ORIGINAL ARTICLE

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Colocalization of estrogen β-receptor messenger RNA with orphanin FQ, vasopressin and oxytocin in the rat hypothalamic paraventricular and supraoptic nuclei

Abstract The functional significance of the novel estrogen receptor β in brain areas that exclusively contain the ERβ receptor subtype such as the paraventricular (PVN) and the supraoptic (SON) nuclei of the hypothalamus is not yet fully understood. The present study attempts to characterize the peptidergic nature of the ERβ-containing neuronal population in the PVN and the SON using the double in situ histochemistry method in the female rat. Using this method, the ERβ mRNA coexpressions with the novel opioid neuropeptide (orphanin FQ and its receptor ORL1) mRNA in addition to the previously reported neuropeptide (arginine vasopressin-AVP, oxytocin-OXY, corticotropin releasing hormone-CRH, enkephalin-ENK) mRNAs were assessed. In the PVN, roughly half of the ERβ expression was colocalized with the prepro-orphanin FQ mRNA, which was comparable to the colocalization observed between the ERβ and AVP mRNAs in the same region. In addition, there was 20% overlap between the ERβ and ORL1 receptor mRNAs, and 10% overlap between the ERβ and OXY mRNAs in the PVN. By contrast, the coexpression between the prepro-orphanin FQ and ERβ mRNAs was less striking in the SON. Potential interactions between the ERβ and the well-characterized AVP-OXY neurosecretory system as well as the novel OFQ-ORL1 opioid neuropeptide system may provide new leads for the functional significance of ERβ, specifically in stress/autonomic responses.

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Introduction

In addition to its widely accepted reproductive function (Larsson and Heimer 1964; Nance et al. 1977; Pfaff and Sakuma 1995), estrogen is implicated in nonreproductive functions such as cognitive (Birge 1996; Tange et al. 1996), neuroprotective (Singh et al. 1995; Fader et al. 1998) and learning and memory (Luine et al. 1998; Gibbs 2000). It was thought that the mechanism through which estrogen could activate such nonreproductive functions was more likely to be non-genomic, since the classically-defined estrogen receptor (ERα) was found to be either low in abundance or absent in regions of the brain that were thought to be important for such functions. More recently, however, a second nuclear estrogen receptor (ERβ) was isolated in the prostate and ovary (Kuiper et al. 1996; Kuiper et al. 1998), cloned and mapped in the rat brain (Shughrue et al. 1997). In situ hybridization histochemistry of ERα and β mRNAs in consecutive sections showed that although there were some overlaps between the mRNA expressions of the two receptor subtypes in some areas of the rat brain, there were distinct regions such as the olfactory bulb, cortex, the paraventricular (PVN) and the supraoptic (SON) nuclei of the hypothalamus which contained high levels of ERβ mRNA and either very scarce or no ERα mRNA (Shughrue et al. 1997). Moreover, although ERα knock-out mice were

greatly impaired in male- or female-typical reproductive behavior, ERβ knock-out mice, in contrast, were found to be functionally intact in these behavioral dimensions (Ogawa et al. 1999). Collectively, these data suggest not only that estrogen, through binding to its own receptors, can activate various brain circuitries and behaviors, but also that the ERβ receptor subtype is a good candidate for direct estrogen regulation of behaviors that are not typically linked to reproduction.

The functional significance of the ERβ receptor subtype remains to be fully understood. Differential expressions of the ERα and ERβ in the brain may be used to shed light on the function of ERβ, especially in regions such as the PVN and SON, which are reported to exclusively contain the ERβ mRNA (Shughrue et al. 1997). These two brain regions have been repeatedly implicated in the regulation of stress response, partly via stress-induced arginine vasopressin (AVP) and oxytocin (OXY) release (Jezova et al. 1993; Miyata et al. 1995; Neumann et al. 1995; Rivest and Laflamme 1995). Using a combined in situ hybridization and immunohistochemistry technique, it has been shown that the ERβ mRNA is colocalized with AVP and OXY immunoreactivity in the PVN and the SON, suggesting a significant role for estrogen in regulating these neuropeptides (Hrabovszky et al. 1998; Laflamme et al. 1998), and by doing so, regulating the hypothalamic-pituitary-adrenal (HPA) axis. The magnitude of any detected colocalization of ERβ neurons with AVP and OXY was roughly 40% at best, leaving more than half of the ERβ neurons in these brain regions still unidentified in neuropeptide origin.

In the present study, using a double in situ hybridization histochemistry technique, we further investigated the neuropeptidergic characterization of the ERβ receptor subtype in the PVN and SON. We asked whether, aside from classical neuropeptides such as AVP, OXY, corticotropin releasing hormone (CRH) and enkephalin (ENK) that are abundantly expressed in the PVN and/or SON, there were other neuropeptides that may critically overlap with the ERβ expression in these regions. Another neuropeptide that is chosen as a likely candidate is the orphanin FQ (OFQ), which belongs to the opioid peptide family. The OFQ or nociceptin is a heptadecapeptide (Mollereau et al. 1994; Meunier et al. 1995), which binds to its own receptor (ORL1) (Mohr and Schmitz 1991) but does not act on μ, κ, δ opioid receptor subtypes. The OFQ-ORL1 system was a likely candidate in this investigation since not only were the peptide and its receptor both found abundantly expressed in the PVN and SON (Neal et al. 1999a, 1999b), but also the distribution of both molecules was very dense in areas that roughly corresponded to the ERβ-positive neuronal population. Our present findings indicate that the OFQ and AVP neuropeptide systems may be equally important in their interactions with the estrogen-ERβ system in the PVN.

