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Distribution and chemical coding pattern of the cocaine- and amphetamine-regulated transcript (CART) immunoreactivity in the preoptic area of the pig

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Abstract: This study provides a detailed description of cocaine-and amphetamine-regulated transcript (CART) distribution and the co-localization pattern of CART and gonadotropin releasing hormone (GnRH), somatostatin (SOM), neuropeptide Y (NPY), cholecystokinin (CCK), and substance P (SP) in the preoptic area (POA) of the domestic pig. The POA displays a low density of immunoreactive cells and rich immunoreactivity for CART in fibers. CART-immunoreactive (CART-IR) cell bodies were single and faintly stained, and located in the medial preoptic area (MPA) and the periventricular region of the POA. A high density of immunoreactive fibers was observed in the periventricular preoptic nucleus (PPN); a high to moderate density of fibers was observed in the MPA; but in the dorso-medial region of the MPA the highest density of fibers in the whole POA was observed. The lateral preoptic area (LPA) exhibited a less dense concentration of CART-immunoreactive fibers than the MPA. The median preoptic nucleus (MPN) showed moderate to low expression of staining fibers. In the present study, dual-labeling immunohistochemistry was used to show that CART-IR cell bodies do not contain any GnRH and SP. CART-positive fibers were identified in close apposition with GnRH neurons. This suggests that CART may influence GnRH secretion. Double staining revealed that CART-IR structures do not co-express any of the substances we studied, but a very small population of CART-IR fibers also contain SOM, CCK or SP. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 4, pp. 604–614*)

Key words: cocaine- and amphetamine-regulated transcript, gonadotropin releasing hormone, somatostatin, neuropeptide Y, cholecystokinin, substance P, preoptic area, pig

Introduction

Cocaine- and amphetamine-regulated transcript (CART) peptides are novel neuropeptides that are involved in multiple physiological functions, including feeding, endocrine regulation, and mediation of the stress response [1]. CART was originally described as mRNA. CART levels increase in the rat striatum following acute administration of psychomotor stimulants such as cocaine and amphetamine [2]. The amino acid sequence of CART mRNA corresponds to an unknown peptide, which was isolated from the ovine hypothalamus and first referred to as 'somatostatin-like' [3]. cDNA encoding has been cloned in some mammals, including rats [2], mice [4] and humans [5].

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CART peptides are abundantly expressed in the mesolimbic dopamine system in cases of drug abuse [6–8]. The presence of CART in the brain areas that are associated with reward and reinforcement may indicate the relevance of CART peptide to drug addiction.

CART-immunoreactivity is present in the hippocampal formation [9, 10]; in the amygdala [11]; and in the medulla, particularly in the nucleus of the solitary tract and area postrema [10], which constitute some of the most important cardiovascular centers of the brain. CART-immunoreactivity has also been reported in the pituitary gland, the autonomic ganglia, the spinal cord, and the myenteric plexus of the intestines [10, 12, 13].

Furthermore, CART mRNA and CART peptide--immunoreactivity have been found to be massively expressed within the hypothalamic regions involved in physiological control of feeding [10, 13–15]. Intracerebroventricular administration of CART peptide fragments causes dose-dependent inhibition of food intake [16] and prevents the orexigenic effects of neuropeptide Y [16, 17], which suggests that these peptides participate in the regulation of food intake and feeding behaviors. Central blockade of CART by administration of CART antiserum results in hyperphagia [17] and CART knockout animals have been found to increase in weight [18, 19]. Therefore, CART is considered to be an anorexigenic peptide. Although most studies have looked at CART action in the brain areas related to feeding behavior, a recent investigation has suggested that CART may play a role in the regulation of gonadotropin release [20], as well as in the mechanism involved in the initiation of maternal behavior [21].

Although the distribution of CART within the hypothalamic nuclei involved in feeding behavior is well documented, little is known about CART distribution in regions related to reproduction, such as the preoptic area. This area is reciprocally strictly connected with many hypothalamic nuclei [22–24], and it influences behavioral, neuroendocrine, and autonomic nervous system activity associated with reproduction, controlling male sexual behavior [25–27], regulating the cyclic release of pituitary gonadotropins [28] and helping regulate parental behavior [29]. There are only a few papers concerning CART peptide occurrence in the preoptic area [30–32] and the neuroanatomical distribution of CART and its chemical coding in the preoptic area has not been studied in detail.

Therefore, the aim of this work was to describe CART distribution and the co-localization pattern of CART with GnRH, SOM, CCK, NPY, and SP in the preoptic area of the domestic pig, which is a very important animal in biomedical studies [33].

Material and methods

Animals. This study used six sexually immature female pigs (approximately 8–12 weeks old), bred by a commercial fattening farm in Niedzwiedz, Poland. All experiments were carried out in accordance with the Local Ethical Committee's rules. All efforts were made to minimize animal suffering and to use the minimum number of animals necessary to produce reliable data.

Tissue fixation and preparation. The pigs were anesthetized by an intravenous injection of pentobarbital (Vetbutal, Biowet, Poland; 25 mg/kg b.w.). Thirty minutes before giving the anesthetic, the animals were pre-treated with propionylopromasine (Combelen, Bayer, Germany; 0.4 mg/kg b.w. i.m.). Then they were perfused transcardially with 1 l of pre-perfusion solution containing 0.9% sodium chloride (Chemia, Gliwice, Poland), 2.5% polyvinylpyrrolidone (Sigma, Deisenhofen, Germany), 0.5 procaine hydrochloride (Polfa, Warsaw, Poland) and 20,000 i.U. of heparin (Heparinum; Polfa, Warsaw, Poland; added *ex tempore*), followed by 4 l of 4% ice-cold buffered paraformaldehyde (pH = 7.4). It should be stressed that the animals in this study were not injected with colchicine.

