THE JOURNAL OF COMPARATIVE NEUROLOGY 403:261–280 (1999)

Distribution of Pre-Pro-Glucagon and Glucagon-Like Peptide-1 Receptor Messenger RNAs in the Rat Central Nervous System

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) is derived from the peptide precursor pre-pro-glucagon (PPG) by enzymatic cleavage and acts via its receptor, glucagon-like peptide-1 receptor (GLP-1R). By using riboprobes complementary to PPG and GLP-1R, we described the distribution of PPG and GLP-1R messenger RNAs (mRNAs) in the central nervous system of the rat. PPG mRNA-expressing perikarya were restricted to the nucleus of the solitary tact or to the dorsal and ventral medulla and olfactory bulb. GLP-1R mRNA was detected in numerous brain regions, including the mitral cell layer of the olfactory bulb; temporal cortex; caudal hippocampus; lateral septum; amygdala; nucleus accumbens; ventral pallium; nucleus basalis Meynert; bed nucleus of the stria terminalis; preoptic area; paraventricular, supraoptic, arcuate, and dorsomedial nuclei of the hypothalamus; lateral habenula; zona incerta; substantia innominata; posterior thalamic nuclei; ventral tegmental area; dorsal tegmental, posterodorsal tegmental, and interpeduncular nuclei; substantia nigra, central gray; raphe nuclei; parabrachial nuclei; locus ceruleus, nucleus of the solitary tract; area postrema; dorsal nucleus of the vagus; lateral reticular nucleus; and spinal cord. These studies, in addition to describing the sites of GLP-1 and GLP-1R synthesis, suggest that the efferent connections from the nucleus of the solitary tract are more widespread than previously reported. Although the current role of GLP-1 in regulating neuronal physiology is not known, these studies provide detailed information about the sites of GLP-1 synthesis and potential sites of action, an important first step in evaluating the function of GLP-1 in the brain. The widespread distribution of GLP-1R mRNA-containing cells strongly suggests that GLP-1 not only functions as a satiety factor but also acts as a neurotransmitter or neuromodulator in anatomically and functionally distinct areas of the central nervous system. J. Comp. Neurol. 403:261–280, 1999. © 1999 Wiley-Liss, Inc.

Indexing terms: in situ hybridization; brain mapping; glucagon-like peptide-1 receptor mRNA; ingestive behavior; obesity; pre-pro-glucagon

Glucagon is a 27-amino acid peptide and a member of a family of structurally-related peptides that include vasoactive intestinal polypeptide (Mutt and Said, 1974), gastric inhibitory peptide (Brown and Dryburgh, 1971), secretin (Mutt et al., 1970), and growth hormone-releasing hormone (Spiess et al., 1982). In the pancreas, glucagon is derived from a peptide precursor, pre-pro-glucagon (PPG), which contains a single glucagon sequence; two glucagon-like sequences: glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2; Bell et al., 1983; Heinrich et al., 1984; Lopez et al., 1983); and a peptide sequence unrelated to glucagon. The post-translational processing of the mammalian PPG precursor is tissue specific and exhibits clear differences between the pancreas and brain (Larsen et al., 1997a).

In the pancreas, glucagon is the major splicing product; in the brain, the major splicing products are glucagon-like peptide-1 and glicentin (Orskov et al., 1987; Larsen et al., 1997b). The amino acid sequence of GLP-1 is identical in rodents and man (Bell et al., 1983; Heinrich et al., 1984), suggesting that GLP-1 has an important biological role. In the central nervous system (CNS), GLP-1 is a potent inhibitor of feeding and drinking behavior (Schick et al.,

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Received 20 February 1998; Revised 18 August 1998; Accepted 27 August 1998

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Abbreviations

1–6	cortical layers
2n	optic nerve
7n	facial nerve
3V	3rd ventricle
4V	4th ventricle
4th	4th ventricle
10,12	cranial nerves
I–IX	laminae of the spinal cord
AAD	anterior amygdaloid area, dorsal part
AAV	anterior amygdaloid area, ventral part
ac	anterior commissure
Acb	accumbens nucleus
ACo	anterior cortical amygdaloid nucleus
AHiA	amygdalohippocampal area
AN	arcuate nucleus
AOB	accessory olfactory bulb
AOE	anterior olfactory nucleus, external part
AOL	anterior olfactory nucleus, lateral part
AOM	anterior olfactory nucleus, medial part
AOV	anterior olfactory nucleus, ventral part
AP	area postrema
APir	amygdalopiriform transition
aq	aqueductus cerebri
ATg	anterior tegmental nucleus
B	basal nucleus of Meynert
BLA	basolateral amygdaloid nucleus, anterior part
BLP	basolateral amygdaloid nucleus, posterior part
BLV	basolateral amygdaloid nucleus, ventral part
BM	basomedial amygdaloid nucleus
BMP	basomedial amygdaloid nucleus, posterior part
BST	bed nucleus of the stria terminalis
BSTIA	bed nucleus of the stria terminalis, intraamygdaloid division
CC	central canal
cc	corpus callosum
CA1–CA3	fields CA1–3 of Ammons horn
Ce	central amygdaloid nucleus
CeC	central cervical nucleus
Cer	cerebellum
CG	central (periaqueductal) gray
Cl	caudal interstitial nucleus of the medial longitudinal fascicle
CLi	caudal linear nucleus of the raphe
CnF	cuneiform nucleus
CNS	central nervous system
cp	cerebral peduncle, basal part
CPu	caudate putamen (striatum)
Cu	cuneate nucleus
DCIC	dorsal cortex of the inferior colliculus
DEn	dorsal endopiriform nucleus
DG	dentate gyrus
Dk	nucleus of Darkschewitsch
DMN	dorsomedial nucleus
DMSp5	dorsomedial spinal trigeminal nucleus
DMTg	dorsomedial tegmental area
DPGi	dorsal paragigantocellular nucleus
DpME	deep mesencephalic nucleus
DTg	dorsal tegmental nucleus
dtgx	dorsal tegmental decussation
DTT	dithiothreitol
ECIC	external cortex of the inferior colliculus
Ent	entorhinal cortex
F	fields of Forel
f	fornix
fr	fasciculus retroflexus
GI	granular insular cortex
Gi	gigantocellular reticular nucleus
GLP-1	glucagon-like peptide 1
GLP-1i	GLP-1–immunoreactive
GLP-1R	glucagon-like peptide 1 receptor
GLP-2	glucagon-like peptide 2
GP	globus pallidus
Gr	gracile nucleus
HDB	nucleus of the horizontal limb of the diagonal band
HiF	hippocampal fissure
ic	internal capsule
IG	indusium griseum
IPC	interpeduncular nucleus, central subnucleus
IPI	interpeduncular nucleus, intermediate subnucleus
IPN	interpeduncular nucleus
IRt	intermediate reticular nucleus
LC	locus ceruleus
LDTg	laterodorsal tegmental nucleus
LH	lateral hypothalamic area
LHb	lateral habenular nucleus
LGN	lateral geniculate nuxleus
lo	lateral olfactory tract
LPB	lateral parabrachial nucleus
LPOA	lateral preoptic area
LRt	lateral reticular nucleus
LRtPc	lateral reticular nucleus, parvocellular part
LS	lateral septum
LSI	lateral septal nucleus, intermediate part
LSO	lateral superior olive
LSV	lateral septal nucleus, ventral part
LV	lateral ventricle
MCPO	magnocellular preoptic nucleus
MdD	medullary reticular nucleus, dorsal part
MdV	medullary reticular nucleus, ventral part
ME	median eminence
Me	medial amygdaloid nucleus
MGN	medial geniculate nucleus
MHb	medial habenular nucleus
ml	medial lemniscus
mlf	medial longitudinal fasciculus
MMN	medial mammillary nucleus, median part
MnR	median raphe nucleus
mo	molecular layer, cerebellum
Mo5	motor trigeminal nucleus
MPA	medial preoptic area
MPB	medial parabrachial nucleus
MPON	medial preoptic nucleus
mRNA	messenger RNA
MS	medial septus nucleus
mt	mammillothalamic tract
MTu	medial tuberal nucleus
MVPO	medioventral periolivary nucleus
NTS	nucleus tractus solitarii
ON	olfactory nerve layer
Op	optic nerve layer of the superior colliculus
opt	optic tract
ot	optic tract
ox	optic chiasm
pc	posterior commissure
PCRt	parvocellular reticular nucleus
PDTg	posterodorsal tegmental nucleus
Pe	periventricular hypothalamic nucleus
Pir	piriform cortex
PLCo	posterolateral cortical amygdaloid nucleus
PMCo	posteromedial cortical amygdaloid nucleus
PMD	premammillary nucleus, dorsal part
PMV	premammillary nucleus, ventral part
PnC	pontine reticular nucleus, caudal part
PON	periolivary nucleus
PPG	pre-pro-glucagon
Pr	principal nucleus of trigeminal nerve
PTN	posterior thalamic nuclei
py	pyramidal tract
pyx	pyramidal decussation
RCh	retrochiasmatic area
RD	raphe dorsalis nucleus
RF	rhinal fissure
RLi	rostral linear nucleus of the raphe
RMg	raphe magnus nucleus
ROb	raphe obscurus nucleus
RPn	raphe pontis nucleus
Rt	reticular thalamic nucleus
S	subiculum
scp	superior cerebellar peduncle (brachium conjunctivum)
SFO	subfornical organ
SHy	septohypothalamic nucleus
SI	substantia innominata
smt	mammillothalamic nucleus
SON	supraoptic nucleus
SNC	substantia nigra, compact part
SNR	substantia nigra, reticular part
Sp5	spinal trigeminal nucleus
sp5	spinal trigeminal tract
SpVe	spinal vestibular nucleus
SSC	standard saline citrate
st	stria terminalis
STh	subthalamic nucleus

Abbreviations (continued)

Subl	subincertal nucleus
SuM	supramammillary nucleus
SuO	superior olive
SuVe	superior vestibular nucleus
tpf	transverse fibers, pons
TT	tenia tecta
Tz	nucleus of the trapezoid body
tz	trapezoid body
VDB	nucleus of the vertical limb of the diagonal band
VL	ventrolateral thalamic nucleus
VMN	ventromedial nucleus
VP	ventral pallidum
VTA	ventral tegmental area
X	vagus nerve
ZI	zona incerta

1993; Tang-Christensen et al., 1994; Turton et al., 1996), although there may be other actions of the peptide that are not related to the regulation of ingestive behavior. The central administration of GLP-1 significantly inhibits food intake in both hungry and fed rats in dose-dependent manner via a mechanism that does not involve taste aversion (Tang-Christensen et al., 1996). Studies have demonstrated that GLP-1 activates adenylate cyclase in brain tissue and membrane preparations (Hoosein and Gurd, 1984; Shimizu et al., 1987), providing additional evidence for the importance of a brain-derived glucagon system.

It is known from immunocytochemical studies that the perikarya of GLP-1–immunoreactive (GLP-1i) neurons are located in the nucleus of the solitary tract and the ventral and dorsal parts of the medullary reticular formation and that these neurons innervate the hypothalamus (Larsen et al., 1997b; Jin et al., 1988). The paraventricular, supraoptic, ventromedial, and dorsomedial nuclei are heavily innervated by GLP-1i fibers, but scattered fibers also occur in the preoptic area, medial septum, lateral septum, bed nucleus of the stria terminalis, midline thalamic nuclei, lateral hypothalamus, and several brainstem areas (Jin et al., 1988). The widespread distribution indicates that GLP-1 functions as a neurotransmitter in the CNS. The distribution of GLP-1 binding sites, as detected with quantitative autoradiography (Uttenthal et al., 1992; Göke et al., 1995), further suggest the presence of receptors in regions where the immunoreactive fibers were seen. It is worth noting that GLP-1 binding sites were also seen in the subfornical organ, arcuate nucleus of the hypothalamus, interpeduncular nucleus, parategmental nucleus, inferior olive, and nucleus of the solitary tract (Göke et al., 1995). Now that the rat GLP-1 receptor (GLP-1R; Thorens et al., 1992) has been cloned, it is possible to ascertain the anatomical distribution of GLP-1R messenger RNA (mRNA)-expressing perikarya in the CNS by using in situ hybridization histochemistry. The hypothalamic distribution of GLP-1R mRNA was reported previously as a short communication (Shughrue et al., 1996a).

