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Immunocytochemical Investigation of Nuclear Progestin Receptor Expression within Dopaminergic Neurones of the Female Rat Brain

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Abstract

Progesterone influences most processes involved in female reproduction, including ovulation, sexual behaviour, pregnancy, parturition, lactation and maternal behaviour. One neurotransmitter through which progesterone might regulate many of these functions is dopamine. To determine where in the brain progesterone might alter dopaminergic activity necessary for these and other processes in rats via cell nuclear progestin receptors, ovariectomized rats were injected subcutaneously with either 4 μg oestradiol benzoate to induce high levels of hypothalamic progestin receptor expression, or oil, and perfused 48 h later. Dual-label immunocytochemistry was used to visualize cells having immunoreactivity (ir) for progestin receptors and tyrosine hydroxylase, a rate-limiting enzyme for dopamine synthesis. Many cells containing both progestin receptor-ir and tyrosine hydroxylase-ir were found throughout the periventricular hypothalamus of oestradiol-treated females. Conversely, very few cells in the hypothalamus of oil-treated controls contained progestin receptor-ir and, consequently, few dual-labelled cells were found in this group. The greatest percentage of tyrosine hydroxylase immunoreactive cells expressing progestin receptors in oestradiol-treated females was in, or near, the arcuate nucleus (A12 group), where up to 55% of tyrosine hydroxylase-expressing cells coexpressed progestin receptors. Notably, dual-labelled cells in oestradiol-treated females were also found more rostrally than previously reported, with approximately 15–20% of tyrosine hydroxylase-ir cells in the preoptic area/anterior hypothalamus (A14 group) also containing progestin receptor-ir. No dual-labelled cells were found for either group in the posterodorsal hypothalamus (A11 group), zona incerta (A13 group), retrorubral field (A8 group), ventral tegmental area (A10 group) or substantia nigra (A9 group) because little or no progestin receptor-ir was found in these sites. These data provide new information about the neural substrate where progesterone might regulate dopamine release in the preoptic area/anterior hypothalamus. Using more sensitive techniques than those used previously, they also confirm the relationship between progestin receptor and tyrosine hydroxylase in the arcuate nucleus, which could be important for the regulation of prolactin release throughout the female reproductive cycle. Additionally, although progesterone alters mesolimbic and nigrostriatal dopamine release, and the numerous behaviours that these pathways influence, these data again suggest that it does not do so via nuclear progestin receptor in dopaminergic cells of the ventral tegmental area and substantia nigra.

All facets of reproduction in female rodents involve fluctuations in ovarian and pituitary hormones, including essential and sometimes dramatic changes in circulating progesterone. Indeed, ovulation (1), copulation (2), pregnancy (3), lactation (4, 5) and maternal behaviour (6) are all critically influenced by progesterone. Progesterone surely impacts many physiological systems to produce its effects, but its interactions with neural dopamine systems might be particularly important for its effects on copulatory and maternal behaviours in rats. Increased levels of circulating progesterone facilitate female sexual behaviour concomitant with increased dopamine release in the preoptic area and striatum, two areas necessary for the display of some sexual behaviours in female rats (7, 8). By contrast, declining levels of progesterone around the time of parturition are associated with the onset of maternal behaviour, as well as decreased dopaminergic activity in the

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preoptic area and increased dopaminergic activity in the dorsolateral striatum (9).

Relatively little is known about how and where progesterone influences dopaminergic neurotransmission in a manner that could influence sexual and maternal behaviours, or any other reproductive processes. Because the well-characterized effects of progesterone on some behavioural end-points (2) require nuclear progestin receptors acting as transcriptional regulators, a straightforward mechanism would be for it to bind directly to intracellular progestin receptors expressed by dopamine-synthesizing neurones of the hypothalamus and midbrain. Two previous studies have investigated whether dopamine cells of the female rat brain express progestin receptors, both using immunocytochemistry for tyrosine hydroxylase (the rate-limiting enzyme for catecholamine synthesis) combined with autoradiography for progestin receptors (10, 11). These studies have produced somewhat discrepant results, both in terms of what populations of dopaminergic cells express progestin receptors, as well as the percentage of cells in some populations that are dual-labelled. Furthermore, the number of cells expressing progestin receptors might be underestimated by autoradiography (12). We have therefore readdressed this issue and used dual-label immunocytochemistry for progestin receptors and tyrosine hydroxylase to examine the distribution of progestin receptor expression in dopaminergic neurones in the brains of oestradiol benzoate- and oil-treated female rats.

Materials and methods

Animals

Adult virgin female Sprague–Dawley rats (Taconic Farms, Germantown, NY, USA), weighing approximately 225 g and aged 90–110 days, were housed in pairs in clear polypropylene cages with wood shavings for bedding, food and water available *ad libitum*, and a 14 : 10 h light/dark cycle. One week after arrival, rats were anaesthetized with ketamine and xylazine and bilaterally ovariectomized. One week later, they were injected subcutaneously with either 4 μg oestradiol benzoate (Sigma, St Louis, MO, USA) in 100 μl sesame oil (n = 7), or 100 μl sesame oil (n = 6). Rats were killed 48 h after injection. This dose of oestradiol benzoate induces high levels of hypothalamic progestin receptor expression at 48 h after injection, which is necessary for the ability of exogenous progesterone to elicit high levels of female sexual behaviour (2). All procedures were in accordance with the standards for animal use and care set by the National Institutes of Health and Michigan State University.

