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Progesterone Receptor Immunoreactivity in Aromatic L-Amino Acid Decarboxylase-Containing Neurons of the Guinea Pig Hypothalamus and Preoptic Area

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ABSTRACT

A double-labeling immunofluorescence procedure was used to determine whether progesterone receptor (PR)-immunoreactive (IR) neurons in the preoptic area and hypothalamus of female guinea pigs also contained aromatic L-amino acid decarboxylase (AADC), an enzyme involved in the synthesis of both catecholamines and serotonin. Immunostaining was performed on cryostat sections prepared from ovariectomized guinea pigs primed by estradiol to induce PR. The nuclear presence of PR was visualized by a red fluorescence while the AADC-containing perikarya showed a yellow-green fluorescence. The topographic distribution of AADC-IR neurons was investigated by using a specific antiserum obtained by immunization of rabbits with a recombinant protein β-galactosidase-AADC in the two regions known to contain the densest populations of estradiol-induced PR-IR cells: the preoptic area and the mediobasal hypothalamus. The localization of PR-IR and AADC-IR cell populations showed considerable overlap in these areas, mainly in the medial and periventricular preoptic nuclei and in the arcuate nucleus. A quantitative analysis of double-labeled cells estimated that about 15% to 23% of AADC-IR cells in the preoptic area and about 11% to 21% of AADC-IR cells in the arcuate nucleus possessed PR. This colocalization persisted throughout the rostrocaudal extent of these areas and represented 3% to 9% of the population of PR-IR cells.

These findings provide neuroanatomical evidence that a subset of AADC neurons is directly regulated by progesterone. The exact physiological role of this enzyme in target cells for progesterone is not understood. AADC may be involved in functions other than that for the synthesis of the classical neurotransmitters.

Indexing terms: arcuate nucleus, diencephalon, immunohistochemistry, biogenic amines, trace amines

Progesterone, acting at the level of the hypothalamus, plays an important role in the neuroendocrine regulation of a variety of neuronal systems. Many of these actions are believed to be mediated by the interaction of progesterone with neural intracellular progesterone receptors (PR).

Immunohistochemical studies using a monoclonal antibody to PR have shown that the immunoreactive (IR) cells are mainly located in the preoptic area and the mediobasal hypothalamus (Warembourg et al., 1986; Blaustein et al., 1988; Don Carlos et al., 1989). Nevertheless, in these regions that control gonadotropin secretion and reproductive behavior, we have only a little knowledge of which molecular components of specific neural circuits are regulated by progesterone.

In an attempt to identify the neurochemicals synthesized by PR-containing neurons, we have elected to focus on the enzyme, aromatic L-amino acid decarboxylase (AADC; EC 4 1.1.28). This enzyme is involved in biosynthesis of the catecholamine (dopamine, norepinephrine, epinephrine) and indolamine (serotonin) neurotransmitters. AADC catalyzes the decarboxylation of L-3,4 dihydroxyphenylalanine (L-DOPA) to dopamine (DA) and of 5-hydroxytryptophan (5HTP) to 5-hydroxytryptamine (5HT, serotonin). It may also catalyze directly the conversion of the

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aromatic amino acids, tyrosine, tryptophan or phenylalanine to their respective amines tyramine, tryptamine or phenylethylamine (Jaeger et al., 1984a). Immunocytochemical studies using antiserum anti-AADC have revealed AADC-IR neurons in the brain of rat (Jaeger et al., 1984a,b; Jaeger, 1986) cat (Kitahama et al., 1988) and goldfish (Beltramo et al., 1994). AADC is contained not only in well-known catecholaminergic and indoleaminergic systems but also in many other nonmonoaminergic neurons. These neurons, named D cells (Jaeger et al., 1984a,b) and containing AADC but lacking either tyrosine hydroxylase (TH) or 5HT, were found in the spinal cord and the hypothalamus (Jaeger et al., 1983a,b; Nagatsu et al., 1988; Karasawa et al., 1991; Tillet et al., 1994) and might belong to the amine precursor uptake and decarboxylation (APUD) system (Karasawa et al., 1994). The aim of the studies reported here was to determine whether there is a relationship between the AADC system and the cells that contain PR.

As gonadotropin releasing hormone (GnRH) neurons do not appear to have receptors for steroids (Shivers et al., 1983; Herbison and Theodosis, 1992; Leranth et al., 1992), other neurotransmitter systems must be involved in the gonadal steroid regulation of anterior pituitary luteinizing hormone (LH) secretion. It is known that DA has an effect on gonadotropin secretion (Fuxe et al., 1978) and that the activity of TH, the rate-limiting enzyme in catecholamine biosynthesis, is regulated by estrogen (Luine et al., 1977; Jones and Naftolin, 1989; Simerly, 1989). TH mRNA levels have been shown to be affected by ovarian steroid hormones in tuberoinfundibular dopaminergic neurons (Morrell et al., 1989; Pasqualini et al., 1991; Arbogast and Voogt, 1993, 1994). Some of these neurons contain estrogen and progesterone receptors but no colocalization has been found in the TH-IR preoptic neurons in the rat (Sar, 1984, 1988), in the guinea pig (Blaustein and Turcotte, 1989; Warembourg et al., 1993), in the sheep (Batailler et al., 1992; Lehman and Karsch, 1993) or in the monkey (Kohama et al., 1992; Horvath et al., 1993). At present, little is known about the monoamine transmitters used by preoptic neurons containing PR.

Therefore, in the present account, immunocytochemistry was used in order to examine the distribution of AADC-IR neurons within these regions of the guinea pig preoptic area and hypothalamus that possess PR, and to establish whether PR and AADC immunoreactivities coexist in the same neurons.

MATERIALS AND METHODS

Animals and treatment

A total of 8 adult female Hartley guinea pigs, weighing 450 to 550 g, were used in this study. Of these, 3 animals were used to develop the optimal immunocytochemical protocol (to test fixatives, postfixation times, antibody dilutions and specificity); the other 5 animals were used for data analysis. The guinea pigs, obtained from Bertho (Yffiniac, France), were group-housed on a 14:10 light/dark cycle. Food and water were available ad libitum. All animals were ovariectomized through bilateral dorsal incisions under a combination of ketamine (8 mg/Kg) and xylazine (40 mg/Kg) anesthesic which was given intramuscularly. Animals received subcutaneous injections of 10 μg of estradiol benzoate dissolved in 0.20 ml of sesame oil daily for 5 days prior to perfusion.

Tissue preparation

At least 10 days following ovariectomy, animals were deeply anesthetized with ketamine plus xylazine and transcardially perfused by using an intraventricular catheter attached to a peristaltic pump. Animals were perfused with 100 ml of heparinized 0.9% saline followed by 500 ml of cold 4% paraformaldehyde and 15% satured picric acid in a 0.1 M sodium phosphate buffer, pH 7.4. Following perfusion, each brain was then dissected; the cerebellum, the telencephalon and the brainstem removed and the diencephalon postfixed in the same solution for 4–6 hours at 4°C. The tissues were then soaked in 15% buffered sucrose for at least overnight at 4°C, subsequently frozen by immersion in liquid nitrogen-cooled isopentane and stored at −80°C until sectioning. Frozen sections (15 μm) were cut in a coronal plane through the preoptic region and hypothalamus with a cryostat at −20°C and mounted on gelatin-coated slides. Sections were washed in 0.01 M sodium phosphate-buffered saline (PBS) pH 7.4 and processed for immunostaining or stored at −80°C until further processing.

Primary antisera

The sections were incubated with a mixture of two primary antisera. The antibody used to detect progesterone receptors was let 64, a monoclonal antibody raised in mouse against rabbit uterine receptors. A working dilution of 2 μg/ml was used. The characteristics and specificity of this antiserum have been previously published (Logeat et al., 1983, 1985; Loosfelt et al., 1984; Lorenzo et al., 1988). The specificity of immunoreaction was checked by comparing sections stained with anti-receptor antiserum and control antibody (mouse receptor-unrelated monoclonal antibody) used at the same concentration, respectively. Sections were also incubated with monoclonal anti-progesterone receptor antibody which had been preabsorbed before the immunostaining procedure with highly purified progesterone receptor, as previously described (Warembourg et al., 1986).

