Histochemistry (1992) 98:155–164

Histochemistry

Nuclear receptor sites for vitamin D-soltriol in midbrain and hindbrain of Siberian hamster (*Phodopus sungorus*) assessed by autoradiography

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Accepted May 12, 1992

Summary. Autoradiograms were prepared from midbrains and hindbrains of male and female Siberian hamsters (*Phodopus sungorus*), kept under short-day or long-day illumination, after injection of tritium-labeled 1,25-dihydroxycholecalciferol (vitamin D, soltriol). Concentration and retention of radioactivity was noted in nuclei of certain neurons, glial cells, and ependymal cells, and in choroid epithelium. Labeled neurons of varying intensity were found throughout the brainstem in distinct populations at characteristic topographical sites, which include cranial nerve motor nuclei, the nucleus (n.) reticularis tegmenti pontis, the caudoventral region of the n. raphe dorsalis, the n. trapezoides, the n. vestibularis lateralis and n. vestibularis superior, neurons in the various nuclei of the sensory trigeminus, accessory optic nuclei, scattered neurons in nuclei of the reticular formation, the n. ambiguus, certain cells in the area postrema, and many others. Glial cells with nuclear labeling, probably microglia, were scattered predominantly in or near myelinated nerve fascicles. The choroid epithelium showed strong nuclear labeling throughout the ventricle. Nuclear labeling of ependyma was variable and weak, mainly at ventral and lateral extensions (recesses) of the ventricle. The extensive presence of nuclear binding in select neural structures indicates that vitamin D exerts specific genomic effects on cell populations that are known to be involved in the regulation of motor, sensory, autonomic, neuroendocrine, metabolic, and immune functions. The results of these studies, in conjunction with those from other brain and peripheral tissues, recognize vitamin D-soltriol as a steroid hormone with a wide scope of hormone-specific target cells, similar to estrogen, androgen, and adrenal steroids, and which are topographically distinct and characteristic for its functions as the steroid hormone of sunlight.

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Introduction

Evidence for vitamin D-specific nuclear binding in brain and spinal cord (Stumpf et al. 1979, 1980) has been obtained in the same experiments that provided the first evidence for the existence of target cells in endocrine organs (pituitary, pancreatic B-cells, thyroid, adrenal, parathyroid chief cells, gonads, thymus), in heart, in skin, and in other tissues (for review see Stumpf 1988). These discoveries were based on the use of our autoradiographic technique (Stumpf 1976), which is highly sensitive and permits cellular and subcellular resolution of receptor sites in non-disrupted tissues within a maintained topographical context. This extensive new information on vitamin D target sites and the related follow-up led to the recognition of a "vitamin D endocrine system" (Stumpf et al. 1979, 1980, 1981; Norman et al. 1982; Bell 1985; Merke et al. 1986) and to the development of the concept of vitamin D-soltriol as a heliogenic seasonal activator and regulator. In contrast to the traditional concept of vitamin D as "the calcium homeostatic steroid hormone", and a vitamin D endocrine system that subserves mainly calcium regulation, we propose that regulation of calcium levels is but one of the many effects of vitamin D. This skin-derived solar zeitgeber-steroid hormone serves primarily the need for biological systems to adapt to solstitial changes and the related maintenance and procreation of life (Stumpf 1988; Stumpf and Privette 1991).

Among the tissues with highest affinities to this heliogenic steroid are thyrotropes in the pituitary (Sar et al. 1980; Stumpf et al. 1987) and neurons in the stria terminalis brain circuit with the central amygdaloid nucleus and the lateral bed nucleus of the stria terminalis (Stumpf and O'Brien 1987) in all vertebrate phyla studied (Stumpf and Bidmon 1990; Bidmon and Stumpf 1991). Motor neurons of cranial nerves and spinal cord, and sensory neurons in spinal ganglia (Stumpf and O'Brien 1987; Stumpf et al. 1988a), as well as epithelial cells of the choroid plexus (Bidmon et al. 1991) have also been noted as species-specific sites with high nuclear

binding of vitamin D. The present experiments were aimed at investigating further the central nervous system of a species with high seasonal adaptation, in order to complement the data for certain forebrain regions obtained in the same species (Musiol et al. 1991, 1992a) and to compare them with those obtained for laboratory rats and mice.

Materials and methods

Fourteen 6-month-old Siberian hamsters (*Phodopus sungorus*), seven males and seven females, weighing 29–35 g were raised on a normal diet. Animals were kept under long-day conditions [light/dark (L/D) 14:10 h, four males and three females] or transferred to short-day conditions for 2 months (L/D 10:14 h, three males and four females). The short-day cycle causes testis regression and in both sexes weight loss and changes in hair color.

During daylight the animals were injected subcutaneously with 1,25-[26,27-^{3}H]dihydroxycholecalciferol (^{3}H-1,25D$_3$, DuPont, Boston, Mass., USA, sp. act. 160 Ci/mmol), dissolved in 20% ethanol-isotonic saline, two pulses each of 0.2 µg/100 g body wt. at an interval of 1 h. One female (long-day) and one male (short-day) were injected with a 1000-fold excess of unlabeled 1,25-D$_3$ (Hoffmann-LaRoche Nutley, NJ, USA), 50% at 30 min before and 50% simultaneously with the first tracer injection. The animals were decapitated 4 h after the first tracer injection. Mid- and hindbrains were mounted on tissue holders, frozen in liquified Freon 22 cooled by liquid nitrogen, and the freeze-mounted samples stored in liquid nitrogen.

Serial section autoradiograms were prepared after the thaw-mount technique (Stumpf 1976). Frozen sections 4 µm thick were cut in a cryostat (Frigocut 2800, Jung, Heidelberg, FRG) and under safe-light were thaw-mounted onto nuclear emulsion (Kodak NTB 3)-coated slides. The section-mounted slides were stored in light-proof desiccator boxes at −15° C. Exposure times between 10 and 26 months were utilized in order to be able to detect target tissues with strong and weak nuclear concentrations of radiolabeled compound. At the end of the exposure, the slides were photographically processed, stained with methylgreen-pyronin, and coverslipped with Entellan (Hoechst).

The autoradiograms were evaluated qualitatively and quantitatively by assessing microscopically the silver grain concentration over selected compartments and cell nuclei. A cell was considered labeled if the density of nuclear silver grains exceeded at least twice the extracellular silver grain density. The relative intensity of nuclear labeling was assessed as weak, intermediate, or strong, as depicted in the schematic drawings in Fig. 1A–K.