Immunocytochemistry for progestin receptor and tyrosine hydroxylase

After perfusion with 200 ml saline and 200 ml 4% paraformaldehyde in sodium phosphate buffer (pH 7.6), brains were immediately submerged in 20% sucrose, later cut into 35 μm sections and stored in cryoprotectant. A one-in-three series of sections through the entire brain was later rinsed in Trisma-buffered saline, incubated in 0.1% sodium borohydride for 10 min, 1% hydrogen peroxide in 0.3% Triton-X for 15 min, 20% normal goat serum in 0.3% Triton-X for 30 min, a solution of 2% normal goat serum in 0.3% Triton-X containing a rabbit polyclonal primary antiserum for progestin receptor that recognizes both A and B isoforms (dilution 1 : 1000; DAKO A0098, Carpenteria, CA, USA) for 3 days at 4 °C. Sections were then rinsed in Trisma-buffered saline, incubated in a goat anti-rabbit biotinylated secondary antiserum (dilution 1 : 500; Jackson ImmunoResearch, West Grove, PA, USA) for 60 min, incubated in avidin–biotin complex (Vector Elite; Vector Laboratories, Burlingame, CA, USA) for 60 min, and 0.05% 3–3′ diaminobenzadine plus 0.06% hydrogen peroxide as the chromagen, which provided a dark brown nuclear label. After rinsing in Trisma-buffered saline, sections were blocked again in 20% normal goat serum in Trisma-buffered saline and incubated in a rabbit polyclonal primary antiserum for tyrosine hydroxylase (dilution 1 : 2000; Chemicon, Temecula, CA, USA) overnight at 4 °C. The next day, sections were rinsed in Trisma-buffered saline, incubated in a goat anti-rabbit biotinylated secondary antiserum for 60 min, incubated in avidin–biotin complex for 60 min, and the antigen visualized with Vector SG chromagen, which provided a light blue cytoplasmic label. Omission of primary or secondary antisera resulted in absence of blue cytoplasmic and/or brown nuclear labelling. Other control sections were incubated in progestin receptor antisera that had been previously preabsorbed overnight at 4 °C with 200 μg/ml of the antigen peptide (amino acids 533–547; Genosys Biotechnologies, Inc., The Woodlands, TX, USA), which eliminated brown nuclear labelling throughout the preoptic area and hypothalamus. Unless otherwise stated, all reagents and chemicals were purchased from Sigma.

Analyses

Three sections through the intermediate preoptic area (POA) separated from the next by 70 μm beginning at approximately 0.260 mm from bregma (13), and three sections through the caudal POA/anterior hypothalamus (where both the POA and anterior hypothalamus appear on the same coronal section) beginning at appoximately 1.300 mm from bregma (13), were analysed bilaterally for the number of cells that expressed only tyrosine hydroxylase, or tyrosine hydroxylase and progestin receptor (indicated by a light blue cytoplasmic label surrounding a dark brown nuclear label). These sites contain the rostral and caudal portions of the A14 dopamine cell group, respectively, and the rostral intermediate POA sections included the dopamine cells of the anteroventral periventricular preoptic area. Nine sections through the rest of the hypothalamus, beginning at approximately 2.300 mm from bregma and each separated from the next by 70 μm, contained the arcuate nucleus (A12) and were also analysed bilaterally. Other areas of brain that contain dopamine-synthesizing cell groups, including the zona incerta (A13), posterodorsal hypothalamus (A11), retrorubral field (A8), ventral tegmental area (A10) and substantia nigra pars compacta (A9), were examined visually. The intermediate POA of one oestradiol benzoate-treated female was not analysable due to damaged brain sections. Data were analysed with two-way analysis of variance, using group (oil or oestradiol benzoate) and rostrocaudal level as factors, followed by Fisher's LSD post-hoc tests. $P < 0.05$ was considered statistically significant.

Results

Periventricular POA (rostral A14)

The distribution of cells that express tyrosine hydroxylase and progestin receptors was found to be similar to that reported previously (10, 14–16). Many tyrosine hydroxylase- and progestin receptor-labelled cells were found at the level of the POA of oestradiol benzoate-treated females, with approximately 20% of all tyrosine hydroxylase-labelled cells also expressing progestin receptor, compared to approximately 10% for oil-treated females [$F(1,30) = 34.8$, $P \leq 0.0001$] (Figs 1 and 2). As was true for the entire hypothalamic region, almost all tyrosine hydroxylase-labelled cells were found adjacent or very close to the third ventricle and many more dual-labelled cells were found ventrally in each section. There were no significant effects of rostrocaudal level [$F(2,30) = 1.1$, $P \geq 0.3$] or group by rostrocaudal level interaction [$F(2,30) = 1.7$, $P \geq 0.2$] on the percentage of tyrosine hydroxylase-containing cells in the POA that also expressed progestin receptor. Oestradiol benzoate- and oil-treated females did not differ in the total number of tyrosine hydroxylase-containing cells [$F(1,30) = 0.2$, $P \geq 0.6$], and