Following perfusion, the forebrain was removed and postfixed by immersion in the same fixative, washed twice in 0.1 M phosphate buffer (pH = 7.4), and then stored in 30% sucrose until sectioning.

Immunofluorescence experiments. The frozen tissue blocks containing the preoptic area were cut into 20 μm-thick cryostat coronal sections. The tissue samples were processed for double-labeling immunofluorescence by means of primary antisera raised in different species.

Briefly, sections were air-dried for 45 min, washed three times in PBS prior to immunohistochemical staining and then processed for routine double-labeling immunofluorescence using a mouse polyclonal antibody against CART (1:2,000; code MAB 163, R&D Systems, USA), which was combined with a rat polyclonal antiserum against SOM (1:600; code 8330-0009, Biogenesis, UK) or rabbit polyclonal antisera against GnRH (1:4,000; code ab5617, Abcam, UK), NPY (1:4,000; code NA1233, Affinity, UK), CCK (1:6,000; code H-069-04, Phoenix, UK), or SP (1:4,000, code 21080751, Biogenesis, UK). In order to show the binding sites of the antigens and the antisera, sections were then incubated (1 h, at room temperature) with a mixture of FITC-conjugated donkey anti-mouse (1:600, code 715-095--150, Jackson ImmunoLabs, USA) combined with either Cy3-conjugated donkey anti-rat (1:6,000; code 712-165-153, Jackson ImmunoLabs, USA) or Cy3-conjugated donkey anti-rabbit (1:6,000; code 711-165-152, Jackson ImmunoLabs, USA). All antibodies were diluted in PBS containing Triton X-100 (0.3–0.5%) and 1% normal donkey serum. The sections were then washed three times in 0.1 M PBS and were

coverslipped in buffered carboxyglycerol (pH = 7.8) and analyzed under an Olympus B X51 microscope equipped with a CCD camera connected to a PC. Images were acquired with AnalySIS software (ver. 3.02; Soft Images Systems GmbH, Germany).

Controls. Standard controls, i.e. the omission as well as replacement of all primary antisera by non-immune sera as well as preabsorption for the neuropeptide antisera, were applied to test both antibody- and method-specificity. For the preabsorption control, each peptide was diluted in the working dilution (see above) of appropriate antibody to obtain the following concentrations of the peptides 0.1 μM for CART (code C5977, Sigma, USA); 0.6 μM for SOM (code S9129, Sigma USA); 0.8 μM for GnRH (code H-6728, Bachem, Switzerland); 0.2 μM for NPY (code PEP-87135, Dianova, Germany); 0.9 μM for CCK (code PEP-87035, Dianova, Germany) and 0.7 μM for SP (code S6883, Sigma, USA).

Results

The preoptic area of the pig is located in the transition zone between the telencephalon and diencephalon. It is situated in front of the hypothalamus, bilaterally to the third ventricle and extends to the level of the paraventricular nucleus of the hypothalamus. It consists of four parts: the medial preoptic area (MPA), lateral preoptic area (LPA), median preoptic nucleus (MPN) and periventricular preoptic nucleus (PPN). The location of the various parts in the pig POA is shown in Figure 1.

The MPA is found rostral to the decussation of the optic nerves and comprises the most rostral part of the POA. The lateral preoptic area is slightly posterior to this. The LPA and MPA have a similar cell structure, constitute extensive cell groups and occupy the majority of the POA, ending at the level of the anterior sector of the supraoptic nucleus. The PPN appears just behind the anterior pole of the LPA, is situated laterally to the third ventricle, and extends to the supraptic nucleus. The MPN, just behind the PPN, is an unpaired band of tightly packed cells located in the midline of the POA. It forms the posterior pole of the POA and ends at the anterior level of the periventricular nucleus of the hypothalamus.

In this study, all four parts of the POA were studied to determine the distribution of CART-IR as well as its co-expression with GnRH, SOM, CCK, NPY and SP.

The distribution pattern and morphology of CART-, GnRH-, SOM-, NPY-, CCK-, and SP-IR structures in the pig preoptic area

All parts of the POA contained CART-immunoreactive fibers and a large number of immunoreactive grains and tiny fiber terminals. The POA exhibited