Materials and methods

Animals and experimental procedures

Twenty-four adult female Sprague-Dawley rats, weighing 280-330 g, were purchased from Charles River Laboratories, Wilmington, Mass. Animals were housed, three per cage, in a temperature (23°C)- and light (lights on from 0600 to 1800 h)-controlled room with free access to rat chow and tap water. Animals were housed at housing conditions for at least 7 days before any experimental manipulation and treated in accordance with the National Institute of Health guidelines on laboratory animal use and care. The oestrus cycle was assessed via vaginal smears, and only rats in diestrus 1 when gonadal hormone levels were at basal, were used in the study.

Animals were killed by rapid decapitation around 9:00 A.M. The brains were removed and frozen in isopentane (-40°C), and stored at -80°C. The frozen brains were sectioned using a cryostat. Thin (20 μm), coronal brain sections were cut, from Bregma 1.4 to 1.6 mm for the SON, and from Bregma 1.6 to 1.8 mm for the PVN, then thaw-mounted onto poly-lysine-subbed glass slides, and stored at -80°C until used. Four animals and 3-4 sections per animal were used for each double in situ probe combination for percentage calculations.

In situ hybridization histochemistry

Sections were removed from the -80°C freezer, fixed in 4% paraformaldehyde for 1 h, and rinsed three times in 2X SSC (1X SSC: 0.15 M NaCl, 0.015 M Na citrate, pH 7.2). Then the sections were placed in a solution containing acetic anhydride (0.25%) in triethanolamine (0.1 M, pH 8.0) at room temperature for 10 min, rinsed in distilled water, and dehydrated through graded ethanol (50, 70, 80, 95, and 100%), and subsequently air-dried. The 35 S-labeled antisense cRNA probe for estrogen β receptor [360 base pairs (bp)] was transcribed via incubation with the transcription enzyme T3 (ERβ mRNA was generously donated by GGJM Kuiper). The digoxigenin-labeled antisense cRNA probes for prepro-OFQ (580 bp), ORL1 (700 bp), CRH (680 bp), enkephalin (ENK, 693 bp), AVP (235 bp) and OXY (524 bp) were prepared with digoxigenin-UTP (Dig-UTP; Boehringer Mannheim, Indianapolis) and transcribed using the SP6 (for prepro-OFQ, AVP, OXY) or the T7 (for ORL1, CRH, ENK) transcription enzymes. Brain sections were hybridized overnight with both probes at 55°C. After hybridization, coverslips were removed and slides were rinsed three times in 2X SSC, and incubated in RNase A (20 μg/ml) at 37°C for 1 h. Slides were washed sequentially in 2X SSC, 1X SSC, 0.5X SSC, and 0.1X SSC for 5 min and 0.1X SSC at 65°C for 1 h, and then processed for visualization of the Dig-labeled probe. Briefly, sections were incubated overnight with an antibody against Dig, conjugated to alkaline phosphatase (sheep anti-dig-AP; Fab fragments; Boehringer Mannheim), diluted 1:20,000. After extensive washing, sections underwent a color reaction by addition of 0.45% nitro blue tetrazolium chloride (Boehringer) and 0.35% 5-bromo-4-chloro-3-indoylphosphate, 4-toluidine salt (Boehringer). After completion of the color reaction (8-24 h) sections were rinsed and stripped of antibody by incubation with 0.1 M glycine and 0.5% Triton-X 100, pH 2.2, for 10 min. Finally sections were fixed in 2.5% glutaraldehyde for 1-2 h, and then dehydrated through a graded series of ethanol. After exposure to Kodak XAR film (Eastman Kodak, Rochester, NY), sections were dipped in liquid emulsion (Ilford KD-5; Polysciences, Warrington, Pa.) and stored in light-tight boxes for 21-30 days. Hereafter, sections were developed (Kodak D-19; Eastman Kodak, Rochester, NY), dehydrated, and coverslipped in a xylene-based mounting medium (Permount). The cellular distribution was determined using a Leica (Leitz, Nussloch, Germany;) microscope. Nonradioactive probes (prepro-OFQ, ORL1, CRH, ENK, AVP and OXY) were visualized under the bright-field illumination as a blue-purple precipitate, whereas the radioactive probe (ERβ) was visualized under the dark-field illumination as silver grain distribution. It should be noted that the nonradioactive in situ hybridization technique is less sensitive

than the radioactive one, hence the dig-labeled cell population tends to be under-represented. All combinations of radioactive and nonradioactive probes were analyzed.

Cell profile counts were determined at 60X or 100X magnification, with the aid of an eyepiece grid. No attempt was made to establish absolute numbers of cells within these structures. Rather, the numbers of cell profiles counted for each animal were used to approximate the relative percent colocalization of mRNA for each animal.

Photography and image processing

Cell counts were performed on digitized images. Bright-field and dark-field images were captured with a Sony CCD video camera (DXC-970MD, Sony, Tokyo, Japan) attached to a Leica (Leitz,) microscope using the Micro Computer Imaging Device (Ontario, Canada) image analysis system. Composites were formed within Adobe Photoshop 5.5. Brightness and contrast were slightly altered to generate photographic quality prints.

