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Cellular Localization of Vasopressin V1a Receptor Messenger Ribonucleic Acid in Adult Male Rat Brain, Pineal, and Brain Vasculature

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ABSTRACT

Vasopressin V1a receptor (V1aR) transcripts were localized in brain, pineal, and superficial brain vascular tissues of adult male rats using hybridization histochemistry and an [^{35}S]riboprobe complementary to the messenger ribonucleic acid (mRNA) encoding the fifth to the midseventh transmembrane regions of the receptor. V1aR mRNA was extensively distributed throughout brain and was expressed in 1) superficial cells of the granule cell layers of the main olfactory bulb, hippocampal dentate gyrus, and cerebellum; 2) numerous anatomically distinct brain nuclei; 3) isolated cells dispersed throughout the central nervous system; 4) cells of the choroid plexus, occasional blood vessels in the olfactory bulb and interpeduncular nucleus, and extraparenchymal intracranial vasculature; and 5) some white matter structures. Numerous cells expressing V1aR transcripts were found in forebrain structures, including primary olfactory (piriform) cortex, the anterior and posterior olfactory nuclei; dorsal, intermediate, and ventral lateral septal nuclei; the septo-fimbrial nucleus and accumbens nucleus; and numerous hypothalamic regions with the most intense hypothalamic labeling in the arcuate, stigmoid, suprachiasmatic, and periventricular nuclei and the lateral hypothalamic area. Cells expressing V1aR transcripts were ubiquitous throughout the midbrain, pontine, and medullary regions. A lower intensity signal was found in cells of the parvocellular paraventricular and anteroventral nucleus of the thalamus, circumventricular organs including the pineal, and the subfornical organ. V1aR transcripts were not generally detected in parenchymal vasculature, but could be found over large blood vessels in the interpeduncular nucleus and medial olfactory bulb; transcripts were commonly detected in perivascular brain cells. V1aR mRNA was abundantly expressed by choroid plexus, endothelial cells of midline blood vessels between the main olfactory bulbs, and superficial vascular tissue on all brain surfaces. These data confirm the presence of the vascular/hepatic-type V1aR gene in brain tissue and document an extensive expression. The distribution of V1aR mRNA suggests that there are at least two types of vasopressin-responsive cells in brain: one type exemplified by lateral septal area neurons innervated by classical axodendritic/somatic synaptic vasopressinergic terminals and a second, perivascular/vascular type that would facilitate humoral vasopressinergic signaling in the brain. (*Endocrinology* **135:** 1511–1528, 1994)

VASOPRESSIN (VP)-containing magnocellular neurons in the hypothalamic paraventricular and supraoptic nuclei project to the neurohypophysis and release VP into the peripheral circulation to regulate salt and fluid balance. Parvocellular cells in the paraventricular nucleus (PVN), bed nucleus of the stria terminalis, and suprachiasmatic nucleus give rise to an extensive network of VP fibers throughout brain (1, 2).

VP binds to at least three distinct subtypes of receptors, two of which, the V1a and V2 receptors, have been cloned from rat libraries (3, 4). Binding to the vascular/hepatic-type V1a receptor (V1aR) stimulates phosphatidylinositol turnover and mobilizes intracellular calcium in liver (5) and brain tissue (6, 7). As expected, the messenger RNA (mRNA) encoding the V1aR is abundant in rat liver and kidney and present in brain (8, 9). Stimulation of cAMP is linked to binding of VP to the renal-type V2 receptor (V2R) (10), and V2R transcripts are heavily expressed in cells of the renal collecting ducts and thick ascending limbs of the loops of Henle (9), structures involved in mediating the antidiuretic effects of VP. A unique pharmacological binding profile suggests the existence of a third VP receptor, V1b (11, 12), which is involved in corticotropin release by VP alone and in combination with corticotropin-releasing hormone. There is little information on the distribution of this receptor outside of the pituitary.

The use of subtype-selective vasopressinergic radioligands suggests that the majority of binding in brain is to the V1aR (13–18). Brain VP receptors have been proposed to mediate the effects of VP on memory and learning (19), antipyresis (20–22), brain development (23–25), selective aggression and partner preferences in rodents (26, 27), cardiovascular responsivity (28–31), blood flow to the choroid plexus and cerebrospinal fluid production (32), regulation of smooth muscle tone in superficial brain vasculature (33, 34), and analgesia (35, 36). The nonspecificity of VP and oxytocin for binding sites, the nonspecificity of synthetically derived agonists and antagonists for their receptors (37–39), and the possibility that multiple subtypes of VP receptors or multiple paths for posttranslational receptor modifications exist in the brain have made it difficult to confirm the identity of putative V1a VP receptors. Thus, localization of V1aR transcripts in brain regions implicated in the central nervous system effects of VP would strengthen the likelihood that the V1aR mediates vasopressinergic actions in the brain and stimulates further pharmacological development of selective V1aR agonists and antagonists. We provide herein the detailed dis-

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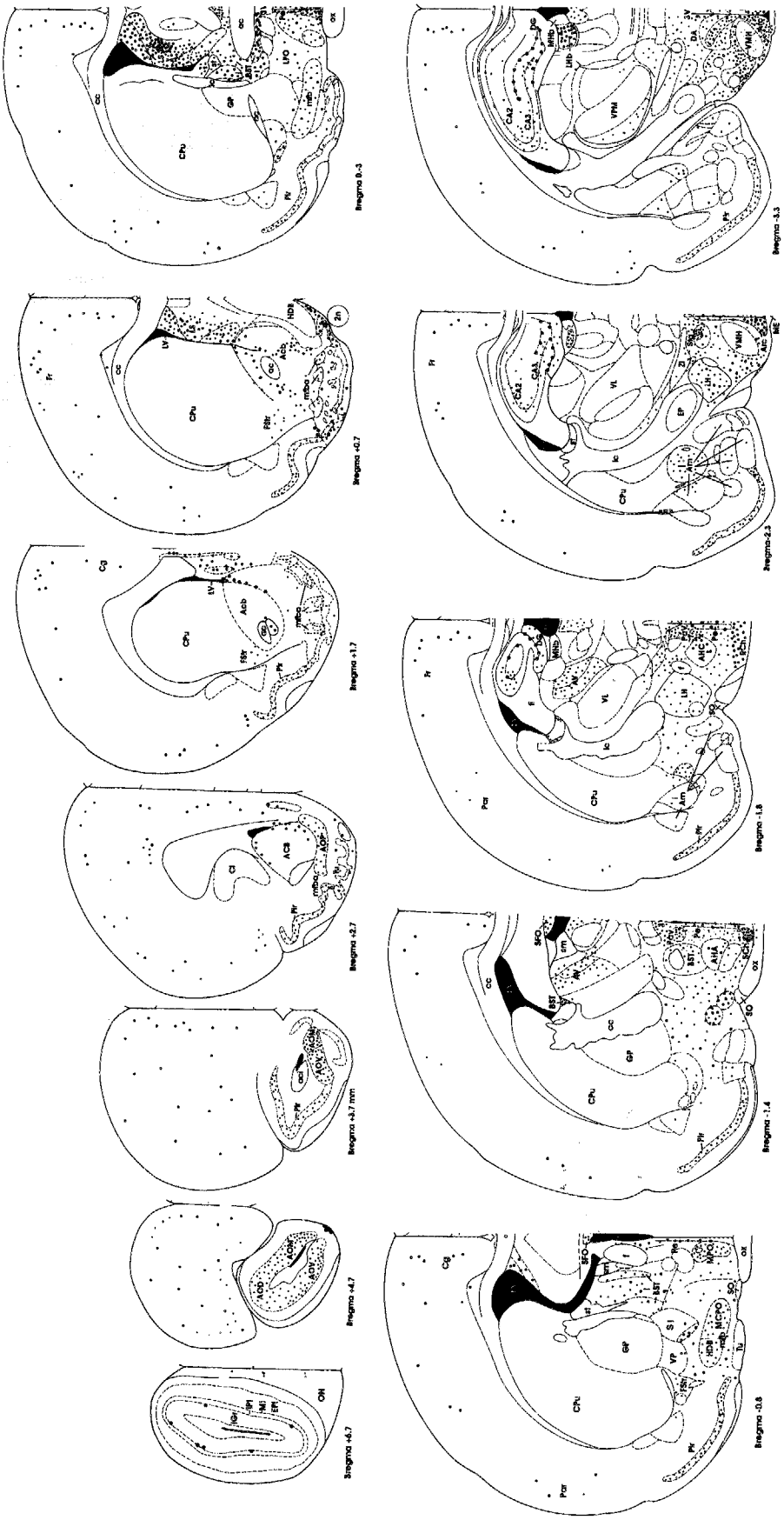
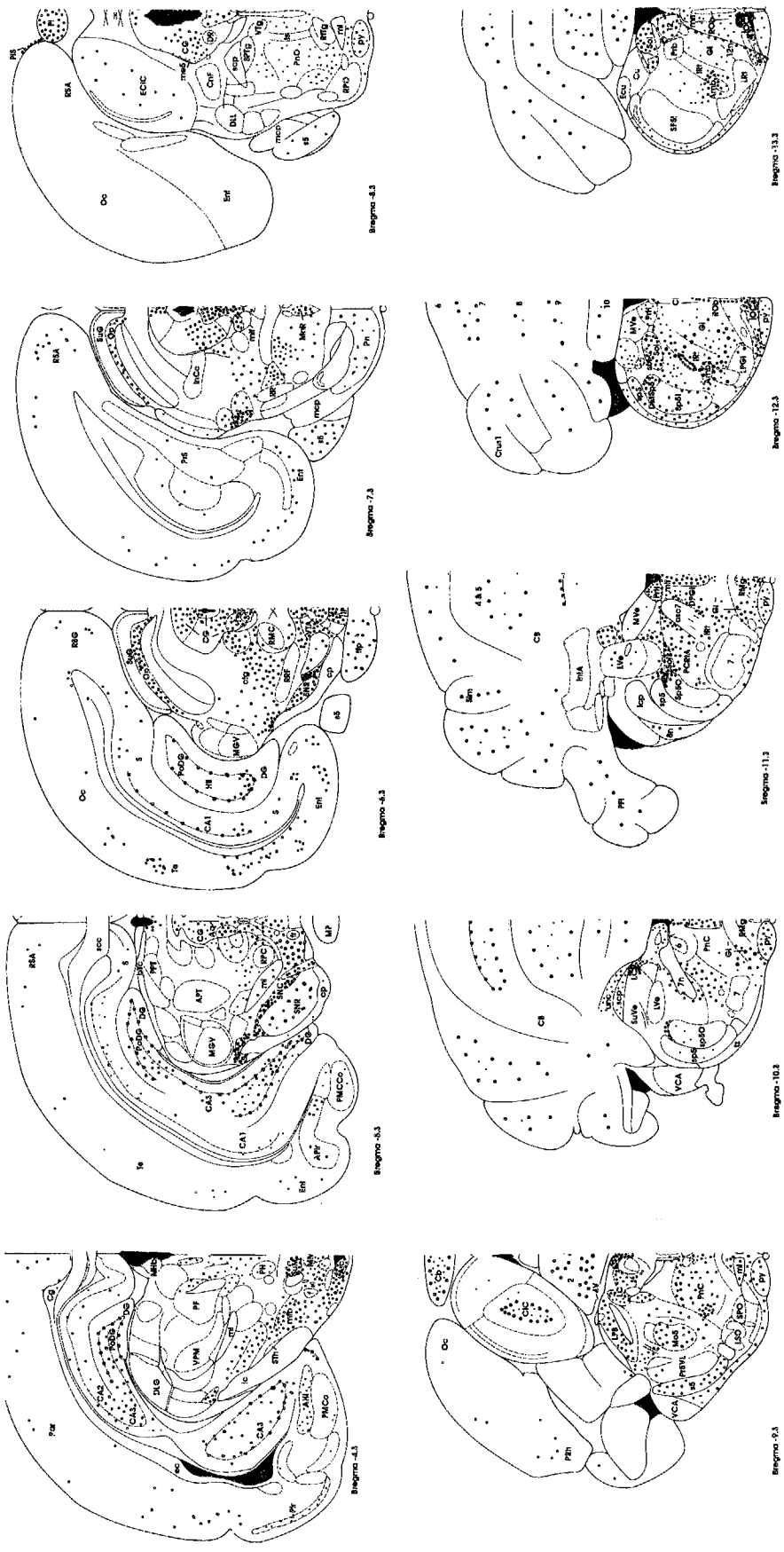