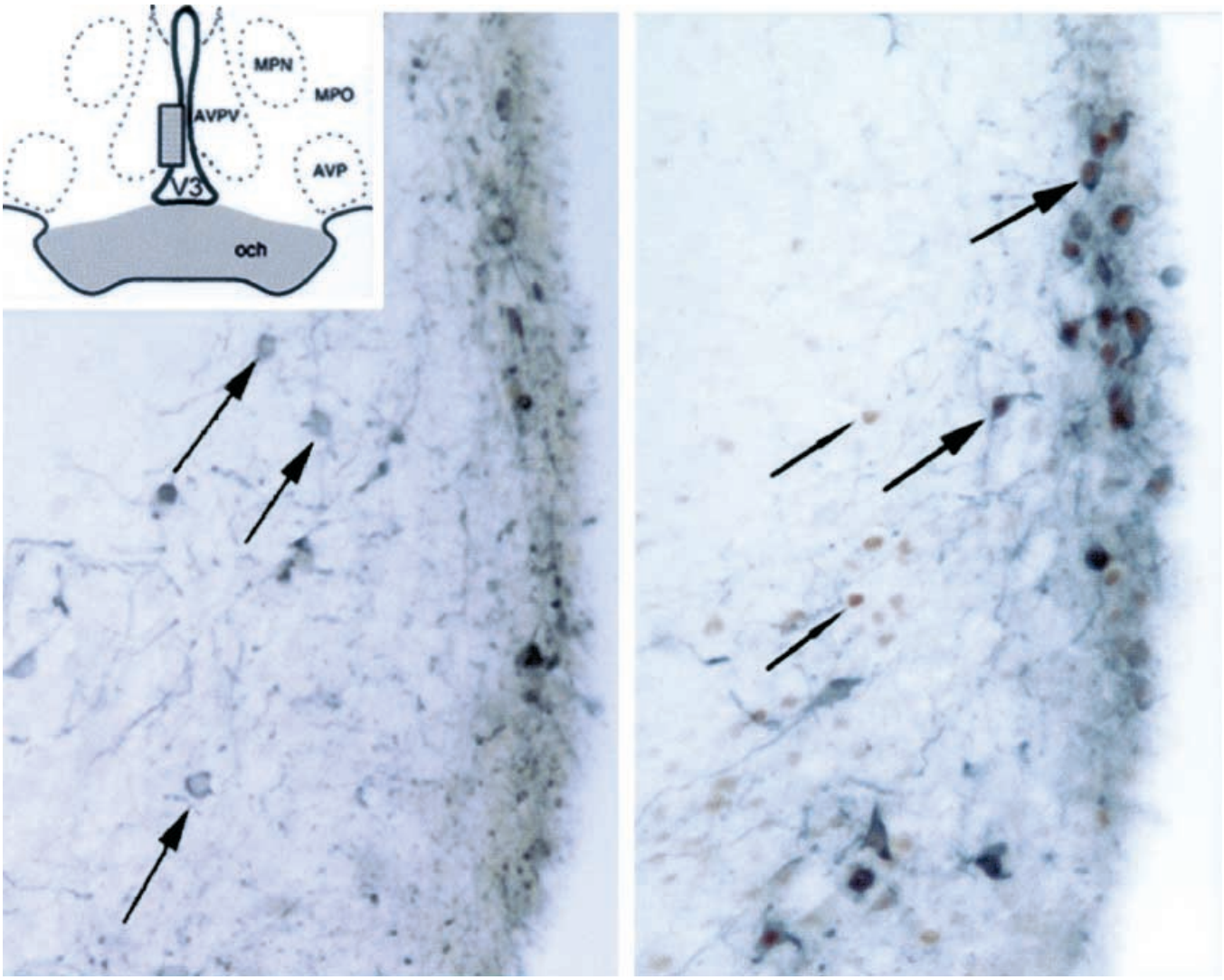

FIG. 1. High-magnification (× 20) photomicrographs of tyrosine hydroxylase-immunoreactivity (ir) and progestin receptor-ir in the periventricular preoptic area of oil-treated (left) and oestradiol benzoate-treated (right) female rats. Grey arrows in the left panel indicate cells containing tyrosine hydroxylase-ir, small black arrows on the right panel indicate nuclei containing progestin receptor-ir, and large black arrows on the right panel indicate dual-labelled cells. Grey-shaded rectangle on inset represents approximate area shown in photomicrographs; modified from (13). AVP, Anteroventral preoptic nucleus; och, optic chiasm; MPN, medial preoptic nucleus; MPO, medial preoptic area; V3, third ventricle.

there were no significant effects of rostrocaudal level [$F(2,30) = 1.3$, $P \geq 0.2$] or the interaction between group and rostrocaudal level [$F(2,30) = 0.4$, $P \geq 0.7$].

Caudal periventricular POA/anterior hypothalamus (caudal A14)

At the level of the caudal POA/anterior hypothalamus, approximately 15% of tyrosine hydroxylase-containing cells of oestradiol benzoate-treated females also expressed progestin receptor, compared to approximately 3% in oil-treated controls [$F(1,33) = 35.5$, $P \leq 0.0001$] (Fig. 3). No significant rostrocaudal level [$F(2,33) = 0.4$, $P \geq 0.6$] or group by level interaction [$F(2,33) = 0.1$, $P \geq 0.9$] was found for the number of dual-labelled cells. Oestradiol benzoate treatment did not affect the total number of tyrosine hydroxylase-containing cells [$F(1,33) = 2.6$, $P \geq 0.1$]. There was a significant effect of rostrocaudal level [$F(2,33) = 7.3$, $P \leq 0.005$], such that more tyrosine hydroxylase-containing cells were found at the most caudal level of analysis compared to the most rostral level. The group by level interaction was not significant [$F(2,33) = 0.1$, $P \geq 0.8$].

Posteroventral hypothalamus/arcuate nucleus (A12)

Similar to the POA and the caudal POA/anterior hypothalamus, many tyrosine hydroxylase-containing cells of more posterior areas of the periventricular hypothalamus of oestradiol benzoate -treated females also contained progestin receptors [group: $F(1,99) = 460.3$, $P \leq 0.0001$] (Figs 4 and 5). An increasing number of tyrosine hydroxylase-containing cells expressed progestin receptors rostrocaudally, with 26% of the tyrosine hydroxylase cells being dual-labelled at the most rostral level of analysis while almost 55% of tyrosine