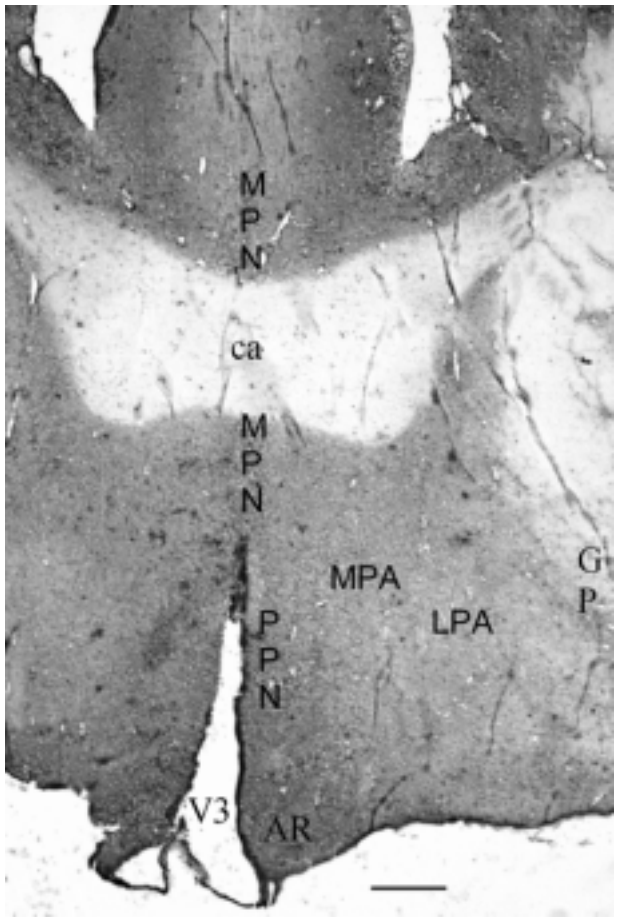


Figure 1. Microphotograph of Nissl-stained section through the middle portion of the preoptic area in the pig. AR — arcuate nucleus; GP — globus pallidus; MPA — medial preoptic area; MPN — median preoptic nucleus; LPA — lateral preoptic area; PPN — periventricular preoptic nucleus; V3 — ventriculus tertius; ca — commisura anterior. Scale bar: 1,000 μm

a low density of immunoreactive cells and only single, faintly stained CART-positive cell bodies were located in the MPA and the periventricular region of the POA (Figures 2A–D; 3A, C, E; 4A–7A; 8A–10A). A high density of staining fibers was found in the periventricular region, high to moderate expression was seen in the medial preoptic area, and the strongest CART staining was observed in the dorso-medial region of the MPA. CART-immunoreactive fibers were less dense in the LPA than in the MPA. Relatively fewer positively stained fibers occupied the anterior part of the MPA and the LPA in comparison with their middle and posterior sections. Fibers were expressed in moderate to low amounts in the median preoptic nucleus. Two types of CART-immunoreactive fibers were observed: thick fibers of varying length, with large varicosities and thin fibers, usually short (although sometimes longer fibers were present) with tiny varicosities. Both fiber types displayed im-

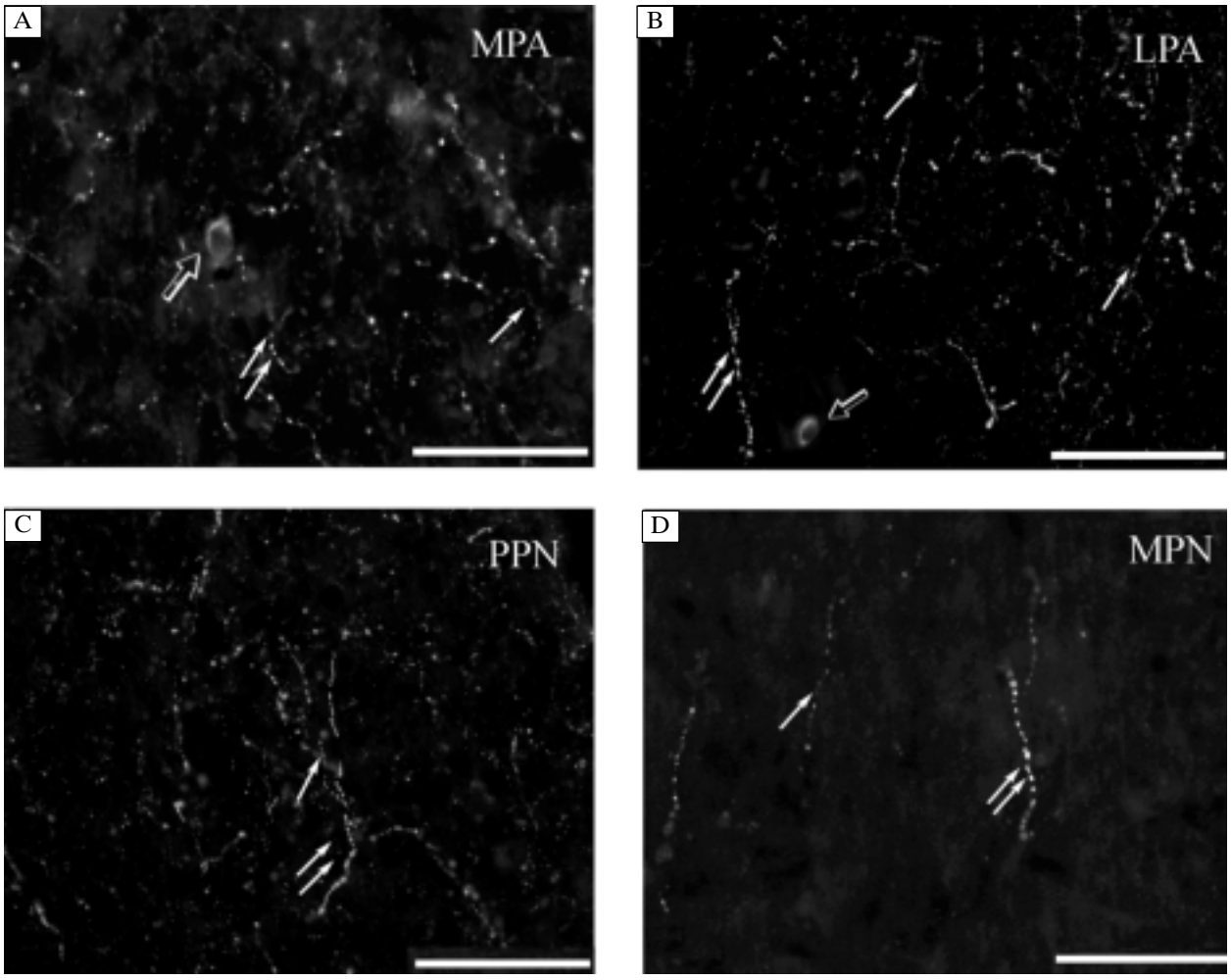


Figure 2. CART-immunoreactivity in the preoptic area of the pig. CART-IR in the medial preoptic area (**A**), lateral preoptic area (**B**), periventricular preoptic nucleus (**C**) and median preoptic nucleus (**D**). Hollow arrows indicate CART-IR neurons, double arrows indicate examples of thick fibers, and single arrows indicate examples of thin fibers. Scale bars: **A**, **D** —50 μm; **B**, **C** — 100 μm