MATERIALS AND METHODS

Animals and tissue preparation

Sixty-day-old female Sprague-Dawley rats (Taconic, Germantown, NY) were housed in the Wyeth-Ayerst animal care facility with a 12-hour light, 12-hour dark photoperiod and free access to tap water and rodent chow. After acclimation, the animals were transferred to the necropsy suit, where they were exposed to a lethal dose of CO_2. Brains, spinal cords, and eyes were removed, frozen on dry ice, and stored at –80°C until sectioning. The studies described in this paper were reviewed and approved by the Radnor Animal Care and Use Committee (RACUC) at Wyeth-Ayerst.

Coronal cryostat sections (20 µm) were cut and collected on silane-coated microscope slides (Histology Control Systems, Glen Head, NY), dried on a slide warmer maintained at 42°C (Lab-Line Instruments, Melrose Park, IL), and stored in slide boxes desiccated with Dricap (Ted Pella, Redding, CA) at –80°C. Before processing, the desiccated slide boxes were slowly warmed to room temperature (–20°C, 12–16 hours; 4°C, 2 hours; room temperature for 1 hour) to eliminate the formation of condensation on slides and thus minimize tissue and RNA degradation. The dry slides were loaded into metal racks, postfixed in 4% paraformaldehyde (pH 9.0) for 5 minutes, and processed as previously described (Shughrue et al., 1996b).

In Situ hybridization

A pGEM plasmid containing 1,046 base pairs of the rat pro-glucagon cDNA (exon 1 to exon 6; a gift from D.J. Drucker, Toronto, Canada; Heinrich et al., 1984) was linearized with EcoR1 and used to generate a S^{35}-UTP–labeled probe that was complimentary to a portion of the rat pro-glucagon, including GLP-1. A pGEM plasmid containing a portion of the rat GLP-1R cDNA (bases 288–1,164 of the coding region; a gift from M.B. Wheeler, Toronto, Canada) was linearized with EcoR1 and used to generate a S^{35}-UTP–labeled probe that was complementary to a portion of the rat GLP-1R mRNA. Processed section-mounted slides were hybridized with 100–150 µl of an antisense or sense (control) riboprobe (4.7×10^6 disintegration per minute/slide), –50% formamide hybridization mix with 5% dextran sulfate and 200 mM dithiothreitol (DTT), and incubated overnight at 55°C in a chamber humidified with 50% formamide/600 mM NaCl.

In the morning, the slides were placed in metal racks and immersed in 2× standard saline citrate (SSC; 0.3 M NaCl, 0.03 M sodium citrate; pH 7.0)/10 mM DTT to remove the excess hybridization mixture. The racks were then transferred to a large container (Rubbermaid #3863; Winchester, VA) and filled with 2× SSC/10 mM DTT. When all the slides were in the large container, the racks were transferred to a new container and washed in 2× SSC/10 mM DTT for 15 minutes at room temperature with gentle agitation. The slides were then washed in RNase buffer, pH 8.0, at 37°C for 30 minutes, treated with RNase A (20 µg/ml) for 30 minutes at 37°C, and washed for 15 minutes in room-temperature 1× SSC. Subsequently, the slides were washed (twice, for 30 minutes) in 0.1× SSC at 65°C to remove nonspecific label, rinsed with 0.1× SSC at room temperature for 15 minutes, and dehydrated with a graded series of alcohol:ammonium acetate (70%, 95%, and 100%).

Air-dried slides were apposed to X-ray film (Amersham, Arlington Heights, IL) for 9 days and then dipped in NTB2 nuclear emulsion (Eastman Kodak, Rochester, NY; diluted 1:1 with 600 mM ammonium acetate). The slides were exposed for 24–64 days in light-tight black desiccated boxes, photographically processed, stained in cresyl violet, and coverslipped. The slides from all animals were hybridized, washed, exposed, and photographically processed together to eliminate differences due to interassay variation in conditions. Details concerning the preparation of tissue

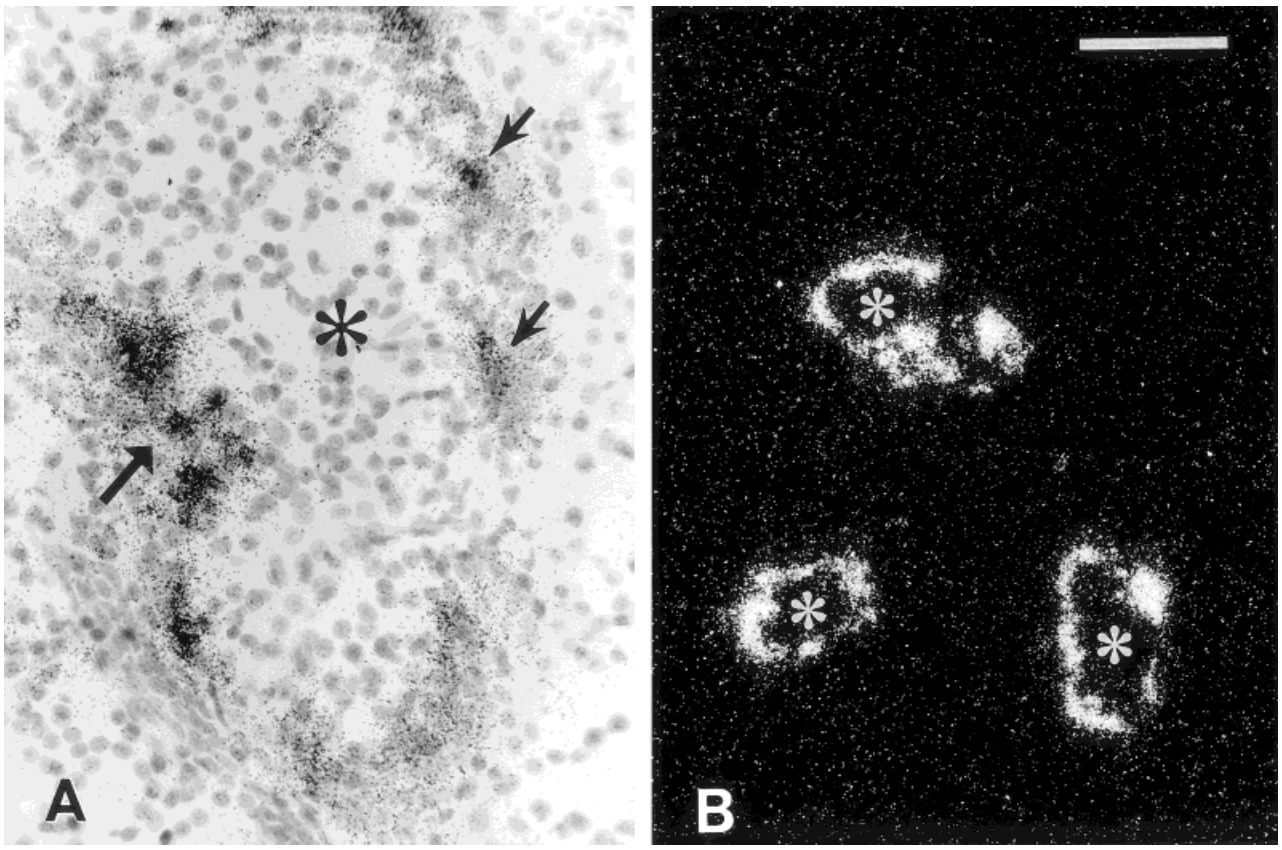

Fig. 1. Pre-pro-glucagon (PPG) messenger RNA (mRNA)-containing cells in the islets of the pancreas. Both brightfield (**A**) and darkfield (**B**) photomicrographs indicate that the hybridization signal is present in cells known to synthesize glucagon, thus verifying the specificity of hybridization for PPG mRNA. Left arrow shows a cluster of labeled cells; right arrows indicate individual cells with PPG mRNA. Asterisks indicate centers of the islets. Scale bars = 100 μm (A), 400 μm (B).

and riboprobes and the in situ hybridization method have been reported previously (Shughrue et al., 1996b).

Evaluation

Section-mounted slides, used to generate film autoradiographic images, were scanned at low magnification with a light microscope to determine the regional distribution of silver grains in the brain, spinal cord, and eye. Adjacent sections, exposed for shorter periods of time, were then viewed with high magnification to determine the cellular distribution of radioactivity and to verify that the silver grains were concentrated over cells. Cells with a radioactive concentration >5× radioactivity in neuropil were considered labeled and were mapped onto computer scans of the rat brain; their size and distribution—representing the number of GLP-1 and its receptor mRNA-containing cells in a particular brain region—were plotted.

Film autoradiograms also were used to assist in generating a map of GLP-1R mRNA in the female rat brain. The brain autoradiograms were digitized with a computer-assisted image-analysis system (C-Imaging Inc., Pittsburgh, PA) and imported into Canvas (Deneba Systems, Miami, FL), and the image was excised from the background and arranged into plates.

RESULTS

The specificity studies, hybridization with sense probes and preincubation of section-mounted slides with RNase, indicated that the signals detected in these studies were the result of hybridization of our radiolabeled riboprobe to GLP-1R and to PPG mRNAs, respectively. In addition, PPG mRNA was expressed in the cells located in the periphery of islets of the pancreas, providing additional support for the specificity of our hybridization method (Fig. 1A,B). Moreover, the distribution of PPG mRNA-containing cells was in good agreement with the distribution of PPG-immunoreactive cells (Jin et al., 1988), even though no immunocytochemical data are available in the olfactory bulb.

Distribution of PPG mRNA-containing perikarya

PPG mRNA-containing perikarya were restricted to two regions of the brain. The majority of the labeled cells were seen in the nucleus of the solitary tract (Fig. 2) or in close vicinity (dorsal and ventral caudal medullae) and in the olfactory bulb (Fig. 3). The neurons in the nucleus of the solitary tract and surrounding vicinity had a dense accumulation of silver grain, whereas the perikarya of neurons in the olfactory bulb showed weaker labeling (Figs. 2C,D vs 3A,B).