FIG. 1. VP V1a receptor mRNA distribution in male rat brain. This is a schematic diagram representing brain regions where V1aR mRNA signal was localized in cells using *in situ* hybridization histochemistry. Plates from the brain atlas of Paxinos and Watson (40) were scanned and modified using Silverscan, Adobe Photoshop (2.5), and V1aR mRNA-expressing cells were plotted onto these maps. The point size represents the relative intensity of cellular label; the point density represents the relative density of labeled cells. Coordinates rostral and caudal to Bregma are given at the *lower left* of each brain diagram and correspond to those of the plates in the atlas (40). Maps were plotted bilaterally. As no apparent hemispheric differences were noted, data were collapsed onto the left hemispheric diagrams. Abbreviations (there are some exceptions, including midline structures) are from the atlas of Paxinos and Watson (40), and the reader is referred to that reference for more detailed neuroanatomical information (40). 6–10, Cerebellar lobules; 2n, optic nerve; 3, oculomotor nucleus; 4V, fourth ventricle; 4&5, cerebellar lobules; 6, abducens nucleus; 7, facial nucleus; 8n, vestibulocochlear nerve; 12, hypoglossal nucleus; 12n, root of hypoglossal nerve; A, cerebral aqueduct; ac, anterior commissure; ACB, accumbens nucleus; Aci, anterior commissure, intrabulbar part; AHC, anterior hypothalamic area; AHi, amygdalo-hippocampal area; Am, amygdaloid nuclei; Amb, ambiguus nucleus; AOD, anterior olfactory nucleus, dorsal part; AOM, anterior olfactory nucleus, medial part; AOP, anterior olfactory nucleus, posterior part; AOV, anterior olfactory nucleus, ventral part; APir, amygdalo-piriform transition area; APT, anterior pretectal nucleus; Arc, arcuate hypothalamic nucleus; AV, anteroventral thalamic nucleus; BST, bed nucleus of the stria terminalis; bsc, brachium of the superior colliculus; CA 1–3, fields CA 1–3 of Ammon's horn; CB, cerebellum; cc, corpus callosum; CG, central gray; Cg, cingulate cortex; CIC, central nucleus of the inferior colliculus; Cl, claustrum; CnF, cuneiform nucleus; cp, cerebral peduncle; CPu, caudate putamen (striatum); Crus 1, Crus 1 of the ansiform lobule; ctg, central tegmental tract; D3V, dorsal third ventricle; DA, dorsal hypothalamic area; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; DLL, dorsal nucleus of the lateral lemniscus; DR, dorsal raphe nucleus; ec, external capsule; ECIC, external cortex of the inferior colliculus; Ecu, external cuneate nucleus; Ent, entorhinal cortex; EP, entopeduncular nucleus; EPI, external plexiform layer of the olfactory bulb; f, fornix; fi, fimbria; Fr, frontal cortex; FStr, fundus striati (ventral striatum); Gi, gigantocellular reticular nucleus; GP, globus pallidus; HDB, nucleus of the horizontal limb of the diagonal band; Hil, hilus of the dentate gyrus; I, intercalated nuclei of the amygdala; ic, internal capsule; icp, inferior cerebellar peduncle; IGr, internal granular layer of the olfactory bulb; InCo, intercollicular nucleus; IntA, interposed cerebellar nucleus; IOC, inferior olive, subnucleus C of medial nucleus; IPI, interpeduncular nucleus, intermediate; IPC, interpeduncular nucleus, caudal; IRt, intermediate reticular nucleus; LC, locus coeruleus; LH, lateral hypothalamic area; LHb, lateral



habenula nucleus; LPB, lateral parabrachial nucleus; LPGi, lateral paragigantocellular nucleus; LR4V, lateral recess of the fourth ventricle; LRt, lateral reticular nucleus; LS, lateral septal nuclei; LSO, lateral superior olive; LV, lateral ventricle; LVe, lateral vestibular nucleus; mcp, middle cerebellar peduncle; MCPO, magnocellular preoptic nucleus; me5, mesencephalic trigeminal tract; mfb, medial forebrain bundle; mfba, medial forebrain bundle "a" component; MGV, medial geniculate nucleus; MHb, medial habenular nucleus; MI, mitral cell layer of the olfactory bulb; ml, medial lemniscus; mlf, medial longitudinal fasciculus; MM, medial mammillary nucleus, medial part; MnR, median raphe nucleus; Mo5, motor trigeminal nucleus; MP, medial mammillary nucleus, posterior part; MPO, medial preoptic nucleus; MVe, medial vestibular nucleus; Oc, occipital cortex; ON, olfactory nerve layer; Op, optic nerve layer of the superior colliculus; ox, optic chiasm; Par, parietal cortex; PaV, paraventricular hypothalamic nucleus; PCRtA, parvocellular reticular nucleus, α part; Pe, periventricular hypothalamic nucleus; PF, parafascicular thalamic nucleus; PFl, paraflocculus; PH, posterior hypothalamic area; Pi, pineal gland; Pir, piriform cortex; PiS, pineal stalk; PMCo, posteromedial cortical amygdaloid nucleus; Pn, pontine nuclei; PnC, pontine reticular nucleus, caudal part; PnO, pontine reticular nucleus, oral part; PoDG, polymorph layer of the dentate gyrus; PPT, posterior pretectal nucleus; Pr5VL, principal trigeminal sensory nucleus, ventrolateral part; Prh, nucleus of Probst's bundle; PRh, perirhinal cortex; PrH, prepositus hypoglossal nucleus; PrS, presubiculum; py, pyramidal tract; RCh, retrochiasmatic area; RMC, red nucleus, magnocellular part; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPC, red nucleus, parvocellular part; RPO, rostral periolivary region; RR, retrorubral nucleus; RRF, retrorubral field; RSA, retrosplenial agranular cortex; RtTg, reticulo-tegmental nucleus of the pons; S, subiculum; s5, sensory root of the trigeminal nerve; scc, splenium of the corpus callosum; SCh, suprachiasmatic nucleus; scp, superior cerebellar peduncle, brachium conjunctivum; SFO, subfornical organ; Sim, simple lobule; sm, stria medullaris of the thalamus; SNR, substantia nigra, reticular part; SNC, substantia nigra, compact part; SO, supraoptic nucleus; Sol, nucleus of the solitary tract; sol, solitary tract; SPTg, subpeduncular tegmental nucleus; SPO, superior periolivary nucleus; sp5, spinal trigeminal nucleus; Sp5I, spinal trigeminal nucleus, caudal part; Sp50, spinal trigeminal nucleus, oral part; st, stria terminalis; Stg, stigmoid hypothalamic nucleus; SuG, superficial gray layer of the superior colliculus; SuVe, superior vestibular nucleus; SuM, supramammillary nucleus; Te, terete hypothalamic nucleus; tfp, transverse fibers of pons; ts, tectospinal tract; Tu, olfactory tubercle; tz, trapezoid body; unc, uncinate fasciculus; VCA, ventral cochlear nucleus, anterior part; VL, ventrolateral thalamic nucleus; VMH, ventromedial hypothalamic nucleus; VP, ventral pallidum; VPM, ventral posteromedial thalamic nucleus; VTA, ventral tegmental nucleus (Tsai); VTg, ventral tegmental nucleus (Gudden); ZI, zona incerta.

tribution of V1aR mRNA in rat brain. We show that the mRNA for the V1a receptor subtype is extensively distributed throughout the brain, both supporting previously proposed roles for centrally acting VP and suggesting additional undefined roles for this neuropeptide.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were decapitated, and their brains were removed and frozen on powdered dry ice. The brains were stored at −80 C until cut into 12- and 24-μm thick brain sections at approximately −20 C. Sections were thaw-mounted onto gelatin-coated slides and stored at −80 C before hybridization. Separate hybridization histochemistry experiments were conducted six times on tissues from different animals and yielded similar distributions of V1aR mRNAs.

Hybridization histochemistry

The hybridization histochemical procedures have been described previously (8, 40). Briefly, tissue sections were thawed, immersed in 4% formaldehyde in PBS, treated with acetic anhydride, and delipidated in a graded series of alcohols and chloroform. Sections were incubated for 20–23 h at 55 C with approximately 1.5×10^6 dpm/60 μl hybridization buffer. Tissue sections were then rinsed in 4 × saline-sodium citrate buffer (SSC), incubated with 20 μg/ml ribonuclease-A at 37 C for 30 min, and washed finally in 0.1 × SSC containing 10 mM dithiothreitol at 65 C. Tissue sections were dehydrated in alcohols containing 300 mM ammonium acetate and dried. Some sections were apposed to Hyperfilm-[^{3}H] (Amersham, Arlington Heights, IL) or imaging plates (Fuji, Stamford, CT) for macroscopic localization and then dipped in Ilford K.5D nuclear emulsion (Polysciences, Inc., Warrington, PA) and exposed for 3–6 months for microscopic localization of V1aR transcripts. To facilitate morphological analyses, tissue sections were stained with thionin or toluidine blue.

Figures 2–14 were photographed directly from emulsion-coated tissue sections of either 12- or 24-μm thickness. Bright- and darkfield images are of the same tissue section. High power magnifications of cells were typically ×400 or ×640 under oil.

Results

VP V1aR transcripts

VP V1aR mRNAs were extensively distributed throughout the brain. Figure 1 summarizes the distribution of V1aR transcripts over cells plotted using the atlas of Paxinos and Watson (41). For ease of presentation, V1aR mRNA distributions were grouped into five types of labeling patterns: 1) laminar labeling, 2) labeling of cells within an anatomical nucleus or circumscribed region (nuclear labeling), 3) random