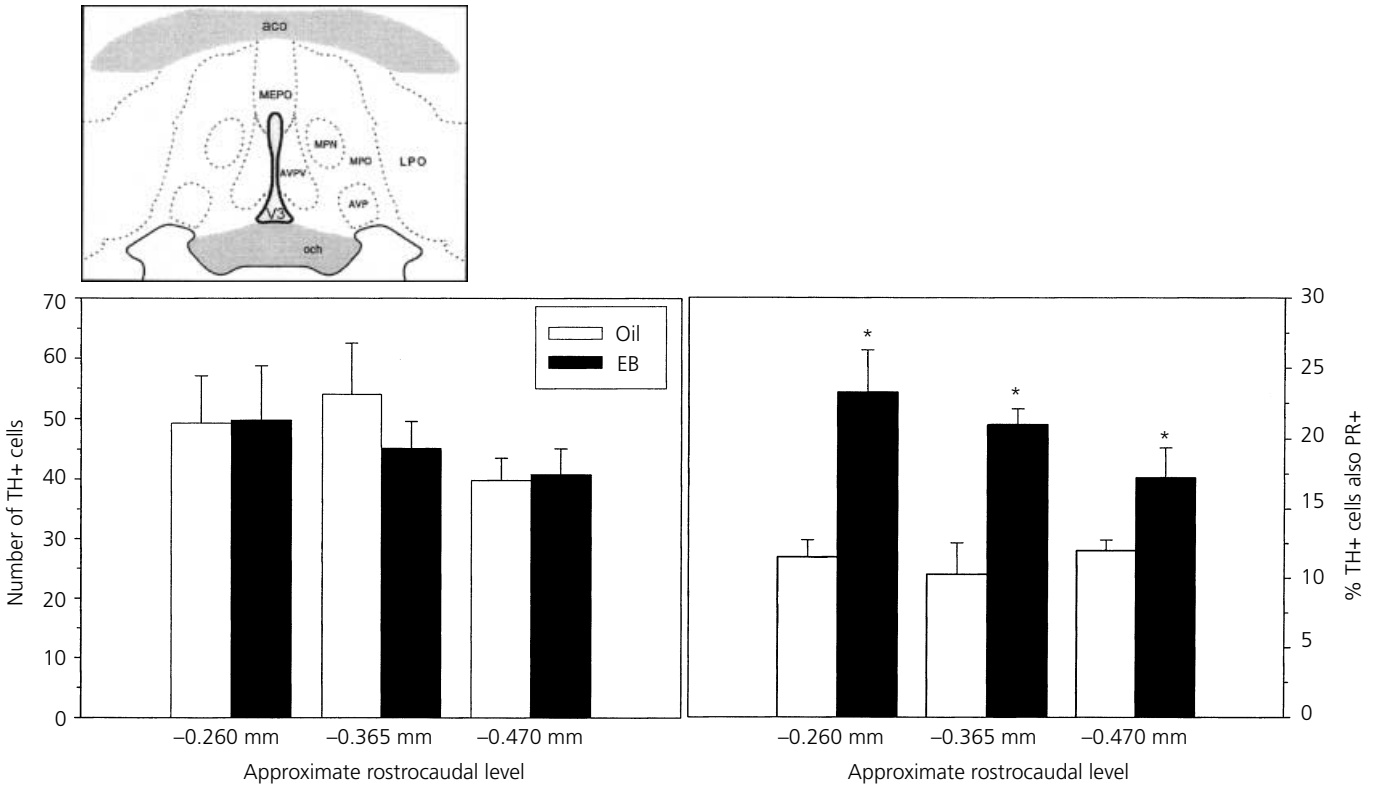

FIG. 2. Mean ± SEM number of cells containing tyrosine hydroxylase-immunoreactivity (ir) (left) and percentage of tyrosine hydroxylase immunoreactive cells also expressing progestin receptor-ir (right) in the intermediate preoptic area of oil- and oestradiol benzoate-treated female rats. Section location in reference to bregma according to Swanson's atlas of the rat brain (13), with modified atlas figure showing the approximate level of the first section analysed. *$P \leq 0.05$. aco, Anterior commisure; AVP, anteroventral preoptic nucleus; AVPV, anteroventral periventricular nucleus; LPO, lateral preoptic area; och, optic chiasm; MEPO, median preoptic nucleus; MPN, medial preoptic nucleus; MPO, medial preoptic area; V3, third ventricle.

hydroxylase-containing cells also expressed progestin receptor at the most caudal level analysed [level: $F(8,99) = 3.433$, $P \leq 0.002$]. The group by level interaction was not significant [$F(8,99) = 2.0$, $P \geq 0.05$]. Groups did not differ in the number of tyrosine hydroxylase-containing cells [group: $F(1,99) = 0.1$, $P \geq 0.8$; group by level: $F(8,99) = 0.4$, $P \geq 0.9$]. There was a significant effect of rostrocaudal level, though, with fewer tyrosine hydroxylase-labelled cells caudally than rostrally [$F(8,99) = 27.6$, $P \leq 0.0001$].

Other sites

Although many tyrosine hydroxylase-labelled cells were found in the zona incerta (A13), dorsomedial hypothalamus (A11), retrorubral field (A8), ventral tegmental area (A10) and substantia nigra (A9), no dual-labelled cells were found in these sites because progestin receptor-immunoreactivity (ir) was rare or absent. We did observe what appeared to be light progestin receptor-ir dorsolateral to the tyrosine hydroxylase-ir cells in the ventral tegmental area and in the lateral substantia nigra pars compacta (Fig. 6). However, labelling in these two areas may be artifactual because, unlike progestin receptor-ir in the hypothalamus, it was not prevented by preabsorption of the primary antisera for progestin receptor with its antigen. No progestin receptor labelling was found in the tyrosine hydroxylase-expressing cells of the dorsocaudal component of the A10 group in the ventral periaqueductal grey and dorsal raphe.

Discussion

Progestin receptor and tyrosine hydroxylase expression in the zona incerta, retrorubral field, ventral tegmental area and substantia nigra

Although progestin receptors were found in many dopaminergic cell groups after oestradiol treatment, we found no evidence of progestin receptor expression in dopaminergic cells of the zona incerta, consistent with previous reports in female rats (10, 11), guinea-pigs (17) and macaques (18). The dopamine projections from the zona incerta have been studied for a role in the control of gonadotropin release necessary for ovulation (19), as well as for sexual receptivity in female rats (20). However, it is not clear if progesterone is capable of altering dopaminergic activity of the zona incerta (19–23). Even if progesterone does affect dopaminergic activity in the zona incerta, the absence of progestin receptors in dopaminergic cells suggests that it does not do this by binding to intracellular progestin receptor within these cells. Similarly, apparently little is known about how hormones modulate activity of the A8 dopaminergic cells in the retrorubral field. Although we found that none express progestin receptors, a small number of these cells do express oestrogen receptors (24, 25). These cells contribute to the mesolimbic dopamine system and innervate a variety of neural sites important for the display of reproductive behaviours in rats (26), as well as influence activity of dopaminergic