munoreactivity in all of the parts of the POA; however, the thick fibers predominated in the MPA and the periventricular region. Some of the thick fibers, especially the long ones, ran in a dorsomedial direction in the periventricular region, the median preoptic nucleus and the medial part of MPA. The remaining fibers ran in every direction throughout the whole POA. Some fibers were arranged in basket-like structures that surrounded unstained cell bodies.

GnRH-IR structures were present in the whole preoptic area (Figures 3B, D, F). The highest number of GnRH-positive neurons was present in the PPN and the MNP. Fewer of them were observed in the medial MPA, while in the lateral part of the MPA and the LPA only single cell bodies showed GnRH-immunoreactivity. Cell bodies were bipolar, unipolar or multipolar, and had smooth contours. Immunoreactivity was usually confined to the neuroplasm of the cell bodies as well as to their extending processes, although sometimes it was observed in the primary ramifications of the dendritic tree. Fibers that were strongly stained for GnRH were abundantly expressed in the MPN and the PPN. These fibers were usually long, thick and varicose; most of them were oriented dorso-ventrally. In the medial and lateral preoptic area, some positive fibers were present. They were thinner and usually much shorter than those of PPN and MPN, although some long fibers were also visible. The fibers were beaded and ran in all directions.

SOM-IR was present to a different extent in every part of the POA (Figures 5B; 8B). The highest density of immunopositive fibers and puncta was seen in the MPN. Moderate to high expression of fibers was observed in the PPN and in the medial part of the MPA. In the lateral part of the MPA, SOM-IR fibers were observed less frequently and staining ranged from moderate to low, whereas in the LPA the smallest number of SOM-positive fibers was ob-

served. In every part of the POA, the SOM-IR fibers were varicose and of different lengths; longer ones predominated in the PPN and MPN. Moderate immunoreactivity of puncta in the neuropil was visible in the PPN, MPA and LPA.

NPY-IR was found throughout the whole POA (Figure 4B). The highest number of strongly NPY-IR nerve fibers was located in the dorsal MPA and PPN. Fewer NPY-positive fibers were found in the medial MPA and MPN, whereas the LPA contained a moderate number of immunostaining fibers. NPY-containing fibers were varicose, oriented in all directions and ranged from long to short. Dense to moderate plexuses of NPY-IR terminals were seen in all parts of the POA.

CCK shows lower immunoreactivity in the POA than NPY, SOM or CART (Figures 6B; 9B). A moderate number of CCK-IR fibers as well as immunoreactive puncta were present in the MPA and LPA. In the PPN and MPN, low to moderate staining for CCK was found. The fibers containing CCK were usually thinner than those described above. They were short and had tiny swellings. Some fibers, especially in the MPA, were longer, thicker and possessed varicosities.

SP in the POA displays low to moderate immunoreactivity (Figures 7B; 10B), similar to the CCK-IR. Immunostaining was observed in single, dispersed somata, mostly in the MPA. SP-IR boutons were diffusely arranged in all parts of the POA. Fibers stained for SP were finely structured and varicose. In general, these fibers were relatively short, although thicker and longer fibers were irregularly distributed among them. The highest number of SP-immunoreactive fibers was seen in the dorsomedial part of the MPA. The morphology of SP-IR fibers was similar to the fibers that contain CCK.

Co-localization pattern of CART and GnRH, SOM, NPY, CCK, or SP in the preoptic area of the pig

Double-labeling staining showed that CART-IR cell bodies contained neither GnRH nor SP. There was also no co-expression of CART-IR fibers with GnRH. However, CART-IR fibers were observed in apposition to GnRH-IR neurons (Figures 3A–F). Taking into account this apposition, three types of GnRH-IR neurons were distinguished. The first and most numerous type consisted of GnRH-IR neurons in close apposition with CART-IR structures (Figures 3A–D). These structures were either boutons which terminated on GnRH-positive cell bodies and their dendrites, or fibers which contacted GnRH-IR neurons. The second type of GnRH-IR neurons occurred in the vicinity of CART-IR fibers without any visible contact between them. The third and least numerous type had no CART-positive fibers nearby (Figures 3E, F). The close apposition of CART-IR fibers and GnRH--IR cell bodies was observed more often in the PPN and MPN, whereas the GnRH-positive neurons without CART-positive fibers in their vicinity occurred mostly in the MPA and LPA. The GnRH-positive neurons of the second group were seen in all regions of the preoptic area.

Double staining for CART and GnRH or CART and NPY revealed that although CART-IR fibers overlap with GnRH- and NPY-IR fibers, they were never observed in the same fiber, so they showed no co-expression (Figures 3A–F; 4A, B). The majority of CART-IR fibers did not contain any of following neuropeptides: CCK, SOM and SP (Figures 5–7). However, double-labeled CART/SOM (Figures 8A, B), CART/CCK (Figures 9A, B) and CART/SP (Figures 10A, B) fibers were occasionally observed. CART-IR fibers co-expressed with CCK, SOM or SP in the medial preoptic area and the periventricular region. These coexpressions were irregularly dispersed in these parts of the preoptic area and observed in every section that was studied.