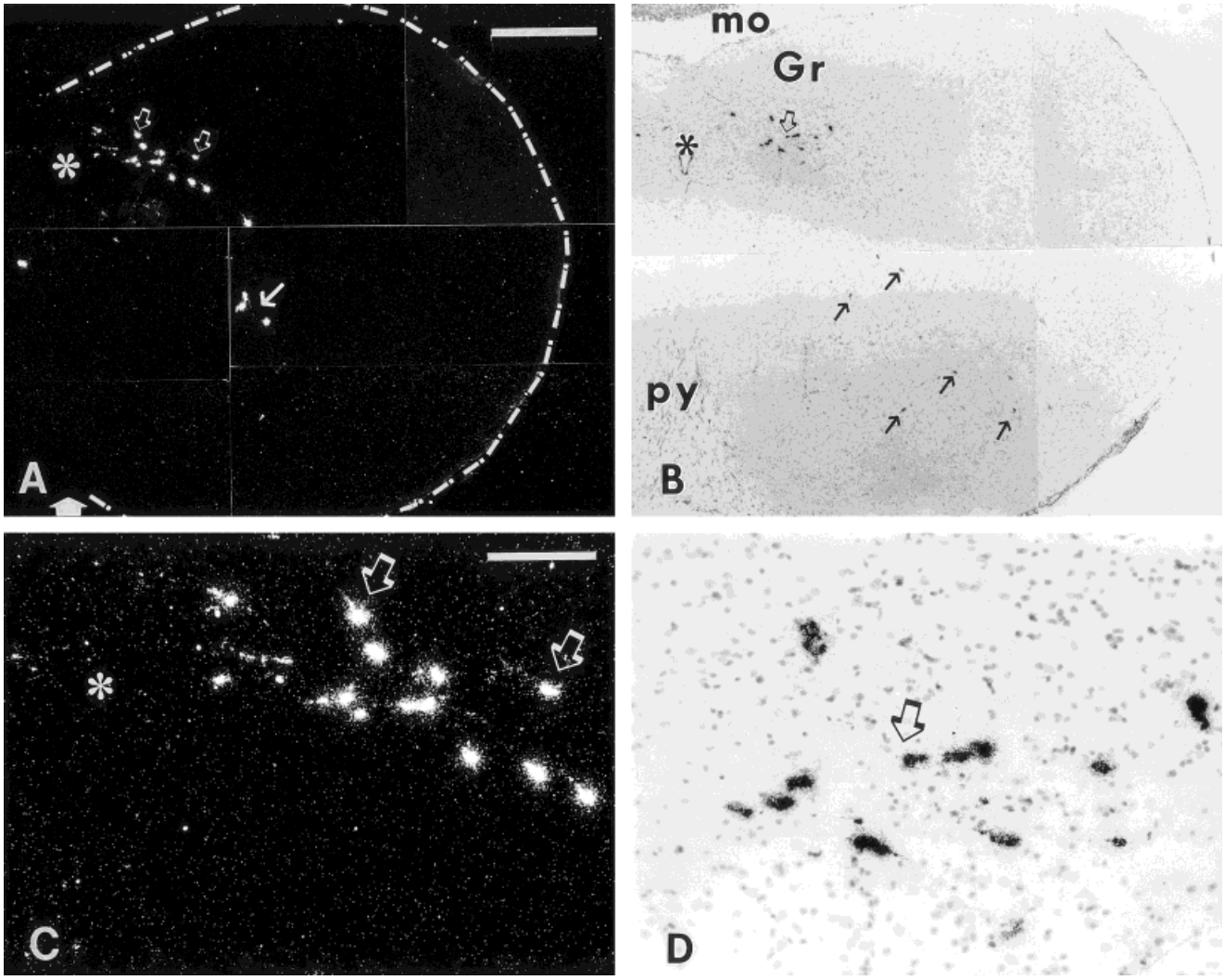

Fig. 2. Pre-pro-glucagon (PPG) messenger RNA (mRNA)-containing cells in the nucleus of the solitary tract. Low magnification darkfield (**A**) and brightfield (**B**) photomicrographs show that the majority of PPG mRNA-expressing cells are located in the medial subdivision of the nucleus of the solitary tract (open arrows); however, additional, scattered labeled cells are also present in deeper medullary areas (closed arrows). Higher magnification darkfield (**C**) and brightfield (**D**) photographs show that the majority of PPG mRNA-expressing cells are located in the medial subdivision of the nucleus of the solitary tract (open arrows). Wide white arrow in A points to the midline; broken line delineates the contour of the medulla. Asterisks indicate the central canal. mo, molecular layer, cerebellum; Gr, gracile nucleus; py, pyramidal tract. Scale bars = 400 μm (A,C), 100 μm (B,D).

In the caudal medulla, the PPG mRNA-containing cells were distributed in an arc, in which the nucleus of the solitary tract and additional positively labeled cells extending ventral to it formed the curve (Fig. 2). Within the nucleus of the solitary tract, most labeled cells were located in the medial nucleus (Fig. 2A,B). A few scattered cells were also seen in the more deeper regions of the caudal medulla, between the dorsal part of the medullary reticular field and the ventral part of the medullary reticular nucleus (Fig. 2A,B). An average of 60 labeled cells were counted per section of the caudal medulla. PPG mRNA-containing cells in the glomerular layer of the olfactory bulb were located among the olfactory glomeruli (Fig. 3). Based on their size and specific location, it is likely that they were either short axon cells or external tufted cells.

Distribution of GLP-1R mRNA-containing perikarya

The distribution of GLP-1R mRNA-containing perikarya is schematically represented in a series of frontal sections of the brain and spinal cord, shown in Figure 4. In the telencephalon, GLP-1R mRNA-containing perikarya were present in the mitral cell layer of the olfactory bulb; basal portion of the frontal cortex; nucleus accumbens; basal nucleus of Meynert; substantia innominata; ventral pallium (Fig. 5A); lateral septum (Fig. 5A); diagonal band of Broca (Fig. 5A); medial, central, and basolateral nuclei of the amygdala (Fig. 5B); septohippocampal nucleus; and posterior portions of the hippocampal formation (CA2–CA3) (Fig. 5C). A few labeled cells were seen in the in bed nucleus of the stria terminalis, particularly in the interme-

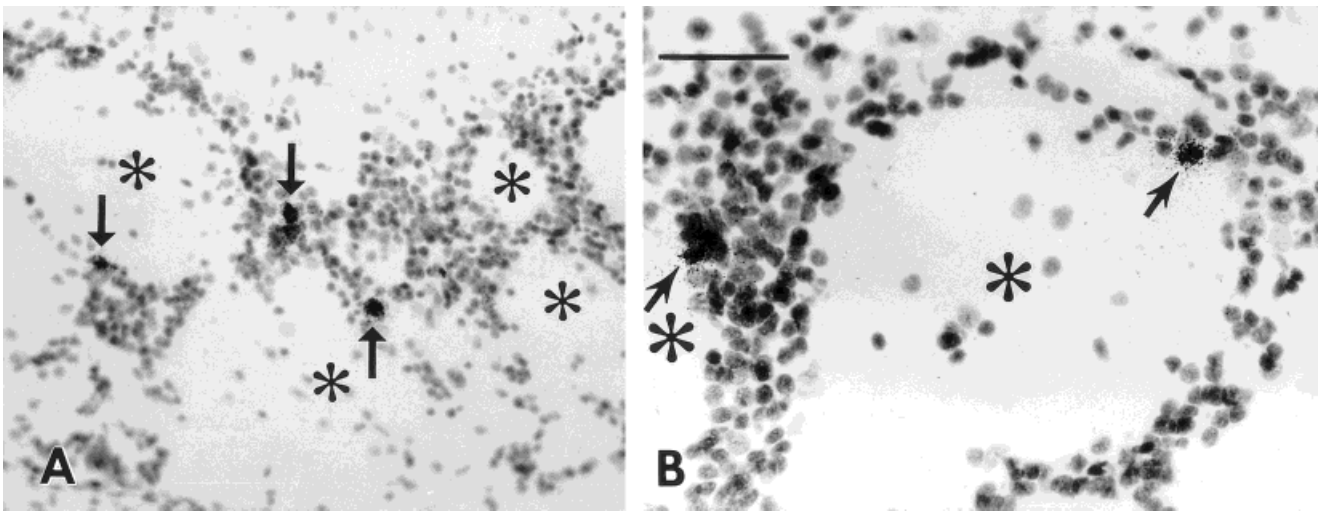

Fig. 3. Pre-pro-glucagon (PPG) messenger RNA (mRNA)-containing cells in the olfactory bulb. The low-magnification photomicrograph (**A**) shows scattered PPG mRNA-expressing cells located among olfactory glomeruli (arrows). At higher magnification (**B**), heavily labeled cells (arrows) are apparent among interglomerular cells. Asterisks indicate the centers of glomeruli. Scale bar = 400 μm (A), 200 μm (B).

diate subnuclei of the posterior bed nucleus of the stria terminalis (Fig. 4). In addition, scattered cells with GLP-1R mRNA were found in the medial septum (Fig. 5A) and in the subfornical organ. Within the hippocampus, the number of labeled cells increased caudally, reaching the highest density in the most caudal portion of the CA3 region (Fig. 5C).

In the diencephalon, the densest accumulation of labeled cells was in the preoptic area (Fig. 6A,B); supraoptic (Fig. 6C), paraventricular (Fig. 6D), arcuate (Fig. 6E), and dorsomedial nuclei of the hypothalamus; and posterior lateral nuclei of the thalamus (Fig. 7A). Scattered labeled cells were also seen in the subfornical organ, lateral hypothalamus, medial habenula, zona incerta, and subthalamic nucleus. Within the preoptic area, two regions had high concentrations of labeled cells: one group occupied the anterior medial preoptic nucleus (Fig. 6B, arrow), the other was located at the lateral border of the medial preoptic area (Fig. 6A,B). Within the paraventricular nucleus, most of the magnocellular neurons were labeled; however, scattered cells could seen in the medial parvocellular subdivision as well (Fig. 6D). In the arcuate nucleus, each subdivision (dorsal, medial, and lateral) contained GLP-1R–expressing cells; the medial and lateral subdivisions contained the most cells (Fig. 6E). The ventromedial nucleus did not contain labeled cells.

In the thalamus, a dense accumulation of GLP-1R mRNA-containing cells was seen in the posterior nuclei, including the dorsal and ventral subnuclei of the pretectal nucleus and the lateral posterior thalamic nuclei (Fig. 7A). Only scattered labeled cells were found in other thalamic nuclei, such as the paraventricular and central medial nuclei. A few GLP-1R–expressing cells were seen in the medial habenula. There was a dense accumulation of labeled cells in the subthalamic nucleus.

The distribution of GLP-1R mRNA-expressing perikarya in the mesencephalon was restricted to the ventral tegmental area (Fig. 7A), fields of Forel (Fig. 7B), nucleus Darkschewitsch (Fig. 7C), central gray (Fig. 8A,C), interstitial nucleus of the tegmentum (Fig. 8C), intermediate subnucleus of the interpeduncular nucleus (Fig. 8A,B) and superficial layers of the superior colliculus. Scattered labeled cells were also present in the raphe nuclei, substantia nigra pars reticulata, and pretectum.

In the rhombencephalon, GLP-1R mRNA-containing perikarya were detected in several pontine areas, including the anterior (Fig. 9A), lateral (Fig. 9B), dorsal (Fig. 9C), and posterodorsal (Fig. 9F) tegmental nuclei; pontine nuclei (Fig. 9E); dorsal and ventral parabrachial nuclei, locus ceruleus, and raphe nuclei (Fig. 9A,B). A high concentration of GLP-1R mRNA expressing perikarya were also seen in regions of the medulla, including the area postrema (Fig. 10C), lateral reticular nucleus (Fig. 10D), raphe nuclei (Fig. 10B), and the nucleus of the solitary tract (Fig. 10A). Scattered labeled cells were present in the periolivary nucleus, sensory nucleus of spinal trigeminal nerve, dorsal nucleus of the vagus nerve, and deep nuclei of the reticular formation.

Within the spinal cord, GLP-1R mRNA-expressing perikarya were found in laminae V–X (Fig. 10E,F), in each major segment (cervical, thoracic, lumbar, and sacral) of the cord, the vast majority of cells being in laminae V and X.

DISCUSSION

Mapping of the distribution of PPG and GLP-1R mRNAs within the CNS has provided information about the sites of GLP-1 action in the brain and represents a critical first step toward understanding the function of GLP-1. The results of the present in situ hybridization histochemical studies have provided detailed and novel information about the distribution of PPG and GLP-1R mRNA-expressing perikarya in the CNS. Our observations are in good agreement with previous immunocytochemical observations on the location of GLP-1i cells (Jin et al., 1988) and with binding studies using ^{125}I-GLP-1 (Göke et al., 1995), although several differences were found (Table 1).