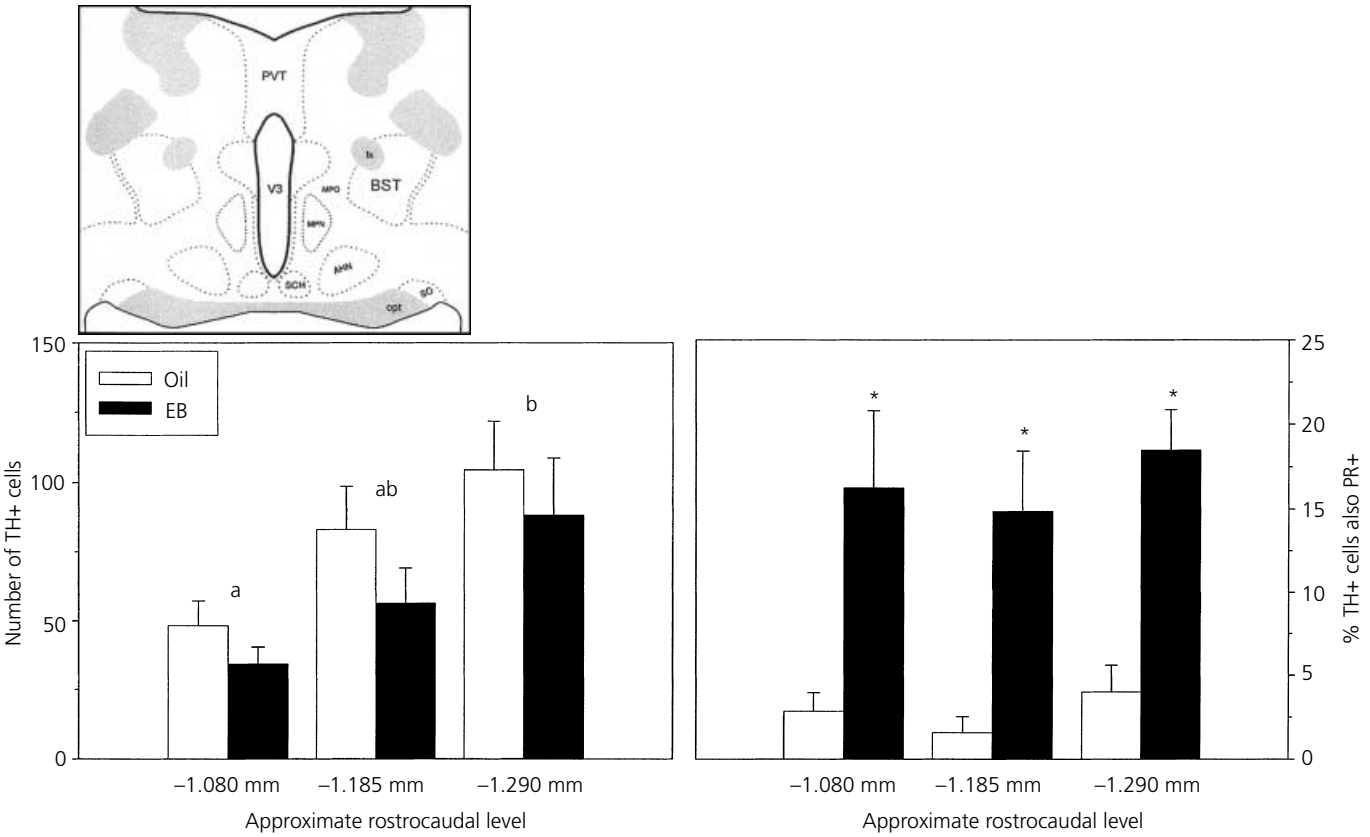

FIG. 3. Mean ± SEM number of tyrosine hydroxylase-immunoreactivity (ir) cells (left) and percentage of tyrosine hydroxylase-ir cells also containing progestin receptor-ir (right) in the caudal preoptic area/anterior hypothalamus of oil- and oestradiol benzoate-treated female rats. Section location in reference to bregma according to Swanson's atlas of the rat brain (13), with modified atlas figure showing the approximate level of the first section analysed. In the left panel, there are significant differences between rostrocaudal levels collapsed across group (indicated by different letters above bars). In the right panel, there are significant differences between EB and oil groups (*$P \leq 0.05$). AHN, Anterior hypothalamic nucleus; BST, bed nucleus of the stria terminalis; fx, fornix; PVT, paraventricular thalamic nucleus; SCH, suprachiasmatic nucleus; MPN, medial preoptic nucleus; MPO, medial preoptic area; so, supraoptic nucleus.

cells in the substantia nigra and ventral tegmental area (27). The retrorubral field also receives a projection from cells in the POA and bed nucleus of the stria terminalis that are activated during the performance of sexual or maternal behaviours (28, 29), and which might alter dopamine release necessary for these behaviours.

We also observed no progestin receptor-ir in tyrosine hydroxylase-containing cells of the ventral tegmental area or substantia nigra, consistent with that reported by Sar (11). This is despite the well-known effects of progesterone on dopamine release within the mesolimbic and nigrostriatal pathways (30–33), and the reproductive behaviours they influence (2, 34). This absence of progestin receptor-ir is not exclusive to ovariectomized rats treated with oil or oestradiol, because rats sacrificed on either day 18 of pregnancy or within 24 h after parturition also do not show progestin receptor-ir within tyrosine hydroxylase-ir cells in these sites (nor in the zona incerta and retrorubral field; Lonstein and Blaustein, preliminary data). The dopaminergic cells of the ventral tegmental area and substantia nigra also do not express progestin receptor-ir during neonatal development in rats, even though progestin receptor expression in these areas is at its developmental peak (35). We did observe what appeared to be progestin receptor-ir labelling dorsolateral to the ventral tegmental area and in the dorsolateral substantia nigra, but because this labelling was not prevented by preabsorbing the primary antisera with its antigen, we cannot conclude that this labelling reflects nuclear progestin receptors. However, there is a report of high progestin receptor mRNA expression in the ventral tegmental area and substantia nigra (36), and this issue may require further attention.

