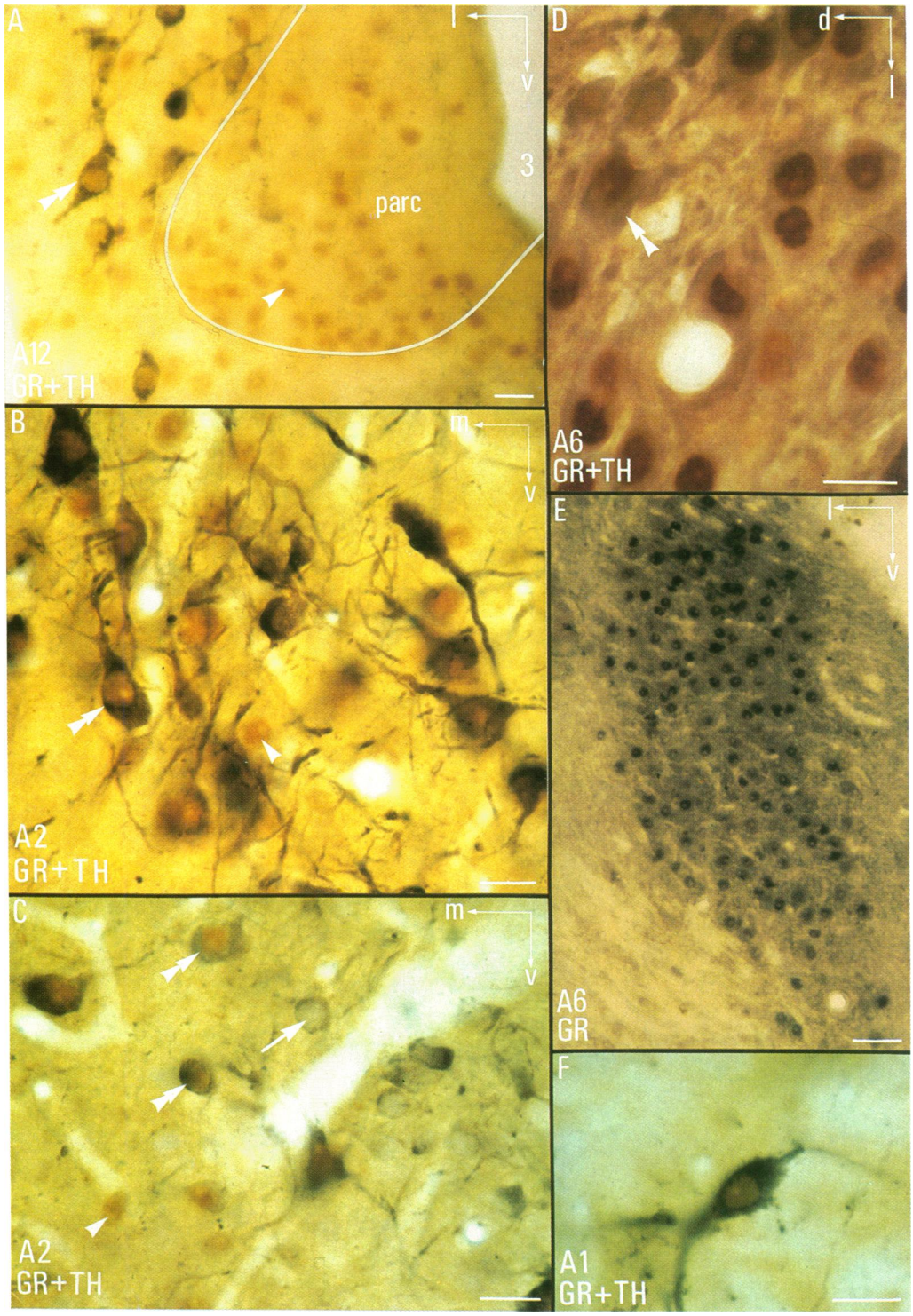

FIG. 1. Two-color immunocytochemistry, using a mouse monoclonal antibody against rat liver glucocorticoid receptor (GR) and a rabbit antiserum against TyrOHase (TH), of coronal sections at various levels of the brain stem of the rat. Only GR immunoreactivity is shown in *E*. (*A*) GR immunoreactivity (brown) is shown in TH-immunoreactive cell bodies (bluish) of the magnocellular part of the arcuate nucleus of the rat hypothalamus (double arrowhead). In the parvocellular part of the arcuate nucleus (parc) a large number of single-labeled GR-immunoreactive nuclei are shown (arrowhead). Arrows: l, lateral; v, ventral. Level: bregma −2.8 mm. (Bar = 25 μm.) (*B*) Noradrenergic cell group A2 of the medial subnucleus of the nucleus tractus solitarius (nTS). A double-labeled neuron is indicated [brown nucleus (GR) and bluish cytoplasm (TH), double arrowhead]. A GR-immunoreactive nucleus without double-labeling is indicated (arrowhead). Arrows: m, medial; v, ventral. Level: bregma −13.8 mm. (Bar = 25 μm.) (*C*) GR immunoreactivity in TH-immunoreactive cells in the dorsal strip and the dorsal parasolitary region of the nTS. Double-labeled (double arrowhead) and non-double-labeled (large arrow) TH-immunoreactive nerve cell bodies are shown. A non-double-labeled GR-immunoreactive nucleus is also indicated (arrowhead). Level: bregma −13.8 mm. (Bar = 25 μm.) (*D*) Double-labeled neurons are seen (e.g., double arrowhead) in the locus coeruleus (A6). Arrows: d, dorsal; l, lateral. Level: bregma −9.8 mm. (Bar = 25 μm.) (*E*) Only GR immunoreactivity is shown by means of α-chloronaphthol (blue) and is mainly located in the nuclei of the nerve cells of the locus coeruleus (A6). Level: bregma −9.8 mm. (Bar = 50 μm.) (*F*) Double-labeled noradrenergic nerve cell body of the caudal ventrolateral medulla oblongata (A1 group). Level: bregma −13.8 mm. (Bar = 20 μm.)

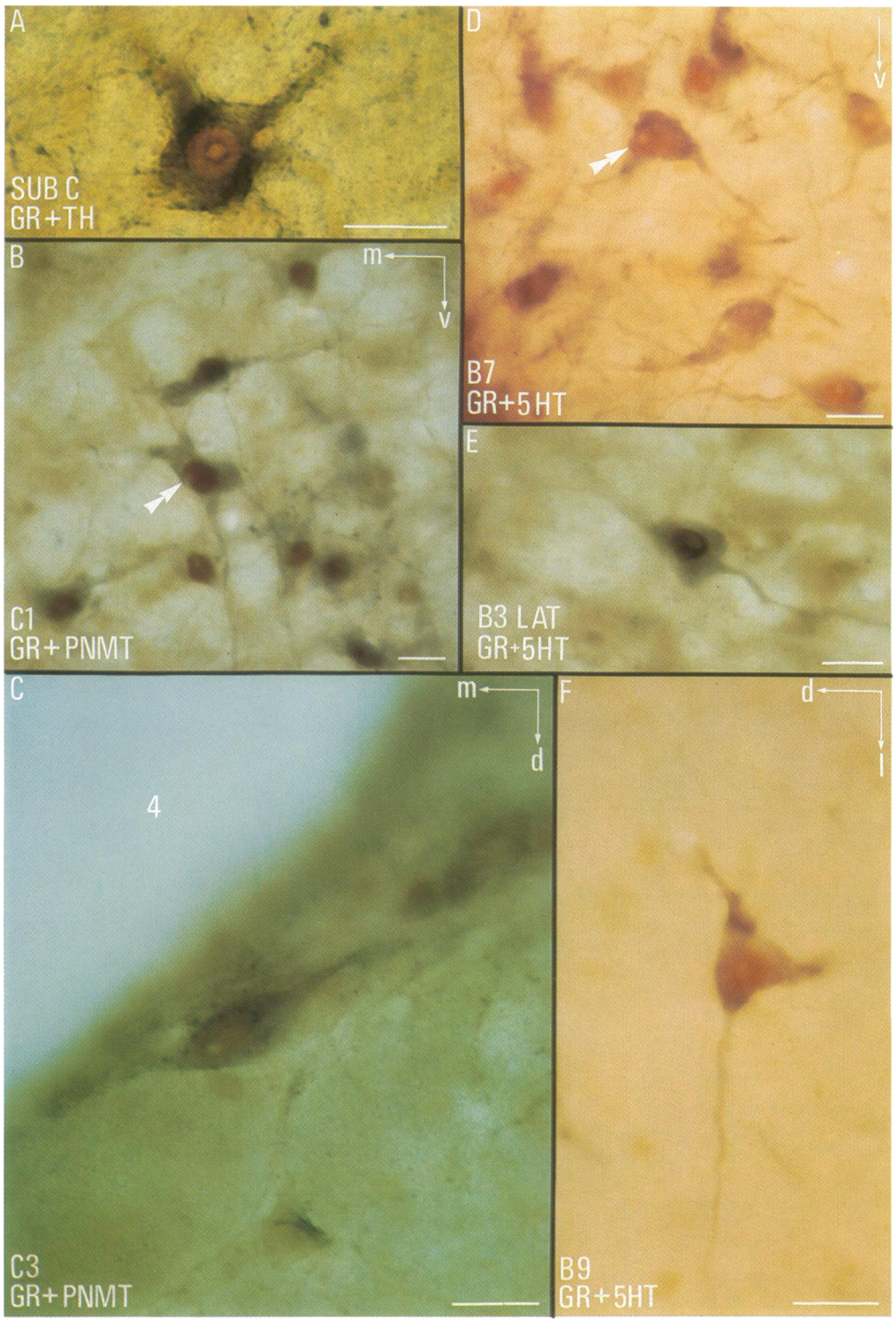

FIG. 2. Glucocorticoid receptor (GR) immunoreactivity in monoaminergic neurons, shown by two-color immunocytochemistry of coronal sections at various levels of the lower brain stem. GR immunoreactivity (brown) is seen in the nuclei, whereas TyrOHase (TH), PNMeTase (PNMT), and 5-hydroxytryptamine (5HT) (bluish) are observed in the cytoplasm. (*A*) A double-labeled nerve cell of the subcoeruleus noradrenergic nerve cell population. Level: bregma −9.8 mm. (Bar = 30 μm.) (*B*) GR immunoreactivity (brown) in PNMT-immunoreactive (bluish) cells of the rostral part of the ventrolateral medulla oblongata (C1 cell group) lateral to the paragigantocellular reticular nucleus. Double arrowhead indicates a double-labeled neuron. Arrows: m, medial; v, ventral. Level: bregma −12.8 mm. (Bar = 25 μm.) (*C*) GR immunoreactivity (brown) is shown in PNMT-immunoreactive (bluish) nerve cells in the rostral midline periventricular area dorsal to the medial longitudinal fasciculus (part of the C3 cell group). Arrows: m, medial; d, dorsal. Level: bregma −12.8 mm. (Bar = 25 μm.) (*D*) GR immunoreactivity (brown) in 5HT-immunoreactive (bluish) cells of the nucleus raphe dorsalis. Double arrowhead indicates a double-labeled neuron. Arrow: v, ventral. Level: bregma −7.8 mm. (Bar = 25 μm.) (*E*) GR immunoreactivity (brown) in nucleus of a 5HT-immunoreactive nerve cell body (bluish cytoplasm) in the nucleus paragigantocellularis pars alpha (lateral B3). Level: bregma −10.8 mm. (Bar = 25 μm.) (*F*) GR immunoreactivity (brown) in a 5HT-immunoreactive (bluish) nerve cell of the ventromedial reticular formation dorsal to the medial lemniscus (B9 cell group). Arrows: d, dorsal; l, lateral. Level: bregma −7.3 mm. (Bar = 25 μm.)

not all of the hypothalamic dopaminergic cell bodies of groups A12 and A14 showed glucocorticoid receptor immunoreactivity, and the majority of the A13 cells were glucocorticoid receptor-immunoreactive. However, no receptor immunoreactivity was observed in the A11 cell group. Further, a varying degree of coexistence was observed in the

dopaminergic cell groups A8, A9, and A10.