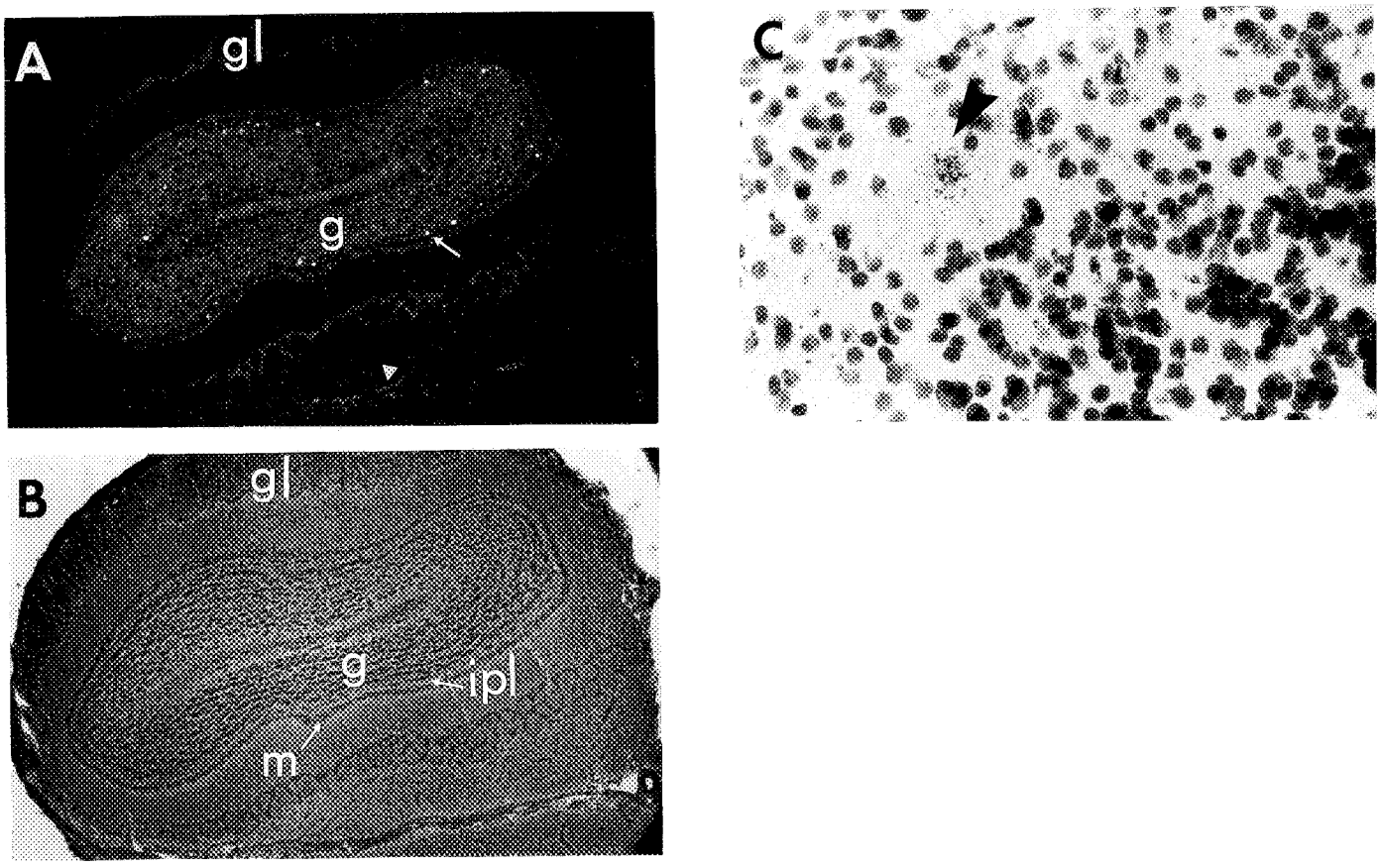
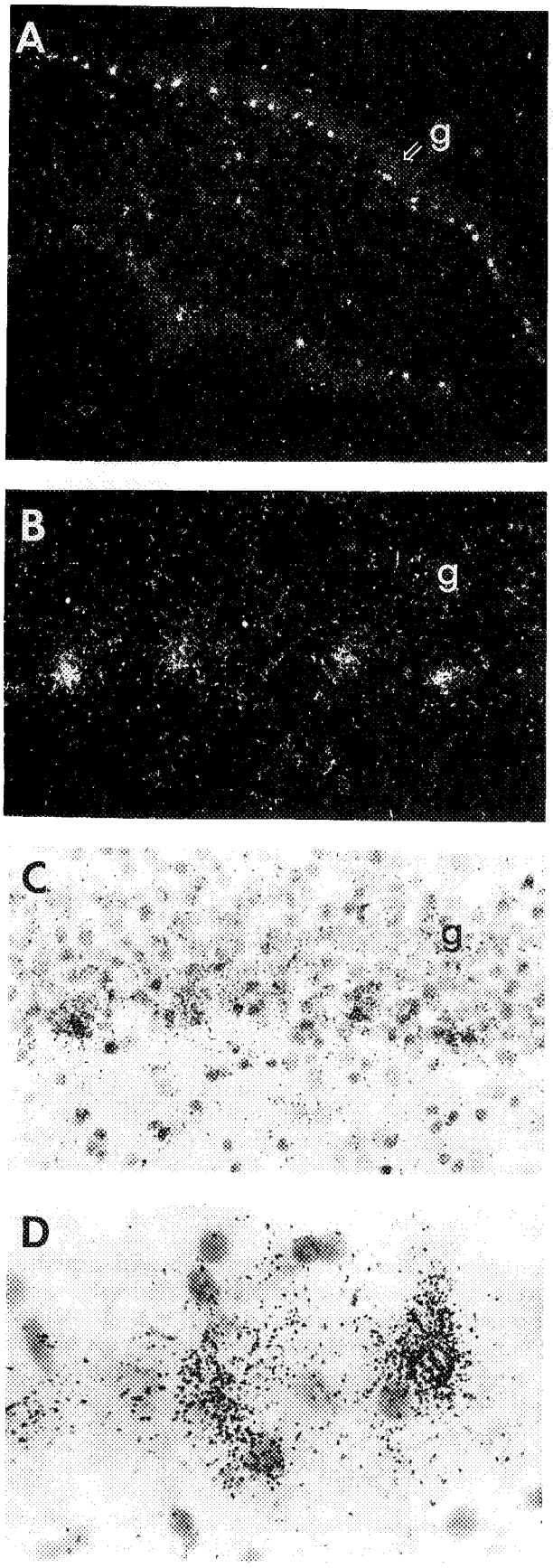


FIG. 2. V1aR mRNA in rat main olfactory bulb. A, This darkfield, low magnification photograph of a coronal section of the main olfactory bulb was overexposed to show the localization of V1aR transcripts in cells at the border of the internal granule cell layer and the internal plexiform layer. Note the rarity of the labeled cells and their laminar organization (*arrow*). Also note label along the midline between the two olfactory bulbs (*arrowhead*). V1aR transcript-producing cells were not found in other regions of the olfactory bulb. B, Brightfield image corresponding to A. C, High magnification of a single V1aR transcript-expressing cell (*arrowhead*). Note the large, light Nissl-stained nuclei and surrounding cell-poor zone. The granule cell layer is at the *bottom right*. gl, Glomerulus; g, internal granule cell layer; ipl, internal plexiform layer; m, mitral cell layer.



sparse cellular labeling, 4) vascular labeling, and 5) label within white matter regions.

Laminar labeling pattern. Three brain regions, the main olfactory bulb, the hippocampus, and the cerebellum, demonstrated V1aR transcript-expressing cells associated with granule cell layers.

Main olfactory bulb. Single cells located at the border between the internal granule cell layer and the internal plexiform layer showed intense hybridization signal (Fig. 2). Cell nuclei were light-staining and tended to be larger than those of surrounding granule cells, and the cells were sometimes surrounded by a cell-poor zone (see Fig. 2C). Only rarely were cells expressing V1aR transcripts detected in deeper areas of the internal granular layer or other subdivisions of the main olfactory bulb (see Fig. 2A).

Hippocampus. Whereas most dentate gyrus granule cells showed near-background levels of hybridization signal, periodically spaced cells along the hilar surfaces showed high intensity V1aR mRNA labeling (see Figs. 3, A, B, and C). Other hippocampal cells that expressed V1aR transcripts (Fig. 3, A and D; and those described below) did not show strict laminar organization.

Cerebellum. In the cerebellum, V1aR transcript-expressing cells were often detected intermittently along the outer edges of the granule cell layers (Fig. 4). Similar to the cells in the olfactory bulb, some V1aR transcript-producing cells had large ovoid nuclei that stained less densely with Nissl stain than the surrounding cells, whereas other V1aR-expressing cells resembled granule cells (Fig. 4B). Occasional cells deeper within the granule cell layer were also labeled.

Nuclear labeling pattern. Numerous brain regions demonstrated uniform or nonuniform patterns of V1aR expression. An overview of the V1aR mRNA-containing cells is given below.

Forebrain olfactory systems. In addition to the cells labeled along the outermost layer of the internal granule cell layer of the main olfactory bulb (described above), V1aR mRNA was abundant in cells of the anterior olfactory nucleus; the medial and ventral subdivisions showed slightly greater numbers of V1a-expressing cells than the dorsal subdivision. The posterior olfactory nucleus showed uniform labeling of a smaller number of cells than the anterior olfactory nucleus. A cluster of extremely densely labeled cells was located on the surface of the brain ventromedial to the anterior olfactory nucleus. The piriform cortex showed a low to moderate

FIG. 3. Laminar organization of V1aR mRNA in hippocampal dentate gyrus cells. A, Darkfield image of a caudal section of the hippocampal formation showing the pattern of V1aR mRNA labeling. Note the "string of pearls" pattern of V1aR expression in cells on the hilar surface of the granule cell layer (A, *double arrow*), which is more clearly seen in the higher magnification photographs, *below*. B, High magnification darkfield. C, corresponding brightfield image showing localization of V1aR hybridization signal in cells along the superficial surface of the caudal dentate gyrus granule cell layer in the rat hippocampal formation. D, V1aR transcript-expressing cells in the polymorph region. g, Granule cell layer of the dentate gyrus.

intensity uniform signal that decreased in intensity at very caudal levels. Most of the olfactory tubercle was poorly labeled, although some cells in the extreme medial regions expressed V1aR transcripts. A few V1aR transcript-expressing cells were found in dorsal regions of the olfactory tubercle (polymorph layer), but most densely labeled cells were scattered above and around the tubercle.

Septo-hippocampal system. The bed nucleus of the stria terminalis was nonuniformly labeled, with cells showing moderate to high intensity hybridization signal. Most cells of the intermediate, dorsal, and ventral subdivisions of the lateral septal nucleus expressed V1aR transcripts (Fig. 5, B and E), with some cells showing very high levels (Fig. 5, A and D). More caudally, label was also detected in the septofimbrial nucleus. Cells of the horizontal limb of the diagonal band of Broca were moderately labeled. The medial septum, fornix, triangular nucleus, and vertical limb of the diagonal band showed background signal.

Labeling of the hippocampus proper showed variability between animals. In some subjects, virtually all regions had a low abundance of labeled cells, whereas in other animals, only a few labeled cells were found, usually in caudal regions. The most consistent and intense hybridization signal was periodic along the hilar surface of the dentate gyrus (described above). However, apparently randomly situated cells throughout the hippocampal formation showed hybridization signals of variable intensities, ranging from extremely dense to just above background. The sparsest signal was over the molecular layer adjacent to the granule cells of the dentate gyrus. Labeling in the polymorph layer of the dentate and CA3 appeared of greater intensity in mid and caudal brain sections containing hippocampus (see Fig. 4). The number of labeled cells in the pyramidal cell layer of CA1 decreased caudally.

Amygdalo-hippocampal system. In the anterior amygdaloid area and the central nucleus, numerous cells expressed uniformly moderate levels of V1a transcripts. Well labeled cells were in the amygdalo-striatal and amygdalo-hippocampal transitional areas.

Basal ganglia. Discrete strings of V1aR-expressing cells, originating primarily on the medial side of the ventral tips of each lateral ventricle (probably cells of the bed nucleus of the stria terminalis), coursed ventrally along its dorsolateral edges before penetrating the accumbens nucleus. Clusters of well labeled cells were found in the core of the accumbens nucleus proximal to the anterior limb of the anterior commissure. Labeled cells were also dispersed throughout the ventral forebrain, including the ventral pallidum, regions dorsal to the olfactory tubercle, the fundus striati (ventral striatum and substriatal area), and substantia innominata, with denser aggregations of moderately labeled cells appearing along the path of the medial forebrain bundle and in the basal nucleus of Meynert. V1aR transcripts were absent from other structures of the basal ganglia, including the caudate-putamen (Fig. 5, C and F), subthalamic nucleus, and globus pallidus, and could not be identified in cells of the islands of Calleja.

Hypothalamus. Numerous hypothalamic nuclei showed moderate to dense V1aR transcript expression. In addition, cells demonstrating low to moderate levels of V1aR mRNA were found throughout most of the hypothalamic gray matter with the exception of the supraoptic nucleus, where no signal was detected (see Fig. 8, for example). Interestingly, a cluster of cells that densely expressed V1aR transcripts was found just dorsal to the supraoptic nucleus.

High intensity dense label was evident in the suprachiasmatic (Fig. 6), arcuate (Fig. 7), dorsomedial, and stigmoid nuclei and in the retrochiasmatic area and the lateral hypothalamus along the path of the medial forebrain bundle. Well spaced, intensely labeled cells were found in the hypothalamic periventricular nucleus (see Fig. 6B, rostral periventricular nucleus; Fig. 7A, caudal periventricular nucleus), each about equidistant from the ventricle wall.

Hypothalamic regions showing fewer intensely labeled cells and more moderately labeled cells included the medial preoptic, anterior, dorsal, and posterior hypothalamic areas; the parvocellular PVN (Fig. 6; including the medium-sized cells of the cap of the PVN); and ventral premammillary and supramammillary nuclei.

The ventromedial nucleus of the hypothalamus (VMH) typically appeared on autoradiographs as an ovoid region of negligible label surrounded by moderately high levels of hybridization signal in the dorsomedial hypothalamic nucleus, perifornical region, medial tuberal nucleus, arcuate nucleus, and lateral hypothalamus (Fig. 8). Inspection of emulsion-coated tissue that was exposed for 4–6 months using high magnification microscopy revealed that although the VMH initially appeared devoid of signal, in some animals one or two cells were detected that expressed extremely low levels of V1aR transcripts.

Thalamus and subthalamic areas. Fewer cells in thalamic nuclei expressed the gene encoding the V1aR than in the hypothalamus, and those cells that did label were typically of much lower intensity. For example, the anteroventral and paraventricular thalamic nuclei showed uniform, low intensity V1aR mRNA expression. Some of the highest intensity labeling was in cells along the third ventricle (the periventricular nucleus) and in the parafascicular thalamic nucleus. Lower densities of transcript-expressing cells were in the reticular nucleus, posterior thalamic areas, including the ventroposterolateral and ventromedial nuclei, and Forel's fields, whereas the zona incerta showed labeling of moderate intensity. Thalamic V1aR expression was otherwise undetectable.