Results

In all of the analyses below, sections corresponding to the PVN are restricted to Bregma 1.6 to 1.8 mm, because the ERβ mRNA signal is most abundantly detected at this rostro-caudal level.

Colocalization between ERβ and AVP

The ERβ mRNA radioactive signal within the PVN is found concentrated in the ventromedial PVN (the transitional zone between the magno- and parvocellular regions), whereas the AVP mRNA nonradioactive (digoxigenin) signal is found concentrated mainly in the magnocellular region (Fig. 2A, B). Dual in situ hybridization revealed that 45 ± 3% of the ERβ cells in this region also expressed the AVP mRNA, whereas in the same sections, 20 ± 2% of AVP mRNA-positive cells also showed radioactive labeling for the ERβ mRNA (Fig. 1A, Fig. 2C). The total number of AVP mRNA-positive cells counted under bright-field illumination was roughly twice as high as the total number of ERβ mRNA-labeled cells counted under dark-field illumination in the analyzed

sections. In comparison, in the SON, 40 ± 4% of ERβ mRNA cells also expressed the AVP mRNA (Fig. 1B), whereas in the same sections, only 5 ± 1% of AVP mRNA-positive cells also expressed the radioactive signal for the ERβ mRNA. Moreover, the total number of AVP-mRNA containing cells in the SON was roughly five times greater than the total number of ERβ -signal containing cells. Hence, in both the PVN and the SON, the ERβ mRNA signal is contained in a smaller cell population than the AVP mRNA signal, and the colocalization between the two probes is expressed in 40-45% of the total ERβ -positive cell population.

Colocalization between ERβ and prepro-OFQ

The distribution of the prepro-OFQ-digoxigenin label is abundantly encountered in both the magno- and parvocellular regions of the PVN (Fig. 3A). Dual in situ hybridization showed that 50 ± 3% of ERβ mRNA-positive cells in the ventromedial PVN also expressed the prepro-OFQ mRNA, whereas in the same sections, only 10 ± 2% of prepro-OFQ-positive cells also showed radioactive labeling for the ERβ mRNA (Fig. 1A; Fig. 3B,C). The total number of prepro-OFQ-labeled cells in the PVN under bright-field illumination was five times greater than the number of ERβ-labeled cells under dark-field illumination in the analyzed sections. In the SON, however, even though the relative ratio of bright-field and dark-field counts revealed twice as many prepro-OFQ mRNA-positive cells than the ERβ mRNA-positive cells in the analyzed sections, only 20 ± 4% of the ERβ mRNA-containing cells also showed expression of the prepro-OFQ mRNA (Fig. 1B; Fig. 5A). Similar to the PVN, only 10 ± 1% of prepro-OFQ-positive cells in the SON remained colocalized with the ERβ mRNA signal. Thus, as in the case of AVP, in both the PVN and SON, the ERβ mRNA signal is contained in a smaller cell population than the pre-pro-OFQ mRNA signal. Moreover, although roughly 50% of the ERβ mRNA-positive cells were also pre-pro-OFQ-positive in the PVN, this number dropped to 20% in the SON.
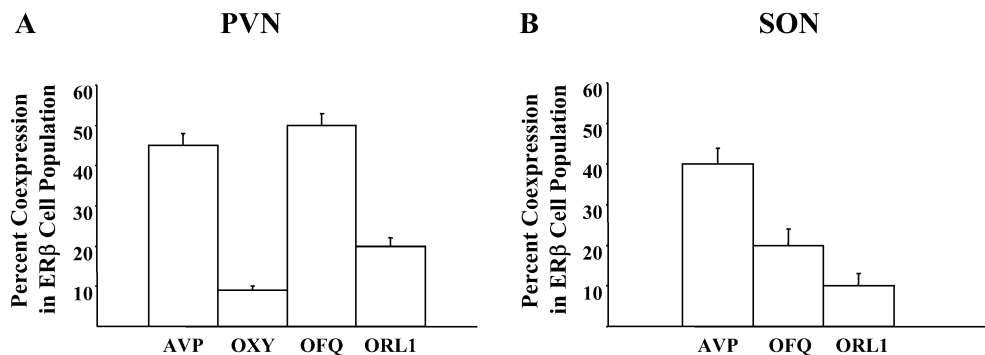