Our results in these sites are consistent with the idea that progesterone, similar to oestradiol, acts through a variety of cellular mechanisms in addition to any nuclear progestin receptor-mediated effects on gene regulation. For example, dopaminergic cells of the substantia nigra and ventral tegmental area may have membrane-bound progestin receptors that influence the display of sexual and other behaviours (37, 38).

Progestin receptor expression in dopaminergic cells of the arcuate nucleus

Our results suggest that many dopaminergic cells found near the third ventricle of female rats are potential targets of circulating progesterone, with possible relevance to numerous reproductive processes. The greatest percentage of tyrosine hydroxylase-containing cells that expressed

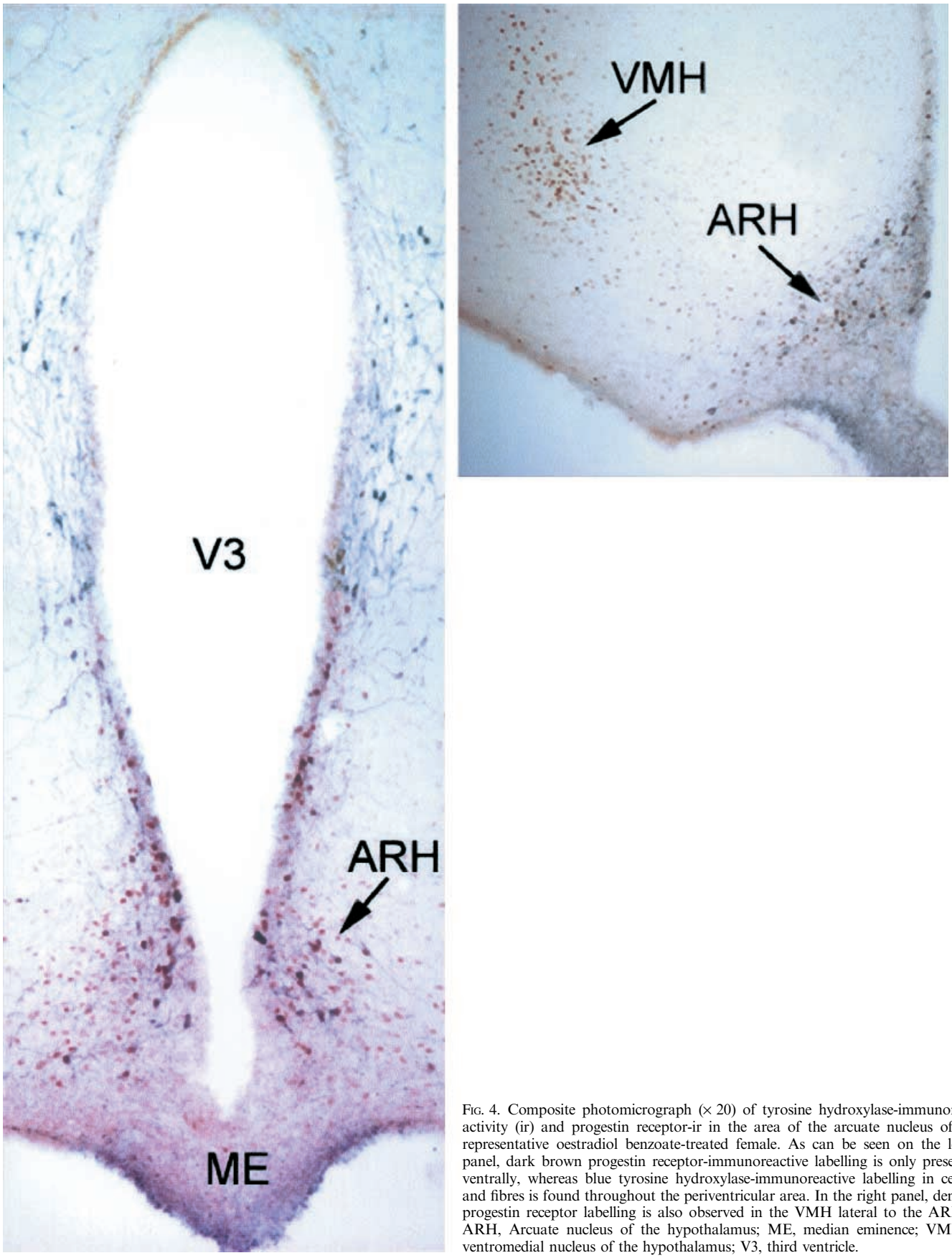

FIG. 4. Composite photomicrograph (× 20) of tyrosine hydroxylase-immunoreactivity (ir) and progestin receptor-ir in the area of the arcuate nucleus of a representative oestradiol benzoate-treated female. As can be seen on the left panel, dark brown progestin receptor-immunoreactive labelling is only present ventrally, whereas blue tyrosine hydroxylase-immunoreactive labelling in cells and fibres is found throughout the periventricular area. In the right panel, dense progestin receptor labelling is also observed in the VMH lateral to the ARH. ARH, Arcuate nucleus of the hypothalamus; ME, median eminence; VMH, ventromedial nucleus of the hypothalamus; V3, third ventricle.