Epithalamus. The lateral habenula showed a moderately high level of transcription, with the highest density of labeled cells concentrated in the medial division of the lateral habenula. No label was found in the medial habenula or the stria medullaris. Pinealocytes and the vascular tissue around the pineal had numerous V1aR transcript-expressing cells (Fig. 9). Interestingly, V1aR-producing cells were sometimes embedded within the lumenal walls of the subcommissural rosettes within the pineal gland (Fig. 9F).

Circumventricular structures. A few labeled cells were found in the subfornical organ (Fig. 10). Cells dorsal to the

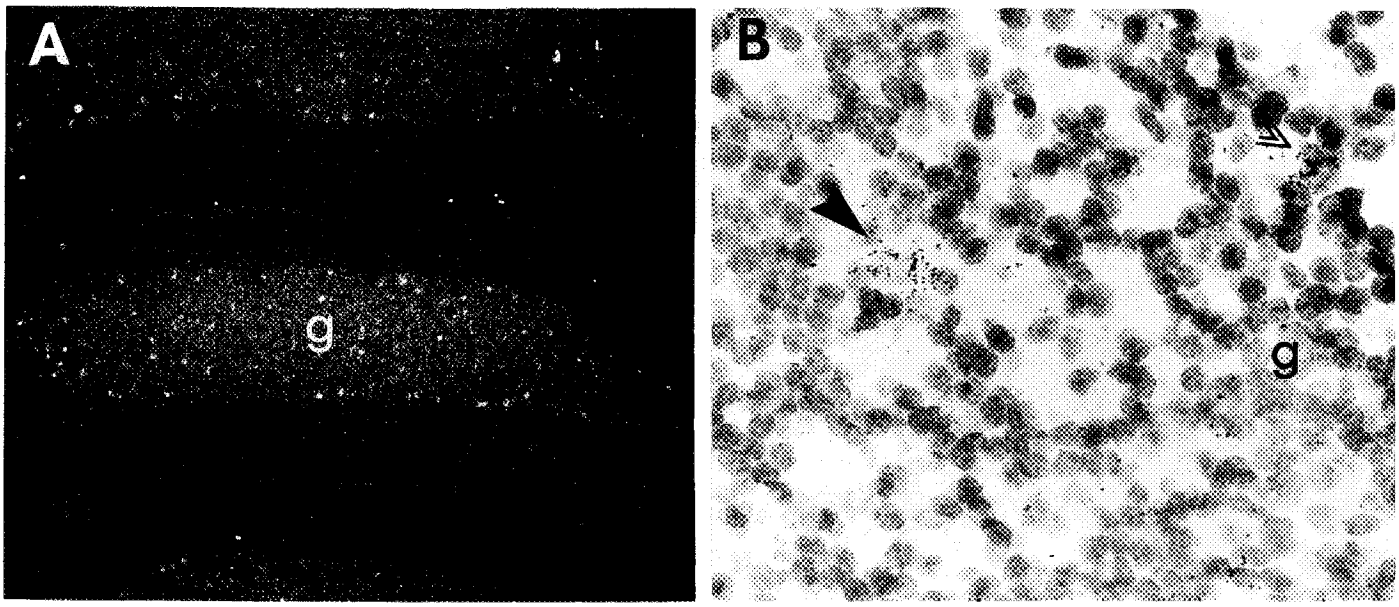


FIG. 4. V1aR hybridization signal in the cerebellar granule cell layers. A, Overexposed darkfield, low magnification photograph of emulsion-coated cerebellar section showing localization of individual V1aR-expressing cells. The highest intensity hybridization signal was often associated with cells dispersed along the peripheral borders of the cerebellar granule cell layers. B, High magnification brightfield image of labeled cells from a different cerebellar section. Note lightly Nissl-stained ovoid nuclei of deep V1aR-expressing cell (*solid arrowhead*) and round nuclei of cell at outer edge of granule cell layer (*double arrowhead*). g, Granule cell layer of the cerebellum.

anterior portion of the third ventricle showed moderately high hybridization signal, in contrast to the lower intensity signal detected in the paraventricular thalamic nucleus (see above). The area postrema (Fig. 11) had numerous high intensity, uniformly labeled cells. Signal was not detected over cells of the ventricular walls, in the vascular organ of the lamina terminalis, the subcommissural organ, or the median eminence.

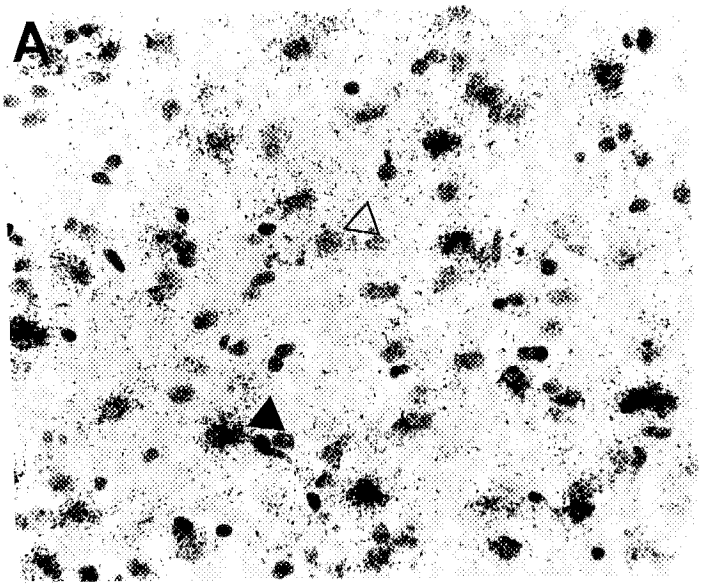
Midbrain nuclei. In general, there was a decrease in the anatomical specificity and an increase in the number of V1aR transcript-expressing cells in midbrain regions and more caudally (see Fig. 1). Moderate to high levels of nonuniformly expressed V1aR transcripts were found throughout the ventral tegmental area, zona compacta, caudal zona reticulata, and pars lateralis of the substantia nigra; the medial subdivisions of the interpeduncular nucleus; and the nucleus commissuralis of Darkschevich. The optic nerve layer of the superior colliculus showed homogenous moderate receptor transcript expression. Receptor transcripts were found in the midbrain dorsal nucleus of the raphe, and the rostral and caudal linear raphe. Generally, V1aR transcript-expressing cells were found throughout most of the ventral half of midbrain gray matter, including the periaqueductal gray. The red nucleus, retrorubral fields, and medial and lateral geniculates did not express V1aR message.

Pons and medulla. High expression of V1aR transcripts was detected in the medial inferior olivary nuclei (Fig. 12) of some animals, but not others. Labeled cells were found in most pontine and medullary raphe nuclei, including the median and paramedian, pallidus, and raphe magnus, and in cells surrounding the raphe obscurus and the raphe pontis nuclei. Cells in the locus ceruleus and both small and very large cells in some nuclei (intermediate reticular nucleus and paragigantocellular reticular nucleus) expressed V1aR mRNA.

With the exception of the optic chiasm (see below), V1aR mRNA production was not typically seen in the cranial nerve bundles. A moderate to high level of signal was frequently detected in cells of parenchymal tissue adjacent to the nerve roots, and this was particularly notable along the fibers of the facial nerve root. Cells expressing the V1aR gene were in the nucleus of the solitary tract (see Fig. 11) and the large cells of the nucleus ambiguus; in nuclei of the oculomotor, facial, accessory facial, motor hypoglossal, and prepositus hypoglossal; and in the sensory and motor nuclei of the trigeminal, supratrigeminal, and dorsal motor nucleus of the vagus. A wide range of label intensity was demonstrated, with densely labeled large cells (*e.g.* motor hypoglossal) and smaller cells dispersed throughout most gray matter regions.

Cerebellum. Occasional cells within the cerebellar granule cell layer expressed V1aR mRNA. In addition to the superficial laminar localization of most V1aR transcript-expressing cells in the cerebellum (described above), randomly distributed cells labeled deep within the granule cell layer. They frequently had large ovoid nuclei, were proximal to cell-free zones, and stained less densely than neighboring unlabeled cells with Nissl stain (see Fig. 4B, *solid arrowhead*). However, not all cells fitting this description (which may correspond to Golgi cells) expressed V1aR transcripts, even within the same tissue section, and some dark-staining round-nucleated cells, probably granule cells, also appeared to express low levels of V1aR mRNA (Fig. 4B, see *double arrowhead*). No label was detected over deep cerebellar nuclei.