Fig. 1 Percent double labeling of ERβ mRNA-positive cells in the PVN **A** and SON **B** with AVP, OXY, OFQ and ORL1 mRNAs. Values are expressed as means ± SEMs and represent quantifications from four animals and 3-4 sections per animal for each double in situ hybridization observation. No colocalization is observed between ERβ and OXY mRNAs in the SON
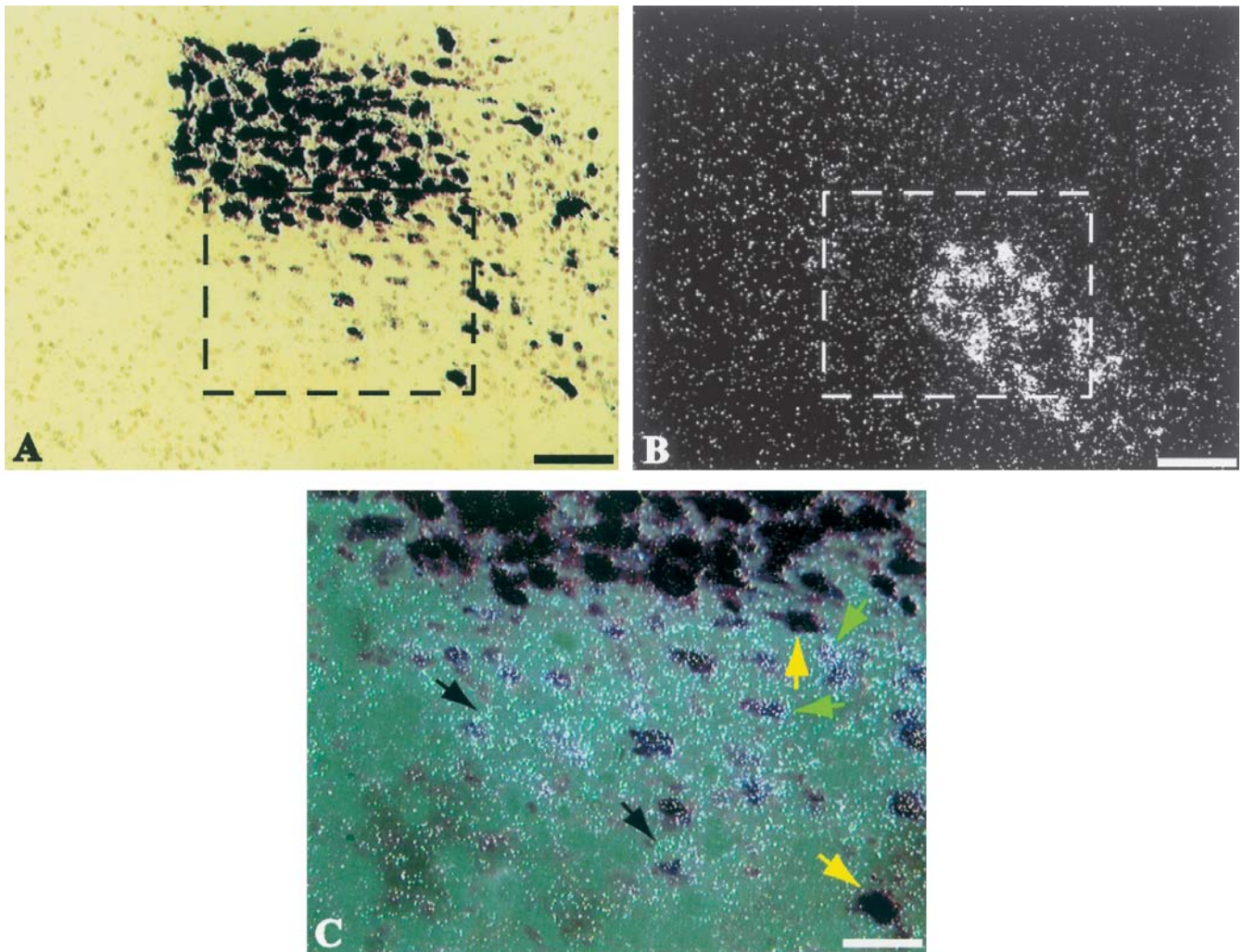
Fig. 2 Dual in situ hybridization of the ERβ mRNA labeled with a radioactive (35 S) probe and the AVP mRNA labeled with a nonradioactive (digoxigenin) probe in the PVN. **A** The AVP mRNA immunoreactivity in the magnocellular region of the PVN viewed under bright-field illumination. The black box corresponds to the ventromedial portion of the PVN. *Scale bar* = 100 μm. **B** The same section as in A viewed under dark-field illumination. *Scale bar* = 100 μm. **C.** The ventromedial PVN viewed under high magnification. *Green arrows* correspond to examples of ERβ mRNA-positive cells that also contain the AVP mRNA immunoreactivity. *Black arrows* correspond to examples of single ERβ mRNA-labeled cells. *Yellow arrows* correspond to examples of single AVP mRNA-labeled cells. *Scale bar* = 50 μm

Colocalization between ERβ and ORL1

The distribution of the ORL1 non-radioactive mRNA signal in the ventromedial PVN was identical to that of the prepro-OFQ as described above (Fig. 4A). Double in situ hybridization revealed that $20 \pm 2\%$ of ERβ-mRNA containing cells in the PVN also expressed ORL1 mRNA, whereas in the same sections, only $10 \pm 3\%$ of ORL1 mRNA-positive cells also expressed ERβ mRNA (Fig. 1A; Fig. 4B, C). The total number of ORL1-labeled cells in the PVN counted under bright-field illumination was three times greater than the total number of ERβ mRNA-positive cells counted under dark-field illumination in the analyzed sections. In comparison, in the SON, the percentage of ERβ mRNA-positive cells that also contained the ORL1 mRNA dropped to $10 \pm 3\%$ (Fig. 1B;