AADC antibody was raised in rabbit against a fusion protein AADC-β galactosidase produced in *E. coli* and previously characterized (Krieger et al., 1993). AADC antibody was diluted 1/2,000. The procedure to test the antibody specificity has been previously discussed (Krieger et al., 1993; Beltramo et al., 1993) and consisted of performing the immunohistochemistry with a primary serum preabsorbed either with the fusion protein (AADC-β-galactosidase) or, alternatively, with the β-galactosidase alone.

Immunocytochemical processing

Immunocytochemistry was performed following the procedure previously reported by us (Warembourg and Jolivet, 1994). All antibodies were diluted in PBS containing 0.3% Triton X-100. PBS was also used to wash sections for 20 minutes between all steps of immunocytochemical procedures (minimum three changes). To diminish nonspecific staining the sections were incubated at room temperature for 30 minutes in a mixture containing 10% normal sheep serum and 10% normal donkey serum. Sections were incubated for 70 hours at 4°C in a humid atmosphere in a cocktail of primary antibodies described in the previous paragraph. AADC immunoreactivity was revealed by incubating the sections in fluorescein-conjugated donkey anti-rabbit immunoglobulins (dilution 1:50; Amersham, les Ulis, France) for 1 hour 30 minutes at 4°C. PR immunoreactivity was revealed by incubating the sections in biotinyl-

TABLE 1. Estimation of the Number of PR-IR, AADC-IR and Double-Immunoreactive (PR-IR + AADC-IR) Neurons in Different Areas of the Preoptic Area and Hypothalamus[1]

Areas[2]	Number of PR-IR neurons	Number of AADC-IR neurons	Number of PR-IR + AADC-IR neurons	% of AADC-IR neurons with PR	% of PR-IR neurons with AADC
Preoptic area					
Medial preoptic nucleus					
rostral level (Fig. 1a,d)	1,225 ± 183	305 ± 68	48 ± 12	15.4 ± 0.9	3.7 ± 0.5
medial level (Fig. 1b,e)	1,683 ± 161	483 ± 91	77 ± 10	16.8 ± 1.5	4.5 ± 0.2
caudal level (Fig. 1c,f)	1,538 ± 183	382 ± 39	81 ± 6	21.6 ± 1.4	5.4 ± 0.5
Hypothalamus					
Periventricular nucleus (Fig. 2a,c)	389 ± 34	239 ± 30	20 ± 3	8.9 ± 1.0	5.5 ± 1.0
Arcuate nucleus					
rostral level (Fig. 2b,d)	884 ± 70	540 ± 96	60 ± 4	12.3 ± 1.7	7.0 ± 0.5
medial level (Fig. 3a,d)	1,971 ± 136	875 ± 61	136 ± 8	15.6 ± 0.5	7.0 ± 0.6
caudal level (Fig. 3b,e)	1,908 ± 104	652 ± 13	102 ± 5	15.6 ± 0.7	5.3 ± 0.2
premamillary region (Fig. 3c,f)	1,071 ± 66	442 ± 21	89 ± 7	20.1 ± 1.0	8.3 ± 0.6

[1]Values represent mean numbers (±S.E.M.) of cells counted in 5 to 6 sections of each area (n = 5).
[2]The areas depicted are shown in Figures 1–3.

ated sheep anti-mouse immunoglobulins (dilution 1:200; Amersham) for 1 hour 30 minutes at 4°C then in Texas red streptavidin (dilution 1:200; Amersham) for 1 hour at 4°C. Next, the sections were coverslipped with glycerine PBS (3:1 v/v).

For control purposes, neither nuclear staining for PR nor AADC immunoreactivity was obtained when: (a) primary antibodies were omitted; (b) primary antibodies were replaced by normal rabbit or mouse serum; and (c) a secondary antiserum was used after the primary antiserum raised in an inappropriate species.

Analysis of results

Labeled sections were examined with a Leitz Orthoplan fluorescent microscope equipped with appropriate Pleomopak filters. Fluorescein-labeled neurons were observed under I_2 filter (excitation filter 450–490 nm) and Texas red-labeled neurons were examined with N_2 filter (excitation filter: 530–560 nm). Identification of double-labeled cells was made by switching from one filter block to the other during the observation. The distribution of immunoreactive structures is shown on schematic drawings of frontal sections through the preoptic area and the mediobasal hypothalamus and labeled according to the anatomical nomenclature from the guinea pig atlas of Bleier (1983). To provide an estimate of the double-immunoreactive cell population (Table I), we counted all PR-IR, AADC-IR, and AADC-IR + PR-IR cells within the boundaries of regions as delineated by PR immunoreactivity using the terminology of the atlas (Bleier, 1983). The immunoreactive cells were counted in eight selected areas, shown in Figures 1–3, in five animals. Cell counting was carried out on 5 to 6 sections per area. The total number of PR-IR, AADC-IR, and AADC-IR + PR-IR cells was calculated for each animal. From these raw data, we then calculated the percentage of the total AADC-IR cells that contained PR and the percentage of the total PR-IR cells that contained AADC. Statistical comparison was carried out with analysis of variance and Student's t test.

RESULTS

Our immunocytochemical technique made it is possible to distinguish three populations of labeled cells: single PR-IR cells, single AADC-IR cells and doubly immunoreactive cells. The PR-IR cells displayed a red fluorescence from Texas red and the AADC-IR cells and fibers showed a yellow-green fluorescence from fluorescein. Figures 1–3 are schematic drawings from sections including pronounced immunoreactivity and characteristic distribution patterns of PR-IR and AADC-IR neurons from one representative brain. Dots illustrate the distribution of immunoreactive perikarya but fibers are not depicted. The description is made from the rostral to the caudal level. Figure 4 demonstrates the appearance of PR and AADC immunoreactivities within cells in different areas.

It was often observed that, whereas PR immunoreactivity was mainly confined to the cell nucleus and absent from the nucleolus, AADC immunoreactivity had often a more diffuse distribution within the cell, without exhibiting a distinct stained cytoplasm (Figs. 5–7). With the filter combination used, there was no interference between the fluorescence of PR-IR cells when illuminated through filter block N_2 and the fluorescence of AADC-IR cells when illuminated through filter block I_2 (Fig. 8).

Distribution of PR-IR neurons

Preoptic area. In general, PR-IR cells were found along the entire length of the third ventricle and extending laterally (Figs. 1a–c; 4a). At the rostral level, PR-IR cells were scattered in the areas of the *lamina terminalis* immediately anterior to the opening of the third ventricle. They were concentrated in ventromedial regions related to the preventricular portion of the periventricular preoptic nucleus (Figs. 4a, 5a). PR-IR cells were also seen in the magnocellular nucleus of the dorsal chiasm (Figs. 1a, 6a). Intensely fluorescent cells were observed within the medial preoptic nucleus extending in columns parallel to the ventricular surface (Fig. 1b). In this region, the labeled cells were more numerous medially than laterally and their number was among the highest in the preoptic area (Table I). More caudally, PR-IR cells were observed extending dorsally and laterally from the third ventricle towards the medial preoptic area (Figs. 1c, 5b).