Controls against chemographic artefacts included development of autoradiograms immediately after thaw-mounting or short exposure for a few hours or days, at which time positive chemography would be apparent (Stumpf and Pilgrim 1992).

The topographic designation of labeled cells was aided by information from several rat and mouse atlases (König and Klippel 1963; Wünscher et al. 1965; Stumpf and Sar 1975a; Paxinos and Watson 1986). Frequently, the latin nomenclature was found more precise and convenient and has been preferred in most instances. The abbreviations utilize small letters for nuclei and capital letters for proper names and nerve fiber tracts; thus, the designation n for nucleus can be omitted and a fiber tract with identical name can be recognized (e.g., st = bed nucleus of the stria terminalis, ST = stria terminalis).

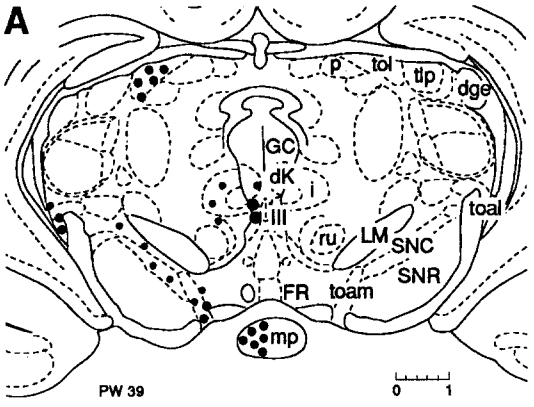
Results

Results are depicted schematically in Fig. 1A–M and as related autoradiograms in Figs. 2–27. After the injection of tritium-labeled 1,25D$_3$ and exposure times between 11 and 26 months, low levels of radioactivity were found throughout the tissue, with accumulation of radioactivity in the lumina of blood vessels and in nuclei of certain cells within anatomically distinct regions (topographic nuclei) or dispersed. Nuclear labeling was re-

Fig. 1A–M. Schematic representation of rodent mid- and hindbrain in rostrocaudal sequence, showing the topographical distribution of neurons with nuclear concentration and retention of radioactivity after injection of ^{3}H-1,25-dihydroxycholecalciferol (^{3}H-1,25D$_3$) in Siberian hamster (*Phodopus sungorus*). Transposed from thaw-mounted autoradiograms. *Left side: Size* and *number of dots* indicate intensity of nuclear labeling and frequency of occurrence of target neurons respectively. *Right side:* Designations of structures. The schematic outlines were adjusted from atlases by Wünscher et al. (1965), Stumpf and Sar (1975a, b), and Paxinos and Watson (1986). The frontal planes correspond with those of the atlas of Paxinos and Watson, indicated as PW number. Terminology and abbreviations are based on traditional Latin usage

Figs. 2–7. Autoradiograms (Figs. 2–27) selected from Siberian hamster brains frozen 4 h after injection of ^{3}H 1,25D$_3$. Varying concentrations of nuclear radioactivity exist in different target cell populations throughout the midbrain and hindbrain. Nucleus (n.) of the oculomotorius nerve (Fig. 2), n. paralemniscalis dorsomedial to the lateral lemniscus (*LL*, Fig. 3), n. subtrigeminalis (Fig. 4), ventrolateral cell group in n. principalis sensibilis of nervus trigeminus (Fig. 5), n. trapezoides (Fig. 6), choroid plexus of fourth ventricle with nuclear labeling of choroid epithelium and radioactivity in lumina of capillaries (Fig. 7)