Fig. 5B). In addition, less than 5% of ORL1-positive cells are also found positive for the ERβ mRNA. The relative ratio of the bright-field (ORL1) and dark-field (ERβ) cell counts was similar to that of the prepro-OFQ in the SON as described above. As in all the comparisons before, the ERβ mRNA label is found to be located in a smaller cell population than the ORL1 digoxigenin label in both the PVN and the SON. Consistent with prepro-OFQ findings above, the percentage of ERβ mRNA-positive cells that also contained ORL1 digoxigenin label was higher in the PVN (around 20%) than in the SON (around 10%).
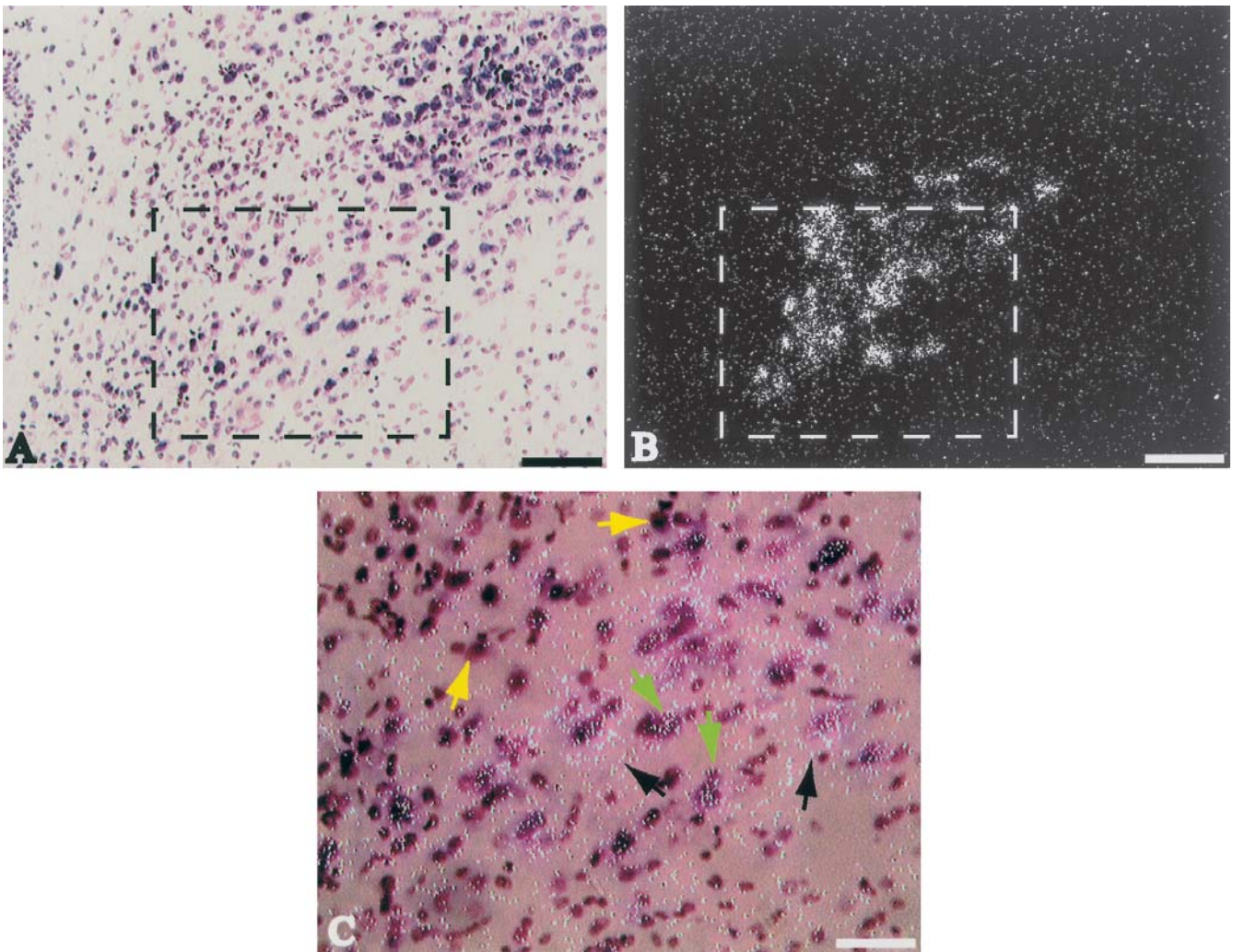
Fig. 3 Dual in situ hybridization of the ERβ mRNA labeled with a radioactive (35 S) probe and the prepro-OFQ mRNA labeled with a nonradioactive (digoxigenin) probe in the PVN. **A** The prepro-OFQ mRNA immunoreactivity distributed in the magno- and parvocellular regions of the PVN viewed under bright-field illumination. The black box corresponds to the ventromedial portion of the PVN. *Scale bar* = 100 μm. **B** The same section as in A viewed under dark-field illumination. *Scale bar* = 100 μm. **C** The ventromedial PVN viewed under high magnification. *Green arrows* correspond to examples of ERβ mRNA-positive cells that also contain the prepro-OFQ mRNA immunoreactivity. *Black arrows* correspond to examples of single ERβ mRNA-labeled cells. *Yellow arrows* correspond to examples of single prepro-OFQ mRNA-labeled cells. *Scale bar* = 50 μm

Colocalization between ERβ and OXY, CRH and ENK

Although, the OXY mRNA signal is encountered in the same sections as the ERβ mRNA signal in the PVN, double in situ hybridization showed that less than 10 ± 1% of ERβ mRNA-positive cells also expressed the OXY mRNA (Fig. 1A; Fig. 6 A, B). Moreover, in the same sections, less than 5 ± 2% of OXY mRNA-positive cells also expressed the ERβ mRNA. No colocalization between the ERβ and OXY mRNA signals is detected in the SON. Double in situ hybridization showed that only a few scattered ERβ mRNA-positive cells also expressed the CRH mRNA in the ventromedial PVN. Throughout the PVN, the ERβ and the CRH mRNAs seemed to be distributed in a non-overlapping fashion. No CRH mRNA digoxigenin labeling is detected in the SON. No colocal-ization between the ERβ mRNA and the ENK mRNA positive cells was detected due to non-overlapping distribution patterns in the PVN. No ENK mRNA digoxigenin labeling is detected in the SON.