Hypothalamus. A large collection of PR-IR cells was also located in a periventricular position throughout the extent of the hypothalamus (Figs. 2a,b; 3a–c). At the rostral level of the hypothalamus (Figs. 2a, 6b), the number of fluorescent cells in the periventricular zone was always relatively low (Table I). A few cells were seen in the magnocellular paraventricular nucleus immediately adjacent to the ventricular border. The suprachiasmatic nucleus did not contain PR-IR cells. The arcuate nucleus contained PR-IR cells throughout its rostrocaudal extent (Figs. 2b, 3a–c; Table I). In the anterior region of the arcuate nucleus

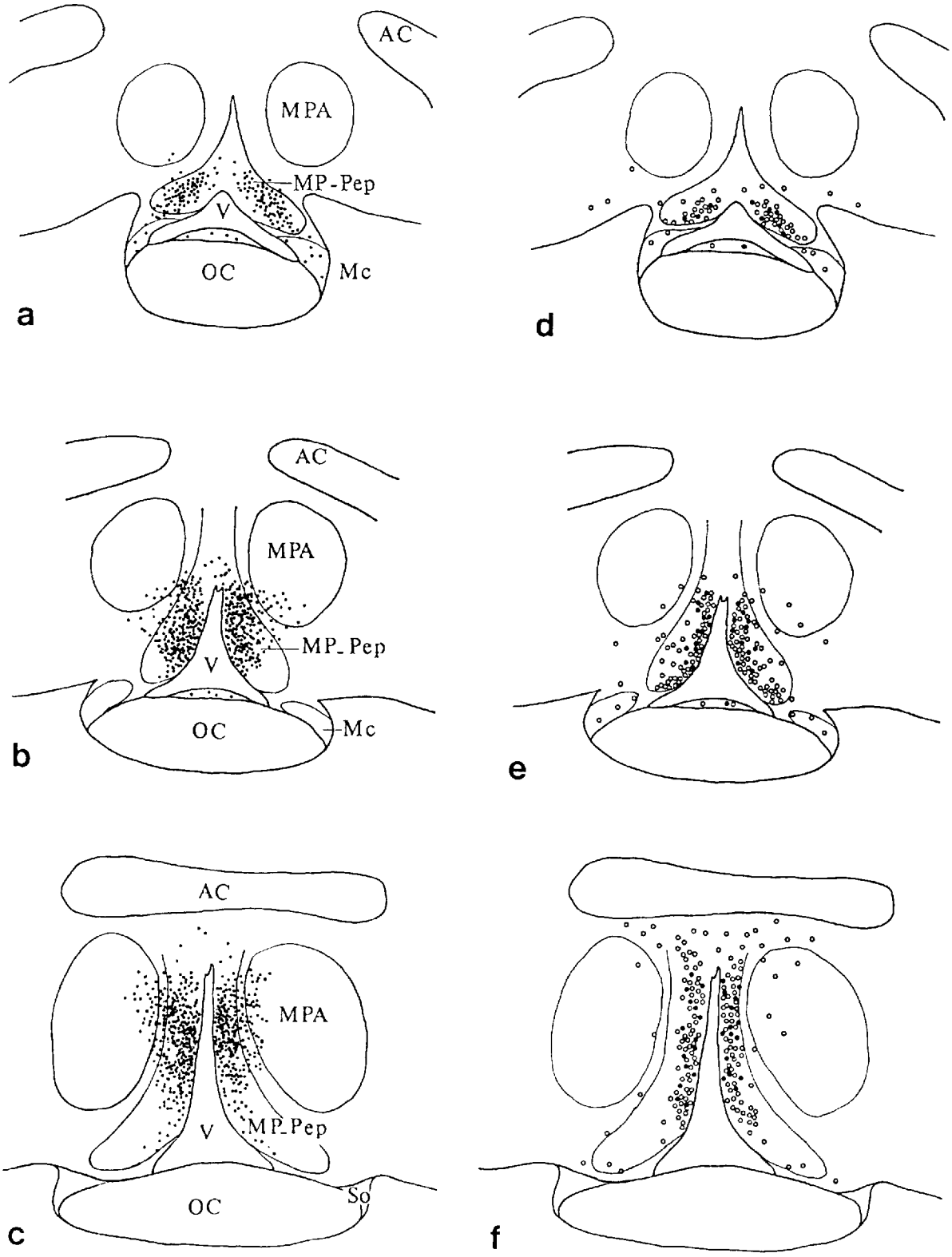

Fig. 1. Schematic drawings of the distribution of progesterone receptor-immunoreactive (PR-IR) cells (**a–c,** fine dots), aromatic L-amine acid decarboxylase immunoreactive (AADC-IR) cells (**d–f,** open circles) and double-immunoreactive cells (d–f, large dots) in sections from the preoptic area. Each dot represents one immunoreactive cell. AC, anterior commissure; Mc, magnocellular nucleus of the dorsal chiasm and lamina terminalis; MPA, medial preoptic area; MP-Pep, medial preoptic nucleus-periventricular nucleus, preventricular portion; OC, optic chiasm; So, supraoptic nucleus; V, ventricle.