Abbreviations: (Lower case for nuclei; upper case for pathways and special structures; *n* = nucleus)
A5 noradrenalin cell group; *amb* n. ambiguus; *ap* area postrema; *Aq* aqueduct; *cc* central canal; *CI* colliculus inferior; *co* n. cochlearis; *cu* n. cuneiformis; *cul* n. cuneatus lateralis; *cum* n. cuneatus medialis; *d* n. Darkschewitsch; *dgl* n. dorsalis (corporis) geniculati lateralis; *FLM* fasciculus longitudinalis medialis; *FR* fasciculus retroflexus; *GC* griseum centrale; *gi* n. gigantocellularis; *giv* n. gigantocellularis ventralis; *gr* n. gracilis; *i* n. interstitialis (Cajal); *ip* n. interpeduncularis; *KF* n. Kölliker-Fuse; *lc* locus ceruleus; *LC* lingula cerebelli; *LM* lemniscus medialis; *llv* n. lemnisci lateralis ventralis; *mp* n. mammillaris posterior; *olv* n. olivaris; *p* n. pretectalis; *pbg* n. parabigeminalis; *pbl* n. parabrachialis lateralis; *pbm* n. parabrachialis medialis; *PCS* pedunculus cerebelli superior; *po* n. pontis; *polv* n. paraolivaris; *prh* n. prepositus hypoglossi; *Py* tractus corticospinalis; *rd* n. raphe dorsalis; *rdm* n. reticularis dorsalis medullae oblongatae; *rgi* n. reticularis gigantocellularis; *rlm* n. reticularis lateralis magnocellularis; *rlp* n. reticularis lateralis parvocellularis; *rma* n. raphe magnus; *rme* n. raphe medianus; *rp* n. raphe pontis; *rpa* n. reticularis parvocellularis; *rpc* n. reticularis pontis caudalis; *rpo* n. reticularis pontis oralis; *rpt* n. reticularis pontis tegementalis; *rpv* n. reticularis pontis ventralis; *rtp* n. reticularis tegmenti pontis; *ru* n. ruber; *rvm* n. reticularis ventralis medulae oblongatae; *sbb* n. subbrachialis; *SNC* substantia