Discussion

This study describes a significant overlap in the gene expressions of the orphanin FQ and estrogen-ERβ systems, particularly in the hypothalamic nucleus that coordinates the stress/autonomic response, the PVN. Our double in situ histochemistry findings showed that roughly half of the ERβ neurons in the ventromedial PVN also contained the OFQ mRNA. In addition, there was 20% overlap between the ERβ and the ORL1
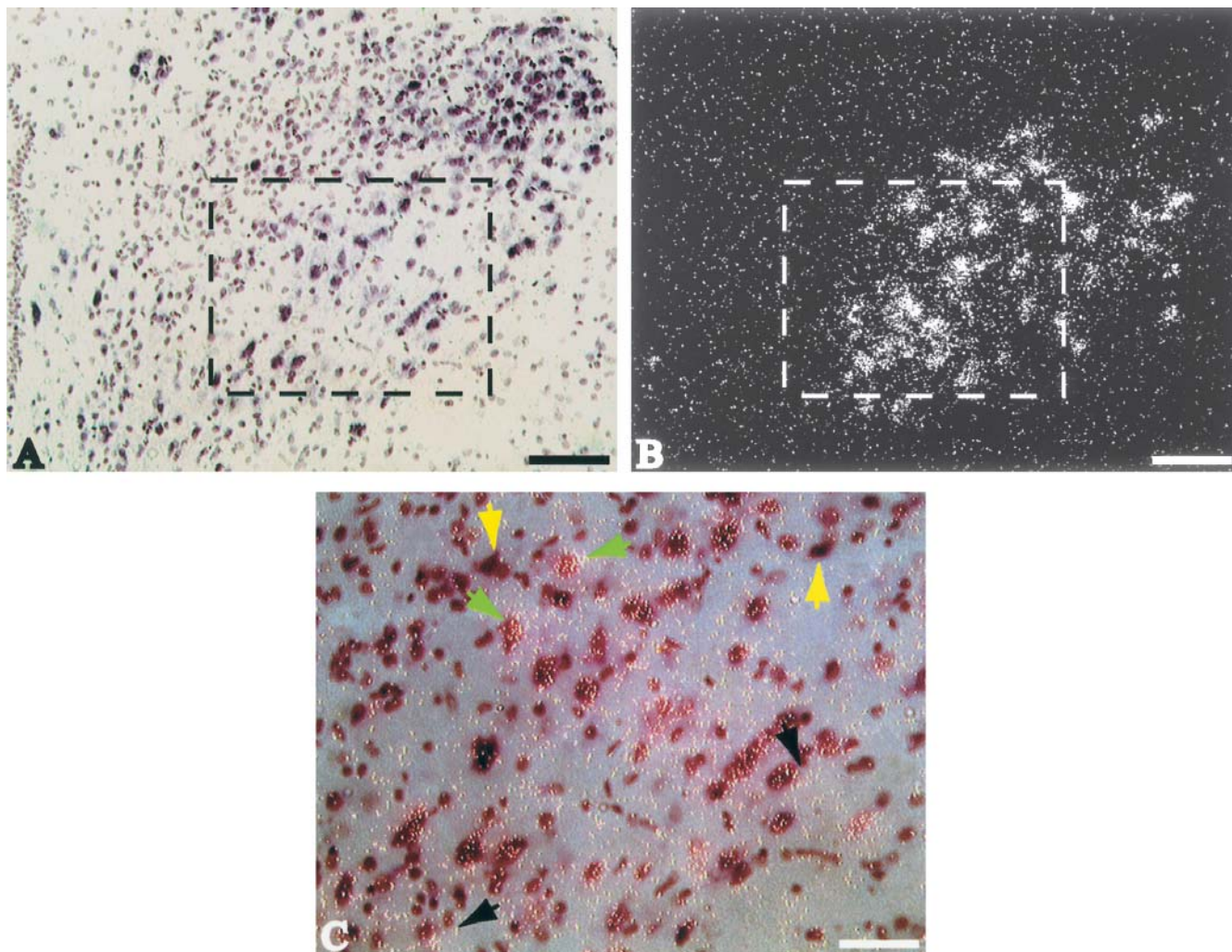
Fig. 4 Dual in situ hybridization of the ERβ mRNA labeled with a radioactive (35 S) probe and the ORL1 mRNA labeled with a nonradioactive (digoxigenin) probe in the PVN. **A** The ORL1 mRNA immunoreactivity distributed in the magno- and parvocellular regions of the PVN viewed under bright-field illumination. The black box corresponds to the ventromedial portion of the PVN. *Scale bar* = 100 μm. **B** The same section as in A viewed under dark-field illumination. *Scale bar* = 100 μm. **C** The ventromedial PVN viewed under high magnification. *Green arrows* correspond to examples of ERβ mRNA-positive cells that also contain the ORL1 mRNA immunoreactivity. *Black arrows* correspond to examples of single ERβ mRNA-labeled cells. *Yellow arrows* correspond to examples of single ORL1 mRNA-labeled cells. *Scale bar* = 50 μm