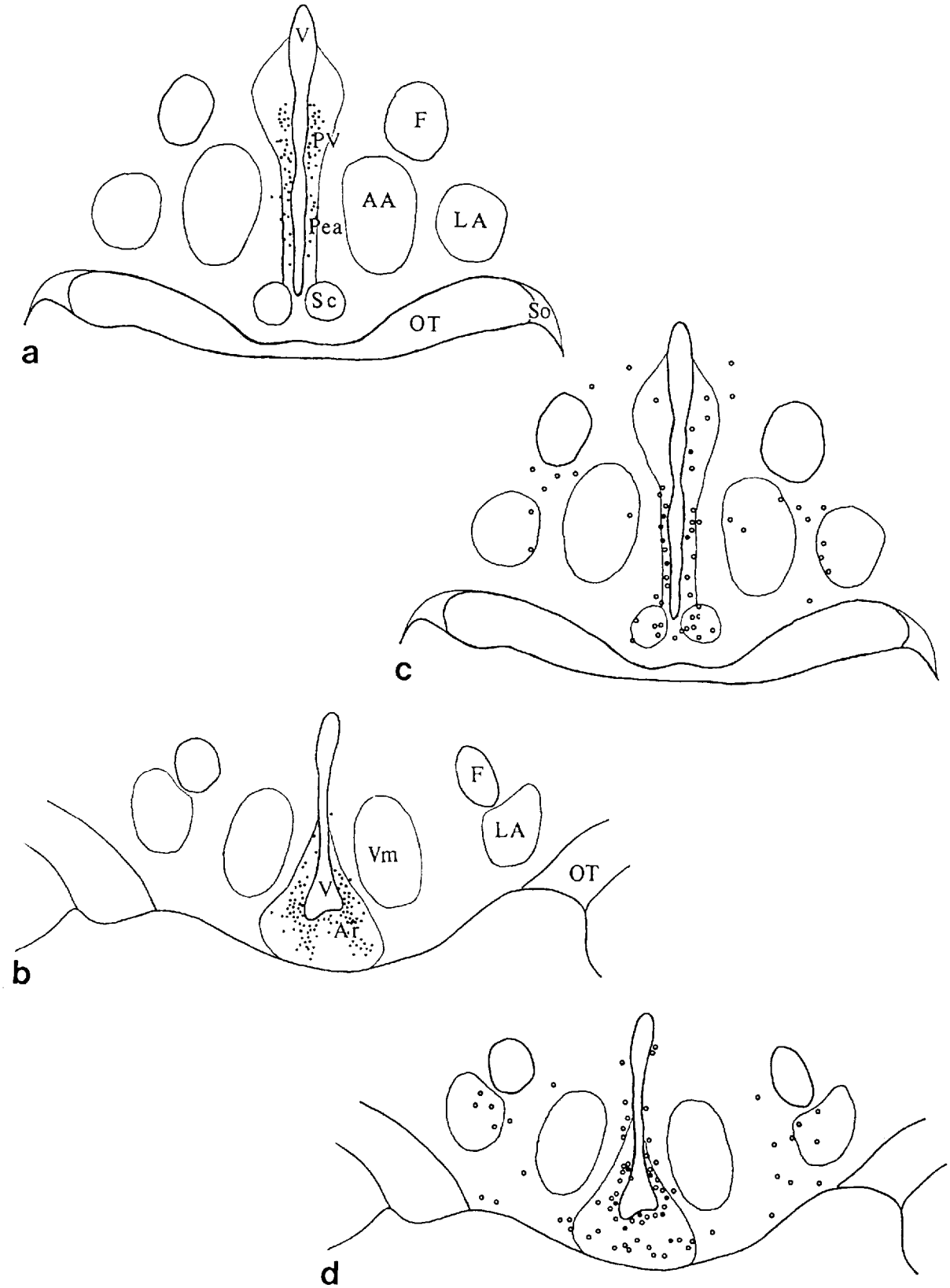

Fig. 2. Schematic drawings of the distribution of PR-IR cells (**a** and **b**, fine dots), AADC-IR cells (**c** and **d**, open circles) and double-immunoreactive cells (c and d, large dots) in sections from the anterior region of the hypothalamus. Each dot represents one immunoreactive cell. AA: anterior hypothalamic area; Ar, arcuate nucleus or infundibular nucleus; F, fornix; LA. lateral hypothalamic area; OT, optic tract; Pea, periventricular nucleus, anterior portion; PV, paraventricular nucleus; Sc, suprachiasmatic nucleus; So, supraoptic nucleus; V, ventricle; Vm, ventromedial nucleus.

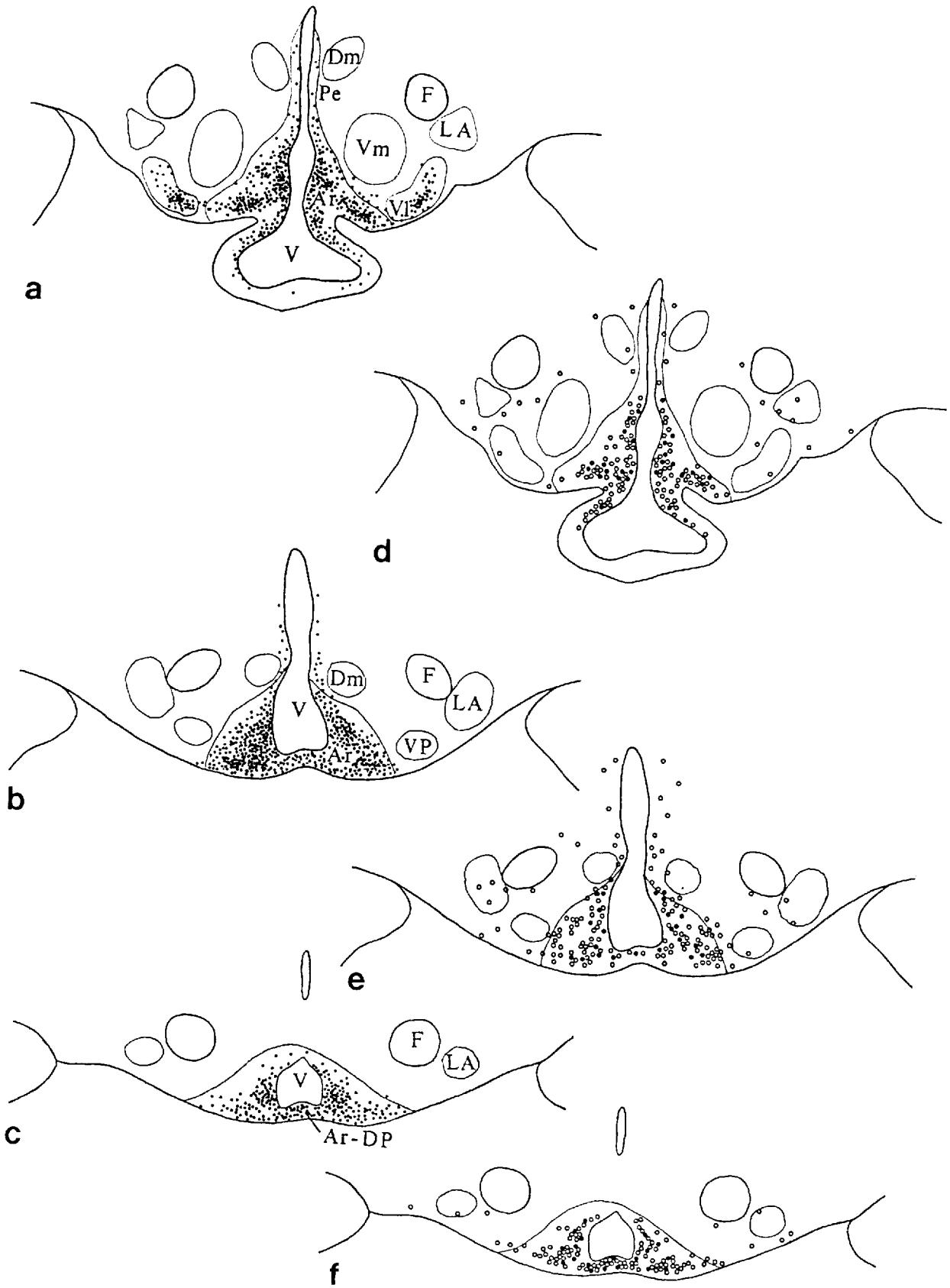

Fig. 3. Schematic drawings of the distribution of PR-IR cells (**a–c,** fine dots), AADC-IR cells (**d–f,** open circles) and double-immunoreactive cells (d–f, large dots) in sections from medial and posterior regions of the hypothalamus. Each dot represents one immunoreactive cell. Ar, arcuate nucleus; Ar-DP, arcuate and dorsal premamillary nucleus; Dm, dorsomedial nucleus; F, fornix; LA, lateral hypothalamic area; Pe, periventricular nucleus; V, ventricle; VI, ventrolateral nucleus; Vm, ventromedial nucleus; VP, ventral premamillary nucleus.