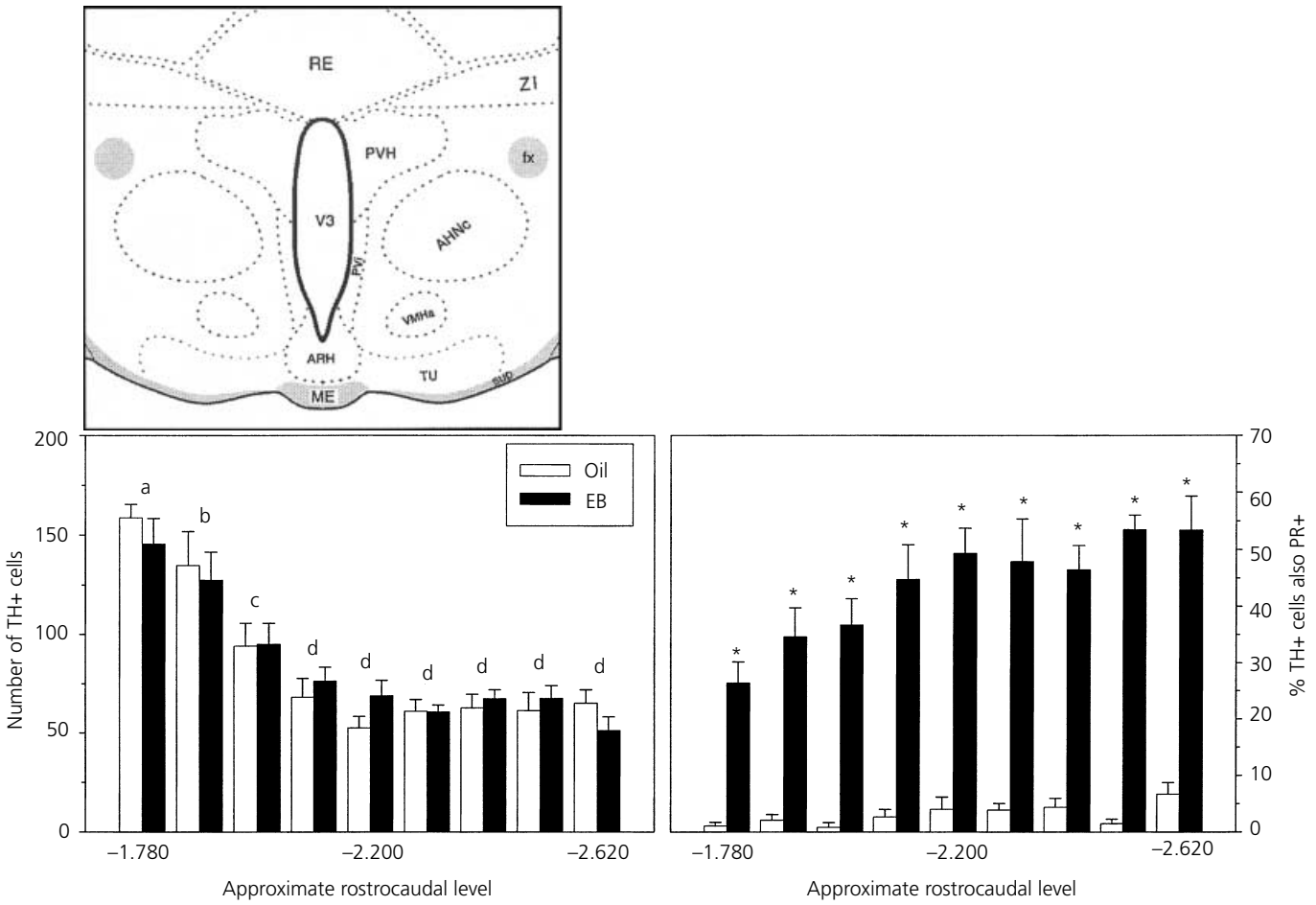

FIG. 5. Mean ± SEM number of cells containing tyrosine hydroxylase-immunoreactivity (ir) (left) and percentage of tyrosine hydroxylase immunoreactive cells also containing progestin receptor-ir (right) at the level of the arcuate nucleus of oil- and oestradiol benzoate-treated female rats. Dopamine cells surrounding the entire third ventricle were included in analysis. Section location in reference to bregma according to Swanson's atlas of the rat brain (13), with modified atlas figure showing the approximate level of the first section analysed. In the left panel, there are significant differences between rostrocaudal levels collapsed across group (indicated by different letters above bars). In the right panel, there are significant differences between EB and oil groups (*$P \leq 0.05$). AHNc, Anterior hypothalamic nucleus, central part; ARH, arcuate nucleus of the hypothalamus; fx, fornix; PVH, paraventricular nucleus of the hypothalamus; RE, nucleus reuniens; VMHa, ventromedial nucleus of the hypothalamus; anterior part; V3, third ventricle; ZI, zona incerta.

progestin receptor was in the area of the arcuate nucleus (A12), with over half of these cells expressing progestin receptor at some rostrocaudal levels. The relatively high coexpression of tyrosine hydroxylase with progestin receptor in the rat arcuate nucleus has been reported previously by Fox *et al.* (10), who used immunocytochemistry for tyrosine hydroxylase and autoradiography for progestin receptor, and found that approximately 90% of tyrosine hydroxylase-immunoreactive neurones in the arcuate bind a synthetic progestin. Using similar methods, Sar (11) also reported that arcuate dopamine neurones bind progesterone, but with lower frequency (30–40%). There are methodological differences between the present study and the two previous studies that likely explain the differing number and percentage of tyrosine hydroxylase-expressing cells coexpressing progestin receptor. These include the use of adult (10, present study) versus prepubertal females (11), the use of progestin receptor immunocytochemistry (present study) versus progestin receptor autoradiography (10, 11), and the extent of the periventricular area analysed. In the present study and that of Sar (11), the entire periventricular area was analysed, whereas Fox *et al.* (10) analysed just the ventral periventricular area, which contains the majority of dopaminergic cells. We also noted that many more tyrosine hydroxylase-expressing cells of the ventral arcuate nucleus were dual labelled compared with tyrosine hydroxylase-expressing cells positioned dorsally.