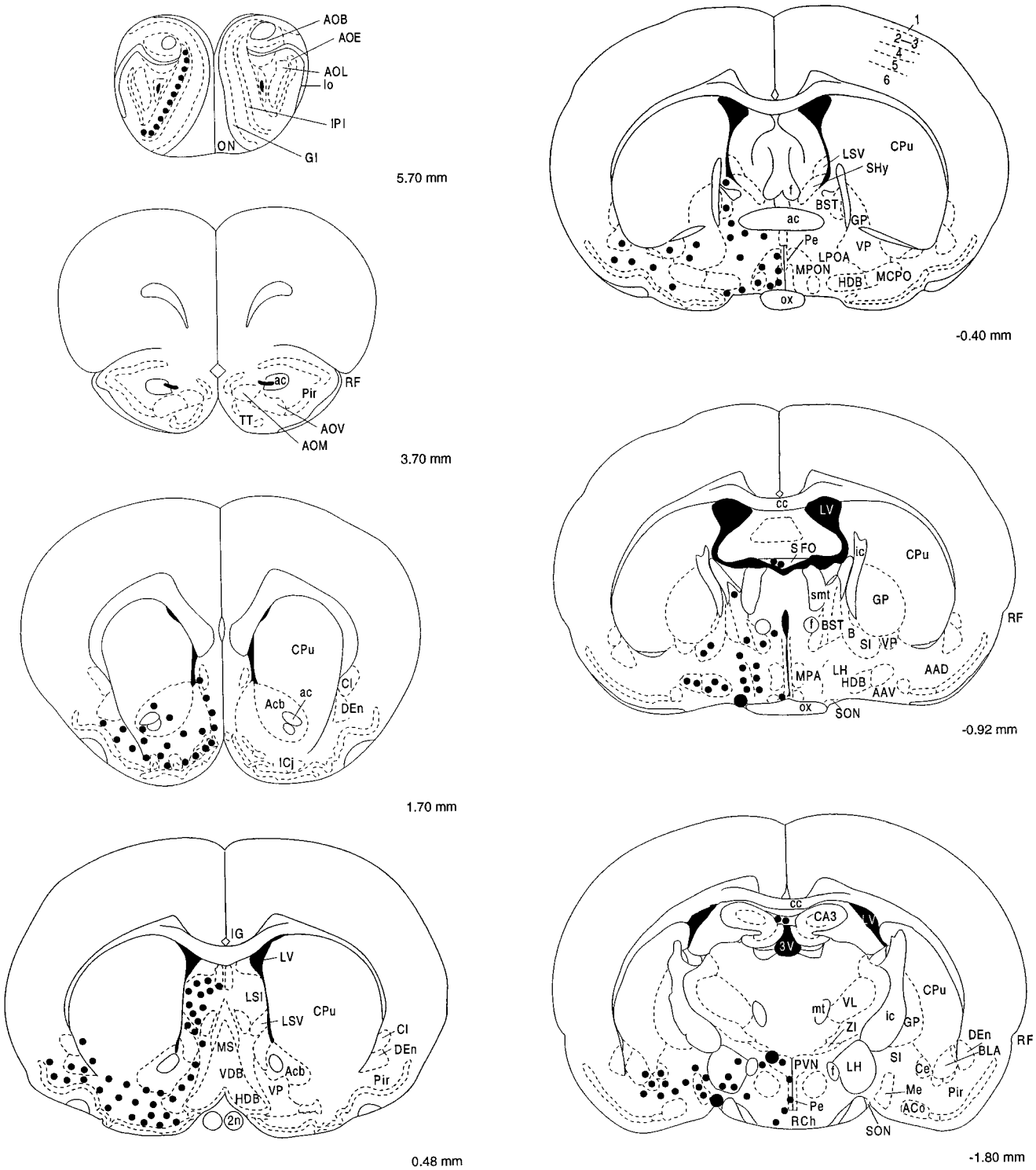

Fig. 4. (Figure continued on following pages.) Schematic representation of coronal sections depicting the distribution of glucagon-like peptide-1 receptor (GLP-1R) messenger RNA in the rat brain and spinal cord. The information has been simplified so that the distribution of labeled cells is depicted by dots that represent both the number and distribution of labeled cells. Signal intensity is not indicated here but is given in Table 1. Small dots, 1–20 labeled cells; large dots, ~50 labeled cells. Schematics and stereotaxic reference points (distance from bregma) were modified from the atlas of Paxinos and Watson (1986). For abbreviations, see list.

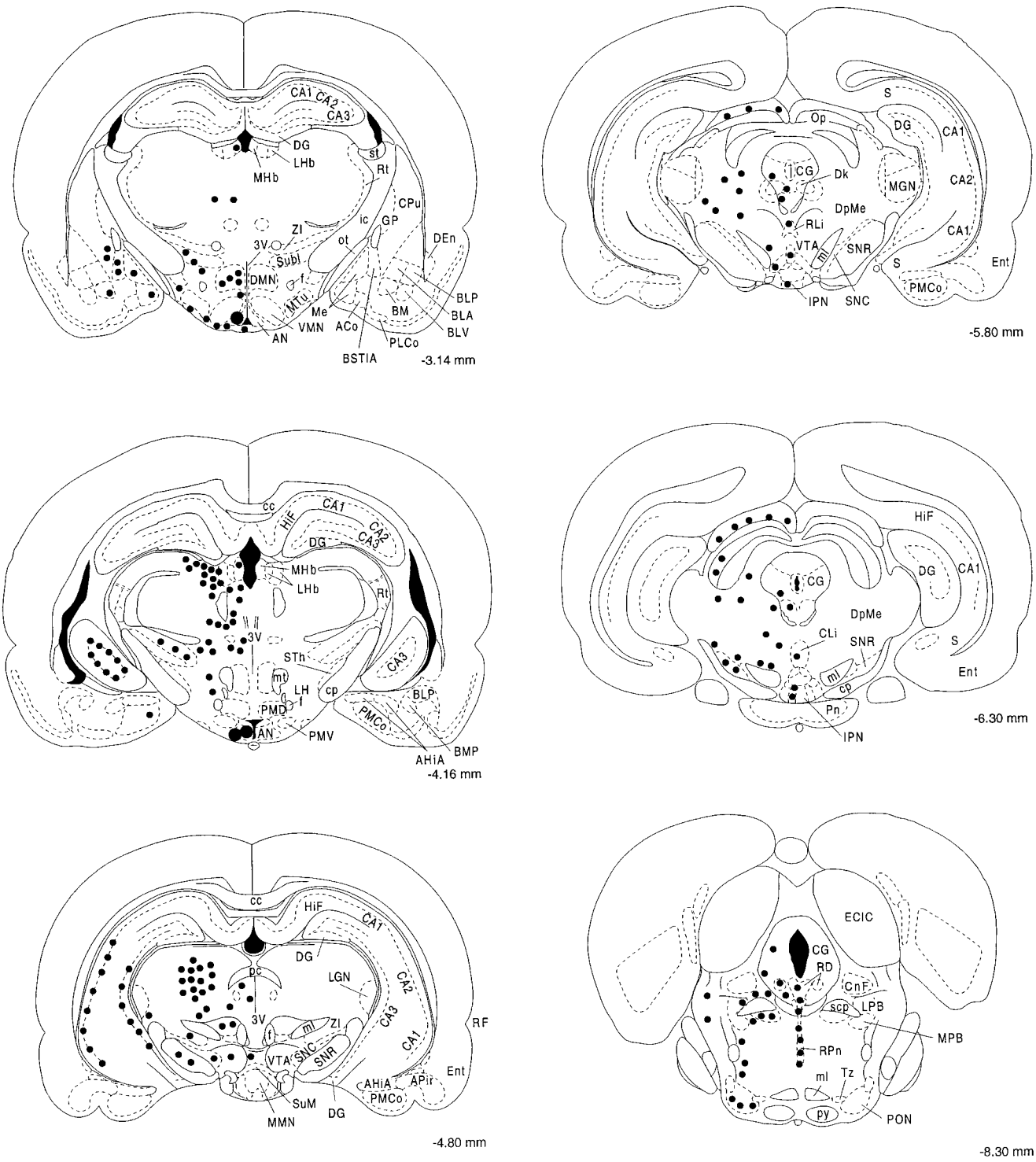

Figure 4 (Continued)

Comparison of the distribution of GLP-1R mRNA-containing perikarya with binding data

In the telencephalon, numerous GLP-1R mRNA-containing perikarya were seen in the mitral cell layer of the olfactory bulb, although no binding data were reported in this region. In contrast, GLP-1 binding was reported in the insulae of Calleja (Göke et al., 1995), but we saw cells with GLP-1R mRNA only above the insulae of Calleja, in the ventral pallium. In addition, medium-density binding was reported in the vertical but not the horizontal limb of the

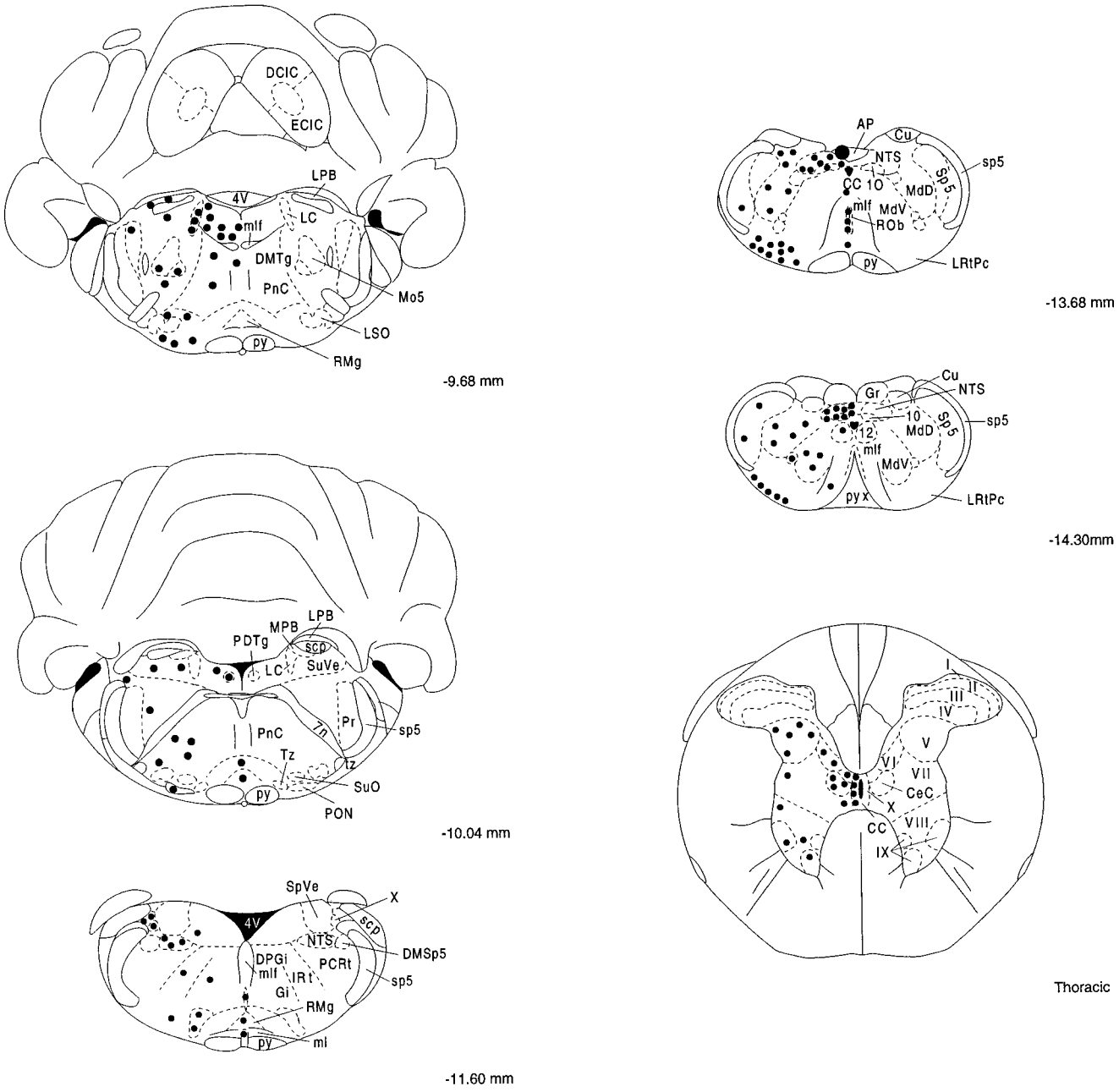

Figure 4 (Continued)

diagonal band of Borca. We however, detected labeled cells in both limbs, although more in the posterior portion of the horizontal limb then in the vertical limb of the diagonal band of Borca.

In the diencephalon, Göke et al. (1995) reported large number of GLP-1 binding in the mammillary nuclei and the median eminence. We saw a few labeled cells in the median eminence but did not see GLP-1R mRNA-containing cells in the mammillary nuclei. However, we found numerous intensely labeled perikarya in the paraventricular and supraoptic nuclei, whereas Göke et al. (1995) reported low or no binding in these hypothalamic nuclei. Interestingly, however, Göke et al. (1995) demonstrated binding in the posterior pituitary. It is possible that GLP-1R protein is transported to distant terminals in the neurohypophysis and the biologically active form is formed during the axonal transportation.