Sparse cellular labeling pattern. Although labeled cells could be found in most cortical areas, slightly higher incidences of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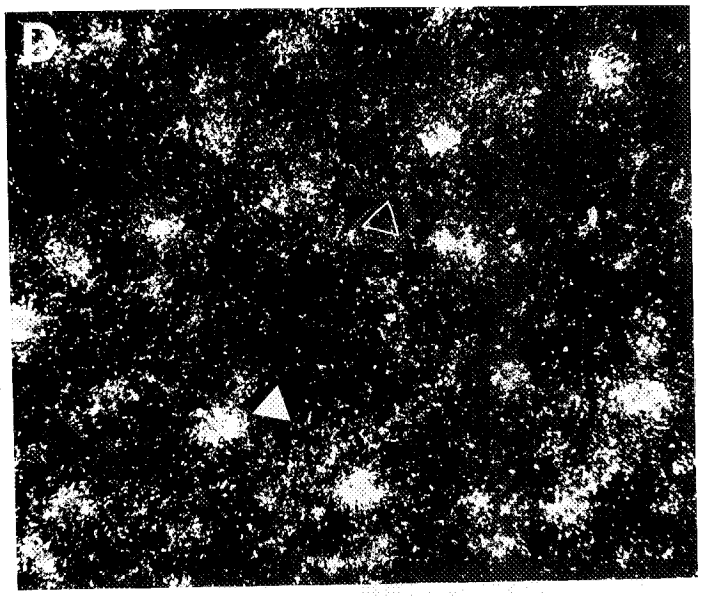
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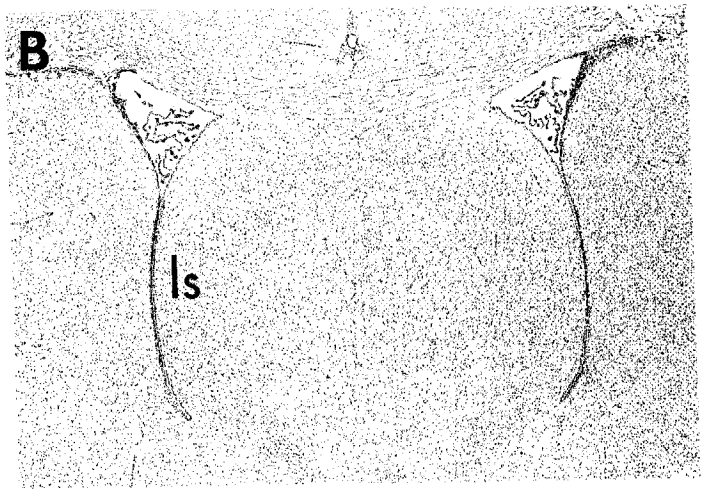
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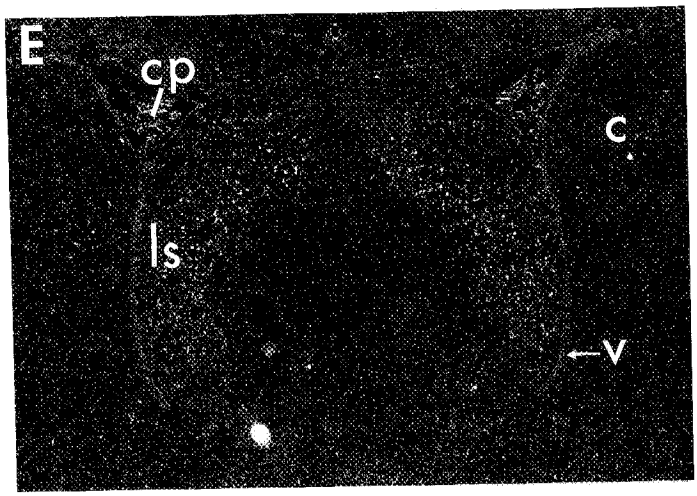
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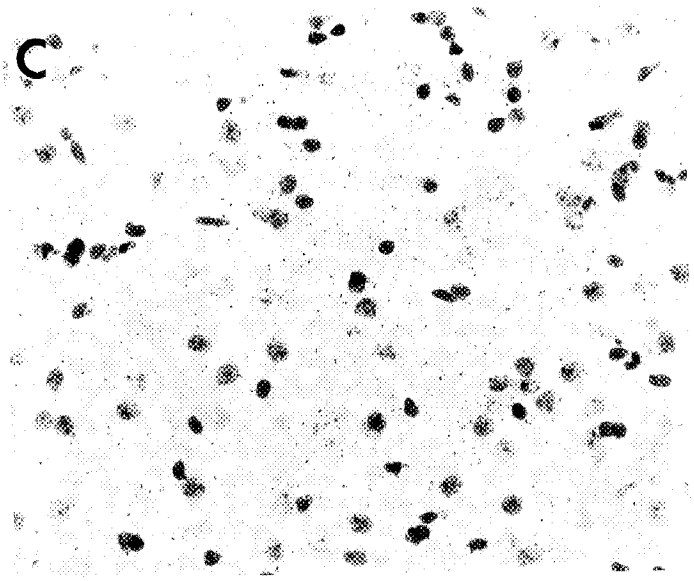
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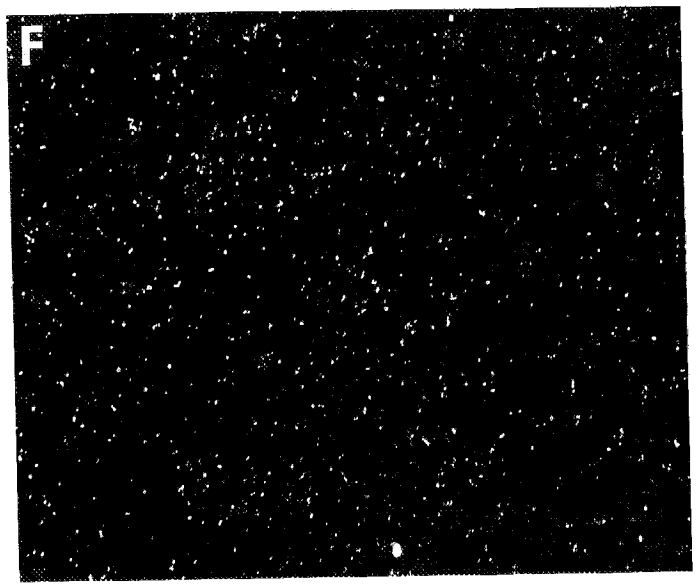
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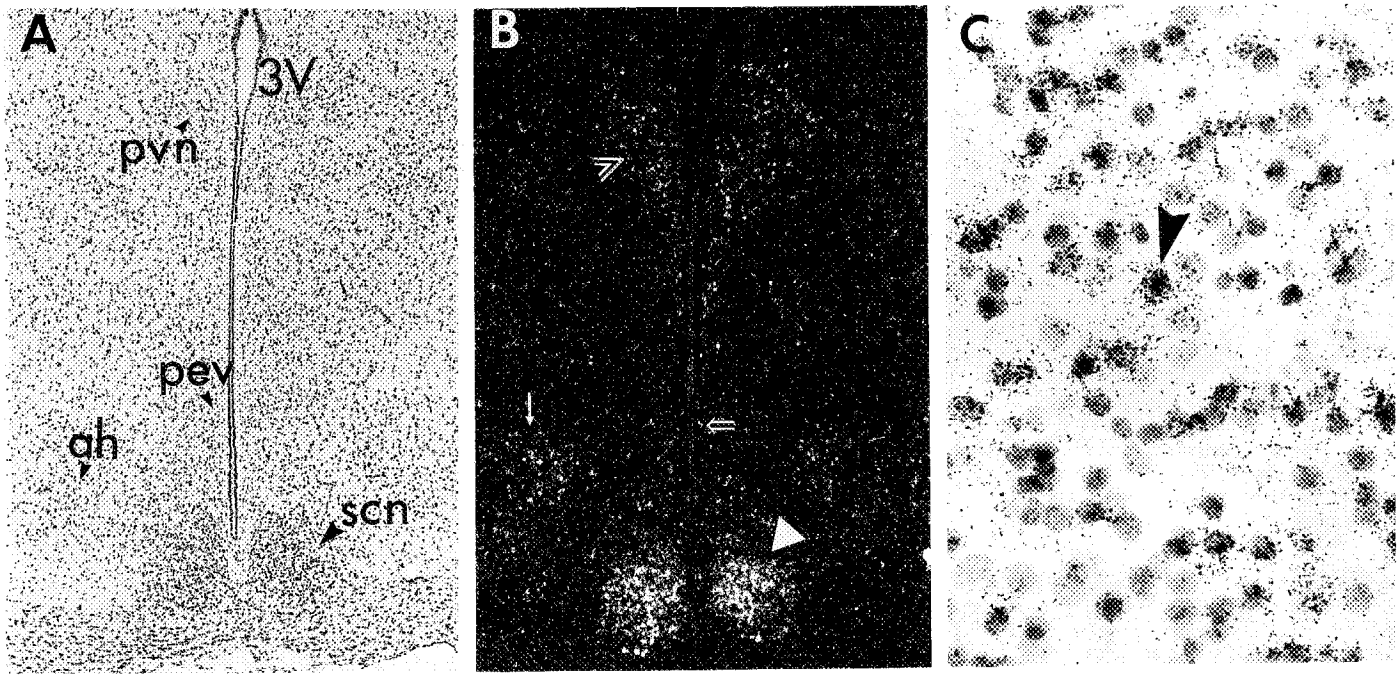

FIG. 6. V1aR transcripts detected in VP-synthesizing nuclei. A, Nissl-stained tissue section from a male rat showing the suprachiasmatic and paraventricular nuclei of the hypothalamus, both of which contain VP-synthesizing cells and V1aR transcripts. B, Corresponding darkfield image from the same tissue section that shows high intensity labeling of cells of the suprachiasmatic nucleus (*solid arrowhead*) and moderate intensity labeling of parvocellular and medium-sized cells in the PVN (*double arrowhead*). Isolated V1aR transcript-expressing cells in the periventricular hypothalamic nucleus could also be detected (*double arrow*). A few labeled cells were found in the anterior hypothalamic nucleus, which does not have VP-containing cells (*single arrow*). C, High magnification photomicrograph of Nissl-stained cells within the suprachiasmatic nucleus. Note high levels of silver grains, indicating V1a receptor mRNA expression in cells (*e.g. arrowhead*). ah, Anterior hypothalamic nucleus; pev, periventricular hypothalamic nucleus; pvn, paraventricular nucleus; scn, suprachiasmatic nucleus; 3V, third ventricle.

labeling occurred in cingulate, temporal, perirhinal, and entorhinal cortex. Single isolated V1aR-expressing cells were detected, usually in cortical layer III, using high power microscopy in emulsion-coated tissue sections.

Outside of the anterior amygdala, central nucleus, and transitional areas (discussed above), amygdaloid labeling was rare. Individual cells or small clusters (*e.g.* two or three cells) expressing low hybridization signal were occasionally seen in the medial nucleus, posteromedial cortical nucleus, lateral nucleus (dorsolateral part), basolateral nucleus (posterior part), and basomedial nucleus (anterior part). V1aR transcripts were also expressed by cells of the subiculum and presubiculum.

Vascular labeling. VP receptor mRNAs were detected over cells of superficial brain blood vessels. Label was notably heavy over vessels between the hemispheres (Fig. 13), on the ventral and dorsal surfaces of the brain, between the midbrain and the hippocampal formation, and ventrolateral to the median eminence. Labeling of blood vessels within the brain parenchyma was uncommon, but in several animals, cells of large vessels that penetrated the ventral surface and coursed through the interpeduncular nucleus expressed V1aR transcripts. V1aR mRNA transcripts were also found within brain tissue on a few blood vessels in the olfactory nerve layer of the main olfactory bulb and in brain cells in the immediate vicinity of midline vessels (Fig. 13, A and B, *double arrowheads*). Otherwise, V1aR transcripts were not observed in blood vessels. Brain cells expressing V1aR transcripts were sometimes apposed to vascular elements.

Cells within the choroid plexus were labeled, and the densest label was associated with cells that stained purplish with thionin and were frequently close to the origin (Fig. 14; also see Fig. 10B).

Label in white matter structures. Occasional V1aR transcript-labeled cell bodies were in the anterior limb of the anterior commisure, optic chiasm, cingulum, posterior commisure, and fimbria. Occasional strings of cells and single cells in the brainstem embedded within the cerebral peduncle, pyramid, and pyramidal decussation expressed V1aR message. These cells did not resemble neurons.

FIG. 5. V1aR transcripts are abundant in the lateral septal nucleus and absent from the caudate-putamen in the rat. A and D are matched, high magnification, brightfield and darkfield images of V1aR transcripts labeled in the male lateral septal nucleus. Note that most cells express V1aR transcripts, ranging from very low levels (*open arrowhead*) to very high levels (*solid arrowhead*). Also note the low background of labeling between cells. B and D, Corresponding low magnification brightfield and darkfield images showing the pattern of V1aR transcript-expressing cells in the lateral septal nucleus; V1aR transcripts were not seen in the medial septum. C and F, Corresponding bright- and darkfield photomicrographs showing cells from the caudate-putamen at the same magnification as A and D. Images were obtained from the same tissue section as A and D, but approximately 1.5 mm lateral to the septal nucleus. Note the virtual absence of hybridization signal in caudate nucleus from the same animal. cp, Choroid plexus; ls, lateral septal nucleus; c, caudate nucleus; v, lateral ventricle.

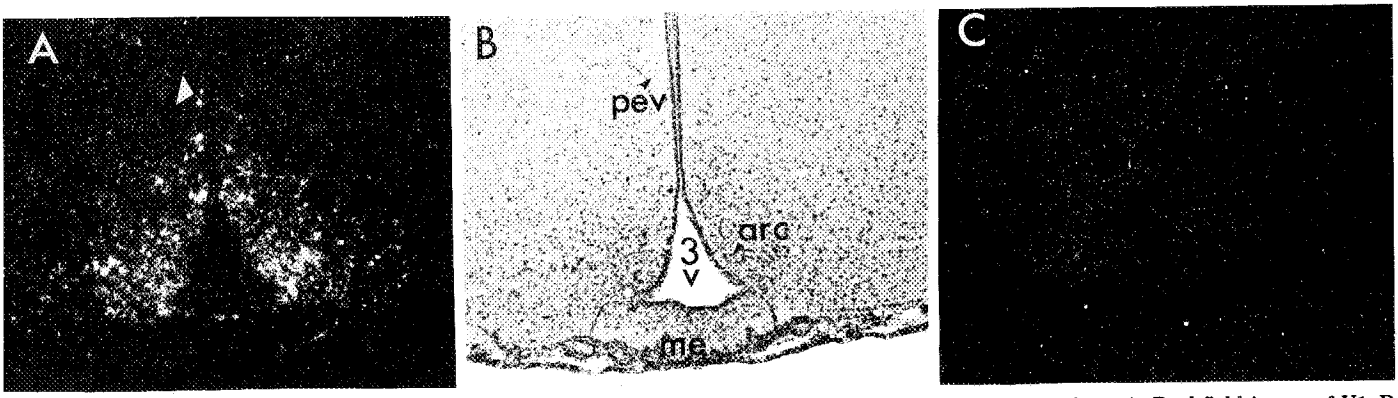

FIG. 7. Hybridization signal of ^{35}S-labeled sense and antisense riboprobes to V1aR mRNA in the arcuate nucleus. A, Darkfield image of V1aR hybridization signal in the arcuate nucleus detected with the ^{35}S-labeled antisense probe. B, Corresponding brightfield image of Nissl-stained tissue section. C, Adjacent control section that was hybridized under identical conditions in the presence of the ^{35}S-labeled sense riboprobe. Note the high intensity, specific hybridization of V1aR transcripts in the arcuate nucleus in A and the absence of hybridization signal in the control section, C. Also note signal in occasional cells along the third ventricle in the periventricular hypothalamic nucleus and the absence of detectable signal in the median eminence. arc, Arcuate nucleus; pev, periventricular hypothalamic nucleus; 3 v, third ventricle; me, median eminence.