nigra, zona compacta; *SNR* substantia nigra, zona reticulata; *sol* n. tractus solitarii; *spt* n. subpeduncularis tegmenti; *tdo* n. tegmentalis dorsalis; *tlp* n. lateralis thalami, pars posterior; *toal* n. tractus optici accessorii lateralis; *toam* n. tractus optici accessorii medialis; *tol* n. tractus optici, pars lateralis; *tv* n. tegmentalis ventralis; *tz* n. trapezoides; *vt* n. ventralis tegmenti; *x* n. x; *III* n. nervi oculomotorii; *4V* fourth ventricle; *V* n. motorius nervi trigemi; *Vs* n. sensibilis nervi trigemini; *Vspc* n. caudalis tractus spinalis nervi trigemi; *Vspo* n. oralis tractus spinalis nervi trigemini; *VI* n. nervi abducentis; *VII* n. nervi facialis; *VIIg* genu nervi facialis; *VIIIm* n. vestibularis medialis; *VIIIl* n. vestibularis lateralis; *VIIIs* n. vestibularis superior; *VIIIsp* n. vestibularis spinalis; *X* n. dorsalis motorius nervi vagi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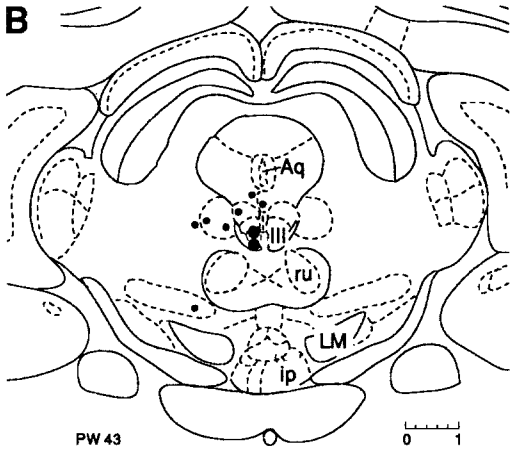
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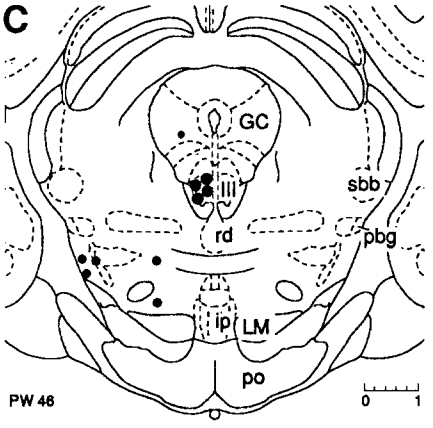
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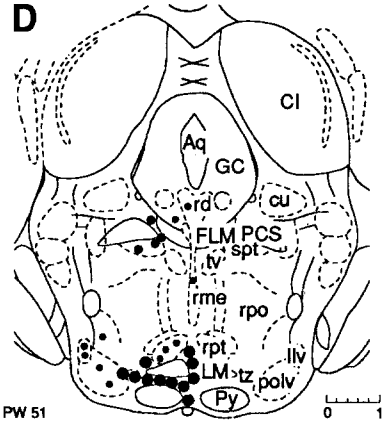
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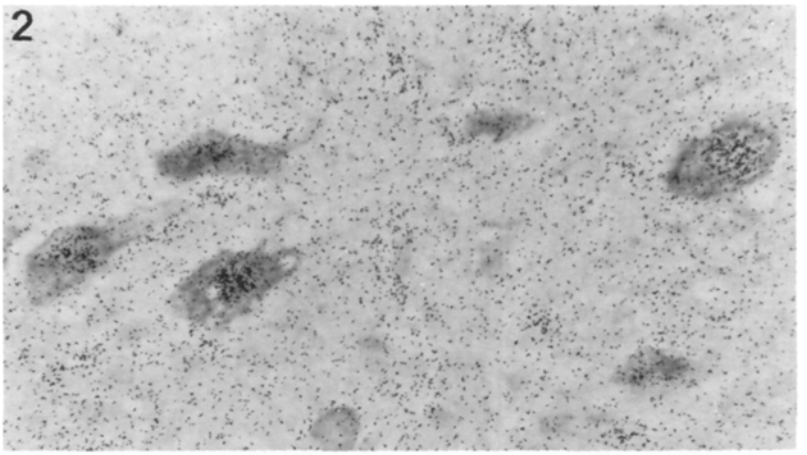
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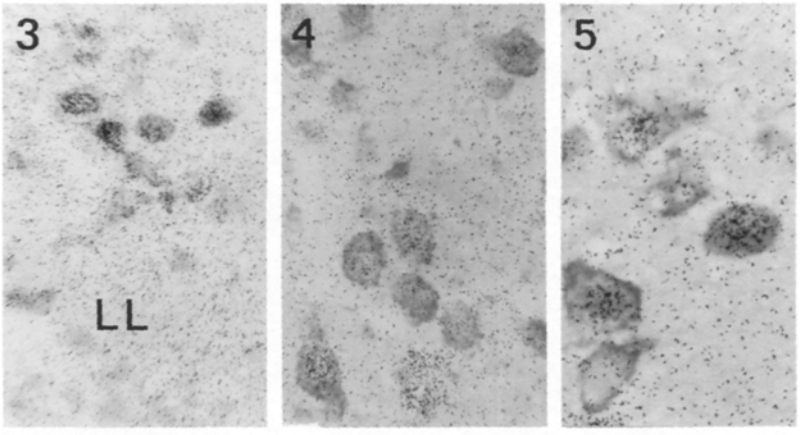
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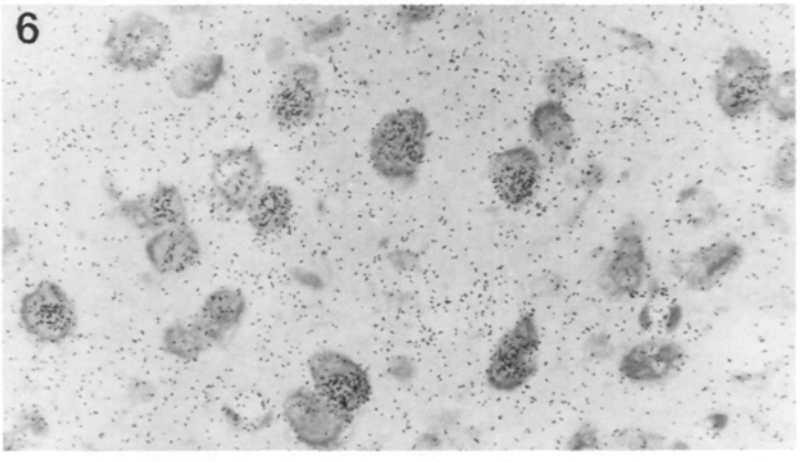
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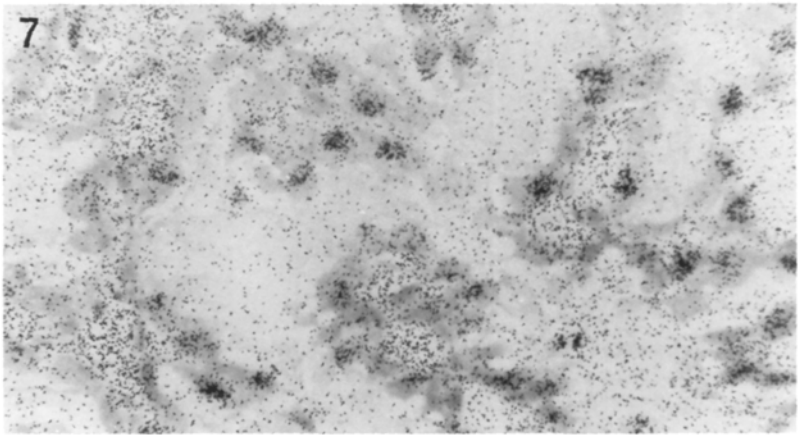
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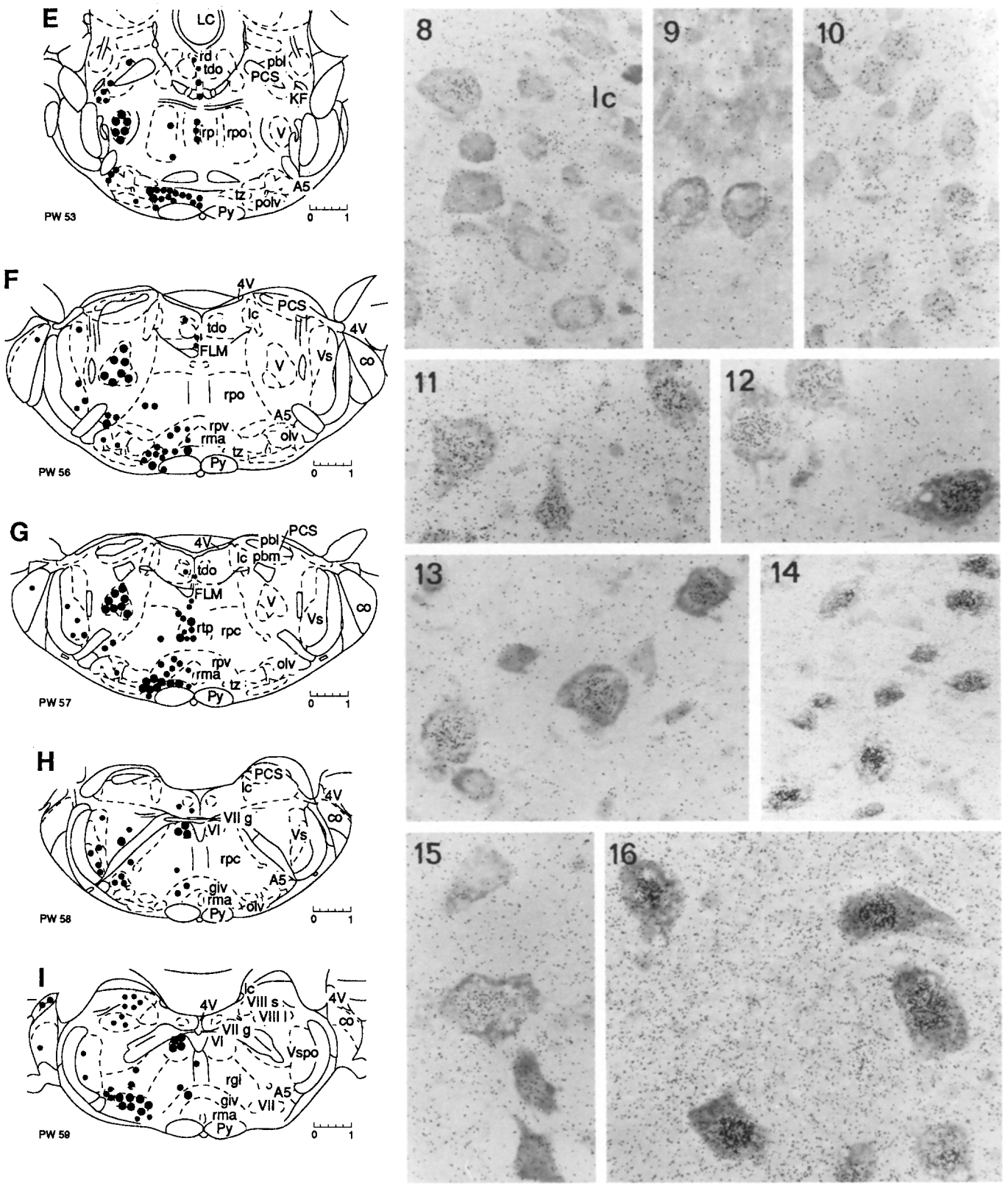