Considering the results of the present study, it seems likely that the glucocorticoid receptor concentration in the nuclei of the monoaminergic nerve cells is particularly high. They should therefore represent an important site of action of glucocorticoid hormones secreted from the adrenal glands. A combined autoradiographic and immunocytochemical study (26) of [^{3}H]corticosterone target neurons and catecholaminergic neurons did not show, but did not exclude, a direct genomic action of corticosterone on catecholaminergic neurons. Previous studies (4, 5, 8) have shown that the noradrenergic, adrenergic, and 5-hydroxytryptaminergic neurons respond to stress by changing their amine levels and utilization. Of particular interest is the finding that, of all the TyrOHase-positive cells in the midbrain, the medially located dopaminergic cells of the A10 group showed the strongest glucocorticoid receptor immunoreactivity in its medial parts. This part of the A10 dopaminergic cell group has been shown to project to the anteromedial frontal cortex and to the anterior cingulate cortex (27). A recent study (6) showed that these cortical dopaminergic systems are highly responsive to stress.

The findings of the present study suggest that the diffusely projecting and stress-responsive monoaminergic brain stem systems involved in the regulation of, *inter alia*, sleep–wakefulness (28), emotional behaviors (29), neuroendocrine (30) and cardiovascular (31) functions, and mood (32) are under influence of circulating glucocorticoid hormones. Thus, the catecholaminergic and 5-hydroxytryptaminergic neurons of the brain stem represent important adaptive systems for the individual. Further, in view of the inhibitory role of the tuberoinfundibular dopaminergic neurons in the control of prolactin, luteinizing hormone, and thyrotropin secretion (33), glucocorticoids may in part control and modulate secretion of these pituitary hormones via actions on the tuberoinfundibular dopaminergic neurons.

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1. Fuxe, K., Wikström, A.-C., Okret, S., Agnati, L. F., Härfstrand, A., Yu, Z.-Y., Granholm, L., Zoli, M., Vale, W. & Gustafsson, J.-Å. (1985) *Endocrinology* **117**, 1803–1812.
2. Fuxe, K., Härfstrand, A., Agnati, L. F., Yu, Z.-Y., Cintra, A., Wikström, A.-C., Okret, S., Cantoni, E. & Gustafsson, J.-Å. (1985) *Neurosci. Lett.* **60**, 1–6.
3. Agnati, L. F., Fuxe, K., Yu, Z.-Y., Härfstrand, A., Okret, S., Wikström, A.-C., Goldstein, M., Zoli, M., Vale, W. & Gustafsson, J.-Å. (1985) *Neurosci. Lett.* **54**, 147–152.
4. Corrodi, H., Fuxe, K. & Hökfelt, T. (1968) *Life Sci.* **7**, 107–112.
5. Sauter, A., Ueta, K. & Goldstein, M. (1980) in *Central Adrenaline Neurons: Basic Aspects and Their Role in Cardiovascular Functions*, eds. Fuxe, K., Goldstein, M., Hökfelt, B. & Hökfelt, T. (Pergamon, Oxford), pp. 97–111.
6. Thierry, A. M., Tassin, J. P., Blanc, G., Stinus, L., Scatton, B. & Glowinski, J. (1977) *Adv. Biochem. Psychopharmacol.* **16**, 5–12.
7. Fuxe, K., Gustafsson, J.-Å. & Wetterberg, L. (1981) in *Proceedings of an International Symposium Held at the Wenner-Gren Center, Stockholm, 27–28 October 1980*, eds. Fuxe, K., Gustafsson, J.-Å. & Wetterberg, L. (Pergamon, Oxford).
8. Moore, R. Y. & Bloom, F. E. (1979) *Annu. Rev. Neurosci.* **2**, 113–168.
9. Oertel, W. H., Tappaz, M. L., Berod, A. & Mugnaini, E. (1982) *Brain Res. Bull.* **9**, 463–474.
10. Wrange, Ö., Carlstedt-Duke, J. & Gustafsson, J.-Å. (1979) *J. Biol. Chem.* **254**, 9284–9290.
11. Wrange, Ö., Okret, S., Radojcic, M., Carldstedt-Duke, J. & Gustafsson, J.-Å. (1984) *J. Biol. Chem.* **259**, 4534–4541.