Discussion

This is the first study to describe the distribution of CART-IR in pigs. It shows that CART-IR is low in neurons and high to very high in fibers in the porcine preoptic area.

A dense network of CART-IR fibers was seen throughout the periventricular region, high to moderate staining was seen in the medial preoptic area, with the strongest staining seen in the dorso-medial region of the medial preoptic area. A lower expression of CART characterizes the porcine LPA and the MPN. Generally, porcine CART distribution is similar to the distribution described in previous studies in rats [20], humans [32] and lower vertebrates [30–31]. It is very difficult to precisely compare the distribution of CART in the porcine POA with the distribution in other species because there are only a few papers that deal with this issue and the division of the preoptic area differs in each species. Koutcherov et al. [32] described CART-IR only in the human medial preoptic nucleus (MPO), which seems to correspond to a part of the medial preoptic area in the pig. Immunoreactivity for CART was prominent in the human medial preoptic nucleus, as in the porcine medial preoptic area. The dorso-medial region of the porcine medial preoptic area corresponds to the medial subnucleus of the human medial preoptic nucleus and both regions displayed the strongest CART staining, with many densely arranged immunoreactive fibers and terminals [present study and 32]. The

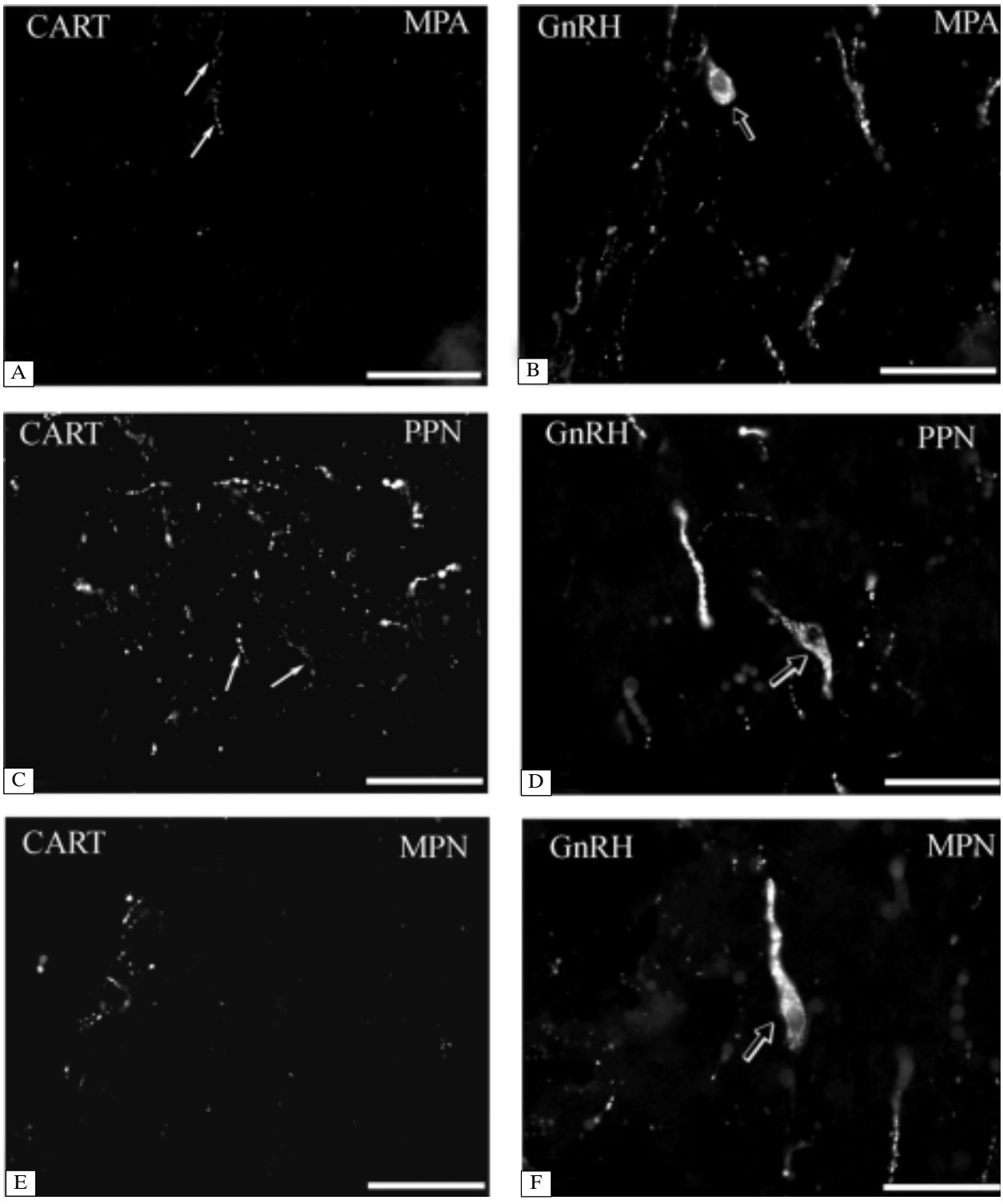
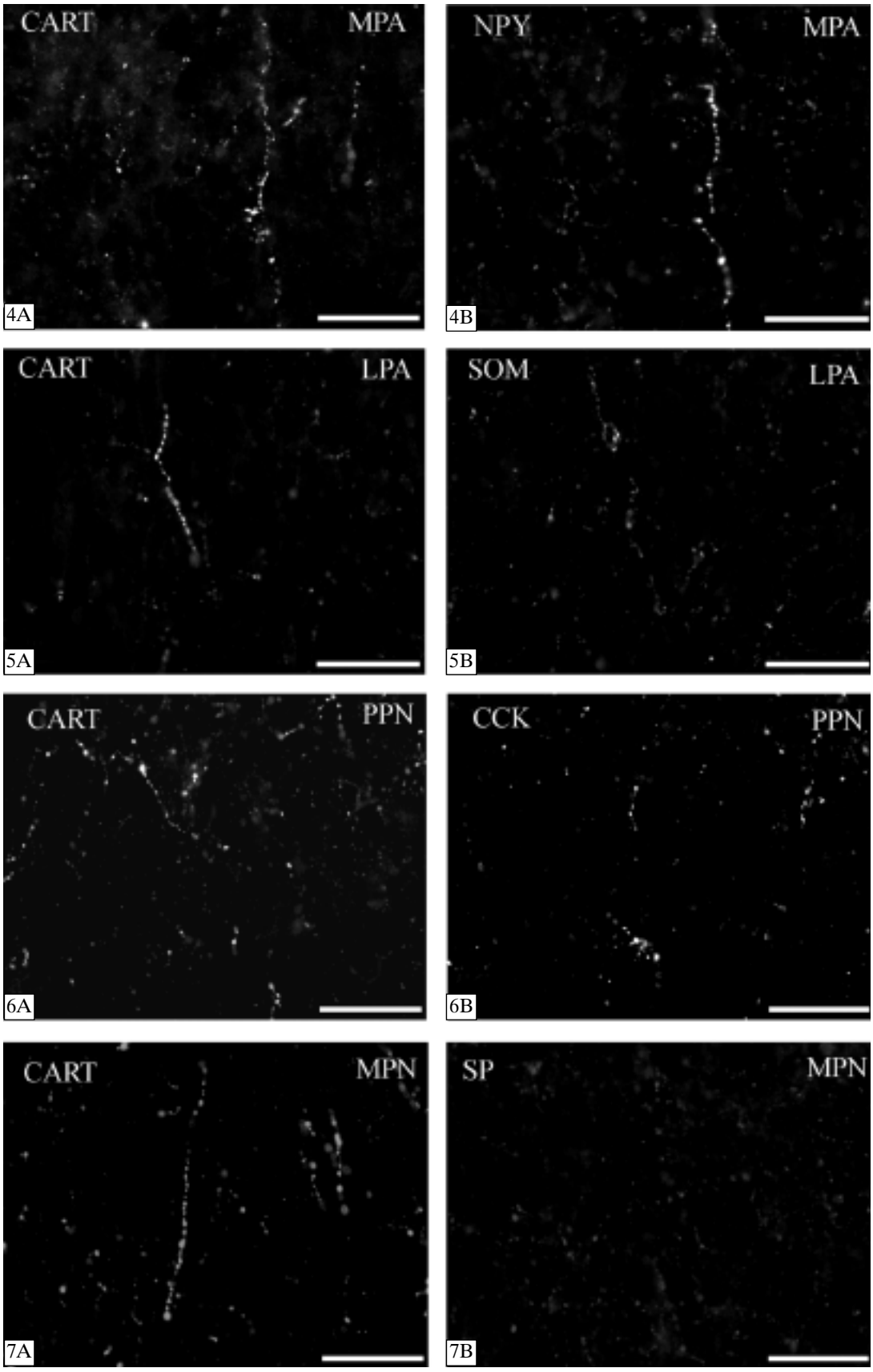