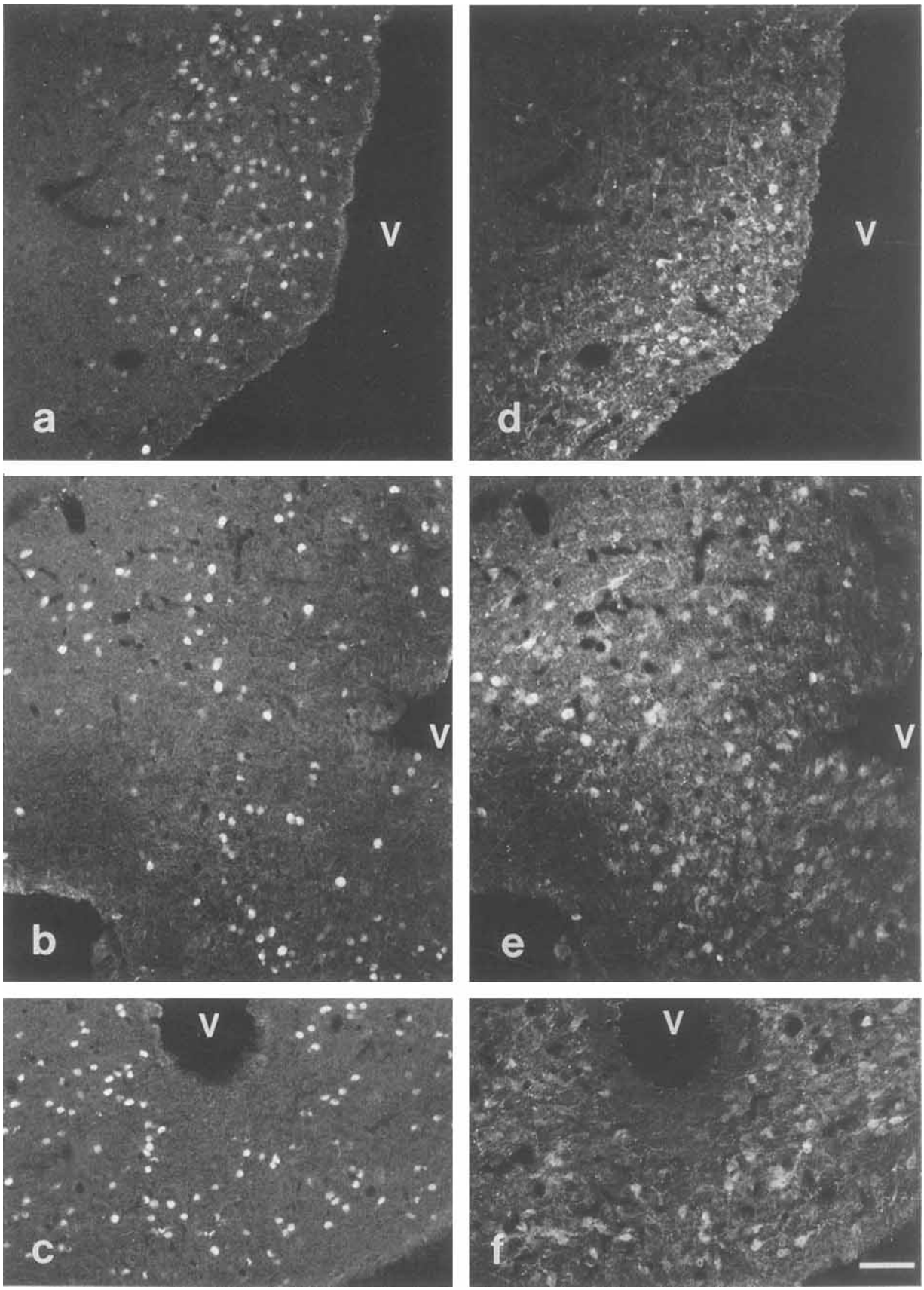

Fig. 4. Low power fluorescent photomicrographs of coronal sections to show the distribution of PR-IR neurons (**a–c**) and the distribution of AADC-IR neurons (**d–f**) through the medial preoptic nucleus (a and d), the rostral arcuate nucleus (b and e) and the caudal arcuate nucleus (c and f). V: third ventricle. Scale bar = 62 μm for a, b, d, e and 75 μm for c and f.

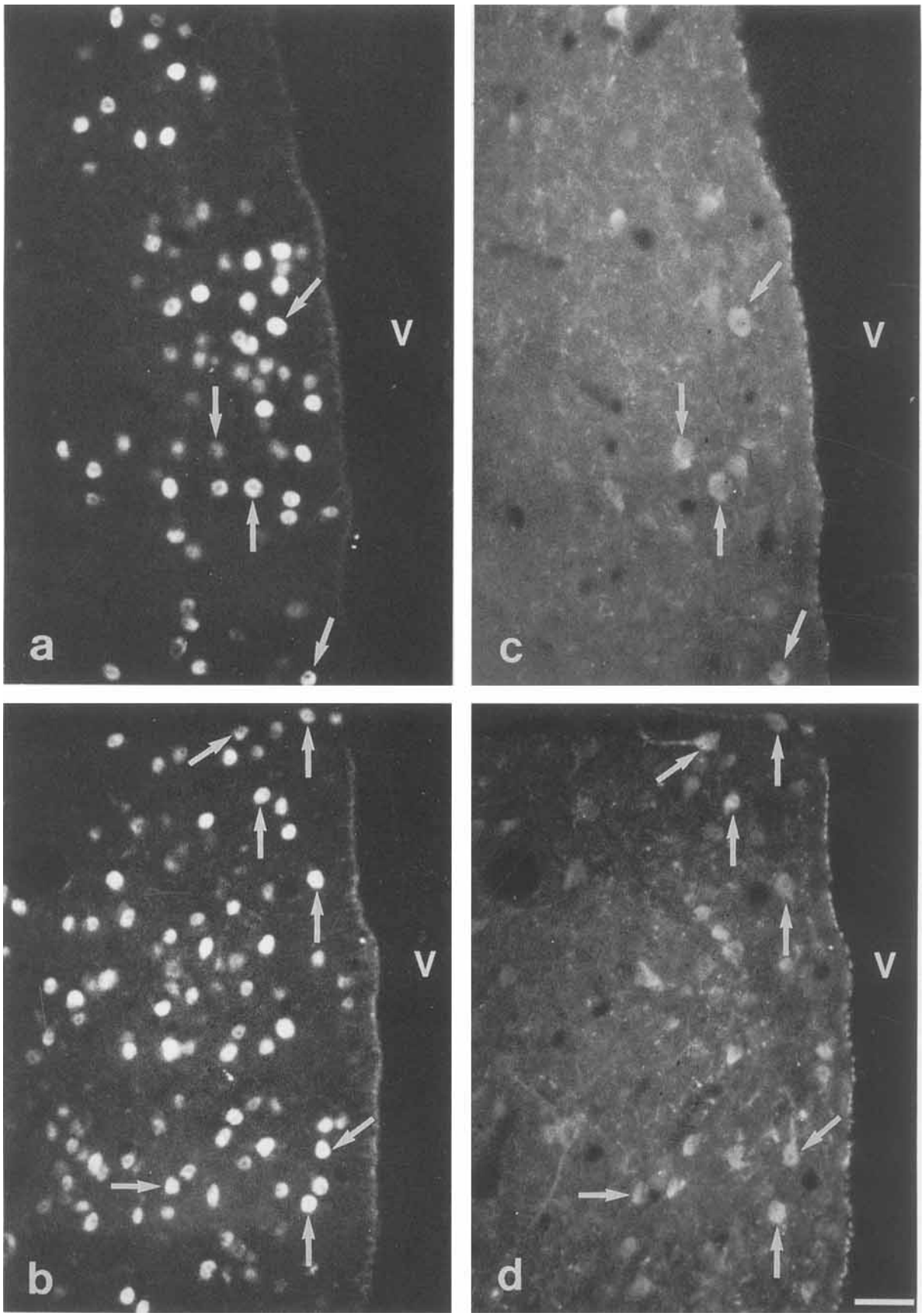

Fig. 5. Photomicrographs of the same coronal sections through the medial preoptic nucleus, at the rostral level (**a** and **c**) and at the caudal level (**b** and **d**) after double labeling with mouse antiserum to PR (a,b) and rabbit antiserum to AADC (c,d). Comparison of a and b, respectively, with c and d reveals PR-IR cells which are also AADC-IR (arrows indicate representative samples). V: third ventricle. Scale bar = 32 μm.