In the brainstem, our findings are similar to those of Göke et al. (1995), although some quantitative differences are present. For example, we saw more GLP-1R mRNA-expressing cells than cells binding GLP-1 (Göke et al. 1995) in the parabrachial nucleus. No comparative data are available for the spinal cord, in which we saw a moderate number of neurons with GLP-1R mRNA.

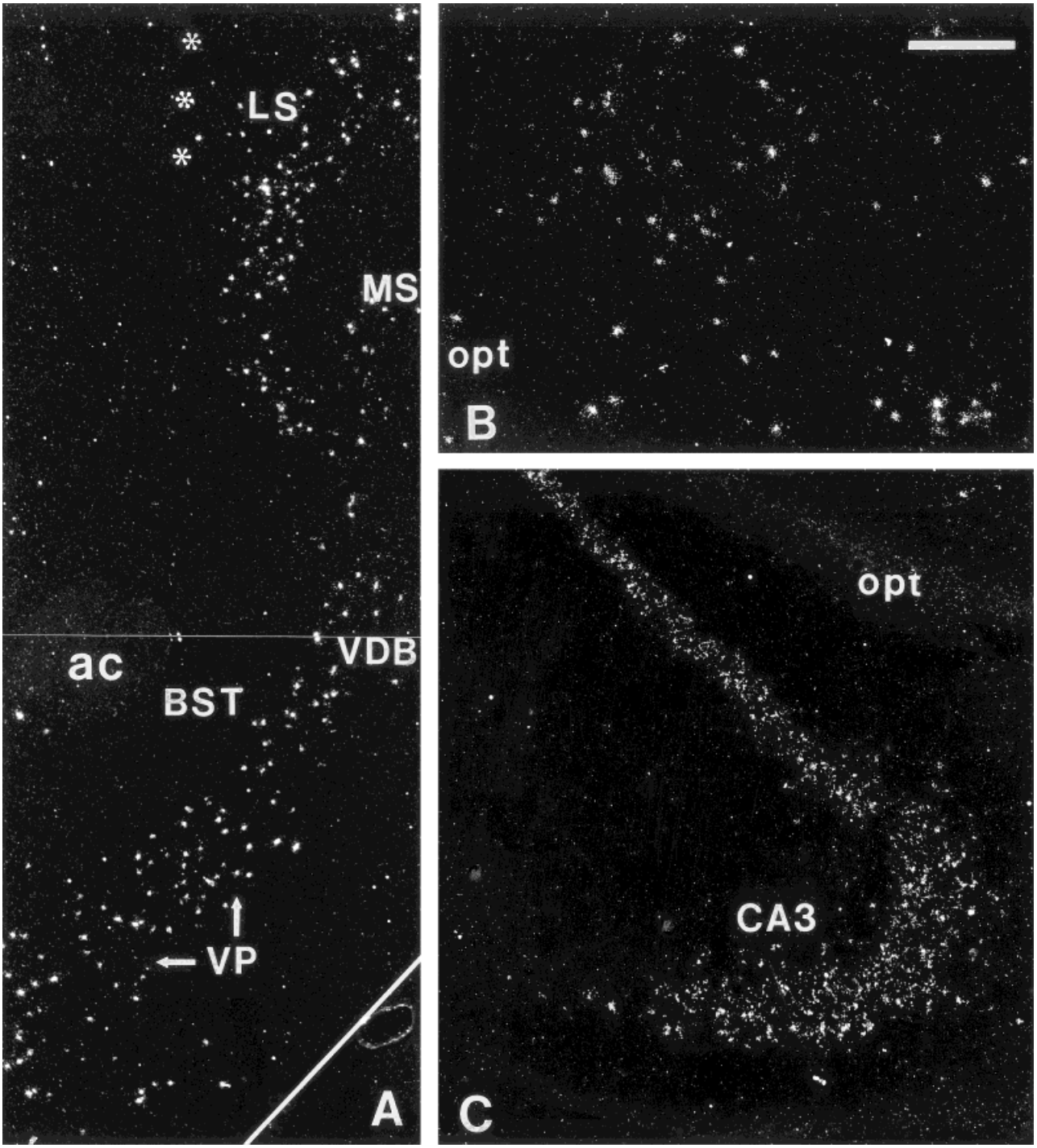

Fig. 5. Glucagon-like peptide-1 receptor (GLP-1R) messenger RNA in telencephalon of rat brain. Hybridization signal was detected in ventral pallidum (VP), lateral septum (LS), medial septum, and vertical limb of the diagonal band of Broca (**A**). Additional labeled cells were seen in the amygdala (**B**) and in ventral portions of the CA3 region of hippocampus (**C**). Asterisks indicate lateral ventricle. MS, medial septus nucleus; ac, anterior commissure; BST, bed nucleus of the stria terminalis; VDB, nucleus of the vertical limb of the diagonal band; opt, optic tract. Scale bars = 400 μm (A,C), 200 μm (B).

Comparison of the distribution of GLP-1R mRNA-containing perikarya with localization of GLP-1–immunoreactive nerve terminals

Despite some discrepancies, the overall match between immunocytochemical data, showing GLP-1i fibers and-nerve terminals, and the present paper, showing GLP-1R mRNA-containing cells in the hypothalamus and the brainstem, is good—with a few exceptions (see below). Similar to two reports in which immunocytochemistry was used to detect PPG immunoreactivity (Kauth and Metz, 1987; Jin et al., 1988), we did not observe either PPG mRNA-

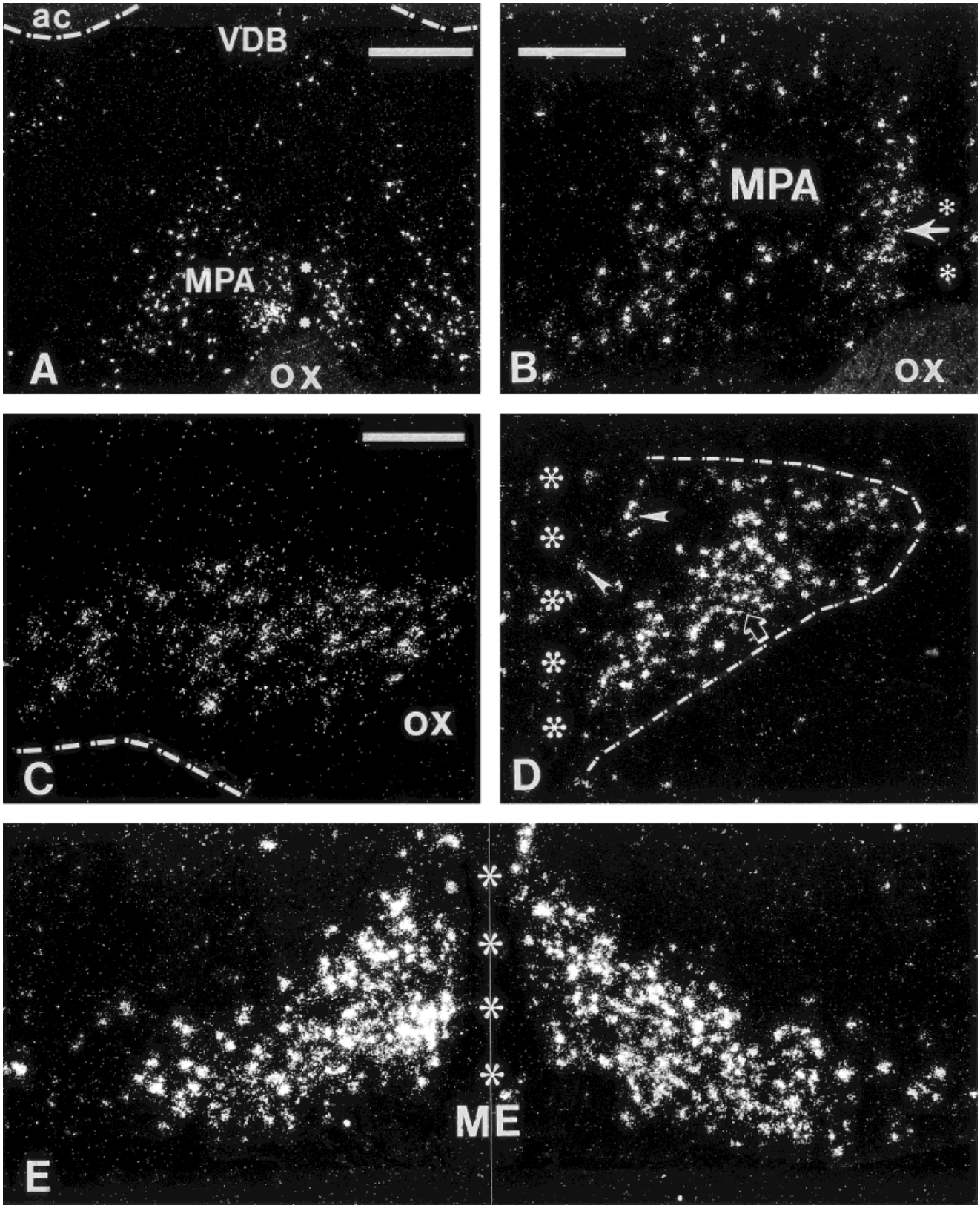

Fig. 6. Glucagon-like peptide-1 receptor (GLP-1R) messenger RNA (mRNA) in hypothalamus of rat brain. Hybridization signal was detected in the medial preoptic area, with dense accumulation in the periventricular preoptic nucleus and the lateral portion of preoptic area (**A,B**). Numerous labeled cells were also seen in the supraoptic (**C**), paraventricular (**D**), and arcuate (**E**) nuclei. Arrow in B shows labeled cells in the anterior periventricular preoptic nucleus. Arrowheads in D indicate labeled perikarya in the parvicellular subdivision of the paraventricular nucleus. Open arrow in D shows GLP-1R mRNA-containing cells in the magnocellular portion of the paraventricular nucleus. Asterisks in A, B, D, and E indicate the third ventricle. Broken lines delineate the anterior commissure (A), the base of the hypothalamus (C), or the lateral-superior extension of the paraventricular nucleus (D). ac, anterior commissure; VDB, nucleus of the vertical limb of the diagonal band; MPA, medial preoptic area; ox, optic chiasm; ME, median eminence. Scale bars = 400 μm (A), 200 μm (B–E).