Figure 3. Examples of appositions of CART-IR fibers or boutons on GnRH-IR perikarya (hollow arrows) in the porcine preoptic area, **A–D**, close appositions of CART-IR processes (arrows) on GnRH-IR perikarya, **E**, **F**, no CART positive fibers in the neighborhood of GnRH-IR neurons. Scale bars: **A**, **C–F** — 50 μm; **B** — 100 μm

preoptic periventricular nucleus in the porcine preoptic area, which is located in the area occupied by the uncinate nucleus in humans [32], showed strong CART-immunoreactivity, with many densely packed positive fibers and terminals as well as some sparsely distributed CART-positive cells. This is similar to CART-IR fibers and cells which characterize the periventricular preoptic nucleus of the pig. In the rat, the medial preoptic nucleus and the anteroventral periventricular preoptic nucleus displayed dense



Figures 4–7. A double-labeled structures of CART and NPY (**4A**, **B**), CART and SOM (**5A**, **B**), CART and CCK (**6A**, **B**), CART and SP (**7A**, **B**) in the porcine preoptic area. Note a lack of co-localization of CART and NPY, SOM, CCK and SP. Scale bars: 50 μm

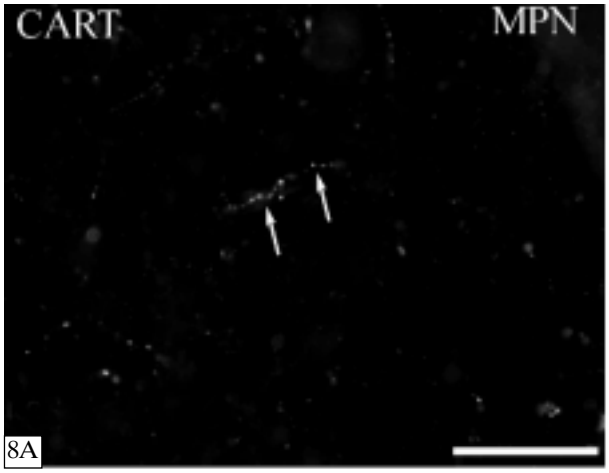
CART-immunoreactivity whereas the median preoptic nucleus showed moderate immunoreactivity [20]. Taking into account that the medial preoptic nucleus and anteroventral periventricular preoptic nucleus of the rat correspond to the medial preoptic area and periventricular preoptic nucleus of the pig, respectively, CART-immunoreactivity is very similar in the two species [20, present results]. In lower vertebrates, the general CART-immunoreactivity of the preoptic area is also similar to that described above. For example, in the frog, a large number of immunoreactive grains and fine varicose fiber terminals occupied the whole preoptic area and only a few CART-IR cells were observed [31]. In the catfish, most CART fibers were located in the lateral portion of the preoptic area with some CART-IR neurons in the preoptic periventricular nucleus [30].