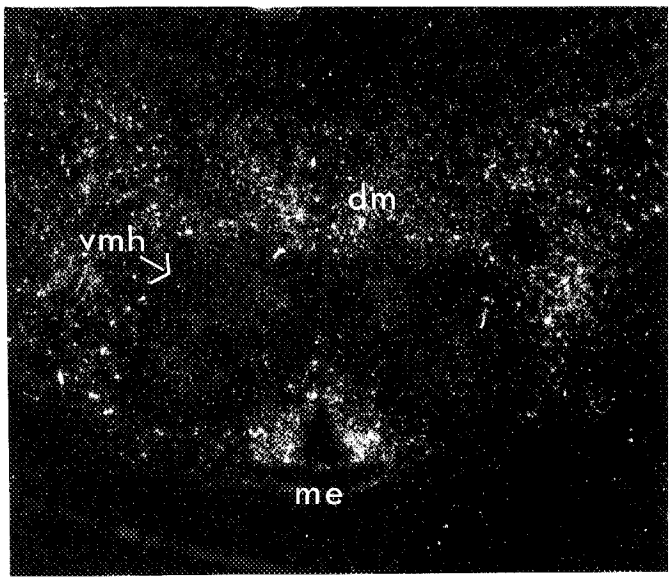

FIG. 8. Darkfield, low magnification photomicrograph showing the V1aR mRNA in cells surrounding the VMH. Cells in the VMH proper show extremely low levels of V1aR transcript expression relative to that in the surrounding hypothalamus, including the arcuate nucleus (dorsolateral to the median eminence) and the dorsomedial hypothalamic nucleus. Silver grains appear white and can be seen surrounding the bilateral, ovoid, poorly labeled nucleus. me, Median eminence; vmh, ventromedial hypothalamic nucleus; dm, dorsomedial hypothalamic nucleus.

Nonspecific labeling. In all experiments, adjacent tissue sections were incubated with the control ^{35}S-labeled V1a sense probe under identical conditions. As shown in Figs. 6 and 14, the sense probes yielded negligible hybridization signal. The labeling pattern of each batch of probe synthesized was also characterized in hepatic and renal tissue to verify V1aR transcript specificity.

Discussion

We provide here a detailed description of the distribution of the mRNA encoding the V1aR for VP in brain, pineal, and cerebral vasculature. These data confirm the existence of the V1aR subtype in brain tissue. Moreover, we show that V1aR transcripts are extensively distributed throughout the brain and are present in numerous regions where VP-immunoreactive fibers have been detected and where binding of VP and related compounds has been demonstrated. These data support the ideas that the calcium-mobilizing effects of VP in brain are mediated by the V1aR subtype and that it is the same subtype that is found in hepatic tissue. This subtype may be the predominant VP receptor in the central nervous system, as V1aR radioligands selectively bind in brain (12, 13, 42), and to date, we have been unable to demonstrate specific hybridization of the V2R cRNA in rat brain.

Specificity of probes for V1aR

Arginine VP binds to mammalian receptors in the kidney, liver, brain, and other tissues (13–16, 43–45). Recently, combinations of selective receptor ligands have been used to attempt to discriminate binding among putative V1a, V1b, V2, and oxytocin receptors (14, 18, 45). Here, instead of targeting the receptor proteins, we labeled the unique mRNA encoding the V1a receptor. Specificity was achieved in the present study by using riboprobes, ribonuclease treatment, and high stringency washes. In addition, control sense probes were used in all experiments and yielded negligible hybridization signal. Ongoing experiments in pituitary, liver, kidney, and brain show no evidence for cross-hybridization of labeled V1aR cRNA with rat V1b, V2, or oxytocin receptor mRNAs. Further, riboprobes constructed to label V2R, V1bR, and oxytocin receptor mRNAs each yield distinct and frequently nonoverlapping patterns of labeling (8) (Ostrowski, N. L., and S. J. Lolait, unpublished). Finally, the correspondence among the patterns of V1aR transcript labeling, VP-immunoreactive fibers in brain, and selective V1a receptor binding strongly suggest that the hybridization signal represents the localization of cells that synthesize the G-protein-linked V1a receptor that selectively binds VP in brain.

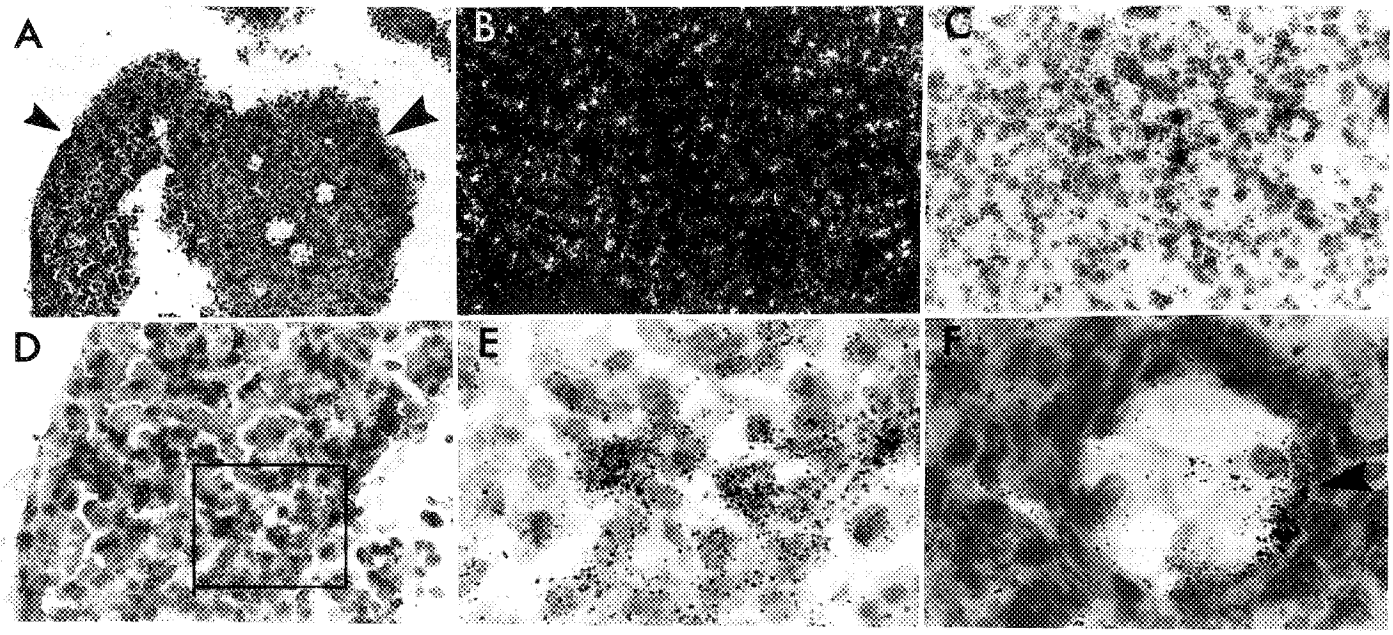

FIG. 9. V1aR mRNA in the pineal. A, Low magnification image of an adult male rat pineal. The *right arrowhead* designates the parenchyma; the *left arrowhead* designates a more vascularized region, which may be contiguous with the pineal stalk. The large round subcommissural rosettes can be viewed in this section (see *small arrowhead* and F). B, Higher magnification darkfield image depicting V1aR hybridization signal in the pinealocytes. White silver grains represent positively labeled cells. C, Brightfield, higher magnification photomicrograph showing nonuniform labeling of individual pinealocytes. V1aR transcripts are represented by black grains overlying Nissl-stained cells. D, Brightfield photomicrograph showing a different, more vascular-like pattern of cell labeling in the segment of the pineal indicated by the *left arrow* in A; in E, individual cells, *within the bracketed area* of D, can be seen at high magnification to express V1aR mRNA and appear to include vascular tissue. F, High magnification of a subcommissural rosette from the pineal (see *small arrowhead* in A) and a V1aR-expressing cell on the lumenal surface (*large arrowhead at right*).

Cellular localization of V1aR transcripts

The majority of V1aR mRNA labeling was in gray matter, and transcripts were most often associated with regionally distinct neuronal morphology (*i.e.* cells in the septal, suprachiasmatic, paraventricular, and arcuate hypothalamic nuclei and the dopamine cell-containing layer of the substantia nigra). There is also evidence that V1aR may be expressed in nonneuronal cells (46). Our data indicate that the V1aR gene is expressed by cell types in addition to neurons in the brain, because 1) cells expressing V1aR mRNA are sometimes found in white matter structures; 2) V1aR transcripts are in the pineal, which does not contain neurons (47); and 3) V1aR are expressed in cells of vascular origin.

V1aR mRNA distribution includes regions with high densities of VP-binding sites and VP-synthesizing cells

There is a striking correspondence between those regions where specific [^{3}H]arginine VP binding (particularly V1a receptor labeling) has previously been reported (14, 15, 17, 18, 42, 44, 45) and the distribution of V1aR transcripts. Receptor transcripts tended to be more discretely localized (*e.g.* superficial granule cells on the hilar surface of the dentate gyrus and optic nerve layer of the superior colliculus) and receptor proteins more diffusely distributed [throughout the polymorph and molecular layers of the dentate gyrus (17, 18) and dorsal layers of the superior colliculus (15)]. Nonetheless, numerous brain regions that show high receptor densities using receptor autoradiography also show abundant V1aR transcripts, including the anterior olfactory nucleus; portions of the accumbens nucleus; the lateral septal nuclei; most subdivisions of the bed nucleus of the stria terminalis; ventral forebrain regions, including the fundis striati and the horizontal limb of the diagonal band; the suprachiasmatic, stigmoid, and arcuate nuclei; the hippocampal dentate gyrus; the central nucleus of the amygdala; the interpeduncular nucleus; central gray; the nucleus of the solitary tract; the area postrema; medial inferior olivary nuclei; and the choroid plexus (18, 42, 45).

The close correspondence between the hybridization signal and VP receptor distribution also suggests that V1aR transcripts are frequently translated into receptors locally (*i.e.* near somatic regions) rather than being transported significant distances in the brain. The few differences that exist at the cellular level (17) may be accounted for by 1) well described neuronal circuitry (*e.g.* the V1aR transcript-containing granule cells of the dentate gyrus have dendrites throughout the molecular layer of the dentate, where receptor labeling is reported) (17), 2) imperfect discrimination between oxytocin and VP receptors by currently available radioreceptor ligands (37–39), 3) the reduced detectability of labeled receptors (which are dispersed over cellular membranes) compared to labeled receptor transcripts (which accumulate near the soma), and 4) unknown and possibly variable rates of translation of transcripts into receptor proteins. A possible localization of V1aR on cell bodies and/or