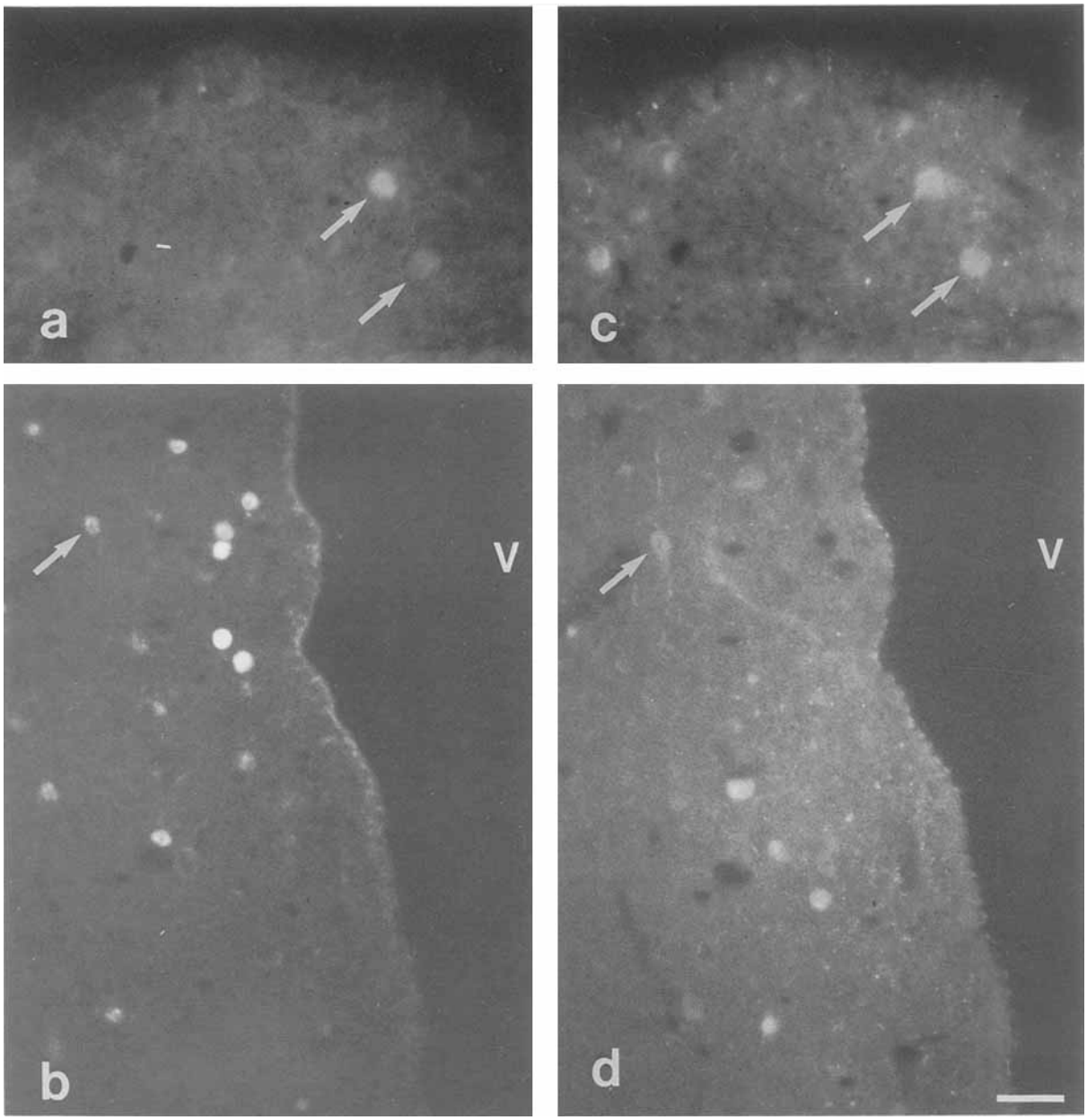

Fig. 6. Photomicrographs of the same coronal sections through the magnocellular nucleus of the dorsal chiasm between the floor of the third ventricle and the dorsal chiasm (**a** and **c**) and through the hypothalamic periventricular nucleus (**b** and **d**) after double labeling with mouse antiserum to PR (a,b) and rabbit antiserum to AADC (c,d). Arrows indicate PR-IR/AADC-IR cells. V: third ventricle. Scale bar = 25 μm for a and c, and 32 μm for b and d.

(Figs. 2b, 4b), PR-IR neurons were scattered near the midline between the third ventricle and the base of the brain. In the middle portion of the hypothalamus (Figs. 3a, 7a), PR-IR cells extended laterally from the periventricular hypothalamic nucleus to the dorsomedial and ventrolateral nucleus. We observed immunoreactive cells in the internal zone of the median eminence. We noted many PR-IR cells in the ventrolateral nucleus but not in the lateral hypothalamus area. The ventromedial nucleus was devoid of fluorescent cells. Caudally, an intense immunoreactivity was found surrounding the third ventricle extending to the premamillary nucleus (Figs. 3c; 4c; 7b).

Distribution of AADC-IR neurons

Preoptic area. AADC-IR neurons formed a rich population within the different regions of the preoptic area (Figs. 1d–f; 4d; Table I). Neurons were located periventricularly in the region of the *organum vasculosum* of the *lamina terminalis* and in the magnocellular nucleus of the dorsal chiasm (Fig. 1d). In the medial preoptic nucleus, fluorescent cells were observed arranged in rows parallel to the ventricular edge (Figs. 1e; 5c,d). These cells extended in the lateral direction into the medial preoptic area and dorsally into the area ventral to the anterior commissure where they

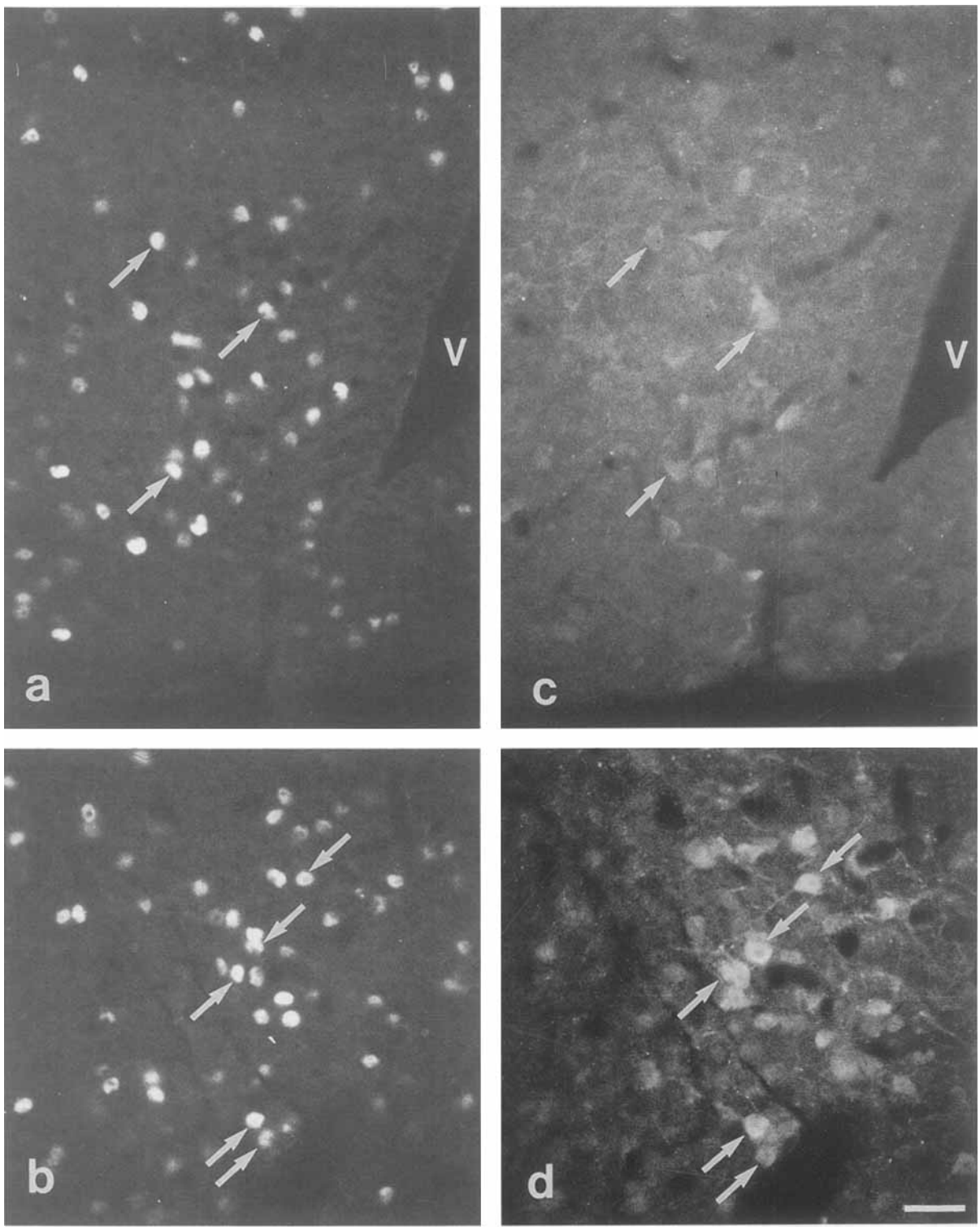

Fig. 7. Photomicrographs of the same coronal sections through the arcuate nucleus at the medial level (**a** and **c**) and at the caudal level near the premamillary region (**b** and **d**) after double labeling with mouse antiserum to PR (a,b) and rabbit antiserum to AADC (c,d). Arrows indicate PR-IR/AADC-IR cells. V: third ventricle. Scale bar = 32 μm.

were sparsely distributed (Fig. 1f). We noted a few fluorescent cells between the floor of the third ventricle and the dorsal chiasm (Figs. 1d,e; 6c).