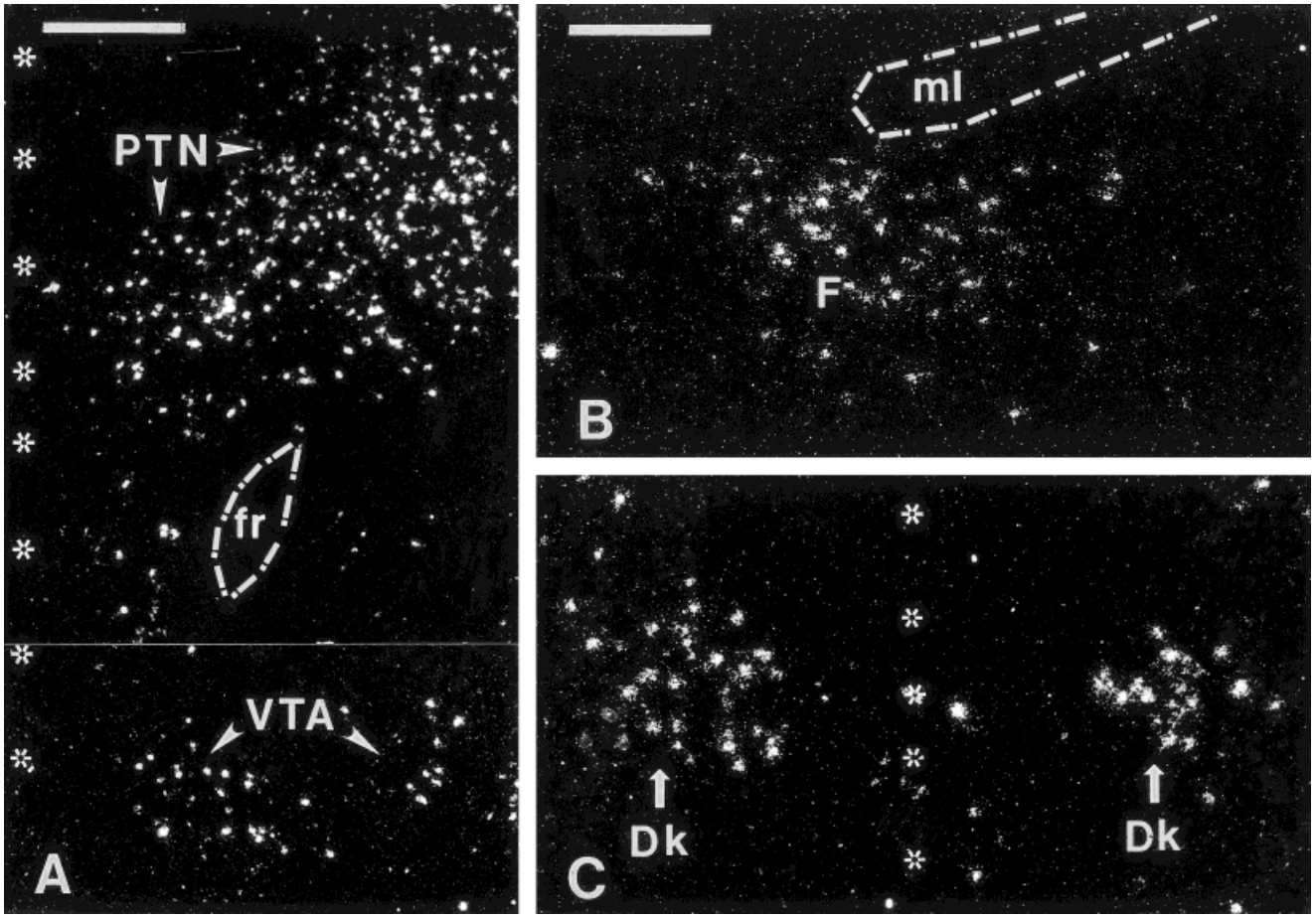

Fig. 7. Glucagon-like peptide-1 receptor (GLP-1R) messenger RNA in thalamus and mesencephalon of rat brain. Hybridization signal was detected in pretectal nuclei of the thalamus and ventral tegmental area (**A**), fields of Forel (**B**), and Darkschewitsch nucleus (**C**). Asterisks indicate the third ventricle (A) or the midline (C). Broken lines delineate the fasciculus retroflexus (A) or the medial lemniscus (B). PTN, posterior thalamic nuclei; fr, fasciculus retroflexus; VTA, ventral tegmental area; ml, medial lemniscus; F, fields of Forel; Dk, nucleus of Darkschewitsch. Scale bars = 400 μm (A), 200 μm (B,C).

expressing cells in the hypothalamus. Therefore, the immunocytochemical detection of GLP-1–immunostained cells in rat hypothalamus by others is doubtful (cf. Orskov et al., 1987).

Although Kauth and Metz (1987) and Jin et al. (1988) reported GLP-1i fibers in the cortex, we did not see any GLP-1R mRNA-expressing cells in this telencephalic region, except for labeled cells in the anterior temporal cortex. In contrast, neither Kauth and Metz (1987) or Jin et al. (1988) reported GLP-1i fibers in the olfactory bulb, where we observed GLP-1R mRNA-containing cells in the mitral cell layer of the olfactory bulb. Although no GLP-1i fibers or terminals were reported in the hippocampus, we detected GLP-1R mRNA-containing cells in the most posterior portions of CA3 region of the Ammons horn. We also detected scattered labeled cells in the CA1–CA2 regions of the posterior hippocampus.

Within the hypothalamus, the most obvious difference between the immunocytochemical and in situ hybridization data is the presence of numerous GLP-1i fibers or terminals in the ventromedial nucleus and lack of GLP-1R mRNA in this region.

In the thalamus, the distribution of GLP-1i fibers or terminals is more widespread than the distribution of GLP-1R mRNA. According to Jin et al. (1988), most of the medially located thalamic (anteromedial, reuniens, rhomboid, central, intermediodorsal, and paraventricular) nuclei contained immunoreactive fibers or terminals. However, only a few GLP-1R mRNA-containing cells were found in these regions. The immunocytochemical and in situ hybridization data both agree that the posterior thalamic nuclei contain the densest accumulation of GLP-1i fibers and GLP-1R mRNA-containing cells.

In the mesencephalon, the distribution of GLP-1i fibers or terminals and GLP-1R mRNA-containing cells is similar.

In the pons and medulla, the distribution of GLP-1R mRNA-expressing cells was more extensive than the reported distribution of GLP-1i fibers or terminals. Jin et al. (1988) found a low density of immunoreactive fibers and terminals only in the central gray of the pons, lateral parabrachial nucleus, reticular formation, facial nucleus, paragigantocellular nucleus, reticular nucleus, and nucleus of the solitary tract. The present study observed hybridization signal in these regions as well as in the locus ceruleus, posterodorsal tegmental nucleus, area postrema, and raphe nuclei.

No immunocytochemical data are available from the spinal cord.

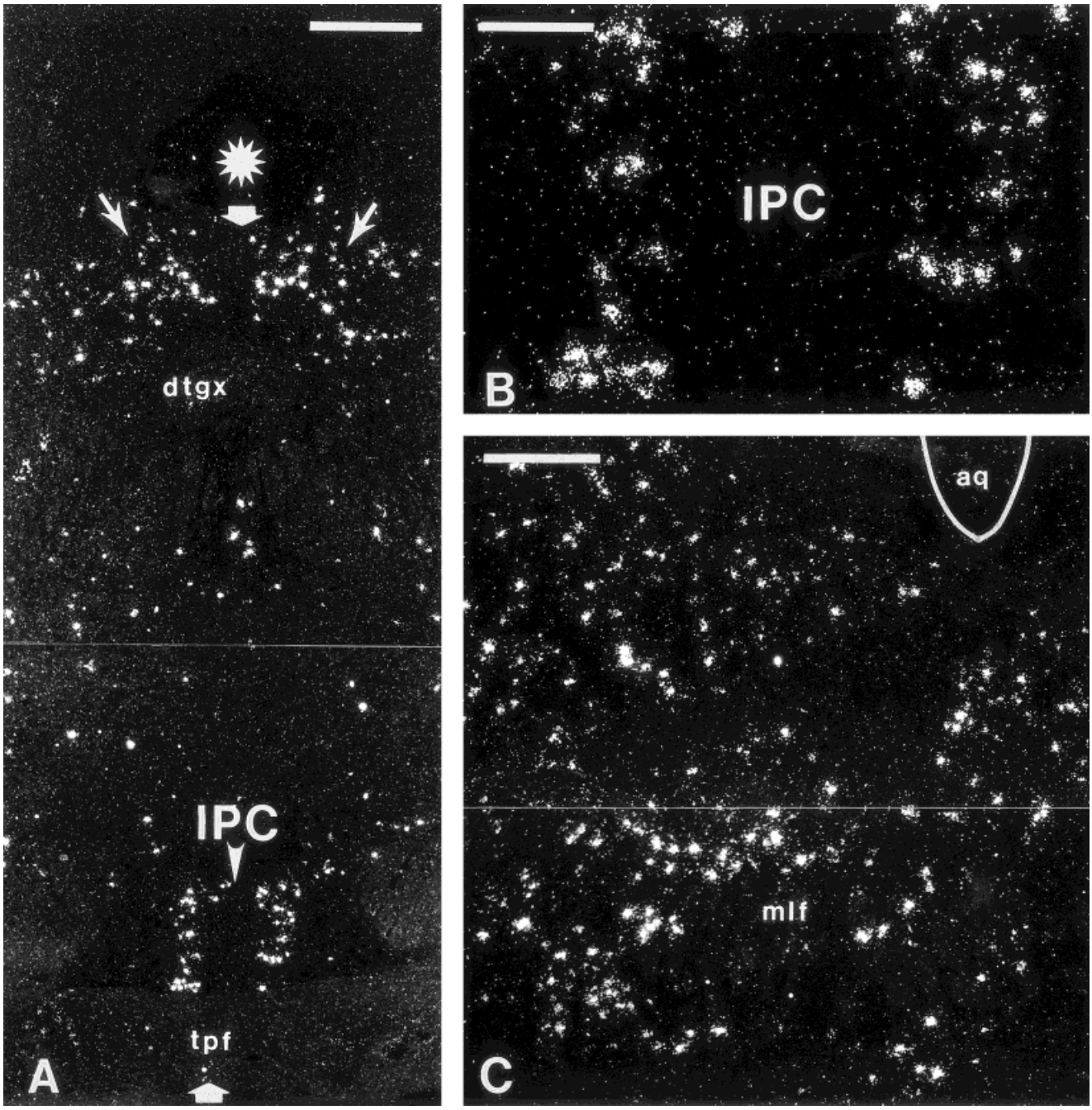

Fig. 8. Glucagon-like peptide-1 receptor (GLP-1R) messenger RNA in mesencephalon of rat brain. Hybridization signal was detected in the ventral aspect of the central gray, including oculomotor nuclei, interstitial nuclei of the medial longitudinal fascicle (arrows in A), and lateral aspect of the interpeduncular nucleus (**A,B**). Additional scattered cells were also seen throughout the mesencephalon (A) including tegmentum (**C**), dorsal raphe, and around the medial lemniscus. Asterisk in A indicates the cerebral aqueduct; wide white arrows point to the midline. Solid line in C delineates the cerebral aqueduct. dtgx, dorsal tegmental decussation; IPC, interpeduncular nucleus, central subnucleus; aq, aqueductus cerebri; mlf, medial longitudinal fasciculus. Scale bar = 400 μm (A,C), 200 μm (B).

In general, the immunocytochemical data, showing GLP-1i fibers or terminals and the in situ hybridization data, showing GLP-1R mRNA-expressing cells, are in good agreement, although there are areas that do not contain GLP-1 immunoreactivity or GLP-1 mRNA. This discrepancy is probably due to the inability of the immunocytochemical technique to distinguish between immunoreactive fibers and nerve terminals. Therefore, several areas may contain immunoreactive fibers without forming nerve terminals and thereby indicate a more widespread distribution of GLP-1 than seen with in situ hybridization.

Functional considerations

In the present studies, we report the presence of PPG mRNA-containing cells in the nucleus of the solitary tract