The relatively large number of dopamine cells in the arcuate nucleus that are targets for progesterone suggests that this area as a whole is more sensitive to the potential actions of progesterone at the cell nucleus than most other dopaminergic cell groups in the brain. Furthermore, there was almost no constitutive progestin receptor expression in the arcuate dopaminergic cells, as indicated by the lack of progestin receptor and tyrosine hydroxylase coexpression in oil-treated females, indicating that many dopamine cells that express progestin receptor must also be sensitive to oestradiol (39, 40). There are distinct species differences in oestradiol-induced progestin receptor expression within arcuate dopamine cells. By contrast to female rats, oestradiol-treated female

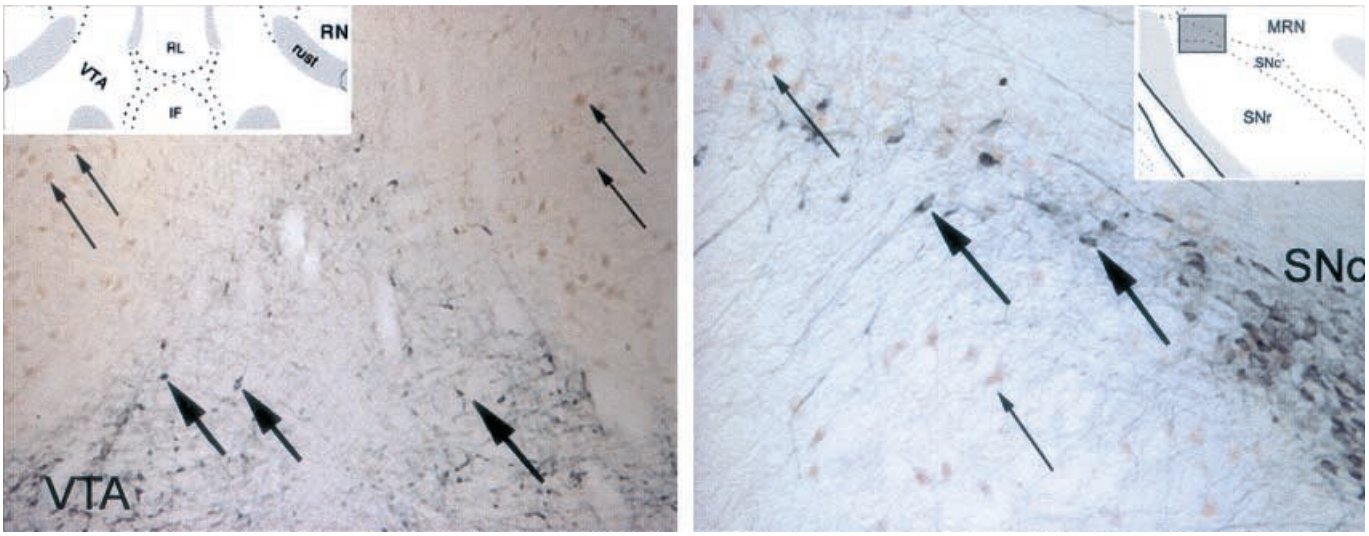

FIG. 6. Photomicrographs of tyrosine hydroxylase-immunoreactivity (ir) and putative progestin receptor-ir dorsolateral to the VTA (left; 10 × magnification) and in the dorsolateral SN pars compacta (SNc) (right; 20 × magnification). Insets modified from (13). Grey-shaded area on right panel indicates approximate area shown in photomicrograph. Larger black arrows show single-labelled tyrosine hydroxylase-ir cells, smaller arrows show putative progestin receptor-ir cells. IF, Interfascicular raphe nucleus; MRN, mesencephalic reticular nucleus; RL, rostral linear raphe nucleus; RN, red nucleus; rust, rubrospinal tract; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area.

macaques show no progestin receptor expression in arcuate dopamine cells but have many dual-labelled cells more dorsally (18), and only approximately 10% of tyrosine hydroxylase-expressing cells in the arcuate nucleus of oestradiol-treated female guinea-pigs express progestin receptors (17).

The dopamine cells of the arcuate nucleus are the source of the neurones of the tuberoinfundibular dopamine system, which are well-known for regulating prolactin synthesis and release (41). Progesterone contributes to the oestradiol-induced prolactin surge during proestrus (42), maintains surges of prolactin during early pregnancy (43, 44 45) and inhibits them during late pregnancy (45), and can inhibit prolactin release during lactation (46). It is apparently not known which dopamine-synthesizing cells of the arcuate nucleus are involved in regulating prolactin secretion, but most (over 70%) of them project to the median eminence (47). The presence of progestin receptors in tuberoinfundibular dopamine system cells provides a possible mechanism by which progesterone can directly affect this system and the variety of processes influenced by prolactin. As noted previously, many of the tyrosine hydroxylase immunoreactive cells in the rat arcuate nucleus also express oestrogen receptor α (39, 40), offering yet another mechanism by which ovarian hormones can modulate prolactin release.

Progestin receptor expression in dopamine cells of the POA and anterior hypothalamus

By contrast to previous reports (10, 11) indicating that dopaminergic cells of the periventricular POA and anterior hypothalamus do not bind progestins, we found that a moderate number (approximately 15–20%) of tyrosine hydroxylase immunoreactive cells do express progestin receptor. Similar results have been found in the female macaque (18). Again, it is possible that our immunocytochemical methods allowed greater ability to detect progestin receptors in the POA and anterior hypothalamus than the autoradiographic techniques used by Fox *et al.* (10) and Sar (11). As far as we are aware, this is the first demonstration of a neural substrate within the POA/anterior hypothalamus that is both sensitive to the actions of progesterone on the cell nucleus and involved with dopaminergic activity. The function for any progesterone effects on dopamine cells of the periventricular POA/anterior hypothalamus is unknown, partly because it is uncertain where these cells project. Some project to the median eminence, and a very small number project to the posterior pituitary (47). Preliminary data also show that a small number of them project back to more posterior parts of the POA (48). The POA is important for maternal and sexual behaviours in rats, and the effects of progesterone on these dopaminergic neurones might be critical for their display (8, 9). Progesterone may also alter dopamine release in the POA indirectly through projections received from other progestin receptor-sensitive sites in the brain, such as the amygdala (49). Dopamine release in the POA is also important for nonbehavioural functions. Some of the POA sections analysed included the anteroventral periventricular area, which contains a group of sexually dimorphic dopamine cells involved in gonadotropin release in rats (50, 51), and progesterone may influence gonadotropin release (52) via direct actions on dopaminergic cells in this site.

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