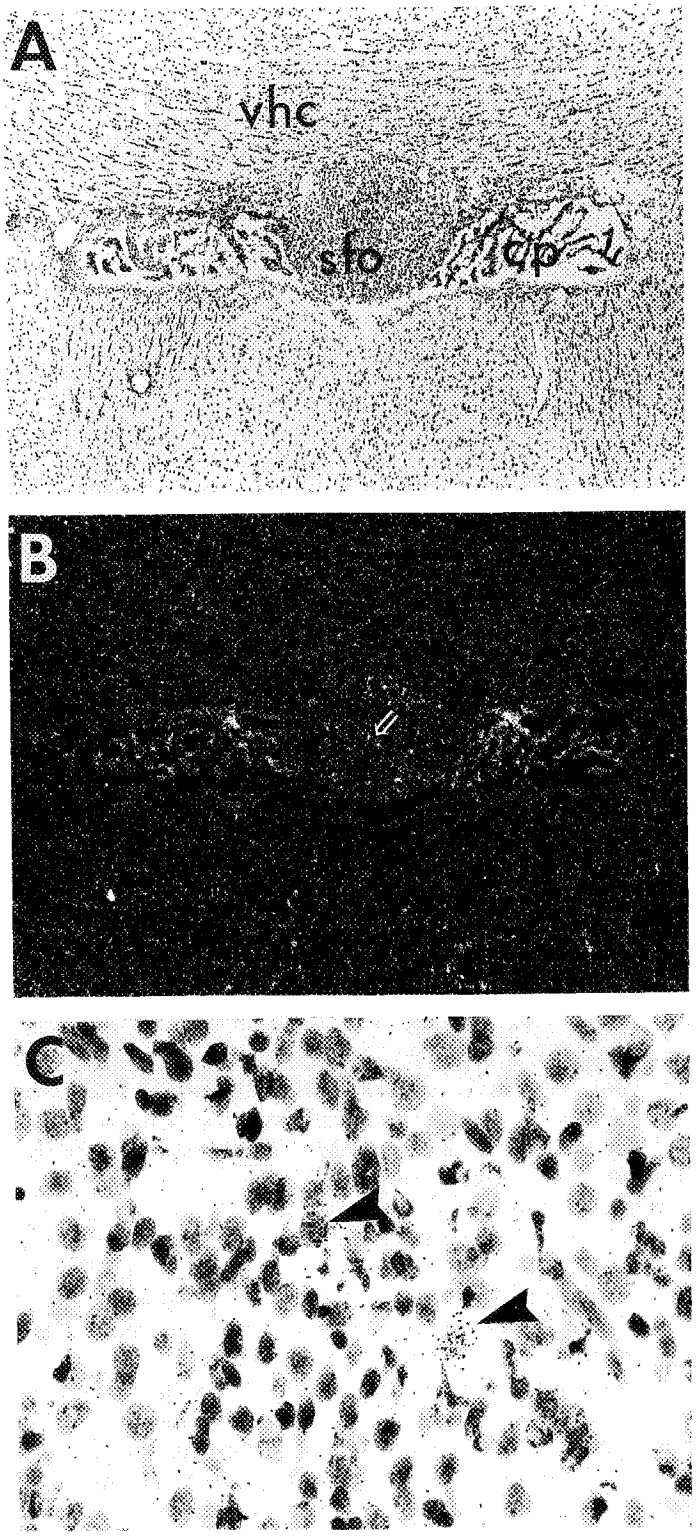

FIG. 10. Subfornical organ cells encode V1aR. A, Brightfield, low magnification view of the subfornical organ, bordered dorsally by the ventral hippocampal commisure and laterally by the ventricles containing choroid plexus. B, Corresponding darkfield image showing the central location of several V1aR transcript-expressing cells in the subfornical organ and label in the choroid plexus (*white arrow*). Note additional labeled cells at the dorsal border of the subfornical organ. C, High magnification brightfield photomicrograph showing two cells from the parenchyma of the subfornical organ that label with the [^{35}S] V1aR riboprobe (*black arrowheads*). cp, Choroid plexus; sfo, subfornical organ; vhc, ventral hippocampal commissure.

proximal dendrites is consistent with data from a previous ultrastructural study showing that most vasopressinergic terminal boutons in the lateral septum make synaptic contacts with dendritic shafts either near or on the soma of somatospiny and other septal neurons (48). VP regulatory actions are suggested, as several areas containing VP cell bodies, such as the suprachiasmatic nucleus, PVN, and bed nucleus of the stria terminalis show moderate to high hybridization signal. Although there is little evidence to support the idea that VP influences V1aR transcript expression (48a), Landgraf *et al.* (49) showed positive feedback control of calcium-dependent arginine VP release from septal fragments, suggesting local signal amplification mechanisms. VP is also released locally by PVN cells (50), can stimulate PVN neuronal cell firing (51), and may regulate its own release from PVN neurons in the rat (52). The presence of V1aR mRNA in the parvocellular PVN as well as in other VP-synthesizing regions, including the suprachiasmatic nucleus, bed nucleus of the stria terminalis, locus ceruleus, and amygdala, strengthens the possibility of a local site for VP regulatory actions. Interestingly, not all sites that synthesize VP express the V1aR gene. For example, no V1aR transcripts were found in the supraoptic nucleus, which synthesizes much of the VP destined for release from the posterior pituitary, or in the median eminence.

V1aR transcript expression in isolated cells and regions previously shown to demonstrate nonspecific radioligand binding

In addition to regions where both receptor transcripts and binding are seen, many other areas were found to contain V1aR transcript-expressing cells. For example, V1aR transcript label was over single, rare, and occasionally highly expressing cell bodies in the internal granule cell layer of the main olfactory bulb; in all cortical areas; and in the subiculum and presubiculum. This accumulation of signal over cell bodies was seen in emulsion-coated tissue sections microscopically, but was not visible using film autoradiography in our initial *in situ* hybridization experiments (8). Receptor binding techniques using film would be unlikely to detect these receptors. Interestingly, [^{3}H]VP receptor binding in the olfactory bulb has been reported in sheep (53) and guinea pigs (54) and in the cingulate cortex in the neonatal (55, 56), but rarely the adult (1, 55, 57), rat, suggesting that in some instances, developmental and species variations in regional distributions of VP receptors may be quantitative, rather than qualitative.

VP-binding sites have previously been detected in association with the vascularized areas of the subfornical organ and the pineal (45). Here, we show V1aR transcript-expressing cells in the parenchyma of these structures as well. Similarly, we found several isolated examples of V1aR transcripts in brain blood vessels, in addition to the heavy expression in superficial vasculature.

Previous investigators concluded that receptor binding in three brain regions, the arcuate nucleus, the parvocellular

region of the paraventricular nucleus of the hypothalamus, and the polymorph layer of the dentate gyrus of the hippocampus, may have been nonspecific, because either high concentrations of VP were required to obtain binding or these concentrations did not eliminate radiolabeled VP binding (14, 18). Although we cannot determine from these experiments whether receptors are expressed at the site of mRNA detection, we found moderate to heavy V1aR transcript expression in these areas, strengthening the idea that cells in these areas synthesize the V1aR.

In the course of these experiments we noted that the detectability of V1aR mRNA in some brain regions varied from animal to animal. Whereas most brain regions (*e.g.* the arcuate nucleus, suprachiasmatic nucleus, lateral septal nuclei, and dentate gyrus) varied little among animals, some anatomical structures showed much greater variability. For example, animals typically showed labeled cells in the cerebellum, Ammon's horn of the hippocampal formation, and medial inferior olivary nuclei, but a few animals showed little or no label in these regions. Although we cannot easily account for these observations, they suggest that transcription may be more dynamically regulated in some brain regions than in others.

Regions that bind arginine VP, but express few V1aR transcripts

The ventral subiculum, amygdaloid nuclei, olfactory tubercle, VMH, and thalamus have been occasionally reported to show VP receptor binding (13, 18, 58), but we show very low levels of V1aR transcript labeling. Although, the VMH initially appeared devoid of V1aR mRNA compared to the surrounding hypothalamus, high power microscopy suggested that a few cells may express extremely low levels of V1aR transcripts. Nonetheless, the apparent differences between receptor transcript densities and receptor binding may be accounted for in several brain regions mentioned (the subiculum, olfactory tubercle, and VMH) if, as suggested by the data of Kremarik, Tribollet, Yoshimura, and associates (17, 18, 45, 59), the receptor binding was to oxytocin, and not VP, receptors. Alternatively, relatively few cells may be sufficient to synthesize large amounts of the receptor protein. Overall, however, the distribution of V1aR transcripts and their relative densities are in good agreement with the published distribution of VP-binding sites when oxytocin receptors are selectively blocked (17, 18, 44).

With the exception of labeled cells concentrated in the central nucleus and amygdalo-striatal transitional areas, very few cells showed signal in the amygdala. Instead of moderate to heavy V1aR label in the thalamus, as has been suggested by some investigators (13, 58), we found low levels of transcripts restricted primarily to the anteroventral, ventral reticular, and posterior nuclei at levels proportional to the density of VP binding reported in the presence of competitive ligands (45). The reason for the low abundance of V1aR mRNA in amygdaloid and thalamic nuclei is not clear, but

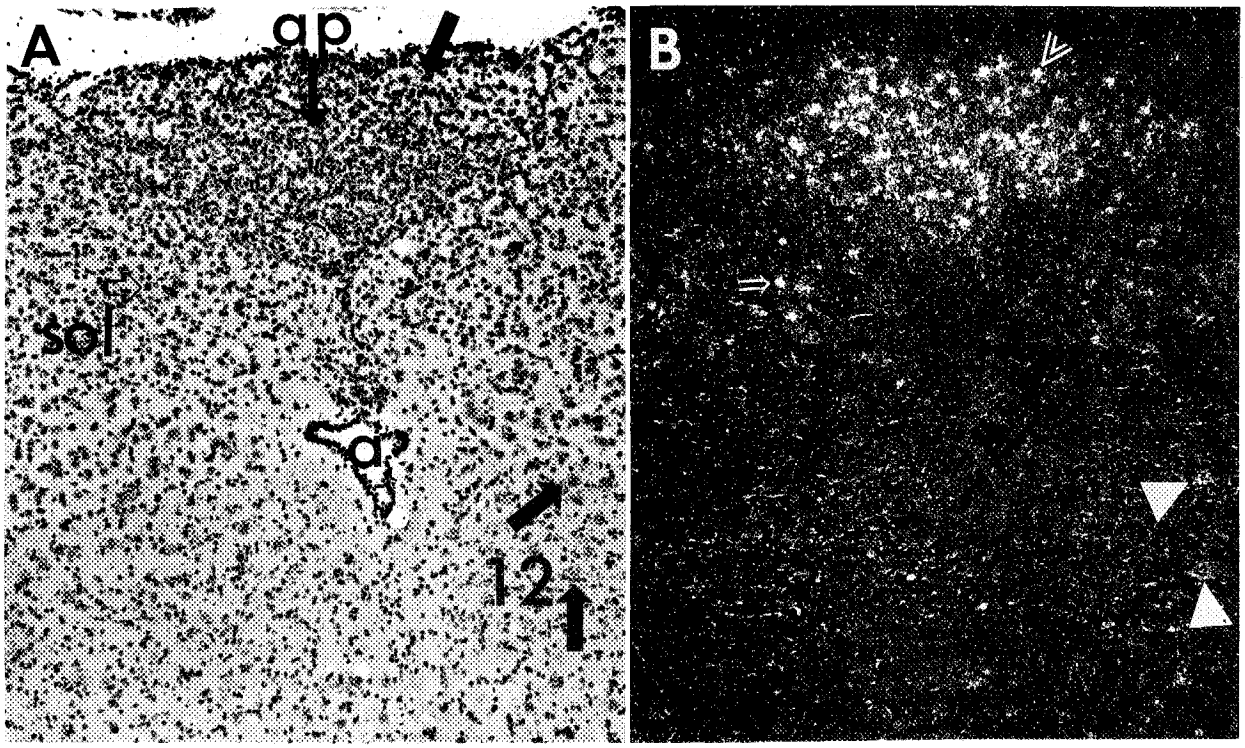

FIG. 11. V1aR hybridization signal in the area postrema, nucleus of the solitary tract, and motor hypoglossal nucleus. A and B are corresponding bright- and darkfield photomicrographs, respectively. A, A Nissl-stained tissue section containing cells of the area postrema (*top, dark arrow*), nucleus of the solitary tract (*left, open arrow*), and motor hypoglossal nucleus (12; *bottom, two arrows*). B, Uniformly high hybridization signal in the area postrema (*top, double arrowhead*). Several intensely labeled cells in the nucleus of the solitary tract (*open arrow*) and two large cells in the hypoglossal nucleus (cranial nerve 12; *white triangles at bottom right*) show hybridization signal. Note that cells surrounding the cerebral aqueduct are not labeled. ap, Area postrema; sol, nucleus of the solitary tract; a, cerebral aqueduct; 12, nucleus of the 12th cranial nerve, the motor hypoglossal nucleus.