receptor mRNAs in the PVN. Interestingly, in the same region, the ERβ mRNA coexpressions with the AVP and OXY mRNAs were 45% and 10% respectively. By contrast, the coexpression between the prepro-OFQ and the ERβ mRNAs was less striking in the SON (10-20%), although the ERβ mRNA was found colocalized the highest with the AVP mRNA in this region (40%). No colocalizations were detected between the ERβ mRNA and the CRH and ENK mRNAs in the ventromedial PVN, and between the ERβ mRNA and the OXY mRNA in the SON. These results confirm that, in addition to the AVP and OXY, estrogen may directly regulate the OFQ-ORL1 neuropeptide system in the PVN, and to a lesser degree in the SON, via its nuclear receptor β.

Both OFQ and ORL1 are found widely distributed in the central nervous system with exceptionally high densities in the hypothalamus including arcuate nucleus, suprachiasmatic nucleus, SON, PVN, ventromedial hypothalamus, and zona incerta (Neal et al. 1999a, 1999b). Moreover, the OFQ is shown to act as an endogenous anxiolytic in the central nervous system in mice and rats, and to attenuate behavioral response to stressors (Jenck et al. 1997; Griebel et al. 1999). In contrast, however, the OFQ-deficient mice have been shown to be impaired in adaptation to repeated stress (Koster et al. 1999). In spite of the reported anxiolytic effects, in our laboratories we have shown that the OFQ administration activates the HPA axis and augments plasma adrenocorticotropin and corticosterone responses in both unstressed and stressed rats (Devine et al. 2001). It is also shown that the OFQ is released endogenously in response to an acute stress exposure (Devine et al. 1998). Together, these data
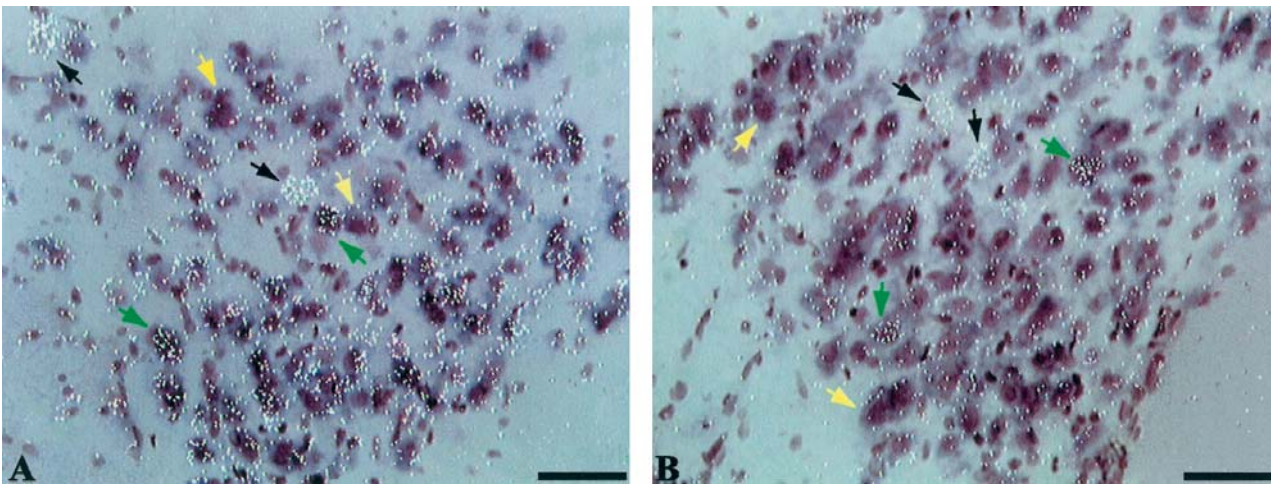
Fig. 5 Dual in situ hybridization of the ERβ mRNA labeled with a radioactive (35 S) probe and the OFQ mRNA **A** or the ORL1 mRNA **B** labeled with a nonradioactive (digoxigenin) probe in the SON. *Green arrows* correspond to examples of ERβ mRNA-positive cells that also contain OFQ **A** or ORL1 **B** mRNA immunoreactivities. *Black arrows* correspond to examples of single ERβ mRNA-labeled cells. *Yellow arrows* correspond to examples of single OFQ **A** or ORL1 **B** mRNA-labeled cells. *Scale bar =* 50 μm