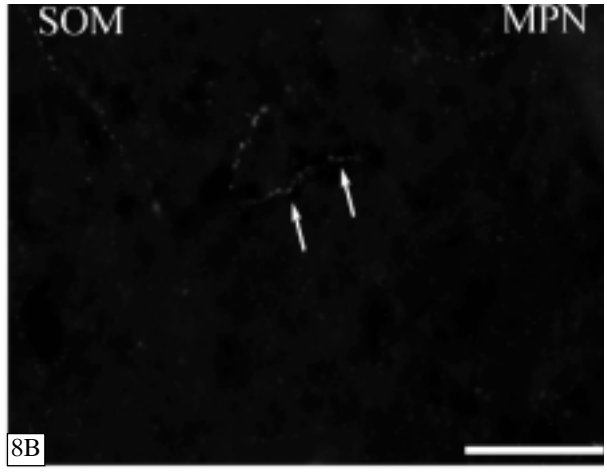
The low CART-immunoreactivity of the neurons in the preoptic area of the pig and in other animals that have been studied [31, 32], and the very rich CART innervation of the region may suggest that most CART-IR fibers are extrinsic in nature. It should also be noted that the animals in our studies were not injected with colchicine. According to Vrang et al. [34], the antibody used to detect cells that contained CART peptide did stain neuronal-like cells in rats that were not treated with colchicine, but cellular staining was greatly facilitated by colchicine. In the lateral hypothalamic area of the mouse, cell bodies that contained CART appeared to be similar both in controls that were injected with colchicine and controls that were injected with monosodium glutamate [35]. However, when these controls were compared, cell bodies that contained CART appeared to be more numerous, more intensely stained, and extend more branched dendritic arborizations in the mice that were injected with monosodium glutamate [35]. When frogs that had been treated with colchicine were compared to those that were left untreated, a similar pattern of CART staining was seen in both groups [31].

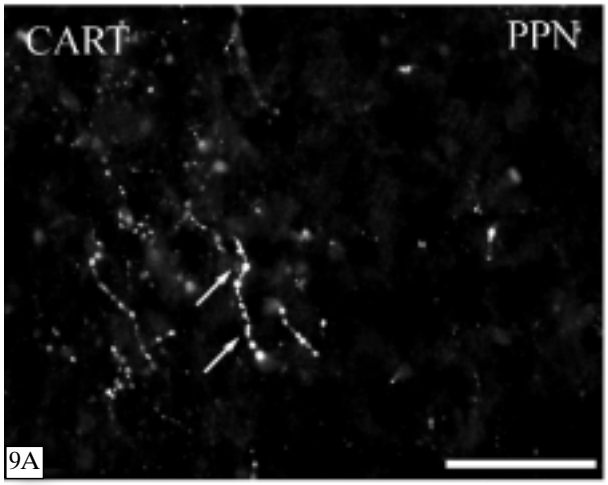
The present results show that although the CART-immunoreactive structures overlap with structures that contain GnRH, SOM, NPY, CCK, and SP, they generally do not co-express with any of them in the porcine preoptic area. However, there is a very small population of CART-immunoreactive fibers that do contain SOM, CCK or SP in this area. CART and NPY are both major regulatory peptides involved in feeding behavior and other neurobiologic effects [16, 21, 36]. They are both found in areas associated with feeding in mammals, but they act inversely to each other [37, 38]. Fibers that contain CART and fibers that contain NPY are present in the porcine preoptic area, in the porcine amygdala and in the infundibular nucleus of the rat, but these substances do not co-express within the same fibers [11, 34]. However, these peptides were recently observed to co-localize in the infundibular nucleus in the human hypothalamus [39], and in the preoptic periventricular nucleus of the catfish [30]. CART and NPY innervation in the preoptic area of the pig, especially in the medial preoptic area, is very similar to the relationship described in the medial preoptic nucleus in humans [32] and in some hypothalamic nuclei in mice [35]. The innervations that project to the preoptic area and contain CART and NPY-positive neurons are most likely to originate from the hypothalamus and the telencephalic areas. They include the nucleus accumbens, the striatopallidal transition area, the striatum, the lateral septum, and the medial pallium [10, 15, 40–42]. In addition, afferents that contain CART derive from the arcuate nucleus and reach the preoptic area. They help integrate feeding and energy-balancing circuitry with neural relays that control reproductive behavior [20].