Fig. 1E–I; Figs. 8–16. An occasional labeled cell in the mostly unlabeled n. tractus mesencephali nervi trigemini with adjacent unlabeled locus ceruleus (*lc*, Fig. 8). Purkinje cells and granule cells in the cerebellum are unlabeled (Fig. 9). The n. raphe dorsalis contains weakly labeled cells in its ventrocaudal portion (Fig. 10). Nuclear labeling weak to intermediate in neurons of the n. vestibularis lateralis and n. vestibularis superior (Fig. 11). Strongly labeled cells in motor nucleus of trigeminal nerve (Fig. 12), in n. reticularis tegmenti pontis (Fig. 13), and in n. of abducens nerve (Fig. 14). Scattered large labeled neurons in the ventral portion of n. reticularis gigantocellularis (Fig. 15). Strongly labeled neurons in n. nervi facialis (Fig. 16)

cognized by a concentration of radioactivity, that is, of silver grains that underly the nuclear compartment. This concentration varied among labeled cells and was designated as strong, intermediate, or weak. A cell was considered labeled if the nuclear density of silver grains exceeded at least twice the perinuclear cytoplasmic and neuropil density of silver grains.

Nuclear labeling was recognizable in strongly labeled cells after relatively short exposure times of 2 months, at which time weakly labeled cells were undetectable. At about 10 months of exposure, under the conditions of the experiment, most of the pattern of labeled cells was recognizable and nearly "complete".

Longer exposure times were utilized in order to assure a complete or near-complete topographical pattern of target cells. The longest exposure times were favorable for low-magnification surveys, although quantification would then be impaired because of non-linearity of image formation due to coincidence quenching. After long exposure times, the whole nuclear area may have been covered with silver grains, except for the region of the nucleolus which remained free. Also, variations in the distribution of radioactivity in different regions of the neuropil became apparent. For instance, the silver grain density was low in the neuropil surrounding the unlabeled neurons of the nucleus of the solitari tract and of the vagus nerve, but high in the adjacent ventral neuropil surrounding the strongly labeled neurons of the nucleus nervi hypoglossi (Fig. 17).

In competition studies with excess unlabeled hormone, the nuclear concentration of radioactivity was abolished. Evidence has been published concerning competition studies in identical animals (Bidmon et al. 1991a, b; Musiol et al. 1992a, b) and in other animals (Stumpf and O'Brien 1987). Among the most strongly labeled cells in the midbrain and hindbrain were motor neurons of cranial nerve nuclei (Figs. 2, 12, 14, 16) and cells of the choroid epithelium (Fig. 7). While most of the labeled cells appeared to be neurons of different sizes, as judged by size, presence of a conspicuous nucleolus, or a relatively large pyronin-positive perikaryon, there were also small labeled cells with only a small rim of pale cytoplasm.

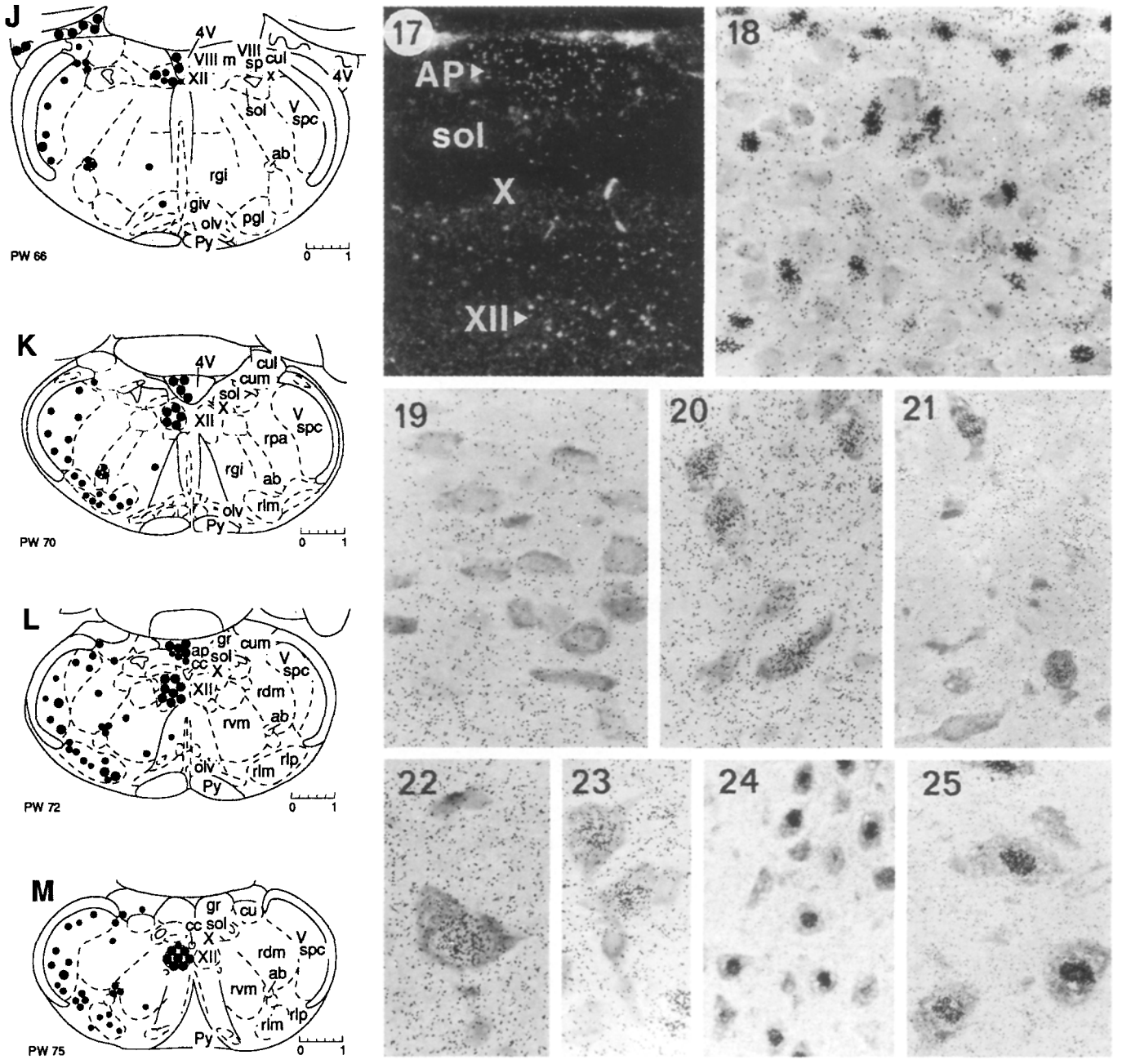
Topography of neurons with nuclear concentration of hormone

In most of the regions and nuclei described, the identification of labeled cells and their topographical attribution appeared with a sufficient degree of certainty. There were, however, several regions in which the delineation and attribution of labeled cells was uncertain and focused follow-up studies were indicated. Also, for several of the regions studied, available brain atlases provided unclear or conflicting information. In the present experiment, for instance, the boundaries were uncertain between the n. raphe magnus and the n. reticularis pontis ventralis, or between the n. vestibularis lateralis and n. vestibularis superior. These and other uncertainties were in part due to the use of thin sections, the 100 μm spacing of sections for the serial autoradiograms, variations in the plane of cutting and in the quality of sections, possible species differences in the topography gleaned from available rodent atlases, and unavailability of a specific atlas for the Siberian hamster brain. In general, a topographic correspondence of structures among rat, mouse, and *Phodopus* was noted.