Hypothalamus. Many AADC-IR neurons were always seen in the periventricular zone of the hypothalamus (Figs. 2c,d; 3d–f; Table I). Periventricular immunoreactive cells formed a vertical column and joined with the suprachiasmatic cell group (Figs. 2c, 6d). We found a few cells in the magnocellular portion of the paraventricular nucleus. Few cells were observed in the anterior hypothalamic area. In

the anterior region of the hypothalamus, AADC-IR were distributed vertically in the periventricular hypothalamic area and extended ventromedially under the floor of the third ventricle into the rostral arcuate nucleus (Fig. 2d). Cells were dispersed in the lateral hypothalamic area and the perifornical region. A dense collection of AADC-IR cells was found in the arcuate nucleus (Figs. 4e,f; 7c,d; Table I). They were scattered throughout the nucleus (Fig. 3d–f) but were more abundant ventrally where they extended into the median eminence (Fig. 3d). AADC-IR neurons were identified in the space between the fornix and the premamillary nucleus (Fig. 3e,f).

Distribution of double-immunoreactive cells

The distribution of PR-IR and AADC-IR cell populations showed considerable overlap in the preoptic area and mediobasal hypothalamus (Figs. 1–3). Within these areas, we identified a large number of AADC-IR cells with PR immunoreactivity (Table I and Figs. 5–8), and we found PR-IR cells in close proximity or in some cases surrounding AADC-IR cells. Within the preoptic area (Fig. 1d–f) the highest proportion (21.6%) of AADC-IR cells that were targets of progesterone was found in the caudal aspect of the medial preoptic nucleus, in a periventricular position (Figs. 1f; 8a,d). A few double-labeled neurons were also observed in the magnocellular nucleus of the dorsal chiasm between the floor of the third ventricle and the dorsal chiasm. Only occasional cases of coexistence were detected in the medial preoptic area. While both populations were present in the hypothalamic periventricular nucleus, only 8.9% of AADC-IR cells contained PR immunoreactivity (Fig. 2c). AADC-IR neurons in the region of the suprachiasmatic nucleus showed an absence of PR immunoreactivity (Fig. 2c). Throughout the mediobasal hypothalamus, we found extensive colocalization of PR and AADC immunoreactivities in the arcuate nucleus (Figs. 2d; 3d–f; 8b,c,e,f). The distribution of double-immunoreactive cells fluctuated slightly throughout the rostrocaudal extent of the nucleus (Table I). The proportion of cells displaying colocalization was higher ($P < 0.05$) in the posterior half (caudal level and premamillary region) compared to the anterior half (rostral and medial levels) and the highest percentage of colocalization occurred in the very posterior portion of the arcuate nucleus near the premamillary nucleus (20.1%). The complete lack of double-labeling in the ventrolateral nucleus was clearly due to the scarcity of AADC-containing cells. Cell count estimates showed that AADC-IR neurons accounted for 3.7% to 8.3% of all PR-IR cells in these areas containing double-immunoreactive neurons (Table I).

DISCUSSION

The present study was the first to localize AADC neurons in the guinea pig preoptic area and mediobasal hypothalamus and to demonstrate by using a double-antibody immunocytochemical technique that a large population of AADC neurons in these regions contained progesterone receptors.

Employing a monoclonal antibody against PR from rabbit uterus raised in mouse, immunoreactivity to the PR was observed only in ovariectomized guinea pigs primed with estradiol. In agreement with earlier studies (Warembourg et al., 1986; Blaustein et al., 1988; Don Carlos et al., 1989) we identified a large population of PR-IR cells in the preoptic area and hypothalamus. In these areas, we studied the cellular distribution of the AADC enzyme with an antibody raised in rabbit using as immunogen a fusion protein (β-galactosidase-AADC) made in *E. coli*. This antibody was biochemically characterized and tested for its specificity by Krieger et al. (1993). It was then applied to recognize both catecholaminergic and serotoninergic neurons in the brainstem of different vertebrates (Beltramo et al., 1993). Our results on the principal pattern of this enzyme in the guinea pig diencephalon were consistent with those obtained by immunocytochemistry in the rat (Jaeger, 1986; Skagerberg et al., 1988) in the cat (Kitahama et al., 1988) and by *in situ hybridization in the mouse* (Eaton et al., 1993). In spite of the close similarity in the general arrangement of AADC neurons in guinea pig and rat, the number of cells as well as areas occupied by AADC-IR cells seemed greater in the guinea pig than in the rat. These discrepancies may be due to a species difference or/and to the use of a more effective AADC antiserum. Moreover, in the present study, ovariectomized guinea pigs primed by estradiol were used for optimal staining of PR while the previous studies used intact rats. Thus, we cannot rule out the possibility that the extent and intensity of immunostaining may vary depending on endocrine state.

In the present study, the anatomical distribution of AADC-IR neurons identified in the guinea pig diencephalon was generally in good agreement with the systems known to contain catecholamine cells. These cells were first demonstrated in the rat brain with the histofluorescence technique (Falck et al., 1962). Using this method, Björklund et al. (1973) distinguished in the diencephalon four principal cell groups: the rostral periventricular group (A14), the arcuate group (A12), the dorsal hypothalamic group (A13), and the caudal thalamic and hypothalamic group (A11). Later, the introduction of immunocytochemical techniques using antibodies raised against TH confirmed the dopaminergic nature of these neurons and a new group of small TH-positive cells, termed A15 group, was delineated in the preoptic area. It joined the dorsal part of the A14 group, rostrally extending to the ventral part of the bed nucleus of the stria terminalis, underlying the anterior commissure and ventrally located above the optic chiasm (Hökfelt et al., 1984). In our study, although the distribution of AADC-IR cells overlapped the TH immunoreactivity, it should be noted that it was far more extensive than the distribution of TH-IR cells, particularly in the preoptic area and the ventrolateral hypothalamus.

The distributions of PR-IR and AADC-IR neurons were compared in the preoptic area and hypothalamus, and a considerable overlap was found in certain nuclei. We showed that a subpopulation of AADC-IR cells do contain PR. It is likely that these double-labeled cells also contain estrogen receptors since virtually all estradiol-induced PR-IR neurons in the diencephalon also express estrogen receptor (Warembourg et al., 1989). Our quantification revealed that approximately 15% to 23% of AADC-IR cells in the medial preoptic nucleus and 11% to 21% in the arcuate nucleus contained PR. Cells colocalizing PR and AADC were estimated to account for 3% to 9% of the total PR population in these regions.