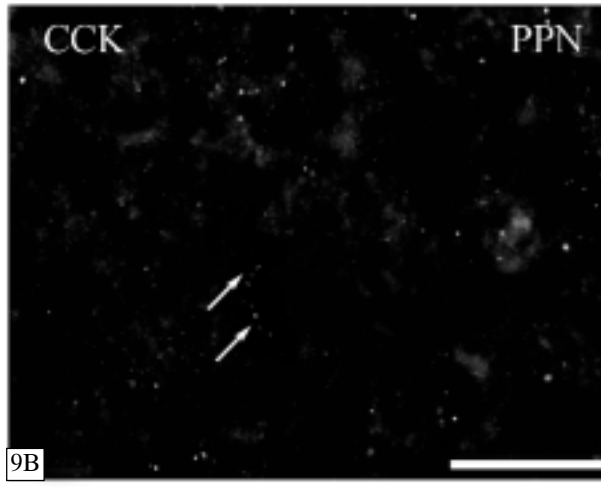
So far, data on the chemical nature of CART-IR neurons and fibers in the preoptic area has not been available in the scientific literature. However, there is some evidence that CART peptides may colocalize with other neuropeptides and classical neurotransmitters in certain other neuronal populations. CART-immunoreactive structures have been found to colocalize with somatostatin, oxytocin, vasopressin, galanin, pro-opiomelanocortin, melanin-concentrating hormone or thyrotropin-releasing hormone in hypothalamic nuclei [34, 35]; and with GABA and dynorphin in nerve terminals in the ventral tegmental area and the substantia nigra [43].

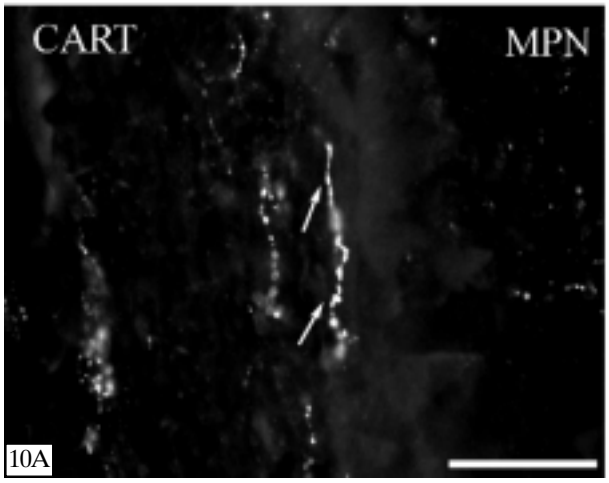
The preoptic area is thought to play a role in the regulation of reproduction. This is because it contains neurons that release gonadotropin, innervate the pituitary gland, and influence the secretion of lutenizing hormone [44]. Neurons in the preoptic region also serve as binding sites for sex steroids [45]. Since CART is expressed in the preoptic area, it may help process information related to reproduction. CART-immunoreactive terminals make close contact with GnRH neurons that express Fos-immunoreactivity on the afternoon of the proestrous day in rats [20]. In this study, we have shown that CART-immunoreactive fibers and boutons terminate on GnRH-positive neurons, especially in the PPN and the MPN. A close association of CART terminals with GnRH neurons in the preoptic area has also been reported in female hamsters [46] and in male and female rats [20]. Fibers that express CART and make visible synaptic contact with GnRH-immunoreactive neurons in the preoptic area derive from neurons that express CART in the infundibular and ventral premammillary nu-

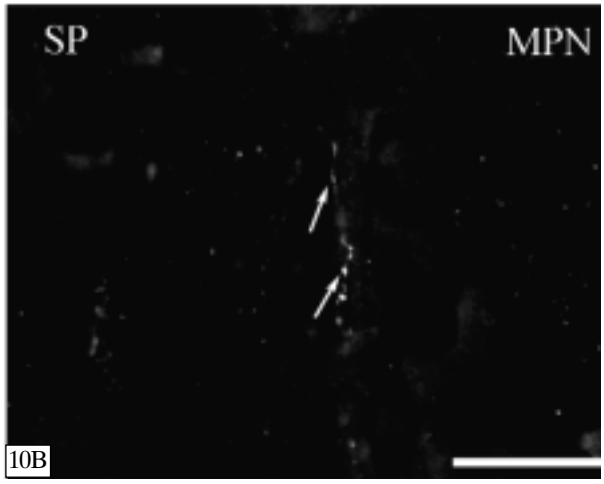












Figures 8–10. Examples of the co-expression of CART and SOM, (**8A**, **B**), CART and CCK (**9A**, **B**) as well as CART and SP (**10A**, **B**) within the porcine preoptic area. Note that single CART-IR nerve fibers (arrows) contain simultaneously SOM, CCK, or SP (arrows). Scale bars: 50 μm

clei [20]. These CART-expressing neurons in the infundibular and ventral premammillary nuclei are regulated by leptin [47]. Leptin helps control food intake and reproductive processes and may be a candidate for an endocrine link between nutrition and reproduction [48]. Thus, CART, which signals GnRH secretion, may convey metabolic information to the reproductive axis [46]. Other researchers have sug-

gested that CART and leptin may reduce GnRH interpulse intervals in prepubertal rats and may increase GnRH amplitude in normally cycling rats [49]. It has also been suggested that CART may mediate leptin's action on neurons that secrete GnRH [50]. CART may also modulate GnRH secretion and the resulting lutenizing hormone surge through projections to a subset of GnRH neurons that are 'active' during the proestrous period [20].

In summary, this study has shown that nearly all CART-immunoreactive structures in the porcine preoptic area do not cotransmit with GnRH, SOM, NPY, CCK and SP, and that CART-immunoreactive fibers in this area are in close apposition with GnRH neurons. However, a very small population of CART-immunoreactive fibers also contain SOM, CCK or SP. CART is considered a modulatory hypophysiotrophic transmitter.

CART is thought to be a neurohypophysial modulatory peptide, because it spills over into the portal circulation [51]. The isolation and identification of CART receptors, and further studies of CART's physiological effects, may help to a better understanding of its functional significance.

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