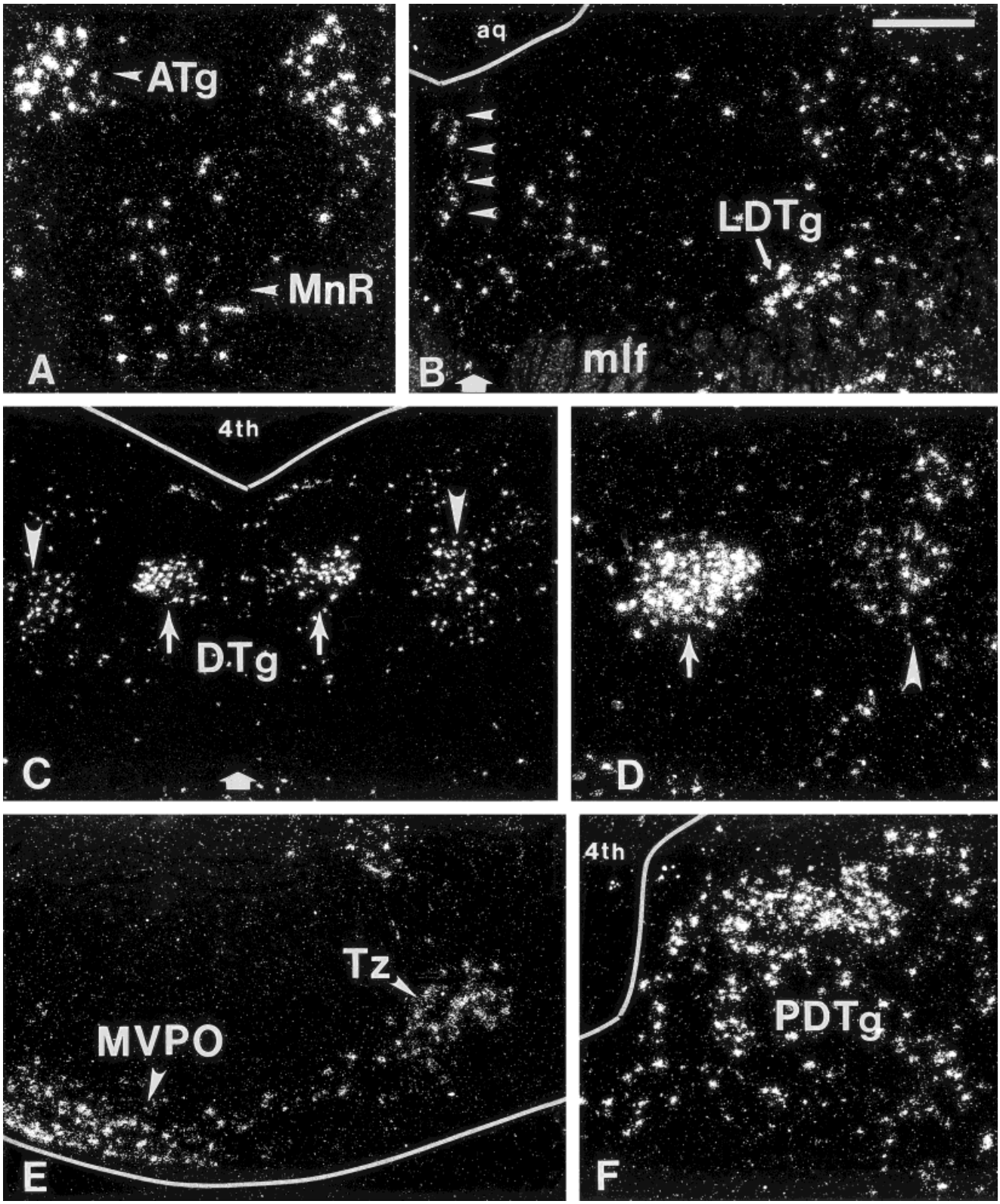

Fig. 9. Glucagon-like peptide-1 receptor (GLP-1R) messenger RNA in pons of rat brain. A dense accumulation of labeled cells was seen in anterior, lateral, dorsal, and posterodorsal tegmental nuclei (**A–D** and **F**). Additional hybridization signal was detected in raphe magnus (A), dorsal raphe (arrowheads in B), trapezoid nucleus, and medioventral periolivary nucleus (**E**). Solid white line delineates cerebral aqueduct (B), 4th ventricle (4th; C,F) or base of the brain (E), and white arrow (B,C) shows the midline. Arrows denote dorsal tegmental nucleus (DTg; C,D), and arrowheads denote laterodorsal tegmental nucleus (LDTg; C,D). ATg, anterior tegmental nucleus; MnR, median raphe nucleus; aq, aqueductus cerebri; mlf, medial longitudinal fasciculus; MVPO, medioventral periolivary nucleus. Scale bar = 200 μm.

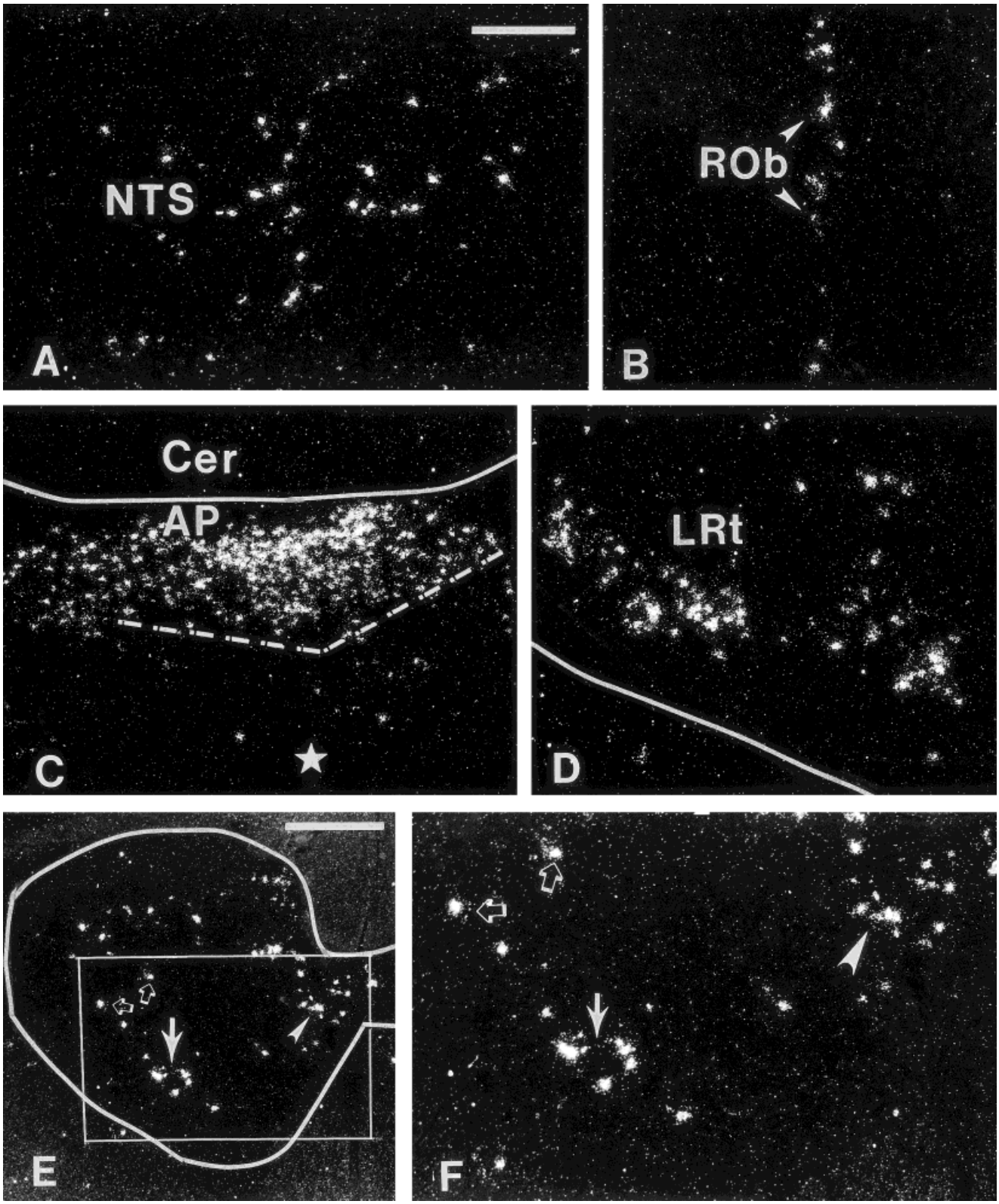

Fig. 10. Glucagon-like peptide-1 receptor (GLP-1R) messenger RNA (mRNA) in the medulla oblongata and spinal cord. Hybridization signal was detected in the nucleus of the solitary tract (NTS; **A**), raphe obscurus (ROb; **B**), area postrema (AP; **C**), and lateral reticular nucleus (LRt; **D**). In the spinal cord (**E**), scattered GLP-1R mRNA-containing cells were seen in the laminae III–X, with a dense accumulation in lamina X around the central canal. **F:** A higher magnification of the area indicated in E. Large arrows show motor neurons with the label; open arrows indicate labeled perikarya in lamina VI; arrowhead shows GLP-1R mRNA-containing cells around the central canal (E,F). Solid white lines indicate the contour of the gray matter (E) or upper and lower borders of the medulla (C and D, respectively). Star in C shows the cerebral aqueduct. Cer, cerebellum. Scale bars = 400 μm (E), 200 (A–D,F).

TABLE 1. Location of GLP-1–Binding Neurons, GLP-1 Immunoreactivity, and GLP-1R mRNA-Containing Perikarya in Rat Brain and Spinal Cord

Location	Binding[1]	Immunoreactivity[2]	mRNA[3]
Telencephalon			
Olfactory bulb			
Mitral cell layer	–	–	+
Cortex	–	+	–
Hippocampus	–	–	–
Anterior	–	–	–
Posterior	–	–	+
Basal Ganglia			
Nucleus accumbens	++	+	+
Caudate-putamen	–	NA	+
Striatum	++	NA	++
Amygdala			
Medial nuclei	++	+	–
Central nucleus	++	+	–
Islands of Calleja	++	NA	–
Diagonal band of Broca	++	++	+
Bed nucleus of stria terminalis	+	++	+
Septum			
Lateral	+++	++	++
Medial	+	+	+
Diencephalon			
Thalamus			
Anterodorsal nuclei	++	+	–
Centromedial nucleus	++	+	–
Laterodorsal nuclei	++	+	+
Paraventricular nucleus	+	+++	++
Posterior nuclei	++	+	+++
Zona incerta	++	+	+
Hypothalamus			
Arcuate nucleus	+++	++	++++
Dorsomedial nucleus	++	++++	+
Dorsal hypothalamic area	+	++	+
Lateral hypothalamus	++	+++	+
Lateral preoptic area	++	++	++
Medial mammillary nucleus	++++	+	+
Medial preoptic area	+	+++	+++
Periventricular preoptic nucleus	NA	+	+++
Paraventricular nucleus	++	+++	++++
Supraoptic nucleus	NA	+++	+++
Ventromedial nucleus	+	+++	–
Mesencephalon			
Dorsal raphe	++	+	+
Interpeduncular nucleus, lateral	++++	+	+
Periaqueductal gray	++	++	+++
Posterodorsal tegmental nucleus	+++	++	+++
Superior colliculus			
Superficial grey layer	+	++	++
Optic nerve layer	+	NA	+
Intermediate grey layer	++	+	+
Ventral tegmental area	++	+	++
Pons			
Central gray matter	+++	+	+
Dorsal raphe	+++	–	+
Lateral reticular nucleus	++	–	+
Parabrachial nuclei	+	+	+
Reticular formation	NA	+	+
Medulla			
Area postrema	++++	–	++++
Inferior olive	+++	–	++
Nucleus of the solitary tract	++++	++	+++

[1]+, 1–5 pmol/mg wet tissue; ++, 6–10 pmol/mg wet tissue; +++, 11–15 pmol/mg wet tissue; ++++, >15 pmol/mg wet tissue. Data are from Göke et al., 1995.
[2]Defined as the number of immunoreactive fibers or terminals, from low (+) to high (++++) density of innervation. Data are from Jin et al., 1988.
[3]Measured as the number of GLP-1R mRNA-expressing cells in situ, from low (+) to high (++++) density of labeled cells.
NA: No data available.

as well as the glomerular layer of the olfactory bulb, a region that was not detected in previous immunocytochemical (Kautz and Metz, 1987) and in situ hybridization (Han et al., 1986) studies. Although the phenotype and function of these neurons is not known, their characteristic location among glomeruli suggest that they are interneurons. In rodents, the interneurons of the olfactory bulb send short dendrites and axons to neighboring glomeruli, where they receive synaptic inputs from the olfactory nerves and mitral and tufted cells (Nieuwenhuys, 1985). The presence of GLP-1R mRNA-containing cells in the mitral cell layer supports the notion that PPG mRNA-positive cells in the glomerular layer are indeed interneurons and that they may innervate mitral cells in the olfactory bulb. Via these interactions, GLP-1 may function as a neurotransmitter, controlling information flow from the olfactory epithelium to the brain.

The only other brain regions in which PPG mRNA-expressing neurons were detected was the nucleus of the solitary tract and an adjacent area, described here as the dorsal and ventral medulla. The nucleus of the solitary tract is a region of the brain that contains the highest density and highest concentration of neurotransmitters (Nieuwenhuys, 1985). The nucleus of the solitary tract is a complex integration center subserving many diverse functions, including the processing and relaying of gustatory sensation (Doetsch and Erickson, 1970; Norgren, 1978; Whitehead, 1986); the regulation of gastrointestinal (Gwyn et al., 1979), respiratory (Spyer and Richter, 1986), and cardiovascular (Snyder et al., 1978; Talman et al., 1980; Healy et al., 1981; Ross et al., 1981) functions; the regulation of blood pressure (Cohen and Schnall, 1970; Crill and Reis, 1968; Palkovits and Zaborszky, 1977), and relaying baro- and chemoreceptor information from the carotid sinus and aortic depressor nerves to autonomic centers of the brain (Ciriello et al., 1981) (cf. Paxinos, 1990).