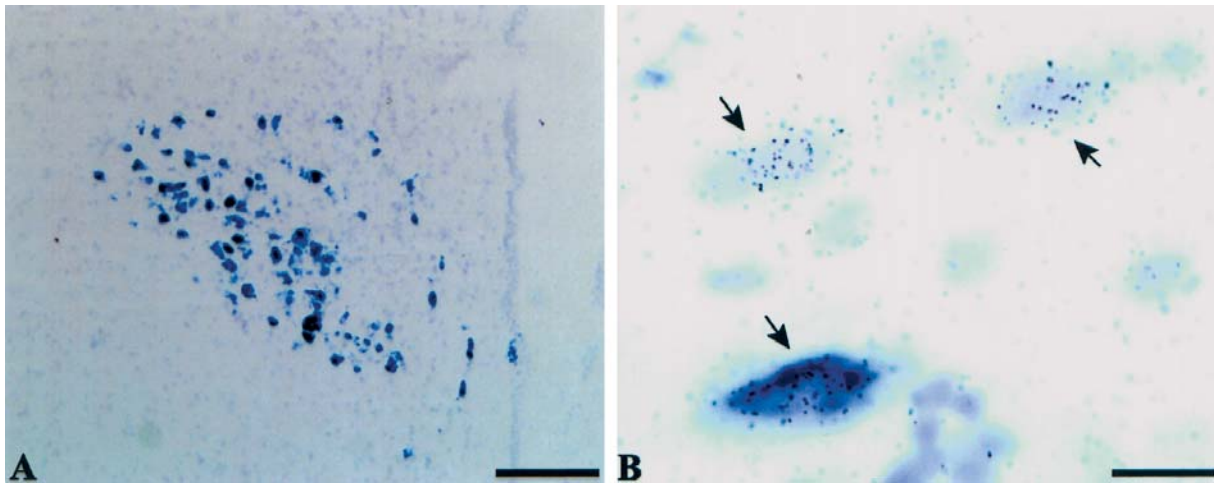**Fig. 6** Dual in situ hybridization of the ERβ mRNA labeled with a radioactive (35 S) probe and the OXY mRNA labeled with a nonradioactive (digoxigenin) probe in the PVN. **A** A low magnification image of the OXY mRNA distribution in the PVN viewed under bright-field illumination. *Scale bar = 50 μm*. **B** A high magnification image of an ERβ mRNA-positive neuron containing OXY mRNA immunoreactivity *bottom arrow* and two single ERβ mRNA-labeled neurons *top arrows* under bright-field illumination. *Scale bar = 5 μm*

suggest that the OFQ system, although far from being fully understood in function, may regulate stress and anxiety responses and perhaps due to its interactions with the ERβ.

It is possible that the ERβ mRNA-positive neurons in the PVN and SON that colocalize with AVP, OFQ, ORL1 and OXY mRNAs constitute to overlapping neuronal populations. It is also possible that these neuropeptide systems occupy separate neuronal populations. In either case, estrogen seems to play an intricate role in regulating multiple neuropeptide systems that are synthesized in the same or neighboring neurons in the PVN and SON. It is unknown whether the OFQ-ORL1-containing neurons in the PVN and SON also express AVP and/or OXY. In case of such an overlap, it is possible that estrogen can activate different genes with different time courses so that a functional differentiation and/or integration can be achieved at projection sites. A closer look at the projection sites of specific neurons that contain the ERβ mRNA in these hypothalamic regions is helpful for understanding the possible functional significance of the ERβ mRNA overlap with the above neuropeptides.

The ventromedial PVN where the ERβ mRNA signal is most abundant, contains parvocellular cells (i.e., medial parvocellular part) as well as magnocellular cells (i.e., posterior magnocellular part). The neuronal cell popula-

tion that is ERβ-positive has little or no overlap with the neuroendocrine part of the PVN. In addition to the neuroendocrine-related projections to the posterior lobe of the pituitary gland (Sherlock et al. 1975) and the median eminence (Vandesande et al. 1977), the PVN also sends projections directly to a number of extrahypothalamic nuclei (Conrad and Pfaff 1976; Swanson 1977; Swanson and Kuypers 1980; Sawchenko and Swanson 1982), which are implicated in multiple aspects of autonomic nervous system function. For example, retrograde fluorescence double-labeling studies previously showed that a significant amount of medial parvocellular and posterior magnocellular neurons that are AVP- and OXY-positive also send long descending projections to the autonomic cell groups in the medulla and spinal cord (Swanson and Kuypers 1980; Sawchenko and Swanson 1982).

The lack of a significant colocalization between the ERβ mRNA and the CRH mRNA and at the same time a high percentage of colocalization between the ERβ mRNA and the prepro-OFQ mRNA in the PVN may suggest that ERβ is not directly regulating the HPA axis via traditional neuroendocrine-mediated pathways. Since the ERβ mRNA showed the highest expression in the ventromedial parts of the PVN, and this zone is mainly involved in the regulation of autonomic functions via caudal projections of medulla and spinal cord, it is logical to suggest that ERβ, through activation of the endogenous OFQ neurons in the PVN, may regulate the autonomic nervous system to yield behaviors such as fight or flight response.

In summary, estrogen through its receptor β subtype can directly activate AVP- and OXY-containing neurons as well as a novel neuropeptide system, the OFQ-ORL1, in the two hypothalamic nuclei, the PVN and the SON. The extent of colocalization between the ERβ mRNA and AVP mRNA is comparable to that of ERβ mRNA and OFQ mRNA in the PVN, hence suggesting that estrogen may have as significant a role in the regulation of OFQ in this brain region. In the SON, however, the magnitude of colocalization is lower for OFQ-ORL1 but remains high with AVP. This may provide a mechanism for differential neuropeptidergic regulation of the PVN and SON by estrogen-binding to the β receptor. In both cases, the interaction between estrogen and the well-characterized AVP-OXY neurosecretory system and the novel OFQ-ORL1 opioid neuropeptide system may provide novel leads to the functional significance of ERβ, specifically in stress/autonomic responses.

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