In the midbrain, at the most rostral and transitional level to the forebrain, labeled neurons accumulated dorsally in the region of the n. tractus optici pars lateralis. In the ventral griseum centrale, scattered labeled neurons were seen ventrolaterally, especially in the n. interstitialis Cajal and also in the Edinger-Westphal nucleus, and with particularly strong labeling in the n. of the nervus oculomotorius (Fig. 2). In the ventrolateral rostral midbrain, a small group of labeled neurons was seen close to the surface dorsolateral to the substantia nigra. Whether or not this group is identical with the lateral nucleus of the accessory optic tract, as we suspect, remains to be studied further. In the pars compacta of the substantia nigra a few weakly labeled neurons could be identified. Ventrally, between the substantia nigra and the interpeduncular nucleus, a small group of neurons, probably belonging to the medial nucleus of the accessory optic tract, showed weak nuclear labeling. At this level and further caudally, the n. ruber and the n. interpeduncularis were unlabeled. A cut through the most caudal portion of the mamillary body revealed intermediate intensity labeling of most of the neurons of the n. mammillaris posterior. Both the colliculus superior and inferior appeared unlabeled, except for a few scattered, labeled cells in the ventral and ventrolateral annulus.

At the level of the rostral pons, scattered labeled cells were present in the n. reticularis pontis. A small group of strongly labeled cells was seen ventrolaterally immediately dorsolateral to the crus cerebri, ventral to the n. parabigeminalis, which we tentatively identified as n. paralemniscalis (Fig. 3). Cells of the n. pontis were unlabeled. Neurons in the rostral n. raphe dorsalis were essentially unlabeled; only in its more caudal pontine part were a variable number of mostly weakly labeled cells apparent (Fig. 10). In other raphe nuclei, which include the n. raphe medianus, pontis, pallidus, and obscurus, only an occasional labeled cell was present. In the region of the n. raphe magnus, labeled cells were more numerous and continuous with the labeled cells in the dorsal n. pontis ventralis and in the ventral n. trapezoides.

There were a few scattered labeled neurons in the griseum pontis, and one or two labeled neurons in the n. of Gudden, while the n. tegmenti ventralis remained free of labeled cells. Intermediate to strong nuclear labeling was conspicuous in the near-midline n. reticularis tegmenti pontis (Fig. 13). Scattered large labeled neurons appeared in the n. reticularis pontis oralis and caudalis, while a more dense labeling was seen of the medium-sized neurons in the n. reticularis pontis ventralis, immediately dorsal to the strongly labeled, small- to medium-sized cells of the n. trapezoides (Fig. 6). Lateral to the relatively extensive n. trapezoides, a small group of neurons, probably belonging to the n. olivaris superi-



Figs. 17–25. Dark-field low magnification survey (Fig. 17) with labeled cells in area postrema (*AP*, also Fig. 18) and n. nervi hypoglossi (*XII*, also Fig. 24), but no nuclear labeling in n. tractus solitarii (*sol*) and n. nervi vagi (*X*, also Fig. 19). Strong nuclear labeling in n. reticularis lateralis parvocellularis (Fig. 20) and in scattered neurons in n. spinalis caudalis of the trigeminus nerve (Fig. 21), in n. reticularis lateralis magnocellularis (Fig. 22), and in n. reticularis gigantocellularis (Fig. 23). Strong nuclear labeling in spinal cord motor neurons of laminae VIII and IX (Fig. 25)

or, contained a few weakly labeled, small neurons that were continuous with the scattered labeled cells in the rostral periolivary area. Other and more caudal components of the extensive n. olivaris were essentially unlabeled.

Neurons of the locus ceruleus and of the n. tractus mesencephali nervi trigemini were unlabeled. As an exception, in two of the animals, in one section each, one of the conspicuous large neurons of this latter nucleus displayed nuclear concentration of radioactivity (Fig. 8). Neurons of the nucleus parabrachialis lateralis and medialis were also mostly unlabeled, with the exception of its most rostral part where several labeled neurons could be noted probably still within the area of this nucleus.

All of the cranial nerve motor nuclei showed strong nuclear labeling of most of their neurons, including motor n. III, IV, V, VI, VII, and XII, as well as motor neurons of the spinal cord laminae IX (Fig. 25) and VIII. Neurons of the various components of the n. ambiguus

showed weak to intermediate nuclear labeling. The dorsal n. vagus was unlabeled (Fig. 19) as was the n. of the solitary tract, except for a small cell in its lateral and caudal portion, which was very occasionally labeled. The sensory components of the n. trigeminus contained medium intensity-labeled, small- to medium-sized neurons especially in its ventral aspects, throughout its oral, principal, and caudal parts (Fig. 21). The representation of labeled neurons in this nucleus varied in its rostrocaudal extent, with the highest density in its caudal levels and in a ventral group of the n. principalis sensibilis of the trigeminus. At the levels of the n. cuneatus, a group of intermediate to strongly labeled cells was conspicuous at the cranial extent of the n. spinalis caudalis V immediately ventral to the n. cuneatus lateralis, corresponding in part to n. x in the atlas of Paxinos and Watson (1986). In the n. vestibularis lateralis and superior, weakly labeled neurons were present in all of the animals studied. Neuronal groups of weak to intermediate intensity of labeling existed in the region of the n. Koelliker-Fuse and of A5 ventral to the motor n. of the nervus trigeminus. Also, more caudally in the medulla oblongata, labeled neurons were present in the A1 region of the n. reticularis lateralis, partes parvocellularis, and magnocellularis (Figs. 20 and 22).