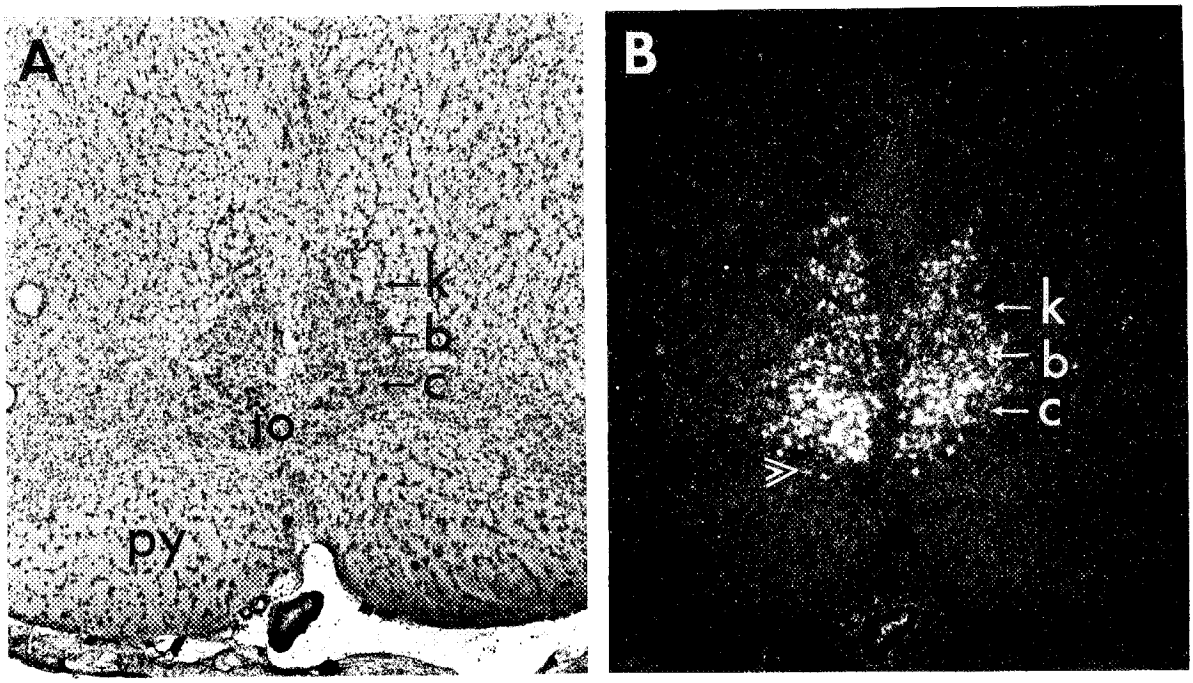

FIG. 12. V1a receptor mRNA in medial subnuclei of the inferior olive. A and B are bright- and darkfield photomicrographs, respectively, of emulsion-coated tissue sections hybridized with [^{35}S]V1aR antisense probe. Note the varying intensities of labeled cells in different subnuclei and cells labeled at the dorsal edge of the pyramid (*double arrowhead*). io, Medial inferior olive; c, subnucleus C, medial nucleus; b, β-subnucleus; k, cap of Kooy; py, pyramid.

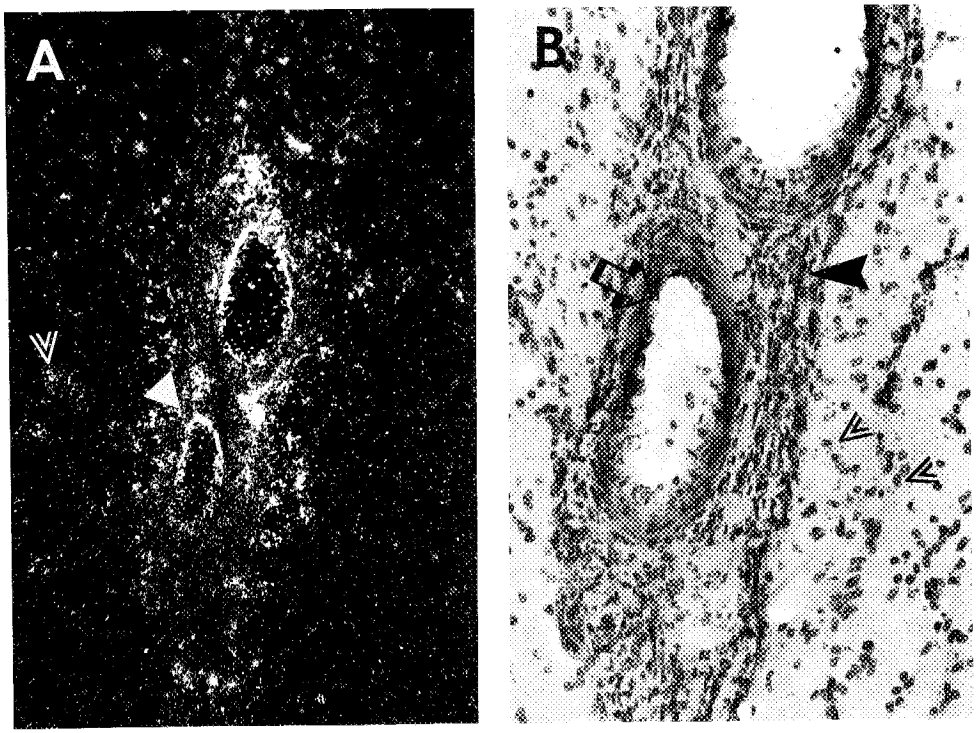

FIG. 13. V1aR in and around midline blood vessels. A, Darkfield photomicrograph showing intense labeling of vascular (*arrowhead*) and perivascular cells between and in (*double arrowhead*) the anterior olfactory bulbs. B, Higher magnification, brightfield image showing localization of grains over endothelial vascular cells (*e.g. open arrow*), over surrounding cells (*solid arrowhead*), and over cells within the olfactory bulb (*double arrowheads*).

may include 1) unknown influences of factors regulating receptor expression (*e.g.* steroid hormones); 2) nonoptimal standardization of variables, such as seasonal or diurnal variations in receptor transcription (48a, 60); 3) the presence of posttranslationally modified receptor subtypes; 4) slow turnover of the receptor proteins; and of course, 5) differences between the sites at which mRNA is detected and the receptor protein is functional.

Cells of the bed nucleus of the stria terminalis and medial amygdala give rise to VP-containing fibers in the amygdala, lateral septum, lateral habenula, ventral hippocampus, central gray, and other regions (1, 61, 62), regions where V1aR transcript-expressing cells were abundant. Interestingly, the vasopressinergic innervation of these regions is sexually dimorphic and dependent on the presence of gonadal steroids (61, 63–65). The presence of V1aR mRNA in these brain

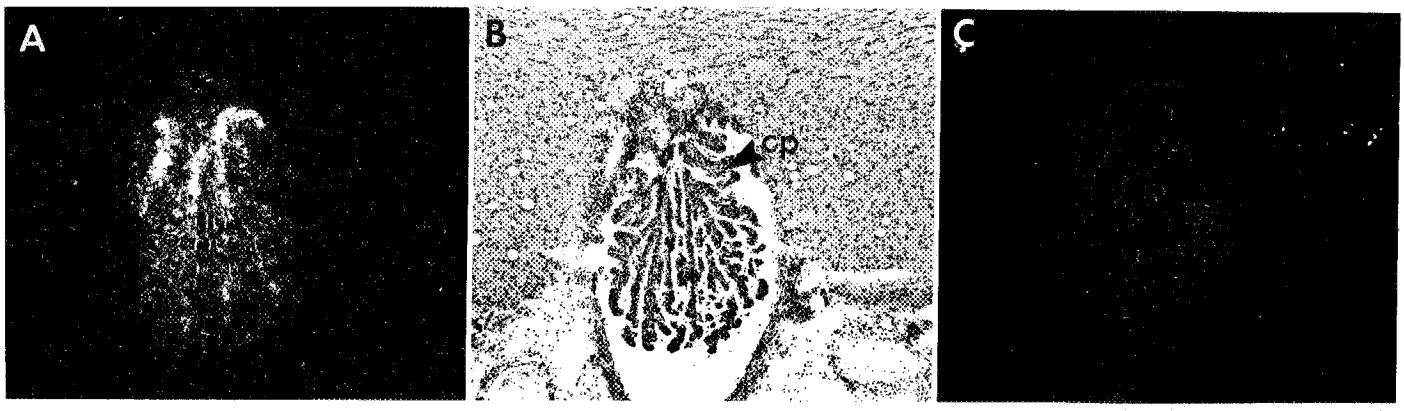

FIG. 14. Cells of the choroid plexus express high levels of V1aR transcripts. A, Low magnification, darkfield image showing the localization of silver grains (which appear white) over cells of the choroid plexus embedded within the third ventricle. B, Corresponding Nissl-stained image. C, Darkfield image of the adjacent section of choroid plexus that was incubated with the control V1a sense probe and exposed for the same amount of time. Note high accumulation of V1aR-hybridized transcript and negligible accumulation of silver grains in control tissue. cp, Choroid plexus.

areas suggests that this receptor may play a role in sexually dimorphic functions.

Role of V1a receptors

From a functional perspective, the distribution of the V1aR-synthesizing cells throughout the brain supports several well established hypotheses about VP's role in homeostasis and behavior and suggests new and as yet undetermined roles for VP.

One of the earliest recognized functions of VP was vasoconstriction, and V1aR transcripts were heavily expressed by cells of superficial brain vasculature, indicating a likely target for regulation of cerebral blood flow (28–31, 33). The nucleus of the solitary tract, nucleus ambiguus, and area postrema, regions important in mediating VP's peripheral vasoregulatory and autonomic effects (31), also expressed V1aR transcripts. The presence of V1aR mRNA in the choroid plexus and some circumventricular organs (*i.e.* subfornical organ and area postrema) is consistent with a role for VP receptors in regulating electrolyte balance and the synthesis of cerebrospinal fluid (32, 66). Perhaps as important, the absence of V1aR transcripts in other circumventricular organs, such as the organum vasculosum of the lamina terminalis, the median eminence, and the subcommissural organ, substantially decreases the likelihood that these structures play any significant role in transducing the V1aR-mediated effects of VP. Conversely, the abundance of V1aR transcripts in pineal cells provides supportive evidence for a V1aR site of action of VP in the regulation of pineal melatonin secretion (67). Finally, the effects of VP and related peptides on learning and memory could well be through actions at the numerous V1aR sites in the hippocampus or septum (19, 68–70).

We confirm here that V1a receptor transcripts are expressed abundantly in the lateral septum, increasing the likelihood that the V1aR mediates the effects of endogenous and exogenous VP in this nucleus. This limbic structure receives dense, sexually dimorphic vasopressinergic input (61, 62, 64, 71), binds radiolabeled arginine VP (13, 45, 72) and responds to iontophoretic application of VP with increased cellular firing rates (73). Septal neurons integrate afferent signals from the hypothalamus, brain stem, spinal cord nuclei, and hippocampus and are reciprocally connected to the telencephalon (47, 74). Microinjections of VP into septal nuclei delay the extinction of conditioned avoidance behavior in rats (75), alter social memory, and facilitate the perception, storage, and retrieval of olfactory information (76). The ventral septal area has also been found to be a primary antipyretic site of action of VP (77). The high density of V1aR transcripts in the septum strengthens the likelihood that the V1aR plays a critical role in these and other behavioral and physiological responses.

The majority of granule cells in the brain are negative for V1aR expression. However, high concentrations of V1aR transcripts were found in superficial, periodically spaced cells of granule cell layers of the main olfactory bulb, hippocampal dentate gyrus, and cerebellum. We are not aware of any previously described cell types specific to granule cell layers that might correspond to those labeled in these experiments; this may be the first evidence to suggest that these cells are functionally distinct from surrounding granule cells. The morphology and staining characteristics of some of these cells labeled in the olfactory bulb and cerebellum were similar, suggesting the possibility of a cell type localized to the boundaries of granule cell layers. It will be interesting to determine whether or in what manner the V1aR mRNA-producing cells influence the well described local circuitry and signaling of surrounding cells of the olfactory bulb, dentate gyrus, and cerebellum. Moreover, because of the well characterized nature of hippocampal neurotransmission, the dentate gyrus may provide a useful *in vivo* or *in situ* model system by which synaptic, hormonal, or pharmacological signals that promote V1aR transcription can be identified.

Important questions remain regarding whether V1a brain receptors respond to VP released within the brain proper or whether the receptors also respond to VP from the peripheral circulation. Recent work (78, 79) suggests that a specific vasopressinergic transporter and/or V1a-like receptor may mediate significant bidirectional transport of VP across the blood-brain barrier. With two exceptions, the main olfactory bulb and the interpeduncular nucleus, V1aR transcripts were notably absent from cells of vasculature identifiable within

brain parenchyma in our experiments. However, in several brain regions, cells expressing V1aR transcripts were sometimes visualized abutting vascular elements. Although electron microscopic experiments are required to confirm whether processes of brain cells expressing V1a receptors penetrate blood-brain or brain-cerebrospinal fluid boundaries, these initial observations are consistent with the possibility that some brain regions possess the ability to transduce signals derived from VP in the peripheral circulation.

Considering the extensive distribution of V1aR transcripts, it is difficult to propose an integrative hypothesis regarding the role for VP at V1a receptors in brain. In a recent review, Dantzer and Bluthe (80) speculate that VP regulates the access of sensory cues to neural circuits involved in various aspects of reproductive function. Although it might be argued that virtually all sensory, learning, and memory, endocrine, autonomic, and motor systems are required for integrated reproductive functions, the heavy limbic, hypothalamic, and hind brain distributions of the V1a receptor mRNA shown here, the sex differences in immunoreactive VP distributions (67, 81), and the steroid hormone sensitivities of the vasopressinergic innervation in brain (48a, 57) are consistent with this very interesting proposal.

Acknowledgments

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