12. Okret, S., Wikström, A.-C., Wrange, Ö., Andersson, B. & Gustafsson, J.-Å. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1609–1613.
13. Köhler, C. & Milstein, G. (1975) *Nature (London)* **256**, 495–497.
14. Goldstein, M., Anagnoste, B., Freedman, L. S., Roffman, M., Ebstein, R. P., Park, D., Fuxe, K. & Hökfelt, T. (1973) in *Frontiers in Catecholamine Research*, eds. Usdin, E. & Snyder, S. (Pergamon, New York), pp. 69–78.
15. Goldstein, M., Fuxe, K. & Hökfelt, T. (1982) *Pharmacol. Rev.* **24**, 293–309.
16. Hökfelt, T., Goldstein, M., Fuxe, K., Johansson, P., Verhofstad, A., Steinbusch, H., Penke, B. & Vargas, J. (1980) in *Central Adrenaline Neurons: Basic Aspects and Their Role in Cardiovascular Functions*, eds. Fuxe, K., Goldstein, M., Hökfelt, B. & Hökfelt, T. (Pergamon, Oxford), pp. 19–47.
17. Steinbusch, H. W. M., Verhofstad, A. A. j. & Joosten, H. W. J. (1978) *Neuroscience* **3**, 811–819.
18. Steinbusch, H. W. M. (1981) *Neuroscience* **6**, 557–618.
19. Papaioannou, J. (1970) *J. Physiol. (London)* **210**, 77–79.
20. König, J. & Klippel, R. (1963) *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* (Williams & Wilkins, Baltimore, MD).
21. Sternberger, L. A. (1979) *Immunocytochemistry* (Wiley, New York), 2nd Ed.
22. Dahlström, A. & Fuxe, K. (1964) *Acta Physiol. Scand.* **62**, Suppl. 232, 1–55.
23. Hökfelt, T., Everitt, B. J., Fuxe, K., Kalia, M., Agnati, L. F., Johansson, O., Härfstrand, A., Lundberg, J.-M., Terenius, L., Theodorsson-Norheim, E. & Goldstein, M. (1984) *Clin. Exp. Hypertens. Part A* **6**, 23–41.
24. Fuxe, K., Goldstein, M., Hökfelt, T. & Joh, T. H. (1970) *Res. Commun. Chem. Pharmacol.* **1**, 627–636.
25. Kalia, M., Fuxe, K. & Goldstein, M. (1985) *J. Comp. Neurol.* **233**, 308–322.
26. Duncan, G. E. & Stumpf, W. E. (1985) *Neuroendocrinology* **40**, 262–271.
27. Fuxe, K., Agnati, L. F., Kalia, M., Goldstein, M., Anderson, K. & Härfstrand, A. (1985) in *Basic and Clinical Aspects of Neuroscience*, eds. Fluckiger, E., Muller, E. & Thorner, M. (Springer, Amsterdam), pp. 11–25.
28. Jouvet, M. (1969) *Science* **163**, 32–41.
29. Reis, D. J. & Fuxe, K. (1969) *Proc. Natl. Acad. Sci. USA* **64**, 108–112.
30. Fuxe, K. & Hökfelt, T. (1969) in *Frontiers in Neuroendocrinology*, eds. Ganong, W. F. & Martin, L. (Oxford Univ. Press. Oxford), pp. 47–96.
31. Fuxe, K., Goldstein, M., Hökfelt, B. & Hökfelt, T., eds. (1980) *Central Adrenaline Neurons: Basic Aspects and Their Role in Cardiovascular Functions* (Pergamon, Oxford).
32. Carroll, B. J. (1982) *Br. J. Psychiatry* **140**, 292–304.
33. Fuxe, K., Andersson, K., Härfstrand, A., Agnati, L. S., Eneroth, P., Janson, A.-M., Vale, W., Thorner, T. & Goldstein, M. (1986) *Neurochem. Int.* **9**, 155–170.