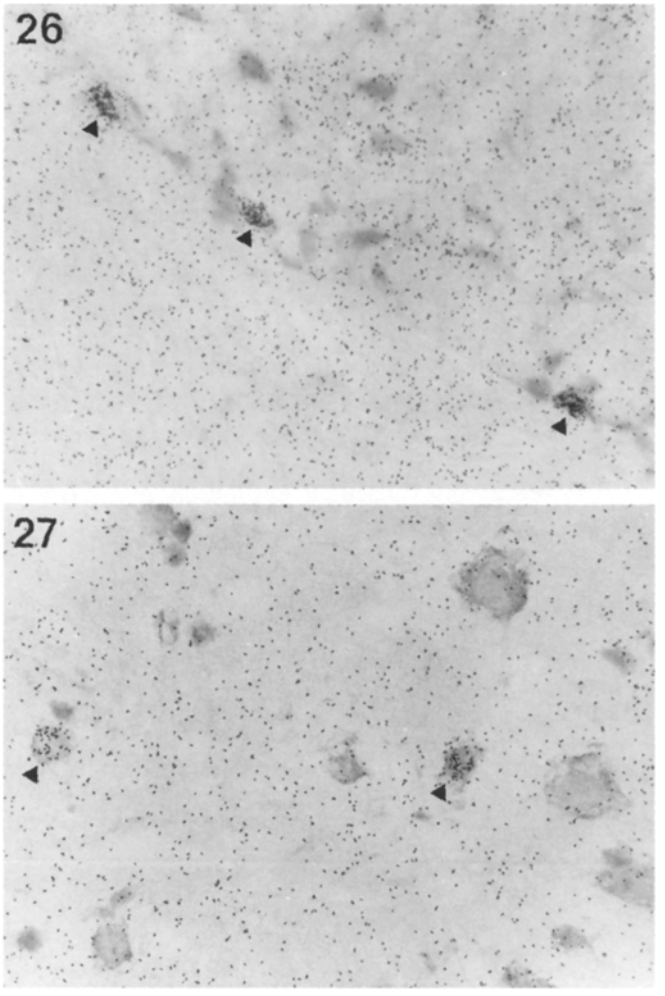
Within the area postrema (Figs. 17 and 18), a subpopulation of strongly labeled cells could be noted, which were mostly small in size and therefore probably mostly of glial origin; also a few somewhat larger labeled cells, possibly small neurons, could be seen. In the cervical spinal cord, in regions of laminae IX and VIII, strongly labeled, large neurons could be identified. In lamina X, ventrally and dorsally to the central canal, also in laminae II and III, on occasion a single labeled small neuron was visible.

Ependyma, choroid plexus, pia mater

Ependyma that lines the aqueduct and fourth ventricle was mostly unlabeled. Occasionally ependymal cells displayed weak nuclear labeling, especially at the floor of the fourth ventricle at the recess of the locus ceruleus and laterally to the medial eminence of the floor of the fourth ventricle at the level of the pontine sulcus medianus. The choroid epithelium was labeled throughout with a strong nuclear concentration of radioactivity in more than 90% of all epithelial cells (Fig. 7). Cells of the stroma of the choroid plexus, which include fibroblasts and endothelial cells, as well as cells of the pia mater, appeared generally unlabeled.

Nerve fiber tract-associated glia (probably microglia)

The majority of small cells, probably astrocytes and oligodendrocytes, appeared unlabeled. A small subpopulation of the small-sized cells, either round or fusiform with a small rim of cytoplasm, displayed nuclear labeling with intermediate or strong intensity. These cells were unevenly distributed. They were most frequently associated with myelinated nerve fibers within and near nerve fiber tracts, such as the superior cerebellar peduncle, fasciculus longitudinalis medialis, genu and radix nervi facialis (Figs. 26 and 27), motor and sensory radices nervi trigemini, and velum medullare anterius. These small scattered cells resembled glial cells, but were sometimes difficult to distinguish from small neurons.



Figs. 26, 27. Small intensely labeled cells, most frequently seen in or near myelinated nerve fasciles, such as genu nervi facialis (VII), are probably microglia (indicated by *arrowheads*)
Figs. 2–27 frozen, thaw-mounted, 4-μm-thick sections stained with methylgreen-pyronin for DNA and RNA. *Magnification:* ×550 (Figs. 2, 5, 6–13, 15, 16, 18–20, 22, 23, 26, 27), ×350 (Figs. 3, 4, 14, 25), ×220 (Figs. 21, 24), ×22 (Fig. 17). *Exposure times:* 11 months (Figs. 2, 6, 9, 12), 12 months (Figs. 14, 20, 26, 27), 14 months (Figs. 10, 13, 23), 16 months (Figs. 4, 15), 18 months (Figs. 3, 5–7), 20 months (Figs. 11, 25), and 26 months (Figs. 17–19, 21, 24)

Discussion

The present studies reveal the selective and extensive presence of nuclear binding of tritiated vitamin D-soltriol in the brain stem of the Siberian hamster. The results of the competition studies with excess unlabeled vitamin D and the special topographical pattern of la-

beled neurons in the various brain stem levels indicate that the observed binding represents nuclear receptors specific for vitamin D. The pattern of distribution of the vitamin D target neurons with nuclear labeling is characteristic for that hormone, when compared to the patterns of target neurons in the midbrain and hindbrain reported previously for other hormonal ligands of the receptor family, which include estradiol, glucocorticoid, progestin, androgen, thyroid hormone, and retinoic acid (Stumpf and Grant 1975; Stumpf et al. 1991).

A near-comprehensive mapping of vitamin D nuclear binding sites has been achieved in *Phodopus* under defined experimental conditions. This is due to the high resolution and high sensitivity of our thaw-mounting technique in order to record high affinity-binding and also low-affinity cells. The use of thin sections accounts for the cellular and subcellular resolution, and the long exposure times allowed storage and accumulation of weak signals derived from only a few labeled molecules, which would remain undetected with thick sections and with short exposure times. Whether or not the presence of low levels of endogenous unlabeled ligand decreases or increases the amount of nuclear receptor remains to be studied, since any change in the level of tissue ligands is likely to affect the expression and detection of the receptor. This study reveals different target cells and target cell groups with strong or intense binding and others with intermediate or low binding. This diversity of nuclear binding, also observed in the pituitary (Stumpf et al. 1987) and peripheral tissues with this and other steroid hormones, is probably of functional significance and deserves further investigation. With the information provided, further studies can now be conducted and need to focus on individual brain regions and nuclei for both anatomical and functional follow-up.

The present results indicate a qualitative similarity of the topographical pattern of vitamin D target neurons between male and female, and between short-day and long-day animals. Quantitative differences among these groups are possible or even likely. More extensive and detailed quantitative studies, which include monitoring of blood and tissue hormone levels, would be required for further clarification. The emphasis in the present experiments was on the identification of neural targets; gross differences between long- and short-day animals have not been detected. A higher amount of nuclear binding in long-day animals, compared to short-day animals, was apparent, however, for choroid epithelium in the same animals (Bidmon et al. 1991).