In addition to information arising from the periphery via the cranial nerves, higher autonomic centers, including the hypothalamus, also reach the nucleus of the solitary tract via descending neuronal pathways (cf. Paxinos, 1990).

Because GLP-1 in the CNS is thought to inhibit eating and drinking behavior, it is interesting to speculate how the nucleus of the solitary tract regulates these behaviors via GLP-1. It is likely that the nucleus of the solitary tract integrates gustatory information transmitted via cranial nerves VII, IX, and X with sensory information transmitted via the vagal nerve from the stomach and blood-born signals, such as leptin. Following integration, GLP-1, synthesized by nucleus of the solitary tract and dorsal and ventral medullary neurons, may alter the activity of neurons innervated by GLP-1 throughout the brain and spinal cord. The activation of these postsynaptic neurons would result in reduced ingestive behavior.

Several brain regions contain neurons that express GLP-1R mRNA and are thought to be important in the regulation of food intake. Among these, the arcuate nucleus and paraventricular nucleus seem to be the best characterized. Lesions in the arcuate and paraventricular nuclei, two nuclei with a high concentration of GLP-1R mRNA, and the ventromedial nucleus, an area lacking GLP-1R mRNA, affect body weight and feeding (Mayer et al., 1955; Gold et al., 1977). In rats, it has been shown that damage to the paraventricular nucleus results in a large increase in food intake and body weight gain (Gold et al., 1977). Lesions within the ventromedial nucleus are associated with hyperphagia and severe obesity, which can be inhibited by vagotomy, implicating the autonomic nervous system in ventromedial nucleus-lesioned obesity (Bray, 1984).

The medial subdivision of the arcuate nucleus contains numerous neuropeptide Y- (NPY-) synthesizing neurons, which project to the paraventricular nucleus (Bai et al., 1985). NPY is the most powerful known stimulant of feeding behavior (Levine and Morley, 1984; Clark et al., 1985; Stanley and Leibowitz, 1985). Intracerebroventricularly administered GLP-1 immediately before NPY admin-

istration greatly reduces food intake (Turton et al., 1996), and intracerebroventricular administration of exendin 9–39, a GLP-1 antagonist, immediately before NPY significantly increases food intake compared with treatment with NPY alone (Turton et al., 1996). Interestingly, intracerebroventricular administration of GLP-1 does not alter the level of hypothalamic NPY mRNA (Turton et al., 1996), but GLP-1 may affect the release of NPY from nerve terminals in the paraventricular nucleus. Although GLP-1 does not stimulate NPY gene expression in the arcuate nucleus, when administered intracerebroventricularly, it does stimulate the expression of the immediate early gene c-*fos*, an indicator of neuronal activity, in the arcuate nucleus (Larsen et al., 1997a). Because the administration of exendin before GLP-1 prevented c-*fos* expression, these observations suggest that the effect of GLP-1 on c-*fos* expression is mediated via GLP-1 receptors.

Another neuropeptide that dramatically reduces food intake is corticotropin-releasing hormone (Schwartz et al., 1996), which is produced in the paraventricular nucleus (Merchenthaler et al., 1982). The recent observations that GLP-1 elicits c-*fos* expression within corticotropin-releasing factor neurons in the paraventricular nucleus suggests that the inhibitory effect of GLP-1 on food intake may involve the corticotropin-releasing hormone-containing neuronal system in the paraventricular nucleus (Turton et al., 1996; Larsen et al., 1997a). GLP-1R mRNA is also present in the magnocellular neurosecretory system of the paraventricular nucleus and supraoptic nuclei, suggesting a functional correlation between GLP-1 and some of the neuropeptides (e.g., oxytocin, vasopressin, dynorphin, and galanin) produced by paraventricular nucleus neurons. Indeed, intracerebroventricular administration of GLP-1 induces c-*fos* expression in 40% of oxytocin neurons, but not in vasopressin neurons (Larsen et al., 1997a). It is known that centrally and peripherally administered GLP-1 dose-dependently inhibits basal drinking and induces a paradoxical increase of arginine vasopressin output (Tang-Christensen, 1996). Because GLP-1 administration is not accompanied by c-*fos* induction in vasopressin neurons, GLP-1 does not exert its effects directly on arginine vasopressin neurons in the paraventricular or supraoptic nuclei. Rather, the secretory response may be a secondary water-conserving mechanism that occurs as a consequence of a pronounced natriuresis elicited by GLP-1. Because GLP-1 activates oxytocin neurons and oxytocin acts as a natriuretic agent (Sawyer 1958; Verbalis et al., 1991b), natriuresis seen after intracerebroventricular administration of GLP-1 may be caused by a direct activation of oxytocin cells in the magnocellular nuclei.

In the nucleus of the solitary tract, several neuropeptides and neurotransmitters are co-localized in the same neuron, which projects to the hypothalamic paraventricular nucleus. For example, a subpopulation of neurons co-localizes β-inhibin, somatostatin-28, and enkephalin (Sawchenko et al., 1990). Because the location of GLP-1-expressing cells and these peptidergic neurons is similar in the nucleus of the solitary tract, it is possible that GLP-1 is also co-synthesized in these peptidergic neurons. Although the functional significance of this putative co-localization is not known, a complex interaction may take place at the synaptic site in the paraventricular nucleus. The majority of these peptidergic neurons in the nucleus of the solitary tract innervate oxytocin neurons in the paraventricular nucleus (Sawchenko et al., 1988). β-Inhibin (Sawchenko et al., 1988), somatostatin (Brown et al., 1988), and enkephalin (Wright and Clarke, 1984; cf. Sawchenko et al., 1990), all regulate oxytocin secretion, and GLP-1 also activates oxytocin neurons (Sawyer, 1958; Verbalis et al., 1991b). Therefore, an interaction among these co-localizing components on the postsynaptic site is possible.

A similar relationship seems to exist between GLP-1R and leptin receptors. The *ob* gene product, leptin, is an important circulating signal for the regulation of body weight (Zhang et al., 1994). Leptin, a 16-KDa protein, enters the brain via a specific transport mechanism (Banks et al., 1996) and binds to its receptors (Tartaglia et al., 1995). There are multiple leptin receptor forms encoded by distinct transcripts (reviewed in Tartaglia, 1997). The mRNA species encoding the short intracellular domain has been found in choroid plexus and in several extrahypothalamic areas, including habenula, hippocampus, and cortex (Campfield et al., 1995; Hakansson et al., 1996).

The mRNA species encoding the long intracellular domain is highly expressed in the hypothalamic arcuate, paraventricular, ventromedial, and dorsomedial nuclei (Ghilardi et al., 1996; Huang et al., 1996; Schwartz et al., 1996). The long form is thought to be the form that signals and mediates the biological effects of leptin, whereas the short form functions as a transporter for the OB protein. Leptin's effects on body weight appear to be mediated primarily via effects on the hypothalamus (Campfield et al., 1995). Some of leptin's effects on energy homeostasis are apparently conveyed by suppression or stimulation of NPY in the arcuate nucleus (Schwartz et al., 1996) of NPY, a potent stimulator of food intake. Using semiadjacent sections, Hakansson et al. (1996) showed that leptin receptor mRNA is co-localized with NPY in the arcuate nucleus. Moreover, intracerebroventricular injection of leptin to fasting animals significantly decreases the levels of NPY mRNA in the arcuate nucleus and increases the levels of mRNA for corticotropin-releasing hormone (an inhibitor of food intake) in the paraventricular nucleus (Schwartz et al., 1996), supporting the critical role of leptin in hypothalamic response to fasting.

Leptin administration to *ob/ob* mice induces c-*fos* expression in the hypothalamus, including the arcuate, paraventricular, ventromedial, and dorsomedial nuclei and the zona incerta, areas where—with the exception of ventromedial nucleus—GLP-1R is also expressed. All of these hypothalamic regions are important in the neuroendocrine control of energy homeostasis. The accumulation of leptin mRNA-expressing neurons in the arcuate and paraventricular nuclei suggests that leptin, NPY, and GLP-1 may interact in these hypothalamic nuclei to modulate ingestive behavior. In addition to the hypothalamus, another site where leptin and GLP-1 may interact is the nucleus of the solitary tract. Goldstone et al. (1997) reported that PPG-expressing neurons in the nucleus of the solitary tract co-express leptin receptor mRNA. Based on these findings, it is possible that GLP-1-producing neurons may be directly influenced by circulating leptin.

GLP-1R mRNA, however, is present not only in the hypothalamus but in several other brain regions. Efferents from GLP-1–synthesizing neurons in the nucleus of the solitary tract and dorsal and ventral medulla may innervate several brainstem, diencephalic, and telencephalic areas (Ricardo and Koh, 1978). Because the processes of periglomerular PPG mRNA-containing neurons do not

leave the olfactory bulb, the location of GLP-1 receptor mRNA-containing regions in the brain provides indirect data on the terminal fields of PPG-synthesizing neurons in the nucleus of the solitary tract and dorsal and ventral medulla. The distribution of GLP-1 receptor mRNA-expressing cells is in good agreement with data based on retrograde and anterograde tracing experiments aimed at mapping the efferent connections of the nucleus of the solitary tract. These terminal fields include parabrachial nuclei (Norgren, 1978); nucleus of the vagus nerve (Norgren, 1978; Palkovits and Zaborszky, 1977); hypoglossal nerve (Travers and Norgren, 1983); medullar reticular formation (Morest, 1967); ventrolateral medulla, including the lateral reticular nucleus or noradrenergic A1 area (Morest, 1967; Loewy and Burton, 1978; Norgren 1978; Sawchenko and Swanson, 1982; Ross et al., 1985); C1 adrenergic area (Dampney et al., 1982; Ross et al., 1985); paraventricular nucleus of the hypothalamus (Sawchenko and Swanson, 1981, 1982); and the spinal cord (Torvik 1956; Satoh et al., 1977; Loewy and Burton, 1978; Blessing et al., 1981). Each of these regions contains GLP-1R mRNA-expressing neurons. Although the number of PPG mRNA-expressing neurons in the nucleus of the solitary tract is low (present studies), our data strongly suggest that the efferent connections of this nucleus are more widespread than previously reported.

In addition to the regions mentioned above, we saw GLP-1R mRNA-expressing cells in several telencephalic, diencephalic, mesencephalic, and rhombencephalic regions in which neurons have not been shown to be connected to the nucleus of the solitary tract. In the telencephalon, these regions include the nucleus accumbens, basal nucleus of Mynert, ventral pallium, temporal cortex, posterior hippocampus, septohippocampal nucleus, bed nucleus of the stria terminalis, and diagonal band of Broca; in the diencephalon, the preoptic area, arcuate and dorsomedial nuclei, lateral habenula, posterior lateral thalamus, zona incerta, and substantia innominata; in the mesencephalon, the raphe nuclei, ventral tegmental area, substantia nigra, interpeduncular nucleus, pretectal nuclei, and central gray; in the rhombencephalon, the posterodorsal tegmental nucleus, area postrema, raphe nuclei, and periolivary nuclei.

Besides the obvious quantitative differences in the numbers of GLP-1R mRNA-containing neurons in these areas, the present observations suggest that the efferent connections of the nucleus of the solitary tract are broader than we believed previously. More importantly, these in situ hybridization data suggest that the function of GLP-1 as a neurotransmitter in the CNS is not only for the regulation of ingestive behavior; it appears to be more widespread. Alternatively, it is possible that GLP-1, produced peripherally, crosses the blood-brain barrier and binds to receptors of neurons that do not receive afferents from the nucleus of the solitary tract or the dorsal and ventral medulla. Although intraperitoneally administered GLP-1 does not reduce ingestive behavior (Turton et al., 1996), a yet undiscovered transporter may deliver GLP-1 to receptor cells within the CNS.

The present studies demonstrated the distribution of GLP-1R mRNA-expressing cells in the CNS, the first step toward understanding the function of GLP-1. The widespread distribution of GLP-1R mRNA-containing cells strongly suggests that GLP-1 functions not only as a satiety factor but also as a neurotransmitter or neuromodulator in anatomically and functionally distinct areas of the CNS.

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