In the guinea pig preoptic area, neither our studies (Warembourg et al., 1993) nor those of Blaustein and Turcotte (1989) revealed the presence of PR in TH-IR somata of A14 and A15 groups. In contrast, in the present report we found that 15% to 23% of AADC-IR cells contained PR immunoreactivity. This suggests that AADC-IR cells containing PR were TH-negative. In fact, some cells of the A14 (periventricular-preoptic region) and A15 groups which have been previously identified as TH-positive were AADC-negative. Our observations point to the hypothesis that these cells are not dopaminergic but L-DOPA-

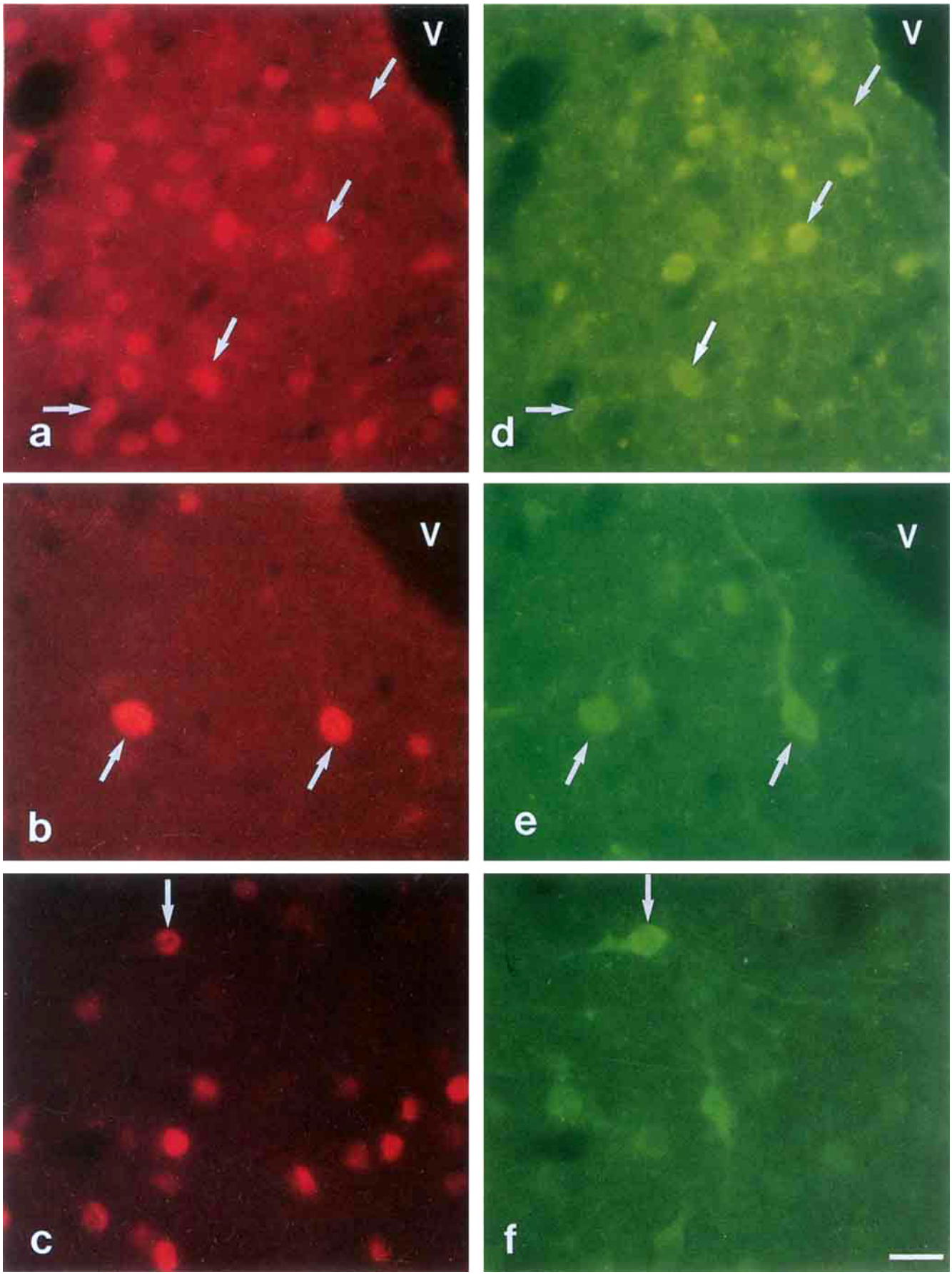

Figure 8

producing. This latter hypothesis is supported by results of Smits et al. (1990) who, using an antibody anti-DA, found no dopaminergic cell bodies in the rostral A14 and A15 groups of the guinea pig and is in accordance with study of Mons et al. (1990) showing the presence of L-DOPA-immunoreactive neurons in the rat preoptic area.

From our results, it is suggested that the PR-IR cells containing AADC enzyme in the preoptic area belong to D group neurons but the exact role of AADC in these cells is unknown. They may catalyze synthesis of other putative transmitters such as trace amines (tyramine, tryptamine, phenylethylamine) by direct decarboxylation of L-aromatic amino acids to biogenic amines (Jaeger et al., 1984a). They may also take up L-DOPA which has passed through the blood-brain barrier and decarboxylate it into DA (Jaeger et al., 1983b; Arai et al., 1994). The neuroanatomical evidence of the presence of PR in a subset of preoptic AADC-IR neurons is an interesting finding but the exact significance of this type of coexistence remains to be further studied.

In the guinea pig hypothalamus, the AADC-IR neurons containing PR immunoreactivity were mainly observed throughout the extent of the arcuate nucleus with a distribution very similar to that described for TH-IR cells containing PR in the guinea pig (Blaustein and Turcotte, 1989) and other species (Sar, 1988; Kohama et al., 1992; Horvath et al., 1993). These cells were thought to belong to the A12 cell group and were dopaminergic. In the present study, the percentage of AADC-IR cells that we found to contain PR (11% to 23%) was greater than that observed in the colocalization of TH and PR, particularly in the caudal portion of the arcuate nucleus (Warembourg et al., 1993). PR-containing AADC-IR cells found in the A12 area appeared to contain TH judging from the comparison of percentages of coexistence. But these double-labeled cells were more numerous in this area than the cells both PR-IR and TH-IR. It is likely that AADC-IR but TH-negative cells are present and intermingle with dopamine-containing cells. We suggest the existence of some D cells containing PR in this region. Phenylethylamine and p-tyramine have been shown to be present in the brains of various species, and the highest concentrations of phenylethylamine in rat brain regions have been found in the hypothalamus and caudate nucleus (Boulton and Juorio, 1982). It may be hypothesized that these amines, which function as neurotransmitters or neuromodulators in the brain (Paterson et al., 1990), are synthesized in those AADC-IR cells which also contain PR immunoreactivity.

It has been also shown that AADC was involved in the decarboxylation of 5HTP to 5HT. So far, no 5HT nerve cells in untreated animals have been found rostrally to the mesencephalon by both the classic histofluorescence method (Dahlström and Fuxe, 1964) and more recent immunohistochemical techniques (Steinbush, 1981; Warembourg and Poulain, 1985). However, 5HT immunoreactivity has been identified in well-known hypothalamic nuclei of rat and cat after intraperitoneal injection of 5HTP, the immediate precursor of 5HT (Steinbusch, 1984; Sakumoto et al., 1984). Further, 5HT immunoreactivity was found in large numbers of parvicellular neurons in the entire extent of the cat hypothalamus after hypothalamic microinjection of 5HTP and pargyline. The distribution pattern of these cells was similar to that of AADC-IR cells (Denoyer et al., 1989). It may be postulated that AADC-IR cells which possess PR may also present, under some physiological conditions, the capacity to take up 5HTP and decarboxylate it to 5HT.

Taken together, these findings suggest that the activity of a subset of AADC-IR neurons could be controlled by progesterone. Although studies on physiological significance of a direct action of the steroid on these neurons are needed, these observations provide new information on the morphological relationships between AADC enzyme and progesterone in the guinea pig preoptic area and hypothalamus.

Fig. 8. Photomicrographs of the same coronal sections through the medial preoptic nucleus at the caudal level (**a,d**) and through the arcuate nucleus at the anterior level (**b,e**) and at the caudal level (**c,f**) after double labeling with mouse antiserum to PR (a–c) and rabbit antiserum to AADC (d–f). Comparison of a, b and c, respectively, with d, e and f reveals PR-IR cells which are also AADC-IR (arrows) Scale bar = 18 μm.

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