The extent of vitamin D nuclear binding at all levels in rat, mouse, and hamster forebrain, midbrain, and hindbrain (Stumpf and O'Brien 1987; Musiol et al. 1991, 1992), suggests that vitamin D circulating in the blood has ready access to all sites within central nervous tissue and, nevertheless, exerts extensive effects on specific components of diverse neural systems. The precise identification and characterization of the steroid hormone-specific neural systems, as in the present experiments, is a prerequisite for the study and understanding of the mechanisms of action of a hormonal messenger and for clarification to the question of hormone-specific functions. Such considerations have been made with the information from our previous studies, which provided the basis for the action concept for the three major groups of mammalian steroids as chief regulators and adaptors to the most vital tenets for survival: control of reproduction (sex steroids); securing of food, territory, and partner (adrenal steroids); and seasonal adjustement (solar-integumental steroid vitamin D-soltriol).

The nuclear target cell types for vitamin D include choroid epithelium, regions of ependymal cells, certain glial cells, and different types of neurons present in distinct brain nuclei. The autoradiographic data indicate direct effects of vitamin D on neurons of all cranial nerve and spinal cord motor nuclei, which suggests neurotrophic effects on skeletal muscles. Muscle weakness associated with clinical conditions of vitamin D deficiency, therefore, may be due predominantly to altered neurotrophic input, rather than to a direct genomic action of vitamin D on muscle cells. In the experimental rodents studied by autoradiography, nuclear uptake and retention of ^{3}H-1,25DHCC has not been observed in skeletal muscle nuclei under conditions when strong nuclear labeling existed in motor neurons, intestinal absorptive epithelium, pituitary thyrotropes, and other target cells (Stumpf 1988). Evidence for nuclear receptor binding to cranial and spinal motor neurons has been furnished earlier in rat and mouse for vitamin D (Stumpf et al. 1988b) and for other steroid hormones, including dihydrotestosterone (Sar and Stumpf 1977) and corticosterone (Duncan and Stumpf 1984), but not for estradiol (Keefer et al. 1973). The presence of nuclear receptors for androgen, glucocorticoid, and soltriol in identical cells point to interactive mechanisms of steroid hormone effects that may be supportive and potentiating, or possibly antagonistic.

The present data indicate direct effects of soltriol not only on somatomotor neurons, but also on neurons of somatosensory systems. Accordingly, labeled cells in lamina II of the spinal cord and in the various components of the sensory nuclei of the trigeminus suggest modulatory effects on touch and pain perception. Similarly, the strong nuclear binding in the trapezoid body suggests a modulation of acoustic input and processing, selectively of that component of acoustic relays, while no or little direct effects are indicated at the levels of the nuclei of the inferior colliculus and the geniculate body. Nuclear binding in accessory optic nuclei suggests modulatory effects of vitamin D on autonomic transduction of retinal light input.

Effects on autonomic centers in the medulla and pons are indicated by the presence of target neurons in the nucleus ambiguus and in various regions of the reticular formation. Nuclear labeling in the caudal nucleus raphe dorsalis suggests some regulatory influence on certain serotoninergic-peptidergic neurons. Labeling with vitamin D of neurons in regions of areas A5 and A1 suggests modulation of catecholamine manufacture and secretion in these cell populations. Since neurons in area A2 appear to be free of nuclear vitamin D receptors, this catecholamine cell population is most likely not to be directly addressed by vitamin D. The latter contrasts with

localized estradiol and co-localized catecholamine (Heritage et al. 1980) in the nucleus of the solitary tract. In the area postrema, a subpopulation of cells is strongly labeled with vitamin D, similar to estradiol and dihydrotestosterone. The functional significance of this circumventricular organ and the effects of vitamin D on this conspicuous region remain to be studied.

The general population of small glia cells, probably mostly astrocytes and oligodendrocytes, does not show nuclear labeling. However, a subpopulation of the small cells is strongly labeled. These rounded or fusiform, small, labeled cells are scattered mainly in or near regions of myelinated fasciles. Estimations of their size, their topographical distribution, and their negative staining with ED1 macrophage-specific antibodies (unpublished data), the latter in agreement with similar findings published in the literature (Sminia et al. 1987), strongly suggest that these small labeled cells are microglia and that vitamin D is involved in modulating neuroimmunological processes and metabolism of myelin (Lamper and Kies 1967; Polman et al. 1986). While the results of the present studies provide important anatomical information and related functional clues, follow-up studies are required to clarify further the functions of the hormone in the identified target regions and also further to define the anatomical target sites, focusing on individual nuclei and regions.

In the Siberian hamster, compared to the laboratory rats and mice, a generally more extensive and, in some brain regions, anatomically different representation of vitamin D target cell populations is observed. Such differences have been reported for the choroid plexus (Bidmon et al. 1991a) and for certain septal and thalamic nuclei (Musiol et al. 1991, 1992). Species-specific target cell distribution, that is, expression of receptors for vitamin D, may be linked to the species-specific habitat and related differing kinds and degrees of seasonal adaptation and response.

A comparison was made of our autoradiographic data with sites of expression of 28 kDa-calcium binding protein (Clark et al. 1986; Musiol et al. 1992); it has become increasingly apparent that there is limited correspondence between vitamin D nuclear binding and expression of the cytoplasmic 28 kDa-calcium binding protein. There is, however, correspondence between nuclear vitamin D-soltriol binding and other cellular products such as insulin in pancreatic island B-cells (Clark et al. 1980), thyroid-stimulating hormone in the pituitary and blood (Sar et al. 1980, 1981), gastrin in the pyloric stomach (Stumpf et al. 1988), ANF (atrial natriuretic factor) in the heart and blood (Bidmon et al. 1991b), and methyl transferase and tyrosine hydroxylase mRNA expression in adrenal medulla (Puchacz et al. 1991). Vitamin D has been reported to be a potent inducer of nerve growth factor synthesis in murine L-929 fibroblasts in vitro (Wion et al. 1991) which raises the possibility that vitamin D plays a role in the in vivo regulation of the nerve growth factor gene.

The results of our present experiments together with those from our previous studies with vitamin D in rodents and in representatives of other vertebrate phyla (Stumpf and Bidmon 1990; Bidmon and Stumpf 1991), indicate a wide scope of effects of the hormone of sunlight and support our concept of vitamin D-soltriol as a seasonal regulator of vital functions.

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